of 5 mg/kg given intravenously 40 min apart. Arterial blood pressure was taken at 1-, 3-, and 5-h intervals after dosing to assess drug effects. All animals were habituated to the test procedure through their long history of testing.

To investigate the mechanism of antihypertensive action, male SHR of the Okamoto-Aoki strain (Taconic Farms, Germantown, NY) of approximately 350 g body weight were restrained in a supine position with elastic tape (Elastikon, Johnson & Johnson, New Brunswick, NJ). The area at the base of the tail was locally anesthetized by subcutaneous infiltration with 2% procaine. The ventral caudal artery was isolated and a cannula of PE 10 and PE 20 fused tubing was passed into the lower abdominal aorta. The cannula was secured, heparinized (1000 IU/mL), and sealed and the wound closed. A second cannula was introduced into the lateral caudal vein for the intravenous administration of challenge substances. Control responses to the challenges were recorded prior to drug administration, after which each animal received either vehicle (10 mL/kg) or compound under study orally. One hour later, the challenges were repeated. The animals were tilted 75° for 30 s for effect on BP and the challenges administered intravenously. The challenges consisted of epinephrine (Epi) 1 and 2 μg/kg, norepinephrine (NE) 1 μg/kg, isoproterenol (Iso) 1 μg/kg, acetylcholine (ACh) 2 μg/kg, angiotensin II (Angio) 0.2 μg/kg, tyramine (Tyr) 250 μg/kg, and 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP) 25 μg/kg. All challenges were calculated as the free base. Each challenge concentration was adjusted such that the volume required was 0.5 mL/kg body weight and followed by 0.1 mL of 0.9% sodium chloride to wash any residual agent from the cannula.

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Synthesis and Hypertensive Activity of Neuropeptide Y Fragments and Analogues with Modified N- or C-Termini or D-Substitutions†

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Porcine neuropeptide Y (NPY), NPY fragments, and analogues with D-Xaa^n, Ala^9, D-Ala^9, and Met^{17} substitutions or modifications to the C- or N-termini were synthesized. The synthesis and purification of these peptides was achieved by using routine laboratory strategies and techniques. The ability of these peptides to alter mean arterial pressure (MAP) and heart rate (HR) in conscious rats was monitored for 15 min following intraarterial administration. Potencies and efficacies of these peptides relative to NPY were determined by comparison of dose-response curves. Administration of 40 μg/kg NPY resulted in a rapid, though short-lived, rise in mean arterial pressure from a basal value of 107.0 ± 2.6 to 157 ± 5.5 mmHg (means ± sem, n = 13). The ED_{50} (±SE) for this response was 3.04 ± 0.88 μg/kg. Peptide YY (PYY) elicited a response that was similar in magnitude but with an ED_{50} (±SE, n = 3) of 0.76 ± 0.24 μg/kg while porcine pancreatic polypeptide (pPP) was inactive when tested at 40 μg/kg (n = 4). Relative potencies for [Ac-Tyr^1]NPY, [Ac-D-Tyr^1]NPY, [des-amino-Tyr^1]NPY, and [Me-Tyr^1]NPY ranged from 1.1 to 2.2. Potencies relative to NPY for D-substitutions at positions 2–6 and 8–13 inclusive ranged from 0.1 to 1.0. Analogues with D-substitutions at positions 1–3 exhibited an extended duration of action. Analogues with D-substitutions at positions 33–35 inclusive were inactive at 40 μg/kg, and [D-Tyr^{36}]NPY was 10-fold less potent than NPY, suggesting that the integrity of the C-terminal region is critical to the overall biological action of NPY. This conclusion is supported by studies with C- and N-terminal deletion peptides. NPY_{2-36} showed full intrinsic activity at 40 μg/kg and retains 40% of the hypertensive potency of NPY. There was a sequential decrease in efficacy upon further N-terminal deletion. In contrast to the finding with NPY_{2-36}, modification of the C-terminus either from the native carboxamide to the free carboxylic acid or by deletion of the C-terminal residue resulted in analogues which were inactive at 40 μg/kg. These data indicate that an essentially full-length, C-terminally amidated NPY structure is required for the hypertensive activity observed in conscious rats upon intraarterial administration of NPY and NPY analogues.

