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Contractile Responses to Epinephrine, Serotonin, and Potassium in Arterial Smooth Muscle[1] (39239)

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Many studies dealing with identification of autonomic and other types of agonist-receptor systems in vascular smooth muscle have been reported (1-7). Whether or not contractile responses produced with cationic agonists such as Ba^{2+} or K^+ involve specific receptor systems is uncertain.

Tests of the applicability of agonist-receptor theory based on the law of mass action to contractile responses in vascular smooth muscle have been limited to only a few types of vascular smooth muscle and a few agonists (1-7). In this context, Johannson *et al.* (5) recently developed a model based on receptor-occupancy theory which elegantly described contractile responses elicited by nerve stimulation or administration of norepinephrine to isolated preparations of the rat portal vein. Similarly, Rioux *et al.* (6) concluded that contractile responses produced with angiotensin in rabbit aortic strips could be described quantitatively by utilizing concepts of receptor occupancy including intrinsic activity and affinity.

The purpose of this study was to test the applicability of receptor occupation theory to contractile responses elicited with epinephrine, serotonin, and potassium chloride in isolated strips of vascular smooth muscle prepared from the femoral and ventral tail arteries of rats.

Methods. Sprague-Dawley rats of either sex (350-450 g) were anesthetized with ether, decapitated, and exsanguinated. Helical strips of arterial smooth muscle, measuring about 4 to 6 mm in length and about 400 μm wide, were prepared from the ventral tail artery according to methods described by Holloway and Bohr (7). Usually two or three strips were mounted in a single muscle chamber. One end of each strip was fixed to a separate post, while its other end was attached to a precalibrated isometric force transducer (Grass, FT-03) by means of a silk thread. Each strip was stretched to a resting tension which, on the basis of preliminary experiments, permitted optimal responses to a supramaximal dose of epinephrine (10^{-4} *M*). Presumably, the passive tension stretched the muscle to its optimal length, thereby allowing accurate assessment of differences between responsiveness to different agonists, and differences between responsiveness of different strips to the same agonist (3-5, 7). According, femoral strips were stretched to a resting tension of 300 mg, whereas tail strips were stretched until 500 mg of passive tension developed. All muscle strips were equilibrated for 2 hr in physiological salt solution (20 ml, PSS) that was maintained at 37° and aerated continuously with a mixture of 95% O_2 and 5% CO_2. The pH of the PSS was 7.3 to 7.4, and its composition (m*M*) was NaCl 130, KCl 4.7, KH_2PO_4 1.18, $MgSO_4 \cdot 7H_2O$ 1.17, $CaCl_2$ 1.6, $NaHCO_3$ 14.9, $CaNa_2$ versenate 0.026, and dextrose 5.5.

Drugs were dissolved in PSS immediately prior to performing experiments. Cumulative dose-response curves were prepared for epinephrine (Parke-Davis, 10^{-9} to 3×10^{-5} *M*), serotonin (Schwarz-Mann, 10^{-9} to 3×10^{-5} *M*), and KCl (10 to 150×10^{-3} *M*), as described by Van Rossum (8). Each dose of the particular agonist tested was in the tissue bath for 3 min. At least 30 min elapsed between challenges with different agonists. In all experiments, the muscle strips were challenged with a maximal dose of epinephrine (10^{-5} *M*) at the beginning and end of the study (2-4 hr). The experiment was discarded if initial

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and final responses to epinephrine differed by more than 10%.

Statistical significance of differences between responses produced by epinephrine, serotonin, and KCl were assessed with Student's t test and required that $P < 0.05$.

