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THE PRESENCE OF BETA-RECEPTORS IN THE SUBMAXILLARY GLAND OF THE DOG

BY

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Salivary secretion is regulated mainly by parasympathetic nerves but in some glands, notably the submaxillary gland of the cat, secretion can be evoked by stimulation of sympathetic fibres also. According to the classical studies by Dale (1906) this effect can be abolished by ergot preparations. Receptors of α-type, in Ahlquist's (1948) sense, are thus present in this gland. This is supported by the fact that whereas adrenaline and noradrenaline usually cause a lively secretion from the gland, isoprenaline has very little secretory effect (Emmelin & Muren, 1951); furthermore, a number of different α-receptor blocking agents have been found to abolish secretion from the cat's submaxillary gland caused by sympathetic stimulation (see Emmelin, 1967). There is, however, evidence showing that β-receptors may occur in some salivary glands, although α-receptors obviously dominate in these glands also. Salivation elicited by sympathetic stimulation or adrenaline in the parotid gland of the rabbit (Nordenfelt & Ohlin, 1957) or by noradrenaline in the submaxillary gland of the rat (Emmelin & Strömblad, 1963) cannot be completely abolished by dihydroergotamine. In these glands isoprenaline causes salivation (Ohlin, 1964). Secretory responses to sympathetic stimulation or noradrenaline which persist in the rat's submaxillary gland after dihydroergotamine can be reduced by injection of pronethalol (Emmelin, Holmberg & Ohlin, 1965).

In experiments on the innervation of the salivary glands of dogs (Emmelin & Holmberg, 1967) it was noticed that dihydroergotamine did not at all reduce the secretory effect of sympathetic stimulation in the submaxillary gland. The present experiments show that the catecholamine receptors of the secretory cells of this gland belong exclusively to the β-type.

METHODS

The observations were made on nine dogs anaesthetized with chloralose and urethane (50+500 mg/kg intravenously) after induction with ether. The submaxillary, and in some cases the parotid or sublingual ducts were exposed and cannulated. Glass cannulae of the widest possible bore were inserted, since cannulae were otherwise easily blocked by the often very viscous saliva produced by sympathetic stimulation. The cannula was usually connected to a bottle in which saliva replaced distilled water. The outlet from this bottle gave 32 drops for 1 ml. water. The drops were recorded on a smoked drum using an electromagnetic signal operated manually. To estimate the flow of blood through the submaxillary gland, the gland was exposed and its main vein was cannulated and connected to a phototube counter, operating an ordinate recorder. The blood was returned to the dog at intervals through a femoral vein. Heparin was given to prevent clotting. Drugs were administered through a femoral cannula or into the submaxillary gland by injection through the

duct in retrograde direction (Emmelin, Muren & Strömblad, 1954). The vago-sympathetic nerve was exposed in the neck and cut and its cranial end stimulated using 5–20 shocks/sec of a duration of 2 msec and a strength of 8–15 V. The chorda-lingual nerve was usually cut and prepared for stimulation, and sometimes the auriculotemporal nerve as well (Burgen, 1964).

RESULTS

Secretory effects of vago-sympathetic stimulation

When the parasympathetic secretory fibres of the salivary glands were intact, stimulation of the cranial end of the cut vago-sympathetic trunk often caused a very marked secretion from the ipsilateral submaxillary and parotid glands, and some secretion from the glands of the other side as well. This occurred particularly when respiratory changes, borborygmus or vomiting revealed that stimulation of vagal afferents had a pronounced reflex effect, which happened especially when the anaesthesia was light. After an additional dose of urethane, or after section of the auriculotemporal and chorda-lingual nerves no secretion was obtained contralaterally. On the side of vago-sympathetic stimulation no saliva, or occasionally a small amount, less than a drop, now flowed from the parotid gland, but from the submaxillary gland secretion was regularly obtained (Figs. 1–4). Very little saliva, or none at all, was delivered through the sublingual cannula, even when the chorda-lingual nerve was intact. The experiments which follow were carried out on the submaxillary gland after acute parasympathetic decentralization.

Submaxillary secretion could be obtained when the vago-sympathetic trunk was stimulated at a frequency of 5 shocks/sec, but usually 20 shocks/sec were given. The flow was never as fast as that evoked by maximal chorda-lingual stimulation, and during a stimulation period of 1–2 min it usually tended to decrease. The saliva was much more viscous than that produced by parasympathetic stimulation.

