Emerging role of relaxin in renal and cardiovascular function

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Conrad, Kirk P., and Jacqueline Novak. Emerging role of relaxin in renal and cardiovascular function. Am J Physiol Regul Integr Comp Physiol 287: R250–R261, 2004; 10.1152/ajpregu.00672.2003.—Although traditionally associated with reproductive processes, relaxin is emerging as an important player in renal and cardiovascular function. Much of our recently acquired understanding of relaxin in this new context has arisen from studies of maternal renal and cardiovascular adaptations to pregnancy in rats where the hormone is turning out to be an important mediator. First, we highlight the influence of relaxin on renal hemodynamics and glomerular filtration rate, as well as on other peripheral circulations. Second, we discuss the effect of relaxin on both the steady and pulsatile systemic arterial load, as well as on the heart, in particular, coronary blood flow. Third, we consider the impact of the hormone on cultured endothelial and vascular smooth muscle cells. Fourth, we address the interaction of relaxin with renal and cardiac disease, as well as its role in angiogenesis. Finally, in Perspectives, we point out several key research questions in need of investigation that relate to a potential autocrine/paracrine role of relaxin in renal and cardiovascular tissues. Furthermore, on the basis of its potent vasodilatory and matrix-degrading attributes, we speculate about the therapeutic potential of relaxin in renal and cardiovascular diseases.

pregnancy; kidney circulation; glomerular filtration; osmoregulation; peripheral circulation; systemic hemodynamics; vasodilation; arterial compliance; heart; myogenic reactivity; angiogenesis; relaxin receptors; matrix metalloproteinase; gelatinase; endothelin B receptor; nitric oxide; angiotensin II

THE OBJECTIVE OF THIS REVIEW is to highlight the new and emerging roles of relaxin (RLX) in the renal and cardiovascular systems. Traditionally, RLX has been tied to reproductive processes during pregnancy, because most investigations conducted by reproductive biologists have dealt with the hormone in this context. The traditional sources of RLX have been reproductive tissues such as the corpus luteum of the ovary from which the hormone emanates and circulates during the luteal phase of the menstrual cycle and during pregnancy in women (95). Furthermore, there has been no well-established role in the male. However, the concept of a role for RLX in cardiovascular function actually has its antecedents early on in the history of RLX research, when Frederick L. Hisaw, who discovered the hormone (47, 113), noted that after administration of RLX to castrated monkeys, there were marked morphological changes in the endothelial cells of the endometrium consistent with hypertrophy and hyperplasia (48). The concept that RLX can affect blood vessel structure and function has since gained considerable support, particularly in the last decade. Much of our recently gained knowledge of RLX in this new light has arisen from studies of maternal renal and cardiovascular function in the gravid rat model where RLX is emerging as a prominent player. In fact, most findings described in this review were obtained from studies in species other than humans. As such, their applicability to humans is in urgent need of confirmation.

Section 1 of this review addresses the influence of RLX on the physiology of 1) the renal circulation; 2) the circulation of other organs; 3) steady and pulsatile systemic arterial load; 4) the heart, especially coronary blood flow, as well as the impact of the hormone on 5) cultured endothelial and vascular smooth muscle cells. Section 2 will deal with the interaction of RLX with renal and cardiac disease, as well as its role in angiogenesis.

SECTION 1: INFLUENCE OF RLX ON PHYSIOLOGICAL PROCESSES

Influence of RLX on the Kidney: Lessons From Pregnancy

Renal circulation during pregnancy. The discovery of the renal vasodilatory action of RLX arose from work on the renal circulation during pregnancy (16). Decreased vascular resistance of nonreproductive organs is one of the earliest physiological adaptations to occur in normal pregnancy leading to a profound decrease in systemic vascular resistance (SVR). The kidneys make a major contribution to this reduction in SVR: a nadir in renal vascular resistance and peak in renal blood flow and glomerular filtration rate (GFR) are reached by the end of the first trimester in women. Thus the kidneys and presumably other nonreproductive organs of typically high vascular conductance vasodilate even further during early gestation, effectively serving as large “arteriovenous shunts.” In turn, the reduced SVR initiates the gestational rise in cardiac output by reducing cardiac afterload and abets the expansion of plasma volume by instigating arterial underfilling, thereby stimulating renal sodium and water retention, the latter by increasing
sympathetic activity and activating the renin-angiotensin-aldosterone axis. This early gestational rise in cardiac output anticipates the tremendous increase in uteroplacental blood flow, as well as the oxygen and nutrient demands of the nascent fetoplacental unit(s). Indeed, the arterial-mixed venous oxygen content difference narrows during early pregnancy in both humans and rats, indicating that oxygen delivery exceeds demand (see Ref. 42 and citations therein).

**Does RLX contribute to the changes in the renal circulation during pregnancy?** There were several compelling, albeit circumstantial, reasons to consider RLX as the mediator of renal vasodilation and hyperfiltration during pregnancy as recently reviewed (16). To begin testing this question directly, however, Danielson and colleagues (22) first investigated whether RLX could vasodilate the renal circulation when chronically administered to conscious, nonpregnant rats. They reported that prolonged administration of porcine RLX (pRLX) or of recombinant human RLX (rhRLX) to chronically instrumented, conscious female rats increased both effective renal plasma flow (ERPF) and GFR to levels observed during midterm pregnancy when renal function peaks in this species (12). This response to rhRLX was not dependent on the presence of ovaries (22) and, of additional interest, was observed in male rats (21).

The osmoregulatory changes of pregnancy as determined by reductions in plasma sodium concentration and osmolality were recapitulated in these studies (12, 21, 22, 62). Chronic pRLX administration diminished the renal vasoconstrictor response to angiotensin II infusion (22)—a phenomenon also observed during rat gestation (13, 20). Furthermore, the myogenic reactivity of small renal arteries isolated from the rhRLX-treated rats was reduced (77) and comparable to the reductions observed in small renal arteries isolated from midterm pregnant rats (35).

The time course and dose response of rhRLX-mediated renal circulatory and osmoregulatory changes in conscious rats were recently reported (19). The effect of RLX was biphasic, insofar as low doses were vasodilatory and produced reductions in plasma osmolality, whereas high doses were relatively inactive. In fact, the relative inactivity of high-dose RLX, which produced circulating levels similar to those observed in late rat pregnancy, may explain the waning of renal function at this gestational stage (12). The onset of action was found to be considerably more rapid than previously believed, within 1–2 h of starting an intravenous infusion.

Finally, by using RLX neutralizing antibodies or removing circulating RLX by ovariectomy, gestational renal vasodilation, hyperfiltration, and reduced myogenic reactivity of small renal arteries isolated from midterm pregnant rats on gestational days 11 and 14 were totally abolished (75). The osmoregulatory adaptations of pregnancy at midterm were also prevented (75). Thus RLX is essential for the renal circulatory and osmoregulatory changes of pregnancy in the gravid rat model. The increases in GFR and ERPF that occur during rat pregnancy before gestational day 9 or during pseudopregnancy when circulating RLX is undetectable are likely to be mediated by other, as of yet, undefined mechanisms.

