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Heparin inhibits Angiotensin II-induced vasoconstriction on isolated mouse mesenteric resistance arteries through Rho-A- and PKA-dependent pathways

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Abstract

Heparin is commonly used to treat intravascular thrombosis in children undergoing extracorporeal membrane oxygenation or cardiopulmonary bypass. These clinical circumstances are associated with elevated plasma levels of angiotensin II (Ang II). However, the mechanisms by which heparin modulates vascular reactivity of Ang II remain unclear. We hypothesized that heparin may offset Ang II-induced vasoconstriction on mesenteric resistance arteries through modulating the Rho-A/Rho kinase pathway. Vascular contractility was studied using pressurized, resistance-sized mesenteric arteries from mice. Rho-A activation was measured by pull-down assay, and myosin light chain or PKA phosphorylation by immunoblotting. We found that heparin significantly attenuated vasoconstriction induced by Ang II but not that by KCl. The combined effect of Ang II with heparin was almost abolished by a specific Rho kinase inhibitor Y27632. Ang II stimulated Rho-A activation and myosin light chain phosphorylation, both responses were antagonized by heparin. Moreover, the inhibitory effect of heparin on Ang II-induced vasoconstriction was reversed by Rp-cAMPS (cAMP-dependent PKA inhibitor), blunted by ODQ (soluble guanylate cyclase inhibitor), and mimicked by a cell-permeable cGMP analogue, 8-Br-cGMP, but not by a cAMP analogue. PKC and Src kinase were not involved. We conclude that heparin inhibits Ang II-induced vasoconstriction through Rho-A/Rho kinase- and cGMP/PKA-dependent pathways.

Keywords

Ang II; heparin; resistance arteries; Rho-A/Rho kinase; vascular tone

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1. Introduction

Heparin is commonly used to treat thromboembolic diseases and critically ill children undergoing extracorporeal membrane oxygenation (ECMO) and cardiopulmonary bypass (CPB). In addition to its well-known anticoagulant and anti-thrombin properties, heparin has multiple actions on different cardiovascular tissues including anti-proliferative, anti-inflammatory, blood pressure lowering and vasodilatory effects (Akimoto et al., 1996; Dilley and Nataatmadja, 1998; Lindner et al., 1992; Salas et al., 2000; Tiefenbacher and Chilian, 1997; Paredes-Gamero et al., 2010). These effects are regulated in a tissue specific manner through the interactions between heparin and a variety of receptors, proteins, and cellular molecules (such as nitric oxide, reactive oxygen species, etc) (Akimoto et al., 1996; Lindner et al., 1992; Paredes-Gamero et al., 2010; Bachetti et al., 1999; Sumitomo-Ueda et al., 2010). Because heparin is widely used in children with an activated renin angiotensin system (RAS), it is important to understand the mechanisms by which heparin interacts with angiotensin II (Ang II).

Ang II is well known for its critical role in the regulation of blood pressure and body fluid homeostasis. Renal perfusion is frequently decreased in children undergoing ECMO and CPB procedures. Changes in renal perfusion and related renal ischemia further activate the RAS, subsequently increase circulating Ang II. Ang II is a powerful vasoconstrictor that can also cause a wide spectrum of vascular injury (Ruiz-Ortega et al., 2003). Previous studies have documented that Ang II induces vasoconstriction through multiple cellular signaling pathways (Schmitz and Berk, 1997; Mehta and Griendling, 2007; Garrido and Griendling, 2009; Nguyen Dinh Cat and Touyz, 2011). Accumulating evidence demonstrates that the Rho-A/Rho kinase (ROCK) pathway plays a key role in mediating Ang II-induced vascular effects including vasoconstriction (Uehata et al., 1997; Matrougui et al., 2001; Jin et al., 2006; Nguyen Dinh Cat and Touyz, 2011), and is involved in the pathogenesis of atherosclerosis, heart failure, hypertension, stroke, and ischemia-reperfusion injury (Uehata et al., 1997; Loirand et al., 2006). However, direct evidence for a functional role of Rho-A activation in heparin-mediated effect on an intact blood vessel is lacking.

