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Inhibition of PTH Secretion by Interleukin-1β in Bovine Parathyroid Glands *in Vitro* Is Associated with an Up-Regulation of the Calcium-Sensing Receptor mRNA

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The principal regulator of parathyroid hormone (PTH) secretion is ionized calcium, but other factors are also known to modulate PTH secretion, such as vitamin D, estrogen, and recently inorganic phosphate. Interleukin-1 (IL-1) possesses a wide variety of biological activities and is produced by leukocytes as well as by various other cells including cells from endocrine tissues and might play a role as a paracrine factor in the control of PTH secretion. We investigated the effect *in vitro* of IL-1β on PTH release, PTHmRNA and the mRNA for the extracellular calcium-sensing receptor (CaR) levels in preparations of bovine parathyroid cells. PTH secretion from cultured parathyroid tissue slices was significantly inhibited in a medium containing IL-1β at a concentration of 2000 pg/ml (PTH in % of control: 63.5 ± 5.3), n=10 ($p<0.01$). The inhibitory effect of IL-1β was not found in preparations of dispersed cells. The inhibitory effect of IL-1β could be counteracted by the IL-1 receptor antagonist (IL-1ra), indicating that the inhibitory effect was mediated through the specific IL-1 receptor on the parathyroid cells. IL-1β (2000 pg/ml) up-regulated CaRmRNA levels to 180% of control, whereas no change in PTHmRNA was found. IL-1ra abolished the upregulating effect of IL-1β on the CaRmRNA. This study demonstrates a direct effect *in vitro* of IL-1β on PTH secretion from bovine parathyroid glands, an effect which may be mediated at least in part through the specific IL-1 receptor causing an upregulation of the calcium-sensing receptor mRNA. IL-1 might therefore play a role as a auto- and/or paracrine factor in the regulation of the PTH secretion.

The principal factor in the regulation of parathyroid hormone (PTH) secretion is the extracellular calcium concentration. Acute changes in extracellular calcium (Ca_e^{2+}) concentration are sensed by a G protein-coupled, calcium sensing receptor (CaR), first cloned and characterized in bovine parathyroid cells (1). In addition to extracellular calcium other factors are also known to modulate PTH secretion, such as Vitamin D, estrogen and recently inorganic phosphate (2-6). The regulation of PTH secretion is at present far from completely understood. The parathyroid are not known to be under any higher level, neuroendocrine control, as observed in other endocrine systems. The parathyroids, however might use local paracrine or autocrine regulatory mechanisms (7).

Interleukin-1α (IL-1α) and interleukin-1β (IL-1β) belong to a group of bioactive polypeptides produced primarily by macrophages, but also by a number of other cells (8). IL-1 possesses a wide variety of biological activities, including the ability to alter neuroendocrine and metabolic functions (9,21). IL-1 has been shown to affect directly the function of the beta-cells in rat pancreatic islets of Langerhans (10). At low substrate levels IL-1 increases glucose- stimulated insulin secretion, and at higher concentrations IL-1 markedly inhibits glucose-stimulated insulin secretion (11). A similar biphasic pattern induced by IL-1 has been demonstrated in vitro on human thyroid cell function (12). We have recently shown that phosphate directly stimulates PTH secretion *in vitro* in bovine parathyroid tissue slices, but not in dispersed bovine parathyroid cells. This may suggest that maintenance of a near normal architecture of the parathyroid gland is essential in order to elicit the effect of high phosphate, indicating that a paracrine factor might be involved in the regulation of PTH secretion (6).

In the present study, we have examined, *in vitro,* the effect of IL-1 on PTH secretion in tissue slices as compared to dispersed cells, and further examined the effect of IL-1 on the levels of the mRNA for the extra-

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cellular calcium sensing receptor (CaRmRNA) and on the prepro(PTHmRNA). Our results show for the first time that IL-1 exerts an inhibitory effect on PTH secretion and, in addition, that IL-1 causes an upregulation of the CaRmRNA levels, but had no effect on the PTHmRNA levels.

MATERIAL AND METHODS

Parathyroid Cell and Tissue Preparations

Fresh bovine parathyroid glands were obtained within 10 min. of slaughter at a local abattoir and transported to the laboratory in ice-cold phosphate buffered saline (PBS) with a pH of 7.4, containing penicillin 100 IU/ml (Merck, Darmstadt, Germany) and streptomycin 10 μg/ml (Leo Pharmaceutical Products, Ballerup, Denmark). Using sterile conditions the glands were trimmed free of excess fat and connective tissue and finely minced in Ham's F12 (Gibco BRL, Life Technologies, Roskilde, Denmark) with addition of $CaCl_2$ to a final concentration of 1.2 mM as well as $MgCl_2$ 0.5 mM, insulin 100 IU/l (Novo Nordisk, Bagsvaerd, Denmark), non essential amino-acids and L-glutamine 0.3 mg/ml (Gibco BRL, Life Technologies, Roskilde, Denmark).

