SUPERSENSITIVITY FOLLOWING "PHARMACOLOGICAL DENERVATION"

N. EMMELIN

Institute of Physiology, University of Lund, Lund, Sweden

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INTRODUCTION

The subject of this review is the supersensitivity towards chemical agents which develops in certain innervated structures during long-lasting treatment with drugs interfering with the transmission of the nerve impulse. The increased responsiveness produced in acute experiments by drugs such as cholinesterase inhibitors, cocaine, thiocyanate (2), creatinine (29), or some general anesthetics (16) will not be treated here. Nor will the sensitizing effect of administration of thyroxine be discussed. The drugs dealt with have as their main effect to block the transmission of the nerve impulse at one point or other; like section of the nerve, they deprive the effector cell of the nerve impulse. Their immediate effect is not to sensitize the effector, but when present for days or weeks they produce a supersensitivity which resembles that caused by denervation. Experiments in which such blocking agents are made to act over long periods may therefore throw light on the mechanism of the sensitization due to denervation and, indirectly, on the normal process of transmission.

The earliest observations in this field were made on the submaxillary gland of the cat using atropine as a blocking agent; these experiments will be described first in some detail. In the following sections, also, experiments on salivary glands will often be mentioned. There are several reasons for the fact that these organs have been used extensively for such investigations. First, secretion of saliva is easily abolished by various blocking drugs; dryness of the mouth is one of the more common side-effects of therapeutic agents. Comparative studies on different effectors have shown the salivary reflex to be particularly susceptible to the blocking action of atropine (49) and hexamethonium (48), for instance. Secondly, the gland cells respond to both sympathomimetic and parasympathomimetic agents, and one type of drug can therefore be used to estimate the level of sensitivity when the action of drugs of the other group has been abolished pharmacologically. Thirdly, methods are available which permit repeated observations on the sensitivity of salivary glands over long periods of time.

In experiments of this type cats are anaesthetized with a short-acting barbiturate, such as hexobarbital, given intracardially after preliminary ether. The salivary ducts are cannulated from the mouth. Secretory agents are administered through the needle in the heart, and in the intervals between the injections saline solution is given to prevent clotting. At the end of the experiment the cannulae in the ducts and the needle in the heart are removed, the cat is allowed to awaken, and the experiment can be repeated after some days (21, 24, 35).

EFFECTS OF ATROPINE ON THE CAT'S SUBMAXILLARY GLAND

Observations on "paralytic secretion" of saliva, which were the starting-point of these investigations, led to the conclusion that this phenomenon is obtained only under special experimental conditions. For instance, there has to be a considerable release of catecholamines from the adrenals, as seen in morphine anaesthesia; and the submaxillary gland cells have to be supersensitive because of previous section of the chorda tympani in order to respond to the increased concentration of the amines circulating in the blood (17, 18, 31). Langley (54) assumed the paralytic secretion to be due to an activity in the postganglionic parasympathetic neurone disconnected from the central nervous system by section of the chorda. Fleming and MacIntosh (47) modified this hypothesis, and postulated a subthreshold activity in this neurone in order to explain the supersensitivity towards adrenaline (epinephrine) and sympathetic impulses which they observed after having cut the chorda in advance. In order to test this idea, atropine was administered daily subcutaneously to cats in which the chorda of one side had been severed. The outcome of these experiments was that atropinization did not prevent the development of the supersensitivity towards adrenaline; on the contrary, not only the decentralized, but the contralateral, normally innervated gland also showed an increased sensitivity (32, 34). A paralytic secretion could, in fact, be obtained in morphine anaesthesia from an atropine-treated, but otherwise normal submaxillary gland (33). Of historical interest is the following observation by Metzner in 1912 (56): "wiederholte Atropinevergiftung mit steigenden Dosen ruft aber bei Katzen eine hochgradige Ueberregbarkeit der sympathischen Speichelcentren hervor, so dass bei dem geringsten äusseren Reiz der Speichel fliesst." In the light of present knowledge Metzner seems to have observed a paralytic secretion, prolonged atropinization having caused a supersensitivity and the external disturbances a hyperactivity of the sympatho-adrenal system.

In order to produce a supersensitivity, atropine has to be injected twice a day in increasing doses. If a dose of 1 mg/kg, for instance, is maintained, the sensitivity first increases then falls off again towards the pretreatment level. Such experiments could easily be used to study development of tolerance to atropine-like and other blocking agents. The dose of atropine has to be raised successively to 10 to 20 mg/kg in the course of two to three weeks. During the first two days of atropinization the gland shows scarcely any change in sensitivity to adrenaline. The sensitivity then rises gradually to reach a maximum within

two to three weeks. The acute effect of atropine in high concentration is to depress the responsiveness of the gland towards adrenaline; therefore, the estimation of the sensitivity should not be made shortly after the injection of a large dose of atropine but after the lapse of some hours. One day after the last injection the sensitivity is still at its high level, but it then starts to decline and returns to the low level of a normal gland within four or five days (35).

The supersensitivity caused by atropine resembles very much that brought about by section of the chorda. It develops gradually, and along the same time course. Like denervation supersensitivity, it is characterized by a lowered threshold to the test drug; a dose of the secretory agent which elicits a submaximal response from the normal gland causes a more rapid and long-lasting secretion from the sensitized gland; the maximal rate of secretion, on the other hand, is not increased. After section of the chorda the gland is supersensitive not only to adrenaline but to other sympathomimetic and to parasympathomimetic drugs as well (33); such an unspecificity seems to be typical of denervation supersensitivity. In the atropine-treated gland parasympathomimetic agents cannot be used as test drugs; even 24 hours after an injection of a large dose of atropine the gland is apparently under the acute influence of the drug, for chorda stimulation produces a scanty secretion only, or none at all. The nonspecificity of the sensitization can, however, be demonstrated using various sympathomimetic drugs such as noradrenaline (norepinephrine), dopamine and Synephrin [*p*-hydroxy-α-(methylaminomethyl)benzyl alcohol]. A further similarity between the two types of supersensitivity is that both can be increased further by removal of the superior cervical ganglion or by the acute injection of cocaine (34). On the other hand, the supersensitivity caused by section of the chorda does not increase that produced by atropinization; the fact that the supersensitivity following parasympathetic decentralization can be somewhat raised by treatment with atropine will be discussed in the next section of this review.

