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Brain Research 695 (1995) 250–252

Short communication

Effects of lidocaine injections into the lateral parabrachial nucleus on dipsogenic and pressor responses to central angiotensin II in rats

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Effects of lidocaine injections into the lateral parabrachial nucleus on dipsogenic and pressor responses to central angiotensin II in rats

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Accepted 28 June 1995

Abstract

This study investigated the effects of bilateral injections of the local anesthetic, lidocaine, into the lateral parabrachial nucleus (LPBN) on the dipsogenic and pressor responses induced by intracerebroventricular (i.c.v.) injection of angiotensin II (ANG II). Centrally injected ANG II (50 ng/1 μl) induced water intake (10.2 ± 0.8 ml/h) and pressor responses (22 ± 1 mmHg). Prior bilateral injection of 10% lidocaine (200 nl) into the LPBN increased the water intake (14.2 ± 1.4 ml/h), but did not change the pressor response (17 ± 1 mmHg) to i.c.v. ANG II. Lidocaine alone injected into the LPBN also induced a pressor response (23 ± 3 mmHg). These results showing that bilateral LPBN injection of lidocaine increase water intake induced by i.c.v. ANG II are consistent with electrolytic and neurotoxic lesion studies and suggest that the LPBN is associated with inhibitory mechanisms controlling water intake induced by ANG II. These results also provide evidence that it is feasible to reversibly anesthetize this brain area to facilitate fluid-related ingestive behavior.

Keywords: Lateral parabrachial nucleus; Angiotensin II; Water intake; Arterial pressure; Thirst; Local anesthetic

The lateral parabrachial nucleus (LPBN) is a pontine structure that lies dorsal to the superior cerebellar peduncle. Recent studies from our laboratory have shown that the LPBN is an important hindbrain structure that is involved in the control of water intake [3,7,10–12]. Rats with electrolytic or neurotoxic (ibotenic acid) lesions of the LPBN show an increase in drinking to central or peripheral angiotensin II (ANG II) and to peripheral isoproterenol [3,10]. Since the water intake induced by central carbachol or subcutaneous hypertonic saline is not changed by LPBN lesions, a LPBN-associated mechanism has been proposed to be involved in the control of extracellular fluid depletion-induced thirst [11]. Similar increases in water intake have also been reported after lesions of the area postrema and adjacent medial nucleus of the solitary tract (AP/mNTS) [11]. The LPBN receives afferent projections from the AP/mNTS and sends efferent projections to areas of the forebrain involved in fluid and electrolyte balance such as the median preoptic nucleus and the central nucleus of the amygdala [2,6,9,15]. In this study, we investigated the effects of temporary, reversible lesions of the LPBN produced by bilateral injections of the local anesthetic, lidocaine, on the dipsogenic and pressor responses induced by intracerebroventricular (i.c.v.) ANG II.

Male Sprague–Dawley rats weighing 300–400 g were used. Rats were anesthetized with Equithesin (3.3 ml/kg b. wt.) and placed in a Kopf stereotaxic instrument. The skull was leveled between lambda and bregma. Stainless steel 23 gauge cannulas (12 mm long) were bilaterally implanted into the LPBN using stereotaxic coordinates of 9.4 mm caudal to bregma, 1.9 mm lateral and 4.1 mm below the dura. The tips of the cannulas were positioned in the brain at points 2 mm above the LPBN. A third cannula (23 gauge × 12 mm long) was implanted in the left lateral ventricle (LV) at coordinates of 1.2 mm caudal to bregma, 1.5 mm lateral and 4.0 mm below the dura. The cannulas were fixed in the cranium using dental acrylic resin and small screws, and metal tubing (30 gauge) was used as an obturator to keep the cannulas patent.

