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The pharmacology of salivary myoepithelial cells in dogs

N. EMMELIN, P. OHLIN AND A. THULIN

Institute of Physiology, University of Lund, Sweden

1. Pressure changes in the submaxillary and parotid ducts of dogs, induced by nerve stimulation or intravenous injection of drugs, were studied.

2. Pressure rises could be elicited by parasympathetic stimulation and by acetylcholine and methacholine, even when no secretion was evoked. These effects were abolished by atropine.

3. Similarly, sympathetic stimulation, adrenaline, noradrenaline and phenylephrine raised the pressure in both glands, also in the absence of secretion. Dihydroergotamine abolished these effects. Isoprenaline increased the pressure in the submaxillary duct, but only when it caused secretion. This effect was abolished by propranolol. In the parotid gland isoprenaline caused neither secretion nor pressure rise. It is concluded that the myoepithelial cells of the two glands are supplied with α-adrenoceptors.

4. Doses of histamine, bradykinin, kallidin and physalaemin which caused no salivary secretion raised the duct pressure even when dihydroergotamine, propranolol and atropine had been given.

5. Angiotensin and 5-hydroxytryptamine increased the pressure only in some experiments. Oxytocin caused very little or no pressure rise. Vasopressin had no effect of its own but reduced the pressure raising effects of nerve stimulation or drugs.

Information about the effects of drugs on the myoepithelial cells of salivary glands is scanty. It has been suggested that these cells can be contracted by adrenaline and histamine in dogs and cats (Babkin, 1950), by adrenaline in mice (Travill & Hill, 1963), and by adrenaline and oxytocin in sheep (Kay, 1958). Recent experiments show that in dogs they receive both sympathetic and parasympathetic motor fibres (Emmelin, Garrett & Ohlin, 1969). In the present experiments on dogs the effects of stimulating and blocking drugs on both divisions of the autonomic nervous system were therefore investigated. In addition, various other smooth muscle contracting agents were studied.

Methods

Altogether thirty-seven dogs, weighing 5–12 kg, were used. In fourteen dogs only the submaxillary and in fifteen only the parotid glands were studied, and in the remaining eight animals the submaxillary and the contralateral parotid gland were

examined. The dogs were anaesthetized with chloralose (50 mg/kg) and urethane (500 mg/kg), given through a cannula in a femoral vein after induction with ether. Additional doses of the anaesthetics were given in the course of the experiment when required. The salivary ducts were exposed and cannulated with a polyethylene cannula of widest possible bore. The cannula was connected to a closed system in which the pressure could be set at any desired level from a pressure bottle. The pressure in the duct was recorded by means of a transducer and a polygraph. The system could be opened to study secretion. The details of the method have been given in a previous paper (Emmelin, Garrett & Ohlin, 1968).

At the beginning of each experiment the vagosympathetic trunk was cut. In one exceptional case a completely separate sympathetic nerve was found. The stimulation of the rostral end of this nerve caused the characteristic ocular and salivary effects. These were not obtained when the adjacent, larger nerve, the vagus, was excited ; only respiratory effects were noticed. The auriculotemporal nerve was also cut (Burgen, 1964) for studies on the parotid gland, and the chorda-lingual nerve when the submaxillary gland was being studied. The nerves were stimulated electrically by single stimuli or repetitively. Square wave pulses of supramaximal strength (8–15 V) and a duration of 1·5–2·0 msec were used.

The highly viscous saliva often produced by both glands tended to cause obstruction ; to overcome this it was found convenient to increase the pressure in the system for a short period. In the submaxillary gland it was raised to about 50 mm Hg and then lowered to about 20 mm Hg, at which level observations on the pressure effects of nerve stimulation or drugs were begun ; during the experiment, the pressure gradually fell from this initial level. In the parotid gland, observations started at about 15 mm Hg, and the pressure was now and again raised to this level. In addition it was often found useful to elicit a rapid secretion by stimulation of the parasympathetic nerve for a short while in order to wash the duct system, particularly if the saliva was very thick.

The following drugs were used: acetylcholine chloride, methacholine chloride, atropine sulphate, adrenaline bitartrate, noradrenaline bitartrate, phenylephrine hydrochloride, isoprenaline sulphate, dihydroergotamine methansulphonate, propranolol hydrochloride, histamine dihydrochloride, antazoline hydrochloride, synthetic kallidin (Sandoz) and physalaemin (Farmitalia), bradykinin triacetate (Sigma), angiotensin amide (Hypertensin, Ciba), 5-hydroxytryptamine creatinine sulphate, vasopressin (lysine-8-vasopressin, Sandoz), oxytocin (Sandoz). With the exception of adrenaline and noradrenaline, which were calculated as bases, the doses refer to the compounds as given above. The drugs were injected through the cannula in the femoral vein.

Results

Parasympathetic stimulation

As was found in a previous investigation (Emmelin *et al.*, 1969) the pressure in the salivary duct increased when a single stimulus was applied to the auriculotemporal or the chorda-lingual nerve. A minute secretory response was obtained by such stimulation in only three experiments, all on parotid glands. Typical pressure responses are shown in Figs. 1, 9 and 10. They were abolished or greatly reduced by atropine (50–100 μg/kg) but not affected by propranolol (1 mg/kg) or

dihydroergotamine (200 μg/kg) (Fig. 1). The maximal rises in pressure varied between 1 and 13·5 mm Hg, with a mean of 6·1 ± 0·74 mm Hg, in twenty submaxillary glands and between 0·5 and 9 mm Hg, with a mean of 4·7 ± 0·49 mm Hg, in twenty-two parotid glands.

