Manganese on Calcium Flux and Norepinephrine-Induced Tension in Arterial Smooth Muscle (36303)

JAMES J. KEENE, CHARLES L. SEIDEL, AND DAVID F. BOHR
Department of Physiology, University of Michigan, Ann Arbor, Michigan 48104

Manganese is known to reduce the spontaneous tension developed by atrial muscle (1) and to reduce the effect of catecholamines in both intestinal (2) and vascular (3) smooth muscles. In vascular smooth muscle the catecholamine, norepinephrine, increases the content (4) and reduces the efflux (4, 5) of ^{45}Ca, suggesting that norepinephrine-induced tension results from an imbalance between calcium influx and efflux, with the net effect being an increase in the amount of intracellular calcium. Manganese decreases membrane permeability to calcium in a variety of tissues (6–8) and this action may be responsible for its attenuating effect on norepinephrine-induced tension. To investigate the possibility that a change in membrane permeability to calcium might be responsible for this attenuation in vascular smooth muscle, we determined the effect of manganese on calcium content of the tissue.

Methods. Albino rabbits of either sex, weighing between 1 and 2 kg, were killed by a blow to the back of the head. The thoracic aorta was removed and placed in a physiological salt solution (PSS) comprised of (m*M*): NaCl, 137; $KHCO_3$, 5.9; $CaCl_2$, 1.6; $MgCl_2 \cdot 7H_2O$, 1.2; glucose, 11; sorbitol, 1.0; and $CaNa_2$ Versenate, 0.026.

Vessels to be used for Ca content measurements were slit open lengthwise and cut into transverse strips. Each strip was blotted for 5 sec between two layers of filter paper under 100 g weight, then weighed to the nearest 0.01 mg and returned to PSS. A strip was suspended at a resting tension of 600 mg, in PSS at 37° for 2 hr. There was constant bubbling of the bath with 99% O_2–1% CO_2, holding the pH at 7.4. At the end of this time the strip was placed for 15 min in PSS containing ^{45}Ca (1.6 μCi/ml) and one of the following: (i) norepinephrine (NE, 10^{-6} g/ml; (ii) manganese ($MnCl_2 \cdot 6H_2O$, 2.4 m*M*); (iii) NE and Mn; (iv) neither NE or Mn. At the end of 15 min the strip was transferred to a tube of nonlabeled salt solution with neither Mn or NE for an additional 5 min, then removed, blotted, weighed and digested in a 50:50 trichloracetic–glacial acetic acid solution. The activity of the muscle digest and of the incubation medium were measured and the ^{45}Ca content was calculated using the following equation:

^{45}Ca content (mmoles/kg of wet wt) =
(cpm in muscle)/(kg of wet wt) ×
(mmoles of Ca in labeled medium)/
(cpm in labeled medium).

The ^{45}Ca content as determined above is an estimate of total Ca content.

Vessels to be used for tension measurements were cut into helical strips. A strip was mounted in a bath containing oxygenated PSS at 37° and isometric contractions were recorded by means of a Grass tension transducer. A passive tension of 600 mg was applied to the strip and it was allowed to equilibrate for 2 hr. At the end of this time NE was injected into the bath to give a final bath concentration of 10^{-6} g/ml. Tension development was followed for 5 min, after which the muscle chamber was rinsed with PSS 3 times, at 3-min intervals, returning the muscle to resting tension level. After several cycles of NE stimulation and wash out, a fairly stable control response was obtained.

[1] Supported by Grant HE-03756 and Training Grant 2 T01 GM 353-11 from the National Institutes of Health and Michigan Memorial Phoenix Project 398.

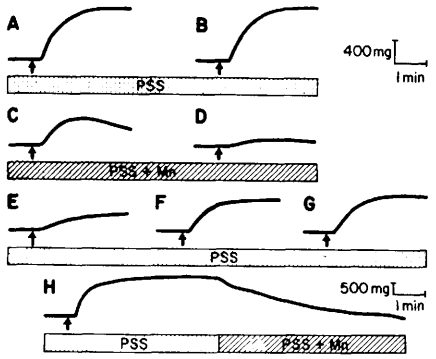

FIG. 1. Mn-attenuation of NE-stimulated tension development: Arrows indicate addition of NE to the bath. (A through G) sections of continuous record showing consecutive responses at 15-min intervals. (A and B) control responses; (C and D) gradual attenuation of response to NE during continued exposure of the muscle to 1.6 m*M* Mn; (E, F and G) gradual recovery of the response after removal of Mn from the bath; (H) tension developed in response to NE is abolished by Mn.

