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SUPERSENSITIVITY DUE TO PROLONGED ADMINISTRATION OF GANGLION-BLOCKING COMPOUNDS

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Repeated subcutaneous injections of ganglionic blocking agents in cats were found to cause a supersensitivity towards adrenaline in the submaxillary gland and the nictitating membrane. The supersensitivity resembled that produced by preganglionic denervation, namely section of the chorda tympani in the case of the gland and of the cervical sympathetic trunk in the case of the membrane. The blocking compounds used were chlorisondamine chloride and hexamethonium. With the former, the sensitization could be obtained in the normally innervated structures. When hexamethonium was used, however, the effect on the gland could be produced only if the synapse had been made particularly susceptible to the blocking action of this agent; this was achieved by cross-suture experiments.

By long-continued treatment of an animal with atropine or a drug with atropine-like actions, it is possible to create a supersensitivity to chemical stimuli in the submaxillary gland (Emmelin and Muren, 1950, 1951, 1952; Emmelin, Jacobsohn and Muren, 1951; Emmelin and Henriksson, 1953; Emmelin and Strömblad, 1957), in the parotid gland (Strömblad, 1956a and b; Nordenfelt and Ohlin, 1957) and in the smooth muscle of the stomach (Muren, 1957). The supersensitivity observed in these cases resembles that which appears after section of the efferent nerve of the structure. Obviously, it would be of importance to know whether, as a general rule, a prolonged administration of blocking agents gives rise to a supersensitivity similar to that which follows denervation. To test this, some experiments were carried out in which the effect of prolonged treatment with a ganglionic blocking agent, hexamethonium, on the sensitivity of the submaxillary gland was investigated (Emmelin, unpublished observations). No sensitization took place. Similarly, Konzett and Rothlin (1953) found no supersensitivity of the nictitating membrane of the cat towards adrenaline or noradrenaline after the administration of hexamethonium for some time.

It seemed possible that these failures could be due to insufficient blocking of ganglionic transmission. In the present investigation, an attempt was made to repeat these experiments under more favourable conditions.

Two types of experiments were carried out. It has been observed that it is possible, under special conditions, to block very effectively and for a considerable period of time the transmission of nerve impulses to the postganglionic parasympathetic neurone which supplies the submaxillary gland with secretory fibres (Emmelin, Muren, and Strömblad, 1957). In cross-suture experiments, the preganglionic fibres of the chorda tympani were exchanged for somatomotor fibres from the hypoglossal nerve. The supersensitivity created by the section of the chorda decreased when the hypoglossal fibres had replaced the chorda fibres functionally. Hexamethonium in very small doses was now found to block the transmission of impulses from the hypoglossal nerve to the postganglionic neurone for a remarkably long period of time. This finding was made use of in the present investigation.

When these experiments had been completed, more suitable ganglionic blocking compounds than hexamethonium became available. Chlorisondamine chloride (ethylene-1-[4, 5, 6, 7-tetrachloro - 2 - methyl*iso*indolinium] - 2 - trimethylammonium dichloride, Ecolid [Ciba]) has a long duration of action; in addition, the striking tolerance which develops to hexamethonium is not seen with chlorisondamine. This

drug was therefore used in a series of experiments in which the sensitivity to adrenaline of the submaxillary gland and the nictitating membrane was examined.

METHODS

In cats, which were anaesthetized with ether and hexobarbitone, the central end of the right hypoglossal nerve was sewn to the distal end of the chorda-lingual nerve. The sensitivity of the two submaxillary glands towards adrenaline was estimated at intervals in hexobarbitone anaesthesia. The salivary ducts were cannulated in the mouth. Hexobarbitone and adrenaline were administered intracardially. When the supersensitivity subsequent to the trans-section of the chorda-lingual nerve had declined (as a result of re-innervation from the hypoglossal nerve by way of the parasympathetic pathway), treatment with hexamethonium was instituted. The drug was injected subcutaneously twice daily over a period of two to three or more weeks. The initial dose, usually 3 mg./kg., was increased successively until a dose of 20 mg./kg. was reached; this dose was then maintained for some days. In some cats, the treatment started with 10 mg./kg. and the dose was raised to 20 mg./kg. The sensitivity was estimated 5 to 19 hr. after an injection of hexamethonium. In some cats, a later treatment with an atropine-like drug was given for a comparison, 1 mg./kg. of the compound Hö 9980 Hoechst (piperidino-ethyl-diphenyl-azetamid) being injected subcutaneously once a day as described by Emmelin and Henriksson (1953).