Acetylcholine and methacholine

A very small dose of acetylcholine (0·15 μg/kg) was found to cause a perceptible pressure rise in the submaxillary and parotid ducts (Table 1); the responses increased with increasing doses. To evoke salivary secretion, on the other hand, doses of 1–10 μg/kg were needed. Similarly, the threshold doses of methacholine for pressure rise were much lower than those for secretion; both were considerably lower than those of acetylcholine (Table 1). Figure 2 demonstrates pressure rises in a submaxillary duct obtained with increasing doses of acetylcholine. A dose of 5 μg/kg or more caused a secondary rise which increased in size with the dose, as did the primary response. It should be pointed out that 5 μg/kg was the threshold dose for secretion. With methacholine, a very small secondary rise occurred after 0·5 μg/kg, a dose which was threshold for secretion (Fig. 3). Such secondary rises were often but not always obtained when secretion-stimulating doses of acetylcholine or methacholine were given. Sometimes, particularly after larger doses, the primary rise, attributed to myoepithelial contraction, and the secondary one, assumed to be due to secretion, seemed to merge.

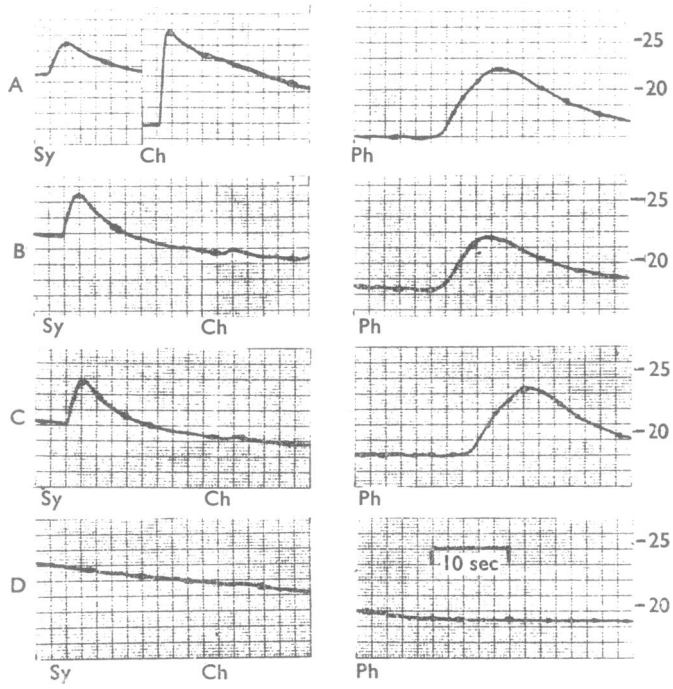

FIG. 1. Pressure responses in a submaxillary duct to single shocks applied to the vagosympathetic (Sy) or the chorda-lingual (Ch) nerve, or to phenylephrine 5 μg/kg (Ph). A, Before blocking drugs; B, after atropine 100 μg/kg; C, after propranolol 1 mg/kg as well; D, after dihydroergotamine 200 μg/kg in addition. Calibration: pressure in mm Hg. Time mark: 10 sec.

Pressure rises elicited by acetylcholine and methacholine were easily abolished by atropine. They were not diminished by propranolol or dihydroergotamine. This applies to the secondary phase of pressure rise also, a fact which excludes the possibility that this rise could be caused by catecholamines released from the adrenals by the injected acetylcholine or methacholine.

Sympathetic stimulation

Single stimuli applied to the vagosympathetic trunks caused pressure rises, varying between 0·5 and 8·5 mm Hg, with a mean of 3·3 ± 0·56 mm Hg, in twenty-one submaxillary glands. In most experiments the responses were smaller than those elicited by parasympathetic stimulation, but in some the reverse was true. To evoke a flow of saliva from the submaxillary gland by excitation of the sympathetic fibres repetitive stimulation was always required, the threshold frequency being usually 2–3 stimuli/sec ; in one experiment it was as low as 0·2/sec, and in two others 5/sec.

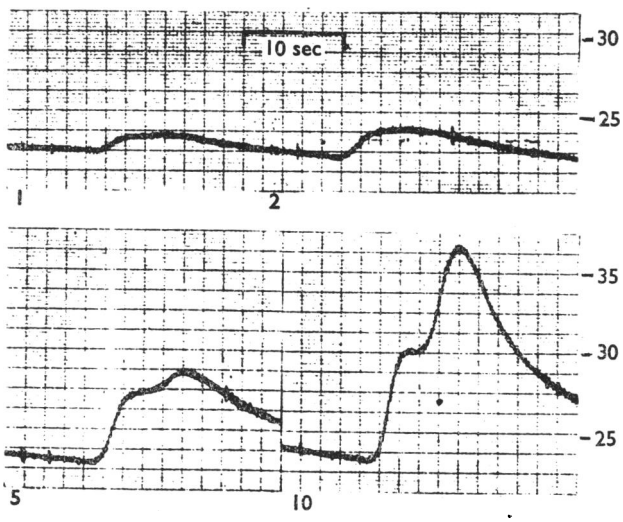

FIG. 2. Pressure responses in a submaxillary duct to acetylcholine ; numbers indicate doses in μg/kg. 5 μg/kg caused secretion. Calibration: pressure in mm Hg. Time mark: 10 sec.