Whether RLX can vasodilate the renal circulation and modify osmoregulation in humans, as well as contribute to the renal circulatory and osmoregulatory changes of pregnancy in women, is currently under investigation. Tantalizing preliminary results that emerged from the rhRLX clinical trials in systemic sclerosis suggested that the hormone increased the predicted creatinine clearance (29).

**Molecular mechanisms of RLX action: a pivotal role for nitric oxide and endothelin.** The renal circulation participates in the overall maternal vasodilatory response to pregnancy. Peak renal vasodilation and hyperfiltration are observed in rats at midgestation (12). In chronically instrumented conscious rats acutely administered analogs of l-arginine such as nitro-L-arginine methyl ester that inhibit nitric oxide synthase (NOS), GFR, ERPF, and effective renal vascular resistance converged in midterm pregnant and virgin control animals (20). That is, compared with virgin rats, the gravid animals responded more robustly to acute NOS inhibition, showing a greater decline in GFR and ERPF and a greater rise in effective renal vascular resistance. Consistent with these in vivo observations were the findings that myogenic reactivity of small renal arteries isolated from midterm pregnant rats was reduced relative to virgin control animals, and inhibitors of NOS restored this reduced myogenic reactivity to virgin levels (35). Subsequently, an essential role for NO in the renal vasodilation, hyperfiltration, and reduced myogenic reactivity of small renal arteries was also established for RLX-treated nonpregnant rats (22, 77).

Paradoxically, endothelin (ET) is also fundamental to the renal vasodilation and hyperfiltration manifested by pregnant and RLX-treated nonpregnant rats. Although ET is widely known as a potent vasoconstrictor by interacting with ETA and ETB receptor subtypes on vascular smooth muscle (44), the peptide has also been shown to raise intracellular calcium in endothelial cells, thereby stimulating prostacyclin, NO, and possibly other relaxing factors through the endothelial ETB receptor subtype (45, 46, 104, 111). In view of the reputation of ET as a potent vasoconstrictor, it was surprising to find that disruption of the ET-1 gene in heterozygous mice elevated blood pressure (60), and specific blockade of the ETA receptor subtype in chronically instrumented conscious male rats with the pharmacological antagonist RES-701–1 produced marked renal vasoconstriction (41). These unexpected findings are consistent with a major role for endogenous ET in maintaining low renal vascular tone via an RES-701–1-sensitive endothelial ETB receptor subtype either by tonic stimulation of endothelial-derived relaxing factors and/or restraint of ET production (45, 46, 59, 104, 111). Similar conclusions were reached by using mixed ETA/ETB antagonists (23, 90), although the renal vasoconstriction was less pronounced most likely because the “vasodilator” ETB receptor subtype on the endothelium and the “vasoconstrictor” ETA and/or ETB receptor subtypes on the vascular smooth muscle were blocked concurrently. RES-701–1 is relatively selective for the “vasodilator” ETB receptor subtype on the endothelium (40). The renal vasoconstriction produced by RES-701–1 or by nitro-l-arginine methyl ester was not significantly affected by BQ-123 (an ETA receptor antagonist), but was markedly attenuated by SB-209670 (a mixed ET, ETA/ETB receptor antagonist), suggesting the presence of an ETB receptor subtype on vascular smooth muscle that primarily mediates renal vasoconstriction when unopposed by the endothelial ETB receptor (40). Moreover, renal vasoconstriction elicited by sarafotoxin 6C (S6C), a selective ETB agonist, was potentiated by RES-701–1 and nitro-l-arginine (Ref. 41 and personal communication from M. Gellai). Thus the RES-701–1-sensitive ETB receptor subtype located on the
endothelial cells. Whether the relaxin receptor also resides in caveolae requires further investigation. See text for further details.

Because endogenous ET acting through the endothelial ETB receptor subtype contributes to the low vascular resistance of the renal circulation most likely by tonic stimulation of NO, it was logical to test whether this mechanism is accentuated during pregnancy. Indeed, acute administration of the endothelial ETB receptor antagonist RES-701-1 inhibited gestational renal vasodilation and hyperfiltration, thereby resulting in a convergence of GFR, ERPF, and effective renal vascular resistance in conscious gravid and virgin rats (15), as well as a reversal of the reduced myogenic reactivity of small renal arteries isolated from gravid rats (35), comparable to the findings using inhibitors of NOS. Moreover, the NO-cGMP pathway was shown to mediate the vasodilatory role of endogenous ET in the renal circulation during pregnancy (15, 35). In analogous studies, an essential role for the endothelial ETB receptor subtype in the renal vasodilation, hyperfiltration, and reduced myogenic reactivity of small renal arteries was also established for rhRLX-treated nonpregnant rats (21, 77).

Molecular mechanisms: a pivotal role for vascular gelatinase activity. Further elucidation of the molecular mechanisms of pregnancy (RLX)-mediated vasodilatory changes in the renal circulation has been sought (54). During pregnancy, RLX may alter one or more of the steps in the vasodilatory pathway depicted in Fig. 1. One possibility to be discussed in detail here is based on the confluence of several observations: 1) the crucial role for RLX, the endothelial ETB receptor, and NO in pregnancy-mediated renal vasodilation as described above, 2) the ability of RLX to upregulate matrix metalloproteinase activity (MMP) activity (at least in fibroblasts; 82, 105, 108), and 3) the potential for vascular MMPs, such as MMP-2, to process big ET at the Gly-Leu bond to yield ET1-32, the latter capable of activating ET receptors (31, 32). Thus RLX may upregulate MMP-2 activity in the renal vasculature during pregnancy, thereby leading to renal vasodilation, hyperfiltration, and reduced myogenic reactivity of small renal arteries in an ET- and NO-dependent fashion.

The best (if not the only) approach to testing the physiological role of MMP-2 in mediating renal vasodilation, hyperfiltration, and reduced myogenic reactivity of small renal arteries induced by RLX is to block MMP-2 production or inhibit its action. The latter approach was taken, first using a newly developed specific peptide inhibitor of gelatinases (57, 86). Cyclic CTTWGFSLTC (cyclic CTT) has some preference for inhibiting MMP-2 relative to MMP-9 and is 10-fold more potent than STHWGFTLTS (STT). After only 30 min of infusion, suppressor doses of cyclic CTT (1 and 3 μg/min), but not the control peptide, STT, completely reversed the renal vasodilation and hyperfiltration induced by chronic administration of rhRLX (54). These results support the concept that gelatinase(s) process big ET to ET1-32, which, in turn, activates the endothelial ETB receptor, thereby mediating renal vasodilation and hyperfiltration via NO (Fig. 1). To corroborate the concept that gelatinase(s) are crucial for the renal vasodilatory response to RLX, a general and well-established inhibitor of MMPs, GM-6001, was also employed (34). GM-6001, but not the dilute DMSO vehicle, totally abrogated the renal vasodilation and hyperfiltration induced by rhRLX (54).

