Chemical stimulation of the dorsomedial hypothalamus elevates plasma ACTH in conscious rats

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Bailey, Timothy W., and Joseph A. Dimicco. Chemical stimulation of the dorsomedial hypothalamus elevates plasma ACTH in conscious rats. Am J Physiol Regulatory Integrative Comp Physiol 280: R8–R15, 2001.—The hallmark neuroendocrine response to stress is increased plasma ACTH. Inhibition of neurons in the region of the dorsomedial hypothalamus (DMH) attenuates experimental air stress-induced elevation of heart rate (HR), mean arterial pressure (MAP), and plasma ACTH. We hypothesized that, under basal conditions, stimulation of the DMH would mimic the neuroendocrine and cardiovascular response to air stress. We examined the effects of unilateral microinjection (100-nl vol) of bicuculline methiodide (BMI, 10 pmol), kainate (KA, 1 or 3 pmol), and N-methyl-D-aspartate (5 pmol) into the DMH or the paraventricular nucleus (PVN) on HR, MAP, locomotor activity, and plasma ACTH in conscious rats. Chemical stimulation of the DMH with KA or BMI produced increased locomotor activity and effects on HR, MAP, and plasma ACTH that together mimicked the pattern seen in experimental stress. Similar treatment in the PVN produced only small increases in MAP. Thus activation of neurons in the region of the DMH results in increased secretion of ACTH along with other changes typically seen in experimental stress.

The salient component of the neuroendocrine response to a variety of stressors is the mobilization of the adrenal cortex through the release of ACTH from the anterior pituitary. The primary secretagogue responsible for triggering this release of ACTH is corticotropin releasing hormone (CRH), which reaches the pituitary by means of a portal system originating in the hypothalamic median eminence (ME). Immunohistochemical studies demonstrate that the majority of CRH-containing neurons in the hypothalamus that project to the ME are located in the medial parvocellular division of the paraventricular nucleus (PVN) (21). Accordingly, electrolytic (3) or knife-cut (18) ablation of the PVN significantly decreases the levels of CRH found in the ME and attenuates stress-induced elevation of plasma ACTH. Similarly, inhibition of the PVN by local microinjection of lidocaine attenuates stress-induced elevation of CRH in the hypophysial-portal blood (25), whereas microinjection of muscimol into the PVN reduces stress-induced increases in plasma ACTH (32). Conversely, electrical stimulation of the PVN increases portal levels of CRH and peripheral levels of ACTH (34), and chemical stimulation of the PVN has been reported to increase plasma levels of ACTH (6, 14, 15, 23). Thus functional and anatomic evidence suggests that CRH originating in the PVN is an important factor controlling ACTH secretion from the anterior pituitary and the initiation point for activation of the hypothalamic-pituitary-adrenal (HPA) axis in stress.

Recently, neurons in the dorsomedial hypothalamus (DMH) have been implicated in the generation of an integrated multisystem response to stress in rats. Chemical stimulation of this region by microinjection of drugs that impair GABA<sub>A</sub> receptor-mediated inhibition or stimulate ionotropic glutamate receptors produces a pattern of physiological and behavioral changes typically seen in experimental stress (7, 27, 29–31). Conversely, microinjection of muscimol, a powerful agonist acting at inhibitory GABA<sub>A</sub> receptors, or kynurenate, a nonspecific ionotropic glutamate receptor antagonist, blocks or markedly reduces experimen- tal stress-induced elevation of heart rate (HR) and blood pressure (BP) (30, 33). Furthermore, air stress-induced increases in plasma ACTH (32) as well as induction of c-Fos in the parvocellular PVN (7a) are markedly attenuated by prior injection of muscimol into the DMH. Neurons in the DMH send efferents directly to the parvocellular PVN where CRH-containing neurons projecting to the ME are concentrated (35, 36), and neurons that both project to the PVN and are activated by footshock or swim stress are concentrated in the DMH (5, 17). Together, these observations suggest that the mobilization of the HPA axis seen in a variety of experimental stress paradigms may be signaled by activation of an excitatory projection originating in the DMH. If so, then the DMH may be an essential component of hypothalamic circuitry that facilitates recruitment of the HPA axis in stress and perhaps other settings.
The present study sought to extend these previous findings relating to the role of the DMH in experimental stress-induced secretion of ACTH. We hypothesized that, in the absence of stress, activation of neurons in the DMH in conscious rats would provoke increases in plasma ACTH, HR, and mean arterial pressure (MAP) resembling those seen in air stress. To address this hypothesis, we examined the effects of microinjection of bicuculline methiodide (BMI), a GABA_A-receptor antagonist, and of the excitatory amino acids kainate (KA) and N-methyl-D-aspartate (NMDA) into the DMH or the PVN on HR, MAP, locomotor activity, and plasma levels of ACTH in conscious rats. For comparison, some of the same animals were subjected to air stress, and the same parameters were measured and compared with observations from microinjection studies.

