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Sodium lactate elicits anxiety in rats after repeated GABA receptor blockade in the basolateral amygdala ☆

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Abstract

Repeated administration of the $GABA_A$ receptor antagonist bicuculline methiodide into the basolateral nucleus of the amygdala at doses subthreshold to eliciting a full response will eventually produce long-term 'priming', such that heart rate, blood pressure as well as anxiety are increased at the lower doses. The present study was conducted in order to determine if the long-term priming of anxiety within the basolateral nucleus is producing a condition similar to that seen in human panic disorder by testing the response elicited by i.v. lactate infusions, since lactate infusions induce a panic attack in patients with panic disorder. Male Wistar rats were fitted with femoral arterial and venous catheters and chronic microinjection cannulae into the basolateral nucleus. Repeated daily injections of a subthreshold dose of bicuculline methiodide into the basolateral nucleus for 4–5 days elicited a primed response, while the same procedure with artificial cerebrospinal fluid vehicle (a-CSF; sham-primed) had no effect. Following priming, rats received both sodium lactate infusions (0.5 N, 10 ml/kg) or 0.9% saline in a random order separated by 48 h. Heart rate and blood pressure were monitored throughout the infusion and the animals were immediately placed in the social interaction test to assess their anxiety response. Only primed and not sham-primed rats responded to a lactate infusion with significant increases in heart rate, blood pressure and experimental anxiety. Thus, rats which are primed with chronic subthreshold GABA receptor blockade in the basolateral nucleus develop a sensitivity to sodium lactate, similar to human panic disorder patients.

Keywords: Anxiety; Blood pressure; Glutamate; Heart rate; Kindling; Long-term potentiation; Panic; Social interaction; Sodium lactate

1. Introduction

Panic disorder is a severe anxiety disorder which is characterized by frequent and unexpected panic attacks consisting of physiological and behavioral arousal associated with a sense of fear and feelings of apprehension. Another characteristic that distinguishes patients with panic disorder from other types of anxiety disorders is their sensitivity to intravenous (i.v.) sodium lactate infusions (0.5 N DL-sodium lactate 10 ml/kg) which can elicit a full blown panic attack (Pitts and McClure, 1967; Reiman et al., 1984, 1989; Liebowitz et al., 1986) and which can be blocked by antipanic treatments (Gorman et al., 1987).

Both animal (Hilton and Zbrozyna, 1963; Kapp et al., 1982; Davis, 1992) and human studies (Feindel and Penfield, 1954) have shown that activation of the amygdala results in behavioral and physiological responses associated with anxiety. In contrast, lesions of this area are associated with a decrease in fear and anxiety (Weiskrantz, 1956; Narabayashi et al., 1963; Phillips and LeDoux, 1992). The basolateral amygdala (basolateral nucleus) is one nucleus in particular that has been implicated in the regulation of anxiety. Blocking $GABA_A$ receptors with bicuculline methiodide in the area of the basolateral nucleus can elicit increases in heart rate, respiration, blood pressure and experimental anxiety (Sanders and Shekhar 1991, 1995a,b), a response very similar to human panic attacks. In addition, the basolateral nucleus contains the

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highest concentration of benzodiazepine receptors (Niehoff and Kuhar, 1983). In fact, the anxiolytic effects of systemic injections of benzodiazepines can be completely blocked by microinjections of the benzodiazepine receptor antagonist flumazenil into the basolateral nucleus (Sanders and Shekhar, 1995a).

Long-term potentiation, a form of synaptic plasticity, has been shown to occur within the basolateral nucleus by both in vitro (Chapman and Bellavance, 1992; Chapman et al., 1990) and in vivo (Clugnet and LeDoux, 1990; Maren and Fanselow, 1995) studies. Induction of long-term potentiation occurs as a result of sufficient stimulation over a given period of time of the glutamate receptors on a neuron. There is a tonic glutamatergic transmission within the basolateral nucleus which is under GABAergic regulation (Rainnie et al., 1991a,b; Smith and Dudek, 1996). Furthermore, it appears that the state of excitability of basolateral nucleus projection neurons is dependent on the GABAergic inhibitory control. Partially removing this GABA inhibition as in rats administered three to five repeated subthreshold doses of bicuculline methiodide directly into the basolateral nucleus will induce a 'priming' effect such that the original subthreshold dose will elicit a panic-like threshold response (Sanders et al., 1995).

