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ACTION OF SOME GANGLION-STIMULATING SUBSTANCES ON THE SECRETION OF SALIVA FROM THE SUBMANDIBULAR GLAND

BY

B. GOMEZ ALONSO DE LA SIERRA*

From the Department of Pharmacology, Harvard Medical School, Boston, Massachusetts, U.S.A.

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An attempt has been made to determine whether the ganglionic actions of pilocarpine and of 4-(m-chlorophenylcarbamoyloxy)-2-butynyltrimethylammonium chloride (McN-A-343) contribute to their effect on salivary secretion. Salivary flow was measured from the submandibular glands of spinal cats. Destruction of the superior cervical ganglion and adrenalectomy failed to reduce the stimulant effect of pilocarpine and McN-A-343. Substances known to interfere with the ganglionic actions of pilocarpine (cocaine, methadone and choline 2:6-xylyl ether bromide) likewise failed to modify the response. It is concluded that stimulation of autonomic ganglia and of the adrenal medulla does not contribute to the salivary secretion observed after intravenous injections of pilocarpine and of McN-A-343. Dimethylphenylpiperazinium, a nicotine-like ganglion-stimulating substance, causes salivary flow by stimulating the adrenal medulla as well as parasympathetic ganglion cells; stimulation of the superior cervical ganglion by this substance does not contribute to the salivary response.

In addition to its direct (" muscarinic ") effects on smooth muscle and on excretory glands, pilocarpine stimulates the adrenal medulla and sympathetic ganglia of the cat and dog (Dale & Laidlaw, 1912 ; Ambache, 1949 ; Root, 1951 ; Trendelenburg, 1954, 1955, 1956, 1961). The stimulation of ganglia by pilocarpine differs from that by nicotine in being resistant to hexamethonium and in being antagonized by cocaine, morphine and methadone (Trendelenburg, 1959).

Since the submandibular gland has a synergistic double innervation and since acetylcholine as well as adrenaline and noradrenaline stimulate the gland, it is possible that the very pronounced action of pilocarpine on salivation is due to multiple sites of action in addition to its " muscarinic " direct effect on the gland cells. Stimulation of the superior cervical ganglion and of the adrenal medulla may enhance the direct secretory action of pilocarpine.

4-(m-Chlorophenylcarbamoyloxy)-2-butynyltrimethylammonium chloride (McN-A-343) has recently been found to have ganglionic actions very similar to those of pilocarpine, while also having " muscarinic " effects (Roszkowski, 1961).

In order to test the above-indicated working hypothesis, experiments were performed in spinal cats. The effects of pilocarpine, McN-A-343 and a nicotine-like

* Present address: Colegio Major Universitario, Cadiz, Spain.

ganglion-stimulating substance, dimethylphenylpiperazinium, were tested before and after destruction of the superior cervical ganglion, before and after adrenalectomy, and before and after the administration of various substances known to interfere with the ganglionic actions of these substances.

METHODS

Cats of 2 to 4 kg body weight and of both sexes were used. Anaesthesia was induced with ether, and spinal preparations were then made as described by Burn (1952). The blood pressure was recorded from the femoral artery by means of a mercury manometer; injections were made into the femoral vein. The ducts of one or both submandibular glands were cannulated with polyethylene tubing at about 10 to 20 mm from their termination. Salivary flow through the tubing caused a displacement of distilled water from two inverted and sealed test-tubes. By using this device, it was possible to eliminate any influence of the viscosity of the saliva on the size of the drops which were displaced from the test-tube. The drops were of very small size (about 100 drops per ml.), and consequently the sensitivity of the recording system was very high. The drops fell on a polyethylene lever connected to a piezo-electric cartridge, the impulses of which were recorded by means of a Grass ink-writing oscillograph.

The movements of the right nictitating membrane were recorded isotonically on the smoked drum by means of a lever fitted with a frontal writing point; its magnification was 12.4 times; the load on the nictitating membrane was 7 g.

The sympathetic preganglionic fibres of both sides were cut. Those of the right side were placed on platinum electrodes and covered with liquid paraffin. Preganglionic stimulation of supramaximal strength was applied with a conventional electronic stimulator (Grass).

The following substances were used: atropine sulphate, 4-(m-chlorophenylcarbamoyloxy)-2-butynyltrimethylammonium chloride (McN-A-343), choline 2:6-xylyl ether bromide, cocaine hydrochloride, dimethylphenylpiperazinium iodide, hexamethonium chloride, methadone hydrochloride, morphine hydrochloride, pilocarpine hydrochloride. All weights refer to the salts.

