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Role of nitric oxide of the median preoptic nucleus (MnPO) in the alterations of salivary flow, arterial pressure and heart rate induced by injection of pilocarpine into the MnPO and intraperitoneally

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Abstract

We investigated the effect of L-NAME, a nitric oxide (NO) inhibitor and sodium nitroprusside (SNP), an NO-donating agent, on pilocarpine-induced alterations in salivary flow, mean arterial blood pressure (MAP) and heart rate (HR) in rats. Male Holtzman rats (250-300 g) were implanted with a stainless steel cannula directly into the median preoptic nucleus (MnPO). Pilocarpine (10, 20, 40, 80, 160 µg) injected into the MnPO induced an increase in salivary secretion ($P<0.01$). Pilocarpine (1, 2, 4, 8, 16 mg/kg) *ip* also increased salivary secretion ($P<0.01$). Injection of L-NAME (40 µg) into the MnPO prior to pilocarpine (10, 20, 40, 80, 160 µg) injected into the MnPO or *ip* (1, 2, 4, 8, 16 mg/kg) increased salivary secretion ($P<0.01$). SNP (30 µg) injected into the MnPO or *ip* prior to pilocarpine attenuated salivary secretion ($P<0.01$). Pilocarpine (40 µg) injection into the MnPO increased MAP and decreased HR ($P<0.01$). Pilocarpine (4 mg/kg body weight) *ip* produced a decrease in MAP and an increase in HR ($P<0.01$). Injection of L-NAME (40 µg) into the MnPO prior to pilocarpine potentiated the increase in MAP and reduced HR ($P<0.01$). SNP (30 µg) injected into the MnPO prior to pilocarpine attenuated (100%) the effect of pilocarpine on MAP, with no effect on HR. Administration of L-NAME (40 µg) into the MnPO potentiated the effect of pilocarpine injected *ip*. SNP (30 µg) injected into the MnPO attenuated the effect of *ip* pilocarpine on MAP and HR. The present study suggests that in the rat MnPO 1) NO is important for the effects of pilocarpine on salivary flow, and 2) pilocarpine interferes with blood pressure and HR (side effects of pilocarpine), that is attenuated by NO.

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- Pilocarpine
- Nitric oxide
- Central nervous system
- Salivation
- Arterial pressure
- Heart rate

Introduction

The overall quality of life is affected by the symptoms of a permanently dry mouth, the inability to enjoy foods, and the trouble and expense of frequent dental treatment. Most systemic treatments have not been tested adequately or have not proved to be effective. Some procedures should be followed to relieve these symptoms, to control oral disease and to improve salivary function. With a systematic approach and aggressive control, most patients with a dry mouth can achieve oral comfort and adequate oral function (1).

Pilocarpine, a muscarinic cholinergic agonist, induces vasodilatation and copious salivation when administered systemically by activating the parasympathetic system (2-4). Pilocarpine stimulates labial salivary gland flow in patients with Sjögren's syndrome (5). The damage produced by irradiation in the salivary glands is reduced by prior administration of pilocarpine (6). Pilocarpine has a sympathomimetic (probably ß-receptor mediated) stimulatory effect, which is also implicated in cardiovascular regulation and salivary secretion. The involvement of hypothalamic areas in the control of salivary secretion in rats has been shown by several studies (7-10). The anteroventral third ventricle (AV3V) region is important for the effect of pilocarpine on salivary secretion in rats (11). Morphological, morphometric and stereological changes of submandibular glands were observed after lesion of the AV3V and ventromedial nucleus of hypothalamus (12).

Nitric oxide (NO) plays an excitatory role in the regulation of parasympathetic nerves inducing salivary secretion in the submandibular gland of rats (13). L-N^G-nitroarginine methyl ester (L-NAME), an NO synthase inhibitor, and L-arginine increase the salivation induced by pilocarpine (14,15). Pilocarpine has been used extensively as the best sialogogue rather than other cholinomimetic agents but produces cardiovascular alterations as side effects. NO plays an important role in the hydromineral and cardiovascular regulation induced by angiotensin (16,17). Different data indicate that the median preoptic nucleus (MnPO) is indeed the target of afferents from osmosensitive and barosensitive systems involved in fluid homeostasis and cardiovascular regulation (18-20).

The aim of the present study was to determine whether NO present in the central nervous system (CNS) interferes with the sialogogue and cardiovascular changes induced by pilocarpine injected into the MnPO or intraperitoneally (*ip*).

Material and Methods

Animals

Male Holtzman rats (250-300 g) were housed in individual metabolic cages, with free access to food pellets and tap water.

