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Functional relationship between subfornical organ cholinergic stimulation and nitrergic activation influencing cardiovascular and body fluid homeostasis

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Abstract

We have studied the effects of L-NG-nitro arginine methyl esther (L-NAME), L-arginine (LAR), inhibitor and a donating nitric oxide agent on the alterations of salivary flow, water intake, arterial blood pressure (MAP) and heart rate (HR) induced by the injection pilocarpine into the subfornical organ (SFO). Rats (Holtzman 250–300 g) were anesthetized with 2, 2, 2-tribromoethanol (20 mg/100 kg b. wt.) and a stainless steel cannula were implanted into their SFO. The volume of injection was 0.2 μl. The amount of saliva secretion was studied over a 5-min period. Pilocarpine (40 μg), L-NAME (40 μg) and LAR (30 μg) were used in all experiments for the injection into the SFO. Pilocarpine (10, 20, 40, 80 and 160 μg) injected into SFO elicited a concentration-dependent increase in salivary secretion. L-NAME injected prior to pilocarpine into the SFO increased salivary secretion and water intake due to the effect of pilocarpine. LAR injected prior to pilocarpine into the SFO attenuated the salivary secretion and water intake. Pilocarpine, injected into the SFO increased the MAP and decreased heart rate (HR). L-NAME injected prior to pilocarpine into the SFO potentiated the pressor effect of pilocarpine with a decrease in HR. LAR injected into the SFO prior to pilocarpine attenuated the increase in MAP with no changes in HR. The present study suggests that the SFO nitrergic cells interfere in the cholinergic pathways implicated in the control of salivary secretion, fluid and cardiovascular homeostasis.

Keywords: Pilocarpine; Subfornical organ; Nitric oxide; Saliva; Fluid and cardiovascular balance

1. Introduction

The SFO is a circumventricular structure that participates in the regulation of body fluid homeostasis [1–3] Neurons situated in the SFO became transneuronal labeled following pseudorabies virus injections into submandibular or into sublingual gland. These neurons are efferently connected to a chain of central neurons directed to secretory or vascular tissue of the submandibular or the sublingual gland [4].

Some procedures should be taken to relieve the symptoms of dry mouth, to control oral disease and to improve salivary functions. With a systematic approach and aggressive control most patients with dry mouth can achieve oral comfort and adequate oral function [5]. Pilocarpine, a muscarinic cholinergic agonist, induces vasodilatation and copious salivation when

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administered systemically, by activation of the parasympathetic system [6–8]. Pilocarpine HCL stimulates labial (minor) salivary gland flow in patients with Sjögren's syndrome [9]. The damage produced in salivary glands after irradiation is reduced by prior administration of pilocarpine [10]. Pilocarpine has a sympathomimetic (probably beta-receptor mediated) stimulatory effect, which is also implicated in the cardiovascular regulation and salivary secretion [11]. One of the questions that remain to be fully answered is if the SFO is important to the sialogogue effect of pilocarpine. We know that pilocarpine when used systemically may act in areas of the central nervous system that lacks the normal blood–brain barrier. The present study will focus on the effect of pilocarpine on salivary flow when injected directly into SFO.

It has been demonstrated that NO of the supraoptic nucleus influences the salivary secretion, sodium renal excretion, urinary volume and arterial blood pressure [12]. L-NAME and FK 409 injections into median preoptic nucleus in conscious rats influence water and salt intake, sodium and urine excretion and arterial blood pressure [13]. It has been demonstrated that moxonidine and rilmenidine injected into medial septal area reduce the salivation induced by pilocarpine [14], implicating vasopressin receptors [15].

Morphological, morphometric and stereological changes of submandibular glands were observed after lesion of AV3V and ventromedial nucleus of the hypothalamus [16]. The function of the submandibular and sublingual glands as thermoregulatory effector organs in rats during extreme heat stress has been clearly established [17].

