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Cardiovascular responses of the anterior claustrum; its mechanism; contribution of medial prefrontal cortex

Masoumeh Hatam [a], Mehrnoosh Sheybanifar [b,*], Ali Nasimi [c]

[a] Dept. of Physiology, Shiraz University of Medical Sciences, Shiraz, Iran
[b] Dept. of Anatomy, Hormozgan University of Medical Sciences, Bandar-Abbas, Iran
[c] Dept. of Physiology, Isfahan University of Medical Sciences, Isfahan, Iran

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ABSTRACT

The anterior claustrum (CLa) has bilateral connections with the areas involved in cardiovascular regulation, though its role in cardiovascular control is not yet understood. This study was performed to find the cardiovascular responsive region of the CLa by stimulating all parts of the CLa with L-glutamate, and to find the possible mechanisms mediating its responses in urethane-anesthetized rats. We also investigated the possible involvement of the medial prefrontal cortex in the cardiovascular responses of the CLa. The effect of microinjection of L-glutamate (50–100 nl, 0.25 M) was tested throughout the Cla and only in one area at 2.7 mm rostral to bregma, 1.8–2.0 midline and 4.5–5.6 mm vertical, significant decreases in arterial pressure were elicited (-21.71 ± 2.1 mmHg, $P < 0.001$, *t*-test) with no significant change in heart rate. Administration (i.v.) of the muscarinic receptor blocker, atropine, had no effect on the change in mean arterial pressure in response to glutamate stimulation, suggesting that the parasympathetic system was not involved in this response. However, administration (i.v.) of the nicotinic receptor blocker, hexamethonium dichloride abolished the depressor response to glutamate, suggesting that CLa stimulation decreases sympathetic outflow to the cardiovascular system. In addition, microinjection of the reversible synaptic blocker, cobalt chloride, into the medial prefrontal cortex greatly attenuated the depressor response elicited by microinjection of glut into the CLa. Thus for the first time, we found the cardiovascular responsive region of the anterior claustrum. Also we showed that its response is mediated through the medial prefrontal cortex.

1. Introduction

The claustrum is a thin subcortical gray matter extending over a large area from the frontal region to the parieto-occipital region. It is reciprocally and topographically connected with all sensory and motor domains of the cerebral cortex. Its exact shape varies among species (Crick and Koch, 2005). The claustrum borders with orbital cortex anteriorly, the perirhinal cortex caudally and insular cortex laterally (Kowianski et al., 1999).

The claustrum has fewer neuronal types compared to those in the cerebral cortex. Its principal cells are called type I, that are medium sized projecting pyramidal like neurons with large spiny dendrites. Type I neurons include fusiform and oval shaped neurons and receive glutamatergic input from the cortices and send glutamatergic projections back to the cortices (Grieve and Sillito, 1995; Perez-Cerda et al., 1996; Rahman and Baizer, 2007). Neurotransmitters of the claustrum include: GABA, the main neurotransmitter of interneurons, and nitric oxide both in interneurons and projecting neurons (Kowianski et al., 2001). Somatostatin (Johansson et al., 1984), vasoactive intestinal peptide (Loren et al., 1979; Sims et al., 1980), neuropeptide Y (Nakagawa et al., 1985; de Quidt and Emson, 1986), glutamate and aspartate were all also reported in the projecting neurons (Perez-Cerda et al., 1996; Rahman and Baizer, 2007).

The claustrum is involved in various functions including nociception (Sloniewski et al., 1995; Persinger et al., 1997), coordination of sensorimotor control (Olson and Graybiel, 1980; Crescimanno et al., 1989), epileptiform activity (Kudo and Wada, 1990; Mohapel et al., 2001; Zhang et al., 2001) and is implicated in Alzheimer's disease (Ogomori et al., 1989; Morys et al., 1996b).

