Effect of Colchicine on Internalization of Prolactin in Female Rat Liver: An In Vivo Radioautographic Study

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ABSTRACT Binding and internalization of 125I-ovine prolactin into hepatocytes of female rats was visualized by the in vivo radioautographic method (Bergeron, J. J. M., G. Levine, R. Sikstrom, D. O'Shaughnesssey, B. Kopriwa, N. J. Nadler, and B. I. Posner, 1977, Proc. Natl. Acad. Sci. USA, 74:5051-5055). Receptor-mediated internalization of label was observed into lipoprotein-filled vesicles in the Golgi/bile canalicular region of the hepatocyte. Colchicine treatment had no effect on the internalization of label into the lipoprotein-filled vesicles. However, the location of the radio-labeled lipoprotein-filled vesicles was altered from the Golgi/bile canalicular region to subsinusoidal. Radioactive content of hepatocytes decreased as a function of time after injection of 125I-prolactin; however, colchicine treatment markedly retarded this loss of label. Subcellular fractionation experiments indicated that colchicine treatment led to decreased levels of 125I-prolactin accumulation in microsomes but augmented the accumulation of label in the L fraction.

It is concluded that in normal female rats prolactin is internalized into lipoprotein-filled vesicles in the Golgi region before degradation of the hormone. Colchicine treatment accumulates labeled lipoprotein-containing vesicles in a subsinusoidal region and retards hormone catabolism. The labeled vesicles observed after colchicine treatment may correspond to the unique vesicles previously observed in the L fraction and found to be enriched in prolactin receptors (Khan, M. N., B. I. Posner, A. K. Verma, R. J. Khan, and J. J. M. Bergeron, 1981, Proc. Natl. Acad. Sci. USA, 78:4980-4981).

Prolactin receptors have been shown to be highly concentrated in microsomal fractions isolated from homogenates of livers from female as opposed to male rats (19, 28). Using subcellular fractionation procedures, we observed that 125I-ovine prolactin (oPRL) administered iv was internalized, via a receptor-mediated process, into rat liver and concentrated in a substantially intact form in Golgi vesicular elements (17, 29). Colchicine treatment was shown to decrease the initial uptake of 125I-oPRL into Golgi vesicles (26). Since other investigators using radioautography have described the uptake of 125I-labeled hormones into secondary lysosomes (1, 10, 15) we examined the uptake and distribution of 125I-oPRL in liver tissue after hormone injection by the in vivo radioautographic technique (5, 8). A major aim was to correlate our observations obtained with subcellular fractionation procedures (17, 26, 29) and those with in vivo radioautography in order to define with increased certainty those structures accumulating internalized hormone.

Our studies demonstrate that 125I-prolactin is bound to female rat hepatocytes to a much greater degree than male, in conformity with previous observations of in vitro binding (19, 28). They further show that 125I-prolactin is rapidly internalized into hepatocytes and concentrated in lipoprotein-containing vesicles in the Golgi region of the cell. Colchicine treatment did not prevent the progressive accumulation of 125I-prolactin into lipoprotein-containing vesicles but altered their cellular location from the Golgi complex to the subsinusoidal region of the hepatocyte.

MATERIALS AND METHODS

Animals: Young adult male and female Sprague-Dawley (Canadian Breeding Farms, St. Constant, Quebec) or Sherman (McGill Anatomy colony) rats (180-280 g) were used. Colchicine was purchased from Sigma Chemical Co. (St. Louis, MO) and a freshly prepared solution of colchicine in saline was administered intraperitoneally s.c. 10 mg/ml per 100 g body weight 3 h before injection of 125I-oPRL.

Hormone: oPRL (NIH-P-S10, 26.4 IU/mg) was generously provided by the Pituitary Hormone Distribution Program of the National Institute of Arthritis, Metabolism and Digestive Diseases and iodinated as described previously (17, 29) to specific activities of 114-152 μCi/μg.