The traditional pathway for ET formation is through the endothelin converting enzyme (ECE), which processes big ET to ET1-21. In addition to providing evidence for a critical role of gelatinase(s) in the formation of ET in RLX-treated rats, evidence was marshaled against the traditional ECE pathway. Toward this end, the ECE inhibitor, phosphoramidon, was employed (70). Consistent with the hypothesis, phosphoramidon did not alter the renal circulation in rhRLX-treated rats despite the complete blockade of the pressor response to big ET-1. Nor did the inhibitor affect renal hemodynamics in vehicle-infused control animals (54, 89). Thus renal vasodilation and hyperfiltration induced by RLX persisted in the presence of pharmacologic blockade of ECE.

The phenomenon of reduced myogenic reactivity of small renal arteries has served as a faithful bioassay for the renal vasodilatory changes induced by pregnancy or RLX treatment of nonpregnant rats (35, 75, 77). Therefore, small renal arteries isolated from midterm pregnant or rhRLX-treated nonpregnant rats were incubated with inhibitors of MMP or ECE to test whether they would reverse the reduction of myogenic reactivity. Analogous to the findings in conscious rats, incubation of small renal arteries isolated from RLX-treated nonpregnant or midterm pregnant rats with either cyclic CTT or GM-6001 reversed the reduced myogenic reactivity (54). Similar results were obtained using the general MMP inhibitor, TIMP-2, and a specific MMP-2 neutralizing antibody. In contrast, and again consistent with the results in the conscious rats, phosphoramidon did not affect the reduction in myogenic reactivity. These results obtained from the study of myogenic reactivity of small renal arteries recapitulate those dealing with renal hemodynamics in the conscious rats, thereby further supporting a pivotal role for MMPs, in particular gelatinase, in the renal circulatory changes of pregnancy or RLX-treated nonpregnant rats (54). Moreover, they implicate a vascular source, rather than circulating enzyme.
Although vascular gelatinase activity may be essential for the renal circulatory changes in pregnancy or RLX-treated nonpregnant rats, vascular gelatinase itself may not be an actual locus of regulation by RLX. In other words, vascular gelatinase activity is clearly in the vasodilatory pathway, but is it vascular gelatinase or some other element in the pathway (e.g., endothelial ETB receptor expression) that is regulated by RLX, thereby enhancing renal vasodilation ultimately via NO (Fig. 1)? To begin addressing this question, gelatinase activity was measured in small renal arteries (54). An ~50% increase in both pro- and active MMP-2 activity was observed in small renal arteries from rhRLX-treated and midterm pregnant rats relative to their respective controls. Of interest, pro-MMP-9 activity was also consistently increased in the small renal arteries from midterm pregnant rats. Whether this upregulation of gelatinase activities occurs in the endothelium (as postulated in Fig. 1), the vascular smooth muscle, or both, is currently unknown.

It should be pointed out that alteration of vascular gelatinase activity as a pivotal step in the circulatory adjustments of pregnancy by RLX, and the potential for concurrent regulation of the ETB receptor or NOS are not mutually exclusive (15, 35). Surprisingly, however, NOS and ETB receptor expression in small renal and other arteries was not significantly different between midterm pregnant and virgin or RLX- and vehicle-treated nonpregnant rats as assessed by Western analysis (Ref. 76; and K. Conrad, L. Kerchner, unpublished observations).

In this review, a pivotal role for vascular MMP-2 activity in the renal vasodilation, hyperfiltration, and reduced myogenic reactivity of small renal arteries during pregnancy or during RLX administration to nonpregnant rats has been presented (54). A crucial role for the endothelial ETB receptor/NO vasodilatory pathway was also described (15, 20–22, 35, 77). Thus it is logical that vascular MMP-2 is in series with, and upstream of, the endothelial ETB receptor subtype and NO (Fig. 1). This linkage is based on the recent finding that vascular MMP-2 can process big ET to ET1–32 at Gly32-Leu33, and ET1–32 in turn, elicits either vasoconstriction or vasorelaxation depending on the experimental circumstance through the vascular smooth muscle or endothelial ETB receptor, respectively, the latter via NO (31, 32). There are two compelling reasons why it is highly unlikely that vascular MMP-2 and the endothelial ETB receptor/NO here are members of separate vasodilatory pathways, which operate in parallel. First, there was no compensation or even partial compensation when either vascular MMP-2 or the endothelial ETB/NO pathway was inhibited separately. Each and every inhibitor of the ETB receptor (15, 21, 35, 77), NOS (15, 20, 22, 35, 77), or MMP (54) totally abolished the renal circulatory changes during pregnancy or RLX treatment of nonpregnant rats. If the vascular MMP-2 or the endothelial ETB/NO pathway are separate and parallel vasodilatory pathways, one might anticipate some degree of compensation of one for the other. Second, small renal arteries from rhRLX-treated nonpregnant rats in which the ETB receptor gene was disrupted (39) failed to manifest reductions in myogenic reactivity; however, the MMP-2 activity of these arteries was increased by the hormone (54). This dissociation of the increased vascular MMP-2 activity from the reduced myogenic reactivity in small renal arteries that lack the ETB receptor strongly argues that vascular MMP-2 is in series with, and upstream of, the endothelial ETB receptor and NO.

Because the endothelial ETB/NO pathway also contributes to the relatively low vascular resistance of the renal circulation in nonpregnant female and male rats (see above), then vascular MMP-2 may play a critical role here, too. Although a 3 μg/min infusion of cyclic CTT did not increase systemic blood pressure, it did significantly decrease GFR, ERPF, and increase ERVR in both vehicle- and rhRLX-treated rats, only more so in the latter (54). It is also noteworthy that phosphoramidon not only failed to alter renal hemodynamics in the RLX-infused rats, but also in the control animals administered the vehicle for RLX (54). Thus vascular MMP-2 may be the major ET converting enzyme responsible for provision of substrate to the endothelial ETB/NO vasodilatory pathway in the nonpregnant condition as well. Possibly, the colocalization of MMP-2 and associated proteins in the caveolae of endothelial cells with the ETB receptor and eNOS facilitates this interaction (Fig. 1, and see Perspectives).

