Pharmacodynamics of the Type II Calcimimetic Compound Cinacalcet HCl

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ABSTRACT

Calcimimetic compounds, which activate the parathyroid cell Ca\(^{2+}\) receptor (CaR) and inhibit parathyroid hormone (PTH) secretion, are under experimental study as a treatment for hyperparathyroidism. This report describes the salient pharmacodynamic properties, using several test systems, of a new calcimimetic compound, cinacalcet HCl. Cinacalcet HCl increased the concentration of cytoplasmic Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_i\)) in human embryonic kidney 293 cells expressing the human parathyroid CaR. Cinacalcet HCl (EC\(_{50}\) = 51 nM) in the presence of 0.5 mM extracellular Ca\(^{2+}\) elicited increases in [Ca\(^{2+}\)]\(_i\), in a dose- and calcium-dependent manner. Similarly, in the presence of 0.5 mM extracellular Ca\(^{2+}\), cinacalcet HCl (IC\(_{50}\) = 28 nM) produced a concentration-dependent decrease in PTH secretion from cultured bovine parathyroid cells. Using rat medullary thyroid carcinoma 6-23 cells expressing the CaR, cinacalcet HCl (EC\(_{50}\) = 34 nM) produced a concentration-dependent increase in calcitonin secretion. In vivo studies in rats demonstrated cinacalcet HCl is orally bioavailable and displays approximately linear pharmacokinetics over the dose range of 1 to 36 mg/kg. Furthermore, this compound suppressed serum PTH and blood-ionized Ca\(^{2+}\) levels and increased serum calcitonin levels in a dose-dependent manner. Cinacalcet was about 30-fold more potent at lowering serum levels of PTH than it was at increasing serum calcitonin levels. The S-enantiomer of cinacalcet (S-AMG 073) was at least 75-fold less active in these assay systems. The present findings provide compelling evidence that cinacalcet HCl is a potent and stereoselective activator of the parathyroid CaR and, as such, might be beneficial in the treatment of hyperparathyroidism.

The secretion of parathyroid hormone (PTH), arguably the major hormone regulating systemic Ca\(^{2+}\) homeostasis, is regulated by small changes in the level of blood Ca\(^{2+}\). Increases in the level of extracellular Ca\(^{2+}\) depress PTH secretion, whereas hormone secretion is elevated in hypocalcemic conditions. The effect of extracellular Ca\(^{2+}\) on PTH secretion is mediated by a cell surface Ca\(^{2+}\) receptor (CaR). The CaR is a G protein-coupled receptor that based on structural homology, is classified within family C. Activation of this receptor by increases in the level of extracellular Ca\(^{2+}\) depresses PTH secretion (for review, see Brown and MacLeod, 2001; Brown et al., 1993). The CaR is the pivotal mechanism regulating PTH secretion and, as such, is an attractive molecular target for drugs capable of altering circulating levels of PTH in disease states such as hyperparathyroidism or osteoporosis (Nemeth, 2002a). Presently, there are no drugs capable of directly altering the secretion of PTH without altering those of plasma Ca\(^{2+}\).

Ligands that mimic or potentiate the effects of extracellular Ca\(^{2+}\) at the CaR have been termed calcimimetics, of which there are two mechanistically distinct types (Nemeth et al., 1998). Type I calcimimetics are agonists and include inorganic and organic polycations, whereas type II calcimimetics are allosteric activators and include certain L-amino acids and phenylalkylamines (Nemeth, 2002b). The phenylalkylamines interact with the membrane-spanning segments of the CaR and enhance signal transduction, presumably by inducing conformational changes in the receptor.
(Hammerland et al., 1999; Hauache et al., 2000). The presumed conformational change reduces the threshold for CaR activation by the endogenous ligand, Ca\(^{2+}\), thereby reducing PTH secretion in the absence of a change in the level of extracellular Ca\(^{2+}\). The first calcimimetic to be evaluated as a drug candidate was NPS R-568, a phenylalkylamine-type II calcimimetic compound. This compound selectively activates the parathyroid CaR and inhibits PTH secretion in vitro and in vivo (Nemeth et al., 1998; Fox et al., 1999a). Significantly, NPS R-568 lowers circulating levels of PTH in patients with primary hyperparathyroidism (Silverberg et al., 1995) and in patients with secondary hyperparathyroidism of end stage renal disease (Antonsen et al., 1998). Despite the safety and efficacy of NPS R-568 in lowering plasma levels of PTH in these patient populations, the pharmacokinetic and metabolic profile of this drug was variable (Goodman et al., 2000a; Frazão et al., 2002). These caveats prompted our search for a compound possessing the safety and efficacy of NPS R-568 but with improved bioavailability and metabolic properties.