Secretory effects of isoprenaline and adrenaline

Isoprenaline given intravenously was found to evoke a lively secretion from the submaxillary gland. The threshold dose was 0.5–2 μg/kg, and after 5–10 μg/kg a flow about as rapid as that caused by sympathetic stimulation at 20 shocks/sec was obtained (Fig. 1). With adrenaline a flow could be produced by injecting 5–10 μg/kg. These agents caused no flow of saliva from the parotid or sublingual glands.

Sympathetic blocking drugs

Dihydroergotamine was given to block α-receptors. A dose of 200–300 μg/kg, which completely abolishes the secretory effect of sympathetic stimulation in the submaxillary gland of the cat, did not affect the response in the dog, even when injected 3 times (Fig. 2).

As β-receptor blocking drugs propranolol (Inderal, ICI) and D-(−)-N-isopropyl-p-nitrophenylethanolamine (INPEA) were used. The secretory effects of a moderate dose of isoprenaline (or adrenaline) could be completely abolished by propranolol (0.5 mg/kg) or INPEA (5 mg/kg). After these blocking doses a high dose of isoprenaline could still cause secretion. The secretion induced by vago-sympathetic stimulation was more resistant, but it could be abolished when the doses of the blocking agents were raised 2–5 times. Figure 1 shows that secretion caused by isoprenaline and adrenaline could be prevented by INPEA (5 mg/kg) whereas the secretory effect of vago-sympathetic

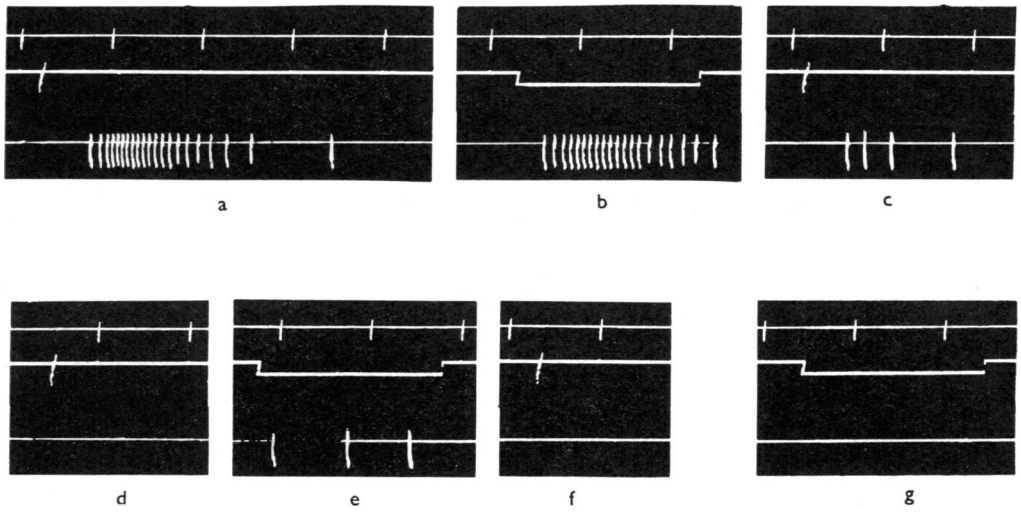

Fig. 1. Effects of INPEA on the secretory responses of the submaxillary gland to isoprenaline, sympathetic stimulation and adrenaline. Dog, 12 kg, chorda-lingual nerve cut acutely. Records from above: min, signal, drops of saliva. a and d: Isoprenaline 5 μg/kg; b, e and g: vago-sympathetic trunk stimulated during 2 min, 20 shocks/sec, 8 V. c and f: Adrenaline 10 μg/kg. Ten minutes before d and g: INPEA 5 mg/kg and 10 mg/kg. All injections given intravenously.

