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Opioid receptors in the prelimbic cortex modulate restraint stress-induced cardiovascular responses in the rat

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ABSTRACT

The prelimbic cortex (PL) is involved in the control of behavioral and autonomic responses to stress. The present study aimed to investigate whether opioid neurotransmission in the PL modulates autonomic responses evoked by restraint stress (RS). Bilateral microinjection of 0.03, 0.3 and 3 nmol/100 nL of the nonselective opioid antagonist naloxone into the PL reduced pressure and tachycardiac responses evoked by RS. However, no effects were observed after its injection at doses of 0.003 and 30 nmol/100 nL, thus resulting in an inverted U-shaped dose–inhibition curve. Similar to naloxone, the selective μ-opioid antagonist CTAP, and the selective κ-opioid antagonist nor-BNI, also reduced MAP and HR increases induced by RS when injected into the PL, whereas treatment with the selective δ-opioid antagonist naltrindole did not affect the pressor and tachycardiac response caused by RS. Blockade of opioid neurotransmission in the PL did not affect the fall in tail temperature and increase in body temperature induced by RS. The present results confirm the involvement of PL opioid neurotransmission in the modulation of cardiovascular responses evoked during the exposure to an aversive situation, and suggest that responses observed after the blockade of local opioid receptors is due to alterations in PL neuronal activity. Furthermore, these results suggest that a distinct circuitry is involved in modulation of the sympathetic output to different vascular territories.

1. Introduction

The medial prefrontal cortex (MPFC) is a limbic structure involved in the integration of stress responses. The prelimbic cortex (PL), an MPFC subdivision, has direct connections with structures related to cardiovascular control (Terreberry and Neafsey, 1987; Vertes, 2004).

Restraint stress (RS) is an acute inescapable and aversive situation, which is characterized by autonomic and endocrine changes such as: sustained increase in mean arterial pressure (MAP) and heart rate (HR); shunting of blood from the periphery to muscles and brain, which results in body temperature increase and skin temperature decrease, as well as activation of the hypothalamus–hypophysis–adrenal axis (Busnardo et al., 2010; Reis et al., 2011; Vianna and Carrive, 2005).

Rats subjected to RS display increased neuronal activity in the PL (Imaki et al., 1993; Yokoyama and Sasaki, 1999). Furthermore, inhibition of PL neurotransmission by local microinjection of the nonselective synapse inhibitor $CoCl_2$ has been reported to increase the tachycardiac response evoked by acute RS, without affecting the RS-evoked MAP response (Tavares and Correa, 2006; Tavares et al., 2009), suggesting a PL inhibitory influence on the evoked HR increase. The inhibitory PL influence on tachycardiac responses has been reported to involve activation of PL NMDA-glutamate receptors (Tavares and Correa, 2006). However, there is no evidence about the neurotransmitter involved in the PL facilitatory modulation of RS-evoked cardiovascular responses.

Among Central Nervous System (CNS) neurotransmitters, opioidergic neurotransmission is known to be activated during stress (Bruchas et al., 2010; Drolet et al., 2001), and to participate in cardiovascular control during aversive situations (Florentino et al., 1987; Houdi et al., 1996; Jimenez et al., 1990; Marson et al., 1989). Three families of classic opioid peptides are known: β-endorphin is mainly produced in cells of the arcuate nucleus and nucleus of the solitary tract projecting to limbic areas (Le Merrer et al., 2009;

Abbreviations: aCSF, artificial cerebrospinal fluid; ANOVA, analysis of variance; HR, heart rate; MAP, mean arterial pressure; MPFC, medial prefrontal cortex; PAP, pulsatile arterial pressure; PL, prelimbic cortex; RS, restraint stress; vMPFC, ventral medial prefrontal cortex.

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Liotta et al., 1984), while enkephalin and dynorphin precursors are widely distributed throughout the CNS (Le Merrer et al., 2009; Weihe et al., 1988). These peptides and the receptors upon which they act are all located in the PL (Leriche et al., 2007; Simantov et al., 1977; Weber et al., 1982). Furthermore, opioid receptors have been reported to regulate neuronal excitability in the MPFC (Giacchino and Henriksen, 1998; Steketee, 2003).

To test the hypothesis that opioid receptors in the PL region modulate autonomic effects of stress, we studied the effect of PL pretreatment with either nonselective or selective μ, κ and δ-opioid receptor antagonists on RS-evoked cardiovascular responses.