Cinacalcet HCl or \((\alpha R)-(\cdots)-\alpha\text{-methyl-N-[3-[3-[trifluoromethylphenyl]propyl]-1-naphthalenemethanamine hydrochloride (Fig. 1)}\) is an analog of NPS R-568 with an improved metabolic profile, and it is now under clinical evaluation for the treatment of secondary HPT. This report describes the salient pharmacodynamic properties of cinacalcet HCl as determined using a combination of in vitro and in vivo test systems.

**Materials and Methods**

**In Vitro Ca\(^{2+}\) Sensing Receptor Assays.** Human embryonic kidney cells (HEK 293 cells) engineered to express the human parathyroid CaR have been described in detail previously (Racke and Nemeth, 1993a; Nemeth et al., 1998). This clonal cell line, referred to as HEK 293 4.0-7 cells, has been used extensively to detect agonists and allosteric activators (calcimimetics) of the CaR using changes in cytoplasmic Ca\(^{2+}\) concentrations ([Ca\(^{2+}\)]\(_{i}\)) as the endpoint (Nemeth et al., 1998; 2001). Changes in the [Ca\(^{2+}\)]\(_{i}\) provide a quantitative and functional assessment of CaR activity in these cells and the results using this assay parallel those obtained using a homologous expression system of bovine parathyroid cells (Nemeth et al., 1998, 2001). Briefly, on-line continuous measurements of fluorescence in fluo-3- or fura-2-loaded HEK 293 4.0-7 cells were obtained using a custom-built spectrofluorometer (Racke and Nemeth, 1993a) or a fluorescence imaging plate reader instrument (Molecular Devices Corp., Sunnyvale, CA; Nemeth et al., 1998, 2001). The EC\(_{50}\) value for cytoplasmic Ca\(^{2+}\) concentration was determined using a range of extracellular Ca\(^{2+}\) concentration. Similarly, the EC\(_{50}\) values for cinacalcet HCl and S-AMG 073 were determined in the presence of 0.5 mM extracellular Ca\(^{2+}\).

For all in vitro assays, cinacalcet HCl and S-AMG 073 were dissolved in DMSO and then diluted in cell culture media. All in vitro vehicle controls consisted of the maximum correlated percentage of DMSO diluted in cell culture media.

The effect of cinacalcet HCl or S-AMG 073 on CaR-dependent regulation of PTH secretion was assessed using primary cultures of dissociated bovine parathyroid cells and has been described in detail previously (Racke and Nemeth, 1993b; Nemeth et al., 1998). The dissociated cells were removed from flasks by decanting and washed with parathyroid cell buffer (126 mM NaCl, 4 mM KCl, 1 mM MgSO\(_4\), 0.7 mM KH\(_2\)PO\(_4\)/KH\(_2\)PO\(_4\), 20 mM Na-Hepes, pH 7.45, and variable amounts of CaCl\(_2\) as specified) containing 0.1% bovine serum albumin, and 1 mg/ml glucose. Portions (0.2 ml) of this cellular suspension were added to polystyrene tubes with or without cinacalcet HCl or S-AMG 073 and/or varying concentrations of CaCl\(_2\). Each experimental condition was performed in triplicate. Incubations at 37°C were for 20 min and were terminated by placing the tubes on ice. Cells were pelleted by centrifugation (500g for 10 min at 4°C), and 0.1 ml of supernatant was immediately assayed for PTH content. A portion of the cells was left on ice during the incubation period and then processed in parallel with other samples. The amount of PTH in the supernatant from tubes maintained on ice was defined as “basal release” and subtracted from other samples. PTH levels were quantified using a rat PTH\(_{1-34}\) immunoradiometric assay kit, which also detects bovine PTH (ImmunoLogic, San Clemente, CA). For each experiment, results were expressed as picograms of PTH released/10⁵ cells and then normalized to PTH released in 0.5 mM Ca\(^{2+}\). Cell numbers were determined by counting nuclei in a hemocytometer after lysing the cells and staining the nuclei with cresyl violet. The IC\(_{50}\) value for cinacalcet HCl and S-AMG 073 were determined in the presence of 0.5 mM extracellular Ca\(^{2+}\).

