Characterization of Vasopressin Actions in Isolated Submucosal Arterioles of the Intestinal Microcirculation

S. Vanner, M.-M. Jiang, V.L. Brooks, and A. Surprenant

Submucosal arterioles are the final resistance vessels of the mesenteric circulation; they supply intestinal mucosa and smooth muscle and contribute significantly to total mesenteric resistance. Characterization of receptors present on submucosal arterioles has not been carried out, because these vessels have not been accessible to study by previous methods. We have used a novel optical method for on-line tracking of outside diameter from in vitro preparations of submucosal arterioles in the ileum and colon of guinea pigs, rabbits, and humans to characterize the vasoconstrictor responses to vasopressin as well as other vasopressor agents along the gastrointestinal tract. All ileal submucosal arterioles showed smoothly graded constrictor responses, whereas colonic arterioles from each species exhibited rhythmic vasoconstrictions. Vasopressin constricted guinea pig and human submucosal arterioles (EC_{50}, 1 nM) by activating classical V1 receptors; dissociation equilibrium constants (K_d) for the V1 antagonist d(CH_2)_4 Tyr (Me) arginine vasopressin were 1–3 nM. This antagonist was 10–50-fold more potent in inhibiting vasopressin constrictions in rabbit submucosal arterioles (K_d=0.05–0.1 nM). No evidence for the presence of V2 receptors was obtained in any arteriole, and no significant differences in the α_1-adrenoceptor-mediated constrictions were observed in these vessels. Results from this study suggest the presence of heterogeneity of V1 receptors in submucosal arterioles; these differences appear to be species dependent. Our results also suggest that intrinsic vasoconstrictor properties of submucosal arterioles differ along the length of the gastrointestinal tract; these differences appear to be species independent. (Circulation Research 1990;67:1017–1026)

The intestinal microcirculation derives primarily from the superior and inferior mesenteric arteries whose branches penetrate the intestinal wall and give rise to the extensive submucosal arteriolar network, whose branches, in turn, penetrate mucosal villi and intestinal smooth muscle along the length of the gastrointestinal (GI) tract. Measurements of intestinal blood flow have revealed that mucosal villi are among the most perfused tissues in the body, and they are the first to be damaged by ischemic diseases.1–3 In spite of the importance of the submucosal vasculature to the GI microcirculation, no quantitative pharmacological data concerning actions of vasoactive substances on these vessels exist, because it has not been possible to prepare isolated preparations of this vascular network that would be amenable to either radioligand binding or contractility measurements.4 Several video measurement–based methods, combined with elegant in vivo preparations such as cranial window techniques,5,6 hamster cheek pouch vessels,7,8 and other exteriorized vascular beds,9,10 have been used to study microvessels. In particular, exteriorized preparations of the rat intestine have provided direct information on submucosal and mucosal blood flow and changes in flow to this microvascular bed in response to applied substances11–14; unfortunately, this in vivo method cannot be applied to a wide range of species, including, obviously, humans. On the other hand, the network of the submucosal vasculature can be isolated by dissecting away the overlying muscle and underlying mucosa, leaving a very thin (30–50-μm-thick) layer of connective tissue in which lie the submucosal arterioles and venules.15 This preparation has been used to examine electrophysiological properties of arteriolar smooth muscle and the correlation between membrane potential and vessel contractility,15 but is has been little exploited as a
microvascular preparation for pharmacological studies because there have not been methods that can adequately and quantitatively monitor diameter or tension from these isolated vessels (see discussions in References 4 and 16). Neilde recently developed a novel optical method for on-line tracking of vessel diameter from submucosal arterioles in vitro. The purpose of the present study was to use this method to characterize arteriolar responses to vasoconstrictor substances, particularly vasopressin, in isolated submucosal vascular networks in different species. Vasopressin generally constricts blood vessels by activating vascular muscle or endothelial V₁ receptors, but it is also known to produce vasodilations through V₂ receptors (the classical antiuretic receptor), particularly in cerebral vessels. There also may be at least two subtypes of the V₁ receptor. Many whole-animal studies have shown that vasopressin is a potent vasoconstrictor of the splanchnic and mesenteric beds, and alterations of this hormone or its receptors in these beds have been implicated in at least one form of hypertension. Therefore, we wanted to determine the actions of this hormone and the associated receptor subtype in submucosal arterioles in the mammalian GI tract; toward this aim, we have examined isolated arterioles in the two major subdivisions of the submucosal vasculature, that of the small intestine (ileum) and that of the large intestine (colon) in guinea pigs, rabbits, and humans.

