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Individualities in post-serotonin attenuation and Na^+/K^+ pump activity in vascular smooth muscle

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Prior treatment with serotonin (10^{-8}-10^{-7} M for 6 min) attenuated responses of rabbit mesenteric arteries to norepinephrine (NE) by 18-62%, but was without effect on the responses of the rabbit aorta. K^+ relaxation responses in the mesenteric arteries were enhanced by serotonin, but in the aortic strips no K^+ relaxation occurred either before or after treatment with serotonin. Maximum relaxation to monensin was similar in the two tissues. Post-serotonin attenuation and K^+ and monensin relaxation were blocked by ouabain, indicating that they depended on Na^+/K^+ pump stimulation. Intracellular Na^+ contents (Na_i) were determined in the vessels by the Li substitution method. Na_i was greater, and was increased to a greater extent by serotonin and K^+-free physiological salt solution in the mesenteric artery compared to the aorta, suggesting that the cell membrane of the mesenteric artery is leakier to Na^+ than is that of the aorta. We conclude that the absence of post-serotonin attenuation in the aorta results from the failure of serotonin to increase Na_i and thereby to stimulate the Na^+/K^+ pump in this tissue. This study demonstrates that important individualities in vascular smooth muscle reactivity even in the same animal may result from differences in membrane permeability to sodium.

Na^+ (intracellular); K^+ relaxation; Norepinephrine; Monensin; Ouabain; Serotonin; Smooth muscle (vascular)

1. Introduction

Serotonin has been shown to have two effects on the actions of other vascular contractile agonists. Contractile responses in vascular tissue are enhanced when the other agonist is administered in the presence of serotonin (De la Lande, 1966; Van Nueten, 1983). On the other hand, after serotonin has been rinsed from the muscle bath, subsequent epinephrine-induced contractions are attenuated (Bohr and Elliot, 1962).

Some observations suggest that post-serotonin attenuation results from α-adrenergic desensitization (Rapoport and Bevan, 1982). However, recent investigations in dog mesenteric arteries have supported the hypothesis that this attenuation results from a serotonin-induced increase in Na^+ influx which stimulates the electrogenic Na^+/K^+ pump causing hyperpolarization and decreasing Ca^{2+} influx induced by norepinephrine (NE; Moreland et al., 1985).

Preliminary observations in our laboratory indicate that post-serotonin attenuation does not occur in all vascular tissue; it is seen in rabbit mesenteric and rat femoral arteries but not in rabbit aorta or rat mesenteric arteries. The purpose of the present study is to determine whether differences in Na^+/K^+ pump activity or intracellular Na content underlie the individualities in post-serotonin attenuation. To make this determination, we compared parameters related to

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Na^+/K^+ pump activity and intracellular sodium content in the rabbit aorta which does not demonstrate post-serotonin attenuation with those of the rabbit mesenteric artery which has a prominent post-serotonin attenuation. The following parameters were monitored: (1) K^+ relaxation, as a measure of Na^+/K^+ pump activity, (2) intracellular Na content, (Na_i), in response to serotonin, (3) Na_i in response to Na^+/K^+ pump inhibition and (4) post-serotonin attenuation. We found that serotonin increases Na_i and augments K^+ relaxation in mesenteric arteries but not in the aortas. These findings indicate that a factor contributing to the difference in post-serotonin attenuation between these two tissues is a greater Na^+ leak in the mesenteric artery compared to that in the aorta. This greater Na^+ leak causes pump stimulation and membrane hyperpolarization.

2. Materials and methods

All experiments were performed on the superior mesenteric artery and thoracic aorta from male New Zealand White rabbits (3-4 kg). Rabbits were anesthetized with xylazine (10 mg/kg Rompun i.m.) and ketamine HCl (50 mg/kg Ketaset i.m.). Six centimetre segments of aorta and mesenteric artery were rapidly excised from each rabbit and stored overnight at 4° C in a beaker of physiological salt solution (PSS, table 1).

TABLE 1

Contents of physiological salt solutions. Composition of the physiological salt solutions used in the present study. All values are listed as mM. PSS = physiological salt solution; K^+-free PSS = physiological salt solution without potassium; Li PSS = physiological salt solution, with equimolar LiCl substituted for NaCl.

