The Disposition of a Human Relaxin (hRlx-2) in Pregnant and Nonpregnant Rats

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The pharmacokinetics and tissue distribution of a human relaxin were investigated after intravenous (iv) bolus administration to pregnant or nonpregnant rats. Human gene-2 relaxin (hRlx-2) serum concentrations after iv bolus administration were described as the sum of three exponentials. The pharmacokinetics were comparable in pregnant and nonpregnant rats. The serum clearance (CL) was 7.4–10.2 ml/min/kg at doses of 46–93 μg/kg and was linear in this range. The half-lives were 1.1–2.0, 15.1–16.4, and 53.7–67.9 min, respectively. The volume of the central compartment (V_c) was 48–79 ml/kg and the volume of distribution at steady state (V_{ss}) was 271–336 ml/kg. Increasing the dose to 463 μg/kg increased the dose-corrected area under the serum concentration–time curve and significantly decreased CL and Vss. The distribution of radioactivity in the tissues of pregnant rats was followed after iv bolus dosing with hRlx-2 internally labeled with ^{35}S-cysteine. Comparison of the extent of organ uptake of radiolabel after ^{35}S-hRlx-2 or ^{35}S-cysteine administration suggested that the kidneys were the principal site of uptake; the liver was of secondary importance. In perfusion experiments utilizing livers isolated from pregnant or nonpregnant rats, 36–52% of the dose of hRlx-2 was cleared from the perfusate in 2 hr. These studies showed that the pharmacokinetics of hRlx-2 in rats appeared to be unaffected by pregnancy and suggested that the kidneys and liver both play a role in the elimination of hRlx-2.

KEY WORDS: human relaxin pharmacokinetics; tissue distribution; pregnant and nonpregnant rats.

INTRODUCTION

Relaxin has been characterized as a hormone of pregnancy; the major source of relaxin in humans during pregnancy appears to be the corpus luteum (1). While relaxin's exact role has not been clearly defined, there are numerous reports that it mediates some of the physiological and structural changes that occur at parturition. Porcine relaxin increases uterine and cervical weights, water content, and total glycosaminoglycans in ovariectomized, estrogen-primed rats (2). The increase in cervical dilatability which occurs in rats during pregnancy correlates with the appearance and rise of blood levels of immunoreactive relaxin (3).

Relaxin has been proposed as a therapeutic agent for use in pregnant women at or near term to increase cervical ripening, i.e., the thinning and softening of the cervix that are necessary to accommodate the passage of the fetus during delivery. Clinical trials with porcine relaxin demonstrated that exogenous administration of relaxin may have beneficial effects. In those studies, relaxin was administered as vaginal and cervical suppositories or gels (4) and by intravenous infusion (5) to pregnant women during or just prior to the induction of labor.

Although there are previous reports of serum concentrations of exogenous porcine relaxin, no pharmacokinetic analyses have been reported. Moreover, there are reports of the tissue distribution of porcine relaxin in mice, rats, and guinea pigs (6,7). The production of significant amounts of human relaxins has recently been accomplished, and there are reports of the disposition of these hormones after administration in mice (8) and rhesus monkeys (9). The present report describes the pharmacokinetics and tissue distribution of a human relaxin (hRlx-2) after iv bolus administration to pregnant (day 19 of gestation) and nonpregnant female rats. In addition, isolated rat liver perfusion experiments were performed in order to elucidate the role of this organ in the clearance of hRlx-2.

MATERIALS AND METHODS

Materials

Human relaxin consists of two polypeptide chains with a molecular weight of approximately 6500 daltons. The A and B chains of hRlx-2 have been chemically synthesized and combined to yield mature relaxin (10). ^{35}S-Labeled hRlx-2 was prepared by a combination of biosynthetically produced ^{35}S-labeled A chain with synthetic B chain. ^{35}S-A chain (labeled on the cysteine residue) was made in *Escherichia coli* containing a plasmid directing the synthesis of a relaxin-A chain fusion protein. Cells were cultured in a defined medium containing ^{35}S-sulfate (Amersham, 40 Ci/mg). The fusion protein was purified from the harvested cells by urea extraction, gel filtration, and reversed-phase HPLC. Relaxin A chain was derived from the fusion protein by cyanogen bromide cleavage followed by reversed-phase HPLC. The labeled A chain was combined with the synthetic B chain to produce ^{35}S-hRlx-2 with a specific activity of 7.2 μCi/μg. ^{35}S-Cysteine (New England Nuclear, 8182 μCi/μg) was used as a means to track the probable distribution of this amino acid after administration of ^{35}S-hRlx-2, assuming that hRlx-2 would be degraded to its constituent amino acids.

