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Angiotensin-II Is a Putative Neurotransmitter in Lactate-Induced Panic-Like Responses in Rats with Disruption of GABAergic Inhibition in the Dorsomedial Hypothalamus

A. Shekhar, P. L. Johnson, T. J. Sajdyk, S. D. Fitz, S. R. Keim, P. E. Kelley, D. R. Gehlert and J. A. DiMicco

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Angiotensin II in dorsomedial hypothalamus modulates cardiovascular arousal caused by stress but not feeding in rabbits

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De Matteo, Robert, Geoffrey A. Head, and Dmitry N. Mayorov. Angiotensin II in dorsomedial hypothalamus modulates cardiovascular arousal caused by stress but not feeding in rabbits. *Am J Physiol Regul Integr Comp Physiol* 290: R257–R264, 2006. First published September 1, 2005; doi:10.1152/ajpregu.00372.2005.—The dorsomedial hypothalamus (DMH) is critically implicated in the cardiovascular response to emotional stress. This study aimed to determine whether the DMH is also important in cardiovascular arousal associated with appetitive feeding behavior and, if so, whether locally released angiotensin II and glutamate are important in this arousal. Emotional (air-jet) stress and feeding elicited similar tachycardic (+51 and +45 beats/min, respectively) and pressor (+16 and +9 mmHg, respectively) responses in conscious rabbits. Bilateral microinjection of GABA_A agonist muscimol (500 pmol) into the DMH, but not nearby hypothalamic regions, attenuated pressor and tachycardic responses to air-jet by 56–63% and evoked anorexia. Conversely, stimulation of the DMH with the glutamate analog kainic acid (250 pmol) elicited hypertension (+25 mmHg) and tachycardia (+114 beats/min) and activated feeding behavior. Local microinjection of a glutamate receptor antagonist, kynurenic acid (10 nmol), decreased pressor responses to stress and eating by 46 and 72%, respectively, without affecting feeding behavior. Bilateral microinjection of a selective AT₁-receptor antagonist, candesartan (500 pmol), into the DMH, but not nearby sites, attenuated pressor and tachycardic stress responses by 31 and 33%, respectively. Candesartan did not alter feeding behavior or circulatory response to feeding. These results indicate that, in addition to its role in mediating stress responses, the DMH may be important in regulating cardiovascular arousal associated with feeding. Local glutamatergic inputs appear to regulate cardiovascular response to both stress and feeding. Conversely, angiotensin II, acting via AT₁ receptors, may selectively modulate, in the DMH, cardiovascular response to stress, but not feeding.

cardiovascular response; tachycardia; glutamate

GROWING EVIDENCE INDICATES that the dorsomedial hypothalamus (DMH) plays a key role in modulating autonomic, neuroendocrine, and behavioral arousal caused by threatening stimuli (12). Activation of inhibitory GABA_A receptors or blockade of excitatory amino acid (EAA) receptors in the DMH, but not nearby hypothalamus regions, markedly decreases the cardiovascular response to emotional stress in rats (38). Conversely, chemical stimulation of the DMH or blockade of local GABA_A receptors elicits increases in blood pressure, heart rate (HR), and behavioral measures of anxiety in rats (32, 33). Moreover, excitotoxic lesions of the DMH cause hypophagia (3) and dramatically reduce circadian rhythms of wakefulness, feeding, and locomotor activity in rats (2, 6), indicating that, apart from the defense response, this region is critically involved in controlling a wide range of other behavioral activities. Al-

though these activities, and particularly feeding, are normally associated in animals with distinct, central nervous system-mediated sympathetic activation (10, 11, 23), the functional role of the DMH in the regulation of the cardiovascular response to nonthreatening, arousing stimuli remains untested.

Forebrain angiotensin II (ANG II) has been implicated in controlling cardiovascular and neuroendocrine responses to various psychological and physical stressors (42). In particular, blockade of AT₁ receptors in the anterior and paraventricular hypothalamic nuclei has been shown to attenuate increases in blood pressure and plasma catecholamines caused by immobilization stress in rats (18, 21). Nevertheless, this role of ANG II appears to be nuclei specific, because, in the neighboring median preoptic nucleus, which contains all elements of the renin-angiotensin system, blockade of AT₁ receptors did not alter the cardiovascular response to air-jet stress (5). The functional role of ANG II in the DMH, which has a relatively high density of AT₁ receptors (1, 40), remains largely untested. However, a recent finding that decreased ANG II receptor binding in several forebrain nuclei, including the DMH, is associated with lactation-induced hyperphagia (37) indicates that local ANG II may be involved in the regulation of ingestive behavior.

In the present study, we first sought to extend the previous findings in rats by showing that the DMH is important in controlling the cardiovascular response to emotional stress in the rabbit. Second, we determined whether the DMH is implicated in the regulation of cardiovascular arousal caused by feeding, using inhibition of this region with a GABA_A agonist muscimol. Finally, we examined whether blockade of AT₁ receptors and ionotropic EAA receptors in the DMH alter cardiovascular arousal elicited by feeding and by emotional stress.

METHODS

General procedures. The experiments were performed in conscious New Zealand White rabbits, weighing 2.2–3.4 kg, that were bred and housed at the Baker Heart Research Institute, in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All procedures were approved by the Alfred Medical Research and Education Precinct Animal Ethics Committee. The rabbits were housed in individual cages and maintained on a 12:12-h light-dark cycle under controlled temperature and humidity. The animals were given water ad libitum but had a controlled pellet and vegetable diet. Rabbits were given vegetables or straw at 0800 and a mix of high-fiber pellets and oaten/lucerne chaff (150 g) at midday. The chow was usually consumed within 3–5 h.