	PSS	K^+-free PSS	Li PSS
NaCl	130	130	–
KCl	4.7	–	4.7
$NaHCO_3$	14.9	14.9	–
Dextrose	5.5	5.5	5.5
KH_2PO_4	1.18	–	1.18
$MgSO_4 \cdot 7H_2O$	1.17	1.17	1.17
$CaCl_2 \cdot 2H_2O$	1.60	1.60	1.60
$CaNa_2$-EDTA	0.03	0.03	0.03
$NaH_2PO_4 \cdot H_2O$	–	1.18	–
LiCl	–	–	130
HEPES	–	–	20
Tris	–	–	20

2.1. Mechanical responses

The mesenteric artery and aorta were cleaned of excess fat and connective tissue. Helical strips (2×10 mm) were cut from the cleaned vessels and mounted vertically in a muscle bath containing PSS at 37° C, pH 7.4 bubbled with 95% O_2-5% CO_2. The lower end of each strip was attached to a stainless steel rod; the upper end to a Grass FT.03 force transducer for isometric force recording. A passive force of 2.5 g was applied to aortic strips and 1.5 g to mesenteric strips. These forces resulted in maximal active force in response to 10^{-5} M NE. Before the start of each experiment, the strips were allowed to equilibrate in the muscle bath for 2 h. We have demonstrated that this is sufficient time for re-establishment of normal electrolyte gradients after overnight storage at 4° C (Dawkins and Bohr, 1960; Barr et al., 1962).

Strip preparations were used for one of four experiments. (1) Post-serotonin atenuation was examined in aortic and mesenteric strips using a protocol previously described (Moreland et al., 1985; fig. 1). The studies were conducted using serotonin concentrations of 10^{-8}, 3×10^{-8} and 10^{-7} M. Briefly, vessel strips were first made to contract with NE (approximately ED_{60}). After rinsing the NE from the muscle bath, serotonin was added. Six minutes later the serotonin was rinsed from the bath followed in 6 min by the addition of NE in the same concentration used before serotonin treatment. Post-serotonin attenuation was measured by calculating the magnitude of the post-serotonin NE contraction as a percent of the magnitude of the pre-serotonin NE contraction. (2) The effects of serotonin on Na^+/K^+ pump activity in mesenteric and aortic strips were compared using the K^+ relaxation method (fig. 3; Webb and Bohr, 1978). To test K^+ relaxation responses the PSS in the muscle bath was replaced by PSS without K^+ (table 1). Two minutes after this replacement, NE (approximately ED_{60}) was added to the bath followed 2 min later by 5 mM KCl. Three minutes later the

strips were rinsed exhaustively with warm, bubbled PSS. Nineteen minutes after NE washout, all strips were exposed to 10^{-8} M serotonin (a concentration that did not cause contraction) for 6 min, followed by three rinses at 2 min intervals with warm, bubbled PSS. The K^+ relaxation procedure was then repeated as described above. K^+ relaxation was quantified as the difference in the magnitude of the NE response before and 3 min after the addition of 5 mM KCl, i.e. in the steady state, divided by the NE response just before K^+ relaxation and expressed as a percent. (3) Monensin dose-response curves (Anderson et al., 1983) were made to assess Na^+/K^+ pump activity after Na^+ loading. For this experiment, denervated strips were prepared using the technique of Aprigliano and Hermsmeyer (1976). Briefly, this involved incubating the artery at 37° C for 10 min in bicarbonate-free PSS with 20 μM glutathione and 300 μg/ml 6-OH dopamine. The arteries were then stored overnight in PSS at 4° C.

2.2. *Measurement of cell sodium (Na_i)*

Measurements of Na^i were made in rabbit mesenteric arteries and aortas in two separate experiments using a modification of the technique of Freidman (Freidman, 1974). In one experiment the effects of serotonin on Na_i in the two tissues were determined. Segments of aorta and mesenteric artery from rabbits were obtained as described above. Cleaned vessels were opened longitudinally, cut into segments weighing 5-15 mg and allowed to equilibrate in beakers of PSS at 37° C, bubbled with 95% O_2-5% CO_2 for 3 h with fresh PSS replacement after 90 min. Na_i was then determined as follows: each segment was rinsed for 5 s in Li PSS with HEPES-Tris buffer (table 1; Brock et al., 1982), pH 7.4, at 2° C then placed in a separate beaker of Li PSS for 25 min at 2° C to remove extracellular sodium. (Pilot studies showed that Li and Na contents reached plateaus in aortic and mesenteric tissues at that time.) Tissues were then removed, gently blotted and their wet weights measured on an analytical balance (Cahn Model G electrobalance, Cahn Instruments, Paramount, CA). The tissue segments were placed in individual tubes which were transferred to an oven and the tissues dried to a constant weight (24 h at 90° C). Dried tissues were weighed, placed in Pyrex tubes containing 4 ml of 0.75 M nitric acid, and stored at 4° C for 7 days to extract cell sodium.

Nitric acid blanks were used for Na_i determinations to account for any Na in the nitric acid. This Na was subtracted from the tissue Na_i determination. Pyrex, rather than borosilicate glass tubes were used in determinations to avoid Na contamination from the glass tubes. All tubes were washed in a 10% HCl solution followed by 20 rinses with triple distilled water before use in the cell Na determinations.

