Effect of Colchicine on the Uptake of Prolactin and Insulin into Golgi Fractions of Rat Liver

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ABSTRACT In previous studies we have shown that ^{125}I-labeled prolactin is taken up by a receptor-dependent process and concentrated in an intact form in Golgi elements from female rat liver (*J. Biol. Chem.*, 1979, 254:209–214). In this study we have examined the effect of colchicine on this uptake process into Golgi elements. Colchicine [25 μmol (10 mg)/100 gm body wt] was injected intraperitoneally in adult female rats, and hepatic Golgi fractions were prepared at 1, 2, and 3 h postinjection. The enzyme recoveries and morphological appearance of fractions from colchicine-treated and control (alcohol alone) animals were similar. At times >1 h after colchicine there was a marked (>60%) inhibition of uptake of ^{125}I-ovine prolactin (^{125}I-oPRL) into Golgi light and intermediate fractions but no inhibition of uptake into Golgi heavy and plasmalemma elements. At times from 2 to 45 min postinjection, ^{125}I-oPRL was extracted from Golgi elements and found to be largely intact as judged by rebinding to receptors. The inhibitory effect of colchicine was seen at doses ranging from 0.25 μmol to 25 μmol/100 g body wt. Vincristine also inhibited ^{125}I-oPRL uptake into the Golgi light and intermediate fractions but lumicolchicine had no inhibitory effect.

There was a smaller effect of colchicine both at early (1 h) and later (3 h) times on the extent and pattern of ^{125}I-insulin uptake. Colchicine treatment did not produce a significant change in lactogen receptor levels in the Golgi fractions. These results demonstrate that colchicine treatment inhibited the transfer of prolactin into Golgi vesicular elements. The much smaller effect on insulin uptake suggests that there may be differences in the manner in which the two hormones are handled in the course of internalization.

In previous studies we have identified intracellular (Golgi) receptors for insulin and prolactin and have shown that their characteristics are similar to those of plasmalemma receptors (5, 34, 35, 38). We have also observed that ^{125}I-ovine prolactin (^{125}I-oPRL) and ^{125}I-insulin are taken up by rat liver in vivo by a receptor-mediated process and concentrated in intact form in Golgi elements (6, 17, 37). These observations of receptor-mediated uptake of hormone have suggested that internalization proceeds via adsorptive endocytosis.

The secretion of plasma proteins via the Golgi apparatus of rat liver has been described (3, 14, 32, 39). Colchicine has been shown to inhibit the secretory process in liver (2, 10, 39–41) and other tissues (15, 20, 21, 30, 31, 47). It has been shown to have a disorganizing effect on Golgi structure in certain tissues (44) and to inhibit endocytosis in chondrocytes (33), fibroblasts (45), and macrophages (44). Because of these observations of colchicine's effects on the Golgi apparatus and endocytosis we have examined the effect of colchicine on the internalization of ^{125}I-labeled insulin and prolactin. Our studies have demonstrated that colchicine treatment markedly inhibited the uptake of ^{125}I-oPRL and, to a lesser extent, ^{125}I-insulin into Golgi vesicular elements [Golgi light (Gl) and Golgi intermediate (Gi)]. The inhibition of ^{125}I-oPRL uptake was not associated with a reduction of lactogen receptor levels in the Golgi fractions.

MATERIALS AND METHODS

Animals

Young adult female Sprague Dawley rats of 9–11 wk of age (180–200 g in weight) were used in these studies. They were fed Purina Chow and caged in groups of two to four animals.

Reagents

Porcine crystalline zinc insulin (24.4 U/mg) was a gift from Connaught Research Laboratories, Willowdale, Ontario. Ovine prolactin (oPRL, NIH P-S,

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26.4 U/mg) was generously provided by the Pituitary Hormone Distribution Program of National Institute of Arthritis, Metabolic, and Digestive Diseases, Bethesda, MD. Carrier-free [^{125}I]iodine and UDP-galactase (U-^{14}C) (209 mCi/mmol) were obtained from New England Nuclear Corp., Boston, MA.

Colchicine, vincristine sulfate (grade II), and ovomucoid were purchased from the Sigma Chemical Co., St. Louis, MO. Lumicolchicine was prepared from colchicine by UV irradiation (48). Colchicine was dissolved (10 mg/ml) in 100% ethanol, and the solution was placed in tightly sealed vials 3 cm from a 30-watt UV light. At regular intervals the UV spectrum of the solution was obtained and the disappearance of colchicine's absorption maximum at 350 nm was followed. After 10 h of irradiation, this had reached a stable minimum so that the estimated residual colchicine was <5% of the original amount. The solution was taken to dryness in vacuo. Colchicine, lumicolchicine, and vincristine were dissolved in 0.9% sodium chloride.

Iodination of insulin and prolactin was done at room temperature using chloramine-T as described previously (34).

Uptake of Labeled Hormone in Vivo

Rats were starved overnight and given ethanol (50% vol/vol) by stomach tube (1.2 ml/100 gm body wt) 90 min before sacrifice (4) except where noted otherwise (Table I). Experimental animals received an intraperitoneal injection of a freshly prepared solution of colchicine (25 μmol (10 mg)/100 g body wt) 3 h before the injection of labeled hormone unless specified otherwise (see Fig. 1 and Table II). Control animals were injected with 0.9% saline. The injection of labeled hormone was performed in animals lightly anesthetized with ether. The liver was exposed through a midline incision, and ^{125}I-oPRL or ^{125}I-insulin (12.5×10^6 cpm) was injected in a 0.4-ml volume of 1% bovine serum albumin (BSA)–phosphate buffered saline via the portal vein. Animals were sacrificed by decapitation 10 min after the injection of ^{125}I-oPRL except in the time course studies depicted in Figs. 2 and 3. Livers were rapidly excised, placed in ice-cold 0.25 M sucrose, and homogenized for the preparation of Golgi fractions or plasmalemma as described in detail elsewhere (5). Labeled fractions were counted in duplicate in a model 3002 Auto Gamma scintillation spectrophotometer (Packard Instrument Co., Downers Grove, IL) at an efficiency of 40% with a counting error of <2%. Protein was determined by the procedure of Lowry et al. (22) with BSA as standard.

