

## ON THE EXISTENCE OF SPECIFIC SECRETORY SYMPATHETIC FIBRES FOR THE CAT'S SUBMAXILLARY GLAND

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Stimulation of the cervical sympathetic trunk in cats causes a secretion from the submaxillary gland. The magnitude of the response varies in different cats. It may be almost as pronounced as that evoked by stimulation of the chorda tympani, but usually it is much smaller and in exceptional cats no secretion at all is obtained. Unlike the secretion elicited by stimulation of the chorda it is usually described as short-lasting, tending to cease in spite of continued stimulation.

Since the time of Ludwig, Claude Bernard, Heidenhain and Langley the sympathetic trunk has been assumed to carry specific secretory fibres for the gland. Kuntz & Richins (1946) found, however, in histological investigations that the innervation of the gland cells is mainly, perhaps exclusively, parasympathetic. In a later paper (Richins & Kuntz, 1953) the same authors expressed the view that the secretion following sympathetic stimulation is caused by the chemical transmitter released from the endings of the vasomotor fibres and diffusing to the gland cells. This conception was based on the observation that the secretory effect of sympathetic stimulation was abolished by occlusion of the external carotid artery. The theory seems to be an extension of the idea discussed by Cannon & Rosenblueth (1937), according to which some cells are excited by transmitter diffusing from nerve endings in contact with certain innervated 'key cells'.

The theory of Kuntz & Richins might afford an explanation of the facts that the sympathetic secretory effect appears with a fairly long latency, shows great variations in different cats, is obtained predominantly at the beginning of a stimulation period, and can be elicited by repetitive stimulation only. There is, however, no direct evidence to show that specific sympathetic secretory fibres do not exist.

Analogous problems have been discussed in connexion with other organs. Celander (1959) found in experiments on adrenalectomized cats no inhibition of the intestinal motility on stimulation of the splanchnic nerves, except when frequencies of stimulation were used which according to Folkow (1955) are above the physiological range. Similar observations

were made by Kock (1959). The conclusion was that the splanchnic nerves contain no specific inhibitory fibres for the intestine.

The experiments of the present paper arose from an investigation on supersensitivity of the gland cells following sympathetic denervation. It is known that removal of the superior cervical ganglion causes an increased responsiveness of the gland cells to chemical agents (Simeone & Maes, 1939). This fact seems to indicate that the sympathetic normally exerts some kind of action on the gland cells; this action could of course be indirect, via the vasomotor fibres, but it must then be due to an impulse rate within the physiological range.

From such considerations experiments on the effect on the gland cells of stimulation of the sympathetic trunk with impulses of low frequency seemed required; for in the earlier investigations on the secretory effect of sympathetic stimulation frequencies above what is now considered as physiological in the vasomotor fibres have been used. Such experiments are the subject of the first part of the present investigation. In the experiments of the second part the effect on the secretory cells of artificial stimulation of the cut sympathetic trunk has been studied by using a frequency of stimulation which restores the tone of the vessels of the gland existing before the nerve was cut. In the third part an intense vasoconstriction via the sympathetic trunk was elicited reflexly and the effect on the gland cells of this constriction and of artificial stimulation causing a similar constriction was investigated. The reflex activity was obtained by bleeding. A preliminary report of these experiments has been given (Emmelin & Engström, 1959).

#### METHODS

The experiments were carried out on cats anaesthetized with chloralose (about 80 mg/kg intravenously) or chloralose-urethane (60+100 mg/kg intravenously). At the beginning of the experiment the chorda tympani was cut. The submaxillary duct was cannulated and the drops secreted were recorded electromagnetically on the smoked drum by an assistant; in some experiments an ordinate recorder was used. The flow of blood from the gland was recorded as drops of blood from a cannula in the external jugular vein, after injection of heparin and ligation of tributaries not draining the gland. The drops passed a phototube counter, which operated an ordinate recorder. The blood was reinjected at intervals through a cannula in a femoral vein. For stimulation of the sympathetic trunk square-wave shocks of supramaximal strength and 1 msec duration were used.