Na_i content of each sample was then determined by atomic absorption spectrophotometry (Perkin-Elmer 2380). Na_i was expressed as mmol/kg tissue dry weight and measured in samples with and without serotonin pretreatment (6 min exposure to 3×10^{-8} M serotonin followed by a 6 min rinse in PSS). Eight additional rabbits were used for experiments to determine the effect of short term exposure to K^+-free PSS on Na_i in the mesenteric artery and aorta. Briefly, vessel segments were denervated, cleaned and incubated in PSS as described above. After the incubation period, vessel segments were transferred to K^+-free PSS containing NE (10^{-7} M mesenteric artery, 10^{-8} M aorta, approximately ED_{60}) for 1, 4 or 8 min. Norepinephrine was included during the K^+-free incubation so that these incubations more closely resembled conditions used during K^+ relaxation experiments (Bukoski et al., 1983a,b). Na_i was then determined as described above for each exposure to K^+-free PSS.

2.3. *Drugs*

The following drugs were used for this study: xylazine (Rompun, Haver-Lockhart), ketamine HCl (Ketaset, Bristol Laboratories), norepinephrine HCl (Levophed, Winthrop-Breon), monensin, ouabain, 6-OH dopamine, glutathione, serotonin creatinine sulphate (all Sigma, Inc.).

2.4. *Statistics*

All data are expressed as the mean ± S.E. Intergroup comparisons were made with the unpaired

two-tailed Student's t-test; the paired t-test was used for comparison within groups. The null hypothesis was rejected at $P < 0.05$.

3. Results

3.1. Mechanical responses

3.1.1. Post-serotonin attenuation

An example of post-serotonin attenuation in the rabbit mesenteric artery is shown in fig. 1A. The magnitude of post-serotonin NE contractions in mesenteric strips was always less than those recorded prior to serotonin treatment. Figure 1B is a time control demonstrating that in the absence of serotonin the response of the mesenteric artery to NE is undiminished. The aortic strips on the other hand exhibit no post-serotonin attenuation (fig. 1C).

Figure 2 demonstrates the effect of ouabain (10^{-5} M) on the post-serotonin attenuation in the mesenteric artery. Figure 2A merely shows the degree of the attenuation in this particular artery before treatment with ouabain. Figure 2B shows that ouabain potentiates the NE contraction. Therefore, in evaluating the effect of ouabain on post-serotonin attenuation (fig. 2C), the percent increase in the NE contraction caused by ouabain was subtracted from the post-serotonin contraction in the presence of ouabain. Post-serotonin NE contraction in the presence of ouabain was measured in three mesenteric strips. After accounting for the potentiation effect of ouabain, post-serotonin NE contraction in all three strips was greater than 99% that of pre-serotonin NE contraction in the same strips, demonstrating that the post-serotonin attenuation can be completely blocked by ouabain (also observed in dog mesenteric arteries by Moreland et al., 1985). This

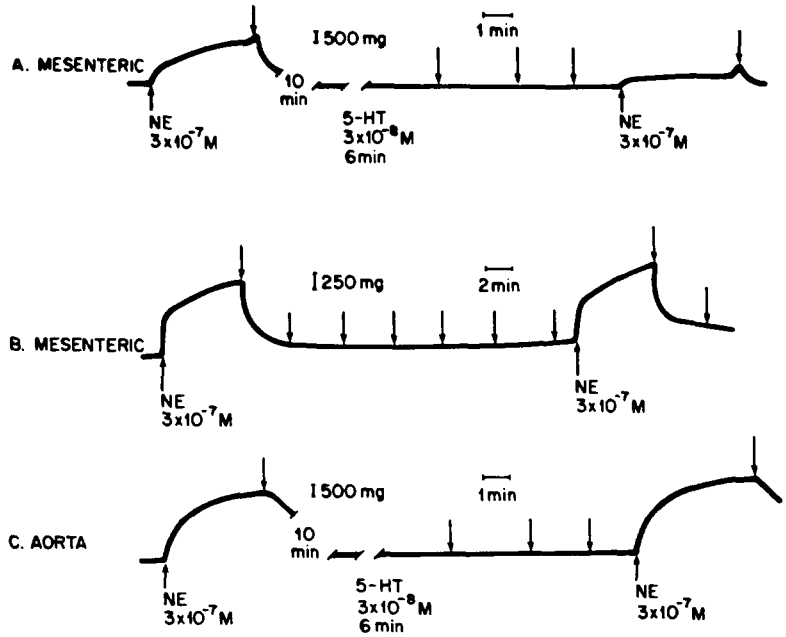

Fig. 1. Serotonin attenuation of NE responses. (A) Protocol used for examining post-serotonin attenuation. This is a tracing showing the effect of serotonin on NE contractions in a helically cut strip of rabbit mesenteric artery. (B) Time control response of a mesenteric strip demonstrating undiminished force development to a repeated NE exposure when the strip had not been treated with serotonin. (C) Tracing from a strip of rabbit aorta treated with the same protocol as the mesenteric artery in (A). Inverted arrows indicate rinses with PSS.

