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A Study of Ganglionic Denervation Supersensitivity Using McN-A-343 and Histamine as Ganglion Stimulating Agents* (33760)

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An increase in sensitivity of the superior cervical ganglion cells to acetylcholine has been purported to occur several days after cutting the preganglionic cervical sympathetic trunk (1–4). More recent evidence from electrophysiological experiments, however, strongly contradicts these earlier studies (5, 6).

The earlier experiments using the contraction of the nictitating membrane as a measure of ganglionic stimulation are difficult to interpret, because acetylcholine may also stimulate the nictitating membrane directly (7). To avoid this problem, two ganglion stimulating agents, which do not directly contract the nictitating membrane, viz, histamine (8, 9) and McN-A-343 (10), [4-(3-chlorphenylcarbamoyloxy)-2-butynyl trimethylammonium chloride], were used in the present study to investigate the possibility that the superior cervical ganglion is an exception to the classical concept of denervation supersensitivity (2).

Methods. Adult cats of either sex were anesthetized with sodium pentobarbital, 40 mg/kg, administered intraperitoneally. Chronic denervation of either the left or right superior cervical ganglion was performed 2–3 weeks prior to the actual experiment. An incision was made parallel to the trachea, the carotid sheath was isolated, and 1-cm segment of the cervical sympathetic trunk, located approximately 3 cm proximal to the ganglion, was removed. The incision was then closed with wound clips. The surgical procedure was followed by an intramuscular injection of 150,000 units of benzathine penicillin (Bicillin).

On the day of the experiment a tracheal cannula was inserted and the intact (control) ganglion on the contralateral side was acutely denervated. The animal was then bilaterally adrenalectomized to prevent the release of catecholamines by histamine or McN-A-343. Both nictitating membranes were attached to separate force-displacement transducers

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TABLE I. Responses of the Nictitating Membranes Following i.v. Administration of Histamine (100 μg) and McN-A-343 (100 μg).

Drug	Animal no.	Force of contraction (g)		Response expressed as % of control
		Control	Decentralized	
Histamine	1	1.6	4.0	250
	2	2.0	5.2	260
	3	1.0	2.7	270
	4	1.2	3.5	290
	5	1.5	4.8	320
	6	1.4	9.8	700
McN-A-343	7	0.4	6.1	1520
	8	0.8	3.0	375
	9	0.9	3.3	365

(Grass, FT-03) by means of silk thread and equal tensions of 5–7 g were applied to each membrane. Movements of the nictitating membranes were recorded on a calibrated ink-writing oscillograph (Grass, model 5 polygraph). From the tracings that were recorded, the tensions in grams were calculated.

Drugs were dissolved in saline and were administered in a volume of 0.5 ml via the jugular vein. At least 45 min elapsed between removal of the adrenal glands and administration of the drugs.

According to the convention set by Cannon and Rosenblueth (2), the superior cervical ganglion deprived of its innervation will be referred to as the denervated ganglion. The nictitating membrane associated with the denervated ganglion will be referred to as the decentralized nictitating membrane.

Statistical methods. Comparison between group means were analyzed using Student's *t* test for paired data unless otherwise specified.

Results. A. Control animals. Experiments were performed to determine whether the left and right ganglia of each animal respond equally to the stimulating agents. In the control animals both normal superior cervical ganglia were denervated acutely at the beginning of each experiment. The intravenous administration of submaximal ganglion stimulating doses of either histamine (4 expts.) in 100 μg doses, or McN-A-343 (3 expts.) in 100 μg doses, was followed after a latency of several seconds by contractions of both nictitating membranes. The mean tensions exerted by the left and right membranes were 1.9 g ± 0.4 (SEM) and 1.9 g ± 0.3, respectively, following ganglionic stimulation by histamine and 0.6 g ± 0.2 and 0.5 g ± 0.1 following McN-A-343. No significant difference was observed between the responses of the left and right nictitating membranes ($p > 0.8$).

The acute removal of the superior cervical ganglion completely abolished the nictitating membrane responses to both drugs, demonstrating that histamine and McN-A-343 did not directly stimulate the nictitating membrane.

