Action of drugs on denervated myoepithelial cells of salivary glands

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Summary

1. The pressure in the ducts of the two submaxillary glands was recorded in anaesthetized dogs in which either the superior cervical ganglion had been removed or the chorda-lingual nerve had been cut on one side, 17–30 days earlier. Noradrenaline, adrenaline, phenylephrine, acetylcholine and methacholine were injected to produce pressure rises attributed to contraction of myoepithelial cells.

2. After sympathectomy increased pressure responses were obtained with all the drugs, but particularly with noradrenaline. Cocaine increased the effect of noradrenaline on the normal gland but slightly less than sympathectomy.

3. Parasympathetic decentralization increased the pressure effects of acetylcholine and methacholine, and in most cases also increased those of the sympathomimetic drugs.

4. It is concluded that the myoepithelial cells of this gland normally receive motor impulses from both divisions of the autonomic nervous system, and that many, if not all, of the cells are innervated by both. Disconnecting the gland from the central nervous system by either pathway causes supersensitivity of the classical post-junctional type, and sympathetic ganglionectomy causes in addition a pre-junctional sensitization.

Introduction

In submaxillary glands of dogs sympathomimetic and parasympathomimetic drugs were found to cause effects which were ascribed to contractions of myoepithelial cells (Emmelin, Ohlin & Thulin, 1969). When the pressure in the salivary duct was recorded in a closed system, the drugs were found to produce pressure rises at doses far below those needed to evoke secretion of saliva. If secretory doses were given, the rise often occurred in two steps, an early, steep response followed by a more gradual rise ; the first phase was attributed to myoepithelial contraction, the second phase to secretion.

The mechanisms which control the activity of the salivary myoepithelial cells are imperfectly known. The cells have been assumed to receive motor nerves from the sympathetic or the parasympathetic system, or from both divisions of the autonomic nervous system (see Babkin, 1950 ; Emmelin, Garrett & Ohlin, 1968 ; Darke & Smaje, 1971). Observations on the effect of nerve section on the chemosensitivity of various structures have in the past served to throw light on the role played by the nerves in the regulation of the effectors (Cannon & Rosenblueth,

1949 ; in the case of salivary glands: Emmelin, 1965). In the present experiments the effects of section of nerves supplying the glands on the responsiveness of salivary myoepithelial cells to drugs were studied.

Methods

Dogs weighing 5–14 kg were used. In 14 dogs the chorda-lingual nerve was cut on one side and in 7 other dogs the superior cervical ganglion of one side was extirpated. These operations were carried out under ether anaesthesia. After 17–30 days chloralose-urethane (50+500 mg/kg) was given intravenously after induction with ether. A tracheal cannula was inserted and both submaxillary ducts were exposed in the neck and cannulated with polyethylene tubing of the widest possible bore. Secretion was studied as flow of saliva in the open, horizontally placed tubes. To record pressure the two tubes were filled with saliva and alternately connected to a closed system containing a pressure bottle, a mercury manometer and a strain gauge transducer, operating a potentiometer writer. The pressure in the duct was set at a suitable level (15–20 mmHg) by means of the pressure bottle. A tap was then turned so that the gland was no longer connected to the bottle and the mercury manometer but only to the transducer. Usually the pressure in the duct gradually fell from the initial level. Drug injections to cause pressure rises were made at basal levels of 8–19 mmHg in different experiments, but in each experiment comparisons between effects on the two glands were always made at the same basal level. As in the previous investigation (Emmelin *et al.*, 1969) it was often found helpful to raise the pressure between drug injections to 50 mmHg for half a minute and then to lower it to 15–20 mmHg, in order to clear obstructions in the duct system caused by the often highly viscous saliva.

Drugs

The following drugs were injected through a cannula in a femoral vein: acetylcholine chloride, methacholine chloride, adrenaline bitartrate, noradrenaline bitartrate, phenylephrine hydrochloride, atropine sulphate, propranolol hydrochloride, dihydroergotamine methansulphonate and cocaine hydrochloride. With the exception of adrenaline and noradrenaline, which were calculated as the base, the doses refer to the salts. The drugs used to cause a rise in pressure were either given in a series of doses, starting near the threshold for pressure rise and increasing the dose, or a standard dose of each drug was chosen for comparison of the effects on the two glands.

Results

In agreement with our earlier observations phenylephrine even in a relatively large dose (0·1 mg/kg) was found to be devoid of secretory effect in the open system, and in the pressure system only one phase of rapid rise was obtained ; in this particular gland sympathetic secretion is mediated by β-adrenoceptors, but myoepithelial contraction via α-receptors (Emmelin & Holmberg, 1967 ; Emmelin *et al.*, 1969). Noradrenaline, adrenaline, acetylcholine and methacholine also produced steep pressure rises, and the doses required were lower than those needed for secretion in the open system. When the secretory threshold was exceeded there was usually a secondary phase in the pressure record.