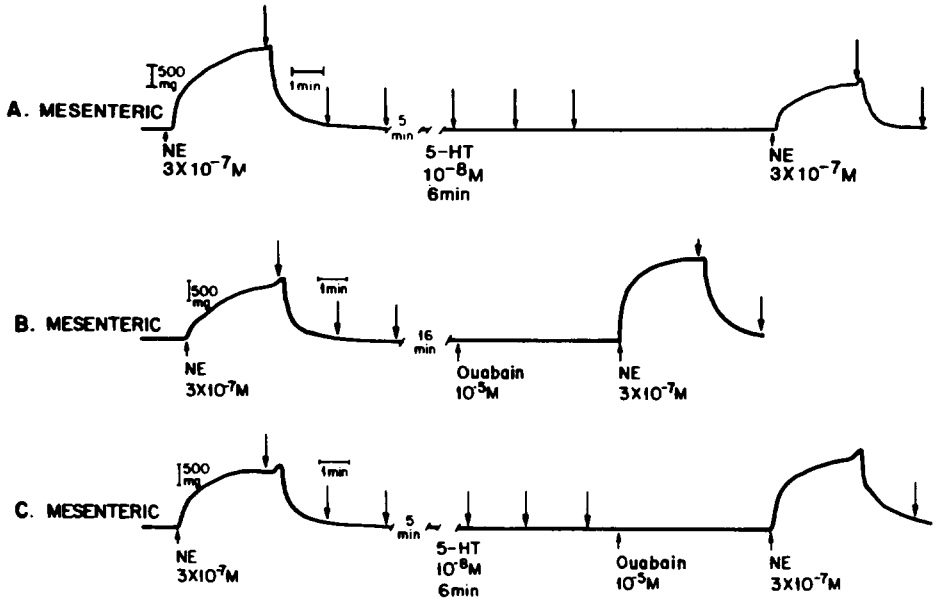

Fig. 2. Effect of ouabain on post-serotonin attenuation in mesenteric artery. (A) Control observation demonstrating the extent of post-serotonin attenuation in this mesenteric artery strip. (B) Potentiating effect of ouabain on the NE contraction. (C) Observation demonstrating that ouabain prevents the post-serotonin attenuation demonstrated in (A). Inverted arrows indicate rinses with PSS.

complete elimination by ouabain of the post-serotonin attenuation of the norepinephrine response could not have been caused by the slightly greater response to norepinephrine in the presence of ouabain. We have observed typical (50%) post-serotonin attenuations of responses to 10^{-6} M norepinephrine which gave an ED_{90} response in contrast to the ED_{60} responses used in our standard studies.

TABLE 2

Effect of serotonin concentration on post-serotonin attenuation of NE contractions. Post-serotonin attenuation = pre-serotonin NE contraction minus post-serotonin NE contraction, divided by pre-serotonin NE contraction, times 100. Data expressed as means ± S.E. [a] $P < 0.001$, unpaired t-test mesenteric artery vs. aorta.

Serotonin concentration	% attenuation	
	Aorta (n = 4)	Mesenteric artery (n = 4)
10^{-8} M	0.0±0.0	18±11 [a]
3×10^{-8} M	1 ±1	46±14 [a]
10^{-7} M	3 ±4	62±13 [a]

Post-serotonin attenuation of NE contractions, using 10^{-8}, 3×10^{-8} and 10^{-7} M concentrations of serotonin, were compared in mesenteric and aortic strips from four rabbits and data are summarized in table 2. Serotonin concentrations greater than 10^{-7} M were not used because they caused large contractions in both mesenteric and aortic strips. In mesenteric arteries post-serotonin attenuation was proportional to serotonin concentration. In contrast, serotonin pretreatment, even at the highest concentration, had virtually no effect on NE contractions in the aorta.

