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ESTUDO DO ENVOLVIMENTO DAS VIAS HISTAMINÉRGICAS CENTRAIS NO CONTROLE DA INGESTÃO DE ÁGUA E SAL EM RATOS

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"ESTUDO DO ENVOLVIMENTO DAS VIAS HISTAMINÉRGICAS CENTRAIS NO CONTROLE DA INGESTÃO DE ÁGUA E SAL EM RATOS"

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"O futuro pertence àqueles que acreditam na beleza dos seus sonhos"

Eleanor Roosevelt

SUMÁRIO

LISTA DE ABREVIATURAS

AMPc	Adenosina monofosfato cíclica
A3V	Região Ântero-Ventral do 3º Ventrículo
AII	Angiotensina II
AVP	Arginina Vasopressina
DAG	Diacilglicerol
GMPc	Guanosina-3',5'-Monofosfato Cíclica
GTP	Guanosina Trifosfato
HA	Histamina
HDC	Histidina Descarboxilase
HTMT	6-[2-(4-Imidazol) etilamino]-N-(4-trifluorometilfenil) heptanocarboxamida)
IP_3	Inositol Trifosfato
NMDA	*N* - metil-D-aspartato
NO	Óxido Nítrico
NOS	Óxido Nítrico Sintetase
OT	Ocitocina
OVLT	Órgão Vasculoso da Lâmina Terminal
OSF	Órgão Subfornical
PEG	Polietileno Glicol
PVN	Núcleo Paraventricular
SNC	Sistema Nervoso Central
VMH	Núcleo Ventromedial Hipotalâmico
IIIV	3º Ventrículo

RESUMO

Os diferentes mecanismos cerebrais de controle do comportamneto de ingestão hídrica desencadeado pela sede, bem como o apetite específico por sódio têm sido objetos de investigação de diversos grupos de pesquisas. A participação das vias histaminérgicas centrais e de seus receptores no controle do equilíbrio hidrossalino do organismo ainda não está completamente esclarecida. Dados da literatura mostram que as vias histaminérgicas centrais através dos seus receptores dos tipos H_1 e H_2 participam dos mecanismos de controle da ingestão hídrica em animais normhidratados e da ingestão de água pós-prandial. Decidiu-se então investigar o papel dos receptores histaminérgicos centrais dos tipos H_1 e H_2 no controle da ingestão hídrica em diferentes situações de desafio homeostático - hiperosmolaridade e hipovolemia, além da verificação da possível interação entre as vias histaminérgicas e colinérgicas centrais no controle da ingestão hídrica.

Investigou-se também a participação desses receptores histaminérgicos nos mecanismos de controle do apetite específico por sódio desencadeado em situações de depleção deste íon, privação de líquidos e estimulação angiotensinérgica central.

Os resultados obtidos neste estudo mostram uma maior funcionalidade dos receptores histaminérgicos do tipo H_1 em situações fisiológicas que provocam modificações nas condições osmóticas dos compartimentos de líquidos corporais, além da existência de uma inter-relação das vias histaminérgicas através dos receptores do tipo H_1 e as vias colinérgicas centrais sobre o controle da ingestão hídrica.

A participação dos receptores do tipo H_2 está relacionada a alterações do volume dos líquidos corporais, bem como à resposta dipsogênica desencadeada pela angiotensina II.

Os resultados obtidos neste trabalho trazem importantes informações que contribuem para o entendimento da participação das vias histaminérgicas no controle do equilíbrio hidrossalin, evidenciando as interações destas vias com outras vias neurotransmissoras centrais no controle da homeostasia hidrossalina do organismo.

Palavras-chaves: 1. Ingestão de água. 2. Ingestão de Sal. 3. Histamina. 4. 3º Ventrículo. 5. Receptores H_1 e H_2.

ABSTRACT

Several research groups have been studying the mechanisms involved in the ingestive behavior and in the control of thirst and sodium appetite. The participation of brain histaminergic pathway in the hydroelectrolyte balance it is not entirely understood. Some studies show that both, H_1 and H_2 histaminergic receptors, may participate in the control of water intake.

In the present study we decide to investigate the role of H_1 and H_2 histaminergic receptors in the control of water intake in different conditions: dehydration, hyperosmolarity, hypovolemia and pharmacological stimulation central cholinergic pathways. Besides, we also studied the role of H_1 and H_2 histaminergic receptors in the controle of sodium appetite after pharmacological stimulation central angiotensinergic pathways and in sodium depleted animals.

The data show H_1 histaminergic receptors main effect in the control of water intake under osmotic challenge, while H_2 histaminergic receptors are associated with fluid volume control. Also, the interaction between histaminergic and angiotensinergic brain pathways, as well as histaminergic and cholinergic brain pathways, seems to be important in the control of water intake.

The results here presented contributed to the knowledge of the mechanisms involved in the regulation of water and salt intake and show an important participation of brain histaminergic pathways in the control of ingestive behavior.

Key-words: 1. Water intake. 2. Salt intake. 3. Histamine. 4. Third ventricle. 5. H_1 and H_2 receptors.

1 INTRODUÇÃO

A histamina encontrada no tecido cerebral tem sua origem na síntese local, e evidências tanto bioquímicas quanto farmacológicas indicam que a histamina cerebral encontra-se em dois tipos celulares: os neurônios e os mastócitos. Embora os mastócitos sejam escassos no sistema nervoso central, seu alto teor de histamina torna-os relevantes para o conteúdo histaminérgico cerebral (GARBARG et al., 1976).

Os neurônios histaminérgicos parecem estar envolvidos em diferentes funções hipotalâmicas como: ciclo sono/vigília (KIYONO et al., 1985), secreção de hormônios (SAKATA et al., 1988), ritmo circadiano (HOFFMAN et al., 1978), termorregulação (FUJIMOTO et al., 1990) e ingestão alimentar (SCHWARTZ et al., 1991). Contudo, a maioria dos estudos funcionais sobre as vias histaminérgicas centrais enfoca seu papel no controle da ingestão alimentar, do balanço metabólico e energético. Dados da literatura sugerem que diferentes áreas cerebrais são responsáveis por este controle.

No que diz respeito às funções da histamina como neuromodulador e neurotransmissor, sabe-se que começaram a ser estudadas a partir de 1970 (TAYLOR & SNYDER, 1972, CALCUTT, 1976, SCHWARTZ et al., 1980).

Em relação ao papel das vias histaminérgicas centrais no controle do balanço hidrossalino, este é pouco conhecido. Alguns estudos têm mostrado que as vias histaminérgicas centrais podem participar do controle da ingestão hídrica pós-prandial (KRALY, 1983, 1990 e KRALY et al., 1995). Entretanto, estudos sistemáticos da participação destas vias neurotransmissoras no balanço hidrossalino ainda são ausentes.

Dessa forma, no presente trabalho investigou-se a participação das vias histaminérgicas centrais no controle da ingestão hídrica, bem como do apetite específico por sódio em diferentes modelos de estudo.

REVISÃO DA LITERATURA

1.1 HISTAMINA COMO NEUROTRANSMISSOR

A localização das vias histaminérgicas centrais tem sido estudada através da utilização de anticorpos monoclonais seletivos para a enzima de síntese da histamina (HA), a histidina descarboxilase (HDC). Os corpos celulares dos neurônios histaminérgicos estão localizados exclusivamente no hipotálamo, especificamente no núcleo tuberomamilar (SCHWARTZ et al., 1991). Empregando-se técnicas de imunohistoquímica, verificou-se que os neurônios deste núcleo apresentam projeções difusas para diversas áreas cerebrais incluindo: córtex, bulbo olfatório, núcleo supraóptico, núcleo paraventricular e núcleo ventromedial hipotalâmico, além de algumas áreas no tronco encefálico e medula espinhal (PANULA et al., 1984, WATANABE et al., 1984). Os neurônios histaminérgicos parecem estar envolvidos em várias funções no sistema nervoso central, tais como: regulação neuroendócrina, controle da pressão sangüínea, regulação do ciclo de sono-vigília, respostas ao estresse, termorregulação e ingestão alimentar (ROBERTS & CALCUT, 1983, STRUMAN, 1996).

Segundo Kruger et al. (1995), em ratos o núcleo tuberomamilar está dividido em três grupos: o núcleo tuberomamilar medial, formado por cerca de 600 neurônios; o núcleo tuberomamilar ventral, com aproximadamente 1500 neurônios, e uma terceira região difusa, classificada como núcleo tuberomamilar difuso, localizado entre vários núcleos hipotalâmicos. Outro grupo de pesquisadores subdivide o núcleo tuberomamilar em dorsal e ventral, sendo este último subdividido em porção rostral e caudal (INAGAKI et al., 1990). Ainda outra divisão do núcleo tuberomamilar é sugerida pelo grupo de Wada et al. (1991). De acordo com eles, este núcleo poderia ser subdividido

em 5 regiões distintas e seriam classificadas de E1 a E5, dependendo das funções que desempenham.

1.1.A - Características Biossintéticas da Histamina

A histamina é sintetizada no cérebro a partir da $_{L}$-histidina, através da enzima $_{L}$-histidina descarboxilase, e catabolizada em 3-metil-histamina, através da enzima histamina $_{N}$-metiltransferase. O processo de síntese da histamina pode ser inibido através da administração de α-fluorometil-histidina, que inibe a ação da enzima $_{L}$-histidina descarboxilase reduzindo assim os níveis de histamina cerebral (KOLLONITSCH et al., 1978).

A síntese e a liberação da histamina apresentam controle inibitório efetuado pelos auto-receptores H_3, localizados no corpo neuronal e nos axônios terminais dos neurônios histaminérgicos (ARRANGE et al., 1983, ITHO et al., 1991, PRAST et al., 1991).

1.1.B - Receptores Histaminérgicos

Os receptores histaminérgicos foram classificados com base nas características farmacológicas e bioquímicas das vias de transdução de sinal e dos segundos mensageiros. Através dessas características estabeleceu-se, até o momento, o perfil farmacológico de quatro tipos de receptores histaminérgicos, classificados como: H_1, H_2, H_3 e H_4 (HILL, 1990, HILL et al., 1997). Diferentes agentes histaminérgicos seletivos (agonistas e antagonistas) têm sido desenvolvidos permitindo a análise da função e da distribuição dos receptores histaminérgicos nos diferentes tecidos (GOOT & TIMMERMAN, 2000, NAKAMURA et al., 2000). No presente estudo utilizaram-se os antagonistas seletivos para os receptores do tipo H_1 e H_2, mepiramina e cimetidina respectivamente. Assim, a seguir apresentaremos as características específicas destes receptores.

O primeiro composto anti-histamínico foi descoberto em 1933. Os primeiros antagonistas histaminérgicos para os receptores do tipo H_1, utilizados na clínica, foram a mepiramina e a difenidramina desenvolvidos a partir de 1977 (LEURS et al., 1995). Os antagonistas para os receptores histaminérgicos do tipo H_2 foram desenvolvidos a partir de 1972. Uma das primeiras substâncias utilizadas foi o burimamida, em 1981, substância essa que originou os principais antagonistas para o receptor do tipo H_2, a exemplo da cimetidina, ranitidina e fomatidina (BLACK et al., 1972, GANELLIN, 1981). Os antagonistas mepiramina e cimetidina apresentam uma alta afinidade para os seus respectivos receptores, sendo que para o do tipo H_1 o pK_d é de 9.4 (ISON, et al., 1973, HILL, 1990) e para o tipo H_2 o pK_d é de 6.1 (GOOT & TIMMERMAN, 2000).

O receptor histaminérgico do tipo H_1 é uma proteína cujo peso molecular de 56 kDa pode variar entre 53 a 58 kDa, dependendo da espécie. Este receptor apresenta 7 alças transmembrânicas e faz parte dos receptores da superfamília acoplada à proteína G (HILL, 1990). Na face intracelular da membrana, o receptor histaminérgico H_1 está associado à proteína $G_{q/11}$, que hidrolisa a guanosina trifosfato, quando o receptor é ativado, e estimula a atividade da fosfolipase C (LEURS et al., 1995). Esta, por sua vez, hidrolisa o fosfatidil inositol, formando, assim, o segundo mensageiro diacilglicerol (DAG) e o inositol trifosfato (IP_3). O DAG potencializa a atividade da proteína cinase C, enquanto o composto IP_3 promove a liberação de cálcio do retículo endoplasmático para o meio intracelular. A ativação do receptor H_1 também pode levar à formação de ácido araquidônico através da ativação da fosfolipase A e a formação de guanosina-3',5'-monofosfato cíclica (GMPc). A formação de GMPc pode ser conseqüência direta da liberação de cálcio no citoplasma, da ativação da enzima óxido nítrico sintetase (NOS), que produz óxido nítrico (NO), e também da estimulação da guanilato ciclase. Nesta via, o receptor H_1 pode ser capaz de modular a liberação de neurotransmissores

na fenda pré-sináptica, desde que o NO e o ácido araquidônico executem o papel de mensageiros retrógrados. A ativação do receptor H_1 também pode interferir na regulação da atividade dos canais de magnésio ativados por receptores $_N$-metil-D-aspartato (NMDA), o que levaria a modificações no potencial de membrana (PAYNE & NEUMEN, 1997).

A distribuição dos receptores H_1 no sistema nervoso central é ampla (CHANG et al., 1979, PALACIOS et al., 1981; BOUTHENET et al., 1988), sendo identificados em áreas como tálamo, córtex, o tegumento e núcleos da rafe. No tegumento os receptores H_1 são colocalizados em neurônios colinérgicos. Os receptores H_1 também estão presentes no sistema límbico e em muitos núcleos do hipotálamo. A maior densidade desses receptores foi evidenciada no núcleo septal, na amígdala e em diferentes áreas do hipocampo. Outras áreas que apresentam alta densidade dos receptores H_1 são: o núcleo accumbens, o núcleo do trato solitário e a área postrema, além do cerebelo (BROWN et al., 2001).

O receptor histaminérgico do tipo H_2 descrito em 1972 por Black e colaboradores, é uma proteína de peso molecular variando entre 40,2 a 40,5 kDa, dependendo da espécie, e também apresenta 7 alças transmembrânicas (HILL, 1990). Quando o receptor H_2 é ativado, a proteína Gs na face intracelular da membrana estimula o adenilato ciclase a produzir o segundo mensageiro, a adenosina monofosfato cíclica (AMPc) (BAUDRY et al., 1975, HEGSTRAND et al.,1976). O segundo mensageiro estimula a proteína cinase A dependente de AMPc que pode fosforilar proteínas no citosol e na membrana celular ou translocar-se para o núcleo e ativar o fator de transcrição CREB (SHENG et al., 1991).

O receptor H_2 está difusamente expresso no cérebro e na medula espinhal (TRAIFFORT et al., 1992, VIZUETE et al., 1997). Estes receptores estão localizados

em alta densidade nos gânglios da base, no sistema límbico, hipocampo, amigdala e nas camadas superficiais do córtex cerebral; e em baixas densidades nas áreas septais, núcleos hipotalâmicos, talâmicos e cerebelo. Podemos encontrar os receptores H_1 e H_2 colocalizados em diversas áreas cerebrais, incluindo células piramidais e granulócitos na formação hipocampal e em outros grupos de células aminérgicas, como os núcleos da rafe e na substância negra (BROWN et al., 2001). Os dados da literatura apresentados evidenciam que, em diferentes trabalhos e sob diferentes condições, as vias histaminérgicas desempenham importante papel no SNC e podem interagir com outras vias neurotransmissoras. Assim, devido às dúvidas ainda existentes a respeito do papel das vias histaminérgicas centrais no controle do balanço hidrossalino, decidiu-se no presente trabalho investigar o papel das vias histaminérgicas no controle da ingestão hídrica e no apetite por sódio, em diferentes condições de estímulos.

1.2 CONTROLE DA INGESTÃO HÍDRICA

O comportamento de ingestão hídrica está associado à necessidade do organismo de manter a osmolaridade e o volume dos líquidos corporais em equilíbrio. A modificação da osmolaridade e do volume dos diferentes compartimentos líquidos levam à sede por dois mecanismos básicos: desidratação intracelular e desidratação extracelular, que podem ser induzidas por alterações na osmolaridade e na volemia plasmática.

Em 1961, Fitzsimons induziu sede em ratos submetidos previamente à administração subcutânea de polietileno-glicol (PEG), que provoca hipovolemia devido ao seqüestro do líquido extracelular e, portanto, desidratação extracelular. Gilman e colaboradores, em 1937, demonstraram o potente efeito dipsogênico causado pela administração orogástrica de soluções hipertônicas em ratos, que leva ao aumento da osmolaridade plasmática, e consequentemente, desidratação intracelular. A sede

também pode ser induzida através da privação hídrica, que ocasiona modificações nos volumes dos líquidos intra e extracelular, afetando também o equilíbrio osmótico do organismo.

Esses dados mostram que diferentes estímulos podem desencadear modificações nos volumes dos líquidos corporais e influenciar diretamente o comportamento de ingestão hídrica. Destarte, estes métodos têm sido utilizados amplamente como paradigmas de estudo das áreas cerebrais e das vias neurotransmissoras envolvidas no controle da ingestão hídrica. Cada um destes métodos leva à ativação de diferentes vias neurotransmissoras centrais gerando o comportamento de busca e ingestão hídrica.

O controle do comportamento de ingestão hídrica está intimamente relacionado à ativação de diferentes vias neurotransmissoras centrais em diversas áreas cerebrais. Entre as vias neurotransmissoras centrais pode-se citar: as vias adrenérgicas, colinérgicas, serotoninérgicas, angiotensinérgicas e opiatérgicas. A ativação das vias adrenérgicas pode levar tanto a estimulação quanto à inibição da ingestão hídrica. A injeção central de agonistas alfa-adrenérgicos leva à inibição da ingestão hídrica, enquanto que a administração de agonistas beta-adrenérgicos resulta em aumento da ingestão de água (GROSSMAN, 1960, LHER et al., 1967, SHARPE & MYERS, 1969; LEIBOWITZ, 1971).

Em relação às vias colinérgicas observou-se que a injeção de carbacol, agonista colinérgico, no III ventrículo, no hipotálamo e na área septal resulta em aumento da ingestão de água em ratos (STRICKER & MILLER, 1968; ANTUNES-RODRIGUES & McCANN, 1970, ANTUNES-RODRIGUES & COVIAN, 1971). Outro neurotransmissor com efeito dipsogênico é a angiotensina II, onde tanto a administração sistêmica quanto a central leva ao estímulo do comportamento de ingestão hídrica (FITZSIMONS & SIMONS, 1969, EPSTEIN et al., 1970, ANDERSSON &

ERICKSSON, 1971). Além do efeito dipsogênico, a angitotensina II também desencadeia potente ação pressora e de estímulo do apetite por sódio. (SAAVEDRA, 1992; WRIGHT & HARDING, 1992; ERIKSON et al., 1995, WISINGER et al., 1996).