Injection of Radio-labeled Hormone and Tissue Fixation: The protocol has been described previously (5, 8). Briefly, the rats were...
anaesthetized with i.p. injections of 0.06 mg Nembutal per 100 g body weight. The radio-labeled hormone, or radio-labeled mixed with unlabeled hormone, was injected into the external jugular vein, usually the left, following a paramedian supraclavicular exposure. The hormone was allowed to circulate for 2 to 60 min. The heart was exposed by a midline ventral incision, anterior incision of the diaphragm and paramedian severance of ribs. Lactated Ringer's solution was perfused via the left ventricle until the liver visibly blanched (~30 s), at which time the perfusate was changed to 2.5% glutaraldehyde in 0.1 M Sorensen's buffer/0.1% sucrose, pH 7.3. Small samples of the midquadrant lobe were removed and diced into 1- to 2-mm cubes. After 2 h in the same fixative as the perfusate, the samples were washed in 0.1 M cacodylate buffer, pH 7.4, and immersed in 2% OsO₄ (0.1 M cacodylate, pH 7.4) or reduced OsO₄ (11) for 2 h. After further rinsing in buffer the samples were stained en bloc with uranyl acetate or galloyl glucose (5, 8). Following dehydration the blocks were embedded in Epon 812.

For some experiments organs were dissected out after glutaraldehyde perfusion, blotted, weighed, and radioactive content was evaluated by gamma spectrometry (Packard autogamma counter; 40.5% efficiency).

**Light and Electron Microscope Radioautography:** For light microscopy, semithin (0.5-μm) sections were stained with iron haematoxylin and coated with Kodak NTB2 emulsion (23) and developed with freshly prepared Kodak D-170 developer (23). For electron microscopy, thin sections (silver-gray) were cut on an LKB-Huxley ultramicrotome (LKB Instruments, Inc., Rockville, MD), and a monolayer of Ilford L-4 emulsion was applied (21). Development was carried out either for large filamentous grains (chemical development) or for fine grains (“solution-physical” development) as described by Kopriwa (22). Sections were poststained with uranyl acetate and/or lead citrate and photographed with either a Siemens IA, Siemens 101 or Philips 400 electron microscope.

Quantitative analysis of light microscope radioautographs was carried out as described previously (5, 8). Exposed light microscope slides were evaluated at ×1,000 using a calibrated ocular equivalent to a frame size of 11 × 11 μm on the slide. For light microscopy greater than 1,000 grains were counted for each determination.

The electron microscope radioautographs when developed for filamentous grains (2 min time point) were photographed (× 10,000) in such a way as to ensure that an hepatocyte cell membrane was in view whenever possible. The distribution of filamentous grains from the nearest cell membrane was determined by placing a pinhole in the geometric center of the grain and measuring the distance to the nearest hepatocyte plasmalemma (5). Filamentous grains were also analyzed by scoring the structure beneath the grain (5, 8). The micrographs developed for fine grain analysis (2-30 min) were photographed differently, i.e. wherever there were grains, with no consideration as to whether a cell membrane was in view. The results were based on the analysis of 266 filamentous grains and 2,943 fine grains. A total of 22 cellular compartments were analyzed, of which only the major labeled compartments are noted in Results.

**Figure 1** Light microscope radioautographs of Epon sections of livers from female (a) and male (b) rats (285 g body weight, 82 d of age) at 2 min after injection of 125I-oPRL (1.8 × 10⁸ dpm; 156 μCi/μg). At 40-d exposure, the silver grain density was 3.54 ± 0.28 grains per frame (11 μm) for the female and 0.81 ± 0.12 grains per frame for the male. Student’s t test revealed a significance of P < 0.001. ×1,000.

**Figure 2** Light microscope radioautographs of Epon sections from livers of adult female rats at 2 min after the injection of 125I-oPRL (5.61 × 10⁸ dpm; 152 μCi/μg) (a) and injected with the same dose of 125I-oPRL but with an excess (1,000 μg) of unlabeled ovine prolactin (b). At 16-d exposure, silver grain density was 8.01 ± 0.36 grains per frame for the sections from the experimental animals (a) as compared to 1.79 ± 0.12 grains per frame for the control (b). Student’s t test revealed this difference as significant (P < 0.001). ×1,000.


Subcellular Fractionation: Liver homogenates from colchicine-treated and control female rats were fractionated into nuclear-mitochondrial pellets, L fraction, microsomes (P), and final supranatant (S) following the protocol described previously (20) based on the method described by Wattiaux et al. (39) and de Duve et al. (12).

