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Ethanol inhibition of stress-related tachycardia involves medullary NMDA receptors

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Abstract

In rats, neurons in the perifornical area of the hypothalamus send descending projections to the commissural part of the nucleus tractus solitarii as demonstrated by an anterograde tracer study. In urethane-anaesthetised rats, stimulation of neurons in the perifornical area by microinjection of bicuculline or 6-OH-saclofen causes tachycardia and inhibits baroreflex bradycardia. The effects elicited from the perifornical area are similar in magnitude to those elicited from the adjacent dorsomedial nucleus, also called the hypothalamic defense area. Microinjection into the nucleus tractus solitarii of the NMDA (*N*-methyl-D-aspartate) receptor antagonist, AP-7 (2-amino-7-phosphonoheptanoic acid), inhibits the tachycardic response to stimulation of the perifornical area. Injection of ethanol intravenously or into the nucleus tractus solitarii also inhibits this tachycardic response, but causes no further inhibition when combined with AP-7. We conclude that the perifornical area is part of the hypothalamic defense area, and it is under strong, tonic GABAergic inhibition mediated by both $GABA_A$ and $GABA_B$ receptors. Furthermore, descending input from the perifornical area to the nucleus tractus solitarii is via an NMDA synapse, and ethanol inhibits stress-related tachycardia by inhibiting these NMDA receptors in the nucleus tractus solitarii.

Keywords: Defense area; Perifornical area; GABA (γ-aminobutyric acid); Glutamate; Nucleus tractus solitarii

1. Introduction

The medullary nucleus of the solitary tract is the principal site of termination of primary afferent fibers arising from peripheral baroreceptors. Recent evidence implicates non-NMDA-type ionotropic (Florentino et al., 1990; Gordon and Leone, 1991; Vardhan et al., 1993) as well as metabotropic glutamate receptors (Glaum and Miller, 1992) in mediating the fast synaptic transmission from baroreceptor primary afferents to second-order neurons in the nucleus tractus solitarii, although NMDA receptors also appear to be involved (Wang et al., 1991; El-Mas and Abdel-Rahman, 1993; Vardhan et al., 1993; Ohta and Tallman, 1994). The barosensitive neurons in the nucleus tractus solitarii also receive descending input from other brain regions involved in cardiovascular regulation, which modulates the transmission of signals from peripheral baroreceptors. An important source of descending inhibitory input to the nucleus tractus solitarii is the hypothalamic defense area, which has been localized to the hypothalamic dorsomedial nucleus (DiMicco et al., 1992). Electrical or chemical stimulation of the dorsomedial nucleus elicits complex behavioral and autonomic responses characteristic of the 'fight or flight' reaction, which includes tachycardia and inhibition of the depressor baroreflex response elicited from peripheral baroreceptors (DiMicco et al., 1992). Some observations indicate that effects similar to those elicited from the dorsomedial nucleus can be also elicited from the perifornical area (Allen and Cechetto, 1992), a cell group adjacent to the dorsomedial nucleus at the junction of the medial and lateral hypothalamus (Palkovits, 1975; Paxinos and Watson, 1986). This suggests that the hypothalamic defense area may include both the dorsomedial nucleus and the perifornical area.

The finding that microinjection of bicuculline into the hypothalamic defense area elicits tachycardic and pressor effects indicates that these neurons are under strong, tonic GABAergic inhibition, probably by neurons originating in the amygdala (Timms, 1981). Stimulation of these neurons in the hypothalamic defense area will, in turn, inhibit the

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activity of barosensitive, vagal premotor neurons in the nucleus tractus solitarii, and this inhibition is also GABAergic (Jordan et al., 1988). However, GABAergic neurons are almost always short interneurons, suggesting that the descending pathway from the hypothalamic defense area is not monosynaptic (Jordan et al., 1988).

There is evidence that endogenous GABA can act via both $GABA_A$ and $GABA_B$ receptors at central sites of cardiovascular regulation (Catelli Sved and Sved, 1989; Florentino et al., 1990; Varga and Kunos, 1992). The purpose of the present experiments is to characterize the nature of the GABA receptors involved in the defense reaction at the level of the perifornical area. Furthermore, we sought to test the hypothesis that activation of GABAergic interneurons in the nucleus tractus solitarii by descending neurons from the perifornical area is via an excitatory glutamatergic synapse. Finally, since ethanol is known to inhibit stress-induced tachycardia (Pohorecky, 1990), and also to inhibit NMDA (Hoffman et al., 1989; Lovinger et al., 1989; Sun and Reis, 1992; El-Mas and Abdel-Rahman, 1993) as well as non-NMDA glutamate receptors (Hoffman et al., 1989; Dildy-Mayfield and Harris, 1992), we examined the effects of acute administration of ethanol on the cardiovascular response to stimulation of neurons in the perifornical area.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (250–350 g) were anesthetized with urethane, 0.8 g/kg i.v. + 0.3 g/kg i.p. Phasic and mean arterial blood pressure were monitored directly via a polyethylene cannula inserted into the femoral artery and connected to a pressure transducer and physiograph. Heart rate was monitored by a tachograph preamplifier driven by the pressure wave. Drugs were injected i.v. through a cannula in the femoral vein.

