The Pharmacokinetics and Metabolism of Human Relaxins in Rhesus Monkeys

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Two forms of chemically synthesized human relaxin (hRlx and hRlx-2) were administered as 88 μg/kg intravenous bolus doses to pregnant and nonpregnant rhesus monkeys. No significant differences in pharmacokinetics were observed between pregnant and nonpregnant animals for either form of relaxin; however, clearance of hRlx (3.1–3.4 ml/min/kg) was significantly slower than clearance of hRlx-2 (6.2–6.5 ml/min/kg) in both pregnant and nonpregnant animals. Although the terminal half-lives for hRlx and hRlx-2 were similar (148–157 min), the initial and steady-state volumes of distribution were somewhat larger for hRlx-2 (71–85 and 398–418 ml/kg, respectively) than for hRlx (61–65 and 294–319 ml/kg, respectively). The metabolism of hRlx-2 was also investigated in pregnant and nonpregnant rhesus monkeys after iv bolus (0.44 mg/kg) or 60-min infusion (1.1 mg/kg) administration. Fast atom bombardment mass spectral analysis of the relaxin immunoreactivity isolated from the plasma indicated that hRlx-2 was partially degraded by removal of amino acids from the C terminus of the B chain. The percentage of intact material declined over a 60-min time course. At 60 min postdose, intact hRlx-2 was ~46–64% of the detected material. Degraded forms representing loss of one and four amino acids (hRlx) from the C terminus of the B chain were ~11–13 and ~19–34% of the detectable material, respectively.

KEY WORDS: relaxin; pharmacokinetics; metabolism; protein; mass spectrometry.

INTRODUCTION

Relaxin is a two-chain (A and B) polypeptide hormone that 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 is necessary to accommodate the passage of the fetus during delivery (1). The major source of relaxin in humans during pregnancy appears to be the corpus luteum (2–4).

In the present study, the pharmacokinetics and metabolism of two forms of chemically synthesized human relaxin (hRlx and hRlx-2) were examined in a series of studies in nonpregnant and pregnant female rhesus monkeys. Allometric analysis of pharmacokinetic data generated in mice, rats, rabbits, and rhesus monkeys has shown that, for this and many other proteins, clearance in these laboratory animals is a function of species size and is highly predictive of the human clearance (5,6). Porcine relaxin has previously been shown to cause physiological changes in the uterus and cervix in nonpregnant rhesus monkeys similar to those induced by pregnancy (7,8). In addition, chemically synthesized relaxin (hRlx-2) has been shown to affect secretion of prolactin and growth hormone in rhesus monkeys (9). A comprehensive review of relaxin's properties has recently been published (10).

hRlx is identical in sequence to the predominant form of relaxin in human corpora lutea and pregnancy sera[6] (11). The A and B chains of hRlx contain 24 and 29 amino acids, respectively. hRlx-2, postulated to be the mature product of human relaxin gene H2 based on sequence homology with porcine relaxin (2,4), is composed of A and B chains containing 24 and 33 amino acids, respectively. hRlx differs from hRlx-2 in the absence of four amino acids from the C terminus of the B chain. Both molecules are active *in vivo* in the mouse pubic symphysis bioassay (6,12). The potential presence of both forms of relaxin *in vivo* prompted an examination of the possible conversion of hRlx-2 to hRlx after administration in primates.

MATERIALS AND METHODS

Materials. The A and B chains of hRlx and hRlx-2 were chemically synthesized by solid-phase methods. The chains were combined, and hRlx and hRlx-2 were purified by chromatographic methods (4). The purity of relaxins used in these studies was ≥98% (13).

Immunoassay Methodology. The concentrations of hRlx-2 and hRlx in serum samples were determined using an enzyme-linked immunosorbent assay (ELISA). This is a modified version of a previously described assay (14). Microtiter plates were coated with affinity-purified, goat anti-hRlx-2 antibodies. The assay diluent used for sample and standard preparation was a pool of normal human, male serum (Peninsula Blood Bank, Burlingame, CA) that had been centrifuged at approximately 625*g* and then filtered through a 0.45-μm filter (Nalgene, Rochester, NY). The standard curve was prepared by diluting hRlx-2 in assay diluent to a final range of 20 to 1250 ng/L. Samples were diluted in the same assay diluent and then nonimmune goat immunoglobulin (Cooper Biomedical, Malvern, PA) was added to each sample and standard to a final concentration of 10 mg/L. Samples and standards were added to the antibody-coated EIA plates and incubated at 4°C for 12 to 18 hr. The plates were washed with PBS/Tween-20 before the addition of horseradish-peroxidase conjugated, affinity-purified, rabbit anti-hRlx-2 antibody. Following a 4-hr incubation at room temperature, the plates were washed and the remainder of the assay was completed as described previously (14). The concentration of hRlx-2 in each sample was calculated relative to a standard curve generated using a nonlinear, four-