Applicability of receptor occupancy theory to contractile responses produced with epinephrine, serotonin, or KCl was examined with procedures outlined by Goldstein *et al.* (9). Briefly, the ED_{50} was graphically determined for each agonist from dose-response curves constructed in the usual manner. As described by Van Rossum (8) and Rioux *et al.* (6), the estimated ED_{50} was used as an approximation for K_A, the apparent dissociation constant of the presumed agonist-receptor complex, and a theoretical dose-response curve was generated using Clark's equation

$$\frac{R}{R_m} = \frac{(A)}{(A) + K_A},$$

where R is contractile response (milligrams of tension), R_m is maximal response produced, and (A) is the concentration of the agonist in the muscle bath. Relationships between dose of agonist (A) and response (R) were explored further by plotting R/R_m against the fraction of maximal stimulus defined by A/A_m where A_m is the dose required to produce a maximal response.

The classic Hill plot (9, 10) was used to determine whether or not more than one molecule of agonist is required to occupy a receptor site or if occupancy of one site promotes occupancy of additional receptor sites. The slope of the line generated when $\log R/(R_m - R)$ is plotted against $\log A$ defines the Hill coefficient. A Hill coefficient greater than 1 suggests that receptor occupancy facilitates further interaction between agonist molecules and receptor sites (9, 10, 14, 15).

Results. Striking differences were detected between the responsiveness of femoral and tail artery smooth muscle to agonists tested (Figs. 1-5). In femoral preparations the maximal increase in isometric tension generated with serotonin was almost 1 + 1/2 times ($P < 0.001$) greater than the maximal tension developed with epinephrine, but responses to K^+ were comparable to those obtained with epinephrine (Fig. 1A). In contrast, maximal responses produced with K^+ in tail artery preparations were only about 1/2 ($P < 0.001$) of those elicited with epinephrine (Fig. 1B). Similarly, maximal tension developed with serotonin was significantly ($P < 0.001$) smaller than the response produced with epinephrine.

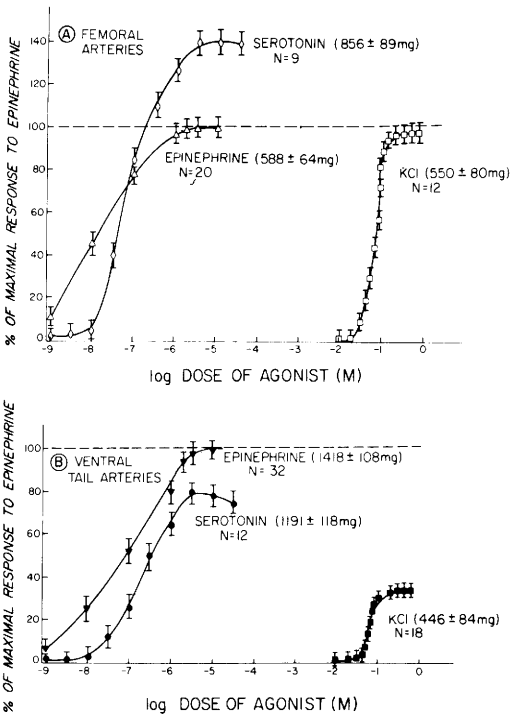

FIG. 1. Increases in isometric tension produced by epinephrine, serotonin, and KCl in rat femoral (panel A) and tail artery smooth muscle (panel B) expressed as percentage of maximal response obtained with epinephrine. Points represent the mean response obtained in the number of preparations indicated, and small verticle bars represent ±1 SE. Maximal tension (milligrams) generated with each agonist is shown for each preparation.

When tension generated by each agonist was expressed in milligrams, the response to either epinephrine or serotonin was significantly greater in tail artery preparations (Fig. 1). No significant difference between the two preparations was detected with respect to force (milligrams) generated in response to maximal doses of KCl.