RESULTS

Initial Binding In Vivo

Injection of 125I-oPRL into female rats resulted in silver grains over liver sections at a 4.4-fold higher grain concentration than over liver sections from identically injected males (Fig. 1). In experiments in which excess unlabeled prolactin was coinjected with labeled hormone into female animals (Fig. 2) a 4.5-fold reduction in grain concentration was observed when compared to sections from female animals injected with 125I-oPRL alone. In contrast no competition by excess unlabeled prolactin was observed over the proximal convoluted tubule of the kidney (Fig. 3).

In a separate experiment carried out to evaluate the proportion of injected 125I-oPRL in kidney and liver it was found (average from two female rats) that 17.2% of the injected dose (393 x 10^6 dpm [disintegrations per minute]) at 2 min was found in kidney and 15.8% in liver as assessed by gamma spectrometry of the fixative-perfused organs (data not shown).

Initial Peripheral Localization

Electron microscope radioautography with the chemical development method revealed filamentous silver grains close to the hepatocyte plasmalemma (Fig. 4). A plot of the distribution of all grains revealed 93% within 2 \mu m of the cell membrane with the first 50% of the these within 1,792 \AA (Fig. 5). Direct scoring indicated 61% of the total grains over the hepatocyte plasmalemma compartment. By directly attributing all grains according to how close they were to either the sinusoidal lateral or bile canaliculus surface, 84.9% of the grains at 2 min were

![FIGURE 3 Kidney cortex from normal female rats injected with 125I-oPRL alone (Experimental, a) or 125I-oPRL and excess unlabeled ovine prolactin (Control, b). Silver grains (30-d exposure) overlay only the proximal convoluted tubules (PCT) but not the distal convoluted tubule (DCT) or glomerular tuft (G). No reduction in grain density is observed over the control (b). \( \times 500. \)]
FIGURE 4 Low power electron microscope radioautograph of liver at 2 min after injection of $^{125}$I-oPRL. Silver grains (arrows) are observed close to the periphery of the hepatocyte (H), with few over endothelial cells (E). The bile canaliculus is indicated (bc). Intracytoplasmic lipid drops (L) are retained by the fixative employed (galloyl glucose). An erythrocyte (RBC) within the liver sinusoid (S) is also indicated. × 9,900.

attributed to the sinudoidal surface with 14% attributed to the lateral surface, and 1.13% attributed to the bile canaliculus.

Hormone Internalization

LIGHT MICROSCOPE RADIOAUTOGRAPHY: Analysis of the radioactive content of liver sections by radioautography (Fig. 6) revealed a loss of radioactivity at late time intervals (30–60 min). Identical studies carried out on colchicine-pretreated rats (Fig. 6) showed a lower radioactive content in liver at 2 to 10 min which progressively increased to levels higher
Distribution of filamentous silver grains about the hepatocyte plasmalemma at 2 min after the injection of $^{125}\text{I}$-oPRL. The perpendicular distance of the geometric center of every grain was measured to the nearest hepatocyte plasmalemma as described previously in Bergeron et al. (5). On the left-hand ordinate is indicated the number of grains scored in histogram form as a function of their distance from the nearest hepatocyte plasmalemma (abscissa). On the right-hand ordinate the data are plotted in cumulative form. 93% of the grains were within 2 $\mu$m of the hepatocyte cell membrane and the first 50% of these were within 1,792 Å. By direct scoring of the 266 silver grains, 87.3% were observed over hepatocytes with 9.8% over endothelial cells and the remaining grains over the lumina of sinusoids. Of the grains over hepatocytes, 75.7% were scored as plasmalemmal, 14.2% over cytoplasm, 7.6% over cytoplasmic vesicles, 6.1% over mitochondria, 1.3% over stacked Golgi saccules, 0.5% over the endoplasmic reticulum and 0.5% over lipid drops.

FIGURE 5 Distribution of filamentous silver grains about the hepatocyte plasmalemma at 2 min after the injection of $^{125}\text{I}$-oPRL. The perpendicular distance of the geometric center of every grain was measured to the nearest hepatocyte plasmalemma as described previously in Bergeron et al. (5). On the left-hand ordinate is indicated the number of grains scored in histogram form as a function of their distance from the nearest hepatocyte plasmalemma (abscissa). On the right-hand ordinate the data are plotted in cumulative form. 93% of the grains were within 2 $\mu$m of the hepatocyte cell membrane and the first 50% of these were within 1,792 Å. By direct scoring of the 266 silver grains, 87.3% were observed over hepatocytes with 9.8% over endothelial cells and the remaining grains over the lumina of sinusoids. Of the grains over hepatocytes, 75.7% were scored as plasmalemmal, 14.2% over cytoplasm, 7.6% over cytoplasmic vesicles, 6.1% over mitochondria, 1.3% over stacked Golgi saccules, 0.5% over the endoplasmic reticulum and 0.5% over lipid drops.