Experimental Methods

Animals. Adult female Sprague Dawley-derived rats (Charles River Breeding Laboratories, Portage, MI) were used. All pregnant rats were at day 19 of gestation. For the pharmacokinetic studies, nonpregnant rats weighing 200–300 g and pregnant rats weighing 281–396 g were used. Pregnant

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rats used in the tissue distribution experiments weighed 230–250 g. Pregnant and nonpregnant rats used in the isolated liver perfusion experiments weighed 240–250 and 190–210 g, respectively.

Pharmacokinetic Dosing. Catheters were implanted into the right jugular vein and left femoral vein under anesthesia 48 hr before the experiment. Intravenous bolus doses (0.1 ml; 46, 93, and 463 μg/kg) were administered to conscious, unrestrained animals through the femoral vein catheter by iv bolus. Serial blood samples were obtained from the jugular vein catheter prior to hRlx-2 injection and at the times indicated in the figures. The blood samples (0.25 ml) were allowed to clot at room temperature, and the serum was harvested and stored at −70°C until assayed for hRlx-2 by an enzyme-linked immunosorbent assay (see below).

Tissue Distribution Dosing. Doses of tracer ^{35}S-hRlx-2 (1.65 μCi, 2 μg/kg), tracer ^{35}S-hRlx-2 plus unlabeled hRlx-2 (1.86 μCi, 1000 μg/kg), or ^{35}S-cysteine (2.17 μCi, 1 ng/kg) were delivered as a bolus injection (0.2 ml) into a tail vein of conscious, restrained, pregnant rats, four animals per dose. At 10, 30, or 120 min after dosing, blood, heart, lungs, liver, kidneys, brain, pituitary, spleen, mammary gland, retroperitoneal fat, biceps femoris muscle, ovaries, uterus, placentae, and fetuses were harvested. Following anesthesia with pentobarbitone, a midline abdominal incision allowed blood to be collected from the aorta via a cannula inserted proximal to the bifurcation. Blood was allowed to clot at room temperature to yield serum. Sixty milliliters of saline was perfused through the aorta to remove residual blood from the organs to be sampled. Known weights of each tissue were solubilized in Soluene 350 (Packard) before the addition of Hionic-Fluor cocktail (Packard) for counting of counts per minute (cpm) in a Tri-Carb 2000CA liquid scintillation analyzer (Packard).

Liver Perfusions. The recirculating liver perfusion system of Billings *et al.* (11) was used in these experiments. Livers were perfused with 150 ml of Krebs-Henseleit buffer (pH 7.4, 37°C) containing 2% bovine serum albumin and saturated with an oxygen–carbon dioxide mixture (95:5). The pH of the buffer remained constant during the perfusions. The perfusion flow rate was approximately 40 ml/min via an inflow cannula in the portal vein. The outflow cannula was placed in the thoracic vena cava. Samples (0.5 ml) of the recirculating perfusate were taken at 0, 2.5, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, and 120 min after hRlx-2 was added to the perfusate. Each liver (four per group) was used for only one experiment. hRlx-2 was added to the perfusate solutions to a concentration of 1 or 10 ng/ml. Livers (four per group) from pregnant and nonpregnant rats were also perfused with buffer containing no hRlx-2.

Analysis of Serum and Perfusate Buffer Levels of hRlx-2. Serum and buffer hRlx-2 levels were measured by an enzyme-linked immunosorbent assay (ELISA) specific for hRlx-2 (12). The assay range was 20–1250 pg/ml.