MATERIALS AND METHODS

Male Sprague-Dawley rats (250–300 g) were used for all experiments. Animals were housed singly under a 12-h light cycle and were permitted free access to food and water. All procedures described were approved by the Institutional Animal Care and Use Committee and followed National Institutes of Health guidelines.

Surgical Procedures

Three separate surgical procedures were performed in all rats. First, each was instrumented with telemetric implants (TA11PA-C40, Data Sciences, St. Paul, MN) under pentobarbital sodium anesthesia (50 mg/kg ip) as previously described (33). Quantitative information describing HR, MAP, and locomotor activity is transmitted from the telemetric probe by AM radio signal and simultaneously received, translated, and written to disk by hardware and software from Data Sciences running a personal computer. The Data Sciences system also detects changes in telemetric probe signal strength as an index of locomotor activity, where one activity unit is approximately equal to 1 cm/s movement. Activity units were quantitated over a given time interval to produce an index of animal movement about the home cage.

After at least 7 days of recovery, rats were instrumented with unilateral stainless steel guide cannulas (26 gauge, 10 mm length, Plastics One, Roanoke, VA) targeting the PVN or the DMH under ketamine/xylazine anesthesia (ketamine, 50 mg/kg ip) as previously described (33). Animals were placed in a stereotaxic instrument with incisor bar 5 mm above the interaural plane. Target coordinates for cannula placement, using bregma as the reference point and 100 nl of saline vehicle (Sal), BMI (10 pmol), KA (1 [KA1] or 3 [KA3] pmol), or NMDA (5 pmol) was injected over 27 s by means of an infusion pump and a 10-μl syringe. Ten minutes after the microinjection, a second blood sample was taken. Cardiovascular and activity parameters were sampled once every minute for the duration of the experiment and were reported as average change from baseline over the 10-min period after microinjection. Baseline for these parameters was determined by averaging recorded HR, MAP, and activity for the 30-min period before the first blood sample. Every animal received vehicle and no more than three additional different treatments in random order at intervals of at least 1 full day.

Air-stress studies. At the conclusion of the microinjection studies, four animals selected at random were tested in an air-stress paradigm. Air-stress study procedures are similar to those outlined above, except that no microinjections were administered. Instead, just after the first blood sample (prestress, baseline), the rat was placed in a narrow tube, and air was blown into the animal’s face (40 l/min) (32). Blood samples were taken at 2, 5, and 10 min after the initiation of air stress for subsequent analysis of ACTH content. Blood samples and cardiovascular data are reported as change from baseline. Blood collection and ACTH radioimmunoassay. Blood samples were collected into plastic microcentrifuge vials containing heparin, stored on ice, and immediately centrifuged. Plasma was then removed and stored at −80°C until assayed for ACTH content with a standard RIA kit (Diagnostic Products, Los Angeles, CA). Inter- and intra-assay variability is <10%. Plasma ACTH is reported as a change from baseline (ACTH content of blood sample 2 minus ACTH content in blood sample 1).

Histology. At the conclusion of experiments, rats were anesthetized with pentobarbital sodium (50 mg/kg ip), and 100 nl of alcian blue dye were injected to mark the precise injection site targeted by the guide cannulas. Rats were then perfused transcardially with 100 ml of 0.1 M PBS (pH 7.4) followed by 150 ml of 4% paraformaldehyde in 0.1 M phosphate buffer (PB; pH 7.4). Animals were then decapitated, and the brains were carefully removed and stored in 4%
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Fig. 1. Coronal schematics depicting approximate locations of unilateral microinjection sites in rats for which data are reported. For clarity, both sides of the hypothalamus depicted in the schematic are used to show unilateral injection sites. For all data presented here, the injection site was in or within 300 µm of the main body of the paraventricular nucleus (PVN) or the dorsomedial hypothalamus (DMH) where a clear zona compacta was evident. Distance from bregma is indicated at each section. DMD, dorsomedial hypothalamus diffuse region; DMC, dorsomedial hypothalamus compact region; f, fornix; mt, mammillothalamic tract.

