Calcium rather than cyclic AMP is an intracellular messenger of parathyroid hormone action on glycogen metabolism in isolated rat hepatocytes

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The synthetic 1–34 fragment of human parathyroid hormone (1–34hPTH) stimulated glucose production in isolated rat hepatocytes. The effect of 1–34hPTH was dose-dependent and 10^{10} M-1–34hPTH elicited the maximum glucose output, which was approx. 80 % of that by glucagon. Although 1–34hPTH induced a small increase in cyclic AMP production at concentrations higher than 10^{-9} M, 10^{-10} M-1–34hPTH induced the maximum glucose output without significant elevation of cyclic AMP. This is in contrast to the action of forskolin, which increased glucose output to the same extent as 10^{-10} M-1–34hPTH by causing a 2-fold elevation of cyclic AMP. In addition to increasing cyclic AMP, 1–34hPTH caused an increase in cytoplasmic free calcium concentration ($[Ca^{2+}]_c$). When the effect of 1–34hPTH on $[Ca^{2+}]_c$ was studied in aequorin-loaded cells, low concentrations of 1–34hPTH increased $[Ca^{2+}]_c$; the 1–34hPTH effect on $[Ca^{2+}]_c$ was detected at as low as 10^{-12} M and increased in a dose-dependent manner. 1–34hPTH increased $[Ca^{2+}]_c$ even in the presence of $1 \mu\text{M}$ extracellular calcium, suggesting that PTH mobilizes calcium from an intracellular pool. In line with these observations, 1–34hPTH increased the production of inositol triphosphate. These results suggest that: (1) PTH activates both cyclic AMP and calcium messenger systems and (2) PTH stimulates glycogenolysis mainly via the calcium messenger system.

INTRODUCTION

Parathyroid hormone (PTH) is a polypeptide containing 84 amino acids and regulates extracellular calcium concentration by acting mainly on kidney and bone, two major target organs of PTH. In addition to these classic target organs, PTH is shown to act on many other tissues. In the liver, specific binding of PTH has been demonstrated both *in vivo* and *in vitro* (D'Amour *et al.*, 1979; Rouleau *et al.*, 1986). Although PTH is degraded in Kupffer cells of liver (Segre *et al.*, 1981), the fact that binding sites for PTH are also found in liver parenchymal cells suggests a possibility that PTH may have an action on liver parenchymal cells. In agreement with these observations, Moxley *et al.* (1974) reported that a relatively high concentration of PTH (10^{-6} M) stimulates glucose output in rat liver parenchymal cells. In stimulating glucose output, PTH has been considered to act on the cyclic AMP messenger system. Canterbury *et al.* (1974) showed that PTH stimulates production of cyclic AMP in liver homogenates. Also, PTH increases cellular cyclic AMP content in isolated hepatocytes (Moxley *et al.*, 1974). Since the role of cyclic AMP as a second messenger of glucagon-induced glycogenolysis is established, a postulate that PTH stimulates glycogenolysis by increasing cyclic AMP seems reasonable. Nevertheless, when the effect of PTH on the production of cyclic AMP is compared with that of glucagon, PTH is considerably less potent than glucagon (Moxley *et al.*, 1974). This observation raises a possibility that PTH may generate

an additional intracellular messenger. In this regard, it has been shown that PTH increases cytoplasmic free calcium concentration, $[Ca^{2+}]_c$, in other target cells, for example, in cells derived from kidney and bone (Hruska *et al.*, 1986; Goligorsky *et al.*, 1986; Yamaguchi *et al.*, 1987).

In the present study, we evaluated the messenger role of cyclic AMP in the action of PTH on glycogenolysis. We also examined whether PTH affects cellular calcium metabolism in rat hepatocytes. The results indicate that PTH increases both cyclic AMP and $[Ca^{2+}]_c$. Our results further suggest that PTH exerts its glycogenolytic action by acting mainly on the calcium messenger system in hepatocytes.

