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EFFECTS OF CROSS-SUTURE AND OF INJECTED ACETYLCHOLINE ON THE SUPERSENSITIVITY OF DENERVATED STRIATED MUSCLE

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The sensitivity of the tongue muscles to acetylcholine was examined in repeated experiments on cats anaesthetized with hexobarbitone. The supersensitivity which developed after section of the hypoglossal nerve was found to decrease when the muscles, after cross-suture, became re-innervated from the vagus or the chorda-lingual nerve. The supersensitivity caused by denervation could also be reduced by injecting acetylcholine repeatedly during six hours in acute experiments.

Re-innervation of the submaxillary gland is accompanied by a decline in the supersensitivity to chemical stimuli which the gland cells develop after section of the chorda tympani. This was found to be the case even when the regenerating nerve was not the chorda but the hypoglossal nerve, indicating that the ordinary low degree of sensitivity is not maintained through some action specific to the normal secretory nerve (Emmelin, Muren, and Strömblad, 1957). However, the fibres cut were preganglionic, and experiments with hexamethonium showed that the hypoglossal fibres, connected to the chorda in a cross-suture, had only regenerated to the synapse. The secretory cells were therefore still innervated by the normal postganglionic fibres. In the present experiments, this complication caused by the presence of an intercalated synapse was avoided by working on striated muscle and its motor nerve. The muscles of the tongue re-innervated from the vagus or chorda-lingual nerves were studied.

The supersensitivity of a parasympathetically decentralized submaxillary gland can, likewise, be abolished by injecting repeatedly over several days a secretory agent such as pilocarpine or carbachol (Emmelin and Muren, 1950, 1951). Corresponding experiments were now carried out for a shorter period on the denervated muscles of the tongue, acetylcholine being used as a stimulating agent.

METHODS

The sensitivity of the tongue muscles of the cat was studied repeatedly in the same animal under standard conditions. The cats were anaesthetized first with ether, then with hexobarbitone given intracardially. With the cat on its back, the head fixed, and the mouth widely opened, a clip was placed at the tip of the tongue and connected by a thread and pulley to a lever writing on a smoked paper. When no hexobarbitone was needed, saline was injected intracardially to prevent clotting in the injection needle, and a series of doses of acetylcholine was administered by the same route. At the beginning of the experiment, 2 mg. of atropine sulphate was given intracardially to abolish the muscarinic effects of acetylcholine.

For the cross-suture experiments the hypoglossal nerve and the cervical vagus or chorda-lingual nerves were exposed on the right side using hexobarbitone anaesthesia. The nerves were cut and the central stump of one of the two latter nerves was sewn to the peripheral stump of the hypoglossal nerve. The sensitivity of the tongue muscles was then estimated at intervals and finally an acute experiment was carried out using chloralose anaesthesia. The result of the cross-suture was now examined by stimulating the nerves electrically.

The effect of often repeated injections of acetylcholine on the level of sensitivity was studied in acute experiments. The hypoglossal nerve was cut and, when a pronounced supersensitivity had developed, the cat was anaesthetized with chloralose. Atropine (2 mg.) was given intravenously, and during about 6 hr. large doses of acetylcholine (50 μg./kg.) were injected intravenously every 5 min. The sensitivity was then estimated at hourly intervals, using a convenient standard dose of acetylcholine, which was given intracardially.

RESULTS

The Development of the Supersensitivity

When the hypoglossal nerve had been cut on one side, a supersensitivity could first be observed

2 or 3 days after the operation. The sensitivity then increased quickly and reached a maximum on the sixth or seventh day. It then remained high, provided that no regeneration took place. The threshold dose of acetylcholine injected into the left ventricle was found to be lowered markedly, from 50 to 100 μg./kg. in the normal innervated, to 0.2 to 0.5 μg./kg. in the denervated structure. The effects of moderate doses of acetylcholine increased markedly during the first week following the denervation. The quick responses to acetylcholine were particularly affected. The slow component, caused in our experiments by doses of acetylcholine of the order of 10 to 20 μg./kg. and above seemed to appear on the fourth day, as described by Brown (1937), and increased in size during the following days; sometimes the slow response was superimposed upon the quick one, sometimes it appeared when the muscles were relaxing after the quick contraction, and sometimes not until the muscles had fully relaxed. The development of the supersensitivity during the first six days is illustrated in Fig. 1.

Cross-suture Experiments

Twelve cats with a vagus-hypoglossal suture and two with a chorda-lingual-hypoglossal suture survived for a month or more after the operation. In all these cats a pronounced supersensitivity developed as a consequence of the denervation, and later in all of them the sensitivity started to decline. In one cat the fall in sensitivity was first observed 38 days after the operation, but usually it was not found until after 6 to 10 weeks. The sensitivity then decreased progressively in the course of the following weeks to near the original level.