The relationship between the two types of sensitization is particularly well illustrated by the following type of experiment (30). In a series of cats the chorda of the right side was cut and atropine treatment instituted. When a high level of sensitivity had been reached on both sides, the contralateral, left chorda was severed and the atropine administration discontinued. Four days later the sensitivity was again estimated. From earlier experience it could be concluded that the sensitivity four days after withdrawal of atropine should be low in a normal gland, and that section of the chorda four days earlier should cause a small supersensitivity only. In the experiments, however, both glands were found to show the same, high level of sensitivity.

These various observations seem to allow the inference that the changes caused by the two procedures, which manifest themselves in a heightened sensitivity, are closely related. Observations on atropinized glands could then serve to elucidate the mechanism responsible for the sensitization following section of the chorda. The main effect of atropine is to deprive the gland cell receptors of the action of acetylcholine, still liberated by the nerve impulse. The sensitization caused by section of the chorda should then be due to the fact that no nerve

impulses liberate acetylcholine; loss of a noncholinergic influence by the nerve (a "trophic" influence) does not seem essential in this connection. When no acetylcholine is liberated by the nerve impulse, after section of the chorda, or when acetylcholine is not allowed to act on the receptors, after atropinization, some changes obviously take place, which manifest themselves in a nonspecific supersensitivity of the gland cells. These changes develop gradually, to reach a maximum in two or three weeks. The experiments with atropine show that they are reversible; they disappear gradually so that the normal state is reached in a few days when no atropine is given. Regeneration of the cut chorda, likewise, restores the normal level of sensitivity (37).

It may be added here that daily injections of pilocarpine or carbachol for some days decrease the supersensitivity caused by section of the chorda to the level of the normal gland, or even lower (35). If it be assumed that the level of sensitivity of a gland cell is, at least to a large extent, determined by the amount of acetylcholine normally released by the secretory impulse, this role of acetylcholine can apparently be taken over by other secretory agents, reaching the gland by the blood.

ACTIONS OF OTHER PARASYMPATHETIC BLOCKING AGENTS ON THE CAT'S SUBMAXILLARY GLAND

Section of the chorda tympani is a preganglionic denervation. Experiences made on various structures and summarized in Cannon's "law of denervation" (10, 11) show that postganglionic denervation generally causes a more pronounced supersensitivity than does destruction of the penultimate neurone. It is not practicable to cut all the postganglionic parasympathetic fibres of the submaxillary gland owing to the fact that many synapses are situated within the gland, but a partial denervation is achieved if the chorda is traced along the salivary duct and cut as close to the hilum as possible. After such an operation a supersensitivity develops which surpasses that caused by section of the chorda (21). It seems reasonable to assume the sensitization due to atropinization to be more like that of a post- than a preganglionic denervation, *i.e.*, to exceed that created by section of the chorda. In the experiments with atropine, a supersensitivity was found only occasionally which was somewhat greater than that seen when the chorda had been cut. Several facts may account for the failure to find this regularly. Because of the tolerance to atropine that develops it is difficult to ascertain that full atropinization is maintained. Furthermore, it is difficult to settle the correct time for an estimation of the sensitivity to be made after an injection of a large dose of atropine; if it is made too early, some of the acute depressant effect of atropine on the responsiveness to adrenaline may remain; if too late, the supersensitivity may have begun to wear off. A parasympathetic blocking agent much more suitable for these experiments was found in the synthetic drug piperidino-ethyl-diphenyl-acetamide (Hoechst) described by Bockmühl *et al.* (4) and Schaumann and Lindner (65). This agent, for brevity called Hö 9980, was found to cause a supersensitivity (27) which regularly surpassed that brought about by section of the chorda (41). The sensitization was higher than that obtained by dissecting the chorda towards the hilum,

which could be expected to give only a partial postganglionic parasympathetic denervation (21). Apart from this quantitative difference the supersensitivity evoked by treatment with Hö 9980 had all the characteristics of the denervation supersensitivity described for atropine. It could, for instance, be further heightened by removal of the superior cervical sympathetic ganglion; the level of sensitivity thus reached after procedures which imitate a complete denervation is probably the highest one of this type that can be attained in a submaxillary gland.

Hö 9980 has the following advantages. It is more long-acting than atropine, and very little tolerance seems to develop. A dose of 1 mg/kg, given once a day for two or three weeks, will thus produce a supersensitivity far above that achieved by section of the chorda. Such a dose, or a much higher dose, does not depress acutely the responsiveness of the gland cells to adrenaline. No ganglionic blocking effect is obtained, as with large doses of atropine. The drug is, in fact, highly specific as an antagonist against the muscarinic effects of acetylcholine. It does not interfere with the release of acetylcholine caused by impulses in the chorda. It can be shown to cause a supersensitivity by local action on the gland if given daily through the submaxillary duct (41).

In view of the high specificity of action of Hö 9980 it is reasonable to assume that this drug owes its sensitizing activity exclusively to its parasympathetic blocking properties. If so, the supersensitivity generated by this drug should partly be due to the fact that the gland is deprived of some action of acetylcholine, liberated by secretory impulses transmitted through the chorda from the central nervous system. Section of the chorda has the same effect. The fact, however, that the level of sensitivity reached during treatment with Hö 9980 is markedly higher than that which ensues when the chorda has been cut, suggests that the parasympathetic blocking drug in addition abolishes some action of a cholinergic mechanism which is still at work when the chorda has been cut.