A participação das vias serotoninérgicas nos processos de controle da ingestão hídrica também tem sido estudada. A administração no III ventrículo do agonista seletivo para os receptores 5-HT1_D, o L-694,247, reduz de forma dose-dependente a ingestão hídrica em animais desidratados ou submetidos à estimulação angiotensinérgica e colinérgica central. Contudo, não modifica a ingestão de água em animais normhidratados. O efeito inibitório desencadeado pelo L-694,247 em animais em condição de desidratação é revertido através do pré-tratamento como o GR 127935, antagonistas seletivos para os receptores 5-HT1_D (DE CASTRO E SILVA et al., 1997).

Em outro estudo, a administração central de GR 113808 e SB 204070, ambos antagonistas seletivos para o receptor serotoninérgico 5-TH$_4$, não é capaz de modificar a ingestão hídrica em animais normhidratados. Contudo, o tratamento com estes antagonistas potencializa a ingestão hídrica em animais induzida pela estimulação colinérgica (CASTRO et al., 2000). Mostra-se dessa forma, a participação das vias serotoninérgicas e sua interação com outras vias no controle e regulação do comportamento de ingestão hídrica.

1.3 CONTROLE DO APETITE POR SÓDIO

Os mecanismos que regulam o apetite por sódio podem, ou não, estar dissociados do comportamento de ingestão hídrica. Diversos métodos foram desenvolvidos para estudar o apetite específico por sódio. A redução nas concentrações de sódio plasmático leva ao apetite específico por sódio e muitos dos métodos de estudo envolvem modificações da natremia. A maior parte do sódio é eliminada através da excreção renal. Todavia, uma perda expressiva deste íon é evidenciada após vômito,

diarréia e desidratação intensa (TAKAMATA et al., 1994). Nos estudos de apetite por sódio é comum a utilização de um paradigma de dupla escolha, isto é, os animais têm acesso a dois bebedouros: um contendo água destilada e o outro contendo salina hipertônica, em doses que normalmente são aversivas aos animais.

O estado hipovolêmico, caracterizado por modificações no volume do líquido extracelular, ativa diferentes mecanismos reguladores, diminuindo a perda de água e sódio e ajustando a distribuição dos líquidos corporais. Entre os mecanismos reguladores inclui-se a liberação de renina, vasopressina, aldosterona, hormônios adrenocorticotrópicos e glicocorticóides (JONHSON & THUNHORST, 1997). A condição hipovolêmica causada pela administração de diuréticos, a exemplo da furosemida, promove perdas significativas, não apenas de volume, mas também de íons como o potássio e o sódio, levando o animal a ingerir grandes quantidades de água e sal (JALOWIEC, 1974).

A administração subcutânea de colóides como o polietileno glicol (PEG) (STRICKER et al., 1992) e a diálise peritoneal (FALK, 1965) também são métodos utilizados para estimular o apetite por sódio em ratos. Outro método de estimulação do apetite por sódio é a administração de hormônios mineralocorticóides (SHELAT et al., 1999) e de angiotensina II (FITZSIMONS, 1998). Ao contrário, em animais em situação de hipovolemia e hiperosmolaridade, a administração central de ocitocina (OT) pode inibir o apetite por sódio (BLACKBURN et al, 1993; STRICKER & VERBALIS, 1996).

Diferentes vias neurotransmissoras centrais parecem estar envolvidas no controle do apetite por sódio. Recentemente, em estudo desenvolvido em nosso laboratório, elas demonstraram que a estimulação farmacológica dos receptores 5-HT_3 através da administração central do agonista específico m-chlorophenylbiguanide

hydrochloride (1-(3-chlorophenyl)biguanide, (m-CPBG), na amígdala medial, reduz a ingestão de sal em animais sódio-depletados. Esse efeito é revertido com o pré-tratamento com ondansetrona, antagonista seletivo para o receptor 5-HT_3. A estimulação de outro tipo de receptor serotoninérgico, o 5-HT_{2C}, localizado na amígdala medial, não modifica a ingestão de sal em animais sódio depletados. Entretanto, o tratamento com antagonista para os receptores serotoninérgicos 5-HT_{2C}, SDZSER082, reduz significativamente a ingestão de sódio em animais depletados deste íon (LUZ et al., 2006).

1.4 VIAS HISTAMINÉRGICAS CENTRAIS E CONTROLE HIDROSSALINO

Poucos estudos foram direcionados para a investigação da participação das vias histaminérgicas centrais no controle da ingestão hídrica e do apetite por sódio. A maioria dos trabalhos foi voltada para o papel destas vias no controle da ingestão alimentar. Diversos trabalhos mostram sua participação tanto no controle metabólico e balanço energético, quanto no comportamento alimentar.

A administração central e periférica de antagonistas seletivos dos receptores H_1 e H_2 histaminérgicos estimulam a ingestão alimentar em ratos (KRALY et al., 1998). Em outros trabalhos observou-se que a administração central, nos núcleos ventromedial (VMH) e paraventricular (PVN), de metoprina, inibidor da enzima de catabolismo da histamina, a N-metiltranferase, eleva os níveis de histamina no cérebro e promove redução da ingestão alimentar, enquanto que em animais pré-tratados com o antagonista do receptor H_1, mepiramina, ocorre estímulo do comportamento de ingestão alimentar (LECKLIN & TUOMISTO, 1998). A administração da tioperamida, antagonista seletivo do auto-receptor H_3, nos núcleos VMH e PVN, provoca a supressão da ingestão alimentar em ratos (SAKATA et al., 1997). O receptor histaminérgico H_3 age como

auto-receptor e pode regular a síntese e liberação da histamina (ARRANGE et al., 1983).

Estes dados mostram que a histamina é um neurotransmissor que controla a saciedade, sugerindo que a histamina participa de forma direta dos mecanismos de regulação do comportamento de ingestão alimentar.

Em muitas espécies a ingestão hídrica e a ingestão alimentar estão associadas. Cerca de 70% do consumo de água ocorre durante ou imediatamente após a alimentação, demonstrando a estreita relação entre os dois processos (FITZSIMONS & LE MAGNEN, 1969, KISSILEFF, 1969). A administração de histamina nos ventrículos laterais, ou em diferentes áreas hipotalâmicas, provoca a estimulação da ingestão hídrica pós-prandial em ratos (GERALD & MAICKEL, 1972; LEIBOWITZ, 1973). A administração periférica ou central dos antagonistas seletivos dos receptores H_1 e H_2 histaminérgicos reduz tanto a ingestão alimentar quanto a ingestão hídrica em animais submetidos a sobrecarga osmótica gastrointestinal (KRALY et al., 1998). A participação dos receptores H_1, H_2 e H_3 no controle da ingestão de água pós-prandial foi evidenciada através da administração no ventrículo lateral de antagonistas específicos para esses receptores o que provocou inibição da ingestão hídrica pós-prandial nesses animais (KRALY et al., 1995). A injeção de histamina por via subcutânea provoca o aumento da ingestão de água em ratos privados de alimento por 12 horas, quadro que pode ser revertido com a utilização de dexbromofeniramina, cimetidina e tioperamida, antagonistas para os receptores H_1, H_2 e H_3 respectivamente (KRALY et al., 1996). A inibição do catabolismo da histamina, através da administração de metoprina, por via intraperitoneal em ratos Wistar, Long-Evans e Brattleboro, deficientes de vasopressina plasmática, mostrou envolvimento deste neurotransmissor na regulação do balanço dos líquidos corporais destes animais (LECKLIN & TUOMISTO, 1995). De fato, observou-

se que a estimulação histaminérgica leva ao aumento da liberação de vasopressina e diminuição da diurese (BHARGAVA et al., 1973; BENNET & PERT, 1974; KJAER et al., 1994).

Tendo em vista que os dados da literatura demonstram a participação das vias histaminérgicas centrais no controle da ingestão hídrica e que há carência de estudos sistematizados mostrando o envolvimento destas vias no controle da ingestão hídrica em diferentes condições de volemia e osmolaridade, bem como na regulação do apetite por sódio, decidiu-se no presente estudo investigar a participação das vias histaminérgicas centrais através dos receptores dos tipos H_1 e H_2 no controle da ingestão hídrica e no apetite específico por sódio em diferentes condições homeostáticas.

2 HIPÓTESES

Hipóteses Nulas

1- As vias histaminérgicas centrais, através dos seus receptores histaminérgicos dos tipos H_1 e H_2, não participam dos processos de regulação e controle do balanço hidrossalino em ratos.

2- As vias histaminérgicas centrais, através dos seus receptores histaminérgicos dos tipos H_1 e H_2, não interagem com as vias colinérgicas e angiotensinérgicas centrais no controle do balanço hidrossalino em ratos.

Controle da ingestão hídrica

Hipótese 1

As vias histaminérgicas centrais, através dos seus receptores histaminérgicos dos tipos H_1 e H_2, estimulam a ingestão hídrica em ratos.

Hipótese 2

As vias histaminérgicas centrais, através dos seus receptores histaminérgicos dos tipos H_1 e H_2, inibem a ingestão hídrica em ratos.

Hipótese 3

As vias histaminérgicas centrais interagem com as vias colinérgicas controlando a ingestão hídrica em ratos

Controle do apetite por sódio

Hipótese 4

As vias histaminérgicas centrais, através dos seus receptores histaminérgicos dos tipos H_1 e H_2, estimulam o apetite específico por sódio em ratos.

Hipótese 5

As vias histaminérgicas centrais, através dos seus receptores histaminérgicos dos tipos H_1 e H_2, inibem o apetite específico por sódio em ratos.

Hipótese 6

As vias histaminérgicas centrais interagem com as vias angiotensinérgicas centrais controlando o apetite por sódio em ratos

3 OBJETIVOS

- Investigar o papel dos receptores histaminérgicos centrais H_1 e H_2 no controle da ingestão hídrica.

Específicos:

a) Investigar a participação dos receptores H_1 e H_2 centrais na resposta da ingestão hídrica em animais induzida por hiperosmolaridade;

b) Investigar o papel dos receptores H_1 e H_2 centrais na resposta da ingestão hídrica induzida por hipovolemia;

c) Verificar a existência de interação entre vias histaminérgicas e colinérgicas centrais no controle da ingestão hídrica.

- Investigar o papel dos receptores histaminérgicos centrais H_1 e H_2 no controle do apetite específico por sódio.

Específicos:

a) Investigar a participação dos receptores H_1 e H_2 centrais na resposta ao apetite específico por sódio em animais depletados deste íon;

b) Estudar o papel dos receptores H_1 e H_2 na resposta de apetite específico por sódio em animais em condições de desidratação;

c) Verificar se ocorre interação entre as vias histaminérgicas e as angiotensinérgicas centrais no controle do apetite específico por sódio.

4 RESULTADOS

ARTIGO 1

"Central H_1 and H_2 receptor participation in the control of water and salt intake in rats"

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Central H_1 and H_2 receptor participation in the control of water and salt intake in rats

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Abstract

The aim of the present study was to evaluate the participation of brain H_1 and H_2 histaminergic receptors on water and salt intake induced by water deprivation (24 h), furosemide-induced sodium depletion and central angiotensinergic pharmacological stimulation in rats. Third ventricle injections of the H_1 and H_2 receptor antagonists, mepyramine (50, 100, 200 and 400 nmol) and cimetidine (100, 200 and 400 nmol), were unable to modify water intake induced by water deprivation and sodium depletion. Salt intake elicited by water deprivation and sodium depletion was reduced by the central administration of mepyramine, while intracerebroventricular administration of cimetidine had no effect. Water and salt intake evoked by central angiotensinergic stimulation (10 ng) was diminished by third ventricle injections of both mepyramine and cimetidine. Inhibition of the ingestive behaviors observed here is not a result of any illness-like effect produced by the intracerebroventricular injections of the histaminergic antagonists used, as demonstrated by an avoidance test. It was also shown that third ventricle injections of these compounds were unable to modify the hedonic behavior that leads rats to drink a tasty saccharin solution. We conclude that central histaminergic receptors participate in the control of salt intake induced by distinct physiological and pharmacological stimuli.

Theme: Endocrine and Autonomic Regulation
Topic: Osmotic and Thermal Regulation

Keywords: Histamine; Salt intake; Water intake; Cimetidine; Mepyramine

1. Introduction

Thirst and salt appetite are important behaviors which help mammals to regulate plasma osmolarity, blood volume and blood pressure. Specialized structures located both in the central nervous system and in strategic peripheral sites detect changes in these parameters continuously and accurately. Based on information obtained by these sensors, the central nervous system yields corrective responses including the stimulation or the inhibition of water and salt intake (for review, see Ref. [1]).

In recent decades, a growing research effort has established the undeniable role of brain neuronal histamine as a specific neuronal circuitry that originates exclusively in the hypothalamic tuberomammilary nucleus and projects to several brain areas. These central histaminergic pathways exert numerous physiological roles, including the control of food intake (for review, see Refs. [2–4]).

The role of brain histamine in the control of fluid balance has received far less attention. However, histamine induces a significant increase in water intake when injected into the cerebral ventricles [5] and dehydration increases hypothalamic histamine synthesis and release [6]. Hista-

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mine has been characterized as one of the key central agents triggering prandial drinking in rats [7]. This behavior is inhibited by the pharmacological blockade of H_1 and H_2 receptors, whose participation is also necessary for the expression of thirst induced by exogenous histamine administration [8].

Several brain aminergic neurotransmitters such as serotonin, acetylcholine and noradrenaline influence salt intake in mammals [9]. An extensive review of the literature shows no data concerning brain histaminergic participation in the control of sodium appetite in laboratory animals.

In the present paper, we investigate the participation of brain H_1 and H_2 histaminergic receptors on water and salt intake induced by two physiological stimuli (sodium depletion and fluid deprivation) and following central pharmacological stimulation with angiotensin II in rats.

2. Material and methods

2.1. Animals

In the present study, we used Wistar male rats weighing 240±20 g. They were housed in individual cages and kept under controlled light (lights on from 7:00 a.m. to 7:00 p.m.) and temperature (22–24 °C) conditions. In all experimental protocols third ventricle injections of saline (controls), and each individual dose of the antagonists were tested in a naïve group of animals. All experiments were conducted between 7:00 and 11:00 a.m.

2.2. Surgical procedures

Third ventricle cannulation was performed under pentobarbital anesthesia (50 mg/kg i.p.). Five days before the experimental sessions, a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA) was used to implant a 15-mm, 22-gauge, stainless steel cannula. The following coordinates were used: anteroposterior=0.5 mm behind bregma; lateral=0.0 mm; vertical 8.5 mm below the skull. To avoid lesions to the midline structures related to body fluid and electrolyte control, the animals were placed in the stereotaxic apparatus with the head inclined 0.2 mm upwards. The cannulas were cemented to the skull bone with dental acrylic and an obturator (28-gauge) was provided to avoid obstruction. After sacrifice by CO_2 inhalation, we verified whether the tip of the cannula was correctly positioned by injecting Blue Evans dye (2.0 μl) into the third ventricle. Only data from animals in which the cannulas were strictly inside the third ventricle were analyzed. After surgery, the animals in all the study groups had free access to two different bottles, one containing distilled water and the other containing 1.5% saline solution. In order to minimize the stress of the experimental maneuvers, the animals were handled everyday.

2.3. Drugs and microinjections

The following drugs were used: mepyramine maleate (*N*-(4-methoxy-phenylmethyl-*N*′,*N*′-dimethyl-*N*-(2-pyridinyl)-1,2-ethanediamine), H_1 histaminergic receptor antagonist, and cimetidine, H_2 histaminergic receptor antagonist, as well as angiotensin II and lithium chloride were purchased from Sigma, St. Louis, MO. Furosemide, a loop diuretic, was purchased from Aventis Pharma, São Paulo, Brazil. Central injections were performed using a Hamilton microsyringe connected to a Myzzy-Slide-Pak needle through polyethylene tubing. All drugs were dissolved in isotonic saline solution. The final volume injected was 2 μl over a period of 90 s. The doses of mepyramine used here were based on previous work from another group [10] in which intracerebroventricular infusions of this compound were used to study the role of central H_1 receptors on food and water intake. In that paper, the authors used a fixed dose of 800 nmol of mepyramine. Another study from a different group states that cimetidine, when injected intracerebroventricularly at similar doses, induces convulsion [11]. Therefore, in order to use both drugs in equimolar amounts, we decided to test mepyramine and cimetidine at smaller doses (50, 100, 200 and 400 nmol) than those used by the group of Lecklin et al.

2.4. Sodium depletion

To induce sodium depletion, the animals were submitted to an experimental protocol in which they had simultaneous access to two bottles (distilled water and 1.5% saline solution) and standard rat chow from the period immediately after third ventricle cannulation until the moment of furosemide administration. To provoke the renal sodium loss that induces sodium depletion, the rats received a subcutaneous injection of furosemide (20 mg/kg) 24 h prior to the experimental sessions. Access to 1.5% saline ceased immediately after the furosemide injection. From that moment on, the animals continued to have free access to distilled water, and normal rat chow was replaced by a low sodium diet (0.001% Na^+ and 0.33% K^+). Control animals not submitted to sodium depletion received subcutaneous injections of isotonic saline solution instead of furosemide. We have previously demonstrated that furosemide administration, at the dose used here, effectively increases urine output and renal sodium excretion and produces hyponatremia [12]. To test the participation of central H_1 and H_2 receptors in water and salt intake in sodium-depleted rats, different groups of sodium-depleted animals received third ventricle injections of different doses (50, 100, 200 and 400 nmol) of mepyramine, a selective H_1 receptor antagonist, or the H_2 receptor antagonist cimetidine (100, 200 and 400 nmol). Sodium-depleted control animals received third ventricle injections of isotonic saline solution. The bottles containing 1.5% saline solution were reintroduced into the cages 15 min after the third ventricle injections. The first

measure of fluid intake was recorded 15 min after this and was continued for the next 120 min. All groups were compared to a control normonatremic group of animals.

2.5. Fluid deprivation

To induce fluid deprivation, bottles were removed from the individual cages 24 h prior to the onset of the experiments. Euhydrated animals served as controls for this experimental group. To investigate the participation of central H_1 and H_2 receptors in water and salt intake in fluid deprivation, different groups of fluid-deprived rats received third ventricle injections of different doses (50, 100 and 200 nmol) of mepyramine, a selective H_1 antagonist, or the H_2 receptor antagonist cimetidine (100, 200 and 400 nmol). The bottles containing 1.5% saline solution were reintroduced into the cages 15 min after the third ventricle injections. The first measure of fluid intake was recorded 15 min after this and was continued for the next 120 min. Fluid-deprived control animals received third ventricle injections of isotonic saline solution. All groups were compared to a group of rats not submitted to fluid deprivation.

2.6. Central angiotensinergic stimulation

To induce a pharmacological stimulation of central angiotensinergic pathways, animals received third ventricle injections of angiotensin II at the dose of 10 ng. Control animals received third ventricle injections of isotonic saline solution. To study the participation of central H_1 and H_2 receptors in water and salt intake after central angiotensinergic stimulation, different groups of rats received third ventricle injections of different doses (100, 200 and 400 nmol) of mepyramine, a selective H_1 antagonist, or the H_2 receptor antagonist cimetidine (100, 200 and 400 nmol) 15 min before receiving angiotensin II (10 ng). Bottles containing 1.5% saline solution were available immediately after the third ventricle injections of angiotensin II. As in the previous experimental sets, the first measure of fluid intake was recorded 15 min after this and was continued for the next 120 min. All groups were compared to a group of rats receiving central administration of saline instead of angiotensin II.