2.2. Baroreflex sensitivity

Baroreflex bradycardia was elicited by bolus i.v. injection of graded doses of phenylephrine (5–40 μg/kg). Peak increases in blood pressure were plotted against the corresponding peak increases in pulse period (1/heart rate). The slope of the regression line was used as an indicator of baroreflex sensitivity.

2.3. Microinjections

The anesthetised animal was placed in the prone position and the head was fixed in a stereotaxic frame (David Kopf). For microinjections into the nucleus tractus solitarii, the head was flexed at 45° and the dorsal surface of the medulla was exposed by transection of the atlanto-occipital membrane. The tip of a drug-filled glass microcannula was inserted into the commissural part of the nucleus tractus solitarii according to the coordinates A/P: 0.0 mm, D/V: −0.5 mm, M/L: 0.5 mm relative to tip of the calamus scriptorius. The proper positioning of the cannula was verified during the experiment by microinjecting 1 nmol sodium glutamate and observing the typical brief hypotensive and bradycardic response. For microinjections into the perifornical area, a hole was drilled in the skull, and the tip of the drug-filled microcannula was inserted into the perifornical area according to the coordinates A/P −3.2–3.6 mm, D/V: −8.4 mm, and M/L: 0.8–1.3 mm relative to the bregma. Drugs were dissolved in saline and microinjected in a volume of 100 nl. Microinjection of 100 nl saline into either the nucleus tractus solitarii or the perifornical area caused no change in blood pressure or heart rate. Ethanol was microinjected into the nucleus tractus solitarii as a 250 mM solution in saline, in a volume of 100 nl. For post-mortem verification of the site of microinjections, 100 nl of 1% Methylene blue was microinjected at the end of the experiments, and the topographical location of the dye was established by light microscopy in 30-μm-thick serial coronal sections of the hypothalamus.

2.4. Anterograde tract tracing

After microinjection into neuronal cell bodies, *Phaseolus vulgaris* leucoagglutinin is transported anterogradely to the nerve terminals where it can be visualized. As there is no further transsynaptic transport, this technique can be used to map monosynaptic descending pathways (Gerfen and Sawchenko, 1984). Five male rats (250 g) were anesthetized by sodium pentobarbital (50 mg/kg i.p.), and the head was fixed in a stereotaxic frame, in a 5° 'nose-down' position. A glass micropipette with a tip diameter of 30 μm was filled with a 2.5% solution of *Phaseolus vulgaris* leucoagglutinin (Vector Labs, Burlingame, CA, USA) in 10 mM phosphate buffer (pH 8.0). The tip of the cannula was inserted into the perifornical area using the coordinates described above. Microinjections of *Phaseolus vulgaris* leucoagglutinin were made using a 7-μA positive current for periods of 7 s separated by 7-s intervals, for a total of 15 min. The micropipette was kept in place for an additional 15 min to minimize backflow of the tracer into the injection tract. Rats were allowed to recover, and on the 4th post-operative day were re-anaesthetised and perfused transcardially with a fixative solution of 4% paraformaldehyde and 15% picric acid in 0.12 M phosphate buffer (pH 7.3). The brain was then removed and fixed in the same solution complemented with 35% sucrose at 4°C overnight. 50-μm-thick coronal sections were made using a cryostat. In order to localize *Phaseolus vulgaris* leucoagglutinin-labeled cells and fibers in the sections, a modification of the avidin biotin peroxidase technique was used (Gerfen and Sawchenko, 1984). Briefly, following a rinse

in phosphate buffer, the sections were immersed in 0.5% Triton for 2 h, and then incubated for 12 h in primary antiserum (goat anti-*Phaseolus vulgaris* leucoagglutinin, 1 : 3000; Vector Labs) containing 2% normal rabbit serum. Following two washes in phosphate-buffered saline containing 2% normal rabbit serum, sections were incubated for 45 min with biotinylated rabbit anti-goat IgG (Vector Labs). Then, the sections were washed again and incubated for 45 min with a 1 : 200 dilution of avidin biotin peroxidase. Finally, the sections were incubated in diamino-benzidine-H_2O_2 in Tris buffer, washed, mounted on gelatine-coated slides, air dried, cleared in xylene and coverslipped.

2.5. Drugs and reagents

(−)Bicuculline methyiodide, muscimol, phenylephrine HCl, and urethane were from Sigma Chemical (St. Louis, MO, USA); 2-hydroxysaclofen (2-OH-saclofen) and (±)-2-amino-7-phosphonoheptanoic acid (AP-7) were from Research Biochemicals International (Natick, MA, USA); *Phaseolus vulgaris* leucoagglutinin was from Vector Labs.