In the second type of experiments the sensitivity of the submaxillary glands was tested repeatedly as described above, but in addition the responses of the nictitating membrane to adrenaline were recorded under standardized conditions. A fine clip was placed at the edge of the membrane and connected to a frontal writing lever by a thread and a pulley. Chlorisondamine was injected subcutaneously twice a day in a dose of 2 mg./kg. over a period of some weeks. In some experiments, the dose was successively raised up to 5 mg./kg. The treatment was then discontinued, and when a pre-treatment degree of sensitivity had again been reached the effect of section of the chorda tympani and the cervical sympathetic trunk and of removal of the superior cervical ganglion was studied.

RESULTS

Cross-suture Experiments

In a series of cats prepared with cross-suture, 7 survived for a sufficiently long period to allow the treatment with hexamethonium. Fig. 1 shows the outcome of one of the experiments. About three months after the hypoglossal nerve had been connected to the chorda-lingual nerve, the

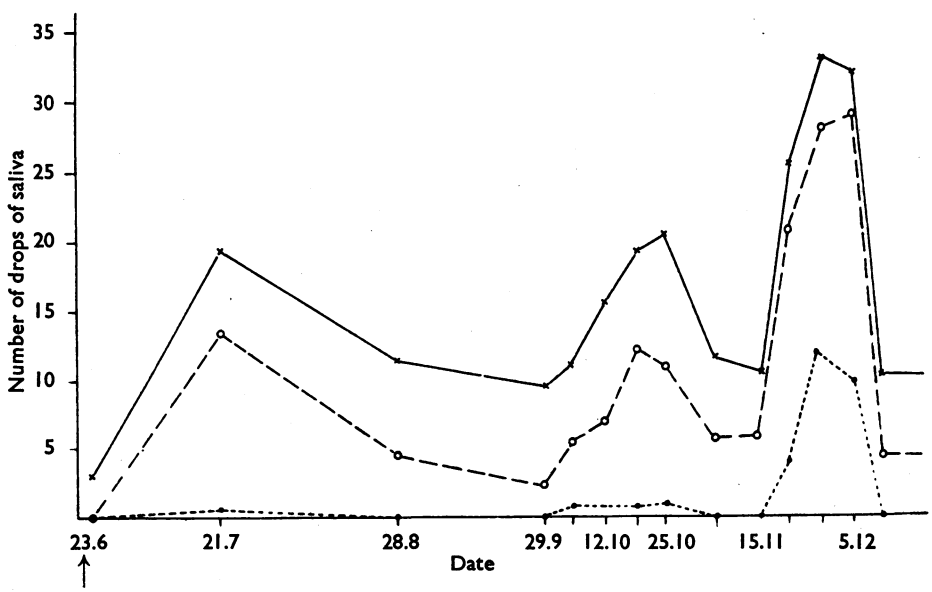

FIG. 1.—The sensitivity of the right submaxillary gland of a cat towards three different standard doses of adrenaline, in the upper curve 10, in the middle curve 5, and in the lower curve 2 μg./kg., tested on 14 occasions during a period of about six months in 1956. The right hypoglossal nerve was cut and its central end sewn to the peripheral end of the cut right chorda-lingual nerve at an operation on 23.6.56 (at arrow). Hexamethonium chloride was injected subcutaneously twice daily between 29.9 and 25.10. The dose given each time was 10 mg./kg. during the first, 20 mg./kg. during the second half of this period. Between 15.11 and 5.12, Hö 9980, 1 mg./kg., was injected once a day. The abscissa gives dates, 21.7 indicating 21st July, 1956.

