

Reactivity of the isolated perfused rat tail vascular bed

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Abstract

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Isolated segments of the perfused rat tail artery display a high basal tone when compared to other isolated arteries such as the mesenteric and are suitable for the assay of vasopressor agents. However, the perfusion of this artery in the entire tail has not yet been used for functional studies. The main purpose of the present study was to identify some aspects of the vascular reactivity of the rat tail vascular bed and validate this method to measure vascular reactivity. The tail severed from the body was perfused with Krebs solution containing different Ca^{2+} concentrations at different flow rates. Rats were anesthetized with sodium pentobarbital (65 mg/kg) and heparinized (500 U). The tail artery was dissected near the tail insertion, cannulated and perfused with Krebs solution plus 30 μM EDTA at 36°C and 2.5 ml/min and the procedures were started after equilibration of the perfusion pressure. In the first group a dose-response curve to phenylephrine (PE) (0.5, 1, 2 and 5 μg , bolus injection) was obtained at different flow rates (1.5, 2.5 and 3.5 ml/min). The mean perfusion pressure increased with flow as well as PE vasopressor responses. In a second group the flow was changed (1.5, 2, 2.5, 3 and 3.5 ml/min) at different Ca^{2+} concentrations (0.62, 1.25, 2.5 and 3.75 mM) in the Krebs solution. Increasing Ca^{2+} concentrations did not alter the flow-pressure relationship. In the third group a similar protocol was performed but the rat tail vascular bed was perfused with Krebs solution containing PE (0.1 $\mu\text{g}/\text{ml}$). There was an enhancement of the effect of PE with increasing external Ca^{2+} and flow. PE vasopressor responses increased after endothelial damage with air and CHAPS, suggesting an endothelial modulation of the tone of the rat tail vascular bed. These experiments validate the perfusion of the rat tail vascular bed as a method to investigate vascular reactivity.

Key words

- Rat tail vascular bed
- Phenylephrine
- Calcium
- Flow
- Endothelium
- Vascular tone
- Perfusion pressure

Introduction

The rat tail artery has been used as a model to study several functional aspects of vascular smooth muscle (VSM). Perfusion of isolated segments of this artery has shown that it is suitable for the assay of vasopressor agents (1-5), in contrast to other vascular beds such as the mesenteric bed, where the

response to vasopressor agents is small (1,6,7). Previous reports have also shown that this artery, when isolated and perfused under constant flow, develops a high basal tone (1) compared to other isolated vascular beds (6,7).

The artery is frequently used as a small isolated dissected segment but there are no reports describing its perfusion *in vitro* as an

entire isolated rat tail vascular bed. Since the tail artery is a suitable preparation for analysis of vasoconstrictor agents and, as previously suggested, has a high intrinsic tone, the whole rat tail vascular bed severed from the body was perfused *in vitro* under constant flow to establish some aspects of its reactivity. Also, the experiments were used to validate the perfusion of this preparation as a method to investigate vascular reactivity. Specifically the present study evaluated the rat tail vascular bed response to changes of flow and external Ca^{2+} concentration both under control conditions and during continuous infusion of phenylephrine (PE). Endothelial participation in vascular tone modulation was also evaluated by comparing PE-induced contractions obtained before and after endothelial lesion.

Material and Methods

Rat tail arteries obtained from 30 male Wistar rats (EPM strain) weighing 250-350 g were used in this study. Care and use of laboratory animals were in accordance with NIH guidelines. All rats had free access to water and rat chow. Six to ten rats were used for each experimental group. For the perfusion experiments the rats were anesthetized with 65 mg/kg sodium pentobarbital *ip* and received 500 units of heparin *ip*. After 10 min, a 1-cm strip of the tail artery was dissected and cannulated near the base of the tail using stretched PE-10 tubing. The rat tail arteries were perfused with Krebs-Henseleit (KH) bicarbonate buffer containing 27.2 mM NaHCO_3 , 119 mM NaCl , 1 mM NaH_2PO_4 , 1.2 mM MgSO_4 , 1.25 mM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 5 mM KCl , 11 mM glucose and 0.03 mM EDTA, pH 7.4, bubbled with 5% CO_2 -95% O_2 at $36 \pm 0.5^\circ\text{C}$ using a peristaltic pump (Milan, Colombo, Paraná, Brazil) at a constant flow of 2.5 ml/min, except when flow rate changes were evaluated. The tail was then severed from the body and placed in a tissue bath containing