Subcellular Fractionation

The temporal accumulation of silver grains over lipoprotein-containing vesicles in colchicine-treated rats (Figs. 8 and 10) was in apparent contradiction to our previous observations (26) where it was found that colchicine treatment inhibited the accumulation of $^{125}\text{I}$-oPRL into purified Golgi fractions. Therefore, in order to resolve this apparent discrepancy, we fractionated homogenates into L, P, and S fractions at various times after $^{125}\text{I}$-oPRL injection into normal or colchicine-treated rats. The radioactive content of liver homogenates from colchicine-treated rats was lower at 5 min postinjection than the radioactive content in control animals (Table I) in accordance with our previous data (26). Moreover, the rate of loss of radioactivity in liver homogenates from colchicine-treated animals was less than that from controls (Table I). At early time intervals, the majority of the homogenate radioactivity was recovered in microsomes (P, Fig. 11). At later time intervals (30 and 60 min), radioactivity accumulated in the cytosol fraction (S, Fig. 11) isolated from control animals but not in the cytosol fraction isolated from the colchicine-treated rats. Rather, colchicine treatment accumulated radio-label in the L fraction at the later time intervals with a slight diminution observed for the microsomal fraction.

DISCUSSION

The utility of the in vivo radioautographic method employed herein has been demonstrated by the extensive mapping studies

FIGURE 6 Radioactive content of liver at various times after $^{125}\text{I}$-oPRL injection. Adult female rats were injected with $^{125}\text{I}$-oPRL (393 $\times$ 10$^6$ dpm; 114 $\mu$Ci/μg) and liver tissue was processed for light microscope radioautography. Grain densities were determined over livers of normal animals (■) compared to those in animals pretreated with colchicine (○). Approximately 1,000 grains were counted for each time interval at a magnification of 1,000 and with a calibrated ocular equivalent to a frame size of 11 $\times$ 11 μm.
Filamentous grains at 2 min revealed a distribution about the hepatocyte cell membrane with 93% within 2 μm. Of these, 50% were within 1,792 Å. Under identical development conditions, a line-source of 125I-thyroglobulin revealed 100% of the grains at 14,400 Å from the center of the line and 50% within 1,568 Å (5). Thus, as for 125I-insulin (5), 125I-oPRL is found close to the hepatocyte cell membrane at 2 min after injection. The peripheral location of the grains was also noted by direct scoring. However, few grains were observed over specific regions of the plasmalemma (e.g., coated pits) even by the fine grain method of silver grain development (1.4% of grains at 2 min in coated vesicles, data not shown). The attribution of silver grains to the various domains of the hepatocyte cell surface (sinusoidal, lateral, and bile canicular) revealed a distinct lack of labeling of the bile canicular membrane but with labeling of the sinusoidal and lateral surfaces which approximated published estimates of their surface areas (8). Thus, as for insulin (8), prolactin seems to be initially bound to receptors dispersed randomly over the sinusoidal and lateral surfaces of the hepatocyte.

Internalization

The radioactive content of liver as assessed by light microscope radioautography was observed to decrease markedly by 20–30 min after injection, presumably due to hormone degradation. The location of the prolactin label was also observed to change quite markedly with time. Thus under the light microscope most silver grains were observed peripherally at 2 min but at 10 and 30 min were observed to cluster near the Golgi complex/bile canicular region of the hepatocyte.

Electron microscope radioautography revealed the progressive accumulation of silver grains over lipoprotein-filled vesicles in the Golgi region of the cell. These lipoprotein-filled vesicles were often in close association with the Golgi apparatus although the Golgi complex stacks were themselves unlabeled. These observations are in general accord with our previous in vivo studies on insulin internalization in which we observed 125I-insulin accumulation into the Golgi region and into Golgi elements which were largely acid-phosphatase negative (8). Lipoprotein-filled structures in the Golgi region have been previously considered as secretory-vesicular elements of Golgi stacks.