2.7. Avoidance test

An avoidance test was carried out to verify whether the central administration of both mepyramine and cimetidine was devoid of nonspecific, inhibitory, "illness-like" effects on water intake. An experimental protocol based on the original design proposed by Nachman [13] was adopted. This protocol uses a temporal association between the novel taste of a 0.25% saccharin solution and the distress induced by lithium chloride administration. Five days after the third ventricle cannulation, the animals had their access to water restricted to 15 min/day (between 12:00 and 12:15 p.m.) for 4 consecutive days. Under these conditions, rats drank water rapidly and reliably. On the fifth day, they were divided into four different groups that, after being submitted to different pharmacological protocols, had access to bottles containing saccharin (no water was offered on this day). The first group (controls) received two consecutive injections, one immediately following the other, of isotonic saline solution, the first being intraperitoneal and the second into the third ventricle. In the second group of animals, 0.15 M lithium chloride intraperitoneal injections (0.6% b.w.) were followed by injections of isotonic saline solution into the third ventricle. In this group the lithium-induced, illness-like effects, a condition that generally disrupts ingestive behaviors in rats, are associated with the novel taste of saccharin. The third and the fourth groups of animals received intraperitoneal injections of saline solution, in the same volume used in the previous group, followed by injections of mepyramine (third group) or cimetidine (fourth group). Either drug was injected at the dose of 400 nmol. In these groups of animals, we investigated whether the blockade of central H_1 and H_2 receptors provokes any degree of discomfort leading to a general reduction in ingestive behavior that the animals could associate with the novel taste of saccharin. On the sixth day, at the same time that bottles had been available on the previous days (12:00 to 12:15 p.m.), saccharin-containing bottles were placed in all cages and the amount ingested recorded. No drugs were injected on this day.

2.8. Dessert test

To investigate whether the histamine antagonists used in the present study were able to modify water and salt intake through a nonspecific, general inhibition of the central nervous system or by a locomotor deficit, we investigated the effect of third ventricle injections of mepyramine and cimetidine on the intake of a 0.1% saccharin solution, a well-established example of hedonic behavior in rats [14]. In this experiment, after third ventricle cannulations, two different groups of animals, kept in the usual individual cages where the only fluid available was water, were transferred (for 2 h each day, for 7 consecutive days) to a different cage (the test cage) in which two bottles, one containing water and the other containing a 0.1% saccharin solution, were accessible. After this period of training, two different groups of fluid-deprived animals received third ventricle injections of mepyramine (400 nmol), cimetidine (400 nmol) or saline (controls) 30 min before being transferred to the test cage. The intake of water and saccharin was then recorded during the following 120 min.

2.9. Statistical analysis

A computer software package (SigmaStat for Windows, Jandel Scientific, San Rafael, CA) was used to carry out

two-way analysis of variance for repeated measures. The post hoc Student–Newman–Keuls test was used for comparison of each treatment with its corresponding time in the control groups (animals receiving third ventricle injections of isotonic saline solution). One-way ANOVA was used to analyze the data concerning the avoidance test and the dessert test. The data are presented as mean±S.E.M. The effects were considered significantly different when $p<0.05$.

3. Results

Fig. 1 (panel A) shows the effect of third ventricle injections of mepyramine at different doses on water intake in fluid-deprived rats. Analysis of variance indicated significant treatment and time main effects and significant treatment×time interaction [$F(4,38)=19.75$; $P<0.0001$; $F(5,20)=46.94$, $P<0.0001$; $F(20,190)=5.52$, $P<0.0001$, respectively]. As expected, there was a significant increase in water intake in saline-treated, fluid-deprived rats when compared to saline-treated normohydrated controls. Third ventricle injections of mepyramine were unable to modify the high water intake displayed by fluid-deprived rats at any of the doses used in this study.

Fig. 1 (panel B) depicts the effect of third ventricle injections of mepyramine at different doses on salt intake in fluid-deprived rats. Analysis of variance indicated significant treatment and time main effects and no significant treatment×time interaction [$F(4,38)=14.83$; $P<0.0001$; $F(5,20)=7.86$, $P<0.0001$; $F(20,190)=0.86$,

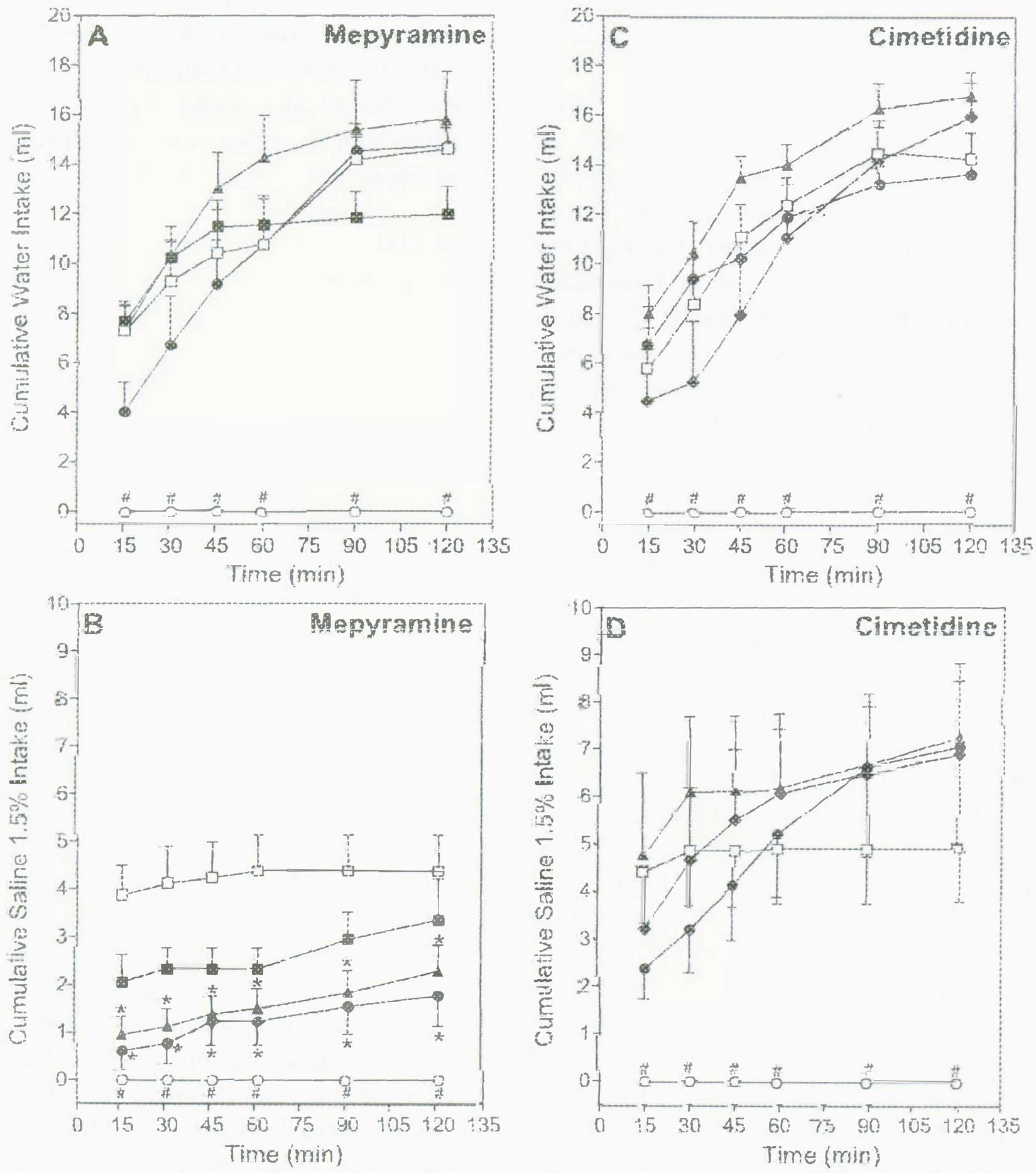

Fig. 1. Cumulative water and salt intake 15 min after third ventricle injections of mepyramine or cimetidine in water-deprived rats. Panels A (water intake) and B (salt intake): saline (□; $n=7$); mepyramine 50 nmol (■; $n=6$); mepyramine 100 nmol (▲; $n=12$); mepyramine 200 nmol (●; $n=9$). An additional group of normohydrated animals receiving third ventricle injections of saline is also shown (○; $n=9$). Panels C (water intake) and D (salt intake): saline (□; $n=7$); cimetidine 100 nmol (▲; $n=8$); cimetidine 200 nmol (●; $n=10$); cimetidine 400 nmol (◆; $n=7$) . An additional group of normohydrated animals receiving third ventricle injections of saline is also shown (○; $n=9$). Data are presented as mean± S.E.M. Asterisks indicate a statistically significant difference ($p<0.05$) when the different groups of water-deprived drug-treated animals are compared to water-deprived animals receiving saline. #indicates a statistically significant difference when the group of normohydrated rats is compared to all other groups. Each curve in the graph has been obtained from a naïve group of animals.

P=0.64, respectively]. Here, a cumulative intake of 1.5% hypertonic was observed for saline-treated, fluid-deprived rats that was significantly higher than the intake of saline-treated, normohydrated controls. Third ventricle injections of mepyramine at the lowest dose used (50 nmol) failed to alter salt intake in fluid-deprived animals. Fluid-deprived animals receiving mepyramine at the two other doses used (100 and 200 nmol) presented a significant reduction in salt intake, as compared to saline-treated, fluid-deprived, control animals.

Fig. 1 (panel C) shows the effect of third ventricle injections of cimetidine at different doses on water intake in fluid-deprived rats. Analysis of variance indicated significant treatment and time main effects and significant treatment×time interaction [$F(4,36)=35.86$; $P<0.0001$; $F(5,20)=63.94$, $P<0.0001$; $F(20,180)=5.60$, $P<0.0001$, respectively]. As expected, saline-treated, fluid-deprived animals that normally present an increase in plasma angiotensin II levels, showed a significant increase in water intake when compared to saline-treated, euhydrated controls. Third ventricle injections of cimetidine at any of the doses used were unable to modify the high water intake of fluid-deprived rats.

Fig. 1 (panel D) shows the effect of third ventricle injections of cimetidine at different doses on salt intake in fluid-deprived rats. Analysis of variance indicated significant treatment and time main effects and significant treatment×time interaction [$F(4,36)=4.79$; $P=0.003$; $F(5,20)=10.79$, $P<0.0001$; $F(20,180)=2.30$, $P=0.002$, respectively]. As expected, saline-treated, fluid-deprived animals exhibited a significant increase in salt intake as compared to saline-treated, normohydrated controls. Third ventricle injections of cimetidine at any of the doses used were unable to modify the high salt intake displayed by fluid-deprived rats.

Fig. 2 (panel A) illustrates the effect of third ventricle injections of mepyramine at different doses on water intake in sodium-depleted animals. Analysis of variance indicated no significant treatment and time main effects and no significant treatment×time interaction [$F(5,37)=0.00$; $P=1.0$; $F(5,25)=0.00$, $P=1.0$; $F(25,185)=0.00$, $P=1.0$, respectively]. As expected, sodium-depleted rats, which normally present a reduced water intake in order to avoid further dilution of the already low plasma sodium concentrations, exhibited no water intake.

Conversely, Fig. 2, panel B shows that furosemide-treated, sodium-depleted rats receiving third ventricle injections of mepyramine at different doses exhibited significantly higher salt intake as compared to normonatremic animals. Analysis of variance indicated significant treatment and time main effects and significant treatment×time interaction [$F(5,37)=16.21$; $P<0.0001$; $F(5,25)=40.56$, $P<0.0001$; $F(25,185)=3.18$, $P<0.0001$, respectively]. Here, third ventricle injections of mepyramine at the doses of 50 and 100 nmol did not modify salt intake in sodium-depleted rats. At the dose of 200 nmol, the central administration of mepyramine significantly inhibited salt intake at 15 and 30 min, whereas the same drug, when administered at the dose of 400 nmol, significantly blunted salt intake for the entire duration of the experiment.

Fig. 2 (panel C) illustrates the effect of third ventricle injections of cimetidine at different doses on water intake in sodium-depleted animals. Analysis of variance indicated no significant treatment effects and no significant treatment×time interaction but a significant time difference [$F(4,31)=0.99$, $P=0.43$; $F(20,155)=0.95$, $P=0.52$; $F(5,20)=4.28$, $P=0,001$, respectively]. As expected, sodium-depleted rats exhibited very little water intake.

Fig. 2 (panel D) illustrates the effect of third ventricle injections of cimetidine at different doses on salt intake in sodium-depleted animals. Analysis of variance indicated significant treatment and time main effects and significant treatment×time interaction [$F(4,31)=11.72$, $P<0.0001$; $F(5,20)=30.27$, $P<0.0001$ $F(20,155)=2.23$, $P=0.003$, respectively]. There was a statistically significant increase in salt intake in all groups of sodium-depleted rats compared to furosemide-free, normonatremic controls. Third ventricle injections of cimetidine failed to modify the high salt intake of sodium-depleted rats at any of the doses used in this study.

Fig. 3 (Panel A) shows the effect of third ventricle injections of mepyramine at different doses on water intake induced by third ventricle injections of angiotensin II (10 ng). Analysis of variance indicated significant treatment and time main effects and significant treatment×time interaction [$F(4,35)=27.43$; $P<0.0001$; $F(5,20)=12.62$, $P<0.0001$; $F(20,175)=2.50$, $P=0.0007$, respectively]. As expected, water intake was significantly increased in animals receiving third ventricle injections of angiotensin II pretreated with third ventricle injections of saline (saline+angiotensin II) compared to control animals receiving two consecutive third ventricle injections of saline (saline+saline). At the lowest dose (100 nmol), third ventricle injections of mepyramine were unable to modify angiotensin II-induced water intake. At the other doses (200 and 400 nmol), the central administration of mepyramine significantly impaired water intake induced by third ventricle injections of angiotensin II.

Fig. 3 (Panel B) shows the effect of third ventricle injections of mepyramine at different doses on salt intake induced by third ventricle injections of angiotensin II (10 ng). Analysis of variance indicated significant treatment and time main effects and significant treatment×time interaction [$F(4,35)=14.42$; $P<0.0001$; $F(5,20)=17.59$, $P<0.0001$; $F(20,175)=7.23$, $P<0.0001$, respectively]. Salt intake of animals that received third ventricle injections of angiotensin II but were pretreated with third ventricle injections of saline (saline+angiotensin II) showed a significant increase when compared with the control group of animals receiving two consecutive injections of saline (saline+saline). At all the doses used (100, 200 and 400 nmol), third ventricle

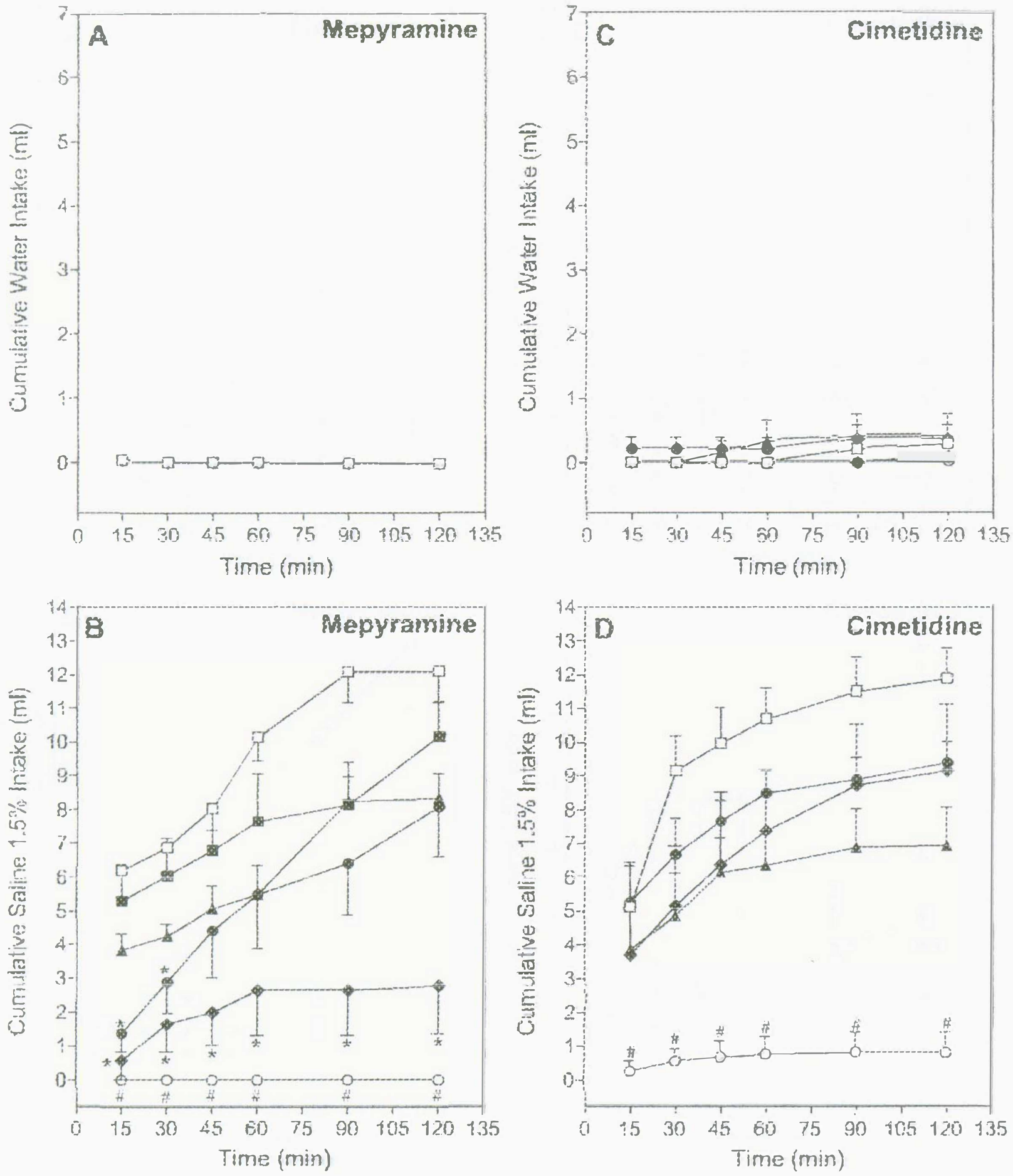

Fig. 2. Cumulative water and salt intake 15 min after third ventricle injections of mepyramine or cimetidine in sodium-depleted rats. Panels A (water intake) and B (salt intake): saline (□; n=7); mepyramine 50 nmol (■; n=10); mepyramine 100 nmol (▲; n=6); mepyramine 200 nmol (●; n=6); mepyramine 400 nmol (♦; n=7). An additional group of animals not submitted to sodium depletion and receiving third ventricle injections of saline is also shown (○; n=7). Panels C (water intake) and D (salt intake): saline (□; n=7); cimetidine 100 nmol (▲; n=6); cimetidine 200 nmol (●; n=9); cimetidine 400 nmol (♦; n=7). An additional group of animals not submitted to sodium depletion and receiving third ventricle injections of saline is also shown (○; n=7). Data are presented as mean±S.E.M. Asterisks indicate a statistically significant difference (p<0.05) when the different groups of sodium-depleted, drug-treated animals are compared to sodium-depleted animals receiving saline. #indicates a statistically significant difference when the group of rats not submitted to sodium depletion is compared to all other groups. Each curve in the graph has been obtained from a naïve group of animals.

injections of mepyramine reduced angiotensin II-induced salt intake throughout the entire duration of the experiment.