the same Krebs buffer at the same temperature. The experimental protocol was initiated after a 30- to 45-min equilibration period with the tails perfused at 2.5 ml/min. The rat tail perfusion pressure was measured with a TP-200T-Nihon-Kohden pressure transducer (connected to an MP-100 FUNBEC preamplifier) inserted between the pump and the arterial cannula and recorded continuously with a polygraphic (ANAMED, AM-820) recorder. Randomized bolus doses of phenylephrine (Sigma) (0.5, 1, 2 and 5 μg in 0.1 ml) were injected into the rubber tube just before the pump and pressor effects were measured. Consecutive doses of PE were administered at 7-10-min intervals and always injected after the perfusion pressure returned to basal values. Since a constant flow was maintained the changes in perfusion pressure represented changes in vascular resistance.

Experimental protocol

Four protocols were used. In the first protocol (N = 8) the effects of flow changes on the rat tail perfusion pressure and pressor responses to PE were studied. Step changes in flow rate were made (1.5, 2.5 and 3.5 ml/min) and the perfusion pressure was evaluated after an equilibration period of 5 min. Pressor responses to PE bolus injections (0.5 μg , 1 μg , 2 μg and 5 μg) were studied at each flow. In the second protocol (N = 6) the effects of flow increments (1.5, 2, 2.5, 3 and 3.5 ml/min) on the rat tail perfusion pressure were studied at several external Ca^{2+} concentrations (0.62 mM, 1.25 mM, 2.5 mM and 3.75 mM) in KH solution. The third protocol (N = 6) was similar to the second protocol except that Krebs solution containing 0.1 $\mu\text{g}/\text{ml}$ PE was used. In the last protocol (N = 10) the participation of the vascular endothelium modulating pressor effects was studied. For this purpose the pressor effect of PE bolus injections was investigated in the perfused rat tail vascular bed (2.5 ml/

min) before and after endothelial damage. Endothelium was damaged by perfusing air and CHAPS buffer (3,3-cholamidopropyl-dimethylammonio-1-propanesulfonate; Sigma) at a flow rate of 3.5 ml/min. Air was perfused for 2 min followed by perfusion with 5 ml KH bicarbonate buffer (1.25 mM Ca^{2+} , 2.5 ml/min) containing 0.015% CHAPS. Endothelial damage was confirmed in 4 preparations used only for histological analysis (hematoxylin/eosin staining of 5- μm transversal and longitudinal paraffin sections of tail arteries fixed in Bouin). In the other 7 preparations acetylcholine infusion (5 μg in 0.01 ml) was used in preparations pre-contracted with 0.1 $\mu\text{g}/\text{ml}$ PE. Acetylcholine promoted changes in perfusion pressure which was reduced after endothelial damage (Δ reduction in perfusion pressure: 41.3 ± 10.9 mmHg before and 8.29 ± 3.68 mmHg after endothelial damage, $P < 0.05$, Student *t*-test).

Statistical analysis was done using repeated-measures ANOVA and the Tukey test was used when statistical significance was attained. The results are reported as mean \pm SEM and were considered to be significant at the 5% level.

Results

The results of the first protocol are shown in Figure 1. The perfusion pressure increased with flow rate. PE bolus injections produced dose-dependent vasopressor responses that were larger at higher flow rates. The pressure-flow curves were displaced upwards (Figure 1) indicating that for the same dose of PE the vasopressor effects were larger at higher flow rates but maintained similar pressure-flow relationships with no difference among the effects of different flows (two-way ANOVA, interaction $P > 0.05$). In Figure 2, the lower group of curves (B) shows the changes in mean perfusion pressure (MPP) induced by increasing flow at various Ca^{2+} concentrations in the KH solution. The in-

crease in perfusion pressure induced by the higher flow was not changed despite the increase of Ca^{2+} in the KH perfusion solution.

The third protocol was performed by pre-contracting the preparations with 0.1 $\mu\text{g}/\text{ml}$ PE. When perfusing the preparations with 1.25 mM Ca^{2+} at a flow rate of 2.5 ml/min the continuous infusion of 0.1 $\mu\text{g}/\text{ml}$ PE almost doubled the perfusion pressure, producing an increase of $95 \pm 0.25\%$. Varying the external Ca^{2+} at several flow rates (Figure 2, upper group of curves, A) caused the pressure-flow curves to be displaced upwards, but with greater MPP changes at higher Ca^{2+} concentrations and flow.

