

## SUPERSENSITIVITY OF SALIVARY GLANDS FOLLOWING TREATMENT WITH BRETILIUM OR GUANETHIDINE

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The sensitivity of the submaxillary glands to noradrenaline was estimated once a week in cats anaesthetized with hexobarbitone. Daily subcutaneous injections of bretylium or guanethidine were found to produce a supersensitivity of the glands, similar to that caused by extirpation of the superior cervical ganglion.

Prolonged treatment with various drugs interfering with the transmission of nerve impulses causes a supersensitivity to chemical stimuli which resembles that brought about by denervation. The salivary glands have proved useful in studies on "pharmacological denervation" since secretion of saliva seems particularly susceptible to blocking agents. So far, drugs acting on the parasympathetic innervation of the glands have been investigated (see Emmelin, 1961a). The salivary gland cells receive, however, sympathetic secretory fibres also, and they become supersensitive to chemical stimuli when the superior cervical ganglion has been removed (Simeone & Maes, 1939; Emmelin & Engström, 1960a and b). In the present experiments attempts were made to find out whether prolonged administration of sympathetic blocking agents can cause a supersensitivity of the submaxillary gland cells to secretory agents.

Treatment with reserpine has been found to sensitize the heart and various smooth muscles to chemical agents (Burn & Rand, 1958a, b, c; 1959; Trendelenburg & Gravenstein, 1958). In our first experiments on salivary glands we also used reserpine; there was some indication that a supersensitivity developed in some of the cats, but in the course of the prolonged treatment the secretory capacity of the glands tended to diminish as the general condition of the experimental animals deteriorated. We therefore used bretylium instead of reserpine, and later guanethidine when this drug became available. These drugs, however, are known to increase the effects of adrenaline and noradrenaline even in acute experiments (Boura & Green, 1959; Maxwell, Mull & Plummer, 1959; Page & Dustan, 1959; Maxwell, Plummer, Schneider, Povalski & Daniel, 1960; Vernikos-Danellis & Zaimis, 1960); we found this to occur in the salivary glands also. The problem was, therefore, to investigate whether, apart from this acute effect, a sensitization similar to that following sympathetic denervation can develop in chronic experiments.

## METHODS

The sensitivity of the two submaxillary glands of cats to noradrenaline was tested in each animal once weekly. The cats were anaesthetized with hexobarbitone sodium (20 mg/kg) administered intracardially after induction with ether and the submaxillary ducts cannulated from the mouth as previously described (Emmelin & Muren, 1952). Further doses of hexobarbitone were given when required. Standard doses of noradrenaline (2, 5, 10 and 20  $\mu\text{g}/\text{kg}$ ) were injected through the needle in the heart into the left ventricle. Noradrenaline was chosen as a test drug since extirpation of the superior cervical ganglion is known regularly to cause a supersensitivity towards this agent (Emmelin & Engström, 1960b). Bretylium tosylate (Darenthin) and guanethidine sulphate (Ismelin) were administered subcutaneously as described below. For comparison the superior cervical ganglion of one side was excised in some experiments.

## RESULTS

The results with bretylium and guanethidine were essentially similar and will be treated together.

Table 1 summarizes the results of a series of experiments in which 10 mg/kg of bretylium (cats 1 to 4) or guanethidine (cats 5 to 11) was given subcutaneously for

TABLE 1  
EFFECT OF BRETYLIUM, GUANETHIDINE AND GANGLIONECTOMY  
Sensitivity of the submaxillary gland to noradrenaline before and after treatment with bretylium (cats 1 to 4) or guanethidine (cats 5 to 11). The effect of extirpation of the superior cervical ganglion is shown in 6 of the cats

Cat	Noradrenaline $\mu\text{g}/\text{kg}$	Total response to noradrenaline (drops of saliva)		
		Before	After treatment	After ganglion- ectomy
1	20	0.3	3.3	3.3
2	10	0.3	6.7	8.7
3	20	0.3	5.7	7.5
4	20	2.3	7.0	9.3
5	20	0.7	6.7	—
6	20	0.7	8.5	—
7	10	0.3	4.7	—
8	10	0.7	8.7	—
9	10	1.7	6.7	6.5
10	20	2.7	7.7	5.3
11	20	0.3	6.7	—

a period of 1 to 3 weeks; the last injection of the drug was given about 24 hr before the sensitivity was estimated. The effect of ganglionectomy in some of the animals is also shown. In all the cases the responses to noradrenaline were found to be augmented after the treatment with bretylium or guanethidine. The increase was of the same order of magnitude as that produced by removal of the superior cervical ganglion. In the table the observation made on each cat is exemplified with one of the standard doses of noradrenaline; the dose chosen was that which caused a small response in the normal gland. When smaller doses of noradrenaline were tested the threshold was found to be similarly lowered by drug treatment and ganglionectomy.