2. Experimental procedures

2.1. Subjects

Experimental procedures were carried out following protocols approved by the Ethical Review Committee of the School of Medicine of Ribeirão Preto following the rules of the European Communities Council Directive of 24 November 1986 (86/609/EEC). One hundred and three male Wistar rats, weighing 250–280 g, were used in the present experiments. Animals were housed individually in plastic cages in a temperature-controlled room (25 °C) of the Animal Care Unit of the Department of Pharmacology, School of Medicine of Ribeirão Preto. Animals were kept under a 12:12 h light–dark cycle (lights on between 6:00 and 18:00 h). Animals had free access to water and standard laboratory food, except during the experimental period. Rats were transported to the experiment room and remained in their own cages until being subjected to restraint. Experiments were performed during the morning period (08:00–12:00) in order to minimize possible circadian rhythm interference.

2.2. Surgical procedures

Seven days before the experiment, animals were anesthetized with tribromoethanol (Aldrich Chemical Co. Inc., Milwaukee, USA) (250 mg/kg i.p.), and their heads fixed to a stereotaxic apparatus (Stoelting, Wood Dale, Illinois, USA). The skull was surgically exposed and perforated with a dental drill at a point located 1.9 mm from the medial line and 11.7 mm anterior to the interaural line (Paxinos and Watson, 2007). Bilateral stainless steel guides cannulas (24G, 11 mm-long) were lowered 2.4 mm from the skull, at a 22° angle, into both sides. Guide cannulas were positioned 1 mm above the intended injection sites and fixed to the skull with a metal screw and dental cement. Stainless occluders, with 0.2 mm outer diameter and 11 mm in length, were introduced into the cannulas in order to avoid clogging during the recovery period after surgery. In a group of 22 rats, intra-abdominal dataloggers (SubCue dataloggers, Calgary, Alberta, Canada) were implanted to record body temperature. All animals received intramuscular injection of a poly-antibiotic containing streptomycins and penicillins (80,000 UI, Pentabiotico®, Fontoura-Wyeth, Brazil,) to prevent infection, and a subcutaneous injection of the nonsteroidal anti-inflammatory flunixin meglumine (2.5 mg/kg, Banamine®, Schering Plough, Brazil) for post operation analgesia. Rats were allowed to recover from the surgery during a period of seven days.

Twenty-four hours before RS session, rats were anesthetized and a polyethylene catheter was implanted into the femoral artery, for MAP and HR recording. The catheter was exposed on the dorsum of the animals and attached to the skin. After surgical procedures, animals were treated with the nonsteroidal anti-inflammatory flunixin meglumine. Cardiovascular and temperature recordings were performed when rats were either in their own cage or during the restraint.

2.3. Cardiovascular recording

Pulsatile arterial pressure (PAP) was recorded using an amplifier (model 7754A, Hewlett Packard, Palo Alto, CA, USA) coupled to a computerized acquisition system (MP100A, Biopac, Santa Barbara, CA, USA). MAP and HR values were derived from the PAP data using the Acknowledge III software (Biopac, USA). MAP was calculated according to the equation: diastolic pressure + (systolic−diastolic)/3. HR (beats/min, bpm) was calculated from PAP peak intervals integrated every 6 s.

2.4. Temperature measurement

Tail temperature was measured using a thermal camera Multi-Purpose Thermal Imager IRI 4010 (InfraRed Integrated Systems Ltd Park Circle, Tithe Barn Way Swan Valley Northampton, UK), placed at a constant 50 cm height above the animal's tail. Tail temperature was the mean of ten measurements recorded at different points throughout the length of the tail. Internal body temperature was recorded using dataloggers (SubCue dataloggers, Calgary, Alberta, Canada) implanted into the abdomen, as described in surgical procedures.

2.5. Drugs

(5α)-4,5-Epoxy-3,14-dihydro-17-(2-propenyl)morphinan-6-one hydrochloride (naloxone, nonselective opioid antagonist, TOCRIS, Westwoods Business Park Ellisville, Missouri, USA), D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH_2 (CTAP, selective μ-opioid antagonist, TOCRIS), 17,17′-(Dicyclopropylmethyl)-6,6′,7,7'-6,6′-imino-7,7′-binorphinan-3,4′,14,14′-tetrol dihydrochloride (nor-BNI, selective κ-opioid antagonist, TOCRIS) and 17-(Cyclopropylmethyl)-6,7-dehydro-4,5α-epoxy-3,14-dihydroxy-6,7-2′,3′-indolomorphinan hydrochloride (naltrindole, selective δ-opioid receptor antagonist, TOCRIS) were dissolved in artificial cerebrospinal fluid (aCSF), which had the following composition: 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.