The priming of anxiety with repeated subthreshold GABA receptor blockade is also phenomenologically similar to the development of panic disorder, where repeated spontaneous panic episodes lead to chronic high levels of anxiety and a panic-prone state (Gorman et al., 1989). If the priming of anxiety in the basolateral nucleus was indeed similar to the development of panic disorder, then primed rats would be predicted to become reactive to sodium lactate infusions which selectively activate a panic response in patients. Therefore, the present study was conducted to test if basolateral nucleus primed rats would show reactivity to i.v. lactate infusions.

2. Materials and methods

2.1. Animals

Experiments were conducted on male Wistar rats (Harlan Laboratories, 275–300 g). They were individually housed in a temperature-controlled room (20°C) on a 12 h day/night cycle and were given food and water ad lib.

2.2. Surgical procedures

2.2.1. Arterial catheterization

Animals were given atropine (1 mg/kg) and anesthetized with pentobarbital (50 mg/kg). Catheters were made up of 5 cm of 0.025-cm Tygon tubing (Fisher Scientific) inside 30 cm of 0.05-cm tubing using cyclohexanone to fuse the tubing together. The 0.025-cm tubing was inserted into the artery or vein while the 0.05-cm tubing was routed subcutaneously to the dorsal aspect of the neck where it was secured with a leather jacket as previously described (Sanders and Shekhar, 1991). In order to improve patency, catheters were soaked in heparinized saline (2.5 units/ml) prior to insertion and filled with the same heparinized saline after placement.

2.2.2. Implantation of chronic injection cannulae into the basolateral nucleus

Immediately following catheterization, animals were placed into a stereotaxic instrument (Kopf Instruments, Tujunga, CA) with the incisor bar set at −3.3 mm. Two stainless steel guide cannulae (26 gauge, length 10 mm) were fixed onto the stereotaxic arms and then lowered into the basolateral nucleus using the coordinates (A, −2.0 mm; L, +5.0 mm; V, −8.0 mm) according to the atlas of Paxinos and Watson (1986). The guide cannulae were secured in place using three 2.4-mm stainless steel screws anchored to the skull and cranioplastic cement. The guide cannulae were sealed with dummy cannulae (Plastic Prod-

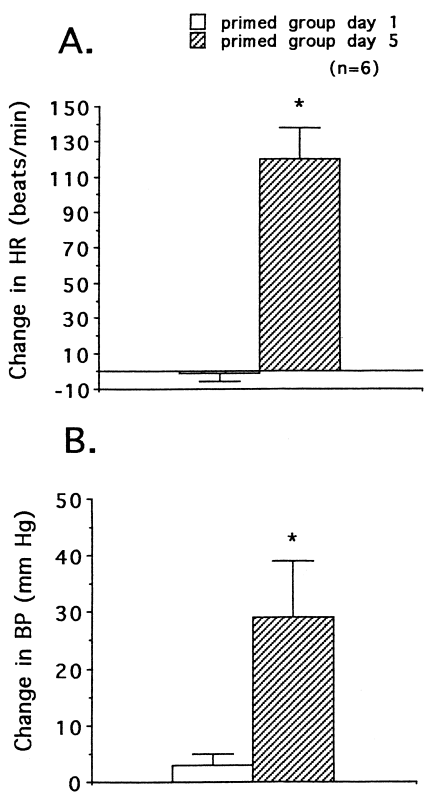

Fig. 1. Changes from day 1 to day 5 in (A) heart rate (heart rate, beats/min) and (B) blood pressure (blood pressure, mmHG) elicited by bilateral injections of bicuculline methiodide (6 pmol/100 nl) daily for 5 days into the basolateral nucleus of rats. Injections of bicuculline methiodide (6 pmol) on day 5 elicited significant increases in heart rate and blood pressure similar to threshold injections of bicuculline methiodide. Data are presented as mean ± S.E.M. Significantly different from * day 1 by repeated measures ANOVA coupled with Fisher's LSD test.