The last protocol was performed by inducing PE vasopressor responses before and after perfusion with air and CHAPS buffer. Histological studies showed that the endothelial layer was damaged by the treatment. After endothelial damage the perfusion pressure increased (49.7 ± 1.98 mmHg for controls and 71.6 ± 3.84 mmHg after endothelial damage in 10 preparations). Figure 3 shows that after endothelial damage PE vasopressor responses were enhanced, suggesting that in this preparation the endothelial layer continuously modulates the VSM tone probably producing vasodilator agents.

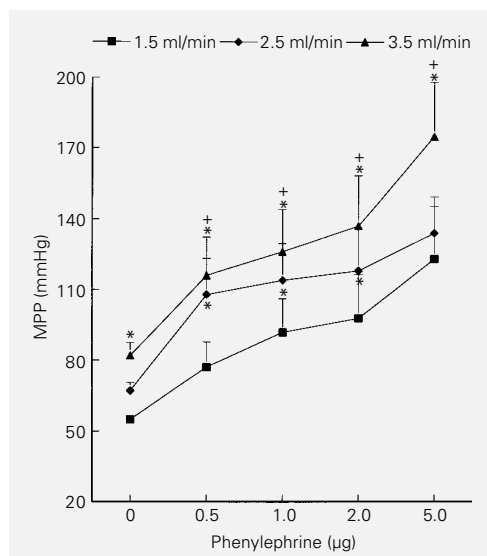
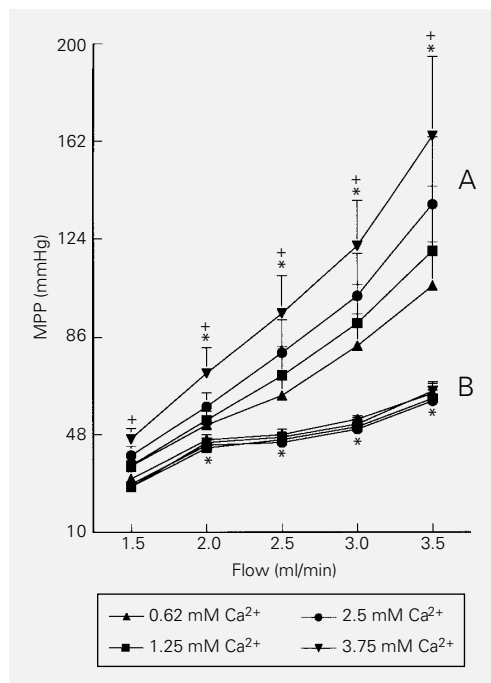


Figure 1 - Effects of 3 different flow rates on the vasopressor responses to phenylephrine (PE) in the rat tail vascular bed perfused with Krebs solution (constant flow, $\text{Ca}^{2+} = 1.25$ mM). Each point represents the means \pm SEM for N = 8. * $P < 0.01$ compared to 1.5 ml/min (Tukey test). Two-way ANOVA was used for comparisons among PE responses at different flow volumes. + $P < 0.01$ compared to zero PE at each flow (Tukey test). The interaction between the PE and flow effects was not significant ($P = 0.99$), suggesting that the three curves are only displaced upwards as the flow increases without changes in their slopes. MPP, Mean perfusion pressure.

Figure 2 - Effects of increasing flow on the mean perfusion pressure (MPP) of the rat tail vascular bed at four different Ca^{2+} concentrations. Group B (lower set of curves) was perfused with phenylephrine (PE)-free Krebs solution and the other group (A, upper set of curves) was pre-contracted with 0.1 $\mu\text{g/ml}$ PE. Each point represents the means \pm SEM for $N = 6$ rat tails. * $P < 0.01$ compared to 1.5 ml/min (two-way ANOVA). Symbols are valid for all curves obtained at different Ca^{2+} concentrations. Observe that changes in extracellular Ca^{2+} concentrations did not alter the flow-perfusion pressure relationship in PE-free Krebs solution. In preparations pre-contracted with PE two-way ANOVA indicated a significant increase in MPP as a function of increasing external Ca^{2+} concentration at each flow (* $P < 0.03$), and a significant difference in the interactions among groups ($P < 0.04$), suggesting that the relationships between PE and flow at different Ca^{2+} concentrations are different; the curves show a different slope.