Tribomoethanol (SIGMA, St. Louis, Missouri, USA) and urethane (SIGMA) were dissolved in saline (NaCl 0.9%). Flunixin meglumine (Banamine®, Schering Plough, Brazil) and a poly-antibiotic preparation of streptomycins and penicillins (Pentabiotico®, Fontoura-Wyeth, SP, Brazil) were used as provided.

2.6. Drug microinjection into the PL

Bilateral injections were performed in a volume of 100 nL using a 1 μL syringe (7001 KH; Hamilton, Reno, Nevada, USA) connected to a microinjection needle (gauge 33 − Small Parts, Miami Lakes, FL, USA) by a piece of PE-10 polyethylene tubing. The microinjection needle was 1 mm longer than the guide cannula (12 mm). Microinjections were performed within a 5-s period. After microinjection, the needle was left within the guide cannula for 1 min before being removed. Drugs were prepared before the experiments and stored at −20 °C. On the day of the experiment, drugs were thawed and kept at 0 °C during experiments.

2.7. Experimental procedure: acute restraint stress

After surgical procedures, animals were kept in individual cages in the Animal Care Unit. Rats were transported to the experimental room in their own home cages. 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 10 min was allowed before microinjections. The injection needle was slowly introduced into the guide cannula without touching or restraining the animals. Bilateral microinjections of opioid receptor antagonists or vehicle were made into the PL. Each animal received only one microinjection per brain side. Ten minutes later, animals were subjected to restraint, which was initiated by introducing animals into a small plastic cylindrical restraining tube (diameter = 6.5 cm and length = 15 cm). Restraint lasted 60 min, and thereafter animals were returned to their cages. Each animal was subjected to one session of restraint to prevent habituation.

Animals were divided into six experimental groups: (1) aCSF-stress group, in which the vehicle was microinjected into the PL and animals were subjected to restraint (evaluation of autonomic parameters $n = 15$ divided into two groups; body temperature evaluation $n = 5$) (2) naloxone-stress group, in which the nonselective opioid receptor antagonist naloxone was microinjected at different doses into the PL and followed by restraint (evaluation of autonomic parameters $n = 33$; body temperature evaluation $n = 17$); (3) CTAP-stress group, in which the selective μ-opioid antagonist CTAP was microinjected into the PL and animals were subjected to restraint ($n = 7$); (4) nor-BNI-stress group, in which the selective κ-opioid antagonist nor-BNI was microinjected into the PL followed by restraint ($n = 6$); (5) naltrindole-stress group, in which the selective δ-opioid antagonist naltrindole was microinjected into the PL and animals were subjected to restraint ($n = 6$); (6) CTAP + nor-BNI-stress group, in which the selective μ and κ-opioid antagonists were microinjected into the PL and followed by restraint ($n = 6$).

2.8. Histological determination of the microinjection sites

At the end of experiments, animals were anesthetized with urethane (1.25 g/Kg i.p.) and 100 nL of 1% Evan's blue dye was injected into the brain as a marker of the injection site. They were subjected to intracardiac perfusion with 0.9% NaCl followed by 10% formalin. Brains were removed and post-fixed for 48 h at 4 °C, and 40 μm-thick serial sections were cut with a cryostat (CM1900, Leica, Wetzlar, Germany). Sections were stained with 1% cresyl violet, for light microscopy analysis. The actual placement of the injection needles was determined by analyzing serial sections, and identified according to the rat brain atlas of Paxinos and Watson (2007).

2.9. Data analysis

Baseline autonomic data, recorded either 10 min before or after pharmacological treatment of the PL, were compared using the Student's *t*-test. Data were expressed as mean ± SEM of MAP, HR, tail and body temperature changes (ΔMAP, ΔHR, Δtail temperature and Δbody temperature, respectively). Points sampled during the 10 min before the test were used as control baseline values. Two-way analysis of variance (two way-ANOVA) for repeated measures was used to compare time curves after aCSF and drugs treatment. Curves for statistical analysis and illustrative figures were generated with points obtained with different data sampling. For statistical purposes, curves were generated from 6 points. Illustrative curves were generated with 36 points for MAP and HR and with 10 points for tail temperature. When an interaction between factors was observed, groups were compared at specific times using Duncan's post-hoc test. Values of $P < 0.05$ indicate statistically significant differences between groups.