In Vitro Activation of Smooth Muscle from the Hog Carotid Artery (37528)

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Very few precise quantitative studies exist on the mechanical properties of vascular smooth muscle (VSM). The lack of data required for an understanding of the basic contractile system in VSM is largely due to the difficulty of obtaining suitable muscle preparations. An arterial smooth muscle preparation obtained from the media of hog carotid arteries was recently developed (1), which because of its large muscle fraction, uniform longitudinal cell orientation, and absence of adventitial connective tissue is well suited for *in vitro* mechanical and biochemical studies. The purpose of the present study was to characterize the response of this preparation to a variety of stimulating agents of physiological significance, and to differentiate direct actions from neurally mediated contractile responses.

Methods and Materials. Carotid arteries were obtained from hogs 20–60 min after slaughter and immediately immersed in ice-cold physiological salt solution (PSS) (composition given below). After removing loose adventitia, the arteries were stored in fresh PSS at 4° until used. Vascular strips were teased from hog carotid arteries as described previously (1). They were mounted vertically between two stainless steel clips. One clip was attached to a Grass FT.03 strain gauge by a stainless steel jewelry chain and the other was connected to a glass rod mounted on a micrometer. The strips were stretched 100% of their initial mounting length and allowed to equilibrate for two hours in PSS. Following equilibration, the strips were stimulated electrically at 15 min intervals until a constant isometric contractile response was obtained. Force was recorded by a Grass Model 7 Polygraph, and length could be determined by means of the micrometer. Bath temperature was regulated at 37°. The composition of the bathing solution was (m*M*): NaCl, 119; KCl, 4.7; KH_2PO_4, 1.18; $MgCl_2$, 1.17; $NaHCO_3$, 14.9; dextrose, 5.5; $CaNa_2$-ethylenediaminetetraacetic acid (EDTA), 0.026; $CaCl_2$, 1.6. The solution was bubbled continuously with 95% O_2 + 5% CO_2 giving a pH of 7.4.

Electric field stimulation was applied to the muscle by means of two (5 × 10 mm) platinum electrodes positioned parallel to the muscle strip. Alternating current was supplied to the electrodes by a power amplifier whose voltage and frequency were determined by a function generator. Stimulus durations of less than 1 sec were obtained using a Grass S4 stimulator which activated a relay in series with the electrodes. Norepinephrine (L-arterenol bitartrate, Sigma) was diluted as needed from a 0.1 *M* stock solution and epinephrine was obtained as the adrenaline chloride solution (Parke, Davis). Acetylcholine chloride (Sigma), bretylium tosylate (Wellcome), and guanethidine monosulphate (CIBA) were prepared fresh for each experiment. Phentolamine mesylate (CIBA) was prepared from the lyophilized powder. All drugs were administered as very concentrated solutions such that the total volume of the drug was less than 1% of the bath volume. Cumulative concentration–response curves were employed.

Results. Figure 1 depicts the response of the strips to changes in frequency, voltage, and duration of sinusoidal electric field stimulation. With constant voltage and stimulus

