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CUTANEOUS VASODILATION ELICITED BY DISINHIBITION OF THE CAUDAL PORTION OF THE ROSTRAL VENTROMEDIAL MEDULLA OF THE FREE-BEHAVING RAT

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Abstract—Putative sympathetic premotor neurons controlling cutaneous vasomotion are contained within the rostral ventromedial medulla (RVMM) between levels corresponding, rostrally, to the rostral portion of the nucleus of the facial nerve (RVMM(fn)) and, caudally, to the rostral pole of the inferior olive (RVMM(io)). Cutaneous vasoconstrictor premotor neurons in the RVMM(fn) play a major role in mediating thermoregulatory changes in cutaneous vasomotion that regulate heat loss. To determine the role of neurons in the RVMM(io) in regulating cutaneous blood flow, we examined the changes in the tail and paw skin temperature of free-behaving rats following chemically-evoked changes in the activity of neurons in the RVMM(io). Microinjection of the $GABA_A$ agonist, muscimol, within either the RVMM(fn) or the RVMM(io) induced a massive peripheral vasodilation; microinjection of the $GABA_A$ antagonist bicuculline methiodide within the RVMM(fn) reversed the increase in cutaneous blood flow induced by warm exposure and, unexpectedly, disinhibition of RVMM(io) neurons produced a rapid cutaneous vasodilation. We conclude that the tonically-active neurons driving cutaneous vasoconstriction, likely sympathetic premotor neurons previously described in the RVMM(fn), are also located in the RVMM(io). However, in the RVMM(io), these are accompanied by a population of neurons that receives a tonically-active GABAergic inhibition in the conscious animal and that promotes a cutaneous vasodilation upon relief of this inhibition. Whether the vasodilator neurons located in the RVMM(io) play a role in thermoregulation remains to be determined.

Key words: infrared thermography, thermoregulation, sympathetic nervous system, cutaneous vasomotion, muscimol, bicuculline methiodide.

The rostral ventromedial medulla (RVMM) contains populations of sympathetic premotor neurons controlling several autonomic functions, including cutaneous vasomotion (Smith et al., 1998; Nagashima et al., 2000; Blessing and Nalivaiko, 2001; Nakamura et al., 2004), brown adipose tissue (BAT) thermogenesis (Morrison, 2001; Cano et al., 2003; Nakamura et al., 2005) and cardiac rate (Cao and Morrison, 2003). Antagonism of $GABA_A$ receptors in the RVMM produces an increase in body temperature in the anaesthetized animal through both a peripheral vasoconstriction (Blessing and Nalivaiko, 2001) and an increase in BAT thermogenesis (Morrison et al., 1999). Conversely, the injection into the same area of a $GABA_A$ agonist leads to vasodilation of the tail skin in the anaesthetized rat (Blessing and Nalivaiko, 2001), and to hypothermia (Zaretsky et al., 2003) and tail vasodilation (Vianna et al., 2008) in conscious rats.

Studies employing injections of the transneuronal retrograde tracer, pseudorabies virus within the rat tail artery wall, have localized putative sympathetic premotor neurons controlling cutaneous blood vessels throughout the RVMM, between the levels corresponding to the rostral portion of nucleus of the facial nerve and the rostral portion of the inferior olive (Smith et al., 1998; Nakamura et al., 2004; Toth et al., 2006). Interestingly, a relative difference in neuronal phenotype has been suggested between the rostral portion of the RVMM, containing more putative glutamatergic neurons expressing the vesicular glutamate transporter, VGLUT3, and the caudal portion of the RVMM, where more serotoninergic neurons have been identified (Nakamura et al., 2004; Stornetta et al., 2005; Toth et al., 2006). From both anatomical and physiological evidence, it has been proposed that the neural substrate in the RVMM for the control of cutaneous vasomotion is represented by a set of VGLUT3-positive, glutamatergic neurons directly projecting to the intermediolateral column (IML) of the spinal cord (Nakamura et al., 2004). These data have led to a theoretical paradigm in which sympathetic outflow to cutaneous blood vessels is proportional to the level of activity of the RVMM glutamatergic sympathetic premotor neurons (Nakamura et al., 2004).

