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Influence of Superior Cervical Ganglion Stimulation Frequency on Salivary Secretion in the Rabbit. Comparative Study of Parotid and Mandibular Glands

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Saliva secretion in response to the stimulation of the superior cervical ganglion (S.C.G.) at different frequencies (2, 3, 5, 10, 15, 20 Hz) has been studied in anaesthetized rabbits. The differences between the two major glands in this species were analyzed, with respect to the flow response, potassium, amylase and total protein content during the sympathetic stimulation. The stimulation of S.C.G. increased the salivary flow rate at all frequencies, on both parotid and mandibular gland. In the parotid gland the flow and stimulation frequency show a positive linear correlation which does not appear in the mandibular gland. In conclusion, the differences observed in the response to sympathetic stimulation in borth glands seem to be due to distinct patterns of sympathetic innervation on different glandular elements.

Key words: Salivary secretion, Rabbit, Superior cervical ganglion, Parotid, Mandibular.

Both major salivary glands in rabbits are sensitive to sympathetic stimulation, although to a greater degree in the parotid than in the mandibular gland (9, 17-20, 23). Nevertheless, there is no systematic study of the effect of this autonomic division on the distinct glandular components.

GJORSTRUP (9) studied the effect of the stimulation frequency on the two glands, with reference to flow, but without referring to the inorganic composition. More recently, their studies have centered on the secretion of amylase by the parotid gland as much in the conscious (11, 12) as in the anaesthetized animals (1, 2, 10) as well as *in vitro* (2). With regard to the inorganic composition of saliva, in rabbits the majority of studies refer to the mandibular gland

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(3, 4, 14, 23), both with parasympathetic and sympathetic stimulation.

The object of this paper is to study the effect that the direct stimulation of the superior cervical ganglion at different frequencies has on fluid secretion and on organic and inorganic composition of the saliva segregated by the two principal glands in rabbits.

Materials and Methods

Animals. A total of 58 rabbits were used, weighing between 1.5 and 4 kg, anesthetized with etilurethane 20 per cent (w/v).

Surgical preparation. In all the rabbits the secretory ducts of the two principal glands were cannulated using polyvinyl tubes (i.d. 0.58 mm × 0.96 mm o.d. in the parotid and i.d. 0.32 mm × 0.58 mm o.d. in the mandibular) and the routine of tracheotomy and drainage of urine bladder was carried out. The femoral artery and vein were cannulated to record the arterial pressure and blood re-entry respectively, in those animals in which blood flow from the mandibular gland was recorded.

Nerve stimulation. The Superior Cervical Ganglion (S.C.G.) was isolated for posterior stimulation at 15 V, 0.5 ms and at frequencies of 2, 3, 5, 10, 15 and 20 Hz. In no case the sympathetic trunk was cut, and the electrodes were always carefully protected to avoid a direct stimulation of the glands, because of the proximity that exists between them and the ganglion. The stimulations were carried out for periods of 5 min, except during the collection of samples in which the stimulations were carried out for a maximun of 1.5 min, with 1.5 min intervals between each stimulation, with the object of minimizing the effect of vasoconstriction on salivary flow.

Salivary flow was measured with a drop counter for the parotid gland, while a 0.1 ml pipette was used to record the flow from the mandibular gland (23). In every case, the flow is expressed as µl/min.

When the output blood flow of the mandibular gland was recorded, it was drained through a cannula located in the external jugular vein, after ligature of all the branches of the facial vein except the glandular one (13). The flow was measured with a drop counter A-978, whith a periodic check to control that the blood had not suffered haemolysis during the assessment of haemoglobin in plasma. Reentry was carried out via the femoral vein. Both the animal and the external circuit were heparinized (500 U/kg) and body temperature was maintained at 38 ± 1°C.

Analytical technique. Potassium concentration was measured by spectrophotometry of atomic absorption (Pye Unican SP90A). Amylase activity was assessed using the technique of NORLTING and BERNFIELD (22).