In the present study on the prolactin receptor of liver, competition for binding of 125I-oPRL was effected by unlabeled oPRL in the target tissue of interest (liver) but not in a site (renal proximal convoluted tubule) known to be nonspecific for other hormones (6, 38). The sex specificity of the binding (Fig. 1) attested to the identification of the binding site as the lactogen receptor (17, 19, 29).

Figure 8 Distribution of radioactivity over major cellular compartments of hepatocytes at various times after the injection of 125I-oPRL (conditions as for Fig. 6) as scored by direct counting of structures beneath silver grains developed by the “fine-grain” method. A total of 22 compartments were scored of which only the major five are shown. The endoplasmic reticulum (ER) compartment includes both smooth and rough ER; LYS, secondary lysosomes defined as possessing a thick membrane, dense heterogeneous content and a complete or partial “halo” between the content and the membrane; SMB, smooth membranous bodies, which are an heterogeneous compartment of membrane-bounded organelles with either clear or moderately dense content and of a considerable variability in size (50–200 nm); LPV, lipoprotein-containing vesicles of 450 ± 150 nm diameter with an easily defined lipoprotein content. An average of 8.7% of total grains were observed over nonhepatocyte structures (i.e., endothelium, Kupffer cells, and sinusoidal lumina) and were excluded from analysis. Remaining grains were found over the plasmalemma, cell cytoplasm, and mitochondria. Gol Stacks, Golgi complex stacks.
vesicles by some investigators (e.g., references 13, 14). However, A. Novikoff (24) and P. Novikoff and Yam (25) consider lipoprotein-containing structures on the trans aspect of the Golgi stack in liver to be part of the distinct organelle GERL. Most recently, these investigators have noted the internalization of ferritin-labeled lactose into lipoprotein-filled GERL structures as well as lipoprotein-filled secretory vacuoles and secondary lysosomes (16). Chao et al. (11) have also documented the internalization to the Golgi region of the ligand LDL into structures which they called “multivesicular-body like” but which would have been scored as lipoprotein-containing vesicles by the criteria of our present study on prolactin internalization. Therefore lipoprotein-filled vesicles close to Golgi stacked cisternae appear to accumulate a variety of ligands besides the hormone insulin (8) and prolactin (present study).

Colchicine Treatment

In an attempt to ascertain with more accuracy the identity of the lipoprotein-containing vesicle, we carried out a parallel internalization study with colchicine-treated female rats. Similar colchicine pretreatment of rats has been documented by Redman et al. (30) to result in a block in the secretion of lipoproteins and other plasma proteins by hepatocytes. Morphologically, this block in secretion was reflected by an accumulation of lipoprotein-filled secretion vacuoles at the sinusoidal front of the cell (30) and an increased accumulation of secretory proteins in Golgi fractions isolated from liver homogenates of colchicine-treated rats (30). Our previous biochemical studies have indicated a decrease in the initial labeling of Golgi fractions at various time intervals after 125I-oPRL injection into colchicine-treated rats (26).

In the present work, colchicine treatment had no observable effect on the internalization of 125I-oPRL into the lipoprotein-containing vesicles (Fig. 8). In contrast to the normal animals, however, these labeled lipoprotein-containing structures were altered from a Golgi/bile canalicular location to a subsinusoidal location. This is exactly what Redman et al. (30) observed in the accumulation of lipoprotein-filled secretion vacuoles following colchicine treatment. In the studies of Redman et al. (30) as well as Bergeron et al. (4), lipoprotein-filled structures were characterized as secretory in nature based on subcellular fractionation studies. The same isolated Golgi fractions with lipoprotein-filled vesicles as their most prevalent component have previously been shown by us to accumulate iv injected 125I-oPRL (17) in normal rats.

The present studies are therefore in apparent contradiction to our previous work in which colchicine treatment was shown to inhibit the accumulation of 125I-oPRL into lipoprotein-filled vesicles in Golgi fractions (26). As galactosyl transferase relates specific activities and recoveries were identical for Golgi fractions isolated from control or colchicine-treated rats (26), then the labeled lipoprotein-containing structures observed in the present work (Fig. 10) may represent a subpopulation of lipoprotein-containing vesicles which do not accumulate in microsomes and are not enriched in galactosyl transferase marker enzyme.