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[6] J. W. Winslow, G. R. Laramee, J. D. Bourell, J. T. Stults, and P. D. Johnston. The structure of human relaxin purified from the corpus luteum of pregnancy (in preparation).

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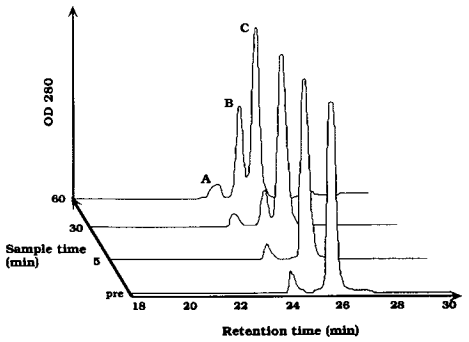

Fig. 1. C4 reversed-phase HPLC elution profile (absorbance at 214 nm) after MAb2 affinity column purification of relaxins from plasma from the nonpregnant monkey that received a 0.44 mg/kg iv bolus dose of hRlx-2. Retention times: peak A, 24.2–24.7 min (N = 7); peak B, 24.9–25.4 min (N = 6); peak C, 25.2–25.4 min (N = 9); standard A24B29, 25.2–25.4 min (N = 3); standard A24B33, 25.8–26.0 min (N = 3).

parameter regression analysis (15). The slope of this standard curve ranged from 1.1 to 1.3 across several assays, and the midpoint of the assay range was approximately 400 pg/ml. The interassay precision was determined for more than 20 assays run over a period of 6 weeks. The coefficient of variation (%CV) was less than 15% for control preparations at 500, 150, and 45 pg/ml. Within-run precision was evaluated by assaying 20 replicates of each control preparation in a single assay, and the %CV ranged from 5 to 12% for high, mid, and low controls. The minimal detectable concentration was calculated to be 7.5 ng/L by the method described by Anicetti *et al.* (16); however, no sample concentration less than 20 ng/L was considered valid in this assay. In rhesus monkey serum, hRlx-2 dilutes linearly throughout the assay range (data not shown). This was true for both hRlx-2 added directly to rhesus serum and hRlx-2 serum samples collected from animals administered three different iv bolus doses of hRlx-2. This linearity of dilution (slope, ~0.98 for dosed hRlx-2, ~0.84 for spiked material) indicated that diluted samples would quantitate accurately throughout the assay range. There was no significant difference in the reactivity of hRlx and hRlx-2 in this assay.

Metabolite Isolation and Identification. Monoclonal antibody (MAb) immunoaffinity columns (4°C, prewashed with PBS buffer containing the protease inhibitor cocktail before loading) consisting of purified anti-relaxin mouse MAb (MAb2) coupled to CNBr-activated Sepharose 4B were prepared (200 μl resin). Plasma samples were thawed

Table I. Pharmacokinetic Parameters for Relaxins in Rhesus Monkeys After Intravenous Administration[a]

