Brit. J. Pharmacol. (1958), **13**, 193.

THE EFFECT OF ANTICHOLINESTERASES ON THE PAROTID GLAND AFTER PARASYMPATHETIC DECENTRALIZATION OR DENERVATION

BY

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(RECEIVED FEBRUARY 24, 1958)

Anticholinesterases (eserine, ethyl pyrophosphate, paraoxon) were injected into the parotid ducts and found to cause a secretion of saliva. After previous preganglionic, parasympathetic denervation the effects were increased above normal; this was probably due to the supersensitivity to acetylcholine which develops after the operation. After previous postganglionic, parasympathetic denervation, on the other hand, the effects were much reduced, in spite of a pronounced supersensitivity towards, for example, acetylcholine. The cause of this " subsensitivity " towards cholinesterase inhibitors is discussed.

In a recent monograph Burn (1956) has quoted several investigations demonstrating, as apparent exceptions of Cannon's law of denervation, that denervated structures may show a diminished responsiveness, or none at all, towards certain chemical agents which stimulate the normally innervated structures. Anderson (1905) found, for instance, in his classical experiments on the pupil that eserine some time after excision of the ciliary ganglion caused no miosis, whereas pilocarpine had a greater effect than normally.

After previous section of the chorda tympani, eserine still has a pronounced secretory effect on the submaxillary gland (Dirnhuber and Evans, 1954; Emmelin, Muren, and Strömblad, 1954). This type of denervation is a preganglionic one. For the present investigation we have used the parotid gland, which can be both pre- and postganglionically denervated.

METHODS

The experiments were carried out on cats under chloralose anaesthesia (80 mg./kg. intravenously after preliminary ether). The parotid ducts were cannulated on both sides using cannulae which delivered 35 drops of distilled water/ml. Drops of saliva falling from the tip of the cannula were recorded on a smoked drum by a signal. Anticholinesterases were injected through the duct towards the gland. This has the advantage of an effect restricted to the injected gland, no interfering general effects are obtained, and the contralateral gland can be used as a control (Emmelin *et al.*, 1954). The following anticholinesterases were used: eserine, ethyl pyrophosphate (TEPP) and paraoxon (Mintacol, *p*-nitrophenyl-diethyl phosphate). These drugs, dissolved in 0.1 ml. saline solution, were injected through a fine rubber tube connected to the salivary cannula. Before the injection the tube was obstructed by a clip; the fluid was injected during 5 sec. and the clip removed 5 sec. later. Control injections of saline solution were made in the same way. Acetylcholine and methacholine were injected through a cannula in a femoral vein.

The parotid gland was parasympathetically decentralized (by destruction of Jacobson's anastomosis) or denervated (by section of the auriculo-temporalis nerves) as described by Strömblad (1955). Further technical details are given below.

RESULTS

Anticholinesterases were found to cause a secretion from the normally innervated parotid gland. Doses of 10 to 50 μg. of the drugs used were required to cause a secretion. One or two drops fell soon after the removal of the clip; this also occurred with control injections of saline solution. After 2 or 3 min., however, a flow started which continued for 10 to 15 min. after a dose of 10 μg. With 50 μg., the flow lasted for $\frac{1}{2}$ to 2 hr. The secretory effects of all three drugs tested were transient and doses repeated after some hours caused similar responses. Doses of the anticholinesterases slightly below those needed for secretion increased the secretory effects of acetylcholine and methacholine given intravenously. When a secretion caused by anticholinesterases had worn off the gland still showed an increased sensitivity to acetylcholine and methacholine for some time (Figs. 1 and 2), but this effect, too, was transient.