Implantation of DMH guide cannulas. Rabbits were premedicated with an intravenous administration of 4 mg of dexamethasone (Dexa-

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son, Troy Laboratories) to prevent inflammation around the guide cannulas. Anesthesia was induced by using an intravenous injection of propofol (Diprivan, 1 mg/kg, Zeneca), after which the rabbits were intubated and then placed on halothane (Fluothane, Zeneca) open-circuit anesthesia with use of a vaporizer (Goldman). The animal was placed in a stereotaxic frame with lambda and bregma parallel to the horizontal stereotaxic arm, and the animal's head was leveled medially and laterally. A burr hole (~5 mm in diameter) was made in the skull, centered at 2.2 mm caudal to bregma. Two stainless steel guide cannulas (23 gauge, 20-mm length) were positioned bilaterally 2.2 mm caudal to bregma and 0.9 mm lateral to the midline, identified as the sagittal separation of the brain hemispheres. The guide cannulas were lowered 8.0 mm from the brain surface so that the tip lay 5 mm dorsal to the anticipated position of the DMH. The guide cannulas were held in place with acrylic cement and three stainless steel screws anchored to the skull. Guide dummies were inserted into the guides to prevent material from entering the cannulas. Animals were allowed to recover for 2 wk after surgery. By the end of the recovery period, all animals regained weight to at least the presurgical level.

Blood pressure and HR measurement. At the beginning of the experiment (0800), the animal was placed in a standard rabbit box (dimensions 15 × 40 × 18 cm, width × length × height) with an adjustable backplate. Under local anesthesia (xylocaine HCl 1%, AstraZeneca), the 4-cm-long catheter was placed into the central ear artery near the ear base to record systemic blood pressure. During the experiment, pulsatile arterial pressure was measured by using a Statham 23Dc pressure transducer, sampled at 500 Hz with the use of an analog-to-digital data-acquisition card (PC Plus, National Instruments) and stored on a computer for future analysis. The beat-to-beat mean arterial pressure (MAP) and HR were detected on-line using the LabVIEW program.

Feeding and emotional stress tests. Food (a small bunch of straw, ~10 g) was first presented at the front of the rabbit box, which typically elicited a prompt behavioral and cardiovascular arousal. Food was then placed inside the box and typically fully consumed within 5–7 min. The duration of the eating bout was used as an inverse index of intensity of eating. The mean change in MAP and HR over the first 5 min was used to estimate the magnitude of cardiovascular response to feeding. The air-jet stress was induced by a fine stream of compressed air (20 psi from 15-cm distance for 8 min) directed at the rabbit through a round 6-cm hole in the front wall of the box, as described previously (26). This stress regimen elicited sustained and highly reproducible increases in MAP and HR, which had similar magnitude to those caused by feeding. After an initial (1–2 min) period of behavioral and locomotor activation, rabbits typically remained quiescent until the conclusion of air-jet. Thus the average change in MAP and HR during the last 5 min of air-jet was used to calculate the response to stress.

Microinjections into the DMH. In 11 rabbits, cardiovascular responses to air-jet stress and feeding were evaluated before and 10–30 min after bilateral microinjections, into the DMH, of muscimol (500 pmol, $n = 8$), kynurenic acid (10 nmol, $n = 4$), candesartan (200 pmol, $n = 3$ and 500 pmol, $n = 9$), or vehicle ($n = 6$). In preliminary experiments, we found that microinjections of these agents caused a stable attenuation in the pressor response to air-jet for at least 1 h. In addition, changes in cardiovascular parameters and feeding behavior in response to local microinjection of kainic acid (250 pmol) were evaluated in five of those rabbits. In 10 other animals, the same doses of muscimol ($n = 8$), candesartan ($n = 4$), kynurenic acid ($n = 2$), and vehicle ($n = 3$) were injected outside the DMH to assess the anatomic specificity of the stress and eating responses. The selected doses of the drugs were based on our laboratory's earlier experiments (22, 24, 25) and on previous studies from other laboratories (13, 35). Each rabbit was subjected to one to two different treatments per experimental day. One recovery day was scheduled between consecutive experimental days ($n = 4$ –5). In the case of two treatments on the same experimental day, a 2- to 3-h period was allowed between treatments, and

full recovery of the stress and feeding responses was observed before proceeding to the next treatment. Each animal received a total of six to eight hypothalamic microinjections during the course of the experiments. The order of treatments was randomized between and within experimental days, except kainic acid was always injected last because of its potential excitotoxic action (13).

Microinjections were made through a 30-gauge stainless steel injector, which extended 5.0 mm beyond the guide cannula. In some cases, microinjections were also made using 2-mm shorter or 2-mm longer injectors. The injector was connected via polyethylene SP8 tubing to a 250- μ l syringe (Hamilton, Reno, NV). Drug injections were made by hand over a period of 1 min, and the injection volume (100 nl) was controlled by measuring the displacement of a small air bubble in the polyethylene tubing. Muscimol, kynurenic acid (both from Sigma), and ANG II (Auspep) were dissolved in Ringer solution (Baxter). Candesartan (gift of AstraZeneca) was dissolved in 1 N Na₂CO₃ solution diluted 80:1 in Ringer, in accordance with AstraZeneca specification.

Localization of injection sites. On completion of the experiment, each animal was deeply anesthetized with pentobarbitone sodium (60 mg/kg, intravenously), and the injection sites were marked with 100 nl of a 2% pontamine sky blue. The animal was then perfused transcardially with 0.1 M phosphate-buffered saline followed by 4% paraformaldehyde in phosphate buffer. The brain was removed and stored in fixative solution containing 20% sucrose. The hypothalamus was cut into either sagittal or coronal sections (40 μ m thick), and every fourth serial section was stained with cresyl violet and taken for histological examination. The atlas of Girgis and Shih-Chang was used as a reference (15).

Statistical analysis. All values are expressed as means \pm SE. The effects of drug treatment and injection site location (i.e., "inside the DMH" vs. "outside the DMH") on the cardiovascular response to stress or feeding were analyzed by a split-plot (nested) ANOVA, which combines within-animal and between-group comparisons (34). The total sums of squares (SS) was divided into between- and within-group SS. The latter contained the between-treatment SS, between-animal SS, and animal \times treatment interaction for each of the two groups (i.e., inside the DMH and outside the DMH groups). Within each group, comparisons of resting and arousal values before and after treatment were made by using a set of orthogonal contrasts. The *F* ratio for each contrast was calculated as the mean square (MS) for the contrast divided by the total residual MS of the two groups. Thus the estimate of the within-group variance was made with a contribution from all of the groups. Between-group comparisons were made using the *F* ratio of the between-group MS divided by the row \times group interaction. $P < 0.05$ was considered significant.

