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BALANÇO TÉRMICO E METABÓLICO DURANTE O EXERCÍCIO FÍSICO: PARTICIPAÇÃO DOS RECEPTORES AT1 PARA ANGIOTENSINA II

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Belo Horizonte, Outubro de 2009

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ÍNDICE

I. RESUMO

A angiotensina II pode interferir no desempenho físico ao induzir ajustes termorregulatórios e metabólicos caracterizados por hipotermia, facilitação da dissipação cutânea de calor, diminuição da taxa metabólica e hiperglicemia. Para avaliar o papel do sistema angiotensinérgico central sobre o equilíbrio térmico, mobilização de substratos energéticos e fadiga central durante o exercício aeróbio em ratos, salina (Sal) ou losartan (Los) foram injetados no ventrículo lateral direito antes do exercício contínuo (velocidade de 18 m/min, 5 % de inclinação; ~66% VO_2) ou progressivo (velocidade inicial de 10 m/min com aumento de 1 m/mim a cada 3 minutos, 10% de inclinação) até a fadiga em esteira. Foram analisados os seguintes parâmetros: (1) consumo de oxigênio durante o exercício contínuo e progressivo; (2) dosagem plasmática de glicose, lactato e ácidos graxos livres durante o exercício progressivo; e (3) temperatura corporal interna e dosagem de serotonina (5-HT), ácido 5-hidroxindoleacético (5-HIAA), dopamina (DA) e ácido 3,4 diidroxifenilacético (DOPAC) na área pré-óptica, hipotálamo, hipocampo e córtex frontal no momento da fadiga após exercício contínuo. Com base nas medidas foram calculados: trabalho realizado, eficiência mecânica e taxa de aquecimento corporal. Independente do tipo de exercício aeróbio, o tratamento com Los reduziu o trabalho realizado pelos animais ($p<0,02$). Esses animais apresentaram maior consumo de oxigênio para ambos os protocolos de exercício ($p<0,05$), além de menor eficiência mecânica ($p<0,05$), a qual se correlacionou inversamente com o trabalho realizado

($p<0,01$). Os animais tratados com Los também apresentaram hiperglicemia, elevação de lactato e ácidos graxos livres até a fadiga, já verificada em baixa intensidade de exercício como 20% do trabalho máximo realizado ($p<0,05$). A elevada taxa de aquecimento corporal verificada nos animais injetados com Los correlacionou-se diretamente com o aumento da concentração de 5-HT na área pré-óptica ($p<0,01$) e hipotálamo ($p<0,01$). A concentração de 5-HT nessas áreas mostrou-se inversamente relacionada com o reduzido tempo de exercício dos ratos Los. Apesar dos níveis de DA não apresentarem alteração em nenhuma das áreas estudadas, o hipotálamo dos animais Los mostrou elevada razão 5-HT:DA ($p<0,01$) que correlacionou-se diretamente com a taxa de aquecimento corporal ($p<0,01$) e inversamente com o tempo de exercício ($p<0,05$). Os dados demonstram que o sistema angiotensinérgico central está envolvido com modulação da produção de calor e melhora da eficiência mecânica durante o exercício físico, exercendo importante efeito sobre o conteúdo central de 5-HT, cuja interação com a DA parece afetar a fadiga central através da modulação da temperatura corporal. O sistema angiotensinérgico central também está envolvido em ajustes metabólicos durante o exercício, alterando a mobilização de substratos energéticos semelhante a situações de ativação simpática intensa e prematura.

Com o intuito de avaliar o envolvimento do núcleo paraventricular do hipotálamo (PVN) nos ajustes cardiovasculares induzidos pelo estresse térmico, os quais são fundamentais para a redistribuição sanguínea para a periferia e dissipação de calor, foram registradas a atividade simpática do nervo renal

(RSNA), pressão arterial média (MAP), frequência cardíaca (HR), temperaturas corporal interna e da cauda em ratos anestesiados durante a exposição ao calor. Antes do aquecimento, CSF, lidocaína ou L-NMMA foram injetados bilateralmente no PVN. O estímulo térmico resultou em bloqueio do aumento da RSNA e da MAP e atenuação da HR após bloqueio do PVN com lidocaína, sugerindo redução da vasoconstrição renal ($p<0,05$). No entanto, o limiar térmico para vasodilatação da cauda não foi afetado pelo tratamento com lidocaína. A RSNA, HR e MAP dos animais injetados com L-NMMA aumentaram proporcionalmente ao estresse térmico. Porém, o limiar térmico de vasodilatação da cauda foi maior nos animais L-NMMA ($p<0,05$), indicando prejuízo na dissipação cutânea de calor. Os dados sugerem que, durante o estresse térmico, o PVN tem ação importante na regulação da atividade simpática que contribui para os ajustes cardiovasculares responsáveis pela redistribuição de sangue interno para a periferia e perda de calor. Além disso, um possível mecanismo através do qual o aquecimento eleva a dissipação cutânea de calor ocorre via disponibilidade de óxido nítrico no PVN.

II. ABSTRACT

Angiotensin II may interfere on physical performance by inducing thermoregulatory and metabolic adjustments characterized by hypothermia, improvement of coetaneous heat loss, decrease of metabolic rate and hyperglycemia. To asses the effect of central angiotensinergic system on heat balance, energetic substrates mobilization and central fatigue during aerobic exercise in rats, saline (Sal) or losartan (Los) were intracerebroventricularly injected before continuous (18 $m.min^{-1}$, 5% inclination; ~66% VO_2) or graded (starting at 10 m/min, increments of 1 m/min every 3 minutes, 10% inclination) running until fatigue. The following parameters were analyzed: (1) oxygen consumption during continuous and graded exercise (2) measurement of glucose, lactate and free fatty acids concentrations during graded exercise; and (3) body temperature and measurement of serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), dopamine (DA) and 3,4-Dihydroxyphenylacetic acid (DOPAC) in preoptic area, hypothalamus, hippocampus and frontal cortex at the moment of fatigue after continuous exercise. Based on the results workload, mechanical efficiency and body heating rate were calculated. Regardless of exercise type, workload of Los-treated rats was lower than control rats ($p<0.02$). These animals had higher oxygen consumption, both during continuous and graded exercise ($p<0.05$), as well as lower mechanical efficiency ($p<0.05$) that correlated inversely with workload ($p<0.01$). Los-animals also showed a faster hyperglycemic response, higher

levels of lactate and free fatty acids until fatigue, already seen at low exercise intensity as 20% of maximal work ($p<0.05$). Los-rats exhibited increased body heating rate that was related to the higher 5-HT concentration in preoptic area ($p<0.01$) and hypothalamus ($p<0.01$). The content of 5-HT in these areas was inversely related with the reduced time to fatigue of Los-rats ($p<0.05$). Although the levels of DA were not altered in any of the studied brain areas, hypothalamus of Los-treated animal showed higher 5-HT:DA ratio ($p<0.01$) that correlated directly with body heating rate ($p<0.01$) and indirectly with time to fatigue ($p<0.05$). The data demonstrate that central angiotensinergic transmission is involved with modulation of heat production and improvement of mechanical efficiency during exercise, performing important effects on brain 5-HT content, whose interaction with DA seems to affect central fatigue through modulation of body temperature. The central angiotensinergic system is also related with metabolic adjustments during exercise, shifting energy balance similarly to situations of enhanced and premature sympathetic activation.

With the purpose of evaluating the involvement of the paraventricular nucleus of the hypothalamus (PVN) on cardiovascular adjustments induced by heat stress, which are critical for the redistribution of blood flow to the periphery and heat loss, renal sympathetic nerve activity (RSNA), mean arterial pressure (MAP), heart rate (HR), body temperature and tail temperature were measured in anesthetized rats during heat stress. Before heating, CSF, lidocaine or L-NMMA were bilaterally injected into the PVN. This heat stimulus resulted in blunted RSNA and MAP and attenuation of HR increase after blockade of the PVN with

lidocaine, suggesting a decreased renal vasoconstriction ($p<0.05$). However, body temperature threshold for tail vasodilation was not affected by lidocaine treatment. RSNA, HR and MAP in L-NMMA injected rats increased according to the heating stress. Still, a higher Δbody temperature until tail vasodilation was shown by L-NMMA injected, which is an indicative of impaired coetaneous heat loss ($p<0.05$). The data suggest that the PVN is critical for enhancing sympathetic activity to heating, contributing to the cardiovascular adjustments elicited by heat stress that influence core blood redistribution to the periphery and heat loss. Furthermore, one possible mechanism by which heating increases heat loss through coetaneous vasodilation is via nitric oxide within the PVN.

III. INTRODUÇÃO

FADIGA E EXERCÍCIO FÍSICO

A fadiga induzida pelo exercício físico é um fenômeno multifatorial que envolve interação complexa entre fatores fisiológicos e psicológicos. Ela pode ser definida como inabilidade em manter força ou potência requerida, ou ainda como dificuldade em manter a taxa de trabalho (Fernstrom & Fernstrom, 2006; Foley & Fleshner, 2008; Meeusen et al., 2007; Nielsen & Nybo, 2003). É importante destacar que a fadiga é considerada mecanismo de defesa por prevenir ameaças à homeostase através de redução forçada da intensidade da atividade física ou a cessação da mesma (Gandevia, 2001; Kay & Marino, 2000; Noakes, 1998). Os fatores que desencadeiam a fadiga têm origem periférica e/ou central, sendo que a última é consequência de falha do sistema nervoso central em proporcionar motivação adequada para manutenção do exercício (Gandevia, 2001; Kay & Marino, 2000; Noakes, 1998). Os mecanismos fisiológicos propostos como precipitadores da fadiga incluem perturbações metabólicas, cardiovasculares e do sistema nervoso central, muitas vezes associados à temperatura corporal interna elevada (Fernstrom & Fernstrom, 2006; Foley & Fleshner, 2008; Meeusen et al., 2007; Nielsen & Nybo, 2003).

Hipertermia

O desbalanço térmico é descrito como fator importante para o estabelecimento da fadiga (Nybo, 2008). O aumento da temperatura corporal interna em função do exercício aeróbio é consequência do descompasso entre a inerente elevação do calor metabolicamente produzido e a dissipação do mesmo (Gleeson, 1998; Webb, 1995). Essas alterações ocorrem em decorrência do metabolismo corporal aumentado dos músculos em atividade (Galbo, 1985; Romijn et al., 1993). O cérebro é especialmente vulnerável a hipertermia, indutora de apoptose neuronal, e a fadiga central constitui mecanismo protetor da integridade do sistema nervoso central (Nielsen & Nybo, 2003). O rápido aumento da temperatura corporal interna e da taxa de acúmulo de calor são fatores considerados limitantes do exercício físico prolongado (González-Alonso et al., 1999; Rodrigues et al., 2003), uma vez que reduzem o impulso do sistema nervoso central para o desempenho físico. Sendo assim, o cérebro e a homeostasia são protegidos pela instalação da fadiga (Nielsen & Nybo, 2003).

A atividade física promove o aumento das taxas de produção e dissipação de calor proporcional à intensidade e duração do exercício (Harri et al., 1982). O consumo de oxigênio durante o exercício aeróbio (i.e., taxa metabólica) é um parâmetro importante que reflete tanto a produção de calor quanto a eficiência mecânica e o desempenho físico (Brooks & White, 1978; Sonne & Galbo, 1980). A eficiência energética do corpo varia durante o exercício físico, sendo aproximadamente 20-27% da energia consumida utilizada para trabalho externo,

enquanto o ATP restante é utilizado para homeostase ou dissipado sob a forma de calor (Brooks et al., 1984). Deve-se destacar que a produção de calor é considerada a variável primária desencadeadora da resposta de dissipação de calor durante a atividade física (Dawson & Keber, 1979; O`Leary et al., 1985, Webb, 1995).

Durante os primeiros minutos do exercício aeróbio, denominada fase dinâmica de balanço térmico, o aumento exagerado da produção de calor, não compensado por sua dissipação, acarreta elevação abrupta da temperatura corporal interna concomitante com o exercício físico (Briese, 1998). A vasoconstrição cutânea mediada pelo sistema nervoso simpático (Hartley et al., 1972; McAllister et al., 1995) dificulta a perda de calor durante este estágio do exercício. A fase estável de balanço térmico do exercício aeróbio inicia-se a partir do momento no qual o tônus simpático periférico cutâneo é superado, isto é, quando o limiar térmico para a vasodilatação cutânea é atingido. Consequentemente, a perda de calor por dissipação é facilitada pela indução da vasodilatação da pele, aproximando-se da taxa de produção de calor e provocando aumento menos acentuado da temperatura corporal interna até a interrupção da atividade física.

A variação do consumo de oxigênio durante a atividade física aeróbia acompanha o mesmo padrão da temperatura corporal interna, isto é, eleva-se consideravelmente na fase dinâmica do exercício até alcançar um platô (Barstow, 1994, Lacerda et al., 2006 a). A partir daí, permanece relativamente inalterada durante a fase estável do exercício devido ao equilíbrio entre a

energia necessária para contração muscular e a produção de ATP pelo metabolismo aeróbico (Barstow, 1994). Entretanto, o consumo de oxigênio na fase estável do exercício apresenta um segundo aumento, menos abrupto, sugerindo uma possível redução na eficiência mecânica nesta fase do esforço (Schrauwen & Hesselink, 2003).

O modelo de balanço térmico durante o exercício aeróbio proposto para humanos se ajusta também para ratos. A figura 1 ilustra dados obtidos durante atividade física aeróbia contínua em esteira em ratos (Lacerda et al., 2005, 2006 a). A interação entre as variáveis de ajuste da temperatura corporal representados pelo consumo de oxigênio (produção de calor) e temperatura da cauda (dissipação de calor), cuja dinâmica afeta a variação da temperatura corporal interna de acordo com o padrão citado anteriormente, pode ser facilmente constatada. A rápida elevação da taxa metabólica (variável primária), observada na fase dinâmica do exercício, é acompanhada por breve queda da temperatura da cauda, indicativa de vasoconstrição cutânea (variável secundária). Essa relação culmina em aumento exacerbado da temperatura corporal interna nessa fase devido ao desequilíbrio entre produção e dissipação de calor. Quando o limiar térmico para vasodilatação cutânea é atingido, a troca de calor ocorre em intensidade suficiente para dissipar grande parte do calor produzido. Dessa forma, a temperatura corporal eleva-se lentamente e gradualmente durante a fase estável do exercício até atingir o valor crítico que promove a interrupção da atividade física.

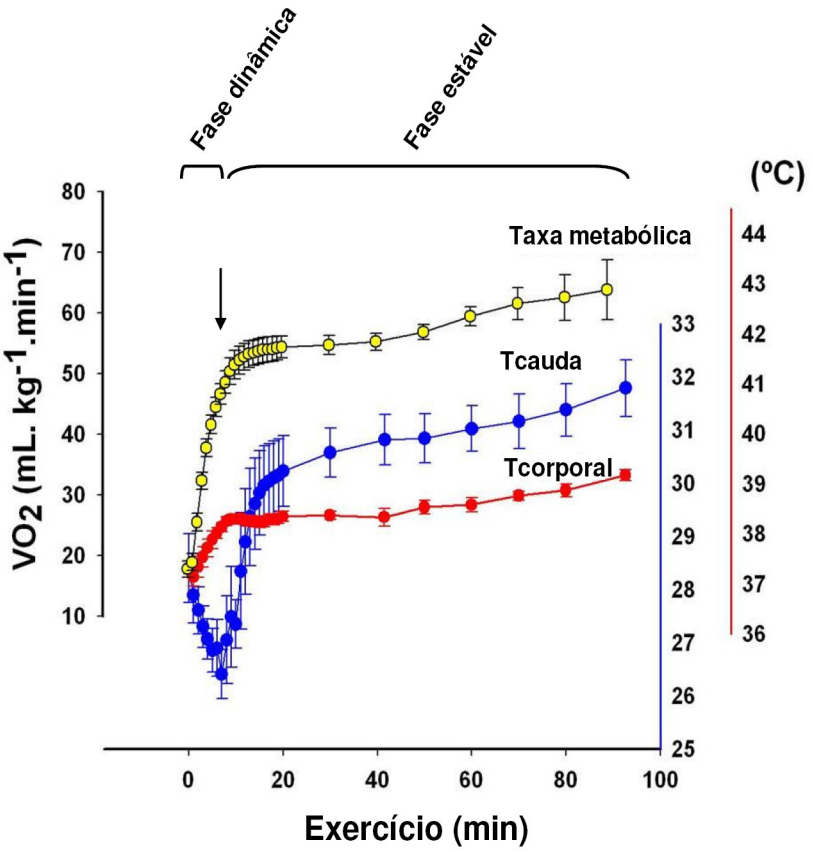

FIGURA 1. Gráfico ilustrativo da dinâmica entre a produção e a dissipação de calor durante as diferentes fases do exercício físico. Adaptado de Lacerda et al., 2005, 2006 a.

Há uma relação inversamente proporcional entre a temperatura corporal interna antes do início da atividade física e o tempo total de exercício, assim como forte correlação negativa entre a taxa de aquecimento e o tempo para a fadiga (González-Alonso et al., 1999; Lacerda et al., 2005; Leite et al., 2006; Walters et al., 2000). Além disso, embora alguns autores proponham a existência de um valor absoluto de temperatura corporal interna crítico que levaria a interrupção da atividade física (Fuller et al., 1998; Walters et al., 2000), a taxa de acúmulo de calor parece ser importante predisponente da antecipação

da fadiga, apresentando relação inversa com o desempenho físico (González-Alonso et al., 1999; Rodrigues et al., 2003). Esses achados indicam que tanto o valor da taxa de aquecimento corporal quanto a quantidade de calor acumulado parecem ser parte dos fatores limitantes da continuidade da atividade física, levando à fadiga (González-Alonso et al., 1999; Lacerda et al., 2005; Leite et al., 2006; Rodrigues et al., 2003).

Estudos do laboratório têm contribuído para estabelecer essa hipótese ao demonstrar a influência de diversos sistemas centrais sobre o equilíbrio térmico e o desempenho físico (Balthazar et al., 2009; Lacerda et al., 2005, 2006 a; Leite et al., 2006; Pires et al., 2007; Prímola-Gomes et al., 2007; Rodrigues et al., 2004). Dentre esses trabalhos, alguns indicaram que o bloqueio central dos sistemas óxido nitrérgico e angiotensinérgico induz redução do desempenho físico em função do aumento do limiar térmico para a vasodilatação cutânea, o qual eleva a taxa de aquecimento corporal e rapidamente produz hipertermia durante o exercício físico (Lacerda et al., 2005; Leite et al., 2006). A elevada taxa metabólica e menor eficiência mecânica, verificadas nos animais tratados com L-NAME (metil N^{ω}-nitro-L-arginina; bloqueador da óxido nítrico sintase), também elevam a taxa de aquecimento e contribuem para a fadiga precoce (Lacerda et al., 2006 a). Por outro lado, essa relação inversa entre taxa de aquecimento e desempenho físico deixa de existir durante a ativação colinérgica e dopaminérgica central (Balthazar et al., 2009; Pires et al., 2007; Prímola-Gomes et al., 2007; Rodrigues et al., 2004). O sistema colinérgico também exerce efeito termorregulatório durante o exercício por facilitar a perda de calor

através da vasodilatação cutânea, atenuando o aumento da temperatura corporal interna, porém, sem afetar o desempenho físico, prevalecendo o esforço cardiovascular como principal fator indutor da fadiga (Pires et al., 2007; Prímola-Gomes et al., 2007; Rodrigues et al., 2004). Já o sistema dopaminérgico central apresenta efeito ergogênico, apesar de induzir elevação da taxa metabólica, acompanhada por hipertermia e aumento do acúmulo de calor (Balthazar et al., 2009). Esses achados sugerem que a dopamina central melhora a tolerância ao calor através da atenuação da percepção de esforço, aumentando a capacidade física (Balthazar et al., 2009).

Conteúdo de neurotransmissores centrais

Entre os processos que levam à interrupção da atividade física se incluem alterações da atividade de centros localizados no sistema nervoso. A fadiga central durante o exercício físico prolongado pode envolver o acúmulo ou a depleção de neurotransmissores centralmente (Nielsen & Nybo, 2003). A síntese e metabolismo de monoaminas, particularmente da serotonina (5-HT), são influenciados durante o exercício físico (Blomstrand, 2006; Meeusen et al., 2007). O aumento da atividade serotonérgica está associada à letargia e perda de motivação, resultando em alteração do desempenho físico (Blomstrand, 2006; Meeusen et al., 2007). Evidências indicam que o exercício físico prolongado induz o aumento da atividade serotonérgica no cérebro, abreviando o tempo para o estabelecimento da fadiga (Davis & Bailey, 1997; Rodrigues et

al., 2009; Soares et al., 2007). Tal fato é apoiado por indícios de que a administração de agonistas serotonérgicos prejudica o desempenho físico de maneira dose dependente (Bailey et al., 1993; Blomstrand, 2006). Em contrapartida, a capacidade física é beneficiada pelo tratamento com antagonistas da 5-HT (Bailey et al., 1993; Blomstrand, 2006).

O aumento do conteúdo de 5-HT nas principais regiões responsáveis pela termorregulação, como a área pré-óptica e o hipotálamo, também está relacionado com produção de calor e precipitação da fadiga (Blomstrand, 2006; Caperuto et al., 2009; Rodrigues et al., 2009; Soares et al., 2007). Como citado anteriormente, a temperatura interna elevada, assim como o acúmulo de calor, estão entre os fatores considerados limitantes para a manutenção da atividade física por reduzir o estímulo a partir do sistema nervoso central, dessa forma protegendo o cérebro contra a hipertermia (Fuller et al., 1998; Rodrigues et al., 2003; Walters et al., 2000). Recentemente foi demonstrado que a fadiga central devido a hipertermia e acúmulo de calor elevado em ratos em exercício estão relacionadas ao aumento de 5-HT na área pré-óptica (Caperuto et al., 2009; Rodrigues et al., 2009; Soares et al., 2007). Além disso, verificou-se que a estimulação central colinérgica e o aumento da disponibilidade central de triptofano em ratos em exercício, uma vez que induzem ações termorregulatórias antagônicas, também apresentam efeitos opostos em relação ao conteúdo de 5-HT na área pré-óptica, hipotálamo e hipocampo (Rodrigues et al., 2009; Soares et al., 2007). Em ambos os casos, a concentração de 5-HT na área pré-óptica mostrou-se estar inversamente relacionada com o aumento da temperatura

corporal interna e acúmulo de calor durante o exercício (Rodrigues et al., 2009; Soares et al., 2007). Em relação ao hipocampo, concentrações diminuídas de 5-HT foram observadas após tratamento com triptofano enquanto o contrário ocorreu em função da ativação colinérgica (Rodrigues et al., 2009; Soares et al., 2007). O hipocampo está diretamente envolvido com o controle da atividade motora (Takahashi et al., 2000), sendo possível que sua ação na determinação da fadiga durante o exercício ocorra através de outro mecanismo além da termorregulação.

Embora o envolvimento da 5-HT na fadiga central seja melhor documentado, é provável que outros neurotransmissores sejam capazes de influenciar a fadiga, tais como a dopamina (DA) (Blomstrand, 2006; Fernstrom & Fernstrom, 2006; Foley & Fleshner, 2008; Meeusen et al., 2007). Há indicações de que a 5-HT interage com a DA durante o exercício (Foley & Fleshner, 2008; Meeusen et al., 2007). Isso se justifica pelo fato da ativação serotonérgica em função do exercício contribuir para a fadiga através da inibição do sistema dopaminérgico (Foley & Fleshner, 2008; Meeusen et al., 2007). A neurotrasmissão dopaminérgica está associada com várias funções fisiológicas como o estado de alerta, recompensa e motivação, as quais poderiam modificar a capacidade física (Foley & Fleshner, 2008; Hasegawa et al., 2008; Meeusen et al., 2007). O metabolismo central da DA aumenta durante o exercício em animais, inclusive na área pré-óptica, hipotálamo e hipocampo, (Balthazar et al., 2009; Hasegawa et al., 2008; Foley & Fleshner, 2008), sendo que sua ação está associada com melhora do desempenho físico apesar da elevação da

temperatura corporal no ponto de fadiga e do acúmulo de calor durante o exercício (Balthazar et al., 2009; Foley & Fleshner, 2008; Hasegawa et al., 2008). Em função disso, o efeito ergogênico da DA parece implicar seu envolvimento no controle do movimento e recompensa, ao invés da termorregulação (Balthazar et al., 2009; Foley & Fleshner, 2008; Hasegawa et al., 2008). Atuando no sistema mesolímbico de recompensa, considera-se que a DA facilita a ultrapassagem dos limites seguros de temperatura (Balthazar et al., 2009; Foley & Fleshner, 2008; Hasegawa et al., 2008). Sinais oriundos do sistema límbico sobreporiam os sinais térmicos, atenuando a intensidade de percepção de esforço e aumentando o desempenho físico (Balthazar et al., 2009; Foley & Fleshner, 2008; Hasegawa et al., 2008).