ID	AUC (μg/ml/min)	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)
Pregnant hRlx	25.0	3.5	59	301	3.2	20.8	132
	27.7	3.2	59	272	3.3	23.4	134
	29.0	3.0	53	270	2.3	22.7	134
	23.9	3.7	74	358	3.7	21.1	158
	28.2	3.1	52	269	2.8	23.7	164
	22.6	3.9	72	295	5.1	37.4	164
Mean	26.1*	3.4*	61	294	3.7[b]	23.9[b]	139[b]
SD	±2.4	±0.3	± 9	±31			
Pregnant hRlx-2	10.2	8.6	105	540	3.8	24.0	138
	19.4	4.5	80	328	4.6	39.2	162
	17.7	5.0	88	366	5.9	33.4	160
	11.1	7.9	65	358	3.1	25.5	167
Mean	14.6*	6.5*	85	398	4.1[b]	28.9[b]	139[b]
SD	±4.6	±2.1	±16	±96			
Nonpregnant hRlx	30.9	2.9	71	345	4.9	24.8	151
	34.4	2.6	64	266	7.1	35.1	162
	28.2	3.1	55	315	4.4	26.0	158
	30.9	2.9	70	338	5.1	17.6	163
	28.5	3.1	41	286	1.5	20.3	146
	21.6	4.1	89	362	6.1	48.4	155
Mean	29.1**	3.1**	65	319	3.8[b]	25.7[b]	139[b]
SD	±3.9	±0.5	±15	±34			
Nonpregnant hRlx-2	16.5	5.3	80	414	6.5	42.5	172
	21.4	4.1	53	399	3.7	17.6	193
	15.3	5.7	69	368	3.7	30.9	132
	8.9	9.8	83	490	2.7	24.9	127
Mean	15.5**	6.2**	71	418	3.7[b]	26.7[b]	139[b]
SD	±4.4	±2.2	±12	±45			

[a] No statistically significant differences were found between pregnant and nonpregnant animals receiving the same forms of relaxin.
[b] Mean half-lives calculated by dividing 0.693 by the group mean rate constant.
* Significant statistical difference between pregnant animals receiving hRlx or hRlx-2.
** Significant statistical difference between nonpregnant animals receiving hRlx or hRlx-2.

and insoluble material was removed by centrifugation. Plasma volumes of 1.2–17.3 ml were applied to separate 200-μl MAb2 columns. The flow-through material was reapplied once, and the column was washed with 1 ml PBS and 1 ml PBS/1 *M* NaCl and eluted with two cycles of 0.4 ml 2 *M* guanidinium (Gu) HCl/10 m*M* Tris, pH 7.5, and five cycles of 0.35 ml 4 *M* GuHCl/10 m*M* Tris, pH 7.5.

Greater than 90% of the applied hRlx-2 ELISA activity eluted in the second 2 *M* GuHCl and the 4 *M* GuHCl washes (protease inhibitors were present in all MAb2 steps up to the 4 M GuHCl washes). These washes were combined and analyzed by HPLC (Hewlett Packard HP-1090). Samples were applied in aliquots (<200 μl) to a 1 mm × 10-cm C4 Synchropak RP4-1000 reversed-phase microbore column (1000-A pore size, Synchrom, Inc.) equilibrated with 20% acetonitrile/0.1% trifluoroacetic acid (TFA). The flow rate was 0.1 ml/min. Parent and degraded forms of hRlx-2 were resolved and eluted by application of a linear gradient from 20 to 60% acetonitrile over 40 min. Figure 1 shows the C4 RP HPLC elution profile (absorbance at 214 nm) after MAb2 affinity column purification of relaxins from nonpregnant monkey plasma (0.44 mg/kg hRlx-2 by iv bolus). Individual uv absorbance peaks (214 nm) were collected and analyzed by fast-atom bombardment mass spectrometry (FAB-MS). Authentic relaxin standards (B29 and B33, 200 ng) were run periodically.

HPLC effluent samples were lyophilized in a Savant Speed-Vac to a final volume of 1–3 μl. Successive 1-μl aliquots were applied to the MS probe tip and evaporated to dryness. The sample on the probe was rehydrated and reduced *in situ* for 10–15 min to separate the A and B chains through the addition of 1 μl 0.5 *M* dithiothreitol (DTT) in 100 m*M* *N*-ethylmorpholine buffer, pH 8.5. The sample was then heated to dryness and redissolved with 0.5 μl thioglycerol combined with 0.5 μl 10% oxalic acid. The samples were ionized with a high-energy xenon or cesium source and injected into a JEOL HX110 tandem MS to determine mass values for the monoisotopic protonated molecular ions. Under these conditions, it was possible to detect both A- and B-chain signals from as little as 750 fmol of relaxin.

Single Intravenous (iv) Doses in Pregnant and Nonpregnant Rhesus Monkeys. Six pregnant (5.1- to 9.0-kg; gestational days, 135–148 by ultrasound) and six nonpregnant (4.4- to 8.5-kg) rhesus monkeys were used. Four pregnant and four nonpregnant animals were crossed over and received both forms of relaxin. All animals were chair restrained and received 88 μg/kg relaxin by iv bolus (0.2–0.9 ml) through a catheter in the brachial vein. Blood samples (0.5–1 ml) were collected from a saphenous vein catheter at 0, 1, 2, 3, 4, 5, 7, 10, 15, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 210, 240, 270, 300, 360, 420, 480, 540, 600, 720, and 840 min postdose. Blood samples were allowed to clot, and serum was harvested by centrifugation. Samples were frozen on dry ice and stored at −70°C until assayed.