Dispersed cells were prepared with minor modifications as described previously by Brown (13) from minced parathyroid tissue, by digestion in Ham's F12 (Gibco BRL, Life Technologies, Roskilde, Denmark) containing dispase grade II 0.5 U/ml (Boehringer-Mannheim, GmbH, Germany) and collagenase 0.5 mg/ml (Sigma St. Louis, MO, USA) for 45-60 min. in a 5% CO_2 incubator at 37°C, with vigorous pipetting every 15 min. using a 60 cc syringe. The solution was then filtered through two layers of gauze and the cells were sedimented at 1800 r.p.m. (600×g) for 5 min. at 20 °C and washed three times in Ham's F12 containing 5% heat inactivated fetal calf serum (Biological Industries, Beth Haemek, Israel). Cell viability was more than 95% by trypan blue exclusion. At least 90% of the acutely dispersed cells were parathyroid cells by morphological criteria. Cells prepared by digestion were used immediately after preparation as acutely dispersed cells at a concentration of 1×10^6 cells/ml, and were maintained in suspension by continuos shaking.

Tissue slices were prepared from the same stock of minced parathyroid tissue as used for the preparation of dispersed cells. Tissue slices were trimmed free of excess fat and placed in 10 cc polypropylene tubes containing 4 ml of culture medium. Incubation took place in an incubator (Forma Scientific, OH, USA) at 37 °C, 5 % CO2 with the 10 cc tubes placed on a shaker. When comparing tissue slices and dispersed cells, the parathyroid tissue used for preparing dispersed cells as well as tissue slices were taken from the same stock of minced tissue and incubated in parallel. The supernatants were collected after centrifugation for 5 min. at 600 × g at the end of incubation time for subsequent measurements of PTH. All of the supernatant was removed from the tube, and sucrose-TRIS-HCl-$MgCl_2$ (STM) buffer at pH 7.85 was immediately added to the pellet of dispersed cells and frozen at −20 °C for subsequent measurement of DNA content. Tissue slices were immediately frozen in liquid nitrogen and stored at −20 °C for DNA measurements. In the experiments for RNA extraction and measurements of CaRmRNA and PTHmRNA the tissue was stored at −80 °C and analyzed within 14 days after the experiment.

Cytokines

Human recombinant IL-1α (30 IU/ng) was a kind gift from Dainippon Pharmaceutical Co (Suita/Osaka, Japan). Human recombinant IL-1β (50 IU/ng) was a generous gift from Dr. Kim Hejnæs (Novo-Nordisk, Bagsvaerd, Denmark). The biological activities of these preparations were comfirmed by EL4 bioassay (14), and calibrated by the use of international reference preparations (National Institute of Biological Standards and Control, Potters Bar, Hertfordshire, UK). Human recombinant interleukin-1 receptor antagonist (IL-1ra) was a kind donation from Dr. D. E. Tracey (The Upjohn Co, Kalamazoo, MI, USA). All cytokine preparations were tested for endotoxin content using a chromogenic Limulus amebocyte lysate assay (Whittaker Bioproducts Inc., Walkerville, MD, USA). All contained less than 0.5 fg *E. coli* LPS equivalent/ng cytokine.

Experiments

I: Effect of interleukin-1β on PTH release from dispersed cells and from tissue slices at different calcium concentrations. The effect of interleukin-1 on PTH release was examined in dispersed cells and tissue slices, incubated for 48 hours at low (0.80 mM) normal (1.20 mM) or at high calcium concentrations (1.8 mM) in medium containing IL-1β at concentrations of 20 pg/ml, 200 pg/ml or 2000 pg/ml or a negative control without IL-1β. In order to examine the effect of IL-1α, tissue slices were incubated with IL-1α (200 pg/ml) at a calcium concentration of 1.20 mM.

II: Reversibility of the IL-1β effect. To investigate whether the effect of IL-1β was reversible, the tissue slices were incubated in three different groups: 1) Control: Incubated for 48 hours in Ham's F12 medium without IL-1β then washed three times and incubated for an additional 48 hours without addition of IL-1β. 2) IL-1β for 96 hours: Incubation for 48 hours with IL-1β (20 pg/ml and 2000 pg/ml), then washed three times and incubated with IL-1β (20 pg/ml and 2000 pg/ml) for an additional 48 hours, in the same concentration of IL-1β as for the first 48 hours. 3) Incubation for 48 hours with IL-1β, washed three times and incubation for a subsequent 48 hours without IL-1β.

