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LEAKAGE OF TRANSMITTERS IN SALIVARY GLANDS

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Salivary secretion evoked by sympathetic stimulation or by injection of guanethidine, adrenaline or synephrine is slightly reduced by parasympathetic antagonists in doses which abolish the secretory responses to stimulation of the parasympathetic nerve. Similarly, an adrenaline antagonist caused a small diminution of the salivary flow elicited by parasympathetic stimulation or by injection of methacholine chloride. Secretion caused by pilocarpine could be accelerated by physostigmine. We conclude that transmitter leaks in subliminal concentrations, as far as secretion is concerned, from the sympathetic and parasympathetic postganglionic nerve endings.

Excess salivation is one of the characteristic signs of administration of cholinesterase inhibitors. The fact that this effect can be obtained even when the cholinergic secretory nerves are not being stimulated, and when the action of the drugs is confined to the salivary gland by injection of small doses intra-arterially or through the secretory duct, suggests that there is normally a continuous release of acetylcholine in the gland, subliminal in the absence of the esterase inhibitors (Riker & Wescoe, 1949 ; Dirnhuber & Evans, 1954 ; Emmelin, Muren & Strömblad, 1954). Secretion is obtained even if the postganglionic parasympathetic fibres have been cut acutely, but not if they have been allowed to degenerate or treated with cocaine injected into the gland through the salivary duct ; these observations accord with the view that the transmitter leaks from the endings of the postganglionic parasympathetic fibres (Emmelin & Strömblad, 1958).

The supersensitivity to chemical stimuli which develops in the submaxillary gland following section of the preganglionic parasympathetic (chorda tympani) fibres can be increased by daily subcutaneous injections of a specific parasympathetic antagonist (Emmelin & Strömblad, 1957). This result provides further evidence that some acetylcholine is liberated from the postganglionic nerve endings, apart from that released by the secretory impulses from the central nervous system. It indicates, in addition, that this acetylcholine fraction, even if subthreshold as far as secretion is concerned, exerts some physiological action on the gland cells, the removal of which action by treatment with a parasympathetic antagonist manifests itself in supersensitivity (Emmelin, 1960).

Similarly, the postganglionic sympathetic secretory endings may release sympathin continuously in amounts which are insufficient to cause secretion but which modify the responsiveness of the glandular cells. This conclusion is supported by the finding that postganglionic, but not preganglionic, sympathetic denervation creates

a supersensitivity in the submaxillary gland (Emmelin & Engström, 1960); furthermore, prolonged administration of guanethidine or bretylium gives rise to a supersensitivity in the gland, similar to that following removal of the sympathetic ganglion (Emmelin & Engström, 1961).

In the investigation described here an attempt was made to demonstrate, in acute experiments, a subliminal secretory effect of the two transmitters. It was shown that the secretory effect of parasympathetic stimulation or of injection of parasympathomimetic drugs was slightly reduced by adrenaline antagonists. Correspondingly, atropine-like compounds caused a small diminution of secretion elicited by stimulation of sympathetic fibres or by injection of sympathomimetic drugs. These experiments were based on the assumption that the parasympathetic and the sympathetic nerves act on the same gland cells (Emmelin, 1964). It was further shown that the secretory effect of pilocarpine was increased when physostigmine had been allowed to reach the salivary gland.

METHODS

The experiments were made on cats anaesthetized with chloralose (about 80 mg/kg intravenously) after induction with ether. Both submaxillary ducts and, in some experiments with pilocarpine and physostigmine, both parotid ducts were exposed and cannulated. The appearance of a drop of saliva was recorded on a smoked drum, using an electromagnetic lever or an ordinate recorder; these instruments were operated manually. Secretion was evoked by electrical stimulation of the peripheral cut end of the chorda-lingual nerve or of the cervical sympathetic trunk, or by administration of drugs. Drugs were made up in saline (0.9% w/v) and injections were made intravenously through a cannula in a femoral vein; intra-arterially through a cannula in the central end of the lingual artery, after tying all branches of the carotid artery except that to the submaxillary gland; intramuscularly; or through the salivary ducts, as described by Emmelin *et al.* (1954). In some experiments arterial pressure was recorded with a mercury manometer and artificial ventilation was provided by a pump.

In some cats unilateral section of the chorda-lingual nerve or extirpation of the superior cervical ganglion was carried out aseptically 2 to 4 weeks before the acute experiment.

