ELSEVIER

Pharmacology Biochemistry & Behavior, Vol. 55, No. 2, pp. 249–256, 1996

0091-3057/96 $15.00 + .00

PII S0091-3057(96)00077-9

Dorsomedial Hypothalamic GABA Dysfunction Produces Physiological Arousal Following Sodium Lactate Infusions

A. SHEKHAR,*†[1] S. R. KEIM,* J. R. SIMON*‡ AND W. J. McBRIDE*‡

*Departments of Psychiatry, †Pharmacology & Toxicology, and ‡Biochemistry & Molecular Biology, Institute of Psychiatric Research, Indiana University Medical Center, 791 Union Drive, Indianapolis, IN 46202

Received 25 March 1995; Revised 30 November 1995; Accepted 27 December 1995

SHEKHAR, A., S. R. KEIM, J. R. SIMON AND W. J. McBRIDE. *Dorsomedial hypothalamic GABA dysfunction produces physiological arousal following sodium lactate infusions.* PHARMACOL BIOCHEM BEHAV **55**(2) 249–256, 1996.—Since impairing gamma-aminobutyric acid$_A$ ($GABA_A$) receptor-mediated inhibition in the dorsomedial hypothalamus (DMH) of rats elicits a panic-like response, experiments were conducted to test if rats with GABA dysfunction in the DMH would be vulnerable to precipitation of a panic-like response after intravenous sodium lactate infusions. Rats were implanted with unilateral infusion cannula into the DMH which were connected with Alzet minipumps that chronically infused (3.5 nmol/μl/h) either a-CSF (vehicle), *dl*-(racemic), *l*- (active) or *d*- (inactive) isomers of allylglycine (AG), an inhibitor of GABA synthesis. Another group of rats had *l*-allylglycine pumps implanted in the paraventricular nucleus of the hypothalamus (PVN) as anatomical controls. Animals were tested in the social interaction (SI) test and given sodium lactate infusions (10 ml/kg/15 min) before Alzet pump implantations and on days 4, 7, and 14 after pump placement. Rats were also tested in the elevated plus-maze on treatment day 4. Chronic impairment of GABA function in the DMH and not PVN resulted in rats being more anxious in the SI test on treatment days 4, 7, and 14 and in the elevated plus-maze on day 4 compared to a-CSF and *d*-AG infusions. Further, rats with GABA dysfunction in the DMH, and not PVN, exhibited significant increases in heart rate and blood pressure following IV sodium lactate infusions. There were significant decreases in DMH glutamic acid decarboxylase activity and GABA content in rats receiving 7 days of *dl*-AG or *l*-AG infusions. These results indicate that chronic reduction of GABA function in the DMH leads to the development of panic-like disorder in this animal model. **Copyright © 1996 Elsevier Science Inc.**

Panic disorder Anxiety Alzet pumps Social interaction elevated plus-maze Lactate infusion Dorsomedial hypothalamus

BLOCKING $GABA_A$ receptor-mediated inhibition in the dorsomedial hypothalamus (DMH) of rats elicits increases in heart rate (HR), blood pressure (BP), respiration (RR), intestinal motility, and peripheral blood flow patterns similar to a defense reaction (6,7,37). Further, blocking $GABA_A$ receptor function in the DMH elicits not only sudden, dramatic increases in physiological measures but also behavioral effects of increased locomotion (26), selective enhancement of "fear" responses (27), and an increase in experimental anxiety measured in three different behavioral paradigms (28,29,31). All of these data suggest that diminished activity of GABA neurons in the DMH induces both physiological and behavioral responses similar to human panic attacks. In addition, the panic-like response to $GABA_A$ receptor blockade in the DMH can be blocked by clinically effective antipanic drugs (30).

An interesting characteristic of patients with panic disorder is that in a majority of subjects a panic attack can be reliably elicited by intravenous infusions of provocative agents such as sodium lactate (15,21). It is still unclear as to whether this vulnerability to substances like sodium lactate is a function of putative central nervous system defects or merely an exaggerated response to peripheral stressors (36). If GABA dysfunction in the DMH is, indeed, one of the central defects associated with the syndrome of panic disorder, then rats that have impaired function of GABA in the DMH should be susceptible to physiological arousal by lactate infusions similar to patients with panic disorder. A vulnerability to lactate elicited by selectively impairing GABA in the DMH of rats would not only provide us with a strong animal model of panic disorder but would demonstrate for the first time that a specific

[1] To whom requests for reprints should be addressed.

central defect can induce sensitivity to peripheral lactate infusions. Therefore, the aim of this study was to induce GABA dysfunction in the DMH of rats and then test their physiological response to intravenous sodium lactate infusions.

METHOD

In the present study, Alzet minipumps (Alza Corp., Palo Alto, CA; model 2002) were used to induce chronic unilateral GABA dysfunction in the DMH of rats by chronically infusing (0.5 μl/h for 2 weeks) the GABA synthesis inhibitors. In both the racemic mixture of *d,l*-allylglycine (*dl*-AG) or *l*-allylglycine (*l*-AG; the active isomer), which inhibit glutamic acid decarboxylase (GAD), the synthetic enzyme for GABA was used (1,11,18). Pumps filled with either artificial CSF (a-CSF, vehicle) or the inactive isomer *d*-allylglycine (*d*-AG) were used as controls. Another group of rats implanted with *l*-AG pumps into the paraventricular nucleus of the hypothalamus (PVN) was used to test the anatomical specificity of eliciting a panic-like responses.

Animals

Experiments were conducted on male Sprague–Dawley rats (Harlan, Indianapolis, IN), weighing 275–325 g, which were housed in individual plastic cages at 72°C on a 12 L:12 D cycle with ad lib food and water. All the experiments were conducted according to the guidelines for the use of laboratory animals and after approval from the IUPUI animal welfare committee.