When in some cases the estimation of the sensitivity was carried out on the day after the first injection of bretylium or guanethidine some increase in responsiveness

to noradrenaline could usually be demonstrated. This was probably the sensitizing effect ordinarily seen in the acute experiment. The effect was considerably smaller than that found after treatment with daily injections for one week. The objection could be made that the effect encountered after one week of treatment was still nothing but the acute one, more pronounced after one week than after one day because a large amount of the drug had accumulated in the body during the week; the two drugs are known to be eliminated remarkably slowly (Boura, Green, McCoubrey, Laurence, Moulton & Rosenheim, 1959; Maxwell, Mull & Plummer, 1959; Maxwell, Plummer, Schneider, Povalski & Daniel, 1960; Boura, Copp, Duncombe, Green & McCoubrey, 1960). Against this explanation two types of results may be quoted.

Firstly, in a series of experiments each cat received single injections of different doses of the drugs with intervals of several weeks between the injections so as to leave time enough for the drug to be eliminated; on the day after each injection the sensitivity of the submaxillary glands to noradrenaline was estimated. Single doses of 10, 30 and 50 mg/kg of bretylium or guanethidine were given. In addition, doses of 100 and 150 mg/kg were administered, but they were divided into two injections over two days (50+50 and 50+100 mg/kg); most cats tolerated even these large doses. The experiments showed that the sensitizing effect, observed one day after an injection, was not much more pronounced with the larger than with the smaller doses of bretylium or guanethidine. Even after 150 mg/kg the increase in responsiveness to noradrenaline was smaller than that obtained when 10 mg/kg was given daily for a week, that is, when altogether 70 mg/kg was injected.

Secondly, the slow elimination of bretylium and guanethidine made the following type of experiment possible. On the first day 50 mg/kg of the drug was injected and on the next day 50 or 100 mg/kg. The sensitivity to noradrenaline was estimated on the third day and then with weekly intervals. Five such experiments are shown in Table 2 (cats 1 to 4 with guanethidine, cat 5 with bretylium). It can

TABLE 2  
PROLONGED EFFECTS OF BRETILIUM AND GUANETHIDINE  
Effect on the sensitivity of bretylium 100 mg/kg (cat 5) or guanethidine 150 mg/kg (cats 1 to 4)  
Total response to noradrenaline (drops of saliva)

Cat	Nor- adrenaline $\mu\text{g}/\text{kg}$	Before	Time after treatment				
			1 day	1 week	2 weeks	3 weeks	4 weeks
1	10	1.5	3.7	6.7	4.7	3.0	0.5
2	10	0.3	2.3	5.7	2.3	1.3	0.3
3	10	0.3	2.7	6.3	1.3	—	—
4	5	0	0.3	1.3	0.7	0.3	—
5	10	1.5	0.7	5.7	6.3	2.7	2.5

be seen that the responses to noradrenaline after one week were markedly higher than those after one day. It may be added that in cat 1 the superior cervical ganglion was later extirpated. This caused a sensitization similar to that seen one week after guanethidine 150 mg/kg; noradrenaline 10  $\mu\text{g}/\text{kg}$  caused a secretion of 6.7 drops one week after the drug and 6.5 drops one week after ganglionectomy.

The slow elimination of the drugs is reflected in the long-lasting sensitization, as shown in the table. This was apparent in another experiment also; two weeks after discontinuation of daily treatment with bretylium 10 mg/kg for one week, stimulation of the exposed sympathetic trunk was found to cause no secretion of saliva.

#### DISCUSSION

The results of the experiments described above indicate that treatment with bretylium or guanethidine for some time causes a supersensitivity similar to that created by removal of the superior cervical ganglion, that is, that these drugs in chronic experiments can cause a supersensitivity by producing a prolonged "pharmacological sympathectomy." The mode of action of these interesting drugs is not altogether clear, but the ultimate result of their action on the adrenergic fibres seems to be to prevent the nerve impulse from releasing the chemical transmitter. It therefore seems reasonable to quote the present experiments in support of the view that supersensitivity following extirpation of the superior cervical ganglion develops as a consequence of reduced liberation of transmitter substance. Since section of the preganglionic sympathetic fibres causes no supersensitivity in the submaxillary gland (Emmelin & Engström, 1960b), the conclusion would be that the transmitter substance is released from the postganglionic neurone even if this neurone is decentralized, that is, independent of impulses from the central nervous system.

A similar conclusion was made from experiments on the parasympathetic nervous system showing that botulinum toxin causes a supersensitivity of the submaxillary cells resembling that brought about by parasympathetic denervation (Emmelin, 1961b).

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