RESULTS

Subliminal effects of acetylcholine

Sympathetic nerve stimulation. The synthetic compound Hoechst 9980 ($\alpha\alpha$-diphenyl-γ-piperidinobutyramide, 15 to 50 μg/kg, intravenously), a highly specific parasympathetic antagonist (Emmelin & Strömblad, 1957), reduced the secretory effect of sympathetic stimulation on the submaxillary gland. The effect was small but was obtained regularly and was best seen when the sympathetic trunk was stimulated at a low frequency (3 to 5 shocks/sec) so as to produce a slow secretion. Hyoscine methyl bromide (50 to 100 μg/kg, intravenously) gave the same effect as Hoechst 9980. Similar observations were made when the blocking agent was given through the submaxillary duct in order to restrict its action to the gland. In one of the experiments Hoechst 9980 (0.5 μg), a dose which almost abolished the effect of parasympathetic nerve stimulation, caused a small decrease in the response to stimulation of the sympathetic trunk. After injection of double this amount of Hoechst 9980 the effect of chorda stimulation was completely

abolished, but the sympathetic effect was not further reduced, even when the dose was raised to 100 μg. After injection of dihydroergotamine (1 μg) on the other hand, sympathetic stimulation had no secretory effect.

Guanethidine. Intravenous injection of this drug causes a long-lasting slow flow of saliva which seems to be due to release of sympathin from the postganglionic sympathetic nerves (Emmelin & Strömblad, 1963). This effect is therefore similar to that of prolonged sympathetic stimulation. Fairly high doses of the drug are required and it is advisable to apply artificial ventilation and to record arterial blood pressure. Smaller doses can be used if the gland cells have been sensitized by cutting the parasympathetic nerves some weeks previously. Fig. 1 illustrates such an experiment. The sensitized gland was secreting slowly after an injection

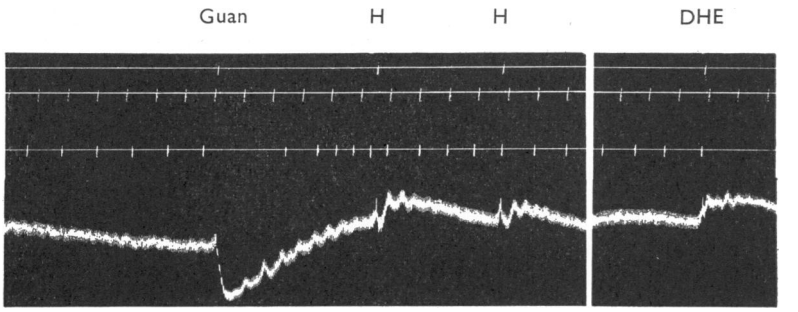

Fig. 1. The effect of αα-diphenyl-γ-piperidinobutyramide (Hoechst 9980) on the secretory responses to guanethidine. Records from above: signal; time in minutes; secretion (drops) from right submaxillary gland; and blood pressure in femoral artery, recorded with mercury manometer. The right chorda-lingual nerve had been cut 15 days previously, and the right superior cervical ganglion and the adrenal glands removed at the start of the experiment, which was with artificial ventilation. Guanethidine (10 mg/kg, intravenously) was injected about 15 min before the record starts, and this dose was repeated at Guan. At H, Hoechst 9980 was injected intravenously, 0.1 mg/kg at the first and 1 mg/kg at the second time. At DHE, dihydroergotamine, 0.5 mg/kg, was injected intravenously.

of guanethidine (10 mg/kg). Repetition of this dose increased the rate of flow, after a period of diminished secretion which was probably due to a fall in blood pressure. Hoechst 9980 (0.1 mg/kg, intravenously) reduced the rate of flow without lowering the blood pressure. This effect on the secretory rate was scarcely increased by ten-times the dose of Hoechst 9980, whereas dihydroergotamine stopped the secretion.

Adrenaline. In these experiments the chorda-lingual nerve of one side had been cut in advance to sensitize the submaxillary gland cells. Adrenaline (0.5 to 2 μg/kg, intravenously) evoked a small secretory response from the sensitized gland, and larger doses (5 to 10 μg/kg) elicited some secretion from the fully innervated gland. The doses were given repeatedly and, when constant responses had been obtained, the parasympathetic antagonist was administered intravenously and adrenaline was again given several times. Hoechst 9980 (15 to 50 μg/kg, intravenously) caused a

small reduction of the responses to adrenaline. This effect could not be increased by raising the dose of Hoechst 9980: in one cat as much as 11 mg/kg was given. Atropine sulphate (0.1 to 1 mg/kg) had a similar effect.

Synephrine. When given intramuscularly (10 to 20 mg/kg) this drug caused a secretion from a submaxillary gland sensitized by previous section of the chorda-lingual nerve. The flow rapidly reached a maximum and then slowly declined. The small reduction in the secretory rate caused by Hoechst 9980 can be studied in Fig. 2.