III: Effect of the specific IL-1 receptor antagonist (IL-1ra) on PTH release. These studies were performed in order to examine whether the effect of IL-1β was mediated through a specific IL-1 receptor on the cell surface. A specific IL-1 receptor antagonist (IL-1ra) (125 ng/ml) was added to the culture medium containing the tissue slices just prior to addition of IL-1β to the culture medium. The effect of IL-1ra in combination with IL-1β on PTH secretion was examined after 48 hours of incubation.

IV: Effect of IL-1 on CaRmRNA levels and PTHmRNA levels. In order to examine the possible effects of IL-1 on PTHmRNA and CaRmRNA levels, parathyroid tissue slices were incubated for 48 hours in a medium containing IL-β (2000 pg/ml), IL-1α (200 pg/ml), IL-1ra (125 ng/ml) and a combination of IL-1β (2000 pg/ml) and IL-1ra (125 ng/ml) at a calcium concentration of 1.2 mM. Northern blots and quantification of mRNA were performed as described. Results are expressed as the ratio of CaRmRNA/β-actin-mRNA and PTHmRNA/β-actin-mRNA, respectively.

Analysis of CaRmRNA and PTHmRNA

Total RNA was extracted by the phenol-chloroform method (Trizol, Amersham, USA) and analyzed by Northern blot analysis. Parathyroid tissue (200-300 mg) was homogenized in 7 ml of Trizol, and total RNA was extracted as directed by the manufacturer. Total RNA (5 μg) was run for two hours in an agarose gel containing formamide and formaldehyde and transferred to a nylon membrane (Qiabrane, Qiagene, Hilden, Germany). The PTH probe was a 517 bp product from a bovine parathyroid cDNA library using the superscript Lambda system for cDNA synthesis and Lambda cloning (Gibco-BRL, Life Technologies, Inc., Gaithersburg, MD). The 517 bp product was incorporated into pCR3-Uni using a Eukaryotic TA Cloning Kit - Unidirectional (Invitrogn, San Diego, CA). A full length probe for the bovine extracellular calcium sensing receptor (BoPCaR) (bp 248-3919) was used. The β-actin probe was a human probe (Clontech labs, Palo Alto, CA). The probes (50 ng) were ^{32}P-labeled according to manufacturers instructions, using the randomly priming Ready-prime kit (Amersham Int, Buckinghamshire, England). The nylon-

membrane was prehybridized at 65 °C for two hours in hybridizationsolution (Hyb-solution, Amersham, USA), followed by 16 hours of hybridization with the respective probes at high stringency, 64 °C. After hybridization the membrane was washed for 20 min. in 2 × SSC + 0.1% SDS twice at room temperature, followed by 2 × 20 min. washes in 0.1 × SSC + 0.1% SDS at 64 °C. After the hybridization with the CaR probe, quantification of the respective bands were performed, exposing the nylonmembrane for two hours to the bioimagine plate (BAS 2000, Fuji, Santax, Roedovre, Denmark) and quantification of was performed using the bio-imagine system BAS 2000 reader (Fuji) and TINA software (Santax, Roedovre, Denmark). The membrane was stripped by boiling it in 0.1 × SSC + 0.1% SDS for 45 sec, and in the same blot, rehybridization with the PTH probe and with the β-actin probe were performed.

Bovine PTH Measurements

Bovine PTH was measured in supernatants by a two site IRMA assay (Allegro, Nichols, San Juan Capistrano, CA), which is specific for intact human 1-84 PTH. This assay also recognizes intact bovine PTH, but does not recognize hormonal fragments (15-16). A standard curve using intact (1-84) bovine PTH (Bachem, CA) was parallel to the human standard curve throughout the range of 7.5-1700 pg/ml, with a recovery of ~20%. The detection limit in our laboratory was 1.5 pg/ml and the interassay coefficient of variation was 7.9% for low levels (40 pg/ml) and 5.9% for intermediate levels of PTH (250 pg/ml) 5.9% (n=6). The intra-assay coefficient of variation at the same levels were 3.5% and 2.1%, respectively (n=6).

