Differential Localization of P2 Receptor Subtypes in Mesenteric Arteries and Veins of Normotensive and Hypertensive Rats

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ABSTRACT

ATP acts at P2 receptors to contract blood vessels and reactivity to vasoconstrictor agents is often altered in hypertension. This study was designed to identify P2 receptors in mesenteric arteries and veins and to determine whether ATP reactivity is altered in deoxycorticosterone acetate (DOCA)-salt hypertensive rats. Computer-assisted video microscopy was used to measure vessel diameter in vitro. ATP was a more potent constrictor of veins (EC50 = 2.7 μM) than arteries (EC50 = 196 μM) from normotensive rats; there was no change in ATP reactivity in vessels from DOCA-salt rats. The P2X1 receptor agonist α,β-methylene ATP (α,β-MeATP, 0.03–3 μM) contracted arteries but not veins. ATP-induced contractions in arteries were blocked by α,β-MeATP-desensitization. There are seven P2X subtypes each containing two membrane spanning domains (Fredholm et al., 1994). P2X receptors are localized to arterial smooth muscle cells where they mediate contraction (Lewis et al., 1998; Hansen et al., 1999). Furthermore, neurogenic contractions of many arteries are inhibited by antagonists that block P2X1 receptors or by selective P2X1 receptor desensitization. Pyridoxal-phosphate-6-azophenyl-2',4-disulfonic acid (PPADS) is an antagonist that blocks P2X1 receptors (Ziganshin et al., 1994) and α,β-MeATP is an agonist at P2X1 receptors (Surprenant and North, 2000). Because P2X1 receptors desensitize rapidly, α,β-MeATP-induced desensitization can be used to identify responses mediated at P2X1 receptors.

There are two classes of receptors for ATP: P2X and P2Y receptors (Fredholm et al., 1994). P2X receptors are ligand-gated channels that mediate fast and, in some cases, rapidly desensitizing responses. There are seven P2X subunits each containing two membrane spanning domains (Fredholm et al., 1994; North and Surprenant, 2000). P2X1 receptors are localized to arterial smooth muscle cells where they mediate contraction (Lewis et al., 1998; Hansen et al., 1999). Furthermore, neurogenic contractions of many arteries are inhibited by antagonists that block P2X1 receptors or by selective P2X1 receptor desensitization. Pyridoxal-phosphate-6-azophenyl-2',4-disulfonic acid (PPADS) is an antagonist that blocks P2X1 receptors (Ziganshin et al., 1994) and α,β-MeATP is an agonist at P2X1 receptors (Surprenant and North, 2000). Because P2X1 receptors desensitize rapidly, α,β-MeATP-induced desensitization can be used to identify responses mediated at P2X1 receptors.

P2Y receptors are a family of G-protein-coupled receptors (Fredholm et al., 1997). In the vasculature, ATP can act at P2Y1, P2Y2, or P2Y4 receptors to alter vasomotor tone (Kunapuli and Daniel, 1998). 2-Methylthio-ATP (2-Me-S-ATP) is a more potent agonist than ATP at P2Y1 receptors than other classes of P2Y receptors (O’Connor et al., 1991) and PPADS blocks P2Y1 receptors (Ralevic and Burnstock, 1996) but not P2Y2 or P2Y4 receptors (Charlton et al., 1996a,b; Bogdanov et al., 1998). UTP is inactive at P2Y1 receptors, whereas UTP and ATP are equipotent as agonists at rat P2Y2 and P2Y4 receptors (Bogdanov et al., 1998; Webb et al., 1998; Williams and Jarvis, 2000). However, suramin blocks P2Y2 but not P2Y4 receptors and can be used to discriminate responses mediated at these two receptors (Bogdanov et al., 1998). Although it is known that ATP contracts veins (Ohara et al., 1998), the receptor mechanism mediating this response has not been clearly established.