B. Chronically denervated animals. 1. Effect of histamine and McN-A-343. The tensions exerted by the nictitating membranes in response to ganglionic stimulation by 100 μg of either histamine (6 animals) or McN-A-343 (3 animals) are reported in Table I. In each instance the response of the chronically decentralized membrane was markedly greater than that of the control. The mean tensions exerted by the control and chronically decentralized membranes following ganglionic stimulation by histamine were 1.5 g ± 0.1 (SEM) and 5.0 g ± 1.0, respectively. The difference between the responses of the two membranes was statistically significant ($p < 0.01$).

The mean tensions exerted by the control and chronically decentralized nictitating membranes following ganglionic stimulation by McN-A-343 were 0.7 g ± 0.15 and 4.1 g ± 1.0, respectively (Table I). Although only

TABLE II. Responses of the Nictitating Membranes Following i.v. Administration of Norepinephrine (10–25 μg).

Animal no.[a]	Force of contraction (g)		Response expressed as % of control
	Control	Decentralized	
1	1.1	4.4	400
2	1.7	6.8	400
3	1.9	4.8	250
4	1.3	4.2	320
5	2.1	4.6	220
6	0.8	9.3	1170
7	0.4	5.6	1400
8	1.0	3.2	320
9	0.8	3.3	410

[a] These animals are the same as those reported in Table I.

3 experiments were conducted the difference between the means was statistically significant ($p = 0.05$). (Due to the small number of experiments, Student's t test for paired data would result in only 2 degrees of freedom and, therefore, could not be employed. Statistical evaluation of the results was made using Student's t test for 2 sample means (unpaired data), which results in 4 degrees of freedom.)

2. *The effect of norepinephrine.* Approximately 30 min after the effects of ganglionic stimulation by histamine or McN-A-343 subsided, norepinephrine (10–25 μg) was administered intravenously. The dose of catecholamine employed was specifically chosen to contract the nictitating membrane to a similar extent as that produced by prior ganglionic stimulation by histamine or McN-A-343. As reported in Table II, in each instance the response of the chronically decentralized membrane to norepinephrine was considerably greater than the response of the contralateral control ($p < 0.005$). The mean responses of the control and chronically decentralized nictitating membranes were 1.2 g ± 0.2 and 5.1 g ± 0.6, respectively.

A comparison was made of the responses of the decentralized nictitating membrane to ganglionic stimulation (by histamine and McN-A-343) and to direct stimulation by norepinephrine. The responses expressed as percentage of control were taken from Tables I and II. The results of the comparison indicate that there is no statistical difference between the increased force of contraction of the chronically decentralized nictitating membrane following direct stimulation of the membrane by norepinephrine or following ganglionic stimulation by either histamine or McN-A-343 ($p = 0.3$).

Discussion. In the present experiments, tensions of the nictitating membranes were used as a valid measure of ganglionic activity since it was clearly shown that the nictitating membrane responses following administration of the ganglion stimulating agents were solely of ganglionic origin. Furthermore, the initial studies left no doubt that the left and right superior cervical ganglia of normal control animals responded equally to the ganglion stimulating actions of McN-A-343 or histamine. Since no difference was evident, the responses of one ganglion served as a control for the responses of the contralateral chronically denervated ganglion.

The nictitating membranes innervated by the chronically denervated superior cervical ganglia exerted considerably more tension than did the control membranes following ganglionic stimulation by McN-A-343 and histamine. The increased membrane responses which in one case was fifteenfold and in another sevenfold, could conceivably have been caused by two mechanisms; an increased sensitivity of the chronically denervated ganglia to the ganglion stimulating agents, or an increased sensitivity of the nictitating membranes to the postganglionic neurotransmitter, norepinephrine, released upon ganglionic stimulation. The following analysis of the results suggests that the latter possibility is correct:

The supersensitivity of the chronically decentralized membrane to exogenous norepinephrine reported here confirms the findings of those previous investigators who have reported that chronic denervation of the superior cervical ganglion produces an increased sensitivity of the nictitating membrane to norepinephrine and other adrenergic amines. (11, 12). The release of equivalent quantities of

the neuromediator, norepinephrine, from the postganglionic nerve terminals of both superior cervical ganglia would be expected, therefore, to cause a greater response of the chronically decentralized nictitating membrane compared with the control.

