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N-methyl-D-aspartate receptors in the insular cortex modulate baroreflex in unanesthetized rats

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ABSTRACT

In the present study, we report the effect of insular cortex (IC) ablation caused by bilateral microinjection of the non-selective synaptic blocker $CoCl_2$ on cardiac baroreflex response in unanesthetized rats as well as the involvement of local glutamatergic neurotransmission.

Unilateral (left or right) microinjection of $CoCl_2$ (1 nmol/ 100 nL) did not affect the bradycardiac response to blood pressure increase evoked by intravenous infusion of phenylephrine nor the tachycardiac response to blood pressure decrease caused by intravenous infusion of sodium nitroprusside, 10 min after $CoCl_2$. Bilateral microinjection of $CoCl_2$ into IC decreased the magnitude of reflex bradycardia without affecting tachycardiac responses. Baroreflex activity returned to control values 60 min after $CoCl_2$ microinjection, confirming its reversible effect. Further we studied the possible involvement of IC-glutamatergic neurotransmission in baroreflex modulation. We observed that bilateral microinjection of the selective NMDA receptor antagonist LY235959 (4 nmol/100 nL) into the IC decreased the magnitude of reflex bradycardia without affecting tachycardiac responses. IC treatment with the selective non-NMDA antagonist NBQX (4 nmol/100 nL) did not affect baroreflex activity.

The results suggest that synapses within the IC have a tonic excitatory influence on the baroreflex parasympathetic component. Moreover, the present data suggest that local NMDA-receptors are involved in the IC-mediated tonic excitatory influence on baroreflex parasympathetic activity.

1. Introduction

Arterial baroreceptors are sensory nerve endings that respond to changes in arterial pressure. Alterations in baroreceptor activity provide essential feedback to the central nervous system that leads to reflex circulatory adjustments, changes in heart rate (HR) and vascular resistance important to maintain arterial pressure within a narrow range. The importance of baroreflex for blood pressure regulation can be easily appreciated by the marked increase in blood pressure variability that occurs after differentiation of sinoaortic baroreceptors (Alper et al., 1987; Buchholz et al., 1986; Cowley et al., 1973; Norman et al., 1981; Trapani et al., 1986).

The insular cortex (IC) is a forebrain limbic structure which integrates sensory and visceral information from peripheral receptors (Saper, 1982). This cortical region receives topographically organized visceral afferent information contained in the vagus nerve and projections from subcortical nuclei involved in autonomic control (Cechetto and Saper, 1987; Ruggiero et al., 1987; Yasui et al., 1991). Moreover, neurons within IC have been shown to innervate nuclei involved in autonomic control, such as the nucleus of the tratus solitary and medullary areas that contain preganglionic parasympathetic motoneurons (Kapp et al., 1985; Neafsey et al., 1986; Shipley, 1982).

Pressor as well as depressor responses were reported after IC electrical or chemical stimulation, depending on IC region stimulated and if animals were anaesthetized or not (Allen and Cechetto, 1995; Hardy and Holmes, 1988; Hardy and Mack, 1990; Ruggiero et al., 1987; Yasui et al., 1991). Also, the IC has been proposed to modulate baroreflex. Unilateral electrolytic lesions or bilateral microinjection of lidocaine into the IC of anaesthetized animals have been reported to affect reflex bradycardia without affecting tachycardiac responses (Saleh and Connell, 1998; Zhang et al., 1998). Moreover, (Saad et al., 1989) reported that IC lesion caused by middle cerebral artery occlusion also affected reflex bradycardia in conscious animals. However, lidocaine is a non-selective blocker that affects both synapses and passage fibers (Sandkuhler et al., 1987). Moreover, previous studies have demonstrated that baroreflex function is affected by the anesthesia (Fluckiger et al., 1985; Shimokawa et al., 1998). Thus, experiments using unanaesthetized animals and compounds that selectively block synapses could offer additional information about the role of IC in baroreflex modulation.

Glutamate (L-glu) is an important central nervous system (CNS) neurotransmitter (Fleck et al., 1993; Khodorov, 2004) which acts on

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metabotropic and ionotropic receptors (Hollmann and Heinemann, 1994; Jingami et al., 2003; Kemp and McKernan, 2002). The ionotropic receptors are nonselective cation-permeable channels and are classified in *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-isoxazole-4-proprionate (AMPA)/ kainite receptors (non-NMDA-receptors) (Hollmann and Heinemann, 1994; Kemp and McKernan, 2002).

Glutamatergic terminals and ionotropic receptors were reported to be present in the IC of rats (Dori et al., 1992), thus suggesting existence of a glutamatergic neurotransmission in the IC. Moreover, previous studies indicated that L-glu microinjection into IC evokes cardiovascular responses (Butcher and Cechetto, 1995; Ruggiero et al., 1987). However, although previous reports suggest an involvement of IC-glutamatergic neurotransmission in cardiovascular control, there is no evidence on the role played by this transmission in baroreflex modulation.

In the present study we evaluated the effect of acute and reversible ablation of IC caused by bilateral microinjection of $CoCl_2$ into that area on the baroreflex cardiac response. Also, we tested the hypothesis that local IC-glutamatergic neurotransmission modulates baroreflex in unanesthetized rats.

2. Methods

2.1. Animal preparation

Twenty-two Wistar rats weighing 230–270 g were used. Animals were kept in the Animal Care Unit of the Department of Pharmacology, School of Medicine of Ribeirão Preto, University of São Paulo. Rats were housed in plastic cages under standard laboratory conditions and had free access to food and water. Animals were kept under a 12 h light/dark cycle (lights on at 06:30 h). The Institution's Animal Ethics Committee authorized housing conditions and experimental procedures, process number 167/2007.

