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Communication

Effect of constant light and immobilization stress on rat submandibular saliva secretory response induced by cholinergic and peptidergic agonists.

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Abstract: The aim of this work was to analyse the parasympathetic control of submandibular saliva secretory response to cholinergic and peptidergic agonists in rats chronically exposed to constant light or repeated immobilization. Thirty two adult male Wistar rats were used: LL (8 rats exposed to constant light for 20 days), IMO (8 rats submitted to 14:10 h light: dark cycle and immobilized 2 hours daily for 7 days), and control (16 rats not exposed to stress and submitted to 14:10 hours light:dark cycle). Saliva was collected under anesthesia from the salivary ducts of submandibular glands under increasing doses of methacholine and substance P. Secretory responses (µg/saliva/mg dry weight gland) to methacholine were significantly higher in LL and IMO groups compared to control for the following doses (µg/kg body weight): 3 (153±9 *versus* 46±3, $p<0.001$ and 76±3 *versus* 40±3, $p<0.001$), 10 (379±23 *versus* 277±8, $p<0.001$ and 275±19 *versus* 250±10, $p<0.01$) and 30 (729±25 *versus* 695±19, $p<0.05$ and 1008±39 *versus* 640±20, $p<0.001$). Also, responses to substance P were significantly increased in LL and IMO groups compared to control for the following doses: 0.2 (80±3 *versus* 30±3, $p<0.01$ and 94±16 *versus* 31±3, $p<0.001$), 0.5 (328±20 *versus* 231±16, $p<0.01$ and 531±31 *versus* 219±25,$p<0.001$), 1 (681±35 *versus* 547±30, $p<0.01$ and 1031±63 *versus* 563±53, $p<0.001$), and 5 (2222±88 *versus* 1868±59, $p<0.01$ and 3230±145 *versus* 1921±218, $p<0.001$). In conclusion, supersensitivity of secretory response to both agonists suggests that chronic exposure of rats to stressors capable of activating the sympathetic adrenal system promotes inhibition of the parasympathetic control of salivary secretion.

Keywords: submandibular gland; saliva, substance P; methacholine; adverse effects.

INTRODUCTION.

The control of salivary secretion is carried out by both branches of the autonomic nervous system working synergistically. The parasympathetic system is controlled by cholinergic and peptidergic agonists, among others, and produces an increase of saliva output rich in water and electrolytes whereas the sympathetic system reduces the release of saliva but increases markedly its protein content.[1]

In humans, chewing and the taste of food release abundant salivary flow to facilitate both chewing and swallowing. During most of the day, when no food intake occurs, other less conspicuous stimuli and the tonic activity itself of the autonomic nerves maintain a scarce, though constant flow of basal saliva which provides moisture, oral comfort and protein components with soft and hard tissue protective and repair properties within the oral cavity.[2] Studies in rats has been extensively used to evaluate

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the influence of environmental changes on the secretion and/or composition of saliva. These studies made it possible to extrapolate the results to humans.[1,3]

Under stressful conditions, when the body is exposed to external or internal stimuli, the sympathetic adrenal system (SAS) and other neuroendocrine systems play a significant homeostatic role both in human and in experimental animals.[4]

The increase in mRNA levels of catecholamine-synthesizing enzymes in sympathetic ganglia and adrenal medulla, in addition to elevated concentrations of adrenaline or nor-adrenaline in tissues and blood, clearly show the activation of one or both SAS components (sympathetic nerves and adrenal medulla) as a result of various kinds of stimuli.[4]

When rats are exposed to either chronic constant light (LL) or repeated immobilization stress (IMO), the activation of adrenal medulla is accompanied by either inhibition in LL or activation in IMO of the neural sympathetic component to the salivary glands.[4,5] Taking advantage of this difference, we have studied SAS control in the rat submandibular gland.

Desensitization of secretory responses to adrenergic agonists (*in vivo*) by α_2 and β-adrenergic receptors down regulation, suggest that in LL rats an increase in sympathetic control of saliva occurs due to circulating catecholamines mainly released by the adrenal medulla. However, in IMO rats, the increase of sympathetic control to the gland would be mediated by both neural and medullar SAS components.[6]

In several tissues the SAS activation by either external or internal stimuli is accompanied by coordinated changes of the parasympathetic nervous system (PNS).[4,7] Considering the PNS relevance in the control of saliva secretion, this study aims to analyze the submandibular salivary secretory response mediated by cholinergic and peptidergic agonists in rats chronically exposed to LL or IMO.

MATERIALS AND METHODS.

Animals

All procedures were carried out in accordance with the standards set by the Bioethics Committee of the School of Medicine, Universidad Nacional de Córdoba, Argentina.

Thirty-two male Wistar rats (300-350g) 120 days old were housed in transparent polycarbonate boxes held in a room at 24±2ºC and provided with food (GEPSA SA, Argentina) and tap water *ad libitum* until 18 hours before testing the secretory response, when food but not water was withheld.

Animal groups

Constant light group (LL) included rats (n=8) continuously exposed to cool white light (150-200lx) for 20 days.