Analysis of dose-response relations. Marked differences between agonist-receptor mechanisms in femoral and tail artery

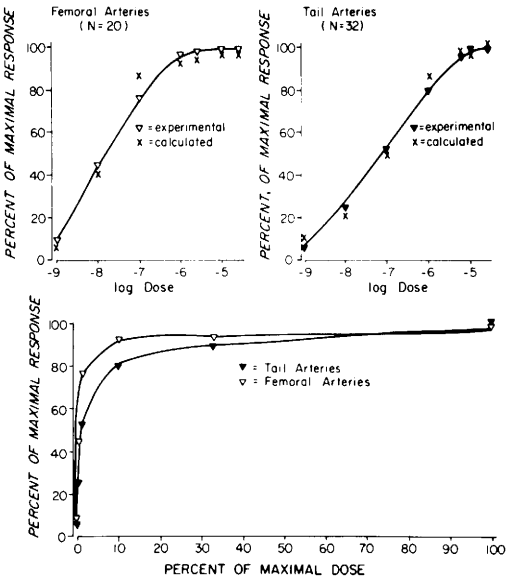

FIG. 2. Dose-response curves for epinephrine in femoral (upper left) and tail artery smooth muscle (upper right). Each triangle depicts the mean response expressed as percentage of the maximal increase in isometric tension produced with the dose of epinephrine given on the abscissa. The cross marks (x) represent values for response calculated from Clark's equation (see text). The lower figure shows the relationship between response (ordinate) and stimulus (abscissa) expressed as percentage of dose required to produce the maximal response.

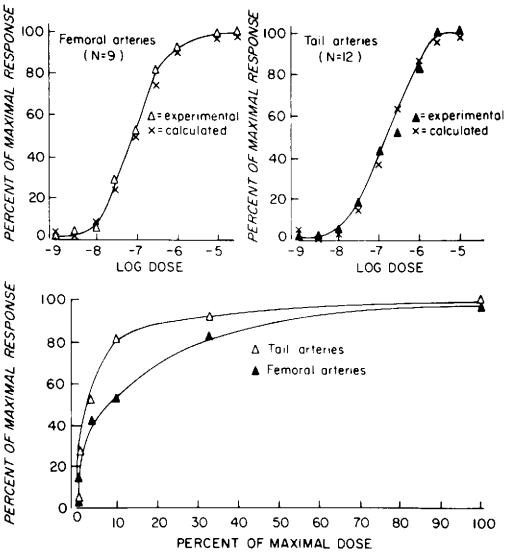

FIG. 3. Dose-response curves for serotonin in smooth muscle preparations from femoral (top left) and tail arteries (top right). Relations between response and stimulus are shown at bottom. Symbols and coordinates are the same as in Fig. 2.

smooth muscle were revealed by subsequent analysis of dose-response relations (Figs. 2–5). In these analyses the response to each dose of a given agonist was expressed as a percentage of the maximal tension attained in the particular type of arterial smooth muscle examined with the agonist (See Methods section and refs. 8, 12).

The ED_{50} for responses to epinephrine in tail preparations (8×10^{-8} *M*) was about $7^1/_2$ times greater than in femoral preparations (1.4×10^{-6} *M;* Fig. 2). Moreover, the concentration of epinephrine required to produce maximal contractions in tail smooth muscle (10^{-5} *M*) was twice that required in femoral smooth muscle (5×10^{-6} *M*). The greater responsiveness of femoral preparations was also reflected by the hyperbolic relationship between response and stimulus (Fig. 2, bottom). Thus, 10% of the maximal stimulus was required to elicit 80% of the maximal response in the tail preparations, whereas only 1% of the maximal stimulus was needed to elicit the same fractional response in femoral preparations.

Femoral preparations were also more responsive to serotonin than were tail preparations (Fig. 3). Although the concentration of serotonin required to produce a maximal contraction was similar (3 to 4×10^{-6} *M*) in both types of smooth muscle, the ED_{50} was greater in tail (1.6×10^{-7} *M*) than in femoral (9×10^{-8} *M*) preparations.