TABLE 1. *Threshold doses (μg/kg) for rises in pressure (P) and secretion (S)*

	Submaxillary gland		Parotid gland	
Drugs	P	S	P	S
Adrenaline	0·035 ± 0·0091	8 ± 1·4	0·11 ± 0·031	—
Noradrenaline	0·14 ± 0·038	15 ± 2·9	0·43 ± 0·075	—
Phenylephrine	0·35 ± 0·087	—	1·0 ± 0·35	—
Acetylcholine	0·15 ± 0·029	4·3 ± 0·75	0·13 ± 0·043	7 ± 2·2
Methacholine	0·06 ± 0·017	1·3 ± 0·43	0·05 ± 0·019	0·43 ± 0·075

Drugs were injected intravenously. Each value is the mean ± S.E.M. of four experiments.

In the parotid gland, single stimuli applied to the vagosympathetic trunk caused pressure rises of 0·3–4 mm Hg, with a mean of $1·2 \pm 0·27$ mm Hg, in eighteen glands and no response in four glands. A response was always obtained when the nerve was excited repetitively. In agreement with common experience, vagosympathetic stimulation was usually found to lack a secretory effect on the parotid gland, even if a stimulation frequency of 10/sec was used (Emmelin *et al.*, 1969). In some experiments, however, a small secretory response could be elicited from the parotid gland when the vagosympathetic trunk was stimulated at a frequency of 5–10/sec. The facts that (*a*) no secretion was obtained even with large doses of adrenaline or noradrenaline, (*b*) the secretion was obtained only when vagosympathetic stimulation caused increased intestinal movements, the contralateral vagosympathetic trunk being intact, and (*c*) this secretion was abolished by atropine or by deepened anaesthesia, suggest that the salivary flow was not due to direct stimulation of sympathetic secretory fibres but was evoked reflexly by way of vagal afferents and parasympathetic secretory fibres. Although the auriculotemporal nerve had been cut in these experiments, this reflex secretion was possibly mediated by parasympathetic fibres reaching the parotid gland of the dog by some other route (Emmelin, Garrett & Holmberg, 1968).

Pressure responses in submaxillary glands to single stimuli acting on the vagosympathetic trunk are illustrated in Figs. 1 and 10; they were not affected by atropine and propranolol but were abolished by dihydroergotamine (200 μg/kg). This finding also applies to repetitive stimulation in the parotid gland. On the other hand, when a pressure rise in the submaxillary gland was due to secretion, dihydroergotamine was without effect but propranolol abolished the rise.

Adrenaline, noradrenaline and phenylephrine

All three drugs were able to raise the pressure in the submaxillary and parotid ducts on intravenous injection (Figs. 1, 3–5, 9). Threshold doses for adrenaline, noradrenaline and phenylephrine obtained in four submaxillary and four parotid glands are shown in Table 1; the parotid seemed somewhat less sensitive to these drugs than the submaxillary glands.

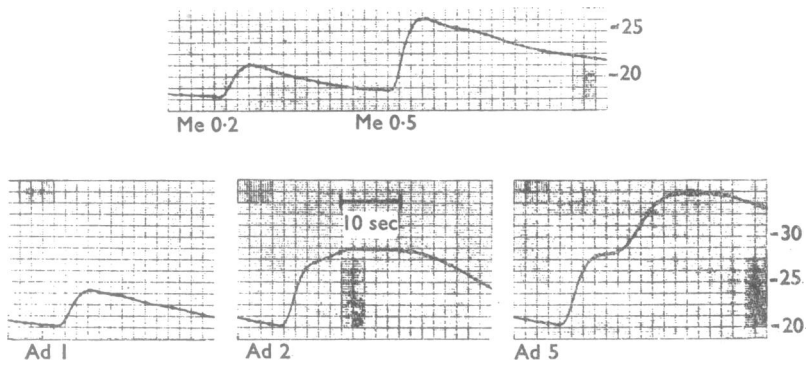

FIG. 3. Pressure responses in a submaxillary duct to methacholine (Me) and adrenaline (Ad); numbers indicate doses in μg/kg. The lowest doses causing secretion were 0·5 μg/kg of methacholine and 5 μg/kg of adrenaline. Calibration: pressure in mm Hg. Time mark: 10 sec.

In the submaxillary gland, the three drugs could cause a flow of saliva, but the thresholds were high, 5–10 μg/kg for adrenaline and 10–20 μg/kg for noradrenaline. With large doses of phenylephrine, 50–100 μg/kg, a minute secretion could be elicited only sometimes. When present, this response was scarcely increased by raising the dose; it was abolished by dihydroergotamine but not by atropine or propranolol. These observations suggest that some submaxillary glands may have a small number of α-receptors mediating secretion.

