Pergamon

Pharmacology Biochemistry and Behavior, Vol. 50, No. 2, pp. 253–258, 1995

0091-3057/95 $9.50 + .00

0091-3057(94)00307-6

Dorsomedial Hypothalamic GABA Regulates Anxiety in the Social Interaction Test

A. SHEKHAR[1] AND J. S. KATNER

Departments of Psychiatry and Pharmacology and Toxicology, Institute of Psychiatric Research, Indiana University School of Medicine, Indianapolis, IN 46202

Received 2 May 1994

SHEKHAR, A. AND J. S. KATNER. *Dorsomedial hypothalamic GABA regulates anxiety in the social interaction test.* PHARMACOL BIOCHEM BEHAV **50**(2) 253–258, 1995. – Blockade of $GABA_A$ function in the region of the dorsomedial hypothalamus (DMH) of rats is known to elicit a constellation of physiologic responses including increases in heart rate (HR), mean arterial blood pressure (BP), respiratory rate, and plasma catecholamine levels, as well as behavioral responses such as increases in locomotor activity and anxiogenic-like effects as measured in a conflict test and the elevated plus-maze test. The aim of the present study was to test the effects of microinjecting $GABA_A$ antagonists bicuculline methiodide (BMI) and picrotoxin, as well as the $GABA_A$ agonist muscimol, into the DMH of rats placed in the social interaction (SI) test. Muscimol decreased HR and BP but increased SI, whereas the GABA antagonists increased HR and BP but decreased SI time. Blocking the HR changes elicited by GABAergic drugs injected into the DMH with systemic injections of atenolol and atropine methylbromide did not block their effects on SI.

Muscimol Bicuculline Picrotoxin Heart rate Blood pressure Stress Anxiolytics Benzodiazepines

SINCE the early studies of Cannon and Britton (4) and Bard (1), the hypothalamus has been implicated in the regulation of autonomic, neuroendocrine, and behavioral expressions of emotions. More recently, direct injections of neurotransmitter agonists and antagonists have indicated that a number of neurotransmitters may be involved in the aversive responses elicited in this region. Blocking $GABA_A$ neurotransmission in the dorsomedial hypothalamus (DMH) elicited large increases in HR and respiration and smaller increases in BP in anesthetized (6,7) and conscious (28) rats. Similar physiologic responses have also been obtained with injections of excitatory amino acids into the DMH (25). We have shown in behavioral studies with conscious animals that $GABA_A$ blockade in the cardiostimulatory region of the DMH elicits "escape-oriented locomotion (20), a selective enhancement of "aversive" responses without affecting "approach" responding (21) and an increase in experimental anxiety as measured in a "conflict" (22) and the elevated plus-maze test (19). All of these data suggest that the GABA neurons in the DMH regulate a constellation of physiologic and behavioral responses associated with anxiety and stress.

In the present study we chose to look at the effects of enhancing and blocking $GABA_A$ receptors in the cardiostimulatory region of the DMH of rats using the social interaction test (SI) of anxiety (8), an ethologically based test that measures spontaneous behavior of rats (12). Furthermore, we also measured the physiologic responses of HR and BP at the time of behavioral measurements to study the physiologic concomitants of $GABA_A$ receptor modulation along with anxiety-related behavior.

The behavior of rats implanted with bilateral microinjection cannulae in the cardiostimulatory region of the DMH were assessed in SI after microinjecting artificial cerebrospinal fluid (a-CSF), the $GABA_A$ agonist muscimol and the $GABA_A$ antagonists, bicuculline methiodide (BMI) and picrotoxin, into the DMH. Furthermore, we attempted to block the peripheral physiologic responses to the injection of BMI and muscimol into the DMH with IP atenolol and atropine methyl bromide, and studied the effects of BMI and muscimol on SI.

Methods

Animals

Male Sprague–Dawley rats (Harlan Laboratories, Indianapolis, IN, 250–300 g) were housed in individual plastic cages

[1] Address requests for reprints to Anantha Shekhar, M.D., Ph.D., 791 Union Drive, IPR, Indiana University Medical Center, Indianapolis, IN 46202.

in a temperature-controlled room (720°F) kept on a 12-h day-night cycle (lights on at 0600 h and off at 1800 h). Rats were given ad lib food and water. All behavioral testing was done between 0800 and 1200 h.