In further cycles, Mn was added during the response to norepinephrine or continuously throughout several cycles of stimulation and rinse.

^{45}Ca was obtained from New England Nuclear Corporation as $CaCl_2$ in 0.5 *M* HCl. Norepinephrine was supplied by the Winthrop Laboratories. The Student's *t* test was used to determine the statistical significance of all results; $p \leq 0.05$ was considered to indicate significance.

Results and Discussion. In four aortic strips the addition of manganese to the bath attenuated, and with time abolished, tension development induced by norepinephrine (10^{-6} g/ml). Figure 1A and B show the last two of six control responses at 15-min intervals; a relatively constant amount of tension was developed by the muscle after each of two successive stimulations with norepinephrine. Figure 1C and D show the next two responses to NE stimulation, beginning 2 min after the strip was exposed to PSS to which 1.6 m*M* Mn had been added. Less tension was developed with each successive NE stimulation. Figure 1 E, F and G, show gradual recovery after the strip was returned to control PSS. Recovery is complete after brief exposure to Mn. The addition of Mn during a period of norepinephrine-induced tension caused relaxation in spite of the continued presense of norepinephrine (Fig. 1 H).

Figure 2 shows the effect of NE and Mn on radioactive Ca content. A significant ($p < .005, n = 7$) increase (52%) in muscle ^{45}Ca content was observed in the presence of 10^{-6} g/ml of NE. When the bath solution contained both NE (10^{-6} g/ml) and Mn (2.4 m*M*), an average increase of 6% in ^{45}Ca content was observed, but this change was not statistically significant ($n = 7$).

The lack of an increase of ^{45}Ca content with NE stimulation in the presence of Mn could be due to a direct Mn effect on NE, on its muscle receptor, or to an effect on Ca flux. In order to develop evidence bearing on these alternatives, we studied the effect of Mn on ^{45}Ca content in the absence of NE (Fig. 2). In the presence of Mn (2.4 m*M*) the aortic strip contained 21% less ^{45}Ca than did the control strip; this difference was significant ($p < .005, n = 7$).

Both tension and ^{45}Ca content of rabbit aorta strips were increased by NE stimulation. The similar effect of NE on these two variables is consistent with the hypothesis that NE may act by increasing the intracellular Ca content. Mn alone decreased ^{45}Ca content and reduced the NE-stimulated increase in tension and in ^{45}Ca content. These obser-

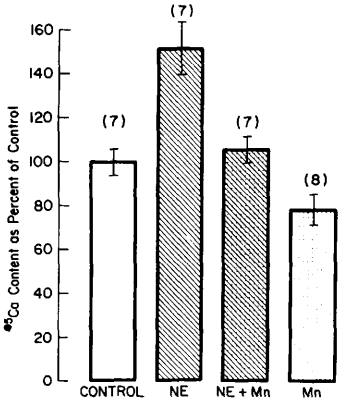

FIG. 2. ^{45}Ca content of vessel strips exposed to NE, NE plus Mn, and Mn, compared with controls: ^{45}Ca content is increased by NE in the absence of Mn, but not in its presence; ^{45}Ca content is reduced by Mn alone. Contents shown are the mean ± SEM for the number of strips shown in parentheses.

vations support the possibility that the reduction of the NE-induced tension by Mn is due to the effect of Mn on membrane permeability to Ca.

Summary. The tension developed by aortic smooth muscle in response to norepinephrine is reduced by Mn. The mechanical responses may be related to the current observations that norepinephrine increases radioactive Ca content and Mn attenuates this increase.

1. Yanga, T., and Holland, W. C., Amer. J. Physiol. **217**, 1280 (1969).

2. Bulbring, E., and Tomita, T., Proc. Roy. Soc., Ser. B **172**, 121 (1969).

3. Sullivan, L. J., and Briggs, A. H., J. Pharmacol. Exp. Ther. **161**, 205 (1968).

4. Seidel, C. L., and Bohr, D. F., Circ. Res. **28 & 29**, Suppl. **2**, II-88 (1971).

5. Hudgins, P. M., and Weiss, G. B., J. Pharmacol. Exp. Ther. **159**, 91 (1967).

6. Hagiwara, S., and Nakajima, S., J. Gen. Physiol. **49**, 793 (1966).

7. Nonomura, Y., Hotta, Y., and Ohashi, H., Science **152**, 97 (1966).

8. Sabatini-Smith, S., and Holland, W. C., Amer. J. Physiol. **216**, 244 (1969).

Received Sept. 27, 1971. P.S.E.B.M., 1972, Vol. 139.