Data Analysis. Individual pharmacokinetic parameters were estimated after iv bolus administration by fitting a three-exponential equation to the individual immunoreactive serum concentration–time data using a nonlinear curve-fitting program (NONLIN84, Statistical Consultants, Inc., Lexington, KY). The area under the serum concentration–time curve (AUC) was computed using the trapezoidal method from $t = 0$ to the last measurable serum concentration (C_t). The remaining AUC (from C_t to infinite time) was estimated by extrapolation using the following equation:

$$AUC_{extrap} = C_t/k_z \quad (1)$$

where k_z was the rate constant for the terminal elimination phase. Serum clearance (CL) was calculated from the equation

$$CL = \text{dose/AUC} \quad (2)$$

The volume of distribution of the central compartment (V_c) was calculated from

$$V_c = \text{dose}/C(0) \quad (3)$$

where $C(0)$ is the sum of the coefficients of the triexponential equation describing the serum concentration–time data. The volume of distribution at steady state (V_{ss}) was calculated from

$$V_{ss} = \text{dose} * \text{AUMC/AUC}^2 \quad (4)$$

where AUMC is the area under the moment curve, i.e., the (serum concentration) * (time) versus time curve. Serum half-lives for each phase were calculated by dividing 0.693 by the respective disposition rate constant.

In the liver perfusion experiments, a monoexponential equation was fitted to the reservoir hRlx-2 concentration–time data. The $AUC_{0-\infty}$ for the perfusate was calculated as described above. Total liver clearance (CL_T) was calculated by

$$CL_T = (\text{perfusate concentration} * 150 \text{ ml})/AUC_{0-\infty} \quad (5)$$

where perfusate concentration is nanograms per milliliter. The hepatic clearance (CL_H) was calculated by correction for the nonspecific disappearance of hRlx-2 during the perfusions by subtracting the mean CL_T obtained in the absence of the liver from the CL_T obtained in experiments with livers in the circuit. CL_H was then corrected for differences in liver weights and expressed as CL_H per gram of liver. The percentage of the hRlx-2 dose in the 150-ml perfusion reservoir specifically cleared in 120 min from the perfusate (D_{CL}) was calculated by

$$\%\, D_{CL} = [(CL_H * 120)/(150)] * 100 \quad (6)$$

For the tissue distribution study, organ/tissue to serum radioactivity ratios were calculated by dividing the cpm per gram of organ/tissue by the cpm per gram of serum. Percentage dose per organ/tissue was calculated by dividing the cpm per organ or tissue by the total dose. It was assumed that muscle and fat represented 39 and 14%, respectively, of the body weight of a pregnant rat (13,14).

Statistical Methods. Omnibus investigation of treatment/dose group differences in each parameter was conducted with an analysis of variance. A one-sided significance level of 0.05 was chosen. When the global F test for treatment/dose group differences was significant ($P < 0.05$), then follow-up multiple comparisons were conducted using least-significant difference t tests. Results are expressed as the mean ± SD.

RESULTS

Figure 1 shows the serum concentration–time profiles after iv bolus administration of 46, 93, and 463 μg/kg hRlx-2 to (A) nonpregnant and (B) pregnant female rats. Table I summarizes the mean calculated pharmacokinetic parameters. Three exponential terms were required to describe the serum concentration–time data for nonpregnant and pregnant rats. Increasing the dose twofold (93 μg/kg) did not result in significant differences in any parameters. Increasing the dose 10-fold (463 μg/kg) did, however, result in a significant increase in the dose-corrected AUC. Both CL and V_{ss} were significantly reduced in nonpregnant and pregnant rats.

For the tissue distribution study, it was assumed that there were no effects of the brief period of anesthesia or the flushing of organs on the distribution of radioactivity. After iv bolus injections of ^{35}S-hRlx-2 and ^{35}S-cysteine in pregnant rats, the kidneys were the main site of localization of the label. Table II shows kidney/serum ratios for the three time points to be consistently larger than other organ/serum ratios. Moreover, the kidney/serum ratios at 10 and 30 min for the two groups receiving the ^{35}S-hRlx-2 were greater than those for the control group receiving ^{35}S-cysteine ($P < 0.05$).

