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Effect of Pilocarpine and Angiotensin II on Salivary Flow, Total Protein and Electrolyte Concentrations of Saliva

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Abstract: Present studies have focused on the effect of intraperitonially i.p. injection of angiotensin II (ANG II) and pilocarpine on the salivary secretion, salivary amylase, saliva total proteins, saliva calcium sodium and potassium. Rats were anesthetized with urethane (1.25 g kg^{-1} b. wt.). The amount of saliva secretion was studied over a 5 min period. Male Holtzman rats (250-300 g) were housed in individual metabolic cages, with free access to food pellets and tap water ad libitum. The basal salivary flow after i.p saline 0.15 M NaCl as control was 17±1 mg/5 min. Angiotensin II (120 ng/0.5 mL), increased the salivary flow. The previous application of losartan (AT_1 ANG II receptors antagonist blocked the sialogogue effect of ANG II. PD123319 (AT_2 ANG II receptors antagonist) blocked with smaller intensity the effect of ANG II. Animals treated with ANG II showed no change in the concentration of total protein, salivary amylase and calcium. The same happened when treated with losartan or PD123319. ANG II increased the concentration of sodium and decreased the concentration of potassium. Losartan blocked the effect of ANG II on sodium and potassium concentration. Pilocarpine increased the salivary flow. Losartan and PD123319 produced no change in salivary flow stimulated by pilocarpine. Pilocarpine did not alter the total protein, salivary amylase and calcium. Previous application of losartan and PD123319 produced no change in this pilocarpine effect. Pilocarpine decreased sodium and potassium concentration. Losartan and PD123319 produced no change in these effects of pilocarpine. In conclusion the results of the present study showed the importance of ANG II and pilocarpine in the control of the mechanism of salivary secretion and in the sodium and potassium-saliva concentration.

Key words: Saliva flow, saliva composition, angiotensin II receptors, pilocarpine

INTRODUCTION

The pilocarpine, a muscarinic cholinergic agonist, acts primarily on receptors of salivary glands inducing intense salivation[1], but intracerebroventricular (i.c.v.) injection of pilocarpine has also been used as a model to induce salivation[2]. Salivation resulting from central or peripheral injections of pilocarpine is impaired by the lesion of the anteroventral of the third ventricle region (AV3V). The AV3V is composed rostrally by the organum vasculosum of the lamina termilalis and extends caudally until the limits of the preoptic area to the hypothalamus, encompassing the ventral median preoptic nucleus, periventricular nucleus of the third ventricle and part of the preoptic area[3]. The use pilocarpine, an important therapeutic agent in cases of xerostomia, cause undesirable secondary cardiovascular effects.

The treatment with captopril, a blocker agent of angiotensin converting enzyme (ECA), increased the salivary secretion, but the composition of saliva is no changed[4]. There is a central circuit dependent on the AV3V region that mediates this salivation and pilocarpine injected i.c.v. activates autonomic efferent that mediate the salivation. There is a compensation in salivary flow when retreat of a parotid gland by tumor[5].

The participation of the Atrial Natriuretic Factor (ANF) in parotid salivary glands is connected with the composition of saliva[6]. The effect of rilmenidine, an imidazolic agonist, when injected by continuous infusion in the vein, decreases the salivary flow[7].

The acinar cells of the patients with Sjögren's Syndrome are able response by acetylcholine because move the calcium intracellular and active the potassium and chloride channels inducing a secretion of salivary

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fluid. The loss of the sensibility by acetylcholine is the reason of the disturbance of salivary flow in the patients with this syndrome[8].

The effects of lithium concentration in submandibulary glands function were studied by Ahmad-Reza *et al.*[9] showing the influence of this ion in salivary flow and in composition of the total proteins, amylase, sodium, potassium and calcium concentration. Baba *et al.*[10] studied the action of several drugs in protein content and in salivary flow.

O'conne *et al.*[11] studied the action of pilocarpine in salivary amylase content. Recently, Almeida *et al.*[12] demonstrate the important function of the Subfornical Organ (SFO) in regulation of the water and sodium intake induced by adrenergic stimulation of the lateral hypothalamus. The importance of AT_1 and AT_2 receptors of angiotensin II of the paraventricular nucleus was also demonstrates in our laboratory[13]. Saad *et al.*[14] demonstrated the participation of nitric oxide in central and peripheral regulation of water intake. The salivary secretion induced by central and peripheral application of pilocarpine has influence of nitric oxide and the angiotensin II has important role in cardiovascular and hidroelectrolyte regulation of the organism.