2.6. Statistical analyses

Comparison of pre- and post-treatment values in the same animals was made using Student's paired or unpaired *t*-test as appropriate.

3. Results

In agreement with published findings (Allen and Cechetto, 1993), the typical cardiovascular component of the defense reaction, i.e. pronounced tachycardia with no significant change in blood pressure, could be elicited by unilateral microinjections of bicuculline methyiodide into the perifornical area. Fig. 1 illustrates the effects of 20 pmol bicuculline methyiodide, which was the maximally effective microinjected dose. Fig. 1 also illustrates the inhibition of the reflex bradycardic response to phenylephrine during the plateau phase of the tachycardia elicited by bicuculline methyiodide.

The specificity of the effect of bicuculline methyiodide is indicated by the ability of the $GABA_A$ receptor agonist muscimol to antagonize the bicuculline methyiodide-induced tachycardia. As shown in Fig. 2, unilateral microinjection of 10 pmol muscimol alone into the perifornical area caused a slight decrease in heart rate. However, when 20 pmol of bicuculline methyiodide was subsequently microinjected into the same site, the tachycardic effect was almost completely blocked as compared to the effect of the same dose of bicuculline methyiodide alone. This suggests that neurons in the perifornical area are under near-maximal tonic GABAergic inhibition.

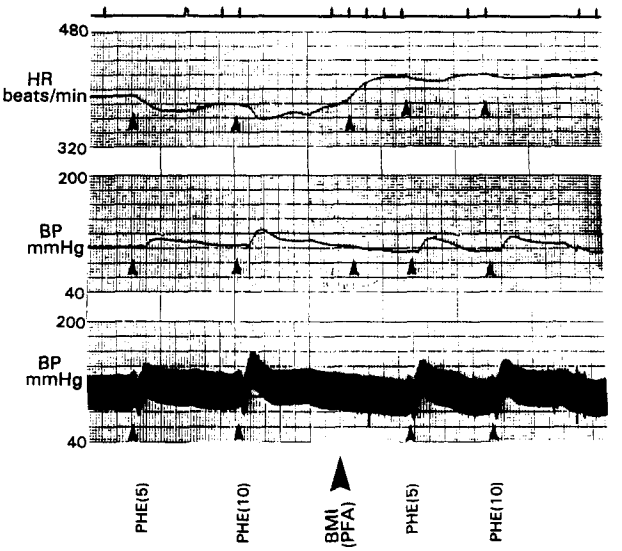

Fig. 1. The effects of defense area activation on heart rate (HR), mean and phasic blood pressure (BP) and baroreflex sensitivity in a urethane-anaesthetised rat. The defense area was activated by unilateral microinjection of 20 pmol bicuculline methyiodide (BMI) into the perifornical area (PFA). The baroreflex was activated by bolus i.v. injections of 5 and 10 μg/kg phenylephrine (PHE). Note that BMI increases basal heart rate, does not affect blood pressure and suppresses the reflex bradycardic response to PHE. Similar results were obtained in 4 additional experiments.

In order to test whether $GABA_B$ receptors in the perifornical area also contribute to the tonic inhibition of these neurons, we tested the effect of the $GABA_B$ receptor antagonist 2-OH saclofen microinjected unilaterally into the perifornical area. As illustrated in Fig. 3, 400 pmol of 2-OH saclofen also elicited tachycardia and inhibited the

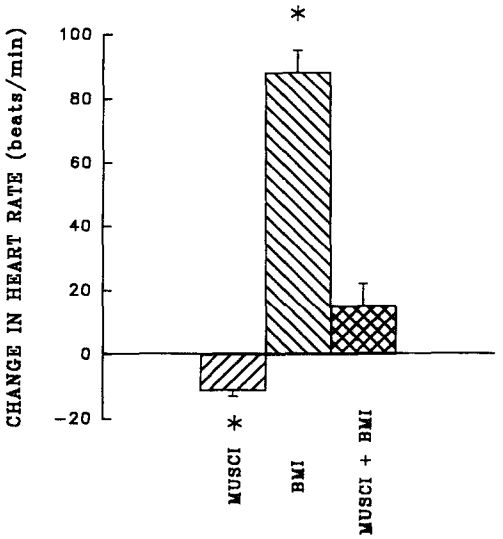

Fig. 2. Muscimol inhibits the tachycardic response to bicuculline in the perifornical area. Bicuculline methyiodide (BMI; 20 pmol), muscimol (MUSCI; 10 pmol) or their combination was microinjected unilaterally in the perifornical area of 5 urethane-anaesthetised rats. Mean ± S.E. values are shown. * Significant difference ($P < 0.05$) from 0.

