L-Amino Acids Regulate Parathyroid Hormone Secretion*

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Parathyroid hormone (PTH) secretion is acutely regulated by the extracellular Ca\(^{2+}\)-sensing receptor (CaR). Thus, Ca\(^{2+}\) ions, and to a lesser extent Mg\(^{2+}\) ions, have been viewed as the principal physiological regulators of PTH secretion. Herein we show that in physiological concentrations, L-amino acids acutely and reversibly activated the extracellular Ca\(^{2+}\)-sensing receptor in normal human parathyroid cells and inhibited parathyroid hormone secretion. Individual L-amino acids, especially of the aromatic and aliphatic classes, as well as plasma-like amino acid mixtures, stereoselectively mobilized Ca\(^{2+}\) ions in normal human parathyroid cells in the presence but not the absence of the CaR agonists, extracellular Ca\(^{2+}\) (Ca\(^{2+}\))o, or spermine. The order of potency was L-Trp > L-Phe > L-His > L-Ala > L-Glu > L-Arg = L-Leu. CaR-active amino acids also acutely and reversibly suppressed PTH secretion at physiological ionized Ca\(^{2+}\) concentrations. At a Ca\(^{2+}\)o of 1.1 mM and an amino acid concentration of 1 mM, CaR-active amino acids (L-Phe = L-Trp > L-His = L-Ala), but not CaR-inactive amino acids (L-Leu and L-Arg), stereoselectively suppressed PTH secretion by up to 40%, similar to the effect of raising Ca\(^{2+}\)o to 1.2 mM. A physiologically relevant increase in the -fold concentration of the plasma-like amino acid mixture (from 1x to 2x) also reversibly suppressed PTH secretion in the Ca\(^{2+}\)o concentration range 1.05-1.25 mM. In conclusion, L-amino acids acutely and reversibly activate endogenous CaR receptors and suppress PTH secretion at physiological concentrations. The results indicate that L-amino acids are physiological regulators of PTH secretion and thus whole body calcium metabolism.

Extracellular Ca\(^{2+}\) ions are recognized as the principal physiological regulators of parathyroid hormone secretion acting to close a classical endocrine feedback loop, whereby PTH1 elevates Ca\(^{2+}\)o, and elevated Ca\(^{2+}\)o in turn, suppresses PTH secretion. A major insight into the mechanism by which Ca\(^{2+}\)o interacts with parathyroid cells was gained with the cloning of the extracellular Ca\(^{2+}\)-sensing receptor (CaR) (1). This receptor, which belongs to subgroup C of the G-protein-coupled receptor superfamily, mediates the Ca\(^{2+}\)o-stimulated activation of intracellular signaling pathways in parathyroid cells leading to the activation of various enzymes including phosphatidyl inositol-specific phospholipase C and attendant intracellular Ca\(^{2+}\) mobilization (for reviews, see Refs. 2 and 3). Targeted deletion of the CaR in the CaR null mouse eliminates feedback control of PTH secretion and results in a severe form of neonatal hyperparathyroidism (4). A similar condition in humans, neonatal severe hyperparathyroidism, has been shown to arise in homozygotes or compound heterozygotes with inactivating mutations of the CaR (5) (for review see Ref. 3).

Molecular analysis of the CaR indicates that it is composed of several functional domains that are conserved across related members of subgroup C. The N-terminal Venus Fly Trap domains of many of these receptors recognize amino acids (especially glutamate) or the amino acid analog, γ-aminobutyric acid. More recent work indicates that several cloned members of the family are also broad-spectrum amino acid sensors. In mammals, these include the CaR, which is allosterically activated by aromatic, aliphatic, and polar amino acids as well as plasma-like amino acid mixtures (6) and a heterodimeric amino acid taste receptor, which has broad selectivity for aliphatic, polar, and charged amino acids but not aromatic amino acids (7). In human embryonic kidney (HEK)293 cells that stably express the human CaR, L-amino acids markedly enhanced the sensitivity of the receptor to Ca\(^{2+}\)o and other cationic agonists including spermine and Gd\(^{3+}\) (6).