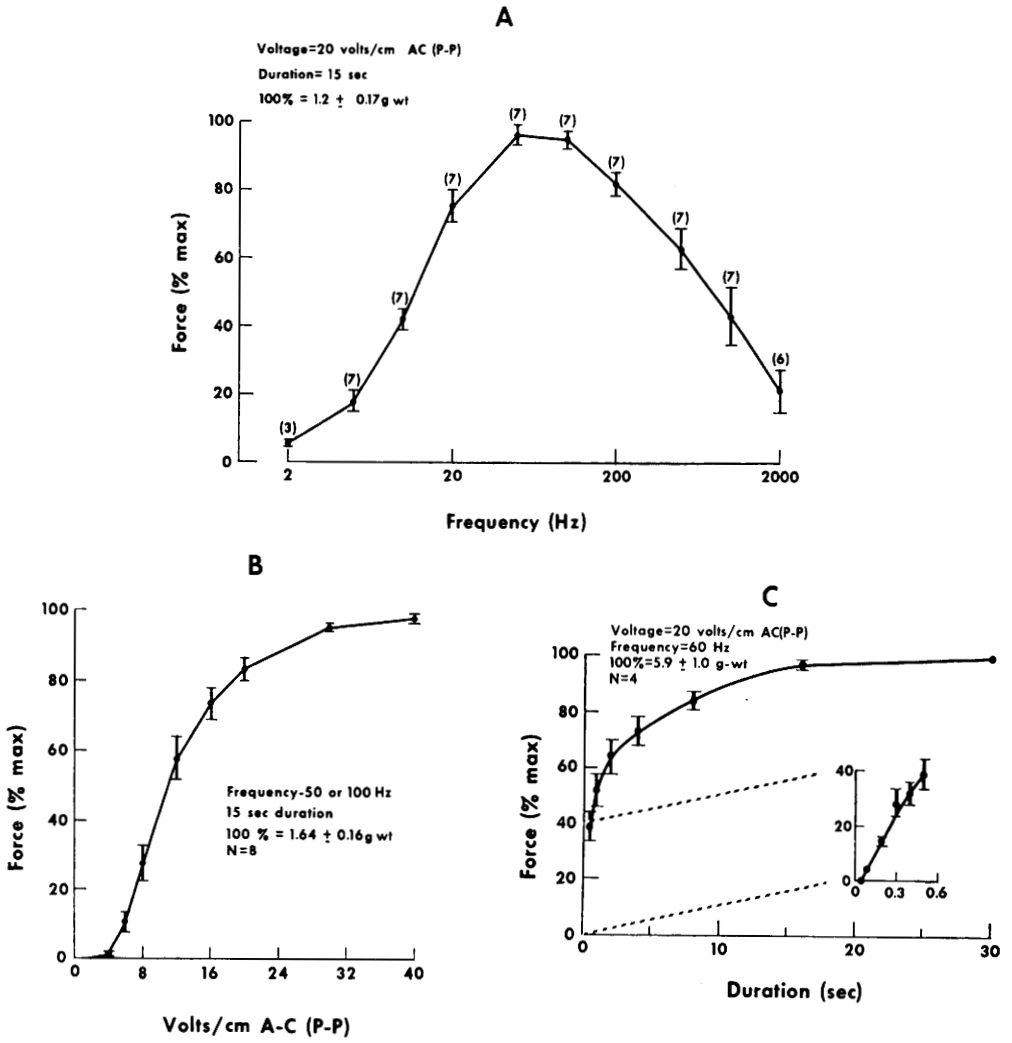

FIG. 1. Dependance of strip response on electrical parameters during field stimulation. Isometric force development (percent of maximum) is plotted on the ordinate in all three panels. Points and bars represent means ± 1 SEM. A. Frequency–response curve. The number of experiments in each group is indicated in parenthesis. B. Voltage–response curve. Voltage was applied across platinum electrodes placed 1 cm apart and expressed as peak to peak values. C. Duration–response curve. Inset shows early responses on an expanded time scale.

duration, the response of the strips varied with changes in frequency (Fig. 1A). Maximum responses were obtained at frequencies between 50 and 100 Hz. With frequency held constant at either 50 or 100 Hz and with durations of 15 sec, maximal responses were obtained at 30 V/cm, peak to peak (Fig. 1B). Some strips were more responsive to electrical stimulation and showed maximum force development at 20 V/cm (Fig. 1C). Maximal responses to changes in stimulus duration were obtained between 15 and 30 sec (Fig. 1C).

Figure 2 shows the response of the strips to electrical stimulation with increasing concentrations of bretylium or guanethidine. With both drugs at maximally effective concentrations, the response of the strips to electrical stimulation was reduced nearly 50%. Although not shown in Fig. 2, phentolamine,

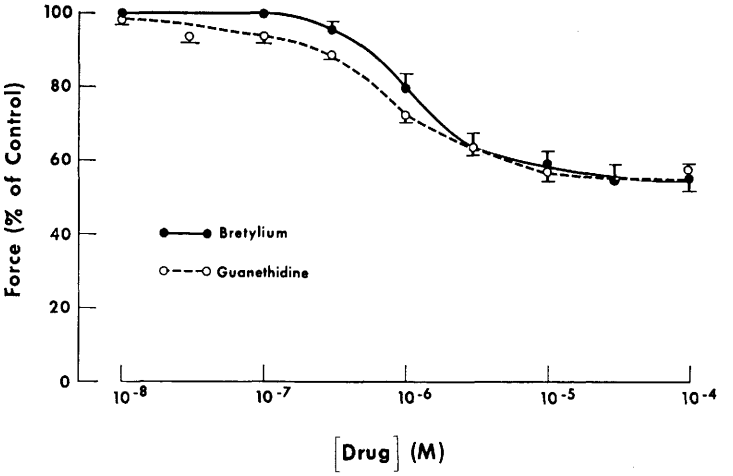

FIG. 2. Inhibitory effect of bretylium (filled circles, solid line) and guanethidine (open circles, broken line) on the isometric force development (percent of control) induced by maximal electrical field stimulation (60 Hz, 30 V/cm peak to peak for 30 sec). Control response was obtained prior to addition of the drug. Means ± 1 SEM are shown for four experiments on each drug. Measurements were made 15 min after drug additions in these cumulative dose–response curves.

(an alpha blocking agent) applied at a maximal dose of 10^{-4} *M*, blocked approximately 80% of the response to electrical field stimulation. Table I lists the norepinephrine content of the various areas of the vessel and shows that 83% of the norepinephrine content of the whole artery resides in the teased muscle strips. The sum of the catecholamine contents of adventitia plus muscle strip (Table I) is not significantly less than that obtained for the whole artery.

The dose–response curves for norepinephrine and acetylcholine are shown in Fig. 3. Maximal responses were obtained at 10^{-5} *M* and 3×10^{-5} *M*, respectively; while half-maximal activation occurred at 3×10^{-7} and 1×10^{-6} *M*. At the end of the acetylcholine experiments the strips were challenged with a maximal dose of norepinephrine. The response to this dose of norepinephrine was always greater than the largest acetylcholine induced contraction.