Electrolyte Measurements

To ensure that the levels of cations in the culture medium were stable throughout the experiments, Ca^{2+}, Mg^{2+}, K^{+}, Na^{+} and pH were measured in the medium just prior to and just after the incubation using ICA2 and KNa analyzers (Radiometer, Copenhagen, Denmark) and a NOVA 8 analyzer (Nova Biomedical, Waltham, MA, USA), the last for measurements of ionized magnesium.

DNA Measurements

Total DNA measurements were performed in order to express PTH secretion as a function of the DNA content. Thus, DNA of the dispersed parathyroid cells was measured in each individual sample using a colorimetric method, as described by Burton (17), and modified by Giles and Myers (18).

DNA measurements in the tissue slices were performed using a DNA extraction Kit (Stratagene, La Jolla, CA, USA), which is a modification of a procedure based on separating contaminating protein from DNA by salt precipitation (19). The tissue slices were homogenized using a polytron in the extraction solution, and the cellular proteins were digested with pronase for 18 hours at 37 C. After removal of the proteins by "salting out" using sodium chloride, and enzymatic degradation of RNA by RNase, colorimetric determination of the DNA yield was carried out in 250 μl of the supernatant. The DNA content of parathyroid tissue was 74.5 ± 2.6 μgDNA/100 mg tissue. In 5 samples (93-305 mg) the coefficient of variation was 3.5%. In our assay the detection limit was 1 μg DNA/ml, intra-assay variation at 30 μg DNA/ml level was 1.6% (n=5) and inter-assay variation was 8.7% at 30 μg DNA/ml and 5.2% at 50 μgDNA/ml level (n=6).

Statistical Analysis

Statistical analyses were performed using Student's unpaired t test. For analysis of multiple comparisons the ANOVA was used. The null hypothesis was rejected when a value of $P<0.05$ was obtained. Statistical calculations were based on an n equal to the number of separate experiments.

RESULTS

We found that IL-1β produced a significant ($p<0.01$) inhibition of PTH secretion after 48 hour of incubation at a calcium concentration of 1.2 mM (fig 1A). The inhibitory effect of IL-1β in tissue slices was also observed at low (0.8 mM) and high (1.8 mM) calcium concentrations (dato not shown). Whereas this inhibitory effect on PTH secretion in tissue slices was not observed in preparations of dispersed parathyroid cells (fig 1B). IL-1α produced an inhibition of PTH secretion similar to that caused by IL-1β (data not shown).

In order to investigate whether the effect of IL-1 on PTH secretion was reversible, the tissue slices were incubated for 96 hours with IL-1β at concentrations of 20 pg/ml and 2000 pg/ml or for 48 hours with IL-1β and after a thorough washing, were incubated for 48 hours in control medium. The effect of IL-1 on PTH secretion after 48 hours of incubation was completely reversible during a subsequent 48 hours of incubation, as there were no significant differences between control preparations and those incubated for the first 48 hours with IL-1 followed by incubation in control medium as well as a significant difference ($p<0.01$) between control and the 96 hours of incubation with IL-1 (fig 2). The inhibitory effect of IL-1 could be completely abolished be a simultaneous incubation with the specific interleukin-1 receptor antagonist (IL-1ra), indicating that the effect of interleukin-1 is mediated via a specific receptor on the parathyroid cells (fig 3).

Addition of IL-1β caused an up-regulation of CaRmRNA to 180% of the control level (fig 4A). A similar upregulation of CaRmRNA was also seen when IL-1α was added, whereas there was no effect of either IL-1β or of IL-1α on CaRmRNA levels when the tissue slices were incubated simultaneously with the IL-1 antagonist IL-1ra. None of the interleukin-1's had any effect on the levels of PTHmRNA (fig 4B).

DISCUSSION

The present study demonstrates clearly that interleukin-1 inhibits PTH secretion in bovine parathyroid tissue slices. A similar effect could however not be found in dispersed parathyroid cells. Recently we have shown that in order to elicit an effect of high phosphate on PTH secretion from bovine parathyroids in vitro, intact or partly intact architecture of the parathyroid tissue was required (6). This interesting direct stimulatory effect of phosphorus on PTH secretion was shown for the first time in 1996 by 3 different groups, all using the in vitro model of totally or partially intact parathyroid glands (4-6).