EXPERIMENTAL

Preparation of liver parenchymal cells

Parenchymal liver cells were prepared by the method of Berry & Friend (1969). Cells were suspended in modified Hanks' solution containing (in mM): NaCl, 137; KCl, 3.5; KH_2PO_4 , 0.44; NaHCO_3 , 4.2; Na_2HPO_4 , 0.33; CaCl_2 , 1.0; Hepes/NaOH (pH 7.4), 20; equilibrated with O_2 gas.

Measurement of cytoplasmic free calcium concentration by aequorin

Changes in cytoplasmic free calcium concentration, $[Ca^{2+}]_c$, were monitored by measuring aequorin biolumin-

Abbreviations used: PTH, parathyroid hormone; *n-mhPTH*, the *n-m* fragment of human PTH; $[Ca^{2+}]_c$, cytoplasmic free calcium concentration; PBS, phosphate-buffered saline (for composition see text).

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escence. Aequorin was loaded into hepatocytes by making plasma membrane reversibly permeable by the method of Borle *et al.* (1986). In short, the cells were briefly washed twice with ice-cold Ca^{2+} -free phosphate-buffered saline (PBS) (in mM: NaCl, 135; KCl, 4; KH_2PO_4 , 0.51; glucose, 11; pH 7.4) and once with PBS containing 1 mM-EGTA for 30 s. Between each wash, the cells were centrifuged at 50 *g* for 2 min and the supernatant was discarded. An aliquot of packed cells was then suspended and incubated for 10 min at 4 °C in 0.5 ml of a buffered salt solution containing 10 μg of aequorin (in mM: NaCl, 140; Hepes, 3; pH 7.4). Finally, the cells were centrifuged at 200 *g* for 30 s. The supernatant was discarded. Aequorin-loaded cells were incubated in modified Hanks' solution. Aequorin signal was measured as described previously (Mine *et al.*, 1986, 1987). A cell suspension containing 10^7 aequorin-loaded cells in 1 ml was applied into a cuvette and incubated at 37 °C under constant stirring. $[\text{Ca}^{2+}]_i$ in unstimulated cells was estimated as described by Snowdowne & Borle (1984) assuming an intracellular magnesium concentration of 1 mM. $[\text{Ca}^{2+}]_i$ in stimulated cells was not calibrated since distribution of calcium in stimulated cells is not known at present. Aequorin-loaded hepatocytes responded to glucagon normally in terms of glycogenolysis (Mine *et al.*, 1986), and more than 95% of aequorin-loaded cells excluded Trypan Blue. In some experiments, we loaded aequorin by the method described by Morgan & Morgan (1982) and results are essentially similar. When extracellular calcium concentration was reduced to 1 μM , Ca^{2+} -EGTA buffer was employed (Mine *et al.*, 1986).

Measurement of cyclic AMP production

A portion of cell suspension (4×10^6 cells/ml) was incubated at 37 °C in modified Hanks' solution oxygenated with 100% O_2 with various agents for 2 min in the presence of 0.5 mM-3-isobutyl-1-methylxanthine. Trichloroacetic acid was then added to the samples to stop the reaction. After the removal of trichloroacetic acid by washing with diethyl ether, cyclic AMP was measured after succinylation by using a radioimmunoassay kit. Protein was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard.

Measurement of inositol trisphosphate production

Hepatocytes (10^7 cells/ml) were labelled with $[\text{H}^3]$ -inositol by incubating cells with 10 μCi of $[\text{H}^3]$ -inositol/ml for 120 min. After the labelling period, cells were washed and incubated at 37 °C in modified Hanks' solution containing 10 mM-LiCl for 10 min. Cells were stimulated for 20 s with 1-34hPTH. The reaction was stopped by adding perchloric acid (final concentration 10%). Cells were homogenized by repetitive aspirations through a 26 gauge needle and centrifuged at 800 *g* for 5 min. The supernatant was taken, neutralized with 5 M-KOH and applied onto an anion-exchange column. Inositol phosphates were separated as described by Berridge *et al.* (1983). Isomers of inositol trisphosphate were not measured.