Levando essas evidências em consideração, sugere-se que o desenvolvimento da fadiga central dependeria da interação entre os sistemas serotonérgico e dopaminérgico, dentre outros fatores (Foley & Fleshner, 2008; Hasegawa et al., 2008). Sendo assim, a fadiga central estaria sujeita ao controle termorregulatório, motivacional e motor a partir desses sistemas, sendo a proporção [5-HT]:[DA], em áreas do sistema nervoso central relacionadas com a termorregulação e motricidade, fundamental para seu estabelecimento (Blomstrand, 2006; Fernstrom & Fernstrom, 2006; Foley & Fleshner, 2008; Meeusen et al., 2007). Isto é, uma alta razão [5-HT]:[DA] estaria diretamente relacionada com a redução do desempenho físico e vice versa (Foley & Fleshner, 2008).

Disponibilidade de substratos energéticos

A oferta, distribuição e utilização adequada de substratos energéticos são indispensáveis para manter o desempenho durante o exercício físico prolongado (Braun & Brooks, 2008). O organismo dispõe de um sistema neuro-hormonal bastante desenvolvido que garante suprimento adequado de substratos para os músculos em atividade (Braun & Brooks, 2008; Coyle, 2000). Esses substratos são carboidrato e gordura, responsáveis por viabilizar as reações químicas geradoras da última fonte de energia para contração muscular, o ATP (Braun & Brooks, 2008; Coyle, 2000). Os estoques intracelulares de ATP são pequenos, portanto, durante o exercício, este deve ser continuamente e rapidamente regenerado para garantir a manutenção da atividade física (Braun & Brooks, 2008; Coyle, 2000). Como citado anteriormente, a energia química liberada pela hidrólise do ATP durante a contração muscular é convertida em força ou calor, resultando em eficiência energética de aproximadamente 20-27% (Brooks et al., 1984).

A regulação da produção hepática de glicose e mobilização de ácidos graxos livres a partir do tecido adiposo, em situações de alta demanda energética como o exercício, já foram descritas como controladas por inervação simpática direta (Galbo et al., 1978; Greiwe et al., 1999; Kjaer, 1998). Esses efeitos são mediados através do aumento da liberação de norepinefrina pelos terminais nervosos e secreção de epinefrina pela medula das adrenais (Greiwe et al., 1999; Kjaer, 1998). Independentemente da duração ou intensidade, o

trabalho físico induz um pico inicial de ativação simpática requerido para adaptar o organismo à nova demanda metabólica (Febbraio et al., 1998; Greiwe et al., 1999). Contudo, a intensidade do exercício é o fator responsável por selecionar a contribuição dos substratos na produção de energia (Greiwe et al., 1999; Zouhal et al., 2008). Exercícios de baixa-moderada intensidade são sustentados primariamente por oxidação de ácidos graxos plasmático (Coyle, 2000). Para intensidades mais elevadas, o substrato utilizado preferencialmente é a glicose (Coker & Kjaer, 2005). O exercício físico, portanto, representa um estado fisiológico no qual são necessárias adaptações metabólicas e hormonais para manter o fornecimento de glicose e ácidos graxos para a musculatura ativa, bem como manter fluxo de glicose adequado para o cérebro (Braun & Brooks, 2008; Galbo et al., 1978).

Apesar da disponibilidade de substrato estar diretamente associada com a manutenção do exercício, a precipitação da fadiga após bloqueio central dos sistemas colinérgico e óxido nitrérgico não relacionou-se com menor oferta de glicose e ácidos graxos livres durante o exercício até a fadiga (Lacerda et al., 2006 b; Lima et al., 1998). Nessas situações, os níveis plasmáticos de glicose e ácidos graxos livres elevaram-se consideravelmente, mesmo em baixa intensidade de esforço, sugerindo que neurônios colinérgicos e óxido nitrérgicos alteram os mecanismos autorreguladores da mobilização de substratos durante o exercício (Lacerda et al., 2006 b; Lima et al., 1998).

O SISTEMA RENINA-ANGIOTENSINA

O sistema renina-angiotensina, além de funcionar como sistema endócrino circulante, apresenta ação local em vários órgãos e tecidos, inclusive o cérebro, caracterizando-o também como sistema parácrino e autócrino (Fyhrquist & Saijonmaa, 2008). A angiotensina II (Ang II) é considerada um dos principais mediadores e efetores do sistema renina-angiotensina, sendo formada pela ação sequencial de duas enzimas, renina e enzima conversora de angiotensina (ECA), atuando sobre o precursor angiotensinogênio. Este é convertido em angiotensina I sob efeito da renina circulante. Por fim, nos capilares pulmonares, a angiotensina I é convertida em Ang II sob ação da ECA (Fyhrquist & Saijonmaa, 2008) (Figura 2).

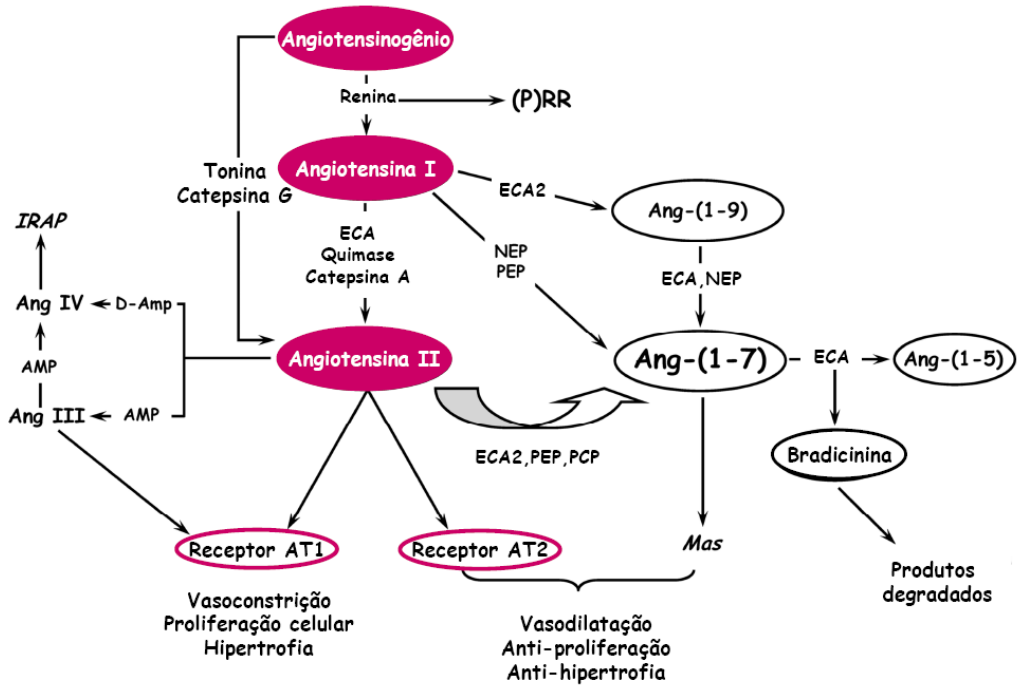

FIGURA 2. Cascata de formação e degradação da angiotensina II. Abreviações: ECA, enzima conversora de angiotensina; Ang, angiotensina; AMP, aminopeptidase; AT1, receptor tipo 1 da Ang II; AT2, receptor tipo 2 da Ang II; Mas, receptor da Ang(1–7); D-Amp, dipeptidil-aminopeptidase; IRAP, aminopeptidase regulada pela insulina; PCP, prolil-carboxipeptidase; PEP, prolil-endopeptidase; NEP, neutral-endopeptidase 24.11; e (P)RR, receptor renina/prorenina. Adaptado de Santos et al., 2008.

A Ang II apresenta várias ações bem definidas e potentes, no entanto, as implicações fisiológicas da Ang II continuam se expandindo (McKinley et al., 2003). Ela exerce seus efeitos ligando-se aos receptores acoplados a proteína G para Ang II do tipo 1 (AT1) ou do tipo 2 (AT2), sendo que a maioria das ações conhecidas da Ang II são mediadas pelos receptores AT1 (Allen et al., 1998; McKinley et al., 2003). Estes receptores, por sua vez, são divididos nos subtipos AT1A e AT1B nos roedores (Allen et al., 1998; McKinley et al., 2003). Dentre as

mais relevantes ações da Ang II estão vasoconstrição da musculatura lisa vascular, retenção de sódio e água nos rins, tanto por efeito direto nesse órgão, como indiretamente através da estimulação da biossíntese de aldosterona adrenal. Ademais, a Ang II age no sistema nervoso central induzindo a sede, o apetite ao sódio, liberação de hormônios pituitários, controle da temperatura corporal e modulação do controle autonômico da função cardiovascular (Allen et al., 1998; McKinley et al., 2003).

É interessante salientar que todos os componentes do sistema renina-angiotensina estão presentes no cérebro, sugerindo haver um sistema renina-angiotensina central (Von Bohlen Und Halbach & Albrecht, 2006). Em alguns casos, as ações locais do sistema nervoso central interagem com aquelas da Ang II sistêmica (Allen et al., 1998; McKinley et al., 2003). Uma vez que a Ang II não atravessa a barreira hemato-encefálica, essa interação ocorre em sítios específicos desprovidos dessa barreira, como os órgãos circumventriculares área postrema e órgão subfornicial, os quais são ricos em receptores AT1 e emitem projeções para outras regiões centrais que se situam atrás da barreira (Allen et al., 1998; Von Bohlen Und Halbach & Albrecht, 2006; McKinley et al., 2003).

A distribuição dos receptores AT1 no sistema nervoso central é difusa, merecendo destaque a localização em outras regiões cerebrais envolvidas no controle cardiovascular, da homeostase dos líquidos corporais e da temperatura corporal (Allen et al., 1998; McKinley et al., 2003), como o núcleo paraventricular

do hipotálamo, núcleo do trato solitário e a área pré-óptica (Allen et al., 1998; McKinley et al., 2003).

Envolvimento da Ang II no equilíbrio térmico

Há várias evidências mostrando que a hipotermia induzida pela Ang II deve-se a facilitação da perda de calor (Fregly & Rowland, 1992, 1993, 1996; Wilson & Fregly, 1985 a,b). Além disso, achados apontam que a ativação angiotensinérgica também resulta em diminuição da produção de calor (Cassis et al., 2002; Fregly & Rowland, 1992, 1993, 1996; Wilson & Fregly, 1985 a,b). A administração tanto periférica quanto central de Ang II mostrou ser capaz de provocar resposta hipotérmica dose-dependente, manifestada através do aumento da temperatura da cauda e diminuição da taxa metabólica (Fregly & Rowland, 1992, 1993, 1996; Mathai et al., 2000; Wilson & Fregly, 1985 a,b). Tais respostas calóricas induzidas pela Ang II são abolidas pelo tratamento com bloqueador do receptor AT1 (Fregly & Rowland, 1992; Horowitz et al., 1999; Wilson & Fregly, 1985 a,b).

Mais recentemente, estudo do laboratório mostrou que a ação da Ang II no balanço térmico não se limita a situações de repouso, mas também durante o exercício físico (Leite et al., 2006). O bloqueio central do receptor AT1 para Ang II utilizando o Los intracerebroventricularmente durante o exercício físico produz aumento significativo das taxas de aquecimento corporal e de acúmulo de calor, agravando a hipertermia do exercício e aumentando o limiar térmico para

vasodilatação cutânea. Além disso, o tratamento com Los reduz o desempenho físico que mostrou-se intimamente associado com a taxa de aquecimento corporal. Esses dados evidenciam que o sistema angiotensinérgico central tem efeitos importantes sobre a termorregulação durante o exercício físico, facilitando a dissipação de calor através da vasodilatação cutânea, atenuando o aumento da temperatura corporal interna e, consequentemente, melhorando a capacidade física (Leite et al., 2006).

Envolvimento da Ang II nos ajustes metabólicos

Além de suas ações sobre o sistema cardiovascular e na regulação do equilíbrio hidroeletrolítico, a Ang II está envolvida com a regulação de funções endócrinas e metabólicas (Machado et al., 2002), especialmente aquelas envolvidas com a homeostase da glicose (Coimbra et al., 1999; Machado et al., 1995 a,b, 1998). Sua ação sobre o metabolismo intermediário ocorre tanto de maneira direta ou através de sua ação sobre a medula adrenal e controle da atividade simpática (Coimbra et al., 1999, Machado et al., 1995 a,b, 1998; Mihessen-Neto et al., 1996). A atuação desse peptídeo na regulação da glicemia é significativa, induzindo hiperglicemia dose dependente atuando sobre receptores AT1 (Machado et al., 1995 a,b). Por outro lado, a injeção intravenosa de antagonista da Ang II resulta em inibição da hiperglicemia induzida por hemorragia (Machado et al., 1995 a,b). A Ang II ainda produz hiperglicemia em animais desmedulados, apesar dessa resposta ser atenuada em comparação

com animais normais (Mihessen-Neto et al., 1996). Portanto, a ação direta da Ang II sobre a produção hepática de glicose, facilitando a gliconeogênese, glicogenólise, e também sobre a atividade da glicogênio fosforilase hepática, soma-se de maneira fundamental à regulação multifatorial da concentração de glicose plasmática por esse peptídeo (Coimbra et al., 1999, Machado et al., 1995 a,b, 1998; Mihessen-Neto et al., 1996) (Figura 3). Não pode ser desconsiderando o efeito inibitório da Ang II sobre a secreção de insulina em resposta a elevação de glicose (Henriksen, 2007; Perkins & Davis, 2008). Uma vez que bloqueadores do sistema renina-angiotensina são capazes de melhorar a sensibilidade à insulina e o controle da glicemia, é possível que a Ang II eleve a glicose sanguínea por interferir na ação desse hormônio (Coimbra et al., 1999, Henriksen, 2007; Perkins & Davis, 2008).

Embora os resultados ainda sejam controversos, evidências apontam que a Ang II também aumenta a lipólise nos tecidos subcutâneo e adiposo de maneira dose dependente (Boschmann et al., 2006; Cabassi et al., 2005). Esse resultado é acompanhado por elevação da concentração intersticial de norepinefrina no tecido adiposo e também no plasma, sugerindo ser a lipólise resultante da elevação da atividade simpática local e sistêmica (Cabassi et al., 2005).

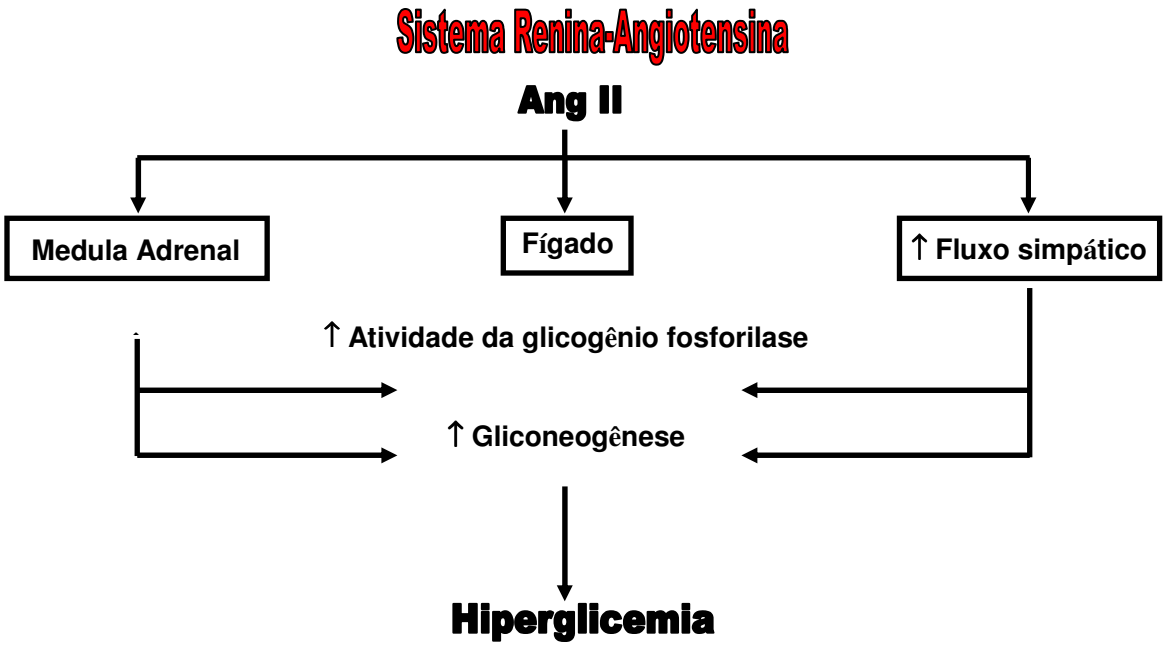

Figura 3. Esquema ilustrativo da regulação multifatorial da concentração de glicose plasmática pela angiotensina II (Ang II).

Interação entre o sistema renina-angiotensina e os sistemas serotonérgico e dopaminérgico

São poucas as evidências que abordam o efeito da Ang II sobre a secreção central de 5-HT ou DA. No entanto, a ativação dos receptores AT1 parece afetar o metabolismo de 5-HT e DA no cérebro (Stadler et al., 1992; Tanaka et al., 2003). Já foi demonstrado que a Ang II injetada dentro do orgão subfornicial causa redução na concentração de 5-HT e ácido 5-hidroxindoleacético (5-HIAA) neste mesmo sítio (Tanaka et al., 2003). Ademais, a injeção intracerebroventricular de Ang II mostrou não modificar a concentração de DA e ácido 3,4 diidroxifenilacético (DOPAC) no PVN ou hipotálamo anterior

(Qadri et al., 1991; Stadler et al., 1992). Portanto, assim como no orgão subfornicial e no PVN, é possível que em outros centros termorregulatórios a interação entre Ang II e 5-HT ou DA interfira no controle da temperatura interna e, consequentemente, no desempenho físico.

INTERAÇÃO ENTRE OS CENTROS REGULADORES DA TEMPERATURA CORPORAL E DO METABOLISMO INTERMEDIÁRIO

A regulação da temperatura corporal deve ser analisada sob o ponto de vista autonômico, endócrino, metabólico e comportamental (Arancibia et al., 1996). As áreas do sistema nervoso central que são ativadas por flutuações na temperatura ambiente e que mediam as respostas fisiológicas a essas flutuações estão localizadas principalmente no hipotálamo (Hasegawa et al., 2000, 2005, 2008; Ishiwata et al., 2001, 2002, 2004; Nagashima et al., 2000; Romanovsky, 2007). A área pré-óptica e o hipotálamo anterior têm sido apontados como os sítios primários da integração de sinais térmicos originados de diferentes partes do corpo e pela coordenação da regulação da temperatura corporal (Hasegawa et al., 2000, 2005, 2008; Ishiwata et al., 2001, 2002, 2004; Nagashima et al., 2000; Romanovsky, 2007). Esses centros contêm neurônios sensíveis ao calor e ao frio que respondem a pequenas variações de temperatura (Ishiwata et al., 2002; Zhang et al., 1997). Os neurônios sensíveis ao calor são os principais efetores tanto da perda quanto da produção de calor. Esses neurônios geram sinais excitatórios para a perda de calor e sinais

inibitórios para a produção de calor através do bloqueio dos neurônios sensíveis ao frio (Nagashima et al., 2000; Romanovsky, 2007). Devido à projeção de vias nervosas a partir da área pré-óptica para outras regiões do sistema nervoso central, diversos sítios têm sido identificados como participantes do controle da atividade termorreguladora (Kazuyuki et al., 1998; Nagashima et al., 2000; Romanovsky, 2007). Propõe-se que as eferências que modulam as respostas efetoras específicas, isto é salivação, tônus vasomotor, tremor, atividade metabólica do tecido adiposo marrom e comportamento, são vias neurais completamente distintas e independentes (Kazuyuki et al., 1998; Nagashima et al., 2000; Romanovsky, 2007). Sendo assim, as respostas termorreguladoras podem ser provocadas pela estimulação térmica, possivelmente de limiares diferentes, de várias áreas do sistema nervoso central, incluindo alguns grupos neuronais do tronco encefálico (formação reticular do mesencéfalo, ponte e bulbo) e da medula espinhal (Kazuyuki et al., 1998; Nagashima et al., 2000; Romanovsky, 2007) (Figura 4).

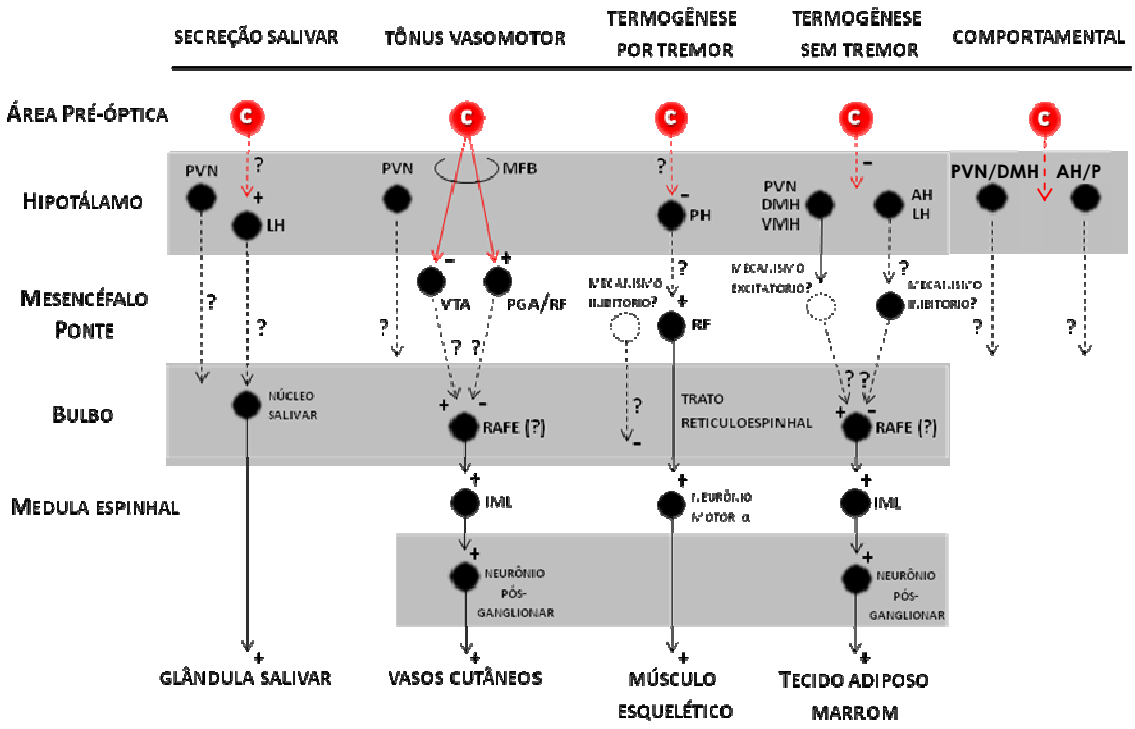

FIGURA 4. Esquema ilustrando as vias termorregulatórias efetoras da área pré-óptica para cada resposta efetora termorregulatória. Linhas contínuas ou tracejadas indicam conexões comprovadas e hipotéticas, respectivamente. A rede neuronal para a termorregulação comportamental é pouco conhecida. Abreviações: (C) neurônios sensíveis ao calor, PVN (núcleo paraventricular do hipotálamo), MFB (feixe prosencefálico medial), VTA (área tegmental ventral), PAG (substância periaquedutal cinzenta), IML (coluna intermediolateral), HL (hipotálamo lateral), RF (formação reticular), AH (hipotálamo anterior), PH (hipotálamo posterior), VMH (hipotálamo ventromedial), DMH (hipotálamo dorsomedial). Adaptado de Nagashima et al., 2000; Romanovsky, 2007.

O PVN é um sítio integrador da atividade nervosa simpática, sabidamente fundamental para regulação cardiovascular (Li et al., 2006; Patel, 2000). Recentemente, este núcleo foi descrito como participante do controle da temperatura corporal interna (Nagashima et al., 2000; Romanovsky, 2007) por

conter neurônios termosensíveis que são ativados durante o estresse térmico (Bratincsak & Palkovits, 2004; Cham & Badoer, 2008, Cham et al., 2006). Além disso, achados apontam que projeções partem do PVN para outros centros termorreguladores, influenciando a atividade simpática de orgãos termoefetores como o tecido adiposo marrom, glândula salivar, vasculatura da cauda, assim como rins e intestino (Cham & Badoer, 2008; Kazuyuki et al., 1998; Smith et al., 1998). Foi descrito também que a inibição neuronal ou lesão do PVN durante exposição ao calor previne a redução do fluxo sanguíneo renal (Cham & Badoer, 2008; Kenney et al., 2001). A redistribuição de sangue do meio interno para a periferia é crítica em situações de hipertermia pois possibilita a perda de calor (Cham & Badoer, 2008; Kenney et al., 2001; Kregel et al., 1994). Ela depende de ajustes cardiovasculares controlados pelo sistema nervoso simpático que induzem vasoconstrição visceral e vasodilatação da pele simultaneamente, acompanhados por elevação da pressão arterial e da freqüência cardíaca (Kanosue et al., 1994; Morrison, 2001; Smith et al., 1998). Considerando essas evidências, o PVN parece estar envolvido na regulação da temperatura corporal interna através do controle do fluxo simpático não uniforme responsável por ajustar respostas cardiovasculares induzidas por estresse térmico determinantes para a dissipação de calor.

