Distribution of Tritiated Human Chorionic Gonadotropin in Superovulated Rat Ovary[1] (37030)

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Specific receptors of steroid hormones in target organs have been demonstrated (1–3) and the occurrence of similar receptors for protein and polypeptide hormones has been reported (4–9). These ovarian cells may contain receptors of gonadotropins. This contention is supported by the reports that LH (5) and HCG (6) labeled with radioiodine were found associated and interacted with subcellular fractions of cells of the corpora lutea. Although radioiodine-labeled hormones were used in autoradiographic studies, the resolution of the method can be improved by the use of tritium-labeled hormones as was demonstrated with tritiated polyadenyl insulin derivatives (9). In the present study tritium-labeled human chorionic gonadotropin ([^{3}H]HCG) was prepared and administered to superovulated rats. The distribution of the hormone in the ovary was analyzed by autoradiography.

Materials and Methods. To 21-day-old female Holtzman rats (50–62 g) 50 IU of PMS were administered followed 56 hr later by 30 IU of HCG. On the 7th day after the HCG injection, [^{3}H]HCG was administered to these rats via the tail vein under light ether anesthesia.

HCG-A (15,000 IU/mg) with LH-like activity was purified by a previously described method (10, 11) and labeled with tritium according to the procedure described by Vaitukaitis *et al.* (12). The prepared [^{3}H]HCG (sp. act. 1.9×10^5 cpm/μg) possessed approximately 75 to 80% of the biological activity.

Groups of 5 rats were sacrificed by decapitation at various time intervals after the administration of labeled HCG. As controls, several rats were injected with labeled HCG together with 100 μg of albumin or with 10 μg of native HCG. The ovaries, livers, and kidneys were removed, weighed and homogenized in Krebs Ringer Bicarbonate Buffer (pH 7.4). The homogenates were digested with Soluene (Packard Co.) and heated at 37° overnight. The radioactivity was measured in a Packard Tri-Carb Scintillation Spectrometer, Model 3003, as previously described (13).

Autoradiography. [^{3}H]HCG (2.0×10^6 cpm) was injected iv into immature pseudopregnant rats which were sacrificed two hr later. The ovaries were excised, fixed in Bouin's solution, embedded in paraffin and cut into five μ sections. The sections were coated with Kodak Nuclear Track Emulsion (NTB3) and exposed for 10–14 days at 4°. The films were developed in Kodak D19 solution and stained with hematoxylin and eosin.

Results. The ovary had the highest uptake of [^{3}H]HCG of any of the organs assayed (Table I). The incorporation by the ovary increased gradually and reached a maximal value at the second hour and declined thereafter. To show that the radioactivity incorporated was due to the uptake of [^{3}H]HCG and not due to a nonspecific uptake of a protein, [^{3}H]HCG was administered with 100 μg of albumin or 10 μg of HCG, and the uptake by the ovary was determined 2 hr later.

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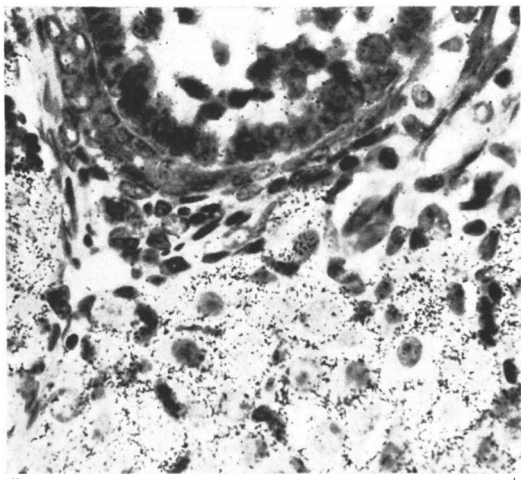

FIG. 1. Autoradiograph of ovary two hr after [^{3}H] HCG. Silver grains are concentrated around the cells of corpus luteum (×320).

The amounts were 4,100 ± 244 and 1,108 ± 40 cpm/100 mg, respectively, suggesting that HCG competed with [^{3}H]HCG for the receptor sites in the cells of the ovary while albumin did not. The amount of radioactivity incorporated by the kidney following the injection of [^{3}H]HCG was lower than the ovary, but greater than the liver. The high uptake by the kidney and the relatively low radioactivity in the serum suggests that the kidney may accumulate HCG for excretion into the urine. It should be noted, however, that the liver showed significant uptake, suggesting that HCG might be utilized or metabolized by this organ.

