

Metabolic Clearance Rate of Rat Prolactin in Preweanling Rat Pups¹ (41679)

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Abstract. The metabolic clearance rate (MCR) of immunoreactive rat prolactin (rPRL) was determined in urethane-anaesthetized 14 to 16-day-old rat pups using the constant infusion to equilibrium method. Two different doses of rPRL were used, 329 and 472 ng/min each at a volume of 0.0025 ml/min for 30 min. The MCR was 0.216 ml/min following infusion of 329 ng PRL/min dose and 0.337 ml/min following infusion of the 472-ng/min dose. When calculated on the basis of metabolic body weight the MCR of rPRL in rat pups was comparable to that of adult rats.

Prolactin (PRL) is rapidly transferred from the circulation of the lactating rat to milk within the alveolar lumina of the mammary gland (1-3). Most of the PRL is retained in the milk, resulting in concentrations of 200-400 ng/ml (1, 2); only a small portion appears to be free to pass back into the circulation (2). Substantial quantities of maternal PRL therefore are potentially available to the pups through the ingestion of milk. Though it is known that maternal PRL can be absorbed into the circulation of the rat pup (4, 5), we have been unable to determine accurately the amounts which are absorbed because we have lacked information on the metabolic clearance rate (MCR) of PRL in the rat pup. The present study is concerned with determination of the MCR of rat PRL in 14- to 16-day-old rat pups.

Materials and Methods. Primiparous rats of the Holtzman strain were housed in a room maintained at 23-25°C and with a lighting schedule of 14 hr light:10 hr darkness. They were fed Purina Lab Chow and given tap water to drink. Each litter was reduced to six pups from postpartum Day 4. On Days 14-16 the pups (male and female) were removed from their mothers and 2-2¹/₂ hr later were weighed and anesthetized with urethane (1 g/kg body wt ip given initially followed 30 min later with a second ip injection of 0.5 g/kg body wt). The skin and tissue area over the jugular vein was infiltrated with carbocaine (1% mepivacaine HCl, Winthrop), then a double-lumen catheter was inserted into an external jugular vein and anchored firmly. The wound was

covered with cotton which was kept moist with 0.9% saline and the pups then were placed on a warmer maintained at 35°C. One channel of the catheter was connected to an infusion pump (Harvard Apparatus Model 600-900); the other was attached to a syringe for withdrawing blood.

Two groups of eight pups each were continuously infused for 30 min with rat PRL (rPRL) (NIAMDD R P-1 PRL, 11 IU/mg) at a rate of 329 ng/min and 472 ng/min, respectively, each at a volume of 0.0025 ml/min. A control group of eight rats was continuously infused at 0.0025 ml/min for 30 min with phosphate-buffered saline + 1% bovine serum albumin (PBS-BSA), the solvent for the rPRL. At 0, 25, and 30 min of infusion the pump was shut off for 15 sec, then a 0.2-ml blood sample was obtained; the blood remaining in the catheter after each sample was taken was flushed back into the rat with heparinized saline along with 0.2 ml saline to compensate for the loss of blood volume. Another group of eight pups was untreated. Each of these was gently removed from the cage and swiftly decapitated; trunk blood was collected to determine the influence of urethane on the baseline level of PRL. The blood samples were quickly chilled on ice, centrifuged, and the plasma stored at -20°C until assayed for PRL using RIA and the NIAMDD kit, as described previously (6).

The MCR of immunoreactive rPRL was calculated for each rat as follows (7):

$$\text{MCR (ml/min)} = \frac{\text{Infusion dose (ng/min)}}{\text{increment in plasma concentration at equilibrium (ng/ml)}}$$

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TABLE I. METABOLIC CLEARANCE RATE OF RAT PRL IN 14- TO 16-DAY-OLD RAT PUPS^a

	Body wt (g)	Plasma PRL concentration (ng/ml) at different times of infusion of rPRL (min)			MCR (ml/min)*
		0	25	30	
Decapitated	28.9 ± 1.4	24 ± 8.7	—	—	—
Infused: PBS-BSA	30.6 ± 1.5	24 ± 11.9	24 ± 8.8	26 ± 5.9	—
Infused: 329 ng/min PRL	31.3 ± 1.5	7 ± 1.4	1325 ± 147	1471 ± 154	0.216 ± 0.024
Infused: 472 mg/min PRL	32.4 ± 1.1	20 ± 7.7	1219 ± 141	1882 ± 208†	0.337 ± 0.028

^a Values are means ± SEM. Eight rats per group.