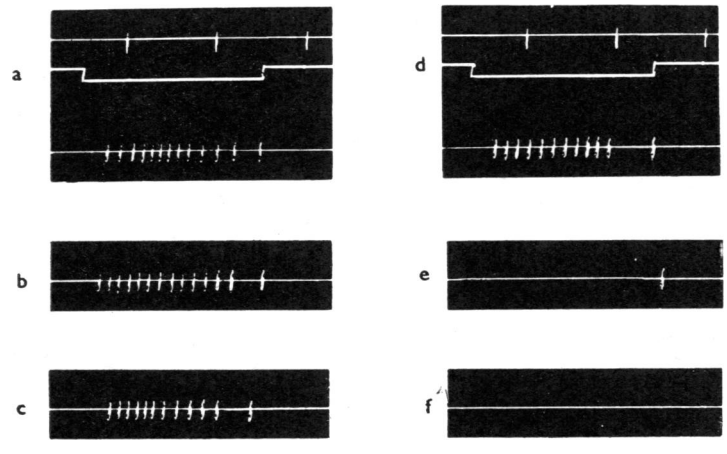

Fig. 2. Effects of dihydroergotamine and INPEA on the secretory responses of the submaxillary gland to sympathetic stimulation. Dog, 11 kg, chorda-lingual nerve cut. a–f: The vago-sympathetic trunk was stimulated each time during 2 min, 20 shocks/sec, 8 V. Five minutes before b, c and d: Dihydroergotamine, each time 200 μg/kg. Ten minutes before e and f: INPEA 5 and 10 mg/kg. Injections intravenously.

stimulation was diminished ; after an additional dose of 10 mg/kg it was abolished. Even high doses of the blocking agents (propranolol 2.5 mg/kg, INPEA 60 mg/kg) were devoid of secretory effect, and they did not affect the secretory responses to chorda-lingual stimulation. Large doses of INPEA often had a marked arousal effect.

Since a local anaesthetic effect has been attributed to some β-blocking agents, for instance to propranolol (Morales-Aguilerá & Vaughan Williams, 1965) but not to INPEA (Somani & Lum, 1965), attempts were made to see whether such an effect can be demonstrated by local application of the drugs in the gland through the submaxillary duct. Doses as high as 300 μg of the two drugs were injected ; the secretory effect of sympathetic stimulation was almost abolished by INPEA and entirely by propranolol, but the effect of chorda-lingual stimulation was not at all affected.

The observation in early experiments that small doses of the β-receptor agents could abolish the secretory effect of isoprenaline but only reduce that of sympathetic stimulation suggested that the secretory cells may contain both α- and β-receptors, and the effect of dihydroergotamine on the remaining response to sympathetic stimulation was investigated. The outcome of such an experiment is shown in Fig. 3. Instead of abolishing

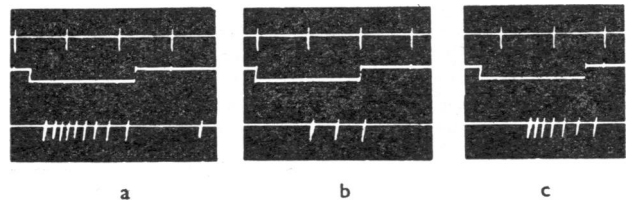

Fig. 3. Submaxillary secretion in response to vago-sympathetic stimulation during 2 min (20 shocks/sec, 12 V) before (a), 10 min after propranolol 1 mg/kg (b), and 5 min after dihydroergotamine 300 μg/kg (c). Injections intravenously. Dog, 6 kg, chorda-lingual nerve cut.

the response dihydroergotamine enhanced it. This was observed both after propranolol, as in Fig. 3, and after INPEA. By raising the doses of the β-receptor blocking agents the secretory effect of sympathetic stimulation could, however, be abolished even when dihydroergotamine had been given.

Vasomotor responses to vago-sympathetic stimulation

In the experiment of Fig. 4 stimulation of the vago-sympathetic trunk caused secretion and a marked vasoconstriction in the submaxillary gland. Dihydroergotamine (200 μg/kg) reduced the constrictor response, and an additional dose of 200 μg/kg abolished it. The diminished flow of blood through the gland after administration of the drug was presumably due to lowered blood pressure as a consequence of a reduction of the general peripheral vascular tone. Vasodilatation in the gland, which occurred when the stimulation of the nerve had ceased, as long as some constrictor effect persisted, already appeared during the stimulation period when the constrictor response had been abolished. After INPEA vago-sympathetic stimulation caused neither secretion nor any vascular response, provided that the anaesthesia was sufficiently deep. Otherwise the flow of blood through the gland could decrease during vago-sympathetic stimulation because of reflexly lowered blood pressure.