Além de serem considerados centros termorreguladores, a área pré-óptica e o PVN são importantes centros mantenedores da homeostase metabólica (Coimbra & Migliorini, 1986; Ferreira et al., 1999; Foscolo et al., 2003; Santos et al., 1991, Silveira et al., 2003). Já foi demonstrado que a

estimulação da área pré-óptica induz rápida elevação da concentração plasmática de ácidos graxos livres, enquanto a estimulação noradrenérgica local resulta em hiperglicemia (Coimbra & Migliorini, 1986; Foscolo et al., 2003). Além disso, evidências comprovaram que as respostas de hiperglicemia e aumento da mobilização de ácidos graxos livres induzidas pelo frio são impedidas após tratamento com bloqueadores adrenérgicos na área pré-óptica (Coimbra & Migliorini 1988; Ferreira et al., 1999). Esses indícios sugerem que o controle termorregulatório e metabólico da área pré-óptica parecem ser integrados. Por sua vez, a ativação noradrenérgica do PVN também induz hiperglicemia (Ionescu et al., 1989). O PVN parece participar do controle metabólico em outras situações de estresse como durante a hemorragia (Silveira et al., 2003). Segundo este estudo, a inibição da atividade colinérgica do PVN, utilizando metilatropina, reduz a resposta hiperglicêmica estimulada por hemorragia.

HIPÓTESE

O sistema angiotensinérgico central provavelmente está envolvido no equilíbrio metabólico e térmico durante o exercício físico por modificar a atividade do sistema monoaminérgico central.

As evidências experimentais apresentadas demonstram que o bloqueio angiotensinérgico central durante o exercício dificulta a dissipação de calor, promove elevação acentuada da temperatura corporal interna resultante do aumento da taxa de aquecimento corporal e do acúmulo de calor. Em função desses efeitos, foi verificada redução significativa do desempenho físico em

experimentos com ratos, caracterizando a Ang II como possível peptídeo ergogênico e termorregulador. Uma vez que o sistema angiotensinérgico está envolvido com a regulação tanto da perda de calor quanto da produção deste, especula-se que o aumento acentuado da temperatura corporal interna observado após bloqueio central da Ang II durante o exercício também se deve a alterações na produção de calor e na eficiência mecânica, os quais afetariam o desempenho físico. Além disso, o fato da elevação da concentração central de Ang II possivelmente promover redução da concentração de 5-HT, cujo aumento no cérebro está diretamente relacionado com elevação da temperatura corporal, permite especular se a redução do tempo de exercício devido a hipertermia após bloqueio angiotensinérgico central ocorreria em função do aumento do conteúdo de 5-HT, interagindo com DA, em centros nervosos termorreguladores e de controle motor. Considerando que a Ang II exerce efeitos metabólicos importantes, principalmente em relação ao metabolismo da glicose e ácidos graxos, é razoável sugerir que o efeito anti-ergogênico do Los central seria desencadeado por alterações na disponibilidade de substratos energéticos. Por fim, devido à ação termorregulatória e de controle da atividade simpática do PVN, questiona-se caso esse sítio esteja envolvido no controle do fluxo simpático não uniforme responsável por ajustar respostas cardiovasculares induzidas por estresse térmico determinantes para a dissipação de calor.

IV. OBJETIVO

OBJETIVO GERAL

Avaliar a influência da transmissão angiotensinérgica central sobre o equilíbrio térmico, mobilização de substratos energéticos e fadiga central durante o exercício físico em ratos, e o envolvimento do PVN nos ajustes cardiovasculares mediados pela atividade simpática, facilitadores da dissipação de calor durante o estresse térmico.

Objetivos específicos

1. Estudar os efeitos do bloqueio central do receptor AT1 para Ang II sobre o consumo de oxigênio, o gasto calórico e a eficiência mecânica em ratos não treinados submetidos ao exercício submáximo na esteira;

2. Estudar os efeitos do bloqueio central do receptor AT1 para Ang II sobre o consumo de oxigênio, o gasto calórico e a eficiência mecânica em ratos não treinados submetidos ao exercício progressivo na esteira;

3. Estudar os efeitos do bloqueio central do receptor AT1 para Ang II sobre a concentração plasmática de substratos energéticos (glicose, lactato e ácidos

graxos livres) em ratos não treinados submetidos ao exercício progressivo na esteira;

4. Estudar os efeitos do bloqueio central do receptor AT1 para Ang II sobre as concentrações centrais de 5-HT, 5-HIAA, DA e DOPAC na área pré-óptica, hipotálamo, hipocampo e córtex frontal em ratos não treinados submetidos ao exercício submáximo na esteira;

5. Estudar os efeitos da inibição bilateral do PVN e do bloqueio óxido nitrérgico nesse mesmo núcleo durante a exposição ao calor sobre a atividade simpática do nervo renal, pressão arterial média, frequência cardíaca, equilíbrio térmico e limiar térmico para vasodilatação cutânea.

V. MÉTODOS

ANIMAIS

Foram utilizados ratos Wistar machos, com peso corporal entre 240-330 g, provenientes do CEBIO-ICB. Os animais foram mantidos em ambiente com temperatura entre 22±2 ºC e fotoperíodo de 14 h luz/10 h escuro, tendo livre acesso à ração e água.

O trabalho foi realizado de acordo com as normas estabelecidas pelo Comitê de Ética em Pesquisa com Animais da UFMG (CETEA/UFMG), segundo o protocolo de número 145/2007.

PROCEDIMENTO CIRÚRGICO

Os animais foram submetidos à cirurgia para implante de cânula guia (16 mm de comprimento x 0,7 mm de diâmetro) no ventrículo cerebral lateral direito, utilizada para microinjeções (2 μL) de solução salina 0,15 M (Sal) ou de solução de Los (60 nmol). Os ratos utilizados foram anestesiados com mistura de 2,2,2-tribromoetanol (300 mg/kg de peso corporal, via intraperitoneal) ou combinação de ketamina (116 mg/kg de peso corporal) e xilazina (5,75 mg/kg de peso corporal, via intraperitoneal) e fixados em estereotáxico. Foram obedecidas as coordenadas estereotáxicas estabelecidas pelo Atlas de De Groot (1959) (A: -1,5 mm; L: -2,5mm; V: -3,0mm). O correto posicionamento da cânula no ventrículo lateral foi verificado pelo deslocamento dos meniscos em um manômetro com salina.

Os animais se recuperaram das cirurgias durante o período de pelo menos uma semana.

EXERCÍCIO FÍSICO

A adaptação ao exercício físico consistiu de uma corrida diária em esteira para roedores (Columbus Instruments, OH, USA, Modular treadmill, serie 96002-2) a uma velocidade de 15 m/min, 5% de inclinação da esteira durante 5 minutos/4 dias consecutivos.

Durante os experimentos, os animais foram submetidos ao exercício contínuo ou progressivo até a fadiga em esteira. O exercício contínuo consistiu de corrida à velocidade constante de 18 m/min e 5% de inclinação. O modelo de exercício progressivo consistiu de velocidade inicial ajustada em 10 m/min, com inclinação de 10% da esteira. A velocidade sofreu acréscimo de 1m/min a cada 3 minutos até a fadiga do animal. O ponto de fadiga foi definido como o momento no qual os animais não conseguiram manter o ritmo da esteira por mais de 10 segundos. A estimulação elétrica utilizada foi estabelecida de acordo com a tolerância de cada animal, a ponto de causar um desconforto, sem causar dor, que o fizesse escolher permanecer na esteira ao invés da grade de estimulação elétrica. O tempo total de exercício e o trabalho realizado foram avaliados como capacidade máxima de trabalho dos animais. O trabalho realizado foi determinado de acordo com a equação:

Trabalho (kgm) = [(intensidade do exercício)x(tempo até a fadiga)x(peso corporal)]. [seno θ (inclinação da esteira)]

PROCEDIMENTO EXPERIMENTAL GERAL

Os animais foram colocados no local do experimento 60 minutos antes do início do exercício. Uma agulha (30G) foi introduzida na cânula guia e conectada a uma seringa Hamilton para injeção das drogas. Imediatamente antes do início da atividade física contínua ou progressiva foi administrado no ventrículo cerebral lateral direito 2 μL de Sal ou de solução de Los (60 nmol), aleatoriamente por método duplo-cego. A temperatura ambiente foi mantida dentro de uma faixa constante, entre 22±2°C. Os experimentos foram realizados entre 10 e 14 horas.

Experimento 1: efeito do bloqueio angiotensinérgico central sobre o consumo de oxigênio durante o exercício contínuo até a fadiga

Neste grupo experimental foi medido o consumo de oxigênio (VO_2, $mL.kg^{-1}.min^{-1}$) durante o exercício contínuo até a fadiga.

O VO_2 foi medido por calorímetro indireto de fluxo aberto (Columbus Instruments), o qual foi calibrado com mistura padrão de gases contendo 95% de O_2 e 5% de CO_2 (White Martins) antes do experimento. O seu registro foi realizado continuamente usando sistema computadorizado em linha com o calorímetro (Oxymax Apparatus, Columbus Instruments). A eficiência mecânica foi determinada de acordo com a equação: (Trabalho realizado/gasto energético) x 100.

Experimento 2: efeito do bloqueio angiotensinérgico central sobre o consumo de oxigênio durante o exercício progressivo até a fadiga

Neste grupo experimental foi medido o VO_2 durante o exercício progressivo até a fadiga de acordo com o protocolo citado anteriormente.

Experimento 3: efeito do bloqueio angiotensinérgico central sobre a concentração plasmática de substratos energéticos durante o exercício progressivo até a fadiga

Neste grupo experimental foi realizada a análise das concentrações plasmáticas de glicose, lactato e ácidos graxos livres durante o exercício progressivo até a fadiga de acordo com o protocolo citado anteriormente. Para tal, além da cirurgia para implante da cânula no ventrículo lateral direito, os animais receberam implante, através da veia jugular, de cateter de silastic no átrio direito do coração, para colheitas seriadas de amostras de sangue (300 μL). Durante o experimento as amostras de sangue foram retiradas pelo cateter atrial imediatamente antes da atividade física, durante o exercício nos tempos de 03, 06, 09, 12, 15, 21 minutos e no momento da fadiga. Para evitar redução no volume sanguíneo do animal, foram feitas reposições do mesmo volume de sangue obtido de um rato doador normal. O sangue para determinação plasmática de substratos foi colhido em seringas heparinizadas. As amostras sanguíneas foram colocadas em gelo até a centrifugação e separação do plasma. Após este procedimento, o sangue foi, então, congelado a -20ºC até a análise bioquímica dos mesmos. As concentrações plasmáticas de glicose e

lactato foram determinadas por método oxidativo utilizando Glucose Analyser (2300 STATPLUS, Yellow Springs Instruments, USA). A concentração dos ácidos graxos livres foi determinada por método enzimático colorimétrico utilizando kit NEFA 30T (Randox Laboratories, USA), adaptado para pequenos volumes de plasma.

Experimento 4: efeito do bloqueio angiotensinérgico central sobre a concentração de monoaminas centrais no momento da fadiga após o exercício contínuo

Imediatamente após o término do exercício físico contínuo o animal foi decapitado para retirada da área pré-óptica, hipotálamo, hipocampo e córtex frontal. Os tecidos cerebrais foram armazenados em freezer -80 °C para análise posterior, por cromatografia líquida de alta eficiência com detecção eletroquímica (Smimadzu, Kyoto, Japan), das concentrações cerebrais de 5-HT, 5-HIAA, DA e DOPAC. Para análise por CLAE, os tecidos foram previamente pesados e homogeneizados com ácido perclórico 0,2 M e centrifugados a 15000 rpm por 20 minutos a 6 °C. Foram, então, injetados 20 μL do sobrenadante no cromatógrafo e a quantificação das substâncias feita pela comparação da área do pico com uma curva padrão utilizando o software CLASSVP em linha com o cromatógrafo.

Durante o exercício físico, a temperatura corporal interna dos animais desse grupo foi registrada continuamente por telemetria utilizando sensor de temperatura intraperitoneal previamente implantado e calibrado (TR3000 VM-FH, Mini Mitter, Sun River, OR). A partir dos dados colhidos foram calculadas:

Taxa de aquecimento corporal (ºC.min^{-1}) = (Δtemperatura corporal interna/tempo de exercício); e

Taxa de acúmulo de calor (cal.min^{-1})= [(Δtemperatura corporal interna).m.c]/(tempo de exercício), sendo m= massa corporal em gramas e c = calor específico dos tecidos do animal (0.826 cal.g^{-1}.ºc^{-1}).

Experimento 5: efeito do bloqueio e inibição óxido nitrérgica do PVN sobre parâmetros cardiovasculares e térmicos durante a exposição ao calor

Para realização desse experimento foram utilizados ratos Sprague-Dawley (220–320 g) anestesiados com combinação de uretana (0,75 g/kg intraperitonealmente) e α-cloralose (70 mg/kg intraperitonealmente). A artéria femoral esquerda foi canulada e conectada a um sistema computadorizado de registro e análise de dados (MacLab; AD Instruments, Mountainview, CA) via transdutor de pressão (modelo P231D; Gould) para registro da pressão arterial e frequência cardíaca. A traquéia foi entubada com o intuito de facilitar a ventilação. Em seguida, o animal foi posicionado em estereotáxico (David Kopf Instruments, Tujunga, CA) para posicionamento da cânula de microinjeção no PVN seguindo as coordenadas de Paxinos e Watson (1986) (A: -1,5 mm; L: -0.4 mm; V: -7.8 mm). As microinjeções foram realizadas com uso de microseringa (0,5 μL; modelo 7000.5; Hamilton).

O posicionamento em eletrodos de platina bipolares de um dos ramos do nervo renal permitiu o registro da atividade simpática do nervo. O sinal foi amplificado (10.000 vezes; Grass amplifier, modelo P55), retificado e integrado.

O sinal registrado após o fim do experimento, quando o animal já havia sido sacrificado, foi considerado ruído. A descarga do nervo foi calculada pela subtração entre o ruído e o valor basal ou o registrado durante o experimento.

Após os procedimentos cirúrgicos e estabilização dos parâmetros por no mínimo 20 minutos, iniciou-se o estresse térmico. A atividade simpática do nervo renal, pressão arterial, frequência cardíaca, assim como as temperaturas corporal interna e da cauda foram registradas continuamente. A temperatura colônica foi considerada como interna e medida usando probe inserido 4 cm após o esfíncter anal (modelo 401, Yellow Springs Instruments, USA). Para determinação da temperatura da cauda, um termistor de cauda (409-B, Yellow Springs Instruments, USA) foi fixado à superfície dorsal da pele, cerca de 10 mm da base da cauda. Essas temperaturas foram usadas para determinação da variação da temperatura interna no momento no qual a temperatura da cauda iniciou-se (vasodilatação). O estresse térmico foi induzido por aumento da temperatura do cobertor térmico (Staco, Model 3PN 1010BV) entre 37 e 43°C, em uma taxa de 1.2°C a cada 6 minutos, durante 30 minutos. Os animais foram aleatoriamente separados nos grupos para receber microinjeção de solução veículo (CSF; 100 nL/lado), lidocaina (1%; 200 nL/lado) ou L-NMMA (inibidor da óxido nítrico sintase; 200 pmol, 100nL/lado) bilateralmente no PVN. Experimento controle também foi realizado, durante o qual os mesmos parâmetros foram registrados durante 30 minutos sem exposição ao calor.

Ao fim dos experimentos, os cérebros foram removidos para avaliação histológica. Somente as injeções localizadas < 0,5 mm dos arredores do PVN foram consideradas efetivas.

ANÁLISE ESTATÍSTICA

Para a análise estatística foi utilizada a análise de variância (ANOVA), seguido do teste de Newman-Keuls. Os dados também foram comparados utilizando teste t de Student pareado ou não pareado, de acordo com a sua aplicabilidade. As correlações foram verificadas por meio do coeficiente de correlação de Pearson. O nível de significância foi estabelecido em 5%.

VI. RESULTADOS ALCANÇADOS

Bloqueio dos receptores centrais AT1 eleva o custo metabólico durante o exercício, reduzindo a eficiência mecânica e o desempenho físico em ratos [Neuropeptides (41): 189-194; 2007].

Foi investigado o efeito do bloqueio central do receptor central AT1 para Ang II sobre a taxa metabólica e desempenho físico em ratos durante o exercício em esteira (18 $m.min^{-1}$, 5 % inclinação). O consumo de oxigênio (VO_2) foi mensurado utilizando sistema calorimétrico indireto, após a injeção de 2 μL de Sal (n=9) ou Los (60 nmol, n=9) no ventrículo cerebral lateral direito, antes dos animais correram até a fadiga. A eficiência mecânica e o trabalho realizado foram calculados. O trabalho realizado pelos animais tratados com Los foi 29% inferior em relação aos animais tratados com Sal ($p<0,02$). Durante os primeiros 10 minutos de exercício (fase dinâmica do exercício), houve um aumento similar do VO_2, enquanto a eficiência mecânica permaneceu a mesma em ambos os grupos. Durante a fase estável do exercício, o VO_2 permaneceu estável no grupo Sal, porém continuou a aumentar e estabilizou-se em um nível mais elevado até a fadiga no grupo de animais tratados com Los. Durante a fase estável do exercício houve uma redução mais acentuada da eficiência mecânica nos ratos tratados com Los quando comparados com os animais tratados com Sal ($p<0,01$), a qual se correlacionou inversamente com o trabalho realizado

(r=0,74; p<0,01). Nossos dados evidenciam que o bloqueio do receptor AT1 aumenta o custo metabólico durante o exercício, reduzindo a eficiência mecânica e o desempenho físico. Os resultados indicam que o sistema angiotensinérgico central modula a produção de calor, aumentando a eficiência mecânica durante a fase estável do exercício.

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Short communication

Central AT_1 receptor blockade increases metabolic cost during exercise reducing mechanical efficiency and running performance in rats ☆

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Abstract

The effect of central angiotensin AT_1 receptor blockade on metabolic rate and running performance in rats during exercise on a treadmill (18 m × min^{-1}, 5% inclination) was investigated. Oxygen consumption (VO_2) was measured, using the indirect calorimetry system, while the animals were exercising until fatigue after injection of 2 μL of losartan (Los; 60 nmol, $n = 9$), an angiotensin II AT_1 receptor antagonist, or 2 μL of 0.15 M NaCl (Sal, $n = 9$) into the right lateral cerebral ventricle. Mechanical efficiency (ME) and workload (W) were calculated. The W performance by Los-treated animals was 29% lesser than in Sal-treated animals ($p < 0.02$). During the first 10 min of exercise (dynamic state of exercise), there was a similar increase in VO_2, while ME remained the same in both groups. Thereafter (steady state of exercise), VO_2 remained stable in the Sal group but continued to increase and stabilized at a higher level in Los-treated animals until fatigue. During the steady state of exercise there was a sharper reduction in ME in Los-treated rats compared to Sal-treated animals ($p < 0.01$) that was closely correlated to W ($r = 0.74$; $p < 0.01$). Our data showed that AT_1 receptor blockade increases metabolic cost during exercise, reducing mechanical efficiency. These results indicate that central angiotensinergic transmission modulates heat production, improving ME during the steady state of exercise.

Keywords: Losartan; AT_1 receptor; Exercise; Oxygen consumption; Mechanical efficiency; Running performance

1. Introduction

The balance between heat production and heat loss determines internal body temperature (T_b) in homeothermic animals. The increase in T_b that occurs in response to continuous exercise results from the temporary imbalance in the rates of metabolic heat production by exercising muscles and heat dissipation during the early stage of exercise (Gleeson, 1998; Webb, 1995). The energy efficiency of body becomes apparent during exercise, when ~20–27% of the energy expended can be used for external work, whereas the remaining adenosine triphosphate (ATP) production is used for homeostasis or dissipated as heat (Brooks et al., 1984). Oxygen consumption during exercise (i.e., total energetic cost) is an important parameter in physical work that reflects both mechanical efficiency and running performance (Brooks and White, 1978; Sonne and Galbo,

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1980). Therefore, any modification in the rates of metabolic heat production and/or heat dissipation will influence the caloric balance during exercise.

We recently showed that intracerebroventricular (icv) infusion of losartan (Los; AT_1 receptor antagonist) induced a significant increase in body heating rate (i.e., rate of increase in T_b) that rapidly produced hyperthermia 0.41 °C with a significant increase in threshold body temperature for tail vasodilation (Leite et al., 2006). Therefore, central angiotensinergic transmission plays an important role on thermoregulation during exercise by increasing heat dissipation through peripheral vasodilation, preventing high levels of heat storage (Leite et al., 2006). Even though the inhibitory effect of angiotensin II (Ang II) on heat dissipation has already been verified, it is still unclear whether it may also interfere on the metabolic cost of exercise. Therefore, the aim of this study was to assess the effects of the central administration of the AT_1 receptor antagonist Los on the metabolic cost of untrained rats submitted to exercise until fatigue.

2. Methods

2.1. Animals

Male Wistar rats (240–310 g) were housed individually at a room temperature of 22 ± 2 °C, under 14/10 h light-dark cycles and had free access to water and rat chow. Following anesthesia achieved using 2,2,2-tribromoethanol (1 mL/100 g ip body weight), the rats were fixed to a stereotaxic apparatus (David Kopf Instruments, M-900, Tujunga, CA, USA) and a guide cannula (22 G) was implanted into the right lateral cerebral ventricle using a previously described technique (Antunes-Rodrigues and McCann, 1970). All animals were allowed to recover for at least 1 week before being submitted to the experiments. The animals were acclimatized to exercise on the motor-driven treadmill by running at a speed of $15\ m \times min^{-1}$ at 5% inclination for 5 min per day during four consecutive days prior to the experiments. The purpose of this preliminary exercise was to show the animals in which direction to run. All experiments were approved by the Ethics Committee of the Federal University of Minas Gerais for the Care and Use of Laboratory Animals and were carried out in accordance with the regulations described in the Committee's Guiding Principles Manual.

2.2. Experimental protocol

The experimental protocol followed the APS Resource Book for the Design of Animal Exercise Protocols (American Physiological Society, 2006). On the day of the experiment, the animals were allowed to rest for 1 h in the rodent treadmill chamber before being submitted to the test. A needle (30 G) protruding 0.3 mm from the tip of the guide cannula was introduced into the right lateral cerebral ventricle by connecting it to a Hamilton syringe. Immediately prior to exercise, 2.0 μL of 0.15 M NaCl (Sal, $n = 9$) or 2.0 μL of Los (Merck Sharpe & Dohme, Campinas, Brazil; 60 nmol, $n = 9$) was injected into the right lateral ventricle. The dose of Los was based on the results of our previous experiments that showed that the response of increase in T_b at fatigue point was clearly Los dose–dependent (Leite et al., 2006). Thus, the chosen dose of Los (60 nmol; icv) decreased time to fatigue and increased in 0.51 °C the T_b at fatigue point of treated-animals (Leite et al., 2006). Rats were randomly assigned to groups receiving either Sal or Los solution. An interval of at least four days was allowed for the animal to recover between the tests. Immediately after the icv injections, the animals were submitted to running exercise until fatigue. Exercise was performed on a motor-driven treadmill (Columbus Instruments, OH, USA, Modular Treadmill, serial number 96002-2) between 10:00 and 14:00 h at a room temperature of 22 ± 2 °C. The intensity of exercise ($18\ m \times min^{-1}$ and 5% inclination) corresponded to an oxygen uptake of ~66% of VO_{2max} (Brooks and White, 1978; Lacerda et al., 2006a,b). Fatigue was defined as the point at which the animals were no longer able to keep pace with the treadmill (Lima et al., 1998; Rodrigues et al., 2004; Soares et al., 2004). Time to fatigue (minutes) and workload (kg m) were considered indexes of exercise performance.

Oxygen consumption (VO_2), was measured by an open-flow indirect calorimeter (Columbus Instruments), calibrated before each use with a certified mixture of gases (20.5% O_2 and 0.5% CO_2). VO_2 ($mL\ O_2 \times kg^{-1} \times min^{-1}$) was continuously recorded on-line, every minute at rest and during exercise until fatigue using a computerized system (Oxymax Apparatus, Columbus Instruments).