Parafomaldehyde in 0.1 M PBS. After a week or more in storage, brains were frozen and cut in the coronal plane on a cryostat (35-µm-thick sections) through the length of the hypothalamus. Sections were later mounted on gelatin-coated or electrostatic slides and allowed to dry. Mounted sections were stained in a 1% neutral red solution, dehydrated, and placed under a coverslip. Injection sites were determined according to the atlas of Paxinos and Watson (24).

Statistics. Results are expressed as mean change from baseline ± SE. Results from microinjection experiments are separated into treatment groups according to injection site (PVN or DMH) and injected solution (Sal, BMI, KA1, KA3, or NMDA). For all experiments for which data are reported, injection sites were in or within 300 µm of either the PVN or the DMH where a clear zona compacta was evident (Fig. 1). Animals subjected to air stress had guide cannulas targeting the PVN or DMH but were grouped into a single “air”-treatment group. Comparisons were made between groups with one-way and/or repeated-measures ANOVA where appropriate (see RESULTS section). Fisher’s protected least-significant difference analysis was used for post hoc analysis. In some cases, paired t-tests were also employed. Limits of probability considered significant were 5% or less.

RESULTS

Microinjection Studies

Approximate sites of injection, as determined from postmortem histology, are shown in Fig. 1 for all animals for which data are included. All injection sites were in, or in the immediate vicinity of, the target nuclei as indicated. Basal HR, MAP, activity, and plasma ACTH were not significantly different among any of the treatment groups (Table 1).

Unilateral injection of BMI (10 pmol) or KA1 or KA3 into the region of the DMH significantly increased HR (averaged for 10 min after injection) when compared with either saline injections in the same region or parallel treatments in the PVN (Fig. 2A). The time course for the effects of BMI or saline on HR is shown in Fig. 2. KA1 or KA3 and NMDA failed to affect HR when injected into the PVN (Fig. 2A). Injection of BMI into the PVN significantly increased HR compared with saline treatment in the same region (Fig. 2B). However, Fig. 3 shows that the time to onset for this change was delayed compared with that for tachycardia produced by injection of BMI into the DMH. Thus, by 1 min after microinjection of BMI into the DMH, HR was elevated by an average of >50 beats/min, whereas this degree of tachycardia was not attained until 5 min after injection into the PVN.

Table 1. Baseline HR, MAP, activity, and plasma ACTH

<table>
<thead>
<tr>
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<th>DMH</th>
<th>PVN</th>
<th>AIR</th>
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<tbody>
<tr>
<td></td>
<td>BMI (10 pmol; n = 11)</td>
<td>KA (1 pmol; n = 9)</td>
<td>KA (3 pmol; n = 9)</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>386 ± 4</td>
<td>376 ± 8</td>
<td>364 ± 8</td>
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<tr>
<td>MAP, mmHg</td>
<td>113 ± 3</td>
<td>111 ± 5</td>
<td>110 ± 6</td>
</tr>
<tr>
<td>Activity, activity units</td>
<td>1 ± 0.5</td>
<td>0.5 ± 0.3</td>
<td>0.9 ± 0.4</td>
</tr>
<tr>
<td>Plasma ACTH, pg/ml</td>
<td>25 ± 5</td>
<td>19 ± 8</td>
<td>20 ± 4</td>
</tr>
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</table>

Values are means ± SE; n = no. of rats. HR, heart rate; MAP, mean arterial pressure; BMI, bicuculline; KA, kainate; NMDA, N-methyl-D-aspartate; DMH, dorsomedial hypothalamus; PVN, paraventricular nucleus; NA, not applicable.
Chemical stimulation of the DMH with any agent produced changes in blood pressure that were no different than those evoked by treatment with saline vehicle (Fig. 2B), although saline alone evoked a small but significant increase over baseline (paired t-test, P < 0.05). In contrast, whereas MAP was unchanged from baseline after injection of saline into the PVN, injection of BMI or KA into this region slightly but significantly elevated MAP compared with that seen after similar injection of saline (Fig. 2B).

