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Activation of α_2-adrenoceptors in the lateral hypothalamus reduces pilocarpine-induced salivation in rats

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ABSTRACT

Anti-hypertensive drugs that act on central α_2-adrenoceptors and imidazoline receptors usually cause dry mouth in patients. A central area important for the control of salivary secretion and also for the effects of α_2-adrenoceptor activation is the lateral hypothalamus (LH). Therefore, in the present study we investigated the effects of the injections of moxonidine (an α_2-adrenoceptor and imidazoline agonist) alone or combined with RX 821002 (α_2-adrenoceptor antagonist) into the LH on the salivation induced by intraperitoneal (i.p.) pilocarpine (cholinergic muscarinic agonist). Male Holtzman rats with stainless steel cannula implanted into the LH were used. Saliva was collected using pre-weighted small cotton balls inserted into the animal's mouth under ketamine anesthesia. Salivation induced by i.p. pilocarpine (4 μmol/kg of body weight) was reduced by the injection of moxonidine (10 and 20 nmol/0.5 μl) into the LH (222 ± 46 and 183 ± 19 mg/7 min, vs. vehicle: 480 ± 30 mg/7 min). The inhibitory effect of moxonidine on pilocarpine-induced salivation was abolished by prior injections of RX 821002 (160 and 320 nmol/0.5 μl) into the LH (357 ± 25 and 446 ± 38 mg/7 min). Injections of the α_1-adrenoceptor antagonist prazosin (320 nmol/0.5 μl) into the LH did not change the effects of moxonidine. The results show that activation of α_2-adrenoceptors in the LH inhibits pilocarpine-induced salivation, suggesting that LH is one of the possible central sites involved in the anti-salivatory effects produced by the treatment with α_2-adrenoceptor agonists.

Cholinergic muscarinic agonists and particularly pilocarpine have been used as important therapeutic agents in cases of xerostomia [33]. Although pilocarpine can induce salivary secretion by acting on muscarinic receptors in the salivary glands, recent results have shown that it may also activates central mechanisms to facilitate salivation [2,7,18,20,21,22,28,29]. Evidence for the existence of central mechanisms activated by pilocarpine is the results showing that intracerebroventricular (i.c.v.) injection of pilocarpine induces salivation in rats and that central lesions, like lesions surrounding the anteroventral third ventricle (AV3V region), in the lateral hypothalamus (LH) or medial preoptic area (MPOA) reduce salivation produced by pilocarpine injected peripherally [18,22,23].

Contrary to pilocarpine, anti-hypertensive drugs that act on α_2-adrenoceptors and imidazoline receptors like clonidine and moxonidine reduce salivary secretion causing dry mouth as a side effect [4,32]. Moxonidine is suggested to reduce sympathetic activity and arterial pressure by acting predominantly on central imidazoline receptors [4]. Because the main action of moxonidine is on imidazoline receptors, it might have fewer side effects related to the activation of α_2-adrenoceptors like dry mouth [32]. However, recent results have shown that moxonidine, similar to noradrenaline injected i.c.v., reduces salivation induced by pilocarpine [20,21].

The α_2-adrenoceptors are distributed throughout the central nervous system and high levels of specific α_2-adrenergic binding sites were found in several hypothalamic and limbic regions, including the paraventricular and arcuate nuclei of the hypothalamus, the central, medial and basal nuclei of the amygdala, lateral septum and the lateral hypothalamus (LH) [8,24,31]. The LH projects to pre-ganglionic parasympathetic nuclei that innervates the salivary glands [14,16] and lesions of the LH produce an immediate hypersalivation that persists for 3 h and then changes to an impairment in basal salivation and reduction of pilocarpine-induced salivation [23,26]. Moreover, a functional inhibitory role of α_2-adrenoceptors in the LH has been reported for central cholinergic induced-water intake [6].

