Structural Requirements for Conserved Arginine of Parathyroid Hormone

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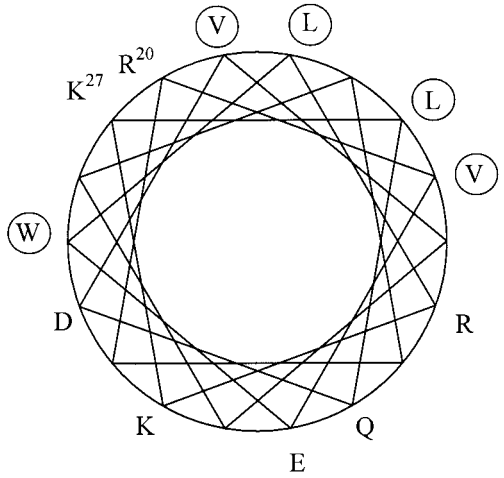
ABSTRACT: Arg-20 is one of two residues conserved in all peptides known to activate the parathyroid hormone (PTH) receptor. Previous studies have failed to find any naturally encoded analogues of residue 20 that had any adenylyl cyclase (AC) stimulating activity. In this work we have studied substitutions of Arg-20 with nonencoded amino acids and conformationally constrained analogues with side chains mimicking that of Arg. No analogue had more than 20% of the AC-stimulating ability of the natural Arg-20-bearing peptide. In descending order of activity, the most active analogues had (*S*)-4-piperidyl-(*N*-amidino)glycine (PipGly), norleucine (Nle), citrulline (Cit), or ornithine (Orn) at residue 20. Analogues with Arg-20 substituted with L-4-piperidyl-(*N*-amidino)alanine, Lys, Glu, Ala, Gln, (*S*)-2-amino-4-[(2-amino)pyrimidinyl]butanoic acid, or L-(4-guanidino)phenylalanine had very low or negligible activity. Low or negligible activities of Lys or Orn analogues suggested ionic interactions play a minor role in the Arg interaction with the receptor. The conformational constraints imposed by the PipGly ring had a negative effect on its ability to substitute for Arg. The side-chain H-bonding potential of the Cit ureimido group was likely an important factor in its mimicry of Arg. The increase in amphiphilicity, as demonstrated by its greater high-performance liquid chromatographic retention, and increased α-helix, as shown by circular dichroic spectroscopy, likely contributed to the activity of the Nle-20 analogue. The data demonstrated that specific H-bonding, hydrophobicity of the side chain, stabilization of α-helix, and possibly specific cation positioning were all important in the interaction of Arg-20 with receptor groups.

Parathyroid hormone (PTH)[1] is a major regulator of extracellular calcium that operates mainly through kidney, bone, and intestinal receptors (*1*, *2*). The hormone, and analogues of it, have been shown to strongly stimulate bone growth in both animals and humans (*3*, *4*). The osteogenic activity of a short PTH analogue, PTH(1−31) (*5*), is similar to that of the well-studied PTH(1−34), but in several kinds of cell it only stimulates adenylyl cyclase (AC), rather than both the AC and phospholipase-C_β stimulated by longer analogues (for a review see ref *6*).

PTH and its paracrine equivalent, parathyroid hormone-related peptide (PTHrP), activate a member of the type II family of G-protein-linked seven-transmembrane receptors (*7*), which also includes those for calcitonin, glucagon, growth hormone-releasing factor, secretin, and vasopressin (*8*). A recent NMR study (*9*) showed that hPTH(1−31)NH_2 has a helix−bend−helix structure like that found in previous studies of PTH(1−34) (*10*). The C-terminal α-helix extending from Ser-17 to Gln-29 is the major structure within the principal receptor-binding region. Residues 21−31 form an amphiphilic part of this C-terminal helix (*2*, *11*, *12*). The remaining part of the hPTH(1−31) contains a partially stable α-helix between residues 3 and 9 (*9*). Although the structure of PTH when bound to its receptor has not been defined, it is likely that at least the hormone's C-terminal region binds to the receptor's extracellular N-terminal domain via the hydrophobic face of an amphiphilic α-helix or a distorted amphiphilic helix (*13*−*15*). Thus, AC stimulation is very sensitive to replacement of residues on the nonpolar face, including Leu-24, Leu-26, and to a lesser extent Trp-23 and Val-31, whereas residues on the polar face are less sensitive to substitution. Arg-20 is at the N-terminus of the hydrophobic face (Figure 1) and is predicted to interact directly with the receptor. Consistent with this, previous reports have shown that analogues in which Arg-20 is replaced by Ser, Gly, Gln, Lys, or Glu (*16*, *17*) are inactive.