In the parotid glands no secretion was obtained even when large doses of adrenaline or noradrenaline were given. One out of nine parotid glands responded to phenylephrine 50 μg/kg with a very small secretion. This effect was abolished by atropine and might have been due to central stimulation, the secretion being elicited by way of an unknown parasympathetic secretory pathway (Emmelin, Garrett & Holmberg, 1968).

The sizes of the responses to adrenaline and phenylephrine increased with the doses; in a submaxillary gland adrenaline 0·5 μg/kg produced a response which could not be raised by doubling the dose, and a similar maximum was reached with phenylephrine 10 μg/kg (Fig. 4). When the dose of adrenaline was raised above the secretory threshold a secondary rise was seen; the maximum rise of the primary phase after adrenaline was about the same as that reached with methacholine 0·5 μg/kg (Fig. 3).

The pressure responses to the three drugs in doses which caused no secretion were unaffected by atropine and propranolol but abolished by dihydroergotamine; this is shown for phenylephrine in Figs. 1 and 5.

Isoprenaline

In dogs, this drug is known to cause salivation from the submaxillary gland but not from the parotid gland (Emmelin & Holmberg, 1967). In the present experiments it was found to produce a pressure rise in the submaxillary duct, with a threshold dose of 0·5–2 μg/kg; this was also the threshold for secretion. The pressure record differed markedly from that obtained with adrenaline, noradrenaline or

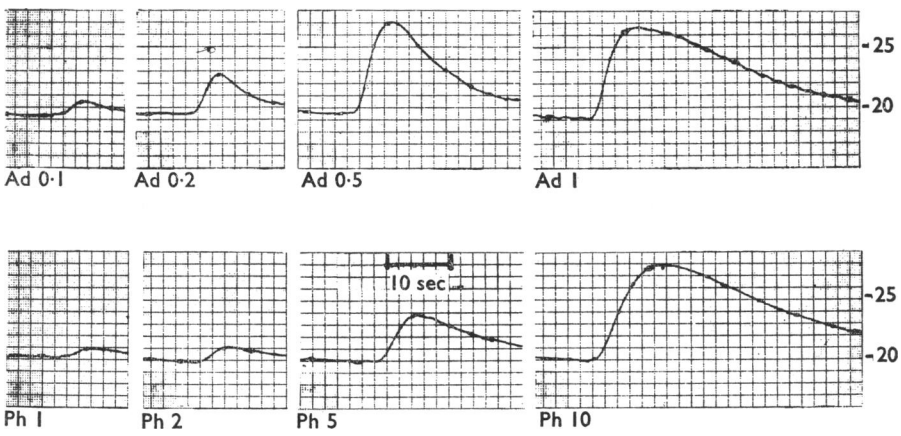

FIG. 4. Pressure responses in a submaxillary duct to adrenaline (Ad) and phenylephrine (Ph); numbers indicate doses in μg/kg. Calibration: pressure in mm Hg. Time mark: 10 sec.

phenylephrine in that the pressure rise after isoprenaline was very slow. Moreover, in contrast to the findings obtained with phenylephrine, the pressure rise was abolished by propranolol (1 mg/kg) but was not affected by dihydroergotamine or atropine (Fig. 5).

In the parotid gland isoprenaline did not cause secretion or a rise in pressure.

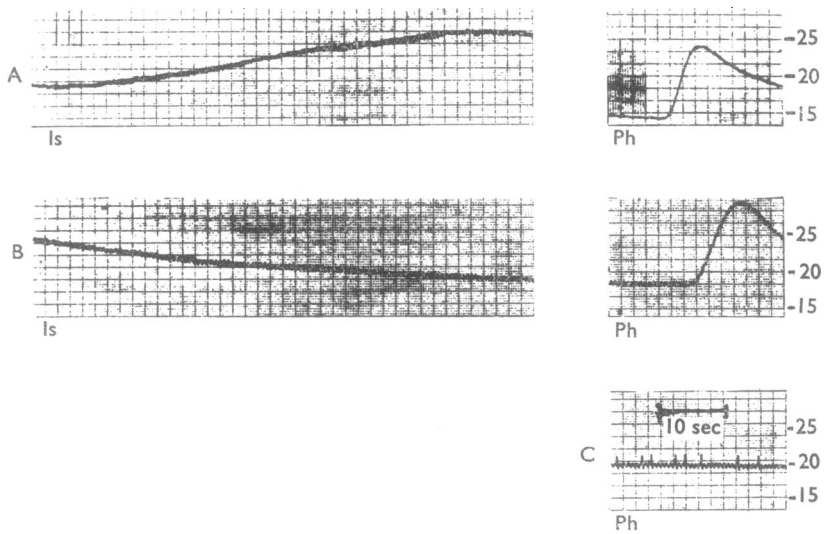

FIG. 5. Pressure responses in a submaxillary duct to isoprenaline (Is), 2 μg/kg, and phenylephrine (Ph), 5 μg/kg, before (A) and after propranolol 1 mg/kg (B) and dihydroergotamine 200 μg/kg (C). The fall in pressure seen when isoprenaline was given after propranolol (B) was that usually seen in salivary glands under our experimental conditions and not caused by isoprenaline. Isoprenaline, 2 μg/kg, but not phenylephrine, 5 μg/kg, caused secretion. Calibration: pressure in mm Hg. Time mark: 10 sec.