Surgical Procedures

Rats were first equipped with femoral arterial and venous catheters by using procedures previously described (23). The opposite end of the catheters were then routed through connective tissue to the dorsal aspect of the neck and stabilized with a leather vest and the rats were allowed to recover from the surgery.

Two days later, after obtaining baseline HR and BP response to lactate infusions as described below, chronic microinjection cannulae connected to Alzet minipumps filled with a-CSF, *dl*-AG, *l*-AG, or *d*-AG (infusion rate 3.5 nmol/0.5 μl/h) were implanted unilaterally in the DMH. Rats were given an IP injection of atropine (1 mg/kg) and anesthetized with an IP injection of pentobarbital (40 mg/kg). The femoral arterial catheter was flushed with saline and connected to a Beckman R511 Dynograph via a pressure transducer to measure HR and BP throughout the surgery. The animal was 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 degrees. A stainless steel L-shaped pump cannula with the side arm attached with a small tygon tube (Plastics One, Ranoake, VA; 29 gauge, 10 mm length) was secured on stereotaxic manipulator arms with a clamping device and connected to a 10 μl syringe with polyethylene tubing (PE-20). A 50 pmole/100 nl solution of the $GABA_A$ antagonist bicuculline methiodide (BMI) was used to fill the tubing and syringe. The syringe was then placed on an infusion pump (Sage Pump, model 355, Fisher Scientific, Pittsburg, PA) and adjusted to deliver 100 nl over a 30-s period. With manipulator arm set at an angle of +10 degrees, the cannula was lowered into the DMH (coordinates in relation to bregma being 1.2 mm posterior, 1.7 mm lateral, 9.0 mm ventral). For the PVN, the incisor bar was set at −3.3 degrees and the cannula placed at the PVN site (0.5 mm anterior, 1.7 mm lateral, 8.5 mm ventral) via a burr hole in the skull. Each site was tested with an injection of 50 pmol of BMI in 100 nl over a 30-s period. Heart rate and BP were recorded prior to injection, and any changes following the injection were noted. Within the DMH, an increase in HR of at least 50 beats/min was considered a reactive site. If injection at the initial 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. Once the implantation site was found, the side tubing was connected to the metal connector in the Alzet minipump that was previously filled with the desired infusion fluid. The pump was then sutured under the skin in the nape of the neck and the connector and cannula were cemented to the skull.

Intravenous Lactate Infusion Procedure

Before pump implantation, rats were given IV infusions of 0.9% saline and 0.5 M sodium lactate (10 ml/kg over 15 min; similar to clinical lactate infusions) (15) in random order with at least 60 min recovery time between infusions to obtain baseline responses. Responses to lactate (HR and BP) reported are the differences between changes elicited by lactate and saline vehicle infusions. Similar IV lactate infusions are conducted at specific times after the Alzet pump implantations to measure the effects of inducing GABA dysfunctions as described below.

Behavioral Tests for Anxiety

Social Interaction (SI) Test. The SI test is a fully validated test of experimental anxiety in rats (8), and the procedure used has been described previously (31). The apparatus consisted of a solid wooden box with an open roof 36′ long by 36′ wide with walls 12′ high. A video camera 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 freely interact 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 by two raters, of whom at least one was blind to any drug treatment. Intraobserver reliability for SI time was 0.9 to 0.97 in our laboratory.

Elevated Plus-Maze Test. The apparatus consisted of a maze with two open arms 50 × 10 cm and two enclosed arms 50 × 10 × 30 cm with an open roof. The arms were placed such that the two open and the two closed arms were opposite each other. The entire maze was elevated to a height of 50 cm above the ground. A video camera was fixed above the maze and all behavioral tests were videotaped. Rats were placed at the center of the four arms of this maze and their exploration of the maze for the next 5 min was recorded on videotape. The measures of time spent in each set of arms was scored from the videotape by a rater unaware of treatment conditions. This well-validated test (20) was used only on postpump day 4, as there are concerns about the reliability of this test with repeated testing (9).

Microdissection of the Hypothalamic Nuclei

Animals were sacrificed after completing their respective experiments by decapitation such that their heads fall into a container full of liquid nitrogen; the heads were removed after 5 s, during which time the brain cools to approximately freezing temperature. The brains were removed in a −20°C

TABLE 1

DETAILS OF THE EXPERIMENTAL PROTOCOL FOR EACH GROUP OF ANIMALS IMPLANTED WITH DIFFERENT ALZET PUMP INTO THE DMH

	Type of Alzet Pump			
Day of Treatment	a-CSF (n=6)	*dl*-Allylglycine (n=11)	*d*-Allylglycine (n=14)	*l*-Allylglycine (n=4)
Day-2	Placement of femoral arterial and venous catheters			
Day-1	Baseline SI; IV saline and lactate infusions	Baseline testing: social interaction test (SI); IV saline and lactate infusions		
Day 0	Alzet pump implantation into the DMH			
Day 4	SI and elevated plus-maze test followed by IV saline and lactate infusion			
Day 7	SI; IV saline and lactate	SI; IV saline and lactate. five rats sacrificed; DMH dissected for GAD assay	SI; IV saline and lactate. nine rats sacrificed; DMH dissected for GAD/GABA assay	SI: IV saline and lactate. All rats sacrificed; DMH dissected for GABA assay
Day 14	SI; IV saline and lactate. All rats sacrificed	SI; IV saline and lactate. Remaining six rats sacrificed	SI; IV saline and lactate. Remaining five rats sacrificed	

a-CSF—artificial cerebrospinal fluid; DMH—dorsomedial hypothalamus; GAD—glutamic acid decarboxylase; SI—social interaction test.

