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OREXIN MICROINJECTION IN THE MEDULLARY RAPHE INCREASES HEART RATE AND ARTERIAL PRESSURE BUT DOES NOT REDUCE TAIL SKIN BLOOD FLOW IN THE AWAKE RAT

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Abstract—The rostral medullary raphe region is an important target of hypothalamic orexin neurons; however, little is known of the effect of orexin in this key autonomic and somatic premotor region. Here we tested the effect of orexin-A (3 and 30 pmol) microinjected in the medullary raphe, on heart rate (HR), mean arterial pressure (MAP), tail skin blood flow, body temperature, and behavior in freely moving, awake rats. HR, MAP, and body activity were recorded by radio-telemetry. Changes in tail skin blood flow and body temperature, as well as potential interscapular brown adipose tissue thermogenesis were recorded indirectly by infrared thermography of the skin of the tail, lumbosacral back, and interscapular back areas, respectively. Compared with saline, orexin-A (30 pmol) evoked significant and long lasting increases in HR (+99 bpm), MAP (+11 mmHg), and body activity (grooming, not locomotor activity). However, it did not reduce tail skin blood flow more than saline, and there was no significant increase in body temperature. A small, though significant, thermogenic effect was observed in the interscapular region, but this effect is more likely to have originated from activity in neck and shoulder muscles than brown adipose tissue. Thus, orexin projections to the rostral medullary raphe can mediate significant cardiovascular changes, but does not seem to affect tail skin vasomotor tone or brown adipose tissue in the awake rat. This important brainstem relay may contribute to the cardiovascular changes evoked by arousal and various forms of stress that are associated with activation of orexin neurons.

Key words: cardiovascular, thermoregulation, brown adipose tissue, psychological stress, arousal, infrared thermography.

The neuropeptide orexin is involved in the control of arousal and has been implicated in the expression of many different states and behaviors, such as wakefulness, emotional stress, feeding, and addiction (de Lecea, 2010; Sakurai et al., 2010). When injected in the cerebral ventricles of awake rats and mice, orexin evokes a marked increase in locomotor activity associated with increases in heart rate and blood pressure (Shirasaka et al., 1999; Matsuzaki et al., 2002; Samson et al., 2007; Samson et al., 2010). This cardiovascular effect is also observed under anesthesia (Chen et al., 2000; Hirota et al., 2003; Huang et al., 2010). Such effects could result from a concerted action of orexin at multiple levels of the neuraxis, as orexin projections are widespread in the CNS (Peyron et al., 1998). However, some targeted structures may play a more important role than others. Of particular interest are orexin projections to the rostral region of the ventral medulla (median, medial, and lateral parts) (Ciriello et al., 2003; Berthoud et al., 2005; Zheng et al., 2005; Puskás et al., 2010) because this region contains important somatic and autonomic premotor centers (Jansen et al., 1995; Kerman, 2008). Indeed, injections of orexin in the vasopressor region of the lateral part of the rostral ventral medulla (RVLM) depolarize vasopressor neurons (Huang et al., 2010) and evoke rises in blood pressure and HR in both awake and anesthetized rats (Chen et al., 2000; Machado et al., 2002). Less is known about the effect of orexin in the medial and median parts of the rostral ventral medulla, which include the medullary raphe magnus (RMg) and pallidus (RPa). Recent work shows that the rostral medullary raphe play an important role in the control of HR, skin blood flow, and brown adipose tissue (BAT) as well as blood pressure, muscle tone, and muscle activity (Blessing, 2003; Cao and Morrison, 2003; Zaretsky et al., 2003a; Zaretsky et al., 2003b; Morrison, 2004; Nason and Mason, 2004; Nakamura et al., 2005; Cao and Morrison, 2006; Morgan et al., 2008; Nakamura and Morrison, 2011). Thus, orexin projections to the rostral medullary raphe could potentially affect all these variables at once. If correct, this region would make an important contribution to the overall effect of orexin.

The aim of this study was to test the behavioral and autonomic effects of orexin-A microinjected in the rostral medullary raphe of awake, freely moving rats, to determine the possible contribution of this region to the effect of orexin in arousal or hyperarousal. We used radio-telemetry to record changes in heart rate, blood pressure, and body activity, and we used infrared thermography to assess changes in tail skin blood flow, body temperature, and, potentially, interscapular BAT (IBAT) thermogenesis.

EXPERIMENTAL PROCEDURES

Subjects

The subjects were 18 naive male Wistar rats (350–550 g) purchased from Monash Animal Services (Melbourne, Australia). The animals were housed in individual home boxes (65×40×22 cm)

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Abbreviations: Activity, body activity; BAT, brown adipose tissue; GiA, gigantocellular nucleus pars alpha; HR, heart rate; IBAT, interscapular brown adipose tissue; MAP, mean arterial pressure; RMg, raphe magnus; RPa, raphe pallidus; RVLM, rostral ventrolateral medulla; TBack, surface temperature of lumbosacral back area; TIscap, surface temperature of interscapular back area; TTail, surface temperature of the tail.

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with *ad libitum* food and water. The room in which they were housed and tested was maintained at a constant temperature of 22–25 °C and kept on a normal 12:12 h light/dark cycle. All procedures were approved by the Animals Ethics Committee of the University of New South Wales and conformed to the rules and guidelines on animal experimentation in Australia.