Because the CaR mediates the acute control of PTH secretion, the finding that it is allosterically activated by L-amino acids raises the possibility that PTH secretion is acutely regulated not only by adjustments in extracellular Ca\(^{2+}\)o but also by physiological changes in amino acid concentration. We have tested and confirmed this hypothesis in the current study on normal human parathyroid cells. The data indicate that L-amino acids and plasma-like mixtures of L-amino acids allosterically activate endogenous parathyroid CaRs contributing to the control of intracellular signaling pathways and PTH secretion. In the presence of physiological concentrations of extracellular Ca\(^{2+}\), L-amino acids (also at physiological concentrations) stereoselectively activated Ca\(^{2+}\) mobilization and inhibited PTH secretion. The data support the view that fluctuations in serum amino acid levels acting via the CaR acutely regulate PTH secretion and thus whole body calcium metabolism.
**EXPERIMENTAL PROCEDURES**

### Origin of Tissue and Preparation of Human Parathyroid Cells—
#### Samples of normal human parathyroid transplants were obtained at necropsy at the Royal North Shore Hospital, St. Leonards, New South Wales, Australia and the Mater Private Hospital, North Sydney, New South Wales, Australia under guidelines established by the relevant institutional ethics committees. The parathyroid tissue, typically taking the form of a single 1.5-1.5 mm diameter “chip,” was transported to the laboratory in ice-cold Hank’s balanced salt solution (Invitrogen) containing CaCl2 1.25 mM. It was either used immediately or stored overnight at 4 °C in MEM (number 11380-037, Invitrogen). The tissue was then transferred into 10 ml of MEM that contained 1 mg/ml collagenase (type I, Worthington, Scimar, Victoria) and 0.2 mg/ml DNase I (type IV, Sigma). The composition of the MEM formulation was as follows: NaCl 137 mM, KCl 5.4 mM, NaHCO3 4.2 mM, CaCl2 1.2 mM, MgSO4 0.81 mM, Na-HPO4 0.34 mM, KH2PO4 0.44 mM, d-glucose 1 g/liter, Phenol Red 10 mg/liter, MEM amino acids, and vitamins. Collagenase was then incubated at –80 °C and brought out for weighing on ice or dry ice. The suspension was briefly oxygenated and then incubated at 37 °C. After 20 min, the enzyme solution was decanted and the parathyroid tissue was transferred into 5 ml of MEM that contained 1 mg/ml of bovine serum albumin (protease-free, Sigma A-3059). The tissue was then triturated by repeated passage (10–15 times) through the tip of a disposable 5-ml syringe (no needle attached). The cloudy suspension containing clumps of parathyroid cells was passed through a 200-μm pore-size nylon filter and then sedimented (Sorvall H1000B rotor, 100 g, 2 min). The cell pellet was gently resuspended and washed twice in 5 ml of bovine serum albumin-containing MEM (Invitrogen, 100 g, 2 min). The cell suspension was finally resuspended in bovine serum albumin-containing MEM. The remaining pieces of undisguised parathyroid tissue were returned to medium containing collagenase and DNase I and incubated for a further 20 min at 37 °C. The enzyme solution was then decanted, and the parathyroid tissue subjected to trituration and centrifugal isolation as above. In general, the second cell harvest was used for analyses of cell function because of greater cell yields; however, both the first and second cell harvests were also frequently combined.

### Amino Acid Solutions—
#### Stock amino acid-containing solutions were routinely made up in physiological saline at 100 or 200 mM with the exception of tryptophan (50 mM because of poor solubility). The amino acid composition of the 1x basal amino acid solution that emulated fasting human amino acids (all L-isomers in physiological saline solution, pH 7.4) was as follows (in μM): 50 Phe, 50 Trp, 80 His, 60 Tyr, 300 Ala, 200 Thr, 30 Cys, 50 Asn, 600 Gln, 125 Ser, 30 Glu, 250 Gly, 180 Pro, 250 Val, 30 Met, 10 Asp, 200 Lys, 150 Arg, 75 Ile, and 150 Leu. Stocks of this basal amino acid solution (40-fold concentrated in amino acids) were made up in physiological saline solution and stored at –20 °C. This stock solution was diluted in an amino acid-buffered physiological saline solution, as required. The control physiological saline used in all microfluorometry experiments had the following composition: 125 mM NaCl, 4.0 mM KCl, 0.2 or 0.5 mM CaCl2, 0.5 mM MgCl2, 20 mM HEPES (NaOH), 0.1% d-glucose, pH 7.4.