chamber, immediately placed in a brain slicer (Zivic-Miller, 1 mm sections), and frozen on dry ice. The brain section containing the DMH [6 mm from the occipital pole according to the atlas of Paxinos and Watson) (19) was placed on a dry ice platform. A square area (1 × 1 mm) of tissue immediately adjacent to the dorsal half of the third ventricle (area corresponding to the DMH) and the 1 mm square area lateral to it (the lateral hypothalamus, LH) were microdissected (30), weighed and stored in a −70° freezer until assayed. The hypothalamic regions on both the pump-treated and untreated sides were microdissected and separately assayed. The PVN was microdissected (from the brain section 8 mm from the occipital pole) in animals that had the pumps implanted into the PVN. In addition, in the PVN group, the regions of the hypothalamus that were anterior and posterior to the site of implantation were also dissected in millimeter segments. The decrease in GABA content of the PVN and the hypothalamic regions anterior and posterior to it were determined, thus helping to determine the degree of spread of l-AG.

Neurochemical Assays

Measurement of GAD Activity. The radiometric assay for GAD [modified from Bostwick and Le (3)] was based on supplying the enzyme with glutamate (substrate) with a ^{14}C labeled carboxyl group and measuring the liberated $^{14}CO_2$. The tissue was homogenized with 20 vol of a solution containing EDTA, Triton X-100, and aminoethanolthiol in phosphate buffer, pH 7. Each well of tissue culture plate (Falcon 3070) receives 10 μl tissue homogenate or blank (in triplicates) and 10 μl buffer substrate, after which the plate was covered with a 14 × 11 cm sheet of gel blot paper, latched shut, incubated in a 37°C water bath for 30 min, and then transferred to a 60°C bath for 45 min. The method for measuring the trapped $^{14}CO_2$ has been previously described (3).

Measurement of Tissue GABA Content. The GABA assay (12) was done with an HPLC analysis (Pump: ISCO 2352; Column: Spherisorb, ODS-2, 4.6 × 100, C_{18}, 3U; Detector: BAS LC-4B; Electrode: BAS glassy carbon; Integrator: HP 3390A; Mobile phase: NaOAc 0.18M, EDTA 1.2 M, 40% Acetonitrile, pH 6.3). Tissue is sonicated in 200 μl of 0.1 M perchloric acid, centrifuged, after which 20 μl of supernatant is combined with 20 μl of reaction buffer (20 mM 0-pthaldialdehide, 0.04 mM *t*-butylthiol, 0.1 M Na_2Co_3, 50% methanol, pH 9.6) for 2 min and GABA measured by injecting on the column. Tissue protein content is determined from the centrifuged pellets using the method of Lowry et al. (16).

Experimental Protocol

The experimental protocol used in the study is represented in Table 1. All rats initially had femoral arterial and venous catheters placed. After at least 24 h following the femoral arterial and venous catheterization, rats were examined in the SI test of anxiety as described above. After obtaining baseline anxiety levels in SI, the arterial catheters of rats were connected to the physiograph for recording HR and BP and the rats were given IV infusions of 0.9% saline and 0.5 M sodium lactate (10 ml/kg over 15 min). Once baseline responses to lactate infusions were recorded, rats were implanted with unilateral Alzet pumps as described above.

On postoperative days 4, 7, and in some animals on day 14 after pump implantation, the performance of the rats were again assessed in the social interaction test. On postoperative day 4, the rats (all except the a-CSF group, which were not tested in the plus-maze on day 4) were placed on the elevated plus-maze for 5 min following the SI test. After the behavioral testing, the arterial catheters were again connected to the physiograph and intravenous infusions of saline and 0.5 M sodium lactate (10 ml/kg, IV in 15 min) were once again

TABLE 2

INCREASES IN HEART RATE AND BLOOD PRESSURE IN RATS ELICITED BY INJECTING BMI (50 pmol/100 nl) INTO THE DORSOMEDIAL AND PARAVENTRICULAR HYPOTHALAMIC NUCLEI UNDER PENTOBARBITAL ANESTHESIA

	Heart Rate (Beats/Minute)		Blood Pressure (mm of Hg)	
Site of Injection	Baseline HR	Change in HR	Baseline BP	Change in BP
DMH (n=35)	311 ± 10	95 ± 7*	122 ± 6	20 ± 3*
PVN (n=6)	295 ± 16	28 ± 16	97 ± 3	13 ± 3*

HR = heart rate; BP = blood pressure; DMH = dorsomedial hypothalamic nuclei; PVN = paraventricular hypothalamic nuclei.

Data are presented as mean ± SEM.

* Significantly different from baseline by paired t-test, $p < 0.05$.

given in random order. The HR and BP responses to lactate infusions were determined for each of these days as described above. Approximately half of the rats with *d*- or *dl*-AG pumps were sacrificed after the postoperative day 7 and their DMH microdissected for determining the tissue GAD activity. All of the rats with *l*-AG pumps and their corresponding *d*-AG pump controls were sacrificed after postoperative day 7, their DMH (or PVN) microdissected and tissue GABA content was determined.

Statistical Analysis

All data were expressed as mean ± SEM. The statistical model used in comparing the means was a mixed analysis of variance with the type of pump (main effect) as the between subject factor and the day of pump (repeated measures) as the within subject factor. The Fisher's least significantly different test (Fisher's LSD) was used for pair-wise post hoc comparison to determine statistical significance. An ANOVA coupled with Student–Newman–Keul's (SNK) test was used when means under similar conditions were being compared.

RESULTS

Table 2 shows the changes in HR and BP elicited by injecting BMI into the DMH and PVN before implanting the Alzet pumps. $GABA_A$ receptor blockade in the DMH elicited significant increases in HR and BP, indicating that the pumps were implanted at the cardiostimulatory sites. Injecting BMI into the PVN elicited significant increases in BP but not HR (Table 2).

