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The role of hypothalamic excitatory amino acid (EAA) receptors in mediating the cardiovascular response to stress was examined using conscious chronically instrumented rats. Microinjection of the EAA agonists *N*-methyl-D-aspartic acid (NMDA; l-10 pmol), α-amino-3-hydroxy-5-methyl-4-isooxazolepropionic acid (AMPA; 0.3-3.0 pmol), or kainic acid (0.1-1.0 pmol) into the dorsomedial hypothalamus (DMH) elicited dose-related increases in heart rate and modest elevations in arterial pressure. Local microinjection of the NMDA antagonist 2-amino-5-phosphonopentanoic acid (AP5; 100 pmol) selectively blocked NMDA-induced cardiovascular changes, whereas the non-NMDA EAA antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 50 pmol) selectively blocked the responses to AMPA and kainic acid. In the stress trials, microinjection of the nonselective EAA antagonist kynurenic acid (l-10 nmol) into the DMH blocked air stress-induced tachycardia in a dose-related manner. Similar injection of kynurenic acid at sites lateral or posterior to the DMH or injection of xanthurenic acid (a structural analogue of kynurenic acid with no antagonistic properties at EAA receptors) into the DMH failed to influence air stress-induced cardiovascular changes. Injection of either AP5 or CNQX into the DMH at dosess hown to be selective for their respective EAA receptor subtypes also attenuated air stress-induced tachycardia. Thus activity at EAA receptors in the DMH appears to be necessary for the generation of stress-induced changes in heart rate.

Historically, the hypothalamus has been implicated repeatedly with central nervous system mechanisms generating the cardiovascular response to emotional arousal and stress. More recently, hypothalamic microinjection of agents that interfere with the postsynaptic actions of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) has been shown to evoke sympathetically mediated elevations in heart rate and arterial pressure in conscious (31) and anesthetized (9, 10) rats. Sites reactive to the GABA antagonists have now been localized to the dorsomedial hypothalamus

(DMH) (24, 25). Conversely, bilateral injection of the GABA agonist muscimol (19) or unilateral microdialysis with the GABA uptake inhibitor nipecotic acid, coupled with contralateral injection of muscimol (1) at this same hypothalamic site, attenuates air stress-induced tachycardia. Thus GABA receptors in the DMH may regulate, in part, neurons involved in mediating the cardiovascular response to stress.

Potential excitatory inputs to this hypothalamic mechanism are as yet undefined. However, microinjection of excitatory amino acids (EAAs) into the DMH of urethan-anesthetized rats elicits marked increases in heart rate and modest elevations in arterial pressure (24). Similar cardiovascular changes caused by injection of GABA antagonists at this same site can be blocked or reversed by local injection of EAA antagonists (25). Therefore these studies are consistent with the notion that EAAs, such as glutamate, may be important excitatory neurotransmitters in the hypothalamus (29). More specifically, a balance between tone at inhibitory $GABA_A$ receptors and EAA receptors may regulate the excitability of a population of neurons in the DMH whose activity is critical for the generation of stress-induced cardiovascular changes in the rat.

The present study addresses the hypothesis that EAA receptors in the DMH mediate the cardiovascular response to acute stress. In the first series of experiments, we examined the effects on heart rate and arterial pressure caused by local injection of the relatively selective EAA agonists *N*-methyl-D-aspartic acid (NMDA), α-amino-3-hydroxy-5-methyl-5-isoxazolepropionic acid (AMPA), and kainic acid (KA) in conscious chronically instrumented rats. The NMDA receptor antagonist 2-amino-5-phosphonopentanoic acid (AP5) and the non-NMDA EAA receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) were used to determine the role of specific EAA receptor subtypes in generating the effects of each agonist. In the final series of experiments, we examined the effects of an air stress paradigm on heart rate and arterial pressure after local injection of the nonselective EAA receptor antagonist kynurenic acid or injection of either AP5 or CNQX at doses shown to be selective for their respective EAA receptor subtypes.

METHODS

Male Sprague-Dawley rats (240-320 g, Harlan Industries Indianapolis, IN) were housed individually under controlled temperature, humidity, and light periodicity with free access to food and water. All procedures employed were approved by the Indiana University Campus Animal Resource Committee. Rats anesthetized with pentobarbital sodium (50 mg/kg ip) and given atropine sulfate (2 mg/kg ip administered to limit bronchial secretions during surgery) were positioned in a stereotaxic frame with the upper incisor bar placed 5 mm above the interaural line. Heart rate was monitored throughout the remainder of the procedure with a cardiotachometer triggered by lead II of the electrocardiogram and recorded on a Beckman 511A strip chart recorder. Once the overlying skin and connective tissue were cleared, holes were drilled into the skull to allow access to the brain. The stereotaxic coordinates for the DMH were 1.2 mm posterior to bregma, 8.8 mm below the surface of the skull, and 0.5 mm lateral from midline. The injector cannula (33 gauge, 12 mm length), fitted in a stainless steel guide cannula (26 gauge, 11 mm length), was lowered unilaterally to the target coordinates at a 10˚ angle with respect to the sagittal plane (entry through a hole in the skull 2 mm from midline) to avoid damaging the midsagittal sinus. Proper placement of the cannula was verified by infusing 10 pmol bicuculline methiodide (BMI) in 100 nl over 12 s. Unilateral injection at active sites in this manner increased heart rate by at least 90 beats/min with an onset of $\leq$ 1 min. If heart rate failed to increase according to these criteria, the cannula was removed from the brain and repositioned by 0.3 mm, and the new placement was then tested. After placement was verified at an active site and heart rate was allowed to return to baseline, the same procedure was performed on the opposite side. (Note that atropine was not given other than during surgery, allowing for the additional contribution of changes in cardiac vagal tone to effects on heart rate seen during experimental protocols in conscious rats.) The coordinates for implantation at sites other than the DMH were as follows: posterior hypothalamus, 2.0 mm posterior to bregma, 9.0 mm below the surface of the skull, and 0.5 mm lateral from midline; lateral hypothalamus, 1.2 mm posterior to bregma, 8.8 mm below the surface of the skull, and 1.5 mm lateral from midline. To verify that these were inactive sites, 10 pmol BMI was infused to demonstrate a lack of effect on heart rate. After both cannulas were positioned, the guide cannulas were cemented in place using cranioplastic cement anchored to the skull with three stainless steel screws. The injector cannulas were removed and replaced with wire dummy cannulas to seal the guide cannula. The animals were removed from the stereotaxic frame and allowed to recover in individual cages.