Determination of glucose output

Isolated hepatocytes (4×10^6 cells/ml) were incubated at 37 °C in modified Hanks' solution oxygenated with 100% O_2 . Glucose concentration in the incubation medium after 11 min was determined according to the

method of Corvera *et al.* (1984). All the results were expressed per mg of protein for 11 min. Protein was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard. Glucose in the incubation medium was found to reflect mainly the activity of glycogenolysis in these conditions (Corvera *et al.*, 1984).

Materials

1-34hPTH and synthetic 3-34hPTH, an antagonist of PTH, were kindly donated by Toyojoko Co. (Tokyo, Japan). These agents were dissolved in 0.1% acetic acid containing 0.1% bovine serum albumin. Aequorin was purchased from Dr. J. R. Blinks (Mayo Foundation, Rochester, MN, U.S.A.). $[\text{H}^3]$ Inositol was obtained from Amersham International and radioimmunoassay kits for cyclic AMP were obtained from Yamasa (Tokyo, Japan).

RESULTS

Glycogenolytic action of 1-34hPTH in isolated hepatocytes

It has been demonstrated that 1-34hPTH is as potent as 1-84hPTH in rat hepatocytes (Moxley *et al.*, 1974). In the present study, we used this analogue of PTH instead of 1-84hPTH.

In a batch incubation system using isolated hepatocytes, 1-34hPTH stimulated glycogenolysis. The increase in glucose output induced by 1-34hPTH was detected at 1 min and the glucose output increased linearly up to 11 min (results not shown). When glucose output was measured at 11 min, 1-34hPTH stimulated glycogenolysis in a dose-dependent manner (Fig. 1). The action of 1-34hPTH was detected at 10^{-11} M and the maximum effect was obtained at 10^{-10} M (Fig. 1). At higher concentrations, the glycogenolytic action of 1-34hPTH is rather smaller. The magnitude of the maximum effect of 1-34hPTH was approx. 80% of that of glucagon.

Fig. 1. Dose-response relationship for 1-34hPTH-induced glucose output in isolated hepatocytes

Aliquots of hepatocyte suspension containing 4×10^6 cells in 1 ml were incubated for 11 min in the presence of varying concentrations of 1-34hPTH. Open column indicates glucose output induced by 5×10^{-9} M-glucagon under a similar condition. Values are the means $\pm$ S.E.M. for six determinations.

Fig. 2. Dose-response relationship for 1-34hPTH- and glucagon-induced cyclic AMP production in isolated hepatocytes

Aliquots of hepatocyte suspension containing 4×10^6 cells in 1 ml were incubated for 2 min with various concentrations of 1-34hPTH (●) or glucagon (○) in the presence of 0.5 mM-3-isobutyl-1-methylxanthine. The reaction was stopped by adding trichloroacetic acid and cyclic AMP was measured as described in the Experimental section. Values are the means $\pm$ S.E.M. for six determinations.

Effect of 1-34hPTH on cyclic AMP in hepatocytes

In the next set of experiments, changes in cyclic AMP content induced by 1-34hPTH were investigated. When the time course of changes in cyclic AMP in response to 1-34hPTH was measured, the maximum effect was obtained at 2 min after the addition of 1-34hPTH (results not shown). We therefore measured cyclic AMP at 2 min after the addition of 1-34hPTH. As shown in Fig. 2, 1-34hPTH had only a small stimulatory effect on cyclic AMP. At 10^{-10} M, 1-34hPTH did not increase cyclic AMP. 1-34hPTH-induced cyclic AMP production was detected at 10^{-9} M, at which concentration 1-34hPTH increased cyclic AMP by 70%. Higher concentrations of 1-34hPTH had no greater effect. In contrast, 5×10^{-9} M-glucagon, which causes the maximum glycogenolytic effect, increased cyclic AMP by 650%. Thus, 1-34hPTH is far less potent than glucagon in stimulating cyclic AMP production.