Neuropeptide Y (NPY; structure shown below) is a 36 amino acid, C-terminally amidated peptide that was first isolated from porcine brain by Tatemoto et al.[1,2] in 1982 using a chemical method for the detection of peptide amides.[3] NPY has 69% sequence homology with peptide YY (PYY)[4] and 50% homology with porcine pancreatic polypeptide (pPP).[5]

5 10
Tyr-Pro-Ser-Lys-Pro-Asp-Asn-Pro-Gly-Glu-
15 20
Asp-Ala-Pro-Ala-Glu-Asp-Leu-Ala-Arg-Tyr-
25 30
Tyr-Ser-Ala-Leu-Arg-His-Tyr-Ile-Asn-Leu-
35
Ile-Thr-Arg-Gln-Arg-Tyr-NH_2

† Abbreviations: The abbreviations for the amino acids are in accord with the recommendations of the IUPAC-IUB Joint Commission on Biochemical nomenclature (*Eur. J. Biochem.* **1984**, *138*, 9-37). The symbols represnt the L-isomer except when indicated otherwise. In addition: NPY, neuropeptide tyrosine; PYY, peptide tyrosine tyrosine; pPP, porcine pancreatic polypeptide; MAP, mean arterial pressure; HR, heart rate; Me-Tyr, *N*-methyltyrosine; Ac-Tyr, *N*-acetyltyrosine; des-amino-Tyr, 4-hydroxyphenylpropanoic acid; BOC, *tert*-butoxycarbonyl; MBHA, 4-methylbenzhydrylamine, CM, chloromethyl; Tos, *p*-toluenesulfonyl; OcHx, cyclohexyl ester; 2ClZ, 2-chlorobenzyloxycarbonyl; Bzl, benzyl ester; 2BrZ, 2-bromobenzyloxycarbonyl; DMF, dimethylformamide; TFA, trifluoroacetic acid; EDT, ethanedithiol; TEA, triethylamine; GRF, growth hormone releasing factor; CRF, corticotropin-releasing factor; NE, norepinephrine; HPLC, high-performance liquid chromatography; C_{18}, octadecyl; TEAP, triethylammonium phosphate; PE, polyethylene.

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NPY possesses potent vasoconstrictor properties and increases blood pressure when injected into animals.[6,7] NPY and norepinephrine (NE) are contained in and co-released from sympathetic nerves;[8] however the NPY-induced vasoconstriction is independent of the release and action of NE.[9] To date, structure–function studies on NPY[10-14] have been limited to the measurement of activities and potencies of fragments. In the present study we have sought to define the domains responsible for bioactivity by the synthesis of NPY analogues with D-substitutions or deletions in the N- and C-terminal regions, and several analogues with modified N- or C-termini. The analogues were administered intraarterially to conscious rats to determine their effects on mean arterial pressure and heart rate.

Results and Discussion

The methodology used for the synthesis of NPY and the NPY analogues resulted in crude materials with a single major peak on analytical HPLC. When comparing the quality of these crude materials with those for other classes of peptides in this size range (20–40 residues, i.e. CRF, GRF) generated under similar conditions, we conclude that NPY is an easily attainable target for solid-phase peptide synthesis. The crude peptides were purified by preparative HPLC in two steps to yield the highly purified peptides. The analytical techniques used for the characterization of NPY and its analogues included analytical HPLC in two buffer systems and amino acid analyses. NPY was further characterized by Edman microsequencing and mass spectrometry. Results from these studies confirmed the identity of the intended structures. While this characterization was acceptable for fragments and non-D-substitutions which differed in composition (and usually retention time) from the standard native peptide against which they were compared, D-substituted analogues had the same composition and often similar retention time to that of the native peptide. It is unlikely that the samples of D-analogues that were prepared contained appreciable amounts of the native (all-L) NPY as optical purities of the BOC-amino acid starting materials were checked prior to their use in the syntheses. Also, the amount of racemization seen in solid-phase synthesis, under the coupling conditions used here, has been shown to be very low (<-0.02%).[15] We did, however, perform HPLC analysis in which each D-analogue was coinjected with NPY. By comparison of the UV traces of coinjection runs and analogue alone runs, it was possible to quantitate the presence of minor impurities coeluting with NPY in the sample of D-analogue. Under the conditions used, six D-analogues were not resolved from NPY. The substantial differences in optical rotations of these compounds however indicated them to be distinct chemical entities. Additional evidence for this contention is the observation that the potencies of many of these compounds differed from that of NPY. Therefore we can be confident that the biological results presented here were not due to contamination of the analogues with NPY.

