ATP-mediated release of arachidonic acid metabolites from venular endothelium causes arteriolar dilation

LEAH W. HAMMER, ALISON L. LIGON, AND ROBERT L. HESTER
Department of Physiology and Biophysics, University of Mississippi Medical Center, Jackson, Mississippi 39216-4505

Received 29 September 2000; accepted in final form 30 January 2001

Hammer, Leah W., Alison L. Ligon, and Robert L. Hester. ATP-mediated release of arachidonic acid metabolites from venular endothelium causes arteriolar dilation. Am J Physiol Heart Circ Physiol 280: H2616–H2622, 2001.—This study was designed to test the hypothesis that venular administration of ATP resulted in endothelium-dependent dilation of adjacent arterioles through a mechanism involving cyclooxygenase products. Forty-three male golden hamsters were anesthetized with pentobarbital sodium (60 mg/kg ip), and the cremaster muscle was prepared for in vivo microscopy. ATP (100 μM) injected into venules dilated adjacent arterioles from a mean diameter of 51 ± 4 to 76 ± 6 μm (P < 0.05, n = 6). To remove the source of endothelial-derived relaxing factors, the venules were then perfused with air bubbles to disrupt the endothelium. Resting arteriolar diameter was not altered after disruption of the venular endothelium (51 ± 5 μm), and the responses to venular ATP infusions were significantly attenuated (59 ± 4 μm, P < 0.05). To determine whether the relaxing factor was a cyclooxygenase product, ATP infusion studies were repeated in the absence and presence of indomethacin (28 μM). Under control conditions, ATP (100 μM) infusion into the venule caused an increase in mean arteriolar diameter from 55 ± 4 to 78 ± 3 μm (P < 0.05, n = 6). In the presence of indomethacin, mean resting arteriolar tone was not significantly altered (49 ± 4 μm), and the response to ATP was significantly attenuated (54 ± 4 μm, P < 0.05, n = 6). These studies show that increases in venular ATP concentrations stimulate the release of cyclooxygenase products, possibly from the venular endothelium, to vasodilate the adjacent arteriole.

microcirculation; cyclooxygenase products; arteriolar diameter

AT THE MICROCIRCULATORY LEVEL local blood flow is tightly coupled to the metabolic demands of the surrounding tissue, suggesting that local mechanisms may play an important role in regulating microcirculatory blood flow. Electrical field stimulation of the hamster cremaster muscle, which increases the metabolic activity of this tissue, has consistently resulted in dilation of cremasteric “feed” arterioles in our laboratory. This response can be partially blocked by disrupting the endothelium of venules paired with arterioles (25), suggesting an obligatory role for the venular endothelium in the regulation of microcirculatory blood flow during functional vasodilation. In addition, functional vasodilation of hamster cremasteric arterioles can be attenuated by inhibiting arachidonic acid metabolism (18, 21). These results suggest that the endothelium of hamster cremasteric venules releases arachidonic acid metabolites in response to muscle stimulation, which subsequently diffuse to the adjacent arterioles to mediate dilation. However, the stimulus for the release of arachidonic acid metabolites from the paired venules remains to be elucidated.

Ellsworth et al. (5) showed that, under conditions of hypoxia and low pH, red blood cells from hamsters released significantly greater amounts of ATP than red blood cells under “normal” conditions. Exercise or increases in skeletal muscle metabolism produce substantial decreases in venous oxygen levels and venous pH (12, 15), and thus may be expected to stimulate the release of ATP from red blood cells in venules. ATP is known to cause the release of endothelial factors such as nitric oxide (NO) and prostacyclin by activating G protein-coupled P2Y receptors on the vascular endothelium (for reviews, see Refs. 8 and 23) and thus may be a stimulus for the release of arachidonic acid metabolites from the venular endothelium. The aim of the present study was to determine whether administration of ATP into hamster cremaster venules results in the dilation of adjacent arterioles and, if so, to determine whether or not arachidonic acid metabolites, specifically, vasodilatory cyclooxygenase products from the venular endothelium, are responsible for this response.