Effect of forskolin on cyclic AMP production and glucose output

Forskolin is a direct activator of adenylate cyclase. Fig. 3 depicts the dose-response relationship of forskolin-induced cyclic AMP production in hepatocytes. Forskolin action was detected at 10^{-5} M and cyclic AMP production was increased as a function of forskolin concentration. Fig. 3 also demonstrates the effect of forskolin on glucose output. At 10^{-5} M, forskolin elicited a 2-fold increase in glucose output and a 1.6-fold increase in cyclic AMP production. The dose-response relation-

Fig. 3. Dose-response relationship for forskolin-induced cyclic AMP production and glucose output in isolated hepatocytes

Aliquots of hepatocyte suspension containing 4×10^6 cells in 1 ml were incubated for 11 min in the presence of varying concentrations of forskolin, and the glucose concentration (○) in the incubation medium was then determined. For measurement of cyclic AMP (●), aliquots of cell suspension (4×10^6 cells/ml) were incubated for 2 min in the presence of 0.5 mM-3-isobutyl-1-methylxanthine and various concentrations of forskolin. Cyclic AMP was measured as described in the Experimental section. Values are the means $\pm$ S.E.M. for six determinations.

ship of forskolin-induced glucose output correlated well with that of forskolin-induced cyclic AMP production.

Action of 1-34hPTH on cytoplasmic free calcium

The effect of 1-34hPTH on $[Ca^{2+}]_c$ was assessed by measuring changes in aequorin luminescence using aequorin-loaded hepatocytes. Fig. 4 depicts the effects of various concentrations of 1-34hPTH on changes in $[Ca^{2+}]_c$. Basal concentration of $[Ca^{2+}]_c$ was 183 nM, assuming an intracellular magnesium concentration of 1 mM and even distribution of calcium inside the cell. The effect of 1-34hPTH on $[Ca^{2+}]_c$ was detected at 10^{-12} M and magnitude of the response was increased in a dose-dependent manner. As demonstrated in Fig. 5(a), addition of 10^{-10} M-1-34hPTH resulted in a prompt increase in aequorin bioluminescence. The action of 1-34hPTH on $[Ca^{2+}]_c$ was transient: $[Ca^{2+}]_c$ returned to a value close to the resting level within 60 s. To identify the source of calcium mobilized by PTH, aequorin-loaded hepatocytes were incubated in modified Hanks' solution containing 1 μ M-calcium. In medium containing this concentration of calcium, basal as well as stimulated calcium influx is negligible (Mauger *et al.*, 1984). When cells were stimulated by 10^{-10} M-1-34hPTH in the presence of 1 μ M extracellular calcium, $[Ca^{2+}]_c$ increased to a level which was smaller than that observed in cells incubated in modified Hanks' solution containing 1.0 mM-calcium.

Effect of an analogue of PTH on 1-34hPTH-induced increase in glucose output and cytoplasmic free calcium concentration

To examine whether the increase in $[Ca^{2+}]_c$ induced by PTH is exerted via the PTH receptor, we tested the effect of a competitive antagonist of parathyroid hormone, 3-34hPTH (Moyle *et al.*, 1973). 3-34hPTH was shown

Fig. 4. Effect of 1-34hPTH on cytoplasmic free calcium concentration

Aequorin-loaded hepatocytes were stimulated by various concentrations of 1-34hPTH in the presence of 1 mM extracellular calcium as indicated by the arrows. Results are representative of at least eight experiments.

