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The Rockefeller University,
New York, N.Y., U.S.A.

PHILIP SEEMAN*

* Present address: Department of Pharmacology, Cambridge University, Cambridge, England.

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Sensitivity to norepinephrine of isolated atria from scorbutic guinea pigs

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IT IS well documented that tissues with postganglionic sympathetic innervation, including the heart, can take up norepinephrine from the circulating blood or from the surrounding fluid.[1] This uptake is probably an important mechanism of rapid inactivation and very likely determines to a large extent the intensity and duration of the effects of norepinephrine. Cocaine efficiently blocks the uptake of exogenous norepinephrine by tissues.[2, 3] Inactivation of norepinephrine is therefore delayed and tissues become more sensitive to it, since norepinephrine may reach higher concentrations at the receptors of the effector organs. Trendelenburg[4] has further suggested that "prevention by cocaine of the uptake of an amine causes a supersensitivity whose magnitude is proportional to the rate of uptake of that amine."

Similarly, chronic denervation of sympathetically innervated organs also causes supersensitivity to norepinephrine. When the nerves degenerate the organ is unable to take up catecholamines. On the basis of these observations some workers have proposed that any procedure, chemical or surgical, which interferes with the uptake of norepinephrine will cause supersensitivity.

It must be realized that uptake is a complex phenomenon, which consists of two steps: (1) transport into the nerve terminals and (2) subsequent binding in the norepinephrine stores.[5] Pretreatment with reserpine leaves the first step intact and prevents the second, while cocaine seems to prevent the transport into the nerve terminal. The first step appears to be important as a factor limiting the concentration of norepinephrine at the receptors of the effector organ; procedures that interfere with it cause supersensitivity to norepinephrine.

It has been demonstrated that the induction of scurvy in guinea pigs is associated with hyperresponsiveness to the pressor and cardiac inotropic effects of injected catecholamines.[6] To the contrary, the present study shows that isolated atria from scorbutic guinea pigs are normal in their response to norepinephrine.

METHODS

Male albino guinea pigs, weighing 300–350 g, were made ascorbic acid-deficient by maintaining them on scorbutogenic diet (rabbit pellets); the controls received ascorbic acid supplement in water in addition to this diet. At the end of 10–14 days, ascorbic acid levels in the tissue were below the detectable limit. The atria were dissected from the hearts of the freshly killed animals (300–450 g body weight) and suspended in a modified Tyrode's solution maintained at 37°C and containing atropine sulfate (0·05 μg/ml). A mixture of 95% oxygen and 5% carbon dioxide was bubbled through the bathing fluid via a sintered glass plate at the bottom of the bath. The Tyrode's solution had the following composition: NaCl, 0·9%; KCl, 0·042%; $CaCl_2$, 0·024%; $NaHCO_3$, 0·05%; glucose, 0·10%. The bicarbonate concentration employed maintained the pH at approximately 7·4. Atria were attached to a Grass force displacement transducer, and isometric contractile force (resting tension of approximately 0·5 g) and rate of spontaneous beat were recorded by a Grass polygraph. The atria were allowed to equilibrate at least 1 hr after being placed in the bath and were washed repeatedly at 15-min intervals. Cumulative dose–response curves of norepinephrine were obtained before and 15 min after cocaine (10 μg/ml). To retard oxidation of norepinephrine, EDTA was added to the bath at a concentration of 15 μg/ml and removed along with it. Five isolated atria from scorbutic guinea pigs were exposed to 0·436 μg of *dl*-7 ^{3}H-norepinephrine HCl per ml (10 μc/ml) at 37° in a bath of 10-ml capacity for 1 or 15 min. After repeated washings with Tyrode's solution, the atria were removed from the bath, blotted with filter paper, weighed, and each assayed for tritiated norepinephrine.[7] Similar experiments were done with atria from control animals.

RESULTS AND DISCUSSION

As seen in Fig. 1, the dose–response curves for norepinephrine on normal atria and on atria from scorbutic animals were superimposed. Cocaine (10 μg/ml) shifted equally both curves of

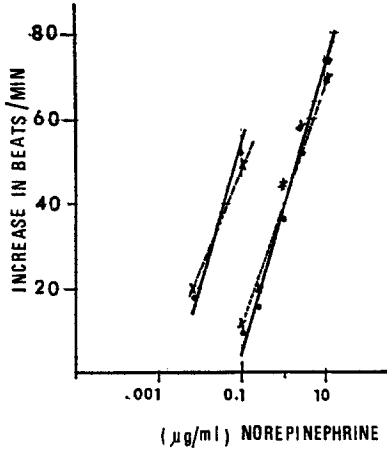

FIG. 1. Effects of cocaine on dose–response curves of norepinephrine on isolated atria from scorbutic guinea pigs. At right,before cocaine; at left, after cocaine (10 μg/ml). Solid lines, controls; broken lines, scorbutic preparations (mean responses, 6 preparations each).

norepinephrine to the left. In some atria cocaine itself had a small to moderate stimulatory effect (these preparations were rejected). The ability to retain ^{3}H-norepinephrine in the atria from scorbutic animals was normal at 1 min but was reduced to 52 ± 8 per cent (mean ± S.D. of five experiments) of the control at 15 min. The reduced retention of ^{3}H-norepinephrine in the atria of scorbutic animals at 15 min is consistent with the findings of Thoa *et al.*[8] The results with the uptake of ^{3}H-norepinephrine indicate that dietary-induced scurvy like reserpine, affects the storage mechanism instead of inhibiting transport of norepinephrine into adrenergic neurons. Thus, norepinephrine is transported into scorbutic tissue at a normal rate but is unable to store it, so that what was taken up at 1 min is then rapidly lost, probably owing to destruction by monoamine oxidase. This explains why scorbutic

atria behave like normal tissue in their sensitivity to norepinephrine and also why cocaine is still able to cause a full sensitizing effect to norepinephrine in these atria. Supersensitivity of the vascular system to injected norepinephrine observed in scorbutic guinea pigs *in vivo*[6] may not be relevant in experiments *in vitro*.

Department of Pharmacology,
College of Medicine, Howard University,
Washington, D.C., U.S.A.

B. BHAGAT
WILLIAM L. WEST
INA M. ROBINSON

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