TABLE I

Effect of Ethanol and Colchicine Treatment on the Protein Yield of Different Subcellular Fractions

	Protein			
	mg/gm liver			
Ethanol	−	−	+	+
Colchicine	−	+	−	+
Homogenate	170	160	157	155
Microsomes	34.0 ± 0.7	32.1 ± 0.7	31.4 ± 2.2	30.9 ± 0.4
Gl	0.1 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.1
Gi	0.4 ± 0.0	0.5 ± 0.1	0.6 ± 0.0	0.7 ± 0.1
Gh	1.8 ± 0.4	1.9 ± 0.0	1.9 ± 0.2	1.8 ± 0.0
Smooth microsomes	13.7 ± 3.0	11.5 ± 0.2	13.2 ± 0.4	14.6 ± 0.0
Rough microsomes	10.7 ± 0.4	10.3 ± 1.1	10.4 ± 0.5	13.7 ± 0.9

Colchicine and ethanol, when administered, were given 3 h and 90 min, respectively, before sacrifice as described in Materials and Methods. For each of the four conditions studied, two animals were used. Each value is the mean ± one-half the range of the two separate fractionations.

Enzymatic Characterization of Golgi Fractions

Enzymes were assayed using previously published methods. 5'-Nucleotidase (EC 3.2.2.4) was determined by the method of Heppel and Hilmoe (16). Acid phosphatase (EC 3.1.3.2) was determined using paranitrophenyl phosphate as substrate as described by Baldijao et al. (1) with 0.1% wt/vol Triton X-100 in the final reaction mixture. Glucose 6-phosphatase (EC 3.1.3.9) was measured by the method of Parkes and Thompson (29). Succinic acid dehydrogenase (EC 1.3.99.1) was assayed by the method of Bernath and Singer (7) in the direction of succinate oxidation using triphenyl tetrazolium chloride as an acceptor dye. After the reaction was stopped with glacial acetic acid, the colored formazone formed was extracted with toluene and the absorbance was read at 495 nm in a Beckman DK 2A spectrophotometer (Beckman Instruments, Inc., Palo Alto, CA). Inorganic phosphate was determined by the method of Chen et al. (12).

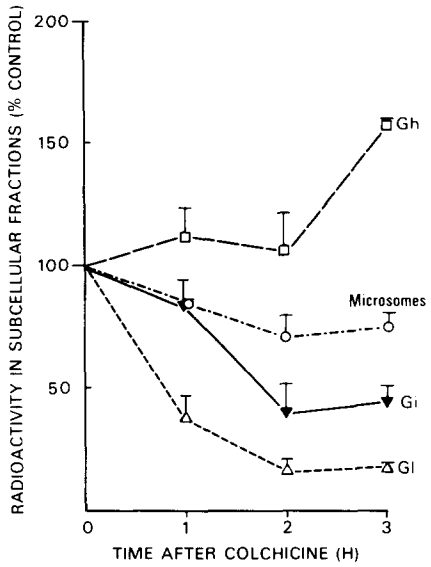

FIGURE 1 Inhibition by colchicine of ^{125}I-oPRL uptake into rat liver homogenate and subcellular fractions as a function of time after colchicine injection. Rats were injected with colchicine (25 μmol/100 g body wt) at the noted times before sacrifice. All animals received ethanol 90 min and ^{125}I-oPRL by injection 10 min before sacrifice. Data are the mean ± SE of three different experiments in each of which three to six rat livers were pooled for fractionation as noted in Materials and Methods. Uptake into whole microsomes (○) and Golgi light (*Gl*, △), intermediate (*Gi*, ▼), and heavy (*Gh*, □) subfractions is depicted.

TABLE II

Effect of Colchicine, Vincristine, and Lumicolchicine on the Uptake of ^{125}I-oPRL into Hepatic Subcellular Fractions

		^{125}I-oPRL uptake, *% control*			
Drug treatment		Homogenate	NMP	Microsomes	Supernatant
	μmol/100 g body wt				
Colchicine	0.25	71.9 ± 6.0	93.5 ± 9.2	80.7 ± 7.9	69.2 ± 6.1
	1.25	68.8 ± 6.6	99.3 ± 11.5	67.8 ± 8.1	60.7 ± 7.4
	12.50	60.6 ± 4.9	90.4 ± 6.4	64.5 ± 6.3	51.5 ± 3.2
Lumicolchicine	1.25	104.8 ± 11.4	108.5 ± 11.0	110.9 ± 9.7	139.1 ± 25.6
Vincristine	1.25	79.4 ± 10.4	97.6 ± 19.4	72.9 ± 9.8	73.4 ± 14.6

Rats received colchicine [0.25 μmol (0.1 mg), 1.25 μmol (0.5 mg), 12.5 μmol (5.0 mg)/100 g body wt]; lumicolchicine [1.25 μmol (0.5 mg)/100 g body wt], or vincristine sulfate [1.25 μmol (1.15 mg/100 g body wt]), and ethanol 3 h and 90 min, respectively, before the injection of ^{125}I-oPRL as described in Materials and Methods. 10 min after ^{125}I-oPRL injection, the animals were sacrificed and their livers subjected to subcellular fractionation. The nuclear-mitochondrial pellet (NMP) was obtained by centrifugation of the homogenate at 10,000 g_{avg} for 10 min. Whole microsomes were prepared from the NMP supernatant as described elsewhere (5). Supernatant refers to the microsomal supernatant. Control animals were handled identically. The radioactivity (cpm/mg protein) in the subcellular fractions from drug-treated rats was expressed as a percent of that in corresponding fractions from control animals. All values are the mean ± SE of two or three different fractionation studies in each of which three to six control and drug-treated animals were used.