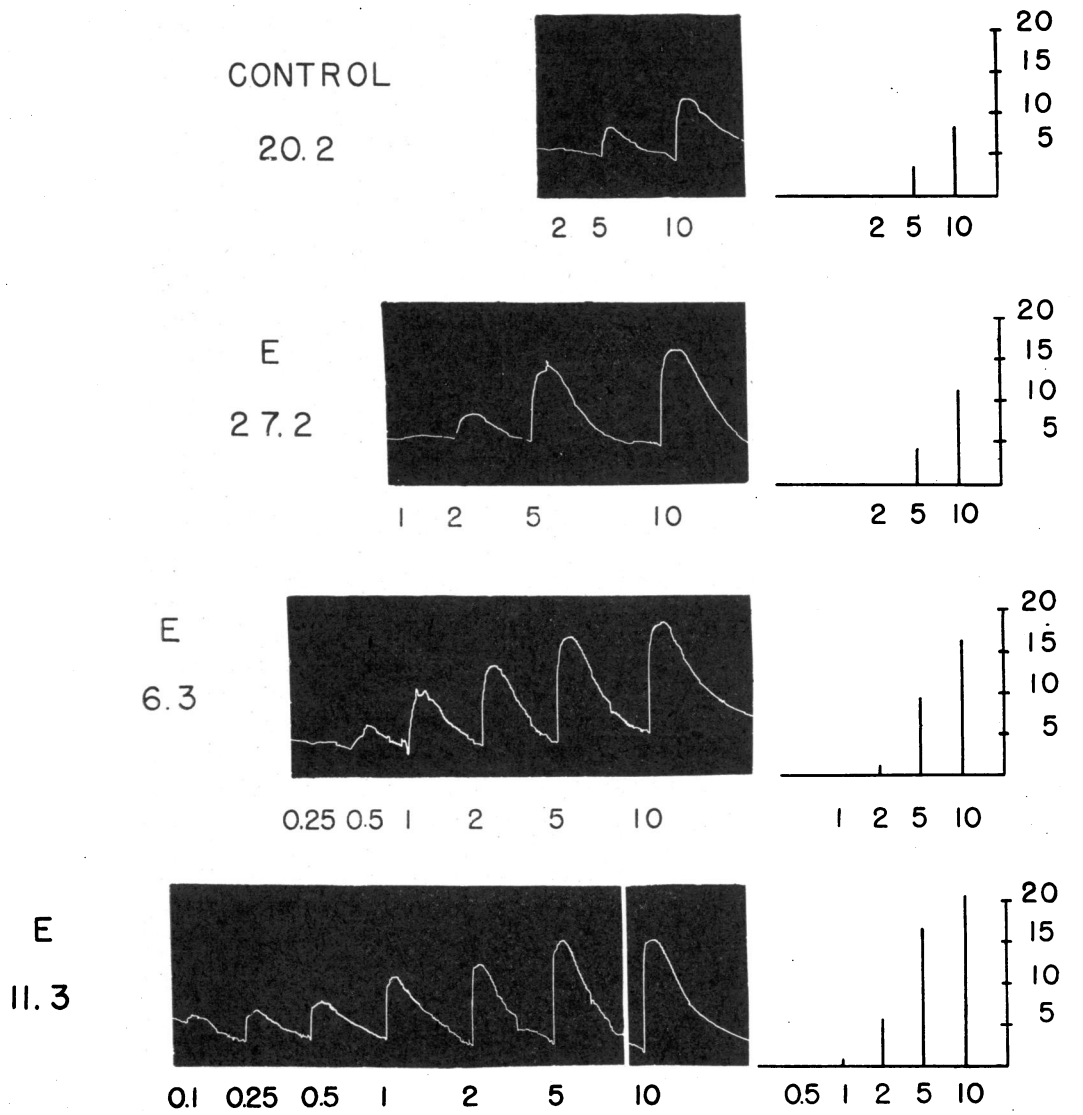

FIG. 2.—The sensitivity of the right nictitating membrane (smoked paper records on left) and of the right submaxillary gland (diagrams on the right) of a cat. The numerals below the tracings and the diagrams represent the doses of adrenaline in μg./kg., used to estimate the sensitivity. The ordinates of the diagrams give the number of drops of saliva secreted. After the control experiment on 20.2.58 (20.2), chlorisondamine (E) treatment was started. Two subcutaneous injections were given daily, the dose being raised successively from 2 to 5 mg./kg.

supersensitivity had been considerably reduced, although not quite abolished. During treatment with hexamethonium the sensitivity again rose and reached a maximum within three weeks of treatment, namely in about the period required for a maximum to be attained after section of the chorda. When the treatment was discontinued, the sensitivity fell and reached its previous level in a few days' time. Treatment with the anti-parasympathetic agent caused a more pronounced rise in sensitivity than that seen during administration of hexamethonium.

It is obvious from Fig. 1 that the supersensitivity caused by the treatment with hexamethonium was very similar to that following parasympathetic decentralization. This was true of all the various doses of adrenaline tested. Similarly the threshold, which had been lowered after the

operation but had risen again in the course of the re-innervation, was lowered when hexamethonium was given; the decrease was the same in both instances. During treatment with Hö 9980 there was again a lowering of the threshold which was more pronounced than on the two previous occasions.