SVSEIQLMHNLGKHLNSMERVEWLRKKLQDV

FIGURE 1: Helical wheel of hPTH(20−31), underlined in the sequence of hPTH(1−31). Hydrophobic residues are circled.

The construction of peptide mimetics requires a detailed understanding of which residues are essential for hormone-receptor binding and activation and the characteristics of

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[1] Abbreviations: PTH, parathyroid hormone; PipGly, 4-[(*N*-amidino)-piperidyl]-(*S*)-glycine; PipAla, 4-[(*N*-amidino)piperidyl]-L-alanine.

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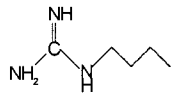
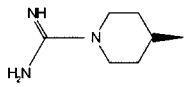
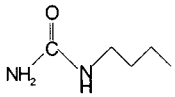
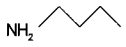
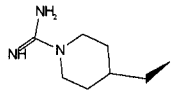
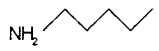
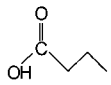
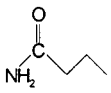
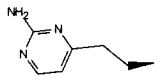
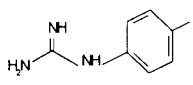
Analogue of Arg[20]	Side-Chain Structure	Mw (obs.)	Mw (calc.)
Arginine		3718.26 (±0.73)	3718.4
PipGly		3743.71 (±0.26)	3745.2
Citrulline		3719.64 (±0.52)	3719.4
Norleucine		3675.61 (±0.36)	3675.4
Ornithine		3676.97 (±0.22)	3676.4
PipAla		3758.20 (±0.30)	3759.2
Lysine		3691.36 (±0.17)	3690.4
Glutamic		3690.06 (±0.96)	3691.4
Alanine		3633.97 (±0.63)	3633.3
Glutamine		3690.67 (±0.51)	3690.4
(S)-2-amino-4-[(2-amino) pyrimidinyl]butanoic		3740.28 (±0.33)	3741.2
L-4-guanidino-Phe		3766.65 (±0.35)	3767.2

FIGURE 2: Substituent side-chain structures and molecular weight data for Arg-20 analogues of hPTH(1−31)NH_2.

these residues responsible for receptor interaction. Arg-20 is one of two residues (Leu-24 is the other) that are present in all of the known peptides that activate or bind tightly to PTH receptors. Apart from simply knowing that Arg-20 is important for receptor activation, there has been no systematic study of the critical properties for receptor interaction. Previous work has been limited to replacing Arg-20 with naturally encoded amino acids because the analogues were obtained by recombinant expression. In this work, we have synthesized hPTH(1−31)NH_2 analogues containing the non-encoded amino acids ornithine (Orn), citrulline (Cit), and norleucine (Nle) and also several synthetic Arg mimetics. We have found three analogues with 10−20% of the natural activity. These results, along with CD analysis to define any secondary structure changes, have enabled us to define which properties of Arg-20 are involved in PTH's specific interaction with the receptor. Such properties can be used to better refine PTH−receptor models, such as that of Jin et al. (*18*).

MATERIALS AND METHODS

Materials. Fmoc-amino acids with the following side-chain protecting groups were purchased from NovaBiochem,

LaJolla, CA, or Peptides International: trityl (Asn, Gln, His, Cit), *tert*-butyl (Ser, Glu, Asp), Boc (Lys, Orn, Trp), and 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl (Arg). The following Arg analogues were obtained from RSP Amino Acid Analogues, Boston, MA: Fmoc-L-4-[guanidino-(Boc)$_2$]Phe, Fmoc-(*S*)-4-piperidyl-[*N*-amidino(Pmc)$_2$]Gly, Fmoc-(*S*)-2-amino-4-[(2-amino)pyrimidinyl]butanoic acid, and Fmoc-L-4-piperidyl-[*N*-amidino(Pmc)$_2$]Ala.