### Microfluorimetry for Determining Cytosolic Ca2+ Concentration—
#### Parathyroid cells were loaded with fura-2/AM (1 μM, 20 min, 37 °C) in physiological saline solution containing bovine serum albumin 1 mg/ml. The cells were then sedimented (Sorvall H-1000B rotor, 100 g, 2 min) and resuspended in albumin-free physiological saline solution. Fura-2/AM-loaded cells were transferred into a superfusion chamber and placed in the light path of a Nikon Diaphot microscope as described previously (8). Excitation was performed at alternating wavelengths (340 and 380 nm), the fluorescent light was detected (peak 510 nm) was detected by a photomultiplier, and its digitized recording was achieved using Acknowledge software for Macintosh. The control superfusion solution had the following composition: 125 mM NaCl, 4.0 mM KCl, 0.2 or 0.5 mM CaCl2, 0.5 mM MgCl2, 20 mM HEPES (NaOH), 0.1% d-glucose, pH 7.4. Data for cytoplasmic free Ca2+ concentrations were expressed either as uncorrected excitation ratios (P340/P380) or converted to ionized Ca2+ concentration using a calibration procedure (8).

### Determination of PTH Secretion—
#### Perfusion of normal human parathyroid cells was undertaken in low molecular mass (4-5 kDa) cut-off gel filtration media so that intact PTH (9 kDa) would appear in the void volume. Gel filtration media were pre-equilibrated with physiological saline that contained 1x basal amino acid mixture and 1 mg/ml bovine albumin (protease-free, Sigma A-2905). 20,000–50,000 cells were loaded onto the surface of a 1-ml bed volume of Bio-Gel P-4 (nominal exclusion limit 4 kDa) and then gently covered with a 1-ml bed volume of Sephadex G-25 (medium, nominal exclusion limit 5 kDa) in a small perfusion column. After tubing connections were established downstream to a peristaltic pump and upstream to a reservoir, the column was suspended in a water bath (37 °C) and perfused at 1.5 ml/min with 37 °C equilibrated control physiological saline, 125 mM NaCl, 4.0 mM KCl, 1.25 mM CaCl2, 1.0 mM MgCl2, 0.8 mM NaH2PO4, 20 mM HEPES (NaOH, pH 7.4), 0.1% d-glucose, 1 mg/ml bovine albumin, 1x basal amino acid mixture. Prior to starting all experiments, the columns were perfused for a 20-min control period. Routinely, 2-min (i.e., 3-ml) samples were collected into tubes immersed in an ice bath, and the tubes were transferred to dry ice upon completion of each collection period. As required, solutions were changed to permit variations in extracellular calcium or amino acid concentration. All samples were stored at –80 °C until the analysis of intact human PTH using an Immulite 2000 autoanalyzer (Diagnostic Products Corporation, CA).

### Statistical Analysis and Curve-Fitting—
#### The Ca2+ mobilization data were expressed as concentration-response curves and fitted to the following form of the Hill equation, $R = b + (a - b) [C/(C + C_0)]$, where $R$ = fractional response, $a$ = maximum fractional response, $b$ = basal fractional response, $C$ = extracellular Ca2+ concentration (in mM), $e$ = EC50, (the concentration of Ca2+ that induced a half-maximal response), and $n$ = Hill coefficient.