We also clearly demonstrated in the present studies that the effect of interleukin-1 on PTH secretion was completely reversible. This result indicates that this effect is not due to a cytotoxic effect, but rather to mod-

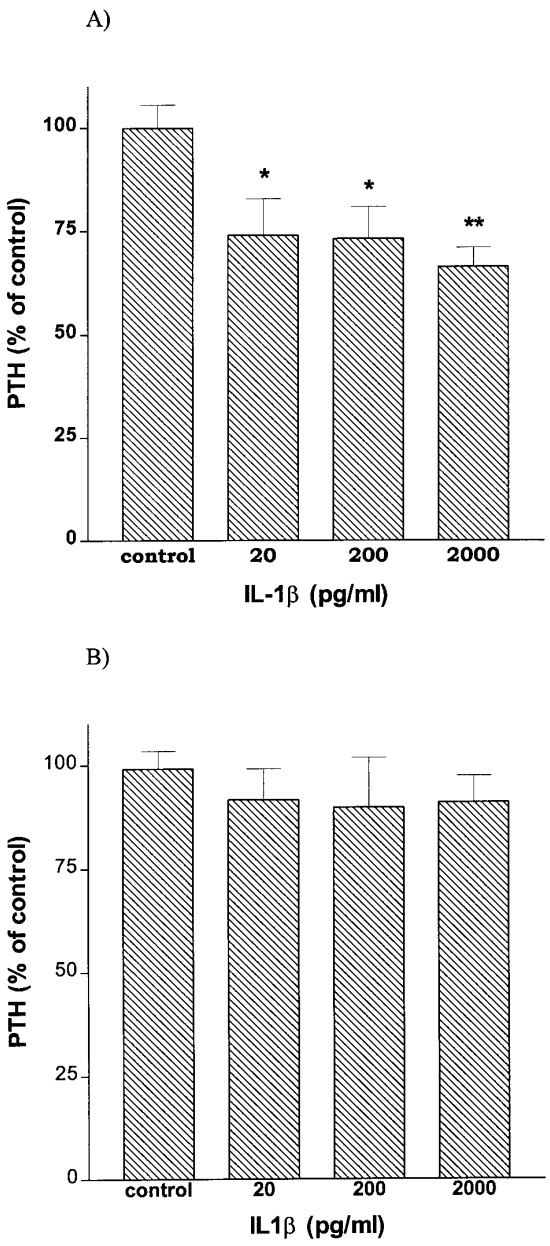

FIG. 1. Effect of IL-1β on PTH secretion in preparations of bovine parathyroid tissue slices and dispersed cells. Parathyroid glands were finely minced in 0.5×0.5 mm pieces and incubated (4-5 pieces in each tube). IL-1β significantly ($p<0.05$) inhibited PTH secretion from preparations of tissue slices at all concentrations tested, as compared to control (panel A). Maximal inhibition was at 2000 pg/ml of IL-1β, here PTH secretion was inhibited to 63% of control ($p<0.01$). (Control=81±6 pgPTH/μgDNA/h). Dispersed cells (1×10^6 cells/ml) were incubated with increasing concentrations of IL-1β (20, 200 and 2000 pg/ml) for 48 hours. No effect of IL-1β was found on the PTH secretion. Results are measured in pgPTH/μgDNA/h and expressed as % of control in means±SEM for 4 independent experiments each performed in triplicate.

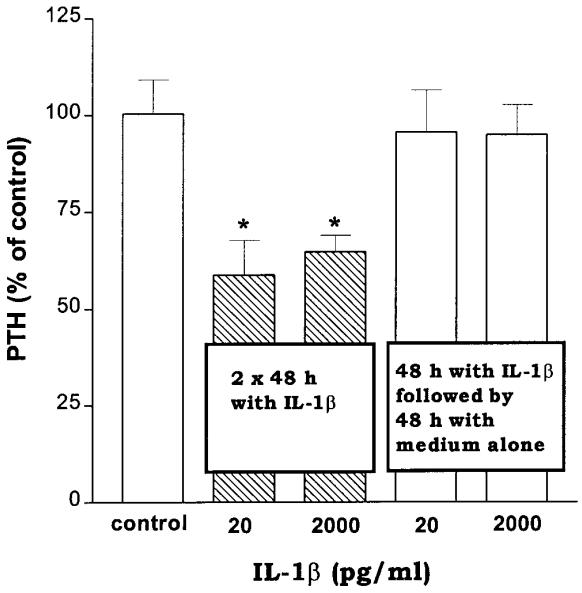

FIG. 2. Reversibility of the IL-1β effect on PTH secretion. Parathyroid tissue slices were incubated for 48 hours with 20 pg/ml or 2000 pg/ml IL-1β, followed by thorough washing and reincubation for 48 hours in either control medium or medium containing IL-1β. Incubation for two sequential 48 hour periods with IL-1β caused significant ($p<0.01$) inhibition of PTH secretion at 20 pg/ml as well as 2000 pg/ml of IL-1β (dotted bars) as compared to control (open bar), whereas PTH secretion was not significantly different from control in the group incubated for 48 hours with IL-1β followed by 48 hours in control medium (dashed bars). Results are given as mean±SEM in 4 independent experiments in triplicate.