Surgical Procedures

The first step consisted of placing a catheter made of Tygon microbore tubing (Fisher Scientific, Pittsburgh, PA) in the femoral artery, as described elsewhere (14). Briefly, rats were first given a 1 mg/kg IP injection of atropine (1 mg/ml) and were then anesthetized with a 50 mg/kg IP injection of pentobarbital (Abbott Laboratories, Chicago, IL; 50 mg/ml). The femoral artery was then separated from the surrounding vein and nerves. A drop of lidocaine (10 mg/ml) was applied over the exposed artery. A small incision was made in the artery, and a catheter was gently forced approximately 5 cm into the artery and flushed with heparinized saline (25,000 U/liter). The catheter was anchored to the artery with silk suture. The opposite end of the catheter was then routed through connective tissue to the dorsal aspect of the neck and stabilized with a leather vest.

Following 24 h, rats were again given an IP injection of atropine and anesthetized with an IP injection of pentobarbital. The femoral arterial catheter was flushed with saline and connected to a Beckman R511 Dynograph (Schiller Park, IL) via a pressure transducer to measure HR and BP throughout the surgery. Rats were then secured within a stereotaxic device (David Kopf Instruments, Tujunga, CA) with the aid of atraumatic ear bars and the incisor bar set at +5°. Stainless-steel microinjection cannulae (33 ga, 12 mm in length) were fitted within guide cannulae (26 ga, 10 mm in length), secured on stereotaxic manipulator arms with a clamping device and connected to a 10-μl syringe with polyethylene tubing (PE-20). A 50 pmol/250 nl solution of the $GABA_A$ antagonist BMI was used to fill the tubing and syringe. The syringe was then placed on an infusion pump (model 355; Sage Instruments, Boston, MA) and adjusted to deliver 250 nl over a 30-s period.

Microinjection cannulae were then bilaterally implanted in the region of the DMH of rats with the manipulator arms set at an angle of 10°. The coordinates in relation to bregma were 1.2 mm posterior, 1.7 mm lateral, and 9.0 mm ventral (13). The injection cannulae, along with the guide cannulae, were then lowered into the DMH on one side according to the above coordinates. Each side was separately tested with an injection of 50 pmol of BMI in 250 nl of a-CSF over a 30-s period. Heart rate and BP were recorded before injection, and any changes after the injection were noted. An increase in HR of at least 50 beats/min was considered a reactive site. If injection at the site did not elicit the expected HR response, the cannulae was then repositioned 0.2 mm anteriorly, posteriorly, medially, or laterally until the physiologically active site was found (maximum four times; most were one to two times).

After waiting for the HR to return to baseline, the same procedure was followed for the opposite side. In a group of control animals, cannulae were placed in brain regions surrounding the DMH where injection of BMI did not cause a 50 beat/min increase in heart rate. After both microinjection cannulae were placed at the active or inactive sites, guide cannulae were fixed at their positions with the help of three stainless-steel screws and cranioplastic cement. The internal cannulae were then withdrawn from the guide cannulae and replaced with steel wire dummy cannulae (screws, cement, and dummy cannulae obtained from Plastics One, Roanoke, VA).

Experimental Protocol

After at least 3 days of recovery, the femoral arterial catheter of the conscious rat was connected to a Beckman R511 Dynograph to measure HR and BP. After a 5-min baseline recording, microinjection cannulae were inserted into the guide cannulae. Rats received a bilateral, 250-nl intracranial (i.c.) microinjection of either a-CSF (n = 15), muscimol (45 or 90 pmole/side; n = 6 and 5, respectively), BMI (20 pmol/side; n = 9), or picrotoxin (33 pmol/side; n = 5). Therefore, the total dose of drug injected into each animal was double the dose per side. All drug solutions were made up in a-CSF and injected in random order. One minute after completion of injection, the injection cannulae were withdrawn. Heart rate