MATERIALS AND METHODS

Animal preparation. The experimental protocols for this study were approved by the Institutional Animal Care and Use Committee of the University of Mississippi Medical Center and were carried out according to both the Guide for the Care and Use of Laboratory Animals from the National Institutes of Health and the guidelines of the Animal Welfare Act.

Forty-three male golden hamsters (120–200 g, Charles River) were anesthetized with an intraperitoneal injection of pentobarbital sodium (60 mg/kg). The left jugular vein was...
cannulated for a continual infusion of pentobarbital sodium in 0.9% saline solution (5 mg/ml at 0.01 ml/min). Deep esophageal temperature was maintained at 37–38°C by convective heating. The hamsters used in these experiments had their tracheas intubated, and the animals spontaneously breathed 30% O₂-balance N₂ to mimic the blood gases typical of conscious animals.

The cremaster muscle was prepared by spreading the muscle over a clear Lucite pedestal and securing the edge of the cremaster muscle with insect pins as previously described (10). During the dissection and experimental period, the cremaster muscle was superfused with warm physiological salt solution (PSS), pH 7.35 at 34°C, containing (in mM) 131.9 NaCl, 4.7 KCl, 2.0 CaCl₂, 1.2 MgSO₄, and 20 NaHCO₃. The superfusion solution was equilibrated with 5% CO₂-balance N₂.

Experimental measurements. The microcirculation of the cremaster muscle was transilluminated and observed with a Leitz Laborlux 12 FS microscope fitted with a ×32 long working distance objective (numerical aperture = 0.40). The microscopic image was televised with a Dage closed-circuit television camera and displayed on a Sony monitor. The magnification of the image was ×900 from the tissue to the monitor screen. Vessel diameter was measured by using a Colorado video 321 analyzer modified to function as a video micrometer. With the use of this device, two movable lines were positioned on the inside walls of the vessel, and a direct current voltage proportional to the line separation was recorded using a computerized data-collecting system. The resolution of this system was ±1 μm.

Criteria for vessel selection. To study the effect of arachidonic acid metabolites from the venular endothelium on adjacent arterioles, only arterioles with a paired venule were selected for study. The majority of arterioles chosen for study were second-order arterioles. However, due to the anatomy of the cremaster microcirculation and the difficulty in consistently obtaining good vessel tone across the branch orders, three of the control groups included a combination of first- and second-order arterioles. We present our results as raw data, and the differences in mean control diameters between experimental groups reflect the different ratios of first- to second-order vessels used within an experimental group. The justification for pooling results from first- and second-order arterioles will be addressed in the appropriate sections in RESULTS. Each preparation served as its own control (responses to ATP were established before and after various experimental treatments), and experiments were discarded if arterioles failed to dilate in response to superfusion of the NO donor sodium nitroprusside (SNP; 10 μM) at the end of the experiment.

Drug administration and venular endothelium denudation. ATP (1 or 100 μM) or SNP (1 μM) were infused (20 s) into a small venule at a site distal to the arteriolar observation site (Fig. 1) via a pressurized glass micropipette. The diameter of the micropipette tips ranged from 5 to 15 μm depending on the size of the venule to be pierced. Infusion solutions were diluted in heparinized saline (100 IU/ml) to prevent clotting at the micropipette tip. To disrupt the venular endothelium, two to three air bubbles were injected into the venule by pressurizing the fluid with short pulses using a picopressurized micropipette as described previously (25). To minimize trauma to the venule, the preferred method of delivery of the air bubbles was via the same glass micropipette that contained the ATP. However, in cases where the pipette tip became blocked, it was necessary to use one or more additional micropipettes to deliver the air bubbles and the second infusion of ATP. Each micropipette was placed immediately proximal to the site of the previous pipette injection (Fig. 1).

