REVISTA ESPAÑOLA DE FISIOLOGIA, 40, 15-18, 1984

# Effect of Sympathetic Stimulation on Salivary Secretion in Submandibular Gland in the Rabbit Stimulated by Pilocarpine

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(Received on April 27, 1983)

M. MORENO, E. MARTINEZ DE VICTORIA and M. A. LOPEZ. *Effect of Sympathetic Stimulation on Salivary Secretion in Submandibular Gland in the Rabbit Stimulated by Pilocarpine.* Rev. esp. Fisiol., 40, 15-18. 1984.

The sympathetic stimulation under slow pilocarpine-induced flow conditions brought about a decrease in this flow rate that could be due to vasoconstriction, since such an effect was not observed after the administration of phentolamine (4 mg/kg i.v.).

Contrariwise the injection of a β-adrenergic blocking agent (propranolol 2-2.5 mg/kg i.v.) produced a decrease of the salivary flow rate that was even greater than in the control animals. These results suggest that the secretory effect in this gland and species is predominantly β-adrenergic. The stated results are related to the changes observed in the blood outflow from the gland.

**Key words:** Rabbit, Saliva, Sympathetic stimulation, Pilocarpine.

At present it is well known that most of the salivary glands are innervated by both divisions of the vegetative nervous system although this does not assume that all the different glandular cells have a double innervation (12).

In cat's parotid and submandibular glands the secretory effect of the sympathetic stimulation is higher when the glands are secreting at as slow parasympathetic flow. This fact seems to be mediated by both types of adrenergic receptors, but with a very high contribution of β-adreneceptors (2). The β-adrenergic receptors also play a role in the increase of salivary flow observed in the dog's parotid gland after sympathetic stimulation under a weak parasympathetic flow. The sympathetic stimulation by itself has not an influence on this gland and species. In the submandibular gland of the same species and under similar experimental conditions, there is a positive effect on salivary flow when the sympathetic trunk is stimulated. These actions could be due either to the secretory action on acinar cells or to a contraction of the myoepithelial cells (3, 4).

In the rabbit the studies carried out in both, parotid and submandibular glands (6) suggest that they have a similar pattern to the other species studied. The re-

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sponse is mediatized through $\alpha$ and $\beta$-adrenoceptors (7, 8, 11).

GJÔRSTRUP (6) found a negative effect of the sympathetic stimulation on the salivary flow of the gland when the parasympathetic division is stimulated simultaneously. This author attributes the results to a vasoconstriction and to the pattern of the sympathetic innervation of these glands in the rabbit.

The object of this work is to study the likely interaction between both vegetative divisions in the submandibular gland of the rabbit to study in depth the mechanism of this interaction and the adrenergic receptors implied; with this purpose, we have stimulated the superior cervical ganglion under different previous salivary flow rates evoked with a parasympathomimetic agent.

## Materials and Methods

*Animals.* 38 rabbits weighing between 1.5-4 kg were anesthetized with ethiluretane (20 % w/v) administered through a cannula placed in the marginal vein of the ear. Surgical preparation, nervous stimulation and blood flow were carried out according to MORENO *et al.* (10).

*Salivation.* Salivary flow was measured using a drop counter (Physiograph E and M). It was expressed as $\mu$l min$^{-1}$ g gland$^{-1}$. Mean values are given for the 5 min stimulation period, for the 5 min period prior to stimulation, and for the three 10 min periods after stimulation. Statistical analysis was made by the Student «t» test.

*Drugs.* Pilocarpine chlorhidrate (Sigma). Phentolamine (Regitine Ciba) propranolol chlorhidrate (Sumial Ici-Pharma).

All experiments were carried out infusing into the animal via femoral vein a solution of pilocarpine at different doses (about 10 to 20 $\mu$g min$^{-1}$ × kg$^{-1}$ for slow flows and 50-100 $\mu$g min$^{-1}$ × kg$^{-1}$ for high floods). The salivary flow rates between 1 and 13 $\mu$l min$^{-1}$ g gland$^{-1}$, were considered slow flows and between 14 and 34 $\mu$l min$^{-1}$ g gland$^{-1}$ high flows. These salivary flow rates have been selected according to previous studies upon doses of pilocarpine and flow of saliva in the submandibular and parotid gland of the rabbit (9).