Fig. 3 (Panel C) shows the effect of third ventricle injections of cimetidine at different doses on water intake induced by third ventricle injections of angiotensin II (10 ng). Analysis of variance indicated significant treatment and time main effects and significant treatment×time interaction [F(4,43)=10.44; P<0.0001; F(5,20)=15.82, P<0.0001; F(20,215)=3.57, P<0.0001, respectively]. As expected, there was a significant increase in water intake in animals receiving third ventricle injections of angiotensin II pretreated with third ventricle injections of saline (saline+angiotensin II) as compared to control animals receiving two consecutive, third ventricle injections of saline (saline+saline). At all doses used in this study (100, 200 and 400 nmol), the central administration of cimetidine significantly impaired water intake induced by third ventricle injections of angiotensin II.

Fig. 3 (Panel D) shows the effect of third ventricle injections of cimetidine at different doses on salt intake induced by third ventricle injections of angiotensin II (10 ng). Analysis of variance indicated significant treatment and time main effects and significant treatment×time interaction [F(4,43)=10.43; P<0.0001; F(5,20)=12.87, P<0.0001; F(20,215)=2.12, P=0.005, respectively]. There was a significant increase in salt intake in animals receiving third ventricle injections of angiotensin II but pretreated with third ventricle injections of saline (saline+angiotensin II)

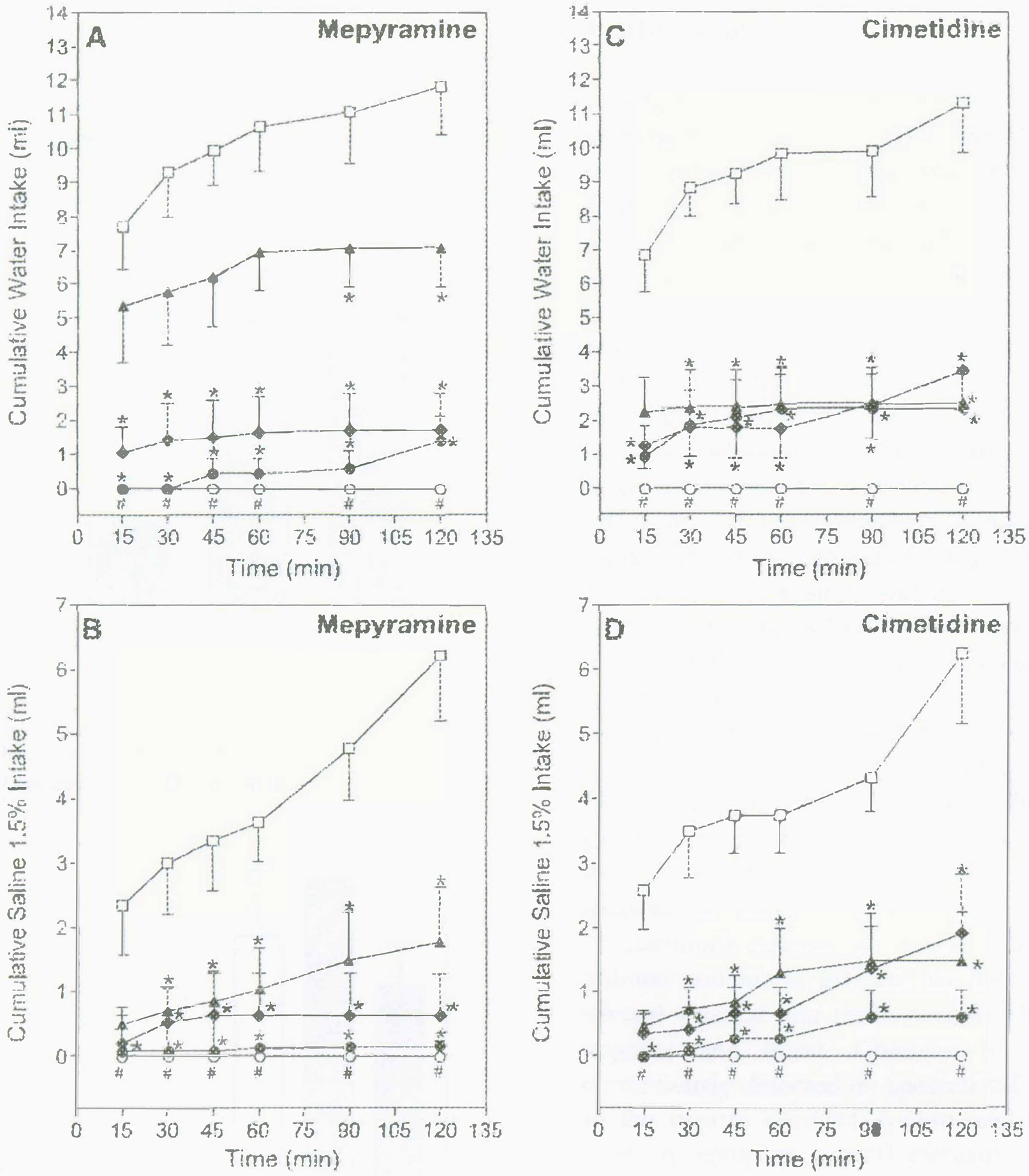

Fig. 3. Cumulative water and salt intake 15 min after third ventricle injections of mepyramine or cimetidine in rats receiving third ventricle injections of angiotensin II (10 ng). Panels A (water intake) and B (salt intake): Saline+angiotensin II (□; n=7); mepyramine 100 nmol+angiotensin II (▲; n=7); mepyramine 200 nmol+angiotensin II (●; n=10); mepyramine 400 nmol+angiotensin II (◆; n=10). An additional group of animals not submitted to central angiotensinergic stimulation (saline+saline) is also shown (○; n=9). Panels C (water intake) and D (salt intake): Saline+angiotensin II (□; n=6); cimetidine 100 nmol+angiotensin II (▲; n=12); cimetidine 200 nmol+angiotensin II (●; n=9); cimetidine 400 nmol+angiotensin II (◆; n=12). An additional group of animals not submitted to central angiotensinergic stimulation (saline+saline) is also shown (○; n=9). Data are presented as mean±S.E.M. Asterisks indicate a statistically significant difference (p<0.05) when the different groups of animals receiving third ventricle injections of angiotensin II but pretreated with mepyramine or cimetidine are compared to animals receiving third ventricle injections of angiotensin II but pretreated with saline. #indicates a statistically significant difference when the group of rats not submitted to central angiotensinergic stimulation is compared to all other groups. Each curve in the graph has been obtained from a naïve group of animals.

when compared with the control group of animals receiving two consecutive injections of saline (saline+saline). At all doses used (100, 200 and 400 nmol) third ventricle injections of cimetidine reduced angiotensin II-induced salt intake throughout the entire duration of the experiment.

Fig. 4 (panel A) depicts the result of the avoidance test performed to verify whether any of the histamine antagonists were able to induce "illness-like" side effects. Analysis of variance indicated a significant treatment difference between the groups [$F(5,40)=8.07$; $P<0.0001$]. As expected, animals establishing a previous association between lithium chloride and saccharin had a significant reduction in saccharin intake on the following day, as compared to saline-treated controls. In contrast, the previous association of each of the histamine antagonists (mepyramine and cimetidine) with saccharin failed to produce any significant reduction in saccharin intake the next day, which suggests that it is unlikely that illness-like effects could explain the results observed here after the injection of these compounds into the third ventricle. Fig. 4, Panel B, shows the results of the dessert test. Here, saline-treated control animals drank more saccharin than water, indicating the hedonic behavior represented by the preferential intake of a "tasty" solution. Third ventricle injections of either mepyramine or cimetidine failed to alter this hedonic preference. Indeed, animals receiving these histamine antagonists drank

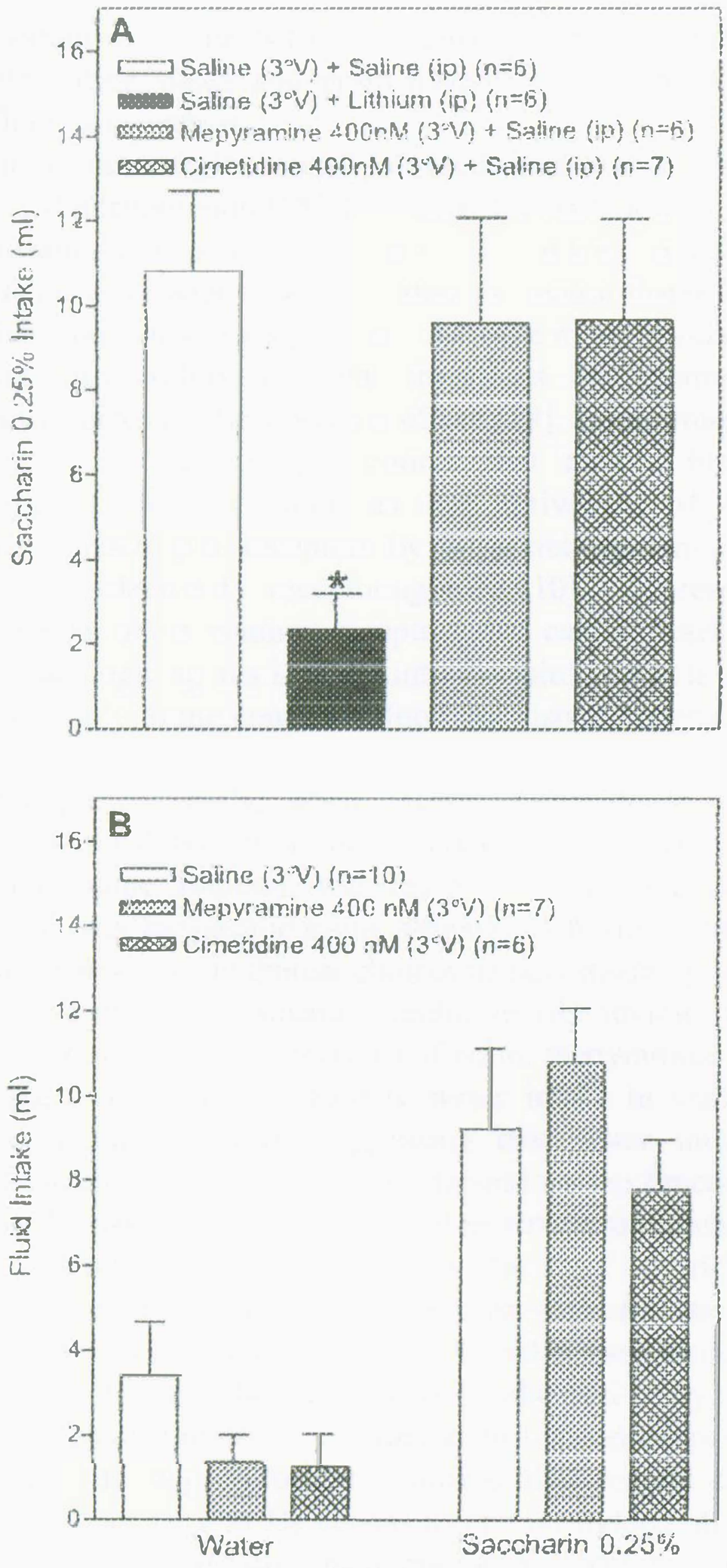

Fig. 4. Panel A (Avoidance test): saccharin solution (0.25%) consumption (ml/100 g body weight) over 15 min at a second offering in animals receiving third ventricle injections of mepyramine (400 nmol), cimetidine (400 nmol) or saline (controls). The sequence of injections used during the first offering of saccharin and the number of animals used are indicated in the figure. The first injection was into the third ventricle and the second via intraperitoneal route. The asterisk indicates a statistically significant difference ($p<0.001$) between that particular group and controls (saline+saline). The number of animals used in each experiment is indicated in the figure. Panel B (Dessert test): saccharin (0.1%) and water intakes (ml/100 g body weight) during 2 h in the test cage in rats receiving third ventricle injections of isotonic saline solution (controls) mepyramine (400 nmol) and cimetidine (400 nmol). The treatment received by each group and the number of animals used is indicated in the graph. There was no significant difference in the ingestion of saccharin and water between groups treated with saline and the histamine antagonists tested. Data are expressed as mean±S.E.M.

the same amount of saccharin [$F(2,20)=0.67$; $P=0.52$] and presented the same water intake [$F(2,20)=1.38$; $P=0.28$] as saline-treated controls.

4. Discussion

The present data show that third ventricle injections of mepyramine, a selective H_1 receptor antagonist, inhibit salt intake in two distinct situations: sodium depletion and water deprivation. Under these conditions, water intake is unaffected by the central administration of mepyramine. Third ventricle injections of cimetidine, an H_2 receptor antagonist, are unable to modify salt intake either in sodium-depleted or water-deprived rats. Third ventricle injections of both mepyramine and cimetidine significantly reduce angiotensin II-induced water and salt intake. The inhibition of the ingestive behaviors after the central administration of either mepyramine or cimetidine seems to be specifically due to an effect on the central pathways regulating water and salt intake and does not reflect a general impairment of the central nervous system. Indeed, we have demonstrated the absence of "illness-like" effects after the central administration of these histaminergic antagonists, and a "dessert" test revealed that the hedonic ingestive behavior of a "tasty" saccharin solution is unaffected by third ventricle injections of either mepyramine or cimetidine. However, we cannot exclude the possibility that an "illness-like" effect could explain the inhibitory effect of the histamine antagonists used here in the group of animals in which salt intake was induced by central angiotensinergic stimulation.

Mammals constantly regulate plasma osmolarity, blood volume and blood pressure by the integrated operation of several visceral and behavioral mechanisms, including salt appetite and thirst. Changes in these parameters are continuously detected by specialized structures located both in the central nervous system and in strategic peripheral sites. A complex central circuitry involving many brain areas and neurotransmitters receives a nonstop flow of information that originates in these sensors and organizes corrective responses that include stimulation or inhibition of sodium appetite and thirst (for review, see Ref. [1]).

Circumventricular structures have a major participation in the control of salt intake. Central areas such as the subfornical organ and the organum vasculosum laminae terminalis are activated by sodium depletion [15] and lesions of these structures induce a significant decrease in sodium appetite in sodium-depleted animals [16]. Furthermore, changes in sodium concentration in the cerebrospinal fluid alter salt intake in rats after sodium depletion [17].

Several structures surrounding the cerebral ventricles that participate in the control of water and salt intake, such as the paraventricular, the supraoptic and the suprachiasmatic nuclei, as well as the ventromedial hypothalamic nuclei and the nucleus of the solitary tract, receive massive histaminergic innervation, and a high density of both H_1 and H_2 receptors is found in these brain regions (for review, see Ref. [2]). This provides the necessary anatomical background to support the present findings. In addition, by having the ability to detect variations in plasma angiotensin II levels, the circumventricular structures may

be important as a link between changes in the peripheral hydroelectrolyte status and brain histaminergic control of body fluid homeostasis.

Brain histaminergic control of food intake is a well-established phenomenon [18]. However, the participation of brain histaminergic pathways in the control of water and salt intake is far less understood. Nonetheless, central histamine administration leads to a significant increase in water intake [5], and intracerebroventricular injections of histamine antagonists produce the opposite effect [19]. Furthermore, drinking is elicited by procedures that reduce brain histaminergic activity, such as the activation of H_3 inhibitory, presynaptic receptors by intracerebroventricular injections of selective H_3 receptor agonists [10]. The present studies reveal that histamine receptors that can be reached by pharmacologic agents injected into the third ventricle are related not only to the control of food but also to water and salt intake.

In the present study, when access to the fluids was allowed, water-deprived animals drank both water and hypertonic saline solution, whereas sodium-depleted animals drank only hypertonic saline although both fluids were available. These are the typical choices usually made by rats submitted to these experimental conditions (for review, see Ref. [1]). Third ventricle injections of either mepyramine or cimetidine were unable to modify water intake in water-deprived animals, thereby suggesting that water intake under these circumstances does not depend on the function of central H_1 and H_2 receptors located in structures reached by anti-histamine agents injected into the third ventricle. Also, the negligible water intake of sodium-depleted rats is unaffected by third ventricle injections of either mepyramine or cimetidine. On the other hand, water intake elicited by the pharmacological stimulation of brain angiotensinergic pathways seems to depend on mechanisms that require the function of both H_1 and H_2 central receptors. Indeed, third ventricle injections of either mepyramine or cimetidine are able to block drinking induced by the central administration of angiotensin II.

The participation of brain H_1 receptors in the regulation of water intake is a rather controversial matter. The lack of participation of brain H_1 receptors in the regulation of water intake found in the present study is in agreement with other data available in the literature, showing that intracerebroventricular infusion of the selective H_1 antagonist mepyramine was unable to modify water intake associated with eating in rats [10]. However, another group of researchers demonstrated that brain H_1 receptors may be involved in the mechanisms leading to water intake induced by intragastric salt load [16]. Nevertheless, salt load-induced thirst is a rather different experimental model, and the authors of that study did not use the same H_1 receptor antagonist used here.

Studies concerning the role of brain H_2 receptors in the control of water intake are also conflicting. Some results indicate that drinking induced by intragastric salt load does not rely on an H_2-receptor dependent mechanism [20,21] while another study shows that prandial drinking during the period of darkness is regulated by brain H_2 receptors [10].

We could not find any other data in the literature analyzing the participation of brain H_1 and H_2 receptors in the dipsogenic response stimulated by the activation of central angiotensinergic pathways. Direct angiotensin/histamine interplay and co-localization of histamine and angiotensin II receptors in the central nervous system have not been documented. Nonetheless, based on the present results, we raise the hypothesis that the dipsogenic effect of brain angiotensinergic stimulation depends on some mechanism related to the function of central H_1 and H_2 receptors. A real possibility is that the blockade of central H_1 and H_2 histaminergic receptors somehow disrupts the functional linking that normally activates the higher integrative areas that promote the motor activities necessary for water and salt intake after brain angiotensinergic stimulation.

Salt intake is elicited by several stimuli (hypovolemia, sodium depletion and hypotension) and some conditions that induce thirst also trigger sodium appetite (for review, see Refs. [22,23]). The regulation of salt intake by the brain is accomplished by means of a complex interplay between the stimulatory drive represented by the cooperative interaction between mineralocorticoids and angiotensin II [24,25] and the negative input represented by the oxytocinergic [26–28] and serotoninergic [12,29,30] components. The present study suggests that the central histaminergic component may also participate in the control of salt intake in rats.