The cyclooxygenase inhibitor indomethacin was used in separate experiments to determine whether arteriolar responses to venular administration of ATP were mediated via vasodilatory prostaglandins or prostacyclin. Indomethacin was administered into the superfusion solution to give a final concentration of 28 μM. This concentration of indomethacin was shown to inhibit arachidonic acid metabolism-mediated vasodilation in the hamster cremaster muscle (24). Indomethacin was dissolved in sodium carbonate (10 mM) and diluted in PSS, with the final solution containing 0.4% sodium carbonate.

Experimental protocols. After a 30-min stabilization period, ATP (1 or 100 μM) was administered into a venule, and the diameter of the adjacent arteriole was measured at a predetermined observation site (Fig. 1). The same concentration of ATP was administered 30 min later to test for reproducibility. In separate animals, ATP (100 μM) was administered into a venule (after a 30-min stabilization period), and the diameter of the adjacent arteriole was measured. When baseline diameter returned to the control value, the venular endothelium was disrupted as described in Drug administration and venular endothelium denudation. A second dose of ATP (100 μM) was administered into the venule after the return of arteriolar diameter to the control value. Arteriolar diameter was again measured at the observation site. To verify that vascular smooth muscle function was not affected by endothelium disruption, these experiments were repeated in different animals using SNP (1 μM) in place of ATP. SNP is a NO donor that directly stimulates vascular smooth muscle in an endothelium-independent manner. Because we do not believe that NO plays an important role in the mechanism examined in this study (see DISCUSSION), we believe that SNP is an appropriate control substance for these experiments. To rule out the possibility that mechanical manipulations contributed to the arteriolar responses to ATP and SNP, heparinized saline was injected into venules paired...
with arterioles before and after removal of the venular endothelium in additional animals.

The role of cyclooxygenase products in mediating the response to ATP was examined in a separate set of experiments. In these experiments, the first infusion of ATP was followed by the administration of indomethacin (or vehicle) as described in Drug administration and venular endothelium denudation. Indomethacin (or vehicle) was superfused for 30 min before administration of a second dose of ATP and remained in the superfusate until the conclusion of the experiment. Again, using different animals, SNP (1 µM) was infused into the paired venule in the absence and presence of indomethacin to confirm that indomethacin had no effect on arteriolar vascular smooth muscle function.

As an additional test of arteriolar vascular smooth muscle function, SNP (10 µM) was superfused at the conclusion of every experiment. Experiments were discarded if this concentration of SNP failed to produce a dilation of arterioles that was at least equal to that produced by the control response to venular ATP or venular SNP.

Drugs. ATP and indomethacin were purchased from Sigma (St. Louis, MO), and SNP was purchased from Gensia Pharmaceuticals (Irvine, CA).

Analytic and statistical methods. Digital data were collected using a Gateway computer equipped with a Metabyte Dash eight-bit analog-to-digital converter. Arterial diameter data were collected at 1 Hz and stored to disk for later analysis. Statistical significance was determined with either a paired t-test or an ANOVA with repeated measures. All data are means ± SE. Statistical significance was accepted at P < 0.05.

RESULTS

Effect of venular administration of ATP on arteriolar diameter. Figure 2 shows the arteriolar response to venular infusions of 1 and 100 µM ATP. Three first-order and two second-order arterioles were selected for study. ATP (1 µM) caused arteriolar diameter to increase from 69 ± 5 to 79 ± 5 µm (n = 5, P < 0.05). This change of ∼14% was reproducible after a 30-min recovery period (Fig. 2A). This 14% change in diameter was observed in both first- and second-order arterioles, and thus the results were pooled. Similarly, ATP (100 µM) caused a reproducible increase in second-order arteriolar diameter of ∼30% (from 50 ± 6 to 65 ± 6 µm, n = 5, P < 0.05; Fig. 2B).

Effect of abluminal administration of ATP on arteriolar diameter. The direct action of ATP on cremasteric arterioles was examined in three animals. ATP (100 µM) injected into the tissue immediately adjacent to the arteriole caused second-order arterioles to occlude, whereas ATP (1 µM) resulted in a constriction from 51 to 38 µm (n = 1). These constrictions were extremely rapid and were followed by dilations that were similar in magnitude to those obtained by venular infusions of ATP (data not shown).