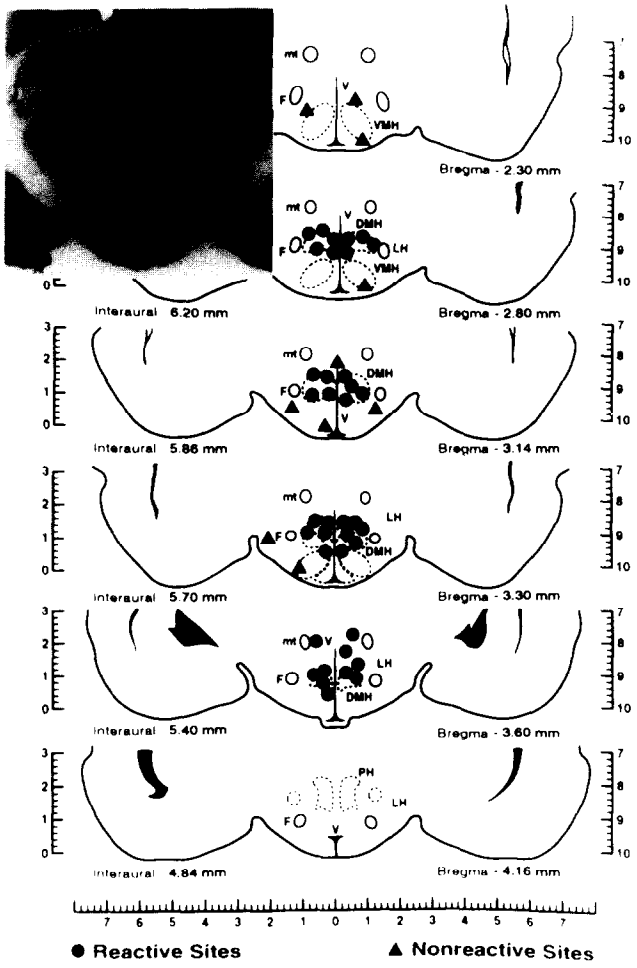

FIG. 1. Schematic representation of the results of histology showing the bilateral injection sites in the hypothalamus and a photomicrograph of an actual histologic section (inset). The sites that were reactive to injection of BMI (●) by eliciting increases in HR under anesthesia were mostly in the region of the DMH; the nonreactive sites (▲) were outside the DMH. Brain sections are represented according to the atlas of Paxinos and Watson. The distances from the interaural line and bregma are given under each section. The scales on the right and left indicate the distances in millimeters from the ventral and dorsal surfaces. The scale at the bottom indicates the distance in millimeters lateral to the midline. DMH, dorsomedial hypothalamus; F, fornix; LH, lateral hypothalamus; mt, mamillothalamic tract; PH, posterior hypothalamus; V, third ventricle; VMH, ventromedial hypothalamus.

TABLE 1

CHANGES (MEAN ± SEM) IN HEART RATE AND BLOOD PRESSURE ELICITED BY BILATERAL MICROINJECTION OF GABAERGIC DRUGS INTO THE DMH OF RATS WITH OR WITHOUT PRETREATMENT WITH IP ATENOLOL AND ATROPINE METHYLBROMIDE

Treatment	Baseline HR (beats/min)	Change in HR (beats/min)	Baseline BP (mmHg)	Change in BP (mmHg)
Vehicle i.c. (n = 15)	382 ± 14	+23 ± 5	124 ± 4	+5 ± 2
BMI 20 pmol i.c. (n = 9)	389 ± 20	+96 ± 12*	128 ± 5	+21 ± 3*
BMI 20 pmol i.c. + atenolol + atropine IP (n = 4)	270 ± 18†	+28 ± 6†	143 ± 6	+16 ± 3*
Picrotoxin 33 pmol i.c. (n = 5)	376 ± 8	+76 ± 12*	137 ± 8	+17 ± 5*
Muscimol 90 pmol i.c. (n = 5)	354 ± 36	−53 ± 16*	125 ± 5	−12 ± 4*
Muscimol 90 pmol i.c. + atenolol + atropine IP (n = 4)	268 ± 15†	−7 ± 1†	126 ± 4	−13 ± 4*
Muscimol 45 pmol i.c. (n = 5)	338 ± 29	−34 ± 9*	121 ± 7	−7 ± 2*

* Significantly different from baseline. †Significantly different from the no-IP treatment group (ANOVA coupled with Student Newman-Keuls test, $p < 0.05$).

and BP were then monitored for a 10-min period in their home cages, and any changes in relation to baseline were noted.

At the end of the 10-min period, the animal was subjected to the SI test (8). The apparatus itself consists of a solid wooden box with an open roof 36 in. long by 36 in. wide, with walls 12 in. high. A videocamera was fixed above the box, and all behavioral tests were videotaped. The "experimental" rat and an unfamiliar "partner" rat were both placed in the center of the box and allowed to interact freely for a period of 5 min. The number of seconds of nonaggressive physical contact (grooming, sniffing, crawling over and under, etc.) initiated by the "experimental" rat was then counted. Sessions were then scored at a later time by two raters, of whom at least one was blinded as to drug treatment. Intra-observer reliability for the time of social interaction was found to be acceptable (r values ranged from 0.971–0.982; n = 6 each). Experimental runs (i.c. injection, physiograph monitoring, and social interaction) with different drug injections were separated by at least 3 days. Most rats received a maximum of three injections, and no rat received more than five i.c. drug injections.