2.3. Calculations

Workload (W; kg m) was calculated as: $W = [\text{body weight (kg)}] \times [\text{TTF}] \times [\text{treadmill speed } (m \times min^{-1})] \times [\sin\theta \text{ (treadmill inclination)}]$ (Brooks and White, 1978; Lima et al., 2001), where TTF is time to fatigue (minutes).

Mechanical efficiency (ME; %) was calculated by the formula: $ME = (W/\text{energetic cost}) \times 100$ (Brooks et al., 1984; Lacerda et al., 2006a; Soares et al., 2003).

2.4. Statistical analysis

The data are reported as mean ± SEM. Differences between groups and the effect of time were evaluated

using the analysis of variance (ANOVA) test, followed by the Newman–Keuls test. The data were also compared using paired or unpaired Student's *t*-test, as applicable. The correlation between the decrease in ME (steady state) and *W* were assessed using Pearson's correlation coefficient. Significance level was set at $p < 0.05$.

3. Results

The icv injection of Los in untrained, normal rats ($n = 9$) induced a 26% and 29% decrease in TTF (18.7 ± 1.2 min, Los, *vs* 25.2 ± 2.9 min, Sal, $p < 0.02$) and *W* (4.4 ± 0.3 kg m, Los *vs* 6.2 ± 0.7 kg m, Sal, $p < 0.01$), respectively, when compared to Sal-treated rats ($n = 9$) (Fig. 1a).

To asses the metabolic cost of exercise in the two treatment groups, we analyzed the metabolic rate data during the two states of exercise: (1) the dynamic state of metabolic adjustment of exercise (first 10 min of exercise, when metabolic rate is still rising), and (2) the steady state of metabolic adjustment of exercise (after 11 min of exercise until fatigue).

Exercise induced a rapid increase in VO_2 in both groups (Fig. 1b). The differences in VO_2 between the treatments were already observed at 7 min and remained different until fatigue. Such increase was more intense during the dynamic state of exercise but remained constant until the fatigue point. However, after 11 min of exercise VO_2 remained stable in the saline group but continued to increase and stabilized at a higher level in Los-treated animals until fatigue. The highest level of metabolic rate in both groups was attained at fatigue point (35.8 ± 2.3 mL $O_2 \times kg^{-1} \times min^{-1}$, Los *vs* 29.0 ± 2.5 mL $O_2 \times kg^{-1} \times min^{-1}$, Sal, $P < 0.05$).

The ME was similar between groups during the dynamic state ($34.0 \pm 2.1\%$, Los *vs* $30.5 \pm 1.3\%$, Sal), however, Los-treated animals showed a lower ME than the Sal-treated animals during the steady phase of exercise ($22.4 \pm 1.1\%$, Los *vs* $26.4 \pm 1.7\%$, Sal; $P < 0.01$) (Fig. 2a). We also observed a close correlation between the decrease in ME during the steady phase of exercise ($r = 0.74$; $P < 0.01$) and *W* (Fig. 2b).

4. Discussion

The present study shows that AT_1 receptor blockade by Los interferes with metabolic rate adjustment in exercising rats. These data suggest that central angiotensin-mediated pathways are involved not only in thermoregulatory heat loss (Leite et al., 2006), but also in the control of metabolic heat production as shown by the higher metabolic rate observed in Los-treated rats. During exercise, the Los-treated rats showed a ~19% higher metabolic cost than controls and a decreased ME during the steady state of exercise that was closely associated with the decrease in W. Therefore, these findings indicate that the increase in the metabolic rate of Los-treated rats, which in turn produced a decrease in ME, may have led to a marked reduction in time to fatigue. To the best of our knowledge, this is

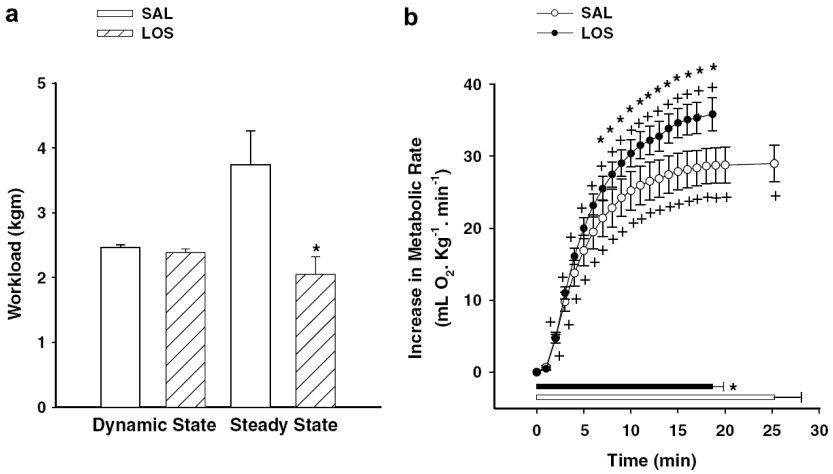

Fig. 1. Effect of icv injection of Los (60 nmol/2 μL) or 0.15 M NaCl (2 μL, Sal) on workload (a) during the first 10 min (dynamic state) and after 11 min of running until fatigue (steady state) and on metabolic rate (b) during exercise. Values are expressed as mean ± SEM, $n = 9$ each group. Time to fatigue is indicated by the horizontal bar at the bottom of graph 1b: Sal (open bar) and Los (filled bar). $^{*}P < 0.05$ compared with saline-treated group. $^{+}P < 0.05$ compared with corresponding basal value.

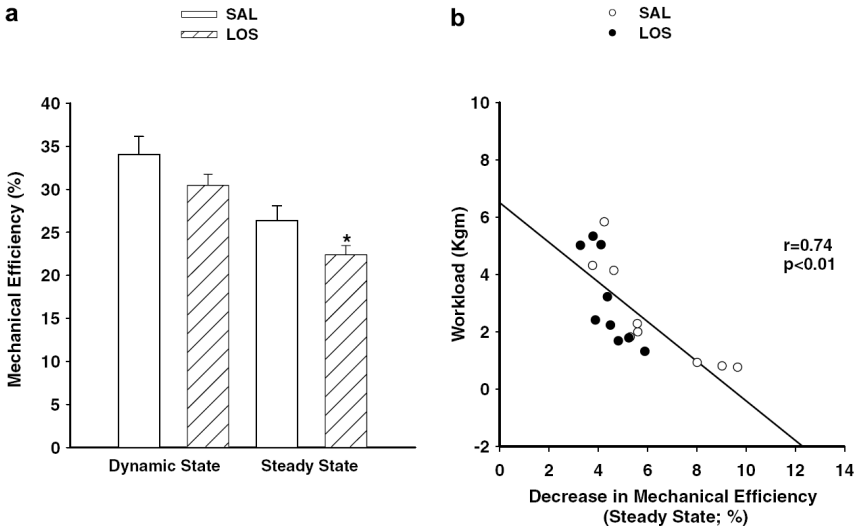

Fig. 2. Effect of icv injection of Los (60 nmol/2 μL) or 0.15 M NaCl (2 μL, Sal) on mechanical efficiency (a) during the first 10 min (dynamic state) and after 11 min of running until fatigue (steady state) and on the correlation between workload and the decrease in mechanical efficiency (b) during the steady state: Sal (open circle) and Los (filled circle). Values are expressed as mean ± SEM, $n = 9$ each group. *Significantly different from the control group ($P < 0.05$).

the first study to describe the role of brain Ang II in modulating heat production to determine metabolic cost of exercise.

The balance between heat production and heat loss determines internal body temperature in homeothermic animals. Increased T_b during exercise is the consequence of an increase in metabolic rate and the failure of heat loss to keep pace with heat production (Gleeson, 1998; Jessen, 1987; Webb, 1995). To maintain the thermal balance, heat production by exercising muscles should be counteracted by increased heat loss, otherwise, activity would result in greater body temperature. Elevated internal body temperature and increased heat storage have been considered limiting factors that reduce the CNS drive for exercise performance and precipitate feelings of fatigue, protecting the brain from thermal damage (Fuller et al., 1998; Nielsen et al., 1997). Furthermore, hyperthermia reduces physical performance in many mammalian species, including rodents (Fuller et al., 1998; Nielsen et al., 1993; Walters et al., 2000).

Some studies provide evidence that central angiotensinergic pathways play an important role in thermoregulation by increasing heat dissipation through skin vasodilation as well as decreasing metabolic rate and T_b (Wilson and Fregly, 1985a,b; Wright and Katovich, 1996). This mechanism would prevent high levels of heat storage and excessive hyperthermia. We have already demonstrated that central angiotensinergic transmission has important effects on thermoregulation during exercise by modulating heat dissipation through peripheral vasodilation (Leite et al., 2006). The present findings bring further evidences that angiotensinergic transmission in CNS is involved in heat balance during exercise. In fact, our data showed that the brain Ang II pathways activation in normal rats may also modulate metabolic heat production since Los-treated rats showed a marked reduction in ME, which reflects a higher amount of energy dissipated as heat. The increased oxygen consumption associated with the reduced ME may have decreased the run-time to fatigue. Therefore, the decrease in TTF observed in our study might have resulted from a higher heat production action of central Los not compensated by heat loss. It is known that part of the metabolic energy consumed during exercise is dissipated as heat and the other part is used to perform mechanical work. This assumption allows us to hypothesize that the brain angiotensin mediated pathways may have a thermolytic effect during exercise, improving heat loss and heat production activation mechanisms, protecting the brain from excessive hyperthermia and improving physical performance.

The exact location and precise pathways involved in the angiotensinergic mediation of normal thermoregulation during exercise still require clarification. However, hypothalamic regions expressing ANG II, such as the preoptic area (POA) and the paraventricular

nucleus (PVN), are possible sites at which ANG II may influence thermoregulation during exercise (McKinley et al., 2003). Therefore, we hypothesized that infusion of Los into the cerebral ventricle would perfuse to the thermoregulatory centers situated in the hypothalamus augmenting heat production response during prolonged exercise. The preoptic area/anterior hypothalamus (POA/AH) is thought to be the primary locus for body temperature regulation (Briese, 1998; Coimbra and Migliorini, 1988; Santos et al., 1990, 1991) due to the fact that it contains both warm-sensitive and cold-sensitive neurons that respond to small changes in temperature (Ishiwata et al., 2002; Zhang et al., 1997). It has been established that the POA/AH is an integrative region for the maintenance of metabolic, vasomotor and thermal homeostasis in resting conditions as well as during exercise (Ferreira et al., 1999; Hasegawa et al., 2005). These data indicate that the POA/AH might be an important mediator of heat production during exercise. Taken together, these results and the fact that POA express receptors for Ang II suggest that the POA is one possible site of Los action. However, further researches are necessary to identify the exact location of angiotensinergic mediation involved in normal thermoregulation during exercise.

In summary, icv infusion of Los induced a significant increase in oxygen consumption, which rapidly produced a ~19% higher metabolic cost than in controls, with a significant decrease in ME and precipitation of fatigue. Therefore, our results provide further evidence that central angiotensinergic transmission has an important role on metabolic adjustment during exercise by modulating heat production and improving ME.

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Receptores centrais AT1 estão envolvidos com ajustes metabólicos em resposta ao exercício progressivo em ratos

[Peptides; (30): 1931-1935; 2009].

Com o intuito de investigar a influência dos receptores AT1 centrais para Ang II sobre os ajustes metabólicos em ratos durante o exercício até a fadiga em esteira, 2 μL de Sal ou Los (60 nmol) foram intracerebroventricularmente injetados imediatamente antes do exercício progressivo (velocidade inicial de 10 m/min, com aumento de 1 m/mim a cada 3 minutos até a fadiga, 10% de inclinação). O consumo de oxigênio (n=6) foi mensurado por calorimetria indireta de fluxo aberto. O mesmo protocolo foi utilizado para coleta de amostras de sangue através de cateter jugular (n=7). As concentrações plasmáticas de glicose, lactato e ácidos graxos livres foram determinadas. A eficiência mecânica e trabalho realizado foram calculados. Os animais tratados com Los apresentaram hiperglicemia até a fadiga, já verificada em baixa intensidade de exercício como 20% do trabalho máximo realizado. Altos níveis plasmáticos de lactato e ácidos graxos livres acompanharam esta resposta hiperglicêmica. Durante os seis primeiros minutos de exercício, aumento similar do VO_2 e eficiência mecânica semelhante foram observados em ambos os grupos. Em seguida, o VO_2 continuou a elevar-se, porém a uma taxa maior nos animais tratados com Los, resultando em redução de 34% na eficiência mecânica ($p<0,01$) associada com redução de 27% no trabalho realizado ($p<0,01$). Os dados mostram que o bloqueio dos receptores AT1 centrais durante o exercício

progressivo induz hiperglicemia e maior mobilização de ácidos graxos livres em baixos níveis de intensidade de exercício. Ademais, eleva o custo metabólico, resultando em menor eficiência mecânica. Conclui-se que o sistema angiotensinérgico central está envolvido em ajustes metabólicos durante o exercício uma vez que o bloqueio dos receptores AT1 altera o equilíbrio energético durante o exercício progressivo, semelhante a situações de ativação simpática intensa e prematura.

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Short communication

Central angiotensin AT_1 receptors are involved in metabolic adjustments in response to graded exercise in rats

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ABSTRACT

To investigate the influence of central angiotensin AT_1-receptors blockade on metabolic adjustments during graded exercise, Losartan (Los) was intracerebroventricularly injected in rats before running until fatigue. Oxygen consumption (VO_2) was measured ($n = 6$) and blood samples collected ($n = 7$) to determine variations of glucose, lactate and free fatty acids (FFA). Los-rats exhibited a hyperglycemic response, already observed at 20% of maximal work, followed by a higher lactate levels and FFA mobilization from adipose tissue. Despite the reduced total time to fatigue and the higher VO_2 associated with reduced mechanical efficiency, exercise led to the attainment of similar levels of effort in both groups. In summary, central AT_1-receptor blockade during graded exercise induces hyperglycemia and higher FFA mobilization from adipose tissue at low exercise intensities in rats running at the same absolute exercise intensity. These data suggest that the central angiotensinergic system is involved in metabolic adjustments during exercise since central blockade of AT_1-receptors shifts energy balance during graded exercise, similarly to situations of higher and premature sympathetic activation.

1. Introduction

We have recently shown that intracerebroventricular (icv) infusion of the angiotensin II (Ang II) AT_1-receptor antagonist Losartan (Los) induces an increase in oxygen consumption, higher metabolic cost and hyperthermia due to reduced peripheral heat loss during continuous exercise (~66% VO_2) [11,12]. Besides the effects of Ang II on thermoregulation and metabolic cost during exercise, it is still not known whether the central angiotensinergic system could also be involved in the regulation of fuel sources, as it happens in situations of high sympathetic activation such as exercise and during hemorrhage [14,15]. Ang II has been found to act in the regulation of metabolic and endocrine functions [18], especially blood glucose homeostasis [4,15–17]. This peptide induces hyperglycemia not only by activating the sympathetic nervous system and the sympathoadrenal system, but also by acting directly on hepatic glucose production, increasing glycogenolysis, gluconeogenesis and also hepatocyte glycogen phosphorylase activity [4,17,20]. Additionally, it has been shown that Ang II increases interstitial norepinephrine and glycerol concentrations in white adipose tissue, which suggests that this is the result of increased local and general sympathetic activity [3]. Therefore, with the purpose of determining metabolic effects of exercise at different intensities, the present study was undertaken to assess the effects of central administration of Los on oxygen consumption, plasma glucose, lactate and free fatty acids (FFA) variations in untrained rats submitted to graded exercise until fatigue.

2. Materials and methods

2.1. Animals

Male Wistar rats (290 ± 10 g) were housed individually at a room temperature of 22 ± 2 °C, under 14/10 h light–dark cycles and had free access to water and rat chow. Following anesthesia achieved using a mixture of ketamine (115 mg/kg body weight i.p.) and xylazine (6 mg/kg body weight i.p.), the rats were fixed to a stereotaxic apparatus (David Kopf Instruments, M-900, Tujunga, CA, USA) and a guide cannula (22 G) was implanted into the right lateral cerebral ventricle using a previously described technique [11,12]. All animals were allowed to recover for at least 1 week before being submitted to the experiments. The animals were acclimatized to exercise on the motor-driven treadmill by running at a speed of

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15 m min^{-1} at 5% inclination for 5 min per day during four consecutive days prior to the experiments. This preliminary exercise did not constitute training. Its purpose was to show the animals in which direction to run [11,12]. All experiments were approved by the Ethics Committee of the Federal University of Minas Gerais for the Care and Use of Laboratory Animals and were carried out in accordance with the regulations described in the Committee's Guiding Principles Manual.

2.2. Experimental protocol

On the day of the experiment, the animals were allowed to rest for 1 h in the rodent treadmill chamber before being submitted to the test. A needle (30 G) protruding 0.3 mm from the tip of the guide cannula was introduced into the right lateral cerebral ventricle by connecting it to a Hamilton syringe. One minute prior to exercise, 2.0 μL of 0.15 M NaCl or 2.0 μL of Los (Merck Sharpe & Dohme, Campinas, Brazil; 60 nmol) were injected into the right lateral cerebral ventricle. The dose of Los was based on the results of our previous experiments [11,12]. Rats were randomly assigned to groups receiving either Sal or Los solution. The investigators were blinded to the randomization scheme. An interval of at least 4 days was allowed for the animal to recover between the tests. Immediately after the icv injections, the animals were submitted to graded running exercise until fatigue. Graded work was performed on a motor-driven treadmill (Columbus Instruments, OH, USA, Modular Treadmill) between 10:00 and 14:00 h, at a room temperature of 22 ± 2 °C and at a constant slope of 10°. The rats started running at 10 m min^{-1} and treadmill speed was increased by 1 m min^{-1} every 3 min until fatigue [10]. Fatigue was defined as the point at which the animals were no longer able to keep pace with the treadmill [10–12]. Total time to fatigue (min) and workload (kg m) were considered indexes of exercise performance.

2.3. Experiment 1: measurement of oxygen consumption during graded exercise until fatigue

In the first group of rats ($n = 6$), oxygen consumption (VO_2) was measured by an open-flow indirect calorimeter (Columbus Instruments), calibrated before each use with a certified mixture of gases (20.5% O_2 and 0.5% CO_2). VO_2 ($mLO_2\ kg^{-1}\ min^{-1}$) was continuously recorded on-line at rest and during graded exercise until fatigue using a computerized system (Oxymax Apparatus, Columbus Instruments). Mechanical efficiency (%) was considered an index of energy efficiency.

2.4. Experiment 2: determination of plasma variations of glucose, lactate and FFA during graded exercise until fatigue

Besides being implanted with a guide cannula, as described above, in another group of animals ($n = 7$), a chronic jugular vein catheter was also implanted during the same surgical procedure [10].

Blood samples (0.3 mL) were collected during exercise through the use of the jugular vein catheter immediately before graded exercise, at 3, 6, 9, 12, 15, and 21 min following the beginning of exercise and at fatigue point. The blood volume collected in each sample was replaced by donated blood to avoid reduction in the animal's blood volume. Three healthy donor rats were kept under standard conditions and used alternately [10]. The blood samples were centrifuged and the plasma separated. The plasma samples were then frozen (−20 °C) until biochemical analyses were carried out. The variations of plasma glucose and lactate were analyzed according to the enzymatic method (oxidase enzyme) with a glucose analyzer (YSI 2300-STAT PLUS, Yellow Springs Instruments). FFA variations were determined using a NEFA 30T kit (Randox Laboratories, USA), adapted for small volumes of plasma.

2.5. Calculations

Workload (kg m) was calculated as: [body weight (kg)] × [total time to fatigue] × [treadmill speed (m min^{-1})] × [sin α (treadmill inclination)] [10,11].

Mechanical efficiency (%) was calculated by the formula: [(workload/energetic cost) × 100] [10,11].

2.6. Statistical analysis

The data are reported as mean ± SEM. Differences between groups and the effect of time were evaluated using analysis of variance (ANOVA), followed by the Newman–Keuls test. The data were also compared using paired or unpaired Student's t-test, as applicable. Significance level was set at $p < 0.05$.

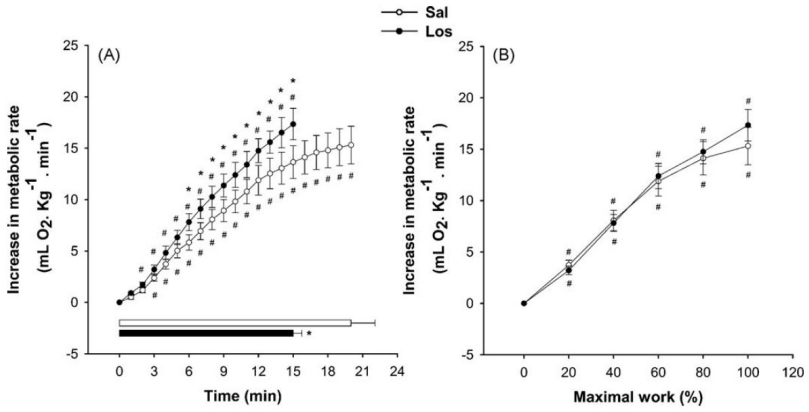

Fig. 1. Effect of icv injection of Los (60 nmol/2 μL) or 0.15 M NaCl (2 μL, Sal) on metabolic rate (A and B) during graded running until fatigue. Values are expressed as mean ± SEM, $n = 6$ each group. Time to fatigue is indicated by the horizontal bar at the bottom of graph 1A. Sal (open bar) and Los (filled bar). Oxygen consumption before exercise: Sal, 13.6 ± 1.5 $mLO_2\ kg^{-1}\ min^{-1}$; Los, 14.4 ± 1.5 $mLO_2\ kg^{-1}\ min^{-1}$. $^{*}p < 0.05$ compared with Sal-treated group. $^{\#}p < 0.05$ compared with corresponding basal value.

3. Results

The icv injection of Los in untrained, healthy rats ($n = 13$) induced a 22% and 27% decrease in total time to fatigue (17.6 ± 1.0 min, Los, vs 22.6 ± 1.5 min, Sal, $p < 0.01$) and workload (13.3 ± 0.9 kg m, Los vs 18.1 ± 1.1 kg m, Sal, $p < 0.01$), respectively, when compared with Sal-treated rats ($n = 13$). Despite the reduced exercise performance and the higher VO_2 already seen at low exercise intensity (12 m min^{-1}, 6 min) until fatigue by Los-treated rats (Fig. 1A), exercise led to the attainment of similar levels of effort in the groups (Fig. 1B). However, Los-treated animals still had a lower mechanical efficiency than Sal-treated animals (16.2 ± 2.2%, Los vs 24.6 ± 3.3%, Sal; $p < 0.01$).

As seen in Fig. 2A and B, plasma glucose levels remained unchanged in control animals. On the other hand, Los-treated rats exhibited a rise in plasma glucose variation throughout the experiment. Such hyperglycemic response was significantly higher than in controls already at 20% of maximal work, being maintained until fatigue.

Sal-treated animals showed an increase in plasma lactate levels in the beginning of exercise. However, such levels remained practically stable during all the experimental protocol. Although Los-treated animals showed a transitory increase in plasma lactate levels, when analyzing the data to maximal work, higher values were recorded throughout the exercise (Fig. 2C and D).

The increase in plasma FFA levels in control animals was only evident at 60% of maximal work and continued high until fatigue (Fig. 2E and F). In contrast, there was a marked increase in plasma variation of FFA in Los-treated rats that was evident as early as 3 min (10 m min^{-1}) until the end of exercise. At fatigue point, plasma FFA levels of Los-treated animals was ~28% higher than that of controls ($p < 0.05$).

4. Discussion

The experiments reported here demonstrate that central Ang II AT_1 receptors are involved in metabolic adjustments during exercise in rats. The data shows that central AT_1-receptor blockade with icv Los produces marked hyperglycemia and increased mobilization of FFA from adipose tissue. In addition, treatment with Los increases glycolytic flux during graded exercise, as indicated by the increase in plasma lactate already observed at low absolute intensity of exercise. Inhibition of the brain angiotensinergic system also induced a higher metabolic cost, decreased mechanical efficiency and exercise performance in rats running at the same absolute exercise intensity. The metabolic response of Los-treated rats to exercise was similar to situations of enhanced peripheral sympathetic outflow such as neurocytoglucopenia [23], hemorrhagic hypotension [25] or cold exposure [6] that also exhibited increased FFA mobilization, hyperglycemia and hyperlactemia. In fact, we recently have shown that icv injection of Los in rats submitted to continuous exercise (~66% VO_2) determine an enhanced sympathetic activation as seen by the increased tail vasoconstriction and elevation of the threshold body temperature for tail vasodilation during exercise [12]. Therefore, it seems reasonable to suggest that the shift of substrate mobilization balance following central AT_1-receptor blockade may be due to this faster activation of the sympathetic system, as previously observed [12]. During graded exercise, such sympathetic activation was more premature, since it was verified at an even lower level of exercise intensity such as 20% of maximal work, leading to increased glucose, lactate and FFA levels in plasma of running rats.