One to three days after implantation of the animals were again anesthetized with pentobarbital sodium. The femoral artery and vein were cannulated with a 4-cm length of Tygon microbore tubing (0.01 in. ID) attached to a 30-cm length of Tygon tubing (0.02 in. ID) and sealed with a stylet. The tubing was then routed subcutaneously to the nape of the neck and through a leather harness fastened around the forequarters of the rat. The animals were allowed to recover in their individual cages for 2-3 days before experiments were performed. By this time, rats had resumed their regular eating, drinking, and grooming habits and exhibited no signs of stress or pain.

Each experiment began with a 10- to 20-min period during which heart rate and arterial pressure were continuously monitored while the animal was allowed to move freely in its home cage. When a stable baseline had been attained, injection cannulas loaded with either artificial cerebrospinal fluid [aCSF; composition in mM: 124 NaCl, 2 KCl, 2 $MgC1_2$, 26 $NaHCO_3$, 1.25 KH_2P0_4, 2 $CaCl_2$, and 11 glucose (18)] or a drug solution (in aCSF) were seated in the guide cannulas. Whenever possible, injection cannulas were seated while the animal sat undisturbed in its home cage, although occasionally the rat was gently hand held for the procedure with every attempt made to minimize undue stress. All injections were bilateral (100 nl/side infused over 12 s) and were made using two l0-μ1 Hamilton syringes mounted in a Harvard infusion pump. Injection cannulas remained in place 1 min after the infusion ended and then were removed. Experiments took place between 10 A.M. and 4 P.M. Individual rats received no more than six injections.

In those experiments examining the effects of EAA receptor agonists on cardiovascular function, rats received injections of three different doses of a single EAA plus injection of aCSF in staggered order over the course of a day. Injections were spaced in time such that any effects on heart rate caused by the previous injection had dissipated before the next injection.

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In experiments assessing the effect of EAA receptor antagonists on air stress-induced cardiovascular changes, each rat received four treatments: injection of aCSF and of a single dose of an EAA antagonist before stressed and unstressed conditions. In unstressed trials, the rat remained in its home cage while heart rate and arterial pressure were monitored for 30 min after injection. In the stress trials rats were confined in a Plexiglas cylinder (21 cm in length and 7 cm diam) immediately after removal of the injection cannulas. An aperture (0.8 cm diam), located at one end of the cylinder ~5 cm from the rat's head, was connected by latex tubing to an air jet so that a stream of air at a constant and specific flow rate (37 l/min) was directed at the rat's head. The stream of air was delivered for an 18-min period starting 2 min after microinjection. At 20 min, the air was turned off and the rat was returned to its home cage. Each rat received four treatments in a 2 × 2 factorial design (aCSFstress, E AA antagonist-stress, aCSF-no stress, and EAA antagonist- no stress) in staggered order. The stress trials were separated by 2-3 days.

At the end of experiments the animals were anesthetized (pentobarbital sodium, 50 mg/kg ip), and the injection site was marked by infusing 100 nl of a suspension of india ink diluted 1:l with saline. The injector remained in place for 10 min after infusion of the marker, at which time the injector was slowly removed. The animals were killed, and the brains were perfused with 50 ml of saline followed by 300-500 ml of fixative solution (1% paraformaldehyde and 1.25% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4) over 20-40 min. The brain was removed and stored in the fixative solution for l-10 days at room temperature. Coronal sections (40 μm) were cut on a freezing microtome, mounted on gelatin-coated slides, and stained with a 1% neutral red solution. The locations of the injection sites were determined according to the atlas of Paxinos and Watson (23).

Results are expressed as means ± SE. Dose-response relationships were analyzed using regression analysis. The effects of EAA antagonists on EAA-induced cardiovascular changes were analyzed using analysis of variance (ANOVA) with repeated measures and Newman-Keuls. The effects of stress, EAA antagonists, and the interaction of stress and EAA antagonists on

cardiovascular function were analyzed using 2 × 2 ANOVA and Scheffé's tests. The effects of xanthurenic acid and aCSF and the effects of kynurenic acid and aCSF injected at sites outside and DMH on stress-induced cardiovascular changes were analyzed using paired *t* tests. Baseline values for heart rate and arterial pressure were compared between groups of animals using one-way ANOVA. The 5% limits of probability were accepted as significant.

RESULTS

Bilateral injection of NMDA, AMPA, or KA into the DMH of conscious rats increased heart rate and arterial pressure (Fig. 1). The three EAAs increased heart rate in a dose-dependent manner and by similar magnitudes (Table 1). However, only NMDA and AMPA produced dose-related increases in arterial pressure at the doses tested. The cardiovascular effects caused by these agents began rapidly; in many instances heart rate started to rise during the infusion, while in the remainder of the cases the tachycardic response began within 45 s of starting the infusion.

Microinjection of each of the EAAs usually produced increases in locomotor activity. Although not quantitated, these changes consisted of intermittent running, rearing, and grooming. This activity generally appeared to follow the same time course as the cardiovascular changes. However, in many animals, the activity was intermittent, and during quiescent periods in these rats, heart rate and arterial pressure remained elevated.

A series of experiments was undertaken to assess the role of different subtypes of EAA receptors in the cardiovascular effects caused by injection of NMDA, AMPA, and KA. Each EAA was injected 4-5 min after local pre-treatment with either 100 nl aCSF, 100 pmol of the NMDA receptor antagonist AP5, or 50 pmol of the non-NMDA EAA receptor antagonist CNQX. AP5 blocked the increases in heart rate and arterial pressure caused by 10 pmol NMDA but did not significantly affect the cardiovascular changes caused by either 3 pmol AMPA or 1 pmol KA. Conversely, CNQX blocked a similar cardiovascular response caused by either AMPA or KA without significantly affecting NMDA-induced changes (Fig. 2).