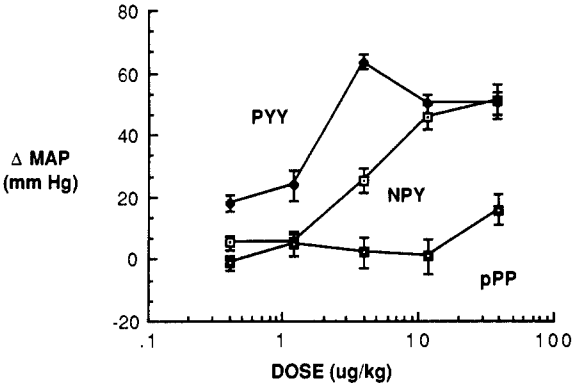

Figure 1. Dose–response curve for NPY, PYY, and pPP. MAP measurements made 1 min after injection of peptide. Points represent means ± SEM (n = 13 for NPY, n = 3 for PYY, and n = 4 for pPP).

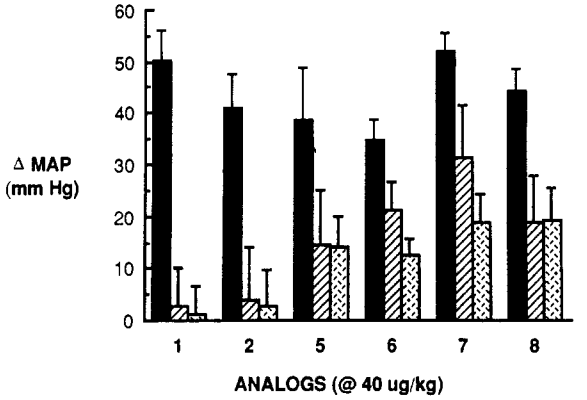

Figure 2. Time-course studies for compounds 1, NPY; 2, [Ac-Tyr[1]]NPY; 5, [D-Tyr[1]]NPY; 6, [Ac-D-Tyr[1]]NPY; 7, [D-Pro[2]]NPY; 8, [D-Ser[3]]NPY. Shown are means ± SEM ($n \geq 3$) of ΔMAP (@ 40 μg/kg) at 1 min (■), 5 min (▨), and 15 min ▨.

The quantities of peptides (greater than 50 mg) that were obtained in highly purified form (greater than 95% pure) have allowed extensive studies (including those described here) to be carried out. We have found that relatively large batches of well-characterized synthetic peptides are extremely important to achieve consistent results. In addition, preliminary studies would suggest that NPY is unstable, for example, when stored in solution at a concentration of 1 mg/mL at 4 °C for 48 h, as evidenced by the appearance of hydrophilic moieties (12%) as characterized by HPLC analysis. For this reason lyophilized bulk material (the stability of which can be checked at regular intervals) is especially valuable.

In the in vivo system used here, NPY caused a substantial, dose-related rise in MAP with an ED_{50} (±SE, n = 13) of 3.04 ± 0.88 μg/kg (Figure 1). Administration of 40 μg/kg of NPY resulted in a rapid, though short-lived, response from a basal MAP of 107.0 ± 2.6 to 157.0 ± 5.5 (means ± SEM, n = 13; Figure 2, panel 1). Readministration of NPY after 10 min (data not shown) caused a repeated and equivalent response with no evidence of tachyphylaxis. The related peptides PYY and pPP were

