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Diagonal band of Broca modulates the cardiac component of the baroreflex in unanesthetized rats

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ABSTRACT

The diagonal band of Broca (DBB) is involved in cardiovascular control in rats. In the present study, we report the effect of acute and reversible neurotransmission inhibition in the DBB by bilateral microinjection of the nonselective neurotransmission blocker $CoCl_2$ (1 mM, 100 nL) on the cardiac baroreflex response in unanesthetized rats. Local DBB neurotransmission inhibition did not affect baseline values of either blood pressure or heart rate, suggesting no tonic DBB influence on cardiovascular system activity. However, $CoCl_2$ microinjections enhanced both the reflex bradycardia associated with blood pressure increases caused by i.v. infusion of phenylephrine and tachycardiac response evoked by blood pressure decreases caused by i.v. infusion of sodium nitroprusside. An increase in baroreflex gain was also observed. Baroreflex returned to control values 60 min after $CoCl_2$ microinjections, confirming its reversible effect. In conclusion, our data suggest that synapses within DBB have a tonic inhibitory influence on both the cardiac parasympathetic and sympathetic components of the baroreflex.

The diagonal band of Broca (DBB) is a basal forebrain structure that plays an important role in cognitive and behavioral processes [26]. Several studies have also shown that the DBB is connected to brain nuclei involved in neurovegetative regulation, such as the medial prefrontal cortex, medial and lateral septal nuclei, central nuclei of the amygdala and most of the hypothalamic nuclei [4,9,29,31].

It has been reported that L-glutamate microinjected into the DBB evokes both arterial blood pressure decreases in anesthetized rats and arterial blood pressure increases in unanaesthetized rats [10,20,28]. The depressor response observed in anesthetized animals is mediated by a local DBB NMDA receptor–nitric oxide–guanylate cyclase pathway [30]. Additionally, DBB stimulation by local injection of adrenergic agonists has also been reported to cause pressor as well as depressor responses in anesthetized rats [1].

Previous studies have also demonstrated a DBB baroreflex modulation in anesthetized rats. Jhamandas and Renaud [14] showed that baroreceptor activation increased firing of DBB neurons projecting to the hypothalamic supraoptic nucleus (SON). Also, lesion or catecholamine depletion in the DBB has been shown to decrease baroreflex-mediated inhibition of SON neuron activity [6,7]. Based on these findings, it was suggested that the DBB is part of a central catecholaminergic pathway that is involved in the baroreflex-induced depression of the spontaneous activity of SON vasopressin-secreting neurons [6,7]. However, the possible DBB modulation of cardiac baroreflex response has not yet been studied.

In this way, in the present study we determined the effect of acute and reversible inhibition of DBB neurotransmission by bilateral microinjection of the nonselective synapse blocker $CoCl_2$ on the cardiac baroreflex response.

Male Wistar rats weighing 230–270 g were used. The animals were kept in the Animal Care Unit of the Department of Pharmacology, University of São Paulo. The rats were housed in plastic cages under a 12 h light–dark cycle, with free access to food and water. Housing conditions and experimental procedures were approved by the institution's animal ethics committee.

Four days before the experiment, the rats were anesthetized with tribromoethanol (250 mg/kg i.p.). After scalp anesthesia with 2% lidocaine, the skull was exposed and guide cannulas were stereotaxically implanted bilaterally in the DBB, using a stereotaxic apparatus (Stoelting, Wood Dale, IL, USA). Cannulas (13 mm long) were handcrafted in the laboratory using 26G stainless hypodermic needles (Becton Dickinson, Curitiba, PR, Brazil). Coordinates for cannula implantation into the DBB were: AP = +9.2 mm; *L* = 0.7 mm

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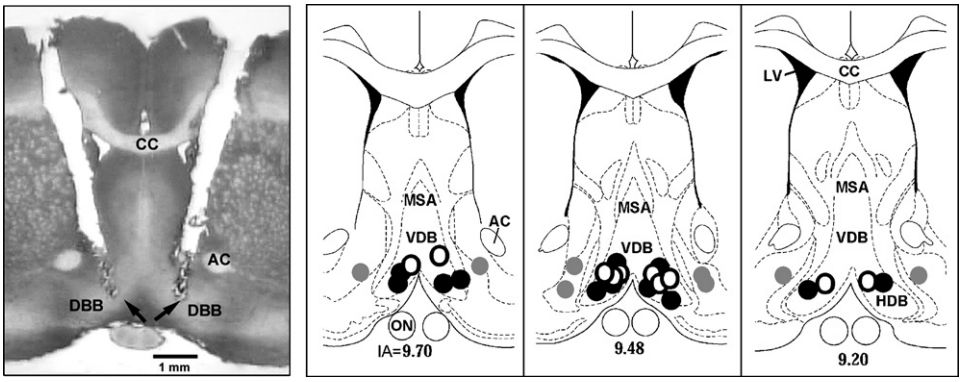

Fig. 1. Left: Photomicrograph of a coronal brain section from a representative rat, showing bilateral injection sites in the DBB. Right: Diagrammatic representation based on the atlas of Paxinos and Watson [22], indicating injection sites of ACF (open circles) and $CoCl_2$ (closed circles) into the DBB and $CoCl_2$ into structures surrounding the DBB (gray circles). AC, anterior commissure; cc, corpus callosum; HDB, horizontal limb of the diagonal band of Broca; IA, interaural; LV, lateral ventricle; MSA, medial septal area; ON, optic nerve; VDB, vertical limb of the diagonal band of Broca.

from the medial suture, $V = -8.0$ mm from the skull with a lateral inclination of 13° [22]. After surgery, the animals received a polyantibiotic (i.m., 0.27 g/kg, Pentabiotico®, Fort Dodge, Brazil) to prevent infection and a nonsteroidal antiinflammatory (i.m., 0.025 g/kg, Banamine®, Schering Plough, Brazil) for post-operative analgesia.