Changes in reactivity to vasoconstrictor substances often occur in tissues obtained from hypertensive animals. However, there are marked differences in the direction of the change (increase, decrease, or no change) that can depend on the hypertension model and on the blood vessels or vascular bed studied. For example, there is an increased sensitivity to

ABBREVIATIONS: PPADS, pyridoxal-phosphate-6-azophenyl-2',4-disulfonic acid; α,β-MeATP, α,β-methylene adenosine triphosphate; 2-Me-S-ATP, 2-methylthioadenosine triphosphate; SHR, spontaneously hypertensive rat; DOCA, deoxycorticosterone acetate; PBS, phosphate-buffered saline.
the contractile effects of norepinephrine in thoracic aortae taken from spontaneously hypertensive rats (SHRs) (Lograno et al., 1989), whereas there are no changes in norepinephrine sensitivity in mesenteric arteries from SHRs (Naito et al., 1998). However, mesenteric arteries from two kidney, one clip or one kidney, one clip (Deng and Schiffrin, 1991) or deoxycorticosterone-acetate (DOCA)-salt hypertensive rats (Suzuki et al., 1994) show enhanced norepinephrine reactivity compared with arteries taken from normotensive rats. There is also variation in reactivity to ATP in veins from hypertensive animals or humans. Mesenteric veins, but not arteries, from SHRs exhibit increased reactivity to ATP (Naito et al., 1998), whereas cutaneous hand veins from hypertensive human subjects were less reactive to α,β-MeATP compared with responses in tissues from normotensive subjects (Lind et al., 1997). Therefore, it not clear whether there is a general change in vascular reactivity in hypertension or whether changes are specific for individual vascular beds and the hypertensive model. The purpose of the present study was 2-fold. First, these studies were done to identify the receptor mechanism mediating ATP-induced contractions of mesenteric veins and to compare this receptor mechanism with that in mesenteric arteries. Second, these studies were done to determine whether there is change in reactivity to ATP in mesenteric arteries and veins in the DOCA-salt model of experimental hypertension in rats. These latter studies were undertaken because sympathetic tone to the venous side of the circulation is elevated in DOCA-salt hypertensive rats (Fink et al., 2000). As ATP is released from perivascular sympathetic nerves (Burnstock et al., 1991) or deoxycorticosterone-acetate (DOCA)-salt hypertensive rats (Fink et al., 1990) to the mesenteric veins, but not arteries taken from spontaneously hypertensive rats (Suzuki et al., 1994) show enhanced norepinephrine sensitivity in mesenteric arteries and veins in the DOCA-salt model of experimental hypertension in rats. These latter studies were undertaken because sympathetic tone to the venous side of the circulation is elevated in DOCA-salt hypertensive rats (Fink et al., 1990). As ATP is released from perivascular sympathetic nerves (Burnstock and Kennedy, 1986; Kennedy, 1996), there may be changes in venous reactivity to ATP.

Materials and Methods

DOCA-Salt Hypertension. Male Sprague-Dawley rats (Charles River, Inc., Portage, MI) weighing 175 to 225 g were maintained according to standards approved by the Michigan State University All-University Committee on Animal Care and Use. Standards were in strict accordance with Michigan State University and National Institutes of Health animal care guidelines. All rats were kept in a light- and temperature-controlled room and housed in clear plastic boxes in groups of three with free access to standard pelleted rat chow (HarlanTeklad 8640 Rodent Diet) and tap water.

After arrival in the animal care facility, rats were acclimatized to their environment for 2 days before surgical manipulation. DOCA-salt hypertension was induced by using established methods (Ormsbee and Ryan, 1973). Briefly, rats were unilaterally nephrectomized under anesthesia with sodium pentobarbital (45 mg/kg; Abbott Laboratories, Chicago, IL) administered i.p. Bronchiolar secretions were controlled by administration of atropine sulfate i.p. (0.04 mg/kg; Sigma, St. Louis, MO). Silicone rubber patches (Dor Cowring, Fendale, MI) impregnated with DOCA (Sigma) were implanted s.c. in rats providing DOCA at 150 mg/kg. Postoperative analgesia was provided by a single injection of butorphanol tartrate s.c. (0.5 mg/kg; Abbott Laboratories). All DOCA-implanted rats were placed on saltwater containing 1% NaCl and 0.2% KCl (DOCA-salt). Normotensive control rats (SHAM) were unilaterally nephrectomized and placed on tap water. All rats were provided standard pelleted rat chow. Blood pressure was measured using the tail cuff method 4 weeks after surgery and rats were used for in vitro studies at this time.