Methods used in determining calcitonin secretion from rat MTC cells have been described in detail elsewhere (Gagel et al., 1980; Lavigne et al., 1998). Briefly, rat MTC 6-23 cells (clone 6) were purchased from American Type Culture Collection (Manassas, VA). Rat MTC 6-23 cells were maintained in growth media (Dulbecco’s modified Eagle’s medium high glucose with Ca\(^{2+}\)/15% heat-inactivated horse serum) that was replaced every 3 to 4 days. The cultures were passaged weekly at a 1:4 split ratio. Ca\(^{2+}\) concentration in the formulated growth media was calculated to be 3.2 mM. Cells were incubated in an atmosphere of 90% O\(_2\)/10% CO\(_2\), at 37°C. Before the experiment, cells from subconfluent cultures were aspirated and rinsed once with trypsin solution. The flasks were aspirated again and incubated at room temperature with fresh trypsin solution for 5 to 10 min. The detached cells were suspended at a density of 3.0 × 10⁵ cells/ml in growth media and seeded at a density of 1.5 × 10⁵ cells/well (0.5 ml of cell suspension) in collagen-coated 48-well plates (BD Labware, Bedford, MA). The cells were allowed to adhere to 56 h postseeding, after which the growth media were aspirated and replaced with 0.5 ml of assay media (Dulbecco’s modified Eagle’s medium high glucose with Ca\(^{2+}\) but with 2% fetal bovine serum). The cells were then incubated for 16 h before determination of Ca\(^{2+}\)-stimulated calcitonin release. The actual Ca\(^{2+}\) concentration in this medium was calculated to be less than 0.07 mM. To measure calcitonin release, 0.35 ml of test agent in assay media was added to each well and incubated for 4 h before determination of calcitonin content in the media. Calcitonin levels were quantified according to the vendor’s instructions using a rat calcitonin immunoradiometric assay kit (Immunotech). An EC\(_{50}\) value for cinacalcet HCl and S-AMG 073 was generated.

**CaR RNase Protection Assay.** Total RNA was extracted from normal rat tissues and cultured cells following standard protocols provided with the STAT60 reagent (Tel-Test Inc., Friendswood, TX) and quantified by OD 260/280 measurement. Radiolabeled antisense RNA probes were transcribed from linearized plasmid templates using T7 RNA polymerase (Promega, Madison, WI) and (\(^{32}\)P)UTP (3000 Ci/mol; Amersham Biosciences Inc., Piscataway, NJ). The rat CaR probe corresponds to nucleotides 2154 to 2435 of the published sequence Gb: U10345. The rat calciphilin probe was transcribed...
from a commercially available template (Ambion, Austin, TX). Ten micrograms of total RNA and $1 \times 10^6$ cpm of each probe were hybridized at 55°C overnight followed by RNase digestion and precipitation. Samples were run on a 6% Tris borate-EDTA urea gel (Invtrogen, Carlsbad, CA). Gels were dried at 80°C and exposed on a phosphorscreen overnight. Screens were scanned with a Storm 840 PhosphorImager (Amerham Biosciences Inc., Sunnyvale, CA), and density of the protected bands was calculated with ImageQuant software (Amerham Biosciences Inc.) using local average background correction.

**In Vivo Evaluation of Cinacalcet HCl.** Male Sprague-Dawley rats weighing 400 to 450 g were given free access to food and water. The protocol was approved by the Institutional Animal Care and Use Committee of Amgen, Inc. (Thousand Oaks, CA). Unanesthetized rats were gavaged with an 18-gauge balled needle at a volume between 0.65 and 0.8 ml. The solubility of cinacalcet HCl in water was not adequate ($<1$ mg/ml) for in vivo studies; therefore, cinacalcet HCl was formulated in 20% capisol in water at 18 mg/ml at pH 7.0. Cinacalcet HCl was administered at doses of 1, 3, 10, and 30 mg/kg in 20% capisol. Vehicle-treated rats (controls) received 20% capisol (gavaged) in a volume of 0.8 ml. Rats were anesthetized with 2% isoflurane in O2. Each rat was bled at time 0 (pre-cinacalcet HCl or vehicle (20% capisol) administration) and 1, 2, 4, 8, and 24 h after oral gavage of cinacalcet HCl or vehicle. For measurements of blood-ionized Ca$^{2+}$ levels, blood (50 μl) was collected from the orbital sinus of anesthetized rats with heparinized capillary tubes. Blood samples were analyzed within seconds of collection using a Ciba-Corning 634 ISE Ca$^{2+}$/pH analyzer. For measurements of serum PTH, phosphorus, and calcitonin levels, a nonheparinized capillary tube was inserted into the orbital sinus and blood (0.5 ml) was collected into SST (clot activator) brand blood tubes. Blood samples were allowed to clot for 15 to 30 min and were centrifuged (3000 rpm; Sorvall RT 600B) and the supernatant was removed. Serum PTH and calcitonin levels were quantified according to the vendor's instructions using rat PTH (1–34) and calcitonin immunoradiometric assay kits (Immutopics). Serum phosphorus levels were determined using a blood chemistry analyzer (AU 400; Olympus, Melville, NY).