We used two different stimuli, sodium depletion and water deprivation, that led to an increase in salt intake. Sodium depletion may trigger sodium appetite-inducing mechanisms through a reduction in extracellular volume and osmolality, whereas water deprivation increases salt intake mainly by reducing extracellular volume without producing significant changes in osmolality [31–33]. As a result, sodium-depleted animals present a higher increase in salt intake as compared to the water-deprived group, as shown in the present study. In the present study, salt appetite was evaluated by measuring the cumulative intake of a 1.5% hypertonic saline solution. The ingestion of this type of solution is strongly avoided by normonatremic rats. Only sodium-depleted animals ingest hypertonic saline in concentrations above 1% and this reflects sodium appetite and not a hedonic ingestion associated with a "tasty", salty solution [1,9,23].

It is important to note that the pharmacological agents used in the present study are rather selective at the doses at which they were administered. Indeed, mepyramine possesses a high affinity for H_1 receptors (pK_d=9.4) and may interact with cholinergic receptors only when used at micromolar concentrations [34,35], a much higher dose than the nanomolar doses used here. Also, cimetidine may exhibit agonistic properties at $GABA_A$ receptors only when used at doses that are significantly higher than the doses we have used in the present study [36].

The pharmacological blockade of brain H_1 receptors significantly inhibited salt intake in all salt intake-inducing experimental protocols used here: sodium depletion, water deprivation and central angiotensinergic stimulation. On the other hand, the blockade of central H_2 receptors was able to decrease angiotensin II-induced salt intake, but failed to modify the rise in sodium appetite elicited by water deprivation and sodium depletion. Therefore, it is reasonable to suggest that brain H_1 receptors located in central structures reached by pharmacological agents injected into the third ventricle participate in the salt intake-triggering mechanisms activated during sodium depletion, water deprivation and central angiotensin II stimulation, while brain H_2 receptors seem to be associated uniquely with angiotensin II-induced sodium appetite. However, it must be observed that sodium depletion and water deprivation are physiological stimuli that activate a loop of events that are probably functionally located before central angiotensin pathways and may even depend on central angiotensinergic activation to induce salt intake. On the other hand, the pharmacological stimulation of central angiotensinercic circuitries induces salt intake by the activation of systems that are functionally positioned ahead. This may explain why the different histamine receptors studied present distinct effects on salt intake, depending on the nature of the salt intake-inducing stimulus used.

Both water and salt intake may be significantly reduced during the course of a hypertensive response. However, a hypertension-induced decrease in water and salt intake can be excluded as the cause of the inhibitory ingestive behaviors observed in the present study since intracerebroventricular injections of histamine increase blood pressure and this effect is blocked by the central administration of both H_1 and H_2 receptor antagonists [4,25,37–39].

The histamine antagonists used have been administered in nanomolar concentrations into the third ventricle. It is obviously impossible to exclude the possibility that a small portion of the total amount injected may have passed to the periphery. However, as histamine is not a neurotransmitter outside the brain, it is difficult to conceive how its putative peripheral actions could explain the specific inhibition of an ingestive behavior such as salt intake.

Brain histamine constitutes a neurochemical component involved in the control of several visceral and behavioral responses. Central histaminergic pathways participate in the regulation of the sleep/arousal cycle, food intake, hypophysial hormone secretion, temperature regulation and blood pressure. On the other hand, H_1 and H_2 receptor antagonists that easily cross the blood–brain barrier are used as therapeutic agents in a myriad of clinical conditions. The widespread use of H_1 receptor antagonists as anti-allergic agents and H_2-receptor antagonists in the treatment of many gastric disorders frequently targets the brain histaminergic system and studies revealing new aspects of brain histaminergic component are of physiological and clinical importance.

In conclusion, the present results indicate that central H_1 receptors are functionally important in the mechanisms that trigger salt intake in sodium-depleted and water-deprived rats, as well as in rats whose sodium appetite was induced by central angiotensinergic stimulation. We have also demonstrated that central H_1 receptors participate in the dipsogenic response elicited by brain angiotensin II stimulation. On the other hand, central H_2 receptors seem to play a pivotal role in the mechanisms that activate both water and salt intake in rats after pharmacological stimulation of central angiotensinergic pathways.

Acknowledgments

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ARTIGO 2

"Involvement of central H_1 and H_2 receptors in water intake induced by hyperosmolarity, hypovolemia and central cholinergic stimulation"

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Involvement of central H_1 and H_2 receptors in water intake induced by hyperosmolarity, hypovolemia and central cholinergic stimulation

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Abstract

In the present study we investigated the participation of central H_1 and H_2 histaminergic receptors in water intake induced by hyperosmolarity (evoked by intragastric salt load), by hypovolemia (promoted by the subcutaneous administration of polyethyleneglycol) and by the pharmacological stimulation of central cholinergic pathways by the muscarinic agonist carbachol in male Wistar rats. The data presented here show that the pharmacological blockade of central H_1 histaminergic receptors by third ventricle injections of mepyramine significantly decreased water intake induced by hyperosmolarity, hypovolemia and by the intracerebroventricular injections of carbachol. On the other hand, the pharmacological blockade of central H_2 histaminergic receptors by third ventricle injections of cimetidine significantly reduced water intake in hypovolemic and hyperosmotic animals, but failed to alter water intake induced by central cholinergic stimulation by carbachol. We conclude that H_1 and H_2 brain histaminergic receptors are involved in inducing thirst during hyperosmolarity and hypovolemia and that H_1 histaminergic receptors located post-synaptically in relation to cholinergic pathways seem to be important in triggering drinking following central pharmacological cholinergic stimulation.

Endocrine and autonomic regulation; Osmotic and thermal regulation
Keywords: Histamine; Water intake; Cimetidine; Mepyramine; Hypovolemia; Hyperosmolarity; Carbachol

1. Introduction

Central histaminergic pathways are involved in the control of numerous visceral and behavioral responses. Indeed, brain histamine participates in the control of body temperature, modulates pain perception and the sleep/wake cycle, affects the synthesis and release of hypothalamic products and pituitary hormones and strongly influences food intake [1,2].

Less attention has been given to the role of brain histaminergic circuitries in the control of fluid balance. Central injections of histamine have been shown to induce water intake [3,4] and brain histamine has also been reported to influence urine output by modulating vasopressin release through its action on the paraventricular nucleus [5,6].

We have been investigating the role of brain histaminergic pathways and histaminergic receptor subtypes in the control of water and salt intake, and recently reported that the pharmacological blockade of central H_1 and H_2 histaminergic receptors, induced by third ventricle injections of histamine antagonists, inhibits water and salt intake induced by central angiotensinergic stimulation, while this same pharmacological procedure fails to modify water intake induced by water deprivation [7]. In another study, we showed that the pharmacological blockade of H_1 and H_2 histaminergic receptors located within the ventromedial hypothalamus (VMH) significantly decreases water intake during the overnight period. In this same study, we also demonstrated that the pharmacological blockade of central H_1 receptors attenuates water intake elicited by hyperosmolarity, while the blockade of central H_2 receptors has no effect on this condition. Additionally, we showed that the pharmacological blockade of central H_1 and H_2 receptors impairs water intake produced by water deprivation [8].

In the present study, we investigated the role of central H_1 and H_2 receptors in the control of water intake elicited by two different

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thirst-inducing physiological stimuli: hyperosmolarity (induced by intragastric salt load) and hypovolemia (produced by subcutaneous polyethyleneglycol administration). Additionally, the existence of a well-documented histamine/cholinergic interplay in the central nervous system [9–12], in which histamine seems to modulate cholinergic transmission, prompted us to investigate the participation of histaminergic receptors in water intake induced by central cholinergic stimulation, a classical thirst-inducing pharmacological approach.

2. Material and methods

2.1. Animals

In the present study, we used male Wistar rats weighing 240± 20 g. They were housed in individual cages and kept under controlled light (lights on from 7 A.M. to 7 P.M.) and temperature (22–24 °C) conditions. In all experimental protocols central injections of saline (controls) and each individual dose of the histaminergic agents were tested in a naïve group of animals. All experiments were conducted between 7 A.M. and 12 P.M. The experimental protocols were conducted according to the rules suggested by the National Institutes of Health (USA) and were approved by a local committee that analyzes ethical aspects of research with laboratory animals.

2.2. Surgical procedures

The cannulation of the third ventricle was performed under pentobarbital anesthesia (50 mg/kg i.p.) 5 days before the experimental sessions. A stereotaxic apparatus (David Kopf Instruments, Tujunga, CA) was used to implant a 15 mm, 22-gauge, stainless steel cannula. The following coordinates were used: anteroposterior=0.5 mm behind the bregma; lateral=0.0 mm; vertical 8.5 mm below the skull. The animals were placed in the stereotaxic apparatus with the head inclined 2.0 mm upwards to avoid lesions to the midline structures related to body fluid and electrolyte control. A microphotograph showing third ventricle cannulation using these procedures does not produce any damage to the brain structures involved in water and salt intake regulation has been previously published by our group [13]. The cannulas were cemented to the skull bone with dental acrylic and an obturator (28-gauge) was provided to avoid obstruction. After sacrifice by CO_2 inhalation, we verified whether the tip of the cannula was correctly positioned by injecting Blue Evans dye (2.0 μl) into the third ventricle. Only data from animals in which the cannulas were strictly inside the third ventricle were analyzed. In order to minimize the stress of the experimental maneuvers, the animals were handled every day.

2.3. Drugs and microinjections

The following drugs were used: mepyramine maleate (*N*-(4-methoxy-phenylmethyl-*N'*,*N'*-dimethyl-*N*-(2-pyridinyl)-1,2-ethanediamine), an H_1 histaminergic receptor antagonist, cimetidine, an H_2 histaminergic receptor antagonist, and polyethylene glycol (m.w. 15.000–20.000; PEG) were purchased from Sigma Co., St. Louis, MO. Central injections were performed using a Hamilton microsyringe connected to a Myzzy-Slide-Pak needle through polyethylene tubing. All drugs were dissolved in isotonic saline solution. The final volume injected was 2 μl over a period of 90 s.

The pharmacological agents used in the present study are selective at the doses at which they were administered. Mepyramine, which has a high affinity for H_1 receptors (pK_d=9.4) may interact with cholinergic receptors at micromolar concentrations [14,15], however, in the present experiment, the compound was used at nanomolar doses. Cimetidine exhibits agonistic properties in $GABA_A$ receptors only when used at doses significantly higher than those used in the present study [16]. The doses of mepyramine used here were based on a previous work carried out by another group [17] in which intracerebroventricular infusions of this compound were used to study the role of central H_1 receptors on food and water intake. In that paper, the authors used a fixed dose of 800 nmol of mepyramine. Another study from a different group states that cimetidine, when injected intracerebroventricularly at similar doses, induces convulsion [18]. Therefore, in order to use both drugs in equimolar amounts, we decided to test mepyramine and cimetidine at smaller doses (50, 100, 200 and 400 nmol) than those used by the group of Lecklin [17].

2.4. Intragastric salt load

To study the role of central H_1 and H_2 receptors in water intake induced by hyperosmolarity, different groups of animals submitted to an acute intragastric salt load received third ventricle injections of H_1 or H_2 receptor antagonists (mepyramine and cimetidine, respectively), and had their water intake monitored during 120 min. Intragastric salt load was achieved by administering 1 ml/100 g of a hypertonic saline solution (1.5 M) via orogastric tubing. In this case, the animals were fasted for 14 h (from 6 P.M. to 8 A.M.) the night preceding the experiments, in order to obtain a uniform electrolyte absorption in all individuals. They received an intragastric salt load 10 min after third ventricle injections of mepyramine or cimetidine at different doses. These groups of animals were compared to an additional group receiving intragastric administration of isotonic saline solution followed by third ventricle injections of isotonic saline solution.

2.5. Polyethylene glycol administration

A 30% PEG solution was prepared in 0.15 M sodium chloride by heating the mixture to approximately 50 °C while stirring constantly. This solution was administered subcutaneously (2 ml/100 g) 4 h before the third ventricle injections of the histaminergic antagonists (mepyramine and cimetidine) or the isotonic saline solution (controls). Graduated bottles were removed from the cages immediately before PEG administration and reintroduced 30 min after the icv injections. Cumulative water intake was measured over the following 120 min. These groups of animals were also compared to an additional group receiving subcutaneous injections of isotonic saline solution in the same volume as the PEG solution followed by third ventricle injections of saline. The dose of PEG used in the present study is identical to that successfully used in previous experiments carried out at this laboratory [19].

2.6. Central cholinergic stimulation

To induce pharmacological stimulation of central cholinergic pathways, animals received third ventricle injections of carbachol at the dose of 2 μg, a dose that has been previously used in other studies [20–22]. Control animals received third ventricle injections of isotonic saline solution. To study the participation of central H_1 and H_2 receptors in water after central cholinergic stimulation, different groups of rats received third ventricle injections of different doses of mepyramine, a selective H_1 antagonist, or the H_2 receptor antagonist cimetidine 15 min before receiving carbachol (2 μg). As in the previous experimental sets, fluid intake was first measured 15 min later and thereafter for the following 120 min. All groups were compared to a group of rats receiving central administration of saline instead of carbachol.

2.7. Hematocrit measurement

Two separate groups of animals, one receiving subcutaneous injections of polyethyleneglycol and the other treated subcutaneously with isotonic saline solution, were used to estimate the efficacy of PEG in inducing hypovolemia. In these groups blood samples were collected from the tip of the tail into microhematocrit tubes 4 h and 30 min after these subcutaneous injections. The blood samples were centrifuged and read immediately following collection.

2.8. Open field test

To test whether the third ventricle injections of either mepyramine or cimetidine were able to induce any significant reduction in locomotor activity that could explain the inhibition of water intake observed here, we submitted different groups of rats to an open field test 30 min after receiving third ventricle injections of either one of the two compounds or saline.

The apparatus consisted of a circular wooden box (60 cm in diameter and 60 cm high) with an open top. The floor was divided into eight areas of equal size with a circle at the center (42.43 cm). Hand-operated counters and stopwatches were used to score locomotion (measured as the number of floor units entered by the animal with all four paws) for 10 min.

The behavioral experiments took place in a sound-attenuated, temperature-controlled (24±1 °C) room between 7 A.M. and 12 P.M. Two 40 W fluorescent lights placed 1.50 m away from the apparatus illuminated the environment. A white-noise generator provided constant background noise and the apparatus was cleaned with 70% ethanol and dried before each session to minimize olfactory cues.

2.9. Statistical analysis

A computer software package (SigmaStat for Windows, Jandel Scientific, San Rafael-CA) was used to carry out the one-way analysis of variance for each time point. The post-hoc Student–Newman–Keuls test was used for comparison among the distinct treatments. The data are presented as the mean± SEM. The effects were considered significantly different when $p<0.05$.

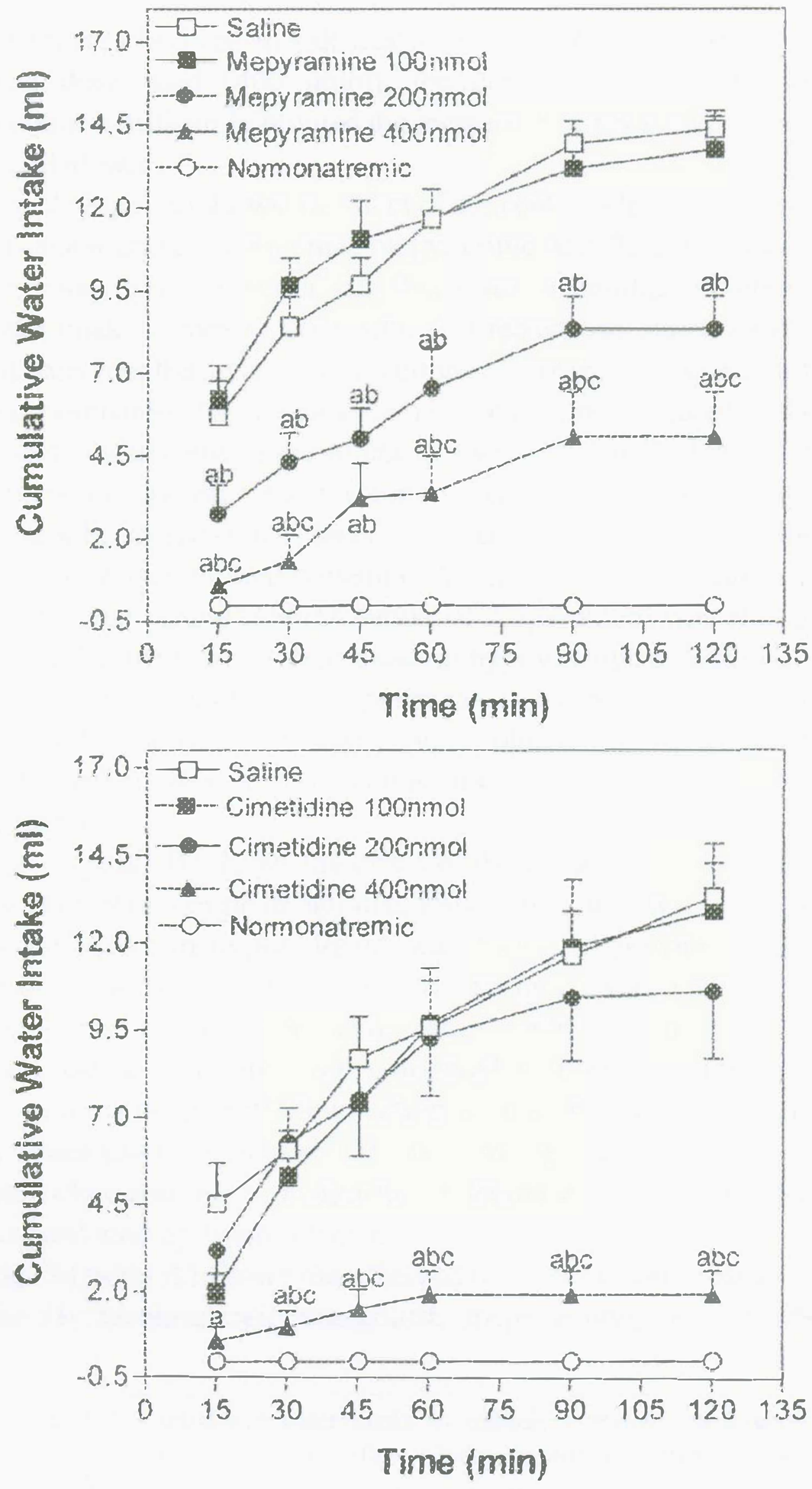

Fig. 1. Panel A: Cumulative water intake in animals receiving an acute intragastric salt load (hyperosmotic animals) or intragastric isotonic saline treated with third ventricle injections of mepyramine. (○, $n=8$) no salt load + icv saline; (□, $n=7$) hyperosmotic + icv saline; (■, $n=8$) salt load + icv mepyramine 100 nmol; (●, $n=9$) hyperosmotic + icv mepyramine 200 nmol; (▲, $n=8$) hyperosmotic + icv mepyramine 400 nmol; Panel B: Cumulative water intake in animals receiving an acute intragastric salt load or intragastric isotonic saline treated with third ventricle injections of cimetidine. (○, $n=7$) no salt load + icv saline; (□, $n=11$) hyperosmotic + icv saline; (■, $n=8$) hyperosmotic + icv cimetidine 100 nmol; (●, $n=6$) hyperosmotic + icv cimetidine 200 nmol; (▲, $n=11$) hyperosmotic + icv cimetidine 400 nmol. Data are presented as the mean±SEM. "a" indicates a statistically significant difference ($p<0.05$) when salt-loaded animals receiving third ventricle injections of mepyramine or cimetidine are compared to salt-loaded animals receiving third ventricle injections of saline. "b" indicates a statistically significant difference ($p<0.05$) when salt-loaded animals receiving third ventricle injections of mepyramine or cimetidine at the doses of 200 and 400 nmol are compared to salt-loaded animals receiving mepyramine or cimetidine at the dose 100 nmol. "c" indicates a statistically significant difference ($p<0.05$) when animals receiving third ventricle injections of mepyramine or cimetidine at the dose of 400 nmol are compared to animals receiving cimetidine at the dose 200 nmol. Each curve in the graph has been obtained from a naïve group of animals.