In the acute experiment, stimulation of the right vagus nerve in the cats with the vagus-hypoglossal suture was observed to cause a contraction of the tongue, often as pronounced as that caused by excitation of the left hypoglossal nerve. Stimulation of the left vagus gave no contraction. However, in most experiments it was possible to obtain a small contraction by stimulating the right hypoglossal nerve, indicating some degree of re-innervation from the normal motor nerve. When this was first observed the precaution was taken in the later experiments to free a long stretch of the hypoglossal nerve by careful dissection, to cut it as far distally as possible and to displace the central stump so that it was separated from the cross-suture by cervical muscles and was remote from the suture. Even then, however, some hypoglossal fibres were able

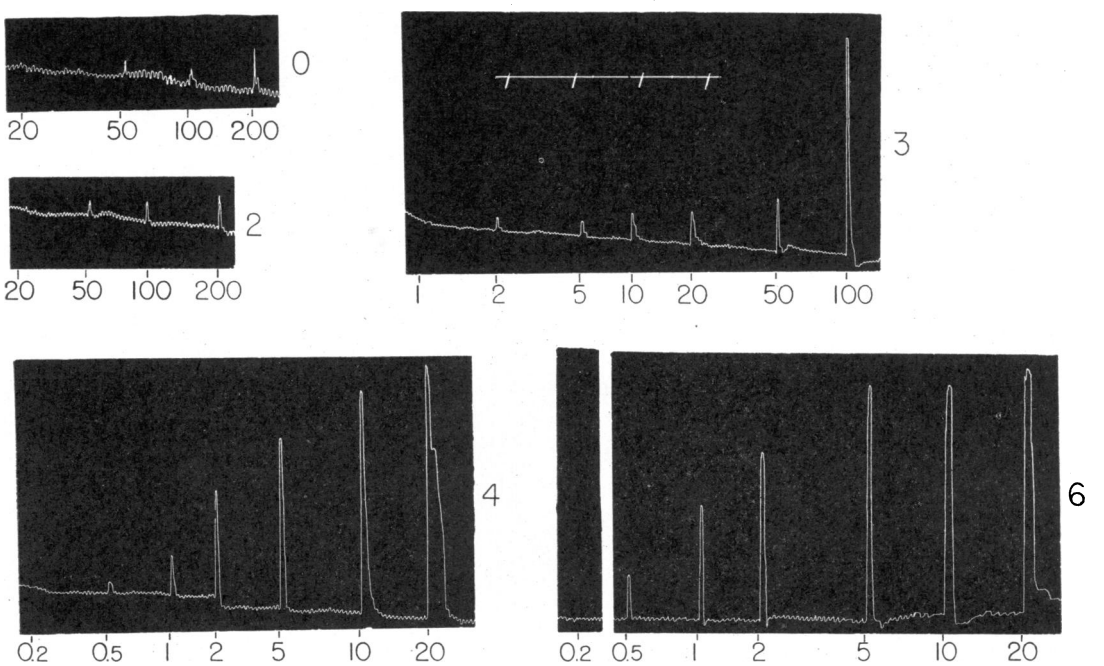

FIG. 1.—The sensitivity of the tongue muscles to acetylcholine before (**0**) and **2**, **3**, **4**, and **6** days after section of the right hypoglossal nerve. The numerals below each tracing give the doses of acetylcholine (in μg./kg.) injected intracardially. Time (on day **3**), min.