Based on this study, the control of the salivary flow and composition is influenced by several factors and then we decide to test the effects of AT_1 and AT_2 receptors of angiotensin II on the salivary flow and composition when after administration of pilocarpine and angiotensin II.

The saliva is made up of 99% water and 1% inorganic and organic components and was demonstrated that 45% of variation in rate of salivary flow is normal but values below 45% are deficient (salivary hipofunction)[15].

MATERIALS AND METHODS

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

Intraperitoneal injection of the solution drugs: Angiotensin II (120 ng/0.5 mL), pilocarpine (4 mg kg^{-1}), Losartan (40 ng/0.5 mL), PD 123319 (40 ng/0.5 mL), Sigma Chemical Company, St. Louis, MO and NaCl 0.15 M (0.5 mL intraperitoneal (i.p.) as control).

Salivary secretion: The flow studied was of the three salivary glands. The animals were anaesthetized with 2, 2, 2-tribromoethanol and the saliva was collected with pre weighed small cotton wool balls inserted into the animal's mouth, a technique slightly different from that used by Schallet *et al.*[16]. Such technique led us to collect the whole 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 and with the other two placed under the tongue. The amount of saliva secreted was measured 7 min before the injection of ANG II (baseline saliva secretion) and 7 min after the injection of ANG II. The losartan (AT_1 receptors antagonist) was injected 5 min before of ANG II as well as the PD123319 (AT_2 receptors antagonist).

Biochemical analyses: The salivary amylase was determined by Carawary's method[17]. The total proteins were determined by Bradford's method[18]. The calcium was determined by Cresolftalein's method[19]. The sodium and potassium were determined by photometer of flame (model IL 143 Lexigton, Massachuttes USA).

Statistical analysis: The results are reported as mean±EPM. The ANOVA and Dunnett's t test were used to determine the significance. The values were considered statistically significant where $p>0.05$.

Experimental protocol: The parameters were obtained from different experimental sessions and from several groups of animals after the injection of the following drugs.

- Intraperitoneal saline solution
- Intraperitoneal ANG II
- Losartan 5 min before ANG II
- PD123319 5 minutes before ANG II

The same experiments with the ANGII antagonists also were used for pilocarpine.

RESULTS

Effect of intraperitoneal application of ANG II in salivary functions

Salivary flow: The basal salivary flow after i.p of saline 0.15 M NaCl as control was 17±1 mg/5 min. The application of ANG II increased the salivary flow to 26±0.6 mg/5 min. This value was statistically different when compared with the control value. The previous application of losartan blocked the effect of ANG II to 16±3 mg/5 min. The previous administration of PD123319 blocked with smaller intensity the effect of ANG II (20±1 mg/5 min) (Table 1).

Salivary total protein: Animals treated with ANG II showed no change in the concentration of total protein when compared with the control value (6.2±0.8 mg kg^{-1}).

Table 1: Effect of angiotensin II on saliva function

Treatments	Flow (mg/5 min)	Total protein (mg kg^{-1})	Amylase (U mL^{-1})	Ca^{++} (mEq Ll^{-1})	Na^{+}	K^{+} (mEq L^{-1})
SALINE	17±1	5.9±1.0	854±12.33	3.12±0.50	6.3±0.3	13.0±0.4
ANG II	26±0.6*	6.2±0.8	852±12.21	3.11±0.12	11.8±0.2*	1.9±0.4*
LOS+ANG II	16±3.0*	6.5±0.7	891±11.12	3.19±0.4	6.8±0.7*	12.0±0.9*
PD+ANG II	20±1.0*	5.9±1.0	865±10.36	2.97±0.6	9.0±0.7*	8.0±0.8*

*Different from control and ANGII $p<0.01$

Table 2: Effect of pilocarpine on saliva function

Treatments	Flow (mg/5 min)	Total protein (mg kg^{-1})	Amylase (U m Ll^{-1})	Ca^{++} (mEq L^{-1})	Na^{+}	K^{+} (mEq L^{-1})
SALINE	19±2	5.9±0.2	821±6.9	3.1±0.5	5.99±0.3	12.0±0.3
PILO	490±12*	6.3±0.32	812±9.10	3.9±0.2	3.20±0.1*	6.6±0.4*
LOS+PILO	521±13*	6.0±0.21	798±11.60	3.2±0.4	4.10±0.9*	4.1±0. 9*
PD+PIL	499±12*	6.2±0.30	844±14.90	3.3±0.3	4.30±0.4*	5.3±0.4*

*Different from control and Pilocarpine $p<0.001$

The same happened when treated with losartan or PD123319 with values of 6.5±0.7 mg kg^{-1}. The control value of total protein was 5.9±1 mg kg^{-1} (Table 1).