In the experiment on the reflex response to bleeding blood was withdrawn from a cannula in a femoral artery. In order to prevent the bleeding from interfering mechanically with the flow of blood through the gland, and to prevent catechol amines released from the adrenals during the bleeding from reaching the gland, a separate perfusion of the gland from another cat was arranged. For that purpose an anaesthetized cat was heparinized and blood directed from the central end of a carotid artery through a polythene tube into the peripheral carotid of the recipient animal; in this cat all the branches of the carotid except that of the gland had been tied. The polythene tube was of such a size that it could be pushed through the

carotid up into the external maxillary artery to fit tightly into this vessel. A ligature round this artery and the tip of the tube was then often not necessary, which diminished the risk of injury to the sympathetic fibres of the gland. Other technical details are given in the text.

## RESULTS

### *Electrical stimulation of the sympathetic trunk*

Stimulation of the peripheral end of the cut sympathetic was found to cause a flow of saliva when a rate of stimulation well within the physiological range was used. Among fifteen cats studied, one responded with secretion at a rate of only 0.2/sec. In three cats 0.5/sec was found to be effective, in two others 1/sec; four responded at 2/sec, two at 3/sec, one at 4/sec and two at 5/sec.

When low frequencies were used a continuous secretion could be maintained. At a rate of 3–10/sec or more the flow diminished in spite of continued stimulation, and at rates of 10 or 20 sec the picture usually described as typical of sympathetically induced secretion was obtained: often the flow ceased completely within the first minute or minutes; sometimes a very slow flow persisted during the whole stimulation period; in some instances the flow ceased early, but periods of secretory activity reappeared now and again.

An attempt was made to find the cause of the decrease or cessation of the flow at high rates of stimulation. A reasonable explanation could be that the vasoconstriction simultaneously produced seriously interfered with the secretion, depriving the gland cells of blood necessary for the activity. It is well known, for instance, that a high rate of secretion cannot be maintained for a long period by chorda stimulation if the artery of the gland is partially clamped. It can, in fact, be seen that the secretory effect of sympathetic stimulation is more pronounced if the constriction is in some way counteracted. This can be achieved by stimulating the chorda after injection of atropine in a dose which entirely abolishes the secretory effect of parasympathetic stimulation.

Figure 1 shows such an experiment. Sympathetic stimulation caused secretion at a rapidly decreasing rate and a pronounced constriction. Chorda stimulation, after atropine, produced its marked vasodilatation but no secretion. When sympathetic stimulation was added, the flow of blood through the gland diminished to some extent, particularly initially; but it was much less reduced than was the case when the chorda was not stimulated. The secretory response to sympathetic stimulation was greater than before, suggesting that vasoconstriction might contribute to the decline in secretory rate. However, it could only be a contributing factor, for even in the period of increasing blood flow the secretion diminished and eventually ceased.

The falling off of the secretion at high rates of stimulation was observed even when the post-ganglionic sympathetic fibres running along the artery were stimulated, and could therefore not be due to failure of ganglionic transmission. A well-maintained secretion could, on the other hand, be elicited by continuous intravenous injection of adrenaline, and when secretion had ceased during sympathetic stimulation the injection of adrenaline was found to produce a flow of saliva. Consequently, the decline could not be attributed to some process in the glandular elements.

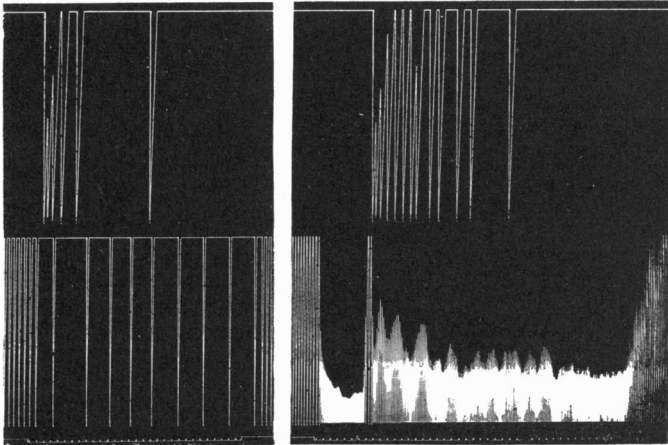


Fig. 1. Records from above down: drops of saliva; drops of blood from the gland; signal, which also marks time intervals of 10 sec. For secretion and blood flow, ordinate recorders were used, the upper one operated manually, the lower one by a phototube. In the left section of the tracing the effect of sympathetic stimulation for 5 min at a rate of 20/sec. Between the sections 2 mg atropine sulphate was given intravenously. In the right section the chorda was stimulated during the whole period marked by the signal (5 min). After 1 min, sympathetic stimulation was added and continued for 5 min.