Measurement of Venoconstriction in Vitro. Rats were killed using a lethal pentobarbital injection. The ileum was removed from the animal and placed in oxygenated (95% O2, 5% CO2) Krebs’ solution of the following composition: 117 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl2, 1.2 mM MgCl2, 25 mM NaHCO3, 1.2 mM NaH2PO4, and 11 mM glucose. An ileal segment was placed in a Petri dish and the mesentery was stretched gently and pinned flat. A section of mesentery close to the ileal wall was carefully cut free from the intestine and the mesentery was then transferred to a small silastic-lined recording bath (1.5-ml volume). Mesenteric fat was carefully dissected away from a secondary artery or vein to expose the edges of the blood vessel. The recording chamber was mounted on the stage of an inverted microscope (Olympus CK-2) and the chamber was superperfused continuously with warm (36°C) Krebs’ solution at a flow rate of 7 ml/min. The output of a black and white video camera (KP-111; Hitachi, Tokyo, Japan) attached to the microscope was fed to a PCVision Plus frame-grabber board (Imaging Technology Inc., Bedford, MA) mounted in a personal computer. The video images were analyzed using Diamtrak software (Nield, 1989), which tracks the distance between the outer edges of the blood vessel in the observation field. The digitized signal was converted to an analog output (DAC-02 board; Keithley Metabyte, Taunton, MA) and fed to a strip chart recorder (EasyGraph; Gould Inc., Cleveland, OH). The sampling rate was 10 Hz and changes in blood vessel diameter of 0.5 μm were resolved.

Experimental Protocols. After mounting on the microscope stage and beginning superfusion of Krebs’ solution, the preparations were allowed to equilibrate for 20 to 30 min. During this time, arteries and veins relaxed to a stable resting diameter of between 150 and 220 μm. Drugs were added in known concentrations to the superfusing Krebs’ solution and were applied using an array of three-way stopcocks. In most experiments, agonists were applied for 2 to 4 min and there was a 10-min interval between successive applications of agonist. A single agonist concentration-response curve, either in the absence or presence of antagonist was obtained in each preparation. Antagonists were applied for a minimum of 20 min before testing agonist effects in the continued presence of antagonist. Concentrations of antagonists causing half-maximal inhibition of ATP-induced contractions were obtained by testing increasing concentrations of antagonist, applied in a cumulative manner, against a maximum ATP concentration (100 μM in veins, 1 mM in arteries).

Data Analysis. Agonist-induced contractions were measured in micrometers and are expressed as a percentage of the initial resting diameter of the blood vessel. Half-maximal effective agonist concentrations (EC50) and maximum responses (Ymax) were calculated from a least-squares fit of individual agonist concentration response curves using the following logistic function from Origin 5.0 (Microcal Software Inc., Northampton, MA):

\[ Y = \left( Y_{\text{min}} - Y_{\text{max}} \right) \left( 1 + \left( x / EC_{50} \right) \right) + Y_{\text{max}} \]

where Ymin and n are the minimum response and slope factor, respectively. All data are expressed as the mean ± S.E.M. Differences between groups were assessed by the Kruskal-Wallis nonparametric analysis of variance and Dunn’s multiple comparison test using GraphPad InStat, version 3.0, for Windows 95 (GraphPad Software, San Diego, CA). The n values refer to the number of animals from which the data were obtained.

Immunohistochemical Procedures. Mesenteric arcades were stretched tightly in a Sylgard-lined Petri dish using insect pins. A 30-gauge hypodermic needle was used to cannulate the primary vein and the arcade was flushed with phosphate-buffered saline (PBS, 0.01 M, pH 7.2). The tissues were then stretched tightly on small piece of balsa wood using insect pins and were immersed in Zamboni fixative (4% formaldehyde, 2% picric acid in 0.1 M phosphate buffer, pH 7.4) overnight at 4°C. The tissues were then cleared three times at 10-min intervals with dimethyl sulfoxide and washed three times at 10-min intervals with PBS. Mesenteric arteries and veins were then dissected from mesenteric fat and incubated overnight in dilute (1:200 in PBS) antisera raised in rabbits against amino acids 392 to 399 of the rat P2X2 receptor sequence (Alomone Laboratories, Jerusalem, Israel). Tissues were washed in PBS and then incubated...
with goat anti-rabbit IgG conjugated to tetramethyl rhodamine isothiocyanate (1:40 dilution in PBS; Chemicon International, Temecula, CA) or to fluorescein isothiocyanate (1:40 dilution in PBS; Sigma) for 1 h. The tissues were washed again with PBS and then mounted on microscope slides and coverslipped using buffered glycerol (pH 8.6). Control studies were conducted by omitting the primary antibody from the protocol or by incubating tissues with primary antibody that had been preincubated for 1 h with 1 μg of the antigen peptide (obtained from Alomone Laboratories). Fluorescent images were acquired using a Leitz Laborlux S upright microscope, a PL Fluorar 40× objective (0.7 numerical aperture), a SPOT-2 cooled color digital camera (Diagnostic Instruments, Sterling Heights, MI), and Adobe Photoshop 5.5 software (Adobe Systems, Inc., San Jose, CA).