Rats With DMH Pumps

There were no significant differences in baseline anxiety among the four groups of rats as measured in the SI time (Fig. 1A, baseline). However, following 4 and 7 days of treatment, the *l*-AG and the *dl*-AG groups had significantly lower SI times [type of pump: $F(3, 33) = 14.245$, $p < 0.0001$; day: $F(3, 75) = 7.605$, $p < 0.0002$; pump × day: $F(8, 75) = 6.209$, $p < 0.0001$], compared to the *d*-AG and a-CSF groups. A shorter SI time was also observed after 14 days of infusion with *dl*-AG (Fig. 1A). In the elevated plus-maze, the rats receiving *l*- and *dl*-AG infusions for 4 days showed decreased open arm time, $F(2, 22) = 8.61$, $p = 0.0017$, compared with those given *d*-AG infusions (Fig. 1B). The total number of entries into the two arms of the plus-maze were similar in the *l*-AG (open = 3 ± 1; closed = 7 ± 1; total = 10 ± 2), *dl*-AG (open = 3 ± 1; closed = 6 ± 1; total = 9 ± 2), and *d*-AG (open = 5 ± 1; closed = 4 ± 1; total = 9 ± 1) groups.

Prior to implanting the minipumps, there were no changes in the HR or BP responses following lactate infusions in any of the four groups of rats (Fig. 2A and B, baseline). However, after pump implantations, lactate infusions elicited significant increases in HR [type of pump: $F(3, 31) = 52.106$, $p < 0.0001$; day: $F(3, 76) = 15.103$, $p < 0.0001$; pump × day: $F(8, 76) = 8.581$, $p < 0.0001$] and BP [type of pump: $F(3, 31) = 16.135$, $p < 0.0001$; day: $F(3, 76) = 11.233$, $p < 0.0001$; pump × day: $F(8, 76) = 3.354$, $p < 0.0025$] in rats treated for 4 and 7 days with *l*- and *dl*-AG but not in rats with a-CSF or *d*-AG pumps (Fig. 2A and B). This physiological reactivity to lactate was seen on treatment day 14 in the remaining group of rats with *dl*-AG pumps that were not sacrificed on day 7 (Fig. 2A and B).

In rats that were sacrificed on treatment day 7, a decrease in GAD activity, $F(1, 8) = 8.04$, $p = 0.022$, and GABA content, $F(1, 3) = 11.231$, $p = 0.05$, in the DMH was seen in rats with *dl*-AG and *l*-AG pumps, respectively, when compared to the nonpump side (Table 3).

Rats With PVN Pumps

In contrast to rats receiving infusions into the DMH, rats given *l*-AG infusions into the PVN for 4 or 7 days did not show any change from baseline in the SI test (Fig. 3A), $F(2, 15) = 0.902$, $p = 0.425$. Further, infusions of lactate in these rats did not elicit any significant changes in HR (Fig. 3B), $F(2, 15) = 1.064$, $p = 0.396$, or BP (Fig. 3C), $F(2, 15) = 1.575$, $p = 0.2393$, compared to baseline responses. Determination of GABA levels in the PVN of these rats revealed that tissue GABA levels on the side with *l*-AG pumps (2.1 ± 0.3 nmol/mg tissue) were significantly lower than GABA levels on the nonpump side [3.2 ± 0.5 nmol/mg tissue; $t(5) = 3.231$, $p < 0.05$]. Further, GABA levels were not significantly decreased in regions of the hypothalamus 1 mm from the site of implantation although some spread was evident at this distance (Fig. 3D).

DISCUSSION

The results of the present study suggest that chronic dysfunction of the GABAergic system within the DMH results in a significant increase in anxiety and produces symptoms of a panic-like state, for instance, physiological arousal following IV infusions of sodium lactate. Rats that have a chronic GABA dysfunction in the DMH spend significantly less time in SI test and the open-arm of the plus-maze test (Fig. 1A and B), suggesting an increase in the level of anxiety. Further, when these animals were given sodium lactate infusions, they exhibited significant increases in HR and BP compared to the rats