To test the hypothesis that the sensitization caused by Hö 9980 is entirely due to the loss of an action by acetylcholine on the gland cells, some other parasympathetic blocking agents have been tried: methylscopolamine, Lachesine [ethyl(2-hydroxyethyl)dimethylammonium benzilate chloride], and Isopropamide (diphenyl-carbamoyl-propyl-di*iso*propyl-methylammonium iodide). All these drugs create a supersensitivity to adrenaline in the submaxillary gland (21). A marked tolerance develops to Lachesine, and some to methylscopolamine as well. The doses of these drugs therefore have to be raised in the course of treatment. It is possible, however, to show that all these drugs create a supersensitivity which surpasses that caused by section of the chorda, and even that following partial postganglionic parasympathetic denervation. Since the supersensitivity produced by these various parasympathetic blocking agents is reversible, they can all be tested on the same cat. Such experiments show that all the four different agents tried sensitize the gland to the same level, a fact which supports the view that they act exclusively by virtue of their parasympathetic blocking properties.

Arguments in favour of the conception that acetylcholine is released from

endings of cholinergic nerves in general, even in the absence of impulses from the central nervous system, have been given by several authors (44, 45, 46, 64).[1] In eserinized plasma, perfused through the submaxillary gland, some acetylcholine appears even when the chorda is not excited. The fact that eserine and other cholinesterase inhibitors, injected through the salivary duct towards the gland cause a secretion may, likewise, indicate that some acetylcholine is released continuously (36, 42); this secretion is not obtained when the postganglionic fibres have degenerated (as can be seen in the parotid gland). The acute section of these postganglionic fibres does not change the secretory response to eserine, and therefore the acetylcholine cannot be released by impulses from the cell body of the postganglionic neurone; a leakage due to local events at the nerve endings seems more likely.

Acetylcholine leaking from the terminals of the postganglionic parasympathetic neurone does not cause any secretion of saliva under ordinary conditions. It does when preserved by a cholinesterase inhibitor. In the course of degeneration of the postganglionic fibres the release may be large enough to cause a flow of saliva, possibly because unusually large packets of acetylcholine are broken up in bursts (22, 43). Even if the effector cells have been sensitized by previous section of the chorda, the leakage from the intact postganglionic fibres is insufficient to cause any secretion. The paralytic secretion is not dependent on some activity in the postganglionic neurone, as assumed by Langley (53). This is apparent from the fact that it is not abolished by atropine. Instead, it is brought to an end by adrenergic blocking agents or extirpation of the adrenals, and the fact that it is caused by catecholamines from the adrenals under exceptional conditions has already been mentioned. Dirnhuber and Lovatt Evans (15) have suggested that slowly liberated acetylcholine causes a subliminal excitation of the gland. It can, in fact, be shown that a small dose of a parasympathetic blocking agent, given in an acute experiment, slightly raises the secretory threshold for adrenaline (23). This can be seen in the gland sensitized by previous section of the chorda also; assuming an unchanged leakage of acetylcholine in such a gland, the subliminal effect of the acetylcholine ought to be larger. This brings the discussion back to the hypothesis of Fleming and MacIntosh (47), according to which the supersensitivity caused by section of the chorda is due to a subthreshold activity in the postganglionic parasympathetic neurone. However, the fact that the previously decentralized gland is still found to be highly supersensitive to adrenaline after the acute injection of a parasympathetic blocking agent shows that a subliminal activity of leaking acetylcholine cannot be the essential cause of the supersensitivity. The leaking acetylcholine must be assumed to have a quite different effect on the level of sensitivity of the effector cell, as witnessed by observations on glands chronically deprived of the action of this acetylcholine by prolonged treatment with parasympathetic blocking drugs. The normal effect of this acetylcholine, subthreshold as regards secretion, must be to keep the sensitivity of the effector at a low level. When the receptors

[1] Dr. Thies of the Rockefeller Institute, New York, kindly drew my attention to the early discussions in this field.

of the gland cells are no longer under the influence of this leaking acetylcholine, because of long-lasting "atropinization" or degenerative section of the postganglionic fibres, a supersensitivity develops which exceeds that merely due to lack of acetylcholine released by secretory impulses.

ACTIONS OF PARASYMPATHETIC BLOCKING AGENTS ON OTHER STRUCTURES

The parotid gland of the cat, treated with Hö 9980 for some time, acquires a supersensitivity towards adrenaline, as shown by Strömblad (67, 68, 69). In this gland a complete postganglionic parasympathetic denervation can be made by cutting the auriculo-temporal fibres. Section of these fibres produces a greater supersensitivity towards acetylcholine than does section of the preganglionic fibres in the tympanic nerves (66). The reverse is true, however, for adrenaline. This is very likely due to the fact that large doses of adrenaline have to be given to produce secretion even in sensitized parotid glands. The vascular effects of the adrenaline dominate and interfere with the secretory responses, and the postganglionically denervated vessels of the gland are particularly sensitive to adrenaline, in accordance with the law of denervation (62). Quantitative comparisons between the levels of sensitivity, as regards secretion, in pre- and postganglionically denervated glands on one hand, and "atropinized" glands on the other, may therefore be misleading. Presumably "atropinization" does not affect the vessels in the same way as does postganglionic section.

The sublingual gland of the cat is about as sensitive to adrenaline as the submaxillary gland. Observations made in the course of experiments on the secretion of saliva, mentioned above, suggest that this gland can be more extensively denervated postganglionically than the submaxillary gland. During treatment with Hö 9980 the sublingual gland becomes supersensitive to adrenaline (23); comparisons between such glands and pre- and postganglionically denervated glands would probably be profitable.

Nordenfelt and Ohlin (61) found the parotid gland of the rabbit to be fairly sensitive to adrenaline, contrary to the submaxillary gland in this species. They found that treatment with Hö 9980 caused a supersensitivity of the parotid gland towards adrenaline and sympathetic nerve impulses. This observation they took as evidence to show that the gland is supplied with parasympathetic cholinergic fibres, a disputed question, which they were unable to settle in stimulation experiments. Later on, they in fact succeeded in finding parasympathetic secretory fibres for the rabbit's parotid gland. Considering the high specificity of Hö 9980 as a parasympathetic blocking agent, a sensitizing action following treatment with this drug may be used to reveal the existence of unknown cholinergic mechanisms in other structures as well.

