Activation of central α₂-adrenoceptors mediates salivary gland vasoconstriction

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A R T I C L E   I N F O

Article history:
Accepted 24 June 2012

Keywords:
α₂-Adrenergic receptor
Pilocarpine
Parasympathetic
Blood flow and vascular resistance
Salivary gland
Moxonidine

A B S T R A C T

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 (α₂-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 α₂-adrenergic 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 α₂-adrenoceptors may oppose to the effects of central cholinergic receptor activation in the SSG vascular resistance.

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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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http://dx.doi.org/10.1016/j.archoralbio.2012.06.017
Moxonidine (α2-agonist) is an anti-hypertensive drug that acts centrally to reduce sympathetic nerve discharge.\textsuperscript{11–14} Moxonidine injected i.c.v. reduces peripheral pilocarpine-induced salivation and vasodilation in the SSG.\textsuperscript{4,10,15} The reduction of pilocarpine-induced salivation produced by moxonidine injected i.c.v depends on the activation of central α2-agonists,\textsuperscript{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 (α2-agonist 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 ± 10%). Lights were on from 7:00 am to 7:00 pm. Guabi rat chow (Paulinia, 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.\textsuperscript{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.\textsuperscript{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\textsubscript{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.\textsuperscript{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
mesenteric (SM) artery and the low abdominal aorta for measurement of mesenteric and hindlimb blood flow, respectively. The probes were fixed to the surrounding tissues with suture thread. Data from animals in which the probes moved during the experiment were not considered for analysis.

The flow probes were connected to a Doppler flowmeter (Dept of Bioengineering, University of Iowa, Iowa City, IA) coupled to a Powerlab computer record system (model Powerlab 16SP, ADInstruments) for blood flow recording. Details of the Doppler flow recording technique, including the reliability of the method for estimation of flow velocity, have been described previously. Relative SSG, mesenteric and hindlimb vascular resistance changes were calculated as the ratio of MAP and Doppler shift.

Blood flow was continuously recorded for 1 h. Blood flow and vascular resistance were analysed for every 5 min. Baseline values were recorded for 10 min and were analysed immediately before yohimbine or vehicle injection (first injection). The values were used as reference to calculate the changes produced by the treatments.

2.7. Histology

After the experiments, Evans blue (100 nl of a 2% solution) was microinjected into the LV for histological analysis. The animals were deeply anaesthetized with urethane (1.2 g/kg of body weight i.v.) and α-chloralose (60 mg/kg of body weight i.v.). Saline followed by 10% buffered formalin was perfused through the heart. The brains were frozen, cut coronally into 50 μm sections and stained with Giemsa stain. Only animals with injections into the LV were considered for statistical analysis.

2.8. Statistical analysis

All values were expressed as means ± SEM. Statistical analysis was performed using two-way analysis of variance (ANOVA) with repeated measures followed by Student-Newman–Keuls post hoc tests to determine significant differences between groups. Significance level was set at p < 0.05.

2.9. Experimental protocols

All studies were performed in rats anaesthetized with urethane (1.2 g/kg of body weight i.v.) and α-chloralose (60 mg/kg of body weight i.v.). After 10 min of control (baseline) recording of MAP, HR and blood flow velocity in SSG, SM and abdominal aorta arteries, yohimbine (320 nmol/2 μl) or vehicle was injected i.c.v. Moxonidine (20 nmol/1 μl) or vehicle was injected i.c.v. 15 min after central injection of yohimbine or vehicle. Pilocarpine (500 nmol/1 μl) or saline was injected i.c.v. 15 min after the i.c.v. injection of moxonidine or vehicle. The recordings stopped 30 min after the last injection.

To study the involvement of central α2-adrenoceptor on the association of cardiovascular effects of central moxonidine and pilocarpine, 4 groups of rats were used: (1) a control group that received vehicle i.c.v. followed by vehicle and saline i.c.v.; (2) a group injected with yohimbine i.c.v. followed by moxonidine and pilocarpine i.c.v.; (3) a group treated with vehicle i.c.v. Followed by moxonidine and pilocarpine i.c.v.; (4) a group that received vehicle i.c.v. Followed by vehicle and pilocarpine i.c.v.