In another group of control rats (n = 4), the effects of IP injections of drugs on physiologic responses and social interaction changes elicited by i.c. injections of GABAergic drugs were recorded. Rats were injected IP with both atenolol (10 mg/kg) and atropine methyl bromide (1 mg/kg). After 20 min, rats were given a random 250 nl i.c. injection of either a-CSF, BMI (20 pmol), or muscimol (90 pmol), and any change in HR, BP, and SI were recorded. Time intervals between experimental runs were at least 3 days.

Upon completion of the experiments, rats were injected IP with a lethal dose of pentobarbital. Microinjection sites were marked by injecting 250 nl of 50% india ink through the microinjection cannulae. The brains were then removed, fixed in 10% neutral buffered formalin, and later sectioned into 50-μm sections and stained with neutral red. The exact site of injection was then determined by comparing the sections with the atlas of Paxinos and Watson (13). All data are presented as mean ± SEM. Statistical analyses were done by using either t-tests or ANOVA coupled with Student Newman-Keuls (SNK) test.

RESULTS

Histologic examination of the sites of injections in the rats used in the present study showed that all reactive sites where BMI injection elicited increases in HR and BP were in the region of the DMH, whereas the nonreactive sites were generally outside the boundaries of the DMH (Fig. 1).

Table 1 shows the effects of microinjecting GABAergic drugs into the DMH on the HR and BP of conscious rats during the first 10 min after the injections. Injections of both 20 pmol of BMI and 33 pmol of picrotoxin elicited a significant increase in the HR of rats compared with a-CSF injection (F = 21.478; df = 2, 26; p = 0.0001). On the other hand, injections of muscimol (45 and 90 pmol) into the DMH elicited a dose-dependent decrease in HR compared with a-CSF injections (F = 25.884; df = 2, 23; p = 0.0001). Similarly, injection of BMI and picrotoxin into the DMH elicited an increase in BP (F = 9.662; df = 2, 26; p = 0.0006), whereas the injection of muscimol caused a dose-dependent decrease in BP (F = 13.00; df = 2, 23; p = 0.0002) compared with a-CSF injections (Table 1).

After 10 min of recording HR and BP, rats were placed in the SI test set. The effects of injecting GABAergic drugs into the DMH on SI are shown in Fig. 2. Microinjection of both BMI and picrotoxin into the DMH elicited a significant decrease in SI time, which suggests an anxiogenic-like effect (Fig. 2A; F = 29.641; df = 2, 26; p = 0.0001). Muscimol injection into the DMH elicited a significant increase in the SI time at the 90 pmol dose, which suggests an anxiolytic-like effect (Fig. 2B; F = 9.686; df = 2, 23; p = 0.0009). In contrast, microinjection of the $GABA_A$ antagonist BMI (20 pmol) into the areas of the hypothalamus outside the DMH (which were nonreactive under anesthesia), did not increase experimental anxiety, as measured by SI time (91 ± 7% of baseline, n = 5).

Pretreatment with atenolol (10 mg/kg) and atropine methylbromide (1 mg/kg) significantly blocked the increases in HR obtained with BMI microinjections into the DMH compared with rats with no IP treatment (Table 1; F = 11.17; df = 1, 11; $p < 0.005$). Similarly, the decrease in HR elicited by muscimol microinjections into the DMH of rats without prior IP treatments was also significantly blocked with pretreatment with IP atenolol and atropine (F = 5.75; df = 1, 7; $p < 0.046$). The BP changes elicited by injecting BMI and muscimol into the DMH were not significantly blocked by IP atenolol and atropine.