Metabolism Studies in Pregnant and Nonpregnant Rhesus Monkeys. Two pregnant (6.8- to 7.5-kg; gestational days, 137 and 153) and one nonpregnant (6.7-kg) rhesus monkeys were used. Animals were chair restrained and received relaxin as an iv bolus (0.44 mg/kg in 1.7–1.9 ml) or 60-min infusion (1.1 mg/kg in 12.5 ml) through a catheter in the brachial vein.

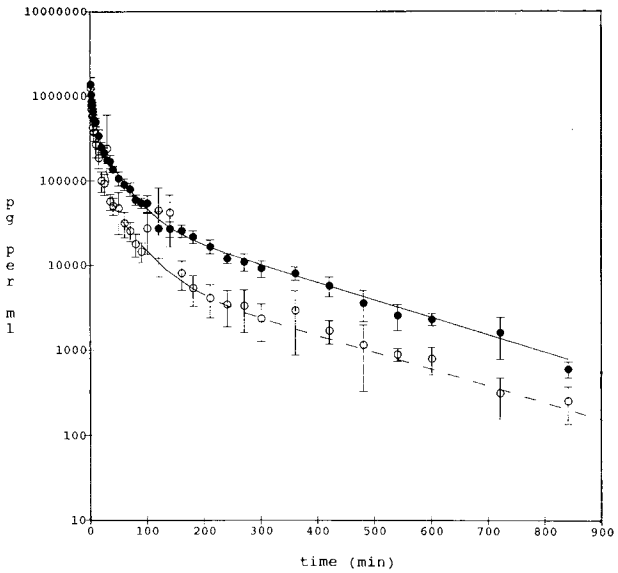

Fig. 2. Mean serum concentration versus time data for hRlx and hRlx-2 in pregnant rhesus monkeys after iv bolus administration. Filled circles, hRlx; open circles, hRlx-2. The mean fitted lines are superimposed on the data (units are ng/ml and min^{-1}). pregnant hRlx: $C = 1050e^{-0.186t} + 365e^{-0.029t} + 42e^{-0.005t}$. Pregnant hRlx-2: $C = 962e^{-0.168t} + 98e^{-0.024t} + 9e^{-0.005t}$.

Preliminary stability studies indicated that hRlx-2 spiked into rhesus plasma or serum was degraded to all forms between full length (A24B33) and −4 amino acids (A24B29); therefore, a protease inhibitor cocktail (suspension) was used to prevent *in vitro* degradation of hRlx-2 in plasma. The cocktail consisted of 10 inhibitors of five differ-

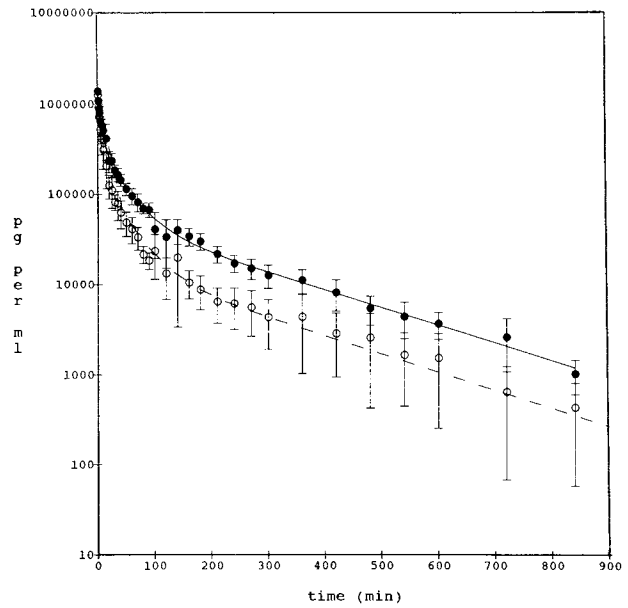

Fig. 3. Mean serum concentration versus time data for hRlx and hRlx-2 in nonpregnant rhesus monkeys after iv bolus administration. Filled circles, hRlx; open circles, hRlx-2. The mean fitted lines are superimposed on the data (units are ng/ml and min^{-1}). Nonpregnant hRlx: $C = 1090e^{-0.184t} + 293e^{-0.027t} + 53e^{-0.005t}$. Nonpregnant hRlx-2: $C = 1100e^{-0.186t} + 148e^{-0.026t} + 17e^{-0.005t}$.