Four days before the experiment, the rats were anesthetized with tribromoethanol (250 mg/kg, i.p.). After local anesthesia with 2% lidocaine, the skull was surgically exposed and stainless steel guide cannulas (26G) were implanted bilaterally into the IC using a stereotaxic apparatus (Stoelting, Wood Dale, Illinois, USA). Stereotaxic coordinates for cannula implantation in the IC were selected from the rat brain atlas of Paxinos and Watson (1997) and were: AP = + 11.7 mm, L = 4.0 mm from the medial suture and V = − 4.5 mm from the skull. Cannulas were fixed to the skull with dental cement and one metal screw. After surgery, the animals were treated with a polyantibiotic preparation of streptomycins and penicillins (i.m., Pentabiotico®, Fort Dodge, Brazil) to prevent infection and with the non-steroidal anti-inflammatory flunixine meglumine (i.m., Banamine®, Schering Plough, Brazil) for post-operative analgesia.

One day before the experiment, the rats were again anesthetized with tribromoethanol (250 mg/kg, i.p.) and a catheter (a 4 cm segment of PE-10 that was heat-bound to a 13 cm segment of PE-50, Clay Adams, Parsippany, NJ, USA) was inserted into the abdominal aorta through the femoral artery, for blood pressure recording. A second catheter was implanted into the femoral vein for infusion of phenylephrine or SNP to evoke baroreflex changes. Both catheters were tunneled under the skin and exteriorized on the animal's dorsum. In the end of the surgery the treatment with polyantibiotic and anti-inflammatory drug was repeated.

2.2. Measurement of cardiovascular responses

Pulsatile arterial pressure of freely moving animals was recorded using an HP-7754A preamplifier (Hewlett Packard, Palo Alto, CA, USA) and an acquisition board (Biopac M-100, Goleta, CA, USA) connected to a computer. Mean arterial pressure (MAP) and heart rate (HR) values were derived from pulsatile recordings and processed on-line.

2.3. Drugs

$CoCl_2$ (Sigma, St. Louis, MO, USA), LY235959 (Tocris, Westwoods Business Park Ellisville, MO, USA) and NBQX (Tocris, Westwoods Business Park Ellisville, MO, USA) were dissolved in sterile artificial cerebrospinal fluid (ACF – composition: 100 mM NaCl; 2 mM Na_3PO_4; 2.5 mM KCl; 1 mM $MgCl_2$; 27 mM $NaHCO_3$; 2.5 mM $CaCl_2$; pH = 7.4). Phenylephrine–HCl (Sigma, St. Louis, MO, USA), sodium nitroprusside (Sigma, St. Louis, MO, USA), tribromoethanol (Sigma, St. Louis, MO, USA) and urethane (Sigma, St. Louis, MO, USA) were dissolved in saline (0.9% NaCl).

2.4. Drug injection

The needle (33G, Small Parts, Miami Lakes, FL, USA) used for microinjection into the IC were 1 mm longer than guide cannula and was connected to a 1 μL syringe (7002-H, Hamilton Co., Reno, NV, USA) through PE-10 tubing. The needle was carefully inserted into the guide cannula and drugs were injected in a final volume of 100 nL over a 5 s period. After a 15 s period, the needle was removed and inserted into the contralateral guide cannula for bilateral microinjections into the IC.

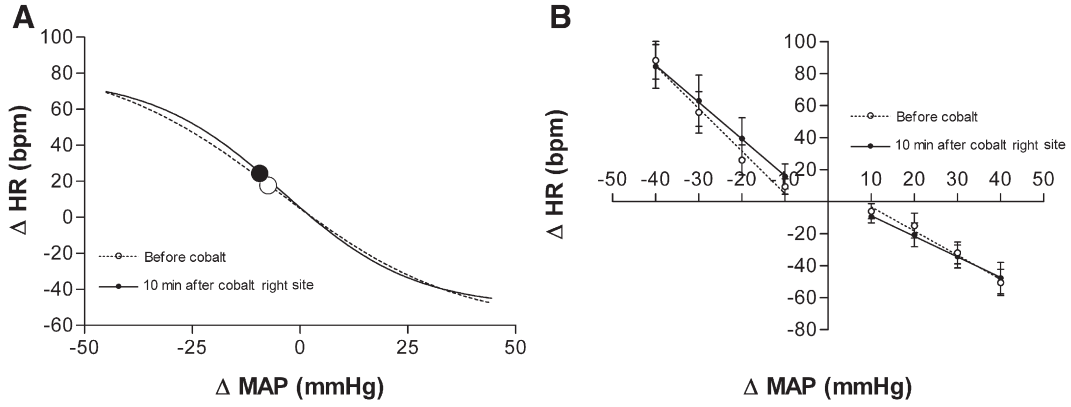

Fig. 1. A – Sigmoid baroreflex curves correlating mean arterial pressure (Δ MAP) and heart rate responses (Δ HR) before ($r^2 = 0.83$) or 10 min ($r^2 = 0.83$) after unilateral microinjection of $CoCl_2$ (1 nmol) into the right insular cortex ($n = 5$). B – Linear regression baroreflex curves correlating Δ MAP and Δ HR responses, before (○) or 10 min (●) after unilateral microinjection of $CoCl_2$ ($n = 5$). Increases or decreases in mean arterial pressure were induced by i.v. infusion of respectively phenylephrine and sodium nitroprusside. Correlation r^2 values for bradycardiac regression curves were respectively 0.65 and 0.55, before or 10 min after microinjection of $CoCl_2$. Correlation r^2 values for tachycardiac regression curves were respectively 0.72 and 0.56, before or 10 min after $CoCl_2$.