hCG: its pancreatic and duodenal receptors and in vivo electrolyte secretion in female rats

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Panesar, N. S., and C. W. Poon. hCG: its pancreatic and duodenal receptors and in vivo electrolyte secretion in female rats. Am. J. Physiol. 275 (Gastrointest. Liver Physiol. 38): G1430–G1436, 1998.—A state of fluid flux probably resulting from ion movement across the plasma membrane occurs during early pregnancy or trophoblastic disease, manifesting as emesis or hyperemesis gravidarum or hydatidiform moles. In emesis or hyperemesis gravidarum, excessive secretion induced by a humoral agent may trigger vomiting by distending and activating the gastrointestinal (GI) tract mechanoreceptors. This agent may be human chorionic gonadotropin (hCG). High-affinity hCG binding sites similar to those in the receptors. This agent may be human chorionic gonadotropin (hCG). High-affinity hCG binding sites similar to those in the ovary were found in the duodenum and pancreas of female (hCG). Administration of hCG to female rats in vivo caused a significant increase in HCO\(_3\)\(^{-}\) secretion from the duodenum and pancreas. Under nonreducing conditions, 150- and 170-kDa proteins were seen, through Western blot analysis, in the pancreas, duodenum, and ovary. Administration of hCG to female rats in vivo caused a significant increase in HCO\(_3\)\(^{-}\) and K\(^{+}\) secretion from the duodenum and pancreas. We hypothesize that during pregnancy hCG stimulates excessive secretion of electrolytes (and fluid) into the upper GI tract, which culminates in the vomiting during pregnancy. We believe the Cl\(^{-}\) conductance may be involved in halide ion efflux; ovarian hyperstimulation syndrome.

The physiological basis for cellular secretion is the efflux of ions across the cell membrane, accompanied by the passive diffusion of fluid, with the former process most likely mediated by humoral agents (17). The gastrointestinal (GI) tract is an active secretory organ, secreting a wide range of substances in response to various stimuli for a variety of reasons. Pathophysiological secretions from the lower GI tract may result in diarrhea, a classic example being the secretory diarrhea caused by cholera toxin. From the upper GI tract, pathophysiological secretions may manifest as vomiting. Vomiting, though, can be triggered by humoral agents acting on the chemoreceptors located in the brain (area postrema) and the GI tract or by the mechanoreceptors located in the GI tract that respond to the distension of the gut (1, 9). Mechanical triggering of vomiting through distension can logically occur as a result of excessive secretion of fluid and electrolytes into the gut lumen in response to a humoral agent.

The first trimester of human pregnancy is a period when ~50% of pregnant women suffer from vomiting and in 0.2% of all pregnancies hyperemesis gravidarum, severe vomiting, causes loss of fluid and electrolytes such as Na\(^{+}\), K\(^{+}\), and Cl\(^{-}\), resulting in weight loss and acidosis partly as a result of loss of alkaline intestinal juices (8). Vomiting during pregnancy coincides with high human chorionic gonadotropin (hCG) levels, which are implicated in the etiology (8, 15, 21), but the mechanism is uncertain. The ovarian hyperstimulation syndrome is also associated with severe fluid and electrolyte disturbances with the exacerbation of the condition by hCG (26), so there seems to be some link between hCG and fluid and electrolyte balance.

