SUPERSENSITIVITY OF THE SUBMAXILLARY GLAND FOLLOWING EXCLUSION OF THE POSTGANGLIONIC PARASYMPATHETIC NEURONE

BY

N. EMMELIN

From the Institute of Physiology, University of Lund, Sweden

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The repeated subcutaneous injection of four different parasympatholytic agents into cats has been found to cause a supersensitivity of the submaxillary glands to adrenaline. The fact that the level of sensitivity reached is the same with all four drugs is taken as evidence that the supersensitivity is due to the absence of an action of acetylcholine on the gland cells. This acetylcholine can only in part be that released by the secretory impulse, for the supersensitivity produced is more marked than that caused by preganglionic parasympathetic denervation. It is assumed that the postganglionic cholinergic neurone exerts some action of its own on the gland cell. Sensitization above the level reached after decentralization ensues when such an action is abolished by parasympatholytic agents. Degenerative section of the postganglionic fibres would also exclude this action, but for anatomical reasons this cannot be made complete. The supersensitivity which follows this operation is greater than after decentralization but less than that produced by treatment with atropine-like drugs.

The supersensitivity to various secretory agents acquired by denervated submaxillary gland cells can to a great extent be attributed to the fact that the cells have been deprived of the secretory impulses from the central nervous system, which normally impinge upon them. Evidence for this view can be found in the following observations: supersensitivity develops, both when the flow of secretory agents is reduced by cutting some of the afferent fibres of the secretory reflex arc, and when it is abolished by treatment of the experimental animal for some time with ganglionic blocking or parasympatholytic agents; furthermore, the supersensitivity caused by denervation disappears when the gland is supplied with injected secretory agents such as pilocarpine, carbachol, or adrenaline (Emmelin and Muren, 1950, 1951, 1952; Emmelin, 1952, 1956, 1959).

Those experiments in which parasympatholytic agents were used to create a supersensitivity deserve special attention. When such a drug, Hoechst 9980 ($\alpha\alpha$ - diphenyl - γ - piperidinobutamide), was given subcutaneously for some weeks to cats, the supersensitivity of the submaxillary gland to adrenaline which ensued was found to exceed that caused by cutting the preganglionic parasympathetic fibres of the chorda tympani (Emmelin and Strömblad, 1957).

The hypothesis was put forward that the drug not only deprives the gland cells of secretory impulses but in addition abolishes an action on the cells of acetylcholine continuously leaking from the endings of the postganglionic neurone (Emmelin and Strömblad, 1957, 1958).

In order to test this hypothesis two types of experiments, described in the present paper, were carried out.

The effect of postganglionic parasympathetic denervation on sensitivity was studied. The ideal experiment would be to compare the level of sensitivity produced by a parasympatholytic drug with that attained after complete section of the postganglionic parasympathetic fibres. Unfortunately this is not feasible, since many of the synapses are situated within the gland and the postganglionic fibres are therefore not anatomically accessible. An attempt could only be made to remove those nerve cells which are found close to the hilum of the gland and to compare the effect of this operation with that caused by section of the preganglionic fibres or treatment with atropine-like drugs.

In the second series of experiments a number of parasympatholytic agents were given to produce a supersensitivity. It was thought that if various different drugs of this pharmacological

group were found to create a supersensitivity surpassing that caused by section of the chorda this would support the view that the additional sensitization could be attributed to the abolition of the action of a cholinergic mechanism on the gland cells.

METHODS

Cats were used as experimental animals. The level of sensitivity of the submaxillary glands to secretory drugs was estimated in one and the same cat about once weekly over a period of several months; after initial ether the cats were anaesthetized with hexobarbitone, injected intracardially, and the two submaxillary ducts cannulated from the mouth (Emmelin and Muren, 1952). To elicit secretion, standard doses of adrenaline were injected intracardially. This was the only test drug used in cats treated with parasympatholytic agents. In the denervation experiments acetylcholine, methacholine, and synephrine were given as well. Care was taken not to inject acetylcholine and methacholine immediately after the administration of hexobarbitone because of the slight atropine-like effect of the latter drug (Emmelin, 1941).