The objective of the present study was to determine whether heparin modulates Ang II-induced vasoconstriction of resistance arteries and if so, what the underlying cellular mechanisms are. Because the Rho-A/ROCK pathway plays a critical role in vascular contractility (Somlyo and Somlyo, 2003), we hypothesized that heparin may offset Ang II-induced vasoconstriction on mesenteric resistance arteries via modulating the Rho-A/Rho kinase pathway. Additionally, based on evidence that different upstream pathways can converge toward regulation of the Rho-A/ROCK pathway (Seasholtz et al., 1999; Qiao et al., 2003), we explored whether other cellular signaling mechanisms can also contribute to the effect of heparin. Our results demonstrated that heparin inhibits Ang II-induced vasoconstriction of mesenteric resistance arteries from mice through the Rho-A/ROCK pathway and, possibly, cGMP/PKA-dependent mechanisms.

2. Material and Methods

2.1 General Preparations

All study procedures were in accordance with institutional guidelines, and approved by the Institutional Animal Care and Use Committee at Children's National Medical Center. Male mice (FVB/N, weight 30-35 g) were purchased from Jackson Labs (Bar Harbor, Maine). Mice were heparinized (500 IU/kg, i.p.) and killed by cervical dislocation after brief isoflurane inhalation. The entire intestine was rapidly removed and placed in cold physiological salt solution (PSS), composed of the following (in mmol/L): NaCl, 119; KCl, 4.7; CaCl₂, 1.6; MgSO₄, 1.17; KH₂PO₄, 1.18; NaHCO₃, 24.9; EDTA, 0.026; and dextrose,

5.5; pH was 7.4. The mesenteric arteries (MA; first- to second-order) were carefully isolated and adipose tissue cleared out.

For protein assays, the entire mesenteric vascular bed was used. The vessels were divided into the following experimental groups: 1) control (incubated with PSS, 20 min), 2) Ang II (50 nmol/L, 20 min), 3) heparin (140 µg/ml, 20 min), and 4) Ang II (50 nmol/L, 20 min) + heparin (140 µg/ml, another 20 min). These samples were then snap-frozen in liquid nitrogen and stored at -70°C until use.

2.2 Measurement of Arterial Diameter via Pressure-Perfusion Arteriograph

As previously described (Xie and Bevan, 1999; Xie et al., 2005), segments of the mouse MA (~2 mm in length, 150±5 µm in internal diameter) were placed in an arteriograph containing PSS constantly aerated with 16% O₂/5% CO₂/79% N₂ (pH = 7.4) at room temperature. Vessels were cannulated between two glass pipettes and pressurized to 60 mm Hg, and it was ensured that there was no potential leak. The cannulated vessel was warmed to 37°C and allowed to equilibrate for 60 minutes under no flow condition, with a longitudinal stretch to approximate its *in situ* length. The blood vessel was imaged using a video camera attached to an inverted microscope (TMS, Nikon) and a dimension analyzer (Living Systems Instrumentation) linked to a chart recorder (Model 23; Perkin-Elmer). Inner diameter and intravascular pressure were measured continuously throughout the experiments. All pharmacological reagents were added to the superfusion solution.

The viability of each vessel was determined before subsequent experimental protocols. Smooth muscle cell viability was verified by constrictive responses to high KCl (HK, 120 mmol/L) and an adrenergic receptor agonist phenylephrine (PE, 1-3 µmol/L), whereas endothelial cell integrity was confirmed by vasodilator response to acetylcholine (Ach, 5 µmol/L). The vessels that failed to meet the above criteria were discarded. To prevent tachyphylaxis to Ang II, only one concentration-response curve (CRC) of Ang II was performed in each vessel.

2.3 Experimental Protocols

2.3.1 Effect of heparin on Ang II-induced response—Effect of Ang II (0.1 – 30 nmol/L) on MA were examined in the absence and presence of heparin (70 and 140 µg/ml) for 20 min, respectively. The effect of heparin on constrictor response to HK (120 mmol/L) and myogenic tone were compared, respectively.

2.3.2 Role of Rho kinase (ROCK) activation—To assess the involvement of ROCK in the contractility of isolated MA, vascular responses to Ang II alone or in combination with heparin were examined in the absence and presence of Y27632 (1-10 µmol/L), a specific ROCK inhibitor.

To further confirm the modulating effect of heparin on ROCK activity, another series of experiments were performed to examine the relaxation by Y27632 (1 nmol/L – 10 µmol/L) on the vessels pre-constricted with PE (~EC₈₀). CRCs for Y27632 were examined in the absence and presence of heparin (140 µg/ml) and arachidonic acid (AA, 50 µmol/L), a known Rho-A/ROCK stimulator (Fu et al., 1998), respectively. PE was used to pre-constrict the vessels, because Ang II-induced tone is unstable due to tachyphylaxis.