RESULTS

Control observations. The submandibular gland of the spinal cat had no resting secretion. Its response to intravenous injections of pilocarpine was very prolonged. Although the response was dependent on the dose, its long duration made it impossible to obtain meaningful dose-response curves. There was no tachyphylaxis of the gland to pilocarpine; on the contrary, previous injections of this substance tended to increase the response to subsequent injections if the time interval was relatively short (Fig. 1). For instance, in 6 experiments the response of the gland to the intravenous injection of pilocarpine 12 μg/kg was a secretion of 0.7 ± 0.3 drops/min during the first min (mean $\pm$ s.e. of mean); the same dose of pilocarpine given less than 15 min after a previous injection caused a secretion of 5.4 ± 1.6 drops (7 experiments). When, however, the time interval was 30 min and longer reproducible responses were obtained: the mean responses to the first injection of pilocarpine 35 μg/kg was 21.0 ± 3.9 drops (number of drops during first min of response, 5 experiments), that to the second injection (given 30 to 40 min later) was 22.1 ± 3.0 drops.

The response of the submandibular gland to an intravenous injection of pilocarpine consisted of two phases (Fig. 1): a high rate of secretion during the first few minutes was followed by a second phase, during which salivary flow decreased

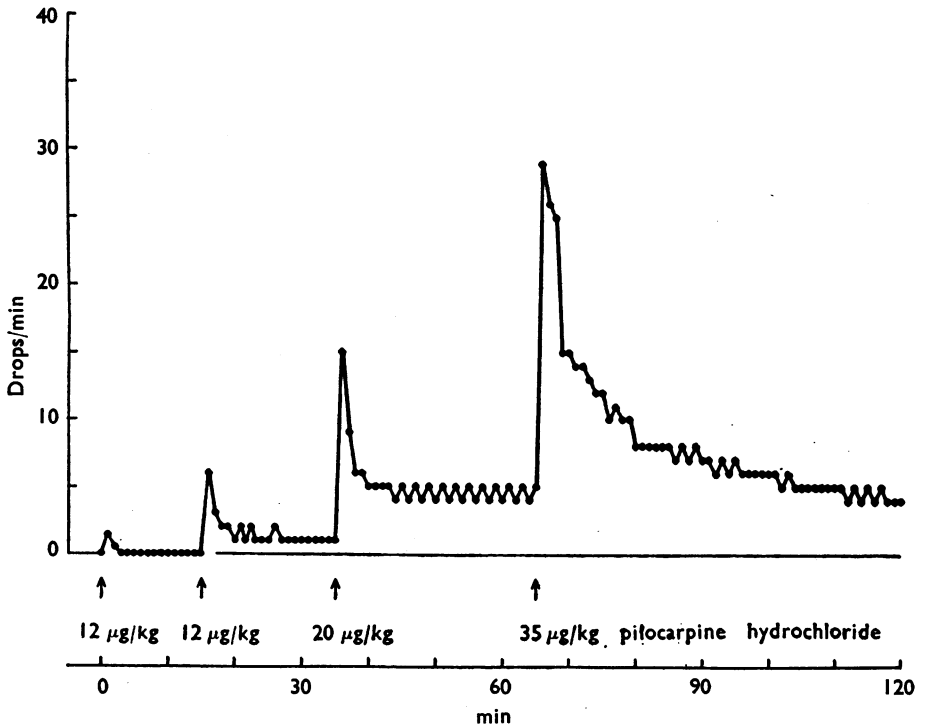

Fig. 1. Spinal cat. Response of submandibular gland to repeated intravenous injections of pilocarpine. Ordinate: salivary secretion in drops/min; abscissa: time in min.

slowly. Simultaneous recording of the movements of the nictitating membrane showed that in about 70% of the preparations pilocarpine (35 μg/kg) caused a contraction of the nictitating membrane which reached its maximum within 1 min.

On the basis of these preliminary experiments it was decided to use the dose of 35 μg/kg pilocarpine as a test dose, to keep time intervals of 30 to 40 min between injections of pilocarpine, and to measure the salivary flow during the first min after the injection.

The response of the gland to the intravenous injection of McN-A-343 30 to 100 μg/kg and of dimethylphenylpiperazinium 100 to 300 μg/kg was of short duration; therefore it was always measured as total number of drops. Furthermore, with both substances it has been found that a time interval of 20 min between two intravenous injections was long enough to ensure reproducibility of the response.