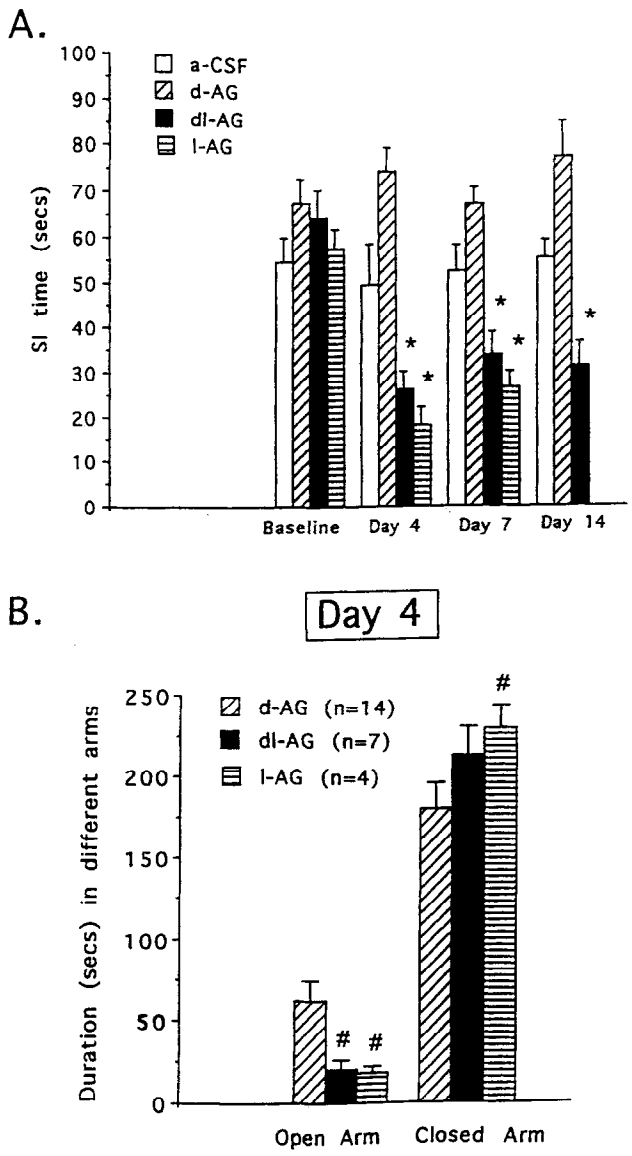

FIG. 1. Effects of implanting Alzet minipumps filled with artificial CSF (a-CSF), *dl*-allylglycine (*dl*-AG), *l*-allylglycine (*l*-AG), and *d*-allylglycine (*d*-AG) into the DMH of rats on the response in the (A) social interaction (SI) and (B) elevated plus-maze tests of "anxiety" prior to lactate infusion. The number of rats per group in the social interaction test were: a-CSF = 6; *d*-AG = 14 except 5 for day 14 (9 were sacrificed on day 7 for GAD and GABA determinations); *l*-AG = 4 (all sacrificed on day 7); *dl*-AG = 11 except 6 for day 14 (5 sacrificed on day 7). Rats with a-CSF pumps were not tested on the elevated plus-maze. Responses of all other rats were measured in the plus-maze on day 4 only. The data are presented as mean ± SEM *Significantly different from baseline and the corresponding day for a-CSF and *d*-AG by two-way ANOVA with least mean square test, $p < 0.05$; significantly different from *d*-AG by ANOVA coupled with SNK, $p < 0.05$.

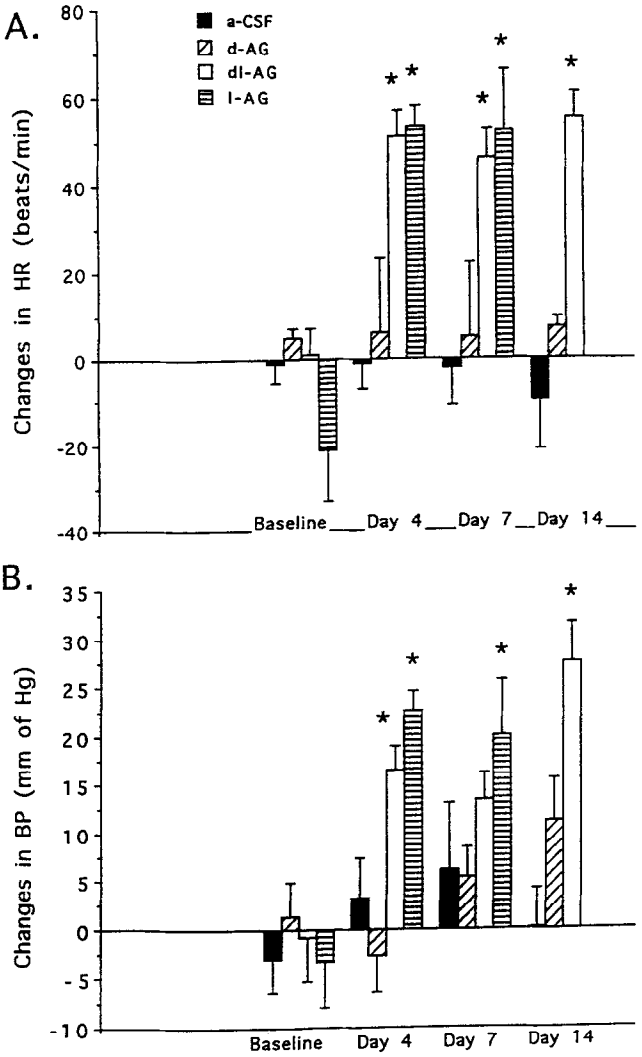

FIG. 2. The effects of intravenous infusion of sodium lactate (0.5 M, 10 ml/kg in 15 min) on (A) heart rate (HR) and (B) blood pressure (BP) of rats implanted with Alzet minipumps into the DMH filled with artificial cerebrospinal fluid (a-CSF), *dl*-allylglycine (*dl*-AG), *l*-allylglycine, and *d*-allylglycine. The change in HR and BP represent mean ± SEM of the difference between the changes elicited by intravenous infusions of 0.9% saline and sodium lactate. See Fig. 1, SI test for the number of rats in each group. *Significantly different from baseline and the corresponding day for a-CSF and *d*-AG by two-way ANOVA with least mean square test, $p < 0.05$.

with no GABA dysfunction in the DMH (Fig. 2A and 2B). This physiological response to lactate infusions in rats with GABA dysfunction in the DMH is consistent with responses elicited by lactate infusions in human panic disorder patients. An increase in HR is one of the most frequently observed physiological change in patients during a panic attack, whether it is naturally occurring (10) or induced by lactate infusions (15). Although lactate infusions elicit physiological arousal suggesting a panic-like response, it would be desirable to show that lactate further potentiates the anxiety seen in the rats with GABA dysfunction in the DMH. Unfortunately, with the present experimental protocol and the tests of anxiety used, testing for anxiety states following IV saline and lactate infusions could not be accomplished. Future studies would be conducted to specifically test this possibility.