### RESULTS

#### Effects of Individual Amino Acids on Cytosolic Free Ca2+ Concentration and Sensitivity to Ca2+—
#### Exposure of Fura-2/AM-Loaded normal human parathyroid cells to active amino acids including L-Arg (0.1–10 mM) at an extracellular Ca2+ concentration ([Ca2+]o) below 0.2 mM had no effect on cytosolic free Ca2+ concentration [Ca2+]i (not shown). However, at [Ca2+]o > 0.2 mM, L-Arg (0.1–10 mM) acutely elevated [Ca2+]i (Fig. 1A), and similar effects were observed with L-Trp (Fig. 1B), L-His, and L-Ala (not shown) but not L-Ile, L-Leu (Fig. 1B), or L-Arg (not shown). CaR-active amino acids exhibited similar effects in the presence of the polycationic CaR agonist, spermine (not shown). The effects of amino acids on intracellular Ca2+ mobilization were stereoselective i.e. L-amino acids were much more effective than D-amino acids (Fig. 1). In addition, the effects of L-amino acids were concentration-dependent (Table I, Fig. 2). At a Ca2+ concentration of 2.0 mM, the apparent EC50 for L-Trp, L-Phe, L-His, and L-Ala were 0.04 ± 0.01 mM, 0.06 ± 0.01 mM, 0.1 ± 0.01 mM, and 0.41 ± 0.02 mM, respectively, and the order of potency was L-Trp > L-His > L-Ala > L-Glu > L-Leu = L-Arg (Table I). Individual l-amino acids were also effective at lower extracellular Ca2+ concentrations (1.5 and 1.0 mM); however, the EC50 values increased progressively, and the amino acid-induced maximum responses fell progressively as the Ca2+ concentration fell (Fig. 2, C and D, Table I). An appreciation of the physiological significance of these effects can be gained by dividing the fasting plasma concentration of an amino acid by its EC50 value (conce/EC50). At 2.0 mM Ca2+o, four of the seven amino acids tested, L-Trp, L-Phe, L-His, and L-Ala all exhibited conce/EC50 ratios close to 1.0. At 1.5 and 1.0 mM Ca2+o, the apparent effectiveness of these individual amino acids fell progressively (Table I). Between 1.0 and 1.5 mM Ca2+o, encompassing the normal physiological range, the data indicate that physiologically significant modulation of the CaR could only arise if multiple CaR-active amino acids acted together in concert.
Effects of Individual Amino Acids and Plasma-like Amino Acid Mixtures on Cytoplasmic Free Ca\(^{2+}\) Mobilization. At 10 mM, both D-Trp and L-Trp also suppressed the sustained phase of the cytoplasmic free Ca\(^{2+}\) response as demonstrated in this record by the suppression in the base line upon the addition of D-Trp and transient overshoot upon the removal of L-Trp.

**TABLE I**
EC\(_{50}\) data for effects of various l-amino acids in fura-2/AM-loaded normal human parathyroid cells

| Amino acid | Ca\(^{2+}\) = 1.0 mM | Ca\(^{2+}\) = 1.5 mM | Ca\(^{2+}\) = 2.0 mM |
|------------|---------------------|---------------------|---------------------|
| L-Trp      | 0.30 ± 0.08 (0.17)   | 0.13 ± 0.02 (0.38)   | 0.04 ± 0.01 (1.25)   |
| L-Phe      | 0.69 ± 0.17 (0.07)   | 0.12 ± 0.02 (0.42)   | 0.06 ± 0.01 (0.83)   |
| L-His      | 1.2 ± 0.6 (0.07)     | 0.28 ± 0.02 (0.29)   | 0.10 ± 0.01 (0.80)   |
| L-Ala      | 5.4 ± 0.1 (0.06)     | 0.81 ± 0.1 (0.37)    | 0.41 ± 0.02 (0.73)   |
| L-Glu      | 2.7 ± 0.2 (0.01)     | 1.8 ± 0.1 (0.02)     | 0.71 ± 0.04 (0.04)   |
| L-Leu      | NR                  | NR                  | 1.4 ± 0.2 (0.11)     |
| L-Arg      | NR                  | NR                  | 2.7 ± 0.3 (0.04)     |

* NR, no response up to an amino acid concentration of 10 mM.

Active L-amino acids also left-shifted the concentration response curves for extracellular Ca\(^{2+}\). In the absence of amino acids, the apparent EC\(_{50}\) for Ca\(^{2+}\) was 2.6 ± 0.2 mM (n = 4); in the presence of 3 mM L-Phe, the concentration response was markedly left-shifted, and the apparent EC\(_{50}\) for Ca\(^{2+}\) was 1.5 ± 0.2 mM (Fig. 3, n = 4). F-test analysis confirmed that the difference was statistically significant (F [1,28] = 30.5, p < 0.001). In addition, L-Phe induced an increase in the maximum response as determined by curve-fitting the cumulative fluorescence ratio data. In the absence of L-Phe, the maximum response was 1.08 ± 0.05, in the presence of 3 mM L-Phe, the maximum response was 1.33 ± 0.08 (F [1,28] = 18.7, p < 0.01). However, L-Phe had no effect on the basal fluorescence ratio at an extracellular Ca\(^{2+}\) of 0.2 mM (F [1,28] = 1.95, p > 0.1). Similar findings were obtained using another CaR-active amino acid, L-Ala, and L-Phe also left-shifted the concentration response curve for spermine (not shown).