Radio-telemetric probe implantation

Rats were first implanted with radio-telemetric probes (PA-C40, Data Sciences International, St. Paul, MN, USA) for recording of arterial pressure, HR, and Activity. The surgery was done in aseptic conditions and under anesthesia with a mixture of ketamine (Ketamil, 120 mg/kg, i.p.) and xylazine (Ilium Xylazil-20, 6.5 mh/kg, i.p.). The rats were also pretreated with the analgesic carprofen (Rimadyl, 5 mg/kg, s.c.) and received antibiotics (Benicillin, 0.3 ml, i.p.) at the end of the surgery. The probes were implanted in the peritoneal cavity, with the catheter sitting in the descending aorta at the level of the iliac bifurcation, as previously described (Carrive, 2000). During the recovery period (1 week), the animals were handled every day to habituate to the experimenter.

Guide cannula implantation

The guide cannulae were implanted 1 week after the radio-telemetric probes. The surgery was done under the same anesthetic, analgesic, and aseptic regimen as the radio-telemetric probe implantations. Once anesthetized, the animal's head was secured in a stereotaxic frame in the flat skull position. The scalp was cut and the skull exposed. Four holes were drilled: three for the screws and one for a single guide cannula (26 G, Plastics One, Roanoke, VA, USA), which was implanted 1 mm above the target region, aimed at the rostral medullary raphe region (RMg/RPa). The coordinates were AP=−2.4, DV=−9.5 along the midline from Lambda according to the stereotaxic atlas of Paxinos and Watson (2005). The guide cannula was finally anchored to the screws with dental cement. Animals were allowed to recover for at least 1 week before testing began.

Drug and testing

Each site of injection was tested with two doses of orexin-A (3 pmol and 30 pmol, Tocris BioScience) and physiological saline (vehicle solution). The three injections were made in a counterbalanced order and on separate days. The procedure was as follows: after 30 min of baseline recording, the animal was gently removed from its home box and restrained with a soft cloth wrapped around its body. An injection cannula (33 G, Plastics One), connected to a 5 μl Hamilton syringe, was inserted into the guide cannula and the injection was made. The volume was 0.4 μl and was injected over 30 s. The cannula was left in place for a further 30 s. The injection cannula was then removed and the animal returned to its home box where recording continued for a further 90 min. To minimize the number of rats and optimize the use of the telemetric probes, we used up to three sets of injection cannulae of different lengths (+0.5 mm, +1.0 mm, and +1.5 mm beyond the tip of guide cannula). This allowed testing of up to three sites per animal (on average 1.7).

Infrared thermography

The surface temperature of the tail was recorded with an infrared digital thermographic camera (ThermaCAM P45, FLIR, Sweden) placed 1 m above the animal as previously described (Vianna et al., 2008). The home box lids were removed and replaced with 60 cm tall Plexiglas walls opened at the top to allow an unobstructed view of the animal for the camera. In about half of the animals, the skin of the lumbosacral back and interscapular back area were also imaged. These regions were shaved on the eve of the experiment under brief anesthesia with isoflurane.

Data collection and analysis

Up to six parameters were recorded simultaneously: heart rate (HR), mean arterial pressure (MAP), body activity (Activity), and surface temperatures of the tail (middle portion, TTail), lumbosacral back (TBack), and interscapular back (TIscap) areas. HR, MAP, and Activity were extracted automatically from the pulsatile blood pressure signal of the telemetric probes using the ART gold software (Data Sciences International). HR and MAP were sampled every 20 s from 3 s time windows. Activity was a cumulated measure of body movements over the same 20 s period. These values were then averaged every minute. Infrared thermographic images were captured automatically every minute, starting 30 min before and ending 90 min after administration of the drugs. The thermal sensitivity of the camera is approximately 0.1 °C with a spatial resolution of 320×240 pixels. The emissivity factor was set at 0.98, which corresponds to the emissivity of the skin. The images were analyzed with the ThermaCAM reporter 7.0 Professional SR-4 software (FLIR). The temperature value of the hottest pixel from each area of interest was extracted and recorded. Occasionally, depending on the posture of the animal, an area would be momentarily concealed and therefore not imaged. Thermogenesis in the interscapular area (potentially of IBAT origin) was estimated by computing the difference between the two surface temperatures TIscap and TBack, as previously reported (Marks et al., 2009).

Statistical analysis

The data were analyzed with Prism 5 (GraphPad Software, Inc.) using a one-way repeated measure of analysis of variance (ANOVA). The independent factor was drug or saline, and the repeated factor was time. Statistical significance was set at $P<0.05$. Unless specified, all comparisons were made between the 1st and 90th min after animals were returned to their home box.

Verification of cannula placement

At the end of the experiment, the animals were given an overdose of pentobarbitone (120 mg/kg i.p.), intracranially microinjected with a dye (Pontamine Sky Blue, 0.4 μl), and transcardially perfused with saline. The brains were removed, post-fixed in 10% formalin solution, and the brainstems sectioned at 50 μm. The centers of the sites of injection were identified and plotted on standard plates from the atlas of Paxinos and Watson (2005).

RESULTS

A total of 31 sites were tested. After histology, 24 sites of injection were found to be located in the target region of RMg and RPa or immediately adjacent to it in the gigantocellular nucleus pars alpha (GiA) (Fig. 1). These sites were selected for analysis. HR, MAP, Activity, and TTail were recorded at each of these 24 sites. TIscap and TBack were also recorded at 17 of them. One site was excluded from the analysis of MAP due to faulty recording of this parameter. Another site was not included in the analysis of TTail due to concealment of the tail during infrared imaging.

The average changes evoked by the two doses of orexin and the vehicle control are shown on Fig. 2. Before injection, the animals were usually at rest in their home box. As can be seen, there was no difference in the base-