Fig. 5. Effect of 1-34hPTH on cytoplasmic free calcium concentration in the presence of 1 μ M extracellular calcium

Aequorin-loaded hepatocytes were stimulated by 10^{-10} M 1-34hPTH in the presence of 1 mM (a) or 1 μ M (b) extracellular calcium. When extracellular calcium was reduced to 1 μ M, the concentration of calcium was fixed by using Ca^{2+} -EGTA buffer. Results are representative of four experiments.

to have little biological action by itself but to block the action of PTH when added at 1000-fold higher concentration. In hepatocytes, 3-34hPTH did not cause glucose output by itself (Table 1). However, when 3-34hPTH

Table 1. Effect of 3-34hPTH on 1-34hPTH-induced glucose output

Hepatocytes were incubated with or without 10^{-10} M 1-34hPTH in the presence or absence of 10^{-7} M 3-34hPTH for 11 min. Glucose output was determined as described in the Experimental section. Values are means $\pm$ S.E.M. for six determinations.

Addition	Glucose output (nmol/11 min per mg of protein)
None	4.14 ± 0.86
3-34hPTH (10^{-7} M)	4.30 ± 0.66
1-34hPTH (10^{-10} M)	14.85 ± 0.60
1-34hPTH (10^{-10} M) + 3-34hPTH (10^{-7} M)	4.88 ± 0.61

Fig. 6. Effect of 3-34hPTH on 1-34hPTH-induced changes in cytoplasmic free calcium concentration

Aequorin-loaded cells were stimulated by 10^{-10} M 1-34hPTH in the absence (a) or presence (b) of 10^{-7} M 3-34hPTH, an antagonist of 1-34hPTH, as indicated by the arrow. In (b), cells were then stimulated with 10^{-7} M 1-34hPTH, as indicated by the arrow. Results are representative of three experiments.

was added concomitantly with 1-34hPTH, the action of 1-34hPTH on glucose output was completely blocked. Thus, 3-34hPTH is capable of blocking the action of 1-34hPTH in hepatocytes. In the next set of experiments, we examined the effect of 3-34hPTH on 1-34hPTH-induced increase in $[\text{Ca}^{2+}]_c$. As demonstrated in Fig. 6(a), addition of 10^{-10} M 1-34hPTH resulted in a prompt increase in aequorin bioluminescence. Addition of 10^{-7} M 3-34hPTH did not cause any change in $[\text{Ca}^{2+}]_c$ by itself but inhibited the elevation of $[\text{Ca}^{2+}]_c$ induced by subsequent 1-34hPTH (10^{-10} M). This inhibition was reversed by an addition of higher concentration of 1-34hPTH (10^{-7} M).

Effect of 1-34hPTH on inositol phosphate production

The effect of 1-34hPTH on inositol phosphate production was examined using [^3H]inositol-labelled cells. When time course of changes in [^3H]inositol trisphosphate was measured, the effect was detected at 10 s and was maximal at 20 s (results not shown). As shown in Table 2, when changes in [^3H]inositol phosphates were

Table 2. Effect of 1-34hPTH on production of inositol phosphates

[³H]Inositol-labelled hepatocytes (10⁷ cells/ml) were incubated for 20 s with or without 10⁻¹⁰ M-1-34hPTH. Inositol phosphates were determined as described in the Experimental section. Values are means ± S.E.M. of four determinations.

Addition	Inositol phosphates (d.p.m.)		
	Inositol monophosphate	Inositol bisphosphate	Inositol trisphosphate
None	26250 ± 1909	1562 ± 760	445 ± 78
1-34hPTH	30541 ± 3728	2887 ± 996	1121 ± 248

Fig. 7. Dose-response relationship for 1-34hPTH-induced inositol phosphates production in isolated hepatocytes

Hepatocytes (10⁷ cells/ml) were labelled with [³H]inositol by incubating cells with 10 μ Ci of [³H]inositol/ml for 120 min. After washing, cells were stimulated for 20 s with various concentrations of 1-34hPTH in the presence of 10 mM-LiCl. Inositol phosphates were determined as described in the Experimental section. Values are the means ± S.E.M. for four determinations.

measured at 20 s, 10⁻¹⁰ M-1-34hPTH induced a 2.5-fold increase in [³H]inositol trisphosphate. 1-34hPTH also increased the content of [³H]inositol bisphosphate and monophosphate. The dose-response relationship for 1-34hPTH-induced inositol phosphate production reveals that the effect of 1-34hPTH was detected at 10⁻¹¹ M. 1-34hPTH action increased in a dose-dependent manner and was the maximum at 10⁻⁹ M (Fig. 7). At 10⁻⁸ M, 1-34hPTH-induced inositol phosphate production was greatly reduced.