To examine the role of hypothalamic EAA receptors in mediating air stress-induced cardiovascular changes, EAA antagonists and aCSF were microinjected bilaterally into the DMH before stress trials. After injection of aCSF, air stress caused immediate and sustained increases in heart rate and arterial pressure ranging from 130 to 170 beats/min and from 18 to 37 mmHg, respectively (Fig. 3). Typically, after a brief initial period of struggling (i.e., during the first l-2 min of air stress, 3-4 min postinjection), rats remained relatively quiescent during the remainder of the stress trial. In rats receiving bilateral injections of 1, 3, or 10 nmol kynurenic acid, air stress-induced tachycardia was attenuated in a dose-dependent manner at 5 min after injection (Fig. 4; see Fig. 8), and the l0-nmol dose of kynurenic acid also significantly attenuated the increase in arterial pressure at this time point. The effect of kynurenic acid on these cardiovascular changes was not sustained throughout the stress period as indicated by the rise in heart rate and arterial pressure to levels approaching those seen after vehicle treatment. The mean baseline heart rates in these three groups of animals ranged from 327 ± 12 to 355 ± 16 beats/min and were not significantly different before any stress trial. Likewise, mean baseline arterial pressures ranged from 108 ± 3 to 114 ± 4 mmHg and were not significantly different before any stress trial.

Given the significant effects of kynurenic acid on stress-induced cardiovascular changes, experiments were undertaken to determine the effects of this agent on baseline cardiovascular function in the same animals (Fig. 5). In these experiments kynurenic acid and aCSF were injected under unstressed, or resting, conditions. Individual changes from baseline in heart rate and arterial pressure occurring 5 min after injection of aCSF ranged from -10 to +20 beats/min and from -5 to +11 mmHg, respectively. Five minutes after injection of 10 nmol kynurenic acid, individual changes from baseline heart rate and arterial pressure ranged from -30 to +10 beats/min and from -13 to -2 mmHg, respectively. The effects of the l0-nmol kynurenic acid treatment on heart rate were not significantly different from the effects of vehicle treatment, whereas kynurenic acid did produce a modest but significant depression of arterial pressure (-8 ± 2 mmHg) compared with vehicle treatment (0 ± 2 mmHg). The l- and 3-nmol doses of kynurenic acid did not produce effects on heart rate or arterial pressure different from those produced by aCSF at 5 min after injection in the unstressed trials. However, at time points beyond 5 min, 3 and 10 nmol kynurenic acid did produce modest but significant decreases in heart rate.

In a series of control experiments, the pharmacological and anatomic specificity of kynurenic acid was examined. In one group of animals aCSF and xanthurenic acid, a structural analogue of kynurenic acid with no antagonistic properties at EAA receptors, were microinjected into the DMH before air stress trials. The air stress-induced tachycardia (+156 ± 19 beats/min) and hypertension (+33 ± 3 mmHg) occurring 5 min after injection of aCSF were not different from the changes in heart rate and arterial pressure caused by air stress in the same animals treated with 10 nmol xanthurenic acid (+155 ± 13 beats/min and +30 ± 3 mmHg, respectively; $n = 3$). To determine whether the effects of kynurenic acid on air stress-induced tachycardia were localized to the DMH, 10 nmol kynurenic acid was injected bilaterally at sites lateral ($n = 3$) or posterior ($n = 4$) to this area (squares in Fig. 6). Injection of kynurenic acid at sites lateral to the DMH failed to attenuate stress-induced tachycardia (+158 ± 16 beats/min) or hypertension (+29 ± 3 mmHg) compared with these same animals treated with aCSF (+167 ± 17 beats/min and +33 ± 6 mmHg). Similarly, injection of kynurenic acid at sites posterior to the DMH failed to attenuate air stress-induced tachycardia (+146 ± 9 beats/min) or hypertension (+33 +3 mmHg) compared with these same animals treated with aCSF (+150 ± 10 beats/min and +32 ± 2 mmHg).

The above experiments suggest experiments suggest that EAA receptors in the DMH are involved in mediating the cardiovascular response to air stress. To examine the role of specific subtypes of EAA receptors in this response, AP5 and CNQX were injected at doses shown in the previous experiments (see above and Fig. 2) to block selectively NMDA and non-NMDA EAA receptor subtypes, respectively. After injection of aCSF, air stress caused immediate and sustained increases in heart rate and arterial pressure as was seen in previous experiments. However, 5 min after injection of either 100 pmol AP5 or 50 pmol CNQX, stress-induced tachycardia was attenuated by ~30% (Figs. 7 and 8). When the same doses of AP5 and CNQX were combined as a single treatment, the tachycardic response was reduced by ~60%. Neither antagonist, alone or in combination, significantly reduced the hypertension caused by air stress at the 5-min time point. The mean baseline heart rates in these three groups ranged from 331 ± 16 to 370 ± 17 beats/min and were not significantly different before any stress trial. Similarly, the mean baseline arterial pressures ranged from 105 ± 5 to 114 ± 5 mmHg and were not significantly different before any stress trial.

In experiments similar to those using kynurenic acid, the effects of the selective antagonists on baseline cardiovascular function were examined (Fig. 9). In these experiments aCSF, AP5, CNQX, and the combination of AP5 and CNQX were injected under unstressed conditions in the same animals used in the above stress trials. Under unstressed conditions, the individual changes in heart rate and arterial pressure from baseline occurring 5 min after injection of aCSF ranged from -5 to +30 beats/min and from -5 to +14 mmHg, respectively. Five minutes after injection of the combination of AP5 and CNQX, individual changes from baseline in heart rate and arterial blood pressure ranged from -40 to 0 beats/min and from -15 to -6 mmHg, respectively. Injection of these two antagonists together resulted in significant lowering of basal heart rate (-23 ± 11 beats/min) and arterial pressure (-10 ±2 mmHg) compared with vehicle treatment (+8 ± 2 beats/min and +3 ± 2 mmHg). However, injection of either antagonist alone produced no significant effects on basal heart rate or arterial pressure under resting conditions.

Histological analysis indicated that cannula placements for sites defined as active (see METHODS) were localized to an area within or immediately adjacent to the hypothalamic dorsomedial nucleus as defined by the atlas of Paxinos and Watson (circles in Fig. 6). Sites defined as inactive were found to be located l-2 mm away from the dorsomedial nucleus (squares in Fig. 6).

DISCUSSION

The present study demonstrates the *1)* stimulation of EAA receptors in the DMH of conscious rats elicits increases in heart rate and arterial pressure and *2)* blockade of these receptors prevents similar cardiovascular changes in response to an air stress paradigm.