Immobilized group (IMO) included rats (n=8) submitted to 14:10 hours light-dark cycle and immobilized 2 hours daily (from 8 to 10 am) for 7 successive days. Immobilization stress was accomplished by taping the fore limbs and hind limbs of the rat in a prone position with surgical tape to metal mounts attached to a board.[4]

Control group (C) included rats (n=16) not exposed to stress and submitted to 14:10 hours light-dark cycle. The onset of the daily light phase (0600 hours) was defined as zeitgeber time 0 (ZT0). During the light period, the light intensity was 150-200lx at the level of the cages. Half of the rats were used as control animals of the LL group. The other half corresponded to the control animals of the IMO group, which were handled every day but not immobilized.

Saliva secretory responses

In all groups, the submandibular salivary secretion was analyzed during the light phase (ZT2-ZT5) of the diurnal cycle. Besides, for animals in the IMO group, the secretory response was tested 24 hours after the last immobilization. Rats were anesthetized (*ip*) with ketamine (8mg/100g body weight) and xylazine (1.28 mg/100 g body weight).

Secretory ducts from both submandibular glands were exposed and cannulated with thin glass tubes.[6]

Cholinergic and peptidergic salivary secretion responses were obtained by injecting, through a femoral cannula, increased doses of methacholine (1, 3, 10 and 30 μg/kg body weight) or substance P (0.2, 0.5, 1 and 5μg/kg body weight).

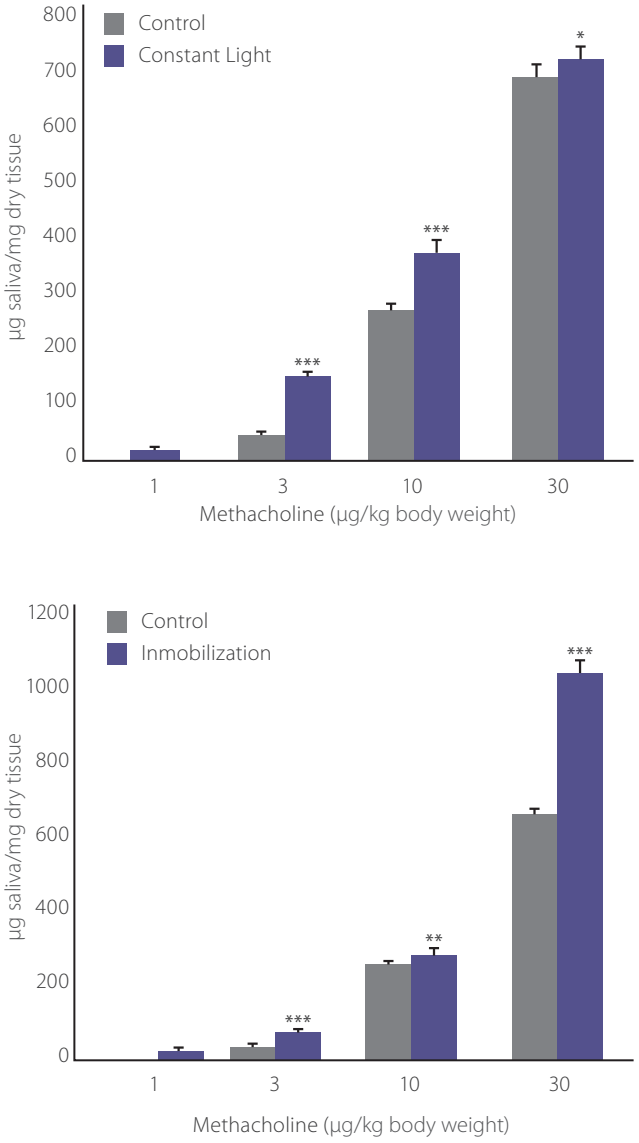
The saliva was collected in pre-weighed plastic tubes during the 3 minutes following drug administration and weighed. After all the doses had been injected and responses recorded, submandibular glands were dissected, weighed, and dried at 110ºC for 72 hours. The secretory response was expressed as μg of saliva per mg of dry tissue.

Methacholine and substance P were purchased from Sigma Chemical Co. (St Louis, MO, U.S.A).