Discussion

The rat tail artery is an important model for the study of several aspects of vascular function such as perfusion experiments (1-5) and analysis of the Na^+ -pump activity in preparations obtained from hypertensive animals (8). All of those studies were performed using small isolated segments of this artery (1-5,8). Although important contributions were made by those studies, the participation of an entire vascular bed, such as the mesenteric bed, with vessels of different diameters and behavior is not possible. We therefore decided to use the entire rat tail vascular bed, with the tail severed from the body, to establish some aspects of its vascular reactivity in response to changes in perfusion pressure in a constant flow model. The rat tail vascular bed developed a large perfusion pressure in response to the 2.5 ml/min flow compared to other vascular beds (1-3). The perfusion pressure increased as a function of flow and, as previously suggested for isolated segments of the tail artery (1-5), is suitable for the assay of vasopressor agents.

The arteries responded to PE with dose-dependent pressor responses. Also, PE pressor responses were larger with increasing flow. So, as previously described for the small segments of the rat tail artery (1-5), we also observed an important intrinsic tone and a large vasopressor response to PE in the rat tail vascular bed.

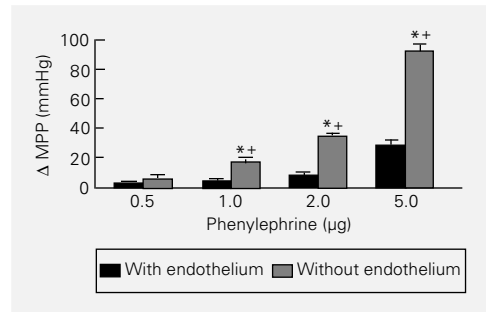
The flow increase did not generate sarcolemmal Ca^{2+} influx into the VSM. This conclusion is based on the fact that external Ca^{2+} increment did not displace the pressure-flow curves. If the increasing flow produced a sarcolemmal Ca^{2+} influx, the pressure-flow relationships should be displaced upwards at higher Ca^{2+} concentration.

However, if the sarcolemmal Ca^{2+} influx into the VSM was elicited with PE under similar conditions (Figure 2), the flow-induced pressure change was potentiated. This fact can be confirmed by comparing the values of the perfusion pressures in the pressure-flow curves in Figure 2. Although our results did not explain the underlying mechanism, they suggest that PE potentiates the pressure increase produced by flow.

The fourth protocol investigated the effects of PE after endothelial damage. Flow has been described as a stimulus for the endothelial cells to liberate vasodilator substances (9-11). On the other hand, the increase in pressure stretches the endothelial layer, inducing the release of endothelium-derived contraction factor (EDCF) (9,10,12). In our protocol, the increase in pressure may release endothelial EDCF that acts synergistically with PE by potentiating the pressure increment produced by flow. Results obtained after endothelial damage showed an increase of the PE vasopressor response together with an increase of the intrinsic basal tone. These findings suggest that the endothelium modulates the intrinsic basal tone and the VSM pressor responses of the rat tail vascular bed. These mechanisms confirm previous reports showing that the endotheli-

al cells produce vasodilator substances, such as endothelium-derived relaxing factor (EDRF) and endothelium-derived hyperpolarizing factor (EDHF) (11). EDRF has been identified as nitric oxide (13,14) produced in response to several stimuli acting on the endothelial cell including flow. Endothelial disruption then reduces the production of vasodilator substances, permitting the increase of basal tone and enhancing the vasoconstrictor effects of PE.

In conclusion, our results suggest that the entire rat tail vascular bed perfused under constant pressure has a higher intrinsic basal tone than other isolated perfused vascular beds. The preparation shows a suitable vasopressor response to PE and its mean perfusion pressure is increased when the flow is enhanced. An increase in extracellular Ca^{2+} does not alter the pressure-flow relationship if the VSM is not stimulated to contract. If



PE is perfused continuously it potentiates the flow effects. Also, in this vascular bed the endothelial layer continuously modulates the VSM tone by producing vasodilator substances; endothelial damage increased the intrinsic basal tone and enhanced PE-induced vasopressor responses. Taken together, these findings validate the perfusion of the entire rat tail vascular bed as a method to investigate vascular reactivity.

Figure 3 - Effects of increasing doses of phenylephrine (PE) on the increase in mean perfusion pressure (MPP) of the rat tail vascular bed before and after endothelial damage with air and CHAPS buffer. Preparations were perfused at a flow rate of 2.5 ml/min with Krebs solution containing 1.25 mM Ca^{2+} . Each column represents the means \pm SEM for N = 10. * $P < 0.01$ for comparisons between PE effects before and after endothelial damage (ANOVA). * $P < 0.01$ compared to 0.5 μg PE (two-way ANOVA). The interaction between the two conditions was significant ($P < 0.0001$), suggesting an enhancement of PE effects after endothelial damage.

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