Brain surgery

The rats were anesthetized with 2,2,2-tribromoethanol (20 mg/100 kg body weight) *ip* and restrained in a stereotaxic apparatus (David Kopf model for rats). A longitudinal incision was made in the skin of the animals' head, the subcutaneous tissue was pulled back, the skull was perforated with a spherical drill, and a stainless steel cannula (14 × 0.7 mm, OD) was introduced into the MnPO. The skull was positioned with the bregma and lambda at the same level. The coordinates for approaching the MnPO were obtained from the Paxinos and Watson atlas (21) (coordinates: AP = -0.30 to 0.40 mm to the bregma; V = 4.3 mm from the dura mater; L = 0.0 mm from the sagittal midline). The cannula was fixed to the skull with screws and acrylic resin. A prophylactic dose of 60,000 IU penicillin (Pentabiotic Fountoura Wyeth, São Paulo, SP, Brazil) was injected intramuscularly after brain surgery.

Intracerebral injection

Pilocarpine, L-NAME, sodium nitroprusside (SNP), and 0.15 M NaCl (as control) were injected into the MnPO with a Hamilton microsyringe (5 µl) connected by a PE-10 polyethylene tubing (25 cm) to a needle (0.3 mm, OD) which was introduced into the brain through the cannula previously fixed to the animals' head. The volume of injection was always 1 µl injected over a period of 30 to 60 s.

Salivary secretion

Salivary flow was stimulated with pilocarpine (10, 20, 40, 80 and 160 µg) in a volume of 1.0 µl injected into the MnPO and with pilocarpine injected *ip* (1, 2, 4, 8 and 16 mg/kg). The animals were anesthetized with urethane, 1.25 g/kg body weight, injected *ip*. Saliva was collected with preweighed small cotton wool balls inserted into the animals' mouth, according to the technique used by Schallert et al. (8) which permitted us to collect the saliva. Saliva was collected with four cotton balls weighing approximately 20 mg each, two of which were placed on either side of the oral cavity, with the other two placed under the tongue. The amount of saliva secreted was measured 5 min before the injection of pilocarpine (baseline saliva secretion) and 5 min after the injection of pilocarpine into the MnPO or *ip* (stimulated salivary secretion). L-NAME and SNP were injected into the MnPO 5 min before pilocarpine injection into the MnPO or *ip* or were injected alone, without pilocarpine.

Mean arterial blood pressure and heart rate recordings

Mean arterial blood pressure (MAP) and heart rate (HR) were recorded after injection of pilocarpine into the MnPO or *ip* and after injection of L-NAME or SNP into the MnPO prior to injection of pilocarpine into the MnPO or *ip*. Polyethylene tubing (PE-10) connected to PE-50 tubing was inserted into the abdominal aorta through the femoral artery under 2,2,2-tribromoethanol anesthesia (20 mg/100 kg body weight) on the day before the recordings. The polyethylene tube was connected to a Statham (P23 Db) pressure transducer (Statham-Gould, Valley View, OH, USA), coupled to a multichannel recorder (DATAq Instruments, Inc., Akron, OH, USA) and connected to a computer system. HR was obtained from arterial pressure pulses using a biotachometer. The values are reported as MAP increase or decrease and changes in HR, as absolute values.

Histology

At the end of each experiment, the animal was anesthetized with ether and injected with 1 µl fast green dye via an intracranial cannula, followed by perfusion with saline and buffered formalin. The brains were removed, fixed in 10% formalin, frozen at -20ºC and sections of 20 to 30 µm were prepared. The presence or absence of dye in the regions near the MnPO was determined visually. Only the data from animals in which the presence of the dye was restricted to the MnPO were used. Figure 1 illustrates the histology at the site of injection into the MnPO.

The MnPO injection sites for the salivary flow, MAP and HR responses terminated in the dorsal portion of the MnPO and in portions of the anterior commissure. Data from animals whose injection sites were anterior

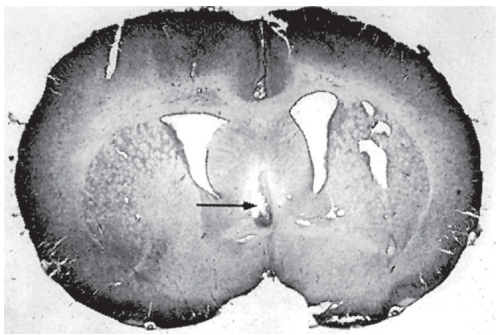

Figure 1. Photomicrograph of hematoxylin-stained transverse sections of the rat brain showing the site of injections into the median preoptic nucleus (MnPO, arrow). The site of injection was located in the dorsal portions of the MnPO and in portions of the anterior commissure (36X).

or anterior and dorsal to the MnPO in the medial septal area were excluded from analysis. It has been demonstrated that the neuronal elements mediating pressor activity are confined to a small region along the midline centered near the anterior commissure (22). The animals whose injection sites were outside the MnPO did not show any changes in the salivary or cardiovascular parameters.

