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Effects of Tetraethylammonium and Manganese on Mesenteric Vasoconstrictor Escape[1] (40354)

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The *in vitro* contractile response of cat mesenteric arterial rings to norepinephrine (NE) is frequently a phasic contraction which reaches a peak in 1–2 min and then fades ("escapes") despite the continuing presence of NE. An earlier study from this laboratory (1) showed that the phasic contraction could be converted to a tonic (nonescaping) response by (a) reducing the external calcium concentration, (b) pretreating with verapamil or (c) depolarizing the vessel by increasing the external potassium–ion concentration.

These observations suggested that the phasic response of the cat mesenteric artery might be associated with calcium-dependent action potentials ("calcium spikes"). If this were so, tetraethylammonium which augments calcium spikes should enhance the phasic contraction whereas manganese, which inhibits calcium-spikes, should diminish it (2).

Methods. Male cats weighing 3–5 kg were anesthetized with intraperitoneal sodium pentobarbital 40 mg/kg. The superior mesenteric artery was dissected free of connective tissue *in situ* and then removed. Rings 2–5 mm long and about 1 mm in diameter were cut from the artery and placed in a physiological salt solution (PSS) containing (in m*M*): NaCl 123, KCL 5, $CaCl_2$ 1.6, $MgCl_2$ 1.2, $NaHCO_3$ 25, $CaNa_2EDTA$ 0.026, ascorbic acid 0.01 and glucose 11.1. This solution, referred to as regular PSS, was aerated with 95% O_2, 5% CO_2; its pH was 7.4. The arterial ring was mounted between a stationary stainless steel rod and a Statham UC-2 strain gauge connected to a Hewlett-Packard 7700 recorder. The mounted ring was immersed in a 20 ml bath containing PSS solution at 37° and was stretched during the equilibration period to maintain a force of approximately 500 dynes. Every 20 min, NE (Levophed, Winthrop Laboratories) was added to the bath and washed out after 5 min. Two more washes were performed before the next NE dose was applied. Two to four hours were required to achieve stable responses. The effects of tetraethylammonium (TEA) 0.06–10.0 m*M* were studied by adding TEA chloride (J. T. Baker Chemical Company) to the bath 5 min before each NE test dose. The effects of higher TEA concentrations were studied by substituting equimolar amounts of NaCl by TEA Cl.

Some experiments were performed after depolarizing the vessel rings by substituting the regular PSS in the bath with a depolarizing solution containing (m*M*) KCl 3, $KHCO_3$ 25, K_2SO_4 86, $CaCl_2$ 1.6, $MgCl_2$ 1.2, $CaNa_2$, EDTA 0.026, ascorbic acid 0.01 and glucose 1.1.

Statistical significance was determined by Student's *t* test for paired comparisons.

Results. Effects of TEA alone. Concentrations of TEA below 40 m*M* had no effect on resting tension in any artery. Higher concentrations induced weak tonic contractions in arteries from six of seven animals. The threshold was between 40 to 80 m*M* in four arteries and between 80 to 120 m*M* in two. The TEA contractions never exceeded 15% of the maximum NE response.

Effects of TEA on the NE response. Figure 1 shows the responses of a mesenteric arterial ring to increasing doses of NE and the effects of 2 m*M* TEA. Note that before TEA, NE 10^{-7} g/ml, a dose close to threshold, produced a tonic contraction of 200 mg. The same NE dose, after pretreatment with TEA, caused a series of phasic contractions with a peak force of 2.8 g after 2 min. Force then declined, despite the continuing presence of NE, to a steady-state force of 300 mg. The figure also shows that TEA enhances the initial component of phasic contractions but not the steady-state response. Additionally, it is seen that the maximum phasic response to NE (3×10^{-5} g/ml) was 4 g before TEA and was

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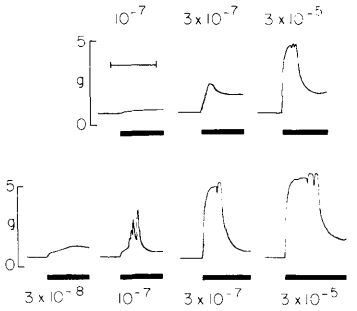

FIG. 1. Contractile responses of cat mesenteric arterial ring to NE before and after TEA. Numbers indicate NE concentrations (g/ml). Upper traces: before TEA, lower traces: after pretreatment with 2 m*M* TEA. Time bar equals 5 min. The black bar below each trace indicates that period during which NE remained in contact with the vessel.

augmented to 5 g after TEA.