DISCUSSION

In the present study, we have studied the effect of PTH on hepatic glycogenolysis using 1-34hPTH. Our results confirm the report by Moxley *et al.* (1974) that PTH

stimulates glucose output in hepatocytes. We extend their observations by showing that much lower concentrations of PTH (10⁻¹¹–10⁻⁹ M) stimulate glucose output in isolated rat hepatocytes. The concentration of PTH to induce glycogenolysis is close to physiological concentrations found in peripheral circulation. Given the fact that liver is the site where PTH is degraded (Segre *et al.*, 1981), it may be possible that PTH affects glycogen metabolism *in vivo* at least under some conditions.

Previous studies have shown that PTH activates adenylate cyclase in hepatocytes and it has been considered that cyclic AMP acts as an intracellular messenger of PTH in the liver (Moxley *et al.*, 1974). To evaluate this postulate, we have compared dose-response relationships for PTH-induced cyclic AMP production and glucose output. The results, however, argue against the postulate. As shown in Figs. 1 and 2, 1-34hPTH-induced cyclic AMP production dissociates from glucose output in two ways. First, compared with 1-34hPTH-induced glucose output, 1-34hPTH increases cyclic AMP only at higher concentrations. Thus, glucose output is maximal at 10⁻¹⁰ M-1-34hPTH whereas 1-34hPTH stimulates cyclic AMP production at concentrations higher than 10⁻⁹ M. Second, even at higher concentrations, 1-34hPTH increases cyclic AMP to a small extent. Although the magnitude of PTH-induced glucose output is comparable with the effect of glucagon, the PTH-induced cyclic AMP response observed at high concentrations is approx. 20% of that elicited by glucagon. The most striking observation is that 10⁻¹⁰ M-1-34hPTH elicits the maximal effect on glucose output without significant increase in cyclic AMP production. Similar dissociations have been often pointed out when agonist-induced production of cyclic AMP is compared with its final effect in other cell systems (Moyle *et al.*, 1973; Dufau *et al.*, 1973; Percherlet *et al.*, 1978). In general, two approaches have been taken to solve the issue. One is based on an idea that cyclic AMP is the sole messenger of the agonist action. It is proposed that only a small, sometimes negligible, increase in cyclic AMP is enough to activate cyclic AMP-dependent protein kinase, and thereby, to induce the final response (Litvin *et al.*, 1984). However, this possibility is quite unlikely at least in the case of PTH action in hepatocytes because of the observation shown in Fig. 3. When glucose output is stimulated by forskolin, a direct activator of adenylate cyclase, there are parallel increases in glucose output and cyclic AMP. A detectable increase in cyclic AMP is, therefore, required to stimulate glycogenolysis. These results exclude the possibility that an undetectable small increase in cyclic AMP mediates PTH action on glucose output. An alternative approach is to identify a different messenger which acts independently or synergistically with cyclic AMP. In the case of adrenocorticotropin, for example, such a messenger is calcium (Kojima *et al.*, 1985). Since PTH affects cellular calcium metabolism in other cells (Hruska *et al.*, 1986; Goligorsky *et al.*, 1986; Yamaguchi *et al.*, 1987), it is reasonable to examine that PTH affects cellular calcium metabolism. Consistent with these considerations, PTH increases [Ca²⁺]_i at reasonably low concentrations. Considering the fact that phosphorylase *b* kinase is activated by calcium, calcium may play a messenger role in PTH-induced glycogenolysis. To increase [Ca²⁺]_i, PTH causes a release of calcium from an intracellular pool and this release reaction is presumably brought about by an increase in inositol trisphosphate. Hence, low concen-

trations of PTH activate the calcium messenger system.