It seems reasonable to assume that at the high rates of stimulation transmission for some reason failed at the endings of the post-ganglionic secretory fibres. This apparently did not occur at the vasoconstrictor nerve endings, for constriction persisted throughout the period of stimulation, as can be seen in Fig. 1. The only exception from this rule is that secretion may cause a temporary vasodilatation. In those cats in which secretion ceased initially but again appeared after some time, corresponding fluctuations in blood flow could be seen, as shown in the experiment of Fig. 2. Even in such cats, however, the secretion finally ceased definitely and constriction persisted.

*Effect of restoring the resting vasomotor tone*

In the experiment of Fig. 3 section of the sympathetic trunk caused an increased flow of blood through the gland. When the peripheral end of the trunk was stimulated electrically at a rate of 1/sec, a constriction was evoked slightly larger than that prevailing before the nerve was cut.

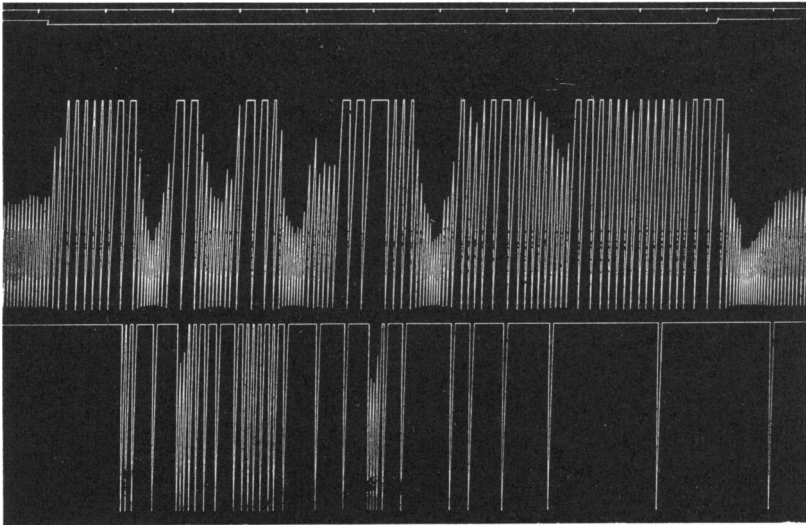


Fig. 2. Records from above down: time marker, minutes; signal, which marks the period of sympathetic stimulation at a rate of 20/sec; blood flow, and secretion; these were recorded as in Fig. 1.

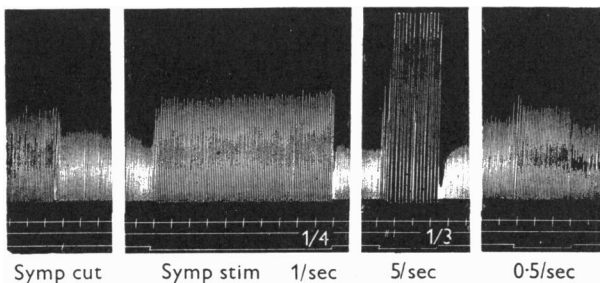


Fig. 3. From above down: blood flow through the gland; time marker, minutes; drops of saliva; signal. The first section of the tracing shows the effect of cutting the sympathetic trunk. In the next sections the peripheral end of the trunk was stimulated at different rates (1, 5 and 0.5/sec).

Already at this rate a slow secretion was obtained. Stimulation at 0.5/sec caused a smaller constriction but no secretion. This was a type of effect seen in most cats; but there were some experiments in which secretion was observed at a rate which was too small to restore the sympathetic resting

constrictor tone. It should be added that there is never any secretion normally which can be attributed to the resting constrictor activity.