Microinjection of BMI, KA, or NMDA into the PVN did not change plasma levels of ACTH compared with injection of saline in the same region (Fig. 2D). Microinjection of BMI or KA into the DMH significantly increased plasma levels of ACTH compared with saline injection in the DMH and similar injections in the PVN. Regression analysis revealed that increases in plasma ACTH and in HR elicited by BMI in different rats were strongly correlated (F = 11.57; r = 0.769; P = 0.0093). Microinjection of KA into the DMH significantly elevated ACTH levels compared with injection of saline into the DMH but not compared with similar treatment in the PVN (Fig. 2D).

Locomotor activity was unaffected by any of the agents microinjected into the PVN (Fig. 2C). Microinjection of BMI or KA into the DMH increased locomotor activity compared with saline treatment of the DMH or similar injections in the PVN, and microinjec-
tion of KA$_3$ into the DMH significantly elevated locomotor activity compared with saline injections in the DMH (Fig. 2C).

**Air-Stress Studies**

To compare the effects of experimental stress and chemical activation of the DMH on HR, MAP, and plasma ACTH, the effect of 20 min of air stress was assessed in four of the same animals used in microinjection studies. The time course of air stress-induced increases in HR, BP, and plasma ACTH sampled 2, 5, and 10 min after initiation of air stress is shown in Fig. 4. Figure 5 shows the changes in HR and MAP averaged through 10 min and changes in plasma ACTH at 10 min after the initiation of air stress or the injection of BMI into the DMH or the PVN. Air stress and injection of BMI into the DMH produced a pattern of changes in HR, MAP, and plasma ACTH that were nearly identical and contrasted sharply with those seen after identical injection of BMI into the PVN (Fig. 5).

**DISCUSSION**

The data presented here indicate that microinjection of KA or BMI into the DMH of conscious rats provokes increases in plasma ACTH similar to those seen in experimental stress. We have previously reported that microinjection of agents that excite or disinhibit neurons into the region of the DMH produces a variety of physiological responses resembling those seen in stress, including sympathetically mediated increases in HR, MAP (7, 8, 29), hindlimb blood flow (8), escape behavior (28), experimental anxiety (27), and increased gastrointestinal motility (11). Activation of the HPA axis is the hallmark of the neuroendocrine response to stress, and elevation of ACTH is a measure of activity of this system. Keim and Shekhar (16) previously demonstrated that microinjection of 80 pmol BMI into the DMH in anesthetized rats elevates plasma ACTH and corticosteroids. The current study has refined and extended this finding by 1) employing smaller doses and volumes of BMI to localize more precisely the site of action in the hypothalamus, 2) testing the effects of agents acting at ionotropic glutamate receptors, and 3) using a conscious rat preparation to eliminate the potentially confounding effects of anesthesia. The data provided here do not specifically preclude the possibility that the agents injected into the DMH may have produced their effects on ACTH after spread or diffusion to another nearby region. However, because of the low doses of BMI and KA that were employed, it is unlikely that these agents could have reached more distant sites at concentrations sufficient to provoke effects. Also, BMI-induced increases in plasma ACTH correlated significantly with the associated increases in HR that have been clearly localized to the region of the DMH in previous studies (7, 29, 30). Finally, the hypophysiotropic region of the PVN is densely innervated by neurons in the DMH (35, 36), which has been shown to provide excitatory input to neurons in the
PVN (1). Together, these findings point to neurons in the same region of the DMH as the specific targets mediating the effects of BMI and KA on plasma ACTH and HR. Thus these observations add recruitment of the major neuroendocrine response to stress, activation of the HPA axis, to the spectrum of stresslike responses produced by stimulation of the DMH in un-anesthetized rats.

Interestingly, the same doses of KA or BMI shown to elevate plasma ACTH when injected into the DMH failed to increase plasma ACTH when injected into the PVN (Fig. 1D). Despite the body of work demonstrating the importance of the PVN in the HPA axis, only two reports have examined the effect of microinjection of glutamate or a glutamate analog into the PVN on circulating levels of ACTH. In 1989, Darlington and colleagues (6) reported that glutamate (25,000 pmol/50 nl) increased plasma ACTH when injected into the PVN. More recently, Feldman and Weidenfeld (10) reported that bilateral microinjection of glutamate (32,000 pmol/1,000 nl per side) increased circulating ACTH in anesthetized rats. In the current study, microinjection of as little as 1 pmol of KA into the DMH (unilateral injection, 100 nl total vol) increased circulating ACTH, whereas similar treatment in the PVN with 1 or 3 pmol of this agent failed to do so (Fig. 2D). Thus the relatively high doses and/or volumes of glutamate injected into the PVN in the previous studies may well have elevated plasma levels of ACTH as a result of spread or diffusion to the DMH.