The placental hCG takes over the role of pituitary lutropin (LH) in maintaining corpus luteal steroidogenesis, which is essential for the integrity of the uterine linings and thus continuance of pregnancy. The effect of hCG on steroidogenesis is mediated via LH-chorionic gonadotropin receptor (LH-CGR)-coupled G protein activation (19) and a cascade of second messengers (25). Duchatelle and J offre (5) reported in 1990 that the gonadotropin stimulated Cl\(^{-}\) conductance from Leydig cells. The significance of the conductance in steroidogenesis is unknown, but Duchatelle and J offre (5) speculated about the possible role in steroid hormone secretion. We believe the Cl\(^{-}\) conductance may be involved in the activation of the adenylyl cyclase-cAMP pathway leading to steroidogenesis (Panesar, unpublished results). Furthermore, we have now demonstrated that hCG causes the efflux of halide ions from cells with functional LH-CGR (C. W. Poon, A. Hidaka, L. D. Kohn, and N. S. Panesar, unpublished data). This is not surprising, since a closely related glycoprotein hormone, thyrotropin (TSH), promotes the uptake of iodide into thyrocytes and then its efflux from the apical surface before the halide is organified and incorporated into thyroglobulin, a precursor of thyroid hormones (18). This action of TSH is also mimicked by hCG, which stimulates the thyroid gland during pregnancy (31, 33) and causes iodide uptake (12, 14). Therefore, the phenomenon of cellular ion efflux in response to hCG, perhaps accompanied by passive fluid diffusion, may be the basis for the fluid and electrolyte disturbances when hCG levels are high (24).

To substantiate our hypothesis that hCG can act on the upper GI tract and cause secretion of ions, we studied the hCG binding activity along the GI tract of a rat by means of a radioreceptor assay. Our preliminary study revealed substantial specific binding in the duo-
denum and pancreas. We now report the characterization of the hCG binding protein from the duodenum and pancreas and the localization of LH-CGR by means of immunohistochemical technique using antibodies directed against an epitope of LH-CGR. Finally, we have studied the effect of acute administration of hCG on the secretion of ions from the pancreas and duodenum of a female rat in vivo. On the basis of our findings, we propose a hypothesis outlining the mechanism by which hCG may induce pregnancy-associated vomiting.

MATERIALS AND METHODS

Materials

hCG (14,000 IU/mg for iodination and 3,000 IU/mg for perfusion study), Freund’s adjuvant (complete and incomplete), and cyanogen bromide-activated Sepharose 4B gel were obtained from Sigma Chemical (St. Louis, MO). [125I]hCG (sp act 17.2 mCi/µg) was obtained from Amersham International (Amersham, UK). The oligopeptides spanning amino acids 24–33 (GPRAGLARLS, termed N1) and 239–249 (SKEKFTSSLVA, termed N2) of rat LH-CGR (19) were synthesized and conjugated to diphtheria toxoid by A. Chiron (Clayton, Australia). All other chemicals were reagent grade compounds obtained from various commercial sources. Female Sprague-Dawley rats and New Zealand White rabbits were obtained from the animal house of our institution.

Methods

Immunization and production of affinity-purified polyclonal anti-LH-CGR antibodies. The immunization protocol for LH-CGR using the oligopeptides N1 and N2 conjugated to diphtheria toxoid was essentially as described previously for another antigen (23). For each of the oligopeptides, antisera was produced in a male (M) and a female (F) rabbit and termed N1M, N1F, N2M, and N2F. At the end of the immunization period, blood was collected from the ear vein and the serum separated and stored at −70°C until used. The free synthetic peptide was conjugated to cyanogen bromide-activated Sepharose 4B gel according to the manufacturer’s instructions. The antisera was incubated with the affinity gel for 2 h at room temperature, followed by sequential washing with PBS and then PBS containing 0.5 M NaCl. The antibody was eluted from the gel with 0.2 M Na2CO3 and used for immunohistochemistry and Western blotting studies.

Scatchard analysis and isoelectric focusing of hCG binding proteins in ovary, duodenum, and pancreas. The female Sprague-Dawley rats (250–300 g) at metestrus/diestrus, as determined by vaginal smears, were killed by cervical dislocation. The duodenum, liver, pancreas, and ovaries were removed. The tissues were homogenized with a Polytron, and the membrane proteins were extracted with 1% Triton X-100. Sixteen micrograms of Triton X-100-extracted membrane proteins from different rat organs.