2.3.3 Involvement of other cellular mechanisms—The following pharmacological tools were used to study other cellular signaling pathway(s) that may contribute to the effect of heparin: Rp-Adenosine 3',5'-cyclic monophosphorothioate triethylammonium salt hydrate (Rp-cAMPS, 10 µmol/L), a specific inhibitor of cAMP-dependent protein kinase A

(PKA); chelerythrine (1 $\mu\text{mol/L}$), a specific inhibitor of protein kinase C (PKC); SQ 22,536 (100 $\mu\text{mol/L}$), a selective inhibitor of adenylyl cyclase; [1,2,4]oxadiazolo[4,3-*b*]quinoxalin-1-one (ODQ, 10 $\mu\text{mol/L}$), a selective inhibitor of soluble guanylate cyclase, 8-Bromoadenosine 3',5'-cyclic monophosphate sodium salt (8-Br-cAMP, 10 $\mu\text{mol/L}$), a cell-permeable cAMP analogue, and 8-Bromoguanosine 3',5'-cyclic monophosphate sodium salt (8-Br-cGMP, 10 $\mu\text{mol/L}$), a cell-permeable cGMP analogue. These drugs at the concentrations mentioned above were administered 20 min prior to the pretreatment with heparin (140 $\mu\text{g/ml}$) and followed by Ang II CRCs.

2.4 Rho-A Activation Assay

Rho-A activation of the mesenteric vessels was evaluated by pull-down assays using GST-Rhotekin bound to glutathione slurry resin, as previously described (Marinissen et al., 2004).

2.5 Western Blot Analysis

The samples from mesenteric vessels were analyzed for protein expression of total and phosphorylated Rho-A, myosin light chain 2 (pMLC₂), and PKA. Western blotting was performed using standard techniques and primary antibodies from Cell Signaling Technology.

2.6 Statistical Analysis

All data are presented as Mean $\pm$ S.E., and n indicates the number of animals used in each group. Ang II- and PE-induced constriction was expressed as a percentage of the maximal response to HK (120 mmol/L). Vasodilation was expressed as a percentage of PE (EC₇₀₋₈₀) pre-constricted tone. Data were analyzed by the paired Student's *t* test for single comparisons and by one-way ANOVA followed by Bonferroni's test for multiple comparisons. The difference was considered significant when $p < 0.05$.

3. Results

3.1 Heparin inhibited Ang II-induced vasoconstriction

Ang II (0.1 – 30 nmol/L) induced concentration-dependent constriction of isolated MA with a maximum response ($52.5 \pm 3.1\%$) of that initiated by HK (Fig. 1A). Heparin at a lower concentration (70 $\mu\text{g/ml}$, 20 min) had little effect on Ang II-induced response. Pretreatment with heparin (140 $\mu\text{g/ml}$, 20 min) significantly attenuated Ang II-induced constriction by 30-54% at various concentrations (Fig. 1A), whereas constrictor response to HK (Fig. 1B) and myogenic tone (Table 1) remained essentially unchanged before and after heparin treatment. Overall the inhibitory effect of heparin lasted for at least one hour (Fig 1B).

3.2 The Rho-A/ROCK pathway contributed to the inhibitory effect of heparin

The ROCK inhibitor Y27632 (1 and 10 $\mu\text{mol/L}$) suppressed the combined effect of Ang II along with heparin in a concentration-dependent manner ($p < 0.05$, $p < 0.001$; see Fig. 2A), suggesting the involvement of ROCK in these responses. In addition, Y27632 (10 $\mu\text{mol/L}$) almost abolished Ang II-induced constriction of MA, in agreement with other studies showing that Ang II-induced constriction is mediated by ROCK activation (Uehata et al., 1997; Matrougui et al., 2001; Nguyen Dinh Cat and Touyz, 2011). Effect of Y27632 alone on the arterial tone was shown in Table 1. In comparison, the inhibitory effect of heparin was unaffected by chelerythrine (1 $\mu\text{mol/L}$, 20 min), the selective inhibitor of PKC (21) (Fig. 2A).

To confirm the involvement of the Rho-A/ROCK pathway in heparin-dependent effect, we assessed the relaxation induced by cumulative increase in the concentrations of the ROCK

inhibitor Y27632 (1 nmol/L – 10 μ mol/L). PE-induced tone was inhibited by Y27632 in a concentration-dependent manner, and complete inhibition was achieved with Y27632 at 10 μ mol/L (Fig. 2B). Heparin enhanced the sensitivity of the vessels to ROCK inhibition at lower concentrations. In contrast, AA markedly antagonized the effect of Y27632 (Fig. 2B). Effects of heparin and AA alone are shown in Table 1. These data supported that ROCK activity played a role in the inhibitory effect of heparin.