Materials and Methods

Preparations

Animals and human tissues. Young albino guinea pigs (150–250 g) and rabbits (1,000–1,500 g) were used in these experiments; they were killed by halothane anesthesia and exsanguinated by carotid transection. Human colonic tissue from surgical samples that had been diagnosed as normal were obtained in the operating room and placed in physiological saline within 5 minutes after removal.

Guinea pig submucosal preparations. The dissection of the ileum has been described in detail previously; in brief, 2–3-cm-long segments of the small intestine were removed and cut open along the mesenteric border and pinned out mucosal side up; the mucosa was stripped away, and the underlying submucosal plexus was peeled off the circular muscle. Segments (2–3 cm long) of the mid-distal colon were removed and opened along the mesenteric border, the mucosa was pulled away in several large sheets, and then the muscularis mucosae was cut where it covered the submucosal blood vessels and was gently removed in small strips. The entire preparation was then repinned serosal-side up, and all of the muscle overlying the submucosal plexus was pulled away.

Rabbit and human submucosal preparations. Identical procedures as those carried out on guinea pig intestine were followed for segments obtained from the rabbit small intestine and large intestine. Identical procedures as those carried out on guinea pig and rabbit colon were followed for segments obtained from human colonic tissue.

All human submucosal preparations had approximately the same dimensions, about 2×2 cm (30–50 μm thick); all arterioles had the same appearance (see photomicrographs in References 15, 16, and 22). All preparations were pinned out in an organ bath (0.5–1 ml volume) and rapidly superfused (10–15 ml/min) with a physiological saline solution at 36°C of the following composition (mM): NaCl 126, NaH₂PO₄ 1.2, MgCl₂ 1.2, CaCl₂ 2.5, KCl 5, NaHCO₃ 25, and glucose 11, gassed with 95% O₂ and 5% CO₂. Preparations were pinned in the organ bath mucosal-side up in approximately half the experiments and serosal-side up in the remaining experiments; pinning the preparation serosa up had the practical advantage of allowing any adherent strips of visceral smooth muscle to be pulled off with fine-tipped forceps. A 30-minute equilibration period was allowed before application of any drug.

Video Monitoring of Outside Diameter

The outside diameter of a segment of arteriole was monitored using the Diamtrak system, which has been described in detail previously. This is a system that can track small vessel diameter by on-line computer analysis of TV images with an Imaging Technology PCVisionPlus frame grabber board (Imaging Technology Inc., Woburn, Mass.) in an AT computer. Briefly, the microscope focus was set so that the two edges of the blood vessel each formed a black line on the TV screen; the TV camera was then rotated until the vessel was horizontal on the screen. With a mouse-controlled screen cursor, cursors were set on the black lines defining the walls of the blood vessel. The area between the cursors (i.e., the vessel diameter) was measured by Diamtrak software, converted to an analog signal, and displayed on a conventional strip chart recorder. The measurement rate was 10–20/second, and the resolution of the system was less than 1 μm.

It is well known that the sensitivity of vascular smooth muscle to many agents depends on the length-tension relation of the vessel, such that vasoconstriction in response to any given agonist concentration would be expected to be less for preloads less than optimum length (Lₒ). On the other hand, there is a rather wide range of preloads greater than Lₒ over which agonist sensitivity varies little or insignificantly. Thus, it is important in terms of physiological significance and interpretation of our results that the submucosal arterioles be under sufficient active stress that they can be considered to be at, or above, Lₒ. The major disadvantage to the Diamtrak monitoring system is that it does not provide absolute values for tension and length-tension relations, a problem that can be overcome by pressurizing the vessel. It should be noted that all vessels in our studies are “under tension” in that the connective tissue in which they lie are pinned out tightly so that the vessels can be delineated under the
microscope. Previous results with vasoconstrictor substances\cite{22,23} showed that guinea pig ileal arterioles produce consistent, reproducible vasoconstrictions with norepinephrine dose-response relations being similar to those reported for many other peripheral arteries\cite{24,25}; such results would not be expected if the preload on the arterioles was significantly less than $L_o$. Nevertheless, we carried out a series of preliminary experiments designed to examine whether increasing the intraluminal pressure of submucosal arterioles would alter the agonist sensitivity.