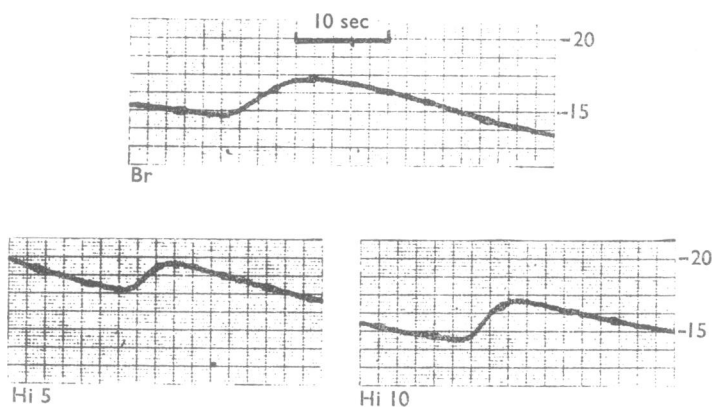

FIG. 6. Pressure responses in a parotid duct to bradykinin (Br), 2 μg/kg, and histamine (Hi), 5 and 10 μg/kg, after atropine 100 μg/kg, propranolol 1 mg/kg and dihydroergotamine 200 μg/kg. Calibration: pressure in mm Hg. Time mark: 10 sec.

Histamine

Histamine produced a pressure rise in the submaxillary and the parotid glands. The threshold dose was 0·5–1·0 μg/kg and the response increased with the dose. To evoke a small secretory response from the submaxillary gland a dose of 20 μg/kg was needed ; no parotid secretion was obtained with this dose. Atropine and dihydroergotamine reduced the pressure rises elicited by histamine in the submaxillary gland ; when both these drugs had been given, histamine had very little or no pressure raising effect. They did not change the responses in the parotid gland, which, however, were abolished by antazoline (10 mg/kg). Propranolol was without

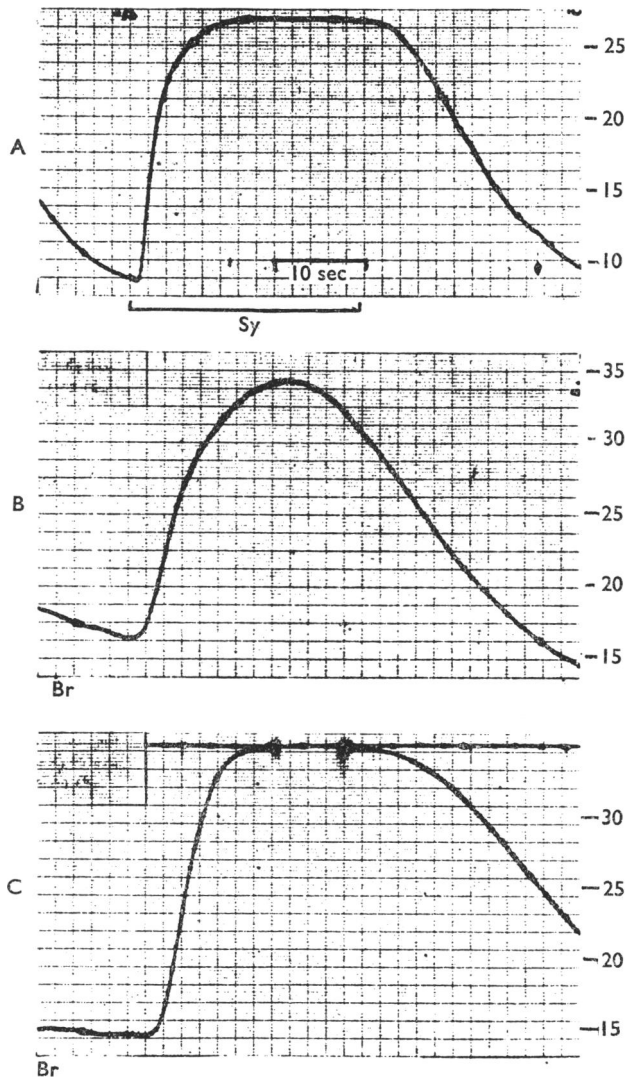

FIG. 7. Pressure responses in a parotid duct to vagosympathetic stimulation at 5/sec (A), and to bradykinin, 20 μg/kg, before (B) and after propranolol 1 mg/kg and dihydroergotamine 200 μg/kg (C). Calibration: pressure in mm Hg. Time mark: 10 sec.

effect in both glands. Thus in the parotid gland, pressure rises could be elicited by histamine even when atropine, propranolol and dihydroergotamine had been administered (Fig. 6).