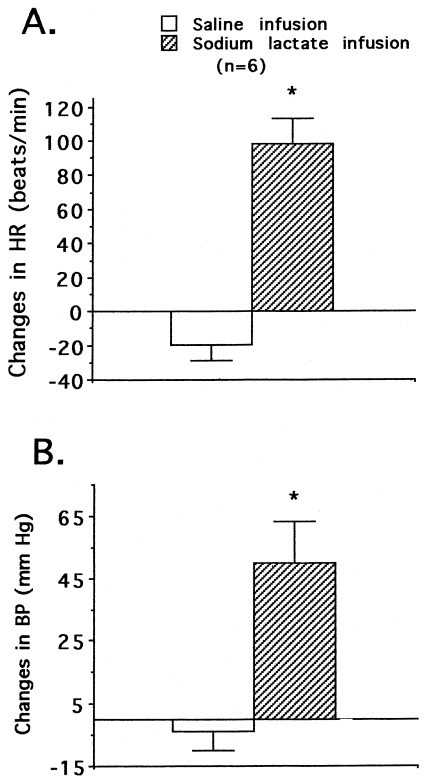

Fig. 2. Changes in (A) heart rate (heart rate, beats/min) and (B) blood pressure (blood pressure, mmHG) elicited by either intravenous sodium lactate (0.5 N, 10 mg/kg) or saline (0.9%, 10 mg/kg) infusions in primed rats. Lactate infusions significantly increased the heart rate and blood pressure in primed rats. Data are presented as mean ± S.E.M. Significantly different from * saline by repeated measures ANOVA coupled with Fisher's LSD test.

ucts, Roanoke, VA). Animals were removed from the stereotaxic instrument and allowed 72 h to recover.

2.3. Intracranial (i.c.) drug infusions

Acute microinjections of drugs into the basolateral nucleus, utilizing injection cannulae (33 gauge) which fitted into and extended 1 mm beyond the guide cannulae, were delivered bilaterally in 100 nl of artificial cerebrospinal fluid (a-CSF). The animals were transported in their home cages from the animal housing room to the experimental room and allowed 30 min to acclimate. The rat's arterial line was connected to a Beckman physiograph (model 511A) and heart rate and blood pressure were monitored. To minimize stress, the injection cannulae were inserted into the guide cannulae without removing the animal from its home cage or placing it under any type of restraint. The heart rate and blood pressure were carefully monitored throughout placement of the injection cannulae. Once the cannulae were in place, the rat was observed and administration of the compounds was not begun until a steady heart rate baseline was obtained for 10–15 min. A 10-μl Hamilton syringe placed on an infusion pump (Sage Instruments, model 355) was connected to the injection cannulae via polyethylene (PE-50) tubing (Fisher Scientific, Pittsburgh, PA). The pump was subsequently turned on for 30 s during which time the 100 nl of solution per site was delivered. The injection cannulae remained in place for an additional minute before being removed.

2.4. Intravenous (i.v.) infusion

The venous tubing secured to the jacket of the animal was carefully untied while the rat was freely moving in his home cage. The venous line was gently flushed with heparinized saline (2.5.units/ml). A 5-cc syringe placed on an infusion pump was connected to the venous catheter via 0.05-cm tubing. Once baseline heart rate was established the pump was turned on and either 0.9% saline or 0.5 N sodium lactate solutions (10 ml/kg) was infused over approximately 15 min as previously described (Shekhar et al., 1996).

2.5. Behavioral measurement (social interaction)

Experimental anxiety was measured by the social interaction test, a fully validated test of anxiety (File, 1980)