Effects of Amino Acid Mixtures on Cytoplasmic Free Ca\(^{2+}\) Concentration and Sensitivity to Ca\(^{2+}\). To further assess the physiological significance of the above results, the effects of plasma-like amino acid mixtures were also examined. Plasma-like amino acid mixtures activated intracellular Ca\(^{2+}\) mobilization in a -fold concentration-dependent fashion (Fig. 4A) and markedly left-shifted the Ca\(^{2+}\) sensitivity of the fura-2/AM-loaded normal human parathyroid cells (Fig. 4B and C). In the absence of amino acids, the EC\(_{50}\) for Ca\(^{2+}\) was 2.6 ± 0.2 mM (n = 8). In the presence of a fasting plasma-like l-amino acid mixture (total amino acid concentration, 2.8 mM), the EC\(_{50}\) for Ca\(^{2+}\) was 1.6 ± 0.1 mM (n = 4, F [1,28] = 78, p < 0.001). As the -fold concentration was increased from 0.5 to 2×, encompassing the range normally described between protein restriction and high dietary protein intake in humans, respectively, a small apparent decrease in the EC\(_{50}\) for Ca\(^{2+}\) and an increase in the maximum response were observed. Under these conditions, the EC\(_{50}\) for Ca\(^{2+}\) dropped from 1.7 ± 0.2 mM (n = 4) to 1.6 ± 0.1 mM (n = 4), and the maximum response, in the form of a cumulative fluorescence ratio measured from baseline, increased from 0.62 ± 0.04 (n = 4) to 0.69 ± 0.04 (n = 4, Table II). To further evaluate whether receptor response is sensitive to physiologically relevant variations in amino acid concentration, the data were replotted as a function of -fold concentration (Fig. 4D). A threshold for the amino acid-dependent responses was observed at a Ca\(^{2+}\) of -0.5 mM. At both 1.0 and 1.5 mM Ca\(^{2+}\), i.e. either side of the normal range (1.1–1.3 mM), the receptor response increased as the -fold concentration rose above 0.5× (Fig. 4D).

**Effects of Individual Amino Acids and Plasma-like Amino Acid Mixtures on PTH Secretion**—In control experiments, normal human parathyroid cells were perfused with a physiological saline solution that contained a fasting plasma-like l-amino acid mixture (total concentration 2.8 mM) and 1 mg/ml bovine serum albumin. Under these conditions, the apparent IC\(_{50}\) for the effect of extracellular Ca\(^{2+}\) on the secretion of intact PTH was 1.12 ± 0.08 mM. A similar left-shift in the concentration response as determined by curve-fitting the cumulative fluorescence ratio data. In the absence of L-Phe, the maximum response was 1.33 ± 0.08 (F [1,28] = 18.7, p < 0.01). In the presence of 3 mM L-Phe, the maximum response was 1.08 ± 0.05, in the presence of 3 mM L-Phe, the maximum response was 1.33 ± 0.08 (F [1,28] = 18.7, p < 0.01). However, L-Phe had no effect on the basal fluorescence ratio at an extracellular Ca\(^{2+}\) of 0.2 mM (F [1,28] = 1.95, p > 0.1). Similar findings were obtained using another CaR-active amino acid, L-Ala, and L-Phe also left-shifted the concentration response curve for spermine (not shown).
concentration plateau. Prior to L-Phe, for example, the low Ca²⁺/HCl concentration plateau secretory rate (in fg min⁻¹ cell⁻¹, n = 4) was 1.95 ± 0.08; in the presence of 3 mM L-Phe, the base-line extracellular Ca²⁺ concentration was 0.2 mM. The arrowhead indicates the point of addition of 3 mM L-Phe in the presence of 0.2 mM Ca²⁺. B, concentration response curves generated from four experiments in which the data like those shown in (A) were obtained.

The effects of various individual amino acids were evaluated in cells exposed to a physiologically significant drop in extracellular Ca²⁺ concentration from 1.2 to 1.1 mM in the presence of the 1x amino acid mixture (Fig. 6, A and B, Table III). Reducing Ca²⁺₀ concentration in this manner resulted in a prompt 2-fold increase in intact PTH secretion. Exposure of cells to various amino acids (all 1.0 mM) under these conditions resulted in a rapid and reversible suppression of PTH secretion in the cases of the CaR-active amino acids L-Phe, L-Trp, L-His,

in the presence of 1.5 mM Ca²⁺. B, the effect of stepwise increments in L-Trp concentration in the presence of 1.5 mM Ca²⁺. C, concentration dependence of L-Phe-induced intracellular Ca²⁺ mobilization at various extracellular Ca²⁺ concentrations (1.0, 1.5, and 2.0 mM). D, concentration dependence of L-Trp-induced intracellular Ca²⁺ mobilization at various extracellular Ca²⁺ concentrations (1.0, 1.5, and 2.0 mM).
The minimum (min) and maximum (max) values refer to the change in the cumulative fluorescence ratio from baseline (i.e. in the presence of low extracellular Ca\(^{2+}\) concentration, 0.2 mM). All data were obtained as returned parameters using MacCurveFit for Macintosh and are expressed as mean ± S.E. The control data (fold concentration zero) were obtained in eight experiments. The remaining data (0.2–5×) were obtained in four experiments/fold concentration.