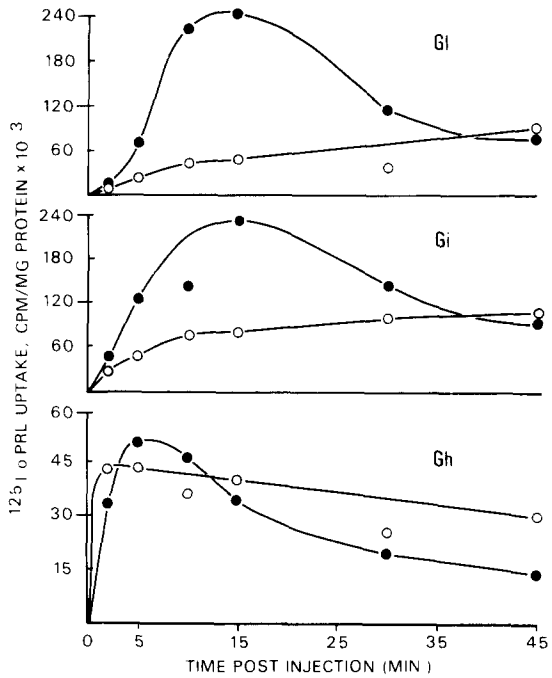

FIGURE 2 Effect of colchicine on the time course of ^{125}I-oPRL uptake into Golgi fractions. Colchicine and ethanol were given 3 h and 90 min, respectively, before the intraportal injection of ^{125}I-oPRL. Each time point represents a fractionation on three pooled rat livers for both control (●) and colchicine-treated (○) animals.

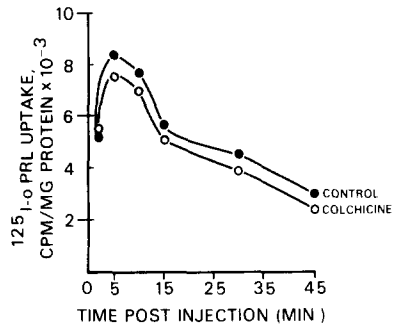

FIGURE 3 Effect of colchicine on the association of ^{125}I-oPRL with a plasma membrane fraction. The experiment was conducted as noted in the legend of Fig. 2. Each point represents one fractionation on two pooled female rat livers from either control (●) or colchicine-treated (○) animals.

Galactosyl transferase (UDP-galactose:ovomucoid transferase) activity was determined using ovomucoid (trypsin inhibitor; Sigma Chemical Co.) as the galactose acceptor (8). 0.1 ml of reaction mixture contained 40 mM cacodylate buffer pH 6.6, 40 mM magnesium chloride, 40 mM mercaptoethanol, 175 μg of ovomucoid, 5 mM ATP, 0.2% (wt/vol) Triton X-100 and 0.1 μCi of UDP-galactose (U-^{14}C) along with unlabeled UDP-galactose to bring the final specific activity to 0.95 Ci/mmol. After addition of subcellular fraction (protein up to 150 μg), the mixture was incubated at 37°C for 60 min. 1 ml of ice-cold 1% (wt/vol) phosphotungstic acid in 0.5 N HCl was added to stop the reaction. After 15 min at 4°C, the tubes were centrifuged at 3,000 rpm for 15 min. Pellets were washed twice with 5% (wt/vol) ice-cold TCA, once with 1 ml of ethanol:ether (1:1), and finally dissolved in 1 ml of 2 M ammonium hydroxide. This was neutralized with glacial acetic acid and was transferred to scintillation vials which contained 15 ml of Instagel (Packard Instrument Co.). Counting was done in a refrigerated liquid scintillation spectrometer (Packard Tricarb, model 3375) at an efficiency of 89%. Suitable enzyme controls were run with each assay by adding membranes to the reaction mixture after stopping the reaction. Specific activity was expressed as nmol of galactose transferred/60 min per mg of protein.

Integrity Studies

The integrity of ^{125}I-oPRL found in the various subcellular fractions of liver after in vivo injection was evaluated by extracting the radioactivity and determining its capacity to rebind to lactogen receptors. ^{125}I-oPRL was extracted with 0.01 N HCl containing 1% BSA by incubating on a shaker at 4°C for 16 h. The suspension was centrifuged at 100,000 g_{avg} for 60 min. An aliquot of supernatant was counted to determine the percent radioactivity extracted. After neutralization with dilute sodium hydroxide, an aliquot was chromatographed on Sephadex G-100 to check the molecular weight of the extracted material. Another aliquot was tested for binding to female rat liver microsomes. Controls were run in which freshly prepared ^{125}I-oPRL was treated in the same way.

Determination of Specific Binding

Microsomes and Golgi fractions were frozen and thawed four times before using in binding assays (38). Plasmalemma was frozen and thawed once only (38). Labeled hormone (5–10 × 10^4 cpm) was incubated in triplicate with 50 μg of Golgi or plasmalemma fraction in a final 0.5-ml volume containing 25 mM Tris-HCl, 10 mM magnesium chloride, and 0.1% (wt/vol) BSA, pH 7.4. Incubations were done at 4°C for 48 h (34, 35). Parallel incubations were done in the presence of excess hormone (5 μg). After completion of the incubation, the reaction mixture was diluted with 3 ml of ice-cold Tris-Mg-BSA buffer, and bound hormone was separated from free by centrifugation at 3,000 rpm for 20 min. Specific binding is defined as the difference between radioactivity bound in the absence (total binding) and that bound in the presence (nonspecific binding) of excess unlabeled hormone and is expressed as a percent of the total radioactivity in the incubation.