Effects of sympathetic ganglionectomy

After extirpation of the sympathetic ganglion the pressure effects of all the drugs, at low doses, were found to be increased, particularly the responsiveness to noradrenaline. Figure 1 shows that increases in pressure induced by a series of doses of noradrenaline were much larger in the denervated than in the normal gland. A dose of 2 μg/kg produced a secondary rise of pressure in the denervated but not in the normal gland (Fig. 1); in the open system this dose was found to be the secretory threshold of the denervated gland, whereas a dose as high as 10 μg/kg failed to evoke secretion in the normal gland. The secondary rise was not obtained after administration of the β-receptor blocking agent, propranolol (1 mg/kg), whereas the first phase was unaltered (Figure 1). The first response, on the other hand, was abolished by dihydroergotamine (0·2 mg/kg). In 6 experiments the mean pressure response to a standard dose of noradrenaline, 0·2 μg/kg, was found to be 3·1 ±0·07 mmHg (mean ± S.E.M.) in the denervated, and 0·5 ±0·10 mmHg in the normal gland. These pressure responses were abolished by dihydroergotamine, 0·2 mg/kg.
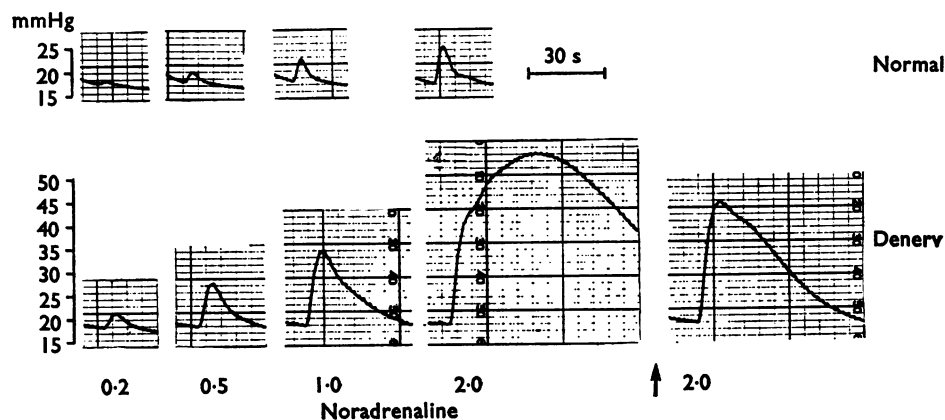
FIG. 1. Pressure effects of noradrenaline in dog salivary glands. Doses (μg/kg) are shown beneath the lower records. Upper records: normal gland, lower records: contralateral gland, 30 days after removal of the superior cervical ganglion. At the arrow propranolol 1 mg/kg was injected.
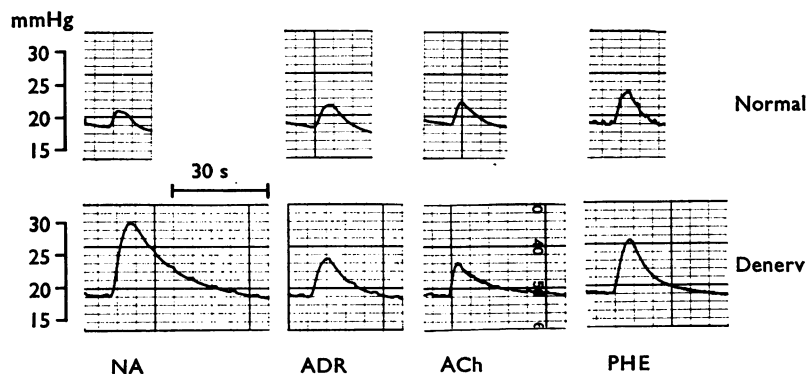
FIG. 2. Pressure effects of noradrenaline, 0·5 μg/kg (NA), adrenaline, 0·1 μg/kg (ADR), acetylcholine, 1·0 μg/kg (ACh) and phenylephrine, 2·0 μg/kg (PHE) in dog salivary gland. Above: normal gland. Below: contralateral gland 3 weeks after sympathectomy.

Supersensitivity to adrenaline and phenylephrine was also obtained but, as illustrated in Fig. 2, it was less than that to noradrenaline. As shown in the figure the response to acetylcholine was also somewhat increased as was that to methacholine (Figure 3). The increased effects of acetylcholine and methacholine were not indirect (caused by release of catecholamines by these drugs), for they remained after the injection of propranolol and dihydroergotamine.

