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The Actions of Growth Hormone and Prolactin on Rat Hepatocytes are not Mediated by Changes in Cytoplasmic Ca^{2+}

Morgan Emtner,[1] Erik Gylfe[2] and Paul Roos[1]

[1]*Department of Biochemistry and* [2]*Department of Medical Cell Biology, University of Uppsala, Sweden*

ABSTRACT

In connection with measurement of the binding of growth hormone and prolactin to rat hepatocytes we investigated whether such binding is associated with changes in the cytoplasmic Ca^{2+} concentration. Whereas hepatocytes from male animals were found to have essentially only somatogenic receptors, lactogenic receptors were dominant in females. All hepatocyte preparations responded to epinephrine and vasopressin with transient peaks of cytoplasmic Ca^{2+}. However, no effects on cytoplasmic Ca^{2+} were obtained when cells from female or male animals were exposed to growth hormone or prolactin. We therefore conclude that signal transduction of the growth hormone and prolactin responses in the rat liver does not involve an early messenger function for Ca^{2+}.

INTRODUCTION

Growth hormone (GH) and prolactin (PRL) are two structurally related polypeptide hormones produced in the pituitary gland. The main action of PRL in female mammals is promotion of mammary development and lactation. GH is involved in somatic growth and its effect appears, at least in part, to be mediated by formation of insulin-like growth factor 1 in the liver. Both hormones exert their effects by interacting with membrane-bound receptors. Although the lactogenic and somatogenic receptors of rat liver bind rat PRL (rPRL) and rat GH (rGH), respectively, with high specificity, interpretation of our previous studies of the receptors were complicated by the fact that human GH (hGH) binds to both

types of receptors (9,14). Somatogenic receptors are most abundant in the livers of male rats, whereas lactogenic ones predominate in females (12). However, the physiological role of the lactogenic liver receptors is not obvious. It is not even clear whether rPRL is the natural ligand, since the affinity for rPRL seems to be considerably lower than the affinity between the hormone and the prolactin receptors of mammary cells. In fact, hGH binds better to the lactogenic receptors of rat liver than does rPRL (5,14).

Since the function of lactogenic liver receptors is unclear, it seems appropriate to look for effects of receptor binding on possible signal transduction pathways. Different experimental approaches have supported the involvement of intracellular calcium ions (Ca^{2+}_i) in the PRL response of mammary cells (13). In contrast, the somatogenic receptors of adipocytes, 3T3-F442A fibroblasts and possibly hepatocytes have been reported to become phosphorylated on tyrosine residues on exposure to GH (7).

We have now studied whether changes of Ca^{2+}_i take part in signal transduction of GH and PRL effects in rat hepatocytes. We shall show that male and female rat liver cells which respond appropriately to epinephrine and vasopressin by mobilizing Ca^{2+} from a common intracellular pool are unaffected by exposure to rPRL, rGH and hGH.

METHODS

Preparation of hepatocytes

Hepatocytes were prepared from Sprague-Dawley rats by collagenase perfusion and subsequent purification in a Percoll gradient as described previously (11). The viability of the cells exceeded 85% as judged by trypan blue exclusion.

Measurement of cytoplasmic calcium

For each experiment $5\text{-}6\text{x}10^6$ cells were washed in a HEPES-buffered medium (HEPES 10 mM, NaCl 136 mM, KCl 4.7 mM, $MgSO_4$ 0.65 mM, $CaCl_2$ 1.2 mM, bovine serum albumin 0.1% (w/v), pH 7.4). The cells were subsequently suspended in 5 ml of the washing medium and 15 µl of 1 mM fura-2 acetoxymethylester (Molecular Probes Inc. Eugene, OR) in dimethylsulfoxide were added. After incubation at 37°C for 40 min, the cells were spun down and washed in identical medium lacking the indicator. The cells were then suspended in 1 ml of the

medium and transferred to a 1 cm quartz cuvette placed in the thermostatically controlled (37°C) cuvette holder of a time-sharing multichannel spectrophotofluorometer (4). The suspension was stirred continuously from the top by a rotating plastic spiral not interfering with the light path. The 340/380 nm fluorescence excitation ratio, together with the fluorescence excitation at 380 nm, were continuously recorded on a strip chart recorder, and these signals were used to calculate Ca^{2+}_i as described previously (8). Hormones were added as 1000-fold concentrated aqueous solutions.