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Table I. HPLC Retention Times, Purities, Specific Rotations, and Relative Hypertensive Potencies of NPY Analogues

no.	NPY analogue	iso RT @ % MeCN[a]	purity,[b] %	$[\alpha]^{25}_D$,[c] deg	ED_{50} (SE)[d]	RP[e]
1	NPY	4.3 @ 33.6	>97	−58.2	3.04 (0.88)	1.0
2	[Ac-Tyr^1]	5.1 @ 33.6	>95	−57.2	1.72 (0.60)	1.8
3	[Me-Tyr^1]	3.6 @ 35.4	>95	−54.2	1.48 (0.48)	2.1
4	[des-amino-Tyr^1]	3.7 @ 36.0	>97	−58.0	2.88 (0.88)	1.1
5	[D-Tyr^1]	4.4 @ 33.6	>98	−67.7	1.40 (0.40)	2.2
6	[Ac-D-Tyr^1]	4.9 @ 33.6	>95	−62.8	2.84 (1.84)	1.1
7	[D-Pro^2]	4.4 @ 33.6	>98	−46.6	8.25 (1.72)	0.4
8	[D-Ser^3]	4.4 @ 33.6	>98	−47.8	11.36 (4.48)	0.3
9	[D-Lys^4]	4.2 @ 33.6	>98	−53.7	18.84 (4.72)	0.2
10	[D-Pro^5]	4.0 @ 33.6	>95	−53.9	28.24 (8.84)	0.1
11	[D-Asp^6]	4.1 @ 33.6	>95	−55.9	12.00 (4.76)	0.3
12	[D-Pro^8]	3.7 @ 33.6	>98	−53.9	5.52 (1.24)	0.6
13	[Ala^9]	4.1 @ 33.6	>98	−56.7	4.28 (1.36)	0.7
14	[D-Ala^9]	4.1 @ 33.6	>95	−64.3	5.76 (1.04)	0.5
15	[D-Glu^{10}]	3.7 @ 33.0	>98	−58.0	2.92 (0.72)	1.0
16	[D-Asp^{11}]	3.4 @ 33.0	>98	−52.2	15.04 (2.32)	0.2
17	[D-Ala^{12}]	3.4 @ 34.2	>95	−45.0	11.56 (3.84)	0.3
18	[D-Pro^{13}]	3.8 @ 33.0	>98	−52.1	16.16 (3.68)	0.2
19	[Met^{17}]	4.4 @ 33.6	>98	−60.9	4.12 (1.72)	0.7
20	[D-Arg^{25}]	3.4 @ 33.0	>98	−65.0	4.36 (1.36)	0.7
21	[D-Arg^{33}]	3.4 @ 36.6	>98	−58.4	>400	
22	[D-Gln^{34}]	3.5 @ 36.0	>98	−55.6	>400	
23	[D-Arg^{35}]	3.8 @ 35.4	>98	−54.5	>400	
24	[D-Tyr^{36}]	3.4 @ 35.4	>98	−59.7	23.72 (7.72)	0.1
25	NPY_{2-36}	4.0 @ 35.4	>98	−59.4	7.72 (3.64)	0.4
26	NPY_{8-36}	3.7 @ 35.4	>98	−45.3	>400	
27	NPY_{11-36}	3.8 @ 34.8	>98	−43.2	>400	
28	NPY_{14-36}	4.0 @ 31.2	>95	−40.1	>400	
29	NPY_{26-36}	3.5 @ 19.8	>95	−44.2	>400	
30	NPY_{1-14}-OH	3.7 @ 24.6	>98	−125.4	>400	
31	NPY_{1-25}-OH	3.3 @ 10.8	>95	−88.9	>400	
32	NPY_{1-36}-OH	3.9 @ 36.0	>98	−61.1	>400	
33	NPY_{1-35}-OH	3.4 @ 35.4	>98	−55.7	>400	

[a] Isocratic retention times in minutes at the specified % MeCN. [b] Consensus of values from three analytical determinations. [c] Determined at 25 °C in 1.0 M AcOH ($c \sim 0.5$). [d] ED_{50}'s from dose–response curves ± SE in μg/kg ($n \geq 3$). [e] Relative potency: ED_{50}(NPY)/ED_{50}(analogue).