RESULTS

Cardiovascular arousal elicited by stress and feeding. Resting cardiovascular parameters were not different between treatment groups before microinjections of test agents into the DMH of conscious rabbits (Table 1). Air-jet stress evoked an increase in MAP and HR, which typically reached a plateau within the first minute (Fig. 1). Before microinjections of test agents, there was no difference between treatment groups in the air-jet-induced changes in MAP and HR, with the overall average response being $+16 \pm 1$ mmHg and $+51 \pm 6$ beats/min, respectively. Rabbits showed no habituation to air-jet stress over the course of the study, so that stress-induced increases in MAP and HR were similar on the first ($+17 \pm 1$ mmHg and $+48 \pm 8$ beats/min) and last ($+16 \pm 1$ mmHg and $+55 \pm 10$ beats/min) experimental days. Food presentation and eating elicited sustained tachycardic and pressor responses, which were similar to those caused by air-jet stress (Fig. 1).

Table 1. Resting hemodynamic parameters and their changes after bilateral microinjections into the dorsomedial hypothalamus of conscious rabbits

Treatment Group	n	MAP, mmHg		HR, beats/min	
		Baseline	Δ	Baseline	Δ
Muscimol	8	76 \pm 3	+3 \pm 1	156 \pm 10	+1 \pm 5
Kynurenic acid	4	70 \pm 3	-7 \pm 6	169 \pm 14	-3 \pm 6
Kainic acid	5	69 \pm 2	+25 \pm 4*	164 \pm 11	+114 \pm 27*
Candesartan	9	69 \pm 2	-1 \pm 1	153 \pm 3	-3 \pm 3
Vehicle	6	71 \pm 3	-4 \pm 1	147 \pm 5	-5 \pm 3

Values are means \pm SE; n, number of experiments. MAP, mean arterial pressure; HR, heart rate; Δ , drug-induced change from baseline. *Statistically different change from baseline, $P < 0.05$.

The overall average HR response to feeding (+45 \pm 4 beats/min) was not statistically different from that induced by air-jet stress ($n = 17$; Fig. 1). However, the overall average MAP response to feeding (+9 \pm 1 mmHg) was lower than that caused by air-jet stress ($P < 0.05$). Baseline MAP and HR values were not different before stress and feeding tests. There was no habituation of the eating-induced arousal during the course of the study, with pressor and tachycardic responses being similar in the first (+9 \pm 1 mmHg and +46 \pm 5 beats/min) and last (+7 \pm 1 mmHg and +44 \pm 6 beats/min) experiments.

Distribution of injection sites in the hypothalamus. In 11 rabbits, injection sites were found in the DMH (Figs. 2A and 5A). In all of these animals, injection sites were located 0.6 mm to 1.3 mm lateral to midline, with the average medial-lateral bias between the left and right sites being 0.37 \pm 0.08 mm, so that no sites were found at or lateral to the level of the fornix-mammillothalamic tract (i.e., in the lateral hypothalamus). In 10 other rabbits, injection sites were also located in the medial hypothalamus (0.5 mm to 1.2 mm lateral to midline), but outside the DMH (Figs. 2A and 5A). In four of those animals, injection sites were found just dorsal to the DMH, in a region known as the dorsal hypothalamic area (DA; $n = 4$; Fig. 2A), which is considered to be a major relay site in mediating the DMH-induced tachycardia (30).

Role of the DMH in cardiovascular arousal caused by stress and feeding. Inhibition of the DMH with bilateral microinjection of GABA_A agonist muscimol (500 pmol, $n = 8$) into the DMH did not change resting hemodynamic parameters (Table 1). Muscimol attenuated the pressor and tachycardic response to air-jet stress by 56 \pm 11 and 63 \pm 24%, respectively ($P < 0.01$; Fig. 2B). Microinjection of muscimol into the DMH also inhibited feeding behavior, so that rabbits showed little or no interest in food. Accordingly, cardiovascular arousal associated with food presentation was nearly abolished in these animals (Fig. 3A). Feeding behavior returned to normal by the following day.

In eight other rabbits, microinjection of muscimol at sites outside the DMH did not affect resting MAP and HR (average change: +1 \pm 1 mmHg and -11 \pm 5 beats/min, respectively). Similarly, the pressor response to air-jet stress remained unaltered by muscimol (Fig. 2C). However, the stress-induced tachycardia tended to decrease by 31 \pm 12% after the injection. In four of those animals, in which injection sites were located in the DA (Fig. 2A), the tachycardic response to stress was attenuated by 42 \pm 7% (from 48 \pm 8 to 28 \pm 7 beats/min; $P < 0.05$). By contrast, in four remaining rabbits, in which injection sites were located anterior, posterior, or ventral to the DMH (Fig. 2A), the tachycardic response to stress was not altered after the treatment. Similarly, in a subset of these animals, the pressor response to feeding was not different before and after microinjections of muscimol at sites outside the DMH ($n = 7$; Fig. 3B), while the feeding-induced tachycardia tended to decrease from 50 \pm 8 to 31 \pm 8 beats/min ($P = 0.06$). The duration of eating bouts was not different before (5.3 \pm 0.4 min) and after (5.2 \pm 0.5 min) microinjection of muscimol outside the DMH.

Role of EAAs in the DMH in cardiovascular response to stress and feeding. Stimulation of the DMH with bilateral microinjection of the glutamate analog kainic acid (250 pmol; $n = 5$) elicited a rapid activation of food-seeking behavior and cardiovascular arousal (Fig. 4A). When presented with food, rabbits exhibited a vigorous drive to eat, which lasted ~30 min and was accompanied by tachycardia (Table 1; time to peak: 26 \pm 2 min) and hypertension (Table 1; time to peak: 25 \pm 4