Like the salivary glands, the lachrymal gland is supplied with both parasympathetic and sympathetic secretory fibres, and it can be excited by adrenaline, even in the presence of atropine (54). In cats anaesthetized with hexobarbital, repeated observations were made on the level of sensitivity of the lachrymal gland towards adrenaline (40). Prolonged treatment with Hö 9980 was found to create a pronounced supersensitivity, which could be superimposed upon

that caused by previous removal of the superior cervical ganglion. It is not known whether parasympathetic denervation sensitizes the lachrymal gland, but these observations make it likely.

So far only glandular structures have been discussed. Muren (57, 58) found, however, that smooth muscles also can be rendered hypersensitive by treatment with parasympathetic blocking agents. He experimented on the motility of the stomach of dogs supplied with a chronic gastric or oesophageal fistula. Repeated observations on the motility were made using a balloon in the stomach, connected to a bromoform manometer. After transthoracic vagotomy the motor responses to carbachol and mecholyl, given intravenously, were found to be increased (59). Experiments with Hö 9980 treatment were possible, thanks to the observation that adrenaline and noradrenaline under certain conditions regularly had a pure motor effect on the stomach (60). For instance, when the dog was anaesthetized with pentobarbital, the gastric tone fell and the effect of the two sympathomimetic agents changed from inhibitory to excitatory. Repeated observations on such dogs showed that during treatment with Hö 9980 a supersensitivity towards adrenaline and noradrenaline developed. The sensitivity increased along the same time course as after vagotomy. It was found to reach a somewhat higher level during "atropinization" than after vagotomy (57). The analogy with the findings on salivary glands is obvious, since vagotomy is a preganglionic denervation. The observations may suggest the existence of a leakage of acetylcholine from the postganglionic endings. In addition, it should be borne in mind that the drug could have abolished the action of acetylcholine released from the postganglionic endings in local reflexes, intact after vagotomy.

In structures like the heart conditions are probably more complex. Isolated hearts from guinea pigs, treated with Hö 9980, did not show any increased responsiveness to adrenaline (39); the significance of this finding is difficult to assess, however, since it is not known whether vagotomy sensitizes the heart (11).

GANGLIONIC BLOCKING SUBSTANCES

The supersensitivity so far described has been the result of a peripheral blockade, *i.e.*, parasympathetic blocking drugs abolishing a muscarinic action of acetylcholine on glandular or plain muscle cells; it resembles that caused by postganglionic denervation. It would obviously be important to know whether a "pharmacological denervation," as a general rule, produces the gradual changes in the effector cells which manifest themselves in an enhanced sensitivity to chemical agents. For instance, if the conclusion of the preceding sections is correct, that the supersensitivity following treatment with atropine-like drugs is in part due to lack of an action of acetylcholine released by the secretory or motor nerve impulse, it could be predicted that prolonged ganglionic blockade should cause a supersensitivity, similar to that caused by preganglionic section. When hexamethonium was given repeatedly in subcutaneous injections to cats, no sensitization of the submaxillary glands could be detected (23). Similarly, Konzett and Rothlin (51) found no supersensitivity of the nictitating membrane

after treatment with this drug. In view of the pronounced tolerance to hexamethonium which develops, it seemed possible that an insufficient blocking of ganglionic transmission might account for these results. In the course of an investigation on cross-sutured nerves a chance observation was made which turned out to be useful in this connection (37). The central end of the cut hypoglossal nerve was sewn to the distal end of the chorda-lingual nerve. Hypoglossal fibres regenerated into the chorda, and the supersensitivity of the submaxillary gland due to section of the chorda was found to be temporary only. When the hypoglossal nerve was stimulated some months after the cross-suture operation this was found to evoke a flow of saliva from the submaxillary gland. The effect was abolished by hexamethonium, and it was observed that very small doses of the drug blocked transmission of impulses from the hypoglossal nerve to the postganglionic neurone for a remarkably long period of time. Thus an intravenous dose of 0.1 mg of hexamethonium per kg abolished the secretory effect of hypoglossal stimulation for one hour and a half, whereas the effects of stimulating the sympathetic trunk or the contralateral, normal chorda were not influenced.

This finding was utilized in the following manner (19). Anastomosis was made unilaterally between the hypoglossal and chorda-lingual nerves, as described above. The sensitivity of the two submaxillary glands to adrenaline was estimated repeatedly in the course of some months. When it had declined on the operated side, after its temporary rise, to a low and constant level, treatment with hexamethonium was instituted. The drug was injected subcutaneously twice daily over a period of two to three weeks or longer. In most experiments the initial dose was 3 mg/kg, and it was raised successively until a dose of 20 mg/kg was reached; this dose was then maintained for some days. The level of sensitivity was estimated 5 to 19 hours after an injection of hexamethonium.

During this treatment the gland of the operated side acquired a supersensitivity, whereas the contralateral gland was not noticeably affected. The supersensitivity produced resembled in all respects that already seen in the same glands as a result of section of the chorda. The threshold dose of adrenaline was lowered, and a moderate dose of the test drug evoked a more rapid and long-lasting secretion on the operated than on the normal side. The supersensitivity developed gradually and reached a maximum in two to three weeks. The maximum level attained in two cats was just the same as that seen previously in the same animals before the gland had been reinnervated by hypoglossal fibres; in five other cats the rise in sensitivity was not quite as large as that seen after cutting the chorda. When the treatment with hexamethonium was discontinued the sensitivity fell and reached its pretreatment level in a few days' time, just as observed when atropine treatment of a normally innervated gland ceases, or when pilocarpine is administered to an already denervated gland. When the effect of hexamethonium had worn off, the parasympathetic blocking drug Hö 9980 was given, and a rise in sensitivity ensued which was much more pronounced than that caused by hexamethonium or preganglionic denervation.