Table II. ΔMAP (Measured at 1 min, Means ± SEM, $n \geq 3$) for NPY Fragments at 40 and 400 μg/kg

NPY/fragment	40 μg/kg	400 μg/kg[a]
NPY	50 ± 6	ND
NPY_{2-36}	49 ± 14	ND
NPY_{8-36}	20 ± 5	ND
NPY_{11-36}	15 ± 5	ND
NPY_{14-36}	<5	16 ± 10
NPY_{26-36}	<5	7 ± 6
NPY_{1-14}-OH	<5	<5
NPY_{1-25}-OH	<5	<5

[a] ND = not determined.

also tested in this system. Administration of PYY resulted in an increase in MAP which was similar in magnitude but with an ED_{50} of 0.76 ± 0.24 (mean ± SEM, $n = 3$; Figure 1). pPP was inactive when tested at 40 μg/kg ($n = 4$; Figure 1). Administration of NPY slowed heart rate in this model, but the difference between 0 and 40 μg/kg doses was not significant. Other peptides described in this study produced similar decreases in heart rate. This decrease in heart rate was presumed to be baroreflex mediated; however, this has not been proven. Deletion of the N-terminal Tyr residue resulted in a 2–3-fold reduction in hypertensive potency though full intrinsic activity was maintained (Tables I and II). This finding is supported by data from membrane binding studies[12] and studies using in vitro tissue preparations[14] in which a decrease in the potency of NPY_{2-36} compared with that of NPY was observed. Further N-terminal deletions resulted in further incremental reductions in potency (Table II) such that NPY_{14-36} and NPY_{26-36} were inactive at 40 μg/kg and were only marginally potent at 400 μg/kg. Deletion of the C-terminal carboxamide to generate NPY_{1-36}-OH or deletion of the Tyr^{36} residue to generate NPY_{1-35}-OH resulted in complete loss of hypertensive activity in the rat in vivo. These findings are supported by studies that indicate that NPY_{1-36}-OH is unable to inhibit electrically stimulated smooth muscle contractions in vitro[16] and did not compete with [^{125}I]NPY in a brain membrane radioreceptor assay.[17] Additionally, other fragments of NPY that were altered at their C-terminus, NPY_{1-14}-OH and NPY_{1-25}-OH, did not raise MAP compared to pretreatment levels when administered at 400 μg/kg (Table II). It is therefore clear that the C-terminal carboxamide is essential for the hypertensive activity of NPY.

N-Acetylation, N-methylation, and D-substitution at position 1 resulted in analogues that were 2-fold more potent than NPY. Desamination of Tyr^1 or the combination of N-acetylation and D-Tyr^1 substitution resulted in analogues with relative potencies of 1.1. One possible explanation for the lack of a significant change in potency with these modifications is that they did not perturb the native conformation of the molecule and thus resulted in a similar structure with equivalent potency. Another explanation is that D-substitutions resulted in analogues whose resistance to proteolytic degradation or elimination offset any deleterious change in configuration engendered by the substitution. This is consistent with the findings of time-course studies (Figure 2) that showed that NPY action was short lived in vivo. Upon administration [D-Tyr^1]NPY, [D-Pro^2]NPY, and [D-Ser^3]NPY, however, MAP remained elevated at 5 and 15 min, consistent with, but not necessarily indicative of, enhanced in vivo stability of these analogues. N-Terminal acetylation did not appear

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to additionally enhance the duration of action of either NPY or [D-Tyr[1]]NPY.

D-Substitutions from positions 2–6 and 8–13 resulted in analogues with ED_{50}'s relative to that of NPY ranging from 0.1 to 1.0. In these cases, the D-amino acid may have been too remote from the N-terminus to offer protection against aminopeptidase action and the fragments thus generated in vivo had decreased biological potency consistent with the present finding of stepwise reductions in potency with N-terminal deletion. An alternative explanation is that D-substitution at positions more distal to the N-terminus do indeed engender a deleterious change in the conformation of the molecule that is sufficient to overcome any protection from proteolytic degradation that may result from the modification.

C-Terminal D-substitutions of the Arg[33], Gln[34], and Arg[35] residues resulted in analogues that were inactive at 40 μg/kg. [D-Tyr[36]]NPY did elicit a response in this assay; however the potency is 10-fold less than that of NPY. These findings correlate well with our results with C-terminally truncated fragments and supports one hypothesis that the C-terminal region of NPY is essential for hypertensive activity of the molecule.