Radioautography

Animals were handled as described above except that they received 180–210 × 10^6 cpm of ^{125}I-oPRL at 10 and 30 min before sacrifice. Portions of freshly prepared Golgi fractions were briefly fixed with 2% glutaraldehyde/0.1 M cacodylate buffer and processed for radioautographic analysis as described in detail previously (17).

RESULTS

Influence of Colchicine Treatment on the Yield and Purity of Golgi Fractions

The recovery of the different subcellular fractions (yield of protein) as a function of treatment with ethanol and/or colchicine is summarized in Table I. Colchicine alone had an effect comparable to that of ethanol alone. The combination of colchicine and ethanol administration produced a minimal increase in the yield obtained with either agent alone.

The yield and purity of the Golgi fractions from colchicine-treated rats was evaluated by examining the Golgi marker enzyme, galactosyl transferase (4). The assay employed was based on the procedure in which ovomucoid, an oviduct glycoprotein with terminal *N*-acetylglucosamine residues, was used as an acceptor of galactose from UDP-galactose (8). We obtained recoveries of 29.4 and 33.4% of homogenate activity in the total Golgi elements form control and colchicine-treated rats, respectively (Table III). The specific activity and relative specific activity of galactosyl transferase in the subcellular fractions from both control and colchicine-treated rats were comparable except for lower activity in the Gl from the latter. In the experiment of Table III, the overall recovery of homogenate galactosyl transferase activity was 100.7% for control and 107.3% for colchicine-treated animals.

The relative specific activities and recoveries of typical marker enzymes, 5′-AMPase, glucose 6-phosphatase, acid phosphatase, and succinic dehydrogenase for plasmalemma, endoplasmic reticulum (ER), and lysosomal and mitochondrial elements are summarized in Table IV. Colchicine treatment did not change the relative specific activity or the recovery of any enzyme activity from that seen in control animals. The recovery of homogenate activity in the generated fractions for control and colchicine-treated rats, respectively was: 5′-nucleotidase, 98.2% and 92.7%; glucose 6-phosphatase, 131.6% and 131.9%; acid phosphatase, 104.9% and 96.8%.

Effect of Colchicine, Lumicolchicine, and Vincristine Treatment on the Hepatic Uptake of ^{125}I-oPRL

In early studies we noted an inhibitory effect of colchicine on the uptake of ^{125}I-oPRL into Golgi fractions. In Fig. 1, the effect of colchicine on ^{125}I-oPRL uptake was examined as a function of time after injection of the drug. The inhibition of uptake into Gl was noted by 1 h after colchicine, whereas that into Gi was not seen until 2 h postinjection. There was no effect on uptake into Golgi heavy (Gh) at early times postinjection, but by 3 h there was an increase of radioactivity in Gh with levels reaching ~150% that of controls. Uptake into microsomes was inhibited in a fashion parallel to, though less marked than, uptake into Gi. In all subsequent studies we have examined ^{125}I-oPRL uptake at 3 h after drug injection.

The effect of three doses of colchicine and one dose each of lumicolchicine and vincristine on the uptake of ^{125}I-oPRL into whole liver homogenates and subcellular fractions was examined (Table II). Colchicine inhibited uptake into liver as judged by levels of radioactivity in liver homogenate. Inhibition was more marked at a dose of colchicine of 12.5 μmol/100 g body wt than at one of 0.25 μmol/100 g body wt. Examination of subcellular fractions indicated no significant inhibition by colchicine of uptake into the nuclear-mitochondrial pellet (NMP) but readily observable inhibition of uptake into the microsome and microsomal supernatant fractions. Vincristine produced a pattern of inhibition comparable to that of colchicine whereas lumicolchicine was without inhibitory effect.

We then examined the effect of colchicine, lumicolchicine and vincristine on the uptake of ^{125}I-oPRL into microsomal subfractions (Table V). Colchicine and vincristine inhibited ^{125}I-oPRL uptake into Gl and Gi. Some inhibition of uptake into rough microsomes was also observed. There was no effect on uptake into Gh but uptake into this fraction was highly variable as reflected in the large standard errors. Inhibition of uptake was seen at a dose of colchicine as low as 0.25 μmol/100 g body wt. Lumicolchicine was without an inhibitory effect on uptake into any of the fractions and actually stimulated uptake into Gl ($P < 0.01$). Subsequent studies have been carried out with colchicine at a dose of 25 μmol/100 g body wt.

TABLE III

Effect of Colchicine Treatment on Yield and Purity of Galactosyl Transferase Activity in Hepatic Subcellular Fractions

	Protein per gm liver	Activity per gm liver	Sp act	Relative sp act	% Recovery from homogenate	
Control rats						
Homogenate	148.60	882.6	5.9	1.0	100	
Microsomes	40.20	606.3	15.1	2.5	68.7	
Gl	0.25	48.5	197.1	33.2	5.5	
Gi	0.42	70.9	168.5	28.4	8.0	29.4
Gh	2.11	140.6	66.5	11.2	15.9	
Smooth microsomes	12.50	252.0	20.1	3.4	28.5	
Colchine-treated rats						
Homogenate	178.30	793.6	4.5	1.0	100	
Microsomes	48.30	519.0	10.7	2.4	65.4	
Gl	0.31	32.3	105.5	23.7	4.1	
Gi	0.48	82.0	172.2	38.7	10.3	33.4
Gh	2.34	150.8	64.5	14.5	19.0	
Smooth microsomes	15.90	249.3	15.7	3.5	31.4	

Colchicine and ethanol were given 3 h and 90 min, respectively, before sacrifice as described in Materials and Methods. Control animals received ethanol only. The livers from three control and three colchicine-treated rats were respectively pooled for homogenization and subsequent fractionation. Galactosyl transferase assays were done on freshly prepared fractions and specific activity was nmol galactose transferred/60 min per mg of protein as described in Materials and Methods. Relative specific activity is the ratio of the enzyme specific activity in a subcellular fraction to that in the whole homogenate.

