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Activation of central α_2-adrenoceptors mediates salivary gland vasoconstriction

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ABSTRACT

Objective: Peripheral treatment with the cholinergic agonist pilocarpine increases salivary gland blood flow and induces intense salivation that is reduced by the central injection of moxonidine (α_2-adrenoceptors/imidazoline agonist). In the present study, we investigated the effects of the intracerebroventricular (i.c.v.) injection of pilocarpine alone or combined with moxonidine also injected i.c.v. On submandibular/sublingual gland (SSG) vascular resistance. In addition, the effects of these treatments on arterial pressure, heart rate and on mesenteric and hindlimb vascular resistance were also tested.

Design: Male Holtzman rats with stainless steel cannula implanted into lateral ventricle and anaesthetized with urethane + α-chloralose were used.

Results: Pilocarpine (500 nmol/1 μl) injected i.c.v. Reduced SSG vascular resistance and increased arterial pressure, heart rate and mesenteric vascular resistance. Contrary to pilocarpine alone, the combination of moxonidine (20 nmol/1 μl) and pilocarpine injected i.c.v. Increased SSG vascular resistance, an effect abolished by the pre-treatment with the α_2-adrenoceptor antagonist yohimbine (320 nmol/2 μl). The increase in arterial pressure, heart rate and mesenteric resistance was not modified by the combination of moxonidine and pilocarpine i.c.v.

Conclusion: These results suggest that the activation of central α_2-adrenoceptors may oppose to the effects of central cholinergic receptor activation in the SSG vascular resistance.

1. Introduction

Pilocarpine is a muscarinic cholinergic agonist used to reduce dryness of the oral mucosa in patients affected by salivary gland diseases.[1,2] It is well accepted that pilocarpine stimulates salivary secretion by acting on cholinergic receptors in the salivary gland.[1,2] This idea is supported by the sialogogue effect of pilocarpine in isolated salivary glands.[3]

However, recent evidence suggests that peripheral administered pilocarpine can also activate muscarinic receptors in the brain to stimulate salivation.[4,5,6] The suggestion that pilocarpine may act centrally to stimulate salivary secretion is also reinforced by studies that have shown that salivation induced by pilocarpine injected peripherally is reduced by focal lesions in the forebrain.[7–9] Pilocarpine injected peripherally also induces submandibular/sublingual gland (SSG) vasodilation,[10] an effect due to the direct action of pilocarpine

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in the salivary glands and perhaps also to the activation of central mechanisms.[6]

Moxonidine (α_2-adrenoceptor/imidazoline receptor agonist) is an anti-hypertensive drug that acts centrally to reduce sympathetic nerve discharge.[11–14] Moxonidine injected i.c.v. reduces peripheral pilocarpine-induced salivation and vasodilation in the SSG.[4,10,15] The reduction of pilocarpine-induced salivation produced by moxonidine injected i.c.v depends on the activation of central α_2-adrenoceptors,[4,15] however, the receptor subtypes involved in the moxonidine inhibition of pilocarpine-induced SSG vasodilation have not been characterized.

Therefore, in the present study we investigated the effects of i.c.v. injection of pilocarpine alone or combined with i.c.v. moxonidine on SSG, mesenteric and hindlimb blood flow and vascular resistance, mean arterial pressure (MAP) and heart rate (HR). Additionally, we also investigated the effects of yohimbine (α_2-adrenoceptor antagonist) injected i.c.v. combined with moxonidine and pilocarpine i.c.v. on MAP, HR and SSG, mesenteric and hindlimb blood flow and vascular resistance.

2. Methods

2.1. Animals

Male Holtzman rats weighing 300–350 g were used. The animals were housed individually in stainless steel cages in a room with controlled temperature (23 ± 2 °C) and humidity ($55 \pm 10\%$). Lights were on from 7:00 am to 7:00 pm. Guabi rat chow (Paulínia, SP, Brazil) and tap water were available ad libitum. The experimental protocols were approved by the Animal Experimentation Ethics Committee of the Federal University of São Paulo.