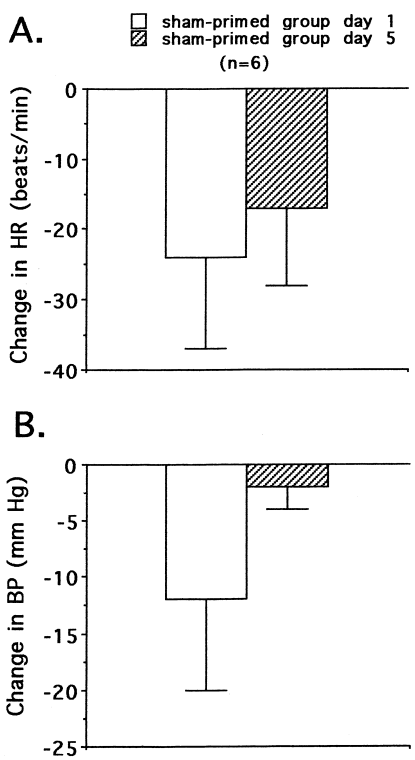

Fig. 3. Changes from days 1 to 5 in (A) heart rate (heart rate, beats/min) and (B) blood pressure (blood pressure, mmHG) elicited by bilateral injections of a-CSF (100 nl) daily for 5 days into the basolateral nucleus of rats. Repeated injections of a-CSF did not induce increases in heart rate and blood pressure. Significantly different from * day 1 by repeated measures ANOVA coupled with Fisher's LSD test.

which has been previously used in our laboratory (Shekhar and Katner, 1995; Sanders and Shekhar, 1995a). The apparatus used was a solid wood box 91.44 cm $L \times 91.44$ cm $W \times 30.48$ cm H with an open roof. A video camera was fixed above the social interaction box and all behavioral tests were recorded. During the test session, the 'experimental' rat was placed into the social interaction box with a 'partner' rat for a total of 5 min. The amount of time the 'experimental' rat spent interacting, i.e., making physical contact (grooming, sniffing, crawling upon, etc.) with the 'partner' rat was recorded. Sessions were scored at a later time by two raters of whom at least one was blind to any drug treatment. Inter-observer reliability for the time of social interaction has been 0.9 to 0.97 in our laboratory. A decrease in interaction time was taken as an increase in 'anxiety' and vice versa.

2.6. Experimental protocol

Seventy-two hours after recovery from surgery, animals were divided into a priming or a sham-priming group ($n = 6$ each). All experiments were conducted between the hours of 7:00 and 11:30 AM. On experimental day 1, each rat was connected to the physiograph and heart rate and blood pressure were recorded. Once a steady baseline was attained, either bicuculline methiodide (6 pmol/ 100 nl) for the priming group or a-CSF (100 nl) for the sham-priming group was administered. The animal remained connected to the physiograph and heart rate and blood pressure were recorded for an additional 15 min. The animals were given their assigned injections daily without heart rate or blood pressure recordings until day 5 when the same protocol as day 1 was repeated.

Following the priming procedure, on experimental day 6, the femoral arterial catheters were again connected to the physiograph and a steady baseline of heart rate and blood pressure was attained. Rats were infused randomly with either 0.9% saline or 0.5 N sodium lactate (10 ml/kg) via the venous catheter such that half of each group received saline first and the other half received lactate infusions. At the end of the infusion, catheters were disconnected from the physiograph and the animals were then immediately placed into the social interaction test. On experimental day 8, rats were given the remaining i.v. infusion (saline or lactate) for their group and the same procedure as on day 6 was repeated.

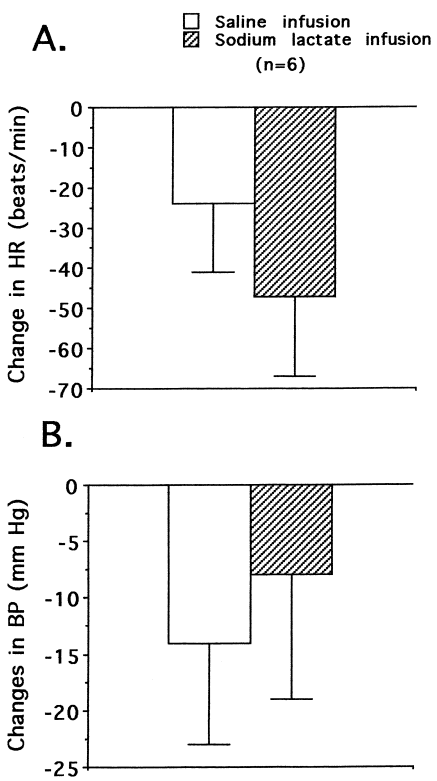

Fig. 4. Changes in (A) heart rate (heart rate, beats/min) and (B) blood pressure (blood pressure, mmHG) elicited by either intravenous sodium lactate (0.5 N, 10 mg/kg) or saline (0.9%, 10 mg/kg) infusions in sham-primed rats. Lactate infusions failed to increase heart rate or blood pressure in sham-primed animals. Significantly different from * saline by repeated measures ANOVA coupled with Fisher's LSD test.