TABLE IV

The Relative Specific Activity and Recovery of Various Marker Enzymes in Total Golgi Elements from Colchicine-treated and Control Rats

	Relative sp act	
Enzymes	Control	Colchicine
5'-Nucleotidase	3.2	3.7
	(6.0)	(6.4)
Glucose 6-phosphatase	1.9	2.0
	(3.5)	(3.5)
Acid phosphatase	1.6	2.0
	(3.0)	(3.5)
Succinic dehydrogenase	ND	ND

Rats received colchicine and ethanol or ethanol alone (control) as described in Materials and Methods. Three colchicine-treated and three control animals were sacrificed and their livers were pooled respectively and homogenized. The individual Golgi fractions, isolated as described before (1), were pooled to yield a total Golgi fraction and assayed for the denoted enzymes as described in Materials and Methods. Relative specific activity was determined as noted in the legend of Table III. The numbers in parentheses refer to percent recovery. *ND*, none detected.

TABLE V

Effect of Colchicine, Lumicolchicine, and Vincristine on the Uptake of ^{125}I-oPRL into Hepatic Microsomal Subfractions

		^{125}I-oPRL uptake, % *control*				
Drug treatment		Gl	Gi	Gh	Smooth microsomes	Rough microsomes
μmol/100 g body wt						
Colchicine	0.25	34.8 ± 4.3	64.6 ± 5.5	74.3 ± 13.9	86.1 ± 6.6	83.3 ± 8.8
	1.25	18.4 ± 3.2	46.5 ± 5.0	111.9 ± 19.8	85.7 ± 14.1	75.7 ± 9.0
	12.50	19.3 ± 5.0	34.8 ± 6.4	84.1 ± 11.4	70.5 ± 8.9	74.7 ± 12.5
	25.00	23.3 ± 6.5	34.6 ± 4.7	100.4 ± 24.7	100.3 ± 4.1	68.0 ± 20.5
Lumicolchicine	1.25	151.3 ± 15.5	99.7 ± 7.8	104.5 ± 18.1	106.4 ± 14.4	107.9 ± 8.3
Vincristine	1.25	28.4 ± 4.7	40.0 ± 6.0	86.4 ± 13.4	63.1 ± 3.0	66.2 ± 7.8

Rats received colchicine, lumicolchicine, or vincristine sulfate and ethanol 3 h and 90 min, respectively, before the injection of ^{125}I-oPRL. Whole microsomes were prepared and subfractionated as described previously (5). The experimental procedure was as described in the legend of Table II. All values are the mean ±SE of three or four different studies in each of which three to six control and drug-treated animals were used.

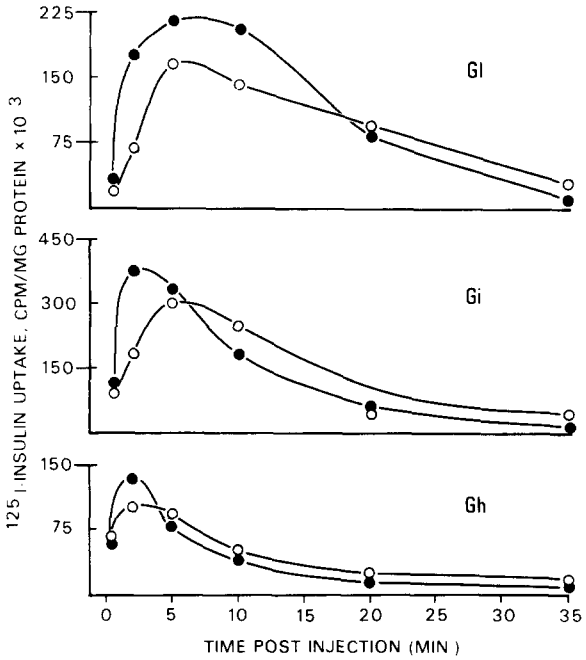

FIGURE 4 Effect of colchicine on the time course of ^{125}I-insulin uptake into Golgi fractions. Experimental protocol was the same as noted in the legend of Fig. 2. Each time point represents a fractionation on three pooled rat livers from both control (●) and colchicine-treated (○) rats.

Effect of Colchicine on the Time Course of Uptake of ^{125}I-oPRL

The time course of ^{125}I-oPRL uptake into Golgi fractions was compared in control and colchicine-treated rats (Fig. 2). Uptake into Gl and Gi was inhibited at times earlier than 45 min after ^{125}I-oPRL administration. Colchicine inhibited the uptake of ^{125}I-oPRL by 77 and 62% in Gl and Gi, respectively, at 15 min, the peak of ^{125}I-oPRL uptake in control rats. Colchicine treatment appeared to cause some accumulation of radiolabel in Gh at times >15 min after ^{125}I-oPRL injection (Fig. 2). At earlier times no such difference between control and colchicine treatment was noted. A second experiment performed in a manner identical to that depicted in Fig. 2 resulted in similar observations. Thus, the uptake of ^{125}I-oPRL was inhibited by 77% in Gl and 64% in Gi by 15 min postinjection. Again, no inhibition of uptake into Gh was observed and indeed some accumulation above control was noted at times 15 min postinjection. Colchicine treatment had minimal, if any, effect on the uptake of ^{125}I-oPRL into a purified plasma membrane fraction (Fig. 3).