Dose-response relations obtained with KCl differed from those for either epinephrine or serotonin in several important respects (Fig. 4). First, graphic estimates of the ED_{50} were similar in femoral (62×10^{-3} *M*) and tail preparations (76×10^{-3} *M*). Second, the concentration of KCl which produced maximal contractions was also similar in both types of arterial smooth muscle (120 to 140×10^{-3} *M*). Third, the relationship between response and stimulus was sigmoid for KCl contrasting sharply with the hyperbolic curves generated with epinephrine or serotonin. Fourth, theoretical dose-response curves for KCl, derived from Clark's equation and graphic estimates of ED_{50}, differed markedly from the experimental dose-response curves. In contrast, calculated and experimental dose-response curves for epinephrine or serotonin agreed closely in both preparations (Figs. 2 and 3).

Fifth, the Hill coefficient for responses to KCl was about 4 in femoral smooth muscle and 5 in tail smooth muscle, whereas the coefficient was about 1 for responses to serotonin and slightly less than 1 for responses to epinephrine. It is important to emphasize that the Hill coefficients for KCl were significantly ($P < 0.001$) greater than 1.

Discussion. The present studies with rat femoral and tail artery smooth muscle suggest that: (a) contractile responses elicited with epinephrine and serotonin are quantitatively compatible with classic receptor occupancy theory of drug action, (b) responses to K^+ may involve a cooperative receptor mechanism such that binding of K^+ facilitates additional binding of agonist, and (c) marked differences exist between the reactivity of arterial smooth muscle from different vascular regions.

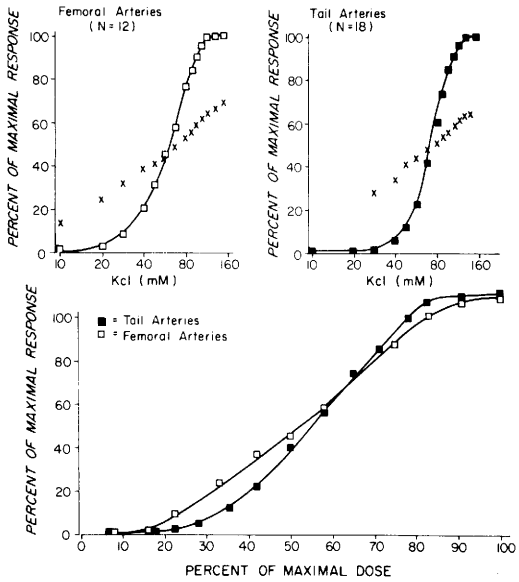

FIG. 4. Dose-response curves for KCl (top) and relationship between response and stimulus (bottom). Symbols and coordinates are the same as in Fig. 2.

If agonist-induced contractions are due to receptor occupancy, a theoretical dose-response curve can be calculated from Clark's equation providing K_A, the dissociation constant of the agonist-receptor complex, is known (see Methods). Clark's equation predicts that the dose of agonist required to elicit half maximal response approximates K_A, and that the relationships between response and stimulus are described by a hyperbolic curve (5, 6, 8, 9, 11). In accord with these predictions, we found that experimental and theoretical dose-response curves for epinephrine and serotonin were in remarkably close agreement in both femoral and tail preparations (Figs. 2, 3). Moreover, hyperbolic curves were generated when contractile response in either type of arterial smooth muscle was plotted against the stimulus offered with epinephrine or serotonin. Therefore, contractile responses produced with epinephrine or serotonin appear to be quantitatively compatible with classic receptor occupancy theory of drug action.

In contrast, calculated and experimental dose-response curves for K^+ were widely disparate in either femoral or tail preparations (Fig. 4). These disparities along with the sigmoidal character of response-stimu-