Salivary amylase: The salivary amylase is not changed with the treatment by ANG II or previous treatment with losartan and PD123319 (852±12.21; 891±11.12 and 865±10.36 U mL^{-1}, respectively) compared the control value (854±12.32 U mL^{-1}) (Table 1).

Calcium: The ANG II produced no change in the calcium concentration compared to the control value. The same happened with losartan and PD123319 (Table 1).

Sodium and potassium: ANG II increased the concentration of sodium and decreased the concentration of potassium in the saliva compared with the control values (18±0.2 and 1.9±0.41 mEq L^{-1}, respectively). Concentration of sodium and potassium in the control animals was 6.33±0.29 and 13.41±0.38 mEq L^{-1}, respectively. Losartan blocked the effect of ANG II on sodium and potassium concentration with values 6.83±0.69 and 12±0.2 mEq L^{-1}, respectively. The pretreatment with PD123319 changed the sodium and potassium concentration with smaller intensity than losartan (9±0.67 and 8±0.81 mEq L^{-1}, respectively) (Table 1).

Effect of intraperitoneal application of pilocarpine in salivary functions

Salivary flow: Pilocarpine increased the salivary flow (490±12 mg/5 min) compared to the control value (19±2 mg/5 min). The pretreatment with losartan produced no change in salivary flow stimulated by pilocarpine (521±13 mg/5 min). Previous administration of PD123319 also produced no change in the salivary flow determined by pilocarpine (499±12 mg/5 min) (Table 2).

Total protein: Pilocarpine did not alter the total protein (6.3±0.32 mg kg^{-1}) in relation to the control group (5.9±0.22 mg kg^{-1}). Previous application of losartan produced no change in the pilocarpine effect (6.0±0.21 mg kg^{-1}). The pretreatment with PD123319 also did not change the effects effect of pilocarpine (6.2±0.3 mg kg^{-1}) (Table 2).

Salivary amylase: Pilocarpine produced no change in the salivary amylase with relation the control group (812±9.1 U mL^{-1}) vs (821±6.9 U mL^{-1}). Pretreatment with losartan and PD123319 produced no change in the effects of pilocarpine (798±11.6 and 844±14.9 U mL^{-1}, respectively) (Table 2).

Calcium: No alteration in the calcium concentration in the saliva was produced by pilocarpine (3.98±0.18 mEq L^{-1}) when compared the control group (3.12±0.14 mEq L^{-1}). Previous application of losartan and PD123319 did not change the effects of pilocarpine (Table 2).

Sodium and potassium: Pilocarpine decreased sodium concentration (3.22±0.12 mEq L^{-1}) with relation of the control group (5.99±0.3 mEq L^{-1}). Losartan and PD123319 produced no change in the effects of pilocarpine. The potassium concentrations decreased when pilocarpine was administered (6.6±0.81 mEq L^{-1}) with relation to the control group (12.32±0.33 mEq L^{-1}). Previous administration of losartan and PD123319 produced no change in the effects of pilocarpine (Table 2).

DISCUSSION

These results demonstrated that the ANG II injected i.p. increased the concentration of sodium and decrease the concentration of potassium in saliva when compared with control group. The ANG II also increases the salivary

flow. These results find support in the effects obtained by Abdollahi and Safarhamidi[20]. The effect of aldosterone on the parotid glands in the regulation of sodium metabolism has been demonstrated[21].