† Significantly greater ($P < 0.05$) than PRL concentration after 25 min of infusion (Student's t test).

* Computed for each pup using average of PRL concentration at 25 and 30 min of infusion minus concentration before start of infusion.

The plasma PRL concentration used in the denominator for each rat was the average of the individual values after 25 and 30 min of infusion minus the plasma concentration at the start of PRL infusion.

Results and Discussion. The plasma PRL concentration of the eight urethane-anesthetized pups infused with PBS-BSA did not change during the 30 min of infusion and was essentially the same on average as that of the eight pups which were decapitated while conscious (Table I). Thus neither the infusion procedure nor urethane anesthesia appeared to affect basal PRL levels in rat pups. We had demonstrated previously that urethane did not alter either the basal PRL concentration or the MCR of rat PRL in the mature lactating rat (8). In rat pups the plasma concentration of PRL appeared to reach equilibrium levels within 25 min of continuous infusion of the 329-ng/min dose. However, the average plasma PRL concentration was significantly higher after 30 min than after 25 min of in-

fusion in those pups infused with the 472-ng/min dose (Table I), but this was due to higher levels in three of the eight rat pups. In previous studies the plasma rPRL concentration in adult female rats was shown to reach equilibrium after about 20 min of continuous infusion (6).

It was demonstrated in adult rats (9) that the MCR of rPRL tended to increase as a function of the infusion rate over a 9.6-fold range of dosage. This tendency was also observed in the rat pups in the present study, being 0.216 ml/min on average (0.185–0.351 ml/min range) after infusion of the 329-ng/min dose and 0.337 ml/min on average (0.225–0.434 ml/min ranges) after the 472-ng/min dose. This tendency of the MCR of PRL to increase with infusion rate may reflect an increased capability of the liver and kidneys to degrade and/or excrete PRL in response to higher PRL levels [see (9)] secondary to enhanced blood flow (10) or PRL receptor induction (11). This assumes that the PRL dis-

TABLE II. COMPARISON OF MCR OF PREWEANLING RAT PUPS WITH ADULT FEMALE RATS ON THE BASIS OF METABOLIC BODY WEIGHT (MBW)^a

Rat	Body wt (kg)	Infusion dose (ng/min)	MCR (ml/min)	MCR/MBW		
				ml/min/kg ^{0.75}	ml/min/kg ^{1.0}	ml/min/kg ^{0.67}
Adults	0.300	329	1.60*	4.00	5.33	
Pups	0.031	329	0.216	5.22		4.66
Adults	0.300	472	1.75	4.44	5.83	
Pups	0.032	472	0.337	7.90		7.06

^a Adult rat data from Ref. (9).

* Extrapolated value from dose-response curve ranging from 100–960 ng/min infusion dose rate.

tributed evenly throughout the various PRL compartments with each dose and that distribution volume was not altered substantially. Table II shows a comparison of the MCR of rPRL in preweaning pups with that of adult female rats determined previously (9) with the infusion to equilibrium method and employing RP-1 rat PRL as the infusate. The MCR has been calculated for all rats in one instance per unit of metabolic body weight (MBW) using an average $MBW = kg^{0.75}$ as used by Davis and Borger (12). Since the factor 0.75 may vary from 0.67–1.00, being lower in developing animals than in adults (13), we have also compared the MCR of adult rats using $MBW = kg^{1.0}$ and for rat pups using $MBW = kg^{0.67}$. With the latter method in particular it can be seen that no real difference exists between the MCR of rPRL of the rat pup and adult rat. Using the average $MBW = kg^{0.75}$, rat pups are calculated to have a somewhat higher MCR than their adult counterparts.

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