Presumably intravenous administration of exogenous norepinephrine presents equal amounts of the drug at both control and decentralized nictitating membranes. The enhanced response of the chronically decentralized membrane to exogenous norepinephrine, therefore, should be of a comparable magnitude to the enhanced responses of the decentralized membrane following the release of equal amounts of norepinephrine from the postganglionic terminals of the left and right ganglia. If the chronically denervated superior cervical ganglion were also supersensitive to histamine and McN-A-343, ganglionic stimulation by these agents would result in considerably more norepinephrine released from the nerve terminals of the chronically denervated ganglion than from the control ganglion. The magnitude of the increase in the response of the chronically decentralized nictitating membrane would be far greater following ganglionic stimulation than following intravenous administration of norepinephrine (which would present equal amounts of the catecholamines to both membranes).

The results of the present investigation clearly show that the increased force of contraction exerted by the chronically decentralized nictitating membrane following ganglionic stimulation was of a similar magnitude to the increased response following direct stimulation of the membranes by norepinephrine. Thus, the increased tension following ganglionic stimulation by McN-A-343 and histamine can be entirely accounted for on the basis of an increased sensitivity of the chronically decentralized nictitating membrane to the postganglionic neurotransmitter released upon ganglionic stimulation.

The reports to date of ganglionic denervation supersensitivity are based on an increased response of the chronically decentralized nictitating membrane following the administration of acetylcholine (1–4). Unlike McN-A-343 and histamine, ganglion stimulating doses of acetylcholine directly contract the nictitating membrane. Furthermore, the chronically decentralized membrane is also supersensitive to the direct actions of acetylcholine (7). The responses of the nicitating membranes following ganglionic stimulation by acetylcholine are, therefore, often difficult to interpret. Thus, it is important to note that when electrical activity was used as the criterion for ganglionic stimulation, eliminating any artifacts imposed by the nictitating membrane, Volle and Koelle (6) showed that the chronically denervated superior cervical ganglion did not become supersensitive to acetylcholine.

The sites of action of McN-A-343 and histamine differ both from acetylcholine and from each other. Thus, while ganglionic stimulation by acetylcholine is abolished by "nicotinic" blocking drugs such as hexamethonium, the effects of McN-A-343 are specifically antagonized by atropine (8) while the ganglionic effects of histamine are inhibited by the antihistamines Mepyramine (7), chlorpheniramine (Brezenoff, unpublished results), and pyrilamine (12).

Bokri *et al.* (4) suggested that the ganglionic atropine-sensitive receptors for acetylcholine are modified by chronic denervation, resulting in an increased sensitivity of the ganglion to acetylcholine. Since McN-A-343 specifically activates these atropine-sensitive receptors, changes in receptor response should be reflected in changes in the activity of McN-A-343. The absence of any observed modification in the ganglionic activity of McN-A-343 by chronic denervation makes it unlikely that the atropine-sensitive receptors are involved in the reported increase in the sensitivity of the chronically denervated ganglion to acetylcholine.

It appears, therefore, that the sensitivity of the superior cervical ganglion to three drugs (McN-A-343, histamine and acetylcholine), acting at three different receptors, is unchanged by chronic denervation of the ganglion cells. In view of these findings it is suggested that the "Law of Denervation Supersensitivity" proposed by Cannon and

Rosenblueth (*2*), be modified to exclude the superior cervical ganglion.

Summary. The ganglion stimulating properties of histamine and McN-A-343 were used to investigate whether the superior cervical ganglion becomes supersensitive to drugs following chronic denervation. Nictitating membrane contractions were used to measure ganglionic stimulation. The results strongly indicate that the chronically denervated superior cervical ganglion does not become supersensitive to either histamine or McN-A-343. The increased response of the chronically decentralized nictitating membrane following ganglionic stimulation by the two agents can be completely accounted for by an increased sensitivity of the membrane to relased postganglionic norepinephrine. It is suggested that the classical law of denervation supersensitivity of Cannon and Rosenblueth does not pertain to the superior cervical ganglion.

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