Effect of venular endothelium disruption on responses to ATP. Venular infusion of ATP (100 µM) resulted in a significant dilation of second-order arterioles from 51 ± 4 to 76 ± 6 µm (Fig. 3A; n = 6, P < 0.05). After disruption of the venular endothelium, ATP failed to cause a significant dilation of the adjacent arteriole (from 51 ± 5 to 59 ± 4 µm; Fig. 3A). SNP (1 µM) infused into venules caused a very rapid and transient increase in the diameter of one first-order and three second-order arterioles (from 67 ± 7 to 90 ± 5 µm, n = 4, P < 0.05), which, in contrast to the arteriolar response to ATP, was not abolished by disruption of the venular endothelium (Fig. 3B). While the percent change in diameter from control was greater for second-order arterioles (average of 42% for the three second-order arterioles and 16% for the one first-order arteriole), removal of the venular endothelium did not affect this change in diameter of arterioles of either order. Infusions of heparinized saline had no effect on arteriolar diameter before or after endothelium disruption (data not shown).

Effect of indomethacin on responses to ATP. In this set of experiments, ATP (100 µM) infused into the venule caused significant dilation of second-order arterioles from 55 ± 4 to 78 ± 3 µm (Fig. 4A; n = 6, P < 0.05). When indomethacin was added to the superfusate, arteriolar diameter averaged 49 ± 4 µm, which was not significantly different from the baseline value observed in the absence of indomethacin. Venular administration of ATP failed to increase arteriolar diameter in the presence of indomethacin (from 49 ± 4 to 54 ± 4 µm). In a separate group of animals, the vehicle for indomethacin, sodium carbonate, was shown to have no effect upon responses to ATP (Fig. 4B; n = 6) in second-order arterioles. SNP was infused into...
venules, and responses were observed in two first-order and two second-order arterioles. SNP elicited similar percent changes in diameter (from control) in both first- and second-order arterioles, which were not attenuated by indomethacin.

**DISCUSSION**

This study tested the hypothesis that increases in venular ATP levels influence the tone of adjacent feed arterioles through a mechanism involving the venular endothelium and arachidonic acid metabolites, particularly cyclooxygenase products. Venular administration of ATP caused a significant increase in the diameter of adjacent arterioles, which was totally blocked by either disruption of the venular endothelium or administration of the cyclooxygenase inhibitor indomethacin. These results support a role for ATP in the regulation of the diameter of upstream feed arterioles.

Increases in muscle metabolism are associated with dilation of terminal arterioles (27) and upstream feed arterioles (14, 31). While the diameter of terminal arterioles may be directly influenced by metabolic factors, the mechanism of dilation of the upstream arterioles remains to be fully elucidated. In virtually all organ vascular beds, large- and most intermediate-sized arterioles are paired with a venule. Previous studies from our laboratory have demonstrated that paired arterioles of the hamster cremaster muscle dilate in response to electrical field stimulation. Disruption of the endothelium of a paired venule has been shown to significantly attenuate the functional dilation of the adjacent arteriole (25), demonstrating an obligatory role for the venular endothelium in functional hyperemia. This leads to the suggestion that, in

---

**Fig. 3.** Effect of venular application of ATP (100 μM; A) and sodium nitroprusside (SNP; 1 μM; B) on diameter of adjacent arterioles before and after disruption of the venular endothelium (n = 6). Data are means ± SE. *Significant difference (P < 0.05) compared with control diameter in the presence of an intact endothelium. + and −, Presence and absence of the endothelium, respectively.

---

**Fig. 4.** Effect of venular application of ATP (100 μM) on diameter of adjacent arterioles in the absence (−) and presence (+) of indomethacin (28 μM, n = 6; A) or sodium carbonate (n = 6; B). C: effect of venular application of SNP (1 μM) on diameter of adjacent arterioles in the absence and presence of indomethacin (28 μM, n = 4). Data are means ± SE. *Significant difference (P < 0.05) compared with control diameter in the absence of indomethacin or sodium carbonate.
response to an increase in muscle activity, the venular endothelium releases a vasoactive substance(s) that diffuses to the adjacent arterioles to cause vasodilation. Thus venular-arteriolar diffusion, which has previously been reported by several laboratories (7, 9, 30), may provide an important mechanism via which the diameter of upstream arterioles is regulated during increases in muscle metabolism.