Peptide Synthesis. Peptides were synthesized by standard Fmoc protocols as described in detail previously (*19*). All peptides were purified to greater than 95% homogeneity by gradient elution with acetonitrile in 0.1% TFA/water from a C_{18}-silica column (Vydac, 10 μ, 1 × 25 cm), and their purities and identities were confirmed by analytical HPLC and electrospray mass spectrometry (SCIEX) (Figure 2).

Adenylyl Cyclase Assays. AC-stimulating assays were performed with 4−5 day cultures of rat ROS 17/2 cells as described earlier. Activities were estimated from the rate of formation of [^{3}H]cAMP from the cellular ATP pools, which had been labeled with [^{3}H]adenine before exposure to the hPTH analogue (*5*). Data were expressed as a percentage of the maximum observed incorporation in the particular experiment. The effective dose for half-maximal activity (ED_{50}) was calculated from a three-variable sigmoid fit or in some cases estimated by extrapolation of a cubic fit (SigmaPlot, SPSS Inc.).

Analysis of Amphiphilicities. Relative amphiphilicities of the Arg-20 analogues were determined from their retention times on elution from a C_{18}-silica column (Vydac) as described by Zhou et al. (*20*). In each run, the Arg-20 analogue was corun with hPTH(1−31)-NH_2.

Circular Dichroism. CD spectroscopy was performed with a Jasco J-600 spectropolarimeter at ambient temperature (21−23 °C). At least four spectra were averaged, and the data were smoothed by the Jasco software. The instrument was calibrated with ammonium (+)-10-camphorsulfonate. Peptides were dissolved in water, and sodium phosphate buffer, pH 7.2, was added to 25 mM. Peptide concentrations were calculated from the absorption at 280 nm, with an extinction coefficient of 5700 M^{-1} for the single tryptophan. Data are expressed per peptide bond.

RESULTS

Analogue Secondary Structures. The integrity of an α-helix between residues 17 and 29 is thought to be necessary for correct receptor binding and activation (*14*, *19*). Lactams that stabilize the α-helix of PTH analogues, especially in the region of residues 17−27, have been found to increase the AC-stimulating activity of the analogue (*19*, *21*). Thus, the stability of the α-helix of an analogue may be an important contributor to its receptor activation activity.

CD is particularly useful for monitoring α-helix in peptides, and the 17−29 α-helix has a strong signal in hPTH and subfragments containing this sequence (*12*, *22*). The key CD parameters are the ellipticities at 209 and 222 nm and their ratio. A perfect α-helix has a $\theta_{222}/\theta_{209}$ of about 1.09 (*23*). The CD spectra of the Cit, Lys, and PipGly analogues are shown in Figure 3 and those of Gln, Ala, and Orn are shown in Figure 4. The spectrum of hPTH(1−31)NH_2 is shown for reference in both Figures 3 and 4. Table 1 summarizes the α-helical parameters of all the analogues.

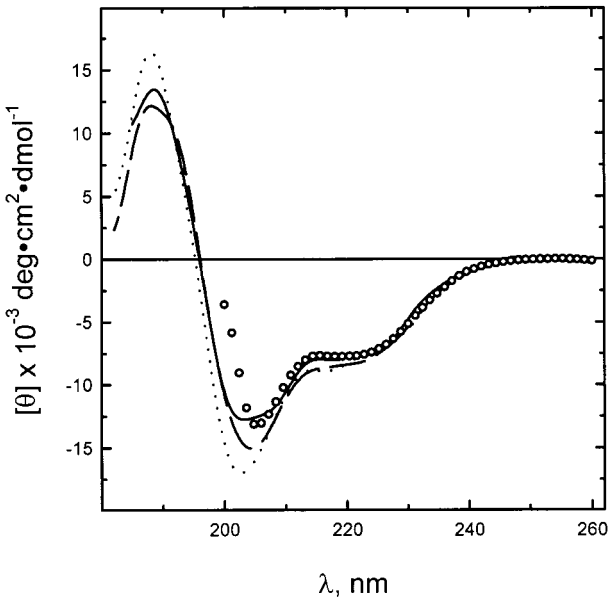


FIGURE 3: CD spectra of hPTH(1−31)NH_2, 0.12 mM (—); [Cit^{20}]-hPTH(1−31)NH_2, 3.2 mM (− − −); [Lys^{20}]hPTH(1−31)NH_2, 1.5 mM (···); and [PipGly^{20}]hPTH(1−31)NH_2, 0.3 mM (○).