When these experiments had been completed, more suitable ganglionic block-

ing compounds than hexamethonium became available. An attempt was made to sensitize the normally innervated submaxillary gland by treatment with chlorisondamine (Ecolid); this drug has a long duration of action and less tolerance seems to develop to it than to hexamethonium. A dose of 2 mg/kg was injected twice a day over a period of some weeks. In some experiments the dose was gradually raised to 5 mg/kg. Unlike hexamethonium, chlorisondamine was found to sensitize the normal submaxillary gland, the effect being maximal within three weeks. When the drug was withdrawn the sensitivity returned to the original level within a week. For a comparison, the chorda was then cut; the sensitivity thereby produced was found to be somewhat higher than that caused by chlorisondamine. In these experiments the responses of the nictitating membrane to adrenaline were recorded as well. This was done 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. Treatment with chlorisondamine caused a gradually increasing supersensitivity of the membrane. When this effect had worn off after discontinuation of treatment, the preganglionic sympathetic fibres were severed. The ensuing supersensitivity was of the same order of magnitude as that seen during treatment with the ganglion-blocking compound; subsequent extirpation of the superior cervical ganglion produced a much higher level of sensitivity.

It seemed somewhat easier to sensitize the membrane than the gland with chlorisondamine, as shown by the following experiment in which two periods of treatment were given. In the first, the highest dose of the drug was 2 mg/kg twice a day. The gland became only slightly sensitized, but the membrane was sensitized to the level reached later after preganglionic denervation. In a second period of treatment the dose of chlorisondamine was raised to 5 mg/kg twice daily. The sensitivity of the membrane did not increase more than in the first period; it was raised further by sympathetic ganglionectomy. The gland, on the other hand, was much more sensitized than when the smaller dose was given, but even now chorda section was 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 larger doses of chlorisondamine, but no cats survived such treatment.

In acute experiments Mantegazza *et al.* (55) found hexamethonium and pentolinium, given intravenously or intraarterially, to cause increases in the responses of the nictitating membrane and the blood vessels to adrenaline. These effects were described as small, and they were obtained with small doses of the blocking agents; the effects were not increased by raising the doses of the drugs. This is clearly an effect quite different from that seen during prolonged treatment with ganglion-blocking substances, when the sensitivity gradually increases in the course of days and weeks to a high level.

The reasonable conclusion from these experiments seems to be that when drugs which abolish the nicotinic effect of acetylcholine at autonomic ganglionic synapses cause a supersensitivity, they do so by depriving, for some time, the effector cell of motor or secretory impulses from the central nervous system. A

"denervation" is thereby produced, comparable to that caused by surgical destruction of the preganglionic neurone.

No experiments have so far been published to elucidate whether abolition of the nicotinic action of acetylcholine in the neuromuscular junction by prolonged curarization gives rise to a supersensitivity. The difficulty of such experiments is obvious. When it had been found that a ganglionic synapse particularly susceptible to hexamethonium can be created by cross-suture, analogous experiments on skeletal muscle were attempted (38). Contractions of the tongue muscles in response to acetylcholine were recorded in repeated experiments on cats under hexobarbital anaesthesia. Cross-suture was established between the central end of the vagus or the chorda-lingual nerve and the distal end of the hypoglossal nerve. A supersensitivity developed because the motor nerve had been severed, but it disappeared when the muscle had been reinnervated. It was hoped that the muscle would now prove particularly susceptible to curarization (or other blocking) agents, so that the tongue could be kept curarized for a long time by doses which did not affect respiration. Unfortunately no such high susceptibility was found. A clinical observation may, however, be of interest in this connection. Churchill-Davidson and Richardson (13) described a patient who in a myasthenic crisis was given large doses of tubocurarine (and artificial respiration) for eight days. This led to a dramatic improvement which was sustained with the aid of reduced neostigmine therapy. It is tempting to suggest that the prolonged curarization had sensitized the muscles to the transmitter of the motor nerves.

A localized, prolonged interference with transmission at the myoneural junction has, however, been found possible by using botulinum toxin (72), as described in the next section.

BOTULINUM TOXIN

Recently Thesleff (72) has shown that botulinum toxin produces a supersensitivity of mammalian skeletal muscle which closely resembles that caused by section of the motor nerve. Botulinum toxin, type A, was injected into the musculature of the cat's hindleg or applied to the exposed surface of the tenuissimus muscle. One to four weeks later this muscle was isolated. Acetylcholine was applied iontophoretically to the individual muscle fibre from a micropipette as described by Castillo and Katz (12). Membrane depolarization caused by acetylcholine was recorded with an intracellular electrode. In botulinum-intoxicated fibres the area sensitive to acetylcholine was found to be increased beyond the limits of the end-plate just as observed by Axelsson and Thesleff (1) in denervated fibres. Acetylcholine also produced a graded and "electrically silent" contracture in the botulinum-treated muscle, as in denervated muscle. The time course of the increase in the acetylcholine-sensitive area was the same in denervated and botulinum-treated muscles. In control experiments no changes in the ultrastructure of the nerve terminals could be detected after chronic botulinum intoxication, unlike the pattern seen after denervation. Nerve stimulation caused no end-plate potential and spontaneous miniature potentials were absent.

Mechanical injury to the nerve terminals during insertion of the microelectrode could, however, produce a burst of miniature end-plate potentials. From these observations, and those of earlier investigators, Thesleff concluded that botulinum toxin acts by interfering with the release of the transmitter of the cholinergic motor fibres. When small amounts of the toxin had been administered, some muscle fibres showed miniature potentials of high frequency; in such fibres the acetylcholine sensitivity was confined to the end-plate region. In other fibres a lower frequency of discharge was encountered, and the sensitive area was enlarged. Some fibres showed no potentials, or potentials of a very low frequency only, and the whole membrane was sensitive to acetylcholine.

The similarity between the effects of denervation and of chronic botulinum intoxication suggests that similar mechanisms are at work. The inference should then be that the supersensitivity in both cases is due to lack of liberation of acetylcholine. As in the postganglionically denervated autonomic effector, this could be the acetylcholine released by the impulse from the central nervous system and in addition that set free by local events at the nerve endings. The supersensitivity of mammalian skeletal muscle is certainly very marked. This may be related to the fact that denervation or treatment with botulinum toxin deprives the muscle fibre of acetylcholine from both these sources.