The administration of pilocarpine at doses mentioned above does not modified significantly the blood pressure.

## Results

When the gland was secreting at slow flow, the stimulation of the superior cervical ganglion, induced in all the cases, except one (n = 15) a decrease of salivary

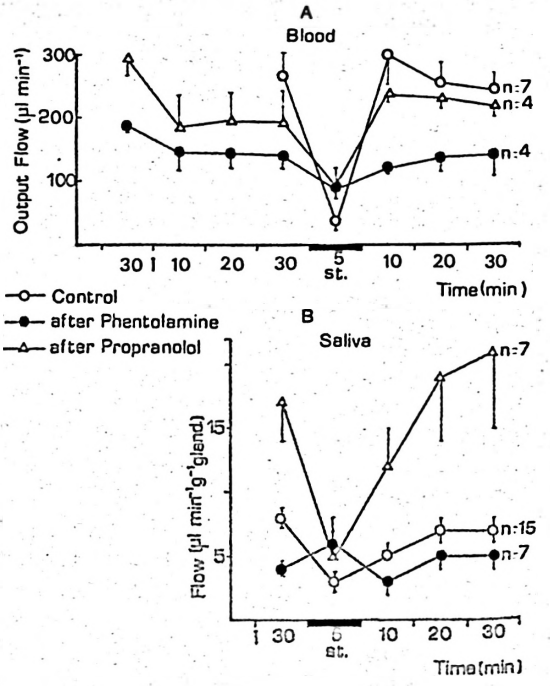


Fig. 1. *Effect of stimulation of the superior cervical ganglion (25 HZ) on salivary (B) and blood flow (A) when the gland is stimulated with pilocarpine and secreting at a slow flow.*
Influence of $\alpha$ and $\beta$ adrenergic blocking agents (→). The values represented in the figure are the mean ± S.E.M. (st: sympathetic stimulation).

flow that became in 8 animals. Obviously the mean values before and after stimulation are statistically significant ($p < 0.001$) the output of blood from the gland, during the stimulation, clearly felt, returning to the previous of superior values within ten minutes after stimulation (figure 1A).

Phentolamine (4 mg/kg i. v.), slightly decreased the flow of saliva in 6 out 7 test carried out. The sympathetic stimulation, in these conditions, produced a small increase of salivary flow that is not significative (fig. 1B). The output blood flow slightly decreased during the stimulation, but these values are not significant if they are compared to the inmediately previous values before stimulation (fig. 1A). This flow get back quickly to these values.

Propranolol (2-2.5 mg/kg i.v.) induced a significant increase in the salivary flow, and it happened in all the animals. The sympathetic stimulation, after a $\beta$-blocking agent, reduced drastically the flow of saliva, being zero in 4 out of 7 test carried out (fig. 1B) returning to previous values within the 20 following minutes the output blood flow has a similar pattern to the control rabbit.

When the submandibular glands are secreting at high flow, produced by a parasympathomimetic agent, the stimulation of superior cervical ganglion, led to a fall of the salivary flow in both, the control animals and those administered with adrenergic blocking agent ($\alpha$ or $\beta$) (figure 2). This decrease was statistically significant ($p < 0.001$) in the control and propranolol treated rabbits, but not in those ones injected with phentolamine.

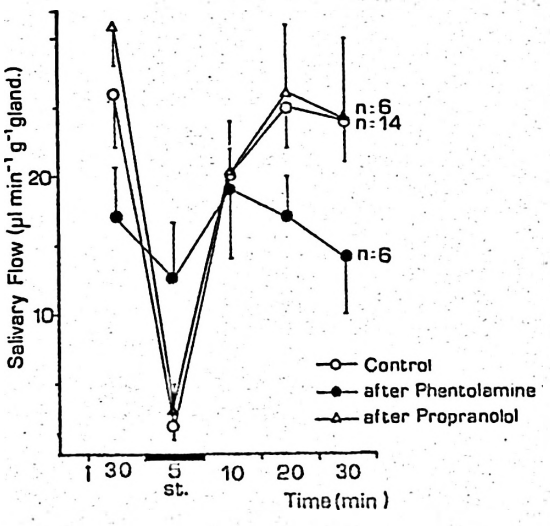


Fig. 2. *Effect of stimulation of superior cervical ganglion (25 HZ) on flow of saliva when the gland is stimulated with pilocarpine and secreting at a high flow.*