Influence of RLX on the Vasculature of Other Organs

**Uterus.** The first evidence suggesting that RLX affects the vasculature was based on histological studies conducted in the uterus. In 1966, Dallenbach-Hellweg and colleagues (18) described an enlargement of the arterioles and capillaries in the superficial portions of the endometrium of castrated monkeys treated with RLX (18). In a later study also in monkeys, they reported not only an enlargement of the vessels but also endometrial cell proliferation in the endometrial vessels (48). Vasilenko and colleagues (109) described similar findings in ovariectomized rats administered porcine RLX. Immunohistochemical studies demonstrated binding sites for RLX on cells associated with blood vessels in the uterus, cervix, and vagina of pigs and humans (56, 71). These studies are consistent with a vasodilatory role for RLX in the uterine circulation.

The results of functional studies are less clear. rhRLX treatment has been shown to increase uterine blood flow in conscious ovariectomized female rats (74); however, porcine RLX had no effect on endometrial and myometrial blood flow in mature anesthetized sheep (93). It is likely that species differences explain this discrepancy. Sheep do not produce RLX because the mRNA contains numerous stop codons in the C-peptide region, which prevent the translation of a functional RLX molecule (91). In humans, there are no studies directly examining the effects of RLX on uterine blood flow. However, in phase II/III trial of rhRLX in the treatment of scleroderma, the most frequent adverse event reported by women receiving the hormone was heavy or irregular menstrual bleeding, suggesting increased endometrial vascularization. In addition, rhRLX increases expression of VEGF in human endometrial cells in culture (106). These findings support a role for RLX in the regulation of human uterine blood flow. In contrast, a study in pregnant women showed a positive correlation between plasma RLX levels and the resistance or pulsatility index measured by ultrasound early in gestation. This suggests that RLX may increase uterine artery resistance during pregnancy (53). Finally in an in vitro study of human intramyometrial arteries, rhRLX had no effect on either resting tension or tension induced by U-46619, ET, or PGF2α (83). The role for RLX in the modulation of uterine blood flow requires further investigation.
Placenta. RLX binding was found on blood vessels in the amnion and within the placental villi (56). Despite the fact that binding sites for RLX were identified, rhRLX did not affect resting tension or tension induced by U-46619, ET-1, or PGF$_{2\alpha}$ in human placental stem villous arteries studied in vitro (83). Another study involving human umbilical arteries also failed to demonstrate an effect of in vitro treatment with RLX or RLX and progesterone on serotonin- and KCl-stimulated contractions (24).

Mammary gland. Evidence that RLX may act as a vasodilator in the mammary circulation includes a morphometric analysis of the dimensions of microvessellumina from ovariec- tomized mice 18–20 h after a single injection of porcine RLX. In this study, the mean diameters of arterioles, capillaries, and postcapillary venules in the mammary glands of the RLX-treated mice were significantly larger compared with ovariec- tomized control mice (7). It is possible that this effect of RLX is not limited to the mouse because RLX binding sites have been identified in blood vessels of the human mammary gland and nipple (56). The pigeon crop sac is a structure analogous to the mammary gland, and there are several advantages to studying this organ. One is that hormones can be administered by intradermal injection that allows for the study of the local effects. Another advantage is that the structure of the crop sac allows for treatment of only one hemi-crop, whereas the contralateral crop from the same animal serves as the control. In this tissue, there was a striking dilation of the blood vessels in the lamina propria of mucosa after 6 h in porcine RLX-treated (1 $\mu$g) pigeon crop sac compared with the vehicle-treated hemi-crop (10).

Liver and mesentery. The effect of RLX on the hepatic vasculature was investigated in male rats. In this study, prRLX dilated the liver sinusoids. This effect was reduced by NO synthesis inhibition, suggesting a role for NO (6).

In the mesenteric circulation, RLX modulates the vascular responses to vasoconstrictor agents. In the perfused mesentery of the spontaneously hypertensive rat in situ, the vasoconstric- tor responses to arginine vasopressin and norepinephrine were decreased after a 42-h infusion of purified rat RLX (67). In the same study, the sensitivity to norepinephrine was also de- creased in the isolated portal vein from RLX-treated rats, whereas the sensitivity to angiotensin was unchanged (67). A 6-h incubation of isolated mesenteric and renal arteries, as well as aorta, from rats with rhRLX decreased the maximal con- tractile response and sensitivity to ET-1, whereas the relaxation response to ET-3 was increased (25). ET-1 contracts arteries by interacting with ET$_A$ and ET$_B$ receptor subtypes on vascular smooth muscle. ET-3 relaxes arteries by interacting with the ET$_B$ receptor subtype on endothelium. These functional re- sponses suggested that RLX upregulated the endothelial ET$_B$ receptor, thereby offsetting the contractile response to ET-1 and potentiating the relaxation response to ET-3. Mesenteric arteries isolated from rhRLX-treated rats were also less responsive to changes in intraluminal pressure (reduced myogenic reactivity) compared with vehicle-treated rats when studied in a pressure arteriograph system (77). In the mesocccum, admin- istration of prRLX to male Wistar rats induced a rapid dose-dependent dilation of the veins; however, arteriolar and capill- lary flows were unchanged (11). RLX treatment also opposed the vasospasm induced by norepinephrine or promethazine (an anticholinergic agent) in the arteries of the mesocccum (11).

Gluteal and pulmonary arteries. Arteries isolated from hu- man gluteal biopsies and mounted in a wire myograph vaso- dilated to rhRLX after preconstriction with norepinephrine in an endothelium-dependent fashion. However, human pulmo- nary resistance arteries failed to vasodilate in response to rhRLX (33). Thus the ability of RLX to produce rapid relax- ation in isolated human blood vessels is apparently dependent on their anatomical source.

Influence of RLX on Steady and Pulsatile Systemic Arterial Load: Insights From Pregnancy

The systemic arterial load is defined as the mechanical opposition to movement of blood flow out of the left ventricle (96). There are two components. One is the steady arterial load commonly known as SVR, which is simply calculated by the quotient of mean arterial pressure and cardiac output (CO) and results mainly from arteriolar properties. The other is pulsatile arterial load, which arises solely as a consequence of the inherently pulsatile nature of the cardiac pump and is deter- mined by vessel geometry and wall viscoelasticity, the branch- ing property of the vasculature that gives rise to wave propaga- tions and reflections and mechanical properties of the blood. Global arterial compliance (global AC) is one measure of pulsatile arterial load, which is derived from CO and the diastolic decay of the aortic pressure waveform.