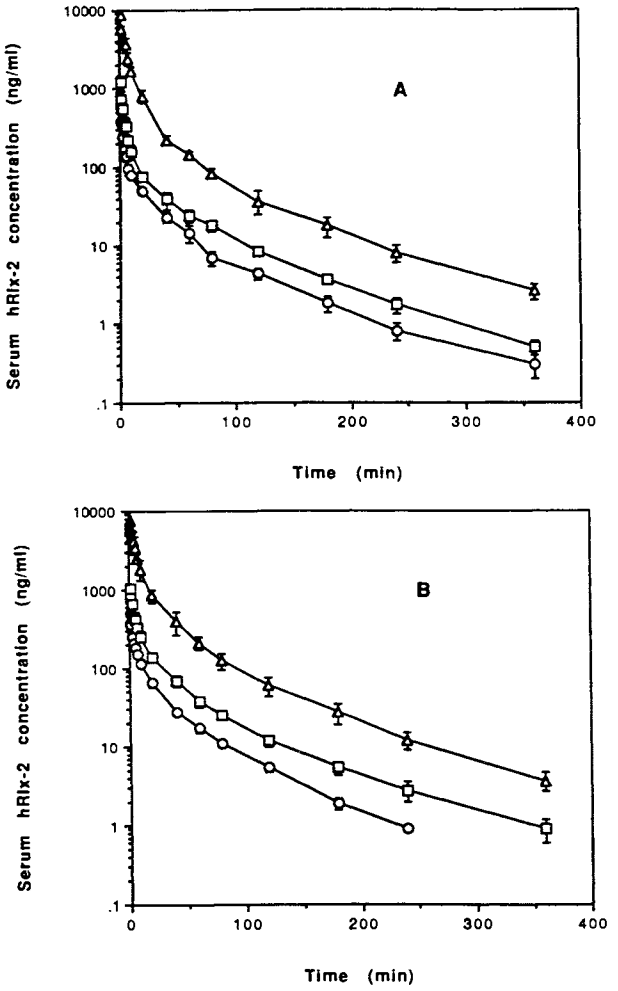

Fig. 1. Mean (±SD; n = 5 or 6) serum concentration–time profiles for hRlx-2 in (A) nonpregnant rats and (B) pregnant rats after iv bolus administration: (○) 46.3 μg/kg; (□) 92.5 μg/kg; (△) 463 μg/kg.

When the results were expressed as percentage of the dose/organ, the kidneys were still the organ of greatest uptake of radioactivity for both hRlx-2 doses at the earliest sampling time (Table III). At all times after the administration of ^{35}S-hRlx-2 with excess unlabeled protein, there was a slightly greater percentage of the dose in the kidneys when compared to the tracer dose alone, although only for the 30- and 120-min time points was this difference statistically significant ($P < 0.05$). Only about 5–7% of the radioactivity from the ^{35}S-cysteine dose was detected in the kidneys at any time.

Although liver/serum radioactivity ratios indicate that the liver accumulated radioactivity to a moderate degree (Table II), hepatic uptake of radioactivity was significant when considered as whole-organ uptake (Table III). The large excess of unlabeled hRlx-2 caused a small decrease ($P < 0.05$) in the percentage of the ^{35}S-hRlx-2 recovered from the liver, compared to tracer alone, at the 10-min time point; at 30 min there were no differences and at 120 min there was a significantly greater ($P < 0.05$) percentage of the dose recovered from the livers of animals that received the larger dose.

Total muscle radioactivity accounted for about 7–12% of the tracer and tracer plus unlabeled doses of hRlx-2 over the time course of the experiment and about 8–16% of the dose of ^{35}S-cysteine (Table III). Fetal uptake of radioactivity after administration of free ^{35}S-cysteine and ^{35}S-hRlx-2 was 2.8–3.3 and 0.21–3.2% of the administered doses, respectively. Placental uptake of radioactivity from free ^{35}S-cysteine and ^{35}S-hRlx-2 was 0.61–3.0 and 0.39–4.0% of the administered doses, respectively. Radioactivity in each of the other sampled organs represented about 1% or less of the doses administered to the pregnant rats, and therefore these data have not been included in Tables II and III.

Clearance values for hRlx-2 perfusions with nonpregnant and pregnant female rat livers are shown in Table IV. The amount of hRlx-2 cleared by livers from nonpregnant rats during the 120-min perfusions was 52 and 36% of the dose for perfusions containing 1 and 10 ng hRlx-2/ml, respectively, and that for livers from pregnant rats was 40% for both the 1- and the 10-ng/ml perfusions.

DISCUSSION

After iv bolus administration, hRlx-2 serum pharmacokinetics in pregnant and nonpregnant rats was described as the sum of three exponentials. Three exponentials were also required to describe the pharmacokinetics of relaxins in mice and rhesus monkeys (8,9) and may be characteristic of many proteins when assayed using a sensitive assay and sampled at appropriate intervals for a sufficient length of time. hRlx-2 plasma clearance appeared to be saturable with increasing doses. There were no significant differences in pharmacokinetic parameters between pregnant and nonpregnant rats up to 93 μg/kg with the exception of the dose-corrected AUC (and therefore CL) for the 93-μg/kg dose. This difference was not observed at any other dose or in any of the other pharmacokinetic parameters and is, therefore, assumed to be a statistical artifact.