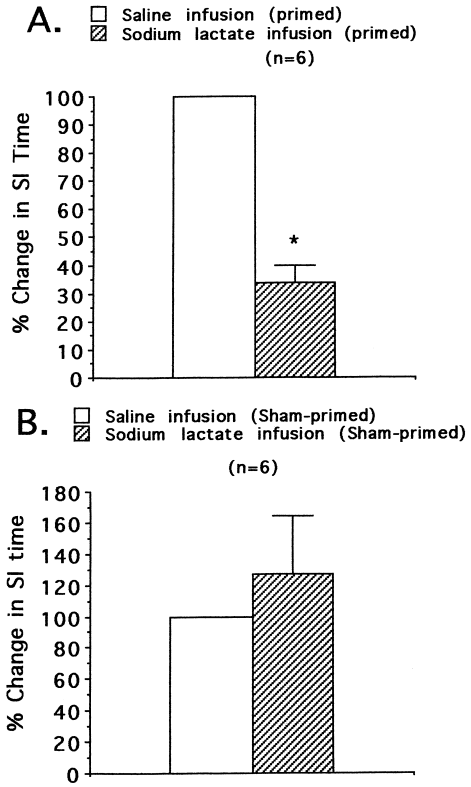

Fig. 5. Changes in (A) social interaction time (social interaction, % of saline, time in s) elicited either intravenous sodium lactate (0.5 N, 10 mg/kg) or saline (0.9%, 10 mg/kg) in primed animals and (B) social interaction time (social interaction, % of saline, time in s) elicited either intravenous sodium lactate (0.5 N, 10 mg/kg) or saline (0.9%, 10 mg/kg) in sham-primed animals. Infusions of sodium lactate elicited increased activity (i.e., decreased social interaction time) in only primed and not sham-primed rats. Significantly different from * saline by repeated measures ANOVA coupled with Fisher's LSD test.

Upon completion of the experiment, animals were anesthetized with halothane and 100 nl of 50% solution of India ink was injected via the amygdalar cannulae. They were sacrificed using a guillotine. The brains were removed, frozen in a cryostat, sectioned (40 μm) and mounted on slides. Slides were subsequently stained with Neutral Red to determine the location of the i.c. injection by comparing them with the atlas of Paxinos and Watson (1986). Only data from the rats that had successful implants into the basolateral nucleus were utilized in the analyses.

The results of the heart rate and blood pressure data were recorded as the maximal change from baseline. Social interaction data were converted to percent change in social interaction time as compared to saline (which was represented as 100% for each rat). All data are presented as mean ± S.E.M. A statistical analysis was done by using a repeated measures analysis of variance (ANOVA) test with a Fisher's least significant difference (LSD) post hoc test. Statistical significance was accepted at $P < 0.05$.

3. Results

Fig. 1A shows that animals that went through the priming procedure had a significant increase in the heart rate response following bicuculline methiodide (6 pmol) injections from days 1 to 5 [$F(1,5) = 59.709$; $P = 0.0006$], while Fig. 1B shows the blood pressure responses that occurred in these same animals [$F(1,5) = 5.925$; $P = 0.0590$]. Primed animals had a significant increase in heart rate [Fig. 2A; $F(1,5) = 33.708$; $P = 0.0021$] as well as blood pressure [Fig. 2B; $F(1,5) = 9.565$; $P = 0.0271$] when infused with sodium lactate as compared with saline. The initial increase in heart rate and blood pressure responses