In the remaining six cats which had hypoglossal-chorda-lingual anastomosis, hexamethonium was also found to cause a rise in sensitivity. In one of these cats, it reached the degree observed earlier after section of the chorda, just as in the experiment shown in Fig. 1. In the other five cats, the rise was not quite as large as that caused by the operation.

In the seven cats of this series there was no increase in the sensitivity of the contralateral, normally innervated gland during the treatment with hexamethonium.

Treatment with Chlorisondamine

During the period when chlorisondamine was injected, the mucous membrane of the mouth was found to be very dry and no salivation seemed to occur when ether was given as the preliminary to the hexobarbitone anaesthesia.

Chlorisondamine, contrary to hexamethonium, was found to sensitize normally innervated structures. Fig. 2 shows responses to a series of doses of adrenaline of the right submaxillary gland (diagram to the right of Fig. 2) and the right nictitating membrane (records on the left of Fig. 2) on four different occasions, with weekly intervals. Treatment with chlorisondamine increased the sensitivity of both structures, the effect being maximal after about three weeks. The threshold doses were lowered, and the effects of moderate doses increased. When the treatment was discontinued the sensitivity fell again. In the submaxillary gland, the pre-treatment level was reached within a week; in the nictitating membrane some supersensitivity persisted at that time, but after about two weeks the original level had been resumed. When the preganglionic nerves (the chorda and the cervical sympathetic) were then cut, it was found that the treatment with chlorisondamine had invariably sensitized the membrane to the same degree as had section of the cervical sympathetic, whereas in the submaxillary gland the chorda supersensitivity level was not quite reached. It seemed somewhat easier to sensitize the membrane than the gland with chlorisondamine. In one cat, two periods of treatment were given. In the first, the highest dose given was 2 mg./kg. twice a day. The gland was only slightly sensitized, but the membrane was sensitized to the level reached later after preganglionic denervation. In a second period of treatment the dose was raised to 5 mg./kg. The sensitivity of the membrane did not increase more than in the first period; that this was not the highest level attainable was shown at the end of the experiment, when removal of the superior cervical ganglion caused a supersensitivity much higher than that produced by treatment with chlorisondamine or section of the preganglionic fibres. During the treatment with 5 mg./kg., the gland was much more sensitized than when the smaller dose was given, but chorda section was then found to cause a somewhat more pronounced supersensitivity. It seems reasonable to assume that the degree of sensitivity following section of the chorda would have been reached with a larger dose of chlorisondamine, but no cats survived such treatment.

Injection of chlorisondamine in an acute experiment did not affect the sensitivity of the membrane or the gland.

Discussion

The experiments showed that treatment with ganglion-blocking agents can produce a supersensitivity in gland cells and smooth muscle. The threshold was lowered by the treatment, and the response to a moderate dose of the stimulating agent was increased. In these respects, and in the time course of development, the supersensitivity resembled that brought about by denervation.

The level of sensitivity reached in these experiments was, at most, that attained after preganglionic denervation (decentralization). This is obviously the degree of supersensitivity which can be produced by depriving the effector cells of impulses from the central nervous system. The blocking action of Hö 9980 which acts more peripherally was followed by a much more pronounced supersensitivity in the submaxillary gland. This latter treatment has been assumed to be equivalent to postganglionic denervation (Emmelin and Strömblad, 1957). Using blocking agents acting in different points of the pathway, it is thus possible to imitate the conditions of surgical decentralization and denervation. Cannon's law of denervation, according to which postganglionic section causes a more pronounced supersensitivity than preganglionic section, seems to be applicable even when the "denervation" is brought about by pharmacological means.

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REFERENCES

Emmelin, N., and Henriksson, K. G. (1953). *Acta physiol. scand.*, **30**, 75.

—— Jacobsohn, D., and Muren, A. (1951). Ibid., **24**, 128.

—— and Muren, A. (1950). *Nature* (*Lond.*), **166**, 610.

—— —— (1951). *Acta physiol. scand.*, **24**, 103.

—— —— (1952). Ibid., **26**, 221.

Emmelin, N., Muren, A., and Strömblad, R. (1957). Ibid., **41**, 18.

—— and Strömblad, B. C. R. (1957). Ibid., **38**, 319.

Konzett, H., and Rothlin, E. (1953). *Abstr. XIX int. physiol. Congr., Montreal*, 1953, p. 525.

Muren, A. (1957). *Acta physiol. scand.*, **39**, 188.

Nordenfelt, I., and Ohlin, P. (1957). Ibid., **41**, 12.

Strömblad, B. C. R. (1956a). Ibid., **36**, 47.

—— (1956b). Ibid., **36**, 158.