Statistical analysis

Data are reported as means ± SEM. ANOVA and the Dunnett *t*-test were used to determine statistical significance. Comparisons were considered to be statistically significant when $P<0.05$.

Experimental procedures

The study of salivary flow and of MAP and HR measurement was initiated 5 days after brain surgery. Each animal was submitted to 3 or 4 experimental sessions at 3-day intervals. The parameters for MnPO were obtained from different experimental sessions and from several groups of animals after the injection of the following drugs into the MnPO or *ip* in satiated animals: i) saline, 0.15 M NaCl injected into the MnPO (control); ii) pilocarpine, 10, 20, 40, 80 and 160 µg injected into the MnPO; iii) pilocarpine, 1, 2, 4, 8 and 16 mg/kg body weight injected *ip*; iv) L-NAME, 40 µg injected into the MnPO alone or before pilocarpine into the MnPO or *ip*; v) SNP, 30 µg injected into the MnPO alone or before pilocarpine into the MnPO or *ip*.

Salivary secretion, MAP and HR were measured in all experiments.

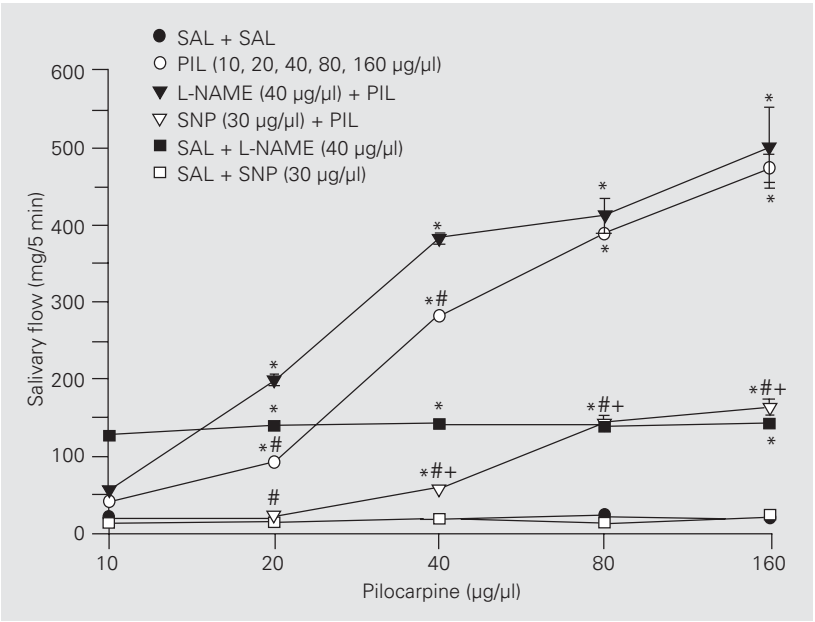

Figure 2. Effect of L-NAME and SNP injected into the median preoptic nucleus (MnPO) on salivary flow induced by injection of pilocarpine into the MnPO. Data are reported as means ± SEM. *P<0.001 compared to SAL + SAL; #P<0.001 compared to L-NAME + pilocarpine; +P<0.001 compared to pilocarpine (Dunnet *t*-test; N = 40 for each group). SAL, saline; PIL, pilocarpine; L-NAME, L-N^G-nitro-arginine methyl ester; SNP, sodium nitroprusside.

Results

Effect of L-NAME and SNP on salivary secretion induced by injection of pilocarpine into the MnPO

Salivary secretion after injection of isotonic saline (0.15 M NaCl) into the MnPO was 19.6 ± 1.6 mg/5 min. The injection of pilocarpine (10, 20, 40, 80, 160 µg) into the MnPO increased salivary secretion to 41 ± 3, 92 ± 4, 282 ± 5, 381 ± 4 and 474 ± 18 mg/5 min, respectively. ANOVA showed that there were significant differences among all concentrations ($F(4,32) = 57.14$, $P<0.01$). The basal values of salivary flow after injection of L-NAME (40 µg) after 5 periods of observations were 126 ± 4, 139 ± 6, 141 ± 7, 139 ± 6 and 142 ± 7 mg/5 min. L-NAME (40 µg/µl) injected into the MnPO before pilocarpine potentiated the sialogogue effect of pilocarpine with values of 54 ± 5, 199 ± 7, 382 ± 6, 412 ± 23 and 501 ± 52 mg/5 min ($F(4,32) = 29.13$, $P<0.01$). The basal values of salivary secretion after SNP (30 µg) injection into the MnPO after 5 periods of observations were 12 ± 1, 14 ± 2, 18 ± 6, 13 ± 4 and 20 ± 7 mg/5 min. SNP (30 µg/µl) injected into the MnPO before pilocarpine attenuated the sialogogue effect of pilocarpine with values of

14 ± 1, 23 ± 2, 58 ± 3, 143 ± 9 and 163 ± 8 mg/5 min. ANOVA showed significant differences among the effects of various concentrations ($F(4,32) = 74.48$, $P<0.01$) (Figure 2).