Therefore, in the present study we sought to investigate the effects of moxonidine alone or combined with α_1- or α_2-

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adrenoceptor antagonists, prazosin and RX 821002 respectively, injected into the LH on intraperitoneal (i.p.) pilocarpine-induced salivation.

Experiments were performed on adult male Holtzman rats weighing 300–320 g. The animals were housed individually in stainless steel cages in a room with controlled temperature (23 ± 2 °C) and humidity (55 ± 10%). Lights were on from 7:00 am to 7:00 pm. Standard Guabi chow (Paulinia, SP, Brazil) and tap water were available *ad libitum*. All experimental protocols were approved by Animal Experimentation Ethics Committee of the School of Dentistry of Araraquara/UNESP.

Rats were anesthetized with ketamine (80 mg/kg of body weight) and xylasine (7 mg/kg) and placed in a Kopf stereotaxic instrument. The skull was leveled between bregma and lambda. A stainless steel cannula (10 mm × 0.6 mm o.d.) was implanted into the lateral hypothalamus (LH) using the coordinates 1.9 mm caudal to bregma, 1.2 mm lateral to midline and 5.6 mm below the dura mater. The cannulas were fixed to the cranium using dental acrylic resin and jeweler screws. The analgesic cetoprophen 1% (0.03 ml/rat) and a prophylactic dose of penicillin (30,000 IU) were injected intramuscularly immediately after the surgery.

Moxonidine hydrochloride (Solvay Pharma, Germany, 5, 10 and 20 nmol/0.5 μl), RX 821002 hydrochloride (RBI, Research Biomedical International, USA, 80, 160 and 320 nmol/0.5 μl) and prazosin (Sigma Chemical Co., USA, 320 nmol/0.5 μl) were injected into the LH. Pilocarpine hydrochloride (Sigma Chemical Co., USA, 4 μmol/kg of body weight) was injected i.p. RX 82112 was dissolved in isotonic saline. Moxonidine and prazosin were dissolved in a mix of propylene glycol–water 2:1. The doses of the drugs used were based on previous studies in which the central effects of the same drugs on salivation and water intake were tested [27,28,29].

Injections into the LH were made using 10 μl Hamilton syringes connected by polyethylene tubing (PE-10) to the injection needle 2 mm longer than the guide cannulas implanted into the brain. The injection volume into the LH was 0.5 μl. The LH injections were placed into the medial and posterior lateral hypothalamic area, ventrolateral to the fornix, reaching at least part of the medial forebrain bundle (Fig. 1). To confirm the specificity of the LH as the site of moxonidine injections that produce effects on pilocarpine-induced salivation, results from rats in which the injections did not reach the LH (misplaced injections) were also analyzed.

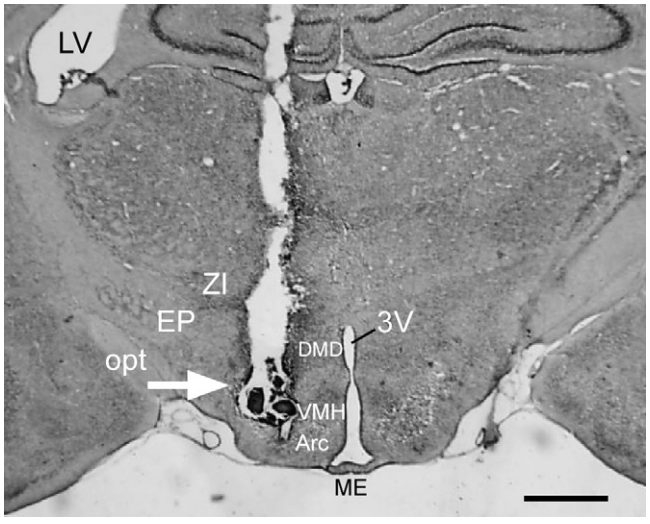

Fig. 1. Photomicrograph of a typical site of injection into lateral hypothalamus (LH) (arrow). Scale = 1 mm. Arc, arcuate hypothalamic nucleus; DMD, dorsomedial hypothalamic nucleus; EP, entopeduncular nucleus; LV, lateral ventricle; ME, median eminence; opt, optic tract; VMH, ventromedial hypothalamic nucleus; ZI, zona incerta; 3V, third ventricle.