Bradykinin

Bradykinin caused pressure rises in both glands. The threshold was about 0·5 μg/kg, and with relatively small doses the pressure increased rather slowly ; a comparison with the effect of histamine in doses causing responses of similar sizes is shown in Fig. 6. With larger doses the pressure rose more steeply (Fig. 7). Sometimes, but not regularly, a dose of 2–5 μg/kg or more caused a secondary rise similar to that seen with large doses of acetylcholine or adrenaline. This secondary rise was not present after adrenalectomy and was therefore attributed to catecholamine release from the adrenals. It was abolished by dihydroergotamine. The primary pressure rise, on the other hand, was unaffected by dihydroergotamine, atropine and

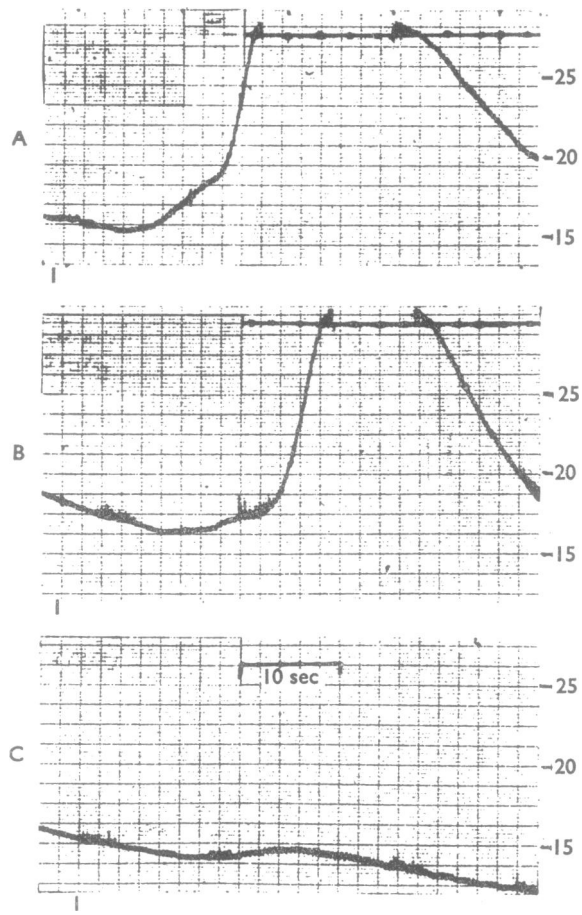

FIG. 8. Pressure responses in a parotid duct to kallidin, 2 μg/kg, before (A) and after atropine 100 μg/kg and propranolol 1 mg/kg (B) and dihydroergotamine 200 μg/kg (C). Calibration: pressure in mm Hg. Time mark: 10 sec.

propranolol. In the experiment on a parotid gland shown in Fig. 7, no secondary rise was obtained. A maximal pressure rise was obtained with bradykinin 20 μg/kg ; it was not reduced by propranolol or dihydroergotamine. This pressure response was of the same magnitude as that produced by sympathetic stimulation at a frequency (5/sec) which resulted in a maximal response.

A dose of bradykinin as large as 20 μg/kg was without secretory effect on the parotid gland. In the submaxillary gland, no secretion was obtained with 5 μg/kg ; higher doses were not used because of the possibility of a release of catecholamine that might have evoked secretion.

Kallidin

Kallidin resembled bradykinin in its action. Small doses (about 0·5 μg/kg) caused a slow rise in pressure after a brief latent period. After a somewhat larger dose, 1–2 μg/kg or more, the pressure curve had a secondary, steep rise which was not affected by atropine and propranolol but was abolished by dihydroergotamine. The primary rise was not affected by these drugs ; there was no secretion during this phase.

Physalaemin

In dogs this endecapeptide causes secretion from the submaxillary (Bertaccini & De Caro, 1965) and the parotid glands (Emmelin & Lenninger, 1967). The secretory threshold was found to be about 0·5 μg/kg in the parotid gland and 1–2 μg/kg in

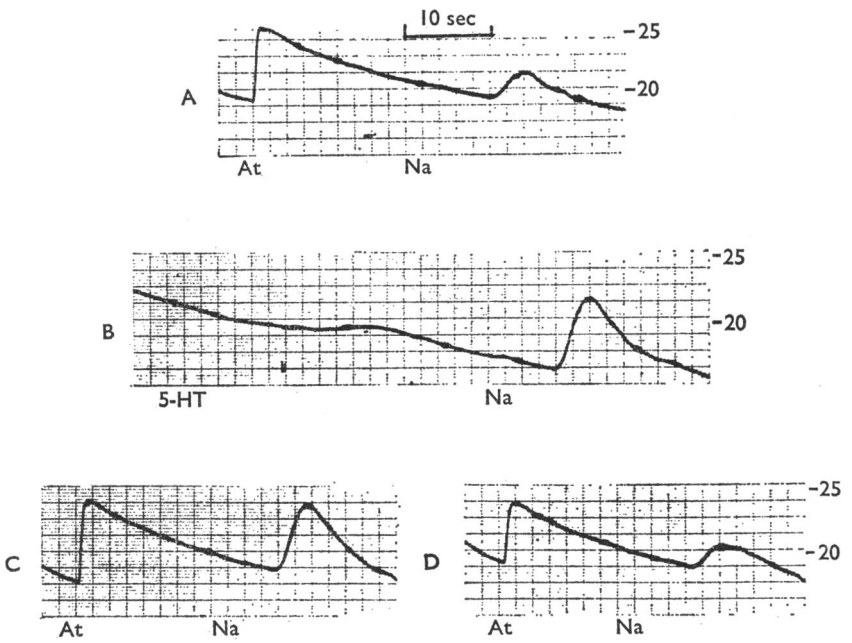

FIG. 9. Pressure responses in a parotid duct to single shocks applied to the auriculotemporal nerve (At) and to intravenous noradrenaline 2 μg/kg (Na). A, Before 5-HT. In B, 5-HT 20 μg/kg was given followed by noradrenaline. C: 10 min and D 15 min after 5-HT. Calibration: pressure in mm Hg. Time mark: 10 sec.