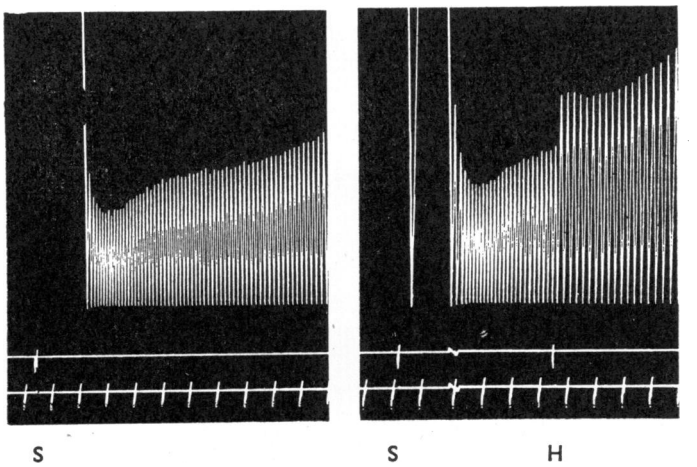

Fig. 2. The effect of αα-diphenyl-γ-piperidinobutyramide (Hoechst 9980) on salivary responses to synephrine. Records from above: secretion from right submaxillary gland (intervals between drops of saliva shown by ordinate recorder); signal; time in minutes. The right chorda-lingual nerve had been cut 2 weeks previously. S, synephrine injected intramuscularly, 20 mg/kg at the first, 10 mg/kg at the second time; H, Hoechst 9980, 1 mg/kg, intravenously. The second synephrine injection was made 1 hr after the first, and saliva was already flowing.

Pilocarpine. These experiments were designed to find out whether acetylcholine, leaking in nonsecretory amounts, could add its effect to that of other parasympathomimetic agents. Some of these experiments were made on the parotid gland of the cat, where conditions are simpler than in the submaxillary gland, the sympathetic secretory innervation being very restricted. Fig. 3 demonstrates the outcome of one of these experiments. Pilocarpine hydrochloride (1 μg) injected into the parotid duct, caused a secretion of five drops of saliva. Physostigmine sulphate (5 μg, administered by the same route) caused no secretion, but increased temporarily the effect of pilocarpine. Similar results were obtained from submaxillary glands.

Subliminal effects of sympathin

Parasympathetic nerve stimulation. A slight reduction of the secretory responses to stimulation of the chorda-lingual nerve was regularly seen after intravenous injection of dihydroergotamine hydrochloride (0.25 mg/kg); the nerve was

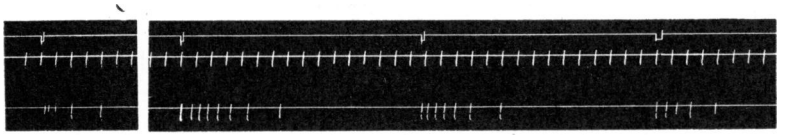

Fig. 3. The effect of physostigmine on secretion caused by pilocarpine. Records from above: signal, each mark showing injection of pilocarpine hydrochloride, 1 μg in 0.1 ml., through the parotid duct; time in minutes; parotid secretion (drops). Physostigmine sulphate, 5$\frac{1}{2}$ μg in 0.1 ml., was given through the duct 13 min before the second part of the tracing starts, and evoked no secretion.

stimulated at a frequency of 0.5 to 2 shocks/sec to produce a slow flow of saliva. In most of the experiments of this series the adrenaline antagonist was administered through the duct or intra-arterially, in order to avoid effects on the blood pressure. When just sufficient dihydroergotamine (0.5 to 1.0 μg) had been given through the submaxillary duct to abolish the response to sympathetic stimulation, there was also a small reduction in the response to chorda-lingual stimulation. This effect wore off gradually, in about 3 hr, and sympathetic stimulation regained its effect at the same time. It seems reasonable, therefore, to attribute this effect on the flow during parasympathetic stimulation to the antiadrenaline action of the drug. This view was supported by the finding that these small doses of dihydroergotamine