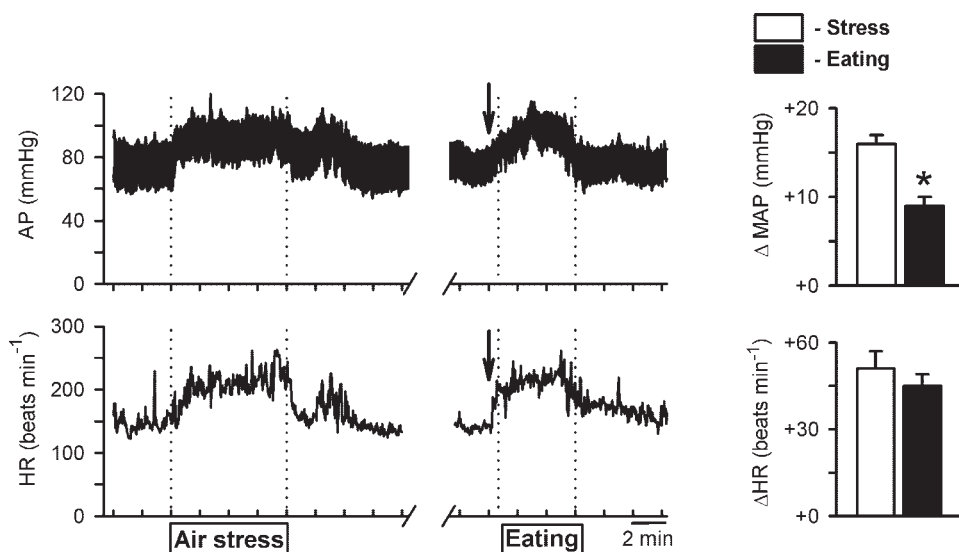


Fig. 1. Left: in a representative, conscious rabbit, aversive (air-jet) and appetitive (straw) stimuli elicited similar increases of arterial pulse pressure (AP) and heart rate (HR). Dotted lines mark the beginning and conclusion of air-jet stress and eating. Arrows mark food presentation, which resulted in prompt cardiovascular arousal before the actual beginning of eating. Right: the overall average responses to air-jet stress and feeding in conscious rabbits ($n = 17$). MAP, mean arterial pressure; Δ , change. * $P < 0.05$ vs. air-jet stress.

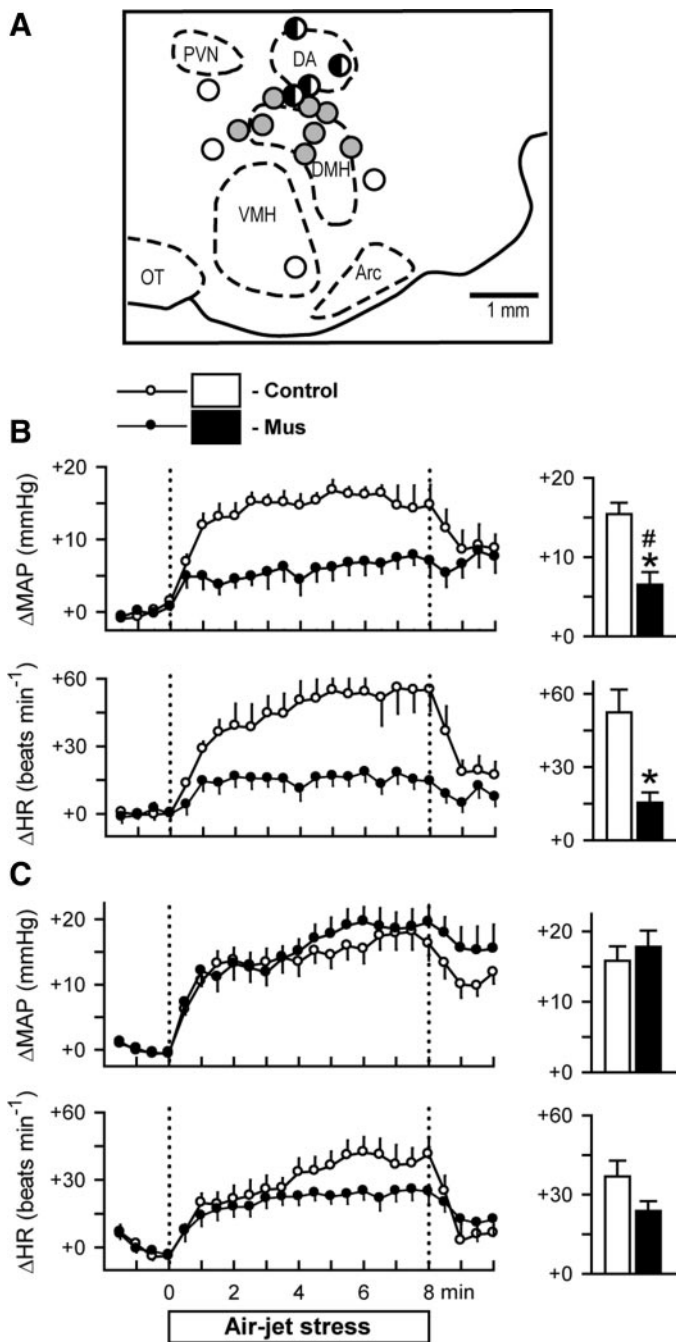


Fig. 2. *A*: representative reconstruction of the sagittal view of the rabbit's brain illustrating location of sites of muscimol injections. Shaded circles, inside the dorsomedial hypothalamus (DMH; $n = 8$); half-shaded circles, inside the dorsal hypothalamic area (DA; $n = 4$); open circles, outside DMH and DA ($n = 4$). Note that, although injection sites were depicted in the same sagittal plane, the average medial-lateral bias between the left and right sites was 0.4 ± 0.1 mm. Because little bias between the left and right injections sites in the sagittal plane was typically detected in the same animal, one circle represents bilateral injection sites. VMH, ventromedial hypothalamus; OT, optic tract; Arc, arcuate hypothalamic nucleus; PVN, paraventricular hypothalamic nuclei. *B*: microinjections of muscimol (Mus) (500 pmol; $n = 8$) into the DMH attenuated increases in MAP and HR caused by air-jet stress in conscious rabbits. *C*: in 8 other rabbits, microinjections of muscimol at sites outside the DMH did not alter the pressor response to stress, but tended to decrease tachycardic response. Each symbol represents an average over a 30-s period. * $P < 0.05$ vs. corresponding control response. # $P < 0.05$ vs. response outside the DMH.