Allen et al.[18] have recently derived a tertiary structure of NPY by application of computer modeling to the X-ray crystal structure of the related avian PP previously solved by Glover et al.[19] The derived structure consists of an N-terminal polyproline II helix (residues 1–8) and an antiparallel α-helix (residues 14–32) connected by a tight bend. Residues 32–36 form a flexible "tail". This structure is highly compact and is stabilized by intramolecular hydrophobic interactions involving, in particular, the proline residues at positions 2, 5, and 8. If this NPY structure indeed accurately reflects the active conformation of NPY, then we can consider the present data to reflect the effect of modifications to the polyproline helix (N-terminally modified analogues and D-substitutions at positions 1–13) or to the flexible tail (D-substitutions at positions 32–36). The reduced potency observed with D-substitution of prolines at positions 2, 5, and 8 may have arisen from a reduction in the stabilizing interactions at these positions. The substitution of Gly[9] with Ala and D-Ala resulted in analogues with relative potencies of 0.7 and 0.5, respectively, suggesting a lack of defined structural requirements at this position. This may reflect a degree of mobility in this region of the molecule as may be expected in the region of a bend. The very low potency of analogues with D-substitution at positions 32–36 suggests that this putative flexible region[19] may in fact have rigid structural requirements. It has been shown previously[20] that pentapeptide analogues of this region bind specifically to brain membrane preparations albeit with low affinity (K_d's: NPY_{32-36}, 5×10^{-6} M; NPY_{1-36}, 1×10^{-8} M), indicating the importance of this region to binding.

These interpretations do not take into account the complications implicit in an in vivo system. Simple structure–activity relationships may be masked by the various competing mechanisms of degradation, internalization, plasma binding, and systemic clearance. Further physicochemical structural and in vitro studies are required in order to dissect the relative contribution to stability, bioavailability, binding affinities, and specificity of site of action made by each of the substitutions introduced so far to generate the analogues reported in Table I. These studies are currently in progress.

Experimental Section

All reagents and solvents were analytical grade (Aldrich Chemical Co., Milwaukee, WI; Fisher Scientific, Springfield, NJ) and were used without further purification except TFA (Halocarbon, Hackensack, NJ) and TEA (Aldrich Chemical Co., Milwaukee, WI), which were reagent grade and used without further purification for peptide syntheses and were distilled to constant boiling point for use in the preparation of chromatographic buffers.

Peptide Synthesis. Peptides were manually synthesized with α-BOC amino protection on MBHA resins prepared in house by the method of Rivier et al.[21] or amino acid-CM resins prepared by the method of Horiki et al.[22] using KF and CM resins (Lab Systems, San Mateo, CA). α-BOC-amino acids (Bachem, Torrance, CA) were coupled via dicyclohexylcarbodiimide in CH_2Cl_2 and/or DMF. Asn and Gln were coupled in the presence of a 2-fold excess of 1-hydroxybenzotriazole. Side-chain protection of α-BOC-amino acids was as follows: Arg(Tos), Asp(OcHx), Glu(OcHx), His(Tos), Lys(2ClZ), Ser(Bzl), Thr(Bzl), and Tyr(2BrZ). Deblocking was accomplished with 50% TFA in CH_2Cl_2 in the presence of 1% EDT for 25 min. The protected peptide-resin was cleaved in liquid HF in the presence of 3% anisole at 0 °C for 45 min for CM resins and 60 min for MBHA resins. The crude peptides were precipitated with anhydrous diethyl ether and separated from ether-soluble nonpeptide material by filtration. The peptides were extracted from the resin with water and the aqueous solutions were then lyophilized.