**Drugs.** 2-Methylthio-ATP and PPADS were obtained from Research Biochemicals International (Natick, MA). All other drugs were obtained from Sigma.

### Results

Data were obtained from 51 SHAM rats and 45 DOCA-salt rats. The mean arterial pressure for SHAM rats was 126 ± 7 mm Hg, whereas the mean arterial pressure for DOCA-salt rats was 193 ± 5 mm Hg. The outside diameter of mesenteric arteries and veins examined in this study ranged between 150 and 230 μm.

**ATP Contracts Mesenteric Veins and Arteries.** ATP produced concentration-dependent contractions of mesenteric veins and arteries from SHAM and DOCA-salt rats. Contractions caused by ATP in arteries desensitized almost completely in the presence of higher concentrations of ATP, whereas in veins the contractions desensitized more slowly and incompletely (Fig. 1). Comparison of ATP concentration-response curves obtained in arteries and veins revealed that ATP was 20- to 70-fold more potent in contracting veins compared with arteries but the maximum contraction caused by ATP was greater in arteries than in veins (Fig. 2; Table 1).

There were no differences in ATP concentration-response curves obtained in veins taken from SHAM rats compared with those obtained from DOCA-salt rats. Similarly, there were no differences between ATP concentration-response curves in arteries from SHAM rats compared with concent-

**Fig. 1.** Representative traces of ATP-induced contractions of mesenteric arteries and veins from SHAM rats. A, responses obtained in a mesenteric artery desensitized in the continued presence of ATP. B, responses in a mesenteric vein were smaller in peak amplitude but were largely maintained throughout the period of ATP application. For both figures, ATP was applied at the indicated concentrations during the period indicated by the bar above each trace.

**Fig. 2.** Concentration-response curves for ATP-induced contractions of mesenteric arteries and veins in preparations taken from SHAM normotensive and DOCA-salt hypertensive rats. ATP was more potent in contracting veins than arteries but there were no differences in sensitivity to ATP associated with DOCA-salt hypertension. Data are mean ± S.E.M. and n indicates the number of animals from which the data were obtained.

### Table 1

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<th>Artery</th>
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<td>SHAM</td>
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<td>SHAM</td>
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<th>EC50 (μM)</th>
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<tr>
<td>196 ± 64</td>
<td>2.8 ± 0.9a</td>
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<tr>
<td>159 ± 51</td>
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<td>57.3 ± 2.2</td>
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<td>61.2 ± 5.3</td>
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*Significantly different from EC50 values obtained in arteries.

**α,β-MeATP Contracts Arteries but Not Veins.** The above-mentioned data indicate that arteries and veins are differentially sensitive to the contractile effects of ATP and this difference could be due to differential expression of P2 receptor subtypes in mesenteric arteries and veins. It has been shown previously that rat mesenteric arteries express the P2X1 receptor subtype and α,β-MeATP is an agonist at this receptor (Lewis et al., 1998). Therefore, α,β-MeATP concentration-response curves were obtained in arteries and veins to determine whether these blood vessels were differentially sensitive to this agonist. α,β-MeATP caused a concentration-dependent contraction of mesenteric arteries but there were no differences in the curves obtained in tissues from SHAM and DOCA-salt rats (Fig. 3). The α,β-MeATP EC50 values obtained from SHAM and DOCA-salt tissues were 0.3 ± 0.1 μM (n = 7) and 0.9 ± 0.4 μM (n = 5), respectively (P > 0.05). The maximum responses caused by α,β-MeATP were not different between SHAM and DOCA-salt arteries (Fig. 3). α,β-MeATP (up to 3 μM) did not cause more than 10% contraction in veins from either SHAM or DOCA-salt rats (Fig. 3).