Forty-eight percent of mesenteric rings did not give phasic contractions but showed sustained tonic responses to all doses of NE. TEA converted these into phasic contractions which attained higher peaks but then escaped to stable levels of force lower than those seen in the absence of TEA.

The differential effects of TEA on the phasic and steady-state components of the NE response were examined quantitatively in arterial rings from 12 cats. An approximately ED_{50} dose of NE was determined for each ring and the effects of pretreating the vessel for 5 min with varying doses of TEA, over the range 0.06–120 m*M*, were measured (Fig. 2). TEA caused a dose-dependent potentiation of the initial component of the NE response. In contrast, TEA inhibited the steady-state response. These effects were maximal at a TEA concentration of 20 m*M*.

Effects of TEA on NE response in calcium-free solution. After 20 min exposure to calcium-free PSS solution, the response to NE was greatly reduced and its phasic character was lost. The response to a maximal NE dose was 4.4 ± 0.4 g ($n = 4$) in regular PSS solution but only 0.1 ± 0.05 g ($n = 4$) in calcium-free PSS.

Pretreatment with TEA had no significant effect ($P > 0.1$) on the NE contractures of four arterial rings in calcium-free PSS. An example is shown in Fig. 3.

Effects of TEA on NE response in depolarizing PSS. When mesenteric arterial rings were transferred to depolarizing PSS a substantial contracture developed. The addition of NE produced a tonic increase in this contracture. Pretreatment of the vessel with 2 m*M* TEA had no significant effect ($P > 0.1$) on the response either to depolarizing PSS or the subsequent addition of NE ($n = 5$). An example is shown in Fig. 4.

Effects of Manganese on the NE response (four cats). Segments which gave phasic responses to NE were exposed to $MnCl_2$ for 10 min prior to and during the addition of NE. Manganese concentrations in the range 0.04–0.1 m*M* reduced the phasic component of the NE response whereas manganese concentrations in the range 0.1–0.3 m*M* abolished them. An example is shown in Fig. 5.

Discussion. A number of investigators (2–6)

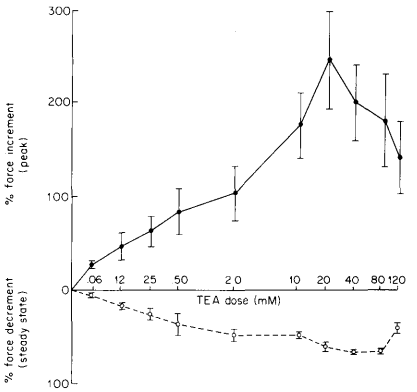

FIG. 2. Effect of pretreatment with various concentrations of TEA on peak (●——●) and steady-state (○- - -○) responses to approximately ED_{50} NE doses. TEA concentrations between 0.06 and 2.0 m*M* were tested in five cats. TEA concentrations between 10 and 20 m*M* were tested in another group of seven cats. The abscissa scale is logarithmic. The effects are shown as percent changes from control. Values are means ± SE.

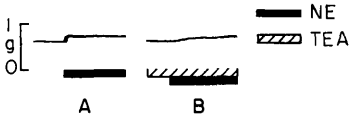

FIG. 3. Failure of TEA to alter NE response in calcium-free solution. A-before TEA, NE 3×10^{-7} g/ml produced a tonic contracture of only 100 mg, B-after 10 min pretreatment with 2 m*M* TEA the NE response was unchanged. Contrast this with the effect of TEA on the same dose of NE in regular PSS (Fig. 1). Time bar = 5 min.

have previously shown that TEA augments the responses of isolated arterial strips to a variety of agonists. Kalsner (5) suggested that the augmentation was due to enhanced calcium mobilization. Haeusler and Thorens (6) obtained direct evidence for this by showing that 10 m*M* TEA enhanced calcium influx in isolated rabbit pulmonary arteries. They also showed that 10–100 m*M* TEA induced a dose-dependent depolarization of pulmonary arterial smooth muscle.