3.1.2. Effect of serotonin on K^+ relaxation

Figure 3 demonstrates the effect of serotonin pretreatment on vascular Na^+/K^+ pump activity, as assessed by K^+ relaxation responses (Webb and Bohr, 1978). In mesenteric strips, the magnitude of contraction to norepinephrine, in K^+-free PSS (where the Na^+/K^+ pump activity is blocked), after serotonin pretreatment, averaged 98% of that prior to serotonin pretreatment. A typical response in a mesenteric artery strip is shown in fig. 3A. Following serotonin treatment, the K^+ relaxa-

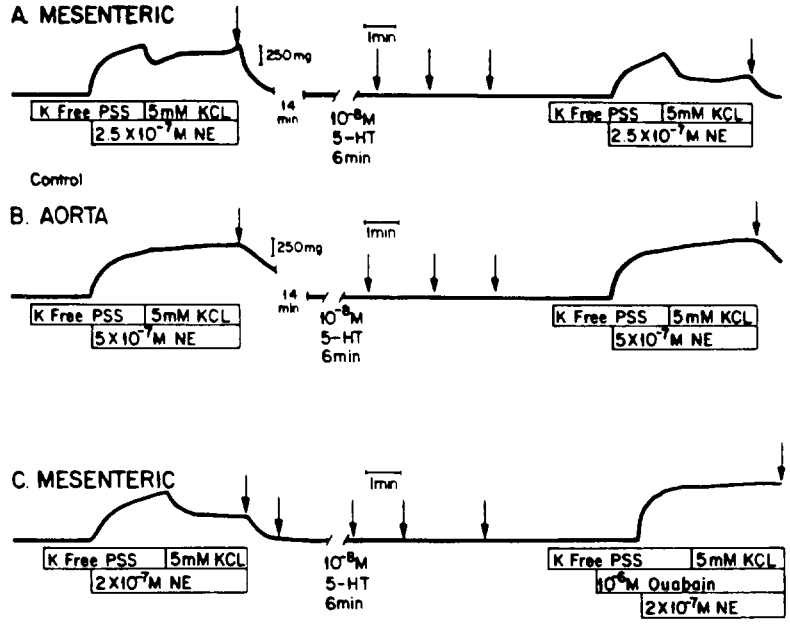

Fig. 3. Effect of serotonin on K^+ relaxation responses. (A) Response in a helically cut strip of mesenteric artery. After 2 min exposure to K^+-free PSS, the strip was contracted with NE ($ED_{60\text{-}70}$). Two minutes later KCl was added to the bath to give a concentration of 5 mM. This addition of potassium caused 'potassium relaxation'. Relaxation was enhanced by serotonin pretreatment. (B) K^+ relaxation procedure on a aortic strip. This procedure did not produce a relaxation in the aorta with or without serotonin pretreatment. (C) An experiment showing that ouabain blocks K^+ relaxation in mesenteric strips even with serotonin pretreatment. Inverted arrows indicate rinses with PSS.

tion was consistently potentiated in the mesenteric artery. In contrast to the responses of the mesenteric artery, K^+ relaxation was not seen in the aorta (fig. 3B). Potassium relaxation in mesenteric arteries could be blocked by ouabain, (10^{-6} M) with or without serotonin pretreatment (fig. 3C).

TABLE 3

Effect of serotonin on K^+ relaxation responses. K^+ relaxation is expressed as the percent relaxation of the NE contraction obtained in K^+-free PSS when 5 mM KCl was added to the bath. Strips were exposed to the K^+-free PSS for 4 min before 5 mM KCl was added. Data expressed as means ± S.E. [a] $P < 0.05$ paired t-test, control vs. serotonin.

	Control (n = 4)	After 10^{-8} M serotonin (n = 4)
Mesenteric artery	51 ± 17	78 ± 7 [a]
Aorta	0 ± 0	0 ± 0

Experiments identical to those shown in fig. 3A and B were performed on four rabbits and are summarized in table 3. K^+ relaxation responses

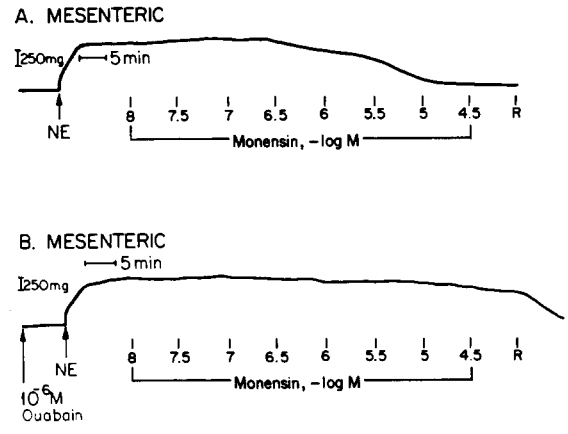

Fig. 4. (A) Monensin relaxation in strips of mesenteric artery contracted with NE, 2×10^{-7} M. (B) Similar study in the presence of ouabain. R = rinse with PSS.