A cross-desensitization protocol was used to determine
whether $\alpha,\beta\text{-MeATP}$ and ATP were acting at the same receptor to cause contraction of mesenteric arteries. A control response to a maximum concentration of ATP (1 mM) was obtained (Fig. 4A, left). After ATP washout and recovery, $\alpha,\beta\text{-MeATP}$ (3 $\mu$M) was applied and the response was allowed to desensitize. In the continued presence of $\alpha,\beta\text{-MeATP}$-induced desensitization, ATP was reapplied (Fig. 4A, middle). In these experiments, the ATP-induced contraction was reduced by more than 80% after $\alpha,\beta\text{-MeATP}$-mediated desensitization (Fig. 4B). After washing out $\alpha,\beta\text{-MeATP}$ for 20 to 40 min, the ATP response fully recovered [Fig. 4, A (right)] and B.

**Fig. 3.** Concentration-response curves for the P2X receptor agonist $\alpha,\beta\text{-MeATP}$ in mesenteric arteries and veins in preparations taken from SHAM normotensive and DOCA-salt hypertensive rats. The data show that $\alpha,\beta\text{-MeATP}$ causes contractions of arteries but not veins and there were no differences in $\alpha,\beta\text{-MeATP}$ sensitivity between SHAM and DOCA arteries. Data are mean ± S.E.M. and n indicates the number of animals from which the data were obtained.

UTP but Not 2-Me-S-ATP Contracts Mesenteric Veins and Arteries. UTP and 2-Me-S-ATP can act as P2Y receptor agonists. Therefore, these drugs were used to determine whether P2Y receptors mediate contraction of mesenteric veins. In a concentration range (0.1–10 $\mu$M) that would activate P2Y receptors (Ralevic and Burnstock, 1996), 2-Me-S-ATP caused less than a 20% constriction of arteries or veins in tissues from SHAM and DOCA-salt rats (Fig. 5A). 2-Me-S-ATP (0.1–10 $\mu$M) concentration-response curves in SHAM arteries and veins were unaffected following pretreatment of tissues for 20 min with indomethacin (10 $\mu$M) and nitro-$l$-arginine (100 $\mu$M). At 10 $\mu$M, 2-Me-S-ATP caused a 16 ± 4% in veins (n = 7) and a 4.5 ± 3% contraction in arteries (n = 3) in indomethacin- and nitro-$l$-arginine-pre-treated tissues. These values were not different from those obtained in normal Krebs’ solution (Fig. 5A, P > 0.05). At concentrations greater than 10 $\mu$M 2-Me-S-ATP did cause contractions of mesenteric arteries studied in normal Krebs’ solution (data not shown).

UTP (1–300 $\mu$M) caused a concentration-dependent contraction of veins that was similar in amplitude to the con-

**Fig. 4.** ATP and $\alpha,\beta\text{-MeATP}$ exhibit cross desensitization in mesenteric arteries. A, representative traces of ATP- and $\alpha,\beta\text{-MeATP}$-induced contractions of mesenteric arteries in the cross-desensitization protocol. In the presence of $\alpha,\beta\text{-MeATP}$-induced desensitization, ATP did not contract arteries. The ATP response recovered 20 min after $\alpha,\beta\text{-MeATP}$ washout. B, pooled data from experiments shown in A, demonstrating that $\alpha,\beta\text{-MeATP}$-induced desensitization significantly inhibited ATP-induced contractions in a reversible manner.

![Fig. 4](https://example.com/f4.png)

**Fig. 5.** UTP but not 2-Me-S-ATP caused contractions of mesenteric veins. A, 2-Me-S-ATP concentration-response curves obtained in mesenteric arteries and veins. 2-Me-S-ATP produced less than 20% maximum contraction in all blood vessels. B, UTP concentration-response curves in mesenteric arteries and veins. Data in both figures are mean ± S.E.M. and n indicates the number of animals from which the data were obtained.
traction caused by ATP. UTP was equipotent in causing contractions of arteries and veins and there was no change in vascular UTP sensitivity associated with DOCA-salt hypertension (Fig. 5B). The EC$_{50}$ values for UTP in SHAM and DOCA-salt veins were $15 \pm 4$ ($n = 5$) and $35 \pm 9$ ($n = 6$) $\mu$M, respectively. The UTP EC$_{50}$ value obtained in SHAM veins was not different from the ATP EC$_{50}$ values obtained in SHAM and DOCA veins ($P > 0.05$, Table 1). The UTP EC$_{50}$ value obtained in DOCA veins was significantly greater than ATP EC$_{50}$ values obtained in SHAM and DOCA veins ($P < 0.05$, Table 1). The EC$_{50}$ values for UTP in SHAM and DOCA-salt arteries were $24 \pm 5$ ($n = 5$) and $23 \pm 4$ ($n = 3$) $\mu$M, respectively. There were no differences in the maximum responses caused by UTP in arteries or veins (Fig. 5B).