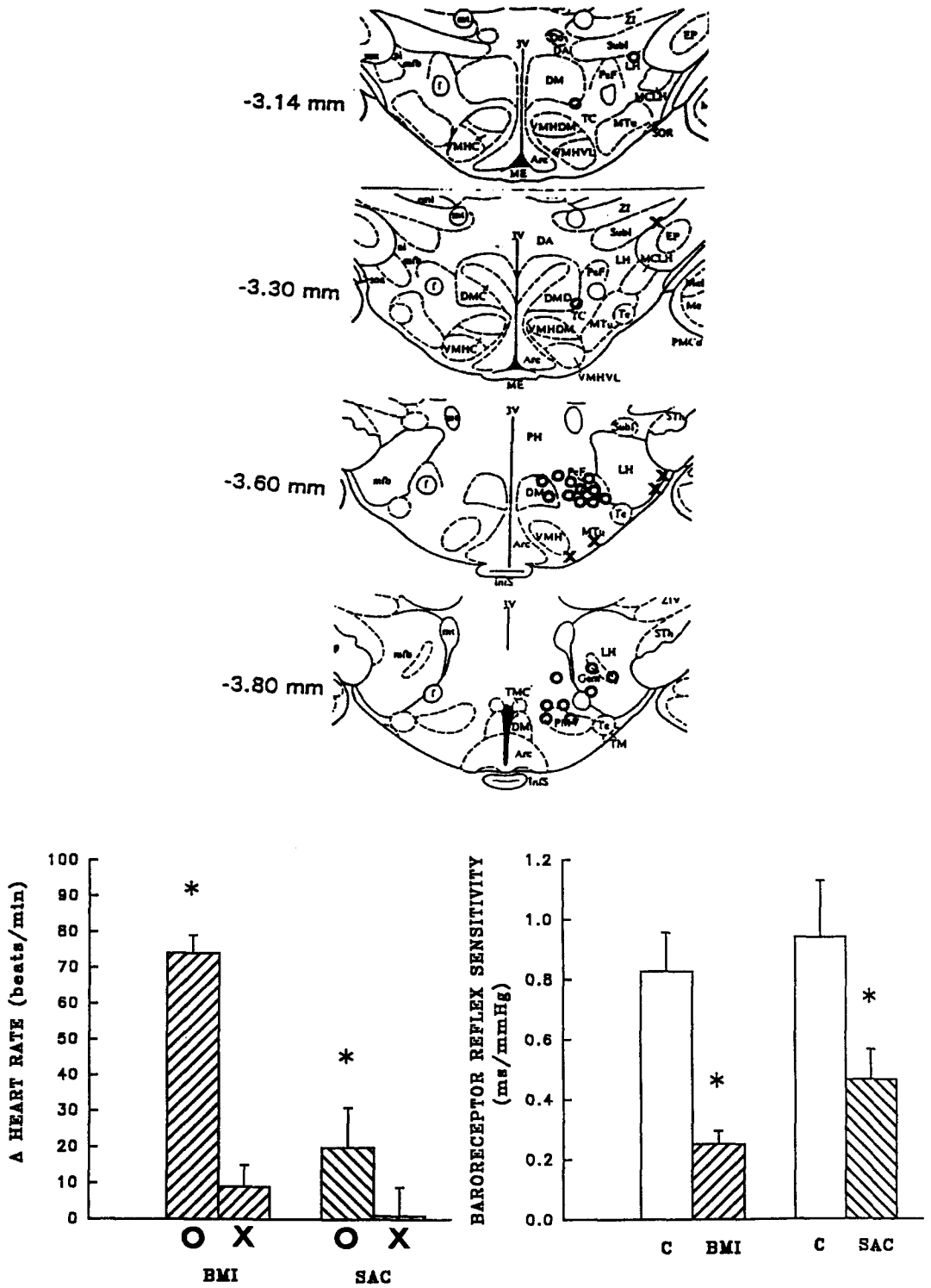

Fig. 3. Tonic inhibition of the defense reaction involves both $GABA_A$ and $GABA_B$ receptors in the perifornical area. Top: anatomical localization of the sites of effective (o) or ineffective (x) microinjections (coronal sections according to Paxinos and Watson (1986). Left lower panel: tachycardic response to bicuculline methyiodide (BMI) or 2-OH-saclofen (SAC) microinjected into various hypothalamic locations (o or x). Note that injections outside the perifornical area or the dorsomedial nucleus (x) caused no tachycardia. Lower right panel: inhibition of baroreflex bradycardia by bicuculline methyiodide (BMI) or 2-OH-saclofen (SAC). Baroreflex bradycardia was elicited by i.v. injection of 3 bolus doses of phenylephrine before (C) or during the tachycardic plateau after BMI or SAC. Mean ± S.E. values from 4–5 experiments are shown. * Significant difference ($P < 0.05$) from 0 (left panel) or from corresponding control value (right panel).

reflex bradycardic response to phenylephrine, although these effects were smaller than those seen after microinjection of bicuculline methyiodide into the perifornical area. The top panel shows the sites where these responses could be elicited from as verified by post-mortem microscopic examination. It can be seen that sites from which bicuculline methyiodide elicited a tachycardic response included both the perifornical area and the dorsomedial nucleus. In 9 experiments where the tip of the cannula was localized in the perifornical area, the mean tachycardic response to bicuculline methyiodide was $+69 \pm 9$ beats/min, whereas in 4 experiments where the microinjection was into the dorsomedial nucleus it was $+55 \pm 3$ beats/min, which was not statistically different from the response elicited from the perifornical area. Microinjections of either bicuculline methyiodide or 2-OH-saclofen outside of these two areas failed to cause any effect on heart rate.