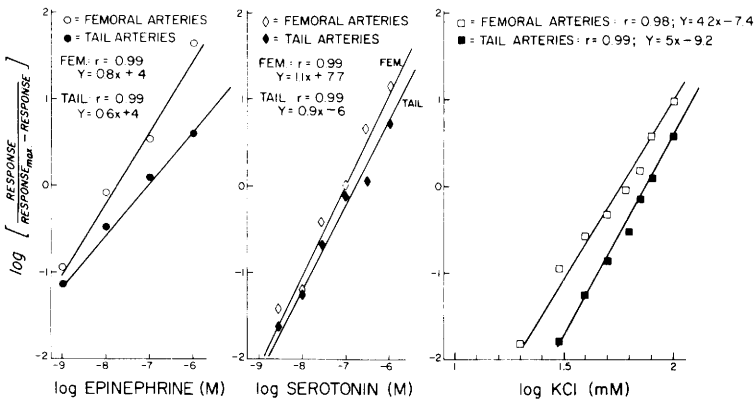

FIG. 5. Classic Hill plots for contractile responses to epinephrine, serotonin, and KCl. Each line is the least-squares line generated from its corresponding equation, while points represent values calculated from experimental data (see text).

lus relations (Fig. 4) and large Hill coefficients (Fig. 5) show that contractile responses to K^+ are mechanistically different from those produced with epinephrine or serotonin.

That contractile responses to K^+ may also involve mechanisms distinct from those involving membrane depolarization is well established (1–3, 12, 13). In this context, the quantitative relations between dose of K^+ and contractile responses detected in this study are strikingly similar to dose–response relations detailed for changes in membrane potential produced with carbamylcholine in electroplax (14). It is tempting to speculate that responses to K^+ elicited in arterial smooth muscle, like responses to carbamylcholine elicited in electroplax, involve interactions between K^+ and specific receptor moieties. The receptors for K^+, unlike those for epinephrine or serotonin, appear to exhibit cooperative properties similar to those revealed for hemoglobin, regulatory proteins, and receptors for carbamylcholine (14–16). Thus, the high Hill coefficients for responses to K^+ indicate that binding of K^+ by its receptor facilitates binding of additional K^+.

Interestingly, the maximal tension generated with K^+ was similar in both femoral and tail preparations. However, when expressed as a percentage of the maximal tension produced with epinephrine, femoral preparations were more responsive to K^+ than were tail preparations (Fig. 1). The significance of these findings simply cannot be deduced from the present studies.

Individuality of vascular smooth muscle reflected as differential responsiveness of a given type of vascular muscle to a variety of agonists, or by differential responsiveness of several types of muscle to a single agonist is well recognized in both *in vitro* and *in vivo* settings (1, 3, 4, 17, 18). The individuality of rat femoral and tail artery smooth muscle was evident in terms of the differential responsiveness of each type of smooth muscle to epinephrine, serotonin, and K^+ (Figs. 1–5), and in terms of differential responsiveness between the preparations. Whether or not the uniqueness of vascular smooth muscle from different regions is due to differences in receptor populations, mechanisms of excitation-contraction coupling, or differences in contractile proteins is unknown (1, 3). Nevertheless, the possibility that variations in receptor mechanisms contribute to regional differences in vascular reactivity merits further study (19).

Summary. Effects of epinephrine, serotonin, and KCl on development of isometric tension were studied in strips of rat femoral and tail artery smooth muscle. Striking differences were detected between the preparations. Tension development in tail artery smooth muscle was greatest with epinephrine, intermediate with serotonin, and least with K^+. In femoral preparations tension developed was greatest with serotonin. Smaller but comparable contractions were elicited with epinephrine and K^+. Theoretical and experimental dose–response curves for epinephrine and serotonin agreed closely, whereas curves for K^+ differed markedly. The relationship between fractional response and fractional stimulus was hyperbolic for epinephrine and serotonin but sigmoid for K^+. The Hill coefficient for serotonin was about 1, slightly smaller than 1 for epinephrine (0.5 to 0.8) and significantly greater for K^+ (4 to 5). These findings show that responses elicited with either epinephrine or serotonin are mechanistically consistent with receptor occupancy theory. They suggest that specific receptors for K^+ exist and that they may involve positive cooperative interactions similar to those described for receptor mechanisms in electroplax.

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