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Inhibition of endothelial cell migration by shear stress

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Angiogenesis is a multistep process involving endothelial cell (EC) migration and proliferation, tightly regulated by a complex system of promoting and inhibiting factors [1]. Stimulation of angiogenesis by increased shear stress *in vivo* is associated with an increase in production of vascular endothelial growth factor (VEGF) and nitric oxide (NO) in skeletal muscle [2,3]. The shear sensor that tranduces the increased shear into an angiogenic signal is poorly understood. Evidence suggests that CD31 expressed on ECs may act as part of a mechanosensory complex [4]. Thus, we aimed to investigate the hypothesis that shear stress and VEGF interact to modulate migration of ECs, and hence control angiogenesis. In addition, we investigated the roles of CD31 as a potential sensor and NO as potential effector of shear-induced changes in this model.

Using real time PCR, changes in expression of genes known to play a role in angiogenesis; VEGF, VEGF receptor (R) 1 & 2, endothelial NO synthase(eNOS), angiopoietin (ANG) 1 & 2, neuropilin (NP) 1 & 2, were investigated in human umbilical vein EC (HUVEC) cultured under stasis or shear stress (1.5 Pa) for 24h, ±VEGF in the final 4h. To assess migration, confluent HUVEC were wounded and then exposed to stasis or shear stress (0.3 Pa or 1.5 Pa) ±VEGF; wound recovery was measured at 0 and 16h. In similar experiments, the effect of NO or CD31 was investigated using the NOS inhibitor L-NAME or siRNA for CD31, respectively.

Gene expression analysis showed that shear alone increased eNOS, VEGFR2 and NP2 expression, but decreased expression of ANG 1 & 2 and NP1. These shear-induced changes were all maintained in the presence of VEGF. Shear decreased EC wound recovery (higher the shear = lower migration; $P<0.001$ 1.5 Pa vs. Static). Nevertheless, EC preferentially migrated in the direction of flow ($P<0.01$). VEGF enhanced wound recovery to the same extent under static and shear ($P<0.05$). Neither NOS inhibition nor reduced expression of CD31 modified the shear-induced inhibition in wound recovery.

Shear stress leads to differential regulation of a number of genes involved in the control of angiogenesis. Interestingly, shear appears to be regulating molecules that modify VEGF effects, rather than VEGF *per se*. VEGF appears to have little effect on any of these key angiogenic genes. Whereas *in vivo* results show that shear stimulates angiogenesis [2], our results show that shear decreases migration of ECs, suggesting that *in vivo* additional cellular responses are being modulated. CD31 does not appear to be the sensor involved in shear-induced inhibition of migration, and NO does not appear to be the effector of this response in this model.

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Intraplatelet L-arginine-nitric oxide and vascular reactivity in an animal model of postnatal stress

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Introduction: Animal models of postnatal stress can be considered depression models, and many of their abnormalities are similar to those revealed in human depression[1-3]. The objective of this study was to investigate the participation of vascular reactivity and the intraplatelet L-arginine-nitric oxide (NO) pathway in atherothrombosis and cardiovascular events common to depression.

Methods: All experiments were reviewed and approved by the Ethics Committee of Animal Experiments of the State University of Rio de Janeiro. Eight male Wistar rats underwent a unique maternal separation (UMS) for 8 minutes at a temperature of 22°C during their second day of life; and nine control rats were included in the study. The animals were anesthethized with pentobarbital (70 mg/kg, i.p.), blood was collected by aortic puncture and the mesenteric arterial bed was rapidly removed. The reactivity of the arterial mesenteric bed was measured as described by McGregor[4]. The basal activity of intraplatelet nitric oxide synthase (NOS) was measured by the conversion of L-[^{3}H]-arginine to L-[^{3}H]-citrulline, and the influx of L-[^{3}H]- arginine was evaluated during an incubation period of 5 minutes at 37°C with L-[^{3}H]–arginine at 100 μM. Mann Whitney test was used to access the statistical significance ($p<0.05$). Data are presented as mean ± EP. Results: There was a difference in vasoconstriction (mmHg) caused by norepinephrine at concentrations of 1, 3 and 10 nmol in UMS rats (12 ± 2.5; 27.2 ± 3.4; 72 ± 9.6%, respectively) compared to controls rats (2.7 ± 2.6; 14.4 ± 3.9; 43.2 ± 7.5%, respectively). The vasodilator effect of acetylcholine was significantly increased in UMS rats compared to controls. In platelets, the basal activity of NOS in UMS rats (0.07 ± 0.01 pmol/10^8 cells) was reduced compared with controls (0.17 ± 0.03 pmol/10^8 cells.). There was also a decrease in total L-arginine transport from 0.42 ± 0.05 μmol/L/h to 0.24 ± 0.02 μmol/L/h in UMS rats.