A major cardiovascular adaptation in human pregnancy is the increase in global AC that reaches a peak by the end of the first trimester just as SVR reaches a nadir (87). At least in theory, the rise in global AC is critical to the maintenance of cardiovascular homeostasis during pregnancy for several rea- sons: 1) prevents excessive decline in diastolic pressure, which otherwise would fall to precariously low levels due to the large fall in SVR; 2) minimizes the pulsatile or oscillatory work wasted by the heart, which otherwise would increase in dis- proportion to the rise in total work required of and expended by the heart during pregnancy; 3) results in arterial underfilling along with the reduction in SVR and, therefore, contributes to renal sodium and water retention and plasma volume expan- sion during early pregnancy; and 4) preserves steady shear- type (or minimizes oscillatory shear type) stress at the blood-endothelial interface despite the hyperdynamic circulation of pregnancy, thereby favoring production of NO rather than superoxide and other damaging reactive oxygen species by the endothelium.

Does RLX contribute to the changes in the steady and pulsatile systemic arterial load during pregnancy? Because RLX mediates maternal renal vasodilation, hyperfiltration, and reduced myogenic reactivity of small renal arteries during pregnancy in rats (75), it was logical to consider whether the hormone might also contribute to the broader cardiovascular changes of pregnancy, i.e., the increases in CO and global AC, as well as the reduction in SVR. To begin investigating this question, Conrad and colleagues (14) first tested whether RLX has the potential to modify systemic cardiovascular function when chronically administered to nonpregnant female rats. A method was developed to quantify global AC in conscious, chronically instrumented, unrestrained rats. rhRLX was admin- istered for 10 days, and serum levels approximated those observed in rats during early to midgestation. Significant in- creases in CO, global AC, and the stroke volume-to-pulse
pressure ratio (another measure of arterial compliance in vivo), as well as reductions in SVR were observed by the earliest time point of measurement (day 2 or 3) and were sustained throughout the 10-day protocol, the magnitude of these changes being ~20%. There was no significant change in mean arterial pressure throughout the 10-day rhRLX infusion, i.e., the decline in SVR was virtually matched by a compensatory rise in CO, the latter mainly due to increases in stroke volume. Finally, small renal arteries dissected from female rats after 5 days of rhRLX infusion and then mounted in a pressure arteriograph and treated with papaverine and EGTA to inhibit smooth muscle function, demonstrated significant increases in compliance relative to those arteries harvested from vehicle-treated animals (14). Similar changes in the passive mechanics of small mesenteric and renal resistance arteries have been reported for late gestation in the rat (36, 69). Recent evidence suggests that small arteries also make an important contribution to the global AC (96a). These results suggest that, in addition to reduction in vascular smooth muscle tone due to the vasodilatory action of RLX (19, 22), the increase in global AC observed in vivo was also due to modification of the vascular structure, e.g., the extracellular matrix in the blood vessel wall. Because vascular gelatinase activity participates in the remodeling of extracellular matrix and plays a pivotal role in the renal vasodilatory response to RLX (54), there are overlapping hormonal and cellular signaling mechanisms for vasodilatory and vascular compliance changes during pregnancy. This sharing of molecular mechanisms is one way to ensure a temporal coincidence of the decrease in both steady and pulsatile systemic arterial loads that, as described above, is critical to the maintenance of cardiovascular homeostasis during pregnancy.

Studies are currently ongoing to determine whether neutralization of circulating RLX by antibodies will prevent these changes in systemic hemodynamics and passive mechanics of small renal arteries during pregnancy in rats (see Ref. 75). Whether the modifications of CO, global AC, and SVR by RLX in rats will pertain to humans requires further investigation. Finally, the exact changes in vascular structure that contribute to the increase in arterial compliance during pregnancy or RLX administration to nonpregnant rats remain to be determined.

Influence of RLX on the Heart

Cardiac expression of RLX binding sites. In 1992, Osheroff and colleagues (78) reported high-affinity binding of 32P-labeled synthetic human RLX H2 to the cardiac atria, but not ventricles of both male and female rats using quantitative autoradiography.1 The dissociation constant was similar to that of the uterus, a known site of RLX binding, being 1.4 and 1.3 nM, respectively. Later work by the same group demonstrated similar high-affinity binding in the atria of both male and female hearts from rats in the neonatal period, postnatal days 1–29, that was comparable to the adult (79). More recently, Tan and colleagues (99) reported similar findings using 33P-labeled recombinant human RLX H2. They found a single population of saturable binding sites with higher receptor densities in the cerebral cortex and atria of adult rats compared with the uterine myometrium.

Cross-linking of 32P-labeled synthetic human RLX H2 to uterine cells and primary rat atrial cardiomyocytes followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis revealed a putative RLX receptor with an apparent molecular mass of 220 kDa and a minor component of 36 kDa (80). In this work, binding was not reduced by GTP, thus mitigating against a G protein-coupled receptor, nor was there evidence for ligand-stimulated tyrosine autophosphorylation. Both similarities and differences to other receptors of the insulin growth factor family were noted. A putative RLX receptor of similar apparent molecular mass was reported by Goldsmith and colleagues (81) in lower uterine segment fibroblasts; however, they did find evidence for ligand-stimulated tyrosine autophosphorylation. Two RLX receptors were recently identified. They are G protein-coupled receptors each of ~87 kDa designated as LGR7 and LGR8 [orphan leucine-rich containing G protein-coupled receptors (50)].

Positive chronotropic and inotropic effects of RLX. Several investigators have reported the chronotropic influence of RLX in the rat heart (17, 43, 55, 98, 102, 103, 110). These results were obtained using male and nonpregnant and pregnant female rats of various strains, isolated whole hearts, isolated left and right atrial preparations, and synthetic and recombinant human RLX generally in the low nanomolar range. The chronotropic effect of RLX was also reported in conscious, freely moving rats by Ward and colleagues (110) and Conrad and coworkers (14). In the latter, the typical reduction in heart rate observed over time in conscious rats that results from progressive training to the experimental conditions was not observed in rats chronically treated with RLX. Interestingly, RLX may also have an independent effect on the ventricles, because a chronotropic action was observed after resection of the atria in the isolated whole heart preparation (102). Thus perhaps Osheroff and coworkers (78) did not look specifically at the AV node or Bundle of His when they reported the absence of RLX binding in the ventricle. In the exhaustive work of Tan and coworkers (98) in which they investigated the potency of various RLX preparations on chronotropy and inotropy using the isolated atrial preparation as a bioassay, they observed that both synthetic and native rat relaxins were less active than human and porcine relaxins, and porcine prorelaxin was comparable to porcine RLX. Finally, using the patch clamp technique on single sinoatrial node cells prepared from rabbits, Han and colleagues reported that synthetic human H2 RLX produced a dose-dependent and reversible increase in the rate of spontaneous action potential and L-type calcium current (43). These responses to RLX were eliminated by pretreatment with cAMP or 8-bromo-cAMP or with an inhibitor of cAMP-dependent protein kinase.