Next, we assessed the activation of Rho-A, a member of Rho family proteins and a upstream activator of ROCK, to evaluate its role in the inhibitory effect of heparin. The small G proteins participate in a wide variety of cellular functions. Activated Rho-GTPases exist in a GTP-bound state, whereas inactive Rho-GTPases are bound to GDP (Burridge and Wennerberg, 2004). As shown on Fig. 3, the activation of Rho-A was remarkably upregulated by Ang II, and heparin antagonized this effect ($p < 0.05$, Fig. 3).

Rho-A/ROCK is a critical component for the regulation of vascular tone through changes in phosphorylation of myosin light chain (Somlyo and Somlyo, 2003). We found that the expression of pMLC was increased in correlation with the activities of Rho-A and ROCK. Particularly, Ang II significantly increased the expression of pMLC, while heparin inhibited this effect ($p < 0.05$, Fig. 3). On the other hand, Src kinase expression was unaltered in response to the above stimuli (data not shown). These results indicate that heparin inhibited Ang II-induced contractility through the Rho-A/ROCK pathway and subsequent change in pMLC.

3.3 PKA was involved in the inhibitory effect of heparin

To investigate the involvement of other cellular mechanisms in the inhibitory effect of heparin, especially those that may converge toward the Rho-A/ROCK pathway, we first examined the effect of Rp-cAMPS, the specific inhibitor of cAMP-dependent PKA. Pretreatment with Rp-cAMPS (10 μ mol/L, 20 min) abolished the inhibitory effect of heparin on Ang II-induced constriction (Fig. 4A). We further assessed whether the specific inhibitors of cAMP and cGMP pathways affected in this process. The combination of heparin with SQ 22,536 (100 μ mol/L), an specific inhibitor of adenylyl cyclase, only restored the maximum constriction initiated by Ang II (Fig. 4A), while pretreatment with the specific inhibitor of soluble guanylate cyclase ODQ (10 μ mol/L) blunted the inhibitory effect of heparin on Ang II-induced sub-maximal and maximal responses (Fig. 4B). These pharmacological tools alone had little influence on basal diameter, as shown in Table 1.

To support the role of a nucleotide in modulating Ang II-induced response, we subsequently examined effects of cAMP and cGMP analogues on Ang II-induced constriction respectively. The stable cAMP analogue 8-Br-cAMP (10 μ mol/L) did not modify the response to Ang II (data not shown). Surprisingly, 8-Br-cGMP (10 μ mol/L), the stable cGMP analogue, caused a similar reduction in arterial constriction to Ang II as obtained with heparin (140 μ g/ml), and this effect was prevented by pretreatment with Rp-cAMPS (10 μ mol/L) (Fig. 4C). These results suggested that heparin inhibited Ang II-induced constriction via the cross-activation of PKA possibly through cGMP. In fact, we found that heparin increased the phosphorylation of PKA (Fig 3). In addition, we showed that the expression of pMLC was increased in correlation with the activities of Rho-A and ROCK. Particularly, Ang II significantly increased the expression of pMLC, while heparin inhibited this effect ($p < 0.05$, Fig. 3).

4. Discussion

The novel findings of the present study include: 1) heparin significantly attenuated Ang II-induced vasoconstriction; 2) this inhibitory effect of heparin was mediated via

downregulation of the Rho-A/ROCK pathway; and 3) the effect was also mediated by the cross activation of PKA possibly through cGMP. These results demonstrate that heparin plays an opposing role in Ang II-mediated vasoconstriction through potentially multiple cellular signaling mechanisms on isolated mouse mesenteric arteries. To the best of our knowledge, we provide the first experimental evidence that heparin regulates vascular tone through the Rho-A/Rho kinase pathway on an intact resistance artery.

Heparin is often used in cardiovascular procedures to prevent or treat thromboembolic disorders. The therapeutic dose of heparin is usually 50-150 U/kg, but may vary greatly from ~1 U/ml to a bolus of 5000 U (Hirsh et al., 2001). We used two different brands of heparin that are routinely used in our hospital to treat critically ill children undergoing ECMO and CPB procedures though staying with one for most of the studies. The concentration of heparin we applied (140 µg/ml, equivalent to 100 U/ml) is comparable to those used in previous work (Thompson et al., 1994; Tiefenbacher and Chilian, 1997; Yu et al., 2011). Lower concentrations of heparin may be effective in cultured cells where heparin is rapidly taken up by heparan sulfate proteoglycans located on the surface of these cells. Higher doses are needed for functional studies due to the non-specific binding of heparin to the extracellular matrix of isolated blood vessels. As the anticoagulant response to heparin varies among patients, different vascular responses to heparin could also be expected due to heterogeneity of vascular beds.

