Endothelin-1 (ET-1) and its receptors on haemorrhoidal tissue: A potential site for therapeutic intervention
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Objectives: Haemorrhoids is a common anorectal condition that affects millions worldwide. We studied the potential role of ET-1 and its (ETA and ETB) receptors in haemorrhoid tissue. Methods: ET-1, ETA and ETB receptor localisation was studied in haemorrhoids using autoradiography and immunohistochemistry. Protein expression was compared between haemorrhoids and normal rectal submucosa using Western blot analysis. ETA and ETB receptor antagonist effects on ET-1- and sarafotoxin 6a-induced contraction of human mesenteric artery and vein were assessed by myography. Results: There was dense [125I]-ET-1 binding to haemorrhoidal sections with ETB > ETA binding (12.7 ± 3 vs 4.4 ± 0.6 pM/mg N, n = 3, NS). Immunohistochemistry revealed a higher ETB than ETA receptor immunostaining in haemorrhoidal than in control rectal tissue. This was confirmed by Western blot analysis where haemorrhoidal ETB receptor protein levels were about 4 times higher than ETA receptors (78.3 ± 28.0 vs 18.8 ± 3.6 densitometric units, p = 0.026). ETA and ETB receptors were localised to smooth muscle of mesenteric arteries and veins with ETB receptors also on endothelium. Myograph studies showed that the sensitivity and maximum contractile response to ET-1 and sarafotoxin 6a were greater in mesenteric veins than in arteries (p < 0.05). Conclusions: ETA and ETB receptors are present in haemorrhoids with ETB receptors predominating. Mesenteric veins are more sensitive than arteries to ET-1-induced contraction, an effect that is blocked to a greater extent by ETB compared with ETA receptor antagonists. Since ETB receptors are located on smooth muscle of both the mesenteric artery and vein ETB agonists may have therapeutic potential via a constrictor action on haemorrhoidal blood vessels.


Endothelin-1 modulates bile secretory function in rats
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Study was conducted in acute trials on rats anaesthetized with urethane 100 mg/100 g of body weight. Endothelin-1 0.1 μg/100 g was injected into the portal vein and 0.9% NaCl (100 μl/100 g) was administrated to the control animals. Choleresis was estimated in microlitres, each for 10 min, during a three-hour experiment. Six half-hour bile samples were collected. Concentration of the bile acids, and lipids in bile was determined by thin-layer chromatography. Endothelin-1 in vasoactive concentration (0.1 μg/100 g) decreased choleresis with maximum reduction by 15.5% (p < 0.05) in 40 min following its administration and exhibited diverse effects on concentration of different bile acids. Taurocholic acid was gradually reduced in control and experimental animals, but its level was higher by 9.3% (p < 0.05) in the sixth sample of experimental animals. Concentration of glycocholic acid was reduced in the control animals, but increased following endothelin-1 administration by 12.3% (p < 0.05), 19.7% (p < 0.01), and 16.3% (p < 0.01) in the last three bile samples correspondingly. Endothelin-1 caused an increase of free bile acids. Concentration of cholic acid increased in the third and sixth samples by 20.6% and 19.8% (p < 0.05) respectively. Phospholipids increased in the fifth bile sample in endothelin-1 action by 16.7% (p < 0.05). Free cholesterol decreased slightly but ether-coupled cholesterol increased in the last two samples by 43.8% (p < 0.05) and 45.7% (p < 0.05) respectively. Triglycerides increased gradually and 50% (p < 0.05) elevation was found in the fourth bile sample. So endothelin-1 intensifies conjugation of the free bile acids and biosynthesis of ether-coupled cholesterol, trihydroxycholane acids and phospholipids in liver that assist in the improvement of bile colloidal system characteristics.


Endothelin system in intestinal villi: A possible role of endothelin-2 in the maintenance of intestinal architecture
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The endothelin system consists of three ligands (ET-1, ET-2 and ET-3) and at least two receptors (ETA and ETB). In intestinal villi, fibroblast-like cells express endothelin receptors and response to ET-1 and ET-3 peptides, changing their cellular shape. Several functions have been attributed to these peptides in the “architecture” maintenance of intestinal villi acting over sub-epithelial fibroblasts. Despite this, ET-2 has not been analyzed in depth. In this work we show the intestine gene expression and immunolocalization of ET-1, ET-2 and ETA and ETB receptors from the duodenum to the rectus and in the villus–crypt axis in mice, allowing a complete analysis of their functions. While ET-1 is expressed uniformly, ET-2 had a particular distribution, being higher at the bottom of the villi of the duodenum, ileum and jejunum and reverting this pattern in the crypts of the colon and rectus, where the higher expression was at the top. We postulated that ET-2 would act in a cooperative manner with ET-1, giving the villus the strength enough to withstand mechanical stress.


Vasoprotective effect of endothelin receptor antagonist in ovariectomized female rats
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The effects of hormone replacement therapy with estrogen on cardiovascular disease in post menopausal women are still controversial. We previously reported that endothelin (ET)-1/ET receptor system is involved in sex differences in the development of neointimal formation after vascular injury. In the present study, we hypothesized that dual ETA/ETB receptor antagonist (ERA) could exhibit vasoprotective effects after menopause. To confirm it, we
examined the effects of ERA and/or angiotensin receptor blocker (ARB) on neointimal formation after vascular injury in intact and ovariectomized (OVX) female rats. The right carotid artery was exposed to balloon injury, and harvested 2 weeks after the injury. In intact female groups, treatment with ARB for two weeks after the injury significantly decreased neointimal formation, whereas treatment with ERA did not affect neointimal formation. On the other hand, in the OVX groups, ET antagonist markedly decreased neointimal formation after the injury although neointimal formation was not significantly improved by ARB. Combined treatment with 17β-estradiol and ARB markedly suppressed neointimal formation after the injury in the OVX groups, whereas there were no additive effects during combined treatment with 17β-estradiol and ERA. These ERA or 17β-estradiol-induced vasoprotective effects in the OVX groups were related to the suppression of NADPH oxidase-dependent reactive oxygen species production. Taken together, ERA has an estrogen-like vasoprotective action on neointimal formation via inhibition of oxidative stress. ERA may be an alternative therapy for the prevention of vascular disease in postmenopausal women.