3. Results

Fig. 1 (panel A) shows the effect of the central administration of the H_1 histaminergic antagonist mepyramine, at different doses, on water intake in rats submitted to an intragastric salt load. As expected, a significant increase in water intake was observed in salt-loaded animals (hyperosmolar intragastric saline) receiving third ventricle injections of saline solution when compared to the group of animals not submitted to salt load (intragastric isotonic saline) also receiving third ventricle injections of isotonic saline solution. At the lowest dose used (100 nmol), the central administration of mepyramine failed to modify the high water intake observed in the group of salt-loaded animals. At the other doses used (200 and 400 nmol), mepyramine significantly inhibited the dipsogenic response induced by intragastric salt load.

Fig. 1 (panel B) shows the effect of the central administration of the H_2 histaminergic antagonist, cimetidine, at different doses, on water intake in rats submitted to an intragastric salt load. As in the previous experiment, intragastric salt load produced a significant increase in water intake. At doses of 100 and 200 nmol, third ventricle injections of cimetidine had no effect on the increase in water intake observed in salt-loaded animals. Conversely, at the highest dose used (400 nmol), the central administration of cimetidine significantly blunted the increase in water intake seen in salt-loaded rats.

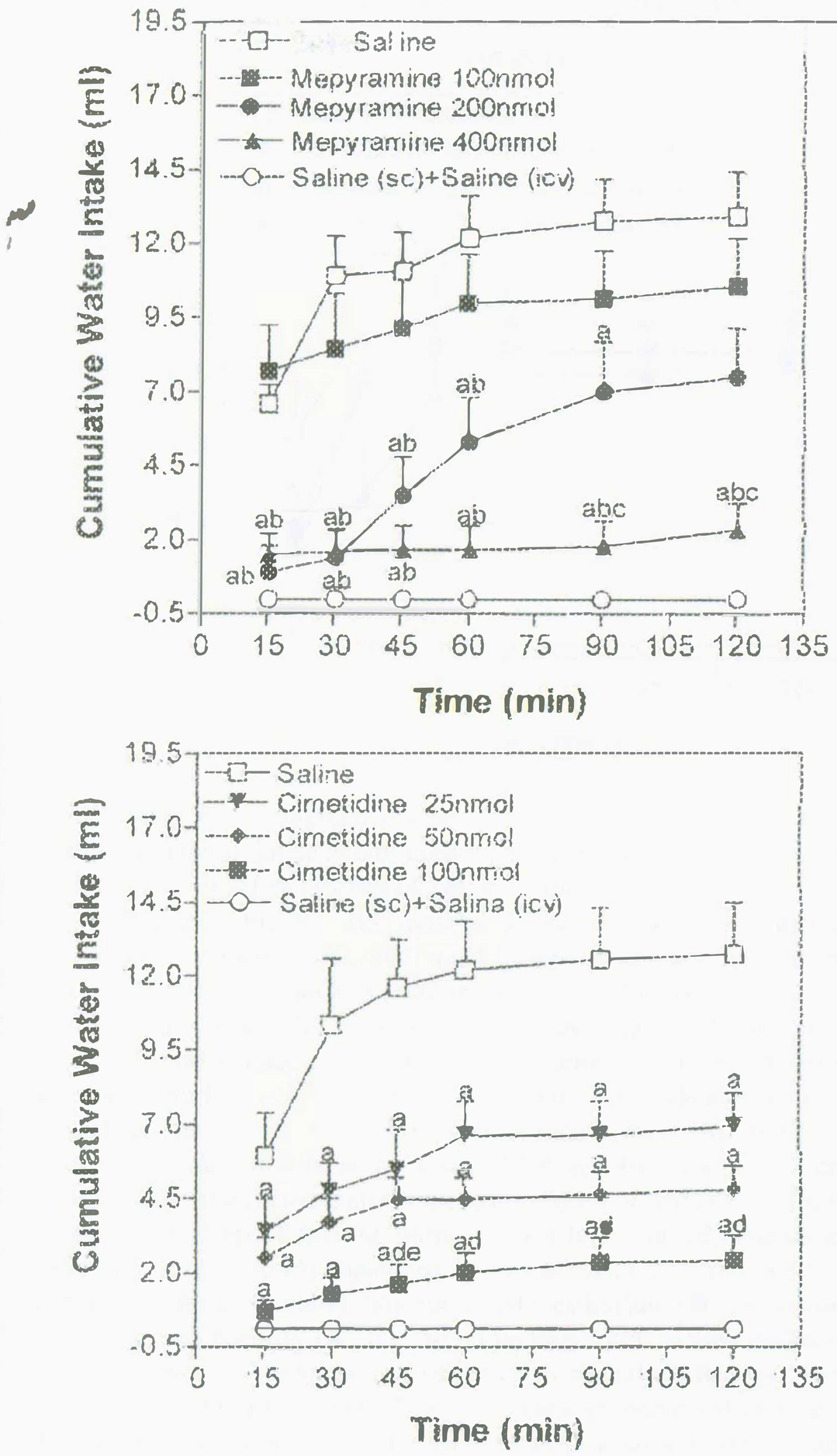

Fig. 2. Panel A: Cumulative water intake in animals receiving subcutaneous administration of PEG or isotonic saline solution treated with third ventricle injections of mepyramine. (○, $n=7$) sc saline solution + icv saline; (□, $n=7$) sc PEG + icv saline; (■, $n=9$) sc PEG + icv mepyramine 100 nmol; (●, $n=6$) sc PEG + icv mepyramine 200 nmol; (▲, $n=9$) sc PEG + icv mepyramine 400 nmol; Data are presented as the mean ± SEM. "a" indicates a statistically significant difference ($p<0.05$) when animals receiving third ventricle injections of mepyramine are compared to animals receiving third ventricle injections of saline. "b" indicates a statistically significant difference ($p<0.05$) when animals receiving third ventricle injections of mepyramine at the doses of 200 and 400 nmol are compared to animals receiving mepyramine at the dose 100 nmol. "c" indicates a statistically significant difference ($p<0.05$) when animals receiving third ventricle injections of mepyramine at the dose of 400 nmol are compared to animals receiving mepyramine at the dose 200 nmol. Panel B: Cumulative water intake in animals receiving subcutaneous administration of PEG or isotonic saline solution treated with third ventricle injections of cimetidine. (○, $n=10$) sc isotonic saline solution + icv saline; (□, $n=9$) sc PEG + icv saline; (■, $n=10$) sc PEG + icv cimetidine 100 nmol; (◆, $n=11$) sc PEG + icv cimetidine 50 nmol; (▼, $n=9$) sc PEG + icv cimetidine 25 nmol. Data are presented as mean ± SEM. "a" indicates a statistically significant difference ($p<0.05$) when animals receiving third ventricle injections of cimetidine are compared to salt-loaded animals receiving third ventricle injections of saline. "d" indicates a statistically significant difference ($p<0.05$) when animals receiving third ventricle injections of cimetidine at the doses of 100 and 50 nmol are compared to salt-loaded animals receiving cimetidine at the dose 25 nmol. "e" indicates a statistically significant difference ($p<0.05$) when animals receiving third ventricle injections of cimetidine at the dose of 100 nmol are compared to animals receiving cimetidine at the dose 50 nmol. Each curve in the graph has been obtained from a naïve group of animals.

Fig. 2 (panel A) shows the effect of the central administration of the H_1 histaminergic antagonist, mepyramine, at different doses, on water intake in hypovolemic rats. There was a significant increase in water intake in hypovolemic animals (treated with subcutaneous PEG) receiving third ventricle injections of isotonic saline solution when compared to normovolemic rats (those treated with subcutaneous isotonic saline solution) also receiving third ventricle injections of isotonic saline solution. At the lowest dose used (100 nmol), mepyramine failed to induce any change in the dipsogenic effect of hypovolemia. At the intermediate dose of 200 nmol, the central administration of mepyramine was able to attenuate the increase in water intake in hypovolemic animals only during the first 60 min of the experiment. At the highest dose used (400 nmol), mepyramine significantly blunted the increase in drinking seen in hypovolemic animals for the entire duration of the experiment.

Fig. 2 (panel B) shows the effect of the central administration of the H_2 histaminergic antagonist, cimetidine, at different doses, on water intake in hypovolemic rats. As in the previous experimental set, a significant increase in water intake was observed in hypovolemic animals receiving third ventricle injections of isotonic saline solution when compared to normovolemic rats also receiving third ventricle injections of isotonic saline solution. At all doses used (25, 50 and 100 nmol), the central administration of cimetidine resulted in a significant decrease in the dipsogenic effect produced by hypovolemia.

Fig. 3 (panel A) shows the effect of the central administration of the H_1 histaminergic antagonist, mepyramine, at different

doses, on water intake induced by third ventricle injections of carbachol (2 ug). In the group of animals receiving central administration of the cholinergic agonist, carbachol, a significant increase in water intake was seen compared to animals receiving third ventricle injections of isotonic saline solution. At the lowest dose used (100 nmol) mepyramine failed to modify carbachol-induced water intake. At the other doses used (200 and 400 nmol),

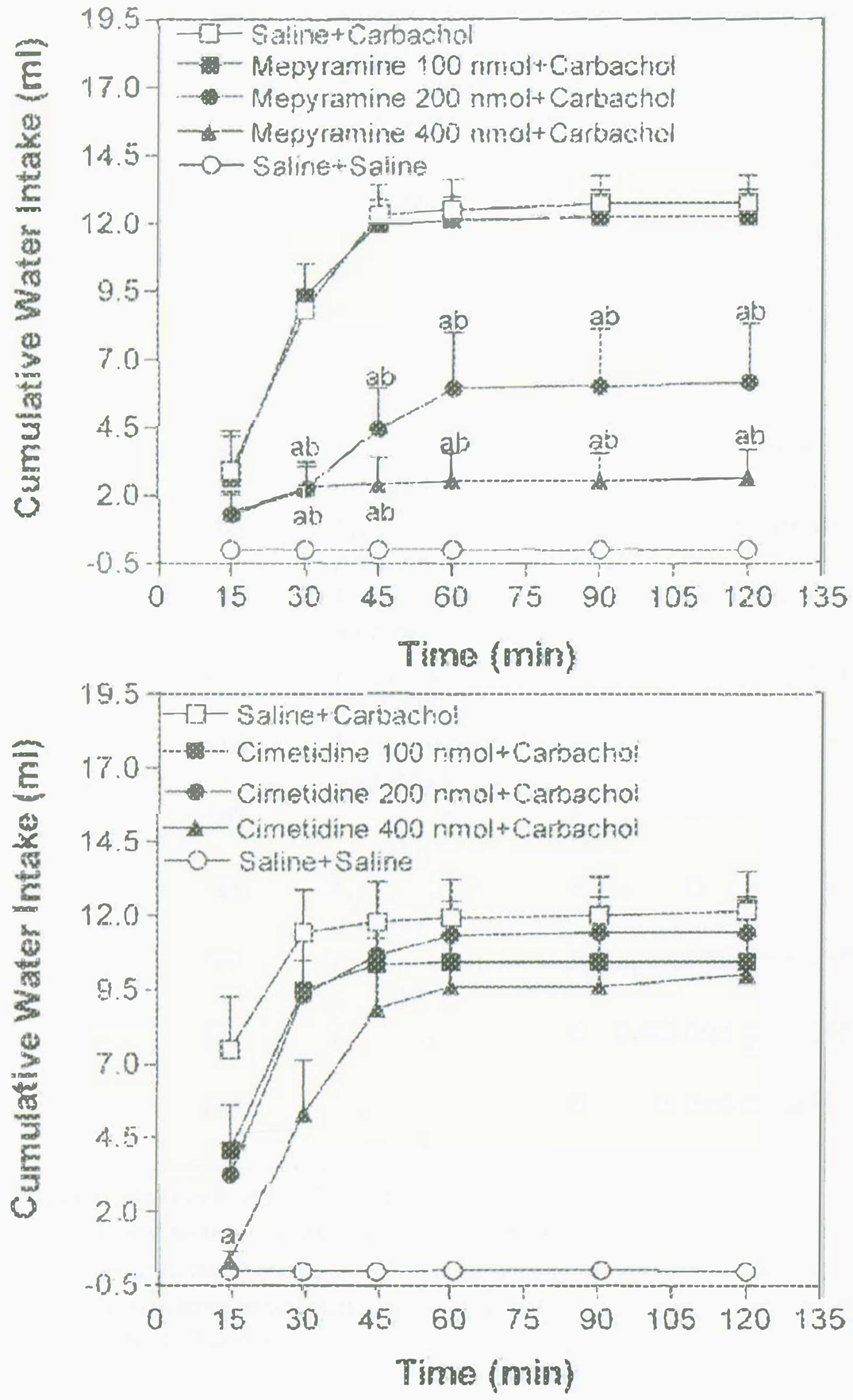

Fig. 3. Panel A — Cumulative water intake 15 min following third ventricle injections of mepyramine in rats receiving third ventricle injections of carbachol (2 μg). (□; $n=6$); saline + carbachol; (■; $n=8$) mepyramine 100 nmol + carbachol; (●; $n=9$) mepyramine 200 nmol + carbachol; (▲; $n=9$) mepyramine 400 nmol + carbachol. An additional group of animals not submitted to central cholinergic stimulation (saline + saline) is also shown (○; $n=12$). "a" indicates a statistically significant difference ($p<0.05$) when animals receiving third ventricle injections of mepyramine are compared to animals receiving third ventricle injections of saline. "b" indicates a statistically significant difference ($p<0.05$) when animals receiving third ventricle injections of mepyramine at the doses of 200 and 400 nmol are compared to animals receiving mepyramine at the dose 100 nmol. Panel B — Cumulative water intake 15 min following third ventricle injections of cimetidine in rats receiving third ventricle injections of carbachol (2 μg). (□; $n=7$) saline + carbachol; (■; $n=7$) cimetidine 100 nmol + carbachol; (●; $n=6$) cimetidine 200 nmol + carbachol; (▲; $n=9$) cimetidine 400 nmol + carbachol. An additional group of animals not submitted to central cholinergic stimulation (saline + saline) is also shown (○; $n=10$). Data are presented as mean ± SEM. Each curve in the graph has been obtained from a naïve group of animals.

Table 1
Effects of third ventricle injections of mepyramine (400 nmol/rat), cimetine (400 nmol/rat), or saline on the behavioral parameters in the open-field test

Behavior	Saline (6)	Mepyramine (6)	Cimetidine (6)	One-way ANOVA
Areas entered	33.67±4.36	33.33±4.63	34.00±4.11	$F_{(2,15)}=0.00582$; $p=0.9942$
Times in the peripheral areas	23.50±2.43	26.00±3.25	26.67±3.77	$F_{(2,15)}=0.271$; $p=0.7665$
Times in the center areas	10.17±2.75	7.33±3.08	7.33±1.41	$F_{(2,15)}=0.421$; $p=0.6637$
Stops	10.67±1.12	10.50±1.23	11.17±2.21	$F_{(2,15)}=0.0472$; $p=0.9541$
Rearing	6.33±0.88	5.17±1.11	7.00±1.98	$F_{(2,15)}=0.435$; $p=0.6552$
Grooming	2.00±0.58	1.33±0.49	1.67±0.33	$F_{(2,15)}=0.484$; $p=0.6257$
Diuresis	0.67±0.21	1.17±0.31	1.33±0.49	$F_{(2,15)}=0.942$; $p=0.4117$
Defecation	1.67±0.56	2.50±0.43	1.33±0.42	$F_{(2,15)}=1.61$; $p=0.2323$

Data is presented as mean±SEM of the number of occurrences of each listed behavior in a 10-minute long open-field test. The number of animals in each treatment group is in parenthesis.

mepyramine significantly decreased the dipsogenic response seen following central administration of carbachol.

Fig. 3 (panel B) shows the effect of the central administration of the H_2 histaminergic antagonist, cimetidine, at different doses, on water intake induced by third ventricle injections of carbachol (2 μg). As in the previous experimental set, the central administration of carbachol induced a significant increase in drinking. Nonetheless, in this case, cimetidine failed to modify the dipsogenic response induced by third ventricle injections of carbachol at any of the doses used (100, 200 or 400 nmol).

A significant increase ($p<0.05$) in hematocrit levels (49.3± 0.23%) was observed 4 h and 30 min after the administration of PEG when compared to a control group receiving subcutaneous injections of isotonic saline solution (43.2±0.72%).

As shown in Table 1, neither third ventricle injections of mepyramine nor cimetidine were able to alter the animals' locomotor activity pattern and the behavior of rats compared to the pattern observed in rats receiving third ventricle injections of saline solution, even at the highest dose used in the experimental sets already described (400 nmol) in an open field.

Table 2, condenses the results of the overall analysis of the effects of third ventricle injections of H_1 and H_2 receptor antagonists or saline on water intake at each time point.