Microinjection of the EAAs NMDA, AMPA, and KA increases in heart rate accompanied by modest elevations in arterial pressure. The ability of these agents to elicit such changes in cardiovascular function is in disagreement with other reports. In these previous studies, focal injection of molar concentrations of the nonselective EAAs glutamate or DL-homocysteic acid at sites throughout the hypothalamus, including the DMH, failed to elicit increases in heart rate and arterial pressure in anesthetized (12) and conscious (14) rats. Injection of these same agents (and concentrations) into the periaqueductal gray elicited increases in heart rate and arterial pressure as

well as stresslike behavioral changes, often termed the defense reaction (3, 14). Therefore these authors concluded that neurons originating in the hypothalamus are not involved in generating or mediating the cardiovascular cardiovascular changes associated with stress. However, the ability of molar concentrations of EAAs to stimulate neurons in certain areas of the central nervous system has been questioned. Lipski and colleagues (18) demonstrated that while relatively low concentrations elicited the expected increases in local unit activity, microinjection of 0.5-1.0 M solutions of glutamate or DL-homocysteate failed to produce long-lasting excitation of the same neurons as would have been predicted. In a similar fashion, we have shown in a previous study that microinjection of relatively high doses of NMDA into the DMH of anesthetized rats produced inconsistent effects on heart rate, whereas lower doses (similar to those used in the present study) consistently elevated heart rate (24). The present study confirms and extends our previous finding that injection of EAAs into the DMH of the rat elicits significant elevations in heart rate and arterial pressure.

To determine whether the cardiovascular responses to microinjection of the different EAAs were the result of activating specific EAA receptor subtypes, EAA receptor antagonists were used. AP5 has been identified as a competitive antagonist selective for the NMDA receptor subtype (8, 30), while CNQX has been shown to act as a competitive antagonist at non-NMDA EAA receptors (6, 15, 30). Selectivity is a potential problem with the latter agent because at higher doses, CNQX can also antagonize NMDA receptor-mediated responses by blocking the strychnine-insensitive glycine site associated with this receptor complex (5,30). Therefore, wherever CNQX is to be employed as a selective antagonist at non-NMDA EAA receptors, appropriate doses of this agent must be established in the particular experimental setting. The doses of AP5 and CNQX used in the present study were chosen based on our previous work with anesthetized rats in which we demonstrated the selectivity of these agents. In the present experiments AP5 blocked the increase in heart rate caused by NMDA without significantly affecting a comparable increase in heart rate elicited by AMPA or KA. Conversely, CNQX attenuated the tachycardic response to AMPA and KA without affecting NMDA-induced tachycardia. In these experiments, the antagonists were injected 4-5 min before injection of the agonist. This protocol was chosen to determine the selectivity and effectiveness of the antagonists 5 min postinjection, a time point that was used in analyzing the effects of these drugs in later experiments involving the air stress paradigm.

The air stress paradigm has been previously used to study the effects of environmental stress on cardiovascular and renal function (1,16,17,19,20). The systemic and regional hemodynamic changes observed during air stress are similar to those reported to occur in conscious rats during other forms of stress (22). In particular, these changes include elevations in heart rate and arterial pressure, increases in mesenteric and renal vascular resistance, and decrease in hindquarter vascular resistance (16). Therefore, in conscious rats, the air stress paradigm can elicit cardiovascular changes characteristic of the defense reaction.

Injection of the nonselective EAA antagonist kynurenic acid into the DMH attenuated air stress-induced tachycardia in a dose-dependent manner. In contrast, xanthurenic acid, a structural analogue of kynurenic acid without significant antagonistic properties at EAA receptors (13), did not reduce the cardiovascular response to air stress when injected at this site. Furthermore, injection of kynurenic acid at sites 0.5-1.0 mm lateral or posterior to the DMH did not reduce the cardiovascular response to stress. Therefore EAA receptors in the DMH may be involved in mediating the cardiovascular response to stress.

To examine the role of specific subtypes of EAA receptors in this response, selective antagonists for EAA receptor subtypes were used. Previous experiments in conscious animals indicated that, under the experimental conditions employed, the doses of the NMDA receptor antagonist AP5 and the non-NMDA EAA receptor antagonist CNQX are selective for their respective EAA receptor subtypes. When either AP5 or CNQX was injected before stress trials, the tachycardic response to stress was reduced by ~30%. When the two EAA antagonists were combined as a pretreatment before stress, the effects on air stress-induced tachycardia appeared additive; the response was reduced by ~60%. Therefore it appears that there are two distinct components of stressinduced tachycardia. One component relies upon activity at NMDA receptors, and the other is mediated through non-NMDA EAA receptors.

The effects of EAA receptor blockade on stress-induced changes in arterial pressure were less clear. Injection of AP5, CNQX, or the combination of AP5 and CNQX before stress failed to significantly reduce the increases in arterial pressure. In fact, the l0-nmol dose of kynurenic acid

was the only treatment that reduced the stress-induced hypertension. Two possibilities seem most likely to be responsible for the inability of these agents to reduce the increases in arterial pressure while at the same time they produce consistent effects on heart rate. First, the changes in arterial pressure may be less sensitive to EAA receptor blockade, and, therefore, higher doses of EAA antagonists may be needed to show a reduction in arterial pressure. Second, given the multitude of systems (neural and humoral) that may be activated or inhibited during stress and the numerous variables that ultimately affect arterial pressure, it may be difficult to produce consistent effects on arterial pressure under this level or degree of stress. A third possibility that should be mentioned is that the hypothalamic region generating stress-induced increases in arterial pressure is more extensive than that responsible for stress-induced tachycardia. As a result, the effects of stress on blood pressure may be more difficult to block with a discrete local microinjection than those on heart rate.

The reduction in treatment with any of the EAA antagonists was not sustained throughout the entire period of stress. Several factors may account for this finding. First, the duration of the effect of these drugs may be limited by their diffusion, metabolism, or uptake. Second, although the air stress-induced increase in heart rate seen in vehicle-treated animals is immediate and sustained at the same level throughout the air stress trial, the concentration of EAAs in the relevant hypothalamic synapses may continue to rise throughout the stress trial and gradually overcome the competitive blockade of EAA receptors. Third, other transmitter systems may play a preferential role in mediating the cardiovascular changes associated with the later stages of the air stress paradigm. Opioids (20), corticotropin-releasing factor (11), norepinephrine (16, 17), and oxytocin (7) have been implicated in central pathways mediating the cardiovascular changes associated with experimental stress, and these signaling systems may be mobilized more slowly and act independently of hypothalamic EAAs.