Influence of $\alpha$ and $\beta$ adrenergic blocking agents (→). The values represented in the figure are the mean ± S.E.M. (st: sympathetic stimulation).

## Discussion

The sympathetic stimulation produces, when the submandibular gland is secreting at slow flow, a clear decrease of the salivary flow. They can be attributed to concomitant vasoconstriction (1, 8, 11); under these conditions the gland is working above basal values, so vasoconstriction could be a limiting factor. Of course it is also possible that a reduced in flow of pilocarpine because the vasoconstriction may contribute to the observed effect. Nevertheless the data exposed by GJÔRSTRUP (6) with stimulation of chorda tympani and superior cervical ganglion are comparable to ours, although the fall in the flow of saliva found by us is greater, perhaps due to the more drastic parameters of sympathetic stimulation and to the higher previous flow rates.

If our assumption is correct, any $\alpha$-blocking agent, that avoids vasoconstriction (fig. 1A) should reduce the fall in the flow of saliva. In fact under these conditions there is a slight increase of flow that is not significant (fig. 1B) but ocurred in 6 out 7 animals. On the contrary the previous administration of propranolol brought about a strong fall of salivary flow during sympathetic stimulation, that was to be expected since the vasoconstrictor effect persist and futher more the

$\beta$-adrenergic receptors, whose role in the secretor process in this gland and species (6, 10, 15) is important are blocked.

- During 10 minutes after stimulation there is a small but steady after dilatation with blood flow values higher than those previous to stimulation. The fact that administration of an $\alpha$-receptor blocking agent, but not of a $\beta$-blocker, prevents this after-dilatation (fig. 1A) suggest that formulation of vasoactive peptides must play an important role (5, 10).

If the sympathetic stimulation is carried out when the gland is secreting at high flow, the flow drops drastically and the administration of phentolamine reduces but does not abolish the decrease of salivary flow suggesting that vasoconstriction is more important under these experimental conditions.

On the other hand the action *per se* of adrenergic blocking agents has been studied. Administration of phentolamine superimposed to pilocarpine infusion lead to a statistically significant decrease in the flow of saliva. This could be due either to a tonic effect of the $\alpha$-adrenoceptors on some glandular elements, or to wide vasodilator effects of this adrenergic blocking agent inducing redistribution of blood flow.

In contrast propranolol produces a rise in the salivary flow, that is statistically significant with either a slow or a high previous flow inferred by pilocarpine. In resting conditions this blocking agent has an opposite action (10). This phenomenon that is no described in the literature has a hard explanation although can be due mainly to the ganglionar action of pilocarpine and its posterior interaction with the $\beta$-adrenoceptors (13, 14).

*Acknowledgements*

The author gratefully acknowledges the help and scientific supervision of this manuscript by Prof. N. Emmelin. Thanks also to Dr. Mañas for his help in the redaction.

## Resumen

Se estudia en conejos el efecto de la estimulación simpática sobre el flujo de saliva inducido por la pilocarpina.

Esta estimulación provoca una disminución del flujo de saliva previo inducido por pilocarpina, efecto que podría deberse a vasoconstricción, ya que no se observa con administración de fentolamina (4 mg/kg i.v.).

Contrariamente, la inyección de un agente bloqueante $\beta$-adrenérgico (propranolol, 2-2,5 mg/kg i.v.) produce una disminución del flujo salival incluso mayor que en los animales control. Estos resultados sugieren que el efecto secretor en esta glándula y especie es predominante $\beta$-adrenérgico. Los resultados expuestos están relacionados con los cambios observados en el flujo de sangre.

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