*Effect of a reflexly induced strong vasoconstriction*

Figure 4 shows an experiment in which a pronounced vasoconstriction was evoked in the gland by bleeding the cat. It can be seen that the two main technical difficulties of these experiments had been overcome in this

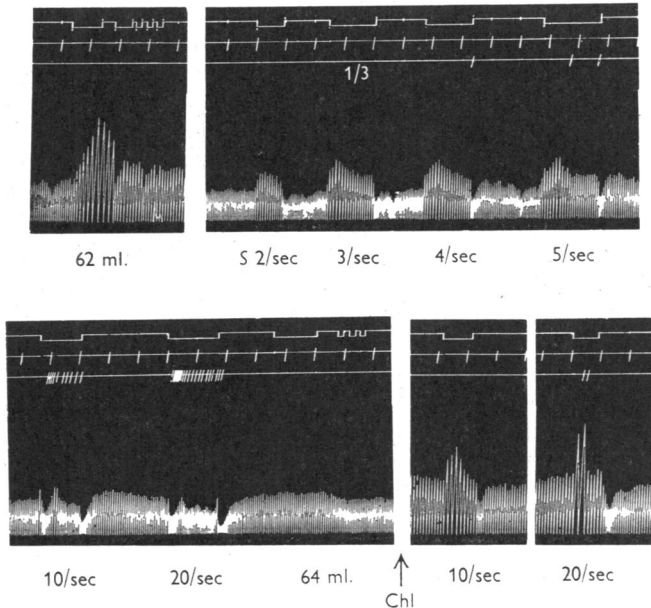


Fig. 4. From above down: signal; time marker, minutes; drops of saliva; blood flow through the gland, which was separately perfused from another cat. The first section shows the effect of withdrawal of blood from the recipient and the reinjection of the blood about 1 min later. This procedure was repeated in section 3. Between the first two sections the cervical sympathetic was cut. S = stimulation of its peripheral stump at different frequencies (2, 3, 4, 5, 10, 20/sec). Chl, chlorpromazine 0.4 mg/kg injected into the donor 30 min before the last section.

preparation. No vascular connexions had been left untied between the gland circulation and the remaining circulatory system of the recipient. This was obvious from the fact that bleeding did not alter the flow of blood from the gland when the cervical sympathetic had been severed. Further, the sympathetic innervation had not been destroyed in the course of the dissection, for stimulation of the sympathetic trunk produced the characteristic responses from the gland.

As is illustrated in Fig. 4, it is possible to produce a marked vasoconstriction in the gland reflexly via the sympathetic vasoconstrictor fibres, without any concomitant secretion. Electrical stimulation of the

sympathetic at a rate which causes a much smaller vasoconstriction is followed by secretion of saliva. It is, in fact, often difficult to reproduce the marked reflex vasoconstriction by artificial stimulation of the sympathetic, since the secretion obtained tends to produce a vasodilatation, obscuring the constrictor response. Attempts were made to get a pure constrictor response by abolishing the secretory effect of sympathetic stimulation. This can be attained by injecting a small dose of chlorpromazine (Emmelin, 1955). In the experiment of Fig. 4 this attempt was only partly successful. When the nerve was stimulated after chlorpromazine at a rate of stimulation of 10/sec, no secretion and a relatively pure constrictor response were obtained; before giving chlorpromazine the dominating vasomotor effect at this rate was vasodilatation. The constriction elicited after injection of the drug was somewhat smaller than that produced by bleeding. When the rate of stimulation was raised to 20/sec, a larger constriction appeared, but it was of short duration, presumably since a small secretion started. Judging from the size of the constriction obtained after chlorpromazine the impulse rate in the sympathetic during bleeding might have been of the order of 10–20/sec.