**ATP and UTP Cross-Desensitize in Mesenteric Veins but Not Arteries.** The response caused by 300 $\mu$M ATP in mesenteric veins desensitized slowly and incompletely (Fig. 6). However, after the ATP response had declined to a stable plateau, the UTP (100 $\mu$M) response was markedly reduced in amplitude and the combined ATP/UTP contraction reached the amplitude of the contraction caused by UTP alone (Fig. 6). The control UTP contraction was 29.5 $\pm$ 2%, whereas after ATP desensitization the response amplitude was 18 $\pm$ 3% ($n = 5$, $P < 0.02$). The UTP response recovered to 26 $\pm$ 3% after ATP washout. In contrast, when $\alpha,\beta$-MeATP (3 $\mu$M) was used to desensitize P2X receptors in arteries, there was no cross-desensitization between the UTP and $\alpha,\beta$-MeATP (Fig. 7). In arteries, the control UTP (100 $\mu$M) response was 42 $\pm$ 9% and after $\alpha,\beta$-MeATP desensitization, the UTP response was 47 $\pm$ 4% ($n = 5$, $P > 0.05$).

**Antagonist Inhibition of ATP- and UTP-Induced Contractions in Arteries and Veins.** To further test the hypothesis that the P2 receptors mediating contractions of arteries and veins are different, PPADS and suramin were used in attempt to block ATP-induced contractions of mesenteric blood vessels. Increasing concentrations of PPADS (0.1–30 $\mu$M) were applied in a cumulative manner to test for inhibition of the contraction caused by maximum concentrations of ATP (1 mM in arteries, 100 $\mu$M in veins). PPADS produced a concentration-dependent inhibition of ATP-induced contractions in arteries (IC$_{50}$ = 4.8 $\pm$ 1.8 $\mu$M, $n = 4$) but not in veins (Fig. 8, A and B). PPADS also did not inhibit UTP (100 $\mu$M)-induced contractions of mesenteric veins (Fig. 8B). Increasing concentrations of suramin (3–300 $\mu$M) were applied in a cumulative manner in an attempt to block ATP- or UTP-induced (each at 100 $\mu$M) contractions of veins. It was found that suramin inhibited the contractions caused by ATP and UTP by a maximum of approximately 50% (Fig. 9).

**Immunohistochemical Localization of P2X$_1$ Receptors in Mesenteric Arteries but Not Veins.** The data summarized above suggest that there is a differential localization of P2 receptors in mesenteric arteries and veins and the arteries express a P2X$_1$ receptor subtype. An antibody raised against the rat P2X$_1$ receptor was used in an attempt to show that this receptor subtype was localized to vascular smooth muscle in mesenteric arteries but not veins. These studies revealed that mesenteric arteries exhibited P2X$_1$ immunoreactivity that was present in a punctate pattern in the wall of arteries but not veins (Fig. 10, observations were
obtained in artery and vein preparations from three SHAM rats and three DOCA-salt rats). There was no obvious difference in the staining pattern of arteries from SHAM or DOCA-salt rats. The staining was absent if the primary antibody was omitted from the staining protocol or if the primary antibody was preincubated with the antigen peptide.