Effect of L-NAME and SNP on salivary secretion induced by *ip* injection of pilocarpine

The *ip* administration of pilocarpine (1, 2, 4, 8, and 16 mg/kg body weight) increased salivary secretion (268 ± 24, 300 ± 20, 392 ± 24, 483 ± 22 and 578 ± 33 mg/5 min, respectively; $F(4,30) = 12.34$, $P<0.01$). After L-NAME injection (40 µg) into the MnPO, the salivary secretion induced by various concentrations of pilocarpine injected *ip* was potentiated, with values of 413 ± 20, 473 ± 27, 541 ± 37, 582 ± 49 and 694 ± 28 mg/5 min, respectively ($F(4,32) = 12.22$, $P<0.01$). SNP (30 µg/µl) injected into the MnPO before various concentrations of pilocarpine injected *ip* attenuated the pilocarpine effect on salivary secretion to values of 132 ± 18, 197 ± 23, 237 ± 14, 255 ± 25 and 272 ± 22 mg/5 min, respectively ($F(4,32) = 1.99$, $P<0.01$) (Figure 3).

Effect of L-NAME and SNP on changes of MAP and HR induced by injection of pilocarpine into the MnPO

Pilocarpine injection into the MnPO induced an increase in MAP of Δ29 ± 6 mmHg and a decrease in HR to 320 ± 4 bpm, that differed significantly ($P<0.05$) from control values (Δ+4 ± 1 mmHg and 365 ± 7 bpm). After administration of L-NAME the basal values were Δ-15 ± 3 mmHg and 305 ± 5 bpm. L-NAME (40 µg) injected into the MnPO before pilocarpine (40 µg in a volume of 1.0 µl) injected into the same region potentiated the pressor effect of pilocarpine (Δ+39 ± 7 mmHg with a decrease in HR to 301 ± 8 bpm, $P<0.05$). The basal values after SNP (30 µg) at a volume of 1.0 µl were Δ+9 ± 2 mmHg and 370 ± 8 bpm. SNP injected into the MnPO before pilocarpine induced a decrease of MAP (-7 ± 2 mmHg, $P<0.05$) without any changes in HR (369 ± 7 bpm), as shown in Figures 4 and 5.

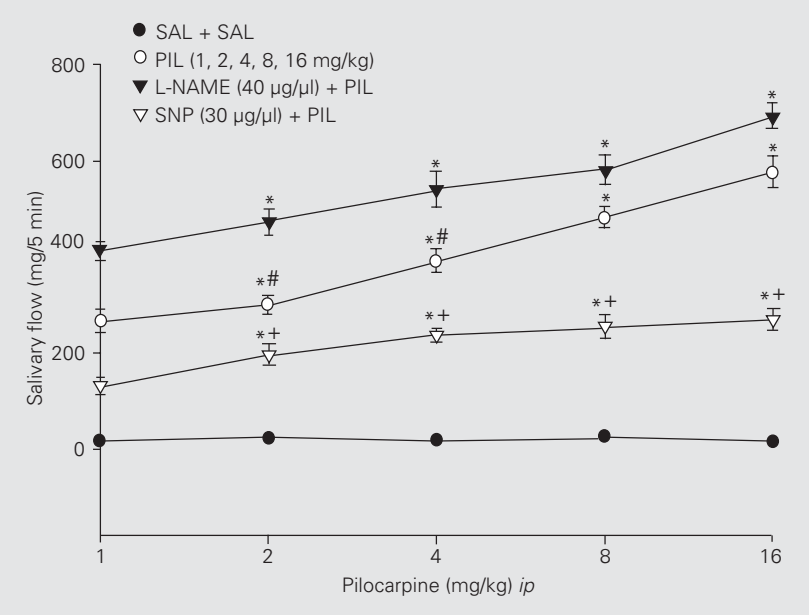

Figure 3. Effect of L-NAME and SNP injected into the median preoptic nucleus on salivary flow induced by intraperitoneal (*ip*) administration of pilocarpine (PIL). Data are reported as means ± SEM. *P<0.001 compared to SAL + SAL; #P<0.001 compared to L-NAME + PIL; +P<0.001 compared to PIL (Dunnet *t*-test; N = 38 for each group). For abbreviations, see legend to Figure 2.