The anterior claustrum (CLa) projects to the prefrontal cortex reciprocally (Druga, 1982; Buchanan et al., 1984; Maxwell et al., 1994; Kowianski et al., 1998), neocortical sensory and motor areas (Minciacchi et al., 1985; Sloniewski et al., 1986; Witter et al., 1988; Sadowski et al., 1997; Kowianski et al., 1998; Mohapel et al., 2001), thalamus (Filimonoff, 1966), entorhinal and subicular cortices (Filimonoff, 1966; Markowitsch et al., 1984; Witter et al., 1988), the amygdala (Filimonoff, 1966; Amaral and Insausti, 1992), the caudate putamen (Arikuni and Kubota, 1985; Filimonoff, 1966), the insular cortex (Filimonoff, 1966; Ruggiero et al., 1987; Witter et al., 1988), the hippocampus (Amaral and Cowan, 1980), the lateral hypothalamic area, the ventral tegmental area, the medial part of the substantia nigra pars

* Corresponding author. Tel.: +98 7616668240.
E-mail address: sheybanifar@gmail.com (M. Sheybanifar).

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compacta (Lindvall et al., 1978; Zhang et al., 2001), the parabrachial, dorsal raphe nucleus (Zhang et al., 2001) and limbic area (Witter et al., 1988; Majak et al., 2000).

Connection to the neocortex is the largest source of inputs to the CLa compared to the subcortical sources (LeVay and Sherk, 1981; Chachich and Powell, 2004). Most of these regions are involved in cardiovascular regulation (Gelsema et al., 1987; Ruggiero et al., 1987; Yasui et al., 1990; Ciriello and Janssen, 1993; Kirouac and Ciriello, 1997; Ciriello and Roder, 1999; Crippa et al., 1999; Kirouac and Pittman, 2000; Ongur and Price, 2000; Fernandes et al., 2003; Kirouac et al., 2004; Resstel and Correa, 2006, 2008; Fortaleza et al., 2011; Muller Ribeiro et al., 2012). These connections probably enable the CLa to affect blood pressure and heart rate. The present study was performed to find whether the CLa is involved in cardiovascular control. In the first series of experiments, all regions of the CLa were chemically stimulated by L-glutamate (glut). In the second series of experiments the possible mechanisms mediating the cardiovascular responses were explored. As one of the major projection areas of CLa is the cingulate region of the medial prefrontal cortex (mPFC) (Druga, 1982; Buchanan et al., 1984; Maxwell et al., 1994; Kowianski et al., 1998), the possible role of the mPFC in the CLa cardiovascular responses was also investigated in urethane anesthetized rats.

2. Materials and methods

2.1. Animals and surgery

Experiments were performed on male Wistar rats (200–300 g). The study was approved by the Animal Use and Care Committee of Hormozgan University of Medical Sciences. The animals were anesthetized with urethane (1.4 g/kg, ip), and supplementary doses (0.7 g/kg) were given as required. The trachea was cannulated to ease ventilation. The body temperature was maintained at 37 ± 1 °C. The femoral artery and vein were cannulated with polyethylene catheter (PE-50) filled with heparinized saline for recording arterial pressure and for intravenous (I.V.) injections respectively. The arterial pressure and heart rate were continuously recorded by both a Harvard polygraph and a computer program written by A. Nasimi. The animals were placed in prone position in a stereotaxic frame (Stoelting, USA) and two small holes were drilled through the frontal bone over the CLa and the mPFC.

2.2. Drug microinjection

Microinjections were performed using a micropipette with internal diameter of 35–45 μm. The stereotaxic coordinates of the CLa were explored from 1–3.7 mm rostral, 1.2–4.8 mm lateral and 3.0–6.8 mm ventral to bregma, and the mPFC was explored using co-ordinates of 1.2 mm rostral, 0.5 mm lateral and 2.8 mm ventral to bregma (Paxinos and Watson, 1998). The injection sites were at least 300 μm apart, and 1–4 injections were made in each animal on both sides. Pressure injection was performed by a pressurized air pulse applicator and the injection volume was measured by direct observation of the fluid meniscus in the micropipette using a microscope fitted with an ocular micrometer. The injection volume of all drugs was 50–100 nl. All drugs were microinjected unilaterally and were dissolved in normal saline except glutamate that was dissolved in phosphate buffered saline (pH = 7.2).