In most cases, RLX was also reported to be a positive inotropic agent on isolated atria or whole heart (17, 55, 58, 98, 110), although some investigators failed to observe this effect (102, 103). Using the patch clamp technique on isolated left and right atrial myocytes from rats, Piedras-Renteria et al. (84, 85) reported that 50 or 100 ng/ml native rat RLX inhibited the transient and sustained outward potassium currents via cAMP-dependent protein kinase and increased calcium entry via both L- and T-type calcium channels. The authors suggested that

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1 Humans have three relaxin genes designated H1, H2, and H3. Mice and rats each have two relaxin genes designated M1 and M3 and R1 and R3, respectively. H2, R1, and M1 gene products are analogous, insofar as they circulate during pregnancy emanating from the corpus luteum (95).
RLX reduces the total potassium current, thereby increasing the duration of the atrial action potential and, consequently, of calcium entry, resulting in positive inotropy.

Using an isolated perfused heart preparation in male rats, Toth and colleagues (103) showed that, when added to the perfusate, 10 nM rhRLX increased the concentration of immunoreactive atrial natriuretic peptide in the coronary venous effluent.

The atrial appendage of human hearts did not demonstrate an inotropic response to rhRLX, despite showing good inotropic responses to catecholamines. Nor were $^{32}$P-labeled RLX binding sites evident by autoradiography in the right and left atria, ventricles, and SA nodal region of the human heart despite strong binding by rat atria used as a positive control (R. Summers, personal communication). In contrast, weak expression of the LGR7 RLX receptor mRNA was detected in whole human heart using RT-PCR (50). Thus, although the human heart may express the LGR7 RLX receptor at low levels, inotropic action of RLX on the human heart was not demonstrable. Whether RLX can elicit a chronotropic response in the human heart has not been tested.

**Coronary blood flow.** Bani-Sacchi and coworkers (8) demonstrated that pRLX was a potent coronary vasodilator. Hearts were obtained from male guinea pigs and rats, mounted in a Langendorff apparatus, and perfused retrograde through the aorta at a constant pressure. The coronary effluents were collected for determination of coronary flow and nitrite (a metabolite of NO). Whether administered by bolus injection into the aortic cannula (1, 5, and 10 nM) or by addition to the perfusion media for constant infusion (5 nM), there was a rapid and parallel increase in coronary blood flow and nitrite concentration. Both responses were blocked by pretreatment with 0.1 mM N\textsuperscript{G}-monomethyl-L-arginine (L-NMMA), an inhibitor of NOS. In these experiments, RLX was found to be considerably more potent than acetylcholine (plus physostigmine to inhibit acetylcholinesterase) and sodium nitroprusside, endothelium-dependent and -independent vasodilators, respectively.

**Influence of RLX on Vascular Cells in Culture**

**Inducible NOS.** Using cultured vascular smooth muscle cells from bovine aorta, Bani and colleagues (4) provided evidence that highly purified porcine RLX upregulated inducible NOS (iNOS). The porcine RLX was reported to be free of endotoxin as determined by the Limulus amebocyte lysate assay. They reported that porcine RLX increased calcium and calmodulin-independent NOS activity, upregulated iNOS protein by immunohistochemistry, increased nitrite in the conditioned media, elevated cGMP in the cultured cells, and inhibited the rise in the calcium transient by thrombin. The same group applied similar approaches to show that porcine RLX could also induce iNOS in cultured rat coronary artery endothelial cells (30). Whether induction of iNOS by RLX occurs in vivo was not investigated in this study, although the authors presented data for induction of iNOS by RLX in hearts challenged with anaphylaxis (normal hearts were apparently not investigated; see above). Not all evidence supports the concept that RLX upregulates iNOS, insofar as 1) infusion of porcine RLX to conscious rats failed to increase the urinary excretion of cGMP and of nitrate plus nitrite (22), 2) calcium-independent NOS activity was not increased in isolated mesenteric arcades or thoracic aortae from rats of gestational days 15–17 when circulating levels of RLX are high (97), and 3) iNOS expression was not induced in renal tissues by pregnancy as evaluated by Western analysis at least in one study (76), although it was in another (1).

**Endothelial ET\textsubscript{B} receptor.** Dschietzig and colleagues (25) reported that rhRLX increased the expression of the ET\textsubscript{B} receptor subtype in cultured human umbilical artery endothelial cells. The hormone increased ET\textsubscript{B} receptor mRNA, protein, and binding sites for radiolabeled ET-1. RLX activated ERK-1/2, but not p38 kinase, and enhanced the DNA binding activity of the transcription factor, nuclear factor (NF)-κB; inhibition of these signal transduction pathways prevented the increase in ET\textsubscript{B} receptor mRNA expression. Many of these findings were duplicated in bovine aortic endothelial cells. The expression of the ET\textsubscript{B} receptor by human vascular smooth muscle cells was not affected by RLX, nor was the ET\textsubscript{A} receptor subtype altered in any of the cultured cells by the hormone. RLX also increased NF-κB reporter activity in bovine aortic endothelial cells, which was not affected by dominant negative Ras (a GTP binding protein coupled to growth factor receptors), but was inhibited by dominant negative forms of mitogen activated protein kinase kinase-1 (MEK-1), Raf-1 (a MEK kinase), and ERK-1/2, thereby providing more clues to the cell signaling mechanisms. The functional relevance of these findings was verified in arteries from rats, insofar as incubation with RLX in vitro resulted in a decrease in the sensitivity and maximal response to ET-1-induced contraction. Moreover, the vasorelaxation to ET-3 was enhanced. All of these functional effects were blocked by the ET\textsubscript{B} receptor antagonist A-192621 or by the MEK-1 inhibitor PD-98059. Overall, these results corroborate those obtained from the renal circulation, where pregnancy or RLX treatment of nonpregnant rats elicited renal vasodilation, hyperfiltration, and reduced myogenic reactivity of small renal arteries via the endothelial ET\textsubscript{B} receptor/NOS vasodilatory pathway (15, 19–22, 35, 54, 75, 77). The study of Dschietzig and colleagues (25) further suggests that one potential mechanism for this vasodilatory role of RLX is through upregulation of the endothelial ET\textsubscript{B} receptor, although to our knowledge, this concept has not been validated in vivo.