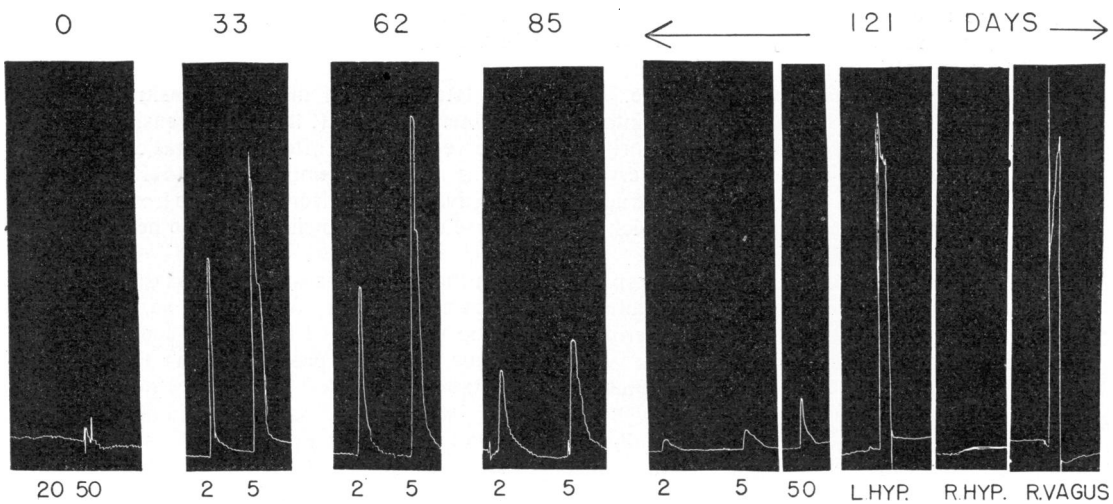

FIG. 2.—Records of sensitivity as in Fig. 1 before (**0**) and **33, 62, 85**, and **121** days after vagus-hypoglossal anastomosis. The doses of acetylcholine used to estimate the sensitivity are given as numerals below the tracings in μg./kg. **L.HYP.**=stimulation of the left, **R.HYP.** of the right hypoglossal, **R.VAGUS** of the right vagus for 10 sec., frequency 20/sec.

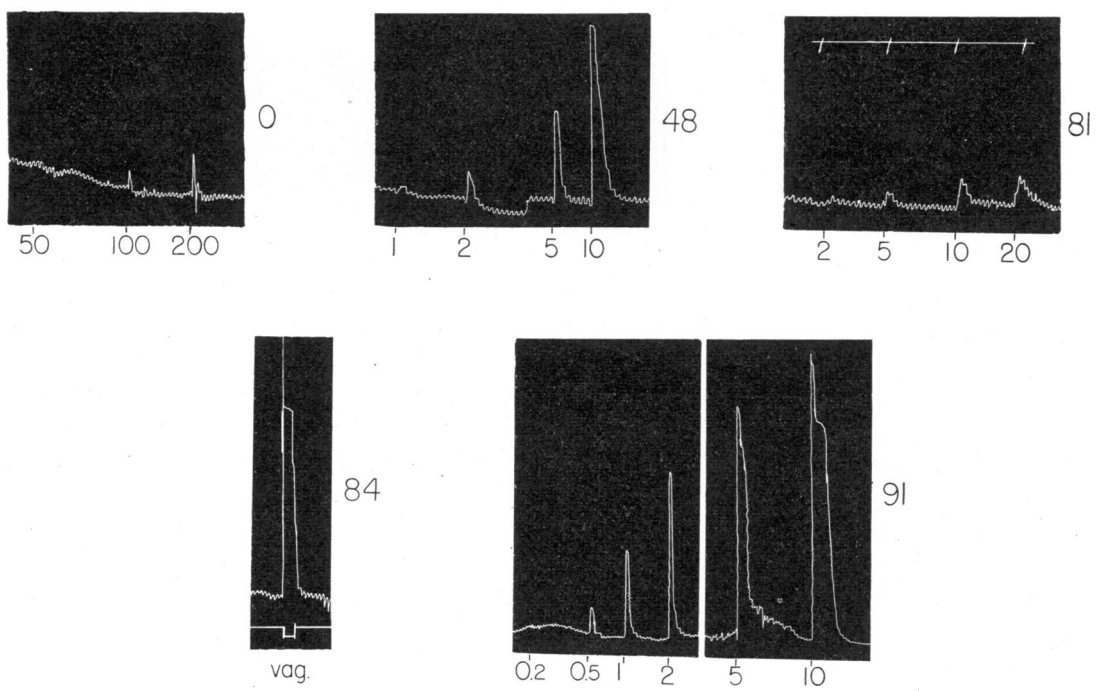

FIG. 3.—Records of sensitivity as in Fig. 1 before (**0**) and **48, 81, 84**, and **91** days after vagus-hypoglossal anastomosis. At **vag.** the right vagus was cut at the base of the skull and stimulated for 10 sec. The numerals below indicate the doses of acetylcholine in μg./kg. Time, min.