| Fold concentration | Min | Max | \(\text{EC}_{50}\) for Ca\(^{2+}\) \(\text{mM}\) | Hill coefficient |
|--------------------|-----|-----|----------------------------|-----------------|
| 0                  | 0.01 ± 0.02 | 0.61 ± 0.04 | 2.6 ± 0.2 | 2.4 ± 0.5 |
| 0.2×               | 0.01 ± 0.05 | 0.62 ± 0.07 | 2.2 ± 0.3 | 3.1 ± 1.2 |
| 0.5×               | 0.01 ± 0.04 | 0.62 ± 0.04 | 1.7 ± 0.2 | 4.0 ± 1.4 |
| 1×                 | 0.01 ± 0.02 | 0.66 ± 0.02 | 1.6 ± 0.1 | 4.9 ± 1.0 |
| 2×                 | 0.02 ± 0.04 | 0.69 ± 0.04 | 1.6 ± 0.1 | 4.3 ± 1.3 |
| 5×                 | 0.02 ± 0.06 | 0.73 ± 0.06 | 1.5 ± 0.2 | 4.4 ± 1.9 |

**DISCUSSION**

The data described in this study support the conclusion that physiologically relevant increases in the concentrations of L-amino acids acutely activate the extracellular Ca\(^{2+}\)-sensing receptor and reversibly inhibit PTH secretion from normal human parathyroid cells. These findings indicate that PTH secretion is under the acute physiological control of serum L-amino acid levels and provide a theoretical basis for a link between protein and calcium metabolism.

L-Amino acids, including L-Phe, L-Trp, and L-Ala but not L-Arg, L-Leu, and L-Ile stereoselectively activated intracellular Ca\(^{2+}\) mobilization from fura-2/AM-loaded normal human parathyroid cells in the presence of the CaR agonist Ca\(^{2+}\)\(_{\infty}\) (Figs. 1 and 2, Table I). In addition, CaR-active amino acids markedly enhanced the sensitivity of the receptor to Ca\(^{2+}\)\(_{\infty}\) (Figs. 3 and 4, Table II). The amino acid selectivity of these responses, L-Trp > L-His > L-Ala > L-Glu > L-Leu > L-Arg, closely resem-

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**Table II**

Effects of various fold concentrations of a plasma-like L-amino acid mixture on the Ca\(^{2+}\) sensitivity of fura-2/AM-loaded normal human parathyroid cells

| Fold concentration | Min | Max | \(\text{EC}_{50}\) for Ca\(^{2+}\) \(\text{mM}\) | Hill coefficient |
|--------------------|-----|-----|----------------------------|-----------------|
| 0                  | 0.01 ± 0.02 | 0.61 ± 0.04 | 2.6 ± 0.2 | 2.4 ± 0.5 |
| 0.2×               | 0.01 ± 0.05 | 0.62 ± 0.07 | 2.2 ± 0.3 | 3.1 ± 1.2 |
| 0.5×               | 0.01 ± 0.04 | 0.62 ± 0.04 | 1.7 ± 0.2 | 4.0 ± 1.4 |
| 1×                 | 0.01 ± 0.02 | 0.66 ± 0.02 | 1.6 ± 0.1 | 4.9 ± 1.0 |
| 2×                 | 0.02 ± 0.04 | 0.69 ± 0.04 | 1.6 ± 0.1 | 4.3 ± 1.3 |
| 5×                 | 0.02 ± 0.06 | 0.73 ± 0.06 | 1.5 ± 0.2 | 4.4 ± 1.9 |

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**Fig. 4.** Impact of various fold concentrations of a plasma-like amino acid mixture on intracellular Ca\(^{2+}\) mobilization and extracellular Ca\(^{2+}\) sensitivity. A, effect of increasing the fold concentration of a plasma-like amino acid mixture on cytoplasmic free Ca\(^{2+}\) concentration in the presence of physiological saline containing 1.25 mM Ca\(^{2+}\), basal amino acid 1× (baso) corresponds to the amino acid composition of fasting plasma. B, effect of stepwise increments in [Ca\(^{2+}\)]\(_{i}\) (from 0.2 to 8.0 mM) in the absence or presence of various fold concentrations of a fasting human plasma-like L-amino acid mixture including and L-Ala. These effects were stereoselective. Furthermore, the CaR-inactive amino acids L-Arg and L-Leu had no effect (Fig. 6, A and B, Table III).