The ANG II did not change the salivary concentration of total protein, salivary amylase and calcium. The organic calcium can be influenced by other mechanisms[22]. The previous application of losartan (antagonist AT_1 receptor) blocked the effects of ANG II about salivary flow as well as the PD123319 (antagonist AT_2 receptor), although with smaller intensity. The data showed that AT_1 receptors are more effective, although AT_2 receptors also are important. The ANG II also increases the urine volume and the sodium urine excretion[23]. These results also showed that the pilocarpine increase the salivary flow when compared the control group. Pretreatment with AT_1 and AT_2 antagonists did not changed the effects of pilocarpine on the salivary flow. The injection of pilocarpine centrally increases the salivary flow that is increase by previous application of L-NAME (an inhibitor of nitric oxide synthase)[24]. The salivary flow induced by pilocarpine is inhibited by centrally injection of noradrenalin[25]. The administration of pilocarpine decreases the salivary sodium concentration. Pretreatment with losartan or PD123319 did not cause any changed in these results. The potassium concentration also decreases and the AT_1 and AT_2 ANGII antagonists receptors produced no alterations in these results, but β-adrenergic receptors influence the effects of pilocarpine[24]. These results were supported by the data obtained by Nagler and Nagler[26]. The electrolyte concentration of saliva depends on salivary production volume[27]. Also the participation of nitric oxide of the central nervous system influence salivary secretion and the electrolyte metabolism[28-30]. The patients with Sjögren´s syndrome had an increase in sodium concentration when compared with the control groups. The increase in the concentration of sodium can be attributed to the reduction of sodium absorption[31]. The concentration of sodium is smaller in saliva than the plasma, around seven times and potassium concentration is around five times smaller in plasma than the saliva[32]. The concentration of sodium in saliva after one year of radiotherapy was smaller than before the treatment, but potassium concentration in saliva did not changed[33]. One important finding of this study is that ANG II has influence in salivary flow and electrolytes salivary concentration, but the effect of pilocarpine is more strongly than that of ANG II. In conclusion the results of the present study showed the importance of ANG II and pilocarpine in the control of the mechanism of salivary secretion and in the sodium and potassium-saliva concentration. Also showed the participation o AT_1 and AT_2 ANG II receptors in this regulatory mechanism.

REFERENCES

1. Wiserman, L.R. and D. Fauds, 1995. Oral pilocarpine: A review of its pharmacological proprieties and clinical potential in xerostomia. Drugs, 49: 143-155.
2. Renzi, A., W.A. Saad, L.A.A. Camargo, L.A. De Lucca and J.V. Menani, 1993. Involvement of the central nervous system in the salivary secretion induced by pilocarpine in rats. J. Dent. Res., 72: 1481-1484.
3. Johnson, AK., 1985. The pereventricular anteroventral third ventricle (AV3V): Its relationship with the subfornical organ and neural systems involved in maintaining body fluid homeostasis. Brain Res. Bull., 15: 595-601.
4. Nederfors, T., C. Dahlof, T. Ericsson and S. Twetman, 1995. Effects of the antihypertensive drug captopril on human salivary secretion rate and composition. Eur. J. Oral Sci., 103: 351-354.
5. Chaushu, G., S. Dori, B.A. Sela, S. Taicher, J. Kronenberg and Y.P. Talmi, 2001. Salivary flow dynamics after parotid surgery: A preliminary report. Otol. Head and Neck Surg., 124: 270-273.
6. Valentino, B., E.F. Lipari, F. Carini and V. Valenza, 1999. Immunohistochemical localization of Atrial Natriuretic Factor (ANF) in the excretory system of the rabbit parotid gland. Europ. J. Histochem., 43: 241-245.
7. De Visser, S.J., J.M.A. van Gerven, R.C. Schoemaker and A.F. Cohen, 2001. Concentration-effect relationship of two infusion rates of the imidazoline antihypertensive agent rilmenidine for blood pressure and development of side effects in healthy subjects. Brist. J. Clinical Pharm., 51: 423-428.
8. Dawson, L.I., E.A. Field, A.R. Harmer and P.M. Smith, 2001. Acetylcholine-evoked calcium mobilization and ion channel activation in human labial gland acinar cells from patients with primary A Jogren's syndrome. Clin. Exp. Immunol., 124: 480-485.
9. Ahmad-Reza, D., A. Mohammad and A. Hekmat, 1995. Effects of lithium on rat parotid submandibulary gland functions. Gen. Pharm., 26: 851-854.
10. Baba, A., K. Tamiguchi, W. Motokawa and K. Abe, 1994. Fluid and protein secretion by the submandibular glands of weanling rats in response to various agonists. Archs. Oral Biol., 39: 979-984.
11. O'Connel, A.C., S.K. Pearson and W.H. Bowen, 1994. Pilocarpine alters caries development in partially desalivated rats. J. Dent. Res., 73: 637-643.