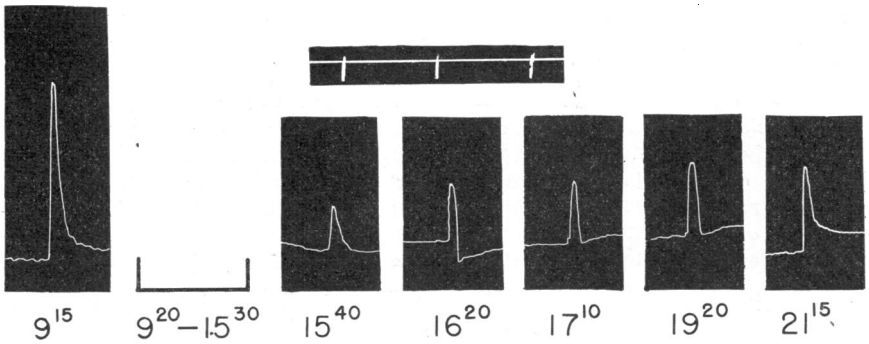

FIG. 4.—The muscles of the tongue had been sensitized by previous section of the hypoglossal nerve, and at 9^{15} the sensitivity to 5 μg./kg. of acetylcholine was high. Acetylcholine, 50 μg./kg., was injected intravenously every 5 min. between 9^{20}–15^{30}, causing strong contractions each time. Each of the following contractions is caused by 5 μg./kg. of acetylcholine, injected intracardially at the time given below the tracings. Time, min.

to find their way to the tongue in some experiments. There were in fact only three experiments in which electrical stimulation of the right hypoglossus had no effect whatsoever on the tongue. Fig. 2 shows one such experiment in which the sensitivity was very high even two months after the cross-suture had been made. After 85 days the sensitivity had markedly decreased and after 121 days it was even less. Stimulation of the right vagus caused a contraction similar to that elicited through the left hypoglossal whereas stimulation of the right hypoglossal had no effect.

The effect of vagal stimulation was abolished by decamethonium and by gallamine in doses which were of the same order of magnitude as those required to abolish the contractions caused by stimulation of the left hypoglossal.

In view of the great tendency to re-innervation from the hypoglossal nerve, it seemed important to ascertain that the decrease in sensitivity observed in the cross-suture experiment was really due to re-innervation from the vagus nerve. In a typical experiment illustrated in Fig. 3, the sensitivity was not tested until 48 days after the vagus-hypoglossal anastomosis when it was found to be high. After 81 days it had greatly decreased. Three days later the vagus nerve was cut as far centrally as possible, using hexobarbitone anaesthesia. Stimulation of the peripheral stump was found to cause contraction of the tongue. The cat was allowed to recover and a week later the sensitivity was found to be very high, even higher than in the experiment after the first denervation, probably because some re-innervation had already taken place at that stage.

In the two experiments in which the chorda-lingual nerve was sewn to the hypoglossal, stimulation of the former nerve was found to give a strong contraction when the sensitivity had declined. In the vagus-hypoglossal suture experiments, however, no contraction of the tongue was obtained when the chorda-lingual nerve was stimulated in the acute experiment, namely the Philipeaux-Vulpian phenomenon was not observed when the tongue had been re-innervated from the vagus nerve.

Treatment with Acetylcholine

Repeated injections of acetylcholine over a period of 6 hr. were found to diminish the supersensitivity caused by denervation. Fig. 4 shows the responses of the tongue muscles to a standard dose of acetylcholine, 5 μg./kg., given intracardially. Ten minutes after the end of the treatment with acetylcholine the sensitivity was markedly decreased, and estimations of the sensitivity about once an hour showed that only a slight recovery took place in the course of nearly 6 hr.

DISCUSSION

As in the submaxillary gland, the high sensitivity to chemical agents created by denervation in the muscles of the tongue is reduced when the structure becomes re-innervated by some other nerve than its own; and the experiments on the tongue have the advantage that there is no synapse in the nervous pathway.

Treatment of the decentralized gland with secretory agents for some days abolishes the supersensitivity. It seemed difficult, for technical reasons, to reproduce similar experiments on skeletal muscle. Since, however, the time course for the sensitization of the tongue muscles was found to be much shorter than that of the gland

it appeared possible that the same might apply to the " desensitization " process, and an attempt was made to " desensitize " the tongue muscles in the course of an acute experiment. The supersensitivity of the muscles was markedly reduced, although not abolished, by often repeated intravenous injections of acetylcholine during 6 hr., and the " desensitization " persisted for many hours.

In a normally innervated salivary gland, a supersensitivity resembling that caused by denervation can be produced by long-continued treatment with an anti-parasympathetic or a ganglion blocking agent (Emmelin and Muren, 1950 ; Emmelin, 1959). No attempts were made in the present investigation to keep a cat curarized for the days necessary for a possible sensitization. In the case of the submaxillary gland, it proved possible to sensitize with hexamethonium in cross-suture experiments, because the new synapse was extremely susceptible to the blocking agent. It was hoped that in the cross-suture experiments with skeletal muscle, decamethonium or gallamine would prove to be particularly active, so that the muscles of the tongue could be kept curarized by doses which did not affect the respiratory muscles. This was, however, not found to be the case.

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