Total protein concentration was determined by absorption at 280 nm and expressed in terms of L-tyrosine (Tyr) standard (5).

Statistical evaluation was performed with linear regression analysis and the Student «t» test. P values less than 0.05 were considered significant. Mean values are expressed as means ± S.E.M.

Results

Mandibular gland. The electrical stimulation of the S.C.G. (15 V/0.5 ms) produced, at all frequencies tested (2, 3, 5, 10, 15 and 20 Hz) an increase in salivary flow (figs. 1 and 2) which was slight in all cases except that of 15 Hz in which the increase was statistically significant ($p < 0.05$). The temporal evolution of the response shows that for frequencies of 2, 15 and 20 Hz there is an increase of flow in the first minute of stimulation, and then it decreases towards the basal values. For the

frequencies of 3, 5 and 10 Hz the increase in flow is less marked, the flow being maintained close to the basal values, and even in some cases below them, except for 3 Hz at which the values were always above the basal values.

The mentioned flow increases were observed in all the animals except in 4, and at all frequencies; the lack of statistical significance can be attributed to the great variety between the individual specimens.

At low frequencies (2, 3 Hz) vasoconstriction is slight and not significant. At 2 Hz vasoconstriction remained constant during the whole period of stimulation, while at 3 Hz it is greater in the first minute and tends to diminish, remaining constant from the second minute. At frequencies of 5 Hz vasoconstriction is greater, although the reduction of blood flow is not significant. This vasoconstriction is marked during the first two minutes of stimulation and after this, blood flow returns towards the basal values, with vasoconstriction practically disappearing in the first minute after stimulation (fig. 1).

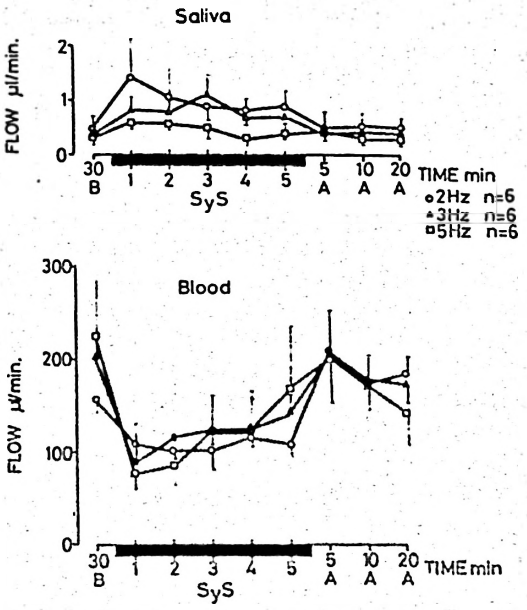

Fig. 1. *Changes in salivary flow and output blood flow in the mandibular gland during the stimulation of the S.C.G. at low frequencies.*
Values are expressed as the mean ± S.E.M. SyS. Sympathetic stimulation. A: After sympathetic stimulation; B: Before sympathetic stimulation (mean of flow during 30 min).

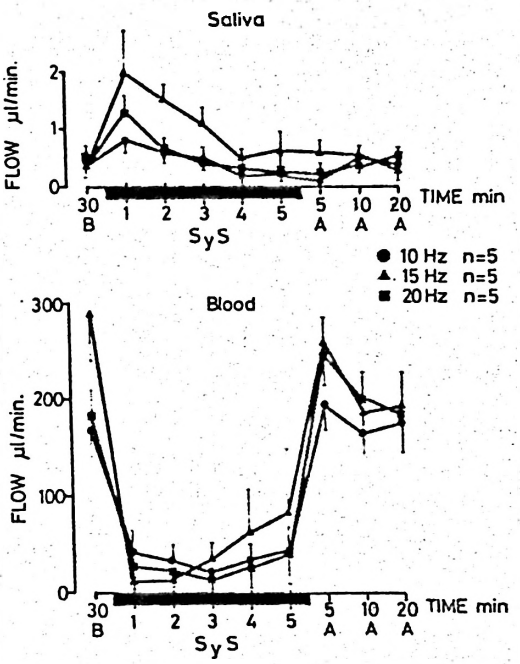

Fig. 2. *Changes in salivary flow and output blood flow in the mandibular gland during the stimulation of the S.C.G. at high frequencies.*
Values are expressed as the mean ± S.E.M. Sy.S. Sympathetic stimulation. A and B as in figure 1.