The negative results obtained after microinjection of any of the agents directly into the PVN in our study are difficult to explain in light of the established role of this nucleus in the HPA axis and the assumption that KA is excitatory at virtually all neurons. One possible explanation is that the permanent guide cannulas damaged the CRH neurons in the PVN whose activation is largely responsible for stimulating the secretion of ACTH. However, in a previous study, similar air stress-induced increases in plasma ACTH were observed in rats without guide cannulas and rats instrumented with bilateral guide cannulas in the PVN through which saline was injected before air stress (32). Thus the effects of guide cannulas on neurons in the PVN can most likely be ruled out as a possible explanation. It is also possible, given the short half-life of ACTH in plasma (2–10 min) (20), that an immediate and short-lived increase in plasma ACTH caused by chemical stimulation of the PVN may not have been detectable in blood samples withdrawn at 10 min. However, according to a recent report, microinjection of glutamate directly into the PVN (1–100 nmol/200 nl unilateral) induced c-fos expression in autonomic-projecting regions of the PVN but little in the area containing hypophysiotropic CRH neurons (4). Thus microinjection of glutamate directly into the PVN apparently failed to produce robust activation of the CRH neurons thought to be responsible for elevation of ACTH. Therefore, the most likely explanation for the negative results is that the treatments targeting the PVN in this study were insufficient to produce direct stimulation of hypophysiotropic neurons regulating the release of ACTH in this region and thus failed to elevate levels of the hormone.

Microinjection of BMI into the PVN increased average HR and MAP, as was first shown by Martin and colleagues (19). Accordingly, one possibility is that blockade of GABA_A receptors in the PVN results in sympathetically mediated increases in HR as these investigators have suggested (19). With the use of much lower doses, our laboratory have reported that the same amounts of BMI, KA, and NMDA that produce marked increases in HR and MAP when injected into the DMH produce lesser effects when injected into the area between the two regions and even smaller responses after microinjection directly into the immediate vicinity of the PVN (7, 8). Thus the tachycardia seen after microinjection of BMI into the PVN in the present study may be a consequence of spread or diffusion to 1) neurons in the DMH, which, although located ~1 mm from the PVN, are exquisitely sensitive to these agents (29, 30), or 2) neurons in the region between the DMH and the PVN. Either of these possibilities is supported in this study by the delayed time course for the increase in HR seen after microinjection of BMI into the PVN compared with that for the tachycardia observed after the same treatment in the DMH (Fig. 3). Conversely, the rapid onset of tachycardia produced by microinjection of BMI into the DMH suggests that the active site is in or very near the site of injection. Thus the data presented here are consistent with the notion that neurons in the DMH, and perhaps, to a lesser extent, in the region between it and the PVN, are responsible for the sympathetically mediated tachycardia seen after microinjection of BMI or excitatory amino acids into either region.

Whereas bilateral microinjection of NMDA (5 pmol, 50 nl) into the DMH significantly elevated HR and BP in conscious rats in a previous study (7), unilateral microinjection of NMDA (5 pmol, 100 nl) into the DMH did not alter HR or MAP in these experiments. However, microinjection of NMDA into the DMH produces robust increases in HR only in a very limited range of doses (29). In the present study, NMDA was injected unilaterally, as opposed to bilaterally previously, and in a greater volume of saline so that the effective local concentration of NMDA here was much lower. These differences may have accounted for the failure of injection of NMDA into the DMH to increase HR and MAP as reported here.

In contrast to the effects on HR and plasma ACTH, MAP was increased after microinjection of BMI or KA into the PVN but apparently not by similar treatment of the DMH (Fig. 1B). In previous studies (7, 30), microinjection of similar doses of KA or BMI into the DMH was found to provoke modest increases in arterial pressure that differed significantly from those produced by vehicle. The failure to demonstrate such an effect in this study may be attributable to the small (i.e., ~8 mmHg) increase in arterial pressure seen after saline treatment that was not apparent previously when artificial cerebrospinal fluid (aCSF) was em-
ployed as a vehicle (30). Similarly, HR was increased over baseline by ~10 beats/min after injection of saline into the PVN, an effect nearly identical to that reported previously after injection of aCSF into the DMH (30), but was elevated by an average of >25 beats/min after injection of saline into the DMH (Fig. 1A). Together, these findings indicate that even saline, widely used as an inactive vehicle for microinjections elsewhere in the central nervous system (CNS), may have small but significant excitatory effects on the critical population of neurons in the DMH responsible for these cardiovascular changes.