**Pharmacokinetics.** A separate study was performed to evaluate the pharmacokinetics of cinacalcet HCl over the dose range used in the pharmacology study. Doses of 1, 10, and 36 mg/kg were administered to male Sprague-Dawley rats, and samples for pharmacokinetic analysis were taken for up to 48 h postdose. Plasma concentrations of cinacalcet HCl (free base) were measured by a validated liquid chromatography coupled with mass spectrometry assay. The lower limit of quantitation was 10 ng/ml. Cinacalcet plasma concentration-time data were analyzed by noncompartmental methods using WinNonlin Professional version 3.1 (Pharsight, Mountain View, CA).

**Statistical Analysis.** To determine EC$_{50}$ or IC$_{50}$ values, concentration-response data were fit to the measured cytoplasmic Ca$^{2+}$ concentration or PTH level with the Levenberg-Marquardt algorithm by using the KaleidaGraph program (Synergy Software, Reading, PA). For calcitonin, curve fitting determination of EC$_{50}$ values were performed using GraphPad Prism software (GraphPad Software Inc., San Diego, CA). The curve fits and EC$_{50}$ values were determined using Prism software's "0 to top, variable slope" curve fit algorithm. The serum PTH, calcitonin, phosphorus, and blood ionized Ca$^{2+}$ levels from the in vivo studies were analyzed by repeated measures analysis of variance, followed by a post hoc test. All results were expressed as the mean ± S.E.M.

**Results**

**Mechanism of Action of Cinacalcet.** To determine the potencies of the enantiomers of cinacalcet HCl, concentration-response relationships were assessed using measurements of [Ca$^{2+}$]. Both enantiomers of cinacalcet HCl evoked concentration-dependent increases in [Ca$^{2+}$] in HEK 293 cells expressing the Ca$^{2+}$ receptor (HEK 293 4.0-7 cells) but not in wild-type cells (Figs. 2 and 3). The effects of cinacalcet HCl on cytoplasmic Ca$^{2+}$ were stereoselective, and cinacalcet HCl (an R-enantiomer) was about 75 times more potent than its corresponding S-enantiomer, AMG S-073 (Fig. 2). The EC$_{50}$ value was 51 nM for cinacalcet HCl and 3.8 μM for AMG S-073.

To assess the relative contributions of extracellular and intracellular sources of Ca$^{2+}$ to the increased [Ca$^{2+}$], induced by cinacalcet HCl, HEK 293 4.0-7 cells were pretreated with a low concentration of La$^{3+}$ (1 μM). This concentration of La$^{3+}$ is sufficient to block the influx of extracellular Ca$^{2+}$, but it is below the minimal concentration required to activate the CaR (Nemeth et al., 1998). Pretreatment with La$^{3+}$ has no effect on the initial rapid, transient increase in [Ca$^{2+}$], but it reduces the long-lasting plateau of increased [Ca$^{2+}$] elicited by extracellular Ca$^{2+}$ (Nemeth and Scarpa, 1987). In the presence of 1 μM La$^{3+}$, the initial rapid and transient increase in [Ca$^{2+}$], evoked by cinacalcet HCl persisted, whereas the subsequent phase returned to baseline levels much faster than in cells not treated with La$^{3+}$ (Fig. 3). Neither R- nor S-enantiomers of cinacalcet HCl altered [Ca$^{2+}$] in wild-type HEK 293 cells, even when tested at concentrations as high as 10 μM (Fig. 3).