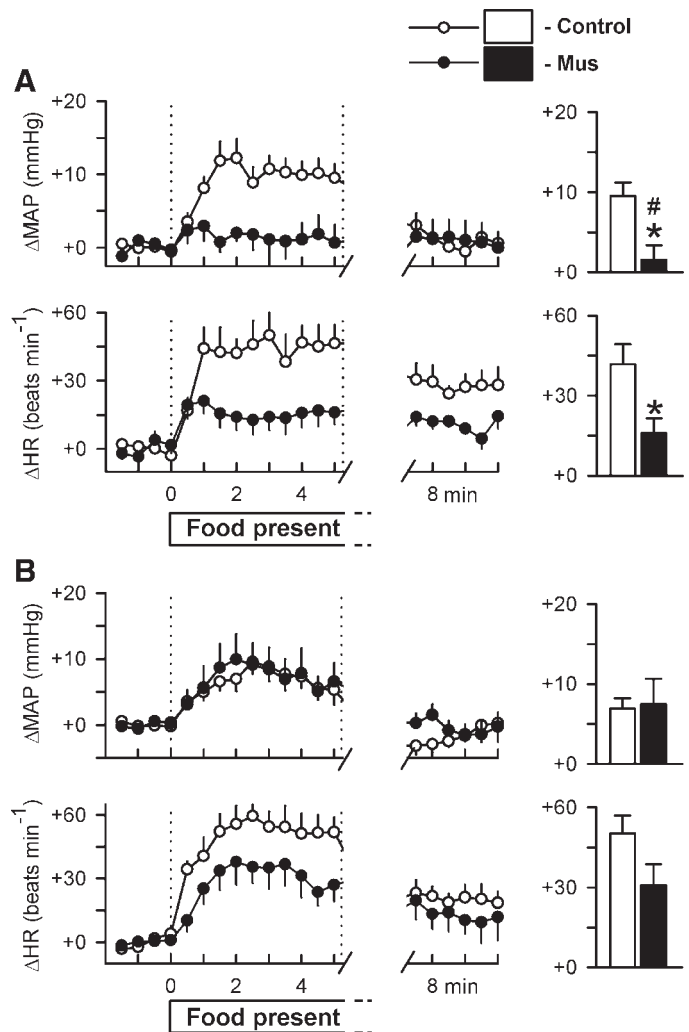


Fig. 3. *A*: microinjections of muscimol (500 pmol; $n = 6$) into the DMH inhibited feeding behavior and nearly abolished the pressor and tachycardic responses to food presentation and feeding. Each symbol represents an average over a 30-s period. *B*: microinjections of muscimol at sites outside the DMH did not alter the pressor response to feeding, but tended to decrease the tachycardic response ($n = 7$). Right panels in *A* and *B* represent the average changes (Δ) in MAP and HR caused by feeding. * $P < 0.05$ vs. corresponding control response. # $P < 0.05$ vs. response outside the DMH.

min). Blood pressure returned to preinjection levels within 2 h, whereas HR remained elevated ($+50 \pm 17$ beats/min, $P < 0.05$) until the end of the experiment (4 h after injection).

Bilateral microinjection of an inotropic glutamate receptor antagonist kynurenic acid (10 nmol; $n = 4$) into the DMH did not statistically change resting cardiovascular parameters (Table 1). Kynurenic acid decreased the pressor response to air-jet stress by $46 \pm 9\%$ ($P < 0.01$; Fig. 4*B*) and tended to reduce the tachycardic response ($-82 \pm 36\%$, $P = 0.08$). Similarly, the MAP and HR responses to eating were attenuated after kynurenic acid by 72 ± 25 and $50 \pm 16\%$, respectively ($P < 0.05$; $n = 4$; Fig. 4*B*). Conversely, the duration of eating bouts was not statistically different before (6.6 ± 1.7 min) and after (5.3 ± 1.1 min) injections of kynurenic acid in these animals. In two other rabbits, microinjection of kynurenic acid at sites outside the DMH had little effect on the MAP and HR responses to air-jet stress and feeding (not shown). The de-

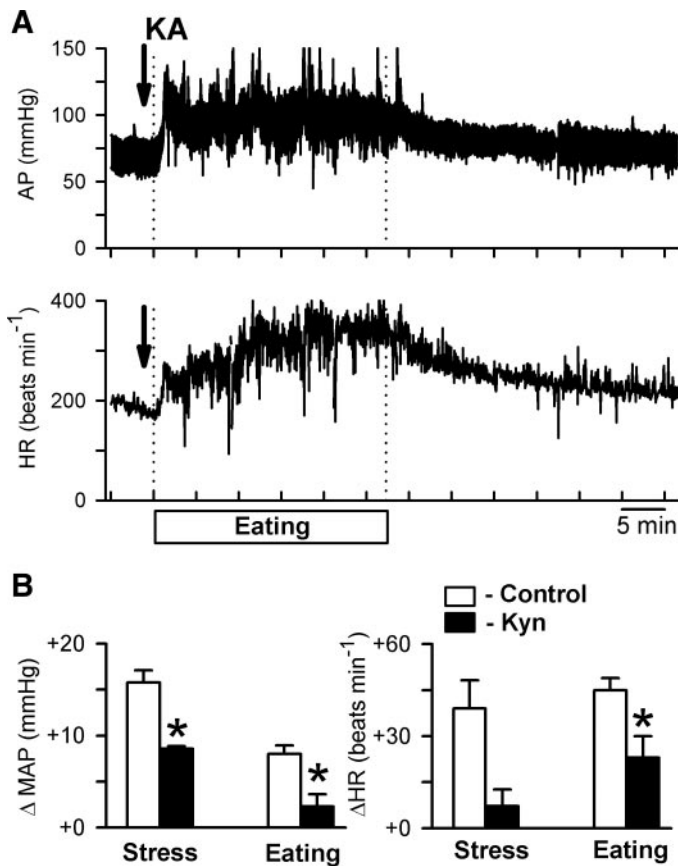


Fig. 4. *A*: microinjection of a glutamate analog kainic acid (KA; 250 pmol, $n = 6$) into the DMH elicited cardiovascular arousal and activated feeding behavior. Arrows mark microinjection of KA. *B*: microinjection of a glutamate receptor antagonist, kynurenic acid (Kyn; 10 nmol, $n = 4$), into the DMH attenuated the pressor response to air-jet stress and eating. * $P < 0.05$ vs. corresponding control response.

creases in the pressor response to emotional stress were strongly correlated with those to feeding (linear regression analysis: $R = 0.93$, $P = 0.008$, $n = 6$). By contrast, there was no correlation between the kynurenate-induced changes in tachycardic responses to stress and feeding ($R = 0.0002$, not significant).