Effect of Colchicine Treatment on the Hepatic Uptake of ^{125}I-Insulin

The effect of colchicine treatment (25 μmol/100 gm body wt) on the time course of uptake of ^{125}I-insulin into Golgi fractions was also studied (Fig. 4). Inhibition of uptake was observed in all three fractions at early times (≤10 min), with the inhibition being most consistent and marked at 2 min after ^{125}I-insulin. In general, the inhibitory effect on uptake into Gl and Gi was of much shorter duration than that observed with ^{125}I-oPRL. These observations were confirmed in a second, comparable study.

TABLE VI

Effect of Colchicine on the Extraction and Integrity of ^{125}I-oPRL Taken up by Rat Liver Subcellular Fractions

Time after injection	Treatment	Specific binding, % *uninjected* ^{125}I-oPRL			
		Gl	Gi	Gh	Smooth microsomes
min					
2	Control	104.2	83.3	100.2	113.4
		(100)	(84.7)	(75.9)	(60.1)
	+ Colchicine	122.9	57.7	109.4	162.7
		(100)	(93.6)	(78.9)	(56.9)
10	Control	81.7	73.4	155.4	137.6
		(83.4)	(91.5)	(78.9)	(57.8)
	+ Colchicine	77.1	79.0	111.4	126.4
		(98.0)	(90.6)	(81.1)	(46.5)
30	Control	106.4	74.4	126.2	134.7
		(86.7)	(83.9)	(98.1)	(60.9)
	+ Colchicine	94.7	89.7	145.4	160.0
		(100)	(79.1)	(93.0)	(49.1)

Rats received colchicine and ethanol or ethanol alone (control) and were injected with ^{125}I-oPRL and sacrificed, after which the subcellular fractions were obtained and extracted for their contained radioactivity as described in Materials and Methods. The extracted radioactivity was evaluated for integrity on the basis of its ability to rebind to female rat liver microsomes. Data are specific binding expressed as a percent of that of uninjected ^{125}I-oPRL. Values in parentheses are the percent of radioactivity extracted from each subcellular fraction.

Integrity of ^{125}I-oPRL Taken up by the Hepatic Subcellular Fractions

The effect of colchicine treatment (25 μmol/100 gm body wt) on the integrity of ^{125}I-oPRL as evaluated by rebinding of extracted radioactivity to female rat liver microsomes is depicted in Table VI. There was no systematic difference attributable to colchicine in either the extractability or integrity of the radiolabeled hormone. In both control and colchicine-treated rats, the radioactivity extracted from both smooth microsomes and Gh was intact at all times examined. In fact the extracted radioactivity showed superior binding to receptor than that seen with fresh uninjected ^{125}I-oPRL, suggesting a selective concentration of intact molecules in these fractions with a corresponding failure to take up less intact ("damaged") ^{125}I-oPRL.[1] In Gl and Gi, the degree of integrity was less than that of control ^{125}I-oPRL but remained remarkably level over the 2- to 30-min time range of this study. Radioactivity associated with smooth microsomes was substantially less extractable than that from other subcellular fractions. Radioactivity from rough microsomes was ≤35% the integrity of uninjected ^{125}I-oPRL at all times studied (data not shown) and accounted for only a small fraction of the total radioactivity taken up by microsomes (17).

Effect of Colchicine Treatment on Binding of ^{125}I-oPRL and ^{125}I-Insulin to Subcellular Fractions from Female Rat Liver

The effect of colchicine treatment on the specific binding of ^{125}I-oPRL and ^{125}I-insulin to subcellular fractions from rat liver is depicted in Table VII. Fractions obtained 1 and 3 h after colchicine treatment show levels of specific binding similar to

[1] Some of the ^{125}I-oPRL may have undergone chemical damage in the course of preparation rendering it less able to bind to receptors.

TABLE VII

Effect of Colchicine Treatment on the Specific Binding of ^{125}I-oPRL and ^{125}I-Insulin to Various Subcellular Fractions

Fraction (μg protein per tube)	Control		Percent Specific Binding in colchicine-treated			
			1 h		3 h	
	oPRL	Insulin	oPRL	Insulin	oPRL	Insulin
Microsomes (150)	7.2 ± 1.6	10.0 ± 2.5	8.9 ± 1.5	9.5 ± 0.8	11.9 ± 2.6	11.6 ± 1.7
Gl (50)	27.8 ± 2.7	7.8 ± 0.7	28.2 ± 2.6	7.4 ± 1.8	26.2 ± 9.5	5.8 ± 1.4
Gi (50)	24.6 ± 1.8	9.8 ± 0.6	42.7 ± 3.9	18.7 ± 0.8	31.9 ± 6.9	13.8 ± 0.5
Gh (50)	3.0 ± 1.8	9.2 ± 1.0	4.9 ± 3.7	8.1 ± 2.6	7.6 ± 1.2	9.7 ± 1.4
Plasmalemma (50)	8.1 ± 4.5	24.6 ± 15.2	8.1 ± 1.4	28.9 ± 1.7	4.4 ± 0.9	26.1 ± 0.1

Rats received colchicine for 1 or 3 h and ethanol for 90 min before sacrifice. Control animals received ethanol alone. Subcellular fractions were prepared and specific binding determined as described in Materials and Methods. Each value is the mean ± one-half the range of two different experiments in each of which three rat livers were pooled for colchicine-treated and control rats, respectively. Nonspecific binding was 6–12% for ^{125}I-oPRL and 4–9% for ^{125}I-insulin.