**Discussion**

**Reactivity to ATP Is Not Altered in DOCA-Salt Hypertension.** It has been shown previously that reactivity to norepinephrine acting at $\alpha_1$-adrenergic receptors is increased in mesenteric arteries from DOCA-salt hypertensive rats (Ekas and Lukhandwala, 1980; Perry and Webb, 1988; Suzuki et al., 1994). As norepinephrine is released from sympathetic nerves associated with mesenteric arteries, increased vascular reactivity would result in increased vasoconstriction and elevated blood pressure (de Champlain, 1990). ATP is a cotransmitter released with norepinephrine from sympathetic nerves and ATP contracts arterial smooth muscle (Burnstock and Kennedy, 1986; Kennedy, 1996). Therefore, it might be expected that ATP reactivity would be increased in arteries from DOCA-salt hypertensive rats as occurs for norepinephrine. However, it was found that there were no differences in the sensitivity of mesenteric arteries to the contractile effects of ATP in tissues from DOCA-salt rats. Similar data have been obtained in mesenteric arteries from SHRs (Naito et al., 1998), indicating that an increase in arterial reactivity to ATP does not contribute to the increase in sympathetic tone to arteries in these two models of hypertension. Although there have been few studies of altered reactivity in veins from hypertensive animals, increased venuconstriction would cause a transient increase in cardiac output and also shift blood from the capacitance to the resistance side of the circulation. Both of these changes would increase blood pressure. Therefore, studies of venous reactivity are potentially important in identifying cardiovascular changes underlying hypertension. In the present study, it was shown that mesenteric veins contract in response to ATP but that there were no differences in reactivity between veins taken from SHAM or DOCA-salt rats. This is an important observation as venomotor tone is elevated in DOCA-salt hypertensive rats and this increased tone is due partly to increased sympathetic input to veins (Fink et al., 2000). These data suggest that, if ATP is a cotransmitter released with norepinephrine from sympathetic nerves associated with mesenteric veins, increases in venous smooth muscle reactivity to ATP do not contribute to increased venomotor tone in DOCA-salt hypertensive rats.

Although receptor mechanisms and functional responses caused by P2 receptor activation in tissues maintained in vitro or in situ have the subject of numerous studies, integrated hemodynamic responses to P2 receptor activation in vivo are less well defined. Activation of P2X receptors increases blood pressure and vascular resistance in hindlimb, mesenteric, and renal circulations in anesthetized rats (Cox and Smits, 1996). Alternatively, P2Y receptor activation following treatment of anesthetized rats with 2-Me-S-ATP decreases blood pressure and vascular resistance (Cox and Smits, 1996). Blockade of P2 receptors following intravenous administration of PPADS to anesthetized rats increased mean arterial pressure fluctuations, suggesting that ATP released from sympathetic nerves functions in part to stabilize arterial pressure (Golubinskaya et al., 1999). Taken together, these data suggest an important contribution of P2X receptor function to the control of arterial diameter and sys-
tomic blood pressure (Golubinskaya et al., 1999). The contribution of P2 receptor activation on venous smooth muscle to integrated venous function and overall hemodynamics has not been studied.

**Differential Localization of P2 Receptors in Arteries and Veins.** ATP acts at P2X receptors to contract arterial smooth muscle (Lewis et al., 1998). These conclusions are supported by data from the present study that show ATP-induced contractions desensitize rapidly, are mimicked by α,β-Me-ATP, cross-desensitize with α,β-Me-ATP, and are blocked by PPADS. These are all properties of responses mediated at vascular P2X receptors (Fredholm et al., 1994; Ziganshin et al., 1994; Kunapali and Daniel, 1998). However, the receptor mechanism for ATP-induced contractions of veins was different. First, ATP responses in veins desensitized slowly and incompletely. Second, α,β-Me-ATP did not contract mesenteric veins and ATP-induced contractions in mesenteric veins were resistant to inhibition by PPADS. Finally, immunohistochemical studies demonstrated the presence of P2X1 immunoreactivity in mesenteric arteries, as also shown by Hansen et al. (1999), but not veins. These data indicate that mesenteric veins do not express P2X1 receptors.