Role of ANG II in the DMH in cardiovascular arousal caused by stress and feeding. Bilateral microinjections of AT₁-receptor antagonist candesartan (500 pmol, $n = 9$; shaded circles in Fig. 5A) into the DMH did not alter resting hemodynamic parameters (Table 1). Candesartan attenuated the pressor and tachycardic response to air-jet stress by 31 ± 5 and $33 \pm 11\%$, respectively ($P < 0.05$; Fig. 5B). Local microinjection of a smaller dose of candesartan (200 pmol; $n = 4$) decreased the pressor ($+23 \pm 2$ mmHg) and tachycardic ($+52 \pm 15$ beats/min) responses to stress by 18 ± 5 and $23 \pm 6\%$, respectively ($P < 0.05$). Microinjection of candesartan outside the DMH (500 pmol; $n = 5$; open circles in Fig. 5A) did not change the cardiovascular response to stress in five other animals (Fig. 5C).

Microinjections of candesartan into the DMH did not alter the circulatory response to feeding (500 pmol; $n = 6$; Fig. 6A). The duration of eating bouts was not different before (6.0 ± 0.7 min) and after (5.9 ± 0.9 min) candesartan. Similarly, in

three other rabbits, microinjections of candesartan outside the DMH (500 pmol; Fig. 6B) did not alter the cardiovascular response to feeding. Microinjections of the candesartan vehicle (1 N Na₂CO₃ solution diluted 80:1 in Ringer) into the DMH ($n = 6$) did not change resting hemodynamic parameters (Table 1) or their responses to air-jet stress (Fig. 7A). Similarly, the eating-induced cardiovascular arousal (Fig. 7B) and dura-

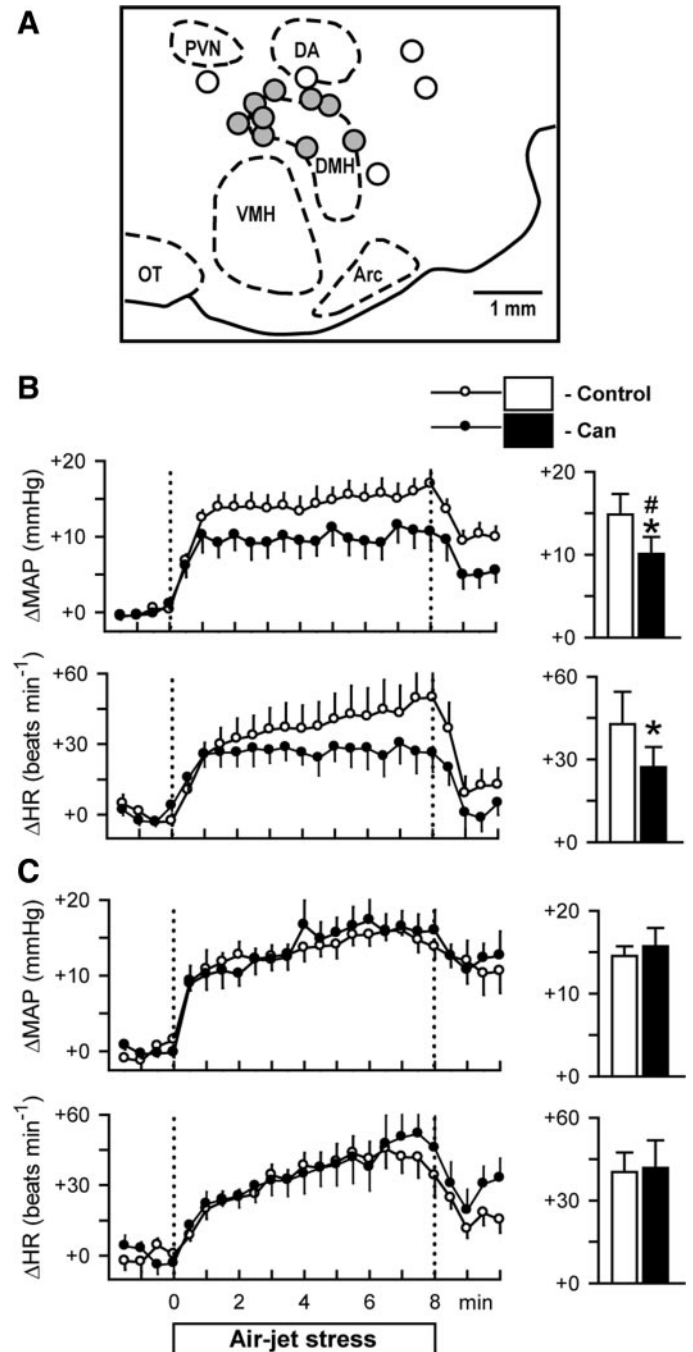


Fig. 5. *A*: representative reconstruction of sagittal view of the rabbit's brain illustrating the location of sites of candesartan injections. Shaded circles, inside DMH ($n = 9$); open circles, outside DMH ($n = 5$). *B*: microinjections of candesartan (500 pmol; $n = 9$) inside the DMH attenuated pressor and tachycardic responses to air-jet stress. *C*: microinjections of candesartan outside the DMH did not alter these responses ($n = 4$). * $P < 0.05$ vs. corresponding control response. # $P < 0.05$ vs. response outside the DMH.