4. Discussion

The present data clearly demonstrate that third ventricle injections of mepyramine, an H_1 antagonist, induced a significant, dose-dependent decrease in water intake induced by hyperosmolarity, hypovolemia or by the pharmacological stimulation of central cholinergic pathways by intracerebroventricular injections of carbachol, a muscarinic agonist. On the other hand, the central administration of cimetidine, an H_2 antagonist, significantly reduced water intake in hypovolemic animals in a dose-dependent

Table 2
Overall analysis of the effects of third ventricle injections of H_1 and H_2 receptors antagonists or saline on water intake at each period of time

	Time (min)	Mepyramine	Cimetidine
Hyperosmolarity	15	$F_{(3,28)}=11.4$; $p<0.0001$	$F_{(3,32)}=3.76$; $p=0.0203$
	30	$F_{(3,28)}=21.8$; $p<0.0001$	$F_{(3,32)}=6.0$; $p=0.0023$
	45	$F_{(3,28)}=13.9$; $p<0.0001$	$F_{(3,32)}=10.4$; $p<0.0001$
	60	$F_{(3,28)}=14.8$; $p<0.0001$	$F_{(3,32)}=9.96$; $p<0.0001$
	90	$F_{(3,28)}=15.0$; $p<0.0001$	$F_{(3,32)}=13.9$; $p<0.0001$
	120	$F_{(3,28)}=17.8$; $p<0.0001$	$F_{(3,32)}=20.0$; $p<0.0001$
Hypovolemia	15	$F_{(3,24)}=10.1$; $p=0.0002$	$F_{(3,32)}=5.52$; $p=0.0036$
	30	$F_{(3,24)}=12.0$; $p<0.0001$	$F_{(3,32)}=10.6$; $p<0.0001$
	45	$F_{(3,24)}=11.9$; $p<0.0001$	$F_{(3,32)}=14.9$; $p<0.0001$
	60	$F_{(3,24)}=13.3$; $p<0.0001$	$F_{(3,32)}=17.2$; $p<0.0001$
	90	$F_{(3,24)}=13.1$; $p<0.0001$	$F_{(3,32)}=15.4$; $p<0.0001$
	120	$F_{(3,24)}=11.9$; $p<0.0001$	$F_{(3,32)}=15.2$; $p<0.0001$
Carbachol	15	$F_{(3,28)}=0.535$; $p=0.6618$	$F_{(3,25)}=6.14$; $p=0.0028$
	30	$F_{(3,28)}=16.8$; $p<0.0001$	$F_{(3,25)}=2.65$; $p=0.0706$
	45	$F_{(3,28)}=15.9$; $p<0.0001$	$F_{(3,25)}=0.497$; $p=0.6871$
	60	$F_{(3,28)}=10.1$; $p=0.0001$	$F_{(3,25)}=0.336$; $p=0.7992$
	90	$F_{(3,28)}=10.1$; $p=0.0001$	$F_{(3,25)}=0.360$; $p=0.7822$
	120	$F_{(3,28)}=9.71$; $p=0.0001$	$F_{(3,25)}=0.296$; $p=0.8280$

The data were analyzed using the one-way ANOVA for each time point. For follow-up statistical tests to contrast specific groups additional post-hoc Student–Newman–Keuls tests were conducted. The group means for the various parameters analyzed were considered to be significantly different when $p<0.05$. These results are shown in Figs. 1, 2 and 3.

way while in hyperosmotic animals this compound was able to inhibit water intake only at the highest dose used. Third ventricle injections of cimetidine failed to alter water intake induced by central cholinergic stimulation by carbachol.

Plasma osmolarity, blood volume and blood pressure are constantly regulated by the central nervous system. Indeed, a complex central circuitry involving many brain areas and neurotransmitters receives a continuous flow of information related to these parameters and operates intricate mechanisms controlling corrective visceral and behavioral responses that include stimulation or inhibition of water and salt intake. Several central neurotransmitters play a significant role in the control of thirst. The cholinergic, adrenergic and serotonergic systems in the brain strongly influence water intake, exerting both positive and negative drives on drinking behavior, depending on the area in which each particular subset of neurons is located, the subtype of receptor activated and the animal's state of hydration [23,24].

The central histaminergic pathways participate in the control of water and salt intake, but the nature of this participation is not yet fully understood [25]. Central administration of histamine into several hypothalamic sites induces a significant increase in water intake [4,26,27], and the decrease in drinking behavior promoted by antihistaminics is reversed by intracerebroventricular injections of histamine [3,28,29].

By modulating not only histamine synthesis but the synthesis and release of several other neurotransmitters, central H_3 receptors may exhibit complex effects on water intake. Indeed, the central administration of H_3 receptor agonists, which decreases brain histaminergic activity, elicits drinking [17], thereby indicating that this effect cannot be attributed to H_3 receptor-dependent modifications in histaminergic activity. In another paper [30] the authors demonstrated that intracerebroventricular injections of a selective H_3 receptor antagonist attenuates drinking elicited by intragastric salt-load. The activation of post-synaptic H_3 receptors or H_3 receptors functioning as heteroceptors modulating the synthesis and release of other neurotransmitters may explain this apparent paradox.

We have previously shown that, in hyperosmotic rats, the activation of histaminergic H_1 receptors located in the ventromedial hypothalamus (VMH) stimulates water intake, while histaminergic H_2 receptors in this same region do not participate in the control of drinking behavior in this same group of animals. In addition, we have also shown that when activated both H_1 and H_2 histaminergic receptors located in the VMH increase overnight water intake and drinking behavior induced by a 14-hour period of water deprivation [8].

In another study, we showed that 1) the activation of central H_1 and H_2 histaminergic receptors stimulates salt intake induced by a 24-hour period of water deprivation, 2) the functional integrity of central H_1 histaminergic receptors is required to trigger salt intake in sodium-depleted rats, while central H_2 histaminergic receptors play no significant role in this mechanism and 3) both H_1 and H_2 histaminergic receptors participate in the mechanisms leading to water and salt intake in rats following central angiotensinergic stimulation [7]. It is important to note that several central structures and neurotransmitters normally linked to the stimulation of salt intake also increase water intake.

In the present study, third ventricle injections of mepyramine and cimetidine significantly blunted water intake induced by hypovolemia, indicating that the functional integrity of these receptors is essential for inducing thirst when blood volume is decreased. Water intake induced by hypovolemia is predominantly triggered by the activation of peripheral and central angiotensinergic components [23,24,31]. However, it should be noted that hypovolemia may induce thirst even in the absence of any increase in angiotensin II levels, as occurs in nephrectomized rats [32]. We have previously shown that the pharmacological blockade of H_1 and H_2 central histaminergic receptors attenuates water intake induced by central angiotensinergic stimulation [7]. Taken together, the data produced by our laboratory indicate that brain H_1 and H_2 histaminergic receptors are required to induce drinking in the presence of a direct pharmacological stimulation

of brain angiotensin II receptors or following hypovolemia, a physiological condition associated with the endogenous activation of central angiotensinergic pathways.

In the present study, third ventricle injections of mepyramine, an H_1 antagonist, significantly reduced water intake in animals receiving an intragastric salt load. This finding is in agreement with previous data from our laboratory showing that this same type of receptor located in the VMH is also necessary for the full expression of water intake in hyperosmotic animals. In addition, in the present study, third ventricle injections of cimetidine, an H_2 antagonist, significantly impaired the dipsogenic response induced by hyperosmolarity, while in the previously mentioned study, injections of cimetidine into the VMH failed to modify drinking behavior induced by this condition. The accumulated data from our previous and current studies indicate that 1) H_1 histaminergic receptors located both in the VMH and in other brain regions reached by injections of histaminergic drugs into the third ventricle participate in the thirst-inducing mechanisms triggered by hyperosmolarity and that 2) the activation of brain H_2 histaminergic receptors located in regions reached by injections of histaminergic drugs into the third ventricle is necessary to induce drinking in hyperosmotic animals, while these receptors located in the VMH play no significant role in this response.

Water intake induced by hyperosmolarity depends strongly on the activation of central cholinergic pathways and different brain areas may be involved [23,24,31,33,34] In this study, the pharmacological blockade of brain H_1 histaminergic receptors significantly decreased water intake both in animals submitted to an intragastric salt load and in animals receiving central cholinergic stimulation. Conversely, the pharmacological blockade of brain H_2 histaminergic receptors attenuated water intake in hyperosmotic animals, but failed to modify water intake in the group of rats receiving central cholinergic stimulation by third ventricle injections of carbachol. This suggests that hyperosmolarity, a physiological condition that certainly induces a myriad of complex alterations in the diverse central nervous system circuitries related to thirst regulation, triggers water intake through a mechanism that requires both H_1 and H_2 receptor involvements, whereas drinking behavior resulting from the pharmacological activation of central cholinergic pathways does not require H_2 histaminergic receptor activation.

A clear interaction has been demonstrated between the central histaminergic and cholinergic pathways in the central nervous system [9,10]. The activation of H_1 histaminergic receptors induces a significant increase in cholinergic transmission and acetylcholine release at many brain sites, and the central administration of histamine H_1 antagonists decreases acetylcholine release [9,12]. In addition, the central administration of selective H_3 histaminergic receptor antagonists, a procedure that increases histaminergic neurotransmission, augments *c-fos* immunoreactivity in cholinergic neurons [11]. In a previous paper, we suggested that H_1 histaminergic receptors located in the VMH may exert a stimulatory drive on acetylcholine release by cholinergic neurons, resulting in an increase in water intake induced by hyperosmolarity. A similar hypothesis may be applied here, linking brain H_1 histaminergic receptors to the release of acetylcholine in some thirst-triggering region of the brain. However, third ventricle injections of mepyramine were also able to attenuate water intake in animals receiving central administration of carbachol. This may indicate that H_1 histaminergic receptors located in circuitries post-synaptically situated in relation to the cholinergic pathways may also be involved in the thirst-inducing mechanisms triggered by central pharmacological cholinergic activation. These mechanisms leading to water intake following central cholinergic activation probably do not depend on histaminergic H_2 receptors located post-synaptically in relation to the cholinergic pathways, since central cimetidine administration failed to modify carbachol-induced drinking behavior.

The thirst-inducing procedures used in the present study generate physiological and pharmacological stimuli that normally trigger water intake. Indeed, we have demonstrated that intragastric salt load, using the same methodology applied in this study, produces a significant increase in plasma osmolarity and in plasma sodium concentration [8]. Furthermore, subcutaneous administration of PEG certainly produced hypovolemia, as indicated by the significant increase in hematocrit in the group submitted to this procedure, as compared to the group of control animals receiving isotonic saline solution subcutaneously. The induction of water intake by central cholinergic stimulation has been largely demonstrated and the use of intracerebroventricular administration of carbachol at the doses used in the present study to induce thirst is in accordance with data produced by other groups of investigators [35].

The inhibitory effects of mepyramine on water intake induced by hyperosmolarity, hypovolemia or by the pharmacological stimulation of central cholinergic pathways by intracerebroventricular injections of carbachol, as well as the inhibitory effect of cimetidine on water intake induced by hypovolemia were typically dose-dependent. This indicates a selective interaction of those compounds with central histaminergic receptors. Analysis of the effects of the various doses of the compounds used in the present study indicates that H_2 histaminergic inhibition of water intake in hyperosmotic animals requires a greater amount of pharmacological stimulation in contrast with the inhibitory influence exerted by central H_1 receptors that is obtained after significantly lower pharmacological stimulation. It is also important to note that, in hypovolemic animals, the magnitude of central H_1 pharmacological stimulation required to inhibit water intake is significantly greater than the central H_2 pharmacological stimulation necessary to produce an antidipsogenic effect.

The inhibition of water intake induced by the central administration of the histamine receptor antagonists seems to result from a specific action of the compounds on the brain circuitries that regulate drinking behavior and does not indicate a general impairment of the central nervous system. Indeed, in previous studies we have used an aversion test to show that third ventricle injections of either mepyramine or cimetidine fail to generate any "illness-like" effects [7]. Moreover, we have previously shown that third ventricle injections of these compounds selectively impair water intake but fail to alter the hedonic behavior represented by the consumption of a tasty saccharin solution [7]. We have also shown that the inhibition of

water intake induced by the central administration of mepyramine and cimetidine is not due to a deficit in locomotor activity since animals receiving the highest dose of the compounds used in this study showed no modification in locomotor activity and behavior as measured by an open field test.

The brain histaminergic system participates in the control of a large number of visceral and behavioral homeostatic processes [1] such as pain perception and the intake of food, water and salt. Central histaminergic circuitries are also involved in thermoregulation and in the control of the sleep/wake cycle. They also influence cardiovascular and neuroendocrine effectors. Furthermore, the central histaminergic system may be involved in important pathological conditions. Indeed, subcortical histaminergic projections have shown significant degeneration in Alzheimer's disease [36,37]; histamine levels and histidine decarboxylase activity are lower in Alzheimer disease and Down's syndrome [38,39]; histamine levels in the brains of patients with Parkinson's disease are selectively higher in the putamen, substantia nigra and globus pallidus [40]; and levels of *t*-methylhistamine, a histamine metabolite, are higher in the spinal fluid of schizophrenic patients [41]. All these facts, associated with the large clinical application of anti-histaminergic agents such as antiallergic or antacid agents that cross the blood–brain barrier, as well as some antipsychotics and recently developed antidepressants, make the brain histaminergic system a target for an extensive list of drugs used in current medical practice. Therefore, the investigation of the physiological roles played by central histaminergic receptors is opportune and relevant.

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5 DISCUSSÃO

Modificações no volume e na osmolaridade dos líquidos corporais, bem como alterações na pressão sanguínea, desencadeiam mecanismos de ajustes do equilíbrio hidroeletrolítico, entre os quais a ingestão hídrica e o apetite por sódio têm papel relevante (JOHNSON et al., 1997). Os mecanismos de controle da ingestão de água e sódio podem ser regulados por diferentes áreas cerebrais e vias neurotransmissoras centrais. A inter-relação entre as diversas áreas e neurotransmissores geram comportamentos como a sede e o apetite por sódio, importantes para a manutenção do equilíbrio hidrossalino do organismo.

Nesta pesquisa foi estudada a participação das vias histaminérgicas centrais no controle da ingestão hídrica e do apetite específico por sódio em diferentes situações: hiperosmolaridade, hipovolemia, privação de líquidos, depleção de sódio e estimulação angiotensinérgica e colinérgica centrais.

Os receptores histaminérgicos encontram-se amplamente distribuídos por todo o sistema nervoso central sendo identificados em diversas áreas, como o tálamo, núcleos da rafe, sistema límbico, núcleos hipotalâmicos, hipocampo, amígdala e substância negra (CHANG et al., 1979, PALACIOS et al., 1981, BOUTHENET et al., 1988, TRAIFFORT et al., 1992, VIZUETE et al., 1997, BROWN et al., 2001). No presente estudo, o terceiro ventrículo foi escolhido como local de administração das drogas devido a sua proximidade com as áreas hipotalâmicas, responsáveis pelo controle hidrossalino, e devido à possibilidade de distribuição ampla das drogas no sistema nervoso central. As áreas e núcleos circunventriculares apresentam alta densidade de receptores histaminérgicos dos tipos H_1 e H_2, além de receberem grande número de projeções histaminérgicas oriundas do núcleo tuberomamilar hipotalâmico.

(WATANABE et al., 1984, THRASHER, 1989, TRAIFFORT et al., 1992, GOOT & TIMMERMAN, 2000, BROWN et al., 2001).

A participação das vias histaminérgicas centrais e de seus receptores no controle do equilíbrio hidrossalino ainda não está totalmente esclarecida. Poucos estudos mostram a participação dessa vias nos comportamentos de ingestão hídrica. O aumento da histamina cerebral em diferentes áreas hipotalâmicas, seja pela redução do seu catabolismo ou por sua administração central diretamente, provoca aumento da ingestão hídrica em ratos (LEIBOWITZ, 1973, KRALY, 1983, SPEACHT & SPEAR, 1989, LECKLIN & TUOMISTO, 1995). Ao contrário, a administração de substâncias anti-histaminérgicas, como a promazina, no hipotálamo lateral, reduz significativamente a ingestão hídrica em animais privados de líquidos por 23h. O efeito inibitório do anti-histamínico sobre a ingestão hídrica é revertido com a administração central de histamina (GERALD & MAICKEL, 1972, KRALY, 1990, CLAPHAN et al., 1993).

Em estudo anterior de nosso grupo observou-se que a administração dos agonistas histaminérgicos HTMT (6-[2-(4-Imidazol)etilamino]-$_N$-(4-trifluorometilfenil heptanocarboxamida) e dimaprita, específicos para os receptores histaminérgicos dos tipos H_1 e H_2 no núcleo ventromedial hipotalâmico (VMH), nas doses de 100 e 200 nmol respectivamente, provoca efeito dipsogênico em animais normhidratados. Investigou-se também a participação dos receptores histaminérgicos no comportamento de ingestão de água pós-prandial. Verificou-se que a administração do antagonista para os receptor histaminérgico dos tipo H_1, mepiramina, no VMH inibe a ingestão hídrica pós-prandial, mas a redução induzida pela cimetidina, antagonista para os receptor histaminérgico dos tipo H_2, não foi estatisticamente significativa (MAGRANI, 2003).

No mesmo estudo, utilizando um outro protocolo experimental, verificou-se que a mepiramina administrada nas doses de 25, 50 e 200 nmol inibe a ingestão hídrica de

forma dose-dependente em animais privados de líquidos por 14 horas, enquanto que a administração de cimetidina reduz significativamente a ingestão hídrica em animais privados de água apenas na maior dose utilizada, 200 nmol. Em animais submetidos à desidratação osmótica induzida pela administração intragástrica de salina hipertônica (1,5%), a administração de mepiramina na dose de 200 nmol reduz significativamente a ingestão hídrica, já a administração de cimetidina não modifica a ingestão de água nos animais nesta condição em nenhuma das doses utilizadas (MAGRANI et al., 2004).

Estes resultados demonstram a participação das vias histaminérgicas e de seus receptores H_1 e H_2 nos mecanismos de controle da ingestão hídrica em diferentes condições. Observa-se um papel mais efetivo dos receptores do tipo H_1 no controle da ingestão hídrica desencadeada por desidratação intracelular. Por outro lado, os receptores do tipo H_2 mostram-se mais efetivos nos mecanismos de ingestão hídrica provocados por desidratação extracelular.

Dando continuidade a este estudo, decidiu-se investigar outros aspectos da participação das vias histaminérgicas e de seus receptores na regulação da ingestão hídrica ainda não esclarecidos pela literatura. Procedeu-se, assim, a administração do antagonista histaminérgico para o receptor do tipo H_1, mepiramina, nas doses de 100, 200 e 400 nmol no III ventrículo de animais submetidos à sobrecarga de sódio intragástrica. Observou-se, então, inibição da ingestão hídrica de forma dose-dependente. Por outro lado, a administração do antagonista para o receptor do tipo H_2, cimetidina, nas mesmas doses, provocou redução significativa na ingestão hídrica somente na maior dose utilizada (400 nmol). Estas respostas foram semelhantes às observadas no estudo anterior, quando em animais submetidos às mesmas condições de hiperosmolaridade, a administração de mepiramina no VMH, apresentou efeito antidipsogênico mais eficaz quando comparado à administração de cimetidina

(MAGRANI et al., 2004). A participação dos receptores histaminérgicos do tipo H_1 nos processos de regulação da ingestão hídrica induzida por hiperosmolaridade parece ser mais efetiva, quando comparadas ao papel dos receptores do tipo H_2.

Chiaraviglio e Perez Guaita, em 1984, demonstraram o envolvimento dos receptores histaminérgicos do tipo H_1, localizados na região ântero-ventral do III ventrículo (A3V), na ingestão hídrica induzida pela sobrecarga de sódio intragástrica. Esta região é conhecida por seu papel no controle da ingestão hídrica. Diversos estudos mostram que, além da histamina, outros neurotransmissores presentes nesta região podem participar da regulação da ingestão hídrica. A lesão eletrolítica da região A3V reduz a resposta dipsogênica induzida por angiotensina II, pela estimulação colinérgica central, pela privação hídrica e pelo aumento da osmolaridade plasmática. Enquanto que a estimulação elétrica dessa região pode desencadear respostas dipsogênicas e natriofílicas (VIEIRA et al., 2006). Esses dados sugerem que a participação de diversos neurotransmissores, incluindo a histamina, nos processos de regulação do balanço hídrico no organismo pode estar diretamente relacionada com a sua localização e distribuição cerebral.