The precise mechanism by which lactate elicits the physiological arousal in rats with GABA dysfunction in the DMH is unknown. Lactate infusions would affect a number of plasma

TABLE 3

EFFECTS OF IMPLANTING *d*-, *l*- AND *dl*-AG PUMPS IN THE DMH OF RATS ON THE ACTIVITY OF GLUTAMIC ACID DECARBOXYLASE, THE GABA SYNTHETIC ENZYME, AND GABA CONTENT IN THE DMH OF RATS

Neurochemical Measurement	Infusion Pump	Nonpump Side	Pump Side	Percent change on Pump Side
GAD activity (pmol/mg protein/30 min)	d-AG pump (n=5)	842 ± 162	903 ± 148	+13 ± 8
	dl-AG pump (n=5)	940 ± 153	595 ± 187	−48 ± 12*
GABA content (nmol/mg tissue)	d-AG pump (n=4)	2.1 ± 0.3	2.3 ± 0.4	+10 ± 4
	l-AG pump (n=4)	2.2 ± 0.1	1.4 ± 0.3*	−37 ± 9*

GAD = glutamic acid decarboxylase.
All the data are expressed as mean ± SEM.
* Significantly different from the nonpump side by ANOVA coupled with SNK, $p < 0.05$.

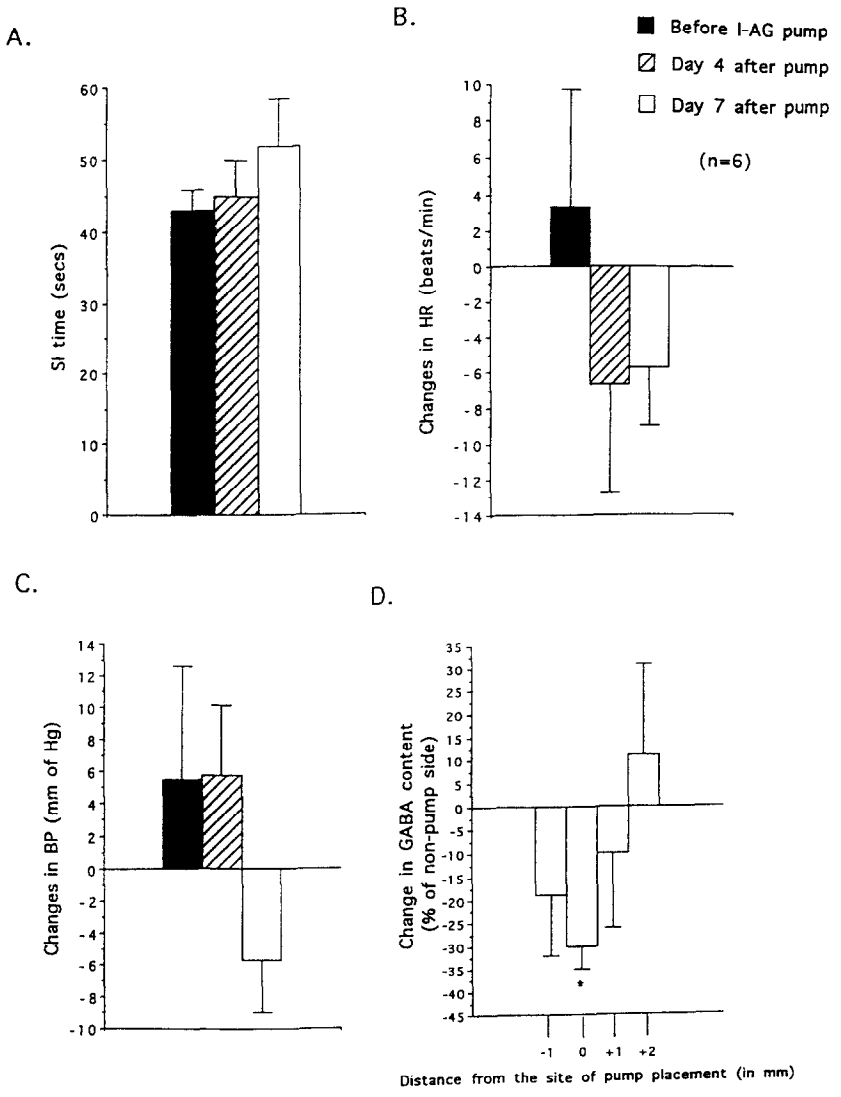

FIG. 3. Effects of implanting Alzet minipumps filled with *l*-allylglycine (*l*-AG) into the paraventricular nucleus (PVN) of rats on the response in (A) the social interaction (SI) before lactate infusions and changes in (B) HR and (C) BP after lactate infusions. D shows the percent decrease in tissue GABA content in 1 mm serial sections from the site of pump implantation in the PVN. The data are presented as mean ± SEM. *Significantly different from baseline by ANOVA coupled with SNK, $p < 0.05$.

parameters such as osmolarity, pH, electrolyte levels, etc. Any such change in plasma is likely to be detected in the CNS via the so-called circumventricular organs that lack an effective blood–brain barrier (22,35). The DMH has extensive connections with three major circumventricular organs, for instance, the organum vasculosum of lamina terminalis, subfornical organ, and area postrema (34). Hence, DMH has the necessary afferent connections to be influenced by peripheral blood parameters and the efferent mechanisms to elicit the physiological responses to lactate. GABA dysfunction in this nucleus could disable the normal homeostatic mechanisms such that a large peripheral osmotic load (or change in some other blood-borne parameter) during lactate infusions would activate the DMH excessively.

It is also suggested that patients with panic disorder may have highly reactive respiratory centers and that lactate infusions activate the medullary respiratory centers by increasing central CO_2 levels, resulting in the triggering of panic response (14). The DMH has projections to the medullary cardiovascular centers and reticular formation (34). Acute GABA blockade in the DMH not only activates the cardiovascular centers (increases in HR and BP) (7), but also respiration (7,29). A dysfunction in the DMH could conceivably make the medullary respiratory centers much more prone to activation.