Following Thesleff's investigations some experiments were made with botulinum toxin on the salivary glands (23). In cats under hexobarbital anaesthesia the toxin was injected towards the gland through a cannula, inserted from the mouth into the salivary duct. When relatively large amounts of the toxin were administered, a supersensitivity was found to ensue, but no cats survived for more than about a week. Since it was possible that the greater proportion of the toxin was quickly removed from the gland through reflex salivation and then swallowed, a small dose of a parasympathetic blocking agent was given prior to the administration of the toxin to suppress salivary secretion. The dose of the toxin could then be considerably reduced and the animals survived. A supersensitivity both to sympathomimetic and parasympathomimetic drugs was found to develop, resembling that found after denervation. This was seen both in submaxillary and parotid glands. It persisted for periods of months, declining gradually; in two cats some increase in sensitivity still remained about seven months after the injection of the toxin. The level of sensitivity reached varied in different experiments. In the submaxillary gland it was somewhat lower than that obtained after section of the chorda. Some secretion was elicited by stimulation of the chorda indicating that the intoxication was incomplete. Hilton and Lewis (50) succeeded in abolishing the secretory and vasodilator effects of chorda stimulation by injection of botulinum toxin into the parenchyma of the submaxillary gland. This was, however, done in short-term experiments and very large doses of the toxin were given, doses far above those which could be used for prolonged treatment. When the chorda of a gland treated with botulinum toxin was severed, a supersensitivity resulted which was more marked than that caused by cutting the nerve of a normal gland. The most reasonable explanation is that the toxin contributed to the supersensitivity by affecting some of the postganglionic

parasympathetic endings. An effect was thus obtained corresponding to that seen when the chorda is dissected and cut close to the hilum. When botulinum toxin is injected through the duct, preganglionic endings are reached as well, and they are, likewise, known to be sensitive to the toxin (50). In such experiments the toxin can be assumed to cause a partial pre- and a partial postganglionic parasympathetic "denervation."

Similarly, parotid glands were usually not sensitized by the toxin treatment to the highest level attainable by parasympathetic denervation. In one case, however, the supersensitivity was as pronounced as that caused by section of the postganglionic parasympathetic fibres in the auriculo-temporal nerves. In the parotid glands the "denervation" was obviously postganglionic, usually partial but in one gland complete.

DRUGS INTERFERING WITH ADRENERGIC TRANSMISSION

A series of investigations, particularly by Burn and colleagues, has shown reserpine to be a valuable tool when investigating supersensitivity of structures innervated by adrenergic nerves. Burn and Rand (7) isolated spiral strips of thoracic aorta of normal rabbits and of rabbits treated for two days with reserpine. Strips from treated animals were found to be supersensitive to adrenaline and noradrenaline. Increased responsiveness after treatment with reserpine was also demonstrated on the blood pressure of the spinal cat (8) and in the perfused hindleg of the dog. Noradrenaline and isoprenaline (isoproterenol) were found to have a more pronounced chronotropic action in heart-lung preparations from dogs treated with reserpine than in such preparations from normal dogs (3). Similarly, Trendelenburg and Gravenstein (73) observed that noradrenaline had a greater effect on the heart rate of an anaesthetized dog if the animal had been pretreated with reserpine; doses of noradrenaline which did not accelerate the heart of a normal dog caused a tachycardia in animals that had been injected previously with reserpine.

In further investigations Burn and Rand (9) compared the effect of denervation and of treatment with reserpine. The mydriatic effects of adrenaline and noradrenaline were observed in anaesthetized cats. Contractions of the spleen enclosed in a plethysmograph were elicited in spinal cats by injection of noradrenaline. The experiments were carried out on normal cats, on cats given reserpine intraperitoneally for two days prior to the experiment, and on cats in which the postganglionic sympathetic fibres of the iris or the spleen had been destroyed about two weeks earlier. Treatment with reserpine was found to cause a supersensitivity of both the iris and the spleen, resembling that produced by denervation. In the irides the degree of supersensitivity appeared to be less than after denervation; in the spleen the threshold dose of noradrenaline was lowered more than ten times both after denervation and during treatment with reserpine.

The similarity between the effect of postganglionic sympathetic denervation and of reserpine treatment was further emphasized when the content of noradrenaline of the various organs was estimated. Denervation is known to cause a loss of noradrenaline from some structures; so does administration of reserpine.

Burn and Rand (9) found that the amount of noradrenaline present in the iris and the spleen of the cat is greatly reduced after injections of reserpine and after denervation. They suggest that the stores of noradrenaline in the tissues are not inactive but that they continually release noradrenaline, which then occupies a proportion of the receptors; the sensitivity is thereby kept low (6, 9). When the nerve fibres degenerate or reserpine is given, the stores are no longer replenished, the leakage of noradrenaline ceases and the sensitivity of the effector organ increases.

Burn and co-workers (3) cited some evidence for a continuous release of noradrenaline. Krayer (52), studying the anti-adrenaline effect of veratramine on the heart, found the alkaloid sometimes to lower the rate of beat and suggested as one possible explanation that catecholamines are liberated in the heart, raising the frequency. Isolated atria from rabbit hearts beat at a lower rate if taken from animals treated with reserpine than if they are taken from normal animals (7). Trendelenburg and Gravenstein (73) found a lower heart rate in their dogs injected with reserpine than in untreated animals.

In experiments on submaxillary glands of cats reserpine treatment was occasionally found to cause a slight increase in the sensitivity towards noradrenaline (26). The level of sensitivity reached after extirpation of the superior cervical ganglion was not attained. Reserpine was considered less suited for experiments on salivary glands since the secretory capacity seemed impaired when the general condition of the animal deteriorated during the prolonged treatment. Drugs like bretylium and guanethidine ([2-(octahydro-1-azocinyl)ethyl]guanidine sulfate) may be more useful for this purpose. By treatment with bretylium a supersensitivity towards noradrenaline can in fact be produced in the submaxillary gland; it approaches that caused by sympathetic ganglionectomy (26). Drugs of this type often seem to increase the effect of sympathomimetic amines already in acute experiments. In the submaxillary gland of the cat, however, such an effect of bretylium is often little pronounced, provided that the slight and evanescent muscarinic effect of the drug (25) is abolished by administration of a small dose of a parasympathetic blocking agent when the level of sensitivity is estimated.