Table II. FAB-MS Analysis of HPLC Effluent[a]

Animal	Sample (min)	Peak area[b] A, % (RT, min)	MS[c] (amu)	MS peak ID	Peak area[b] B, % (RT, min)	MS (amu)	MS peak ID	Peak area[b] C, % (RT, min)	MS (amu)	MS peak ID	Total % ID[d]
Nonpregnant rhesus, 0.44 mg/kg iv bolus	0 spike	6.4 (24.4)	3782 3797 3814 3832 3845 3856	UK B33 B33 – 1ox B33 – 2ox B33 – 3ox UK	NS	NS	NS	93.6 (25.8)	2950 3797	UK B33	100 B33
	5	5.5 (24.2)	NS	NS	NS	NS	NS	94.5 (25.5)	~3798	B33	100 B33
	30	5.8 (24.2)	NS	NS	15.1 (25.3)	~3814	B33-oxt	79.1 (25.8)	3315 3684	B29 B32 (B29:B32 = 1:1)	5.8 UK 79.2 B29:B32 (~1:1) 15.1 B33
	60	8.1 (24.5)	NS	NS	31.2 (25.3)	3039 3312 3685	B27t B29 B32 (B29:B32 = 3:2)	60.7 (25.9)	3225 3313 3798	B28t B29 B33 (B29:B33 = 1:3)	8.1 UK 31.2 B29:B32 (~3:2) 60.7 B33:B29 (~3:1)
Pregnant rhesus, 0.44 mg/kg iv bolus	0 spike	ND[e]	ND	ND	ND	ND	ND	ND	ND	ND	ND
	5	5.2 (24.4)	~3862	B33-4ox	0.2 (25.0)	NS	NS	94.6 (25.6)	3798	B33	0.2 UK 99.8 B33
	30	5.0 (24.5)	3829 3846	B33-2ox B33-3ox	11.5 (25.3)	3314 3330 3346 3361 3378 3685 3702 3720 3733 3748 3814 3830	B29 B29-1ox B29-2ox B29-3ox B29-4ox B32 B32-1ox B32-2ox B32-3ox B32-4ox B33-1ox B33-2ox (B29:B32:B33 = 2:2:1)	83.5 (25.7)	3313 3798	B29 B33 (B29:B33 = 1:10)	11.5 B29:B32:B33 (~2:2:1) 83.5 B29:B33 (~1:10) 5.0 B33
	60	6.4 (24.7)	3040 3055 3072 3088 3105	B27 B27-1ox B27-2ox B27-3ox B27-4ox	22.7 (25.4)	3314 3329 3346 3362 3685 3701 3717 3733 3814	B29 B29-1ox B29-2ox B29-3ox B32 B32-1ox B32-2ox B32-3ox B33-1oxt (B29:B32 = 1:1)	70.9 (25.9)	3314 3685 ~3798	B29 B32t B33 (B29:B33 = 1:10)	6.4 B27 22.8 B29:B32 (~1:1) 70.9 B29:B33 (~1:10)

Table II. Continued

Animal	Sample (min)	Peak area[b] A, % (RT, min)	MS[c] (amu)	MS peak ID	Peak area[b] B, % (RT, min)	MS (amu)	MS peak ID	Peak area[b] C, % (RT, min)	MS (amu)	MS peak ID	Total % ID[d]
Pregnant rhesus, 1.1 mg/kg iv infusion	0 spike	NS	NS	NS	NS	NS	NS	100 (25.2)	3798	B33	100 B33
	60	NS	NS	NS	26 (24.9)	3378 3751	B29-4ox B32-4ox (B29:B32 = 4:3)	74 (25.5)	3314 3799 3846	B29 B33 B33-3ox (B29:B33 = 1:10)	26.0 B29:B32 (~3:2) 74.0 B29:B33 (~1:10)

[a] Theoretical masses of the A- and B-chain molecular ions in amu as follows. A chain: pyroglu-A24 = 2657. B chain: B33 = 3798; B32 = 3685; B29 = 3314; B28 = 3227; B27 = 3041; B26 = 2940. amu = atomic mass units; A = earliest-eluting peak. MS = FAB-MS; NS = insufficient signal; ND = not determined; RT = retention time; t = trace; UK = unknown. Ratios of molecular ions approximate; estimated by visual inspection.