The sampling times in the distribution study were chosen, to a large extent, based upon the profile of the plasma

Table I. Pharmacokinetic Parameters [Mean (SD); n = 3–4] for hRlx-2 in Nonpregnant and Pregnant Rats After Intravenous Bolus Administration

	Dose (μg/kg)	AUC (μg · min/ml)	Dose-corrected AUC	CL (ml/min/kg)	V_c (ml/kg)	V_{ss} (ml/kg)	$t_{1/2\alpha}$ (min)	$t_{1/2\beta}$ (min)	$t_{1/2\gamma}$ (min)
Nonpregnant									
Group 1	46.3	4.9	0.105	9.5	47.5	315	1.1	15.2	67.9
		(0.1)	(0.002)	(0.2)	(8.1)	(35)	(0.2)	(3.9)	(7.2)
Group 2	92.5	9.2	0.099**	10.2**	65.6	336	1.8	16.4	58.9
		(1.0)	(0.011)	(1.4)	(18.0)	(77)	(0.5)	(6.6)	(9.9)
Group 3	463	73.5	0.159*	6.3*	51.8	153*	3.3*	16.7	68.8
		(3.8)	(0.008)	(0.3)	(15.4)	(10)	(1.6)	(5.4)	(7.9)
Pregnant									
Group 4	46.3	5.6	0.121	8.3	78.7	271	2.0	15.1	53.7
		(0.5)	(0.011)	(0.7)	(27.4)	(27)	(1.1)	(7.2)	(17.0)
Group 5	92.5	12.7	0.137	7.4	67.5	275	1.8	14.6	62
		(1.6)	(0.017)	(0.8)	(5.3)	(35)	(0.2)	(2.3)	(5.5)
Group 6	463	84.7	0.183***	5.8***	51.9	172***	2.5	15.7	63.7
		(17.5)	(0.038)	(1.3)	(15.3)	(38)	(0.7)	(2.6)	(3.9)

* Significantly different from Group 1 and Group 2 means.
** Significantly different from Group 5 means.
*** Significantly different from Group 4 and Group 5 means.

concentration–time data gathered in the pharmacokinetic study. The distribution study identified the kidneys, liver, and muscle as the prime locations of the radioactivity recovered up to 2 hr after doses of ^{35}S-hRlx-2 administered to pregnant rats. Urine and feces were not collected and so a complete radioactivity inventory could not be completed. Interpreting the relative importance of the three organs identified as the major locations of radioactivity after ^{35}S-hRlx-2 dosing was attempted through use of the data for the administration of ^{35}S-cysteine, by performing isolated perfused liver experiments and by some limited studies of the pharmacokinetics of hRlx-2 in nephrectomized rats (results not shown).

Rats were dosed with ^{35}S-cysteine in order to obtain data on the distribution of cysteine itself, since this is the amino acid in ^{35}S-hRlx-2 that contained the ^{35}S. This was performed so that localization of free ^{35}S-cysteine and/or the ^{35}S-cysteine incorporated into endogenous proteins, such as would occur after degradation of ^{35}S-hRlx-2, could hopefully be distinguished from the uptake of ^{35}S-hRlx-2. This point underscores the limitation of this methodology; the labeled amino acid of internally labeled proteins can become incorporated into other proteins following the degradation of the starting material, thus potentially confounding the interpretation of the distribution of the starting material. Alternatively, if hRlx-2 were amenable to labeling with ^{125}I (attempts at this procedure resulted in biologically inactive ^{125}I-hRlx-2), there would still be the issue of tracking ^{125}I, in the blood and tissues, resulting from the degradation of the parent compound or from deiodinase action. It is, however, possible, by the use of appropriate controls, to interpret the results of tissue distribution studies using labeled proteins with some confidence.