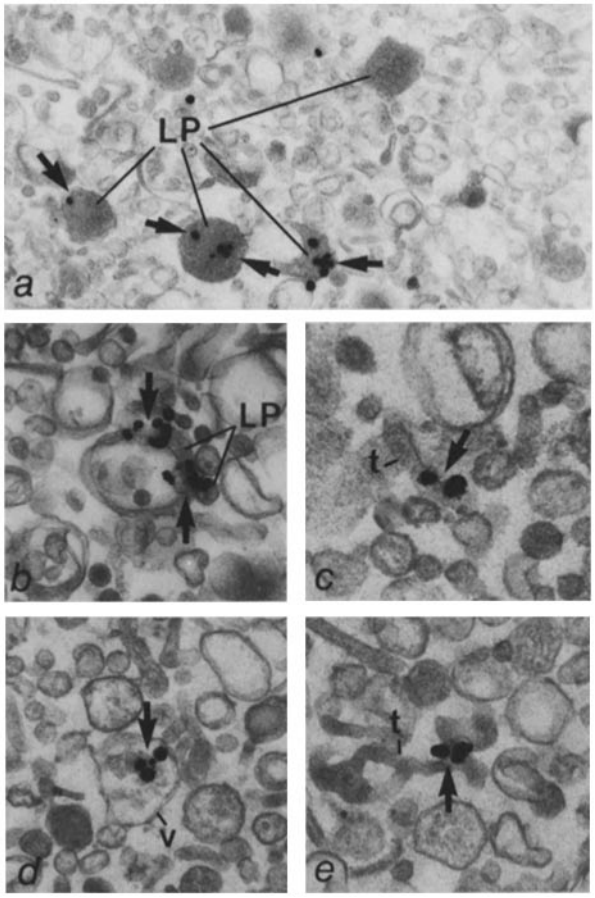

FIGURE 5 Electron microscope radioautographs of Gi (*a* and *b*) and Gh fraction (c–e) at 10 and 30 min, respectively, after injection of ^{125}I-oPRL into colchicine-pretreated rats. Silver grains (arrows) were found mainly (71%) over lipoprotein-filled (*LP*) vesicular and tubular structures in the Gi fraction (*a* and *b*) but over small tubular structures (*t*) in the Gh fraction (c and e), with fewer grains over vesicular (*v*) elements (*d*). Quantitative analysis carried out on the Gh fraction at 10 min and 30 min after hormone injection revealed 61% and 58.4%, respectively, of the total grains (369 and 243, respectively) over tubular structures with 18.4 and 28.4%, respectively, over vesicular structures. For control animals, 53% of the silver grains were observed over small empty tubular structures in the Gh fraction. *(a)* × 25,000; (*b–e*) × 38,000.

that of fractions from control animals. Two exceptions are the higher specific binding of ^{125}I-insulin in Gi from colchicine-treated compared with control animals, and the higher specific binding of ^{125}I-oPRL in Gi and Gh from animals 1 and 3 h, respectively, after colchicine treatment compared with control animals. In particular, colchicine treatment had no significant effect on ^{125}I-oPRL binding in Gl and Gi prepared 3 h after the drug was given when uptake into these fractions was greatly inhibited (Fig. 1).

Electron microscope radioautography was performed on Gh fractions isolated 10 and 30 min after the injection of ^{125}I-oPRL to control and colchicine-treated rats. We observed the same distribution of grains between flattened saccules and small vesicles from colchicine-treated and control rats (Fig. 5).

DISCUSSION

The data of Table I indicate that colchicine treatment increased the yield of the Gl fraction in a manner similar to ethanol pretreatment. The effect on the yield of the other subcellular fractions appeared to be minimal. This differs from the observation of Oda et al. (25) who showed that colchicine pretreatment increased the yield of all the hepatic Golgi fractions compared with the yield from both ethanol-treated and untreated rats. In agreement with Oda et al. is our observation of lower levels of galactosyl transferase in the Gl fraction from colchicine- vs. ethanol-treated rats (Table III). This does not seem to be due to contamination of the Gl fraction by other subcellular elements, since morphologic studies have attested to the similarity of hepatic Golgi fractions from colchicine-treated and control animals at both low (25) and high (39) doses of the drug. In addition, the enzymatic characteristics of fractions from colchicine-treated animals closely resembled those from controls, except for reduced levels of galactosyl transferase in the Golgi light fraction from the former. The recent demonstration of colchicine binding to Golgi fractions and its inhibition of galactosyl and sialyl transferase activities (23) raises the possibility that the observed reduction of galactosyl transferase activity in the Gl fraction after treatment with colchicine derives from a direct effect of Golgi-associated colchicine on this enzyme activity. Taken together, the above considerations suggest that colchicine treatment does not significantly affect the purity of Golgi fractions from rat liver.