There is functional evidence for a descending pathway that projects from the hypothalamic defense area to the

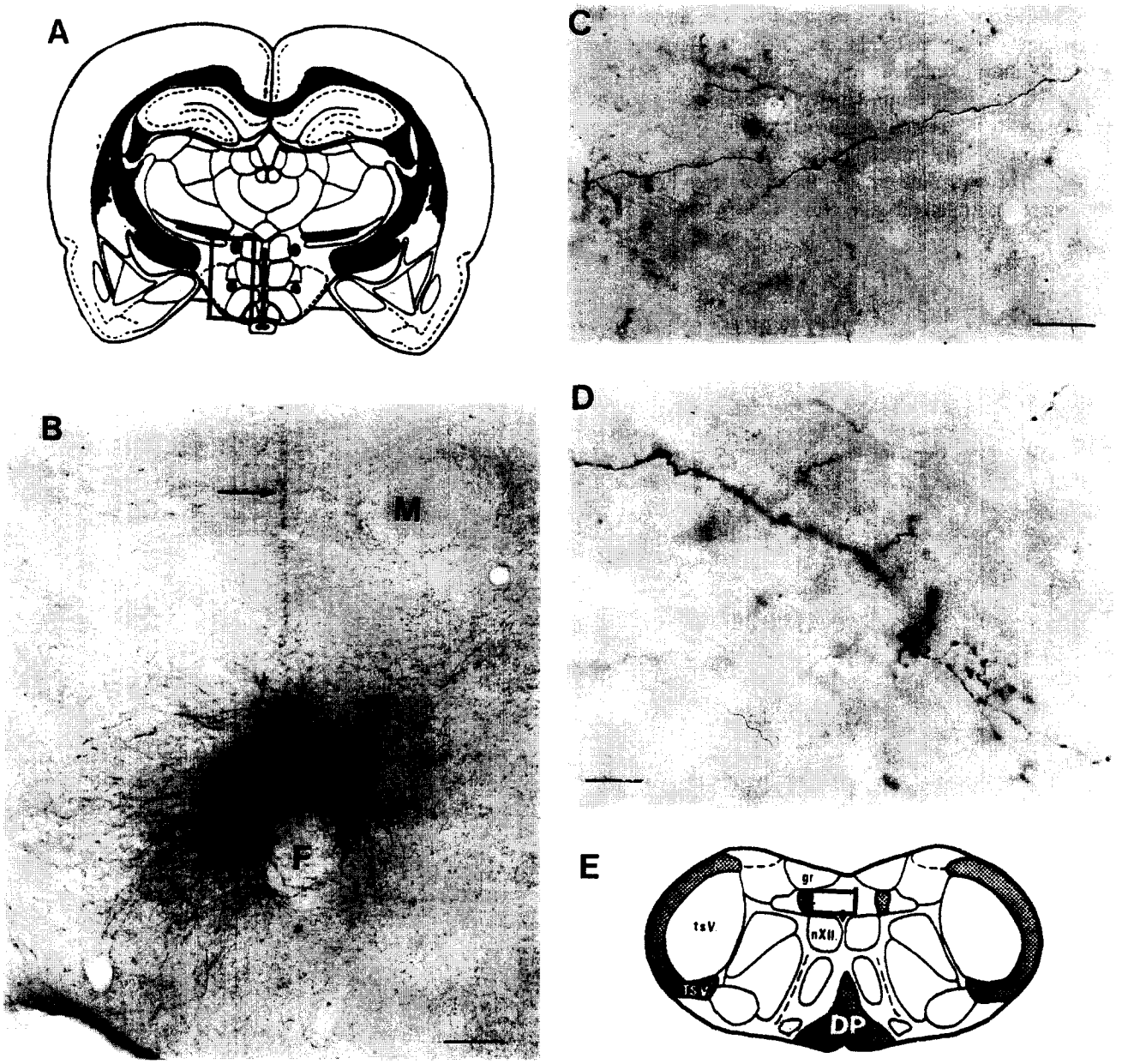

Fig. 4. Demonstration of the existence of direct neuronal projections from the perifornical area (PFA) to the nucleus tractus solitarii by intra-PFA microinjection of *Phaseolus vulgaris* leucoagglutinin. A: coronal section of the forebrain at the level of the perifornical area. Area enlarged in B is bracketed by rectangle. B: site of the microinjection in the perifornical area. The channel of the micropipette is indicated by the arrow. C,D: *Phaseolus vulgaris* leucoagglutinin-positive varicose axons and terminals in the commissural part of the nucleus tractus solitarii (area is indicated in E by a rectangle). DP, pyramidal decussation; F, fornix; gr, gracilis nucleus; M, mamillothalamic tract; nXII, motor hypoglossal nucleus; tsV, spinal trigeminal nucleus; TSV, spinal trigeminal tract; Bar scales: 200 μm (B); 25 μm (C); and 20 μm (D).