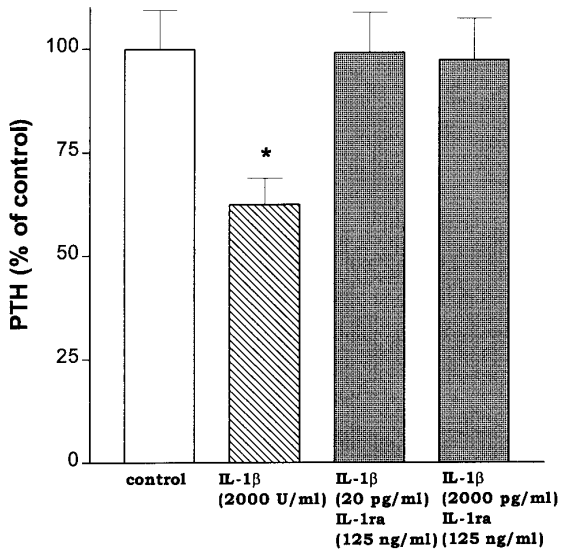

FIG. 3. The effect of IL-1β on PTH secretion is counteracted by interleukin-1 receptor antagonist (IL-1ra). Simultaneous incubation with IL-1ra (125 ng/l) and IL-1β (at 20 pg/ml and 2000 pg/ml) (dashed bars) blocked the effect of IL-1β (closed bar) on PTH secretion. Results are given as mean±SEM for 3 independent experiments in triplicate.

ulation of PTH secretion per se. The regulation of PTH secretion is at present not yet completely understood, and the parathyroid are not under neuroendocrine con-

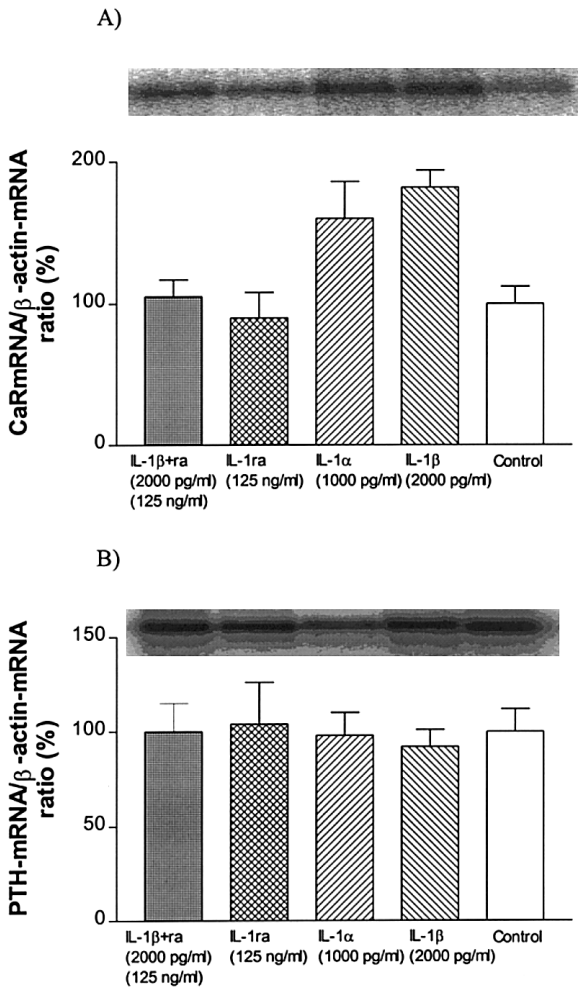

FIG. 4. Influence of IL-1β on CaRmRNA levels and PTHmRNA levels. IL-1α as well as IL-1β causes an up-regulation of CaRmRNA levels to 180% of control (panel A), whereas this up-regulating effect is abolished when the parathyroid tissue is incubated with IL-1β and IL-1ra simultaneously. IL-1 did not cause any change in the PTHmRNA/β-actin-mRNA levels (panel B). Results are the mean±SEM of 3 independent Northern blots.

trol, as observed in other endocrine systems. The parathyroid are, therefore, likely to use local paracrine or autocrine regulatory mechanisms (7).