the submaxillary gland. Considerably smaller doses were found to cause pressure responses, the threshold doses being 0·005–0·01 μg/kg in both glands. After doses which did not evoke any secretion the pressure rose slowly, even more slowly than after a small dose of bradykinin or kallidin. A secondary rise was obtained only after a dose which caused secretion. The pressure rise was still obtained after administration of atropine, propranolol and dihydroergotamine.

Angiotensin

Angiotensin in doses of 2 μg/kg sometimes caused pressure responses in the submaxillary and parotid glands. The responses appeared after a fairly long latent

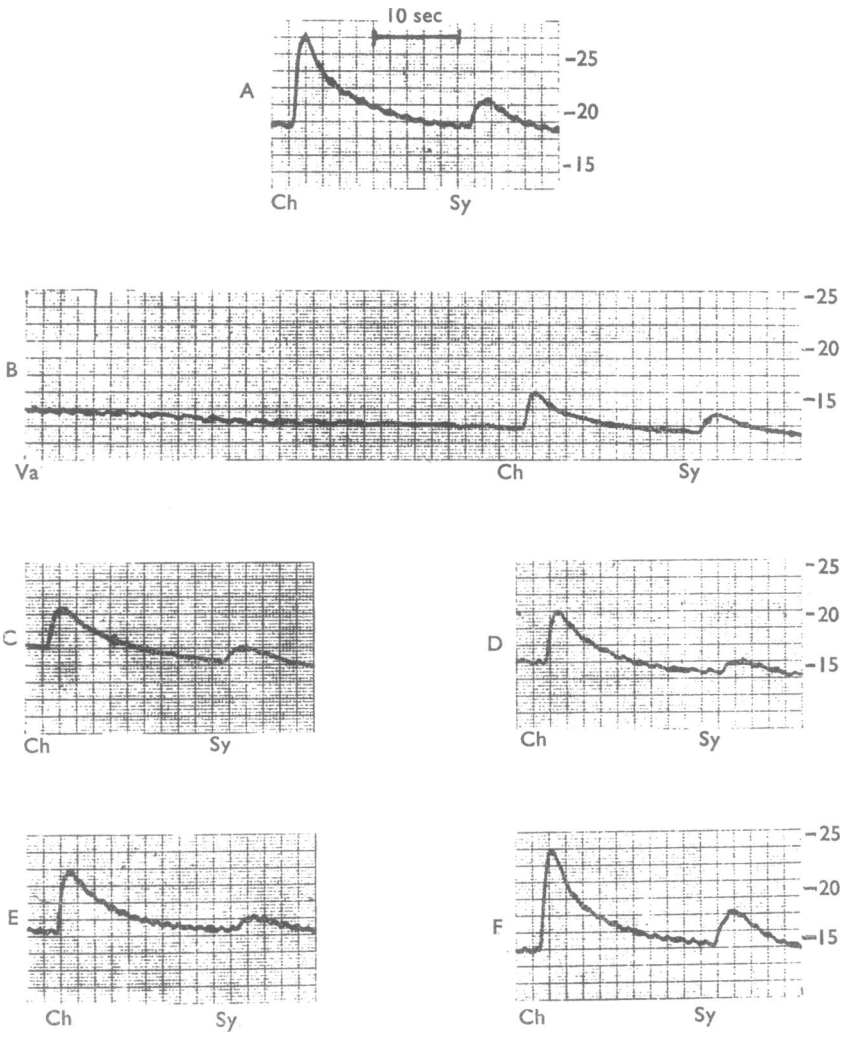

FIG. 10. Pressure responses in a submaxillary duct to single shocks applied to the chorda-lingual (Ch) or to the vagosympathetic (Sy) nerve. A, Before vasopressin. In B, vasopressin 0·5 i.u./kg (Va) was injected, followed by stimulation of the nerves. C is 7 min, D is 18 min, E is 28 min and F in 47 min after vasopressin. Calibration: pressure in mm Hg. Time mark: 10 sec.

period and were not affected by atropine or propranolol, but abolished by dihydroergotamine. Because there was very marked tachyphylaxis, reproducible effects were obtained only when the intervals between doses were about half an hour. Another striking feature was that the effect could be elicited in some dogs but not in others. In some animals even very large doses caused either no, or only a small, rise in pressure.

No secretory response was observed after angiotensin. After large doses of angiotensin, 20 μg/kg or more, the pressure responses to noradrenaline, phenylephrine and sympathetic stimulation were often found to be enhanced, even when angiotensin itself caused no pressure response.