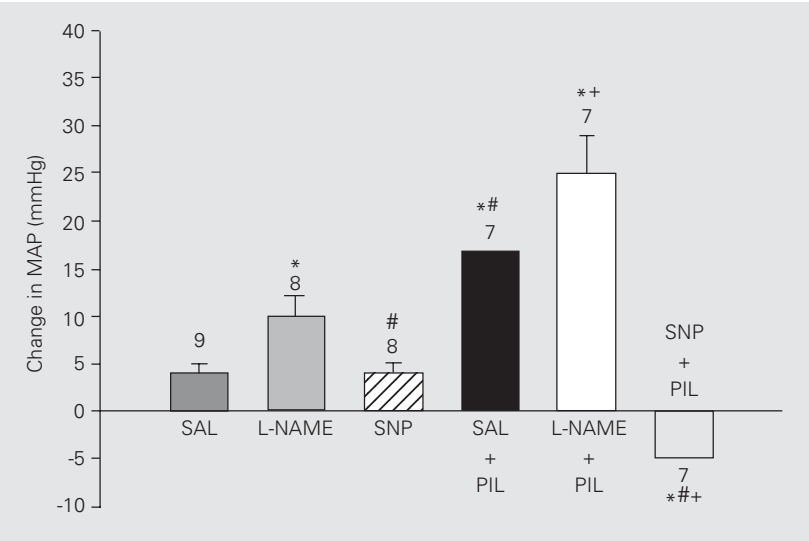

Figure 4. Effect of L-NAME and SNP injected into the median preoptic nucleus (MnPO) on mean arterial pressure (MAP) during injection of pilocarpine (PIL) into the MnPO. The number of animals is given at the top of each column. Data are reported as means ± SEM. *P<0.001 compared to SAL (control); #P<0.001 compared to SAL + L-NAME; +P<0.001 compared to SAL + PIL (Dunnett *t*-test). For abbreviations, see legend to Figure 2.

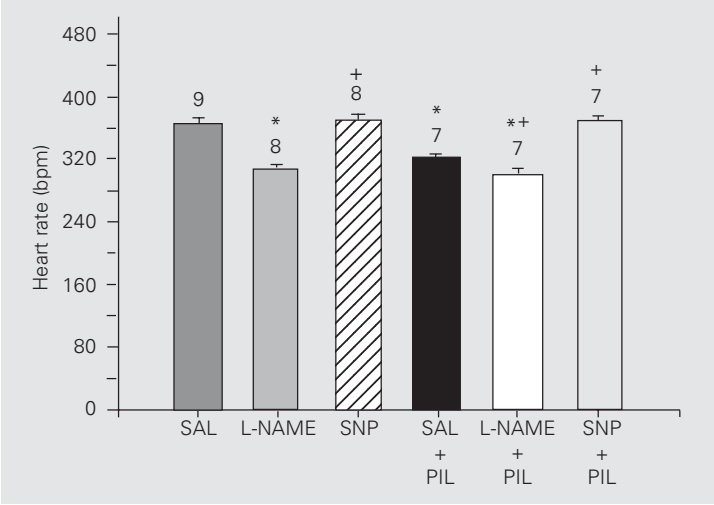

Figure 5. Effect of L-NAME and SNP injected into the median preoptic nucleus (MnPO) on heart rate during injection of pilocarpine (PIL) into the MnPO. The number of animals is given at the top of each column. Data are reported as means ± SEM. *P<0.001 compared to SAL (control); +P<0.001 compared to SAL + PIL (Dunnett *t*-test). For abbreviations, see legend to Figure 2.

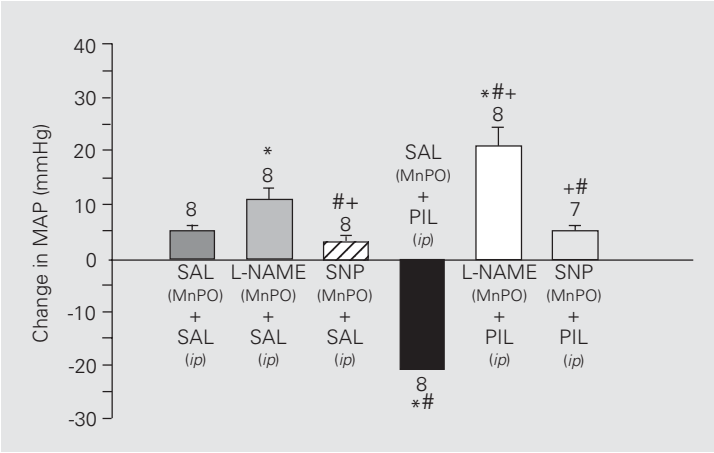

Figure 6. Effect of L-NAME and SNP injected into the median preoptic nucleus (MnPO) on mean arterial pressure (MAP) during intraperitoneal (*ip*) administration of pilocarpine (PIL). The number of animals is given at the top of each column. Data are reported as means ± SEM. *P<0.001 compared to SAL (control); #P<0.001 compared to SAL + L-NAME; +P<0.001 compared to SAL + PIL (Dunnett *t*-test). For abbreviations, see legend to Figure 2.