Reducing the level of extracellular Ca$^{2+}$ by the addition of EGTA eliminated the stimulatory effect of cinacalcet HCl on [Ca$^{2+}$] (Fig. 4). Increasing the concentration of extracellular magnesium to 3 mM still elicited an increase in [Ca$^{2+}$], under these conditions (Fig. 4). These findings demonstrate that cinacalcet HCl causes the mobilization of intracellular Ca$^{2+}$ and that this effect was dependent on the presence of extracellular Ca$^{2+}$ or some substitute inorganic cation.

The effects of cinacalcet HCl on Ca$^{2+}$ responses elicited by various concentrations of extracellular Ca$^{2+}$ were examined...
using fluorescence imaging plate reader. In the presence of 10 or 100 nM cinacalcet HCl, the concentration-response relationship for extracellular Ca\textsuperscript{2+} was shifted to the left, with a greater shift occurring with 100 nM cinacalcet HCl treatment; the maximal response to extracellular Ca\textsuperscript{2+} was not altered by either concentration of cinacalcet HCl (Fig. 5). The EC\textsubscript{50} for extracellular Ca\textsuperscript{2+} changed from 0.87 ± 0.01 to 0.74 ± 0.01 mM or 0.58 ± 0.01 mM in the presence of 10 or 100 nM cinacalcet HCl, respectively. At low levels of extracellular Ca\textsuperscript{2+} (≤0.5 mM), both concentrations of cinacalcet HCl failed to elicit an increase in [Ca\textsuperscript{2+}]\text{\textsubscript{i}} (Fig. 5).

Effects of Cinacalcet on Secretion of PTH and Calcitonin in Vitro. Bovine parathyroid cells were incubated for 20 min with various concentrations of cinacalcet HCl or vehicle control, and the secretion of PTH was assessed by radioimmunoassay. The addition of cinacalcet HCl (3 nM–1 μM) to cells bathed in buffer containing 0.5 mM Ca\textsuperscript{2+} caused a concentration-dependent decrease in PTH secretion (Fig. 6) with an IC\textsubscript{50} of 27 ± 9 nM (n = 3). In contrast, no inhibition of PTH secretion was observed using AMG S-073 up to a concentration of 10 μM (Fig. 6).

In a second series of experiments, parathyroid cells were incubated in buffer containing varying amounts of extracellular Ca\textsuperscript{2+} with or without 10 or 100 nM cinacalcet HCl. Increasing the concentration of extracellular Ca\textsuperscript{2+} from 0.1 to 2 mM inhibited PTH secretion by 80% with a half-maximal effect [IC\textsubscript{50}] at 1.01 ± 0.03 mM. In the presence of cinacalcet HCl, the concentration-response curve for extracellular Ca\textsuperscript{2+} was shifted to the left, but the magnitude of the secretory response obtained at low or high concentrations of extracellular Ca\textsuperscript{2+} was not altered (Fig. 7). The IC\textsubscript{50} value for extracellular Ca\textsuperscript{2+} in the absence of cinacalcet HCl was 1.01 mM.
and was lowered to 0.6 ± 0.02 or 0.41 ± 0.03 mM in the presence of 10 or 100 nM cinacalcet HCl, respectively (Fig. 7).

When normalized to cyclophilin, RNase protection assay analysis revealed significantly higher levels of CaR mRNA in the parathyroid compared to kidney, whereas the levels of CaR mRNA in MTC 6-23 cells were low but detectable. The levels of CaR mRNA in rat parathyroid were approximately 100-fold greater than in MTC 6-23 cells (Fig. 8).

Extracellular Ca$^{2+}$ produced a concentration-dependent increase in calcitonin release from MTC 6-23 cells, with an EC$_{50}$ of approximately 2.0 mM (Table 1). Cinacalcet HCl (10–1000 nM) produced a concentration-dependent shift in the potency of Ca$^{2+}$ to stimulate calcitonin release. At the highest concentration of cinacalcet HCl tested, a 2-fold increase in the potency of Ca$^{2+}$ was observed (Table 1). Cinacalcet HCl did not stimulate calcitonin release in the absence of added Ca$^{2+}$ and did not increase the maximal response to Ca$^{2+}$. The concentration of cinacalcet HCl that produced a half-maximal shift in the Ca$^{2+}$ concentration-response curve was estimated to be 34 nM. At a concentration of 1 μM, no measurable increase in the potency of Ca$^{2+}$ to stimulate calcitonin secretion from MTC 6-23 cells was observed using AMG S-073 (Fig. 9).