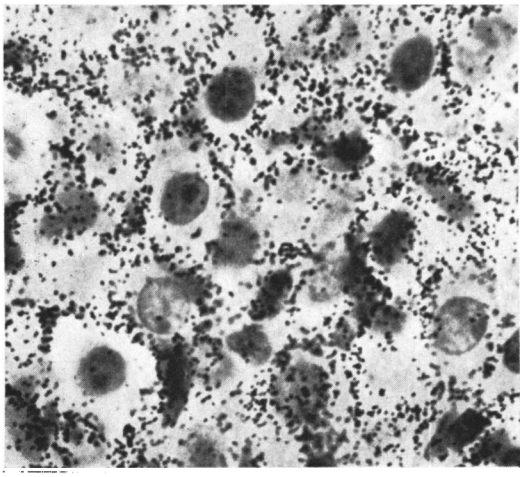

FIG. 2. Autoradiograph of corpus luteum. Silver grains are localized around the periphery of the cells (×600).

Ovarian autoradiography revealed the greatest accumulation of silver grains in the luteal cells while cells of the granulosa and theca layers contained relatively few grains (Fig. 1). The silver grains were found to be localized around the peripheral surface of the luteal cells (Fig. 2).

Discussion. Polypeptide hormones such as Insulin (14), TSH (15), ACTH (16), FSH (5), and LH (5, 17) are selectively concentrated by cells of the target tissues. The results of the present study showed that superovulated ovaries of immature rats had a high uptake of radioactive HCG. Similar observations were made by prior investigations utilizing tritiated HCG (12) and radioiodine-labeled HCG (18–22).

TABLE I. Uptake of Radioactivity in Rat Organs after Intravenous Injection of [^{3}H]HCG.[a]

Time (hr)	Ovary	Kidney	Liver	Serum cpm/0.1 ml
	(cpm/100 mg wet wt)			
0.25	2,330 ± 164[b]	766 ± 120	277 ± 44	563 ± 71
0.5	2,700 ± 280	755 ± 66	169 ± 84	419 ± 26
1	3,921 ± 380	793 ± 31	301 ± 40	262 ± 23
2	4,309 ± 456	619 ± 137	250 ± 8	160 ± 35
4	3,210 ± 460	367 ± 33	208 ± 73	92 ± 24
24	1,724 ± 222	355 ± 50	71 ± 21	31 ± 8

[a] [^{3}H]HCG (50,000 cpm) was administered iv to each rat weighing 50 to 62 g.

[b] Standard error of mean.

In the present study it was found that cells of corpora lutea incorporated [^{3}H]HCG, whereas cells of the theca layer and granulosa cell layer of the follicles of superovulated ovary did not. These results support the findings that luteal cells accumulated [^{125}I] HCG (19, 21). In addition, cells of the theca layer of developing follicles incorporated [^{125}I]HCG (21). The uptake by theca cells may reflect a variation in the sensitivity or ability of follicular cells to interact with HCG during the various stages of the ovarian cycle (21).

The autoradiographs obtained in the present study showed that silver grains of [^{3}H]

HCG were located mainly around the periphery of the luteal cells. Similar localization of other hormones were reported, *e.g.*, [^{3}H]Insulin was bound to the sarcolemmal membranes of striated and cardiac muscle (9). On the other hand, it was reported that [^{125}I] LH was incorporated into cells of the testis and suggested that LH can permeate into the cells and bind to a receptor site in the cytoplasm (8). Although there is a possibility that fragment(s) of gonadotropin might enter the luteal cells, the present autoradiographic study with [^{3}H]HCG suggests that the hormone interacts with some membranous structure and little, if any, penetrated into the cells. It should be pointed out that the results obtained with [^{125}I]HCG and [^{3}H] HCG might not be comparable because the radioiodine label is in the peptide moiety while the tritium label is in the carbohydrate moiety of HCG. It is conceivable though unlikely that HCG might be hydrolyzed and the peptide and carbohydrate moieties may be metabolized via different pathways by the ovarian cells.

Summary. Tritium-labeled human chorionic gonadotropin was administered to superovulated rats. The ovary showed the highest uptake of the organs assayed. The uptake reached a maximal level during the second hr after the administration and declined thereafter. The uptake of [^{3}H]HCG was inhibited when administered with native HCG, suggesting that HCG competed with [^{3}H] HCG.

Ovarian autoradiography showed that the silver grains were localized around the periphery of the luteal cells. The present results suggest that HCG probably acts on the cell membrane of the corpus luteum and only a minute amount, if any, enters the cell.

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