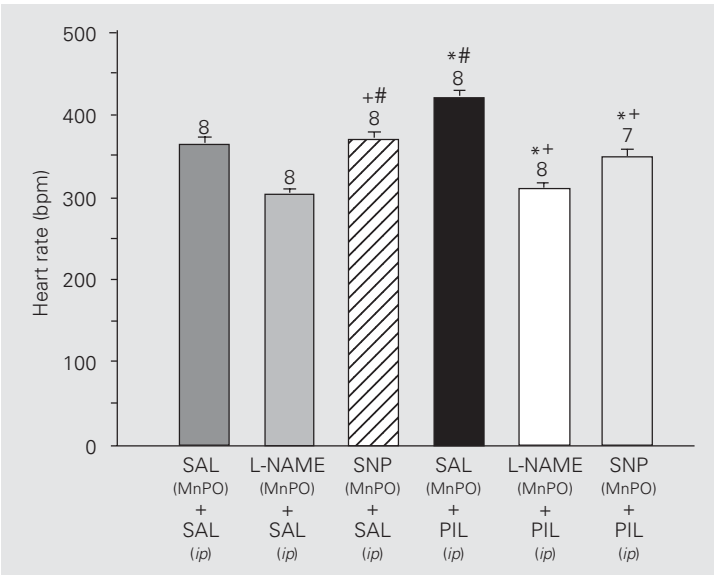

Figure 7. Effect of L-NAME and SNP injected into the median preoptic nucleus (MnPO) on heart rate during intraperitoneal (*ip*) administration of pilocarpine (PIL). The number of animals is given at the top of each column. Data are reported as means ± SEM. *P<0.001 compared to SAL + SAL (control); #P<0.05 compared to SAL + L-NAME; +P<0.001 compared to SAL + PIL (Dunnett *t*-test). For abbreviations, see legend to Figure 2.

Effect of L-NAME and SNP injected into the MnPO on changes in MAP and HR induced by injection of pilocarpine

Pilocarpine injected *ip* induced a decrease in MAP but an increase in HR, with values of Δ-21 ± 3 mmHg and 421 ± 7 bpm. L-NAME injected into the MnPO before pilocarpine injected *ip* reversed the effect of pilocarpine, with values of Δ+21 ± 3 mmHg and 311 ± 6 bpm ($P<0.05$). SNP injected into the MnPO before pilocarpine injected *ip* blocked the effect of pilocarpine, with values of Δ+5 ± 1 mmHg and 349 ± 8 bpm (Figures 6 and 7).

Discussion

The present results show that the injection of pilocarpine into the MnPO and *ip* affects salivary flow in a concentration-dependent manner. Pilocarpine at a concentration of 40 µg produced a medium salivary flow, whereas the flow rate increased in a linear fashion up to 160 µg. It has been demonstrated that pilocarpine, when injected intracerebroventricularly (*icv*), produced salivary secretion at a significantly higher level than control (11,16). It has also been reported that electrolytic lesion of the AV3V produced a decrease in salivary flow induced by pilocarpine injected *icv* (11). These results indicate that other areas of the CNS, such as the areas surrounding the third ventricle, are important for pilocarpine-induced salivary secretion. The areas surrounding the third ventricle are important for the regulation of the water-salt and cardiovascular balance as well as for the control of salivary composition and salivary flow. This is in agreement with results showing that the MnPO is important for the central regulation of salivary gland function (23).

Difficulty in swallowing (dysphagia) is a common upper gastrointestinal disorder caused by salivary malfunction (24). The present results show the participation of the CNS in attenuating these disorders, demonstrating that a controlled-release form of pilocarpine may overcome the therapeutic weaknesses of current pilocarpine preparations by prolonging salivary secretion and reducing undesirable side effects. Pilocarpine injected *ip* or into the MnPO produced changes in salivary flow and in cardiovascular parameters, such as an increase in MAP and a decrease in HR. When injected *ip* it caused a decrease in MAP and an increase in HR. These results are important, since several drugs used by patients with cardiovascular diseases may alter salivary secretion and interfere with the effects of pilocarpine when it is used as a drug. When injected centrally, pilocarpine produced alterations in salivary secretion, MAP and HR. An oral dose of pilocarpine increased salivary flow rates in patients with xerostomia (dry mouth). Pilocarpine induced submandibular and parotid salivary flow, which remained constant over a period of time (25).