Binding assay

The cells were incubated in 200 µl phosphate-buffered saline (PBS; NaCl 137 mM, KCl 3.0 mM, Na_2HPO_4 8.4 mM, KH_2PO_4 1.6 mM, and bovine serum albumin 0.1% (w/v), pH 7.4) for 2 h at room temperature with [^{125}I]-hGH (30,000 cpm) in the absence and presence of excess unlabelled hormone. One ml of the PBS medium lacking tracer was then added and the cells were spun down at 1,500 g for 3 min. The supernatants were discarded and the radioactivities of the pellets were measured. Specific binding was calculated as the difference between cpm in tubes lacking and containing excess unlabelled hormone divided by the total radioactivity and is expressed as per cent.

An identical protocol was used for measuring specific binding of a monoclonal antibody against the female rat liver lactogenic receptor (6) utilizing [^{125}I]-labelled and unlabelled monoclonal antibody.

MATERIAL

The hormones hGH, human PRL (hPRL), rPRL and rGH were prepared as described previously (10,15,16,17). [^{125}I]-hGH was donated by Dr. C.-Å. Isacson and recombinant hGH (Genotropin^R) by Dr. Karin Fhölenhag (KabiVitrum, Stockholm, Sweden). Dr. U. Schröder (Lund, Sweden) supplied bGH (American Cyanamid, USA).

RESULTS

Binding of different hormones to the cells

Fig. 1 shows the specific binding of hGH to hepatocytes isolated from female and male rats as a function of cell numbers. It is

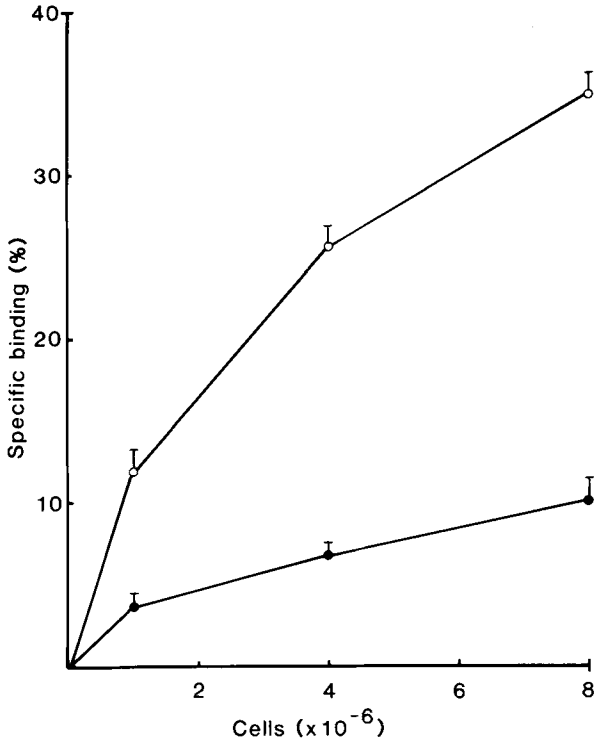

Fig. 1 Specific binding of human growth hormone as a function of the number of male (●) and female (○) rat hepatocytes. Different numbers of hepatocytes were incubated with [^{125}I]-labelled human growth hormone (30,000 cpm) for 2 hours in the absence or presence of excess unlabelled hormone. The cells were spun down and the specific binding calculated. The values are means ± S.E.M., n=5.

apparent that the female cells bound 3-4 times more hGH than did the male cells. The specificity of the binding was also different. Of the specifically bound labelled hGH 85.3 ± 3.5% was displaced from female hepatocytes by hPRL as compared to only 6.5 ± 1.7% from the male cells. The effect of excess bGH was the opposite, 13.3 ± 2.1% and 69.4 ± 1.7% of the labelled hGH being displaced from female and male cells, respectively (mean values ± S.E.M., n = 3). Moreover, only the hepatocytes from female rats bound a monoclonal antibody raised against lactogenic receptors of rat liver.

Influence of hormone-binding on cytoplasmic Ca^{2+}

The male (not shown) and female cells (Fig. 2) gave adequate responses to epinephrine and vasopressin, which are known to increase Ca^{2+}_i by mobilizing intracellular calcium in hepatocytes. There was no obvious sex dependence in the peaks of Ca^{2+}_i induced by these hormones. Epinephrine and vasopressin apparently mobi-