Intravenous injections of cocaine (2–10 mg/kg) did not affect the pressure responses of the denervated gland to noradrenaline but increased those of the normal gland to a level somewhat below that of the denervated gland. This is shown in Fig. 3, which also demonstrates that the responses of the two glands to methacholine were not changed by cocaine.
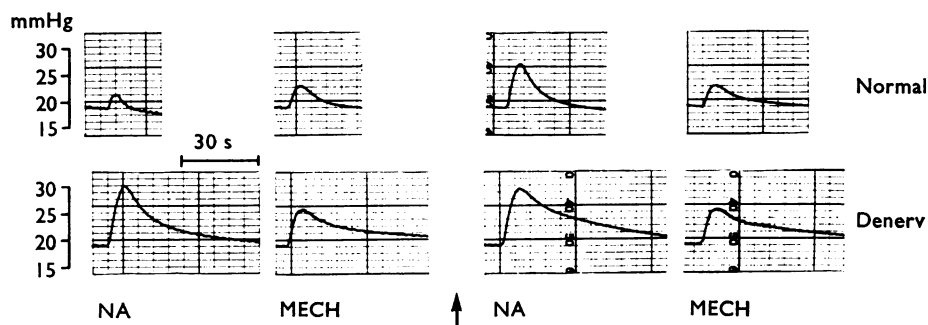

FIG. 3. Pressure effects of noradrenaline, 0·5 μg/kg (NA) and methacholine, 0·2 μg/kg (MECH) in dog salivary gland before and after cocaine, 2 mg/kg, which was injected at the arrow. Above: normal gland. Below: contralateral gland 3 weeks after sympathectomy.

Effects of section of the chorda-lingual nerve

Supersensitivity to methacholine and acetylcholine was regularly obtained after parasympathetic decentralization. The effect of methacholine is shown in Figure 4. On increasing the dose to 0·5 μg/kg a secondary rise was seen in the denervated
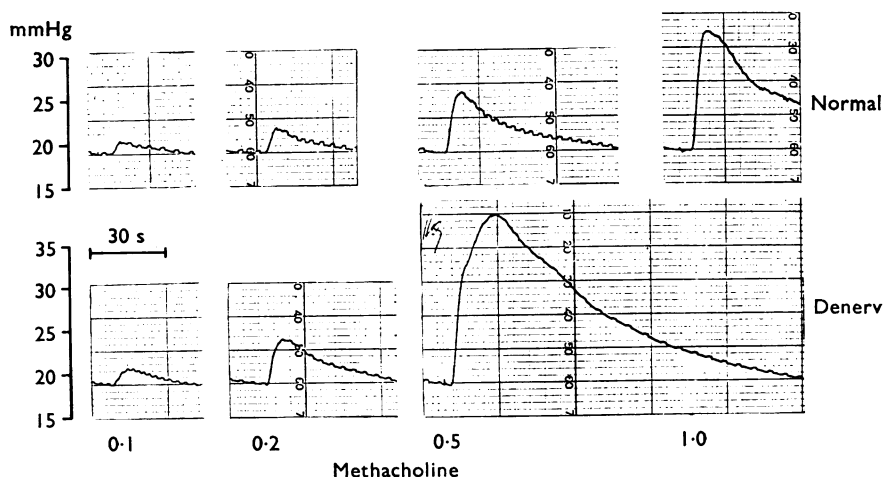

FIG. 4. Pressure responses of dog salivary gland to methacholine in the doses (μg/kg) shown in the figure. Lower records show responses of gland to which the chorda-lingual nerve had been cut 18 days earlier ; the highest dose given to the denervated gland is 0·5 μg/kg.

gland ; this did not occur in the normal gland even when the dose was doubled. In 8 experiments the pressure rise in response to 0·05 μg/kg was 1·3 ±0·29 mmHg in the operated and 0·2 ±0·10 mmHg in the normal gland. This dose of methacholine was below the threshold for secretion which was 0·3 ±0·06 μg/kg in the operated and 1·0 ±0·20 μg/kg in the normal gland. A similar supersensitivity of the pressure responses to acetylcholine was observed. In 6 experiments 0·2 μg/kg raised the pressure 0·9 ±0·20 mmHg in the operated and 0·2 ±0·06 mmHg in the normal gland. Secretion was never obtained below a dose of 2 μg/kg in the operated, and below 5 μg/kg in the normal gland.