All previous studies of TEA potentiation of arterial vasoconstrictor responses have used preparations which show only tonic responses to agonists. The present investigation is the first to examine the effects of TEA and manganese on a vessel which commonly shows a striking "fade" or "escape" of the mechanical response during continuing NE exposure. The principal findings were that (a) TEA potentiated the initial component of the NE response but not the steady-state response; (b) TEA potentiation did not occur in completely depolarized vessels or in vessels exposed to calcium-free solution; (3) manganese inhibited the initial component but not the steady-state component of the NE response. These observations suggest that the steady-state response is dependent upon a different excitation or excitation-contraction coupling mechanism than the initial portion of the response. In a previous paper (1) it was reported that NE-induced phasic contractions of cat mesenteric arteries were blocked by pretreatment with calcium-free solution, verapamil or potassium-rich solutions and it was suggested that the phasic response might be associated with calcium-spikes. The present observations support this view. TEA augments calcium-spikes by blocking the late

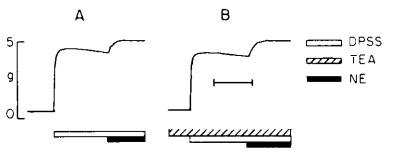

FIG. 4. Effects of TEA on the NE response of a mesenteric arterial ring treated with depolarizing solution (DPSS). Note that DPSS produces a large tonic contracture which is augmented by NE. The response before TEA (A) does not differ significantly from the response after 10 min pretreatment with 2 m*M* TEA (B). Time bar = 5 min.

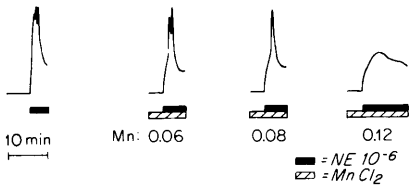

FIG. 5. Effects of $MnCl_2$ (numbers indicate m*M*) on the mesenteric arterial response to NE.

potential-dependent increase in potassium conductance which limits the degree of depolarization which can be induced by calcium influx (2). Thus, contractions dependent upon calcium spikes should be potentiated by TEA. This was clearly the case for the initial component of the mesenteric NE response (Figs. 1, 3). In contrast, the steady-state response was inhibited by TEA. The mechanism of this inhibitory effect is not revealed by these experiments, but the very absence of potentiation indicates that this part of the response is not based on calcium-spikes and may be dependent upon pharmacomechanical coupling. The fact that the mesenteric artery will respond to NE when completely depolarized and that TEA does not alter the response supports this view.

Manganese is known to block calcium-spikes in many tissues (2) and in the low concentrations used in our experiments, it blocked the phasic component of the NE response but not the steady-state response.

The effects of TEA and manganese, therefore, appear to support the hypothesis that mesenteric vasoconstrictor escape may be due to the inability of mesenteric arterial smooth muscle to sustain action potentials for more than a minute or two following NE administration.

Summary. Norepinephrine (NE) induced either phasic or tonic contractions in isolated rings of cat mesenteric arteries. Tetraethylammonium (TEA), 0.6–120 m*M* enhanced the peak contractile response to NE but reduced the steady-state response. Manganese, 0.06–0.12 m*M*, inhibited the peak NE response with no effect on steady-state force development. TEA-potentiation was maximal at 2–20 m*M*. No potentiation occurred in calcium-free solutions or when the vessel was depolarized by high external potassium concentrations. These observations provide

circumstantial evidence that mesenteric vasoconstriction may be associated with "calcium-spike" activity and that vasoconstrictor escape may be due to fading of this activity.

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