![Fig. 1 - Changes in (A) blood flow and (B) vascular resistance in the submandibular/sublingual gland (SSG) produced by pilocarpine (500 nmol/1 μl) injected i.c.v. in rats pre-treated with yohimbine (320 nmol/2 μl) or vehicle combined with moxonidine (20 nmol/1 μl) or vehicle injected i.c.v. The results are represented as means ± SEM. n = number of rats. *Different from vehicle + vehicle + saline (Student-Newman–Keuls test, p < 0.05).](image)
3. Results

3.1. Changes in the SSG vascular resistance and blood flow produced by pilocarpine i.c.v. in rats pre-treated with moxonidine i.c.v. alone or combined with yohimbine i.c.v.

Pilocarpine (500 nmol/1 μl) injected i.c.v. reduced SSG vascular resistance (−34 ± 11%, vs. saline: 5 ± 5%) [F (3, 17) = 118.13; p < 0.01] and increased SSG blood flow (43 ± 18%, vs. saline: 6 ± 3%) [F (3, 17) = 105.66; p < 0.01] (Fig. 1).

Contrary to the effects of pilocarpine injected i.c.v. alone, the SSG vascular resistance increased (80 ± 36%) and the SSG blood flow was reduced (−45 ± 15%) by the treatment with pilocarpine i.c.v. combined with moxonidine (20 nmol/1 μl) i.c.v. (Fig. 1).

The pre-treatment with yohimbine (320 nmol/2 μl) injected i.c.v. abolished the increase in SSG vascular resistance (3 ± 6%, vs: moxo + pilo: 80 ± 36%) and the vasodilatation (7 ± 3%, vs: moxo + pilo: −45 ± 15%) produced by combining moxonidine and pilocarpine i.c.v. (Fig. 1).

3.2. Changes in MAP and HR and in the vascular resistance and blood flow in the SM artery and hindlimb produced by pilocarpine injected i.c.v. in rats pre-treated with moxonidine i.c.v. alone combined with yohimbine i.c.v.

Pilocarpine (500 nmol/1 μl) injected i.c.v. induced pressor responses (21 ± 4 mmHg, vs. saline: 2 ± 2 mmHg) [F (3, 17) = 63.47; p < 0.05] and tachycardia (15 ± 4 bpm, vs. vehicle 3 ± 4 bpm) [F (3, 17) = 44.12; p < 0.05] and increased vascular resistance (28 ± 4% vs. saline: 6 ± 3%) [F (3, 17) = 46.19; p < 0.05] and reduced blood flow in the SM artery (−13 ± 6% vs. saline: 2 ± 1%) [F (3, 17) = 53.07; p < 0.05], without changing hindlimb vascular resistance or blood flow (Figs. 2–4).

Prior injection of moxonidine (20 nmol/1 μl) i.c.v. alone or combined with yohimbine (320 nmol/2 μl) did not modify the pressor response (18 ± 4 and 16 ± 3 mmHg, respectively), the tachycardia (12 ± 4 and 13 ± 3 bpm, respectively), the increase in SM vascular resistance (20 ± 4% and 19 ± 4%, respectively) and the reduction of blood flow (−10 ± 4% and −12 ± 3%, respectively) produced by i.c.v. pilocarpine (Figs. 2 and 3).

The baseline MAP and HR immediately before yohimbine or vehicle injections in each group of rats are presented in Table 1.