Statistical analysis

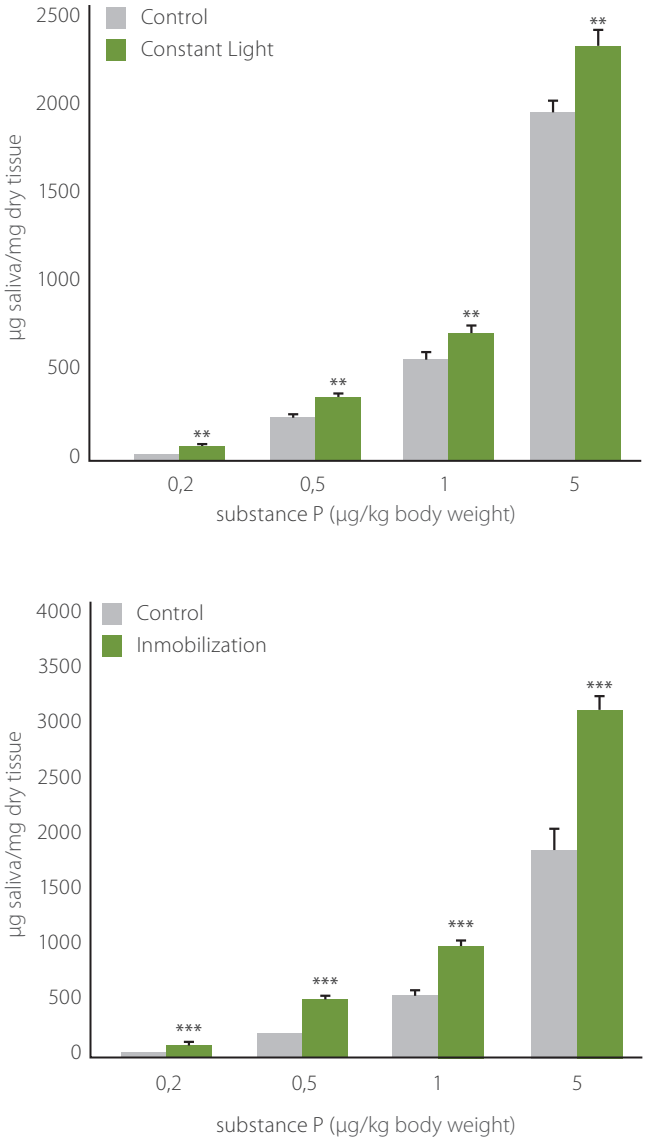
All numerical data are given as mean±SEM. To assess the average value of the secretory response for each dose of methacholine or substance P, the data of each submandibular gland was taken separately. Student's *t*-test (unpaired) was used to compare the data from each experimental group *versus* controls. Statistical significance was set at $p<0.05$.

Figure 1. Submandibular gland secretory response to methacholine in rats exposed to constant light or inmobilization.



Values are means±SEM (n=8). Statistical significance assessed with Student's *t*-test. *$p<0.05$, **$p<0.01$, ***$p<0.001$.

Figure 2. Submandibular gland secretory response to substance P in rats exposed to constant light or inmobilization.



Values are means±SEM (n=8). Statistical significance assessed with Student's *t*-test. **$p<0.01$, ***$p<0.001$.

RESULTS.

Submandibular gland secretory response to methacholine in rats exposed to constant light or immobilization is shown in Figure 1. Submandibular gland secretory response to substance P in rats exposed to constant light or immobilization is shown in Figure 2.

DISCUSSION.

Salivary secretion is the result of secretory cell activation by neurotransmitters released from sympathetic and parasympathetic nerves efferent to salivary glands[1] as well as by the hypothalamic-pituitary-adrenal and gonadal axes, but also by the sympathoadrenal system.[1,5] Electric

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stimulation of these nerves or systemic administration of adrenergic or cholinergic agonists in anesthetized rats promoted saliva release. Threshold dose and dose–response curves define normal sensitivity of the gland. When the activity of the nerves is chronically diminished or increased, the response of glandular effectors cells is modified to make up for the neural input disorder (supersensitivity or subsensitivity).[8]

In previous studies from our laboratory, dose-response curves to adrenergic agonists were used to analyze the sympathetic control of submandibular saliva secretion in rats chronically exposed to stimuli able to trigger SAS, such as prolonged exposure to constant light or continuous immobilization.[5] A significant drop in secretory response (subsensitivity) and in the number of α_2 and β-adrenergic receptors in these animals was regarded as an adaptation of salivary glands to a prolonged rise in the sympathetic control of saliva secretion.[5,6]

In the present study, we used dose-response curves to methacholine and substance P to evaluate the parasympathetic control of submandibular secreted saliva of rats chronically exposed to either LL or IMO. The results showed that in both experimental conditions, stimulation at all tested doses of methacholine produces a significantly higher secretory response compared to control animals. In addition, a similar effect was observed in the dose response curves when the agonist substance P was used.

This study shows, for the first time, the increased (supersensitivity) secretory response to these two sialogogue agents suggesting an inhibition of parasympathetic reflex activity in LL and IMO groups. This impairment would be one of the body responses to an emergency in which the SAS is chronically activated resulting in the activity of salivary glands.[1,7] In humans, it has been shown that in situations of posttraumatic stress disorder the increase in heart rate is related to a decreased parasympathetic activity.[9] Reduced parasympathetic activity is also related to stress in humans provoked by sleep deprivation during night duty work.[10]

Under physiological conditions in both humans and rodents, both branches of the autonomic system act synergistically and simultaneously in the nervous control of saliva secretion. Thus, the parasympathetic innervation provides higher control over salivary flow whereas the concentration and composition of proteins preferentially are dependant upon sympathetic control.[1] However, the existence of basal saliva secretion has not been demonstrated in rats, and so the results of this study were limited to stimulated saliva.

CONCLUSION.

Supersensitivity of secretory response to both agonists suggests that chronic exposure of rats to stressors capable of activating the sympathetic adrenal system promotes inhibition of the parasympathetic control of salivary secretion.

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