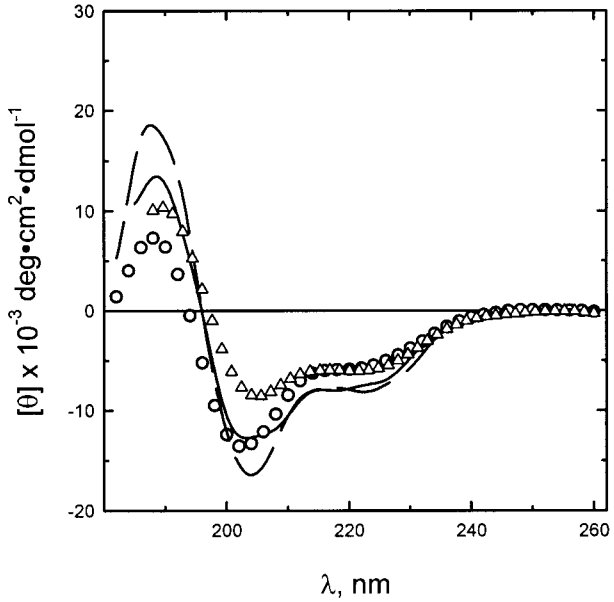


FIGURE 4: CD spectra of hPTH(1−31)NH_2, 0.12 mM (—); [Gln^{20}]-hPTH(1−31)NH_2, 1.5 mM (− − −); [Ala^{20}]hPTH(1−31)NH_2, 0.3 mM (△); and [Orn^{20}]hPTH(1−31)NH_2, 3.4 mM (○).

Several substitutions, including Lys, Cit, and PipGly (Figure 3), Gln (Figure 4), and Glu, had little effect on α-helix stability. Three substitutions (PipAla, Orn, and Ala) resulted in about a 20% loss of α-helix. The Nle^{20} substitution strongly stabilized the α-helix (Figure 5, Table 1) and had a moderate concentration dependence. The increased signal at the higher concentration presumably arose from helix stabilization by dimer formation resulting from hydrophobic interactions of the more amphiphilic C-terminal α-helix.

Amphiphilicity of α-Helix. It has been demonstrated previously that there is an amphiphilic α-helix between residues 21 and 34 (*11*, *12*). It has been speculated that the amphiphilicity is important in itself for binding of the hormone to its receptor or has a secondary effect in promoting binding to the hydrophobic surface of the cell (*16*,

Table 1: CD Helical Parameters

residue 20	$-\theta_{209}$ ($\times 10^{-3}$)	$-\theta_{222}$ ($\times 10^{-3}$)	$\theta_{222}/\theta_{209}$	helical residues[a]
Arg	11.3	7.5	0.66	8
Nle	11.7	11.5	0.98	12
Cit	12.0	8.3	0.69	9
PipGly	10.8	7.7	0.70	8
Gln	11.8	8.2	0.69	9
Lys	11.8	7.7	0.65	8
Orn	9.4	5.8	0.62	6
Ala	7.4	6.0	0.81	6
Glu	9.4	6.6	0.70	7
PipAla	7.8	5.7	0.73	6

[a] Estimated number of peptide bonds as α-helix, from $-\theta_{222} \times 30/28\,000$ (*12*, *23*).

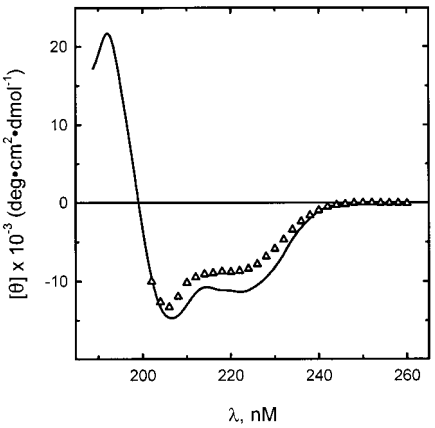


FIGURE 5: CD spectra of [Nle^{20}]hPTH(1−31)NH$_2$ at 70 μM (—) and 3.5 μM (△).