Several P2Y receptor subtypes are present in the vasculature. Biochemical and molecular biological studies have shown that P2Y1, P2Y2, and P2Y4 receptors are expressed by endothelial cells and these receptors couple to the production and release of vasodilator prostanooids and nitric oxide (Kunapali and Daniel, 1998). Data from functional studies in the rat mesenteric vasculature indicate that P2Y1 and P2Y2 receptors couple to activation of nitric-oxide synthase (Ralevic and Burnstock, 1991, 1996). P2Y2 and P2Y4 receptors are expressed by vascular smooth muscle cells and stimulation of these receptors causes smooth muscle contraction (Kunapali and Daniel, 1998). The data presented here are consistent with localization of P2Y4 receptors to the smooth muscle layer of rat mesenteric veins. This conclusion is based on the following observations. 2-Me-S-ATP is a more potent agonist than ATP at P2Y1 receptors (Fredholm et al., 1994) but 2-Me-S-ATP at concentrations that would activate P2Y2 receptors (Ralevic and Burnstock, 1996; Barnard, 2000) produced little or no response in mesenteric veins. High concentrations (>10 μM) of 2-Me-S-ATP contracted mesenteric arteries. However, because ATP and 2-Me-S-ATP are equipotent at stimulating P2X1 receptors (Surprenant and North, 2000), contractions caused by high concentrations of 2-Me-S-ATP were likely due to an action at P2X1 receptors. As P2Y1 receptors on endothelial cells can cause release of vasodilator prostanooids and/or nitric oxide (see above), it is possible that contractions caused by 2-Me-S-ATP acting at P2Y1 receptors were masked by a simultaneous vasodilatory response. However, 2-Me-S-ATP concentration-response curves were unaffected following pretreatment of tissues with indomethacin to block cyclooxygenase and nitro-L-arginine to block nitric-oxide synthase. Finally, PPADS can block P2Y1 receptors (Ralevic and Burnstock, 1996) but ATP-induced contractions of mesenteric veins were resistant to PPADS antagonism. Taken together, these data lead to the conclusion that that smooth muscle cells in rat mesenteric veins do not express contractile P2Y1 receptors.

UPT is equipotent with ATP as an agonist at P2Y2 receptors and rat P2Y4 receptors (Fredholm et al., 1994; Bogdanov et al., 1998) and in the present study UTP and ATP were equipotent in contracting veins from SHAM rats. Suramin blocks P2Y4 but not P2Y1 receptors (Charlton et al., 1996a,b; Bogdanov et al., 1998) and this antagonist was used to identify the contractile P2Y2 receptor in mesenteric veins. It was found that suramin partly inhibited contractions of mesenteric veins caused by both ATP and UTP. Finally, responses caused by ATP and UTP in mesenteric veins showed cross-desensitization, indicating that these agonists acted at the same receptor site. Overall, these data indicate that in the rat mesentery ATP acts at P2Y2 receptors to cause venucrostriction. Ohara et al. (1998) concluded previously that P2U receptors mediate ATP-induced contractions of perfused mesenteric veins from the rat. P2U receptors are now classified as P2Y2 receptors (Communi and Boeynaems, 1997). Because suramin produced only a partial inhibition of ATP- and UTP-induced contractions of mesenteric veins, it is likely that another P2 receptor type contributes to these responses as well.

**UTP Responses in Mesenteric Arteries.** UTP caused contractions of mesenteric arteries that persisted after α,β-Me-ATP-induced desensitization of P2X1 receptors. These data, and those published previously by others (Juul et al., 1992), suggest that UTP acts at a P2 receptor in mesenteric arteries to cause contractions. There is evidence for a UTP preferring “pyrimidine” receptor in sympathetic neurons (Connolly, 1994), cardiac endothelial cells (Yang et al., 1996), and in the rat aorta (Garcia-Velasco et al., 1995). However, the UTP-prefering receptor does not appear to be a member of a separate class of receptors and it is likely to be a P2Y receptor subtype (Communi and Boeynaems, 1997). ATP is a cotransmitter released from peri-arterial sympathetic nerves and P2X1 receptors are the target for nerve-released ATP in mesenteric arteries. The ATP-insensitive, UTP-prefering receptor in mesenteric arteries may be activated by vasoactive nucleotides released by nonadrenergic perivascular nerves or by non-neuronal sources.

**Conclusions.** The data presented here indicate that ATP contracts mesenteric veins and arteries and that reactivity to ATP in mesenteric arteries and veins is not altered in tissues from DOCA-salt hypertensive rats. In addition, the receptor mechanism mediating ATP-induced contraction is different in arteries and veins. The data indicate that P2X1 receptors mediate ATP-induced contractions of mesenteric arteries and P2Y2 receptors mediate ATP-induced contractions of mesenteric veins. These data support the concept that vasomotor activity on the arterial and venous sides of the circulation can be targeted selectively by drugs acting at receptors uniquely expressed by arterial or venous smooth muscle cells.

**References**


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