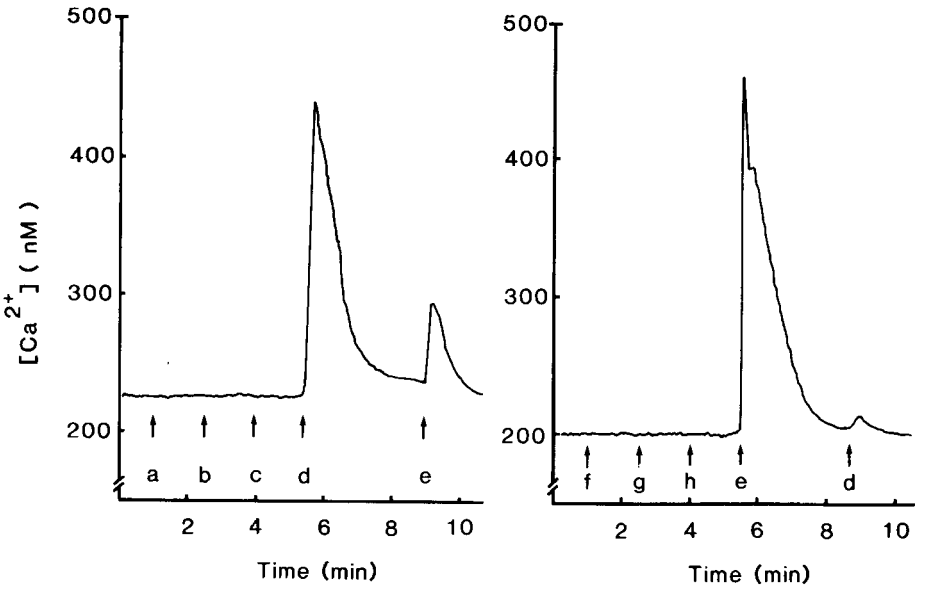

Figure 2. Effect of different hormones on the cytoplasmic Ca^{2+} of hepatocytes from female rats. Hepatocytes in suspension were loaded with fura-2 by a 40 min incubation in medium containing 3 µm fura-2-acetoxymethylester followed by rinsing in medium lacking the indicator. Additions of 0.01 (a), 0.2 (b), 1 µg/ml of rat prolactin (c), 10 µM vasopressin (d), 10 µm epinephrine (e), 0.01 (f), 0.1 (g) and 1 µg/ml human growth hormone (h) are shown by arrows.

lized Ca^{2+} from the same pool, since exposure to either hormone markedly decreased the subsequent response to the other. However, even very high concentrations (1 µg/ml) of rPRL or hGH failed to affect Ca^{2+}_i levels in the epinephrine and vasopressin responsive hepatocytes from male (not shown) or female (Fig. 2) rats. The same lack of response was observed in control experiments with rGH and recombinant hGH (not shown).

DISCUSSION

The physiological role of rat liver lactogenic receptors is not well understood. Although these receptors seem to have lower affinity for rPRL than do the corresponding receptors in mammary cells (5,14), there are reasons to believe that PRL is their natural ligand. The rat liver PRL receptors are thus only found in female animals, and northern blot analyses have shown that a cDNA probe

for the PRL receptor obtained from a rat liver library hybridizes with mRNA from a number of PRL receptor expressing rat cell types, including mammary cells (2). There is a strongly localized sequence identity between rat PRL and GH receptors (2). However, the latter receptors have a much longer cytoplasmic region. It is therefore conceivable that different mechanisms are involved in signal transduction.

The GH responses of different cell types have been proposed to involve activation of a tyrosine kinase activity purported to be part of the cytoplasmic receptor region (7). However, it has also been argued that this region lacks a potential phosphorylation site (2). In the case of PRL-signal transduction there are arguments favoring involvement of Ca^{2+}_i. The removal of extracellular Ca^{2+} or addition of an organic Ca^{2+}-channel blocker have thus been found to abolish several of the effects of PRL on cultured mammary tissue (1,3), and PRL-binding has been found to result in prompt stimulation of calcium accumulation (1).

In the present study it was possible to exclude the involvement of early changes in Ca^{2+}_i in the signal transduction of PRL and GH in rat liver. There were consequently no acute Ca^{2+}_i transients like those observed following addition of epinephrine or vasopressin, which are known to mobilize intracellular Ca^{2+} by inositol 1,4,5-trisphosphate mediated processes (18). Neither were there any slower sustained increases of Ca^{2+}_i, which would be expected if the hormones stimulated calcium influx.
Despite a certain homology at the level of mRNA (2), the PRL receptors of liver and mammary cells seem to have different hormone binding characteristics (5,14). The question arises whether PRL responses in liver and mammary cells are mediated by separate transduction pathways.

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Address for reprints: Paul Roos, Department of Biochemistry, Biomedical Center, P.O.Box 576, S-75123 UPPSALA, Sweden.