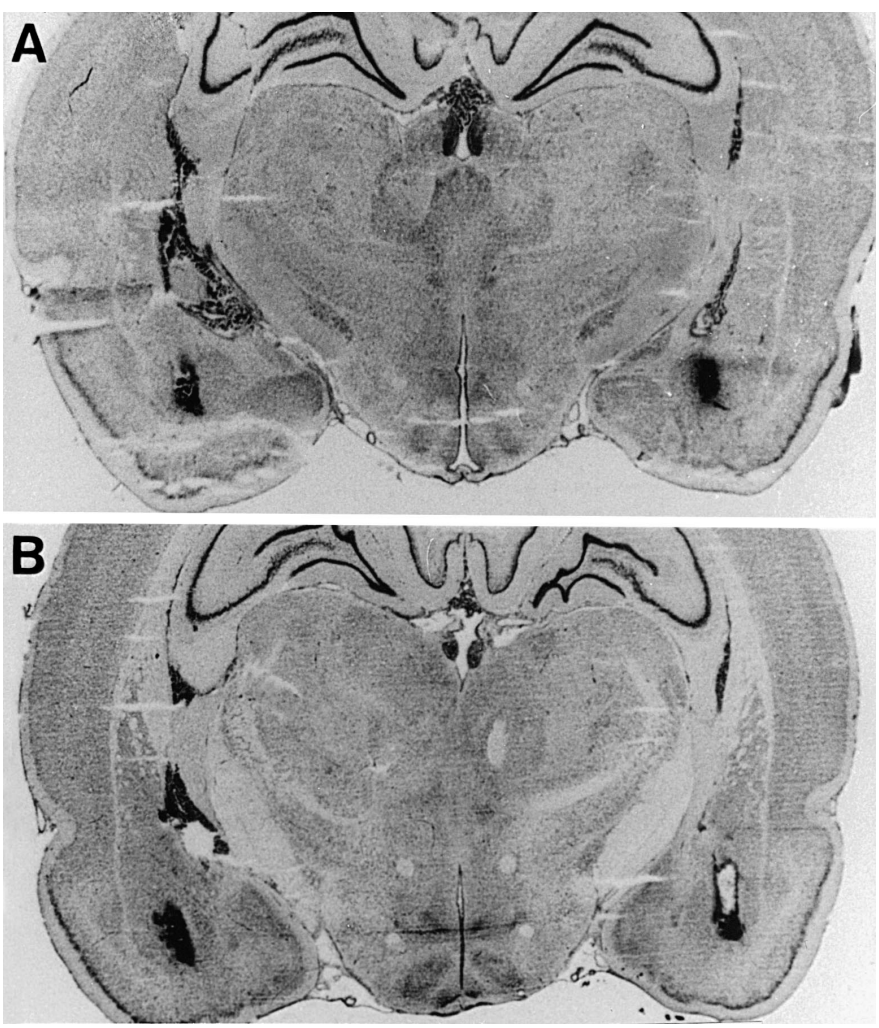

Fig. 6. Representative photomicrographs of histological sections showing the sites of microinjection cannulae implantation in the basolateral nucleus of (A) primed rats, and (B) sham-primed rats. The sites were marked by injecting 50% India ink and confirmed by comparing with the atlas of Paxinos and Watson (1986). Only data from the animals with cannulae placement in the basolateral nucleus were used in the analysis.

were noticed within 1–2 min of the start of the infusion and subsequently increased in a linear manner with peak levels being reached between 8 and 10 min. The increase in heart rate and blood pressure was maintained at peak levels until the infusion was complete. Immediately following the infusion the rats were placed in the social interaction test; thus the time course of the heart rate returning to baseline was not examined. However, in other studies it has been noted that once the infusion is complete the heart rate and blood pressure levels begin to decrease quickly, with levels returning to baseline values within 5–7 min. Additionally, there were no significant changes in baseline values for heart rate or blood pressure between experimental days 1 and 5. There were also no significant changes observed in either heart rate or blood pressure (Fig. 3A and B) between days 1 and 5 with the a-CSF injections. Likewise, there were no significant changes in heart rate and blood pressure of the sham-primed animals during either saline or sodium lactate infusions (Fig. 4A and B).

Animals in the primed group as compared to the sham-primed group showed a significant decrease in social interaction time [Fig. 5A and B; $F(1,5) = 1.382$; $P = 0.0001$] following the sodium lactate infusion.