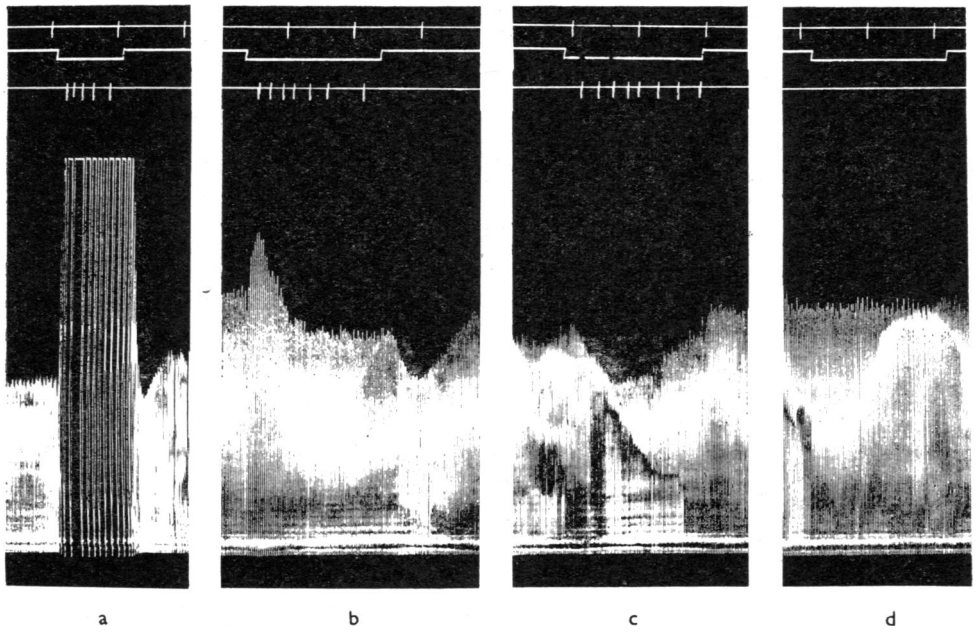

Fig. 4. Effects of dihydroergotamine and INPEA on secretory and vascular responses of the submaxillary gland to sympathetic stimulation. Dog, 10 kg, chorda-lingual nerve cut. Records from above: min, signal, drops of saliva, blood flow recorded with an ordinate recorder (each ordinate marks the time interval between two drops of venous blood from the gland). The vago-sympathetic trunk was stimulated (20 shocks/sec, 10 V) during 1 min in a, during 2 min in b, c and d. Dihydroergotamine (200 μg/kg) was injected twice: 7 min before b and 5 min before c. Ten minutes before d: INPEA 10 mg/kg.

DISCUSSION

In the present experiments vago-sympathetic stimulation and sympathomimetic amines caused a marked salivation from the submaxillary, but very little or none at all from the parotid and sublingual glands. These findings are in agreement with the general opinion, based on classical experiments by Ludwig (1851) and Heidenhain (1878), that in dogs only the submaxillary gland has a fairly rich supply of sympathetic secretory fibres. They differ from observations made by Shimamoto & Inoue (1958) and by Shimamoto, Inoue & Oyaizu (1958), according to which sympathetic stimulation and adrenaline and noradrenaline may evoke a flow of saliva from the parotid gland even larger than that obtained from the submaxillary gland. A marked parotid secretion on vago-sympathetic stimulation was obtained in the present experiments only when the parasympathetic secretory fibres were intact, and the observations described indicate that this flow was evoked reflexly by stimulation of vagal afferent fibres. It is known that excitation of vagal fibres may evoke a flow of saliva which is of reflex origin (Babkin, 1928; Hockman, Hagstrom & Hoff, 1965).