**TABLE 1**

<table>
<thead>
<tr>
<th>Cinacalcet HCl</th>
<th>Ca$^{2+}$ EC$_{50}$</th>
<th>nM</th>
<th>mM</th>
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<tr>
<td>0</td>
<td>2.03 ± 0.17</td>
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<tr>
<td>10</td>
<td>1.63 ± 0.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>1.07 ± 0.1*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1000</td>
<td>0.90 ± 0.06*</td>
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Maximum stimulation was defined as the difference in calcitonin release between stimulated (5.0 mM extracellular Ca$^{2+}$) and unstimulated (0.07 mM extracellular Ca$^{2+}$).

*p < 0.05 vs. 0 nM cinacalcet HCl.
(\(p < 0.001\)) reduction in blood ionized \(Ca^{2+}\) levels was observed in drug-treated animals compared with vehicle-treated animals as early as 1 h after drug treatment. At this dose, a statistically significant \((p < 0.001)\) reduction in blood-ionized \(Ca^{2+}\) was also observed 2 h after drug treatment, but after 4 h the effect of the drug was no longer observed. A dose of 3 mg/kg cinacalcet HCl elicited a greater suppressive effect than the 1-mg/kg dose, producing a statistically significant \((p < 0.02)\) effect at 4 h postdosing. At a dose of 10 mg/kg, cinacalcet HCl produced a statistically significant \((p < 0.001)\) reduction in blood-ionized \(Ca^{2+}\) levels at all time points up to and including 8 h postdrug treatment, and a dose of 30 mg/kg produced a statistically significant reduction in blood-ionized \(Ca^{2+}\) levels for up to 24 h after drug treatment (Fig. 10B). In contrast, oral administration of AMG S-073 (10 mg/kg) had no statistically significant effect on serum PTH and blood-ionized \(Ca^{2+}\) levels compared with animals treated with vehicle (data not shown).

Oral administration of cinacalcet HCl (30 mg/kg) or vehicle meditated a transient decrease in serum phosphorus levels within the first 4 h. By 8 h postdosing, a significant \((p < 0.05)\) elevation in serum phosphorus levels was observed in the cinacalcet HCl-treated animals, whereas the serum phosphorus levels from vehicle-treated animals had returned to the prevehicle baseline (Fig. 11). The elevation in serum phosphorus levels mediated by cinacalcet HCl returned to baseline 36 h postdose.

In a separate study, serum PTH and calcitonin levels were measured after oral administration of various doses of cinacalcet HCl or vehicle. At the highest dose (40 mg/kg), cinacalcet HCl caused a rapid increase in serum calcitonin levels that paralleled the decrease in serum levels of PTH. Serum

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**Fig. 9.** MTC 6-23 cells were incubated in buffer containing 1.5 mM \(Ca^{2+}\) in the presence or absence of the indicated concentration of cinacalcet HCl (\(\bullet\)) or AMG S-073 (\(\downarrow\)). A separate portion of cells was incubated with 5 mM \(Ca^{2+}\) (\(\square\)) to determine maximum calcitonin release. Each point is the mean \(\pm\) S.E.M. of three separate experiments.

**Fig. 10.** Serum levels of PTH (A) and blood-ionized \(Ca^{2+}\) (B) in normal rats administered orally with vehicle (x) or cinacalcet HCl at doses of 1 (\(\bullet\)), 3 (\(\square\)), 10 (\(\triangle\)), or 30 (\(\bullet\)) mg/kg. Values represent mean \(\pm\) S.E.M., \(n = 5\)/group. *, \(p < 0.05\) versus vehicle (see text).
calcitonin levels returned to baseline by 8 h after dosing, although at the higher doses, serum PTH levels were still depressed at this time (Fig. 12). The values of serum calcitonin and PTH at 30 min after dosing were used to construct dose-response relationships and estimates of potency. The EC50 value for cinacalcet HCl for stimulating calcitonin secretion was 16 mg/kg, whereas the IC50 for lowering serum levels of PTH was 0.5 mg/kg.

**Pharmacokinetics in the Rat.** Cinacalcet HCl freebase (cinacalcet) concentration-time profiles after oral administration of cinacalcet HCl are displayed in Fig. 13. Consistent with the increasing effect of cinacalcet HCl, with increasing dose of cinacalcet, concentrations increased approximately proportionally to dose over the dose range of 1 to 36 mg/kg. Maximal serum concentrations were generally attained 1.5 to 3 h postdose, which roughly corresponds to the time at which maximal PTH suppression was achieved. Mean maximal concentrations were 18.1, 72.6, and 124 ng/ml for the 1-, 10-, and 36-mg/kg dose groups, respectively.