An analysis of the effects of raising the fold concentration of the amino acid mixture from 1 to 2× was performed to evaluate the impact of a physiologically relevant variation in total serum amino acid level (Fig. 6C). The 2-fold concentration of the amino acid mixture reversibly suppressed PTH at all three Ca\(^{2+}\)\(_{\infty}\) concentrations tested. With respect to Ca\(^{2+}\)\(_{\infty}\) concentration, the inhibitory effects of the 2-fold amino acid mixture were as follows, 25 ± 4% at 1.05 mM, 36 ± 8% at 1.15 mM, and 34 ± 5% at 1.25 mM (n = 5). Paired t test analyses confirmed that the effects of basal amino acid 2× were statistically significant at all three Ca\(^{2+}\)\(_{\infty}\) concentrations (p = 0.01 for 1.05 mM, p = 0.02 for 1.15 mM, and p = 0.002 for 1.25 mM). These results indicate that the PTH secretion rate is highly sensitive to physiologically relevant fluctuations in serum amino acid concentration and that changes in total amino acid concentration inhibit PTH secretion at all levels of Ca\(^{2+}\)\(_{\infty}\) concentration within the normal range.
bles that described previously for the cloned human CaR stably expressed in HEK293 cells (6, 10). In addition, an L-amino acid mixture that emulated the composition of fasting human plasma also activated intracellular Ca\(^{2+}\) mobilization and enhanced the sensitivity of the receptor to extracellular Ca\(^{2+}\) (Fig. 4). A threshold extracellular Ca\(^{2+}\) concentration for the effects of plasma-like L-amino acid mixtures was detected around 0.5 mM (Fig. 4). The Ca\(^{2+}\) mobilization response to a change in fold concentration of the plasma-like L-amino acid mixture from 0.5 to 2\(^{\text{th}}\) (the range normally described between protein restriction and high protein intake) took the form of a small decrease in EC\(_{50}\) (from 1.7 to 1.6 mM) and an increase in maximal response. A decrease in the EC\(_{50}\) for Ca\(^{2+}\) of 0.1 mM would be expected to induce a physiologically significant reduction in the Ca\(^{2+}\) set-point (i.e. IC\(_{50}\)) for PTH secretion, because the normal range for ionized Ca\(^{2+}\) is extremely tight (1.1–1.3 mM). Furthermore, an increase in the maximum response of the receptor would be expected to lower the minimum level of PTH secretion observed at high Ca\(^{2+}\). The observed effects of CaR-active amino acids on the IC\(_{50}\) for Ca\(^{2+}\) and minimum level of PTH secretion (Figs. 5 and 6) are consistent with both predictions.

CaR-active amino acids including L-Phe, L-Trp, L-His, and L-Ala stereoselectively suppressed PTH secretion from normal human parathyroid cells that were perifused with physiological saline solution that contained a 1-fold mixture of L-amino acids that emulated normal fasting human plasma (11–13). CaR-inactive amino acids including L-Arg and L-Leu had no inhibitory effect (Fig. 6, A and B, Table III). Consistent with the concept that aromatic and aliphatic L-amino acids are allosteric activators of the CaR, L-Phe enhanced the Ca\(^{2+}\) concentration dependence of intact PTH secretion, lowering the IC\(_{50}\) for Ca\(^{2+}\) from 1.15 to 1.05 mM. The inhibitory effects of L-Phe and L-Trp (Fig. 5) as well as the inhibitory effect of raising the