24). Thus, we have observed that replacing Lys^{27} with Leu gives an analogue with an AC-stimulating ED$_{50}$ about 3-fold greater than that of the native sequence. Furthermore, a completely artificial idealized amphiphilic α-helix analogous to the 22−31 region of PTH has been substituted into the C-terminal region of hPTHrP(1−34), and the resulting analogue, although 6-fold less active than hPTHrP(1−34) in AC stimulation, was highly active in vivo in bone restoration (*25*). Therefore, one could argue that a generalized effect on the hydrophobic moment of the helix could contribute to the ability of an amino acid to replace Arg^{20}. The Nle^{20} and Ala^{20} analogues, in particular, have high hydrophobic moments, as calculated by the method of Eisenberg et al. (*26*) using the moment of Leu for Nle (Figure 6A). These two analogues show maxima in the hydrophobic moment plot at about 95°, near the theoretical value of $\delta = 100°$ for a perfect amphiphilic α-helix. The result of the Nle^{20} or Ala^{20} substitution is to extend the amphiphilic helix to include residue 20. Peptides with the same amino acid composition show enhanced retention times in reversed-phase HPLC, depending on their amphiphilic potential (*20*). Those with higher potentials are eluted at higher concentrations of the organic phase. Figure 6B shows the HPLC profiles of hPTH(1−31)NH$_2$ and its Ala^{20} and Nle^{20} analogues. The Nle^{20} analogue had the highest retention time (5.2 min greater than the natural Arg^{20} peptide) of these three and all other analogues (data not shown). The increased relative HPLC retention time of the analogue compared to that of the natural peptide resulted in part from the increased hydrophobicity of the Nle^{20} analogue and in part from its contribution to the amphiphilicity of the C-terminal α-helix. The Ala^{20}

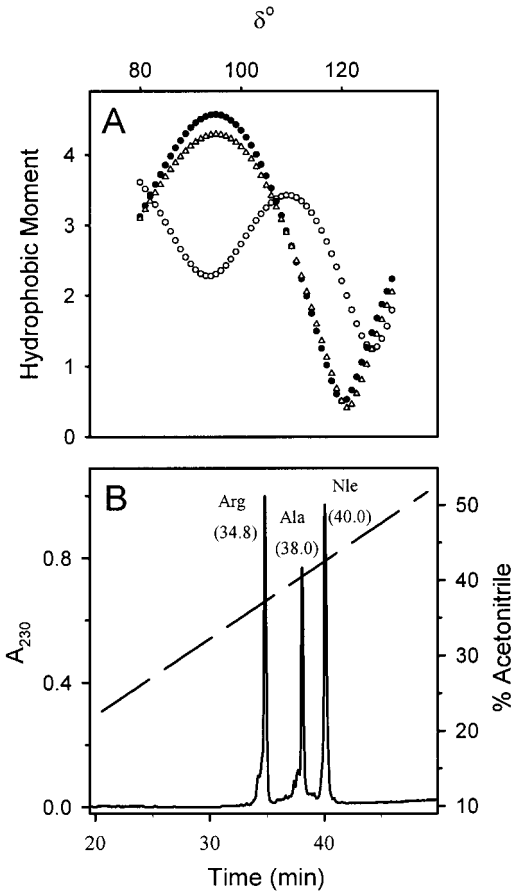


FIGURE 6: (A) Hydrophobic moment plots for hPTH(20−31) (○), Nle^{20} (●), and Ala^{20} analogues (△), calculated according to Eisenberg et al. (*26*). The value for Leu (*26*) was used for Nle in the calculation. (B) HPLC of hPTH(1−31)NH$_2$, Ala^{20}, and Nle^{20} analogues. The gradient is 1%/min acetonitrile in 0.1% TFA/water. Retention times are indicated in minutes below the peak identifications.

