Life Sciences 84 (2009) 444–450

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

The diagonal band of Broca is involved in the pressor pathway activated by noradrenaline microinjected into the periaqueductal gray area of rats

Gislaine Garcia Pelosi *, Rodrigo Fiacadori Tavares, Fernando Morgan Aguiar Corrêa

Department of Pharmacology, School of Medicine of Ribeirão Preto, University of São Paulo, Av. Bandeirantes 3900, 14040-900, Ribeirão Preto, São Paulo, Brazil

ARTICLE INFO

Article history:
Received 28 April 2008
Accepted 22 January 2009

Keywords:
Noradrenaline
Cardiovascular system
Periaqueductal gray area
Diagonal band of Broca
Neural connections

ABSTRACT

Aims: The dorsal periaqueductal gray area (dPAG) is involved in cardiovascular modulation. Previously, we reported that noradrenaline (NA) microinjection into the dPAG caused a pressor response that was mediated by vasopressin release into the circulation. However, the neuronal pathway that mediates this response is as yet unknown. There is evidence that chemical stimulation of the diagonal band of Broca (dbB) also causes a pressor response mediated by systemic vasopressin release. In the present study, we evaluated the participation of the dbB in the pressor response caused by NA microinjection into the dPAG as well as the existence of neural connections between these areas.
Main methods: With the above goal, we verified the effect of the pharmacological ablation of the dbB on the cardiovascular response to NA microinjection into the dPAG of unanesthetized rats. In addition, we microinjected the neuronal tracer biotinylated-dextran-amine (BDA) into the dPAG and looked for efferent projections from the dPAG to the dbB.
Key findings: The pharmacologically reversible ablation of the dbB with local microinjection of $CoCl_2$ significantly reduced the pressor response caused by NA microinjection (15 nmol/50 nL) into the dPAG. In addition, BDA microinjection into the dPAG labeled axons in the dbB, pointing to the existence of direct connections between these areas.
Significance: The present results indicate that synapses within the dbB are involved in the pressor pathway activated by NA microinjection into the dPAG and direct neural projection from the dPAG to the dbB may constitute the neuroanatomic substrate for this pressor pathway.

Introduction

The periaqueductal gray area (PAG) is a mesencephalic region surrounding the aqueduct which is involved in the control of behavioral responses and their cardiovascular correlates (Nashold et al. 1969; Jenck et al. 1989; Huang et al. 2000). Both electrical and chemical PAG stimulations evoke cardiovascular changes (Bandler et al. 2000; Pizzirusso et al. 1998; Lovick 1985; Krieger and Graeff 1985). Previously, we have reported that noradrenaline (NA) microinjection into the dorsal PAG (dPAG) caused dose-related responses associated with bradycardia (Pelosi and Corrêa 2005). The fact that pressor response was blocked by systemic pretreatment with a vasopressin antagonist and was absent in hypophysectomized rats, suggests it is mediated by acute vasopressin release.

The diagonal band of Broca (dbB) is an important basal forebrain nucleus which regulates cognitive and emotional functions (Semba 2000). Studies have also suggested a dbB role in central cardiovascular modulation (Gelsema and Calaresu 1987; Kirouac and Ciriello 1997; Tavares and Corrêa 2003; Tavares et al. 2007). dbB stimulation with homocysteic acid or L-glutamate caused depressor responses in anesthetized rats (Gelsema and Calaresu 1987; Kirouac and Ciriello 1997). In unanesthetized rats, chemical dbB stimulation with glutamate has been reported to evoke a pressor response which was also dependent on systemic vasopressin release (Tavares and Corrêa 2003). Considering these facts, it is timely to study the hypothesis that dbB is a relay in the pressor pathway activated by NA microinjection into the dPAG of unanesthetized rats.