2.2. Brain surgery

Rats were anaesthetized with intraperitoneal (i.p.) injection of ketamine (80 mg/kg of body wt) combined with xylazine (7 mg/kg of body wt) and placed in a stereotaxic frame (model 900, David Kopf Instruments). The skull was levelled between bregma and lambda. A stainless steel cannula (10 mm × 0.6 mm o.d.) was implanted into the lateral cerebral ventricle (LV) using the following stereotaxic coordinates: 0.3 mm caudal to bregma, 1.5 mm lateral to midline and 3.6 mm below the dura mater. The cannula was fixed to the cranium with dental acrylic resin and jeweller screws. Rats received a prophylactic dose of penicillin (30,000 IU) given intramuscularly and a subcutaneous injection of the analgesic Ketoflex (ketoprofen 1%, 0.03 ml/rat) post-surgically. After the surgery, the rats were maintained in individual box with free access of tap water and food pellets for at least 7 days before the tests.

2.3. Drugs

Moxonidine hydrochloride (20 nmol/1 μl), a gift from Solvay Pharma (Germany), pilocarpine hydrochloride (500 nmol/1 μl) and yohimbine hydrochloride (320 nmol/2 μl) from Sigma Chemical Co., USA were injected i.c.v. A mix of propylene glycol/water 2:1 was used as vehicle for yohimbine and moxonidine because these drugs at the doses used are not soluble in saline. Pilocarpine was dissolved in isotonic saline.

The dose of pilocarpine used in the present study was based on a previous study employing pilocarpine i.c.v. to induce salivation in rats.[7] The doses of yohimbine and moxonidine were based on previous studies that have shown the effects of different doses of yohimbine and moxonidine on pilocarpine-induced salivation, water and sodium intake and cardiovascular responses.[4,16,17]

2.4. Cerebral injections

Injections into the LV were made using 10 μl Hamilton syringes connected by polyethylene tubing (PE-10) to injection cannulas 2 mm longer than the guide cannulas implanted into the brain. The volume injected into the LV was 1 or 2 μl.

2.5. Arterial pressure and heart rate recordings

On the day of the experiment rats were anaesthetized with urethane (1.2 g/kg of body weight i.v.) and α-chloralose (60 mg/kg of body weight i.v.) (after the induction with 1% halothane in 100% O_2). A femoral artery catheter (PE-10 connected to PE-50) was implanted for the record of pulsatile arterial pressure, mean arterial pressure (MAP) and heart rate (HR). A femoral vein catheter was implanted for administration of anaesthetic. To record pulsatile arterial pressure, MAP and HR, the arterial catheter was connected to a Statham Gould (P23 Db) pressure transducer coupled to a pre-amplifier (model ETH-200 Bridge Bio Amplifier, CB Sciences) and to a Powerlab computer recording system (model Powerlab 16SP, ADInstruments). Recordings began 10 min after the connection of the arterial line to the pressure transducer. MAP and HR were continuously recorded during 1 h and were analysed at every 5 min. Baseline values were recorded for 10 min and were analysed immediately before yohimbine or vehicle injection (first treatment). These values were used as reference to calculate the changes produced by the treatments.

2.6. Submandibular/sublingual gland, superior mesenteric and low abdominal aorta artery blood flow recordings

Immediately after vein and artery catheterization, an incision was made in ventral midline of the neck to localize the right submandibular/sublingual gland (SSG) complex and the artery that irrigates the SSG complex. The SSG artery, a cervical branch of external maxillary artery is usually larger just above the anterior margin of posterior belly of digastricus.[6,10] The artery that irrigates the SSG complex was isolated and a miniature pulsed Doppler flow probe (Iowa Doppler Products; Iowa City, IA) was perfectly adjusted around the artery to record blood flow.

A midline laparotomy was also performed and miniature pulsed Doppler flow probes were placed around the superior