Previous studies have sought to identify the factor(s) released from the venular endothelium in response to increases in muscle metabolism. Endothelium-derived NO is known to exist in the microcirculation, and several studies have reported its release from venules (2, 7, 13, 22). Although endothelium-derived NO has been shown to be important for coordinating the resting tone of arterioles, Saito et al. (24) and Boegehold (2) reported that it did not contribute to the dilation of arterioles observed during functional hyperemia. In contrast, inhibition of the production of arachidonic acid metabolites attenuated functional dilation of first- and second-order hamster cremasteric arterioles (18, 21), suggesting that metabolites of arachidonic acid may be released from the venular endothelium during muscle stimulation that subsequently diffuse to and dilate the adjacent arterioles. Release of arachidonic acid metabolites from venules has been reported by others (4, 11, 19). However, the stimulus for the release of arachidonic acid metabolites from paired venules remains to be elucidated.

Ellsworth et al. (5) showed that, under conditions of hypoxia and low pH, red blood cells from hamsters released significantly greater amounts of ATP than red blood cells under “normal” conditions. The vascular effects of ATP have been well characterized. ATP-mediated vasodilation is reported to occur predominantly via interaction with specific receptors (P2Y) on the vascular endothelium, which results in the synthesis and release of endothelium-derived vasodilators such as NO, prostacyclin, and endothelium-derived hyperpolarization factor (for reviews, see Refs. 1 and 23). Given that red blood cells can release ATP under conditions of hypoxia and that venous blood becomes selectively hypoxic during muscle stimulation (10, 15), we hypothesize that during periods of increased muscle metabolism, ATP is released from venular red blood cells. This ATP then stimulates receptors on the venular endothelium, resulting in the release of arachidonic acid metabolites.

To test this hypothesis, we infused ATP directly into venules that were paired with arterioles. Venular infusions of ATP resulted in significant increases in the diameters of the adjacent arterioles. To determine whether or not the arteriolar dilatory response to ATP was dependent on the venular endothelium, we infused ATP, in the same preparation, after disruption of the venular endothelium using injections of small air bubbles. Disruption of the venular endothelium abolished the arteriolar response to venular administration of ATP. Disruption of the venular endothelium demonstrates that the arteriolar response elicited by ATP, like the response to field stimulation (25), is dependent on an intact venular endothelium. To rule out the possibility that arteriolar dilation was simply an artifact of mechanically puncturing the venule, we injected saline into paired venules before and after disruption of the venular endothelium (data not shown). Saline did not alter the diameter of the adjacent arterioles regardless of the status of the endothelium.

It is possible that ATP itself was diffusing out of the venule and directly stimulating the arterioles to dilate. To test this hypothesis, we applied ATP (1 and 100 μM) directly onto the abluminal surface of the cremasteric arterioles. ATP (100 μM) applied directly onto the abluminal surface of cremasteric arterioles caused marked constriction (and in some cases total closure of the arteriole) before the dilation. This constriction is proposed to be mediated by ligand-gated ion channels (P2X receptors) located on the vascular smooth muscle, and the subsequent dilation is likely due to G protein-coupled dilatory P2Y receptors located on the arteriolar endothelium [reviewed by Ralevic and Burnstock (23)]. If ATP was diffusing from the venule to the arteriole, it would arrive at the arteriole via the abluminal surface and would be expected to constrict the vessels before dilating them. Constriction was not observed during these experiments. Furthermore, the absence of an arteriolar response to ATP after disruption of the venular endothelium provides strong evidence for an indirect effect via the venular endothelium rather than a direct effect on the arteriole.