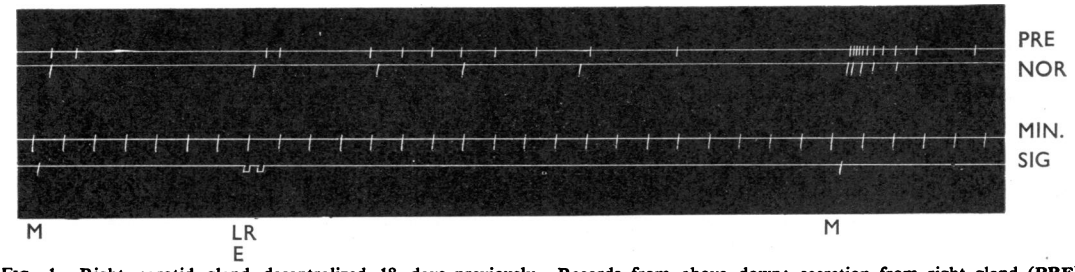

FIG. 1.—Right parotid gland decentralized 18 days previously. Records from above down: secretion from right gland (PRE) and from left gland (NOR); time in min.; signal (SIG); M=methacholine (2 μg./kg.) given intravenously; E=eserine (10 μg.) into the left (L) and into the right (R) duct.

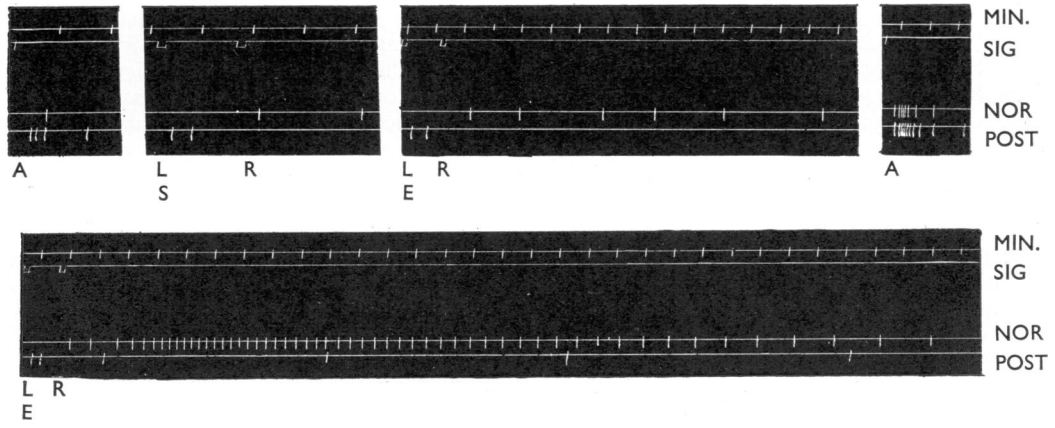

FIG. 2.—Left parotid gland denervated 18 days previously. From above down: time in min.; signal (SIG); secretion from right gland (NOR) and from left gland (POST); A=acetylcholine (2 μg./kg.) intravenously; S=0.1 ml. saline solution into the left (L) and into the right (R) duct; E=eserine (10 μg. in upper and 50 μg. in lower tracing) injected into the ducts.

After previous preganglionic, parasympathetic denervation the parotid gland still responded to anticholinesterases, as shown in the experiment shown in Fig. 1. In this cat the preganglionic fibres had been cut on the right side 18 days before the experiment. Eserine, 10 μg. injected through the ducts, caused a flow of saliva from both glands. The response of the decentralized gland was greater than that of the normally innervated one. As shown in the figure, this gland was supersensitive to methacholine. These observations on the parotid gland are in accord with those made previously on the submaxillary gland (Emmelin *et al.*, 1954).

Fig. 2 shows an experiment in which postganglionic, parasympathetic denervation of the parotid gland had been carried out by cutting the auriculo-temporal fibres on the left side 18 days previously. An injection of 10 μg. of eserine into the parotid duct caused a secretion from the normally innervated gland only; the effect on the left gland equalled that caused by saline solution. An injection of 50 μg. of eserine induced a flow of saliva from both glands, but the response of the denervated gland was much smaller than that of the normally innervated gland. This was the case in spite of the fact that the operation had caused a supersensitivity to acetylcholine, as shown in the upper part of Fig. 2.

When pre- and post-ganglionically denervated parotid glands were compared, the latter was found to be more sensitive to drugs such as acetylcholine and methacholine than the former. Nevertheless, anticholinesterases caused a bigger secretory effect on the decentralized than on the denervated gland.

In most of the cats the postganglionic fibres were cut 2 to 4 weeks before the acute experiment; the secretory effects of anticholinesterases on the denervated glands were in these cases invariably much smaller than on the contralateral, normally innervated glands. The same observation was made on a cat, operated on five days previously. Immediately after the operation, on the other hand, the denervated gland responded like a normal gland.