Despite the blockade of the HR changes, BMI still elicited a significant anxiogenic-like effect in SI test (Fig. 3; F =

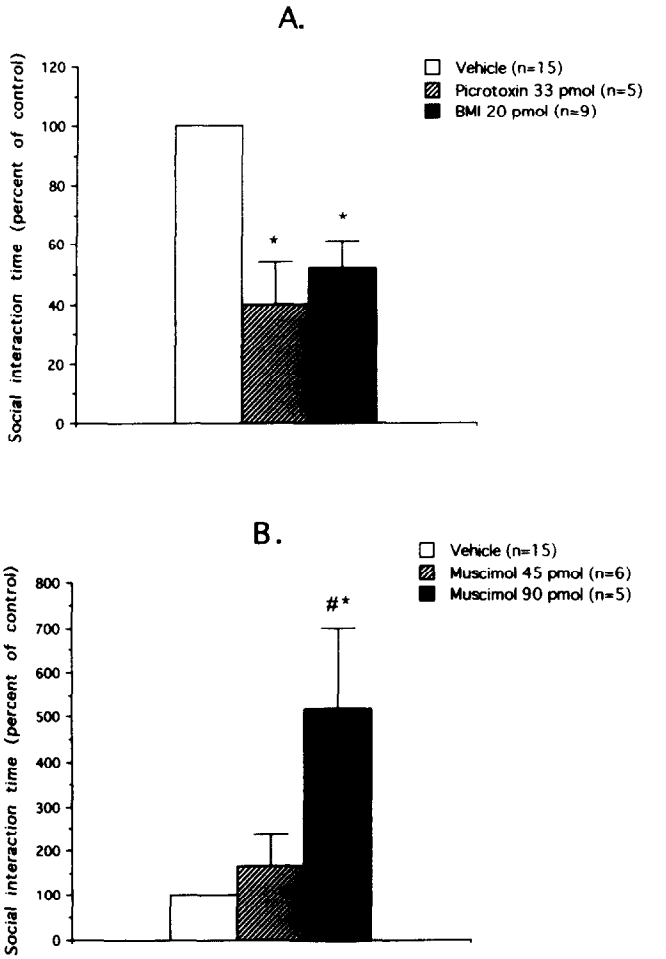

FIG. 2. Changes in the total social interaction time during the 5-min test period elicited by microinjecting GABAergic drugs into the DMH of rats. Drugs were injected bilaterally such that the total dose injected was double the dose indicated. (Panel A). The decreases in social interaction time elicited by injecting the GABA antagonists, BMI and picrotoxin, into the DMH. (Panel B). The effects of injecting two doses of the GABA agonist muscimol into the DMH. Data are presented as mean ± SEM.

516.678; df = 1, 6; p = 0.0001), whereas muscimol significantly increased SI time (Fig. 3; F = 70.90; df = 1, 6; p = 0.0004). Pretreatment with atenolol and atropine did not significantly change the absolute SI values in rats (F = 0.50; df = 1, 11; p = 0.50). The absolute SI after a-CSF injection into the DMH with IP atenolol and atropine was 39 γ 11 s (n = 4); without IP pretreatment it was 49 ± 8 s (n = 9).

DISCUSSION

The results clearly suggest that blocking and enhancing $GABA_A$ receptor function in the DMH of rats respectively elicit anxiogenic- and anxiolytic-like effects in the social interaction test. Taken in conjunction with our previous findings that $GABA_A$ blockade and enhancement in the DMH also elicits anxiogenic- and anxiolytic-like effects in the elevated plus-maze (19) and a conflict test (22), this strongly supports the argument that $GABA_A$ receptors in the region of DMH may regulate anxiety responses. In addition to these anxiogenic-like effects in three different tests of anxiety, $GABA_A$ blockade in the DMH also elicits increases in the physiologic concomitants of anxiety such as HR and BP (Table 1), respiratory rate (6,19), plasma catecholamines (29), and plasma ACTH (7). We have previously shown that blockade of $GABA_A$ function in this region also elicits escape-oriented locomotor behavior (20) and a selective enhancement of aversive behavior in a Sidman avoidance schedule (21).

Similarly, GABA enhancement in the DMH with muscimol elicits anxiolytic-like responses in three tests of anxiety, as well as decreases in HR and BP (Table 1), and blocks the increase in plasma norepinephrine level during stress (23). Furthermore, microinjections of muscimol into the DMH block the increases in HR and BP elicited by air-jet stress (11) and the elevated plus-maze test (23). These findings all suggest that GABA inhibition in the DMH regulates a constellation of stress responses that includes behavioral changes such as anxiety, selective enhancement of aversive behavior, and locomotor activation, and physiologic changes such as increases in HR, BP, respiratory rate, catecholamines, and corticosterone secretion.