Os diferentes estímulos utilizados, privação de líquidos e sobrecarga de sódio provocam modificações tanto do volume quanto da concentração dos íons dos líquidos intracelulares e extracelulares. Estas alterações da volemia e da osmolaridade ativam diferentes mecanismos para a regulação da homeostasia hidrossalina. Modificações da osmolaridade extracelular ativam células neuronais especializadas localizadas especialmente em estruturas circunventriculares. Essas células são sensíveis a pequenas modificações da osmolaridade do líquido extracelular e enviam sinais neuronais para outras áreas centrais, como o núcleo preóptico mediano, núcleo supraóptico e núcleo paraventricular desencadeando assim, modificações da atividade neuronal que

estimulam a sede e o comportamento de ingestão hídrica. A redução do volume dos líquidos corporais ativa receptores de estiramento localizados no arco aórtico e seio carotídeo, além de receptores nos átrios cardíacos, que emitem sinais através dos nervos vago e glossofaríngeo para o núcleo do trato solitário. Este núcleo comunica-se com áreas hipotalâmicas que estimulam a liberação de hormônios, a exemplo da AII e AVP, que irão promover retenção renal de água e sódio, além de estimular a ingestão de água e sal visando ao restabelecimento dos volumes normais (FITZSIMONS, 1998).

Entre as diversas vias neurotransmissoras centrais tem sido demonstrado que as vias colinérgicas têm papel relevante no controle da ingestão hídrica induzida pela hiperosmolaridade. De fato, observou-se que o comportamento de ingestão hídrica desencadeado pela situação de hiperosmolaridade plasmática ativa diretamente as vias colinérgicas centrais em várias áreas cerebrais (HOFFMAN et al., 1978, SAAVEDRA, 1992, FREECE et al., 1995, JOHNSON & THUNHORST, 1997). As vias colinérgicas centrais participam de forma direta dos mecanismos de controle da ingestão hídrica (MENANI et al., 1984). A administração na área septal e no hipotálamo do agonista colinérgico, o carbacol, provoca significativo aumento da ingestão hídrica em ratos (STRICKER & MILLER, 1968, ANTUNES-RODRIGUES & COVIAN, 1971). O mesmo efeito dipsogênico é observado quando este agonista é administrado no III ventrículo (ANTUNES-RODRIGUES & McCANN, 1970).

Tendo em vista que a administração de antagonistas histaminérgicos no III ventrículo modifica a ingestão hídrica induzida por hiperosmolaridade, decidiu-se investigar a possível interação entre as vias histaminéricas e colinérgicas centrais no controle da ingestão hídrica. Verificou-se que o bloqueio dos receptores histaminérgicos do tipo H_1, através da administração de mepiramina, reduz significativamente a ingestão hídrica induzida pela estimulação colinérgica através da administração de carbacol no

III ventrículo. Ao contrário, a administração de cimetidina, antagonista para os receptores histaminérgicos do tipo H_2 não é capaz de bloquear a resposta dipsogênica induzida pela administração central do agonista colinérgico (MAGRANI et al., 2006).

Outros estudos têm demonstrado a relação entre as vias histaminérgicas e colinérgicas no sistema nervoso central. A liberação de acetilcolina no núcleo estriado ventral, hipocampo e áreas hipotalâmicas, sofre modulação dos neurônios histaminérgicos colocalizados nestas áreas (RAO et al., 1987, PRAST et al., 1999, BACCIOTTINI et al., 2000 e 2001). A estimulação dos receptores histaminérgicos do tipo H_1 localizados em neurônios colinérgicos aumenta significativamente a transmissão colinérgica e a liberação de acetilcolina no núcleo estriado ventral. Entretanto, efeito oposto pode ser observado quando são estimulados os receptores histaminérgicos do tipo H_2 localizados em neurônios dopaminérgicos do núcleo estriado ventral (PRAST et al., 1991 e 1999). Esses dados estão de acordo com os resultados aqui apresentados, onde o bloqueio dos receptores histaminérgicos do tipo H_1, mas não dos receptores do tipo H_2, reduz de forma significativa a ingestão hídrica desencadeada pela estimulação colinérgica central. Mostrando uma maior e mais efetiva participação dos receptores do tipo H_1, tanto no controle da ingestão hídrica, quanto na modulação da liberação de agentes colinérgicos em diferentes áreas centrais.

A sede pode ser desencadeada por diferentes situações que alteram tanto volume quanto a osmolaridade dos líquidos corporais, entre estas condições pode-se citar: a hemorragia com redução significativa de volume sanguíneo, a depleção de sódio causada por uso de diuréticos ou por alterações patológicas da excreção e reabsorção de eletrólitos pelos rins e por vômito e diarréia. No caso do estado hipovolêmico desencadeado pela redução do volume dos líquidos corporais ocorre a ativação de diferentes mecanismos centrais e periféricos, como a secreção de hormônios a exemplo

da vasopressina, da aldosterona e da angiotensina II, que, sozinhos ou associados, aumentam a pressão sanguínea, reduzem a excreção renal de água e eletrólitos e estimulam a ingestão hídrica, bem como o apetite por sódio, visando ao restabelcimento das condições homeostáticas normais. (JOHNSON, 1982, REID, 1984, SAAVEDRA, 1992, JOHNSON & THUNHORST, 1997, FITZSIMONS, 1998).

Um dos protocolos experimentais utilizados para desencadear a sede hipovolêmica é a administração subcutânea de polietileno glicol (PEG), que provoca migração do líquido extracelular para a região da injeção, levando a mudanças no volume dos compartimentos de líquidos corporais e causando o estado de hipovolemia e, conseqüentemente, a sede. O estado hipovolêmico causa tanto aumento da ingestão hídrica quando do apetite por sódio, visto que ambos, volume e osmolaridade dos líquidos corporais, precisam ser restabelecidos, muito embora o primeiro fator a ser corrigido seja a volemia.

No presente estudo, animais submetidos à condição hipovolêmica, através da administração subcutânea de PEG, apresentaram redução significativa da ingestão hídrica após a administração no III ventrículo dos antagonistas para os receptores histaminérgicos dos tipos H_1 e H_2, mepiramina e cimetidina. A mepiramina apresentou efeito inibitório da ingestão hídrica somente nas maiores doses utilizadas, 200 e 400 nmol, enquanto a cimetidina bloqueou a resposta dipsogência em todas as doses utilizadas, 25, 50 e 100 nmol, de forma dose-dependente. Esses dados demonstraram uma participação mais efetiva dos receptores do tipo H_2 no estado de hipovolemia do que os receptores do tipo H_1.

Como as vias angiotensinérgicas têm importante papel no controle da sede hipovolêmica, decidiu-se investigar a interação entre as vias histaminérgicas e angiotensinérgicas centrais nesse controle. O aumento da angiotensina endógena, seja

central ou periférica, ativa diferentes mecanismos que provocam o comportamento de ingestão de água. Centralmente, o aumento nos níveis de angiotensina ativa neurônios no OSF e OVLT, áreas estas que fazem parte do circuito de controle da sede (THRASHER et al., 1982). Perifericamente, em situações de hipovolemia, o complexo justaglomerular renal, através da liberação de renina, aumenta os níveis de angiotensina II circulante que atua nas mesmas áreas cerebrais, OSF e OVLT, além da área postrema, também estimulando a sede (FITZSIMONS, 1972).

No presente estudo, a administração dos antagonistas histaminérgicos para os receptores dos tipos H_1 e H_2 foi capaz de reduzir a resposta dipsogênica desencadeada pela administração central de angiotensina II. A administração no III ventrículo de mepiramina apresentou potente efeito inibitório sobre a ingestão hídrica somente nas maiores doses utilizadas (200 e 400 nmol), enquanto que a administração de cimetidina promoveu inibição da ingestão hídrica causada pela angiotensina em todas as doses utilizadas nos experimentos (100, 200 e 400 nmol) (MAGRANI, 2005). Os dados obtidos nesse trabalho sugerem uma possível interação entre as vias histaminérgicas e angiotensinérgicas centrais no controle da sede induzida por variações dos volumes corporais.

Assim, pode-se concluir que os receptores histaminérgicos H_1 e H_2 participam de forma diferenciada dos mecanismos de controle do balanço hidroeletrolítico do organismo. Enquanto os receptores do tipo H_1 parecem ser mais importantes no controle da sede induzida por estímulos osmóticos e por ativação colinérgica, os receptores H_2 mostram maior efetividade no controle da ingestão de água induzida por estímulo volêmico e por ativação de vias angiotensinérgicas.

Outro comportamento importante para manutenção do equilíbrio hidrossalino do organismo é a ingestão de sal. O apetite por sódio pode ser desencadeado em resposta a

diversos fatores como a hipovolemia, a hiponatremia, e por modificações na liberação de renina, mineralocorticóides e outras substâncias hormonais (DENTON, 1982). A modificação na concentração de íons nos líquidos corporais, principalmente a do sódio, é percebida por células sensíveis a este íon localizadas no órgão vasculoso da lâmina terminal (OVLT) e no órgão subfornical (OSF) (NODA, 2006). O mecanismo de percepção dessas alterações é extremamente sensível à ativação desses osmorecptores /receptores de Na^+, desencadeando a ativação de vias neurotransmissoras centrais que estimulam ou inibem o comportamento de apetite específico por sódio (ANTUNES-RODRIGUES et al., 2004, DANIELS & FLUHARTY, 2004).

Nesse estudo decidiu-se investigar também a participação das vias histaminérgicas centrais no controle do apetite específico por sódio. Três modelos experimentais, que têm sido empregados correntemente por diversos grupos de pesquisas dedicados a este tipo de estudo - privação de líquidos; depleção de sódio e estimulação angiotensinérgica central - foram utilizados no presente estudo.

Em condição de privação líquida por 24h nos animais que receberam a administração no III ventrículo do antagonista para o receptor histaminérgico do tipo H_1, mepiramina, observou-se redução significativa da ingestão de sal em todas as doses utilizadas (100, 200 e 400 nmol), sendo esse efeito inibitório dose-dependente. Nos animais submetidos ao mesmo protocolo experimental, que receberam administração do antagonista para os receptores histaminérgicos do tipo H_2, cimetidina, não se observou modificação da ingestão de sal quando comparados aos animais controles.

Nos grupos de animais submetidos à depleção de sódio, através da administração subcutânea do diurético furosemida associada a uma dieta livre de sódio, a administração central de mepiramina levou à redução significativa da ingestão de sódio apenas na dose de 400 nmol. Nos animais tratados com a cimetidina também se

verificou redução da ingestão de sal, contudo, nenhuma das doses utilizadas apresentou diferença estatística significativa quando comparadas com o grupo controle.

Na condição de privação de líquidos ocorre a redução do volume do líquido extracelular e conseqüente modificação na osmolaridade plasmática. Por outro lado, a situação de depleção de sódio, desencadeada pelo o uso de diurético, provoca aumento da excreção urinária com perda tanto de volume quanto de solutos (FITZSIMONS, 1961). A furosemida, assim como a bumetanida, são diuréticos de alça que diminuem a reabsorção de íons no ramo ascendente espesso da alça de Henle através do bloqueio do co-transporte de $Na^+/K^+/2Cl^-$. Esta redução da reabsorção de eletrólitos leva ao aumento da osmolaridade nas porções finais do néfron reduzindo a reabsorção de água neste segmento, aumentando a excreção urinária tanto em volume quanto em concentrações de íons. Desta forma, as privações de líquidos, bem como a depleção de sódio, provoca modificações nas concentrações iônicas plasmáticas e no volume dos líquidos corporais, acionando mecanismos centrais que provocam o apetite por sódio (GREENLEAF, 1992, TAKAMATA et al., 1994). Dados da literatura mostram que as estruturas circunventriculares, como o OSF e o OVLT, são ativadas quando ocorre depleção de sódio no organismo. Por outro lado, lesões nestas áreas reduzem significativamente o apetite por sódio em animais depletados deste íon (HIYAMA et al., 2002; LIEDTKE, 2005A e 2005B; NODA, 2006).

Os resultados apresentados no presente estudo mostram que quando os receptores histaminérgicos do tipo H_1 são bloqueados, a resposta natriofílica desencadeada pelos estímulos de privação de líquidos e depleção de sódio é abolida. No entanto, quando ocorre o bloqueio dos receptores histaminérgicos do tipo H_2, a resposta natriofílica promovida pela depleção de sódio é reduzida, porém não de forma

significativa, enquanto que na situação de privação não ocorre qualquer modificação da ingestão de sódio.

No protocolo experimental em que os animais foram submetidos à estimulação angiotensinérgica central, o bloqueio farmacológico dos receptores histaminérgicos dos tipos H_1 e H_2, em todas as doses utilizadas (100, 200 e 400 nmol), provocou um potente efeito inibitório da ingestão de sódio.

A regulação central do apetite específico por sódio está associada à ativação de diferentes áreas e vias neurotransmissoras centrais que podem estimular a liberação de hormônios tais como: aldosterona e angiotensina II (GALAVERNA et al., 1996, SHELAT et al., 1999), ou inibir outras vias reguladoras a exemplo das vias ocitocinérgicas e serotoninérgicas centrais (MENANI et al 1998, CASTRO et al., 2002 e 2003). Em condições de hiponatremia ocorre estímulo para a liberação tanto da angiotensina II quanto de aldosterona, provocando, assim, aumento da reabsorção renal de sódio. No quadro de hipernatremia ocorre redução da liberação dessas substâncias e, consequentemente, aumento da excreção renal de sódio.

Os dados do presente trabalho sugerem a existência de uma interação entre as vias histaminérgicas e angiotensinérgicas centrais na regulação do apetite específico por sódio. Dessa forma, pode-se concluir que as vias histaminérgicas através dos seus receptores H_1 e H_2 têm papel relevante nos mecanismos de controle tanto da ingestão hídrica, quanto do apetite por sódio. Esses processos de controle da homeostasia hidroeletrolítica do organismo envolvem a interação das vias histaminérgicas com outras vias neurotransmissoras centrais, a exemplo das vias colinérgicas e angiotensinérgicas. Observou-se também que a efetividade dos receptores do tipo H_1 é maior quando ocorrem modificações nas condições osmóticas dos compartimentos de líquidos corporais. Além disso, a atividade deste receptor parece fundamental para o

efeito estimulatório das vias colinérgicas sobre a ingestão hídrica. Por outro lado, a participação dos receptores do tipo H_2 está relacionada a alterações do volume dos líquidos corporais, bem como à reposta dipsogênica desencadeada pela angiotensina II.

Os agentes farmacológicos utilizados nesse trabalho apresentam especificidade para os receptores histaminérgicos H_1 e H_2 (ISON, et al., 1973, HILL, 1990, GOOT & TIMMERMAN, 2000) e as dose administradas estão em concordância com os dados da literatura (KRALY, et al, 1995, LECKLIN & TUOMISTO, 1995). Wyngaarden & Seevers, em 1951, mostraram que altas doses de substâncias anti-histaminérgicas afetam o sistema nervoso central, provocando excitação e convulsão em crianças; depressão e coma em adultos. Dessa forma, optou-se por utilizar doses de, no máximo, 400 nmol, pois doses maiores de agonistas e ou antagonistas histaminérgicos podem causar excitação central e convulsão em ratos (GERALD & MAICKEL, 1972)

O conhecimento do papel funcional dos receptores histaminérgicos H_1 e H_2 no controle hidrossalino é relevante desde que antagonistas desses receptores têm sido empregados na clínica médica. Estudos clínicos mostram a utilização dos antagonistas dos receptores histaminérgicos H_2 no controle e na redução do ganho de peso em pacientes esquizofrênicos tratados com olanzapina (antipsicótico) que tendem a ter aumento de peso corporal durante o tratamento. A utilização de olanzapina em associação com nizatidina (antagonista H_2) tem como resposta estabilização do ganho de peso destes pacientes e até redução do peso corporal posterior (SACHETTI et al., 2000).

O uso dos antagonistas histaminérgicos para o receptor do tipo H_2, cimetidina, ranitidina e fomatidina, quando administrados intragastricamente em ratos provocam redução no consumo alimentar (STOA-BIRKETVEDT et al., 1997). Essa redução da ingestão alimentar pode estar associada ao bloqueio dos receptores histaminérgicos do

tipo H_2 periféricos, como ocorre no tratamento de doenças gástricas, como úlceras, tendo como efeito redução ou mesmo a supressão da produção de ácido gástrico, o que provoca diminuição na absorção de nutrientes pelo intestino e conseqüente perda de peso (STOA-BIRKETVEDT, 1993). O uso de antagonistas histaminérgicos no tratamento de patologias gástricas (BRINBLECOMB et al., 1975) e no controle e redução de peso (STOA-BIRKETVEDT, 1993, STOA-BIRKETVEDT et al., 1996) pode ser futuramente estendido para o tratamento de patologias relacionadas à regulação do balanço hidroeletrolítico do organismo, propiciando maior efetividade nos tratamentos de patologias associadas a alterações na atividade dos receptores histaminérgicos. Acredita-se que o presente estudo contribui significativamente para o desenvolvimento do conhecimento a respeito da participação das vias histaminérgicas centrais no controle da ingestão hídrica e do apetite por sódio.

6 CONCLUSÃO

Os resultados apresentados neste estudo demonstraram a participação dos receptores histaminérgicos centrais nos mecanismos de controle da ingestão hídrica e regulação do apetite por sódio.

Os efeitos inibitórios obtidos com a administração dos antagonistas específicos, mepiramina e cimetidina, na ingestão hídrica induzida pela hiperosmolaridade, hipovolemia e estimulação colinérgica central mostram a participação desses tipos de receptores na regulação do balanço hídrico. Os dados mostram uma participação mais eficiente dos receptores histaminérgicos do tipo H_1 em todos os modelos de sede apresentados, enquanto os receptores histaminérgicos do tipo H_2 apresentaram maior funcionalidade na condição de hipovolemia.

O bloqueio farmacológico dos receptores histaminérgicos do tipo H_1 resultou em eficaz redução da ingestão específica de sal em animais submetidos à depleção de sódio, privação hídrica e estimulação angiotensinérgica. Os mesmos protocolos utilizados com os receptores histaminérgicos do tipo H_2 apresentou resultado apenas nos animais submetidos à estimulação angiotensinérgica. Esses dados sugerem uma participação mais efetiva dos receptores do tipo H_1 nos mecanismos de regulação do apetite por sódio quando comparados aos receptores do tipo H_2. Sugerem também que os receptores do tipo H_2 apresentam funcionalidade mais específica na resposta natriofílica desencadeada pela estimulação angiotensinérgica central.

O conjunto dos dados mostra uma interação seletiva entre vias e receptores histaminérgicos e as demais vias centrais de regulação do balanço hidroeletrolítico do organismo.

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