The evaluation of the physiological role of RVMM neurons controlling cutaneous blood flow has been until now limited to the rostral portion of the RVMM, within the rostro-caudal level of the nucleus of the facial nerve (RVMM(fn)) (Tanaka et al., 2002; Ootsuka and Blessing, 2006), where the more VGLUT3 positive neurons are located. No data are available on the role of the more caudal portion of RVMM neurons, within the rostro-caudal section of the rostral pole of the inferior olive (RVMM(io)), in controlling cutaneous vasomotion.

The aim of the present study is a more extensive characterization of the RVMM physiological role in controlling cutaneous vasomotion, with special focus on the cau-

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Abbreviations: AP, arterial pressure; BAT, brown adipose tissue; EKG, electrocardiogram; HR, heart rate; IML, intermediolateral nucleus of the spinal cord; RVMM, rostral ventromedial medulla; RVMM(fn), rostral ventromedial medulla at the level of the facial nucleus; RVMM(io), rostral ventromedial medulla at the level of the rostral inferior olivary nucleus; Ta, ambient temperature; Thy, hypothalamic temperature; Tpaw, paw temperature; Ttail, tail temperature.

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dal RVMM. To avoid the interference that general anaesthetics produce on thermoregulatory function (Wixson et al., 1987; Refinetti and Carlisle, 1988), experiments have been carried out in free behaving rats. Moreover, thermoregulatory cutaneous vasomotion has been traditionally measured in the tail vascular district, but, since it has been suggested that paws also have a possible functional role as heat exchanger organs (Caputa and Demicka, 1995), both paw and tail vasomotion have been studied, by means of infrared thermography.

EXPERIMENTAL PROCEDURES

Ethical approval

The experiments were carried out with the approval of the Comitato Etico-Scientifico dell'Università di Bologna (Ethical-Scientific Committee of the University of Bologna), in accordance with the European Union Directive (86/609/EEC) and under the supervision of the Central Veterinary Service of the University of Bologna and the National Health Authority. All efforts were made to minimize the number of animals used and their pain and distress.

Surgical procedures

Male CD Sprague–Dawley rats (n=33, Charles River Inc, Lecco, Italy) were deeply anaesthetized (diazepam (Valium; F. Hoffmann-La Roche ltd, Basel, Switzerland), 5mg/kg, intramuscular, followed by ketamine-HCl (Ketalar; Parke-Davis, Detroit, MI, USA), 100 mg/kg, intraperitoneal), placed in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA) with the incisor bar set at −3.8 mm. Since deep brain temperature is critical in modulating sympathetic outflow to cutaneous blood vessels (Tanaka et al., 2002), hypothalamic temperature (Thy) was monitored with a thermistor (B10KA303N, Thermometrics Corporation, Northridge, CA, USA) mounted inside a stainless-steel needle (21 gauge) and stereotaxically implanted above the left anterior hypothalamus. The electrocardiogram (EKG) was recorded between two isolated stainless–steel electrodes (AS 632, Cooner Wire Inc., Chatsworth, CA, USA): one on the ventral surface of the xiphoid process and the other in the upper mediastinic cavity, as described in Sgoifo et al., 1996. Arterial pressure (AP) was telemetrically measured with a femoral artery catheter (PA-C40, Data-Sciences International, St. Paul, MN, USA). For technical reasons, the AP signal was not recordable in the A2 experiment.

Microinjection procedure

A microinjection guide cannula (C315G-SPC, Plastics One Inc, Roanoke, VA, USA; internal cannula extension below guide: +3.5 mm) was stereotaxically positioned to target either the RVMM(fn) (−2.5 to −3.0 mm posterior to lambda, 0.0 mm lateral to the midline, −9.0 to −9.5 mm ventral to the dorsal surface of the cerebellum) or the RVMM(io) (−3.3 to −3.8 mm posterior to lambda, 0.0 mm lateral to the midline, −9.0 to −9.5 mm ventral to the surface of the cerebellum). Under anaesthesia, optimal cannula positioning was assessed with a functional test in which infrared thermography was used to assess changes in tail temperature evoked by injection of the $GABA_A$ agonist, muscimol (100 pmol in 100 nl). The cannula was considered to be appropriately positioned when a rapid increase in tail temperature was observed within 5 min of the muscimol injection (Blessing and Nalivaiko, 2001) and the cannula was then secured to the skull with four stainless steel screws and acrylic dental resin. Rats in which such increases in tail temperature could not be elicited, even after minor movements of the guide cannula position, were not admitted into this experimental plan (n=7). The ineffective injection sites were marked and the brains fixed and sliced as described in the histology section. The location of the ineffective injection sites is shown in Fig. 1.