Segments of intestine were obtained as described above; before the intestine was cut open, the prearteriolar mesenteric artery was cannulated with polyethylene tubing of 0.6 mm diameter. The cannula was fixed in place, and then the usual dissection was undertaken, taking care to dissect the area that was fed by the cannulated mesenteric branch. The cannula was then attached to a 50-ml syringe filled with physiological saline, thus allowing the luminal pressure to be altered by altering the vertical position of the syringe.

Concentration-response curves to norepinephrine were constructed for control arterioles (i.e., no pressure head but stretched to the usual degree); the syringe was then raised until the outside diameter of the vessel being monitored had distended to, and stabilized at, a diameter 10–15% greater than control (termed pressure 1), or to a value 25–40% greater than control (termed pressure 2), and norepinephrine was reapplied as before. Results from all experiments carried out on guinea pig ileal arterioles are shown in Figure 1. $EC_{50}$ values and maximum responses were not significantly different between control arterioles and arterioles maintained at 10–15% above control diameter. Distension of arterioles greater than this reduced the maximum constrictor response by 10% and resulted in a twofold shift to the right in the $EC_{50}$ value.

**Pharmacological Experiments**

All drugs were applied by superfusion for 2–3 minutes (per agonist concentration) with a 20-minute washout period between applications. In initial experiments, all drugs were applied in noncumulative fashion; in later experiments, norepinephrine, phenylephrine, and U46619 were applied cumulatively, because we found there to be no significant differences between dose-response curves obtained by either of these methods. Vasopressin was applied in noncumulative concentrations in most experiments carried out in arteriolar preparations from rabbits and humans because early experiments showed that cumulative additions of vasopressin in these preparations produced smaller vasoconstrictions than when vasopressin was applied in a noncumulative fashion. Cumulative concentrations were used in experiments on guinea pig submucosal arterioles because these vessels responded similarly to either type of application.

Dissociation equilibrium constants ($K_d$ values) for antagonists were obtained by constructing concentration-response curves for agonists in the absence and then presence of three to four increasing concentrations of antagonist; these results were then used to obtain $K_d$ values according to the method of Arunlakshana and Schild.\cite{28} In those arterioles showing oscillatory vasoconstrictor responses (see "Results"), vasoconstriction was measured as the peak diameter change to a given concentration of agonist. Data were included in the analysis only if concentration-response curves obtained after washout of all antagonists returned to within 10% of initial control responses. Antagonists were present in the superfusion fluid for 5–10 minutes before the concurrent application of agonist. All results obtained with vasopressin are expressed as percent of the maximum norepinephrine response, because in all arterioles this maximum norepinephrine response was complete occlusion of the lumen. All responses described in this study were considered to be due to direct actions of agonists activating receptors present on the arterioles, rather than due to indirect neural mediated effects, because the addition of tetrodotoxin (1 $\mu$M, $n=12$), guanethidine (1 $\mu$M, $n=6$), or atropine (100 nM, $n=5$) did not alter agonist responses nor patterns of vasomotion.

Tests for significance were accomplished using Student's $t$ test; differences were considered not significant for values of $p>0.5$. Results are expressed as mean±SEM.

**Drugs**

The following drugs were used in this study: (Arg$^6$)-vasopressin, Fmnp1O-Me-Tyr$^1$- (Arg$^8$)-vasopressin [d(CH$_2$)$_5$]Tyr (Me) AVP], angiotensin II, and endothelin (Peninsula Laboratories, Inc., Belmont,
Results

General Characteristics of Isolated Submucosal Arterioles

The resting outside diameters of arterioles examined in this study ranged from 40 to 87 μm, and there were no significant differences in the range of arteriolar diameters among species. It is to be noted that other methods of monitoring vessel diameter refer to inside diameter,\(^8,11,29\); submucosal arteriolar size corresponds approximately to inside diameters of 15–40 μm. The larger arterioles, or vasa recta, were the first branches from the mesenteric arcade that pierced the serosa and muscularis propria to run within the submucosa toward the antimesenteric border, parallel to the plane of the tissue. Smaller arterioles were first- or second-order branches of these vessels, also running in the plane of the tissue. There were no significant differences in the responses obtained from different-sized arterioles within a given preparation.