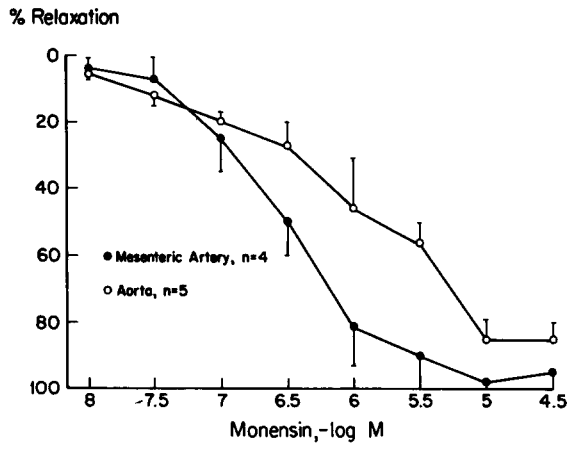

Fig. 5. Concentration response curves of aortic and mesenteric vessels to monensin. Both muscles had been precontracted with NE (2×10^{-7} M). Brackets indicate S.E.M. of % relaxation of the NE response.

were variable in mesenteric strips but were significantly ($P < 0.05$) increased by an average of 50% in these strips by pretreatment with 10^{-8} M serotonin. None of the aortic strips exhibited K^+ relaxation with or without serotonin pretreatment.

3.1.3. Monensin relaxation

Dose-response studies with the carrier ionophore monensin were performed on mesenteric and aortic strips from five additional rabbits. This agent produces relaxation in vascular smooth muscle by increasing intracellular Na^+ and thus stimulating the membrane electrogenic pump to cause hyperpolarization (Anderson et al., 1983; Pressman and De Guzman, 1985; Saini et al., 1979). An example of a vascular response to monensin is shown in fig. 4A. Monensin relaxation can be blocked by ouabain (10^{-6} M) (fig. 4B; Anderson et al., 1983). Figure 5 demonstrates that monensin produces a relaxation of aortic smooth muscle that is almost as great as the relaxation that it causes in the mesenteric artery: this is in striking contrast to K^+ relaxation which has no effect on aortic smooth muscle.

TABLE 4

Effect of serotonin on Na_i in rabbit vessels. 'Serotonin-treated' refers to values obtained after 6 min exposure to 3×10^{-8} M serotonin followed by a 6 min rinse in PSS. All values expressed as mean ± S.E. Na_i is expressed in mmol/kg tissue dry weight. [a] $P < 0.02$, paired t-test, control vs. serotonin-treated. [b] $P < 0.01$, unpaired t-test mesenteric artery vs. aorta. Values are the means and S.E. of eight or nine determinations.

	Control	Serotonin-treated
Mesenteric artery	42.4 ± 2.1 [b]	49.6 ± 3.6 [a,b]
Aorta	30.3 ± 1.7	28.6 ± 1.8

TABLE 5

Effect of exposure to K^+-free PSS on Na_i in rabbit vessels. Na_i is expressed in mmol/kg tissue dry weight. [a] $P < 0.05$ mesenteric artery vs. aorta unpaired t-test. [b] $P < 0.01$ mesenteric artery vs. aorta unpaired t-test. [c] $P < 0.05$ 4 or 8 min vs. 1 min (Student's Newman-Keuls test). All values expressed as means ± S.E.

	Na_i		
	1 min	4 min	8 min
Mesenteric artery	35.6 ± 3.3	45.9 ± 1.8 [b,c]	48.7 ± 3.2 [a,c]
Aorta	31.3 ± 3.6	29.6 ± 3.7	37.9 ± 2.6 [c]

3.2. Na_i in mesenteric arteries and the aorta; effect of serotonin pretreatment and K^+-free PSS

The effects of serotonin (6 min exposure to 3×10^{-8} M followed by 6 min PSS rinse) on mesenteric and aortic Na_i, are summarized in table 4. Na_i was higher in untreated segments of mesenteric artery compared to those of the aorta. Na_i was increased significantly in response to serotonin in mesenteric arteries but not in the aorta.

The effects of a K^+-free PSS on Na_i are summarized in table 5. Increasing the duration of exposures to K^+-free PSS from 1 to 8 min resulted in significant increases in Na_i in mesenteric and aorta tissues. These increases occurred more rapidly and were greater in the mesenteric artery than in the aorta.

4. Discussion

These observations clearly establish that following exposure to low concentration of serotonin the response of the rabbit mesenteric artery to NE is

attenuated, whereas a similar treatment of the rabbit aorta with serotonin fails to attenuate this response. The current study was designed to define mechanistic differences between the vascular smooth muscle from these two sources which could account for the differences in this attenuating effect of serotonin.