In the present study, we found that heparin antagonized Ang II-induced vasoconstriction by regulating the Rho-A/ROCK pathway. These findings are supported by several lines of evidence. First, Ang II-induced vasoconstriction was significantly inhibited by heparin, the combined effect was further reduced and abolished in the presence of the ROCK inhibitor Y27632 at concentrations of 1 and 10 µmol/L, respectively. Additional supporting evidence for the interaction between heparin and ROCK was obtained by our data that heparin significantly enhanced the sensitivity of the vessel to Y27632; in contrast, the Rho-A/ROCK stimulator AA remarkably reduced the Y27632 inhibition. Second, Ang II stimulated the activation of Rho-A, and this effect was normalized by heparin at the same concentration that effectively inhibited Ang II-induced vasoconstriction. Third, both Rho-A and ROCK activities, whether upregulated by Ang II or downregulated by heparin, induced the corresponding changes in MLC phosphorylation and vascular tone. Finally, heparin had little effect on PKC and Src kinases.

In vascular smooth muscle cells, the Rho-A/ROCK pathway can be activated after 5 minutes of stimulation with Ang II (Bregeon et al., 2009), which is in accordance with the acute vascular effect of Ang II we observed. At the concentrations used in our study, Y27632 is a specific ROCK inhibitor and exhibits 10- to 50-fold higher selectivity to Rho kinase than other protein kinases such as PKC or p21-activated protein kinase. Similar concentrations of Y27632 (1-10 µmol/L) were used by others to inhibit the ROCK activity in various vascular beds (Uehata et al., 1997; Fu et al., 1998; Matrougui et al., 2001; Gokina et al., 2005).

Taken together, our findings indicate that the Rho-A/Rho kinase pathway contributes to the vascular effects of both Ang II and heparin on mesenteric resistance arteries. These results are in agreement with previous studies showing that this pathway plays a key role in Ang II-induced vasoconstriction (Uehata et al., 1997; Matrougui et al., 2001; Jin et al., 2006; Nguyen Dinh Cat and Touyz, 2011). It should be mentioned, however, that Rho-A and ROCK may exhibit different activities depending on the nature or duration of stimuli, and experimental models (Zhang et al., 2005; da Silva-Santos et al., 2009). Recently, downregulation of Rho-A/ROCK activation was reported to mediate anti-proliferative effect of heparin, and these results were obtained with cultured pulmonary artery smooth muscle cells (Yu et al., 2011). Our results extended their findings by revealing that heparin can

regulate vascular tone through the Rho-A/ROCK pathway on isolated mesenteric resistance arteries.

We also found that the inhibitory effect of heparin on Ang II-induced vasoconstriction was closely associated with the cross-activation of cAMP-dependent PKA likely via cGMP. Our results showed that the inhibitory effect of heparin on Ang II-mediated response was abolished by Rp-cAMPS, the specific inhibitor of cAMP-dependent PKA, suppressed by ODQ, the selective inhibitor of guanylate cyclase, and mimicked by the cell-permeable cGMP analogue 8-Br-cGMP, but not by the cell-permeable cAMP analogue 8-Br-cAMP. Again, Rp-cAMPS, when administered along with 8-Br-cGMP, prevented the inhibitory effect of 8-Br-cGMP on Ang II-induced constriction. Overall, in agreement with the results from previous studies (Algara-Suarez and Espinosa-Tanguma, 2004; Worner et al., 2007), our data sustain the notion that cross activation of PKA via cGMP may decrease smooth muscle contractility. It has been demonstrated that various cellular pathways can converge toward the regulation of Rho-A/ROCK activation (Seasholtz et al., 1999; McMurtry et al., 2010). Intriguingly, both cAMP- and cGMP-dependent kinases have been reported to phosphorylate Rho-A, resulting in inhibition of Rho-A-dependent functions, which may explain the vasodilatory properties of these kinases (Sauzeau et al., 2000; Ellerbroek et al., 2003). Because PKA can inhibit Rho-A/ROCK activation in endothelial and vascular smooth muscle cells (Qiao et al., 2003; Murthy et al., 2003; Murthy, 2006), an alternative explanation of our findings could be that the inhibition of Rho-A/ROCK activity by PKA might, at least in part, mediate the opposing effect of heparin on Ang II-induced tone. In support of this notion, we found that heparin induced the phosphorylation of PKA on mesenteric resistance arteries. A schematic illustration of the interaction between heparin and Ang II on the mesenteric resistance arteries is shown in Figure 5.