**SECTION 2: INTERACTION OF RLX WITH RENAL AND CARDIAC DISEASES AND ITS ROLE IN ANGIogenesis**

**RLX and Renal Disease**

In view of both the renal vasodilatory (22) and matrix-degrading (82, 105, 108) attributes of RLX, it has been suggested that the hormone may be efficacious in the treatment of renal diseases associated with renal vasoconstriction and fibrosis (9). So far, the hormone has been tested in several rat models of renal disease: bromoethylamine-induced renal interstitial fibrosis (38), renal mass reduction by infarction or surgical excision of both poles (37), cyclosporin nephrotoxicity (52), and Goodpasture’s syndrome (68). In all cases, amelioration of renal injury was observed. Furthermore, the authors of the last study provided evidence suggesting that rhRLX increased the degradation of fibronectin, a major component of the extracellular matrix in renal fibrosis, by the ubiquitin-proteasomal pathway (68).
RLX and Cardiac Disease

Myocardial ischemia-reperfusion. Masini and colleagues (64) showed that RLX ameliorates the myocardial injury induced by ischemia-reperfusion. Hearts were harvested from male guinea pigs, mounted in a Langendorff apparatus, and perfused retrograde through the aorta at constant pressure. The coronary effluents were collected for determination of coronary flow, nitrite, and histamine concentrations. Left ventricular tissue was obtained at the end of the ischemic-reperfusion protocol for determination of malondialdehyde (MDA; an end-product of lipid peroxidation of cell membranes), calcium content, and mast cell degranulation. Myocardial ischemia was produced by ligating the left anterior descending coronary artery for 20 min followed by release of the ligature and reperfusion for 20 min. In some hearts, pRLX was added to the perfusate at the start of the coronary occlusion (30 ng/ml), whereas in others, L-NMMA was added to the perfusate 1 h before addition of pRLX and coronary ligation. Major findings included the preservation of coronary flow during ischemia by RLX treatment, as well as augmentation of flow during the reperfusion period. The concentration of nitrite in the coronary effluent rose progressively throughout the ischemia and reperfusion periods in the RLX-treated hearts, whereas the expected rise in histamine concentration due to activation and degranulation of resident mast cells subsequent to postischemic reoxygenation was blocked by RLX. In addition, the rise in left ventricular MDA, calcium content, and degranulation of mast cells were all prevented by RLX. Mitochondrial morphology as assessed by electron microscopy and the amplitude of myocardial contractions as determined by a force transducer attached to the apex of the heart were also maintained by RLX despite ischemia-reperfusion. All of these salutary effects of RLX were prevented by pretreatment with L-NMMA. The authors concluded that mainly by dilating collateral coronary vessels, which are well developed in the guinea pig heart, thereby preserving coronary flow during the ischemic period, RLX prevented the myocardial damage that ensues on reoxygenation. It should be noted, however, that RLX may have had a direct effect on mast cells to inhibit degranulation and on myocyte calcium metabolism to reduce calcium content. That is, pRLX can stimulate NO in mast cells [and other cell types, e.g., platelets (3)], thereby reducing intracellular calcium and histamine secretion (2, 65).

These studies on the beneficial effects of RLX in myocardial ischemia-reperfusion injury were extended by Bani and colleagues (5) using an in vivo rat model. Male rats were anesthetized with ketamine and ventilated, and the chest was opened to place a ligature around the left anterior descending coronary artery. The ligature was tightened for 30 min followed by 60 min reperfusion. In those animals administered intravenous pRLX 30 min before coronary occlusion (100 ng), the area of myocardial damage was significantly reduced and the number of severe ventricular arrhythmias decreased. Furthermore, mortality was less, the accumulation of neutrophils attenuated as determined histologically and by myeloperoxidase activity, lipid peroxidation decreased as determined by MDA content, calcium content was reduced, endothelial and myocyte injury decreased as assessed by EM, and mast cell degranulation and histamine release were inhibited.

Cardiac anaphylaxis. Using another model of heart disease, Masini and coworkers (66) showed a beneficial effect of RLX in protecting against cardiac anaphylaxis. Male guinea pigs were sensitized by two intraperitoneal injections of ovalbumin administered on consecutive days. They were killed 15–30 days later, and the heart was harvested and then mounted in a Langendorff apparatus and perfused retrograde through the aorta at constant pressure. The coronary effluents were collected for determination of coronary flow, nitrite, and histamine concentrations. Cardiac anaphylaxis was then induced by administration of ovalbumin into the aortic cannula. In some animals, pRLX was added to the perfusate 30 min before challenge (30 ng/ml). The reduction in coronary flow, as well as the increase in inotropy and chronotropy induced by the ovalbumin challenge, was prevented by the pretreatment with RLX. The increase in histamine concentration in the coronary effluent and in mast cell degranulation, as well as the decrease in myocardial histamine content, was also inhibited by RLX pretreatment. Nitrite concentration increased commencing with the addition of RLX to the perfusate and was sustained at elevated levels throughout the protocol. This finding correlated with increased cardiac expression of iNOS determined by Western analysis, increased calcium-independent and -dependent NOS activity, increases in cGMP content, and reduction in calcium content. The authors concluded that a major mechanism of the protection afforded by RLX in cardiac anaphylaxis was inhibition of mast cell degranulation and release of histamine and other mediators, analogous to their earlier work in bronchial hyperresponsiveness and inflammatory lung injury induced by antigen challenge (2).

Heart failure. Dschietzig and colleagues (27) recently provided evidence that RLX may be a compensatory factor in human heart failure. They measured increased circulating levels of RLX that corresponded to the severity of the heart failure (severe > moderate > controls) and noted that in 11 of 14 cases of the severe congestive heart failure (CHF), the RLX concentration in the coronary sinus was greater than in the left ventricle, suggesting a cardiac contribution to the elevated circulating levels. Between 12 and 48 h after a 12-h infusion of sodium nitrouprusside, plasma RLX concentrations fell in the patients with severe CHF to levels observed in moderate CHF; the RLX levels in moderate CHF were not affected by sodium nitrouprusside and remained elevated relative to control subjects without CHF. Although the decline in plasma RLX was clearly delayed in relation to the changes in hemodynamic parameters induced by sodium nitrouprusside, these results suggested a hemodynamic mechanism for increased expression of cardiac RLX in severe CHF. Indeed, plasma RLX correlated significantly with left ventricular end diastolic pressure (r = 0.69) and cardiac index (r = -0.62). By semi-quantitative RT-PCR, the authors reported a significant increase in both H1 and H2 gene expression in the left ventricle and right atrium of the failing heart. On Western analysis, the authors reported detecting the 18-kDa prorelaxin, but not the 6-kDa mature RLX in the right atrium and left ventricle, that was significantly increased in the former but not the latter in CHF. Immunostaining suggested the expression of RLX by myocytes and interstitial cells in the left ventricular tissue from a failing heart. Levels of prohormone convertase-1 mRNA were decreased in right atrium but not left ventricle, possibly accounting for the increase in prorelaxin in the former. Using isolated rat hearts,
the authors further demonstrated that elevation in left ventricular end-diastolic pressure increased preprorelaxin mRNA in the left ventricle but not in the right atrium, whereas elevation in both right and left atrial pressure had no effect. The authors showed that plasma RLX and ET-1 concentrations were significantly and inversely correlated in the patients with severe CHF. Moreover, RLX inhibited the secretion of ET-1 (and preproET-1 mRNA expression) in bovine pulmonary endothelial cells that was induced by a combination of low shear rate and high downstream pressure or by angiotensin II. This inhibitory effect of RLX on ET-1 secretion was prevented by ETB receptor antagonism. The authors suggested that RLX increases ETB receptor expression by the cultured endothelial cells, thereby potentiating the well-known autoregulation of preproET-1 mRNA expression by ET-1 [(26); also see Influence of RLX on cultured vascular endothelial and smooth muscle cells, below].