Many mechanisms must be considered when explaining the cause of a depression of this vascular smooth muscle response to NE: (1) increased release of endothelial-derived relaxing factor; (2) increased release of a neurohumoral dilator agent from intrinsic nerve terminals; (3) decreased release of a neurohumoral constrictor agent: (4) increased membrane permeability to potassium causing hyperpolarization; (5) impairment of some phase of the phosphoinositide system; (6) membrane stabilization; (7) activation of the prostaglandin system; and (8) stimulation of the electrogenic Na^+/K^+ pump. In the current study we chose to evaluate the stimulation of this electrogenic sodium pump because we (Moreland et al., 1985) had presented considerable evidence that post-serotonin attenuation of the NE response in the dog mesenteric artery was caused by an increase in Na^+/K^+ pump activity causing membrane hyperpolarization, that resulted in a decrease in calcium influx in response to NE stimulation. The following evidence was presented in support of this mechanism: (1) inhibition of Na^+ influx by amiloride diminished the attenuation; (2) inhibition of the electrogenic pump with ouabain prevented the attenuation; (3) verapamil, a calcium channel blocker, diminished the attenuation; (4) ryanodine, used to block release of intracellular calcium, had no effect on the attenuation; (5) prior treatment with serotonin caused a reduction in ^{45}Ca influx stimulated by NE; (6) prior treatment with serotonin attenuated responses to all constrictor agents tested (methoxamine, clonidine, prostaglandin F_2, KCl and serotonin itself); the attenuation was not affected by adrenergic denervation, endothelial removal, propranolol, indomethacin or arachidonate.

Based on this mechanism, the greater post-serotonin attenuation observed in the rabbit mesenteric artery could have been caused by either or both of the following characteristics of this artery compared to those of the rabbit aorta: (1) a greater membrane Na^+ leak induced by serotonin or (2) a Na^+/K^+ pump that is more sensitive to stimulation by Na^+. Our observations support the hypothesis that a greater Na^+ leak in the membrane of the mesenteric artery is the cause of the difference.

In the current study, we have shown that serotonin pre-treatment causes dose-dependent attenuation of NE contraction of mesenteric arteries. In contrast, even pretreatment with higher concentrations of serotonin did not cause significant post-serotonin attenuation in aortic strips, although this concentration of serotonin caused a contraction of the strips. (Post-serotonin attenuation and contraction of vascular smooth muscle are mediated by the same serotonin receptor (Black et al., 1981; Moreland et al., 1985). Serotonin caused an enhancement of the K^+ relaxation (an indicator of Na^+/K^+ pump activity) in mesenteric arteries but not the aorta. In addition, post-serotonin attenuation and K^+ relaxation are both blocked by ouabain and post-serotonin attenuation was not seen in K^+-free PSS (present study (fig. 3), Moreland et al., 1985; Webb and Bohr, 1978). Since the Na^+/K^+ pump is inactive in K^+-free PSS, the absence of post-serotonin attenuation in a K^+-free PSS confirms our hypothesis that this attenuation reflects an increase in pump activity. For the same reason, ouabain did not potentiate the response to NE in K^+-free PSS. Post-serotonin attenuation and K^+ relaxation appear to be related phenomena requiring Na^+/K^+ pump stimulation. This stimulation was considerable in mesenteric arteries but did not occur in the aorta.

Although the phenomenon of post-serotonin attenuation and K relaxation appear to depend on the same mechanism, the post-serotonin attenuation lasts over 30 min whereas the K relaxation lasts no more than 5 min. The basis for this discrepancy is not evident; however, it could be accounted for if the duration of K relaxation merely reflected the time required to pump out the excess intracellular sodium; whereas serotonin had some residual effect on membrane permeability to sodium. We postulate that serotonin induces a sodium leak in the vascular smooth muscle cell

membrane. It is not known how this comes about. However, it can be assumed from our data, that the sodium leak does not require the continued contact of serotonin with the artery; post-serotonin attenuation is observed after the serotonin has been rinsed from the muscle bath. The duration of the altered post-serotonin pump activity depends on the dynamics of both the pump activity and of the serotonin-induced sodium leack. This leak appears not to correct itself quickly after exposure to serotonin, thereby contributing to the prolonged post-serotonin attenuation.

Serotonin pretreatment increased Na_i in mesenteric arteries but not the aorta (table 4). Therefore differences in post-serotonin attenuation between the msenteric artery and the aorta probably reflect differences in serotonin mediated increases in Na_i and hence in pump stimulation. Serotonin-induced increases in Na_i probably also contribute to the enhanced K^+ relaxation seen in mesenteric arteries after serotonin pretreatment. The phenomenon of K^+ relaxation is influenced by both the magnitude of the sodium influx during the exposure to the K^+-free environment and the intrinsic Na^+/K^+ pump activity.