5-Hydroxytryptamine

5-Hydroxytryptamine resembled angiotensin as far as shape of the pressure curve and the effect of autonomic blocking drugs on the response were concerned. Moreover, the effect was not obtained regularly, tachyphylaxis developed readily and there was no secretion. There was also an enhancing effect on the responses to noradrenaline (Fig. 9).

Vasopressin

Vasopressin in doses between 0·5 and 2 i.u./kg did not raise the pressure. After injection of the substance, the pressure responses to stimulating agents, nerve stimulation or drugs, were found to be reduced. This effect persisted for 0·5–1 hr, depending on the dose, and disappeared gradually (Fig. 10). It was observed after injection of as little as 0·05–0·1 i.u. vasopressin/kg.

Oxytocin

After 0·5–2 i.u./kg the pressure rose only very little or not at all. Some depression of the responses to stimulating agents was possibly present after injection of oxytocin, but this effect was much smaller and of shorter duration that that observed after injection of vasopressin ; moreover, it was not seen regularly, even after large doses.

Discussion

Pressure rises in the ducts of salivary glands of dogs can be elicited by intravenous injections of acetylcholine, methacholine, adrenaline, noradrenaline and phenylephrine. The fact that the doses needed to raise the pressure are far below those required to evoke salivary secretion indicates that the pressure effect observed is a motor effect, presumably exerted on the myoepithelial cells. Even when doses of the drugs are given which cause secretion it is often possible to recognize a separate motor response appearing on the pressure curve earlier than the pressure rise due to secretion. When sympathomimetic drugs are used the possibility that secretion is the sole cause of the pressure rises is excluded by the following considerations: in the parotid gland these drugs have no secretory effect at all, whereas in the submaxillary gland phenylephrine, which stimulates mainly α-receptors, is likewise almost devoid of secretory activity. In the submaxillary gland, but not in the parotid gland, secretion can be elicited by sympathetic stimulation which activates

β-receptors (Emmelin & Holmberg, 1967); in the submaxillary but not in the parotid gland, isoprenaline causes a pressure rise, but only when doses are used which cause secretion, an effect which is abolished by the β-receptor blocking drug propranolol. When secretion is suppressed by propranolol, a pressure rise can still be elicited by adrenaline, noradrenaline, phenylephrine or sympathetic stimulation. Dihydroergotamine, on the other hand, blocks, in the parotid gland, the pressure effects of all doses of sympathomimetic drugs and, in the submaxillary gland, the pressure effects of doses which do not cause secretion. These observations on the effects of sympathomimetic and parasympathomimetic drugs and the actions of blocking agents support the view that the myoepithelial cells are supplied with motor fibres from both divisions of the autonomic nervous system. It may be concluded that in the submaxillary gland of the dog, sympathetic stimulation causes myoepithelial contraction by activation of α-receptors, whereas secretion is mediated almost exclusively by β-receptors.

The maximal effects on the myoepithelial cells of parasympathomimetic and sympathomimetic drugs and of sympathetic nerve stimulation are similar, but the effects of parasympathetic stimulation cannot be assessed, since secretion occurs at a fairly low frequency of stimulation.

Bradykinin, kallidin and physalaemin have been found to cause contraction of the salivary myoepithelial cells by a " direct " effect, because it is not abolished by the usual autonomic blocking agents. The actions of kallidin and bradykinin are more complex due to a secondary contractor effect caused by catecholamine release from the adrenals. This secondary pressure rise is not associated with secretion, a fact which suggests that the amounts of catecholamines released are too small to evoke secretion. On the other hand, secretion can easily be obtained with physalaemin, which seems to have a " direct " effect on the secretory (Emmelin & Lenninger, 1967) and myoepithelial cells of the two glands. When a pure " direct " myoepithelial effect is elicited with large doses of these polypeptides, the maximal contraction is of a size similar to that obtained by repetitive sympathetic stimulation. It is tempting to consider the possibility that kinins might play a role in the mechanism which causes contraction of salivary myoepithelial cells, but the present experiments throw no light on this question.

Unlike the salivary myoepithelial cells of sheep (Kay, 1958) those of the dog seem very insensitive to oxytocin. It is of interest that, in the dog, vasopressin and, to a less extent, oxytocin reduce the effects of stimulating agents.

The effect of histamine on the salivary myoepithelial cells described by MacKay (1930) and Stavraky (1931) has been confirmed by the present experiments. In the submaxillary gland the effect of histamine seems to be mediated partly by atropine-sensitive receptors and partly by adrenoceptive α-receptors, because the pressure response is reduced by atropine and dihydroergotamine, particularly when they are given together. The pressure response obtained in the parotid gland is unaffected by these blocking agents and therefore reminiscent of the " direct " effects of bradykinin, kallidin and physalaemin. The actions of histamine on the salivary glands are very complicated (Emmelin, 1966) and seem to deserve further analysis.

The myoepithelial cells of salivary glands differ in many respects from those of mammary glands, which are contracted by oxytocin and, to a less extent, by vasopressin. Moreover, adrenaline reduces the responses to oxytocin, and bradykinin,

histamine and acetylcholine cause contraction only in relatively large doses (Bisset, Clark, Haldar, Harris, Lewis & Rocha e Silva, Jr., 1967; Bisset, Clark & Lewis, 1967).

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