Supersensitivity of Decentralized and Denervated Nictitating Membranes to Barium[1] (35225)

J. M. MORRISON[2] AND W. W. FLEMING

Department of Pharmacology, West Virginia University Medical Center, Morgantown, West Virginia 26506

Sympathetic postganglionic denervation of smooth and cardiac muscle leads to two independent types of supersensitivity which Trendelenburg has called presynaptic and postsynaptic (1). Decentralization (preganglionic denervation), however, results only in the postsynaptic type (1). Postsynaptic supersensitivity is characterized by being very nonspecific, the sensitivity being increased to several unrelated agonists (1, 2). It has been suggested that postsynaptic supersensitivity is the result of changes in the responding muscle beyond the level of drug receptors (3–6). An essential aspect of this hypothesis is the consistent increase in sensitivity to ions such as potassium (3, 4) and calcium (5, 6) which has been observed in postsynaptic supersensitivity.

Although the nictitating membrane of the cat is the classical organ for studies of supersensitivity, an increased sensitivity to ions has not been clearly established in that structure. Preliminary experiments indicated that denervation lowered the threshold of the nictitating membrane to potassium (7). However, subsequent experiments have indicated that intra-arterial potassium may stimulate the nictitating membrane indirectly via the release of norepinephrine. Supersensitivity of the denervated nicitating membrane to barium has been reported by Schmidt and Fleming (8). However, those experiments were done before the complicating factor of tone was discovered by Langer (9). Furthermore, the possibility that barium was acting by the release of norepinephrine was not eliminated. Therefore, the experiments reported here were undertaken to determine the sensitivity to barium in the absence of tone, not only in denervated, but also in decentralized nictitating membranes. In addition, experiments were done with phentolamine to test the possibility that barium might be releasing norepinephrine.

Materials and Methods. Cats of 2.0 to 3.7 kg of body weight and of either sex were used. The animals were divided into three groups: (i) controls which underwent no surgery prior to the experiment; (ii) cats in which the right nictitating membrane had been decentralized 14–21 days before the experiment; and (iii) cats in which the right nictitating membrane had been denervated 14–21 days. Denervation was accomplished by surgical removal of the right superior cervical ganglion and decentralization by the excision of about 2 cm of the right cervical sympathetic nerve (preganglionic fibers). Anesthesia for these procedures was produced with pentobarbital sodium given ip in a dose of 35 mg/kg.

Spinal preparations were prepared by the method of Burn (10) under ether anesthesia. Following this operation the animals were maintained on artificial respiration and the ether was discontinued. The responses of the right nictitating membrane were recorded isometrically by means of a Grass strain gauge and polygraph. Resting tension was maintained at 7.5 ± 0.5 g and responses were measured as increases in tension above this level. The carotid arterial pressure was also recorded. Injections of barium and *l*-norepinephrine bitartrate were given intra-arterially through the cannulated central end of the

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[2] Present address: Food and Drug Administration, Bureau of Drugs, Office of Scientific Coordination, BD-224, 200 C. Street, S.W., Washington, D.C. 20204.

lingual artery into the external carotid artery, such that high concentrations could be delivered directly to the nictitating membrane without marked systemic effects. No injection was given until the nictitating membrane and blood pressure had recovered from the effects of the preceding injection. The volume of injection (0.1 ml) was constant for all injections. Injections of phentolamine methanesulfonate were given intravenously via a femoral cannula. The doses of barium chloride are reported as milligrams of barium per kilogram of body weight. Doses of norepinephrine and phentolamine are quoted in terms of the free base.

Tone (9) is the term given to the existence of a partial contraction in denervated or decentralized nictitating membranes due to supersensitivity to small amounts of circulating catecholamines. Since these catecholamines are the result of sympathoadrenal activity emanating from the spinal cord, it was eliminated in all decentralized and denervated cats either by the administration of a ganglionic blocking agent (chlorisondamine, 3.0 mg/kg, iv) or by pithing the spinal cord (9, 11, 12).

Since equieffective doses of a drug on the nictitating membrane are log-normally distributed, geometric means of doses producing a 7 g increase in tension (ED_{7g}) were calculated (13, 14). The dose producing a 7-g response was calculated from each dose–response curve. This dose was then converted to its log and the mean log for each group was determined. The 95% confidence interval was determined for each mean log and the mean log values for each experimental group were compared to the comparable values of the control group by Student's t test. The antilogs of the mean log ED_{7g} and its confidence interval yielded the geometric mean ED_{7g} and its confidence intervals. The antagonistic effects of phentolamine were measured by determining the ED_{7g} for norepinephrine or barium on a nictitating membrane before and after phentolamine. A paired sample t test was therefore used to test the significance of the phentolamine effects.