Preganglionic denervation was made by cutting the chorda or chorda-lingual nerve. For partial post-ganglionic denervation the chorda fibres were traced along the salivary ducts, under the dissecting microscope, and cut as near the gland as possible. The distal end of the piece of nerve cut out was examined histologically for ganglion cells. This was kindly done by Dr. Dora Jacobsohn of Lund. The operation was done aseptically under hexobarbitone anaesthesia.

The following parasympatholytic drugs were used: lachesine chloride [(2-benzoyloxyethyl)ethyl-dimethylammonium chloride], Hoechst 9980, isopropamide iodide [Tyrimid; (3 - carbamoyl - 3,3 - diphenylpropyl)diisopropylmethylammonium iodide], and methscopolamine nitrate (hyoscine methonitrate). I am very grateful to Professor Burn, of Oxford, for a gift of lachesine. Hoechst 9980 was kindly supplied by A. B. Webass, Gothenburg, isopropamide by A. B. Leo, Hålsingborg, and methscopolamine by A. B. Pharmacia, Uppsala. The doses of the drugs used and further technical details are given below.

Some cats were finally studied in acute experiments under chloralose anaesthesia as described below.

RESULTS

Partial Postganglionic Denervation

After the dissection of the chorda towards the gland a supersensitivity developed which was more pronounced than that of a gland preganglionically denervated. This is illustrated in the experiment of Fig. 1, in which the chorda of the right submaxillary gland was dissected and the left chorda-lingual nerve cut at the same time.

The supersensitivity resembled that seen after preganglionic section in the following respects: it developed with the same time course; the threshold dose was lowered, and the secretion time increased; it was unspecific, so that sensitization to adrenaline, synephrine, acetylcholine, and methacholine was seen. The difference was that the threshold was even lower than after preganglionic denervation, the secretion period for a certain dose longer, and the number of drops obtained after a moderate dose greater. With large doses the responses of the dissected and the contralateral, preganglionically denervated gland were about the same. In two cats the glands were cut out more than a month after the operations. They were found to weigh 0.705 (dissected) and 0.756 g. (chorda-lingual cut) in one animal, and 0.596 and 0.610 g. respectively in the other. The weight of a normal gland is about 1 g. (Emmelin, Jacobsohn, and Muren, 1951). Both glands had thus atrophied, and to about the same extent; this corresponds to the fact that the maximal secretory rate of the two glands was about the same.

In the dissection ganglion cells were removed. This was shown by the following observations:

(a) The distal part of the excised piece of nerve was often thicker than the rest of the chorda, and when squeezed in forceps gave the same feeling as, for instance, a superior cervical ganglion. Histological examination revealed the presence of numerous nerve cells.

(b) Eserine, injected through the duct towards the gland, had less secretory effect on the dissected

FIG. 1.—Supersensitivity following dissection and removal of the chorda towards the gland (solid lines) and simultaneous section of the contralateral chorda-lingual nerve (broken lines). Abscissa: dates (day and month) when the sensitivity was tested; ordinate: drops of saliva. *a*, Responses to 5 µg./kg. adrenaline (crosses) and 5 µg./kg. acetylcholine (rings); *b*, responses to 2 mg./kg. synephrine (crosses) and 2 µg./kg. methacholine (rings).

side than on the side of preganglionic section, in spite of the fact that the gland of the former side was more sensitive to acetylcholine than that of the other side. Eserine is assumed to act by preservation of acetylcholine leaking from the endings of the postganglionic neurone (Emmelin and Strömbad, 1958). Such an experiment is shown in Fig. 2. The smaller dose of eserine given