In the majority of experiments, no spontaneous vasomotion was observed in submucosal arterioles after an initial 30–60-minute equilibration period except as a consequence of physical disturbance (e.g., directly touching the arteriole with forceps) or elevated temperature (>38°C). Occasionally (5–10% of experiments), spontaneous vasomotor constrictions continued for greater than 60–90 minutes; such preparations were discarded because they were also associated with an overall constricted appearance and pronounced tachyphylaxis to vasoconstrictor substances.

Characteristics of Vasoconstrictor Responses

All arterioles constricted in response to applications of norepinephrine, the \(\alpha_1\)-adrenergic receptor agonist, phenylephrine, the thromboxane analogue, U46619, and vasopressin. Two distinct patterns of responses to these substances were evident (Figure 2, Table 1). Vasoconstrictor substances produced smoothly graded responses in ileal arterioles from both guinea pig and rabbit; in contrast, colonic arterioles displayed regular oscillating changes in diameter, which were superimposed on the envelope of the graded vasoconstriction (Figures 2 and 3). This type of oscillatory constriction was observed in all human \((n=7)\) and rabbit \((n=17)\) colonic arterioles, but it was less consistent in guinea pig colonic arterioles (Table 1).

The frequency of vasoconstrictor oscillations, measured from peak-to-peak excursion, observed in the presence of a maximum concentration of vasopressin or norepinephrine averaged 15.3±0.3/min \((n=6)\) and 5.68±0.5/min \((n=5)\) in rabbit and human colonic arterioles, respectively. We also determined the fundamental frequency of the oscillations by subjecting the digitized waveforms to power spectrum analysis with discrete fast Fourier transforms as described in detail by Colantuoni et al\(^31\); examples of the results so obtained are shown in Figure 3. The characteristic frequency determined by this calculation was not significantly different from that determined by the former calculation and averaged 15.6±0.4 cycles/min in rabbit colonic arterioles \((n=4)\) and 6.05±0.3 cycles/min in human colonic arterioles \((n=3)\).

The onset of the vasoconstriction in response to application of vasopressin was approximately three times slower than that to norepinephrine, phenylephrine, U46619, or high external potassium solution (110 mM). The delay to onset of the vasoconstrictor response to these latter substances was mostly due to transit time through the superfusing tubes (approximately 15–18 seconds as estimated by dye travel and by time to onset of the high potassium constriction). The average onset of vasoconstrictor responses to norepinephrine or U46619 was 19±0.3 seconds \((n=9\), two from each vessel); the average time to onset of vasopressin constriction was 54±1.3 seconds.
EC$_{50}$ values were determined from averaged dose-response curves obtained from each preparation (n=3–6) in all experiments except human arterioles [n=2–4]). Number of samples is given in parentheses. NE, norepinephrine; PE, phenylephrine; AVP, vasopressin; GP, guinea pig.

*K$_d$ estimates based on single antagonist concentrations according to the equation $[B]/(A'/A+1)=K_d$ where B is antagonist concentration, and A and A' are agonist concentrations giving equivalent responses in the absence and presence of antagonist; n=4–7 for each value.

The adrenoceptor present on submucosal arterioles appeared to be homogeneous based on K$_d$ determinations of the inhibition of norepinephrine or phenylephrine vasoconstrictions by the $\alpha_1$-adrenoceptor antagonist prazosin (Table 1). Prazosin produced a parallel, rightward shift in the norepinephrine or phenylephrine dose-response curve, and Schild analysis yielded slopes that did not differ from unity, indicating that prazosin acts as a competitive antagonist at these submucosal $\alpha_1$-adrenoceptor sites. One example of the results obtained in submucosal arterioles with norepinephrine and prazosin is shown in Figure 4. The K$_d$ values we obtained for prazosin (0.075–0.1 nM) in submucosal arterioles are the same as those found in numerous other vascular tissues,32,33

**Responses to Vasopressin**

Vasopressin produced dose-dependent vasoconstrictions in all submucosal arterioles; maximum vasopressin responses were approximately equivalent to maximum norepinephrine constrictions in all arterioles except those from the rabbit ileum, where the mean maximal response was 50% of the norepinephrine response (Figures 5 and 6). EC$_{50}$ values for vasopressin ranged from 0.5 nM in the guinea pig colon to 11 nM in the rabbit ileum (Table 1). Vasopressin responses were not altered significantly