The results observed in the present study agree that the angiotensinergic system is involved in endocrine and metabolic adaptations in situations of stress [11,15,18]. It brings further evidences that central Ang II participates in the control of sympathetic activity during exercise, being responsible for adjusting the supply of fuel to the working muscles in order to make up for the new demand for ATP. Similarly to the actions of Ang II on prolactin secretion [18], the present results support that Ang II may possess divergent peripheral and central metabolic effects. Peripherally, Ang II is attributed to induce hyperglycemia due to sympathetic activation and by acting directly on hepatic glucose production [4,17,20]. This effect of Ang II seems also to be a consequence of its contribution to insulin resistance [22]. On the other hand, peripheral Ang II receptor blockers have been shown to block sympathetic activation and improve glycemic control by benefiting glucose-induced insulin secretion [22,27]. Herein, central Ang II AT_1-receptor blockade during graded exercise seems to induce sympathetic activation precociously at a lower level of exercise intensity, as verified by the increased FFA mobilization, hyperglycemia and hyperlactemia. Whether such opposite central and peripheral effects of Ang II on metabolic adjustments during exercise are physiologically relevant is still unknown.

The regulation of intramuscular carbohydrate metabolism, hepatic glucose production and mobilization of FFA from the adipose tissue during situations of high energy demands, such as in the case of exercise, have been shown to be modulated by direct sympathetic innervation and catecholamine secretion [5,8,9,30]. Sympathetic activity responds to exercise by inducing the secretion of higher amounts norepinephrine and epinephrine, released by the nerve endings and adrenal medullas, respectively [8,9]. Regardless of its duration or intensity, physical exercise induces an initial sympathetic activity burst required to adapt the organism to the new metabolic demand [5,8,30]. After this first burst of sympathetic activation, subsequent increases in sympathetic nervous system activity are proportional to the exercise intensity [8,30]. It is know that sympathetic nervous system activity rises little during mild to moderate exercise, which means that considerable sympathetic activation occurs mainly at exercise intensities higher than 60% [2]. Our experiments with Los-treated animals showed that glucose and FFA levels increased already at 20% of maximal work. Taking into account that direct sympathetic activation, rather than catecholamines secreted by the adrenal medulla, is the dominant and a potent stimulus to lipolysis and the release of FFA to the plasma [1,24], and that adrenomedullation of rats does not block lipid mobilization, including during exercise [14,24], our results point to a direct action of Ang II in modulating sympathetic outflow to the adipose tissue. This suggests that central AT_1 blockade probably induces a faster activation of the sympathetic system at a lower level of exercise intensity, causing a shift of substrate mobilization balance. Although both groups reached the same level of exercise intensity, the fact that Los-treated rats showed reduced mechanical efficiency and workload also indicates that sympathetic activity response may have been exacerbated and stimulated precociously as an attempt to compensate the reduced exercise performance.

It is important to point out that this regulation of energy metabolism during exercise is a very sensitive feedback mechanism that depends on the metabolic demands of the exercising muscles and the ambient glucose and FFA concentrations in plasma [28]. The experiment reported here with Sal-injected rats supports this assumption. In these animals, the blood variations of glucose and FFA remained practically unchanged during exercise, since the feedback regulation mechanism accurately matched the hepatic glucose production and FFA mobilization from adipose tissue to the peripheral uptake of these metabolites. Our results with Los injection into the cerebral ventricle suggests that this agent disrupts the feedback adjustments during exercise, since there was a clear increase of glucose and FFA in the animal's circulation. Therefore, because of this increase in plasma glucose and FFA levels, the reduction in running performance in Los-

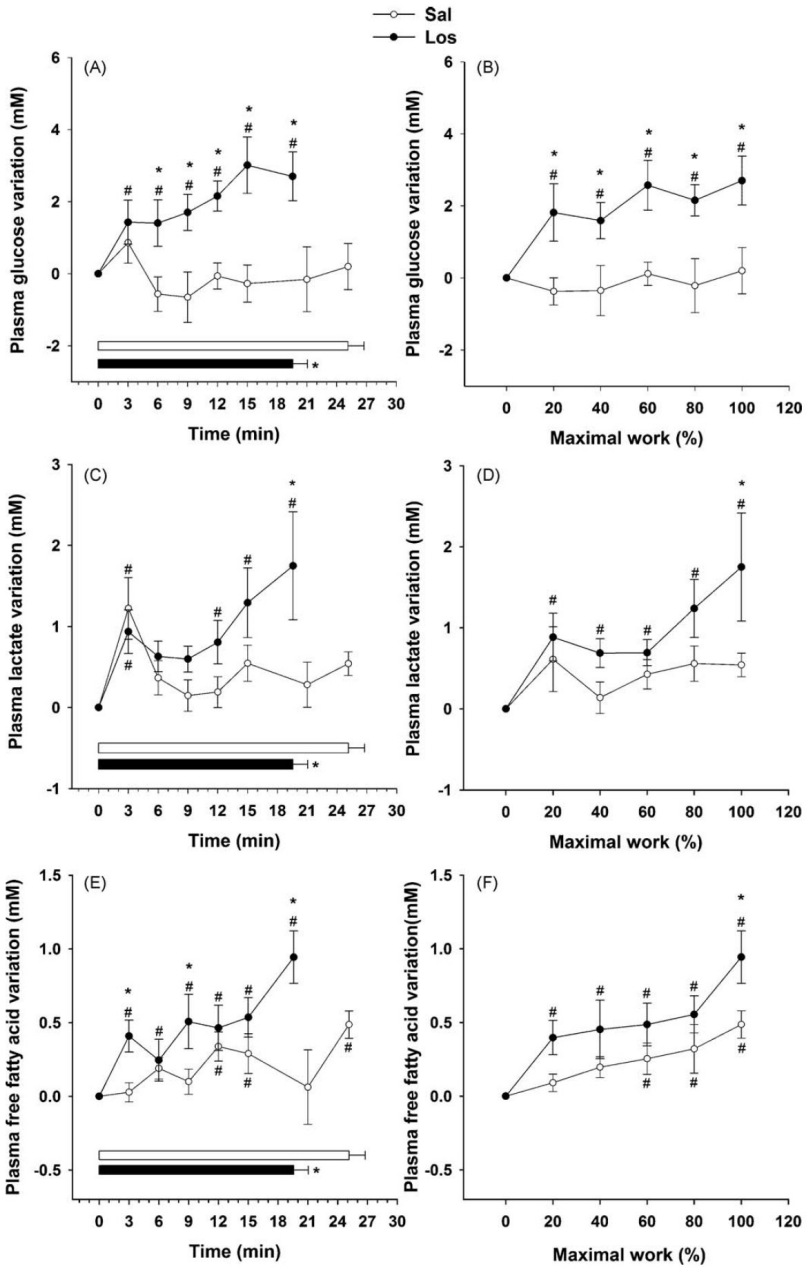

Fig. 2. Effect of icv injection of Los (60 nmol/2 μL) or 0.15 M NaCl (2 μL, Sal) on plasma glucose (A and B), lactate (C and D), and free fatty acids (FFA; E and F) variations during graded running until fatigue. Values are expressed as mean ± SEM, $n = 7$ each group. Time to fatigue is indicated by the horizontal bar at the bottom of graph 3A, C and E: Sal (open bar) and Los (filled bar). Basal values of glucose: Sal, 9.9 ± 0.5 mM; Los, 9.4 ± 0.5 mM; Lactate: Sal, 1.4 ± 0.2 mM; Los, 1.1 ± 0.1 mM; and FFA: Sal, 0.9 ± 0.1 mM; Los, 1.0 ± 0.2 mM. $^{*}p < 0.05$ compared with Sal-treated group. $^{\#}p < 0.05$ compared with corresponding basal value.

treated rats may not be related to an impaired availability of energy substrate.

The exact location and precise pathways involved in angiotensinergic mediation of metabolic homeostasis during exercise still require clarification. Hypothalamic regions expressing Ang II, such as the preoptic area (POA) or the paraventricular nucleus of the hypothalamus (PVN) are possible control sites [19]. In fact, these nuclei have been shown to be involved in both control of

hepatic glucose production and mobilization of FFA from adipose tissue [1,6,7,25]. It has been established that the POA is an integrative region for the maintenance of metabolic, vasomotor and thermal homeostasis [6,7,29]. Regarding PVN, it is considered a central site for the integration of sympathetic activity, including the activation of lipolysis from adipose tissue, induction of hyperglycemia and tail skin vasodilation [21,25,26]. However, further research is necessary to identify the exact location of angiotensinergic mediation involved in normal metabolic homeostasis during exercise.

A possible interaction between Ang II and nitric oxide (NO), already observed in many neuronal structures including the PVN, may be one of the mechanisms that led to the results observed here [13]. It has been shown that within the PVN, Ang II interacts with NO, a sympathetic tonus inhibitor, inducing its secretion, which in turns moderates the Ang II excitatory effect over sympathetic outflow, possibly via an inhibitory feedback mechanism [13]. Previous experiment showed that the inhibition of the brain's nitrergic system disrupts the accuracy of the neural mechanism that regulates plasma glucose and FFA mobilization during exercise in rats [10]. The present study also corroborates with this assumption by reaching similar results. Therefore, there might be an interaction between Ang II and NO in CNS sites responsible for metabolic homeostasis, where hypothetically Los would perfuse after its injection into the cerebral ventricle.

4.1. Conclusions

In summary, central AT_1-receptor blockade by Los during graded exercise results in higher levels of plasma FFA and higher glycolytic flux, as shown by enhanced lactate levels in plasma, even at a low level of exercise intensity. The results of this study indicate that central angiotensinergic transmission modulates metabolic cost and modifies energy balance during exercise in rats, probably by modulating sympathetic outflow as seen by the increased lipolysis.

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Fadiga central induzida por Losartan envolve o conteúdo cerebral de serotonina e dopamina

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A fim de investigar a influência do bloqueio dos receptores AT1 centrais para Ang II sobre a fadiga central devido ao metabolismo de 5-HT e DA durante o exercício físico, 2 μL de Sal (n=6) ou Los (60 nmol, n=6) foram intracerebroventricularmente injetados imediatamente antes de corrida até a fadiga (18 m-min^{-1}, 5 % inclinação). No momento da fadiga, o tecido cerebral foi cuidadosamente removido para dosagem de 5-HT, 5-HIAA, DA e DOPAC por cromatografia líquida de alta eficiência na área pré-óptica, hipotálamo, hipocampo e córtex frontal. Os ratos tratados com Los exibiram aumento do conteúdo de 5-HT na área pré-óptica e hipotálamo que correlacionou-se diretamente com a elevada taxa de aquecimento corporal e indiretamente com o reduzido tempo total de exercício. Ao contrário, o tempo para fadiga correlacionou-se positivamente com o reduzido conteúdo de 5-HT no hipocampo dos animais Los. Apesar dos níveis de DA não terem se alterado em nenhuma das regiões estudadas, o hipotálamo dos animais Los apresentou elevada razão 5-HT:DA. Os resultados indicam que o sistema angiotensinérgico está envolvido com a fadiga central devido a hipertermia uma vez que a elevada taxa de aquecimento corporal resultante do bloqueio dos receptores AT1 centrais em ratos em exercício está relacionada com aumento do conteúdo de 5-HT na área pré-óptica e hipotálamo, assim como sua redução no hipocampo. Além disso, a

interação entre 5-HT e DA no hipotálamo provavelmente contribui significativamente para a hipertermia e fadiga central prematura após inibição angiotensinérgica.

Central fatigue induced by Losartan involves brain serotonin and dopamine content

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ABSTRACT

Purpose: Investigate the influence of angiotensin II (Ang II) AT1 receptors blockade on central fatigue induced by brain content of serotonin (5-HT) and dopamine (DA) during exercise. *Methods:* Losartan (Los) was intracerebroventricularly injected in rats before running until fatigue (n=6/group). At fatigue, brains were quickly removed for measurement of 5-HT, 5-hydroxyindoleacetic acid (5-HIAA), DA and 3,4-Dihydroxyphenylacetic acid (DOPAC) by HPLC in preoptic area, hypothalamus, hippocampus and frontal cortex. *Results:* Intracerebroventricular injection of Los increased 5-HT content in preoptic area and hypothalamus that correlated positively with body heating rate and inversely with total time to fatigue. On the contrary, time to fatigue was directly correlated with the diminished 5-HT concentration in hippocampus of Los-rats. Although the levels of DA were not affected by Los treatment during exercise in any of the studied brain areas, a higher 5-HT:DA ratio was seen in hypothalamus of Los-animals. This higher hypothalamic 5-HT:DA ratio correlated positively with body heating rate and inversely with time to fatigue. *Conclusions:* Our results show that central fatigue due to hyperthermia and increased body heating rate induced by central Ang II AT1 receptors blockade in exercising rats is related with higher 5-HT content in preoptic area and hypothalamus, as well as with decreased level of this neurotransmitter in hippocampus. Furthermore, the interaction between 5-HT and DA within hypothalamus seems to contribute markedly to hyperthermia and premature central fatigue following angiotensinergic inhibition.

Keywords: Preoptic area, hypothalamus, hippocampus, hyperthermia, exercise performance.

INTRODUCTION

Paragraph number 1 Central fatigue during prolonged exercise is considered to be affected by accumulation or depletion of neurotransmitters, particularly serotonin (5-HT) (3, 9, 22, 23). High 5-HT activity is associated with lethargy and loss of central drive/motivation (3, 22). In fact, evidences suggest that a rise in 5-HT content in major brain areas responsible for thermoregulation, such as the preoptic area/anterior hypothalamus, is related with heat production and precipitation of fatigue (3, 5, 26, 28). In view of this last observation, substances that elevate body temperature, such as in the case of fever induced by cytokines, may anticipate central fatigue through elevation of brain 5-HT content (7).

Paragraph number 2 Although the role of 5-HT on central fatigue has been well documented, it is likely that other neurotransmitters are capable of influencing fatigue, such as dopamine (DA) (3, 9, 11, 22). This monoamine has been suggested to interaction with 5-HT during exercise, affecting exercise performance (11, 22). DA neurotransmission is associated with many physiological functions such as arousal, reward and motivation (2, 11, 14, 22), that could modify running performance. Central DA metabolism enhances during exercise in animals (2, 11, 14) and its central elevation has been linked with a delay on fatigue despite higher body temperature at fatigue point and heat storage during exercise (2, 11, 14).

Paragraph number 3 High internal body temperature and increased heat storage have been proposed as limiting factors that reduce the central nervous system drive for exercise performance, thus protecting the brain from thermal damage (12, 27, 34). Therefore, considering that serotonergic and dopaminergic interaction is involved with central fatigue, which is coincident with high body temperature and/or heat storage, the activation of mechanisms that could modulate the activity of such systems would improve exercise performance by inducing thermal adjustments.

` *Paragraph number 4* We have recently shown that intracerebroventricular infusion of the widely used antihypertensive drug Losartan (Los; Angiotensin II AT1 receptor antagonist) reduces running performance in rats due to a heat imbalance characterized by higher metabolic cost and reduced peripheral heat loss. These responses resulted in hyperthermia and increased body heating rate that was indirectly related with time to fatigue (18, 20). Besides the effects of angiotensin II (Ang II) on thermoregulation and metabolic cost during exercise, it is still not known whether this effect of angiotensinergic blockade on heat storage may be linked to serotonergic and/or dopaminergic pathways that exhibit relevant thermoregulatory and exercise performance effects (2, 11, 21, 28). Therefore, this study aimed to investigate the possible interaction between the central angiotensinergic system and 5-HT and DA content in areas of the central nervous system involved in thermoregulation and motor activity, including preoptic area, hypothalamus, frontal cortex and hippocampus, during exercise.

METHODS

Animals

Paragraph number 5 Male Wistar rats, approximately three months old (290 ± 10 g), were housed individually at a room temperature of 22 ± 2 ºC, under 14/10 h light-dark cycles and had free access to water and rat chow. Following anesthesia achieved using a mixture of ketamine (115 mg/kg body weight i.p.) and xylazine (6 mg/kg body weight i.p.), the rats were fixed to a stereotaxic apparatus (David Kopf Instruments, M-900, Tujunga, CA, USA) and a guide cannula (22 G) was implanted into the right lateral cerebral ventricle using a previously described technique (20). Also during this surgical procedure, TR3000 VM-FH temperature sensor (Mini Mitter, Sun River, OR) was implanted into the peritoneal cavity through a small incision in the linea alba. All animals were allowed to recover for at least 1 week before being submitted to the experiments. The animals were acclimatized to exercise on the motor-driven treadmill by running at a speed of 15 $m \cdot min^{-1}$ at 5% inclination for 5 min per day during four consecutive days prior to the experiments. This preliminary exercise did not constitute training. Its purpose was to show the animals in which direction to run (18, 20). Electrical stimulation was determined according to each animal's tolerability. All experiments were approved by the Ethics Committee of the Federal University of Minas Gerais for the Care and Use of Laboratory Animals and were carried out in accordance with the regulations described in the Committee's Guiding Principles Manual. Additionally, the experimental protocol followed the American College of Sports Medicine (ACSM) animal care standards.

Experimental protocol

Paragraph number 6 On the day of the experiment, the animals were individually allowed to rest for 1 h in the rodent treadmill chamber before being submitted to the test. A needle (30 G) protruding 0.3 mm from the tip of the guide cannula was introduced into the right lateral cerebral ventricle by connecting it to a Hamilton syringe. One min prior to exercise, 2 μL of 0.15 M NaCl (Sal) or 2 μL of Los (Merck Sharpe & Dohme, Campinas, Brazil; 60 nmol) were injected into the right lateral ventricle. The dose of Los was based on the results of our previous experiments (18, 20). Rats were randomly assigned to groups receiving either Sal or Los solution (n=6/group). The investigators were blinded to the randomization scheme. Immediately after the intracerebroventricular injections, the animals were submitted to running exercise until fatigue at constant speed and inclination (18 m/min and 5% inclination). Exercise was performed on a motor-driven treadmill (Columbus Instruments, OH, USA, Modular Treadmill) between 10:00 and 14:00 h at a room temperature of 22 ± 2 ºC. The intensity of exercise corresponded to an oxygen uptake of ~66% of maximal oxygen uptake, which represents a physical activity of moderate level (4, 15, 20). Body temperature was recorded continuously by telemetry. Fatigue was defined as the point at which the animals were no longer able to keep pace with the treadmill (18, 20). Total time to fatigue (min) and workload (kgm) were considered indexes of exercise performance.

Paragraph number 7 As soon as the fatigue point was reached, the animals were killed by decapitation. The brain was quickly removed and washed with ice-cold saline. The preoptic area, hypothalamus, hippocampus and frontal cortex were rapidly dissected on an ice-cold plate (26, 28), frozen immediately in dry ice and stored at -80 °C until 5-HT, 5-hydroxyindoleacetic acid (5-HIAA), DA and 3,4-Dihydroxyphenylacetic acid (DOPAC) were measured by high-pressure liquid chromatography (HPLC). The HPLC system was equipped with a reverse-phase column (Shim Pack CLC-OSD; 25 cm, 5 m, Shimadzu). The potential was set at 850 mV versus Ag/AgCl reference electrode. A mobile phase containing 31.4 g citric acid, 584 mg NaCl, 800 mL miliQ water, 140 mg octylsodium sulfate, 48 mL acetylnitrile and 28 mL tetrahydrofurane (pH 3.0) was filtered and pumped through the system at a flow rate of 1.0 mL min^{-1}. The brain samples were weighed and preoptic area and hypothalamus homogenized in 200 µL of perchloric acid (0.1 M), while hippocampus and hypothalamus were homogenized in 300 µL of the same solution. The homogenates were then centrifuged at 15000 × *g* for 20 min at 6 °C, and the supernatants filtered through a Millipore membrane (0.22 pore size; 13 mm; Millex, SP, Brazil). Twenty microliters of the supernatant were injected into the HPLC-EC system for analysis (Shimadzu, Kyoto, Japan). Quantification of 5-HT, 5-HIAA, DA and DOPAC were made by comparing the peak area to a standard curve.

Calculations

Paragraph number 8 Workload (kgm) was calculated as: [body weight (kg)]. [total time to fatigue (min)]. [treadmill speed ($m.min^{-1}$)].[sine θ (treadmill inclination)] (18, 20).

Paragraph number 9 Body heating rate ($ºC.min^{-1}$), i.e. rate of increase in body temperature, was calculated as: Δbody temperature/(running time interval), where Δbody temperature is the change in body temperature (T_f-T_i), T_f = body temperature at fatigue point; and T_i = initial body temperature measured prior to exercise (20).

Paragraph number 10 Heat storage rate ($cal.min^{-1}$) was calculated as: (Δbody temperature).m.c/(total time to fatigue), where "m" is the body weight in grams, and "c" is the specific heat of the body tissues (0.826 $cal.g^{-1}.ºc^{-1}$) (20).

Statistical analysis

Paragraph number 11 The data are reported as mean ± S.E.M. A two-way analysis of variance (ANOVA) was used for determining differences between time and treatment and also interactions between them to evaluate the differences in changes of body temperature. Significant interactions observed by ANOVA were further evaluated by Newman-Keuls post hoc analysis to locate significant differences between means. Time to fatigue, workload, body heating rate, heat storage rate, as well as monoamines concentrations were compared using unpaired Student's *t*-test. The correlations were assessed using Pearson's correlation coefficient. Significance level was set at $p<0.05$.

RESULTS

Paragraph number 12 As previously seen and evidenced in table 1, intracerebroventricular injection of Los reduced time to fatigue and workload by ~59 and 58%, respectively, compared to Sal-treated group ($p<0.01$). Although exercise promoted a rapid increase in body temperature in both groups, at fatigue point Los-treated rats had 0.33 °C higher body temperature than Sal-animals ($p<0.01$). To compare the total thermal effects of exercise in both experimental groups, body heating rate and heat storage rate were calculated. At fatigue, body heating rate and heat storage rate of Los-treated rats were increased by ~150 and 97% in comparison with Sal-treated rats ($p<0.01$).

Paragraph number 13 Fig 1 shows 5-HT and 5-HIAA content in the studied brain areas. At fatigue, 5-HT content was significantly increased in preoptic area (Fig. 1A) and hypothalamus (Fig. 1B) of Los-exercised rats compared to Sal-exercised animals. On the other hand, in fatigued Los-exercised rats, a lower content of 5-HT was observed in hippocampus in comparison with control group ($p<0.05$) (Fig. 1C). Within frontal cortex, 5-HT concentration was not affected by angiotensinergic blockade during exercise (Fig. 1D). As also seen in Fig. 1, the levels of 5-HIAA were enhanced in preoptic area and hypothalamus ($p<0.05$), but not within hippocampus and frontal cortex.

Paragraph number 14 Los treatment during exercise did not interfere with DA concentration in any of the studied brain areas (Fig. 2). Again, the concentration of DOPAC increased in preoptic area and hypothalamus ($p<0.05$), but not in hippocampus and frontal cortex (Fig. 2).

Paragraph number 15 The increased concentration of 5-HT in preoptic area ($r = 0.85$; $p<0.01$) and hypothalamus ($r = 0.75$; $p<0.01$) correlated positively with body heating rate (Fig. 3A and C). Moreover, an inverse relationship between time to fatigue and 5-HT levels in preoptic area ($r = 0.82$; $p<0.01$) and hypothalamus ($r = 0.70$; $p<0.01$) (Fig. 3B and D) was verified. On the contrary, the correlation between 5-HT level in the hippocampus and total time to fatigue ($r = 0.56$; $p<0.05$) was direct (Fig. 3F). However, no correlation between body heating rate and 5-HT content in hippocampus was seen (Fig. 3E). Although, it was not observed any correlation between DA concentration in any of the brain areas and body heating rate or time to fatigue, the ratio between the content of 5-HT and DA (5-HT:DA) increased significantly within the hypothalamus ($p<0.01$) (Fig. 4A). Additionally, the increased 5-HT:DA ratio of the hypothalamus correlated positively with body heating rate ($r = 0.73$; $p<0.01$) and negatively with time to fatigue ($r = 0.56$; $p<0.05$) (Fig. 4B and C).

DISCUSSION

Paragraph number 16 The present study demonstrates that central fatigue due to hyperthermia and increased body heating rate induced by central Ang II AT1 receptors blockade in exercising rats is related with higher 5-HT content in preoptic area and hypothalamus, as well as with decreased level of this neurotransmitter in hippocampus. Additionally, the higher hypothalamic 5-HT:DA ratio shown by Los rats,

which correlated directly with body heating rate and inversely with time to fatigue, indicates that 5-HT and DA interaction in this region may contribute significantly to hyperthermia and premature central fatigue following angiotensinergic inhibition. Taken together, the data indicate that angiotensinergic transmission has important effects on brain 5-HT content during exercise, whose interaction with DA affects central fatigue probably through modulation of body temperature.