The ability of the EAA antagonists to prevent stress-induced tachycardia contrasts with the effects of these agents on basal heart rate and arterial pressure. Hypothalamic injection of EAA antagonists in conscious rats produced only a modest reduction in basal heart rate. Interestingly, the time at which basal heart rate was significantly lowered in unstressed rats (i.e., 10-25 min after injection) did not correspond with the time of maximal suppression of tachycardia seen in the stress

trials (5 min after injection). This observation might suggest that spread of the EAA antagonists to a site other than the point of injection in the DMH may be responsible for lowering heart rate at these later time points. In previous studies involving smaller volumes of injection in urethananesthetized rats, microinjections of kynurenate or AP5 at this site had no effect on either cardiovascular parameter (24). Similar results were reported for the effects of the $GABA_A$ receptor agonist muscimol when microinjetted at this site in stressed and unstressed conscious rats (19) or in anesthetized animals (31). Thus neurons affected by the EAA antagonists or by muscimol in these studies appear to contribute minimally to the maintenance of basal cardiovascular function but are recruited primarily under conditions of stress.

Sites reactive to BMI under anesthesia and subsequently reactive to microinjection of EAAs in conscious animals were within or immediately adjacent to the DMH. A similar pattern of anatomic localization was found for sites at which blockade of EAA receptors effectively antagonized stress-induced tachycardia. Conversely, sites l-2 mm posterior or lateral to the DMH were unreactive to BMI under anesthesia and were not associated with suppression of stress-induced tachycardia upon injection of kynurenic acid. The DMH projects to a number of sites in the central nervous system involved in the control of autonomic nervous system activity (26,28). Fibers originating in the DMH innervate *1*) the nucleus tractus solitarius, the primary site of input from baroreceptor afferents, *2*) the nucleus ambiguus, site of parasympathetic preganglionic neurons projecting to the heart, *3*) the intermediolateral cell column in the spinal cord where cell bodies of sympathetic preganglionic neurons are located, and *4*) the rostra1 ventrolateral medulla, an area thought to contain neurons responsible for sympathetic tone. In addition, the DMH projects to the parvocellular portion of the paraventricular nucleus of the hypothalamus (27), a site recently implicated in the generation of stress-induced tachycardia (7), and to the periaqueductal gray, a region where injection of EAAs elicits the defense reaction (2, 3, 14). Therefore, the known 13. connections of the DMH provide a morphological basis for its participation in the generation of the cardiovascular and perhaps other changes associated with the response to stress.

In summary, stimulation of EAA receptors in the DMH of conscious rate evokes cardiovascular changes that mimic those observed in stress. Conversely, blockade of these receptors prevents stress-induced tachycardia. Therefore the DMH appears to be the site of a

population of neurons whose activation by endogenous ligands for EAA receptors mediates the cardiovascular changes associated with stress.

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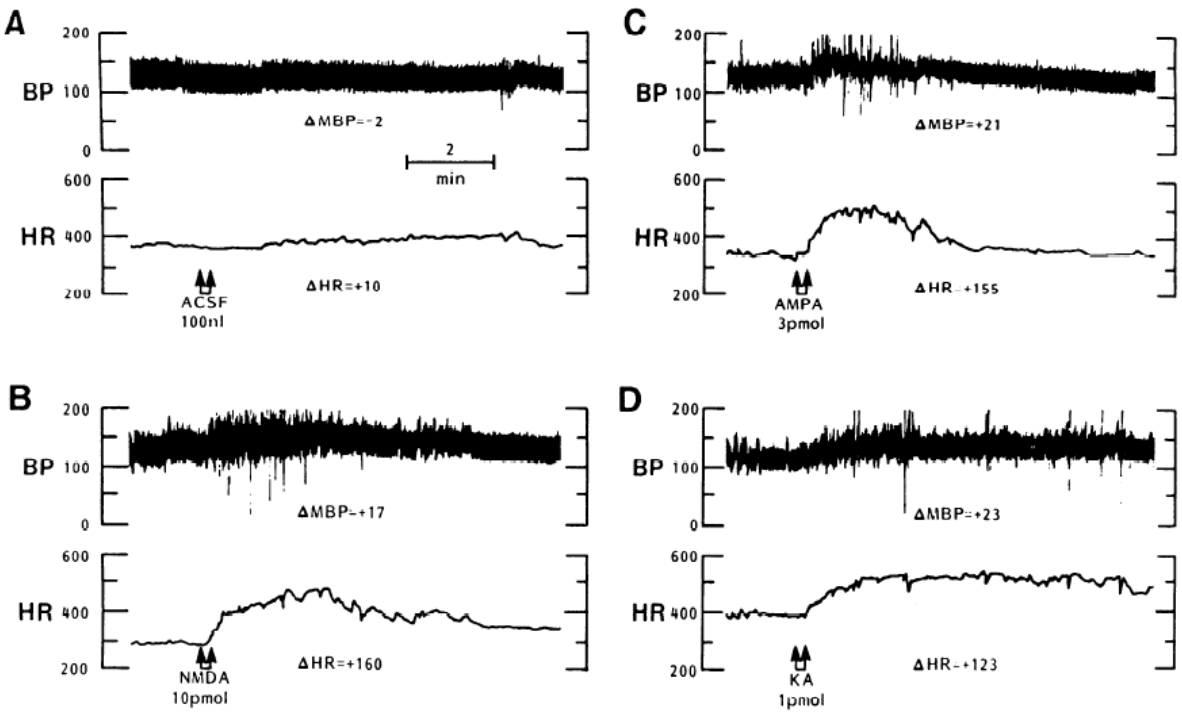

Fig. 1. Tracing of arterial blood pressure (BP, mmHg) and heart rate (HR, beats/min) from 4 conscious chronically instrumented rats depicting effects of bilateral microinjection of artificial CSF (aCSF; A), *N*-methyl-D-aspartic acid (NMDA; *B*), α-amino-3-hydroxy-5-methyl-5-isoxazolepropionic acid (AMPA; C), and kainic acid (KA; *D*) into dorsomedial hypothalamus. Injections were made in 100 nl of aCSF. Maximal changes in mean arterial blood pressure (MBP) and HR are indicated.