### Table 1. Summary of Vasoconstrictor Responses in Isolated Submucosal Arterioles in the Gastrointestinal Tract

<table>
<thead>
<tr>
<th>Arterioles from</th>
<th>% Samples with oscillatory response</th>
<th>NE EC$_{50}$ (µM)</th>
<th>PE EC$_{50}$ (µM)</th>
<th>Prazosin K$_d$ (nM)</th>
<th>AVP EC$_{50}$ (nM)</th>
<th>d(CH$_2$)$_5$Tyr (Me) AVP K$_d$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GP ileum</td>
<td>0 (24)</td>
<td>0.6</td>
<td>1.4</td>
<td>0.08</td>
<td>1.1</td>
<td>1.6</td>
</tr>
<tr>
<td>GP colon</td>
<td>66 (12)</td>
<td>0.6</td>
<td>1.6</td>
<td>0.09</td>
<td>0.5</td>
<td>3.2</td>
</tr>
<tr>
<td>Rabbit ileum</td>
<td>12 (17)</td>
<td>0.8</td>
<td>...</td>
<td>0.12*</td>
<td>10.8</td>
<td>0.06</td>
</tr>
<tr>
<td>Rabbit colon</td>
<td>100 (17)</td>
<td>0.54</td>
<td>1.1</td>
<td>0.09*</td>
<td>1.1</td>
<td>0.05–0.1*</td>
</tr>
<tr>
<td>Human colon</td>
<td>100 (7)</td>
<td>0.7</td>
<td>...</td>
<td>...</td>
<td>1.2</td>
<td>...</td>
</tr>
</tbody>
</table>

(n=14). Thus, the latency to onset of vasoconstriction by norepinephrine, phenylephrine, and U46619 was about 1 second from time of drug arrival, whereas the onset to vasoconstriction by vasopressin was not less than 30 seconds. The inclusion of peptidase inhibitors (see "Materials and Methods") in the superfusion solution did not alter the time course of response to vasopressin (n=4). A similarly prolonged time to onset of vasoconstriction was observed with both angiotensin II (n=3) and endothelin (n=2), but it was noted that the onset of the vasodilation in response to the peptide substance P (19±0.2 seconds, n=12 in guinea pig ileal arterioles) did not differ from that in response to acetylcholine or muscarine in these same vessels (see also References 18 and 21).

**Responses to Norepinephrine and Phenylephrine**

Table 1 lists the EC$_{50}$ values for submucosal arteriolar vasoconstrictions to norepinephrine and phenylephrine; it can be seen that these values are approximately equivalent for each vascular network examined. The maximum response to norepinephrine was the same as the vasoconstriction to high potassium solution, and so, all dose-response curves were subsequently expressed as percent of maximum norepinephrine constriction.
when peptidase inhibitors were included in the perfusion fluid (n = 4); in two guinea pig colonic arterioles, 3 nM vasopressin produced a vasoconstriction of 89% and 89% of maximum before addition of peptidase inhibitors and 89% and 91% of maximum after inclusion of inhibitors. Similarly, EC₅₀ values for vasopressin in guinea pig ileal submucosal arterioles were 0.9 nM before and 1.0 nM after inclusion of peptidase inhibitors (n = 2).

Several experiments were performed in which the effects of gossypol, an irreversible inhibitor of endothelium-derived relaxing factor (EDRF) release and/or synthesis, on the vasopressin and norepinephrine constrictions were examined. Gossypol (1–3 µM) did not alter the dose-response curves to either of these vasoconstrictor substances (n = 5 for each substance). These concentrations of gossypol were considered effective in inhibiting EDRF release because they produced irreversible enhancement of the nitroprusside vasodilation. We have previously found that higher concentrations of gossypol block all vasoconstrictor responses in guinea pig ileal submucosal arterioles; we have similarly observed this nonselective blockade of responses by high concentrations of gossypol in the other submucosal arterioles examined in this study (n = 4).