Western blotting of LH-CGR in membrane protein extracts of different rat organs. The pancreas, duodenum, and ovaries from Sprague-Dawley rats (250–300 g) at metestrus/diestrus stage were homogenized with a Polytron, and the membrane proteins were extracted with 1% Triton X-100. Sixteen micrograms of Triton X-100-extracted membrane proteins from different organs were mixed with fivefold-concentrated loading buffer (0.0625 mol/l Tris-HCl, 2% SDS, 10% glycerol, and bromophenol blue) without or with 5% 2-mercaptoethanol and separated on 4–7.5% discontinuous SDS-PAGE (Laemmli buffer system). The proteins were electrophoresed on to nitrocellulose membrane (Bio-Rad) in 25 mmol/l Tris-HCl, 192 mmol/l glycine, and 20% methanol. The membranes were washed, blocked, and incubated with 20 µg/ml affinity-purified N2F antibodies overnight. The reaction was visualized using anti-rabbit immunoglobulin conjugated to horseradish peroxidase (DAKO, Glostrup, Denmark) and enhanced chemiluminescence Western blotting detection kit (Amersham International).

In vivo electrolyte secretion from pancreas and duodenum in response to hCG. Female Sprague-Dawley rats (200–250 g) at the metestrus/diestrus stage were anesthetized with pento-
barbital sodium (10 mg/100 g) and vagotomized. The bile duct was ligated and catheterized just above its entry into the pancreas. The duodenum was ligated at the pyloric end of the stomach and just below the entry of the common bile/pancreatic duct into the duodenum, catheterized below and above the respective ligation sites, and perfused with PBS containing 50 mM phosphate at 2 ml/h. The perfusate and the bile juice were collected at 4°C every hour. After one basal collection, hCG (to give a blood concentration of 0.2 µmol/l) in saline was injected into the femoral vein, and three further collections were made. Control animals received saline. Each group comprised seven animals. The electrolytes in the perfusate and bile juice were measured using ion-selective electrodes on the Dimension AR clinical chemistry analyzer (Wilmington, DE). Total bicarbonate was measured with Ciba-Corning 238 blood gas analyzer (Medfield, MA). Extreme care was taken in handling of animals during the experiment, and they were humanely killed at the end of the experiment without the animal regaining consciousness.

**Statistics.** Data were analyzed using various statistical analyses. The means ± SD of Kd values for hCG binding protein from the duodenum, pancreas, and ovary were calculated by grouping multiple curves.

**RESULTS**

**Duodenal, Pancreatic, and Ovarian hCG Binding Proteins: Scatchard Analysis and IEF**

The duodenum and pancreas demonstrated specific hCG binding during metestrus/diestrus. The amount of labeled hCG bound was $1.06 \times 10^{-13}$, $1.13 \times 10^{-15}$, and $5.49 \times 10^{-15}$ mol/mg of ovarian, duodenal, and pancreatic membrane proteins, respectively. Kd (mean ± SD) for LH-CGR from duodenum, pancreas, and ovary was $1.9 \pm 0.6$, $4.7 \pm 3.5$, and $0.11 \pm 0.02$ nM (Fig. 1), respectively. Preparative IEF followed by specific hCG binding study revealed a pl for the duodenal and ovarian proteins of 5.5 (Fig. 2).

**Specificity of Antisera for LH-CGR in Ovary and Localization of Receptor in GI Tract**

Of the four antisera developed against the two oligopeptides from LH-CGR, N1M and N2F gave the most intense immunohistochemical staining of the target tissues, namely the corpus luteum and the blood vessels (data not shown). Figure 3 shows the immunohistochemical staining of various organs with N1M antisera. Liver sections (negative control tissue) showed negative immunohistochemical staining for LH-CGR without and with the exclusion of LH-CGR antiserum (Fig. 3, A and B). In the positive control tissue, the ovary, the granulosa cells, and the theca interna were moderately stained, whereas the theca externa was not (Fig. 3C).