Fig. 6A shows a representation of the injection cannulae implantation sites in the basolateral nucleus of rats used in the primed animals, while Fig. 6B represents the cannulae sites in the sham-primed animals.

4. Discussion

Although anxiety is a normal emotional response to a fearful situation, individuals who suffer from anxiety disorders show a greater sensitivity in both their physiological and behavioral reactions in a variety of situations (Marks, 1987; Uhde, 1990). In addition, this exaggerated 'fight-or-flight' reaction can occur as a response to inappropriate stimuli. One unique characteristic that distinguishes patients with panic disorder from normal controls and other anxiety disorders is that these individuals are selectively sensitive to infusions of sodium lactate (Pitts and McClure, 1967; Reiman et al., 1984, 1989; Liebowitz et al., 1986) such that lactate can reliably elicit a full panic attack. Therefore, the development of lactate sensitivity may be utilized to study the underlying mechanisms in the development of panic disorder.

Blocking $GABA_A$ receptors in the basolateral nucleus with repeated subthreshold doses (four to five) of bicuculline methiodide leads to long-term changes in the basolateral nucleus neurons such that a full-panic-like response is elicited by a subthreshold dose of the $GABA_A$ receptor antagonist (Sanders et al., 1995). The long-term changes of increased anxiety and easy arousal of panic responses that occur during the priming response are phenomenologically similar to the development of clinical symptoms of individuals diagnosed with panic disorder. Therefore, a change in amygdalar responses similar to priming may be one potential underlying abnormality with patients suffering from that subtype of anxiety. Further, the present findings show that animals which were given a daily injection of a subthreshold dose of bicuculline methiodide (6 pmol/100 nl) for five consecutive days not only showed a significant increase in heart rate and blood pressure to that subthreshold dose by day 5 (Fig. 1A and B), i.e., became primed, but then rats were also exhibiting physiological and behavioral arousal following i.v. lactate infusions. Animals that received only a-CSF showed neither changes in heart rate or blood pressure (Fig. 3A and B) after 5 days nor were they reactive to lactate infusions. Therefore, the changes that are occurring in the basolateral nucleus to elicit a panic-like state are due to the repeated receptor blockade of GABA inhibition and not the result of tissue damage from repeated injections.

Other types of long-term changes such as long-term potentiation (Chapman et al., 1990; Clugnet and LeDoux, 1990; Gean et al., 1993; Maren and Fanselow, 1995) and kindling (Goddard et al., 1969; Racine and McIntyre, 1986) are also strongly associated with the basolateral nucleus. The anxiety response seen after priming occurred prior to any epileptic-like activity in the basolateral nucleus as measured by EEG activity in this area during priming (Sanders et al., 1995). Therefore, priming is not likely due to seizure activity originating in the basolateral nucleus. However, long-term potentiation in the basolateral nucleus has been shown to be involved in the learning of fear conditioning (Maren and Fanselow, 1995) and thus it is possible that priming is a phenomenon similar to long-term potentiation.

Since the study by Pitts and McClure (1967), it has been known that infusions of sodium lactate (0.5 N, 10 ml/kg) can induce panic attacks in individuals suffering from panic disorder. It has been theorized that the sensitivity to lactate in these individuals could be the result of an exaggerated response to increased CO_2 levels in the medullary respiratory centers thus producing a suffocation false-alarm (Klein, 1993) or a hyperactive locus ceruleus, another brainstem area associated with arousal (Redmond and Huang, 1979). The exact mechanism by which lactate elicits a panic response in patients with panic disorder is still unclear.