Supersensitivity to the sympathomimetic drugs following parasympathetic decentralization was seen in the majority of cases and was usually less pronounced than that to acetylcholine and methacholine. With phenylephrine supersensitivity was obvious in 7 dogs out of 10 ; a clear effect is shown in Figure 5. In 2 of the 10 dogs the effect was doubtful and in 1 it was absent. Supersensitivity to noradrenaline was seen in 4 out of 5 dogs and to adrenaline in 5 out of 7 dogs.
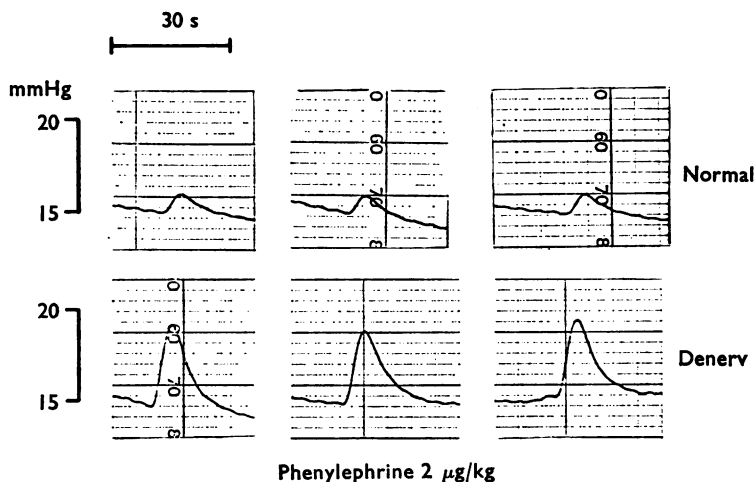

FIG. 5.   Pressure responses of dog salivary gland to phenylephrine, 2 μg/kg, given three times both to the gland to which the chorda-lingual nerve had been cut 28 days earlier (lower records) and to the contralateral gland.

Discussion

The present observations suggest that the salivary myoepithelial cells can be added to the list of effector cells in which supersensitivity to chemical agents develops after denervation.

Sympathetic ganglionectomy was found to give rise to supersensitivity which was greatest to noradrenaline and less to adrenaline and phenylephrine. This indicates a supersensitivity of the prejunctional type, a view supported by the fact that blocking of the axonal uptake mechanism by means of cocaine greatly increased the noradrenaline sensitivity of the normal gland but did not affect that of the gland in which the sympathetic axons had degenerated. However, the level of sensitivity thus reached in the normal gland was not quite as high as that of the denervated gland. By analogy from another extensively studied effector organ, the

nictitating membrane of the cat, it seems reasonable to assume that the supersensitivity of the sympathetically denervated myoepithelial cells has two components: a prejunctional supersensitivity to sympathomimetic amines, particularly great where noradrenaline is concerned, and in addition a postjunctional component, localized to the effector cells and due to the fact that the ganglionectomy disconnects these cells from the central nervous system (see Trendelenburg, 1963). The latter type of supersensitivity, equivalent to that following section of the preganglionic neurone, is responsible for the fact that the sympathectomized gland is more sensitive to noradrenaline than the cocaine-treated normal gland; it also accounts for the heightened responses to acetylcholine and methacholine caused by the sympathectomy.

Preganglionic parasympathetic denervation by section of the chorda-lingual nerve was also found to increase the pressure responses to the drugs tested. In the case of the secretory cells of salivary glands, supersensitivity following decentralization has been assumed to be due to loss of an action on the cells of the chemical transmitter, normally released by the secretory impulse from the central nervous system (Emmelin, 1960, 1961, 1965). If this applies to the salivary myoepithelial cells also, the present observations seem to allow the following conclusions. The myoepithelial cells are supplied with both adrenergic and cholinergic nerves and receive impulses from the central nervous system via both sets of nerves. Furthermore, since section of each type of nerve can produce a supersensitivity to both sympathomimetic and parasympathomimetic drugs it seems likely that many if not all of the myoepithelial cells, like the secretory cells, possess both acetylcholine receptors and adrenoceptors and receive nerves of both types.

After section of the nerves of salivary glands a marked supersensitivity is known to develop in the secretory cells. It is therefore essential to exclude the possibility that increases in the pressure responses observed in the present investigation were due to supersensitivity of the secretory cells. The following observations are against such a possibility. Increased pressure responses were observed when doses of the drugs were used which were far below those which in the open system caused flow of saliva and in the closed system a secondary pressure rise. Supersensitivity could, after both types of operation, be demonstrated to phenylephrine, which because of its lack of β-receptor stimulating activity is devoid of secretory effect on the submaxillary gland of the dog; similarly, supersensitivity to adrenaline and noradrenaline was present even after administration of the β-receptor blocking drug, propranolol.

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