FIG. 2.—Chloralose anaesthesia. Secretory effects of acetylcholine (A 1=1 μ g./kg., A 2=2 μ g./kg.) and eserine (E 25=25 μ g., E 50=50 μ g.) 3 weeks after dissection of the chorda (upper line of the tracing) and section of the contralateral chorda-lingual nerve (lower line). Acetylcholine was given intravenously. Eserine, dissolved in 0.1 ml. saline solution, was injected during 5 sec. through a rubber connexion attached to the salivary cannula. The rubber tube was clamped distally during the injection and the following 5 sec. The dots mark the time of injection of the drugs. For eserine, the first dot corresponds to the injection into the duct of the dissected gland, the second into the other duct. Time in min. (in the lower tracing the drum was run at a higher speed).

in this experiment caused a secretion from the gland on the dissected side only (the single drop from the other side is probably part of the fluid injected which was expelled); this corresponds to the greater sensitivity of that side towards acetylcholine shown in the first part of the tracing. When the dose of eserine was raised, both glands secreted. The flow was now more rapid from the gland subjected to preganglionic denervation only. It could be objected that the higher dose of eserine could have a paralytic effect on the more sensitive gland; this was, however, not the case, for 100 and 200 μ g. of eserine were found to cause a similar secretion to 50 μ g. both on this gland and on the contralateral one. The conclusion from these experiments is that some postganglionic cholinergic fibres had degenerated on the dissected side.

(c) Nicotine, injected intravenously, was found to have less secretory effect on the dissected

side than after mere preganglionic denervation. Dihydroergotamine was given in advance to abolish a secretory action of sympathin released by the nicotine from the adrenergic neurone or the adrenal medulla, and the experiments were carried out immediately after the operations in order to avoid the complication caused by sensitization.

That the postganglionic denervation was incomplete, as expected, was evident from the fact that eserine and nicotine still caused a marked secretion on the dissected side.

Postganglionic sympathetic fibres are known to enter the gland with the artery at the hilum. Section of these fibres is followed by a supersensitivity which can be superimposed upon that caused by cutting the chorda. Although care was taken not to touch the tissue surrounding the artery it seemed desirable to ascertain that the sensitization above the chorda level was not merely due to damage to sympathetic fibres. Two types of control experiments were carried out. (1) In some cats the sympathetic trunk was exposed in the neck but not cut. The trunk was stimulated electrically and the secretory response noted. The chorda was then immediately dissected towards the gland and stimulation of the sympathetic trunk repeated. The second secretory response was found to be as big as the first one. The wounds were then sutured. A supersensitivity was found to develop which surpassed that produced by section of the chorda-lingual nerve. This could not be due to injury of the preganglionic sympathetic fibres during the stimulation, for section of the sympathetic trunk does not sensitize the submaxillary gland cells (Emmelin and Engström, 1960). (2) In some cats the superior cervical ganglion was extirpated bilaterally when the level of supersensitivity had reached a plateau about three weeks after the usual parasympathetic operations. The sensitivity was found to increase on both sides, and the gland on the dissected side still showed a more pronounced supersensitivity than the gland deprived of the preganglionic parasympathetic (and postganglionic sympathetic) innervation.

Treatment with Parasympatholytic Agents

The chorda on one side was cut, and when the supersensitivity had reached a maximum, treatment with the parasympatholytic drugs started. The drugs were given subcutaneously. Hoechst 9980 was injected once a day in a dose of 1 mg./kg. It was found to cause a higher supersensitivity to adrenaline than that produced by preganglionic parasympathetic denervation, as

described earlier (Emmelin and Strömblad, 1957). A similar result was obtained with isopropamide iodide, 1 mg./kg. a day. When the same dose of lachesine chloride was administered, the sensitivity of both glands started to rise, but after two weeks it was found to be lower than after one week. Obviously a tolerance to the drug had developed. The dose had to be raised successively in order to prevent a fall in sensitivity, and doses as high as 10 mg./kg., given twice a day, were eventually used in some cats. Similar results were obtained when atropine was used (Emmelin and Muren, 1950a, 1951). The experiment shown in Fig. 3 demonstrates the decline of the sensitivity