[b] Percentage of sum of peaks A + B + C. See Fig. 2.

[c] All samples showed A-chain peaks at ~2657.

[d] No distinction was made with regard to oxidation; data used for Fig. 5.

[e] No relaxin was detectable; sample was apparently not spiked.

ent classes of proteases (Sigma). Stock solutions of individual inhibitors were made in either 95% ethanol [phenylmethylsulfonyl fluoride (PMSF), *N*-tosyl-L-phenylalanine chloromethyl ketone (TPCK), *N*-tosyl-L-lysine cloromethyl ketone (TLCK), 1,10-phenanthroline, pepstatin A, leupeptin] or water (benzamidine, iodoacetamide, EDTA, EGTA) and mixed together to yield a final concentration of 1 m*M* for each inhibitor when 0.68 ml of cocktail was added to 10 ml of blood (with the exception of pepstatin and leupeptin, which were 0.1 m*M*). The protease inhibitor cocktail was shipped frozen, thawed on ice, and resuspended by mixing just before use.

For iv bolus doses, blood samples were collected from a saphenous vein catheter at 0 (1.4 ml), 5 (2.8 ml), 30 (14 ml), and 60 (33 ml) min postdose. The predose (0) sample (1.4 ml blood plus 0.14 ml protease inhibitor cocktail) was spiked with 0.5 ml of 20 μg/ml hRlx-2 (diluted in sterile saline); the final target hRlx-2 spiked concentration was 5 μg/ml. For iv infusion doses, blood samples were collected before the start of the infusion (0 min, 10 ml) and at the end of the infusion (60 min, 30 ml). The predose sample was spiked with hRlx-2 to 6 μg/ml (9 ml blood + 0.68 ml protease inhibitor cocktail + 0.3 ml 200 μg/ml hRlx-2 diluted in sterile saline). Blood samples were collected into EDTA plasma tubes containing 0.68 ml protease inhibitor cocktail per 10 ml blood. Plasma was harvested by centrifugation. Samples were frozen on dry ice and stored at −70°C until assayed.

Pharmacokinetic Methods. Both compartmental and noncompartmental methods were used in the calculation of pharmacokinetic parameters. Individual iv pharmacokinetic parameters were estimated by fitting a triexponential equation to the immunoreactive serum concentration–time data using a nonlinear curve fitting program (NONLIN84, Statistical Consultants, Inc., Lexington, KY). A three-exponential equation has previously been determined to be required to adequately describe concentration vs time data for relaxin in a variety of laboratory species (6), including rhesus monkeys (BLF, unpublished results). This determination was based primarily on the minimization of the sum of squared residuals. The area under the serum concentration–time curve (AUC) was computed with a computer program (#AUC, Genentech, Inc.) that employed the log trapezoidal method from the first to the last measurable serum concentration, $C(t)$. The AUC from $C(t)$ to infinite time was estimated by extrapolation; $C(t)$ was divided by the terminal elimination rate constant. Clearance (CL) was calculated by the equation CL = dose/AUC (from #AUC). The initial volume of distribution (V_c) was calculated from V_c = dose/$C(0)$, where $C(0)$ was 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 as CL * AUMC/AUC, where AUMC was the area under the moment curve, i.e., the (serum concentration) * (time) vs time curve (from #AUC). Serum half-lives were calculated by dividing 0.693 by the respective disposition rate constants.

Statistics. Investigation of treatment group differences in pharmacokinetic parameters was conducted with an analysis of variance (ANOVA). A (one-sided) significance level of 0.05 was used. If the global F test for treatment group differences was significant in the ANOVA, then follow-up multiple comparisons were conducted using least significant difference t tests (RS/1 procedure ANALYZE, BBN Software Products Corp., Cambridge, MA).