At high frequencies (10, 15 and 20 Hz) there is a marked vasoconstriction, which at 10 and 20 Hz practically annuls the output blood flow from the gland for the whole period of stimulation. At 15 Hz vasoconstriction is very strong during the first two minutes, with blood flow increasing in the last three, although in no case reaching the previous values (fig. 2).

At all the frequencies used there is vasodilatation subsequent to stimulation, which is not always present and when it does appear is slight and transitory.

Total protein concentration and output show an increase, with the sympathetic

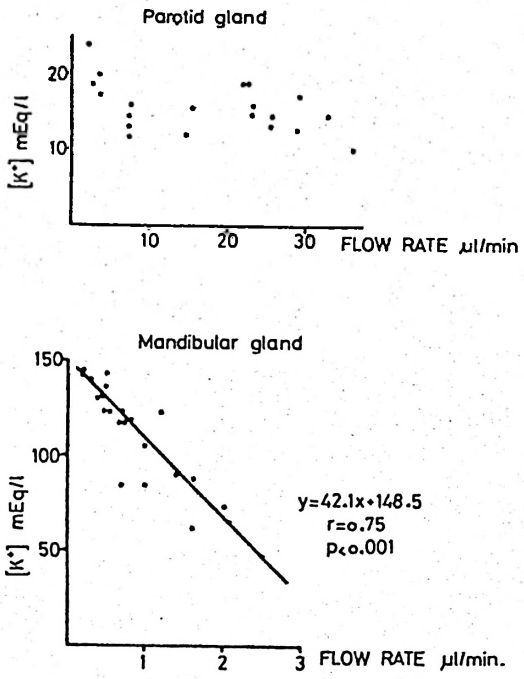

Fig. 3. *Correlation between salivary flow and potassium concentration in the glands of the rabbits.*

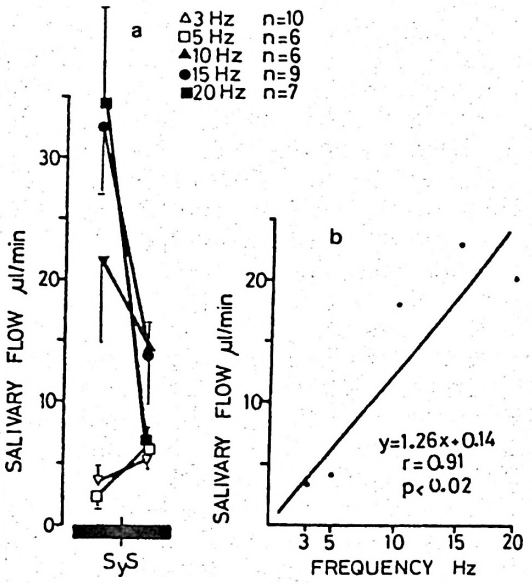

Fig. 4. *Stimulation effects of the S.C.G. at different frequencies on the parotid gland secretion.* A: Temporal evolution of flow response. SyS. Sympathetic stimulation. The stimulation period, 5 min, is divided in two periods of 2.5 min. B: Correlation between salivary flow and stimulation frequency. Values are expressed as the mean ± S.E.M.

stimulation (9.31 ± 0.83, $n = 14$, to 35.6 ± 3.44 µM Tyr/ml, $n = 24$ and 2.5 ± 0.33, $n = 14$ to 23 ± 3.18 µM Tyr/min, $n = 24$ respectively) which in both cases is statistically significant ($p < 0.001$). However, there is no correlation between the stimulation frequency and the total protein concentration or output. Total protein instead of amylase has been determined because in this rabbit gland there are only a few traces of this enzyme.