A common approach to investigate the possible participation of specific brain areas in a functional neural pathway is based on information obtained by acute functional ablation. The technique is based on giving restricted microinjections of compounds that reversibly block neuronal activity over a given period. Microinjections of $CoCl_2$ (cobalt) into discrete brain areas have been used for reversible functional inactivation (Kretz 1984; Deolindo et al. 2008; Ciriello et al. 2008; Pelosi et al. 2007; Fisk and Wyss 2000). In addition, neuronal connections among brain structures may be studied by histological analysis of neuronal tracer distribution after their microinjection into selected brain areas. A useful tracer is the low molecular weight

* Corresponding author. Tel.: +55 16 36023207; fax: +55 16 36332301.
E-mail address: pelosigg@yahoo.com.br (G.G. Pelosi).

0024-3205/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.lfs.2009.01.011

biotinylated-dextran-amine (BDA). BDA is transported bidirectionally, thus evidencing both neural afferents from projecting neurons and efferent projections of a brain region under study (Vercelli et al. 2000).

In the present work we endeavored to verify whether the dbB is involved in the pressor pathway activated by NA microinjection into the dPAG of unanesthetized rats as well as to describe neuronal connections between the dPAG and dbB.

Materials and methods

Subjects

Experimental procedures were carried out following protocols approved by the ethical review committee of the School of Medicine of Ribeirão Preto, University of São Paulo. Male Wistar rats weighing 240–260 g were used in the present experiments. Animals were housed individually in plastic cages in a temperature-controlled room (25 °C) in the Animal Care Unit of the Department of Pharmacology of the School of Medicine of Ribeirão Preto, under a 12:12 h light–dark cycle. Animals had free access to water and standard laboratory chow, except during the experimental period.

Surgical preparation

Animals were anesthetized with tribromoethanol (250 mg/kg, i.p; Aldrich Chemical Co. Inc., USA) before implanting stainless steel guide cannulas in the dPAG and DBB. After local anesthesia with 2% lidocaine, the skull was surgically exposed and stainless steel guide cannulas (24G) were implanted 1 mm above the injection sites using a stereotaxic apparatus (Stoelting, Wood Dale, Illinois, USA). Stereotaxic coordinates for cannula implantation in the dPAG and dbB were selected from the brain atlas of Paxinos and Watson (1997). For implants in the dPAG, the following coordinates were used: AP = +2.7 mm from the interaural line; L = +2.5 mm from the medial suture and V = −4.7 mm from the skull with a lateral inclination of 26°. For implants in the dbB, the following coordinates were used: AP = +9.48 mm from the interaural line; L = +0.5 mm from the medial suture and V = −7.1 mm from the skull. Cannulas were fixed to the skull with dental cement and one metal screw. A tight-fitting mandrill was kept inside the guide cannula to avoid its occlusion.

Two days later, rats were anaesthetized with tribromoethanol (250 mg/kg, i.p.) and a polyethylene catheter was implanted into the left femoral artery for blood pressure recording. The arterial catheter consisted of a piece of PE-10 tubing (4.0 cm) heat-bonded to a longer segment of PE-50 tubing (10–12 cm). The catheter was filled with 0.3% heparin (5,000 UI/mL) in sterile saline (0.9% NaCl). The PE-10 piece was introduced into the femoral artery until the tip reached the aorta. The catheter was secured in position with thread and the PE-50 part was passed under the skin to be extruded at the dorsum of the animals. After surgery, the animals were allowed to recover for 24 h. Animals were kept in individual cages during the post-surgery period.

After surgeries, animals were treated with a polyantibiotic preparation of streptomycins and penicillins i.m. (Pentabiotico®, Fort Dodge, Brazil) to prevent infection and with the nonsteroidal antiinflammatory flunixine meglumine i.m. (Banamine®, Schering Plough, Brazil) for post-operative analgesia.