Paragraph number 17 We have recently shown that intracerebroventricular infusion of Los precipitates fatigue through an increase in metabolic cost and hyperthermia due to reduced peripheral heat loss and increased heat production during exercise (18, 20). Elevated internal body temperature and increased heat storage have been proposed as limiting factors to physical performance (12, 27, 34). It is important to point out that fatigue is considered a defense mechanism that prevents the development of homeostatic imbalances capable of endangering physical integrity, especially of the brain because of its vulnerability to hyperthermia (17, 24). Mechanisms of fatigue include factors of peripheral and central origin, the later being characterized by modifications within the central nervous system that impairs an adequate drive to the muscles (9, 24).

Paragraph number 18 Increased brain serotonergic activity contributes to the development of fatigue possibly by causing lethargy and loss of drive (9, 22, 24). It has been shown that the enhanced 5-HT concentration in preoptic area and hypothalamus verified after exercise is related with exercise-induced hyperthermia and decreased running performance (5, 26, 28). The present findings agree with such observation and bring further evidence that inhibition of central angiotensinergic system during dynamic exercise, which resulted in hyperthermia and higher body heating rate associated with increased 5-HT content in preoptic area and hypothalamus, may have an anti-ergogenic effect, decreasing time to fatigue to protect the organism against thermal damage. The indirect relation between time to fatigue and the enhanced 5-HT concentration in preoptic area and hypothalamus of Los-rats also supports this observation.

Paragraph number 19 We can not disregard that serotonergic neurons have many other important functions in the central nervous system, including the complex control of motor activity (3, 22, 30, 31, 32). Hippocampus is also considered to be related with motor activity (30, 31, 32), as seen by findings that local infusion of 5-HT in this site produces an increase in motor activity of rats (30, 32) and that lower hippocampal serotonergic activity precipitates fatigue during exercise (26, 28). Our data showing decreased 5-HT concentration in hippocampus that correlated directly with time to fatigue, but not with body heating rate, is in accordance with such idea. The decreased level of 5-HT in hippocampus may have contributed to the lower exercise performance shown by Los-treated rats through modulation of motor activity. As previously seen, it seems that the hippocampus involvement with central fatigue is of motor nature, rather than thermal (26, 28). In addition, such opposite 5-HT turnover in areas of central nervous system involved in thermoregulation and motor activity following inhibition of the brain angiotensinergic system agrees with findings that the serotonergic system controls its effects through selective alteration of 5-HT concentration according to specific brain regions (6, 26, 28).

Paragraph number 20 It is well defined that DA content and release is increased during exercise, including within hypothalamus and hippocampus (2, 11, 14). Although increased brain DA results in improvement of exercise performance (ergogenic effect), this response is also followed by hyperthermia and enhanced heat storage (2, 14). DA acting on the mesolimbic reward system is considered to exceed the limits of safe temperature by overruling inhibitory signals from the central nervous system that would alter perceived effort and compromise exercise performance (2, 11, 14). In the present study, although angiotensinergic blockade during exercise resulted in increased DOPAC levels in preoptic area and hypothalamus, which is an indicative of elevated turnover, the treatment did not induce any modification in the concentrations of DA in the studied brain areas. Such increased turnover of DA in the brain may have prevented any marked elevation in the concentration of DA that could improve physical performance (3). This finding corroborates with evidences that intracerebroventricular injection of Ang II does not alter the concentrations of DA in paraventricular nucleus or anterior hypothalamus (25, 29). Nevertheless, there are evidences that brain DA levels are similar to resting levels at fatigue (2, 11), thus, the possibility that the concentration of DA increased differently among the groups in between exercise can not be excluded.

Paragraph number 21 The preoptic area/anterior hypothalamus is the major brain region involved in thermoregulation that integrates thermal inputs with energy-linked metabolic processes (8, 10, 13, 16). This is supported by the fact that this area contains both warm-sensitive and cold-sensitive neurons that respond to small changes in temperature (13, 16) and that lesion or pharmacologic blockade of the preoptic area/anterior hypothalamus produce a severe impairment in thermoregulation (13, 16). Besides preoptic area, the hypothalamus also has influence on body temperature control. Lin et al. (21) demonstrated that elevation of 5-HT levels in the hypothalamus through the infusion of 5-hydroxytryptophan resulted in hyperthermic effects which were brought by increased metabolic heat production and decreased heat loss. Similarly, it has also been shown that exercise increases DA concentration not only in hypothalamus, but also in preoptic area, associated with elevated body temperature (2, 14). Therefore, it seems reasonable to suggest that Los hypothetically perfused to these regions after its injection into the cerebral ventricle, altering 5-HT and DA release and, consequently, thermal control. However, the mechanisms involved in these hyperthermia-induced effects and how brain neurotransmission affects thermoregulation during exercise still require clarification.

Paragraph number 22 The synthesis and turnover of 5-HT in the central nervous system depend on changes in the availability of brain tryptophan, the enzyme responsible for catalyzing the first reaction in the synthesis of 5-HT (3, 9). It is known that the exercise-induced increase in plasma free fatty acids indirectly facilitate the entry of tryptophan into the brain because they compete for the same carrier (albumin) (3, 9). In other words, an increase in free fatty acid concentration in plasma elevates the free tryptophan level. We have recently shown that central angiotensinergic blockade during exercise also shifts energy balance during graded exercise, resulting in higher and premature increase in plasma free fatty acids at low absolute intensity of exercise (19). The association of this last result with the current one indicate

that the increased 5-HT content within preoptic area and hypothalamus induced by Ang II blockade is probably benefitted by the higher free fatty acids mobilization from adipose tissue.

Paragraph number 23 The precursor of DA, tyrosine, also competes with other amino acids for entry into the brain, including tryptophan (11), indicating that the interaction between 5-HT and DA may be an important factor affecting the central component of fatigue (22). Actually, the "central fatigue hypothesis" postulates that high 5-HT:DA ratio is associated with exercise performance loss (11, 22). This is because the increase in 5-HT activity during physical activity contributes to fatigue through inhibition of the dopaminergic system (11, 22). To support such assumption, it has been demonstrated that administration of a 5-HT agonist blocked the exercise-induced increase in DA and that treatment with its antagonist prevented the decrease in DA at exhaustion (1, 11). Therefore, the reduction in exercise performance depends largely on 5-HT level increase, which was induced by central angiotensinergic blockade within preoptic area and hypothalamus at fatigue. Additionally, such effect led to a higher hypothalamic 5-HT:DA ratio. Yet, we can not exclude the possibility that Ang II alters 5-HT and DA metabolism in various brain regions through other factors.

Paragraph number 24 In summary, our results provide evidences that central AT1 receptors blockade promotes an increase in the content of 5-HT in preoptic area and hypothalamus that is related with increased heat production and reduced exercise performance. The decrease in 5-HT levels in hippocampus associated with the reduced time to fatigue of Los-exercised rats suggests that 5-HT acting in this brain area is probably related with motor activity control during exercise. Furthermore, the interaction between 5-HT and DA within hypothalamus of Los rats favors hyperthermia and premature central fatigue following angiotensinergic inhibition. Given that Losartan is widely prescribed to patients with high blood pressure, kidney disease and heart failure (33), this study brings further evidences of additional effects of such drug during exercise. In conclusion, our data indicate that angiotensinergic transmission prevents central fatigue by altering brain 5-HT and DA metabolism during exercise, affecting the control of heat production and exercise capacity.

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Paragraph number 27 The results of the present study do not constitute endorsement by ACSM.

CONFLICT OF INTEREST

Paragraph number 27 There is no conflict of interest.

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FIGURE CAPTIONS

Fig.1. Effect of intracerebroventricular injection of 2 µL of 0.15M NaCl (Sal) or Losartan (Los; 60 nmol) on concentrations of serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) in preoptic area (A), hypothalamus (B), hippocampus (C) and frontal cortex (D) after exercise until fatigue. Values are expressed as mean ± SEM, n = 6 each group. * $p<0.05$ compared with Sal-treated group.

Fig.2. Effect of intracerebroventricular injection of 2 µL of 0.15M NaCl (Sal) or Losartan (Los; 60 nmol) on concentrations of dopamine (DA) and 3,4-Dihydroxyphenylacetic acid (DOPAC) ($ng.mg^{-1}$) in preoptic area (A), hypothalamus (B), hippocampus (C) and frontal cortex (D) after exercise until fatigue. Values are expressed as mean ± SEM, n = 6 each group. * $p<0.05$ compared with Sal-treated group.

Fig. 3. Correlation between serotonin (5-HT) concentration in preoptic area and body heating rate (A) and time to fatigue (B): correlation between 5-HT concentration in hypothalamus and body heating rate (C) and time to fatigue (D); correlation between 5-HT concentration in hippocampus and body heating rate (E) and time to fatigue (F) in rats treated with 2µL of 0.15M NaCl (open circles) or Losartan (Los; 60nmol, filled circle).

Fig.4. (A) Effect of intracerebroventricular injection of 2 µL of 0.15M NaCl (Sal) or Losartan (Los; 60 nmol) on serotonin:dopamine (5-HT:DA) ratio in preoptic area, hypothalamus, hippocampus and frontal cortex after exercise until fatigue. Values are expressed as mean ± SEM, n = 6 each group. * $p<0.05$ compared with Sal-treated group. Correlation between 5-HT:DA ratio in hypothalamus and body heating rate (B) and time to fatigue (C) in rats treated with 2 µL of 0.15M NaCl (Sal) or Losartan (Los; 60 nmol).

TABLE AND FIGURES

Table 1: Effect of intracerebroventricular injection of 2 µL of 0.15 M NaCl (Sal) or Losartan (Los; 60 nmol) on exercise performance and thermoregulatory parameters during exercise until fatigue.

	Sal	**Los**
Time to fatigue, min	70.67 ± 6.55	29.17 ± 4.75*
Workload, Kgm	17.99 ± 2.01	7.56 ± 1.12*
Body temperature at fatigue, ºC	38.71 ± 0.13	39.04 ± 0.15*
Body heating rate, $ºC.min^{-1}$	0.02 ± 0.00	0.05 ± 0.01*
Heat storage rate, $cal.min^{-1}$	5.69 ± 0.87	11.23 ± 1.72*

Values are expressed as mean ± SEM. n=6/group. * Significant diference between groups; $p< 0.01$.

Fig. 1

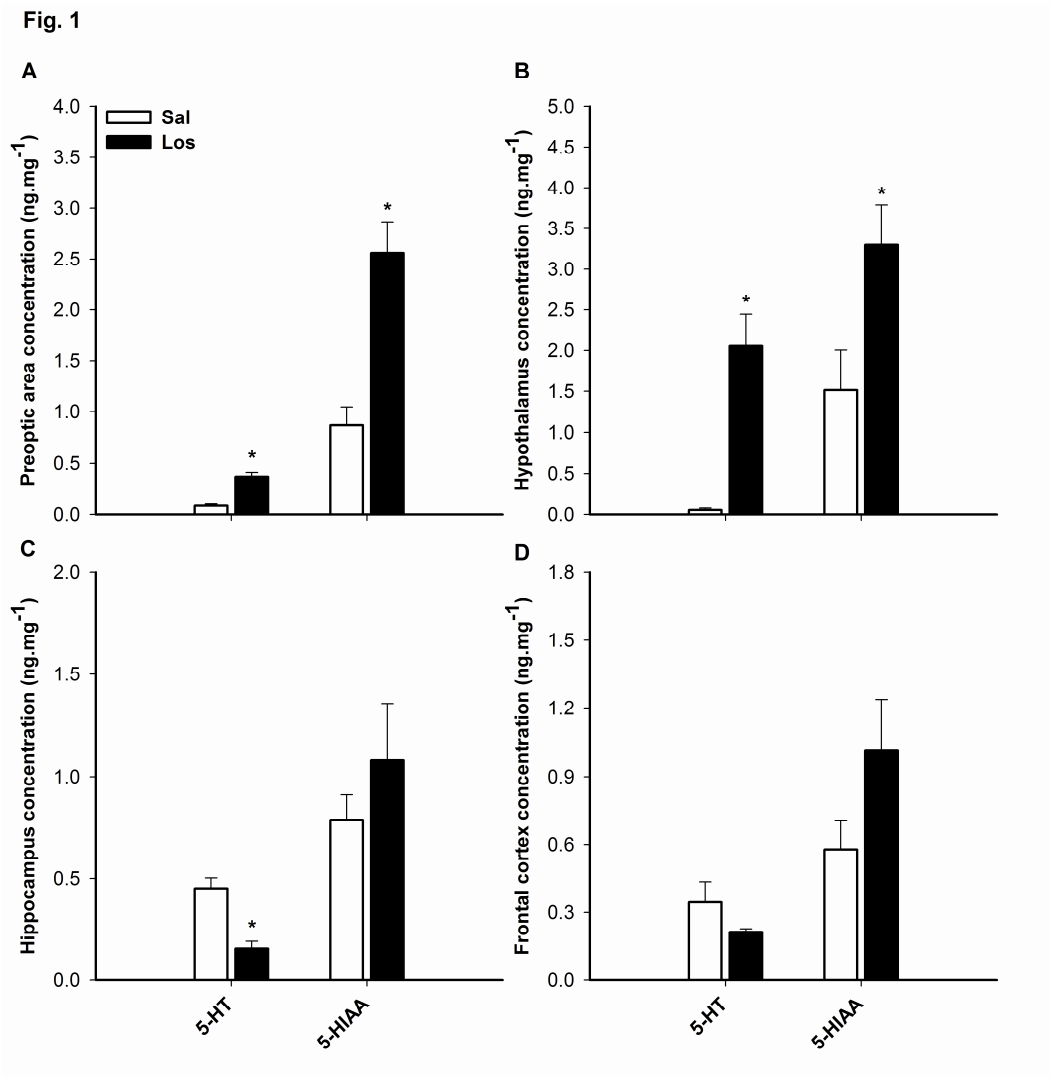

A
B
C
D
Sal
Los
Preoptic area concentration (ng.mg-1)
Hypothalamus concentration (ng.mg-1)
Hippocampus concentration (ng.mg-1)
Frontal cortex concentration (ng.mg-1)
5-HT
5-HIAA
*

Fig. 2

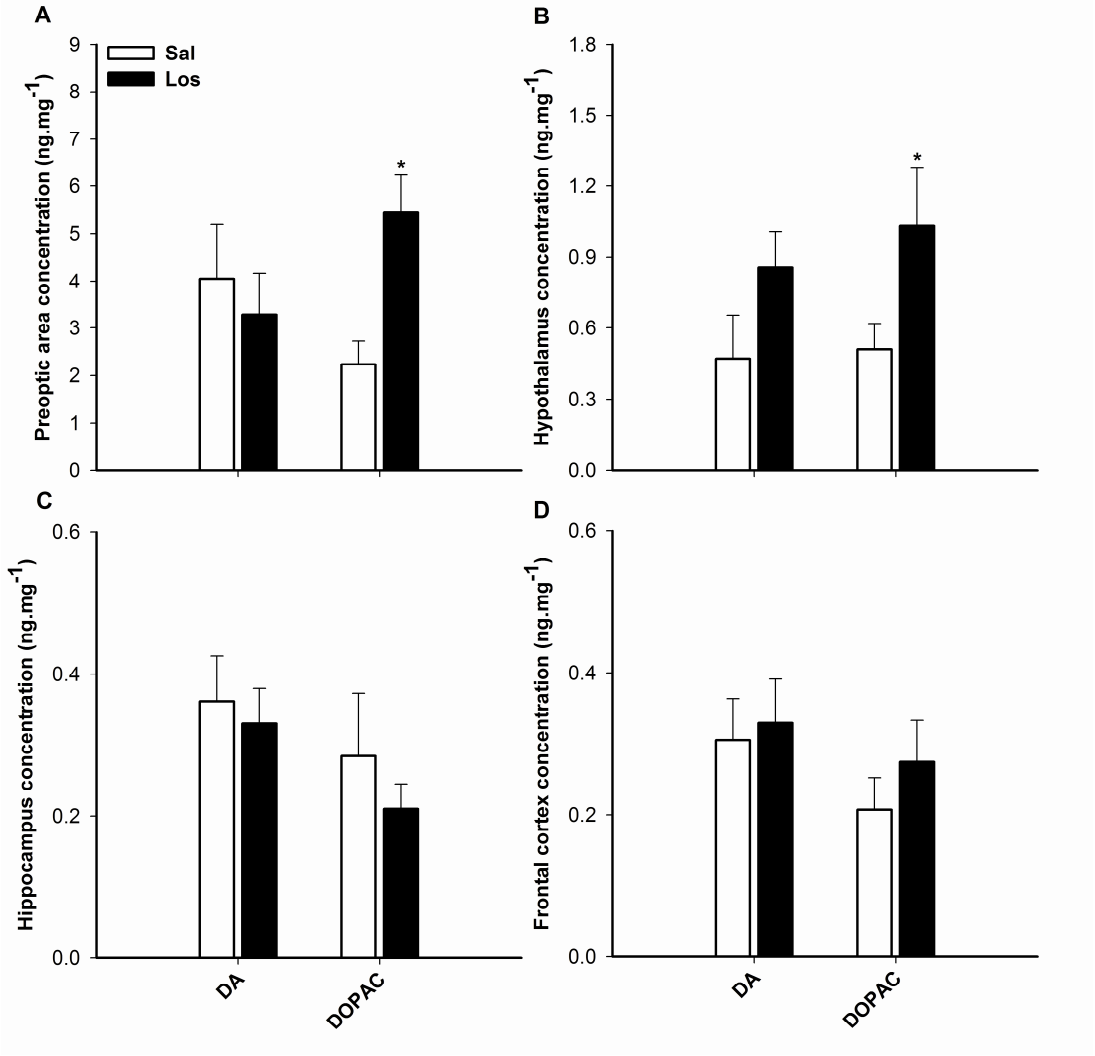

A
B
C
D
Sal
Los
Preoptic area concentration (ng.mg-1)
Hypothalamus concentration (ng.mg-1)
Hippocampus concentration (ng.mg-1)
Frontal cortex concentration (ng.mg-1)
*
DA
DOPAC

Fig. 3

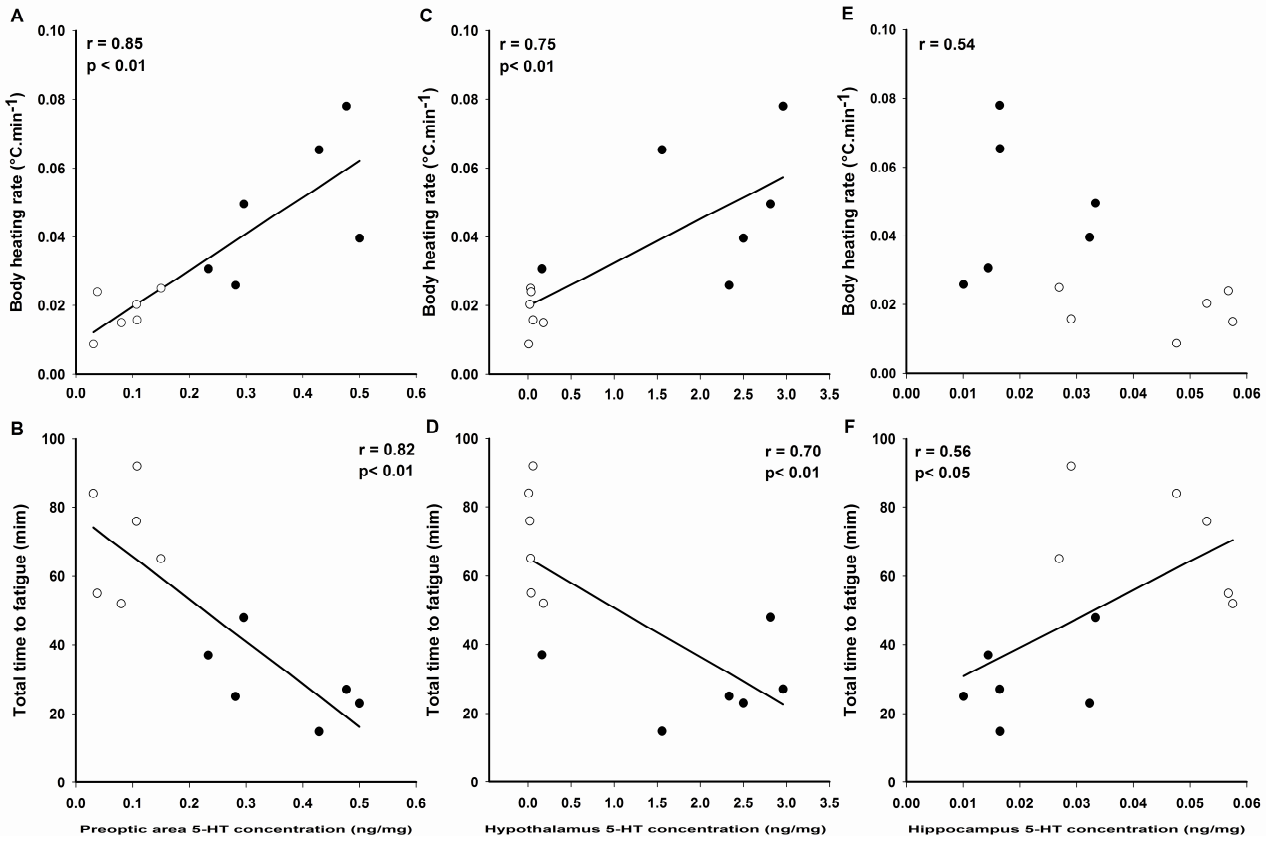
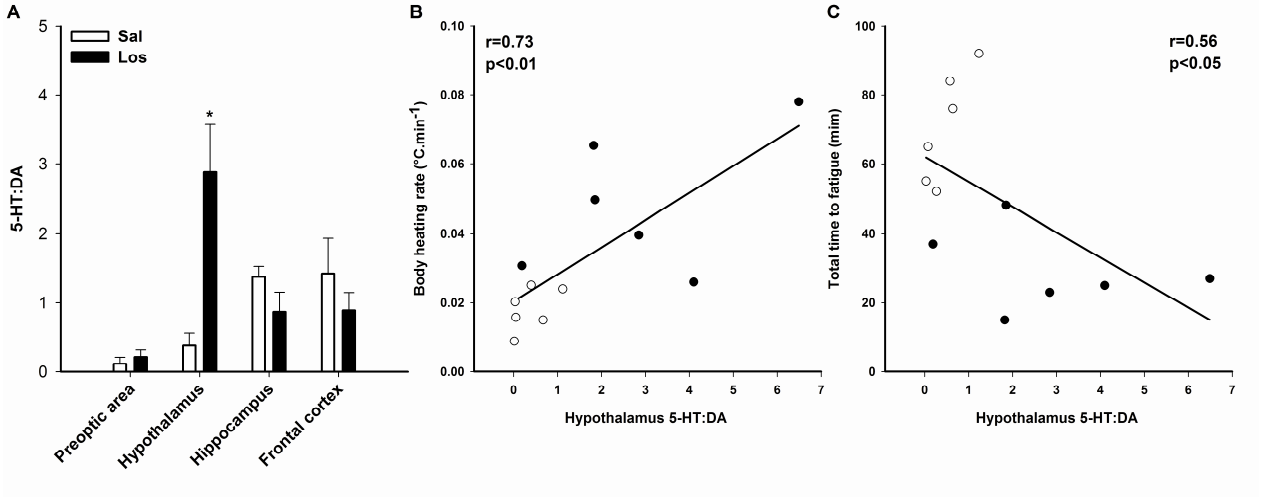

A
r = 0.85
p < 0.01
Body heating rate (°C.min-1)
C
r = 0.75
p< 0.01
Body heating rate (°C.min-1)
E
r = 0.54
Body heating rate (°C.min-1)
B
r = 0.82
p< 0.01
Total time to fatigue (mim)
Preoptic area 5-HT concentration (ng/mg)
D
r = 0.70
p< 0.01
Total time to fatigue (mim)
Hypothalamus 5-HT concentration (ng/mg)
F
r = 0.56
p< 0.05
Total time to fatigue (mim)
Hippocampus 5-HT concentration (ng/mg)

Fig. 4


A
Sal
Los
5-HT:DA
*
Preoptic area
Hypothalamus
Hippocampus
Frontal cortex
B
r=0.73
p<0.01
Body heating rate (°C.min-1)
Hypothalamus 5-HT:DA
C
r=0.56
p<0.05
Total time to fatigue (mim)
Hypothalamus 5-HT:DA

Envolvimento do núcleo paraventricular nos ajustes cardiovasculares induzidos pelo estresse térmico (Em preparação)

O PVN está intimamente envolvido nos ajustes simpáticos cardiovasculares induzidos pelo aquecimento, e conseqüente dissipação de calor através da vasoconstrição visceral e vasodilatação da pele para redistribuição do fluxo sanguíneo para a periferia. No presente estudo, RSNA, MAP, HR, temperatura corporal (Tb) e temperatura da cauda (Ttail) foram mensuradas em ratos anestesiados submetidos ao estresse térmico. Os ratos foram separados aleatoriamente em grupos para receber microinjeção de CSF, lidocaina ou L-NMMA bilateralmente no PVN. O estresse térmico foi induzido por aumento da temperatura do cobertor térmico entre 37 e 43°C durante 30 minutos. Este estímulo térmico resultou em inibição do aumento da RSNA após o bloqueio do PVN com lidocaína, sugerindo redução da vasoconstrição renal. O aumento da MAP também foi bloqueado e o aumento da HR atenuado significativamente, contudo o limiar térmico para vasodilatação da cauda não foi afetado. RSNA, HR e MAP em ratos tratados com L-NMMA aumentaram proporcionalmente ao estresse térmico. Além disso, ΔTb até a vasodilatação da cauda foi superior após injeção de L-NMMA, indicando prejuízo na dissipação de calor. Os dados indicam que o PVN é fundamental nos ajustes da atividade simpática que contribuem para a regulação cardiovascular durante o estresse térmico passível de influenciar a redistribuição sanguínea para a periferia. Além disso, um possível mecanismo pelo qual o aquecimento aumenta a perda de

calor através da vasodilatação cutânea ocorre via disponibilidade de óxido nítrico no PVN.