Results. Figure 1 presents the mean barium dose–response curves for the three groups of cats. The curves in denervated and decentralized nictitating membranes are virtually superimposed and are well to the left of the control curve. There is, therefore, equal supersensitivity to barium in both experimental groups. The magnitude of the increase in sensitivity is shown in Table I. The sensitivity ratios indicate increases in sensitivity to

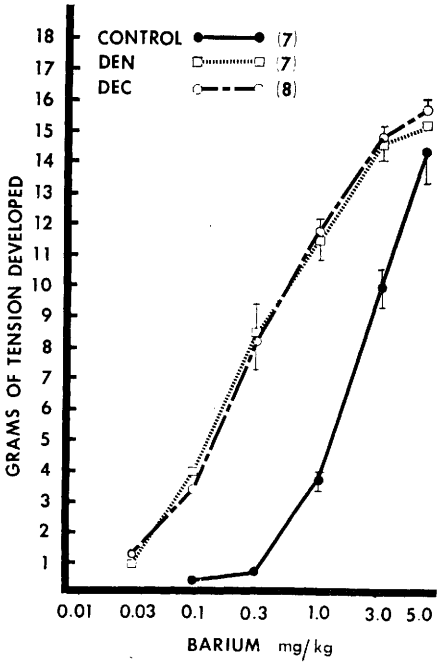

FIG. 1. Dose–response curves of barium in nictitating membranes of cats: DEN = nictitating membranes denervated 14–21 days before the experiment; DEC = nictitating membranes decentralized 14–21 days before the experiment. Numbers in parentheses equal numbers of cats per group. Vertical lines are SEM.

TABLE I. ED_{7g} Values for Barium in Control, Decentralized, and Denervated Nictitating Membranes.

Group	N	ED_{7g}[a] (mg/kg)	Sensitivity ratio[b]
Control	7	1.86 (1.40–2.48)	
Decentralized	8	0.26 (0.14–0.49)[c]	7.1
Denervated	7	0.25 (0.17–0.38)[c]	7.4

[a] Geometric means with 95% confidence interval.

[b] Ratio (geometric mean ED_{7g} control)/(geometric mean ED_{7g} experimental).

[c] Significantly different from control, $p < 0.001$.

TABLE II. Antagonism of Phentolamine (Ph) Versus Norepinephrine and Barium in Denervated Nictitating Membranes.

Agonist	N	ED_{7g}[a] Before Ph	ED_{7g}[a] After Ph[b]	Ratio[c]	p
Norepinephrine[d]	5	9.0 (4.7–17)	760 (194–2960)	84	<0.001
Barium[e]	5	0.39 (0.21–0.71)	0.48 (0.22–1.10)	1.2	0.1

[a] Geometric means with 95% confidence interval.
[b] Dose of phentolamine = 0.6 mg/kg, iv.
[c] Ratio: ED_{7g} after Ph/ED_{7g} before Ph.
[d] Doses of norepinephrine (μg/kg, ia).
[e] Doses of barium (mg/kg, ia).

barium of approximately 7-fold in both decentralized and denervated nictitating membranes.

The dose–response cuve for an agent which acts primarily indirectly by the release of norepinephrine is generally shifted to the left by decentralization but to the right by denervation (2). The fact that both procedures shift the barium curve to the left to an equal degree strongly suggests that barium is acting directly on the nictitating membrane. Nevertheless, to be absolutely certain that the response to barium is not mediated in part by the release of a small quantity of norepinephrine from a few neurons remaining after denervation, the experiments summarized in Table II were undertaken. As shown, a dose of phentolamine which caused an 80-fold shift of the norepinephrine dose–response curve to the right had no significant effect on the ED_{7g} of barium. It seems quite certain, therefore, that release of endogenous norepinephrine plays no significant part in the response of the nictitating membrane to barium.

Discussion. Comparison of the present results in denervated nictitating membrane with those of Schmidt and Fleming (8) indicate that the apparent increase in sensitivity to barium in the presence of tone is considerably less than the true shift (2-fold vs 7-fold). The maximum response is also underestimated in the presence of tone (9.5 vs 16 g). This is consistent with the findings of Langer *et al.* (12) for other agents.

These results illustrate clearly that the postsynaptic supersensitivity of the nictitating membrane extends to an ion whose effect is not mediated by the receptor for the natural transmitter substance. It has been suggested that postsynaptic supersensitivity is the consequence of a change in the physiological events of the excitation and contraction process at a point beyond the drug-receptor combination (3–6). Although present evidence favors a change in membrane function (4, 6) a change in the coupling mechanism between membrane activity and contraction is also a possibility. Supersensitivity to barium is consistent with either alternative since barium may have actions both on the cell membrane and on the coupling mechanism (15). The action of barium on membranes is particularly interesting in relation to the existing hypothesis concerning a membrane site for postsynaptic supersensitivity (4). Barium has been shown to decrease the resting membrane potential of smooth muscle cells (16), to decrease potassium conductance (17–19), and to displace calcium from membrane binding sites (15).

Summary. Denervation and decentralization of the nictitating membrane produced a 7-fold increase in sensitivity to barium. The action of barium was not mediated via the release of endogenous norepinephrine. This supersensitivity to barium is consistent with the hypothesis that the postsynaptic form of supersensitivity in smooth muscle is the result of a functional change beyond the level of drug receptors, perhaps in membrane properties.

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