dorsomedial medulla: stimulation of neurons in the hypothalamic defense area was found to cause GABA-mediated inhibition of barosensitive neurons in the nucleus tractus solitarii (Jordan et al., 1988). We therefore examined whether nerve terminals in the nucleus tractus solitarii can be visualized by anterograde labeling of neurons in the perifornical area. As illustrated in Fig. 4, after unilateral microinjection of *Phaseolus vulgaris* leucoagglutinin into the perifornical area, labeled cells were limited to the perifornical area just dorsal to the fornix (Fig. 4A,B). Neurons in the neighboring lateral hypothalamus and the dorsomedial nucleus were not labeled. *Phaseolus vulgaris* leucoagglutinin-labeled fibres can be followed through the lower brainstem down to the ipsilateral nucleus tractus solitarii. Within the nucleus tractus solitarii, fine, *Phaseolus vulgaris* leucoagglutinin-labeled varicose axons can be seen mostly in the commissural part of the nucleus (Fig. 4D,E). Most of the fibres and terminals were on the ipsilateral side, but some crossed the midline and could be seen on the contralateral side (Fig. 4C). Whether or not axon collaterals of neurons of the perifornical area may also terminate in medullary areas other than the dorsal vagal complex has not been addressed by the present tracing experiments.

We have previously reported that microinjection of the NMDA channel blocker, MK-801 ((+)-5-methyl-10,11-dihydro-5,4-dibenzo[a,d]cyclohepten-5,10-imine maleate, 30 pmol/50 nl), into the nucleus tractus solitarii, inhibited the tachycardic response to microinjection of bicuculline methyiodide into the ipsilateral nucleus tractus solitarii (Kunos and Varga, 1995), suggesting that the pathway from the perifornical area to the nucleus tractus solitarii has an NMDA synapse. Since ethanol is known to inhibit stress-induced tachycardia (Pohorecky, 1990), and it also inhibits NMDA receptors (Hoffman et al., 1989; Lovinger et al., 1989), we tested whether ethanol can inhibit the tachycardic response to stimulation of neurons in the perifornical area. We first tested the effects of 1 g/kg ethanol administered intravenously. This dose has been shown to result in peak blood ethanol levels between 20 and 30 mM