L-NAME significantly increases the discharge of neurons of the SFO showing the importance of NO in the electrical activity of the SFO [18]. The parenteral administration of monosodium glutamate (MSG) to neonatal rats induces specific lesions in the central nervous system that led to a well-known characterized neuroendocrinologial dysfunction. Treatment of neonatal rats with MSG induced a substantial reduction in the volume of the SFO and in the number of its nitrergic cells with regards to control animals [19]. These findings suggest that the SFO could be implicated in some physiological functions such as salivary secretion and cardiovascular alterations observed in MSG-treated rats. Nitric oxide (NO) plays an excitatory role in the regulation of parasympathetic nerve inducing salivary secretion in the submandibular gland of rats [20]. L-NAME and L-arginine increased and decreased the salivation induced by pilocarpine respectively [21,22]. The present paper addresses the possible influence of NO on the effect of pilocarpine-induced salivary secretion when injected into the SFO.

Pilocarpine has been used extensively over the last century as the best sialogogue rather than other cholinomimetics agents but it produces cardiovascular alterations, as side effects. Nitrergic and angiotensinergic cells of SFO play an important role in the hydromineral and cardiovascular regulation [23–25]. Different data indicate that the SFO is indeed the target of afferent from osmosensitive and barosensitive systems concerned with fluid homeostasia and cardiovascular regulation [26–28]. The response mechanism of pilocarine effect, when used as a sialogogue agent, on water intake and cardiovascular regulation remains to be fully understood. In the present studies, we hypothesized that the SFO is one of the most important sites involved in the central effect of pilocarpine for the regulation of salivary secretion, water intake and cardiovascular alterations. In such case the experiments were designed to determine the participation of NO of the SFO with the sialogogue, dipsogenic and cardiovascular alterations induced by pilocarpine injected into the SFO.

2. Materials and methods

2.1. Animals

Male Holtzman rats (250–300) were housed in individual metabolic cages, with free access to food pellets and tap water. The Medical Ethics Committee of the Universidade Estadual Paulista UNESP approved all protocols in this study.

The temperature in the animal colony was maintained at approximately 23 °C. The 12:12-h light–dark cycle began with lights on at 08:00. All animals used in the experiments received the same drugs but at different times. However, each animal was used in 3 experiments at most.

A prophylactic dose of penicillin (30,000 IU) (Pentabiotico Fontoura Wyeth) was given intramuscularly (i.m.) and presurgically.

2.2. Brain surgery

After an acclimatization period of 7 days, the animals were maintained under 2,2,2-tribromoethanol (Aldrich) (20 mg/100 kg b. wt.), intraperitoneally [i.p.] anesthesia throughout surgery and restrained in a stereotaxic apparatus (David Kopf model for rats). A longitudinal incision was made in the skin of each animal's head, the subcutaneous tissue was pulled back and the skull was drilled with a spherical drill. The coordinates for approaching the SFO were obtained from the Paxinos and Watson atlas [29]. A stainless steel cannula (14 × 0.4 mm o. d.) was stereotaxically implanted into the SFO with the following coordinates AP = 1.3 mm caudal to the bregma; V = 4.2 mm from the dura mater; L = 0.0 mm from the sagittal line. The cannula was fixed to the skull with screws and acrylic resin. The insertion of a close-fitting stylet kept the lumen of the cannula free of debris and clots.

2.3. Drugs

- Saline 0.15 M NaCl (control)
- Pilocarpine 10, 20, 40, 80, and 160 μg · 0.2 μl^{-1} purchased from Sigma (Chemical Co., St. Louis, MO)
- L-NAME (40 μg · 0.2 μl^{-1}) purchased from Sigma (Chemical Co., St. Louis, MO)
- L-arginine (30 μg · 0.2 μl^{-1}) purchased from Sigma (Chemical Co., St. Louis, MO).

2.4. Drugs injections

Bolus intracranial injections were made after gently removing the animal from their cage, replacing the stylet by