**Characterization of the Vasopressin Receptor Present in Submucosal Arterioles**

The V₁ receptor antagonist d(CH₃)₅ Tyr (Me) AVP inhibited the vasopressin constrictions and shifted the vasopressin dose-response curves to the right in all submucosal arterioles (Figures 7 and 8). The inhibition was competitive in guinea pig submucosal arterioles with Kᵦ values (1–3 nM) for this antagonist being the same in both ileal and colonic arterioles of this species (Table 1).

d(CH₃)₅ Tyr (Me) AVP was 10–50-fold more potent in inhibiting vasopressin responses in rabbit submucosal arterioles. This inhibition was competitive when concentrations of d(CH₃)₅ Tyr (Me) AVP less than 10 nM were used, but higher antagonist concentrations produced an insurmountable inhibition of the vasopressin response. These results demonstrated the adequacy and usefulness of our diameter tracking method to carry out quantitative pharmacological studies on isolated gastrointestinal microvessels.
restricted with U46619 or phenylephrine in the presence of 10 nM d(CH2)5 Tyr (Me) AVP to block the V1 vasoconstrictor response. Vasopressin (1–30 nM) applied to these V1-blocked preconstricted vessels also did not produce any vasodilation (n=8).

**Discussion**

The main purposes of this study were to characterize vasoconstrictor properties of isolated submucosal arterioles in the mammalian GI microcirculation and to determine the vasopressin receptors present in these microvessels. We examined submucosal arterioles from guinea pig, rabbit, and human intestine and generally found that regional differences along the GI tract (i.e., ileal versus colonic vessels) were more prominent than were species differences.

**Vasoconstrictor Properties of Submucosal Arterioles**

Rhythmic vasoactivity, either spontaneous or in response to vasoconstrictor substances, has been described in several vessels and has most often been associated with arteries from hypertensive animals, primarily small resistance arteries and arterioles. Submucosal arterioles examined in this study did not display resting spontaneous rhythmic vasomotion at physiological temperatures (35–37° C), but the finding that they became rhythmically vasoactive at increased temperature demonstrates that all submucosal arterioles have the capacity to exhibit this type of mechanical activity. On the other hand, colonic submucosal arterioles, but not ileal arterioles, showed this characteristic rhythmic pattern of constriction–dilation (“phasic” activity) superimposed on a sustained constriction (“tonic” activity) when vasoconstrictor substances were applied (Figure 2). Thus, the same stimulus may produce a differential response along the length of the GI microcirculation; this region-dependent response appears to be species independent. Our results would tend to support previous hypotheses that rhythmic vasomotion is an intrinsic property of small resistance vessels, which may play a physiological role in the microcirculation. It is interesting that the characteristic frequency of vasomotor oscillations that we found in human and rabbit colonic arterioles (Figure 3) and that has previously been described in in vivo studies of exteriorized rat submucosal arterioles is approximately the same as that reported for slow waves from visceral smooth muscle of human and rabbit colon. Neither slow waves in visceral smooth muscle nor the submucosal arteriolar oscillations we observed are neurogenic in origin, and the preparations of submucosal plexus used in the present study contained no visceral smooth muscle; thus, neither neural nor release of muscle-derived factors can be involved in the arteriolar oscillations. The mechanisms underlying the intrinsic vasomotion in these vessels remain to be elucidated, but the coincidence in frequency of visceral slow waves and arteriolar oscillatory behavior suggests a physiological interaction involving gut motility. An attractive explanation, albeit one based solely on indirect evidence, for the cellular mechanism underlying this characteristic rhythmic vasomotion is that there exists electrogenic pacemaker activity in arteriolar smooth muscle. The basis of such electrogenic activity is the presence of voltage-dependent, dihydropyridine-sensitive calcium currents and voltage-dependent, calcium-activated potassium currents in submucosal and other arteriolar smooth muscle. Electrophysiological studies on ileal submucosal arteriolar smooth muscle have shown a precise relation between norepinephrine-induced changes in membrane potential and arteriolar constriction; it will be important to combine arteriolar diameter tracking with intracellular recording from submucosal arteriolar muscle under conditions that produce rhythmic

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**FIGURE 6.** Summary of vasopressin constrictions in submucosal arterioles from guinea pig (gp) ileum (■) and colon (▲), rabbit (rb) ileum (○) and colon (▲), and human colon (○). Each point is mean ± SEM for three to eight experiments except for experiments with human arterioles (n=2). NE, norepinephrine.