IL-1 possesses a wide variety of biological activities in addition to its lymphocyte activating properties, including the ability to alter neuroendocrine and metabolic functions. The effects of IL-1 are mediated through specific interleukin-1 receptors. Thus, the inhibitory effect of IL-1 on thyroglobulin secretion from human thyrocytes in vitro could be abolished by the specific interleukin-1 receptor antagonist (20). IL-1ra is closely related to IL-1α and IL-1β and consists of a single polypeptide chain of 17 kDa (21). It is produced by many cells, including activated macrophages and monocytes and binds selectively to IL-1 receptors without activating the target cell. IL-1ra competes with IL-1α and IL-1β for occupancy of the IL-1 receptors, and it selectively blocks the biological activities of IL-1α and IL-1β in several in vivo and in vitro models (22-23). In this study we found that the interleukin-1 receptor antagonist abolished the inhibitory effect of interleukin-1 on PTH secretion. This indicates for the first time that parathyroid cells possess specific interleukin-1 receptors that can mediate the effect of interleukin-1 on PTH secretion.

IL-1β also induced an up-regulation of the mRNA for the extracellular calcium sensing receptor (CaRmRNA) to 180% of control levels. IL-1α produced a similar up-regulation of CaRmRNA levels, whereas when preparations were incubated simultaneously with IL-1ra and IL-1β no effect on the CaR-mRNA levels was found.

Furthermore, no effect of IL-1β on PTHmRNA was seen after 48 hours of incubation, suggesting that the suppressive effect of IL-1β is on the secretory levels and not on the transcription of PTH. The lack of response to IL-1β in preparations of dispersed cells could be due to the fact that dispersed parathyroid cells in vitro undergo rapid dedifferentiation with loss of calcium responsiveness due to a marked decline in calcium sensing receptor mRNA and protein levels (24-25); IL-1 per se, is obviously not able to counteract such a mechanism.

Apart from IL-1, other factors are known to modulate the CaRmRNA levels. Recently, it has been shown that repletion with $1,25(OH)_2D_3$ in vitamin D depleted rats is associated with an upregulation of the CaRmRNA in the parathyroid glands and in the kidneys (26). This upregulation of the CaRmRNA by $1,25(OH)_2D_3$ may be involved in the suppressive effects of vitamin D compounds on PTH secretion.

Gain of function mutations in the calcium sensing receptor have been identified in patients with hypocalcemia associated with hypercalciuria and normal PTH levels. The mutations were identified in the extracellular domain of the calcium sensing receptor gene and shown to cosegregate with the disease. Analysis of the functional expression of the mutant receptors in human embryonic kidney cells (HEK-293) demonstrated shifts in the dose-response curves so that the extracellular calcium concentrations needed to produce half-maximal increases in total inositol phosphate in the cells were significantly lower than those required for the wild-type receptor (27). These findings indicate that the regulation of CaRmRNA levels and the function of the CaR play a crucial role in the calcium homeostasis.

Critically ill patients have increased prevalence of hypocalcemia (28). In patients undergoing surgery with cardiopulmonary bypass a high incidence of hypocalcemia and delayed increase in PTH levels has been shown (29), the reason for this inappropriate PTH secretion during major surgery is unknown, but critically ill patients are known to have high circulating levels

of interleukin-1β and this cytokine may at least in part explain this phenomenon.

In summary, the present study indicates that the inhibitory effect of IL-1 on PTH secretion in bovine parathyroid glands is mediated through specific IL-1 receptors on the parathyroid cells. IL-1 caused an up-regulation of the CaRmRNA, which could increase the calcium sensitivity of the parathyroids resulting in an inhibition of the PTH secretion, an effect which was independent of the PTHmRNA levels. IL-1 might thus act as an auto- or paracrine modulator of parathyroid functions.