Involvement of the paraventricular nucleus on heat stress-induced cardiovascular adjustments

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Running head: PVN mechanisms in thermoregulation

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ABSTRACT

We hypothesized that the paraventricular nucleus (PVN) is intimately involved in the heating mediated sympathetic cardiovascular adjustments and consequent heat dissipation through vasoconstriction of the viscera and vasodilation of the skin to redistribute blood flow to the periphery. In the present study, renal sympathetic nerve activity (RSNA), mean arterial blood pressure (MAP), heart rate (HR), body temperature (Tb), and tail skin temperature (Ttail) were measured in α-chloralose-urethane-anesthetized rats submitted to heat stress. The rats were randomly assigned to groups receiving microinjection of vehicle (CSF), Lidocaine or *N*G-monomethyl-L-arginine (L-NMMA) bilaterally into the PVN. Heat stress was induced by a heating pad with a graded increase in temperature from 37 to 43°C during 30 min. This heat stimulus resulted in blunted RSNA response after blockade of the PVN with Lidocaine, suggesting a decreased renal vasoconstriction. MAP was also blunted and HR increase significantly attenuated, however, body temperature for peripheral tail vasodilation was not affected by Lidocaine treatment. RSNA, HR and MAP in L-NMMA injected rats increased according to the heating stress. Nevertheless, a higher ΔTb until tail vasodilation was shown by L-NMMA injected rats, which is an indicative of impaired peripheral heat loss. In conclusion, the PVN is critical for enhancing sympathetic activity to heating, contributing to cardiovascular adjustments elicited by heat stress that influence core blood redistribution to the periphery. Furthermore, one possible mechanism by which heating increases heat loss through peripheral vasodilation is via nitric oxide within the PVN.

Key words: Paraventricular nucleus, nitric oxide, thermoregulation, renal sympathetic nerve activity, vasodilation threshold.

INTRODUCTION

In order to maintain body temperature (Tb) constant at ~37° C, the balance between heat production and heat loss is accurately adjusted by coordinated thermoregulatory responses controlled by the central nervous system (Webb, 1995). As a result of increases in Tb, heat loss mechanisms are stimulated mainly through modulation of sympathetic activation (Kenney et al., 2001; Kregel et al., 1990). During heat stress, the elevation of Tb is proportionally followed by increases in renal sympathetic nerve activity (RSNA), heart rate (HR), mean arterial pressure (MAP), as well peripheral sympathetic inhibition (Gisolfi et al., 1991; Kenney et al., 2001; Kregel and Gisolfi, 1994). These effects are critical for heat dissipation through blood flow redistribution from the core to the skin surface, being a consequence of sympathetically controlled vasoconstriction of the viscera and vasodilation of the skin (Kanosue et al., 1994; Morrison, 2001; Smith et al., 1998).

The paraventricular nucleus of the hypothalamus (PVN) is a central site for the integration of sympathetic nerve activity (Li and Patel., 2003; Patel, 2000) and recently described to be involved in the control of Tb (Nagashima et al., 2000; Romanovsky, 2007). The PVN contains thermosensitive neurons that are activated during heat stress (Bratincsak and Palkovits, 2004; Cham and Badoer, 2006, 2008). Additionally, evidences indicate that projections arise from the PVN to other thermoregulatory centers to influence the sympathetic activity of thermoregulatory effectors organs like the brown adipose tissue, vasculature of the rat's tail, as well as the kidneys and gut (Kazuyuki et al., 1998; Kenney et al., 2003; Smith et al., 1998). It has also been demonstrated that neuronal inhibition of the PVN prevents the reduction in renal blood flow in response to increased Tb (Cham and Badoer, 2008), supporting that the PVN may be involved in the control of cardiovascular adjustments that influence blood redistribution induced by changes in Tb (Kazuyuki et al., 1998; Nagashima et al., 2000).

Nitric oxide (NO), which is diffusely found in the PVN (Cham et al., 2006, 2007), was proven to exert thermoregulatory effects characterized by hypothermia (Almeida and Branco, 2001; Mathai et al., 2004). Acting centrally, NO plays a tonic role in reducing Tb due to increased heat loss through peripheral vasodilation (Lacerda et al., 2005). Within the PVN, the injection of the NO synthase inhibitor L-NMMA induces vasoconstriction of the kidneys, while treatment with the NO donor sodium nitroprusside elicits the opposite response (Zhang et al., 1997). These results are in agreement with the general idea that NO decreases sympathetic tonus (Patel et al., 2001; Simon, 1998), and suggest that NO may be a potential signaling molecule involved in the control of blood flow redistribution that enables heat dissipation during heat stress.

We hypothesized that the PVN is intimately involved in heating mediated sympathetic cardiovascular adjustments and consequent heat dissipation through blood flow redistribution to the periphery. In the present study, we determined the effect of PVN blockade with Lidocaine during heat stress on RSNA, which was considered an indicator of sympathetic outflow to the kidneys and a reliable representation of renal vasomotor tonus and, consequently, blood flow. At the same moment, tail

temperature (Ttail) was measured as an index of peripheral vasculature tonus. Since NO is a thermoregulatory heat loss effector, as well as sympathoinhibitory, we also investigated if the cardiovascular actions of the PVN due to heat stress would be dependent on the availability of NO within the nucleus. Thus, the purpose of the present study was to examine how the PVN affects RSNA, HR, MAP, heat balance and Tb threshold for peripheral vasodilation during heat stress and if NO within the nucleus is involved in such regulations.

METHODS

All rats were fed and housed according to approved protocol by the Institutional Animal Care and Use Committee of the University of Nebraska Medical Center and conformed to the guidelines for the care and use of laboratory animals of the National Institutes of Health and the American Physiological Society.

Male Sprague-Dawley rats (220–320 g body wt; Sasco Breeding Laboratories, Omaha, NE) were allowed to acclimate to our animal care facility for 1 wk before use. On the day of the experiment, each rat was anesthetized with urethane (0.75 g/kg ip) and α-chloralose (70 mg/kg ip), and the left femoral vein was cannulated with polyethylene (PE-50) tubing for injection of supplemental anesthesia. The left femoral artery was cannulated and connected to a computer-driven data-recording and -analyzing system (MacLab; AD Instruments, Mountainview, CA) via a pressure transducer (model P231D; Gould) for recording of arterial blood pressure and HR. The trachea was intubated to facilitate spontaneous ventilation.

Placement of Microinjection Cannula in the PVN

The anesthetized rat was placed in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA). A longitudinal incision was made on the head and the bregma was exposed. The coordinates for the PVN (1.5 mm posterior to bregma, 0.4 mm lateral to the midline, and 7.8 mm ventral to the dura) were determined from the atlas of Paxinos and Watson (Paxinos and Watson, 1986). A small burr hole was made in the skull. For the microinjections, a thin needle (0.5 mm OD, 0.1 mm ID) connected to a microsyringe (0.5 μL; model 7000.5; Hamilton) was lowered into the PVN bilaterally.

Recording of Renal Sympathetic Nerve Activity

The left kidney was exposed through a retroperitoneal flank incision. A branch of the renal nerve was isolated from the fat and connective tissue. The nerve was placed on a pair of thin bipolar platinum electrodes. The nerve-electrode junction was insulated electrically from the surrounding tissue with silicone gel (Sil-Gel 604 AB; Wacker). The electrical signal was amplified (10.000 times) with a Grass amplifier (model P55) with high- and low-frequency cutoff of 1.000 and 100 Hz, respectively. The output signal from the amplifier was rectified and integrated (20-ms time constant) and stored for later analysis using a computer-run data acquisition system (MacLab). The signal recorded at the end of the experiment (after the rat was dead) was considered background noise. Nerve discharge was calculated by subtraction of the background noise from the actual recorded value. Basal nerve discharge was defined by subtraction of the background noise from the actual nerve discharge before administration of drugs into the PVN. The peak

response of RSNA to administration of drugs into the PVN during the experiment (averaged over 20–30 s) was subsequently expressed as percent change from baseline.

Recording of Colonic Temperature and Tail Skin Temperature

Colonic temperature was taken as Tb and was measured using a thermistor probe (model 401, Yellow Springs Instruments, USA). The thermistor probe was inserted 4 cm past the anal sphincter after fecal pellets had been removed from the colon by gentle external massage. Ttail was measured using a probe (series 409-B,l Yellow Springs Instruments) taped to the dorsal surface of the skin, 10 mm from the base of the tail. Tb and Ttail were used to determine ΔTb until the moment at which Ttail clearly begins to increase (vasodilation threshold).

Experimental Protocol

After the surgical procedures, the animals were allowed to stabilize for at least 20-30 min before being submitted to the experimental protocol. RSNA, MAP, HR, Tb and Ttail of the rats were recorded continuously during heat stress. Heat stress was produced in rats by increasing the temperature of a heating pad (Staco, Model 3PN 1010BV) from 37 to 43°C at a rate of 1.5°C every 6 minutes in an interval of 30 minutes. The rats were randomly assigned to groups receiving bilateral microinjection into the PVN of artificial cerebroespinal fluid (CSF; 100 nL/side), lidocaine (1%; 200 nL/side) or L-NMMA (200 pmol, 100nL/side) immediately before the beginning of heat stress. Throughout the experimental procedure, the rat's tail was maintained away from the surface of the heating pad.

Control experiments were carried out as well. The animals were submitted to similar experimental procedures, but instead of being submitted to heat stress after injection of the drugs, their Tb was kept stable during 30 min while RSNA, MAP, HR and Ttail were recorded.

Brain Histology

After the experiment, the rats were killed and the brains removed and fixed in 10% formalin for at least 24 h. The brains were then frozen, and serial transverse sections (30 μm) were cut with a cryostat (-18°C). The sections were mounted on microscope slides and stained using 1% neutral red. The location of the injection within the PVN was verified under a microscope with X 40 magnification (Fig.1). The microinjections that terminated in the boundaries of the PVN were considered effective. For the purposes of analysis, only the animals in which dye was deposited within or < 0.5 mm from the boundaries of the PVN were considered histologically targeted. The 100- to 200-nL injection volumes targeting the PVN would be expected to distribute the drug in or within < 0.5 mm from the rostrocaudal and mediolateral boundaries of the PVN.

Data Analysis

Responses of RSNA to the drugs are expressed as percent change from baseline. Responses of arterial MAP, HR, Tb, Ttail are expressed as the difference between the basal value and the value after each drugs. Data were subjected to a two-way ANOVA, followed by the Newman–Keuls test. The data were also compared using paired or unpaired Student's t-test, as applicable. Significance level was set at $p<0.05$.

RESULTS

Table 1 shows that after injection of Lidocaine or L-NMMA bilaterally within the PVN of anesthetized rats during 30 min without heat stress, RSNA, MAP, HR and Ttail responses remained stable in both experimental groups.

Heat stress induced an increase in RSNA response after PVN injection of CSF, starting at Δ1.2ºC (6 min) and sustained until the end of heat stress (Figs. 2C and 2D). As seen in Fig. 2C, Lidocaine injection resulted in blunted RSNA response throughout heat stress. Such response was significantly lower than CSF group from 12 min (Δ2.4ºC) of heat stress until the end of the protocol ($p<0.05$). L-NMMA injection resulted in increased RSNA response, observed from 12 min and maintained high until the end of the experiment (Fig. 2D). There was no significant difference in RSNA response between CSF and L-NMMA groups. The highest difference between the CSF group and Lidocaine or L-NMMA groups occurred at the end of the heat stress (117.6 ± 17.0 % CSF vs. 11.3 ± 7.3 % Lidocaine or 105.4 ± 12.6 % L-NMMA, $p<0.05$).

MAP, following PVN injection of CSF, increased after 12 min of heat stress (Fig. 3A and B). Lidocaine injection resulted in blunted MAP response throughout heat stress (Fig. 3A). MAP was only significantly different between CSF and Lidocaine groups at Δ4.8ºC (24 min) and in the end of heat stress ($p<0.05$). MAP of the L-NMMA group reached high values as the CSF group. However, this increase in HR was slower and only significantly higher than baseline value from 24 min of heat stress. MAP was significantly lower in this group in comparison with CSF group just between 6 and 12 min of heat stress (Fig. 3B; $p<0.05$).

HR values enhanced progressively with increases in temperature in all groups of animals (Fig. 3C and D). HR was already higher after 6 min of heat stress in CSF group (Fig. 3C and D). Lidocaine injection induced an increase in HR from the 18th min (Δ3.6ºC) of heat stress. HR after Lidocaine injection within the PVN was lower than following CSF injection from the 12th min until the end of heat stress (Fig. 3C; $p<0.05$). Fig. 3D shows that L-NMMA injection resulted in elevated HR values from the 12th min of heat stress until the end of the protocol. HR of L-NMMA group was lower than CSF only during the 12th min (Fig. 3D; $p<0.05$).

The effect of heat stress on Tb is shown in Fig. 3. Heat stress induced a rapid increase in Tb in all groups, which was already observed from the 9th min after injection of CSF (Fig. 4A and B). From the 8th min of heat stress, Tb was significantly higher than baseline value in the Lidocaine group (Fig. 4A). Similarly to the response observed with CSF injection, L-NMMA induced an increase in Tb from the 9th min until the end of heat stress (Fig. 4B). There were no significant differences between the CSF and Lidocaine groups, as well as between the CSF and L-NMMA groups, throughout heat stress (Fig. 4A and B).

As illustrated in Fig. 4C and D, Ttail increased within 15, 13 and 16 min of heat stress in CSF, Lidocaine and L-NMMA groups, respectively, indicating that heat loss mechanisms had been activated. The increases in Ttail remained similar between CSF and Lidocaine group (Fig. 4C). Although Ttail of L-NMMA increased mostly in a similar way to CSF group, between the 5th and 7th min interval of heat stress Ttail of these rats was significantly lower (Fig. 4C and D). To assess whether heat loss mechanism was affected by PVN injection of the drugs, ΔTb until tail vasodilation was calculated (Fig. 4E and F). Vasodilation threshold was not different between rats that received CSF and Lidocaine injections within the PVN (0.3 ± 0.1°C CSF; 0.4 ± 0.2°C Lidocaine). However, results showed that the value was 0.5°C higher in L-NMMA injected rats compared with CSF injected animals (0.3 ± 0.1°C CSF vs 0.8 ± 0.1°C L-NMMA; $p<0.01$).

DISCUSSION

In the present study, blockade of the PVN with Lidocaine resulted in blunted RSNA, suggesting a decreased renal vasoconstriction, as well as blunted MAP response and attenuated increase in HR to heat stress. Such treatment did not interference on body temperature threshold for peripheral vasodilation. On the other hand, L-NMMA injection into the PVN led to higher ΔTb until tail vasodilation but non-affected RSNA, MAP and HR. Taken together, these data indicate that the PVN influences the responsiveness of sympathetic pathways that mediate cardiovascular adaptations required for the control of Tb during heat stress, particularly by modulating renal sympathetic nerve activity that enables diversion of core blood volume during heat stress. Furthermore, one mechanism by which heating increases heat loss through peripheral vasodilation is via NO within the PVN.

Body heating provides a potent stimulus to sympathetic activity in order to stimulate heat dissipation responses and maintain Tb within safe limits (Kenney et al., 2001; Kregel et al., 1994). During heat stress, the sympathetic nervous system is capable of altering blood flow of multiple organs nonuniformly by selectively altering the pattern of sympathetic outflow according to specific vascular beds (Hirai et al., 1995; Morrison et al., 2001). Heat dissipation through blood redistribution from the viscera to the periphery is critical for the maintenance of Tb during heat stress. The shift of blood volume from the central to the peripheral circulation is the result of cardiovascular responses controlled by the sympathetic nervous system that induce vasoconstriction of the viscera and vasodilation of the skin simultaneously (Kanosue et al., 1994; Morrison et al., 2001; Smith et al., 1998).

The recent involvement of the PVN in thermoregulation awoke the hypothesis that the nucleus may contribute to the maintenance of Tb by influencing sympathetic outflow that regulates cardiovascular responses elicited by heat stress (Li & Patel., 2003; Nagashima et al., 2000; Patel, 2000; Romanovsky, 2007). In fact, there is evidence that neuronal inhibition of the PVN with the $GABA_A$ agonist muscimol prevents the normal reduction in renal blood flow in response to increased Tb (Cham and Badoer, 2008). In the present study, Lidocaine was bilaterally injected to completely block all neuronal transmissions within the PVN during heat stress. This treatment blunted RSNA response to heating, i.e., renal vasoconstriction, which is an evidence of accumulation of blood volume in the kidneys that impairs its redistribution to the skin surface to dissipate heat. During heat stress, up to 60% of cardiac output is redistributed to the surface of the skin to improve heat loss, this effect being depended mostly on decreased peripheral resistance (Rowel, 1983). The fact that rats treated with Lidocaine had their vasodilation threshold not altered is an indicative that the PVN blockade by itself may not interfere with peripheral vasomotor tonus during heat stress. In rodents, tail skin vasodilation is the primary route of heat loss from the body, being responsible for the dissipation of an equivalent of 25% of resting heat production (Shellock and Rubin, 1984; Young and Dawson, 1982). Therefore, although the blood volume of other viscera, such as the gut, are also important for blood redistribution, the decreased renal sympathetic activation shown by Lidocaine rats suggests a thermoregulatory deficit. Nevertheless, the apparent normal body temperature for tail vasodilation possibly made heat loss possible, keeping the increase in Tb proportional to the heat stimulus.

In control rats, heat stress resulted in increased MAP and HR responses. This hemodynamic pattern is the result of sympathetic activation, which increases cardiac output, due to enhanced HR, in combination with a greater redistribution of blood flow from visceral vasculature beds for heat dissipation (Kenney, 2008; Rowell, 1983). In rats treated with Lidocaine, heat stress also resulted in blunted MAP. Although HR increased in this group, such increase was significantly lowered. During heat stress, both the vasoconstrictor and the vasodilator systems are critical in blood pressure regulation via the baroreflex (Crandall et al., 1996). As mentioned before, the redistribution of blood flow to the periphery depends mainly on the reduction of peripheral resistance (Charkoudian, 2003). On the other hand, the maintenance of blood pressure depends particularly on vasoconstriction because a large percentage of the cardiac output is redistributed to the periphery (Rowel, 1983). Since Lidocaine animals had decreased vasoconstriction of the kidneys, it seems that the decreased MAP is the consequence of the lower sympathetic activation that may have not only decreased artery resistance of the kidneys, but also of other vascular beds. Thus, it is reasonable to suggest that the PVN blockade with Lidocaine results in attenuation of sympathetic nervous system activation to heating and inability to elicit the appropriate cardiovascular adjustments.

Evidences showing the involvement of brain NO in thermoregulatory pathways demonstrated that this neurotransmitter mediates autonomic heat loss mechanisms, as indicated by reduction in Tb associated with a rise in skin temperature (Eriksson et al., 1997). Conversely, it has also been shown that central blockade of NO production increases the threshold to activate tail skin vasodilation leading to higher Tb (Lacerda et al., 2005; Mathai et al., 2004). This is in agreement with the current results that L-NMMA injection within

the PVN impairs heat dissipation through the tail during heat stress. Although Tb did not differ and Ttail remained mostly similar with the groups, L-NMMA injected rats exhibited a higher ΔTb until vasodilation, i.e., tail skin vasodilation was induced at a higher Tb than control animals. Skin blood flow is modulated by adrenergic vasoconstrictor activity (Owens et al., 2002) and NO within the PVN causes a pronounced reduction in sympathetic outflow (Krukoff and Khalili, 1999; Patel et al., 2001). Therefore, it is possible that heat stress-induced tail skin vasodilation is probably a consequence of tail sympathetic activity withdrawal and that lower levels of NO within the PVN decreases heat dissipation by augmenting sympathetic outflow to cutaneous vascular beds. It has been shown that neurons expressing NOS within the PVN are activated after exposure to a hot environment (Cham et al., 2006). The present data brings further evidence that one mechanism by which heating increases heat loss through peripheral vasodilation is via NO within the PVN.

After injection of L-NMMA within the PVN, RSNA, MAP and HR remained unaltered. This is not only an indicative of adequate cardiovascular adaptations but also a sign that visceral vasoconstriction induced by heating was not impaired by L-NMMA, which kept the normal supply of blood to the periphery for heat dissipation. The results of many studies have shown that the injection of L-NMMA within the PVN induces an increase in RSNA and that treatment with SNP elicits the opposite response (Horn et al., 1994; Zhang et al., 1997, 1998). As previously described, since NO is sympathoinhibitory, it would be expected that NO blockade within the PVN would result in even higher RSNA to heating, as well as increased MAP and HR. However, if considering that NO is also a heat loss inducer, the blockade of the system would lead to thermoregulatory impairments, similarly to the decreased peripheral vasodilation observed in the present study. Therefore, it is possible that renal blood flow remained unchanged after L-NMMA injection as a way of balancing the dual effects of NO, resulting in normal adaptation of RSNA, MAP and HR responses to heating. It is important to point out that the data from most of the thermoregulatory studies suggest that NO act in the brain to decrease sympathetic outflow to the periphery (Erickson et al., 1997; Lacerda et al., 2005; Mathai et al., 2004). Thus, we cannot exclude the possibility that at least during heat stress, NO within the PVN regulates mainly the sympathetic activity to the periphery, affecting heat loss through skin vasodilation mainly.

When analyzing the current results, the possible interference of the anesthesia on the data cannot be excluded, even though this method is widely used in the study of the sympathetic activity and cardiovascular adjustments during heat stress (Gisolfi et al., 1991; Kenney et al., 2001). It is possible that anesthesia may influence sympathetic nerve discharge responses and, consequently, thermoregulatory effectors during heat stress. Although this cannot be disregarded, the fact that in anesthetized rats heat stress results in increased sympathetic discharge suggest that anesthesia may slightly interfere on sympathetic activity during heat stress (Kenney et al., 2001; Kregel et al., 1988). Additionally, anesthesia eliminates the behavioral modifications that could alter sympathetic nerve discharge during heat stress.

In summary, our results show that the PVN alters sympathetic outflow to thermoeffector organs that mediate cardiovascular adaptations required for the maintenance of body temperature during heat stress.

Furthermore, the PVN is a site in the brain in which NO acts to facilitate heat loss by influencing the sympathetic outflow to the periphery.

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FIGURE LEGENDS

Fig. 1. Schematic transverse sections of the rat hypothalamic PVN showing the center of the microinjection sites of CSF, Lidocaine and L-NMMA .