**FIGURE 7.** Example of antagonism of vasopressin (AVP)-mediated vasoconstriction by d(CH2)5 Tyr (Me) AVP in guinea pig ileal arteriole. Cumulative concentration-constriction curves were obtained in this experiment in the absence (panel A) and presence of 10 nM (panel B) and 100 nM (panel C) d(CH2)5 Tyr (Me) AVP.
vasomotion (e.g., increased temperature in ileal arterioles and agonist applications in colonic arterioles).

Vasopressin Actions and Receptors in Submucosal Arterioles

Vasopressin was a potent vasoconstrictor of all submucosal arterioles except in the rabbit ileum; here vasopressin was 10-fold weaker, and the maximum was only half that observed in the other vessels (Figure 6). It is possible that this difference results from suboptimum preloads placed on the rabbit ileal vessels; however, this seems unlikely, because the norepinephrine vasoconstrictions were similar to those obtained in other submucosal arterioles. Differential agonist potencies are not unexpected in view of numerous similar results obtained in a variety of vascular beds\(^{17,19,36,45}\) and are usually the result of partial agonist actions or differences in receptor density or receptor-effector coupling mechanisms.\(^{46}\)

In contrast, our results with the selective \(V_1\) receptor antagonist \(d(\text{CH}_2)_5\text{Tyr}\) \((\text{Me})\) AVP suggests that there may be subtypes of the \(V_1\) receptor in submucosal arterioles of different species. That is, \(d(\text{CH}_2)_5\text{Tyr}\) \((\text{Me})\) AVP inhibited the vasopressin constrictions in all submucosal arterioles, and in each preparation this inhibition could be shown to be competitive over a given concentration range of the antagonist (Figures 8 and 9). Schild analyses showing unitary slopes and antagonist \(K_d\) values obtained from these data in guinea pig submucosal arterioles (1–3 nM) are consistent with the presence of a single type of receptor, the classical \(V_1\) receptor,\(^{36}\) on these submucosal arterioles (Figure 9). Similar analyses performed on rabbit submucosal arterioles showed significantly different \(K_d\) values (0.05–0.1 nM) for the same antagonist (Figure 9). We could find no evidence (e.g., Schild slopes differing from unity) that this resulted from the presence of more than one population of vasopressin receptor such as a mixed \(V_1-V_2\) response. \(d(\text{CH}_2)_5\text{Tyr}\) \((\text{Me})\) AVP also did not exhibit any properties of a partial agonist in rabbit (or guinea pig) submucosal arterioles. Our findings then are reasonably suggestive that the vasopressin receptor present in rabbit submucosal arterioles represents a distinct subtype of the \(V_1\) receptor. Evidence for the existence of two subtypes of \(V_1\) receptor has been presented in which \(V_{1a}\) is the common receptor subtype and \(V_{1b}\) is a novel subtype present in adenohypophyseal tissue.\(^{19,20}\) The results we obtained in rabbit submucosal arterioles with \(d(\text{CH}_2)_5\text{Tyr}\) \((\text{Me})\) AVP make it unlikely that the vasopressin receptor in these vessels is of the \(V_{1b}\) subtype; \(d(\text{CH}_2)_5\text{Tyr}\) \((\text{Me})\) AVP and its related analogues were less effective in inhibiting vasopressin responses in adenohypophyseal tissue,\(^{20}\) but this compound was more effective as a blocker in rabbit submucosal arterioles. Further experiments with a number of compounds will be required to substantiate the existence of a distinct \(V_1\) receptor subtype in rabbit submucosal arterioles.

![Figure 8. Summary of inhibition of vasopressin responses by \(d(\text{CH}_2)_5\text{Tyr}\) \((\text{Me})\) AVP in guinea pig (GP) (panels A and B) and rabbit (panels C and D) submucosal arterioles. Each point is mean±SEM of three to six experiments.](image)