**Discussion**

The results obtained in the present study are comparable with those described previously for NPS R-568 (Nemeth et al., 1998) and indicate that cinacalcet HCl acts as a stereoselective type II calcimimetic compound with preferential activity at the parathyroid cell CaR. The evidence supporting this derives from several distinct in vitro and in vivo assays. Cinacalcet HCl and its S-enantiomer increase [Ca2+]i in HEK 293 cells expressing the human receptor but not in wild-type HEK 293 cells. Thus, the expression of the CaR is necessary for cinacalcet HCl to increase [Ca2+]i in these cells. Wild-type HEK 293 cells express receptors for ATP, bradykinin, and thrombin that are coupled to the mobilization of intracellular Ca2+. The failure of cinacalcet HCl to elicit changes in [Ca2+]i in wild-type HEK 293 cells indicates that it does not activate these receptors or the transmembrane signaling mechanisms leading to the mobilization of intracellular Ca2+. Although cinacalcet HCl was not specifically tested for inhibitory effects on these receptors, other compounds in this structural class, such as NPS R-467 and NPS R-568, do not act on these receptors nor do they affect the activity of receptors structurally homologous to the CaR, such as metabotropic glutamate receptors or γ-aminobutyric acid receptors.

Cinacalcet HCl acts stereoselectively to increase [Ca2+]i in HEK 293 4.0-7 cells and to inhibit PTH secretion in bovine
parathyroid cells. In HEK 293 cells expressing the CaR, cinacalcet HCl is 75-fold more potent than its corresponding S-enantiomer at increasing $[\text{Ca}^{2+}]_i$. In bovine parathyroid cells, an estimate of the potency difference was not obtained although it is clear that cinacalcet HCl again acted stereoselectively because it inhibited PTH secretion with an IC$_{50}$ value of 27 nM yet the S-enantiomer was without effect even when tested at a concentration as high as 1 $\mu$M.

The stimulatory effect of cinacalcet HCl on $[\text{Ca}^{2+}]_i$ in HEK 293 4.0-7 cells was not affected by blocking the influx of extracellular Ca$^{2+}$ (with La$^{3+}$) but was eliminated by removal of extracellular Ca$^{2+}$ (with EGTA). These findings are consistent with the mechanism of action proposed for type II calcimimetics that act as positive allosteric modulators of the CaR (Nemeth et al., 1998). In contrast, type I calcimimetics, which are mostly inorganic or organic polycations (Nemeth and Fox, 1999), are agonists of the CaR and increase $[\text{Ca}^{2+}]_i$, even in the absence of extracellular Ca$^{2+}$. Type II calcimimetics do not affect CaR-mediated responses in the absence of a type I calcimimetic. Thus, calcimimetics such as cinacalcet HCl shift the concentration-response curves for extracellular Ca$^{2+}$ to the left and increase the sensitivity of the CaR to activation by extracellular Ca$^{2+}$. This is clearly shown in Figs. 5 and 7 for extracellular Ca$^{2+}$-induced increases in $[\text{Ca}^{2+}]_i$ and inhibition of PTH secretion. In either case, cinacalcet HCl lowers the concentration of extracellular Ca$^{2+}$ required to affect a cellular response.

In mammals, calcitonin is secreted primarily by parafollicular cells of the thyroid gland (C-cells), inhibits osteoclastic bone resorption in vitro, and has a serum Ca$^{2+}$-lowering effect in vivo (Defos et al., 1999). C-cells of the thyroid are known to express the CaR (Garrett et al., 1995a,b). In addition, the present studies demonstrate the presence of CaR on MTC-6-23 cells, a clonal cell line derived from a spontaneously occurring rat medullary thyroid carcinoma (Zeytinoğlu et al., 1980). These cells release calcitonin in response to increasing extracellular concentrations of Ca$^{2+}$ and clearly show cinacalcet HCl-induced potentiation of Ca$^{2+}$-stimulated calcitonin release. The increased release of calcitonin by cinacalcet HCl is consistent with previous studies using MTC 44-2 cells and the type II calcimimetics NPS R-568 (Garrett et al., 1995b) or NPS R-467 (Lavigne, 1998).