12. Almeida, N.A.A., V.R. Antunes, W.A. Saad and L.A.A. Camargo, 1999. Effect of the alpha antagonists into the lateral hypothalamus on the water and sodium intake induced by angiotensin II. Brain Res. Bull., 48: 521-525.
13. Camargo, L.A.A. and W.A. Saad, 1999. Effect of angiotensin II receptor subtype 1 and 2-selective ligands injected into the paraventricular nucleus of conscious rats. Reg. Pept., 84: 91-96.
14. Saad, W.A., L.A.A. Camargo, R. Saad, A.F. Pereira and S. Simões, 1999. Effect of injection of L-NAME on drinking response. Brazilian J. Med. Biol. Res., 32: 1413-1416.
15. Chezzi, E.M., L.A. Lange and F.A. Ship, 2000. Determination of variation of stimulated salivary flow rates. J. Den. Res., 79: 1874-1878.
16. Scharllert, R.S., 1978. Saliva hypersecretion during aphagia following lateral hypothalamic lesions. Physiol. Behav., 21: 461-463.
17. Caraway, W.T., 1959. Amilase determination. Am. J. Clin. Pathol., 32: 97.
18. Bradford, M.M., 1976. A rapid method for the quantitation of micrograms quantities of protein utilizing the principle of protein dye binding. Anal. Biochem., 72: 248-254.
19. Connerty, H.V. and A.R. Briggs, 1966. Calcium determination. Am. J. Clin. Pathol., 45: 290.
20. Abdollahi, M. and M. Safarhamidi, 2002. Protection by nitric oxide of morphine-induced inhibition of rat submandibular gland function. Pharm. Res., 45: 88-92.
21. Riad, F., J. Lefaivre, C. Tournaire and J.P. Barlet, 1986. Aldosterone regulates salivary sodium secretion in cattle. J. Endocrinol., 108: 405-411.
22. MacDougall, J.G., E.I. Johnson, J.P. Coglan, D.A. Denton B.A. Scoggins and R.D. Wright, 1984. Calcium antagonists and stimulus-secretion coupling of aldosterone. J. Hypertens. Suppl., 2: S531.
23. Camargo, L.A.A., W.A. Saad, S. Simões, T.A.F.B. Santos and W.A. Saad, 2002. Interaction between paraventricular nucleus and septal area in the control of physiological responses induced by angiotensin II. Braz. J. Med. Biol. Res., 35: 1017-1023.
24. Saad, W.A., I.F.M.S. Guarda, R.S. Guarda, L.A.A. Camargo, T.A.F.B. Santos, S. Simões and W.A. Saad, 2002. Role of nitric oxide and beta adrenoceptors of the central nervous system on the salivary flow induced by pilocarpine injection into the lateral ventricle. Pharm. Bioch. Behav., 72: 229-235.
25. Moreira, T.S., T.A.C. Takura, L.A. De Luca, A. Renzi and J.V. Menani, 2002. Inhibition of pilocarpine-induced salivation in rats by central noradrenaline. Arch. Oral Biol., 47: 429-434.
26. Nagler, M.R. and A. Nagler, 2001. The effect of pilocarpine on salivary constituents in patients with chronic graft-versus-host-disease. Arch. Oral Biol., 46: 689-695.
27. Almstahl, A. and M. Wikstrom, 2003. Electrolytes in stimulated whole saliva in individuals with hyposalivation of different origins. Arch. Oral Biol., 48: 337-344.
28. Saad, W.A., I.F.M.S. Guarda, L.A.A. Camargo, T.A.F.B. Santos, R.S. Guarda, W.A. Saad and S. Simões, 2003. 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 MnPO and intraperitoneally. Braz. J. Med. Biol. Res., 36: 897-905.
29. Saad, W.A., L.I. Gutierrez, I.F.M.S. Guarda, L.A.A. Camargo, T.A.F.B. Santos, W.A. Saad, S. Simões and R.S. Guarda, 2004. Nitric oxide of the supraoptic nucleus influences the salivary secretion, sodium renal excretion, urinary volume and arterial bloods pressure induced by pilocarpine. Life Sci., 74: 1593-1603.
30. Saad, W.A., I.F.M.S. Guarda, L.A.A. Camargo, T.A.F.B. Santos, W.A. Saad, S. Simões and R.S. Guarda, 2002. Novel evidence that nitric oxide of the medial septal area influences the salivary secretion induced by pilocarpine. Life Sci., 70: 2403-2412.
31. Pedersen, A.M., J. Reibel and B. Nauntofte, 1999. Primary Sjogren's syndrome (pSS): Subjective symptoms and salivary findings. J. Oral Pathol., 28: 303-311.
32. Mandel, I.D., 1980. Sialochemistry in diseases and clinical situations affecting salivary glands. Crit. Ver. Clin. Lab. Sci., 12: 321-366.
33. Funegard, U., L. Franzén, T.H. Ericson and R. Henriksson, 1994. Parotid saliva composition during and after irradiation of head and neck cancer. Oral Oncol. Eur. J. Cancer, 30B: 230-233.