One day before the experiment, the rats were again anesthetized with tribromoethanol and a catheter was inserted into the abdominal aorta through the femoral artery for mean arterial pressure (MAP) and heart rate (HR) recording. A second catheter was implanted into the femoral vein for infusion of phenylephrine or sodium nitroprusside to evoke blood pressure changes. Catheters were tunneled under the skin and exteriorized on the animal's dorsum. After surgery, a nonsteroidal antiinflammatory (Banamine®) was administered for post-operative analgesia.

Arterial cannulas were connected to a pressure transducer and pulsatile arterial pressure was recorded using an HP-7754A preamplifier (Hewlett Packard, Palo Alto, CA, USA) and an acquisition board (MP100A, Biopac Systems Inc., Goleta, CA, USA) connected to a computer. MAP and HR values were derived from pulsatile arterial pressure recordings and were processed online.

$CoCl_2$ (SIGMA, St. Louis, MO, USA) was dissolved in artificial cerebrospinal fluid (ACF) (composition: 100 mM NaCl; 2 mM Na_3PO_4; 2.5 mM KCl; 1.0 mM $MgCl_2$; 27 mM $NaHCO_3$; 2.5 mM $CaCl_2$; pH 7.4). Needles (33G, Small Parts, Miami Lakes, FL, USA) used for microinjection into the DBB were 1 mm longer than the guide cannulas and were connected to a 2-μL syringe (7002H, Hamilton Inc., Reno, NV, USA). Drugs or vehicle were injected in 100 nL.

The baroreflex was activated by intravenous phenylephrine (50 μg/kg; 0.34 mL/min) (Sigma, St. Louis, MO, USA) or sodium nitroprusside (50 μg/kg; 0.8 ml/min) (Sigma, St. Louis, MO, USA) infusion using an infusion pump (K.D. Scientific, Holliston, MA, USA) [2]. Infusions lasting 50–60 s caused incremental pressor or depressor responses. Phenylephrine and sodium nitroprusside were dissolved in 0.9% NaCl.

HR values matching MAP variations were determined. Paired values of MAP and HR variations were plotted to create sigmoid curves for each rat to determine the baroreflex [13]. To study bradycardiac and tachycardiac response separately, the slope of the linear regression curves was determined to baroreflex evaluation [25].

Rats were kept in their own cages throughout experiment and the study was conducted in unanesthetized freely moving animals. The experimental room had controlled temperature (25 °C) and was acoustically isolated. Animals were allowed 1 h to adapt to the conditions of the experimental room. At least one additional 30-min period was used to record baseline values.

All animals used in this study received three infusions of both phenylephrine and sodium nitroprusside to find out the control baroreflex response. In sequence, the first group received bilateral microinjections of ACF ($n = 5$, 100 nL) into the DBB and phenylephrine and sodium nitroprusside infusions were repeated 10 min after treatment. The second group received bilateral microinjections of 1 mM $CoCl_2$ ($n = 6$) into the DBB and infusions of the vasoactive drugs were repeated 10 and 60 min after treatment. The third group received bilateral microinjections of $CoCl_2$ ($n = 4$) into the structures surrounding the DBB. The order of infusions of vasoactive drugs was randomized.

At the end of the experiments, rats were anesthetized with urethane (1.25 g/kg, i.p.) and 100 nL of 1% Evan's blue dye was bilaterally injected into the DBB to mark injection sites. The chest was surgically opened, the descending aorta occluded, the right atrium severed, and the brain perfused with 10% formalin through the left ventricle. Brains were postfixed for 24 h at 4 °C, and 40-μm sections were cut using a cryostat (CM-1900, Leica, Wetzlar, Germany). Sections were stained with 1% neutral red and injection sites were identified.

Baroreflex was analyzed using sigmoid curves that were characterized by four parameters: (i) P1 (bpm) lower HR plateau and P2 (bpm) upper plateau; (ii) HR range (bpm), i.e., the difference between upper and lower plateau levels; (iii) median blood pressure (BP_{50}, mmHg) which is the MAP at 50% of the HR range; and (iv) average gain (G, bpm/mmHg) which is the average slope of the curves [13]. Significant differences between sigmoid curve and linear regression parameters were determined using one-way ANOVA followed by Dunnett's test, assuming $P < 0.05$ as significant. Baseline cardiovascular values before and after ACF or $CoCl_2$ microinjected into the DBB and baroreflex parameters in response to ACF microinjection into the DBB and $CoCl_2$ administered into structures surrounding the DBB were compared using Student's t-test.

A photomicrograph of a coronal brain section showing the microinjection site in the DBB is presented in Fig. 1. Fig. 1 also shows a diagrammatic representation of injection sites in the DBB or surrounding structures.

Bilateral microinjections of ACF into the DBB did not affect baseline MAP (99 ± 3 mmHg vs. 100 ± 4 mmHg, $t = 0.3$, $P > 0.05$) or HR (339 ± 10 bpm vs. 330 ± 7 bpm, $t = 1$, $P > 0.05$). Nonlinear and linear regression analysis of baroreflex activity indicated that microinjections of ACF into the DBB did not affect the baroreflex response (Table 1). Linear regression also indicate no differences in the slope of bradycardiac (-1.6 ± 0.3 bpm/mmHg vs. -1.7 ± 0.3 bpm/mmHg, $t = 0.3$, $P > 0.05$) or tachy-