With this consideration in mind we have studied the effect of colchicine on the pattern and extent of uptake of ^{125}I-labeled oPRL and insulin into Golgi subcellular fractions from rat liver. In previous studies we have shown that both labeled peptide hormones are taken up into rat liver by a receptor-mediated process and concentrated in a relatively intact form in Golgi fractions (17, 37). The time course of uptake was shown to peak first in Gh and then later in Gi and Gl fractions. Electron microscope radioautography of Gi and Gl fractions

showed that the labeled hormone was associated with bona fide very low density lipoprotein (VLDL)–filled Golgi vesicles, predominantly the membranes of these vesicles (17, 38). Our present studies have confirmed earlier observations and have indicated that colchicine treatment inhibits the concentrative internalization of labeled hormone into the Golgi vesicular fractions (i.e., Gl and Gi elements). This effect was more marked for ^{125}I-oPRL but was clearly seen for ^{125}I-insulin at 2 min postinjection. The effect on ^{125}I-insulin uptake rapidly disappeared thereafter in contrast to the more prolonged effect on ^{125}I-oPRL uptake. Colchicine treatment did not appear to inhibit uptake of ^{125}I-oPRL into the Gh fraction or association of ^{125}I-oPRL with the plasmalemma (Fig. 3) or with elements in the nuclear-mitochondrial cell fraction (Table II). In the Gh fraction, the majority of components containing labeled hormone have been shown to be structures of uncertain origin which, as we have suggested previously, may be largely endocytotic vesicles (37). Assuming this, we would tentatively suggest that colchicine has a negligible effect on the early phase of endocytosis. Rather, colchicine's major action would seem to be the inhibition of later processes leading to the transfer of ^{125}I-oPRL into Golgi vesicles. As noted in Table VI the radiolabeled material from subcellular fractions of colchicine-treated rats was comparable in integrity to that from subcellular fractions of control rats.

In previous studies it was shown that colchicine administration (0.5–25 μmol/100 g body wt) caused an inhibition of hepatic protein synthesis (40). Since cycloheximide treatment of rats resulted in a rapid decrease in hepatic lactogen receptors levels (18), we considered whether the inhibition of ^{125}I-oPRL uptake into Gl and Gi fractions reflected a depletion of lactogen receptor in these fractions secondary to an inhibition of protein synthesis. The data of Table VII indicate that colchicine treatment did not reduce lactogen or insulin binding levels at either 1 or 3 h after treatment in any of the subcellular fractions.

Previous studies have demonstrated that colchicine inhibits secretion in a number of tissues including the β-cell of the pancreas (20), thyroid (47), anterior pituitary (31), parotid (30), lacrimal glands (11), hypothalamus (15), and the liver (10). In this latter organ, colchicine has been shown to inhibit the release of VLDL (41), albumin, and other plasma proteins (39). The studies of Redman et al. (39) demonstrated that colchicine inhibited the discharge step leading to the accumulation of secretory proteins in all Golgi elements. If, as we have suggested above, the uptake of ^{125}I-oPRL into the Gh fraction reflects endocytosis, then the failure of colchicine to inhibit this uptake would indicate that receptor-mediated endocytosis is not coupled to exocytosis under the conditions of our experiment. This could reflect an influence of colchicine to uncouple endocytosis from exocytosis which may be otherwise coupled processes as previously suggested (28). In addition to inhibiting mitosis and secretion, colchicine has been observed to inhibit various cellular uptake processes. Endocytosis of horseradish peroxidase by chick chondrocytes (33) and rat peritoneal macrophages (44) was inhibited by colchicine. The drug was shown to inhibit amino acid and fucose uptake by transplanted Morris hepatoma cells (42). Effects distal to the uptake process as such have been reported. Thus, colchicine has been shown to inhibit epidermal growth factor (EGF) degradation by 3T3 cells without affecting EGF binding and uptake by these cells (9), and to inhibit both the endocytosis and subsequent degradation of sulfated proteoglycans by cultured human skin fibroblasts (45). Colchicine inhibited the degradation of chylomicron cholesteryl ester after uptake by hepatocytes in monolayer (13). The drug has been shown to decrease asialoglycoprotein uptake by hepatocytes and to impede the access of endocytosed material to lysosomes (19). Finally, it has been shown that colchicine inhibited insulin degradation by hepatocytes at doses of the drug which had no effect on insulin binding (43).

These latter observations suggest that the translocation of internalized substances to intracellular degrading sites has been retarded by colchicine. Since we have observed an apparent inhibition by colchicine of the translocation of internalized peptide hormone to Golgi vesicles, it might be argued that the endocytotic route leading to ultimate degradation passes through Golgi vesicular elements. In support of this are our observations of a chloroquine-induced retention of internalized ^{125}I-insulin in liver with marked accumulation of intact hormone in Golgi elements (36).

The mechanism of colchicine's inhibitory effect on intracellular translocation is not clear. Since colchicine binds to microtubules and promotes their disaggregation (26), one is tempted to suggest that such translocations may be regulated by microtubules and hence subject to inhibition by colchicine. In support of this is our observation that a second microtubular agent, vincristine, inhibited ^{125}I-oPRL uptake, whereas lumicolchicine, a colchicine congener inactive on microtubule aggregation (26), was without inhibitory action. On the other hand, Redman et al. (39) reported seeing microtubules in the Golgi region of hepatocytes from rats treated with doses of colchicine comparable to those used in our study. Consideration should also be given to a possible direct effect of colchicine on membranes and their topography. Colchicine impaired the movement of membrane particles in the alveolar membrane of *Tetrahymena pyriformis* (49) and bound to rat liver Golgi membranes and increased their fluidity (23). Such effects might explain the influence of colchicine in inhibiting the selective uptake of concanavalin A binding sites during phagocytosis by polymorphonuclear leukocytes (27), concanavalin A-induced agglutination of hepatoma cells (24), and the appearance of augmented amino acid transport capacity after partial hepatectomy (46), although an influence via microtubular effects cannot be excluded. It is possible that colchicine binds to intracellular membranes and alters their physical properties so as to reduce the intracellular translocation of peptide hormones. The extent of such inhibition may depend on subtle features of receptor structure and could then vary from one receptor to another. This might explain the differences we observed between colchicine's inhibition of prolactin and insulin uptake into Golgi vesicles.

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