**Fig. 1.** Typical Scatchard plots for human chorionic gonadotropin (hCG) binding proteins from duodenum (A), pancreas (B), and ovary (C) and their respective dissociation constant (Kd) values.

**Fig. 2.** Typical isoelectric focusing profiles of rat duodenal and ovarian hCG binding proteins.
The corpus luteum and the blood vessels gave the most intense staining for LH-CGR (Fig. 3, C and E). When the LH-CGR antibody was omitted, there was no positive immunostaining (Fig. 3, D and F), thus confirming the specificity of LH-CGR immunostaining. In the pancreas, the zymogen granules of the acini and the apical side of the intralobular ducts were heavily immunostained (Fig. 3G). In the duodenum, the smooth
muscles were heavily stained (Fig. 3, K and L), and there was moderate immunostaining of Brunner's glands, the crypt cells, and the parasympathetic ganglion (Fig. 3, K and L). The blood vessels in the pancreas (Fig. 3L) and duodenum (Fig. 3L) also showed strong positive staining. Omission of LH-CGR antibody in duodenal and pancreatic sections again resulted in negative immunostaining (Fig. 3, H, J, and M).

Western Blotting of LH-CGR in Membrane Protein Extracts of Different Rat Organs

With N1F antiserum and using discontinuous SDS-PAGE under nonreducing condition, we detected dimer forms of LH-CGR; predominantly 150- and 170-kDa forms were detected in the pancreas, duodenum, and spleen, and in the control tissues, ovary and uterus (Fig. 4A). Under reducing conditions (Fig. 4B), the predominant forms of LH-CGR were 60 and 65 kDa, which were only detected in control tissues, together with a trace amount of 80-kDa protein. However, an 80-kDa LH-CGR was detected in duodenal extract, probably due to unmasking of epitopes after reduction.

In Vivo Electrolyte Secretion From Pancreas and Duodenum in Response to hCG

The hourly perfusate bicarbonate concentration decreased with time in both groups of animals. However, the animals injected with hCG showed a significantly (P < 0.015) retarded decrease of the secretion (i.e., increased secretion) at 1 h after hCG injection (Fig. 5A). K⁺ secretion was also significantly (P < 0.04) higher in the hCG-treated animals (Fig. 5B). There was no difference in the secretion of Na⁺ and Cl⁻ in the perfusate between the two groups (data not shown). None of the electrolytes in the biliary juice was significantly different between the groups (data not shown).

DISCUSSION

Of the four antisera developed against two oligopeptides spanning amino acids residues 24–33 (N1) and 239–249 (N2) of rat LH-CGR sequence, N1M and N2M strongly reacted with the known target for the receptor, the corpus luteum and the recently described target, the blood vessels (32). Both the duodenum and pancreas of a Sprague-Dawley rat demonstrated the presence of hCG binding activity which appeared to be similar to the ovarian LH-CGR. Because of variable lengths of various stages of an estrus cycle in a rat, it was not possible to study a substantial number of animals at each stage of the cycle, but from our limited preliminary data (data not shown), the
peak period for the occurrence of the duodenal and pancreatic receptor was metestrus/diestrous, a period when the ovarian LH-CGR reappears for luteinization (29). The slightly lower $K_a$ for GI tract receptors may have been due to inhibitory substances or enzymatic degradation, though protease inhibitors were included in the buffer. However, the duodenal and the ovarian hCG binding proteins shared a similar pi of 5.5. The presence of the high-affinity binding in the duodenum and the pancreas was further corroborated by the positive Western blotting and immunohistochemistry for LH-CGR in these two tissues. Moreover, the blood vessels in these two organs also showed positive LH-CGR immunohistochemistry, which is not unique since blood vessels in the uterus have also been reported to possess the receptor (32). This to our knowledge is the first report of LH-CGR in the GI tract, though it is known that pancreatic tumors can secrete hCG subunits (11). An hCG-like substance was reported in an unspecified region of the human small intestine, but Braunstein et al. (2) ruled against the presence being due to LH-CGR. LH-CGR, therefore, is turning out to be quite ubiquitous, having been reported in other gonadal tissues (see Ref. 22).