Conclusion: Our findings demonstrate that postnatal stress presents with a dysfunction of mesenteric vascular reactivity associated with an inhibition of the L-arginine-NO pathway in platelets. These results are a possible additional mechanism to understand the pathophysiology of depression and cardiovascular complications.

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Facilitation of the myogenic response by pressure-induced changes in intracellular pH in small arteries

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The myogenic response plays an important role in blood flow autoregulation. In order to get more insight into the mechanisms of the myogenic response, the hypothesis was tested that changes in intracellular pH (pHi) are involved in the myogenic response. The study was performed on isobaric preparations of rat tail small arteries using videomicroscopic diameter determination and pH fluorimetry. In response to a pressure-increase pHi did not change throughout the early vessel dilation. During the subsequent vessel constriction pHi decreased with a similar time course as the constriction. For example, pHi was reduced by 0.12±0.01 units (n=7; $p<0.05$) upon a pressure increase from 10 to 80 mmHg. Subsequently, pHi recovered by 50.4±4.0% (n=7; $p<0.01$). In order to get insight into the mechanisms involved in the pHi alterations associated with the myogenic response, the effect of inhibition of proton transport mechanisms on the pressure-induced pHi changes was studied. Removal of extracellular bicarbonate did not reduce the early pHi peak or the later recovery phase. Application of EIPA and HOE 694, inhibitors of the Na/H-exchange, did not affect the early pHi peak, but reduced the amount of pHi recovery after a pressure increase from 10 to 80 mmHg by 48.7±3.2% (n=6; $p<0.05$). In addition, at 80 mmHg, but not at 10 mmHg, these inhibitors reduced the pHi recovery after an NH_4Cl-induced acid load. Based on the relationship between pHi and vessel diameter obtained by selectively altering pHi using NH_4Cl, it was determined that the pressure-induced pHi change accelerated the initial phase and increased the amplitude of the myogenic response. In summary, the myogenic response of rat small arteries is associated with changes in pHi, which facilitate the myogenic response. The early pHi peak seems to be coupled to the contractile process. The later pHi recovery is due, at least partly, to a pressure-induced activation of the Na/H-exchange.

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Stimulation of the AMP-activated protein kinase induces dilatation in hamster microvessels

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Recent studies suggest that the AMP-activated protein kinase (AMPK) inhibits BK_{Ca} channels in type I cells of rat carotid bodies (1). It has also been shown recently that AMPK could phosphorylate myosin light chain kinase in macrovascular smooth muscle (2), a mechanism resulting in decreased calcium sensitivity. While the earlier mechanism, when expressed in smooth muscle, would lead to vasoconstriction and inhibition of EDHF mediated dilation, the latter would lead to a vasodilation. We investigated whether a novel activator of the AMPK (3) A769662 (A76), affects microvascular tone and EDHF-dependent dilator pathways in hamster resistance arteries in which we have characterised EDHF mediated dilations on the molecular level (4).

Small resistance arteries (about 250 mm in diameter, n= 17 in total) were isolated from hamster gracilis muscle, cannulated and the smooth muscle layer loaded with the calcium indicator Fura2-AM as described previously (5). Vascular diameters were anlysed by videomicroscopy. All microvessels were pretreated with the COX inhibitor indomethacin (30μM) and the NOS inhibitor L-NAME (30μM).

When exposed to A76 (10^{-6} to 10^{-4} M), resistance arteries (preconstricted with 0.3 μM norepinephrine) showed a dose dependent vasodilatation (94.5 ± 4.0 % at 10-4M, mean ±SEM) associated with a decrease of $[Ca^{2+}]_i$ (94.5 ± 10.9% at 10^{-4}M) This vasodilation was not endothelium dependent. In arteries pre-constricted with 100mM potassium, A76 (10^{-4} M), induced neither relaxation nor decreased smooth muscle calcium. There was also no calcium independent dilation during an observation period of 15 min. Neither inhibition of IK_{Ca} / BK_{Ca} potassium channels with CTX (1μM) – which has been shown to inhibit EDHF mediated dilations previously - nor IBTX(10^{-7} M), a specific inhibitor of IK_{Ca} did alter maximal effects of A76 significantly whereas the K_{ATP} inhibitor glibenclamide (0,1mM) reduced dilation to 10.6 ±5.0 % as well as the decrease of $[Ca^{2+}]_i$ to 35.1 ± 0.5 %.