Increased cerebrovascular sensitivity to endothelin-1 in obstructive sleep apnea rats is endothelin-B receptor mediated


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Obstructive sleep apnea (OSA) has been identified as a significant risk factor for stroke. However, little is known regarding the effects of OSA on the cerebrovascular wall. We hypothesized that OSA augments endothelin-1 (ET-1) induced constrictions of cerebral arteries. Using rats chronically instrumented with an inflatable endotracheal obstruction device we simulated a moderate severity of sleep apnea, 30 apneas/h for 8 h/day (sleep phase) for 1 month. Cerebral arteries were harvested for analysis of gene-expression, immunohistochemistry, and vascular reactivity. Following 1 month of OSA, blood pressure and plasma/cerebral vessel ET-1 levels were similar in sham and OSA rats (n = 4–7, NS). Using the pressurized cerebral artery preparation, we observed a 17.5-fold increase in sensitivity to ET-1 (n = 5–6, p < 0.05). The increased sensitivity of OSA cerebral arteries to ET-1 was abolished by the ET-B receptor antagonist BQ-788 (n = 6, NS). However, increased ET-1 sensitivity of OSA cerebral arteries persisted in the presence of the ET-A receptor antagonist BQ-123 (n = 3–6, p < 0.05). Additionally, constrictions to the ET-B specific agonist IRL-1620 were significantly greater in OSA, versus sham, cerebral arteries (n = 6, p < 0.05). Gene expression analysis revealed no difference in the mRNA levels of ET-1, ET-a or ET-b receptors, or endothelin converting enzyme (ecel1) (n = 6, NS). However, immunohistochemical analysis demonstrated elevated levels of ET-B receptor in the smooth muscle of OSA cerebral arteries. These data demonstrate that OSA increases the sensitivity of cerebral arteries to ET-1, which appears to be driven by increased ET-B receptor signaling. These observations suggest an important role of ET-1 signaling in the adverse cerebrovascular outcomes associated with OSA.


The Akt pathway mediates the neuroprotective effect of IRL-1620 in a rat model of focal cerebral ischemia

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We found that the ETB receptor agonist, IRL-1620, provides significant neuroprotection following permanent cerebral ischemia. The serine/threonine kinase Akt plays a role in regulating cell survival and death cascades, and may mediate the neuroprotective effect of IRL-1620. The present study investigated the effect of IRL-1620 on the phosphorylation state of Akt in a rat model of cerebral ischemia. Male Sprague–Dawley rats underwent permanent middle cerebral artery occlusion. Following surgery, rats received three intravenous injections of vehicle or IRL-1620 (5 μg/kg) at 2, 4, and 6 h post-occlusion. Evaluation of behavioral parameters confirmed the induction of stroke. Animals were sacrificed 7 and 24 h following occlusion and brains were processed to evaluate protein expression of total Akt and Akt phosphorylated at Ser473. There were no significant changes in behavioral parameters between the vehicle and IRL-1620 groups 7 h post-occlusion. However, at 24 h there was a marked improvement in the behavioral parameters of IRL-1620 compared to vehicle treated rats. There were no changes in total Akt expression levels in the brains of sham, vehicle, and IRL-1620 treated rats; however, there was an increase in the phosphorylation of Ser473 of Akt (p473-Akt) in the IRL-1620 treated rats compared to sham (P<0.01) or vehicle treated (P<0.05) rats 7 h post-occlusion. No difference in total Akt or p473-Akt levels was observed 24 h post-occlusion in the brains of sham, vehicle, or IRL-1620 treated rats. It is concluded that IRL-1620 causes a transient elevation in phosphorylation of Ser473 after 7 h of cerebral ischemia, suggesting that the Akt pathway may be involved in mediating the neuroprotective effect of IRL-1620.

Sepsis is a disease of the microcirculation, with endothelial dysfunction playing a key role in its pathogenesis and subsequent associated mortality. Pathophysiology of brain dysfunction due to sepsis remains poorly understood. Cerebral microcirculatory alterations may play a potential role; however, experimental data are scarce. The present study sought to investigate whether key angiogenic pathways are altered in the frontal cortex in a clinically relevant animal model of endotoxemia/sepsis. Male mice at 8 weeks of age were administered either saline or 20 mg/kg lipopolysaccharide (LPS) at different time points (1, 3, 6, and 10 h). Mice that did not receive LPS were considered to be controls. We confirmed the induction of endotoxemia by measuring circulatory TNF-alpha level as well as cerebral mRNA levels of TNF-alpha, IL-1beta, and IL-6. Vascular endothelial growth factor (VEGF), a major vascular multifactorial cytokine involved in all the three types of vascular growth namely, angiogenesis, arteriogenesis and atherogenesis, mediates its angiogenic action through its receptor VEGF-R2. In the frontal cortex...