4. Discussion

The present results show that central injections of pilocarpine reduce SSG vascular resistance and the increase MAP, HR and mesenteric vascular resistance. Contrary to the reduction in the salivary gland vascular resistance, the combination of moxonidine and pilocarpine injected i.c.v. increased SSG vascular resistance, an effect abolished by the previous injection of yohimbine i.c.v. The changes in mesenteric vascular resistance, MAP and HR produced by pilocarpine i.c.v. were not altered by the central injection of moxonidine. Hindlimb vascular resistance was not affected by either treatment. These results suggest that the activation of central α2-adrenoceptors may oppose to the effects of central cholinergic receptor activation in the SSG vascular resistance.
The effects produced by i.c.v. injection of pilocarpine on MAP, HR and on SSG and mesenteric resistances were similar to those produced by peripheral injections of pilocarpine, which reinforces the suggestion that pilocarpine injected peripherally may act centrally to reduce SSG vascular resistance and to increase MAP, HR and mesenteric vascular resistance. In addition to the central effects, pilocarpine injected peripherally may also produce SSG vasodilation by acting directly in the salivary glands. In spite of this direct effect on salivary glands, moxonidine

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**Fig. 3** – Changes in (A) blood flow and (B) vascular resistance in superior mesenteric (SM) artery produced by pilocarpine (500 nmol/1 μl) injected i.c.v. in rats pre-treated with yohimbine (320 nmol/2 μl) or vehicle combined with moxonidine (20 nmol/1 μl) or vehicle injected i.c.v. The results are represented as means ± SEM. n = number of rats. *Different from vehicle + saline (Student-Newman-Keuls test, p < 0.05).

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**Fig. 4** – Changes in (A) blood flow and (B) vascular resistance in hindlimb produced by pilocarpine (500 nmol/1 μl) injected i.c.v. in rats pre-treated with yohimbine (320 nmol/2 μl) or vehicle combined with moxonidine (20 nmol/1 μl) or vehicle injected i.c.v. The results are represented as means ± SEM. n = number of rats. *Different from vehicle + saline (Student-Newman-Keuls test, p < 0.05).
Table 1 – Baseline MAP and HR in the groups of rats that received pilocarpine or saline injected i.c.v. after the pre-treatment with yohimbine or vehicle combined with moxonidine or vehicle injected i.c.v.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Baseline MAP (mmHg)</th>
<th>Baseline HR (bpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle + vehicle + saline (i.c.v.) (n = 6)</td>
<td>102 ± 4</td>
<td>382 ± 9</td>
</tr>
<tr>
<td>Vehicle + vehicle + pilocarpine (i.c.v.) (n = 7)</td>
<td>100 ± 3</td>
<td>373 ± 10</td>
</tr>
<tr>
<td>Vehicle + moxonidine + pilocarpine (i.c.v.) (n = 7)</td>
<td>101 ± 4</td>
<td>388 ± 12</td>
</tr>
<tr>
<td>Yohimbine + moxonidine + pilocarpine (i.c.v.) (n = 8)</td>
<td>103 ± 4</td>
<td>380 ± 11</td>
</tr>
</tbody>
</table>

The results are represented as means ± SEM. n = number of rats. Pilocarpine (500 nmol/1 μl); moxonidine (20 nmol/1 μl); yohimbine (320 nmol/2 μl).

injected i.c.v. combined with pilocarpine injected intravenously also increased SSG vascular resistance,10 similar to the effects of moxonidine combined with pilocarpine i.c.v. (present results). Moxonidine injected i.c.v. alone also increases SSG vascular resistance,10 which suggests that the activation of central α2-adrenoceptors overcomes the effects central cholinergic activation resulting in increased SSG vascular resistance when pilocarpine is combined with moxonidine both injected i.c.v.

The importance and the involvement of the central α2-adrenoceptors in the inhibition of salivation were shown previously by injecting clonidine intracisternally in cats that received electrical stimulation of brainstem parasympathetic nuclei.10 The effect of clonidine was inhibited by prior intracisternal injection of yohimbine. Previous studies also showed that the activation of central α2-adrenoceptors with moxonidine reduces salivation and abolishes the increase in the SSG blood flow produced by peripheral injection of pilocarpine.4,10 The present results extend the conclusion of the previous studies showing that: (1) central injection of pilocarpine reduces SSG vascular resistance, similarly to the activation of the parasympathetic output or to peripheral injection of pilocarpine; (2) the increase in the SSG vascular resistance after combining central injection of pilocarpine and moxonidine results from the activation of central α2-adrenoceptor by moxonidine.