Table 1. *Cardiovascular effects caused by microinjection of NMDA, AMPA, KA, and aCSF into dorsomedial hypothalamus in conscious rats*

	Baseline		Maximum Change		Time to Maximum HR, min	Time to Recovery, min
	HR, beats/min	MAP, mmHg	HR, beats/min	MAP, mmHg		
aCSF (100 nl)	348±9	119±3	+9±4	+2±1		
NMDA						
1.0 pmol	323±15	124±6	+26±8	+4±2	1.1±0.3	3.1±0.6
3.0 pmol	316±15	121±6	+55±11	+12±4	1.5±0.3	3.9±0.9
10.0 pmol	331±9	125±7	+145±12	+22±1	1.4±0.1	6.5±0.6
AMPA						
0.3 pmol	336±4	115±4	+31±7	+4±2	1.0±0.3	3.2±0.4
1.0 pmol	341±9	112±5	+100±18	+21±2	1.0±0.1	3.9±0.4
3.0 pmol	335±8	116±4	+138±6	+23±1	1.2±0.2	6.1±0.7
KA						
0.1 pmol	370±12	113±3	+65±13	+14±3	1.4±0.4	6.0±0.7
0.3 pmol	361±5	117±3	+110±12	+15±3	1.1±0.1	9.0±0.5
1.0 pmol	356±11	121±4	+150±9	+18±2	1.6±0.2	10.3±0.8

Values are means ± SE; $n = 4$ for NMDA, AMPA and KA and 12 for aCSF. HR, heart rate; MAP, mean arterial pressure. See text for other definitions. Injections were bilateral in 100 nl/side. Dose of drug cited in table represents amount injected per side.

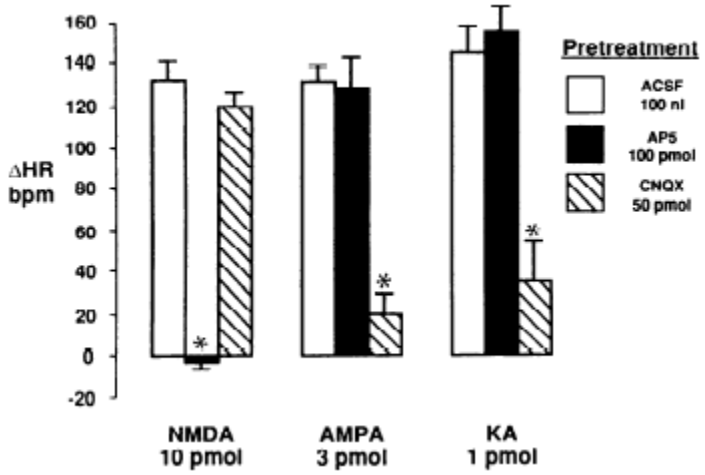

Fig. 2. Maximal changes in HR caused by intrahypothalamic injection of 10 pmol NMDA, 3 pmol AMPA, or 1 pmol KA after local pretreatment with 100 nl aCSF, 100 pmol 2-amino-5-phosphonopentanoic acid (AP5), or 50 pmol 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX); n = 3 rats/bar. Pretreatment injection occurred 4-5 min before injection of the excitatory amino acid (EAA). Injections were made in 100 nl of aCSF. Each set of injections (pretreatment followed by EAA) occurred on a separate day. * Significant difference from aCSF pretreatment.

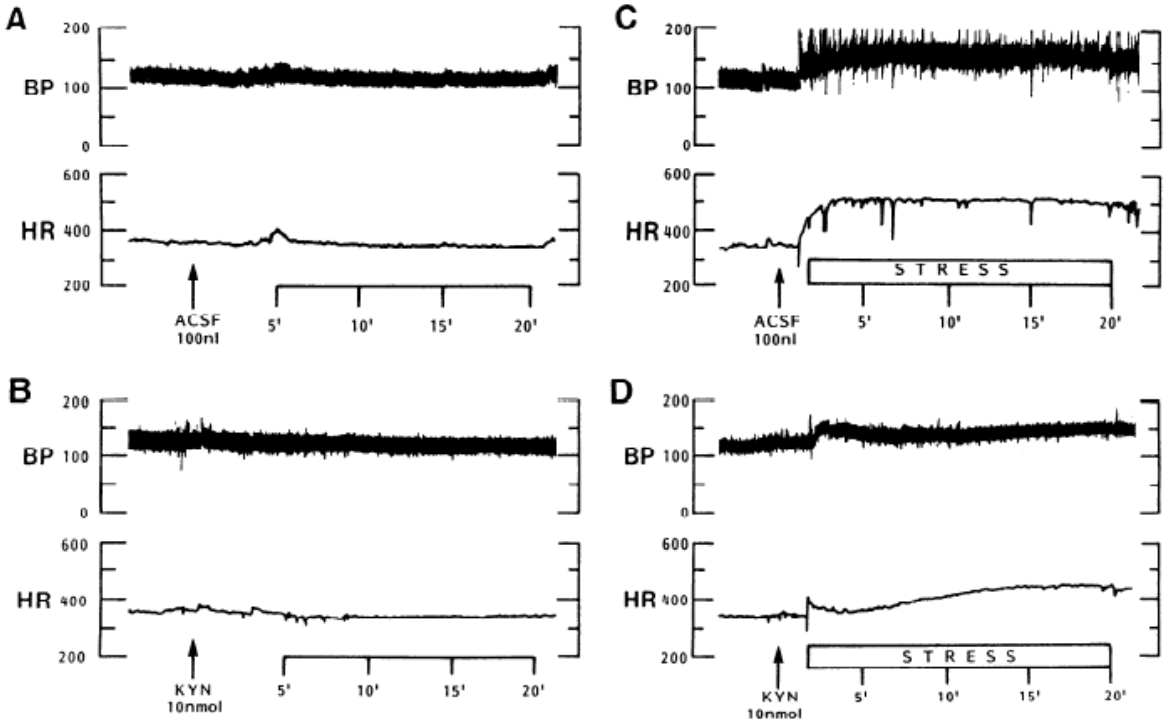

Fig. 3. Tracing of BP and HR from the same conscious chronically instrumented rat under resting conditions (*A* and *B*) or stress conditions (*C* and *D*) after bilateral microinjection of aCSF (*A* and *C*) or 10 nmol kynurenic acid (Kyn; *B* and *D*) into dorsomedial hypothalamus. Injections were made in 100 nl of aCSF. Doses are expressed as amount per side.