Angiotensin II (50 ng/1 μl) and 10% lidocaine hydrochloride (Sigma, St. Louis, MO) were dissolved in 0.15 M NaCl (vehicle). Hamilton syringes (10 μl) were connected by PE-10 polyethylene tubing to needles (30 gauge) which

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SSDI 0006-8993(95)00872-1

were introduced into the brain through the previously affixed guide cannulas. The needles for injection into the LPBN and LV were 2 mm longer than the guide cannulas. Injection volumes into the LPBN were 200 nl and into the LV 1 μl. Vehicle or 10% lidocaine was injected into the LPBN immediately before i.c.v. ANG II was injected into the LV. The volume of water consumed over a period of 1 h after i.c.v. injection of ANG II was measured at 15 min intervals using burettes graduated with 0.1 ml divisions and fitted with metal spouts. Direct mean arterial pressure (MAP) was recorded in unanesthetized, unrestrained rats. On the day before the experiment, the rats were anesthetized with Equithesin (3.3 ml/kg b.wt.) and a PE-10 polyethylene tube connected to PE-50 was inserted into the abdominal aorta through the femoral artery. The cannula was tunneled subcutaneously to the back of the rat and was connected to a pressure transducer coupled to a multichannel recorder (Dynograph Recorder R611, Sensor Medics).

At the end of the experiments, the animals received bilateral injections of a dye (200 nl of methylene blue solution) into the LPBN. They were then deeply anesthetized with sodium pentobarbital (80 mg/kg) and perfused with saline followed by 10% formalin through the heart. The brains were removed, fixed in 10% formalin, frozen, cut in 50 μm sections, stained with Cresyl violet and analyzed by light microscopy with the aid of the brain atlas of Paxinos and Watson [13] to confirm the position of the injections into LV and LPBN.

Histological material was examined without knowledge of the results of the functional tests (i.e. 'blind'). Only animals that met the criteria of (1) ventricular cannulas with access to the LV, (2) caudal injection sites terminating bilaterally in the LPBN, and (3) each of the 200 nl Methylene blue injections confined to the LPBN were considered successful preparations. Functional data from only successful preparations were used for analysis. Experimental results are reported as means ± S.E.M. Analysis of variance and Student t-test were used for comparisons. Differences were considered significant at $P < 0.05$.

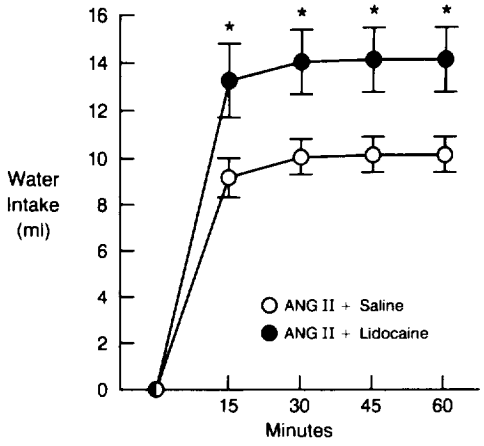

Fig. 1. Cumulative water intake induced by i.c.v. injection of ANG II (50 ng/1 μl) after previous injection of vehicle or 10% lidocaine (200 nl) bilaterally into the LPBN. The results are represented by means ± S.E.M. * Different from ANG II + saline tested by t-tests ($P < 0.05$). ANOVA indicated a significant main effect between treatments ($F_{1,18} = 6.13$; $P < 0.05$). $n = 10$ rats.

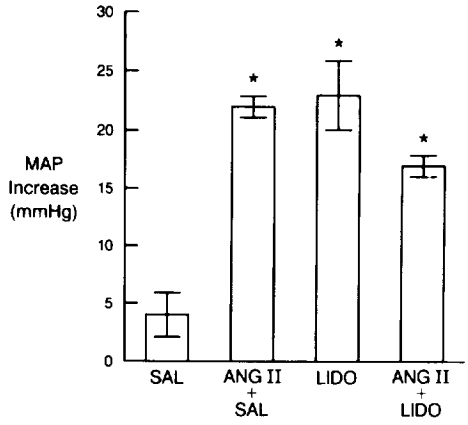

Fig. 2. Increase in MAP induced by i.c.v. ANG II (50 ng/1 μl) before and after bilateral injection of saline (SAL) or 10% lidocaine (LIDO, 20 nl) into the LPBN. The results are represented by means ± S.E.M. * Different from injection of saline tested by t-tests ($P < 0.05$). $n = 5$ rats.