analogue had a retention time 3.3 min greater than that of the natural sequence. Data reported previously for retention coefficients of amino acids in HPLC of short synthetic sequences (*27*) do not strictly apply to longer peptides with secondary structure. However, we suggest it is possible to compare relative values of amino acid retentions. The data from short peptides imply that the hydrophobic effect of substituting Nle should be much greater than Ala, in contrast to what was observed (Figure 6). Data from small peptide retentions gave a difference between Leu and Lys of 10 min (*27*). In contrast to this, we have observed a difference in retention time between hPTH(1−34)NH$_2$, with its natural Lys^{26}, and [Leu^{26}]hPTH(1−34)NH$_2$ of only 2 min (Barbier, unpublished observation). These data suggest that the Ala and Nle substitutions have a large component (about 3 min) in their HPLC retention that is related to increased amphiphilicity of the 20−31 sequence.

Adenylyl Cyclase Activities of Arg-20 Analogues. The AC-stimulating activities of eight of the 11 Arg-20-substituted analogues are shown in Figures 7 (PipGly, Cit, Ala, and Gln) and 8 (Nle, Orn, Lys, and Glu), and all of the estimated ED$_{50}$ values are listed in Table 2. None of the naturally encoded residues that we tested (Lys, Glu, Ala, and Gln) had significant activity, as expected from previous reports (*16*, *17*). One of the constrained analogues, PipGly, was the most active of the remaining analogues tested, but even this one

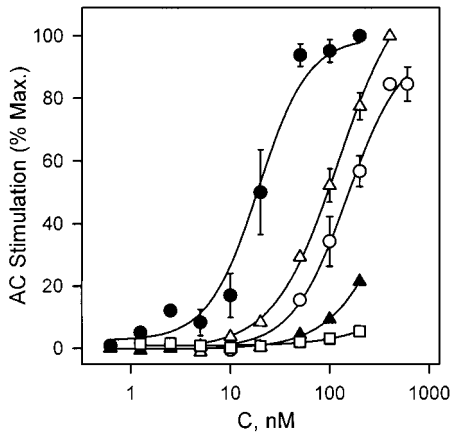


FIGURE 7: AC-stimulating activities of Arg^{20} analogues of hPTH-(1−31)-NH_2: hPTH(1−31)-NH_2 (●), $PipGly^{20}$ (△), Cit^{20} (○), Ala^{20} (solid triangles), Gln^{20} (□).

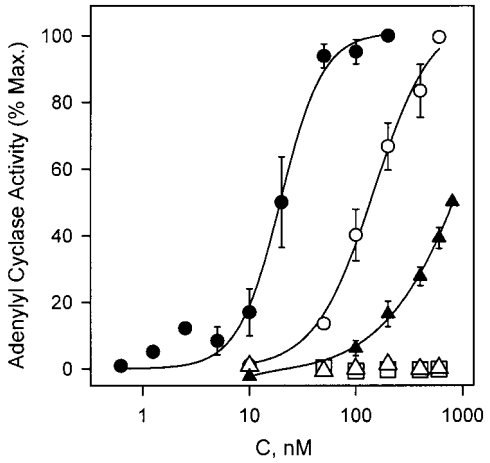


FIGURE 8: AC-stimulating activities of Arg^{20} analogues of hPTH-(1−31)-NH_2: hPTH(1−31)-NH_2 (●), Nle^{20} (○), Orn^{20} (solid triangles), Lys^{20} (□), and Glu^{20} (△).

Table 2: Adenylyl Cyclase Stimulating Activities of hPTH(1−31)NH_2 Analogues

analogue of Arg^{20}	ED_{50} (nM)	relative activity[a]
Arg	19 (±4)	1.0
PipGly	95 (±9)[b]	0.2
Nle	136 (±24)[b]	0.14
Cit	155 (±21)[b]	0.12
Orn	276[c]	0.07
PipAla	>400	<0.05
Lys	>1000	<0.001
Glu	>1000	<0.001
Ala	>1000	<0.001
Gln	nil	
(*S*)-2-amino-4-[(2-amino) pyrimidinyl]butanoic acid	nil	
L-4-guanidino-Phe	nil	