The DMH is one of the hypothalamic nuclei activated when rats are subjected to different stressors as measured by the c-*fos* method (4). The known neuroanatomical connections of the DMH also support its proposed key integrative role in anxiety and panic-like responses. The DMH has ascending afferents from spinal cord, locus ceruleus, raphe nuclei, nucleus of the solitary tract, and periaqueductal gray (32). Afferents are also seen from the septum, bed nucleus of the stria terminalis, PVN (5), lateral hypothalamus (25), hippocampus, subiculum, and amygdala (32). The efferent projections of the DMH are to a number of areas that regulate emotional and physiological responses such as the PVN, lateral hypothalamus, bed nucleus of stria terminalis, hippocampus, periaqueductal gray at the level of cranial nerve III, nucleus tractus solitarius, subretrofacial nucleus, nucleus ambiguus, and the intermediolateral column of the spinal cord (24,32,34). Therefore, the afferent connections of the DMH are such that it has a variety of sensory, limbic, and cognitive input while its efferent connections enable it to be a coordinated output center of stress responses (2).

The lack of physiological responses to lactate infusions in rats with GABA dysfunction in the PVN suggests some anatomical specificity of this effect to the DMH. The PVN is another hypothalamic site that is also frequently associated with autonomic and neuroendocrine responses to stress (33). Blockade of $GABA_A$ receptors in the PVN are reported to elicit increases in cardiovascular responses, particularly a pressor response (17). In the present study, $GABA_A$ receptor blockade in the PVN did not elicit significant increases in heart rate but did increase BP (Table 2). Further, chronic GABA dysfunction in the PVN did not make the rats more anxious in the SI test (Fig. 3A) or make them physiologically reactive to lactate infusions (Fig. 3B and C). $GABA_A$ receptor blockade in the PVN does not, unlike the DMH, elicit consistent increases in corticosterone secretion (13). Therefore, while PVN may be a primary area for regulation of a number of stress responses, a tonic GABAergic system appears to regulate the coordinated autonomic and behavioral responses in the DMH and not the PVN.

The results of the present study indicate that spread to the PVN is not likely to account for the effects of *l*-AG infusions into the DMH. The serial GABA determinations in animals with pumps implanted in the PVN indicate that the spread of *l*-AG beyond 1 mm from the site of implantation is minimal (Fig. 3D). At least in the anterior–posterior direction, spread of *l*-AG to some other area to cause the panic-like response appears unlikely. It is still possible that the drug could spread to other areas posterior or lateral to the DMH. This possibility, while being less likely because acute injections of the GABA antagonist BMI posterior and lateral to the DMH has not yielded any cardiovascular or behavioral responses (29,31), still needs to be tested in a similar series of experiments as with the PVN. Another possibility is that mechanical damage at the site of pump implantation could lead to diffusion of *l*-AG to distant sites. Hisotological examination of the implantation sites conducted in addition to neurochemical analysis in a small group of animals revealed no obvious mechanical damage and the site of implantations in these animals were comparable to histology from chronic implantations in previous studies [see (31)].

In summary, the above results suggest that physiological sensitivity to peripheral lactate infusions can result from a dysfunction of GABA in the DMH of rats. Rats with GABA dysfunction in the DMH resemble human panic disorder in essential ways, and provide a potential animal model in which panic-like responses can be studied in the laboratory much like studies of lactate-induced panic in patients. Further, these results demonstrate for the first time that a discrete central nervous system defect can result in a susceptibility to peripheral infusions of sodium lactate leading to a panic-like response.

ACKNOWLEDGEMENTS

This study was supported by PHS Grant MH 45362 and research funds from the Indiana State Division of Mental Health. The authors thank Dr. Richard Thielen and Brian Bodart for assistance in conducting the GABA assay.

REFERENCES

1. Abshire, V. M.; Hankins, K. D.; Roehr, D. E.; DiMicco, J. A. Injection of L-allylglycine into the posterior hypothalamus in rats causes decreases in local GABA which correlate with increases in heart rate. Neuropharmacology 27(11):1171–1177; 1989.
2. Bernardis, L. L.; Bellinger, L. L. The dorsomedial hypothalamic nucleus revisited: An update. Brain. Res. Rev. 12:321–381; 1987.
3. Bostwick, J. R.; Le, W.-D. A tyrosine hydroxylase assay in microwells using coupled nonenzymatic decarboxylation of DOPA. Anal. Biochem. 192:125–130; 1991.
4. Buijs, R. M.; Markman, M.; Nunes-Cordoso, B.; Hou, Y.; Shinn, S. Projections of the suprachiasmatic nucleus to stress-related areas in the rat hypothalamus: A light and electron microscopic study. J. Comp. Neurol. 335:42–54; 1993.
5. Conrad, L. C.; Pfaff D. W. Efferents from the medial forebrain and hypothalamus of the rat: An autoradiography of the anterior hypothalamus. J. Comp. Neurol. 169:221–262; 1976.
6. DiMicco, J. A.; Abshire, V. M. Evidence for GABAergic inhibition of a hypothalamic sympathoexcitatory mechanism in anesthetized rats. Brain Res. 402(1):1–10; 1987.
7. DiMicco, J. A.; Soltis R. P.; Anderson J. J.; Wible J. H., Jr. Hypothalamic mechanisms and the cardiovascular response to stress. In: Kunos G.; Ciriello J., eds. Central neural mechanisms in cardiovascular regulation, Col. 2. Boston: Birkhauser; 1992:52–79.
8. File, S. E. The use of social interaction as a method for detecting anxiolytic activity of chlordiazepoxide-like drugs. J. Neurosci. Methods 2:219–238; 1980.