After surgery, animals received 20 ml/kg of saline subcutaneously and 0.25 ml of an antibiotic solution (penicillin G, 37500 IU; streptomycin-sulfate, 8750 IU) i.m.. Rats recovered for at least 1 week, initially in their home cage and subsequently, for at least 3 days, in a Plexiglas cage with a wire mesh floor positioned within a thermoregulated, sound-attenuated chamber where the animal remained throughout the experiment. A 12:12 h light (100 lux at cage level)—dark cycle was maintained within the experimental chamber with lights on at 9:00, animals had free access to food and water and, during the recovery period, the ambient temperature (Ta) was maintained at 24 °C±0.5 °C. Tail temperature (Ttail) and paw temperature (Tpaw) were measured by means of an infrared thermocamera (Thermovision A20, FLIR Systems, Boston, MA, USA) placed below the stainless steel grid floor (wire diameter=2 mm, inter-wire distance=10 mm) of animals cage. Such infrared thermography provides an accurate measure of cutaneous vasomotion (Vianna and Carrive, 2005).

All microinjections were performed with the following apparatus located outside the sound-attenuated recording chamber so that animals remained undisturbed by the injection procedure. The injection system consisted of a Hamilton 5 μl gastight syringe (Hamilton Company, Bonaduz, Switzerland) positioned in an infusion pump (MA 01746, Harvard Apparatus, Holliston, MA, USA; infusion rate 0.3 μl/min) and connected to the internal cannula through one meter of microdialysis FEP tubing (ID 0.12 mm OD 0.65 mm, Microbiotech/se AB, Stockholm, Sweden). The cannula and the tube were filled with either the drug dissolved in vehicle solution (saline, NaCl 0.9% w/v) or vehicle solution only while the syringe was filled with colored mineral oil. During each injection (average duration: 30±5 s), the volume of injectate was microscopically-assessed by the movement of the oil-injectate interface over a ruler. The injection cannula was not removed for at least 30 min after the injection. Drugs for microinjection were obtained from Tocris Bioscience, Bristol, UK.

One day prior to each RVMM microinjection, the internal temperature of the experimental chamber was adjusted to provide the desired Ta. At light onset, rats were connected to the recording system and, after a 2-h baseline recording period, the internal cannula was inserted into the guide cannula and a microinjection was performed 30 min later. One day was allowed between multiple injections in the same animal. The order of the injections was shifted across experimental groups, in order to balance the sequence of drug administrations among animals.

Experimental protocols

Experiment A compared the influence of neurons in the RVMM(io) and in the RVMM(fn) on tail and paw vasomotor control in conscious rats.

Experiment A1. With a cannula in the RVMM(fn), four rats were injected with the following: (a) the $GABA_A$ antagonist, bicuculline methiodide (100 pmol in 100 nl) at Ta=32 °C; (b) muscimol (100 pmol in 100 nl) at Ta=24 °C; (c) saline (100 nl) at Ta=24 °C.

Experiment A2. With a cannula in the RVMM(io) and Ta=24 °C, six rats were injected with the following: (a) bicuculline methiodide (100 pmol in 100 nl); (b) muscimol (100 pmol in 100 nl; (c) saline (100 nl).

Experiments B, C and D were based on the results of experiment A.

Experiment B investigated the influence of RVMM(io) neuronal activity on the tail and paw cutaneous vasodilation evoked by a warm Ta in conscious rats.

Experiment B1. With a cannula in the RVMM(io), four rats were injected with the following: (a) the $GABA_A$ antagonist, GABAzine (50 pmol in100 nl) at Ta=24 °C; (b) GABAzine (50 pmol in100 nl) at Ta=32 °C.