The fact that isoprenaline causes secretion from the dog's submaxillary gland suggests the presence of β-receptors in this gland. This view is supported by the observation that the secretory effects of sympathetic nerve stimulation and sympathomimetic amines are

antagonized by propranolol and INPEA, drugs which exert a β-receptor blocking action on other structures (Teotino, Polo Friz, Steis & Della Bella, 1963; Black, Crowther, Shanks, Smith & Dornhorst, 1964). The findings that (a) the α-receptor blocking agent dihydroergotamine even in large doses does not reduce the secretory response to sympathetic stimulation, (b) the β-receptor blocking agents completely abolish the secretory effects of adrenaline and sympathetic stimulation and, (c) isoprenaline can cause a flow as fast as that evoked by maximal sympathetic stimulation (Fig. 1) indicate in fact that the catecholamine receptors of the submaxillary gland of the dog belong entirely to the β-type. In this respect the gland differs from all other salivary glands investigated. Somewhat larger doses of propranolol and INPEA are required to abolish the secretory effect of sympathetic stimulation than that of isoprenaline or adrenaline, but even in these larger doses the drugs obviously act specifically as β-receptor blocking agents; the secretory effect of parasympathetic stimulation is not affected. It is common experience that blocking drugs more easily abolish effects when stimulating agents are injected into the blood than when such agents are released from nerve endings in close contact with the effector cells. When the effect of isoprenaline has been abolished by the β-receptor antagonists, secretion can be resumed by increasing the dose of isoprenaline; the antagonism is apparently of the competitive type, as shown with propranolol on atrial strips by Black, Duncan & Shanks (1965).

Secretory effects of sympathetic stimulation, reduced by β-receptor blocking drugs, were found to be increased by injection of dihydroergotamine. An interaction between α- and β-receptor blocking drugs has recently been demonstrated on the blood pressure (Sharma, 1966). In the present experiments it could be hypothesized that the α-receptor antagonists abolished a vasoconstrictor response to sympathetic stimulation which would otherwise reduce the salivary flow. This seems unlikely, however, in view of the fact that dihydroergotamine did not increase the salivary flow unless a β-receptor antagonist had been given initially ; besides, the relatively slow secretion which can be evoked by sympathetic stimulation is not greatly dependent on a large supply of blood. Another explanation might be that transmitter from the vasoconstrictor nerve endings cannot attach itself to the vascular α-receptors after administration of the α-receptor antagonist (Brown & Gillespie, 1957) and reaches the β-receptors of the gland cells, adding its effect to that of transmitter released from the secretory nerve endings. Secretion caused by sympathetic stimulation can thereby be increased, but only if suppressed by previous injection of a β-receptor antagonist, and not when a maximal response is evoked in the absence of β-receptor antagonists.

In the submaxillary gland of the rat the catecholamine receptors are mainly of the α-type, but β-receptors are also present. Pronethalol was found to exert some β-antagonist action here, but in addition it often evoked a flow of saliva; this was even more striking with dichlorisoprenaline (Emmelin *et al.*, 1965). In the present experiments propranolol and INPEA were devoid of secretory effect on the dog's submaxillary gland. These drugs lack apparently sympathomimetic activity at least on the dog's gland.

Attempts to separate vasoconstrictor and secretory effects of sympathetic stimulation have been to some extent successful in the submaxillary gland of the cat (Emmelin, 1955). It has been found that the secretory α-receptors are more susceptible to the blocking action of chlorpromazine than are the vascular α-receptors, whereas the opposite is true

for tolazoline. To obtain separation the doses of the drugs have to be carefully adjusted, however. The submaxillary gland of the dog is obviously a more suitable preparation for experiments in which constriction and secretion are studied separately, since constriction is brought about entirely by α-receptors, secretion by β-receptors.

The finding that after administration of an α-receptor blocking agent sympathetic stimulation causes vasodilatation, which is abolished by β-receptor antagonists, cannot be taken as evidence to show that there are specific sympathetic vasodilator fibres acting on vascular β-receptors. The vasodilatation may well be secondary to the secretion.

SUMMARY

1. In dogs under chloralose-urethane anaesthesia secretion from the submaxillary gland evoked by stimulation of the vago-sympathetic trunk was not affected by dihydroergotamine, whereas the vasoconstrictor response in the gland was abolished.

2. Isoprenaline caused a lively secretion from the gland, similar to that produced by sympathetic stimulation.

3. The secretory responses to sympathetic stimulation, isoprenaline and adrenaline were abolished by propranolol and N-isopropyl-p-nitrophenylethanolamine (INPEA).

4. It is concluded that the catecholamine receptors of the submaxillary gland cells in dogs belong exclusively to the β-type. This gland differs in that respect from all other salivary glands investigated so far.

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