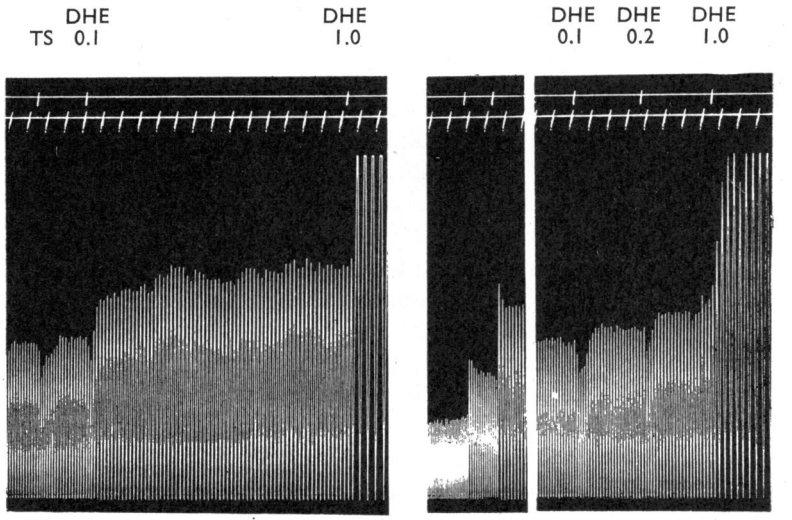

Fig. 4. The effect of dihydroergotamine on secretion elicited by parasympathetic stimulation. Records from above: signal; time in minutes, submaxillary secretion, using an ordinate recorder. First section: secretion from the left (innervated) gland, with the chorda-lingual nerve stimulated at 2 shocks/sec throughout this section. Second and third sections: secretion from the right gland, the right superior cervical ganglion having been removed 2 weeks previously; the chorda-lingual nerve was first stimulated at 2 shocks/sec, changing to 1.5 shocks/sec at the first and to 1.0 shock/sec at the second signal and then kept at 1.25 shocks/sec throughout the third record. Injections were intra-arterial. TS, Tyrode solution, 0.1 ml.; DHE, dehydroergotamine, 0.1, 0.2 and 1.0 μg, respectively.

did not affect the responses to chorda-lingual stimulation when the superior cervical ganglion had been extirpated some weeks earlier. When the dose of dihydroergotamine was raised to 10 to 20 μg, however, the effect of parasympathetic stimulation was strongly diminished or, in some instances, abolished; this result also occurred after previous sympathetic postganglionic denervation.

Similar observations were made when the drug was injected intra-arterially (Fig. 4). In this experiment the right superior cervical ganglion had been removed 2 weeks earlier. Stimulation of the left chorda-lingual nerve at a frequency of 2 shocks/sec caused a slow flow of saliva. The rate of flow was slightly reduced by 0.1 μg intra-arterially of dihydroergotamine, and more by 1.0 μg. The right submaxillary gland, sensitized by sympathetic denervation, responded with a more rapid flow of saliva when 2 shocks/sec were applied to the right chorda-lingual nerve. A stimulation rate of 1.25 shocks/sec was chosen so as to produce a flow similar to that obtained on the left side. Dihydroergotamine (0.1 and 0.2 μg) did not materially affect the flow, but 1.0 μg reduced it.

Methacholine chloride. Methacholine chloride (0.5 to 1 μg/kg, intravenously) was given to evoke secretion of saliva from the submaxillary gland. The responses were slightly diminished after injection of dihydroergotamine (0.25 mg/kg, intravenously).

DISCUSSION

Different parasympathetic antagonists in concentrations sufficient to abolish the secretory effect of parasympathetic stimulation caused a slight reduction of the salivary flow evoked in the submaxillary gland by sympathetic stimulation or by injection of various sympathomimetic compounds. Such an effect of atropine was described long ago by Feldberg & Guimarais (1935). Of particular interest is our finding that higher doses of the specific parasympathetic antagonist Hoechst 9980 do not further reduce the secretion once a dose has been reached which extinguishes the effect of parasympathetic stimulation. This result provides good evidence that the slight blocking effect is not due to a nonspecific action of the drug. Similarly, the flow of saliva elicited by parasympathetic stimulation is slightly diminished by dihydroergotamine in a dose which abolishes the effect of sympathetic stimulation, an effect not seen in preparations where the sympathetic nerves have degenerated. When the dose of this drug is raised, however, some nonspecific action, possibly vascular, comes into play, as witnessed by the fact that the secretion is seriously affected even if the sympathetic postganglionic fibres have degenerated.

Our results support the previously expressed view (Emmelin, 1960, 1961) that there is a continuous leak of transmitters from the postganglionic parasympathetic and sympathetic endings in the salivary glands. They suggest, in addition, that this leak, usually subliminal, exerts some facilitatory action when the secretory cells are activated, particularly noticeable when nerve fibres are excited at a low frequency. In this connexion the pronounced convergence of parasympathetic fibres on to the gland cells (Lundberg, 1955) should be borne in mind. It seems possible that a secretory effect of a low activity in some parasympathetic fibres, elicited from the central nervous system, may be raised because of leak of acetylcholine from other

fibres acting on the same cells; the finding that physostigmine, in a dose which does not cause secretion, increases the secretory effect of pilocarpine may lend some support to this hypothesis.

The fact that drugs that block one division of the autonomic nervous system can reduce the effect of nerves of the other division seems, in addition, to speak in favour of the concept that the single gland cell receives secretory fibres from both sets of nerves (Emmelin, 1964). The salivary glands thus offer a unique opportunity to study leak of transmitters using specific blocking agents, as in the present investigation.

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