The concentration of potassium presents a highly significant ($P < 0.001$) negative linear correlation (fig. 3), with the salivary flow. Nevertheless, there is no correlation between the stimulation frequency used and ion concentration.

Parotid gland. At all the frequencies tested, the stimulation of S.C.G. initiated a secretion of saliva from this gland, as it does not present a resting secretion, although at 2 Hz and due to the measurement method employed, this flow could only be quantified in one animal out of the seven used. Nevertheless, stimulation for successive periods of 5 min permitted the collection of samples from a total of four animals at this frequency.

Figure 4a shows the great difference between the quantitative response at low frequencies (3 and 5 Hz) and high frequencies (10, 15 and 20 Hz). As in the mandibular gland, the maximun response is at 15 Hz. At low frequencies there is a long latent period (about 2 min), the flow being equal or greater during the second half of the stimulation period. At high frequencies, the latency is shorter (never grater than 1 min) and the salivary flow is always greater during the first part of the stimulation.

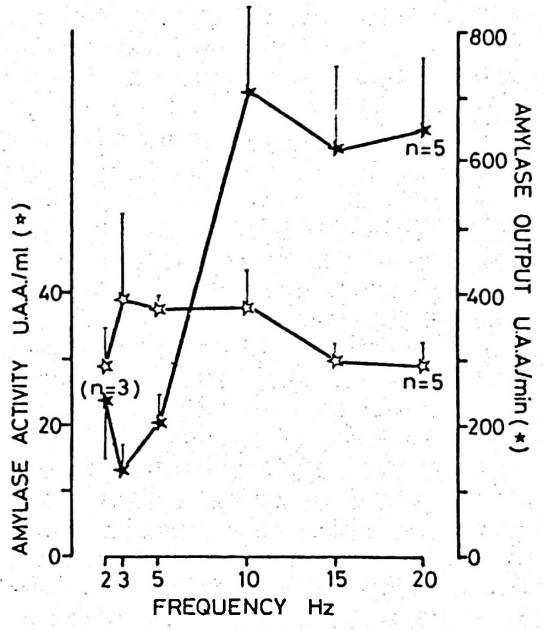

Fig. 5. *Changes in the activity and amylase output with respect to stimulation frequency in the parotid gland of rabbit.*
Values are expressed as the mean ± S.E.M. This figure starts from null values of flow before the stimulation as there is no resting secretion in this gland.

There is a statistically significant correlation ($p < 0.02$) between stimulation frequency and flow obtained (fig. 4b), which does not appear in the case of the mandibular gland.

Potassium concentration is higher when the flow is low, tending to diminish when the flow is greater. There is a statistically significant correlation between potassium concentration and salivary flow below 7.3 µl/min ($p < 0.01$) (fig. 3). For greater flows the concentration of this cation becomes independent of the flow.

Amylase concentration is high (about 40 U/ml) at frequencies of 3,5 and 10 Hz falling slightly at frequencies of 15 and 20 Hz. On the contrary the output of this enzyme markedly increases as stimulation frequency is increased up to 10 Hz where it stabilizes (fig. 5).

Discussion

In the experimental conditions and at all the stimulation frequencies used (2, 3, 5, 10, 15 and 20 Hz) there was an increase in basal salivary flow in the mandibular gland although it was slight in all cases, except at 15 Hz when the increase was significant ($p < 0.05$). These results concur with those described by SMAJE (23) for a frequency of 20 Hz, although quantitatively they differ from those obtained by GJORSTRUP (9) specifically at a frequency of 5 Hz.

The results may indicate the existence of a secretory effect of the sympathetic fibres that inervate this gland. Such a secretory effect described by SMAJE (23), appears to be due to the action of the autonomic division almost exclusively on β-adrenergic receptors, although in accordance with the same researcher and with previous studies from the present authors (19) the possibility of a slight effect attributable to α-adrenergic receptors cannot be discounted, although *in vitro* after stimulation with α-agonists no secretory response was obtained (3). Neither can the contribution of motor effects be discounted through contraction of myoepithelial cells which in all cases would be mediated by α-receptors (8, 23).