To investigate this possibility, we fractionated liver homogenates into L, P, and S fractions following standard protocols (12, 20). Colchicine treatment inhibited the accumulation of radioactivity into microsomes (Fig. 11) in confirmation of our previous results (26). Furthermore colchicine treatment augmented the accumulation of radioactivity in the L fraction and inhibited markedly the accumulation of radioactivity in S (Fig. 11). Based on this limited data, it is suggested that the labeled lipoprotein-containing vesicles in colchicine-treated rats may be equivalent to the “unique” vesicle previously identified in total L fraction (20) and in the L1 subfraction of purified lysosomes (27).

Radioactivity in the S fraction has previously been shown to reflect largely degraded hormone (27), and the inhibition by colchicine is consistent with the present morphologic (Fig. 6) and biochemical (Table I) studies indicating a retention of radioactive content in liver following colchicine treatment.

Lysosome and Endosome Involvement

Using identical definition for lysosomes as used most recently by Haimes et al. (16), namely a thick membrane and partial or complete halo, we observed a maximum of 18.7% of silver grains over secondary lysosome and secondary lysosome-like structures, with no difference noted by colchicine treatment. From 10 to 30 min, the proportion of silver grains over lysosomes was about 15% of the total grains.

A peak in the smooth membranous body compartment was noted at the early time intervals. The smooth membranous body compartment was heterogeneous and consisted of small (50–100 nm diameter) vesicles and heterogeneous larger structures (up to 200 nm diameter). At 2 min most of the silver grains (75%) in the smooth membranous body compartment of normal animals was in the smaller vesicular structures. At later intervals a greater proportion of grains was observed over the larger structures in this compartment. The small vesicles observed in the present study corresponded in size and temporal labeling to the small smooth-surfaced vesicles which accounted for the labeling of the Golgi heavy fraction in previous studies assessing 125I-oPRL internalization by subcellular fractionation (17, 26). Our combined in vivo studies of the present work and previous subcellular fractionation data (17, 26) therefore indicate a sequence of internalization involving initial binding of hormone to the sinusoidal and lateral surfaces of the plasmalemma followed by internalization into small, smooth-surfaced intracytoplasmic vesicles and temporary accumulation within vesicles with a lipoprotein content.

Further analysis of the effect of colchicine on prolactin accumulation and comparison to the effect of chloroquine (27).

Figure 9. Electron microscope radioautographs of liver sections from normal female rats at 20 min after 125I-oPRL injection. In the top panel, silver grains are shown over a lipoprotein-containing vesicle (LPV). A secondary lysosome (LYS) is labeled (arrow) in the center panel, and a small and large smooth membranous body (SMB) is shown labeled in the lower panel. Mitochondria (M), the cell nucleus (N), the stacked Golgi complex saccules (G) and the plasmalemma (PM) are indicated. X 30,000.
TABLE I

Effect of Colchicine Treatment on Radioactive Content of Liver Homogenates

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Control</th>
<th>+ Colchicine</th>
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<tbody>
<tr>
<td>5</td>
<td>100.0 ± 3.6*</td>
<td>64.2 ± 5.0</td>
</tr>
<tr>
<td>10</td>
<td>74.9 ± 8.8</td>
<td>44.1 ± 4.5</td>
</tr>
<tr>
<td>15</td>
<td>116.2 ± 14.9</td>
<td>82.2 ± 21.1</td>
</tr>
<tr>
<td>30</td>
<td>30.4 ± 4.5</td>
<td>58.4 ± 11.2</td>
</tr>
<tr>
<td>60</td>
<td>10.5 ± 0.4</td>
<td>40.7 ± 12.0</td>
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*125I-oPRL (31.25 ± 10^6 dpm, 142 μCi/μg) was injected into each of three animals for each time interval and treatment indicated. Livers were rapidly excised and placed in ice-cold 0.25 M sucrose and homogenized. Radioactive content of liver homogenates from control rats at 5 min after injection averaged 2.05 ± 10^8 dpm/g liver. All other values have been expressed as a proportion of this 5-min value for both control and colchicine-treated rats.

should help in the elucidation of the molecular mechanisms of receptor recycling and perhaps hormone action.

That colchicine treatment may have affected receptor recycling can be inferred from the data in Fig. 6 and Table I in which hormone binding at 2 min (Fig. 6) and 5 min (Table I) is lower in colchicine-pre-treated rats than in the normal animals. However, the continued internalization of 125I-oPRL in the colchicine-treated animals (Fig. 8) argues against a tight coupling between the phenomena of endocytosis and exocytosis, the latter completely inhibited by identical colchicine treatment (30).

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