FIG. 3.—Abscissa; date (day and month) of estimation of the sensitivity, ordinate: drops of saliva secreted in response to 5 µg./kg. (crosses) and 1 µg./kg. (rings) of adrenaline for the right gland (solid lines) and for the left gland (broken line; no response to 1 µg./kg.). At the arrow the right chorda tympani was cut. Treatment with lachesine chloride, A: 1 mg./kg. once a day, B: 1 mg./kg. twice a day, C: 2 mg./kg. twice a day.

curves in spite of a moderate increase in the dose of lachesine. Nevertheless, it is apparent from the Fig. that a higher supersensitivity can be reached by treatment with this drug than that caused by preganglionic denervation. Methscopolamine nitrate was initially given daily in doses of 1 mg./kg. Since there seemed to be a slight tendency for a tolerance to develop, it was often found suitable to raise the dose to 2 mg./kg. a day after 1 or 2 weeks. A supersensitivity above that caused by section of the chorda was found to ensue.

FIG. 4.—Secretory responses from the right (solid line) and the left gland (broken line) to a standard dose of 10 µg./kg. adrenaline. The right chorda had been cut 3 weeks before the experiment started. The dose of the three drugs was 1 mg./kg. a day.

In some cats it was possible to try several parasympatholytic drugs in the same animal. These experiments allow the conclusion to be drawn that all the drugs produce about the same high level of supersensitivity. Such an experiment is shown in Fig. 4, which demonstrates that methscopolamine, Hoechst 9980 and isopropamide sensitize both glands far above the level caused by the section of the chorda and that all three drugs create about the same degree of supersensitivity. There might be a slight difference

FIG. 5.—Secretion from the right (solid line) and left gland (broken line) caused by 5 µg./kg. adrenaline. The left chorda had been cut 3 weeks before the experiment started. Isopropamide iodide was given in a dose of 1 mg./kg. once a day. At the arrow the right chorda was dissected towards the gland.

between the responses of the right and left gland treated with blocking agents in this cat; this could be due to the fact that section of the chorda causes some atrophy of the gland, an effect which treatment with parasympatholytic agents does not seem to give (Emmelin, Jacobsohn, and Muren, 1951).

Comparisons were made between the supersensitivity caused by treatment with parasympatholytic agents and that produced by dissecting the chorda towards the gland. Fig. 5 shows that the operation produced a supersensitivity which was more pronounced than that following section of the chorda. It was, on the other hand, less marked than that caused by treatment with the parasympatholytic agent, which was to be expected since the postganglionic denervation was incomplete.

DISCUSSION

The finding that four different parasympatholytic agents sensitize the submaxillary gland to the same level suggests that they act by virtue of their parasympatholytic properties only. Since the level of supersensitivity reached by far exceeds that attained by disconnecting the gland from the central nervous system via the chorda tympani it must be assumed that the drugs deprive the gland not only of acetylcholine released by the secretory impulse but in addition of some other influence exerted by a cholinergic mechanism. It is reasonable to focus interest on the postganglionic parasympathetic neurone. This neurone must then be assumed to have some action on the glandular cell apart from that due to the secretory impulse from the central nervous system, an action which persists after section of the preganglionic fibres. This postulated action of the neurone on the gland cell must be mediated

by acetylcholine released in subthreshold concentration as regards secretion, since the gland does not secrete under ordinary conditions after section of the chorda. The fact that small amounts of acetylcholine can be detected in a perfusate of an eserinated gland even when the chorda is not stimulated (Emmelin and Muren, 1950b) is of interest in this connexion. Recent experiments with eserine (Emmelin and Strömblad, 1958) support the view that there is a continuous leakage of acetylcholine from the endings of the postganglionic neurone. The sensitization above the chorda level caused by parasympatholytic agents suggests that the leaking acetylcholine, although insufficient to cause secretion, exerts some action on the gland cell, the removal of which manifests itself in supersensitivity. The gland can be deprived of this action by treatment with atropine-like drugs. Another way of removing this influence would be to cause the postganglionic fibres to degenerate. Unfortunately this can only be done with some fibres; correspondingly, the sensitivity is then only raised to a level below that reached after treatment with parasympatholytic drugs.

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