Measurement of cardiovascular responses

After surgery, the animals were kept in individual cages in the Animal Care Unit, which were transported to the experimental room. Animals were allowed 1 h to adapt to the conditions of the experimental room, such as sound and illumination, before starting blood pressure and heart rate recording. The experimental room was acoustically isolated and had constant background noise produced by an air exhauster. At least one extra period of baseline recording of 15 min was allowed before the microinjections. The injection needle was slowly introduced into the guide cannula without touching or restraining the animals. Pulsatile arterial pressure (PAP) of freely moving animals was recorded using an amplifier (model 7754A, Hewlett Packard, Palo Alto, CA, USA) coupled to a computerized acquisition system (MP100, Biopac, Santa Barbara, CA, USA). Mean arterial pressure (MAP) and heart rate (HR) were derived from PAP data using Acknowledge III software (Biopac, USA). Blood pressure baseline values were calculated as the average of the 3 min recording before the injection, whenever blood pressure was considered to be stable. Mean arterial pressure and heart rate responses were measured at the peak of the hypertensive effect, which was observed about 40 s after the NA injection.

Drug microinjection into the dPAG or the dbB

Noradrenaline (NA, 15 nmol/50 nL; Sigma, St. Louis, MO, USA) was dissolved in artificial cerebrospinal fluid (ACF: NaCl 100 mM; Na_3PO_4 2 mM; KCl 2,5 mM; $MgCl_2$ 1,0 mM; $NaHCO_3$ 27 mM; $CaCl_2$ 2,5 mM; pH = 7.4) and cobalt ($CoCl_2$, 1 mM/100 nL; Sigma, St. Louis, MO, USA) was dissolved in sterile 0.9% NaCl. On the first day of the experiment, unanesthetized rats received NA microinjection into the dPAG. On the second day, the same rats received NA microinjection into the dPAG 10 min after dbB pretreatment with cobalt or vehicle.

A 1 µL syringe (KH7001, Hamilton Co., Reno, NV, USA) connected to a 33G injection needle by a segment of PE-10 tubing was used to microinject NA into the dPAG or cobalt into the dbB. The injection needles were 1 mm longer than the guide cannulas.

Histological determination of the microinjection sites

At the end of the experiments, the animals were anesthetized with urethane (1.25 g/kg, i.p.; Sigma, St. Louis, MO, USA) and 50 nL or 100 nL of 1% Evan's blue dye was microinjected into the dPAG or dbB, respectively, as a marker of the injection site. The chest was surgically opened, the descending aorta occluded, the right atrium severed and the brain perfused with 0.9% NaCl followed by 10% formalin through the left ventricle. The brains were removed and post-fixed for 24 h at 4 °C and serial 40 µm-thick sections were cut with a cryostat (CM 1900, Leica, Germany). Brain sections were stained with 0.5% cresyl violet for light microscopy analysis. Injection sites were determined from serial sections and according to the rat brain Atlas of Paxinos and Watson (1997).

Biotinylated-dextran-amine (BDA) microinjection into the dPAG

The anterograde and retrograde tracer BDA (5 µg/50 nL; Molecular Probes Inc., USA) was used to determine connections between the dPAG and the dbB. Male Wistar rats (240–250 g, n = 5) were used in the present experiment. The rats were anesthetized with tribromoethanol (250 mg/kg, i.p.). After local anesthesia with 2% lidocaine, the skull was surgically exposed. The tracer (3000 MW BD; Molecular Probes Inc., USA) was microinjected under pressure into the dPAG using a stereotaxic apparatus (Stoelting, Wood Dale, Illinois, USA). The coordinates AP = +2.7 mm; L = +2.5 mm from the medial suture and V = −4.8 from the skull with a lateral inclination of 26° were selected from the rat brain atlas of Paxinos and Watson (1997). The injection needle (33G) was connected to a 1.0 µL syringe (7001KH, Hamilton, Co., Reno, NV, USA) through a segment of PE-10 polyethylene (Intramedic, Clay-Adams, USA). The skull was surgically exposed and the needle introduced into the brain. A 10% BDA solution in 0.01 M phosphate buffer (pH = 7.4) was injected in a volume of 50 nL. Animals were treated with a polyantibiotic preparation of streptomycins and penicillins i.m. (Pentabiotico®, Fort Dodge, Brazil) to prevent infection and with the nonsteroidal antiinflammatory flunixine