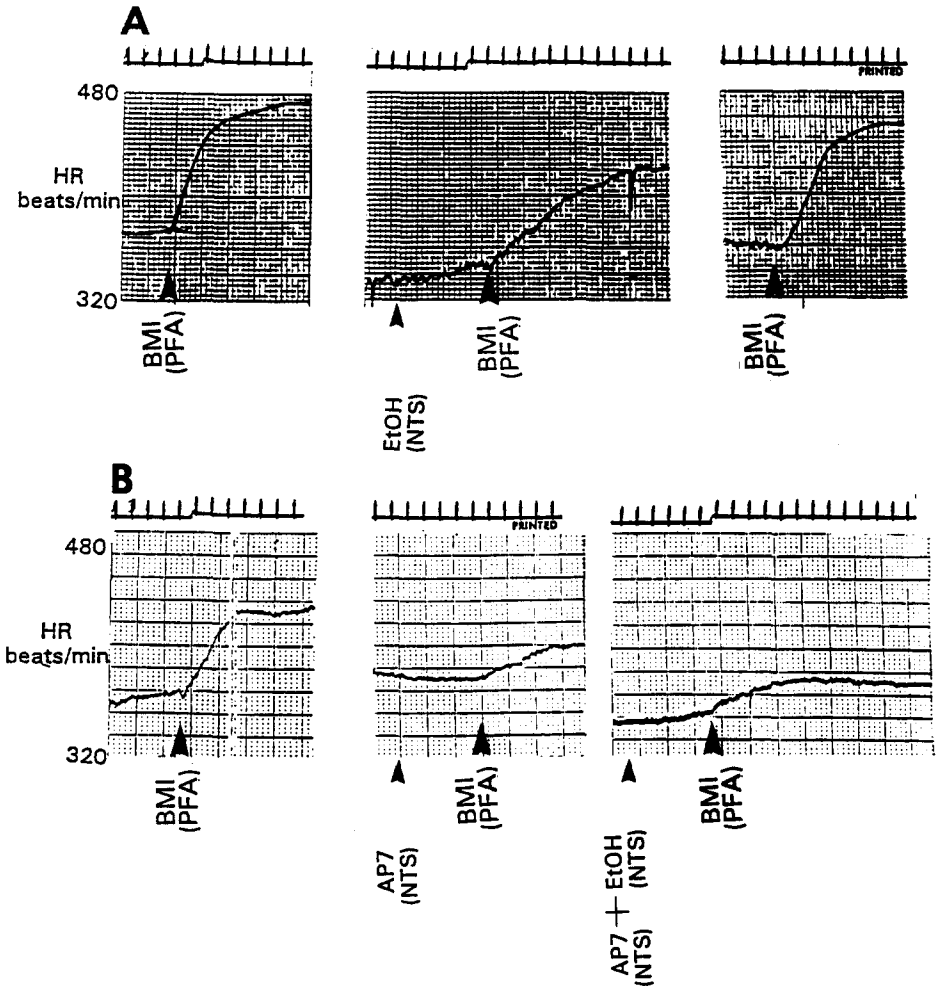

Fig. 5. The tachycardic effect of defense area activation is inhibited by microinjection into the ipsilateral nucleus tractus solitarii of 25 nmol ethanol (A) or the NMDA-receptor antagonist AP-7, 2 nmol/50 nl (B). Note that the effect of ethanol (EtOH) is reversible (A) and that the combination of ethanol and AP-7 causes no further inhibition (B). Similar observations were made in 3 additional experiments (see text).

in rats (Varga and Kunos, 1990), which is equivalent to levels causing moderate inebriation in man. In four separate animals, unilateral microinjection of 20 pmol bicuculline methyiodide into the perifornical area increased heart rate by 68 ± 24 beats/min before ethanol, and by 44 ± 12 beats/min 10 min after the i.v. administration of 1/g/kg ethanol ($P < 0.05$). We next tested the effect of ethanol microinjected into the nucleus tractus solitarii. As illustrated in Fig. 5, the tachycardia elicited by microinjection of bicuculline methyiodide into the perifornical area is markedly and reversibly inhibited by microinjection of 25 nmol ethanol into the ipsilateral nucleus tractus solitarii (Fig. 5A). A similar inhibition of the tachycardia is observed after microinjection into the nucleus tractus solitarii of the NMDA receptor antagonist, AP-7 (2 nmol/50 nl), and additional microinjection of 25 nmol ethanol into the same site does not cause further inhibition (Fig. 5B). Similar observations were made in 3 additional experiments, in which the tachycardic response to unilateral microinjection into the perifornical area of 20 pmol bicuculline methyiodide was $+83 \pm 14$ beats/min under control conditions. When microinjection of bicuculline methyiodide was repeated following the microinjection into the ipsilateral nucleus tractus solitarii of 2 nmol AP-7 alone or 2 nmol AP-7 + 25 nmol ethanol, the response was $+40 \pm 10$ or $+38 \pm 11$ beats/min, respectively. Each of these latter responses were significantly lower than the control response to bicuculline methyiodide, but were not significantly different from one another.

4. Discussion

Sites in the intermediate and posterior hypothalamus from which a tachycardic response can be elicited were morphologically verified to include both the dorsomedial nucleus and the perifornical area. The size of the response elicited by bicuculline methyiodide from these two adjacent areas was similar, which indicates that both structures are involved in the defense reaction, and the response elicited from one area is not simply due to some of the microinjected drug reaching the other area by diffusion. Thus, the present observations are in agreement with previous studies identifying either the dorsomedial nucleus (DiMicco et al., 1992) or the perifornical area as the hypothalamic defense area (Allen and Cechetto, 1992). The existence of neural projections from the dorsomedial nucleus to the nucleus tractus solitarii, dorsal vagal nucleus and nucleus ambiguus has been documented earlier (Ter Horst and Luiten, 1986). We have now demonstrated the existence of a monosynaptic connection from the perifornical area to the nucleus tractus solitarii and the dorsal vagal nucleus (Fig. 4). This provides further support to the notion that neurons of the hypothalamic defense area are located not only in the dorsomedial nucleus but also in the perifornical area.

Medium to large size neuronal perikarya are common in the perifornical area, which in rats is located dorsal and partly lateral to the fornix, at the junction of the medial and the lateral hypothalamus, 3.0–3.8 mm caudal to the level of the bregma (Palkovits, 1975). A great variety of neurotransmitter and neuropeptide synthesizing neurons have been localized in the perifornical area (Palkovits, 1988; Cechetto and Saper, 1988). This area receives multiple afferent input from various areas of the brain involved in neurobehavioral and cardiovascular regulation, including the amygdala, periaqueductal grey, bed nucleus of the stria terminalis, insular cortex, and lateral septum (Allen and Cechetto, 1993). Although all of these inputs may be involved in the strong, tonic GABAergic inhibition of the defense area, the primary source of this inhibitory input is thought to be the amygdala (Timms, 1981). Whereas this inhibitory input appears to be mediated by both $GABA_A$ and $GABA_B$ receptors in the perifornical area (see Fig. 3), activation of the neurons in the perifornical area is most likely due to the action of endogenous glutamate. Once activated, these neurons inhibit the barosensitive neurons in the nucleus tractus solitarii (Timms, 1981), via release from local interneurons of GABA that activates both $GABA_A$ and $GABA_B$ receptors in the nucleus tractus solitarii (Kunos and Varga, 1995).