Intravenous injections of adrenaline caused responses of short duration which were clearly dependent on the dose of adrenaline. Previous injections of pilocarpine tended to increase the response to an injection of adrenaline. The following mean responses to adrenaline given as the total number of drops secreted were obtained in preparations which had not received any previous injection of pilocarpine: 0.1 drops after 1 μg/kg, 1.4 drops after 3 μg/kg and 3.0 drops after 10 μg/kg adrenaline.

Electrical stimulation of the preganglionic fibres of the superior cervical ganglion, by supramaximal stimuli for 5 sec at a rate of 25/sec, caused a salivary flow of a few drops. This response, though small, remained constant when electrical stimulation was repeated. When preganglionic stimulation was applied during the prolonged response to pilocarpine (see above) it frequently resulted in a diminution of secretion ; this inhibitory effect is probably due to the simultaneous stimulation of vasoconstrictor fibres present in the preganglionic nerve.

Destruction of the superior cervical ganglion. In this series of experiments the ducts of the glands of both sides were cannulated, and the movements of the right nictitating membrane were recorded as well. Responses were obtained before and after crushing the right superior cervical ganglion with artery forceps. The destruction of the ganglion was verified by the abolition of the response of the gland and of the nictitating membrane to preganglionic stimulation.

Table 1 summarizes the results ; from the values presented it is evident that the destruction of the ganglion fails to cause a marked reduction of the salivary response to any of the three substances. This is borne out by a comparison of the two responses of the right (operated) side, obtained before and after destruction of the ganglion, as well as by the comparison of the operated with the normal (left) side. The slight reduction in salivary flow observed after pilocarpine was a transient event and probably due to the manipulations necessitated by the exposure of the ganglion ; the response to a subsequent injection of pilocarpine was back to the initial level (right side: 28.0 ± 4.5 ; left side: 25.0 ± 3.8 drops). The response to adrenaline was not affected by destruction of the superior cervical ganglion, since the response remained identical on both sides.

The nictitating membrane of 6 of 9 preparations responded to the intravenous injection of pilocarpine 35 μg/kg, and a response to McN-A-343 30 to 100 μg/kg was observed in 5 out of 6 preparations. Crushing the ganglion greatly reduced or abolished the response of the nictitating membrane (Table 1). These observations confirm that the intravenous injection of pilocarpine and of McN-A-343 is able to stimulate the superior cervical ganglion (Trendelenburg, 1954 ; Roszkowski, 1961) and that the ganglion was destroyed in the present experiments. The normal response of the nictitating membrane to an intravenous injection of dimethylphenylpiperazinium 100 to 300 μg/kg consisted of a very fast contraction which was followed after about 10 sec by a second slower contraction. Destruction of the ganglion abolished the fast phase without affecting the slow contraction.

Exclusion of the adrenal glands from the circulation. This failed to have any marked effect on the salivary response to pilocarpine or McN-A-343 (Table 2). It reduced considerably the response to dimethylphenylpiperazinium. In these experiments there was a reduction from 10 to 6, from 13 to 10 and from 47 to 7 drops. Exclusion of the adrenal glands likewise reduced the slow component of the response of the nictitating membrane to dimethylphenylpiperazinium.

Cocaine, morphine, methadone and choline 2:6-xylyl ether bromide. Previous studies showed that these four substances are able to antagonize the response of the nictitating membrane to pilocarpine as well as the pressor response to pilocarpine ; these studies also demonstrated that both the responses are due to ganglionic actions

TABLE 1

EFFECT OF DESTRUCTION OF THE RIGHT SUPERIOR CERVICAL GANGLION ON THE RESPONSE OF THE SUBMANDIBULAR GLAND AND OF THE NICTITATING MEMBRANE

The response to pilocarpine is expressed as number of drops observed during first min of response; other responses represent total number of drops of saliva. The response of the nictitating membrane is expressed as mm on drum; n=number of observations. All responses expressed as mean ±s.e.