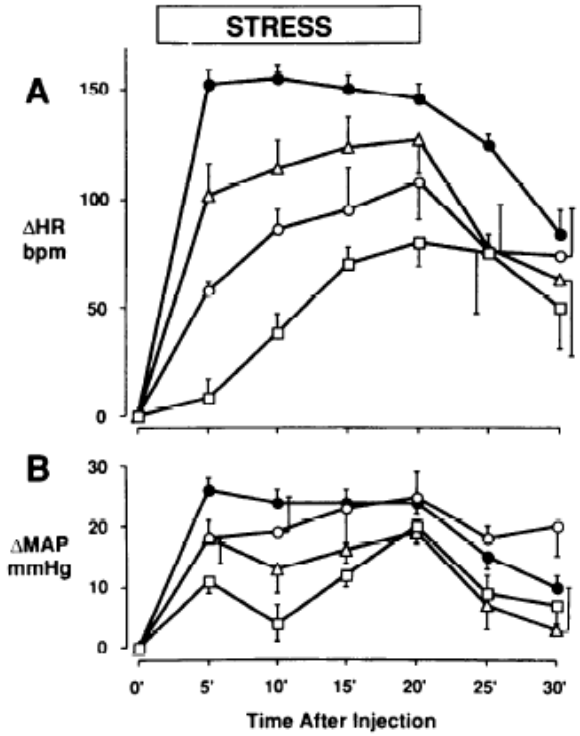

Fig. 4. Changes in HR (*A*) and mean arterial pressure (MAP; *B*) during air stress after bilateral injection of 100 nl aCSF (●; *n* = 12) or 1 (Δ; n = 4), 3 (○; *n* = 4), or 10 nmol kynurenic acid (□; *n* = 4). Air stress began 2 min after injection. Injections were made in 100 nl of aCSF. Doses are expressed as amount per side.

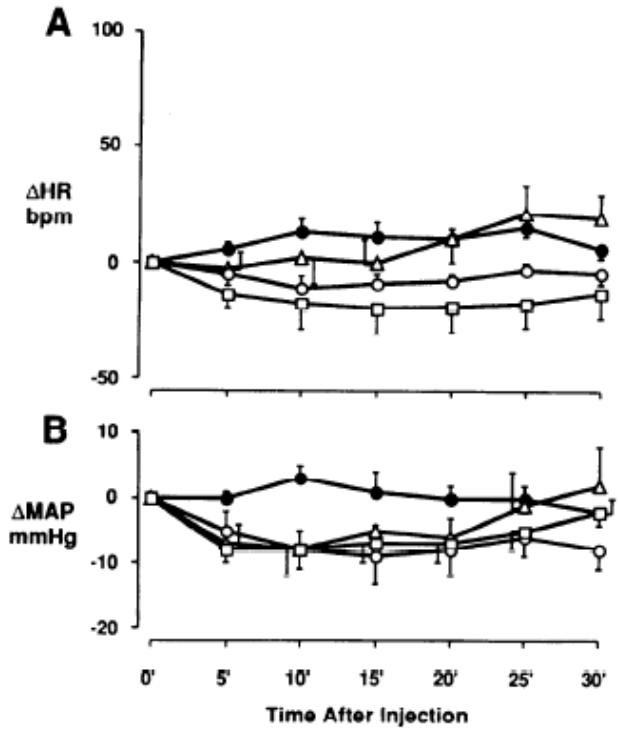

Fig. 5. Changes in HR (*A*) and MAP (*B*) during resting conditions after bilateral injection of 100 nl aCSF (●) or 1(Δ), 3 (○), or 10 nmol kynurenic acid (□). Rats remained in their home cages during these trials. Injections were made in 100 nl of aCSF. Doses are expressed as amount per side.