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REFERENCES

1. Brown, E. M., Gamba, G., Riccardi, R., Lombardi, M., Butters, R., Kifor, O., Sun, A., Hediger, M. A., Lytton, J., and Hebert, J. (1993) *Nature* **366,** 575–580.
2. Silver, J., Russell, J., and Sherwood, L. M. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 4270–4273.
3. Naveh-Many, T., Almogi, G., Livni, N., and Silver, J. (1992) *J. Clin. Invest.* **90,** 2434–2438.
4. Almadén, Y., Canalejo, A., Hernandez, A., Ballesteros, E., Garcia-Navarro, S., Torres, A., and Rodriguez, M. (1996) *J. Bone Miner. Res.* **11,** 963–969.
5. Slatopolsky, E., Finch, J., Denda, M., Ritter, C., Zhong, M., Dusso, A., MacDonald, P., and Brown, A. J. (1996) *J. Clin. Invest.* **97,** 2534–2540.
6. Nielsen, P. K., Feldt-Rasmussen, U., and Olgaard, K. (1996) *Nephrol. Dial. Transplant* **11,** 1762–1768.
7. Lewin, E., Nielsen, P. K., and Olgaard, K. (1995) *Curr. Opin. Nephrol. Hypertension* **4,** 324–333.
8. Bendtzen, K. (1989) *Autoimmunity* **2,** 177–189.
9. Mandrup-Poulsen, T., Nerup, J., Reimers, J. I., Pociot, F., Andersen, H. U., Karlsen, A., Bjerre, U., and Bergholdt, R. (1995) *Eur. J. Endocrinol.* **133,** 660–671.
10. Bendtzen, K., Mandrup-Poulsen, T., Nerup, J., Nielsen, J. H., Dinarello, C. A., and Svenson, M. (1986) *Science* **232,** 1545–1547.
11. Spinas, G. A., Mandrup-Poulsen, T., Molvig, J., Back, L., Bendtzen, K., and Dianrello, C. A. (1986) *Acta Endocrinol.* **113,** 551–558.
12. Rasmussen, Å. K., Feldt-Rasmussen, U., and Bendtzen, K. (1993) *Autoimmunity* **16,** 141–148.
13. Brown, E. M., Hurwitz, S., and Aurbach, G. D. (1976) *Endocrinology* **99,** 1582–1588.
14. Svenson, M., and Bendtzen, K. (1988) *Scand. J. Immunol.* **27,** 593–599.
15. Nussbaum, S. R., Zahradnik, R. J., Lavigne, J. R., Brennan, G. L., Nozawa-Ung, K., Kim, L. Y., Kentmann, H. T., Wang, C. A., Potts, J. T., and Segre, G. V. (1988) *Clin. Chem.* **33,** 1364–1367.
16. Daugaard, H., Egfjord, M., and Olgaard, K. (1988) *Am. J. Physiol.* **254,** E740–E748.
17. Burton, K. (1956) *Biochem. J.* **62,** 315–323.
18. Giles, K. W., and Myers, A. (1965) *Nature* **4979,** 93.
19. Miller, S. A., Dykes, D. D., and Polesky, H. F. (1988) *Nucl. Acids Res.* **16,** 1215.
20. Rasmussen, Å. K., Diamant, M., Blichert-Toft, M., Bendtzen, K., and Feldt-Rasmussen, U. (1997) *Endocrinology* **138,** 2043–2048.
21. Carter, D. B., Deibel, M. R., Dunn, C. J., Tomich, C,-s., Laborde, A. L., Slightom, J. L., Berger, A. E., Bienkowski, M. J., Sun, F. F., McEwan, R. N., Harris, P. K. W., Yem, A. W., Waszak, G. A., Chosay, J. G., Siu, L. C., Hardee, M. M., Zucher-Neely, H. A., Reardon, I. M., Heinrikson, R. L., Truesdell, S. E., Shelly, J. A., Eessalu, T. E., Taylor, B. M., and Tracey, D. E. (1990) *Nature* **344,** 633–638.
22. Dinarello, C. A. (1996) *Blood* **87,** 2095–2147.
23. Arend, W. P. (1993) *Adv. Immunol.* **54,** 167–227.
24. Leboff, M. S., Rennke, G., and Brown, E. M. (1983) *Endocrinology* **113,** 277–284.
25. Ambrish, M., Kifor, O., Kifor, I., Vassilev, P., Butters, R., Krapcho, K., Simin, R., Fuller, F., Hebert, S. C., and Brown, E. M. (1995) *Endocrinology* **136,** 3087–3092.
26. Brown, A. J., Zhong, M., Finch, J., Ritter, C., McCracken, R., Morrissey, J., and Slatopolsky, E. (1996) *Am. J. Phys.* **270,** F454–F460.
27. Pearce, S. H. S., Williamson, C., Kifor, O., Bai, M., Coulthard, M. G., Davies, M., Lewis-Barned, N., McCredie, D., Powel, H., Kendall-Taylor, P., Brown, E. M., and Thakker, R. V. (1996) *N. Engl. J. Med.* **335,** 1115–1122.
28. Zaloga, G. P., and Chernow, B. (1986) *JAMA* **256,** 924.
29. Robertie, P. G., Butterworth, J. F., Royster, R. L., Prielipp, R. C., Dudas, L., Black, K. W., Cole, L. R., and Zaloga, G. P. (1991) *Anesthesiology* **75,** 43–48.