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

Wilson Abrão Saad, Laura Izabel Gutierrez, Regina Célia Vendramini,
André Henrique Freuria de Oliveira, Luiz Antonio de Arruda Camargo and Gustavo Garcia

Basic Institute of Biosciences, UNIANTU, Taubaté, SP, Brazil
Department of Exact and Natural Science, UNIARA, Araraquara, SP Brazil
Department of Physiology and Pathology, School of Dentistry, Paulista State University, UNESP, Araraquara, SP Brazil

Abstract: Present studies have focused on the effect of intraperitoneally 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\textsuperscript{-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, ANG II receptors antagonist blocked the sigalogue effect of ANG II. PD123319 (AT; 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\textsuperscript{1}, but intracerebroventricular (i.c.v.) injection of pilocarpine has also been used as a model to induce salivation\textsuperscript{2}. Salivation resulting from central or peripheral injections of pilocarpine is impaire by the lesion of the anteroverentral of the third Ventricule region (AV3V). The AV3V is composed rostrally by the organum vasculosum of the lamina terminalis 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\textsuperscript{2}. 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\textsuperscript{5}. 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\textsuperscript{5}.

The participation of the Atrial Natriuretic Factor (ANF) in parotid salivary glands is connected with the composition of saliva\textsuperscript{9}. The effect of rilmenidine, an imidazolic agonist, when injected by continuous infusion in the vein, decreases the salivary flow\textsuperscript{9}.

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
fluid. The loss of the sensibility by acetylcholine is the reason of the disturbance of salivary flow in the patients with this syndrome[6].

The effects of lithium concentration in submandibular 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.[14] 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 Subformical Organ (SFO) in regulation of the water and sodium intake induced by adrenergic stimulation of the lateral hypothalamus. The importance of AT1 and AT2 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 AT1 and AT2 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.[10]. 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 (AT1 receptors antagonist) was injected 5 min before of ANG II as well as the PD123319 (AT2 receptors antagonist).

Biochemical analyses: The salivary amylase was determined by Caraway’s method[9]. The total proteins were determined by Bradford’s method[9]. The calcium was determined by Cresolphenol’s method[19]. The sodium and potassium were determined by photometer of flame (model IL 143 Lexighton, Massachusetts 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±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 PD1 23319 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

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Flow (mg/5 min)</th>
<th>Total protein (mg kg⁻¹)</th>
<th>Amylase (U mL⁻¹)</th>
<th>Ca⁺⁺ (mEq L⁻¹)</th>
<th>Na⁺ (mEq L⁻¹)</th>
<th>K⁺ (mEq L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SALINE</td>
<td>17±1</td>
<td>5.9±0.1</td>
<td>854±12.33</td>
<td>3.12±0.50</td>
<td>6.3±0.5</td>
<td>13.0±0.4</td>
</tr>
<tr>
<td>ANG II</td>
<td>26±0.5*</td>
<td>6.2±0.8</td>
<td>852±12.21</td>
<td>3.11±0.12</td>
<td>11.8±0.2*</td>
<td>1.9±0.4*</td>
</tr>
<tr>
<td>LOS+ANG II</td>
<td>16±3.0*</td>
<td>6.5±0.7</td>
<td>895±11.12</td>
<td>3.16±0.4</td>
<td>8.8±0.7*</td>
<td>12.0±0.9*</td>
</tr>
<tr>
<td>PD+ANG II</td>
<td>20±1.0*</td>
<td>5.9±0.1</td>
<td>865±10.36</td>
<td>2.97±0.6</td>
<td>9.6±0.7*</td>
<td>8.0±0.9*</td>
</tr>
</tbody>
</table>

*Different from control and ANGII p<0.01

Table 2: Effect of pilocarpine on saliva function

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Flow (mg/5 min)</th>
<th>Total protein (mg kg⁻¹)</th>
<th>Amylase (U mL⁻¹)</th>
<th>Ca⁺⁺ (mEq L⁻¹)</th>
<th>Na⁺ (mEq L⁻¹)</th>
<th>K⁺ (mEq L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SALINE</td>
<td>19±2</td>
<td>5.9±0.2</td>
<td>821±6.9</td>
<td>3.1±0.5</td>
<td>5.9±0.3</td>
<td>12.0±0.3</td>
</tr>
<tr>
<td>PILO</td>
<td>49±12*</td>
<td>6.3±0.32</td>
<td>812±9.10</td>
<td>3.9±0.2</td>
<td>3.7±0.1*</td>
<td>6.6±0.4*</td>
</tr>
<tr>
<td>LOS+PILO</td>
<td>52±13*</td>
<td>6.0±0.21</td>
<td>798±11.60</td>
<td>3.2±0.4</td>
<td>4.1±0.0*</td>
<td>4.1±0.9*</td>
</tr>
<tr>
<td>PD+PIL</td>
<td>49±12*</td>
<td>6.2±0.30</td>
<td>844±14.90</td>
<td>3.3±0.3</td>
<td>4.3±0.4*</td>
<td>5.3±0.4*</td>
</tr>
</tbody>
</table>

*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; 895±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 6.3±0.29 and 13.4±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 12.6±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 (194±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 to 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 (3.9±0.18 mEq L⁻¹) when compared the control group (3.1±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 (3.2±0.12 mEq L⁻¹) with relation of the control group (5.9±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 (12.3±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\(^{29}\). The effect of aldosterone on the parotid glands in the regulation of sodium metabolism has been demonstrated\(^{11}\).

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\(^{21}\). 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 change 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 change 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 \(\beta\)-adrenergic receptors influence the effects of pilocarpine\(^{26}\). These results were supported by the data obtained by Nagler and Nagler\(^{29}\). 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 of AT\(_1\), and AT\(_2\) ANG II receptors in this regulatory mechanism.

REFERENCES