[a] Relative to ED_{50} of natural Arg-20 hPTH(1−31)NH_2. [b] Estimated by fit to sigmoidal function. [c] Estimated by fit to cubic polynomial.

had only about 20% of the activity of the natural sequence (Table 2 and Figure 7). Two uncoded amino acids, Cit and Nle, were only slightly less active than the constrained one (Table 2, Figures 7 and 8). The constrained analogue lacks some of the H-bonding potential of Arg but places a positive charge at a similar distance (about 6 Å) from the polypeptide backbone. This observation, and the fact that Lys (ED_{50} > 1000 nM) and Orn (ED_{50} = 276 nM) were only weakly active, suggests that charge in itself is not the important determining factor but that the H-bonding potential of the guanidino group plays an important role. The Nle^{20} analogue (ED_{50} = 136 nM) was more active than Orn. The relatively high activity of the Nle^{20} analogue presumably reflects the importance of hydrophobic interactions of CH_2 groups of Arg^{20} of the native sequence with a nearby hydrophobic receptor residue(s) and also suggests the residue is buried in the complex.

Comparison of these observations on AC-stimulating activity and the CD data above suggests that the large increase in α-helix stability of the Nle-20 analogue is likely a factor in its AC-stimulating potency. In contrast, the loss of α-helix in the Orn-20 analogue may have contributed to a lessening of its AC-stimulating activity. The data do not suggest any further effect of secondary structure on the receptor activation activities of these analogues. An additional factor in the activity of the Nle analogue is its increased amphiphilicity, which may have contributed to its receptor binding or to its interaction with the membrane.

DISCUSSION

Numerous structural studies over the past 10 years have demonstrated a stable α-helix between residues 17 and 29 in the C-termini of PTH(1−31)NH_2 (*9*), PTH(1−34) (*10*, *12*, *28*), and PTH(1−37) (*29*). Part of the sequence, specifically residues 21−31, of hPTH(1−31) forms an amphiphilic α-helix, which is stabilized in the presence of lipid (*12*, *15*). Substituting residues, particularly Leu-24, Leu-28, and Val-31, on the hydrophobic face of this helix can strongly affect the resulting analogue's AC-stimulating ability, whereas residues on the polar face can be substituted with much less effect on function (*30*). The presence of Arg^{20} on the predominantly hydrophobic face of the 17−29 α-helix suggests that this residue might be also important for receptor binding and activation (Figure 1). Furthermore, based on evolutionary conservation of sequence, only the Arg^{20}-$XXXL^{24}$ motif is conserved in all PTH receptor-activating moieties, including PTHrP and the tuberoinfundibular peptide. The results of this study of Arg^{20} analogues clearly demonstrate the critical importance of this residue and provide insight into the details of how Arg interacts with receptor residues.

The observation that only a few residues of PTH are critical for receptor activation is analogous to the extensively studied binding of human growth hormone to its receptor. The crystallographic data on that hormone−receptor complex, coupled with analogue data, has made possible a precise analysis of the critical determinants of peptide−receptor recognition (*16*). Clackson and Wells (*31*) have coined the term "functional epitope" to describe this phenomenon. One of the most important polar residues in the functional epitope of the receptor is an Arg that interacts with receptor residues by salt bridge formation, H-bonding, and multiple van der Waals interactions via its side-chain methylenes. In this instance, the bulk of the interaction energy came from the *β*- and *γ*-methylenes of the Arg side chain, since both Leu and Met, but not Ala, substituted well for Arg (*32*).

The observed activity of the Nle^{20} analogue in the present study suggests that the three methylene groups of Arg^{20} side chain contribute substantially to its binding interaction. This

analogue likely had a contribution to its receptor activating activity resulting from the observed stabilization and increased amphiphilicity of the α-helix. This was the only analogue in the present study where an effect on the secondary structure of the hormone was implicated as contributing positively to activity. In contrast, Orn was the only analogue that could have had a negative contribution from helix destabilization. The previous observation that a purely artificial amphiphilic α-helix can partially substitute for residues 22−31 of PTHrP supports the possibility that effects on helical stability and amphiphilicity could contribute to the ability of an analogue to substitute for the critical Arg (*25*). The amphiphilicity contribution to activity may also be related to enhanced membrane binding of the peptide, an explanation invoked earlier to explain the frequency of amphiphilic sequences in bioactive peptides (*24*).