The recognition of the role of NO in cell-to-cell communication has changed the concept of traditional neurotransmission. N-methyl-D-aspartate receptors mediate the dipsogenic response of c-Fos expression induced by *icv* infusion of angiotensin II (26). The presence of NO in many structures of the CNS has been described (27-31). It has been demonstrated that L-NAME increases the salivary flow induced by pilocarpine (14). L-NAME, when injected into the MnPO alone or before pilocarpine injected into the same region, potentiated the salivary flow induced by pilocarpine. NO contributed to the control of vascular tone in the salivary glands of rats. Also, L-NAME, when injected into the MnPO alone or before pilocarpine, potentiated the hypertensive effect of pilocarpine, decreasing HR. Pilocarpine may also act in areas of the CNS that are free from the blood-brain barrier, stimulating the areas that are involved in the control of systemic arterial blood pressure, like the MnPO and the periaqueductal gray region. The injection of NO-donating compounds into the dorsal re-

gion of the periaqueductal gray also decreased MAP, whereas L-NAME increased it (20). SNP injected into the MnPO before pilocarpine reduced the sialogogue effect of pilocarpine, with a decrease in MAP and an increase in HR. We infer that pilocarpine may also act in areas of the CNS such as the MnPO that regulates salivary flow and cardiovascular alterations. A regulatory mechanism may also exist between salivary flow and the cardiovascular parameters. Pilocarpine injected *ip* produced a copious salivary flow that was potentiated by the previous central injection of L-NAME into the MnPO. Therefore we postulate that the systemic effects of pilocarpine on salivary flow and cardiovascular regulation are influenced by central NO release, in agreement with the results reported by others (16,17). Pilocarpine injected *ip* decreased the MAP and increased the HR, with an opposite effect when injected into the MnPO, where it acted by increasing salivary flow and blood pressure, with a decrease in HR. The release of NO in the CNS continuously reduces the salivary secretion, playing an important role in the central and peripheral effects of pilocarpine. The cardiovascular changes produced by pilocarpine were mediated by the release or inhibition of NO. These results, taken together with those reported by others (22,23,32), suggest that the MnPO is involved in body fluid regulation not only by controlling vasopressin secretion and water intake but also by modulating central sympathetic outflow which regulates body fluid balance through an effect on the kidney and on salivary gland function (23,32).

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References

1. Fox PC (1997). Management of dry mouth. *Dental Clinics of North America*, 41: 863-875.
2. Ferguson MM (1993). Pilocarpine and other cholinergic drugs in the management of salivary gland dysfunction. *Oral Surgery, Oral Medicine, and Oral Pathology*, 75: 186-191.
3. Garret JR, Suleiman AM, Anderson LC & Proctor GB (1991). Secretory responses in granular ducts and acini of submandibular glands *in vivo* to parasympathetic nerve stimulation in rats. *Cell and Tissue Research*, 264: 117-126.
4. Baum BJ (1987). Neurotransmitter control secretion. *Journal of Dental Research*, 66: 628-632.
5. Rhodus NL (1997). Oral pilocarpine HCl stimulates labial (minor) salivary gland flow in patients with Sjögren's syndrome. *Oral Diseases*, 3: 93-98.
6. Nagler R, Marmry Y, Fox PC, Baum BJ, Har-El R & Chevion M (1997). Irradiation-induced damage to salivary gland. *Radiation Research*, 147: 468-476.
7. Hainsworth FR & Epstein AN (1966). Severe impairment of heat-induced saliva-spreading in rats recovered from lateral hypothalamic lesions. *Science*, 153: 1255-1257.
8. Schallert T, Leach LR & Braun JJ (1978). Saliva hypersecretion during aphagia following lateral hypothalamic lesions. *Physiology and Behavior*, 21: 461-463.
9. Flynn FW, Evey LA & Mitchell JC (1981). Heat-induced saliva secretion and thermoregulation in female rats with ventromedial hypothalamic lesions. *Physiology and Behavior*, 26: 779-782.
10. Kanosue K, Nakayama T, Tanaka H, Yanase M & Yasuda H (1990). Modes of action of local hypothalamic and skin thermal stimulation on salivary secretion in rats. *Journal of Physiology*, 424: 459-471.
11. Renzi A, Colombari E, Mattos Filho TR, Silveira JE, Saad WA, De Luca Jr LA, Derobio JG & Menani JV (1993). Involvement of the central nervous system in the salivary secretion induced by pilocarpine in rats. *Journal of Dental Research*, 72: 1481-1484.
12. Renzi A, Lopes RA, Sala MA, Camargo LAA, Menani JV, Saad WA & Campos GM (1990). Morphological, morphometric and stereological study of submandibular glands in rats with lesion of the anteroventral region of the third ventricle (AV3V). *Experimental Pathology*, 38: 177-187.
13. Takai N, Uchihashi K, Higuchi K, Yoshida Y & Yamaguchi M (1999). Localization of neuronal-constitutive nitric oxide synthase and secretory regulation by nitric oxide in the rat submandibular and sublingual glands. *Archives of Oral Biology*, 44: 745-750.
14. Damas J (1994). Pilocarpine-induced salivary secretion, kinin system and nitric oxide in rats. *Archives Internationales de Physiologie, de Biochimie et de Biophysique*, 102: 103-105.
15. Saad WA, Guarda IFMS, Guarda RS, Camargo LAA, Santos TAFB &