![Figure 9. Schild analysis of results obtained with the \(V_1\) vasopressin antagonist \(d(\text{CH}_2)_5\text{Tyr}\) \((\text{Me})\) AVP. Linear regression slopes (1.02, 1.1) and \(pA_2\) values (8.8, 8.5) obtained in guinea pig (gp) ileal and colonic arterioles were not significantly different from each other. The \(pA_2\) value for this antagonist in rabbit ileal arteriole was 10.2 with a slope (1.08) that was not significantly different from one.](image)
There have been several reports implicating an interaction between vasopressin as well as norepinephrine and EDRF.\textsuperscript{47-49} Specifically that these agonists can lead to a release of EDRF so that the presence of an intact endothelium the agonist-mediated constriction is considerably less than when endothelium is absent. Submucosal vessels are too small and fragile to permit mechanical disruption of their endothelium or other methods that rely on flushing detergent-type solutions through their lumen,\textsuperscript{22} which meant that we had to rely on pharmacological manipulations to examine possible effects of endothelium on vasopressin responses. Gossypol has been shown to block EDRF actions in many vascular tissues\textsuperscript{34} when applied to the serosal surface, including guinea pig ileal submucosal arterioles;\textsuperscript{22} our results with gossypol would tentatively suggest that EDRF does not play a role in vasoconstrictor responses produced by vasopressin or norepinephrine in submucosal arterioles. It deserves to be emphasized that further studies with several different pharmacological manipulations designed to examine vasomotor roles of the endothelium in submucosal arterioles will be necessary before conclusions concerning the role of endothelium in these arterioles can be drawn.

General Physiological Significance

Results from this and previous studies of submucosal arterioles with the Diamtrak video-monitoring system\textsuperscript{16,22,25} show that these preparations and this method can be a valuable addition to in vitro microvascular research of the GI vasculature. The findings that concentration-response curves to norepinephrine were the same in vessels with and without increased intraluminal pressure (Figure 1) indicate that the isolated, stretched preparations of submucosal plexus provide sufficient active stress, or preload, to the arteriolar walls to allow them to be considered to be within the range of optimum length. Further confidence can be drawn from the observations that little variability in responses to vasoconstrictor agonists were observed from preparation to preparation (e.g., Figures 4, 6, and 8) and that norepinephrine, phenylephrine and vasopressin EC\textsubscript{50} values determined in submucosal arterioles in this study are very similar to values obtained in prearteriolar mesenteric arteries from these and other species.\textsuperscript{18,24,26,27} There are limitations to the use of this outside diameter video-tracking method, the most significant being the inability to accurately estimate vascular resistance changes that may result from thickening or thinning of the arteriolar wall; this limitation will generally preclude studies dealing with vascular muscle mechanics (see References 4 and 29) but should not interfere with, or alter the validity of, experiments designed to examine the ability of nerve-released or exogenously applied substances to produce vasoconstrictions or vasodilations. In this regard, a significant advantage to the use of submucosal arteriolar preparations is that there are minimal diffusion barriers to applied drugs; total wall thickness is less than 10 \(\mu\)m, and there is only one layer of circularly oriented smooth muscle and one layer of endothelial cells.\textsuperscript{15}

Changes in blood flow in the mesenteric-splanchnic circulation can contribute up to one third of total peripheral resistance\textsuperscript{1-3}; the submucosal-mucosal arterioles are the final resistance vessels in this vascular network, and they have been estimated to contribute up to 40% of total mesenteric-splanchnic resistance.\textsuperscript{1} Thus, submucosal arterioles may play a significant role in systemic blood pressure in addition to their major contribution to GI microcirculation and vascular perfusion of intestinal mucosa. This study has provided the first characterization of vasoconstrictor properties of isolated submucosal arterioles in different regions of the GI tract of guinea pigs, rabbits, and humans. We found that vasoconstrictor responses in submucosal arterioles from the small intestine differ from those in the large intestine, the former showing smooth, sustained constrictions, and the latter showing regular, rhythmic oscillations. We also determined the pharmacological profile of vasopressin actions in submucosal arterioles; these actions were all \(V_1\) (guinea pig arterioles) or \(V_2\)-like (rabbit arterioles) vasoconstrictions. Alterations in both patterns of vasomotive rhythmicity and in vasopressin actions in the mesenteric vasculature have been reported to occur in hypertensive animals.\textsuperscript{18,37,38,50} Studies similar to those reported here, carried out on isolated submucosal arterioles obtained from controlled animal models of hypertension as well as from humans with hypertensive diseases, should contribute to a more quantitative description of the changes that may be expected to occur in GI microvessels as a consequence of chronic hypertension.

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S Vanner, M M Jiang, V L Brooks and A Surprenant

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