The identification of the kidneys and liver as major sites of uptake of radioactivity was consistent with previous studies using ^{125}I-labeled porcine relaxin, in which the kidneys of the mouse and estrogen-primed guinea pig (6) and the kidneys and liver of the ovariectomized rat (7) were the major sites of radioactivity uptake. It is probable that in the current study the kidneys were a site of uptake of intact hRlx-2, since only in this organ was the uptake of radioactivity greatly increased after ^{35}S-hRlx-2 dosing, compared to the

Table II. Organ or Tissue-to-Serum Ratios[a] [Mean (SD); n = 3 or 4] at Various Times After Administering ^{35}S-Cysteine, ^{35}S-hRlx-2, or ^{35}S-hRlx-2 with an Excess of Unlabeled hRlx-2, as Intravenous Bolus Injections to Pregnant Rats

	^{35}S-Cysteine			^{35}S-hRlx-2			^{35}S-hRlx-2 + hRlx-2		
	10 min	30 min	120 min	10 min	30 min	120 min	10 min	30 min	120 min
Kidney	4.4 (0.3)	5.4 (0.7)	4.6 (0.6)	8.7 (0.5)	7.7 (0.7)	2.7 (0.2)	5.9 (0.8)	8.2 (2.1)	4.0 (1.6)
Liver	2.0 (0.2)	2.3 (0.2)	1.8 (0.2)	1.8 (0.1)	3.0 (0.4)	1.9 (0.2)	0.7 (0.1)	2.2 (0.3)	2.0 (0.2)
Muscle	0.2 (0.07)	0.2 (0.04)	0.2 (0.04)	0.1 (0.02)	0.2 (0.01)	0.1 (0.01)	0.1 (0.01)	0.2 (0.01)	0.1 (0.01)
Mammary	0.6 (0.1)	0.5 (0.1)	0.6 (0.1)	0.2 (0.01)	0.4 (0.1)	0.3 (0.1)	0.2 (0.03)	0.3 (0.1)	0.3 (0.1)
Fat	0.1 (0.1)	0.1 (0.03)	0.1 (0.02)	0.1 (0.01)	0.1 (0.04)	0.1 (0.01)	0.1 (0.01)	0.1 (0.06)	0.1 (0.06)
Uterus	0.7 (0.01)	0.9 (0.08)	0.8 (0.2)	0.4 (0.02)	0.8 (0.2)	0.5 (0.1)	0.3 (0.01)	0.5 (0.05)	0.6 (0.1)
Placentae	0.1 (0.01)	0.1 (0.02)	0.2 (0.01)	0.1 (0.01)	0.1 (0.04)	0.1 (0.03)	0.1 (0.01)	0.1 (2.02)	0.1 (0.03)
Fetuses	0.4 (0.04)	0.5 (0.1)	0.6 (0.1)	0.1 (0.01)	0.2 (0.03)	0.3 (0.02)	0.1 (0.01)	0.1 (0.01)	0.4 (0.06)

[a] Ratio = cpm per g organ/cpm per g serum. Only data for organs that had at least 1% of the dose at any sampling time are included.

Table III. Percentage [Mean (SD); n = 3 or 4] of the Dose of ^{35}S-Cysteine, ^{35}S-hRlx-2, or ^{35}S-hRlx-2 Plus an Excess of Unlabeled hRlx-2, Administered as Intravenous Bolus Injections, Recovered from Organs[a] of Pregnant Rats

	^{35}S-Cysteine			^{35}S-hRlx-2			^{35}S-hRlx-2 + hRlx-2		
	10 min	30 min	120 min	10 min	30 min	120 min	10 min	30 min	120 min
Kidney	7 (0.7)	6 (0.4)	5 (0.4)	18 (2.4)	8 (1.3)	3 (0.6)	21 (4.8)	14 (2.8)	7 (2.0)
Liver	14 (1.6)	12 (0.5)	9 (0.7)	17 (0.6)	15 (2.5)	10 (1.6)	11 (1.6)	17 (1.4)	16 (2)
Muscle[b]	16 (3.8)	13 (2.8)	8 (2.0)	7 (1.3)	8 (1.0)	4 (2.7)	10 (1.5)	12 (0.9)	9 (2.4)
Mammary	2 (0.3)	1 (0.2)	1 (0.2)	0.7 (0.05)	0.8 (0.1)	0.6 (0.1)	1 (0.1)	0.9 (0.1)	1 (0.1)
Fat[b]	1 (0.3)	0.8 (0.2)	0.7 (0.1)	0.8 (0.1)	1 (0.4)	0.6 (0.1)	1 (0.1)	1 (0.8)	2 (1.7)
Uterus	1 (0.2)	1 (0.1)	0.9 (0.2)	0.8 (0.02)	0.8 (0.2)	0.6 (0.1)	0.8 (0.1)	0.9 (0.1)	1 (0.1)
Placentae	3 (0.5)	0.6 (0.1)	0.6 (0.03)	2 (0.1)	0.4 (0.1)	0.5 (0.1)	3 (0.2)	0.6 (0.01)	4 (0.05)
Fetuses	3 (0.5)	3 (0.3)	3 (0.2)	0.3 (0.01)	1 (0.1)	2 (0.3)	0.2 (0.03)	1 (0.1)	3 (0.5)