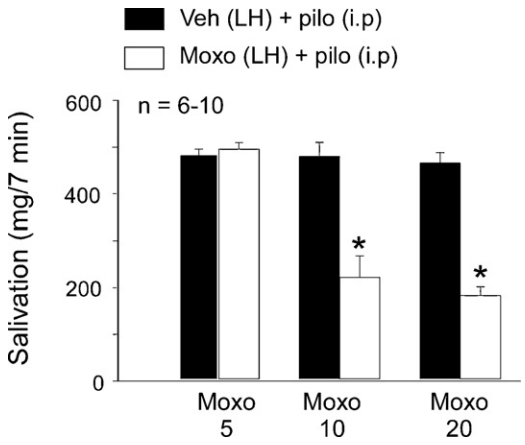

Fig. 2. Salivary secretion (mg/7 min) induced by i.p. pilocarpine (pilo) (4 μmol/kg of body weight) in rats pre-treated with vehicle (Veh) or moxonidine (Moxo, 5, 10 and 20 nmol/0.5 μl) into LH. The results are represented as mean ± S.E.M. *n* = number of rats. (*) Different from vehicle + pilocarpine (Newman–Keuls test, $p < 0.01$).

For the test, rats were anesthetized with ketamine (100 mg/kg of body weight), received injections of vehicle or moxonidine into the LH and 15 min later pilocarpine was injected i.p. Ten minutes after the injection of pilocarpine, the oral cavity was wiped with cotton and four pre-weighed cotton balls were inserted into the oral cavity of each animal: two underneath the tongue and two bilaterally medial to the teeth and oral mucosa [18,20,21,28,29]. The cotton balls were removed 7 min later and weighted again in a balance (Mettler-Toledo, 0.0001 g division). The mass of saliva secreted was calculated by subtracting the initial from the final weight of the four cotton balls. To test the effects of the antagonists, they were injected into the LH 15 min before moxonidine.

To test each dose of moxonidine one group of rats was used. Each group of rats was tested twice with a three-day interval between tests. In each test, one half of the group was treated with vehicle + pilocarpine and the other half was treated with moxonidine + pilocarpine in a counterbalanced design.

One group of rats was also used to test each dose of the adrenoceptor antagonists combined with pilocarpine. Each group of rats was also tested twice with a three-day interval between tests. In each test, one half of the group was treated with vehicle + moxonidine + pilocarpine and the other half was treated with the adrenoceptor antagonist + moxonidine + pilocarpine in a counterbalanced design. At the same time that the rats were tested with the combination of antagonists + moxonidine + pilocarpine, one group of rats was injected with only pilocarpine for control.

At the end of the experiments the animals were deeply anesthetized with sodium thiopental (70 mg/kg of body weight, i.p.) and perfused through the heart with saline followed by 10% formalin. The brains were removed, frozen, cut coronally into 50 μm sections and stained with Giemsa stain. Only animals with injections into the LH were considered for statistical analysis (Fig. 1).

Statistical analysis was done with Sigma Stat version 3.0 (Jandel Corporation, Point Richmond, CA). Data are reported as means ± standard error of means (S.E.M.). One-way parametric analysis of variance followed by Newman–Keuls test was used for comparisons. Significance was set at $p < 0.05$.

Moxonidine (10 and 20 nmol/0.5 μl) injected into LH reduced i.p. pilocarpine-induced salivation (222 ± 46 and 183 ± 19 mg/7 min, vs. vehicle: 480 ± 30 mg/7 min) [$F(5,35) = 118.5$, $p < 0.01$] (Fig. 2). Moxonidine (5 nmol/0.5 μl) injected into LH produced no effect on the salivation induced by i.p. pilocarpine (486 ± 37 mg/7 min