SOME OTHER EFFECTS OF "PHARMACOLOGICAL DENERVATION"

Apart from a supersensitivity, prolonged treatment with blocking agents causes some other changes which will be considered briefly. Such changes are clearly of particular interest if they can be brought about by surgical denervation as well. The depletion of the stores of noradrenaline which follows both denervation and treatment with reserpine has already been mentioned.

Numerous investigators have suggested that supersensitivity following denervation might be due to a reduced activity of enzymes acting upon the transmitters. Strömblad (67, 68, 69) studied the effect of denervation and of prolonged treatment with Hö 9980 on the cholinesterase and amine oxidase activity in submaxillary and parotid glands. One complication arises from the fact that denervation causes an atrophy of the glands which is not seen after treatment with

atropine-like drugs. The enzyme activity must therefore be calculated both for the total gland and as a concentration. Preganglionic parasympathetic denervation reduced the total activity but not the concentration of the cholinesterase in both glands. Postganglionic denervation, carried out in parotid glands, reduced the activity of the enzyme much further, so that the concentration was also lowered. Treatment with Hö 9980, on the other hand, did not affect the activity in either gland. Obviously no supersensitivity towards acetylcholine can be demonstrated in glands under the influence of parasympathetic blocking agents. Experiments on enzymes destroying adrenaline and noradrenaline seem more important. So far only amine oxidase has been examined (68). Preganglionic parasympathetic denervation diminished the amine oxidase activity to about the same extent as it reduced the weight of the gland. Postganglionic denervation lowered the total activity of the enzyme; there was a slight, but not significant, fall in concentration. "Atropinization" reduced both total activity and concentration in both glands; in the parotid gland the decreases in amine oxidase activity were greater than those seen after postganglionic parasympathetic denervation.

The effect of botulinum toxin on the cholinesterase activity of skeletal muscle has also been investigated by Strömblad (71). The enzyme activity in the anterior tibial muscle of the rat was estimated 14 days after injection of the toxin into the muscle. The activity of the whole muscle was found to be lower than that of the contralateral muscle, but the muscle had atrophied and when the comparison was made on a weight basis, no difference in enzyme activity was found. In muscles denervated 14 days earlier the atrophy was less, but the decrease in enzyme activity more pronounced than after injection of botulinum toxin.

The following experiments may be mentioned in this connection. Strömblad (70) examined the oxygen consumption of chopped submaxillary glands *in vitro*. The normal effect of secretory drugs in increasing the oxygen consumption was found to be much reduced, and sometimes absent, if the chorda had been cut two to three weeks earlier; treatment with Hö 9980 during such a time period had essentially the same effects as section of the chorda.

The interesting fact that treatment with parasympathetic blocking agents does not cause the atrophy of the submaxillary gland (28) which is seen regularly after section of the chorda, has already been mentioned. One possible explanation may be that there is some relation between the structure of the gland and the flow of blood through it. Normally the blood supply of the gland is manifoldly increased by impulses in the chorda. This does not occur when the chorda has been cut, but in the normally innervated gland of the atropinized cat there is presumably often, and during meals particularly, a pronounced reflex vasodilation even if there is no secretion. This may serve to prevent atrophy. Some support of this explanation may be found in the fact that treatment with botulinum toxin, which affects both secretion and vasodilatation, is followed by atrophy of the gland (23). It may also be pointed out that removal of the superior cervical ganglion leads to an increase in weight of the salivary gland (5); this operation

increases the flow of blood through the gland. It is, however, possible that there are more complicated reasons for the fact that parasympathetic blocking drugs are unable to imitate denervation when the effect on the weight is considered; among other things, the fact should be kept in mind that some increase in the oxygen consumption can be seen in an atropinized gland when the chorda is activated.

In one respect the structure of the gland is affected by prolonged atropinization; the histological picture is changed in apparently the same way as by denervation (28).

The activity of the kallidin-releasing enzyme of saliva is much reduced during treatment with Hö 9980, just as it is after section of the chorda tympani (27). Further experiments on the composition of saliva from denervated glands, and from glands treated with blocking agents, would be interesting.

DISCUSSION

Experiments quoted in this review indicate that various drugs interfering with storage, release or action of chemical transmitters may increase the reactivity of effector cells to chemical agents if allowed to act for some time. Drugs used in pharmacology and therapeutics may thus, in addition to an immediate blocking effect, have an action to which hitherto little attention has been paid; this action results from the fact that an effector deprived for some time of an unknown action of the transmitter acquires a supersensitivity to stimuli. It may be worth while to have in mind that such a secondary action might account for effects, beneficial or unwelcome, when drugs with a central, ganglionic or peripheral depressant action are administered therapeutically over a long period of time. It could be tempting, for instance, to regard the salivary gland cell, sensitive to both acetylcholine and adrenaline, and highly sensitized to adrenaline when under the influence of atropine, as a model that can be replaced by, say, a cell in the central nervous system, sensitive to excitatory and inhibitory transmitters, and sometimes for long periods subjected to depressant drugs, acting directly upon it or on distant neurones which impinge on it. Admittedly, this point may belong to the realm of speculation, beyond the scope of the author's competence, and it may be more appropriate to consider some physiological implications of the experiments instead.

The many resemblances between the supersensitivity caused by denervation and that created by prolonged treatment with drugs interfering in different ways with transmission at synapses, myoneural junctions or between postganglionic fibre and gland or smooth muscle, have been emphasized already. It seems justified to extend the "law of denervation" and assume it to be valid not only for surgical denervation but for "pharmacological denervation" as well. In this connection it may be stressed that the expression "pharmacological denervation" has been used mainly for the sake of brevity; it should be realized that treatment with blocking agents only to a certain extent imitates a proper denervation, as suggested by the previous section of this review. No doubt exceptions to the law of denervation could be found after pharmacological, just as after surgical, denervation which it would be profitable to explore.