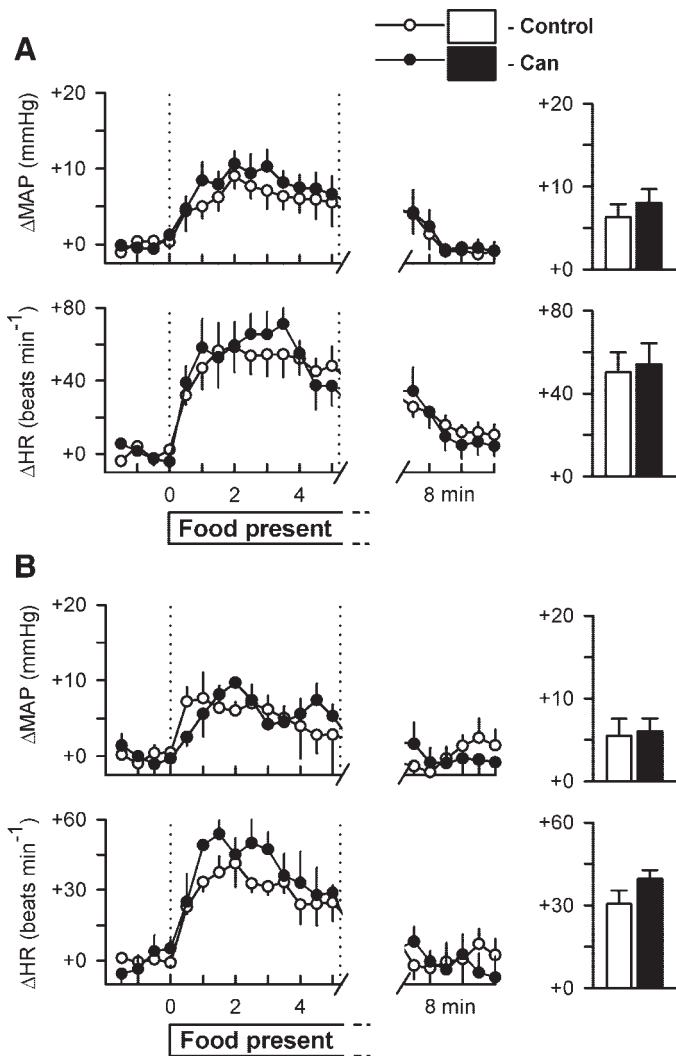


Fig. 6. Microinjections of candesartan (Can; 500 pmol) either inside (A; $n = 6$) or outside (B; $n = 3$) the DMH did not alter pressor and tachycardic responses to feeding. Symbols and abbreviations are as defined in Fig. 3 legend.

tion of eating were similar before and after vehicle injections into the DMH (6.6 ± 1.0 and 5.9 ± 0.5 min, respectively). The pressor and tachycardic responses to stress and feeding were not altered by microinjections of vehicle outside the DMH ($n = 3$; data not shown).

Microinjections of ANG II (100 pmol, unilaterally; $n = 6$) into the DMH increased MAP by 10 ± 4 mmHg (time to peak: 2.8 ± 0.8 min; $P < 0.05$) and HR by 11 ± 4 beats/min ($P < 0.05$). These responses were blocked by preinjections of candesartan (500 pmol; $n = 3$) into the DMH. By contrast, microinjections of ANG II just outside of the DMH (100 pmol; $n = 4$) did not alter MAP and HR (average change: $+4 \pm 1$ mmHg and -2 ± 2 beats/min, respectively).

DISCUSSION

The present study indicates that the DMH plays a crucial role in regulating autonomic cardiovascular arousal caused by emotional stress in rabbits, in line with previous findings in rats (33, 35, 38). The present results also provide, to our knowl-

edge, the first evidence that the DMH is critical in mediating cardiovascular arousal associated with appetitive feeding behavior. Additionally, our results suggest that, despite a similar degree of arousal caused by stress and feeding, different neurotransmitters and pathways within the DMH regulate hemodynamic adjustments to these, presumably oppositely valenced, events. In particular, endogenous ANG II, acting via AT_1 receptors, selectively modulates cardiovascular arousal caused by stress, but not feeding.

In this study, microinjection of a GABA_A agonist, muscimol, into the DMH markedly attenuated pressor and tachycardic responses to air-jet stress, in accord with earlier findings (38). It is unlikely that this attenuation was due to the spread of muscimol into adjacent regions, because microinjections near the paraventricular or posterior hypothalamic nuclei did not alter the stress response. Thus it appears that the DMH is critically involved in regulating both pressor and tachycardic components of the defense response in the rabbit. Conversely, microinjection of muscimol into the DA attenuated the tachycardic, but not pressor, response to air-jet stress. These data are

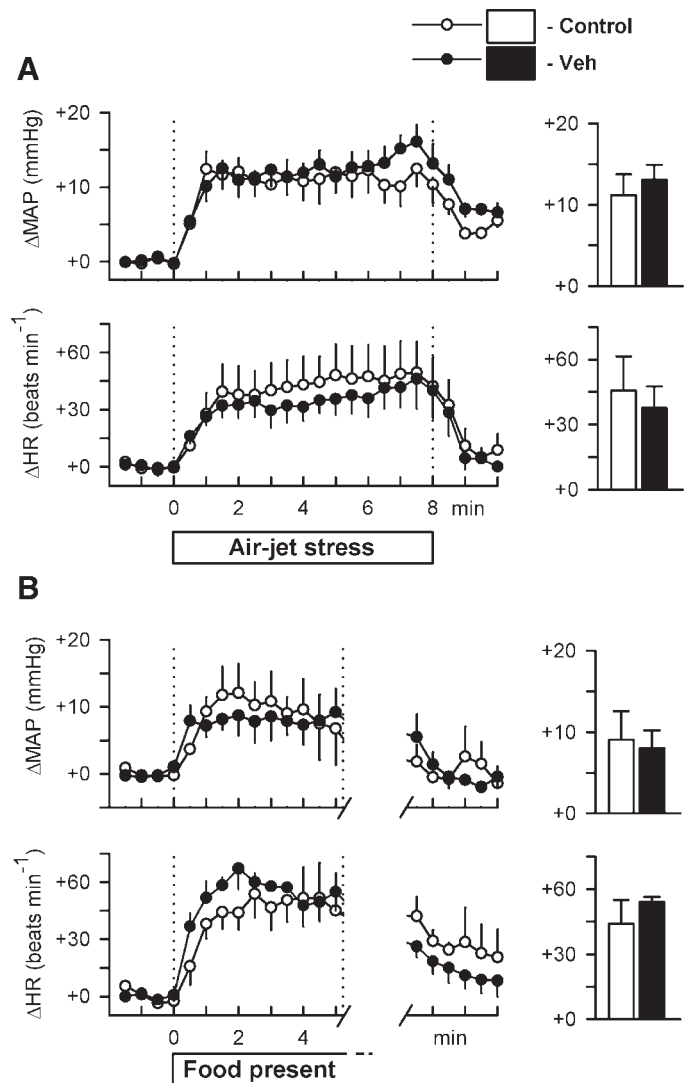


Fig. 7. Microinjections of vehicle (Veh; 100 nl; $n = 6$) into the DMH did not alter the cardiovascular response to air-jet stress (A) or feeding (B).

consistent with previous findings in rats that the DA is a key relay in the descending sympathoexcitatory pathway that mediates the tachycardic, but not pressor, response to DMH stimulation or air-jet stress (30, 31, 43).