9. File, S. E.; Mabbutt, P. S.; Hitchcock, P. K. Characterization of the phenomenon of "one trial tolerance" to the anxiolytic effect of chlordiazepoxide in the elevated plus-maze. Psychopharmacology (Berlin) 102:98–101; 1990.
10. Freedman, R. R.; Ianni, P.; Ettedgui, E.; Puthenzhath, N. Ambulatory monitoring of panic disorder. Arch. Gen. Psychiatry 42:244–248; 1985.
11. Horton, R. W. The role of 2-keto-4-pentenoic acid in seizures induced by allylglycine. Biochem. Pharmacol. 27:1471–1477; 1978.
12. Kehr, J.; Ungerstedt, U. Fast HPLC estimation of gamma-aminobutyric acid in microdialysis perfusates: Effects of nipecotic and 3-mercaptopropionic acids. J. Neurochem. 51:1308–1310; 1988.
13. Keim, S. R.; Shekhar, A. Effects of GABA*A* receptors blockade in the dorsomedial hypothalamus of rats on ACTH and corticosterone secretion. Soc. Neurosci. Abstr. 20:373; 1994.
14. Klein, D. F. False suffocation alarms, spontaneous panics and related conditions: An integrative hypothesis. Arch. Gen. Psychiatry 50:306–317; 1993.
15. Liebowitz, M. R.; Gorman, J. M.; Fyer, A.; Dillon, D.; Levitt, M.; Klein, D. F. Possible mechanisms for lactate's induction of panic. Am. J. Psychiatry 143(4):495–502; 1986.
16. Lowry, O. H.; Rosebrough N. J.; Farr A. L.; Randall R. J. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265–271; 1951.
17. Martin, D. S.; Segura, T.; Haywood, J. R. Cardiovascular responses to bicuculline in the paraventricular nucleus of the rat. Hypertension 18:48–55; 1991.
18. Orlowski, M.; Reingold, D. F.; Stanley, M. E. *d*- and *l*-stereoisomers of allylglycine: Convulsive action and inhibition of brain *l*-glutamate decarboxylase. J. Neurochem. 28:349–353; 1977.
19. Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. 2nd ed. New York: Academic Press; 1986.
20. Pellow, S.; Chopin, P.; File, S. E.; Briley, M. Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J. Neurosci. Methods 14(3):149–167; 1985.
21. Pitts, F. N.; McClure, J. N. Lactate metabolism in anxiety neurosis. N. Engl. J. Med. 227:1329–1336; 1967.
22. Ramsay, D. J.; Thrasher, T. N.; Keil, L. C. The organum vasculosum of stria terminalis: A critical area for osmoreception. In: Cross, B. A.; Leng, G., eds. The neurohypophysis: Structure, function and control. Progress in brain research, vol. 60. Amsterdam: Elsevier/North-Holland; 1983:91–98.
23. Sanders, S. K.; Shekhar, A. Blockade of $GABA_A$ receptors in the anterior basolateral amygdala of rats elicits increases in heart rate and blood pressure. Brain Res. 576:101–110; 1991.
24. Saper, C. B.; Loewy, A. D.; Swanson, L. W.; Cowan, W. M. Direct hypothalamo-autonomic connections. Brain Res. 117:305–312; 1976.
25. Saper C. B.; Swanson L. W.; Cowan W. M. An autoradiographic study of the efferent connections of the lateral hypothalamic area of the rat. J. Comp. Neurol. 183:707–720; 1979.
26. Shekhar, A.; DiMicco, J. A. Defense reaction elicited by injection of GABA antagonists and synthesis inhibitors into the posterior hypothalamus in rats. Neuropharmacology 26:407–417; 1987.
27. Shekhar, A.; Hingtgen, J. N.; DiMicco, J. A. Selective enhancement of shock avoidance responding elicited by GABA blockade in the posterior hypothalamus of rats. Brain Res. 420:118–128; 1987.
28. Shekhar, A.; Hingtgen, J. N.; DiMicco, J. A. GABA receptors in the posterior hypothalamus regulate experimental anxiety in rats. Brain Res. 512:81–88; 1990.
29. Shekhar, A. GABA blockade in the region of the dorsomedial hypothalamus regulates "anxiety" in rats in the elevated plus-maze: I. Behavioral measures. Brain Res. 627:9–16; 1993.
30. Shekhar, A. Effects of chronic treatment with imipramine and clonazepam in an animal model of panic disorder. Biol. Psychiatry 36:748–758; 1994.
31. Shekhar, A.; Katner, J. S. $GABA_A$ receptors in the dorsomedial hypothalamus of rats regulate anxiety in the social interaction test. Pharmacol. Biochem. Behav. 50:253–258; 1995.
32. Swanson, L. W. The hypothalamus. In: Bjorklund, A.; Hokfelt, T.; Swanson, L. W., eds. Handbook of chemical neuroanatomy, vol 5. Amsterdam: Elsevier; 1987:1–104.
33. Swanson, L. W.; Sawchenko, P. E. Hypothalamic integration: Organization of the paraventricular and supraoptic nuclei. Annu. Rev. Neurosci. 6:269–324; 1983.
34. Ter Horst, G. J.; Luiten, P. G. M. Projections of the dorsomedial hypothalamic nucleus of the rat. Brain Res. Bull. 16:231–248; 1986.
35. Van Houten, M.; Posner, B. I. Circumventricular organs: Receptors and mediators of direct peptide hormone action on brain. In: Szabo, A. J., ed. Advances in metabolic disorders, vol. 10. Central nervous system regulation of carbohydrate metabolism. New York: Academic Press; 1984:269–289.
36. Van den Hout, M. A. Explanations of experimental panic. In: Rachman, S.; Maser, J. D., eds., Panic: Psychological perspectives. Hillsdale, NJ: Lawrence Erlbaum Assoc.; 1988:1237–1258.
37. Wible, J. H., Jr.; Luft, F. C.; DiMicco, J. A. Hypothalamic GABA suppresses sympathetic outflow to the cardiovascular system. Am. J. Physiol. 254:680–687; 1988.