 McKay et al. (18) showed that arteriolar dilation in response to muscle stimulation was largely attenuated by the cyclooxygenase inhibitor indomethacin. These authors showed that subsequent removal of the venular endothelium did not further attenuate the response to muscle stimulation, suggesting that the venular endothelium is a major site for the production of cyclooxygenase metabolites. Similarly, in the present experiments, we demonstrated that the arteriolar response to ATP is abolished by indomethacin. Taking into account that the response to ATP was dependent on an intact venular endothelium, our results suggest that ATP injected into venules may interact with receptors on the venular endothelium that stimulate the production of a vasodilatory prostaglandin or prostacyclin through the cyclooxygenase pathway. We propose that this cyclooxygenase product then diffuses to the adjacent arteriole, where it elicits vasodilation. In support of this hypothesis, preliminary experiments from our laboratory (20) have shown that intraluminal administration of ATP into isolated veins results in abluminal release of prostacyclin. The fact that indomethacin abolished the arteriolar response to ATP in the present study provides further evidence against the involvement of NO in the mechanism currently under investigation.

To confirm that the mechanism underlying the arteriolar response to ATP was specific for this dilator, we repeated our experiments using SNP in the place of ATP. Infusions of SNP into venules resulted in rapid dilation of the adjacent arterioles, which was not affected by either endothelial disruption or indometha-
The dilatory response to SNP was transient, with the diameter of vessels returning to baseline values immediately on cessation of the venular infusion. In contrast, arterioles were slower to respond to ATP and remained dilated for up to 5 min after ATP infusions. The rapid response of SNP is probably indicative of a direct action of this compound on the smooth muscle of the arteriole, whereas the slower response to ATP supports the concept that an intermediate step is involved in the arteriolar dilation to ATP. Sustained arteriolar dilation in response to ATP stimulation has been reported by other investigators (16), although the mechanism of action is unclear.

Arterioles have been shown to have the ability to propagate vasomotor responses along arteriole walls (26), and thus conducted vasodilation may be an additional mechanism contributing to the results obtained in the present study. While ATP (3, 17) and ACh (6, 29) have been shown to cause conducted vasodilation, conducted responses have only been observed over distances longer than ~1,000 μm when administered directly onto or into arterioles with few or no branches. These and other studies (28) seem to suggest that branching of resistance networks results in the dissipation of conducted vasomotor responses. In the hamster cremaster preparation in the present study, the distance between the venular ATP application site and the arteriolar observation site was typically between 400 and 700 μm (see Fig. 1). However, the distance between the two sites if traced along the vasculature from the venular site to the arteriolar site was well in excess of 2,000 μm. Therefore, it is difficult to imagine that a signal originating in a large venule could possibly travel thousands of micrometers, through many vessel branches, to result in the dilation of the second-order arterioles (and even first-order arterioles; data not shown), as observed in the present study. In the present study, responses to ATP were abolished in the presence of indomethacin. Prostaglandins and prostanoids have not been implicated in conducted vasodilation to date. In fact, in the studies of McCullough et al. (17) and Collins et al. (3), the conducted responses were completely abolished by inhibition of NO synthase, suggesting a role for NO in conducted vasodilation initiated by ATP. Thus we believe that mechanisms other than or in addition to conducted vasodilation are important in the ATP-mediated responses observed in the present study.

In conclusion, these results show that ATP administered into a venule can result in dilation of the adjacent arteriole. This action is dependent on an intact venular endothelium and the production of cyclooxygenase metabolites. We hypothesize that ATP stimulates the release of a vasodilatory metabolite of arachidonic acid from the venular endothelium via interaction with specific ATP receptors on the venular endothelium. We propose that this metabolite then diffuses to the adjacent arteriole, where it initiates dilation. The similarities between the mechanism of ATP-mediated arteriolar dilation and electrical field stimulation-mediated arteriolar dilation in the hamster cremaster muscle suggest that ATP may be involved in the hyperemic response to increases in skeletal muscle metabolism.

This work was supported by National Heart, Lung, and Blood Institute Grant HL-51971 and by an American Heart Association grant-in-aid.

REFERENCES