Previously, cardiovascular changes caused by peripheral injections of vasopressin have been shown to induce behavioral changes such as conditioned taste aversion (3). In our second set of experiments, blocking the increases in HR elicited by BMI microinjection into the DMH by IP injections of atenolol and atropine methyl bromide (blocking the sympathetic and parasympathetic output to the heart without significant central effects) does not block the behavioral effects of BMI in the social interaction test (Table 1 and Fig. 3). Similarly, IP injections of atenolol and atropine combination do not affect the behavioral effects of muscimol microinjection into the DMH while significantly blocking the decrease in HR (Table 1 and Fig. 3). We were not able to block the modest but significant BP changes elicited by BMI and muscimol injections with atenolol and atropine (Table 1). There is still a possibility that the BP changes elicited by the GABAergic drugs may be responsible for the behavioral effects.

In primates, lesions of the DMH and surrounding hypo-

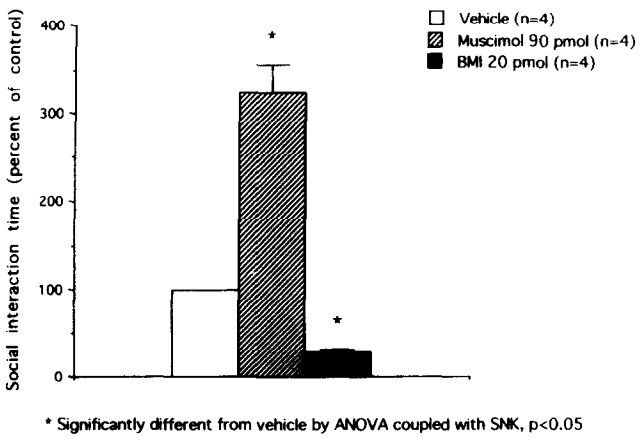

FIG. 3. Summary of the effects of microinjecting the GABA agonist, muscimol, and the antagonist, BMI, into the DMH of rats placed in the social interaction test after pretreatment with IP atenolol and atropine methylbromide to block the HR effects of the GABAergic drug injections. The data are presented as mean ± SEM. Drugs were injected bilaterally such that the total dose injected was double the dose indicated.

thalamic areas cause loss of physiologic responses associated with emotional arousal (24). Human hypothalamotomy involving the posterior hypothalamic area causes a decrease in anxiety and has been termed "sedative surgery" (9). Electric stimulation before lesioning of the posterior region of the hypothalamus under local anesthesia in humans as reported by Schvarcz (18) and Sano et al. (15) consistently elicits an increase in heart rate, respiration, and fear or anxiety. Both stimulation of DMH in rats with GABA blockade and electric stimulation of a corresponding posterior hypothalamic area in humans eliciting a similar pattern of anxiety and physiologic arousal further support the hypothesis that GABA in DMH may regulate anxiety responses.

The known neuroanatomic connections of the DMH also support its proposed key integrative role in anxiety responses. The DMH has ascending afferents from spinal cord, locus ceruleus, raphe nuclei, nucleus of the solitary tract, and periaqueductal grey (26). Afferent projections are also seen from septum and the bed nucleus of the stria terminalis (5). Within the hypothalamus, it receives large projections from paraventricular nucleus (5) and the lateral hypothalamus (17), which receives the amygdalar pathway responsible for autonomic arousal (10). The DMH also has connections with the hippocampus and the subiculum (26).

The major efferent target within the hypothalamus is the paraventricular nucleus (27), which is thought to be responsible for stress-related release of ACTH and sympathetic arousal (16,26). The DMH also projects to the periaqueductal grey; nucleus tractus solitarius, the primary relay center of the baroreceptors; subretrofacial nucleus, the medullary center for sympathetic tone; and the nucleus ambiguus containing the parasympathetic cells projecting to the heart and the intermediolateral column of the spinal cord. Therefore, the afferent connections of the DMH are such that it has a variety of sensory, limbic, and cognitive input, whereas its efferent connections enable it to be a coordinated output center of stress responses (2,7).

In summary, microinjection of the $GABA_A$ agonist, muscimol, and the $GABA_A$ antagonists, BMI and picrotoxin, into the DMH of rats elicits increases and decreases in the social interaction time, suggesting anxiolytic- and anxiogenic-like effects, respectively. The anxiogenic-like effect is not obtained by injecting BMI into other hypothalamic areas surrounding the DMH. The behavioral effects of microinjecting BMI and muscimol into the DMH are obtained even when the changes in HR are blocked by systemic injections of atenolol and atropine, which suggests that these effects do not result from the HR effects.

ACKNOWLEDGEMENTS

This study was supported by PHS Grant R29 MH 45362. The authors thank William Rusche for technical assistance.

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