To clarify the relative contributions of Na^+ leak and intrinsic Na^+/K^+ pump activity to the observed difference in K^+ relaxation and post-serotonin attenuation, we studied the effect of monensin on these two tissues. Monensin is an ionophore that forms membrane pores through which sodium ions may pass (Pressman and De-Guzman, 1975; Saini et al., 1979; Anderson et al., 1983). It causes an increase in intracellular sodium thereby stimulating the electrogenic Na^+/K^+ pump to cause membrane hyperpolarization. We observed that this ionophore causes relaxation of the rabbit aorta that is almost as great as that which it produces in the mesenteric artery (fig. 5). This is in marked contrast to the effects of serotonin or a K^+-free solution, neither of which causes any inhibition of contraction in the rabbit aorta. These contrasting results suggest that Na^+ can enter the aortic smooth muscle cell via the artificial monensin pore but that Na^+ cannot leak into the aortic cells under the conditions of our studies in effective amounts through the physiological Na^+ channels. It appears that the Na^+/K^+ electrogenic pump of the aorta can be stimulated if Na^+ is admitted into the cell with monensin (Anderson et al., 1983). We have not made direct measurement of Na_i in rabbit vessels treated with monensin, but Brock et al. (1982) have shown that the sodium content of rat aorta is maximally increased after incubation with 1.4×10^{-5} M monensin.

Therefore the difference between post-serotonin attenuation response of the aorta and mesenteric artery appears to reside in the lesser sodium leak produced by serotonin in the aortic membrane. We have no evidence that there is a difference in intrinsic Na^+/K^+ pump activity between the aorta and mesenteric artery. The slightly greater relaxing effect of monensin in the mesenteric artery than in the aorta could reflect a greater intrinsic pump function in the mesenteric artery. However, this difference is not sufficient to account for the observed great difference in serotonin attenuation or potassium relaxation between the two tissues.

A greater net influx of Na^+ in mesenteric vessels during brief (4 min) K^+-free conditions is probably responsible for the presence of K^+ relaxation in mesenteric arteries but not the aorta (left portion of fig. 3A and B and see table 4; Schwartz et al., 1975). This difference in sodium leak may also be responsible for the serotonin-enhanced K^+ relaxation in mesenteric vessels compared to the aorta. The mesenteric arteries accumulate more Na during brief exposure to K^+-free PSS thus amplifying the pump-stimulating effect of serotonin-induced Na^+ influx.

Another mechanism that should be considered is that the K^+-free conditions may reduce K^+ conductance and result in cell depolarization (Hirst and Van Helden, 1982). If such an effect were greater in the rabbit aorta, compared to mesenteric artery, then it might contribute to the lack of K^+ relaxation seen in the aorta. Such a mechanism, however, could not account for the lack of sensitivity to serotonin in that tissue with respect to post-serotonin attenuation and changes in Na_i.

We have also considered the report by Lynch et al. (1986), that NE can stimulate Na^+/K^+ ATPase (as measured by ^{86}Rb uptake) in rat and rabbit aortas. They suggested a major role for a Na^+ 'leak' in this NE pump stimulation in rat but not

in rabbit aorta. Thus, the possibility exists that the combination of NE and serotonin contributed to enhanced K^+ relaxation in mesenteric arteries relative to that seen in the aorta. However, as is evident in table 4, we have observed that serotonin treatments alone increase Na_i in the rabbit mesenteric artery but not in the aorta. We conclude that the stimulated smooth muscle membrane of the rabbit mesenteric artery is leakier to Na^+ than is that of the aorta, whereas maximum Na^+/K^+ pump activity is similar in these tissues. This greater Na^+ leak is primarily responsible for enhanced K^+ relaxation responses in the mesenteric vessels.

We conclude that the serotonin- or K^+-free-treated smooth muscle membrane of the rabbit mesenteric artery is leakier to Na^+ than is that of the aorta, whereas maximum Na^+/K^+ pump activity is relatively similar in these tissues. This greater Na^+ leak is primarily responsible for enhanced K^+ relaxation responses in the mesenteric vessels. We also conclude that K^+ relaxation and post-serotonin attenuation are related phenomena due to Na^+/K^+ pump hyperactivity. It is unlikely that the absence of post-serotonin attenuation in the aorta results from a reduced intrinsic Na^+/K^+ pump activity; rather, it results primarily from a reduced Na^+ influx in response to either serotonin or to a K^+-free environment. When artificial channels for Na^+ are introduced in the membrane with monensin the aortic smooth muscle relaxes, demonstrating that its Na^+/K^+ pump is stimulated by an increase in Na_i.

The results of the present study again demonstrate the marked individuality of vascular smooth muscle from different sites even within the same animal. The differences observed in this study are not based on the frequently reported differences in receptor population or sensitivity. Instead they appear to reflect differences in intrinsic and serotonin-induced changes in Na_i, and hence Na^+/K^+ pump activity. Because of these differences one may predict individualities is constrictor and dilator responses between these two vessels, since both Na_i and Na^+/K^+ pump activity influence the contractile response of vascular smooth muscle. Differences in membrane permeability to Na^+ appear to be an important basis for individualities among responses of vascular smooth muscle from different sources.

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