		Salivation						Nictitating membrane		
		Operated side (right)			Control side (left)			Right side		
	n	Before	After	Difference	Before	After	Difference	n	Before	After
Pilocarpine	8	26·4±4·7	21·1±3·7	−5·3±3·1	24·3±4·5	20·9±3·3	−3·6±2·6	6	12·3	1·1
McN-A-343	6	17·7±4·0	15·5±4·1	−2·2±1·4	20·3±6·0	20·3±6·1	0±1·2	5	26·3	0
Dimethylphenyl-piperazinium (after adrenalectomy)	3	7·7	7·3	−0·4	7·3	7·3	0	—	—	—
Pre-ganglionic stimulation	8	5·0	0	—	—	—	—	8	79·0	0

TABLE 2

EFFECT OF ADRENALECTOMY ON THE RESPONSE OF THE SUBMANDIBULAR GLAND

Values as in Table 1

	n	Before adrenalectomy	After adrenalectomy	Difference
Pilocarpine	7	22·3±3·5	19·9±4·3	−2·4±1·8
McN-A-343	8	20·0±3·1	18·4±2·7	−1·6±1·3
Dimethylphenyl-piperazinium	3	23·3	7·7	−15·6±12·2

of pilocarpine (Trendelenburg, 1954, 1955, 1961). It was thus of interest to study the effect of cocaine, morphine, methadone and choline 2:6-xylyl ether bromide on the response of the submandibular gland to pilocarpine, McN-A-343 and dimethylphenylpiperazinium.

The results of these experiments are presented in Table 3, which shows that the response of the submandibular gland to pilocarpine and to McN-A-343 was not significantly affected by any of the above-mentioned drugs. While the intravenous injection of cocaine, morphine and methadone did not cause any salivation, salivation was commonly observed after the intravenous injection of choline 2:6-xylyl ether bromide 5 mg/kg, which also caused a contraction of the nictitating membrane. The response to choline 2:6-xylyl ether bromide was of rather long duration and probably accounted for the slight increase in salivary response to the first subsequent

TABLE 3

EFFECT OF VARIOUS DRUGS ON THE RESPONSE OF THE SUBMANDIBULAR GLAND TO PILOCARPINE, McN-A-343 AND DIMETHYLPHENYLPIPERAZINIUM

Values as in Table 1

	n	Before drug	After drug	Difference	
Cocaine, 1 mg/kg					
Pilocarpine	4	21·8	24·8	+3·0±3·5	
McN-A-343	3	24·3	23·7	−0·6±5·9	
Morphine, 1 mg/kg					
McN-A-343	4	22·3	20·0	−2·3±2·3	
Methadone, 1 mg/kg					
Pilocarpine	2	34·0	32·5	−1·5	
McN-A-343	3	28·0	23·0	−5·0±7·5	
McN-A-343	3	26·7	27·7	+1·0±0·6	(After adrenalectomy)
Dimethylphenyl-piperazinium	2	14·5	3·0	−11·5	
Dimethylphenyl-piperazinium	1	14·0	3·0	−11·0	(After adrenalectomy and destruction of ganglion)
Choline 2:6-xylyl ether bromide, 5 mg/kg					
Pilocarpine	3	19·3	16·0	−3·3±1·2	
McN-A-343	15	26·2	33·0	+6·8±2·4	
Dimethylphenyl-piperazinium	2	16·5	11·5	−5·0	

injection of McN-A-343 (Table 3). Further injections of McN-A-343 caused responses identical to the initial control response. This phenomenon was not observed with pilocarpine, since time intervals between injections of pilocarpine (and therefore also the time interval between the injection of choline 2:6-xylyl ether bromide and the subsequent injection of pilocarpine) were considerably longer.

Cocaine, morphine and methadone were given in amounts which had previously been found to abolish the pressor response as well as the response of the nictitating membrane to pilocarpine. The dose of choline 2:6-xylyl ether bromide employed (5 mg/kg) caused complete block of the response of the submandibular gland and of the nictitating membrane to supramaximal preganglionic stimulation.

In 2 experiments choline 2:6-xylyl ether bromide reduced only slightly the mean response of the submandibular gland to dimethylphenylpiperazinium (Table 3), but 1 mg/kg methadone was quite effective (Table 3). In an additional experiment methadone was equally effective when administered to an adrenalectomized preparation, the superior cervical ganglion of which had been destroyed; the response to 300 μg/kg dimethylphenylpiperazinium was reduced from 14 to 3 drops.

Atropine and hexamethonium. The salivary response to pilocarpine, to McN-A-343 and to dimethylphenylpiperazinium was reduced after an intravenous injection of atropine, 1 μg/kg, and abolished after 10 μg/kg.