The question arises, what is the nature of the descending input to the GABAergic interneurons in the region of the nucleus tractus solitarii. Our recent findings that microinjection into the nucleus tractus solitarii of either 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), a non-NMDA glutamate receptor antagonist, or MK-801, an NMDA channel blocker, inhibit the tachycardic response to activation of neurons in the hypothalamic defense area (Kunos and Varga, 1995) suggest the presence of a glutamatergic synapse in the nucleus tractus solitarii and the involvement of both NMDA and non-NMDA receptors. As part of its 'anxiolytic' effect, ethanol is well known to inhibit stress-induced tachycardia (Pohorecky, 1990). Our finding that systemically administered ethanol inhibits the tachycardia caused by defense area stimulation could be viewed as an experimental model of this effect. The finding that localized microinjection of ethanol into the nucleus tractus solitarii caused similar inhibition of the tachycardic response indicates that the site of action of ethanol is in the nucleus tractus solitarii. Since ethanol inhibits NMDA receptor-mediated biochemical effects (Hoffman et al., 1989), NMDA-gated ion currents (Lovinger et al., 1989) as well as NMDA synapses in the baroreflex arc (Sun and Reis, 1992; El-Mas and Abdel-Rahman, 1993), a likely mechanism of the action of ethanol is inhibition of NMDA receptors mediating descending input from the perifornical area to GABAergic interneurons in the nucleus tractus solitarii. Further evidence to support this notion is the lack of additivity of the inhibition caused by ethanol and the similar inhibitory effect of microinjection of the NMDA receptor antagonist AP-7 into the nucleus tractus solitarii.

The effect of locally administered ethanol cannot be attributed to non-specific tissue damage, since the inhibition was fully reversible (Fig. 5A).

The present observations with ethanol are somewhat paradoxical as another well-documented effect of ethanol is inhibition of baroreflex bradycardia (Abdel-Rahman et al., 1987; Zhang et al., 1989; Varga and Kunos, 1990, 1992; Sun and Reis, 1992; El-Mas and Abdel-Rahman, 1993; Varga et al., 1994), an effect attributed to facilitation of the similar inhibitory action of endogenous GABA at barosensitive neurons in the nucleus tractus solitarii (Varga and Kunos, 1990, 1992; Varga et al., 1994). Thus, ethanol can both inhibit and facilitate (disinhibit) the baroreflex control of heart rate, and both effects are mediated within the nucleus tractus solitarii, although via different neurotransmitters. This apparent paradox may be reconciled if one considers that under resting condition the tonic inhibitory input to the nucleus tractus solitarii from the hypothalamic defense area is minimal, due to the near-maximal GABAergic inhibition of neurons in the perifornical area. This means that the NMDA receptor-mediated input to the GABAergic interneurons of the nucleus tractus solitarii is minimal, and the ethanol-induced potentiation of the action of the small amounts of GABA representing 'resting' release from these interneurons can manifest itself. On the other hand, during stress the descending input from the perifornical area to the nucleus tractus solitarii is increased, leading to increased GABAergic inhibition of the baroreflex at the level of the nucleus tractus solitarii. Since the NMDA synapse is postulated to be upstream from the release of GABA, this input can be interrupted by inhibition of these NMDA receptors by ethanol, which probably more than offsets the potentiation of the effects of the subsequently released GABA.

Whereas the role of glutamate as the transmitter in primary baroreflex afferents is now widely accepted, the nature of the receptor it interacts with in the nucleus tractus solitarii has been more ambiguous. There is evidence for the involvement of metabotropic (Glaum and Miller, 1992) as well as non-NMDA type ionotropic glutamate receptors (Florentino et al., 1990; Gordon and Leone, 1991; Vardhan et al., 1993). The role of NMDA receptors has been less clear, although recent evidence suggests their involvement in fast synaptic transmission in the nucleus tractus solitarii (Wang et al., 1991; El-Mas and Abdel-Rahman, 1993; Vardhan et al., 1993; Ohta and Tallman, 1994). Part of the difficulty in defining the role of NMDA receptors in the nucleus tractus solitarii may be their presence in more than one type of synapse. The present findings indicate the obligatory involvement of NMDA receptors in transmitting the descending input from the hypothalamus that ultimately inhibits the activity of barosensitive neurons in the nucleus tractus solitarii. Depending on the tonic activity of this pathway, blockade of these receptors will facilitate baroreflex responses, which is opposite to the result of blocking the input from primary baroreflex afferents. Therefore, analysis of the role of NMDA receptors in synaptic transmission from baroreflex afferents may require elimination of descending input to the nucleus tractus solitarii from the hypothalamus and other regions in the brain.

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