The basolateral nucleus is one of several sites within the CNS that is capable of eliciting a 'fight-or-flight' response. Many of the other sites within the defense circuit such as the dorsomedial hypothalamus (DiMicco et al., 1992; Shekhar, 1993, 1994) and the periaquaductal gray (DiScala et al., 1984; Graeff et al., 1986) are also under tonic GABAergic inhibition. Blocking GABA inhibition at these sites also elicits a similar panic-like response in rats. The panic response elicited by GABA blockade within the dorsomedial hypothalamus has been extensively studied (Shekhar, 1994; Shekhar and Katner, 1995; Keim and

Shekhar, 1996). It was found that rats with chronic GABA dysfunction in the dorsomedial hypothalamus could elicit panic-like symptoms following i.v. sodium lactate infusions. (Shekhar et al., 1996). Furthermore, it was shown that blocking the neuronal activity within the organum vasculosum lamina terminalis, a circumventricular organ, with tetrodotoxin could completely block the panic response seen during intravenous lactate infusions, while direct injections of sodium lactate into the organum vasculosum lamina terminalis could produce a dose-dependent anxiety response in these rats (Shekhar and Keim, 1996). This suggests that the organum vasculosum lamina terminalis may be a primary afferent site for the lactate response in animals with compromised GABA function in the dorsomedial hypothalamus and the circumventricular organs may be critical sites in detecting the lactate stimulus.

The circumventricular organs are special areas in the central nervous system which lack an intact blood brain barrier and thus are capable of detecting changes in plasma osmolarity, pH, etc. (Johnson and Gross, 1993). The amygdala has connections to the subfornical organ and the organum vasculosum lamina terminalis, both circumventricular organs located along the third ventricle (Johnson and Gross, 1993). Preliminary data suggest that blocking the afferents to the basolateral nucleus from the subfornical organ in primed animals by injecting tetrodotoxin into the subfornical organ completely blocks the panic-like response to sodium lactate infusions (Shekhar et al., unpublished observations). This data taken with the current findings suggest that one possible pathway for the lactate response in the basolateral nucleus primed rats may be via the activation of the subfornical organ, which in turn activates an already excitable basolateral nucleus. Previous studies have also shown a close relationship between the amygdala and the hypothalamus in eliciting the autonomic changes necessary for the defense response (Hilton and Zbrozyna, 1963; LeDoux, 1992). Therefore, the lactate sensitivity seen in rats with a compromised dorsomedial hypothalamus or amygdala suggest at least two important pathways working in parallel to regulate the panic response and, in particular, the lactate sensitivity seen in panic disorder.

The hypothesis that a dysfunctional basolateral nucleus may be one possible cause of panic disorder is highly plausible. The anatomical circuitry of the amygdala is such that it enables this region to integrate emotional information. It has direct input from all sensory modalities via the thalamus and the neocortex (Tigges et al., 1982, 1983; Amaral and Price, 1984). It has appropriate efferents via the hypothalamic neuroendocrine and autonomic sites (Oldfield and Silverman, 1985) as well as the preganglionic autonomic areas of the brainstem (Fallon et al., 1978; Norita and Kawamura, 1980; Price and Amaral, 1981). It has also been suggested that the basolateral nucleus acts as an integration center for sensory and memory information in the anxiety response (LeDoux et al., 1990; Campeau and Davis, 1995). Therefore, if there is change in the basolateral nucleus resulting in abnormal sensory interpretation and emotional responses, it could conceivably result in pathological states such as panic disorder.

Presently, Donald Rainnie and his research group are examining basolateral neurons from primed rats utilizing intracellular recording techniques. Preliminary data suggests that baseline excitability is not altered in these neurons, but under stimulatory conditions there is a decreased inhibitory response. This suggests that repeated blockade of $GABA_A$ receptors will lead to increases in excitation. This is important due to the fact that this is an area that is very sensitive to long-term synaptic changes. Thus, it could be that synaptic alterations are occurring in the basolateral projection neurons (glutamatergic cells) which lead to further downstream changes in other nuclei involved in the stress pathway, the net result being an abnormal stress/anxiety response. From the other studies, it appears that the subfornical organ could be located within this pathway and consequently, may explain the change in sodium lactate sensitivity.

In summary, the data presented show that rats administered repeated subthreshold injections of a $GABA_A$ antagonist into the basolateral nucleus will develop a panic-like response to a previously subthreshold dose, i.e., become primed. In addition, primed as compared to sham-primed animals show an increase in sensitivity to intravenous sodium lactate infusions, as seen in increases in heart rate, blood pressure and experimental anxiety, a response similar to patients with panic disorder.

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