Atropine (1.4×10^{-6} *M*) completely blocked the response of the strips to 10^{-4} *M* acetylcholine. The strips were still able to respond to epinephrine (3×10^{-6} *M*). Guanethidine (10^{-4} *M*) or phentolamine (10^{-4} *M*) did not decrease the response to acetylcholine. However, phentolamine blocked the response to epinephrine.

Discussion. The response characteristics of the hog carotid strips to transverse field stimulation are in general agreement with those reported for other smooth muscles. Stimulus voltage and duration optima (Fig. 1, B and C) are consistent with those seen in mesenteric arteries (3), trachealis smooth muscle (4), and taenia coli (5). The fre-

TABLE I. Norepinephrine Content of the Hog Carotid Artery.[a]

Tissue	Weight (% of total)	μg NE/g tissue	NE (% of total)
Whole artery	100 (5)	1.58 ± .35 (4)	100
Muscle strip	54 ± 3 (5)	2.42 ± .69 (4)	83
Adventitia	46 ± 3 (5)	0.36 ± .08 (2)	10

[a] Norepinephrine measured by the method of Robinson and Watts (2). Values represent means ± 1 SEM. Numbers in parentheses are numbers of samples.

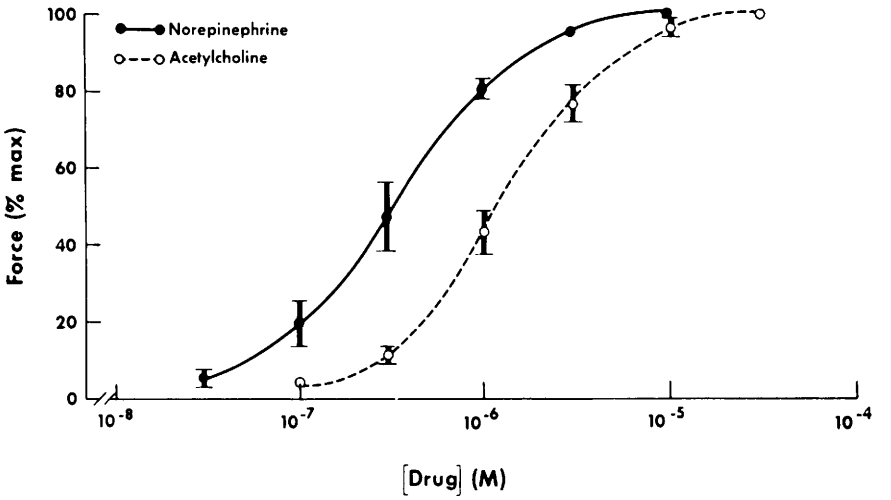

FIG. 3. Cumulative dose–response curves for isometric force development produced by norepinephrine (closed circles, solid line) and acetylcholine (open circles, dashed line). Circles and bars represent means ± 1 SEM for four experiments with each drug.

quency dependence of the response (Fig. 1A) is nearly identical with that seen in mesenteric and umbilical arteries (3). The response of VSM to sinusoidal field stimulation consists of two components: (i) a direct electrical effect on the muscle cells, and (ii) an indirect effect mediated by the release of neurotransmitter from excited nerve endings (6). A direct component in the carotid strips is indicated by the similarity of the frequency–response curve (Fig. 1A) to that of the umbilical artery which is presumably nerve free (3). Moreover, even high doses of the tested blocking agents were unable to inhibit completely the response to electrical stimulation. A large indirect component is also present. Bretylium and guanethidine, agents which presynaptically inhibit the release of catecholamine from electrically excited nerve endings, blocked 50% of the response of the strips to 60 Hz stimulation (Fig. 2). Approximately 80% of the response was inhibited post-synaptically by phentolamine, an α-blocking agent. The presence of nerve endings within the preparation is indicated by the high (*i.e.*, 80% of total) catecholamine content of the strips. Recognition of this indirect response is necessary in experiments where tension development is measured quantitatively. Speden (7) has commented on the rapid degeneration of nerves in vessels which have been stored in the cold and the subsequent deterioration of vascular response to field stimulation. Such deterioration may explain the variability in tension development noted here and previously (1).

Acetylcholine appears to act directly on the muscle cell although it is not as effective as norepinephrine in eliciting tension. Atropine completely blocked the response to 10^{-5} *M* acetylcholine without blocking the epinephrine elicited contraction. Guanethidine and phentolamine did not inhibit the contraction due to acetylcholine administration. These results suggest that acetylcholine-induced contractions are not mediated by catecholamine release from nerve endings.

The variability in the response of VSM to different agents is well documented (8) and presents special problems in determining maximal activation of the muscle. Maximal tension development by the carotid media preparation at the optimum muscle length (P_o) was obtained with potassium depolarization (1). Stimulation with norepinephrine, acetylcholine, or electrical stimulation does not fully activate the carotid smooth muscle contractile system.

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