These experiments indicate that there is no spread of transmitter from the vasoconstrictor endings to the gland cells sufficient to evoke secretion, even if the constrictor fibres are intensely activated reflexly. They do not, however, exclude the possibility that some diffusion of the transmitter may take place. In order to test this possibility we have tried to make the gland cells particularly sensitive to secretory agents. This was achieved by cutting the chorda tympani some weeks before the acute experiment. In one experiment a particularly high supersensitivity had been created in this way. The gland cells responded to adrenaline  $0.5 \mu\text{g}/\text{kg}$  intravenously instead of normally about  $15 \mu\text{g}/\text{kg}$  (Emmelin & Muren, 1951). When a vasoconstriction was elicited in this cat by bleeding, a very slow secretion started after about 1 min; this effect was abolished by section of the sympathetic trunk in the neck.

#### DISCUSSION

When the cervical sympathetic trunk is stimulated electrically at low frequencies, a secretion is obtained which differs from that usually described in the literature in being continuous. The normal resting activity of the sympathetic on the blood vessels seems to be of an order of magnitude comparable to that which causes secretion on artificial stimulation; but it is not accompanied by secretion. According to Folkow (1955) normal resting vasoconstrictor tone is maintained at a rate of about 1–3 impulses/sec. When the cervical sympathetic is stimulated electrically at this rate, a secretion of saliva is obtained in most cats. Even when the

vasoconstrictor fibres are activated reflexly at a rate which probably approaches the limit of what is possible in the course of physiological events, no secretion is elicited in a normal gland. These observations indicate that specific secretory nerve fibres for the submaxillary gland of cats are present in the cervical sympathetic trunk. They do, of course, not exclude the possibility that transmitter diffusing from the terminals of the vasoconstrictor nerves might contribute to some extent to the secretory response provoked by the sympathetic stimulation, at least when the activity in the vasomotor fibres is high. The observations on the sensitized gland support this view.

#### SUMMARY

1. Secretion of saliva can be elicited from the submaxillary gland of cats by stimulating the cervical sympathetic trunk at a rate which is well within physiological limits.

2. If the sympathetic vasoconstrictor tone in the gland is abolished by section of the sympathetic in the neck, it can be restored by stimulating the peripheral end of the nerve trunk electrically at a rate of stimulation which in some cats may cause secretion; in others a slightly higher rate is required to cause a flow of saliva.

3. Even when the vasoconstrictor fibres of the gland are strongly activated by bleeding, no secretion is obtained in a normal gland, although the impulse rate in the constrictor fibres must by far exceed the secretory threshold rate for artificial electrical stimulation.

4. These observations indicate that the cervical sympathetic trunk contains specific secretory fibres for the submaxillary gland.

#### REFERENCES

- CANNON, W. B. & ROSENBLUETH, A. (1937). *Autonomic Neuro-effector Systems*. New York: MacMillan.
- CELANDER, O. (1959). Are there any centrally controlled sympathetic inhibitory fibres to the musculature of the intestine? *Acta physiol. scand.* **47**, 299-309.
- EMMELIN, N. (1955). Sympathicolytic agents used to separate secretory and vascular effects of sympathetic stimulation in the submaxillary gland. *Acta physiol. scand.* **34**, 29-37.
- EMMELIN, N. & ENGSTRÖM, J. (1959). Sympathetic secretory fibres for the cat's submaxillary gland. *J. Physiol.* **149**, 67-68 P.
- EMMELIN, N. & MUREN, A. (1951). Sensitization of the submaxillary gland to chemical stimuli. *Acta physiol. scand.* **24**, 103-127.
- FOLKOW, B. (1955). Nervous control of the blood vessels. *Physiol. Rev.* **35**, 629-663.
- KOCK, N. G. (1959). An experimental analysis of mechanisms engaged in reflex inhibition of intestinal motility. *Acta physiol. scand.* **47**, Suppl. 164, 1-54.
- KUNTZ, A. & RICHINS, C. A. (1946). Components and distribution of the nerves of the parotid and submandibular glands. *J. comp. Neurol.* **85**, 21-32.
- RICHINS, C. A. & KUNTZ, A. (1953). Role of sympathetic nerves in the regulation of salivary secretion. *Amer. J. Physiol.* **173**, 471-473.
- SIMEONE, F. A. & MAES, J. P. (1939). Sensitization of submaxillary gland by sympathetic denervation. *Amer. J. Physiol.* **125**, 674-679.