In the present study, neuronal inhibition by muscimol in the DMH but not in adjacent hypothalamic regions elicited anorexia, so that animals showed little or no interest in food during several hours after injection. Accordingly, cardiovascular arousal, which was normally associated with food anticipation and eating in overnight fasted rabbits, was abolished by muscimol. Again, this effect was unlikely to be caused by the spread of muscimol into the ventromedial or paraventricular hypothalamic nuclei, because it stimulates, not inhibits, ingestive behavior in these nuclei (19, 27). Thus our data suggest that the DMH plays a key role in integrating the behavioral and autonomic components of the feeding response in rabbits. Previous observations in rats that blockade of GABAergic inhibition in the DMH increases intestinal motility (16), whereas chemical lesion of the DMH causes sustained hypophagia (3), are in accord with this possibility.

It has been shown that cardiovascular arousal evoked by blockade of GABAergic inhibition in the DMH or by emotional stress depends on activation of local EAA receptors in rats (35, 36). Similarly, in our study, microinjection of an EAA antagonist, kynurenic acid, into the DMH effectively decreased the pressor response to air-jet stress. An important new finding is that the cardiovascular response to feeding, which had a similar magnitude as that caused by stress, was also attenuated by kynurenic acid. One possibility is that this attenuation was in fact mediated by a partial loss of appetite in response to the blockade of local EAA inputs, and thereby motivational value of the stimulus. This seems unlikely, however, because rabbits ate with the same intensity before and after treatment, as estimated by the time spent eating the standard amount of food. Thus it appears that, in moderately fasted rabbits, local EAA inputs are important in regulating cardiovascular arousal associated with eating, but exert a limited tonic influence on neural circuits that control eating as such. Nevertheless, a prolonged bout of intensive eating caused by the glutamate analog kainic acid suggests that these excitatory inputs may initiate eating behavior under other conditions. Previous findings that electrical stimulation of the DMH elicited a voracious drive to eat in cats and elicited hyperphagia in sheep (3) are in accord with this possibility.

Another novel finding of the present study is that microinjection of a selective AT₁-receptor antagonist, candesartan, into the DMH, but not nearby hypothalamic sites, attenuated cardiovascular effects of emotional stress. Accordingly, forebrain AT₁ receptors have been implicated in physiological arousal caused by various emotional stressors in rats (17, 20, 29, 42). It seems unlikely that the decrease in the stress response in our study was due to a nonspecific inhibitory action of candesartan, associated with microinjecting the highly concentrated solution of this substance. Indeed, injection of a similar dose of candesartan (200 pmol) into the rostral ventrolateral medulla (RVLM), which forms a final common pathway for sympathoexcitation (8), decreased the pressor response to stress, without affecting the sympathoexcitatory response to baroreceptor unloading or local injection of glutamate (24). Given that the RVLM mediates sympathoactivation elicited by

DMH stimulation (14), our data suggest that ANG II-sensitive cells may form an essential part of hypothalamic-medullary circuits that are specifically involved in integrating the cardiovascular component of the defense response, although further experiments are clearly necessary to validate this possibility. The cellular and intracellular mechanisms that underlie the excitatory actions of ANG II during stress also remain to be established. Our preliminary data, however, suggest that the superoxide-sensitive signaling pathway may be implicated in these actions of ANG II in the RVLM (26) and also possibly in the DMH (9), as local microinjections of superoxide scavengers tempol and tiron attenuated the cardiovascular response to air-jet stress in rabbits.

In contrast to the stress-induced arousal, microinjection of candesartan into the DMH did not affect cardiovascular correlates of eating behavior, which is normally regarded as appetitive or positively motivated (28). Conversely, decreased ANG II receptor binding in several forebrain nuclei, including the DMH, has been linked to the lactation-induced hyperphagia (37). However, because lactation is also associated with reduced responsiveness to stress (7, 39), it is plausible that the decreased ANG II binding in lactating rats reflected the latter phenomenon. It could also be argued that the inhibitory effect of candesartan on the feeding-induced arousal was masked by its yet unknown orexinergic action that increased the motivational value and thereby intensity of the stimulus. This seems unlikely, however, because blockade of AT₁ receptors nearly abolished the pressor response to air-jet (24), without altering that to feeding (D. N. Mayorov, unpublished observations) in the RVLM, which has not been implicated so far in appetite regulation (4). Thus our results indicate that, under experimental conditions, AT₁ receptors in the DMH play little role in cardiovascular arousal initiated by appetitive ingestive behavior. Our data cannot exclude that AT₁ receptors in the DMH are more important in modulating cardiovascular arousal elicited by other types of nonaversive stimuli. However, the independence of feeding-induced arousal from local AT₁ receptors may have much broader implications, at least for the rabbit. Indeed, in individually housed laboratory rabbits, feeding appears to be the sole determinant of circadian rhythms of blood pressure, HR, and locomotor activity (41). Neurochemical mechanisms that selectively control, in the DMH, the cardiovascular response to feeding remain to be determined. However, it is tempting to suggest that local orexinergic and anorexigenic peptides, including NPY and leptin, may be important in regulating the cardiovascular correlates of appetitive ingestive behavior.

In summary, the present results indicate that the DMH plays a key role in regulating cardiovascular and behavioral arousal associated with the appetitive feeding response and the defense response in rabbits. The present results also suggest that local glutamatergic inputs may be important in mediating cardiovascular arousal evoked by both stress and feeding, although they appear to play a limited role, in moderately fasted rabbits, in controlling eating as such. Finally, these results provide initial evidence that locally released ANG II, acting through AT₁ receptors, may selectively modulate the activity of a subset of local neurons that are primarily associated with the cardiovascular defense response.

GRANTS

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