Saad WA (2002). Role of nitric oxide and beta receptors of the central nervous system on the salivary flow induced by pilocarpine injection into the lateral ventricle. *Pharmacology, Biochemistry and Behavior*, 72: 220-235.
16. Saad WA, Camargo LAA, Saad R, Pereira AF & Simões S (1999). Effect of injection of L-NAME on drinking response. *Brazilian Journal of Medical and Biological Research*, 32: 1413-1416.
17. Camargo LAA, Saad Wilson A, Simões S, Santos TAFB & Saad Willian A (2002). Interaction between paraventricular nucleus and septal area in the control of physiological responses induced by angiotensin II. *Brazilian Journal of Medical and Biological Research*, 35: 1017-1023.
18. Cunningham JT & Johnson AK (1989). Decreased norepinephrine in the ventral lamina terminalis region is associated with angiotensin II drinking response deficits following local 6-hydroxydopamine injections. *Brain Research*, 480: 65-71.
19. Gardiner TW & Stricker EM (1985). Imparied drinking responses of rats with lesions of nucleus medianus: circadian dependence. *American Journal of Physiology*, 248: R224-R230.
20. Lind RW & Johnson AK (1982). Subfornical organ-median preoptic connections and drinking and pressor responses to angiotensin II. *Journal of Neuroscience*, 2: 1043-1051.
21. Paxinos G & Watson C (1986). *The Rat Brain in Stereotaxic Coordinates*. Academic Press, New York.
22. O'Neill TP & Brody MJ (1987). Role of the median preoptic nucleus in centrally evoked pressor responses. *American Journal of Physiology*, 252: R1165-R1172.
23. Hübschle T, McKinley MJ & Oldfield BJ (1998). Efferent connections of the lamina terminalis, the preoptic area and the insular cortex to submandibular and sublingual gland of the rat traced with pseudorabies virus. *Brain Research*, 806: 219-231.
24. Valdez IH & Fox PC (1991). Interactions of the salivary and gastrointestinal system. II. Effects of salivary gland dysfunction on the gastrointestinal tract. *Digestive Diseases*, 9: 210-218.
25. Fox PC & Mandel ID (1992). Sjogren's syndrome. *Oral Surgery, Oral Medicine, and Oral Pathology*, 74: 315-318.
26. Zhu B & Herbert J (1997). Angiotensin II interacts with nitric oxide-cyclic GMP pathway in the central control of drinking behavior: Mapping with c-fos and NADPH-diaphorase. *Neuroscience*, 79: 23-29.
27. Arnais SL, Coronel MF & Boveris A (1999). Nitric oxide, superoxide, and hydrogen peroxide production in brain mitochondria after haloperidol treatment. *Nitric Oxide*, 3: 235-243.
28. Bredt DS, Glatt CE, Hwang PM, Fotuhi M, Dawson TM & Snyder SH (1991). Nitric oxide protein and mRNA are localized in neuronal populations of the mammalian CNS together with NADPH diaphorase. *Neuron*, 7: 615-624.
29. Ignarro LJ, Bug GM, Wood KS, Byrns RE & Chaudhuri G (1987). Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proceedings of the National Academy of Sciences, USA*, 84: 9265-9269.
30. Kadekaro M, Terrell ML, Harmann P & Summy-Long JY (1994). Central inhibition of nitric oxide synthase attenuates water intake but does not alter enhanced glucose utilization in the hypothalamo-neurohypophysial system of dehydrated rats. *Neuroscience Letters*, 173: 115-118.
31. Rees DD, Palmer RM & Moncada S (1989). Role of endothelium-derived nitric oxide in the regulation of blood pressure. *Proceedings of the National Academy of Sciences, USA*, 86: 3375-3378.
32. Yashuda Y, Honda K, Negoro H, Higuchi T, Goto Y & Fukuda S (2000). The contribution of the median preoptic nucleus to renal sympathetic nerve activity increased by intracerebroventricular injection of hypertonic saline in the rat. *Brain Research*, 867: 107-114.