Special attention was paid to the small secretion caused by the injection of anticholinesterases into a gland, the auriculo-temporal nerves of which had been cut previously. Like the secretion caused from a normal gland, it was abolished by small doses of atropine, given intravenously. Similarly, it was abolished by a dose of cocaine (5 to 10 mg.), injected into the salivary duct, which did not affect the response of the secretory cells to acetylcholine or methacholine. In one experiment the facial nerve was cut, in another the superior cervical ganglion extirpated simultaneously with trans-section of the auriculo-temporal nerves. In both cases the usual small secretory response was obtained when anticholinesterases were given in the acute experiment some weeks later.

Discussion

When cats were treated for two or three weeks by daily subcutaneous injections of the anti-acetylcholine drug Hoechst 9980 (diphenyl-piperidino-ethyl-acetamide), a supersensitivity to chemical agents was found to develop in the submaxillary gland which exceeded and could be superimposed upon that obtained after preganglionic, parasympathetic denervation (Emmelin and Strömblad, 1957). Since this drug was found to be a remarkably specific acetylcholine antagonist, it was postulated that the treatment had caused a superimposed sensitization by excluding the action of acetylcholine released from the postganglionic endings. It was thus presumed that there is a release of acetylcholine from the postganglionic parasympathetic endings even when the postganglionic neurone has been disconnected from the central nervous system.

The present experiments were carried out in an attempt to find further evidence for such a release of acetylcholine.

Anticholinesterases, acting locally in the parotid gland, were found to cause a flow of saliva. This effect was obtained in a normally innervated gland and in accordance with previous experiments was not changed by acute preganglionic denervation (Emmelin *et al.*, 1954). After chronic preganglionic denervation a response was still obtained, and it was even bigger than that of a normally innervated gland. It seems reasonable to connect this with the fact that the effector cells were supersensitive to secretory drugs. The secretory effect of anticholinesterases is obviously not dependent on impulses from the central nervous system.

After chronic postganglionic, parasympathetic denervation, on the other hand, the secretory effect of anticholinesterases was very small in spite of the fact that the gland cells were supersensitive to drugs like acetylcholine and methacholine.

The secretory effects of the anticholinesterases were abolished by atropine. It cannot be excluded that the drugs exerted their effect by a direct, acetylcholine-like action, or by releasing acetylcholine. It is, however, generally assumed that anticholinesterases act mainly by causing an accumulation of acetylcholine, released in the course of physiological events. If so, the results of the present experiments would suggest that there is a liberation of acetylcholine from the postganglionic endings which is not due to impulses from the central nervous system. The finding that acute section of the auriculo-temporal fibres does not affect the secretory response to anticholinesterases would then indicate that such a release is not initiated from the cell body of the postganglionic neurone, but rather is due to a process analogous to that assumed to operate at the endings of somatomotor fibres.

Five days or more after section of the auriculo-temporal nerves, when the fibres could be assumed to have degenerated, anticholinesterases had a small secretory effect only. This seems compatible with the idea that the drugs acted mainly by an acetylcholine mechanism; if they had some direct action exclusively one would rather expect them to have an enhanced effect on the supersensitive gland cells.

The small secretory effect of anticholinesterases remaining after previous postganglionic denervation could be explained in different ways. It could be a slight direct effect on the supersensitive gland cells or it could be due to preserved acetylcholine. Such acetylcholine might be of non-nervous origin, for instance like that found in the gut by Feldberg and Lin (1949). These authors observed, however, a release of acetylcholine even after administration of cocaine, whereas in our experiments cocaine abolished the effect of anticholinesterases. Possibly some cholinergic fibres to the gland were still intact after the operation on the postganglionic nerves, but attempts to eliminate such fibres by section of the facial nerve or excision of the superior cervical ganglion were unsuccessful. It may be added that Chang and Gaddum (1933) showed that acetylcholine is present in the parotid gland after previous section of the auriculo-temporal nerves although in reduced quantity.

We are grateful to Dr. B. Holmstedt, Stockholm, for a gift of ethyl pyrophosphate and Mintacol.

Technical assistance was given by Miss Ursula Delfs.

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