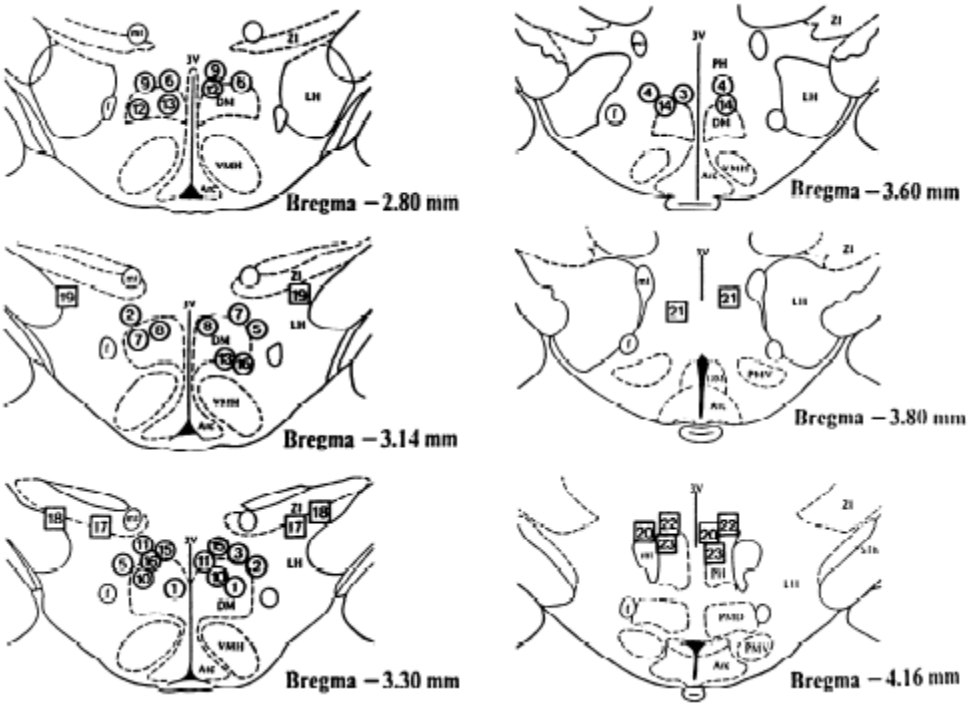

Fig. 6. Schematic coronal sections of rat brain adapted from the atlas of Paxinos and Watson (23). Numbers represent injection sites in each of 23 rats. Numbers in circles represent active sites as defined by tachycardic response to unilateral injection of bicuculline methiodide under anesthesia (see METHODS). Numbers in squares represent inactive sites as defined by these same criteria. *Rats 1-6* received bilateral injections of EAAs (NMDA, AMPA, or KA), *rats 7-11* and *17-23* received bilateral injections of kynurenic acid, and *rats 12-16* received bilateral injections of AP5 and/or CNQX. Arc, arcuate nucleus; DM, dorsomedial hypothalamic nucleus; f, fornix; LH, lateral hypothalamic area; mt, mammillothalamic tract; PH, posterior hypothalamus; PMD, dorsal premammillary nucleus; PMV, ventral premammillary nucleus; STh, subthalamic nucleus; VMH, ventromedial hypothalamus; ZI, zona incerta; 3V, third ventricle.

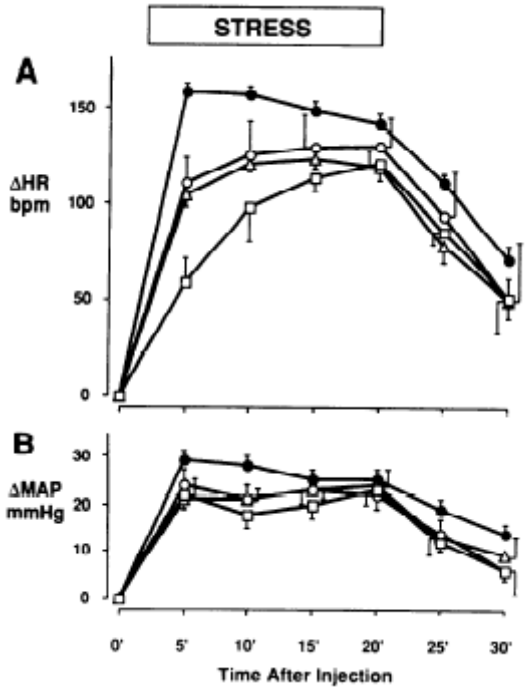

Fig. 7. Changes in HR *(A)* and MAP (*B*) during air stress after bilateral injection of 100 nl aCSF (●; $n = 12$), 100 pmol AP5 (Δ; $n = 4$), 50 pmol CNQX (○; 14 circles; $n = 4$), or the combination of AP5 and CNQX (□; $n = 4$). Air stress began 2 min after injection. Injections were made in 100 nl of aCSF. Doses are expressed as amount per side.

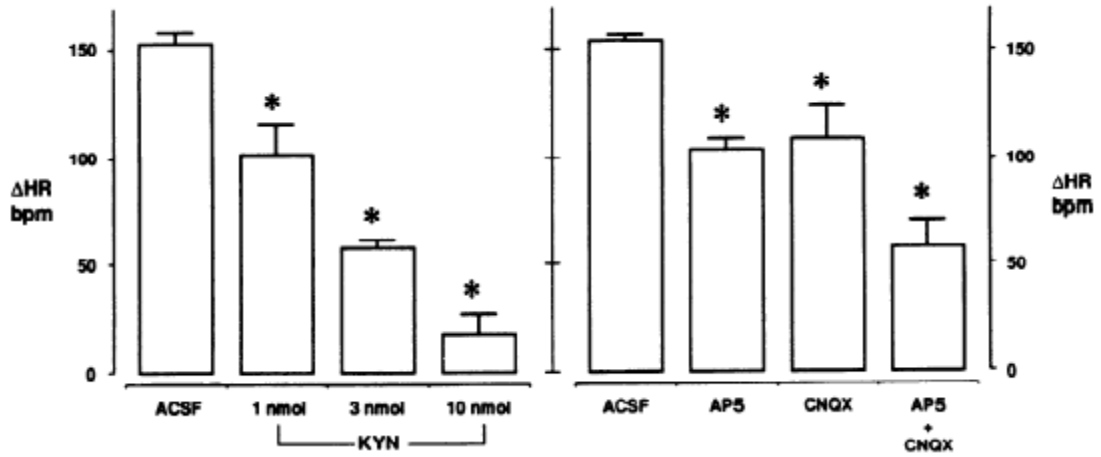

Fig. 8. Changes in HR during air stress 5 min after injection of 100nl aCSF, 1-10 nmol kynurenic acid (Kyn), 100 pmol AP5, 50 pmol CNQX, or the combination of AP5 and CNQX (n = 4 rats/group). Stress began 2 min after injection.
*Significant differences from aCSF compared with own control by 2 x 2 ANOVA and Scheffé's tests . Effects of Kyn were dose related by regression analysis. Effects of the combination of AP5 and CNQX were significantly different from AP5 and CNQX alone by ANOVA and Scheffé's tests.

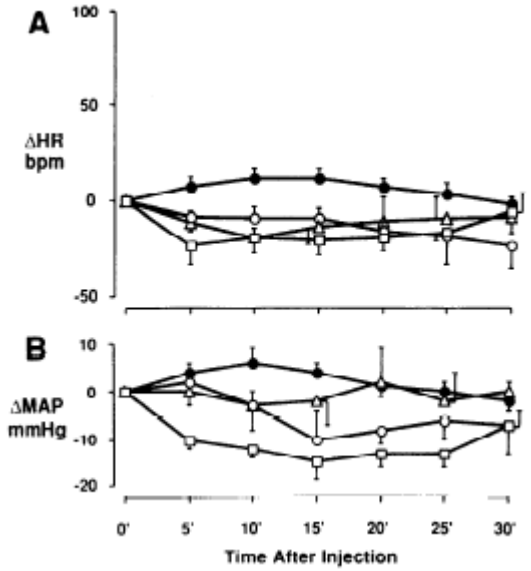

Fig. 9. Changes in HR (*A*) and MAP (*B*) during resting conditions after bilateral injection of 100 nl of aCSF (●; n = 12), 100 pmol AP5 (Δ; n = 4), 50 pmol CNQX (○; n = 4), or the combination of AP5 and CNQX (□; n = 4). Rats remained in their home cages during these trials. Injections were made in 100 nl of aCSF. Doses are expressed as amount per side.