Finally, the possibility that heparin may modulate the vascular response to Ang II through participating in other cellular pathways cannot be excluded. Hence, more studies are needed to determine how does heparin control Rho-A, and whether its effects are mediated by vascular smooth muscle cells, endothelial cells, or both. Moreover, given the complexity of cellular key molecules, kinases and their interactions, as well as their clear relevance to vascular diseases, future studies await these findings to be validated in endothelium-denuded vessels or to be substantiated *in vivo*. For now, the present study provides new insights into the signaling pathways by which heparin regulates Ang II-induced vascular contractility, and advanced our understanding on vascular biology of heparin-mediated vascular tone. Our findings might also have potential significance in the development of therapeutic targets for vascular hyperresponsiveness to Ang II.

5. Conclusions

Our findings indicate that heparin inhibits Ang II-mediated vasoconstriction through Rho-A/ROCK- and possibly cGMP/PKA-dependent pathways on isolated mesenteric resistance arteries.

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Figure 1.

Effects of heparin on constriction induced by angiotensin II (Ang II, **A**) and high KCl (HK, **B**) of mouse mesenteric resistance arteries (MA). Data are presented as mean $\pm$ SE, n=4-8 each group. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with controls.

Figure 2.

Effects of the Rho kinase inhibitor Y27632 on responses to Ang II and heparin (**A**), and the relaxation by Y27632 on mouse MA precontracted with phenylephrine (PE) (**B**). Data are presented as mean±SE, n=4-6. ** $P < 0.01$, *** $P < 0.001$, and # $P < 0.05$ versus the correspondent controls.

Figure 3.

Rho-A activation, and phosphorylation of myosin light chain (pMLC) and PKA on mouse mesenteric vessels shown in SDS-PAGE gel image (**A**) and densitometry analysis (**B**). The results are presented as mean $\pm$ SE, corresponding to three independent experiments. * $P < 0.05$, ** $P < 0.01$ versus controls. # $P < 0.05$ compared with Ang II.

Figure 4.

The combined effect of heparin with Ang II in the absence and presence of Rp-cAMPS, SQ 22,536, chelerythrine (A); or ODQ (B); and effects of 8-Br-cGMP, alone and in combination, with Rp-cAMPS, on Ang II-induced constriction (C). Data are presented as mean±SE, n=4-5. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ compared with Ang II + heparin (A, B), and with Ang II (C).

Schematic illustration of the interaction between heparin and Ang II on mesenteric arteries

Figure 5.

A schematic illustration of the interaction between heparin and Ang II on mesenteric resistance arteries of mice. Ang II activates the RhoA/Rho kinase pathway, which triggers the inhibition of MLC phosphatase and increase in MLC phosphorylation, leading to smooth muscle contraction through Ca^{2+} sensitization. Heparin, may directly or indirectly induce the activation of PKA, and antagonize Ang II-increased Rho-A/ROCK activation and MLC phosphorylation. As a result, vasodilation counteracts vascular contractility induced by Ang II.

Table 1

Effects of pharmacological tools on basal diameter of mouse mesenteric arteries

Reagent	Before	After	N
Heparin (140 µg/ml)	222 ± 5	225 ± 5	21
Rp-cAMPS (10 µmol/L)	210 ± 10	212 ± 9	10
SQ 22,536 (100 µmol/L)	205 ± 12	209 ± 12	6
ODQ (10 µmol/L)	230 ± 7	227 ± 7	7
8-Br-cAMP (10 µmol/L)	224 ± 22	225 ± 22	5
8-Br-cGMP (10 µmol/L)	187 ± 9	192 ± 10 *	9
Chelerythrine (1 µmol/L)	186 ± 6	184 ± 8	4
Arachidonic acid (50 µmol/L)	216 ± 14	229 ± 14 **	5
Y27632 (1 µmol/L)	220 ± 11	236 ± 13 **	5
(10 µmol/L)	213 ± 10	230 ± 8 **	9

Data are expressed in mean±S.E.

*
p 0.05**
p 0.01, compared with the controls.