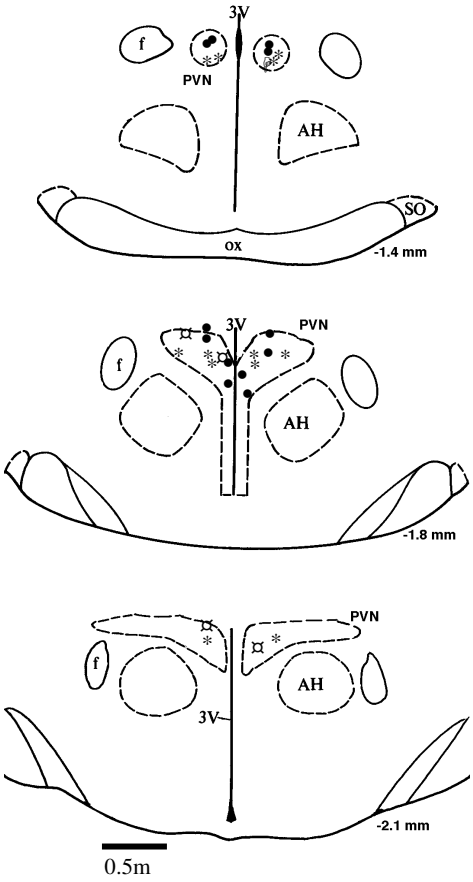
Fig. 2: Effect of heat stress on renal sympathetic nerve activity (ΔRSNA) after bilateral PVN injection of CSF, Lidocaine or L-NMMA. A and B: segments of original recordings from individual rats; C and D: graphical representation. Values expressed as mean ± SEM. n = 6/group. + Significantly different from corresponding basal value. ∗ Significant difference between groups, $p<0.05$.

Fig. 3: Effect of heat stress over time on mean arterial pressure (ΔMAP; A and B) and heart rate (ΔHR; C and D) after bilateral PVN injection of CSF, Lidocaine or L-NMMA. Values expressed as mean ± SEM. n = 6/group. + Significantly different from corresponding basal value. ∗ Significant difference between groups, $p<0.05$.

Fig. 4: Effect of heat stress over time on body temperature (ΔTb; A and B), skin tail temperature (ΔTtail; C and D) and on ΔTb until tail skin vasodilation (E and F) after bilateral PVN injection of CSF, Lidocaine or L-NMMA. Values expressed as mean ± SEM. n = 6/group. + Significantly different from basal value until 30 min. ∗ Significant difference between groups, $p<0.05$.

FIGURES AND TABLES

Fig. 1



¤ CSF

∗ Lidocaine

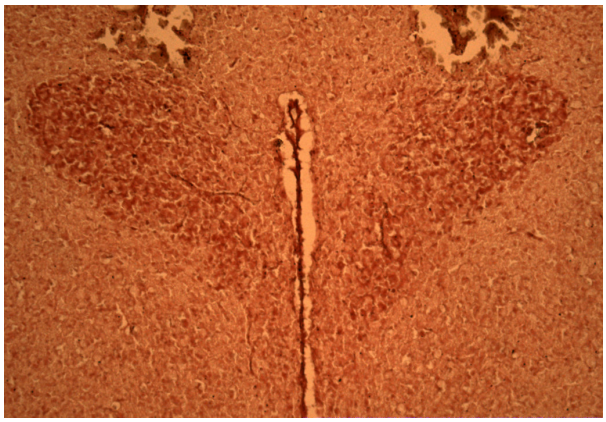


TABLE 1

Table 1. Effect of bilateral injection within the PVN of Lidocaine or L-NMMA on ΔRSNA, MAP, HR, and Ttail over time, without changes in temperature in anesthetized rats.

	Lidocaine (n=3)			**L-NMMA (n=4)**		
	Baseline	**18 min**	**30 min**	**Baseline**	**18 min**	**30min**
ΔRSNA (%)	-	-4.6±2.1	-12.7±4.9	-	18.4±10.6	13.8±8.3
MAP (mmHg)	105±5	108±3	105±4	92±8	99±7	92±9
HR (bpm)	339±29	345±20	350±17	365±11	347±43	369±10
T_{tail} (°C)	28.2±0.7	27.3±0.5	27.8±0.5	28.4±0.6	28.3±0.6	28.2±0.6

Values are expressed as mean ± SEM.

Fig. 2

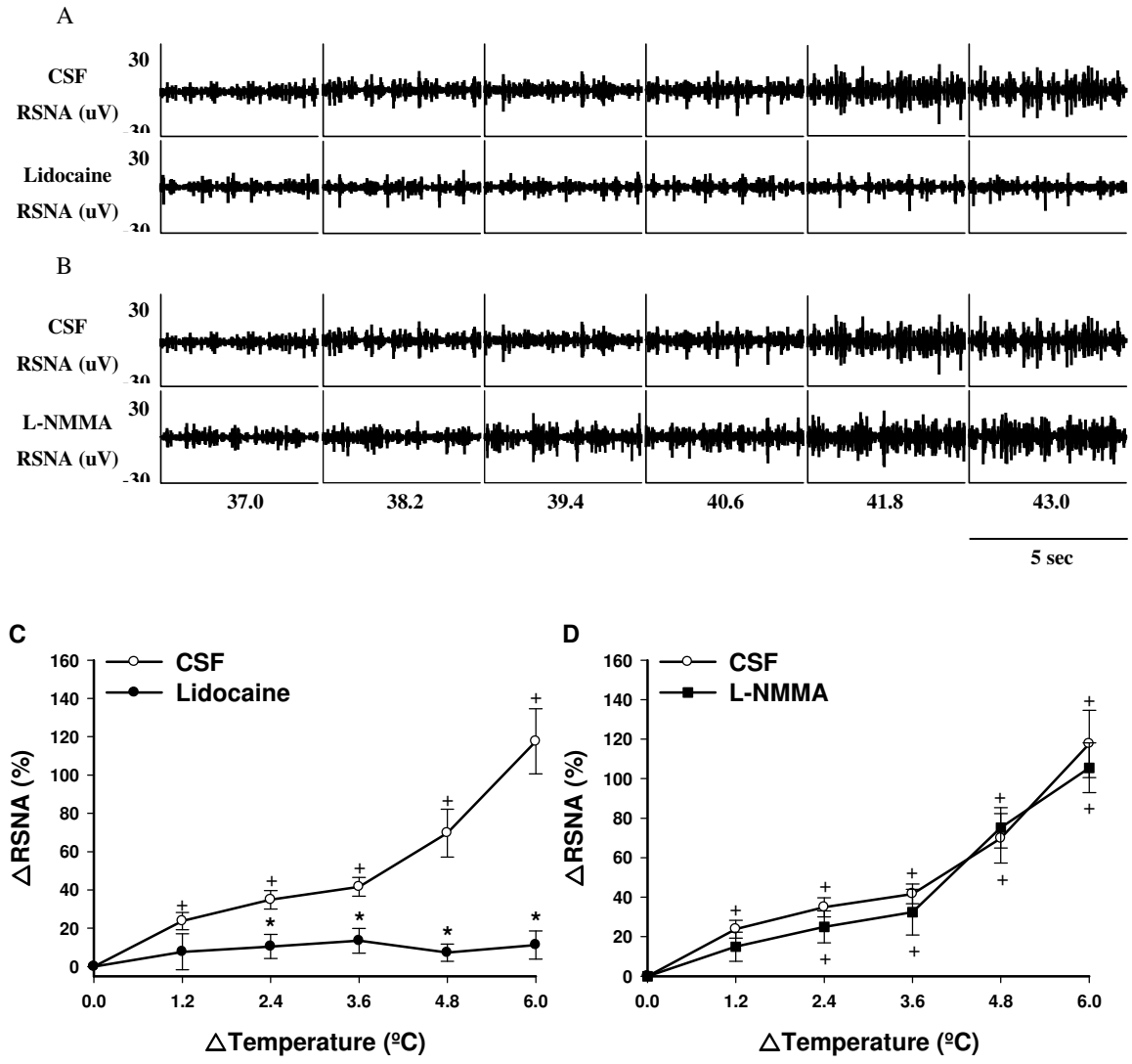

A
CSF
RSNA (uV)
30
-30
Lidocaine
RSNA (uV)
B
CSF
RSNA (uV)
L-NMMA
RSNA (uV)
37.0
38.2
39.4
40.6
41.8
43.0
5 sec
C
CSF
Lidocaine
△RSNA (%)
△Temperature (ºC)
D
CSF
L-NMMA
△RSNA (%)
△Temperature (ºC)

Fig. 3

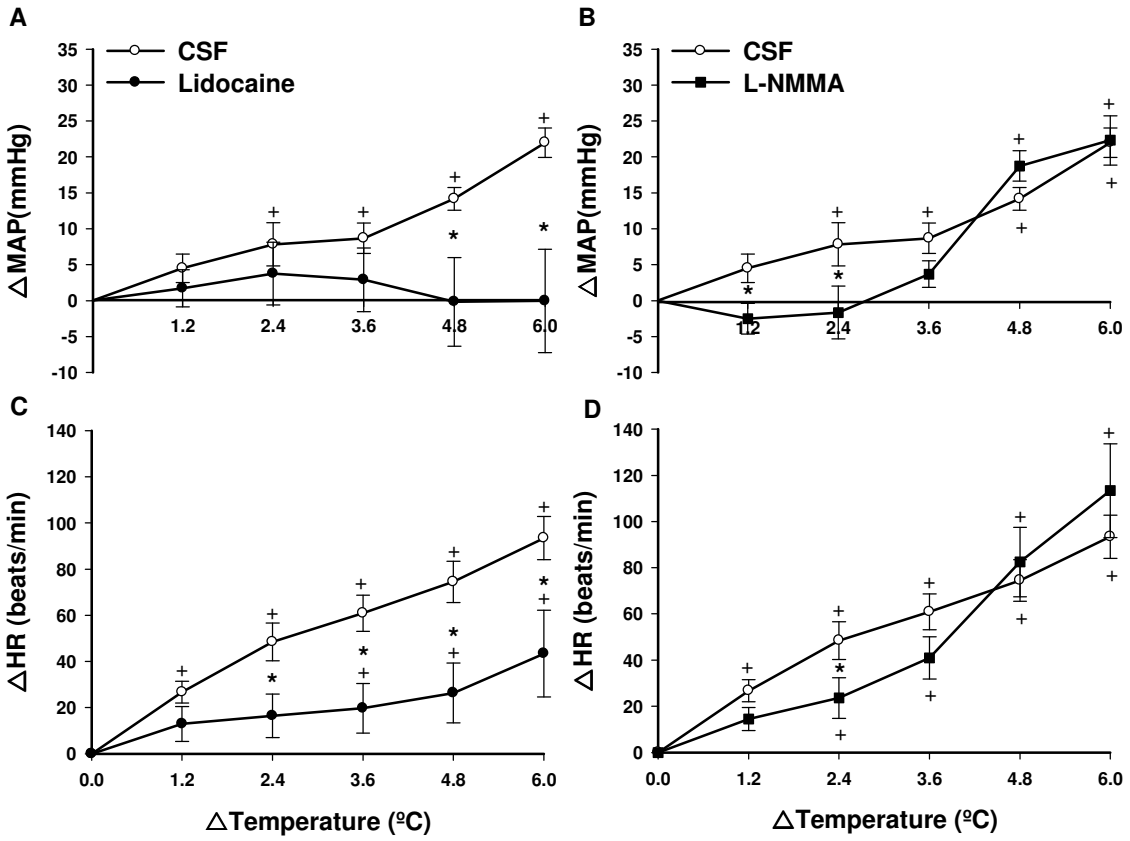
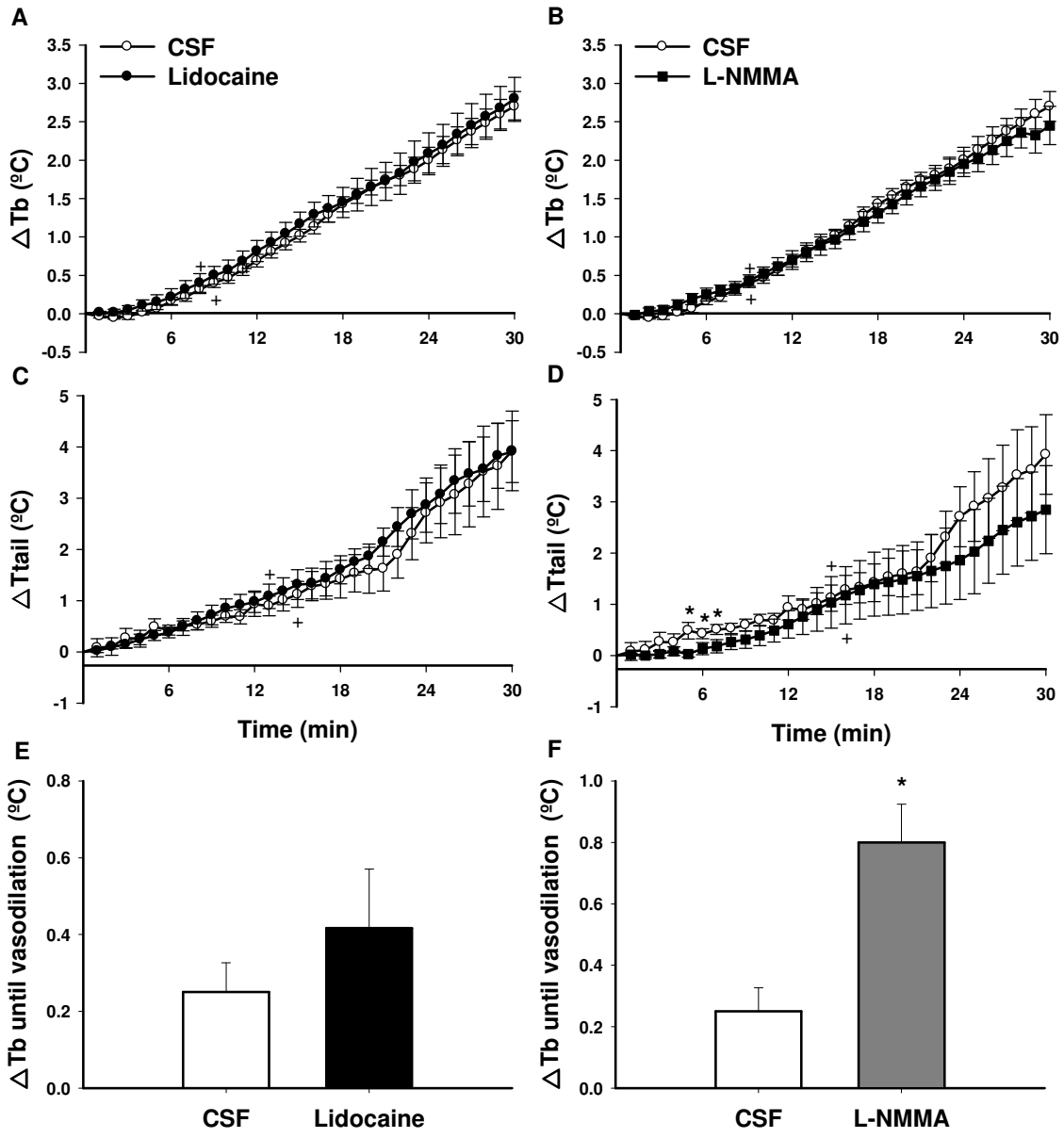

A
CSF
Lidocaine
△MAP(mmHg)
B
CSF
L-NMMA
△MAP(mmHg)
C
△HR (beats/min)
△Temperature (ºC)
D
△HR (beats/min)
△Temperature (ºC)

Fig. 4


A
CSF
Lidocaine
ΔTb (ºC)
B
CSF
L-NMMA
ΔTb (ºC)
C
ΔTtail (ºC)
Time (min)
D
ΔTtail (ºC)
Time (min)
E
ΔTb until vasodilation (ºC)
CSF
Lidocaine
F
ΔTb until vasodilation (ºC)
CSF
L-NMMA

VII. CONSIDERAÇÕES GERAIS

Os achados do presente estudo evidenciam elevação da taxa metabólica e redução da eficiência mecânica associada à fadiga precoce após bloqueio central angiotensinérgico durante o exercício físico. Esses dados demonstram que a Ang II não está somente envolvida com a dissipação e calor (Leite et al., 2006), mas também com a produção deste (Leite et al., 2007). O desbalanço térmico induzido pelo bloqueio central dos receptores AT1 foi determinante para a redução do desempenho físico uma vez que a hipertermia do exercício foi mais intensa devido a maior produção de calor não compensado pela dissipação do mesmo (Leite et al., 2006, 2007). Soma-se a esse efeito a menor eficiência mecânica, indicativa de maior quantidade de energia metabolicamente produzida dissipada sob a forma de calor. Esses dados estão de acordo com a descrição da literatura que relata ser a Ang II, atuando tanto perifericamente quanto centralmente, indutora de hipotermia manifestada através do aumento da temperatura da cauda e diminuição da taxa metabólica (Fregly & Rowland, 1992, 1993, 1996; Mathai et al., 2000; Wilson & Fregly, 1985 a,b).

O efeito hipertérmico e redução do desempenho físico acarretados pelo tratamento com Los central estão integrados ao aumento do conteúdo de 5-HT em importantes centros termorregulatórios, como a área pré-óptica e o hipotálamo. O aumento da atividade serotonérgica está associada à letargia e perda de motivação, resultando em alteração do desempenho físico (Blomstrand, 2006; Meeusen et al., 2007). Além disso, as evidências apontam

que a 5-HT induz efeitos termorregulatórios caracterizados por hipertermia (Soares et al., 2004, 2007), estando a fadiga central devido ao acúmulo de calor elevado em ratos em exercício relacionada ao aumento desse neurotransmissor na área pré-óptica (Caperuto et al., 2009; Rodrigues et al., 2009; Soares et al., 2007). Os dados aqui apresentados corroboram com essas evidências ao mostrar que o sistema angiotensinérgico também regula a temperatura corporal interna através da modulação do conteúdo de 5-HT em núcleos termorregulatórios. O mesmo controle possivelmente ocorre em centros motores como o hipocampo. Nessa região ocorreu diminuição do nível de 5-HT após bloqueio dos receptores AT1 intimamente relacionada ao menor desempenho físico, sugerindo haver uma resposta seletiva da 5-HT específica e dependente do centro nervoso.

A DA é outro neurotransmissor associado à melhora do desempenho físico, apesar de acarretar elevação da temperatura corporal no ponto de fadiga e do acúmulo de calor durante o exercício (Balthazar et al., 2009; Foley & Fleshner, 2008; Hasegawa et al., 2008). No entanto, após inibição angiotensinérgica, não se verificou qualquer alteração do conteúdo de DA nas regiões cerebrais estudadas. Essa resposta condiz com os poucos dados demonstrando que a injeção intracerebroventricular de Ang II não modifica a concentração de DA no PVN ou hipotálamo anterior (Qadri et al., 1991; Stadler et al., 1992). De qualquer forma, essa monoamina interagindo com a 5-HT no hipotálamo parece exercer ação importante no estabelecimento da hipertermia e redução do tempo de fadiga após inibição dos receptores AT1. A alta razão

hipotalâmica 5-HT:DA, compatível com menor desempenho físico, mostrou-se diretamente associada ao reduzido tempo de exercício e alta taxa de aquecimento corporal dos animais Los.

Os efeitos metabólicos da Ang II, principalmente sobre a homeostase da glicose (Coimbra et al., 1999, Machado et al., 1995 a,b, 1998; Mihessen-Neto et al., 1996), também estão atuantes durante o exercício físico. No presente estudo, o bloqueio central dos receptores AT1 resultou em hiperglicemia e maior mobilização de ácidos graxos do tecido adiposo durante o exercício físico. Além disso, o tratamento com Los em ratos em exercício elevou o fluxo glicolítico, evidenciado pelo aumento plasmático de lactato. Em função dessa maior disponibilidade de substratos energéticos, o reduzido desempenho físico desses animais possivelmente não se deveu ao prejuízo na oferta, distribuição e utilização de fontes de energia. Essa resposta metabólica do Los à atividade física aparentemente foi semelhante a situações indutoras de aumento de fluxo simpático periférico, como a neurocitoglicopenia (Coimbra & Migliorini, 1986; Ribeiro-de-Oliveira et al., 1999), hipotensão hemorrágica (Silveira et al., 2003) e exposição ao calor (Ferreira et al., 1999) que também exibiram hiperglicemia aumento de lactato e ácidos graxos livres. É razoável sugerir que a mudança na mobilização de substratos após bloqueio angiotensinérgico deve-se a maior ativação do sistema simpático, como observado previamente através do aumento da vasoconstrição da cauda e do limiar térmico para vasodilatação cutânea em ratos em exercício tratados com Los (Leite et al., 2006). Essa ativação simpática elevada foi verificada mesmo em baixa intensidade de

exercício, como 20% do trabalho máximo, a qual acarretou em maior concentração plasmática de glicose, lactato e ácidos graxos livres. O fato de ambos os grupos atingirem o mesmo nível de intensidade de exercício também sugere que a atividade simpática foi intensificada e estimulada precocemente, evidenciando maior esforço para execução de mesmo trabalho.

Contudo, ainda são desconhecidos os mecanismos através dos quais o bloqueio angiotensinérgico resulta em efeitos semelhantes àqueles consequentes da ativação simpática. Uma possível interação entre a Ang II e o óxido nítrico, já verificada em outros centros nervosos como o PVN, pode ser um dos mecanismos responsáveis por tal efeito (Li et al., 2006). Demonstrou-se que dentro do PVN, a Ang II interage com o óxido nítrico, um inibidor simpático, induzindo sua secreção, a qual modera o efeito excitatório da Ang II sobre o fluxo simpático, possivelmente através de retroalimentação inibitória (Li et al., 2006). Experimentos prévios do laboratório demonstraram que o bloqueio do sistema óxido nitrérgico central resulta em efeitos sobre o balanço térmico e metabólicos semelhantes aos observados após bloqueio angotensinérgico (Lacerda et al., 2005, 2006 a,b; Leite et al., 2006, 2007, 2009). Portanto, é possível que ocorra uma interação entre a Ang II e óxido nítrico em sítios controladores da homeostase térmica e metabólica.

O PVN surge como um sítio que se encaixa nas características citadas acima, particularmente no que concerne a termorregulação. O bloqueio do PVN com lidocaína durante o estresse térmico resultou em inibição da RSNA, assim como da MAP, e aumento menos acentuado da HR. No entanto, tal tratamento

não afetou o limiar térmico para vasodilatação cutânea. Resposta oposta foi verificada após injeção bilateral no PVN de L-NMMA, isto é, RSNA, MAP e HR inalterados, acompanhados de maior limiar térmico para vasodilatação. Os dados sugerem que, durante o estresse térmico, o PVN tem ação importante na regulação da atividade simpática que contribui para os ajustes cardiovasculares responsáveis pela redistribuição de sangue interno para a periferia e perda de calor. Além disso, um possível mecanismo através do qual o aquecimento eleva a dissipação cutânea de calor ocorre via disponibilidade de óxido nítrico no PVN.

VIII. CONCLUSÕES

O sistema angiotensinérgico central está envolvido no equilíbrio metabólico e térmico durante o exercício físico ao induzir modificações na atividade do sistema serotonérgico central.

O bloqueio do receptor AT1 para Ang II durante o exercício físico induz:

1. Aumento do consumo de oxigênio, responsável por elevação de 19% do custo metabólico em comparação com os animais controle, diminuição significativa da eficiência mecânica diretamente relacionada com a precipitação da fadiga. Tal elevação da eficiência mecânica foi verificada já em baixos níveis de intensidade de exercício durante corrida de mesmo alcance de intensidade, apesar do reduzido tempo total de exercício. Esses dados indicam que o sistema angiotensinérgico central induz ajustes metabólicos durante o exercício físico através da modulação da produção de calor e melhora da eficiência mecânica;

2. Alteração da mobilização de substratos energéticos, evidenciada pelo aumento da resposta hiperglicêmica e altos níveis de lactato e ácidos graxos livres plasmáticos durante o exercício progressivo. Os achados indicam que o sistema angiotensinérgico central está envolvido em ajustes metabólicos durante o exercício, alterando a mobilização de

substratos energéticos através do ajuste da atividade simpática, semelhante a situações de ativação simpática intensa e prematura;

3. Elevação do conteúdo de 5-HT no hipotálamo e área pré-óptica, assim como redução no hipocampo, intimamente relacionadas com a taxa de aquecimento corporal e redução do desempenho físico. Adicionalmente, a interação entre 5-HT e DA no hipotálamo parece ser fator determinante para a hipertermia e precipitação da fadiga. Os dados sugerem que o sistema angiotensinérgico exerce importante efeito sobre o conteúdo central de 5-HT durante o exercício, cuja interação com a DA afeta a fadiga central através da modulação da temperatura corporal

A inibição bilateral do PVN induz bloqueio dos ajustes cardiovasculares durante o estresse térmico fundamentais para dissipação de calor. Esses ajustes são preservados após bloqueio óxido nitrérgico no PVN, apesar do elevado limiar para vasodilatação cutânea. Os dados indicam que o controle simpático a partir do PVN é fundamental nos ajustes cardiovasculares durante o estresse térmico passíveis de influenciar a redistribuição sanguínea corporal para a periferia. Além disso, um possível mecanismo pelo qual o aquecimento facilita a perda de calor através da vasodilatação cutânea ocorre via disponibilidade de óxido nítrico no PVN.

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