In the present studies, the increase in calcitonin release occurred over the same concentration range of cinacalcet HCl associated with increases in $[\text{Ca}^{2+}]_i$ and reductions in PTH secretion. Overall, the three cellular systems used generated similar potency estimates for cinacalcet HCl. However, in vivo studies revealed that the potency of cinacalcet HCl to reduce the plasma level of PTH was considerably greater than its ability to increase calcitonin levels, even though the nucleotide sequence of the coding region of the CaR is identical in parathyroid cells and thyroid C-cells (Garrett et al., 1995a). These findings for cinacalcet HCl are consistent with previous in vivo studies using NPS R-568 (Fox et al., 1999b) or NPS R-467 (Lavigne et al., 1998). The possible mechanisms underlying the preferential effects of these particular compounds on serum levels of PTH have been considered previously (Nemeth, 1996). These compounds are prime examples of compounds showing conditional efficacy (Kenakin, 2003) and demonstrate that the same receptor genotype can have different pharmacological phenotypes depending on the cellular environment and explains the predominant effect of PTH rather than calcitonin in vivo.

Oral administration of cinacalcet HCl to normal rats caused a rapid decrease in serum levels of PTH, the magnitude and duration of which was dose- and concentration-dependent. The decrease in plasma levels of PTH was accompanied by a hypocalcemic response in rats. The pharmacological activity (inhibition of PTH secretion) observed at the plasma concentrations of cinacalcet achieved is generally consistent with the in vitro potency data. It has been previously shown using NPS R-568 that plasma levels of Ca$^{2+}$ persist in acutely nephrectomized animals, suggesting that mechanism for the observed hypocalcemia is not mediated through CaR located on the kidney (Fox et al., 1999a). As reported here as well as by other investigators, calcimimetics cause a transient increase in serum levels of calcitonin, in rats, that contributes to the rate of onset of the hypocalcemic response (Lavigne et al., 1998; Fox et al., 1999b). As pointed out by Nemeth and colleagues, calcimi-
metrices depress serum PTH levels at doses that are at least 10 times lower than those that increase plasma calcitonin in rodents, which is generally consistent with the present findings for cinacalcet HCl (Fox et al., 1999b; Nemeth and Fox, 1999). The kidney plays a dominant role in systemic phosphorus homeostasis. Normally, an acute increase in serum phosphorus concentration produces a transient decrease in the concentration of blood ionized Ca$^{2+}$ and stimulates PTH secretion, which reduces phosphate reabsorption in the proximal tubule and leads to a readjustment in serum phosphorus concentrations. Therefore, it is not surprising that rats with an intact or partially intact renal system treated with cinacalcet HCl have an increase in serum phosphorus levels. A cinacalcet HCl-mediated decrease in serum PTH would cause an increase in phosphate reabsorption, resulting in a decrease in phosphorus excretion and higher serum phosphorus levels. Consistent with the present studies, it was noted that serum phosphorus levels were higher in animals treated with NPS R-568 (Fox et al., 1999a). However, clinical studies in patients with secondary HPT of end-stage renal disease have demonstrated that administration of cinacalcet HCl did not exacerbate hyperphosphatemia but instead tended to lower serum phosphorus values. This is presumably due to a renal system that is totally nonfunctional in these patients (Quarles et al., 2003; Lindberg et al., 2003). Hyperphosphatemia and elevated Ca$^{2+}$ × phosphorus levels are prevalent in this population and indicate risk of coronary artery disorders and mortality (Llach 1999; Goodman et al., 2000b; Ganesh et al., 2001). Therefore, a therapy capable of reducing serum Ca$^{2+}$, phosphorus, and Ca$^{2+}$ × phosphorus product as well as PTH could represent a significant therapeutic advance for end-stage renal disease patients.

Cinacalcet HCl potentially provides a novel means of controlling serum levels of PTH in disease states. By decreasing PTH secretion through modulation of the CaR, cinacalcet HCl is a new therapeutic approach with potential for treating both primary and secondary hyperparathyroidism. Recent clinical studies demonstrate that once-daily oral doses of cinacalcet HCl in study patients result in dose-dependent decreases in PTH with concomitant reduction in the Ca$^{2+}$ × phosphorus product when administered to hemodialysis patients with secondary HPT (Lindberg et al., 2003; Quarles et al., 2003). Thus, cinacalcet HCl may offer significant advantages over current therapies (primarily vitamin D sterols) for the treatment of secondary HPT.

References


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