The increases in MAP noted after microinjection of either BMI or KA into the PVN were likely the result of an action in this nucleus. Secretion of vasopressin in the peripheral circulation has been shown to be under tonic GABAergic inhibition (37), and neurohypophysial neurons containing vasopressin are located in the PVN (25). Accordingly, microinjection of glutamate into the PVN increases plasma vasopressin (6), and similar microinjection of clonidine (9) or norepinephrine (12) into the PVN elicits an increase in blood pressure mediated by vasopressin. Therefore, the effects on MAP observed after microinjection of KA or BMI into the PVN may be a result of the release of vasopressin into the peripheral circulation.

As has been concluded previously with regard to increases in HR, the data suggest that increases in plasma ACTH may result from either disinhibition of neurons in the region of the DMH through blockade of tonic activity at GABA$_A$ receptors or excitation of these same neurons through stimulation of glutamate receptors. Quaternary salts of BMI are well accepted as relatively selective antagonists at GABA$_A$ receptors that probably mediate the most common form of synaptic inhibition in the mammalian CNS. Microinjection into the DMH of doses of KA in the range of those used here have been shown previously to produce tachycardia through actions at non-NMDA ionotopic glutamate receptors (29–31), and these are most likely of the AMPA subtype. Thus the same receptors regulate the activity of neurons in the DMH whose excitation results in sympathetically mediated tachycardia and mobilization of ACTH from the adenohypophysis.

The neuronal pathways or signaling mechanisms through which neurons in the DMH may activate the HPA axis have yet to be explicitly defined. However, several key observations define a simple hypothesis. The PVN is densely innervated by neurons in the DMH (34, 35), and many of the same neurons appear to be activated in response to swim (5) or footshock stress (17). Because some of the neurons in the DMH that give rise to this projection have been found to contain glutamate decarboxylase, the enzyme responsible for neuronal synthesis of GABA (2), some have assumed the pathway to be inhibitory (5, 13). However, in a hypothalamic slice containing both the PVN and the DMH, Boudaba and colleagues (1) found the DMH to be one of two hypothalamic sites where chemical stimulation provoked an excitatory response in parvocellular neurons in the PVN. Thus converging anatomic and functional observations suggest that, both in air stress and after chemical stimulation of the DMH, the HPA axis is activated through a direct excitatory projection from neurons in this region to CRH-releasing neurons in the PVN.

In this study, the magnitude of changes in HR, MAP, and plasma ACTH produced by unilateral chemical stimulation of the DMH was similar to those changes produced by air stress (Fig. 5). Conversely, in previous studies, microinjection of the inhibitory agent muscimol, an agonist at GABA$_A$ receptors, into the DMH attenuated or abolished air stress-induced increases in HR, arterial pressure (33), plasma ACTH (32), and c-fos expression in the PVN (7a). Therefore, activity of neurons in the region of the DMH, neurons under tonic inhibition by GABA and capable of being stimulated by glutamate analogs, appear to play a key role in the generation of both the autonomic and neuroendocrine components of the response to air stress in rats. The latter effect is likely to be mediated through a direct projection from neurons in the DMH to the CRH-releasing neurons in the PVN. These results fit into an emerging role of the DMH as a key hypothalamic center initiating both the autonomic and neuroendocrine response to certain modes of stressful stimuli in rats.

**Perspectives**

Together with previous findings, these results point to a key role for neurons in the same discrete region of the DMH in the generation of a varied array of physiological and behavioral responses to an exteroceptive threat, where activation of these neurons appears to play an obligatory role in the generation of tachycardia, increases in plasma ACTH, and “escape” behavior and “anxiety” associated with emotional stress in rats. One possibility is that different subsets of neurons localized in the same area mediate effects on the various endpoints. However, it is tempting to speculate that excitation of a single population of true “command neurons” is responsible for generating the highly integrated and stereotypical response emotional, a mechanism strongly conserved across mammalian species because of its high survival value. Determination of which of these possibilities is more likely will be facilitated by definitive identification of the specific neurons in this region that are relevant to different facets of the response.

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