Joint analysis of the temporal development of the salivary secretory response and output blood flow from the gland, clearly demonstrates that at frequencies of 10 to 20 Hz vasoconstriction is a limiting factor in the observation of the secretory effects of the sympathetic, since at these frequencies the sharp drop in saliva flow coincides with a drastic fall in output blood flow from the gland. In this aspect GJORSTRUP (9) found flow reductions at lower stimulation frequencies (0.3-5 Hz). Nevertheless, in these experimental conditions, at frequencies from 2 to 5 Hz, a slight reduction of blood flow through the gland can be observed although it is not significant and is reflected in the salivary flow values which

in most cases, during the period of stimulation, remain above their previous values.

As in the mandibular, sympathetic stimulation in the parotid gland induces an appearance of flow at all frequencies tested except at 2 Hz which is logical, since according to GJORSTRUP (9, 10) at these frequencies the threshold of fluid secretion in this gland is to be found. Nevertheless, the flow responses in the parotid were always quantitatively greater than in the mandibular which indicates a greater sensitivity of the parotid gland to sympathetic stimuli, a consideration remarked by NORDENFELT and OHLIN (21).

Analysing the temporal development of the response, clear differences for frequencies between 10 and 20 Hz and between 3 and 5 Hz can be seen attributable again to the more intense vasoconstriction which would occur at high sympathetic stimulation frequencies. Contrasting with what is observed in the mandibular gland, in the parotid there is a statistically significant correlation frequency ($p < 0.02$). Probably, these differences between the two glands support the data of GARRET (7) with regard to the peculiar characteristics of sympathetic innervation in the two main glands in rabbits.

The performance of potassium is the same in the two glands, at the same rate flow, decreasing as the flow increases. This pattern is similar to that described by other authors (3, 14) for parasympathetic stimuli. These results indicate that sympathetic stimulation produces an inhibition of ductal potassium secretion. This fact has been described by several authors as an adrenergic effect (6, 15, 16, 24). The output of amylase clearly increases as the stimulation frequency is raised, remaining at high values for frequencies of 15 and 20 Hz. On the contrary, the activity of the enzyme in saliva, although attaining high values, is not affected at the different frequencies. The increase in the amylase output is greatest between 5 and 10 Hz becoming stabilized at higher frequencies, which may be due, according to GJORSTRUP (10) to the vasoconstriction which accompanies stimulation at these frequencies and which may alter the metabolism and glandular function.

Likewise, total protein concentration and output in the mandibular gland show a great increment after sympathetic stimulation, although there is no correlation between these parameters and the stimulation frequency. These results agree with the fact that the stimulation of this autonomic division has a secretory effect on the acinus in both glands, although the flow response is greatest in the parotid gland.

From the above considerations, a distinct response of the main glands in rabbits to sympathetic stimuli may be observed, which suggests the different roles of the two glands within the physiology of the animal and which are probably supported by the differences in the patterns of sympathetic innervation, which differ, according to GARRET (7), both in density and in their more or less intense relationships with the distinct glandular elements: acini, myoepithelial cells, blood vessels and glandular ducts (8).

Resumen

Se estudia en conejos anestesiados la secreción salival en respuesta a la estimulación del ganglio cervical superior (G.C.S.) a diferentes frecuencias (2, 3, 5, 10, 15, 20 Hz). Se comentan los efectos de la estimulación simpática en ambas glándulas sobre el flujo y la concentración de potasio, amilasa y proteína total. La estimulación del G.C.S. aumenta el flujo salival en la glándula mandibular y hace aparecer un flujo en la parótida, para todas las frecuencias usadas. En la glándula parótida aparece una correlación lineal positiva entre el flujo y la frecuencia de estimulación, que no se presenta en la glándula mandibular. Las diferencias observadas en la respuesta a la estimulación simpática en ambas glándulas parecen debidas a los distintos patrones de inervación simpática sobre los diferentes elementos glandulares.

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