RESULTS AND DISCUSSION

Table I lists the calculated pharmacokinetic parameters for hRlx and hRlx-2 in pregnant and nonpregnant rhesus monkeys. These have been shown to be highly reproducible

in a separate group of animals (data not shown). Figures 2 and 3 show the mean serum concentration vs time data; the mean fitted lines are superimposed on the data.

No statistically significant differences in pharmacokinetics were observed between pregnant and nonpregnant animals for either form of relaxin. The difference in clearance between hRlx (3.1–3.4 ml/min/kg) and hRlx-2 (6.2–6.5 ml/min/kg) was statistically significant in both pregnant and nonpregnant animals. V_c and V_{ss} were somewhat larger for hRlx-2 (71–85 and 398–418 ml/kg, respectively) than for hRlx (61–65 and 294–319 ml/kg, respectively), although these differences were not statistically significant. There were no statistically significant differences in the serum half-lives.

Isolation of hRlx-2 and its degradation products was accomplished with an overall immunoreactivity recovery of 24–84% for the nonpregnant iv bolus study, 29–42% for the pregnant iv bolus study, and 9% for the pregnant 60-min iv infusion study. Table II lists the FAB-MS analysis of the HPLC fractions generated from the plasma samples. The FAB-MS analysis indicated that after iv bolus and 60-min infusion administration, hRlx-2 (A24B33) was partially degraded by removal of amino acids from the C terminus of the B chain (Fig. 4); the A chain remained intact with a molecular ion mass of 2657 amu, corresponding to N-terminal pyroglutamic acid A chain. Oxidized forms of the intact and degraded B chains were also observed. A24B33 accounted for all of the detectable material in the spiked samples and at 5 min postintravenous dose. The percentage of material identified as A24B33 declined over the 60-min time course. At 60 min postintravenous bolus dose, A24B33 relaxin was ~46–64% of the detected material. A24B32 and A24B29 appeared in the 30-min samples and increased over the 60-min time course. At 60 min postintravenous dose A24B32 and A24B29 were ~11–13 and ~19–34% of the detectable material, respectively; ~6.4% of the material at 60 min was A24B27. Similar results were observed after the 60-min intravenous infusion. A24B33 was only ~67% of the detected material at the end of the infusion; A24B32 and A24B29 accounted for ~11 and ~22%, respectively. Based on the results of this study, significant conversion of hRlx-2 to hRlx occurs *in vivo* in primates after intravenous bolus or intravenous infusion administration.

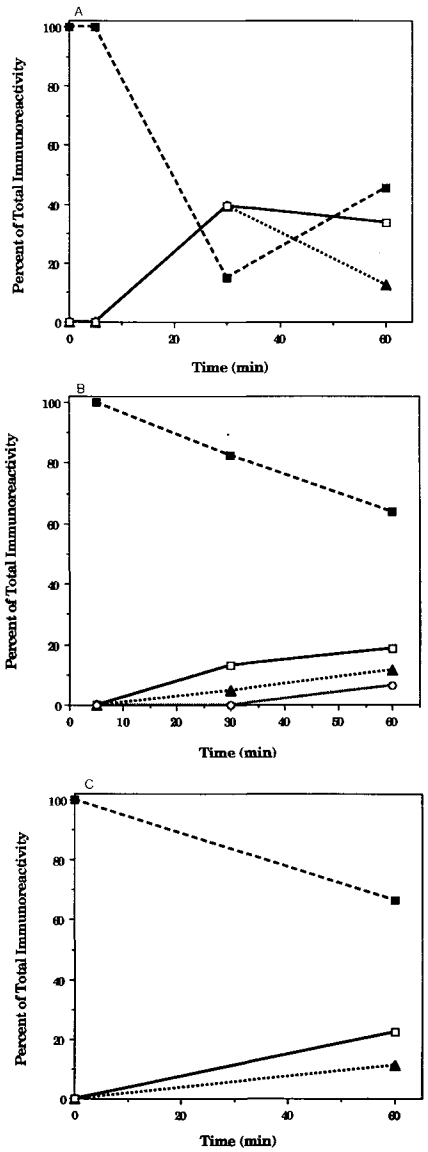

Fig. 4. Time course of relaxin metabolism in rhesus monkey plasma determined by FAB-MS analysis. Filled squares, A24B33; filled triangles, A24B32; open squares, A24B29; open circles, A24B27. A, nonpregnant, iv bolus administration; B, pregnant, iv bolus administration; C, pregnant, 60-min iv infusion.

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