In the duodenum, LH-CGR immunoreactivity was confined to Brunner’s glands and smooth muscles. In the pancreas, the pancreatic acini and intralobular ducts showed strong LH-CGR immunoreactivity, along with the zymogen granules. The significance of the latter is uncertain but might represent the internalized LH-CGR. Brunner’s glands, the acini, and intralobular ducts are known to be involved in electrolyte secretion, especially of $\text{HCO}_3^-$. The acute administration of hCG in vivo caused increased secretion of $\text{HCO}_3^-$ and $K^+$ from the duodenum and pancreas. On the other hand, there was not uptake of the perfused fluid in both the control and hCG-treated animals. One reason for this may have been to compensate for the loss of bile juice and excessive insensible loss from the exposed region. The pancreas and duodenum secrete $\text{HCO}_3^-$ from ductal cells and mucosa, respectively, under the influence of gut hormones, e.g., secretin, released when acidic stomach contents enter the duodenum (3, 13). Our preliminary study in unvagotomized rats demonstrated bicarbonate secretion in response to secretin (data not shown). The pancreas has also been reported to possess steroid hormone receptors (30), and ovariectomy in rats decreased acinar cell zymogen content (28). Conversely, estrogens administered to dogs lowered protein, zinc, and $\text{HCO}_3^-$ in pancreatic juice (10). Because the pituitary gonadotropin secretion is under negative feedback control by sex hormones, the apparent secretory effects on pancreatic zymogen, protein, zinc, and $\text{HCO}_3^-$ in the aforementioned studies could be ascribed to gonadotropins, partly substantiated for bicarbonate in this study. Therefore, it appears that hCG can stimulate the secretion of ions from the duodenum and pancreas, and this action is most likely mediated via the LH-CGR present in these two organs.

The mechanism behind emesis/hyperemesis gravidarum is not known, but many factors have been implicated in the etiology, including neurotic, allergic, metabolic, and endocrine factors (8, 20). More recently, hyperolfaction has been added to the list of factors contributing to the etiology of hyperemesis gravidarum (6). Emesis/hyperemesis occurs predominantly during the first trimester when levels of hCG are high, and therefore hCG is thought to be involved in the etiology (8, 15, 21), but the mechanism is still unresolved. One mechanism by which hCG is thought to induce hyperemesis gravidarum is by stimulating the thyroid gland. However, we have consistently found that <30% of hyperemetic women have raised thyroid hormones (Ref. 31 and unpublished data), which does not support the thyroid connection. Although hCG may induce vomiting by acting on the area postrema chemoreceptors through the recently described LH-CGR (16), it is also possible that the primary site of action may be the upper GI tract, where hCG leads to copious secretion of electrolytes (and fluid) into the gut lumen. Our present findings would lend support to this contention. The active secretion of bicarbonate in response to hCG could then be the reason for hyperemetic women developing metabolic acidosis, which hitherto had been ascribed to the loss of alkaline intestinal juices (8). Similarly, excessive $K^+$ secretion could account for the development of hypokalemia in these patients. The presence of LH-CGR in the smooth muscle of the duodenum implies a role for the gonadotropin there too, which might be to maintain the quiescent state of the intestine and reduce gut motility, as is the case for the uterus (7).

With the tentative findings, we hypothesize that emesis/hyperemesis, which afflicts 50% of pregnant women to some degree and 0.2% more severely, is induced by hCG interacting with secretory cells in the upper GI tract through LH-CGR and stimulating copious secretion of electrolytes (and fluid), resulting in distension of the upper GI tract and the mechanoreceptors located therein, eventually triggering the vomit reflex. The fact that most pregnant women experience “morning sickness” could be related to the accumulation of secretions in the upper GI tract during the night and on arising the sudden distension of the GI tract triggers the vomit reflex.

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