2.3. Experimental groups

The research was split into different experimental groups. Firstly, for the control group, the effects of microinjection of saline (50–100 nl; n = 5 rats; 18 injections) into the Cla were tested. The effects of microinjection of glut (0.25 M/50–100 nl; Sigma) into all parts of the CLa (n = 47 rats, 137 injections) were then investigated. For the atropine group, the effects of injection of the muscarinic receptor blocker atropine (1 mg/kg, i.v.) on responses to CLa were determined. First, glut was microinjected into the CLa, then atropine was injected (i.v.), 2–3 min later the same site was retested by microinjection of glut to assess possible parasympathetic involvement in the response (n = 7 rats). To assess possible sympathetic involvement in the response, the nicotinic receptor blocker hexamethonium dichloride (30–40 mg/kg, i.v., Sigma) was utilized. Secondly, glut was microinjected in the CLa, then hexamethonium dichloride was injected (i.v.), 2–3 min later the same site was retested by microinjection of glut (n = 7 rats). Lastly, it was investigated whether cardiovascular responses of the CLa activation were mediated by the mPFC. For this, glut was firstly injected into the CLa, then after the arterial pressure and heart rate returned to the baseline, the reversible synaptic blocker, cobalt chloride ($CoCl_2$, 5 mM/50 nl,

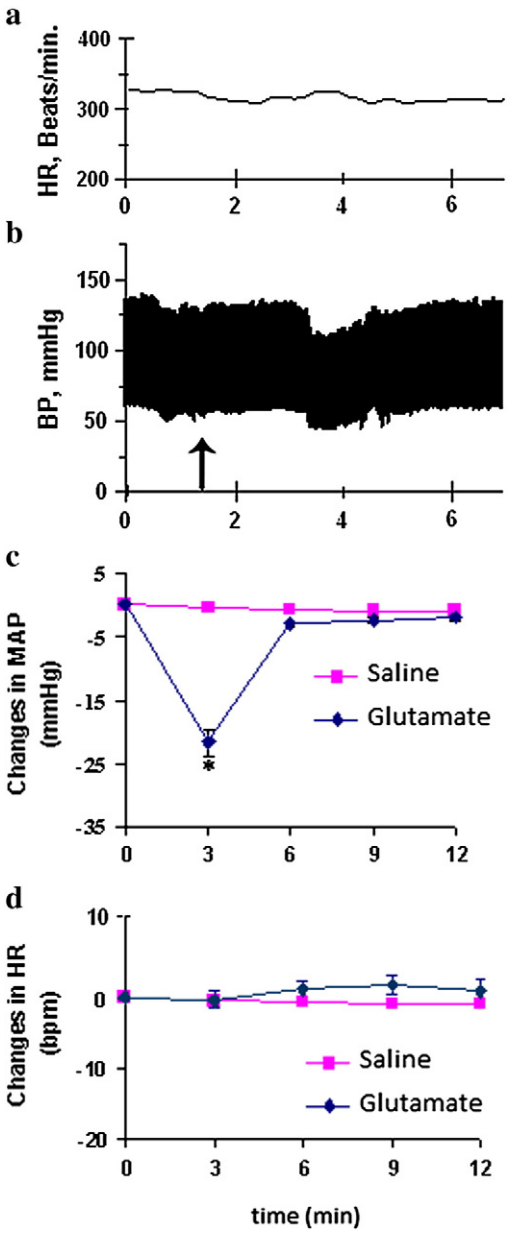

Fig. 1. a–b: Example traces of blood pressure and heart rate, showing the effect of microinjection of L-glutamate into the cardiovascular responsive area of the CLa. The arrow indicates the injection time. c–d: Time–course of the cardiovascular effects of microinjection of L-glutamate and vehicle (control) into the cardiovascular responsive area of the CLa. Glut was injected at time zero. The maximum change was compared to the pre-injection value (*P < 0.001, paired *t*-test) and the corresponding point value of the control group (*P < 0.001, independent *t*-test).