The law of denervation seems to be applicable to "pharmacological denervation" not only in the respect that a supersensitivity develops during treatment with blocking agents; the analogy can be carried further. According to Cannon's law, decentralization of an autonomic effector produces a less pronounced supersensitivity than does denervation (when this term is used in its restricted sense, *i.e.*, for destruction of the postganglionic neurone). Similarly, "pharmacological decentralization" by treatment with ganglionic blocking agents causes less sensitization than "pharmacological denervation" produced by drugs with a more peripheral site of action such as atropine, botulinum toxin (when applied to the postganglionic endings), reserpine, and bretylium. Furthermore, although it is sometimes difficult for technical reasons to secure a complete pharmacological blockade and therefore the maximal sensitization obtainable with a certain drug is not always reached, the conclusion seems correct that "pharmacological decentralization" causes the same degree of supersensitivity as section of the preganglionic neurone, and "pharmacological denervation" (in the limited sense) the same supersensitivity as destruction of the postganglionic neurone. For instance, when a ganglion-blocking compound is administered, the submaxillary gland can be sensitized to the level found after section of the chorda tympani, a preganglionic denervation; likewise, the nictitating membrane is sensitized to the level reached by preganglionic sympathetic denervation. Various parasympathetic blocking agents sensitize the submaxillary gland above the chorda level, and above the level reached by partial postganglionic denervation. They sensitize the plain muscle of the stomach above the level reached by cutting the vagi, which is a preganglionic section. The technical difficulties of such experiments have already been pointed out and also the need for a tissue in which more accurate quantitative comparisons between the sensitizing effects of parasympathetic decentralization, denervation, and treatment with atropine-like drugs is possible. Botulinum toxin was in one parotid gland found to cause the same degree of supersensitivity as section of the postganglionic cholinergic nerves. Reserpine sensitized the spleen to the same extent as postganglionic denervation.

These different experiences may be illustrated schematically in the figure

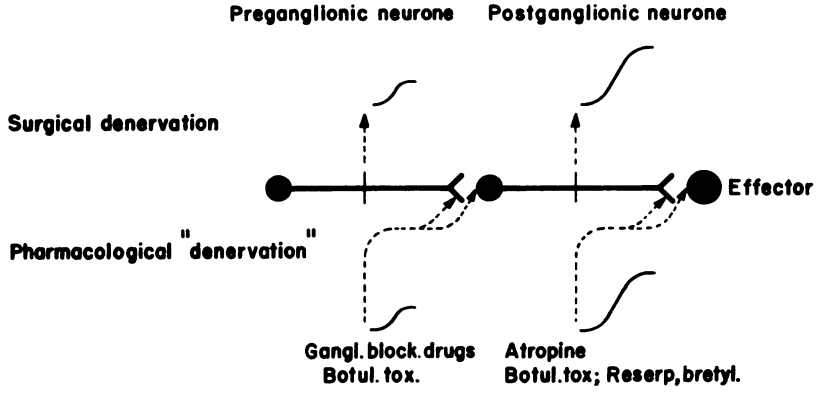

FIG. 1

which shows the sensitizing effects of surgical and pharmacological decentralization and denervation.

When the experiments with atropine-like drugs were described, it was pointed out that these led to the conclusion that there is normally a leakage of acetylcholine from the postganglionic parasympathetic nerve endings and that this leakage in some way keeps the sensitivity of the effector cell low (20, 21). From the reserpine experiments, Burn and Rand (9) inferred that leakage of noradrenaline normally occurs from some store and that this serves to keep the sensitivity of the sympathetically innervated cell low. Presumably such a leakage continues when the preganglionic fibres have been cut or ganglion-blocking compounds given. It is reasonable to assume sensitization following these latter procedures to be due to lack of transmitter normally set free by the impulse in the secretory or motor nerve. The inference seems justified that postganglionic denervation or treatment with atropine-like drugs, reserpine, bretylium, and botulinum toxin produce a more marked sensitization than surgical or pharmacological decentralization because the effector is deprived both of transmitter released by the nerve impulse and transmitter leaking from the endings of the final neurone. The pronounced sensitization following denervation of skeletal muscle or its treatment with botulinum toxin may also have both these causes. The possibility that supersensitivity may result from lack of transmitter has, in fact, been considered long ago. In 1934 Dale (14) pointed out that the depot of transmitter at the nerve endings disappears when the nerve degenerates and wrote: "we may note, in passing, the probability that the exaggerated sensitiveness of the denervated effector cells, to the artificial application of the chemical transmitter, may be conditioned by this disappearance of its depot and failure of its normal release."

After reserpine or botulinum toxin there is no release of the transmitter, and it has been concluded that this causes the supersensitivity (9, 72). In the "atropinized" tissues such a release presumably still occurs, and these experiments allow the reasonable conclusion that sensitization occurs even when there is a release but the transmitter is prevented from attaching itself to the receptors of the effector (18, 32). It may be added that supersensitivity caused by section of the chorda can be prevented or abolished by treatment for some days with pilocarpine, carbachol, or adrenaline (34, 35). The role of the transmitter in keeping the sensitivity low can apparently be taken over by other agents with a similar action. During administration of such agents the sensitivity of the gland, denervated or innervated, may even fall below the "normal" level (35); such observations might suggest that the "normal" sensitivity of an effector may fluctuate, depending on the intensity of the bombardment with impulses from the central nervous system.

Experiments on "pharmacological denervation" may serve further to throw light on the process by which the transmitter acts to keep the sensitivity at a low level. They do not seem to give much support to the "enzyme hypothesis," although further experiments in this field seem required. Of particular interest are the experiments on skeletal muscle by Thesleff (72) showing that both after

denervation and botulinum toxin the area of the muscle fibre sensitive to acetylcholine is extended. Such an increase of the region sensitive to acetylcholine consequent on denervation was postulated by Perry and Zaimis (63), who found a depolarizing agent to cause a greater output of potassium from denervated than from innervated skeletal muscle. How this increase in area is brought about is not known. Thesleff suggests two possibilities. The increase in permeability at the end-plate by the released acetylcholine might allow a chemical agent to enter the muscle, an agent which is able to prevent the formation of acetylcholine receptors outside the end-plate. Alternatively, a substance may escape from the fibre when acetylcholine acts on the end-plate, a substance which would otherwise induce the formation of acetylcholine receptors.

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