The bilateral injection of lidocaine (200 nl) into the LPBN significantly increased the water intake induced by i.c.v. ANG II (50 ng/1 μl; Fig. 1). Lidocaine alone injected into the LPBN induced no water intake in satiated rats. Bilateral injections of 10% lidocaine (200 nl) alone into the LPBN increased MAP (Fig. 2). The pressor response to lidocaine occurred immediately after the injections into the LPBN (peak response in 3 min). No change in the pressor response to i.c.v. ANG II (injected 5 min after lidocaine) was observed after the injection of 10% lidocaine into the LPBN (Fig. 2). The resting baseline MAP prior to lidocaine injection was 117 ± 3 mmHg, and the baseline MAP prior to ANG II after the treatment with lidocaine was 128 ± 3 mmHg.

The present results show that bilateral injections of lidocaine into the LPBN increase the water intake induced by i.c.v. ANG II. These results together with previous studies showing similar increases in water intake to ANG II-related stimuli after electrolytic or neurotoxic lesions of the LPBN [3,10–12] provide evidence of an inhibitory pathway associated with the LPBN that is important for the control of ANG II-induced water intake.

Studies have shown that the increase in blood pressure induced by ANG II impairs its dipsogenic effect [5,14,16]. Since prior injection of lidocaine into the LPBN produced no significant change in the pressor response to i.c.v. ANG II, the increased water intake is unlikely to be due to an impairment of the pressor effect of the peptide.

Lidocaine injected bilaterally alone into the LPBN increased MAP. This observation suggests that cells within or fibers passing through the LPBN may tonically inhibit

increases in arterial pressure. There may be a role for the LPBN in processing blood pressure and/or volume-related information that affects the control of drinking. Central representation of information derived from systemic receptors and afferent pressure/volume pathways may play an inhibitory role in the control of ANG II-induced water intake. Blocking this information by injection of lidocaine into the LPBN may be responsible for facilitating the dipsogenic action of central ANG II. However, it should not be concluded that there are no LPBN-associated excitatory mechanisms. Injection of glutamate into the LPBN increases arterial pressure [17]. The ultimate effects of electrolytic, neurochemical and anesthetic (lidocaine) lesions of the LPBN on functional responses in all likelihood reflect the net result of removing a combination of both excitatory and inhibitory influences.

An increase in water intake to i.c.v. ANG II similar to that produced by bilateral LPBN injections of lidocaine has also been observed [4,11] after AP/mNTS lesions. Ohman and Johnson [11] have suggested that AP/mNTS and LPBN may be linked in a common drinking-related inhibitory pathway. A recent study from our laboratory [12] showed that electrolytic LPBN lesions abolished the inhibition of water intake observed during the inflation of a right atrial balloon. The antidipsogenic effect of atrial stretch may be due to the activation of neural and/or humoral mechanisms. Neural input activated by changes in blood volume reaches the parabrachial nucleus [18]. However, an alternative or parallel (redundant) inhibitory mechanism might be a humoral mechanism that involves circulating atrial natriuretic peptide (ANP). It has been shown that central or peripheral administration of ANP reduces dipsogenic responses [1,8]. The AP is rich in ANP receptors. Since the AP lacks a blood–brain barrier, plasma ANP may activate receptive cells within the AP and this information is, in turn, carried via an ascending projection to the LPBN where it acts to inhibit drinking.

In summary, results showing that inactivation of a cerebral area (bilateral LPBN in this case) increases water intake to i.c.v. ANG II suggest that injection of a local anesthetic into specific areas of the brain can be used to study experimentally-induced dipsogenic responses.

Acknowledgements

This research was supported by National Heart, Lung, and Blood Institute Grants HL-14338 and HL-44546 and Fundacao de Amparo a Pesquisa do Estado de Sao Paulo Proc. 93/0167-7.

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