In this work, we have correlated AC-stimulating activity with productive binding of an analogue to the receptor. In principle, one could have nonproductive binding where the stabilized receptor conformation will not bind Gs protein. Receptor binding studies in the past have not always correlated with AC-stimulating activity (*33*, *34*). This may be an artifact of the competitive binding assay or may be real nonproductive binding. Unfortunately, direct binding studies, such as performed with Biacore technology, are not yet feasible with cell surface receptors.

The weak activity of the Orn^{20} analogue and even weaker activity of the Lys^{20} analogue implies that the precise position of the guanidinium cation of the side chain is critical for full activity. This could result from interaction with nearby receptor anions that pair with the guanidinium cation plus specific H-bonding. An alternate interaction would be cation−π bonding between the guanidinium group and a nearby aromatic chromophore. Specific high-energy interactions between Arg and aromatic residues (Phe, Tyr, Trp) have been described in the literature. Aside from the obvious ion-pair potential of Arg with Asp and Glu, cation−π interactions have been proposed to play an important role in Arg interactions with these aromatic groups (*35*, *36*). A recent study has shown that Lys and Arg cation−π interactions with aromatic residues, particularly Trp, are common in known protein structures and that the side chain of Arg is more likely than Lys to be part of such an interaction (*37*). This Arg preference may be related to the ability of Arg to form multiple H-bonds with Trp. At this point, we speculate that such an interaction may be responsible for the observed specificity of Arg^{20}. Arg^{20} is a conserved residue between PTH and PTHrP, but there is no PTHrP data at present concerning similar substitutions to those presented here for PTH. Furthermore, the tuberoinfundibular peptide, which activates the PTH2 receptor, also has an Arg in the same location as Arg^{20}, when the two peptides are aligned against the common sequence RXXWL (*38*). Although the presence of a critical charged residue might be particularly significant for a peptide hormone−receptor interaction because its long-range effect could enhance the rate of binding of the hormone, the data of the present study do not support it having a major role in the PTH−receptor complex.

Cit was the most active natural amino acid replacement of Arg. Its ureimido functional group retains some of the H-bonding potential of the guanidinium group but lacks the positive charge. This observation further suggests the importance of a stereospecific pairing of Arg^{20} with receptor residues or a cation−π interaction with a Trp, Tyr, or Phe residue of the receptor.

An approach to understanding the nature of Arg side-chain interactions has been to use Arg mimics with constrained side chains (*39*, *40*). One such constrained analogue, L-3-piperidyl-(*N*-guanidino)Ala, was particularly useful for developing a thrombin-specific serine protease inhibitor (*40*). The most active Arg^{20} substitute in our work was PipGly, although even this one had an ED_{50} that was still about 5-fold less than that of the natural Arg^{20} peptide. This analogue constrains the rotation of the three methylenes of the side chain and their orientation with respect to N^{ϵ} but allows the rest of the guanidinium group to rotate freely. The net conformational space available to the guanidinium group has been reduced. The similar analogue PipAla with its additional methylene group was inactive, as was the 4-guanidino-Phe analogue. Both of these analogues place the guanidinium group an additional 1.5 Å from the C_{α} and their inactivities further emphasize a critical positioning of the guanidino group.

It is highly likely that PTH will be in use as an anabolic agent for the treatment of osteoporosis soon (*41*), as a short-term agent for accelerating bone repair (*42*), and possibly for topically treating psoriasis (*43*). All of these applications are related to its agonist activity and specificity of receptor binding. The development of improved analogues, particularly orally available ones, will depend on knowing in detail how it interacts with its receptor. A direct study of the conformation of PTH as part of its receptor complex is not likely to be possible soon. Meanwhile, studies of particular crucial residues will provide important data that must be compatible with any hormone−receptor model. We now have a reasonable understanding of how its C-terminus, with its critical Arg^{20}, and the N-terminus bind to the receptor. However, the orientation of these two segments to each other and the role of intervening sequence remain unknown.

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