As shown in Table 1 and Fig. 6, the action of 1-34hPTH is inhibited by 3-34hPTH, a competitive inhibitor of 1-34hPTH. This observation suggests that PTH activates the calcium messenger system by a receptor-mediated mechanism. Thus, both cyclic AMP formation (Moxley *et al.*, 1974) and inositol phosphate formation are events regulated by receptor occupancy. The fact that markedly different concentrations of PTH induce changes in cyclic AMP and $[Ca^{2+}]_c$ raises a possibility that there are two different classes of PTH receptor in hepatocytes. Further studies are needed to verify this issue.

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REFERENCES

- Berridge, M. J., Dawson R. M. C., Downes, C. P., Heslop, J. P. & Irvine, R. F. (1983) *Biochem. J.* **212**, 473-482
- Berry, M. N. & Friend, D. S. (1969) *J. Cell. Biol.* **43**, 506-520
- Borle, A. B., Freudrich, C. C. & Snowdowne, K. W. (1986) *Am. J. Physiol.* **251**, C323-C326
- Canterbury, J. M., Levy, G., Ruiz, E. & Reiss, E. (1974) *Proc. Soc. Exp. Biol. Med.* **147**, 366-370
- Corvera, S., Huerta-Bahena, J., Pelton, J. T., Hruby, J. V., Trivedi, D. & Garcia-Sainz, J. A. (1984) *Biochim. Biophys. Acta* **804**, 434-441
- D'Amour, P., Segre, G. V., Roth, S. I. & Potts, J. T., Jr. (1979) *J. Clin. Invest.* **63**, 89-98
- Dufau, M. L., Watanabe, K. & Catt, K. J. (1973) *Endocrinology (Baltimore)* **92**, 6-11
- Goligorsky, M. S., Loftus, D. J. & Hruska, K. A. (1986) *Am. J. Physiol.* **251**, F938-F944
- Hruska, K. A., Goligorsky, M. S., Scoble, J., Tsutsumi, M., Westbrook, S. & Moskowitz, D. (1986) *Am. J. Physiol.* **251**, F188-F198
- Kojima, I., Kojima, K. & Rasmussen, H. (1985) *J. Biol. Chem.* **260**, 4248-4256
- Litvin, Y., PasMantier, R., Fleischer, N. & Erlichman, J. (1984) *J. Biol. Chem.* **259**, 10296-10302
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265-275
- Mauger, J. P., Poggioli, J., Guesdon, F. & Claret, M. (1984) *Biochem. J.* **221**, 121-127
- Mine, T., Kojima, I., Kimura, S. & Ogata, E. (1986) *Biochem. Biophys. Res. Commun.* **140**, 107-113
- Mine, T., Kojima, I., Kimura, S. & Ogata, E. (1987) *Biochim. Biophys. Acta* **927**, 229-234
- Morgan, J. P. & Morgan, K. G. (1982) *Pflügers Arch.* **395**, 75-77
- Moxley, M. A., Bell, N. H., Wagle, S. R., Allen, D. O. & Ashmore, J. (1974) *Am. J. Physiol.* **227**, 1058-1061
- Moyle, W. R., King, Y. C. & Ramachandran, J. (1973) *J. Biol. Chem.* **248**, 2409-2417
- Perchellet, J. P., Shanker, G. & Sharma, R. K. (1978) *Science* **199**, 311-312
- Rouleau, M. F., Warshawsky, H. & Goltzman, D. (1986) *Endocrinology (Baltimore)* **118**, 919-931
- Segre, G. V., Perkins, A. S., Witters, L. A. & Potts, J. T., Jr. (1981) *J. Clin. Invest.* **67**, 449-457
- Snowdowne, K. W. & Borle, A. B. (1984) *Am. J. Physiol.* **246**, E198-E201
- Yamaguchi, D. T., Hahn, T. J., Iida-Klein, A., Kleeman, C. R. & Muallem, S. (1987) *J. Biol. Chem.* **262**, 7711-7718

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