**RLX-deficient mice.** Zhao and colleagues (112) developed a mouse deficient in the M1 RLX gene. Using this model, Du and coworkers (28) investigated cardiac structure and function in mice deficient in the M1 RLX gene. They observed increased procollagen type 1 mRNA expression in the ventricles, but not atria, of the RLX-deficient mice beginning at least by 6 mo of age. This increase in mRNA was associated with increased left ventricular collagen content by at least 10 mo. There were no significant changes in the mRNA expression for MMP-2, -9, or -13. Functional studies revealed an increase in the transmural flow velocity, specifically during the late filling phase, beginning at 8–10 mo of age, and a significantly higher left ventricular end-diastolic pressure from 12 to 24 mo. Thus the left ventricle was less compliant in the RLX-deficient mice resulting in the aforementioned functional changes that produced atrial hypertrophy. Interestingly, these findings were manifested in male, but not in female, mice.

**RLX and Angiogenesis**

Vasodilatory hormones frequently have an angiogenic role. One of the many interesting observations to emerge from the phase II/III trial of rhRLX in the treatment of scleroderma was the high incidence of menometrorrhagia (heavy, irregular, or prolonged menstrual bleeding) in those women receiving hormone (106). In cultured human endometrial cells, rhRLX stimulated the production of VEGF in a cAMP-dependent fashion (106). Taken together, these results suggest that RLX contributes to neovascularization of the endometrium.

Unemori and colleagues (107) observed enhanced vessel ingrowth of matrigel plugs containing rhRLX that were implanted subcutaneously in mice. Using the Hunt-Schilling wound chamber assay, they observed increased VEGF 164 and basic fibroblast growth factor (bFGF) mRNA expression by cells (presumably macrophages) in the chamber aspirate of those rats administered rhRLX. This increase corresponded with enhanced expression of factor VIII-related antigen in the granulation tissue of the chamber, indicative of accelerated blood vessel growth. Interestingly, VEGF mRNA expression was not enhanced by rhRLX in resident macrophages in the lung or spleen remote from the site of injury. Nor did rhRLX have any direct angiogenic effects on cultured endothelial cells, confirming an earlier report (73). Finally, rhRLX increased VEGF 165 and 120, as well as bFGF mRNA expression by THP-1 cells, a human monocyte/macrophage cell line. On balance, rhRLX appears to be indirectly angiogenic by enhancing VEGF and bFGF expression in wound macrophages, thereby stimulating angiogenesis. In a subsequent investigation by the same investigators, systemic administration of rhRLX was reported to significantly accelerate wound closure in full thickness skin excisions in db/db mice (51). This improved healing was associated with increased staining for factor VIII-related antigen in the granulation tissue, which was thicker and more cellular than in vehicle-treated mice.

Further evidence for the indirect angiogenic attributes of rhRLX was demonstrated using a model of chronic myocardial infarction in rats (61). Systemic infusion of rhRLX potentiated the increase in bFGF mRNA and protein at 7 and 21 days in the peri-infarct region being expressed by myocytes and fibroblasts. Similar infusions in sham-operated rats showed no change in bFGF or VEGF expression in corresponding regions of the left ventricle or in the right ventricle. The increase in the number of thin-walled, collateral vessels lacking smooth muscle was correspondingly potentiated in the peri-infarct region by systemic rhRLX. With the use of human neonatal heart cells in culture (fibroblasts and myocytes), rhRLX was reported to increase the mRNA and protein for VEGF and bFGF.

**Perspectives**

RLX is emerging as a player in renal and cardiovascular function. An important question in need of further study is whether RLX is produced locally by renal and cardiovascular tissues. The first evidence for such local expression was reported by Taylor and Clark (100), who used a reverse hemolytic plaque assay to detect secretion of immunoreactive RLX (and/or its precursors) from neonatal rat atrial cardiocytes. Subsequently, R3, but not R1, RLX gene expression was detected in the atria and left ventricle of rats by RT-PCR (58), and both H1 and H2 gene expression were detected in the right atrium, left ventricle, saphenous vein, and mammary artery of humans by RT-PCR (27). Irrespective of gender, various arteries and the kidneys isolated from rats and mice, as well as cultured human vascular smooth muscle and endothelial cells, express RLX mRNA by RT-PCR, and rat arteries express RLX protein and precursor forms (K. Conrad, L. Kerchner, K. Inovina, K. Hanley-Yanez, unpublished observations).

Another important future investigation is to identify the RLX receptors in renal and cardiovascular tissues. RLX binding sites were reported to be on blood vessels in pig uteri (72) and in human reproductive organs (56). The LGR7 receptor mRNA was identified in the atria and left ventricle of the rat by RT-PCR (58), and weak mRNA expression was evident in the human heart (50). LGR7 mRNA expression was also observed in a variety of cultured human endothelial and vascular smooth muscle cells (K. Conrad, unpublished observations).

Because RLX and its receptors are likely to be expressed by renal and cardiovascular tissues, the hormone is likely to exert local actions. Thus it will also be important to identify the autocrine and paracrine effects of RLX in renal and cardiovascular tissues. On the basis of the effects of circulating RLX, we speculate that blood vessel-derived RLX is a locally acting compliance and relaxing factor.

Given the wide spectrum of RLX effects in the renal and cardiovascular systems, there is potential for therapeutics. On
the basis of its physiological actions, RLX may be useful in the treatment of vasoconstrictive diseases, renal and cardiovascular pathologies associated with fibrosis, congestive heart failure (via cardiac afterload reduction), and arterial stiffness, to name a few. The data further suggest that the hormone may be useful in potentiating angiogenesis at ischemic sites. Clearly, however, before therapeutics can be seriously contemplated, we need to confirm that the renal and cardiovascular actions of RLX identified in animal models will pertain to humans.

NOTE ADDED IN PROOF

In addition to calcium-mediated activation of endothelial nitric oxide synthase (eNOS) through the endothelial ETB receptor subtype (see Molecular mechanisms of RLX action: a pivotal role for nitric oxide and endothelin herein), a recent publication shows a role for protein kinase B/Akt and subsequent eNOS phosphorylation and activation (Liu S, Fremont RT, Kontos CD, Huang J, and Rockey DC. Endothelin-1 activates endothelial cell nitric-oxide synthase via heterotrimeric G-protein βγ-subunit signaling to protein kinase B/Akt. J Biol Chem 278: 49929–49935, 2003).

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