[a] Only the data for organs that had at least 1% of the dose at any sampling time are included.
[b] Total body fat and total body muscle.

uptake of radioactivity from the dose of ^{35}S-cysteine. The possibility that this radioactivity could also have been associated with partially degraded hRlx-2 cannot be discounted. Further support for the role of the kidneys in the clearance of hRlx-2 in rats came from kidney–serum radioactivity ratios being increased after ^{35}S-hRlx-2, compared to ^{35}S-cysteine. Additionally, a separate study showed the clearance of hRlx-2 to be decreased by 33% in nephrectomized nonpregnant rats but not in sham-operated or ureter-ligated rats (data not shown).

The extent of the hepatic uptake of radioactivity after ^{35}S-hRlx-2 was very similar to that after the administration of ^{35}S-cysteine, and so it was not possible to determine from the distribution experiment whether the liver was a site of uptake of intact hRlx-2; radioactivity may also have been present as a result of the uptake of degraded hRlx-2. Isolated perfused liver experiments clearly demonstrated, however, that intact hRlx-2 was extracted by the liver of pregnant and nonpregnant rats. It was shown that 37–55% of the total dose added to the perfusate was cleared in 2 hr. It is likely, therefore, that the liver plays some role in the *in vivo* clearance of hRlx-2 in rats.

Fetal levels of radioactivity were up to 3% of the administered doses of hRlx-2. These values were not greatly different from those observed for free ^{35}S-cysteine and suggest that at least part of the fetal radioactivity after ^{35}S-hRlx-2 was due to fetal uptake of ^{35}S-cysteine from metabolized ^{35}S-hRlx-2. After continuous iv administration of ^{35}S-hRlx-2 to a pregnant rhesus monkey for 2 hr, hRlx-2 was detectable in fetal plasma by ELISA, but hRlx-2 accounted for only 8–15% of the fetal radioactivity over the 2-hr period (15). hRlx-2 may have, therefore, crossed the rat placenta in the present experiment. A similar uncertainty exists regarding the form of radioactivity in muscle; however, it is suggested that the radioactivity present following administration of ^{35}S-hRlx-2 is due to incorporation of free ^{35}S-cysteine, from degraded ^{35}S-hRlx-2, into protein, and not to the presence of intact ^{35}S-hRlx-2. Alternatively, muscle radioactivity could be due to intact ^{35}S-hRlx-2 present in the blood of the many vessels of muscle.

Table IV. Mean [Mean (SE); n = 4] Hepatic Clearance (CL_H) and Percentage of the Dose of hRlx-2 Cleared (D_{CL}) from Recirculating Perfusions of Isolated Nonpregnant (NP) and Pregnant (P) Female Rat Livers

Perfusate conc.	1 ng/ml		10 ng/ml	
	NP	P	NP	P
CL_H (ml/min/g)	0.10* (0.03)	0.05 (0.02)	0.07 (0.03)	0.05 (0.01)
D_{CL} (%)[a]	52** (14)	40 (16)	36 (11)	40 (10)

[a] D_{CL} is the percentage of hRlx-2 cleared from the perfusate in 120 min.
* $P > 0.05$ for the comparison of CL_H from all groups.
** $P > 0.05$ for the comparison of D_{CL} from all groups.

In conclusion, this study has shown that pregnancy has no effect on the disposition of exogenous hRlx-2 in rats. By use of an internally labeled protein, the pregnant rat kidneys and liver were shown to be sites of substantial uptake of radioactivity following hRlx-2 administration. The livers of pregnant and nonpregnant rats were capable of extracting significant amounts of hRlx-2 *in vitro*, a finding which suggests that the liver, as well as the kidneys, could play a role in the elimination of hRlx-2 *in vivo*.

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