In 3 experiments hexamethonium 6.3 mg/kg was injected intravenously into adrenalectomized preparations, the superior cervical ganglion of which had been destroyed. The salivary response of these preparations to dimethylphenylpiperazinium (7.3 drops) was abolished by hexamethonium.

DISCUSSION

Pilocarpine is well known for its strong stimulant action on salivary secretion. Since the salivary glands are innervated by both the sympathetic and the parasympathetic system, and since both innervations and their peripheral transmitter substances stimulate secretion, it was tempting to speculate that pilocarpine might act on salivary secretion by mechanisms additional to its well-established "muscarinic" actions. Such additional mechanisms are conceivable, since it has been found that pilocarpine stimulates the adrenal medulla and the superior cervical ganglion (Dale & Laidlaw, 1912; Ambache, 1949; Trendelenburg, 1954). Furthermore, in the dog and in the spinal cat, pilocarpine elicits a pressor response (Root, 1951) which is due to general stimulation of sympathetic ganglion cells by pilocarpine (Trendelenburg, 1955, 1961).

Roszkowski (1961) recently found that McN-A-343 has ganglion-stimulating properties in addition to certain "muscarinic" actions. The ganglion-stimulating properties of McN-A-343 resemble those of pilocarpine in being antagonized by small amounts of atropine and in being resistant to hexamethonium (Trendelenburg, 1954; Roszkowski, 1961). Dimethylphenylpiperazinium, on the other hand, is a ganglion-stimulating substance which is pharmacologically closely related to nicotine (Ling, 1959); hence it is not antagonized by small amounts of atropine but is ineffective after hexamethonium.

The evidence presented in this paper indicates that the ganglion-stimulating properties of pilocarpine and of McN-A-343 do not contribute to any significant degree to the salivary response elicited by the intravenous injection of these two substances. Destruction of the superior cervical ganglion and adrenalectomy failed to reduce the response of the submandibular gland to pilocarpine and McN-A-343 ; destruction of the ganglion, however, abolished or reduced the response of the nictitating membrane to these two substances. This observation demonstrates that the substances were injected in amounts sufficient to stimulate the superior cervical ganglion. The same argument applies to the results obtained with dimethylphenylpiperazinium, and the evidence indicates that stimulation of the superior cervical ganglion by ganglion-stimulating agents fails to cause any significant response of the salivary gland. Such a conclusion is further supported by the observation that electrical stimulation of the preganglionic fibres causes only a small response of the submandibular glands. Moreover, preganglionic stimulation had some inhibitory effect when it was applied during the prolonged salivation induced by a large dose of pilocarpine; since vasoconstrictor as well as secretory fibres are stimulated by electrical impulses, it may be assumed that vasoconstriction (by preganglionic stimulation or by ganglion-stimulating agents) counteracts the response to stimulation of neurones innervating the secretory cells.

The view that the ganglion-stimulating properties of pilocarpine and McN-A-343 do not contribute to the salivary response is further supported by the observation that cocaine, morphine, methadone and choline 2:6-xylyl ether bromide failed to affect the response of the submandibular gland. These substances have previously been found either to antagonize the stimulation of the superior cervical ganglion by pilocarpine (Trendelenburg, 1954, 1957) or to abolish the pressor response of the spinal cat to pilocarpine (Trendelenburg, 1955, 1961).

Although the destruction of the superior cervical ganglion failed to affect the response of the submandibular gland to dimethylphenylpiperazinium, adrenalectomy reduced it, and thereafter an injection of hexamethonium abolished it. This observation indicates a contribution of the adrenal medulla to the salivary response to dimethylphenylpiperazinium, the remaining (reduced) response being probably due to stimulation of parasympathetic ganglion cells, since it was fully abolished by hexamethonium. A participation of parasympathetic ganglion cells in the response to dimethylphenylpiperazinium is further indicated by the observation that methadone (in normal preparations as well as after adrenalectomy) significantly reduced the response to dimethylphenylpiperazinium ; such a conclusion is based on the observation of Paton (1957) that morphine and related compounds reduce the liberation of acetylcholine from postganglionic cholinergic fibres—an action which would reduce any response to stimulation of parasympathetic ganglion cells. Kosterlitz & Taylor (1959) also obtained evidence for a reduced response to stimulation of parasympathetic nerves after the administration of morphine-like substances. Finally, injection of dimethylphenylpiperazinium 100 μg/kg caused no flow of saliva after the administration of atropine 10 μg/kg.

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