Pilocarpine at the dose used in the present study reduces salivary gland vascular resistance and increases mesenteric vascular resistance without changing hindlimb vascular resistance. The opposite effects on vascular resistance suggest that pilocarpine activates different mechanisms producing specific adjustments in vascular resistance and blood flow in different regions of the body. Pilocarpine injected centrally produces: (1) decrease in the SSG vascular resistance and no change in the hindlimb; (2) increase in MAP that seems to be dependent on the increase in mesenteric vascular resistance; (3) increase in HR that may be involved in pressor pathway. It is well known that the central activation of cholinergic receptors stimulates sympathetic activity and vasopressin release, causing an increase in MAP.20,21 Combining the increase in MAP and the reduced salivary gland vascular resistance, central injection of pilocarpine produces a strong increase in blood flow to the salivary glands and these effects may explain why pilocarpine is so effective at inducing salivary secretion.

Despite of the anti-hypertensive properties of moxonidine (α2-adrenoceptor and imidazolone agonist12-14,24), no change in pilocarpine-induced vasoconstriction and pressor responses was observed following the treatment with moxonidine into the lateral cerebral ventricle as previously demonstrated for pilocarpine injected peripherally.10 Studies have demonstrated that moxonidine acts in the brainstem, particularly in rostral ventrolateral medulla (RVLM), to reduce sympathetic outflow and blood pressure.11-14 In the present study, moxonidine was injected into the lateral cerebral ventricle and in this case the main areas reached by moxonidine were forebrain areas. The results suggest that the activity of the cholinergic pressor mechanisms in the forebrain is not modified by the activation of α2-adrenoceptors or imidazolone receptors with i.c.v. injection of moxonidine.

The vasoconstriction in the salivary gland induced by moxonidine may be the result of the activation of a vasoconstrictor or removal of the vasodilator mechanism to the salivary gland. The latter seems less likely because vasodilator tone to the salivary glands is rather small.22 Whatever the mechanism activated by central moxonidine, the increase in salivary gland vascular resistance produced by i.c.v. moxonidine was not modified by peripheral10 and central injection (present data) of pilocarpine.

It seems that pilocarpine acting centrally activates both salivary gland secretion and vasodilation.7,8,10 Because salivation depends on secretory mechanisms and on the increase in blood flow to the glands,23 reduction in salivation may occur if one or both mechanisms are affected. The activation of α2-adrenoceptor with moxonidine reduces the salivary secretion and the vasodilation induced by pilocarpine.15,10 Therefore, it is possible that moxonidine inhibits pilocarpine-induced salivation at least partially by reducing salivary gland blood flow. Besides this, the vasoconstriction and the reduction of the blood flow to the salivary glands produced by the activation of the central α2-adrenoceptors is probably important for the sensation of dryness in the mouth by patients treated with moxonidine or the same type of drugs.

In summary, the present results suggest that central cholinergic and α2-adrenergic mechanisms have opposite roles in the control of the salivary gland vascular resistance and blood flow. However, the increase in MAP, HR and mesenteric vascular resistance produced by the cholinergic activation in the forebrain is not affected by central α2-adrenoceptor activation, suggesting that different central mechanisms are activated by pilocarpine to produce the changes in the vascular resistance in different vascular beds.

**Funding**

São Paulo State Foundation (FAPESP).
Competing interests

None declared.

Ethical approval

Experimental protocols were approved by the Animal Experimentation Ethics Committee of the Federal University of Sao Paulo (UNIFESP) and Conselho Nacional de Pesquisa (CNPq/PRONEX).

Acknowledgements

We would like to thank also Solvay Pharma and Dr. P. Ernsberger for the donation of moxonidine. This research was supported by public funding from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and CNPq/PRONEX.

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