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**Fig. 5.** Impact of elevated levels of L-Phe and L-Trp on PTH secretion from perifused parathyroid cells. Normal human parathyroid cells were perifused with physiological saline solution containing the 1× L-amino acid mixture and 1 mg/ml bovine serum albumin at 37 °C. The data in A and B are representative experiments showing acute responses to various concentrations of extracellular Ca\(^{2+}\) in the absence or presence of 3 mM L-Phe or 3 mM L-Trp, respectively. The data in C were derived from four L-Phe experiments and three L-Trp experiments. The experimental series in A and B were undertaken separately and exhibited an ~2-fold difference in the maximum secretion rate when corrected for cell number. Accordingly, the PTH secretion data in C were corrected to the percent of maximum. The symbols are as follows, open circles, pre- and post-controls in cells exposed to L-Phe; open triangles, pre- and post-controls in cells exposed to L-Trp; closed circles, L-Phe; and closed triangles, L-Trp. The control physiological saline solution contained the 1× amino acid mixture as well as bovine serum albumin (1 mg/ml).
FIG. 6. Impact of various amino acids and impact of an increase in the 10-fold concentration of the plasma-like amino acid mixture on PTH secretion. Normal human parathyroid cells were perfused with physiological saline solution containing the 1× L-amino acid mixture and 1 mg/ml bovine serum albumin at 37°C. The data (means ± S.E.) in A and B were derived from three experiments in each case and show acute responses to various amino acids (all 1.0 mM). The cells exhibited marked sensitivity to a small change in extracellular Ca²⁺ concentration (from 1.2 to 1.1 mM) as well as sensitivity to CaR-active L-amino acids (see also Table III). In C the effect of an elevation in the 10-fold concentration of the amino acid mixture from 1 to 2× and the reversibility of this effect is shown from data obtained in five experiments in which cells were exposed to repetitive stepwise changes in ionized Ca²⁺ concentrations 1.25, 1.15, 1.05, and 1.25 mM. Basal amino acid 1× (baa) corresponds to the amino acid composition of fasting plasma.
Amino acid PTH secretion (\% of control)  

| Amino acid | 1 mM | % of control |
|------------|------|--------------|
| Nil        | 101  | ± 4.9 (10)   |
| L-Phe      | 59.8 | ± 6.3 (6)    |
| L-Trp      | 65.1 | ± 6.0 (6)*   |
| L-Ala      | 76.3 | ± 7.3 (4)*   |
| L-His      | 79.7 | ± 4.5 (4)*   |
| L-Arg      | 91.2 | ± 5.7 (6)*   |
| L-Leu      | 102  | ± 7.8 (6)    |
| B-Phe      | 106  | ± 6.8 (6)*   |
| L-Trp      | 97.9 | ± 6.0 (6)    |
| L-Ala      | 83.3 | ± 7.8 (4)**  |
| L-His      | 88.7 | ± 5.9 (4)**  |

* p < 0.001.
** p < 0.01.
*** Not significant.

-10-fold concentration of the plasma-like amino acid mixture from 1 to 2× (Fig. 6) were observed in the physiological Ca\(^{2+}\) concentration range i.e. from 1.1 to 1.3 mM. The data indicate that amino acids are also effective in the wider pathophysiologi- cal range from ~0.8 to 1.5 mM and above.

In the current work the observed effects of amino acids are not simply pharmacological, because plasma-like amino acid mixtures as well as individual amino acids, are effective CaR activators (Figs. 4 and 6, Table II). The effects are also not simply “permissive,” because the response of the receptor was clearly submaximal in the presence of the 1× mixture (Figs. 4 and 6, Table II). The conclusion that physiologically relevant fluctuations in the plasma levels of amino acids modulate the Ca\(^{2+}\) sensitivity of CaR and parathyroid hormone secretion relies on the observed impact of variations in amino acid concentration in the context of a normal fasting amino acid mixture. Most important was the observed impact of an increase in the -fold concentration of the plasma-like amino acid mixture from 1 to 2×, which reversibly suppressed PTH secretion by 25–40% at all ionized Ca\(^{2+}\) concentrations tested in the range 1.05–1.25 mM (Fig. 6C). A -fold concentration change of this magnitude approximates what is observed in response to a protein-rich meal (11, 13–15) or between the 24-h trough and peak levels of the normal circadian rhythm (16, 17). In addition, the observed inhibitory effects of individual L-amino acids on PTH secretion in the presence of the 1× mixture (Figs. 5 and 6, Table III) indicate that there is a “reserve” amino acid-inducible sensitivity even under normal physiological conditions.

Links between protein and calcium metabolism have been previously identified including evidence that elevated dietary protein intake promotes urinary calcium excretion (18, 19) and reduced protein intake induces secondary hyperparathyroidism in the absence of changes in plasma-ionized Ca\(^{2+}\) concentration (20). The finding that the CaR in normal human parathyroid cells is modulated by physiologically relevant

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