Peptide Purification. Crude peptides were purified by preparative reversed-phase HPLC usually in two steps. This methodology has been previously described in detail.[23,24] Briefly, gradient conditions were established by analytical HPLC. Gradient conditions for preparative HPLC were inferred from the analytical results. A Hitachi 655 analytical system was used, comprising a 655A-11 pump, 655A-71 proportioning valve, Rheodyne 7125 injector, Vydac C_{18} column (0.46 × 25 cm, 5-μm particle size, 30-nm pore size), 655A variable-wavelength detector (detection was at 210 nm), and 655-61 system processor. The preparative HPLC system used comprised a modified Waters 500A Prep LC, Waters preparative gradient mixer or Eldex Chomatrol II gradient former, Waters 450 variable-wavelength detector (detection was at 230 nm), and Houston Instruments Omniscribe chart recorder. The cartridges used were hand-packed, in house, with Waters polyethylene sleeves and frits and Vydac bulk C_{18} material, 15–20-μm particle size, 30-nm pore size. The crude lyophilized peptides (0.5–1.5 g) were dissolved in water, loaded onto a C_{18} cartridges, and eluted with linear TEAP (pH 2.25)/MeCN gradients. Fractions (50–100 mL) were collected and monitored by isocratic analytical HPLC. Appropriately enriched fractions (>95% pure) were pooled, diluted, and reloaded onto the preparative cartridge. The peptide was eluted with a linear gradient of 0.1% TFA/MeCN. Fractions were again collected and analyzed, and those containing the purified peptide were pooled and lyophilized. Final products were >95% pure by HPLC analysis. Final yields ranged from 3% to 17% of theory when based on resin substitution.

Peptide Characterization. Purified peptides were subjected to HPLC analysis in two mobile phase systems: 0.1% TFA/MeCN and TEAP (pH 2.25)/MeCN on a C_{18} stationary phase. The analytical HPLC system used was the same as that described in detail above. Additionally, analytical HPLC in the 0.1% TFA/MeCN system was performed on a Waters HPLC system which comprised two M-45 pumps, WISP sample injector, Kratos Spectroflow 773 UV detector, and Waters data module integra-

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tor/recorder. Using this system we also performed coinjection analysis on paired sets of samples containing either analogue or analogue + NPY 1:1 by weight. The purities quoted in Table I are a consensus of values determined in the three analytical systems described. The retention times quoted in Table I were determined from isocratic analysis of each peptide with the Hitachi system described above. The mobile-phase system used was 0.1% TFA/MeCN (percentages as specified).

Amino acid analysis of the peptides was performed following hydrolysis in 4 N methanesulfonic acid at 110 °C for 24 h. A Perkin-Elmer LC system comprising two Series 10 LC pumps, a ISS-100 sample injector, RTC 1 column oven, Kratos Spectroflow 980 fluorescence detector, and LCI-100 integrator was used. A Pierce AA511 ion-exchange column was maintained at 60 °C and post column derivatization with o-phthalaldehyde was performed at 52 °C. Samples containing the internal standard γ-aminobutyric acid were injected and a gradient 0–100% B in 25 min and then 100% B for 15 min was commenced 5 min after injection. The flow rate was 0.5 mL/min, and A and B buffers were Pierce Pico buffer (pH 2.20) and Beckman Microcolumn sodium citrate buffer (pH 4.95), respectively.

Optical rotations of peptides were measured in 1.0 M acetic acid ($c \sim 0.5$, i.e. 5 mg of lyophilized peptide/mL uncorrected for TFA counterions or water present after lyophilization). Values were calculated from the means of 10 successive 5-s integrations determined at 25 °C on a Perkin-Elmer 241 polarimeter (using the D line of Na emission) divided by the concentration of the sample in g/dL and are quoted as uncorrected specific rotations.

Conscious Rat Blood Pressure Assay. Male Sprague–Dawley rats (240–280 g, Bantin-Kingman Laboratories Fremont, CA) were used. All procedures performed on these animals were in accordance with the guidelines of the University of California, San Diego Committee on Investigations Involving Animal Subjects. All animals, when used in experiments were conscious and freely moving. Femoral catheters were constructed by heat bonding of 1-cm lengths of PE 10 tubing to pieces of PE 50 tubing. The PE 10 segment was inserted into the femoral artery. The remaining PE 50 was routed subcutaneously and exteriorized between the scapulae. Catheter placement was performed on the day of the experiment under pentobarbital anesthesia (40 mg/kg). There was at least a 2-h recovery period between the catheter placement and the start of the experiment. Saline solutions of the peptides were administered intraarterially in a 100-μL bolus. MAP and HR were measured with Gould-Statham P23Db pressure transducers and monitored with a Beckman R-611 dynograph or a Gould physiograph. A Cyborg A-D converter linked to an IBM-XT compatible computer was used for data collection. MAP was calculated as: [(systolic − diastolic/3) + diastolic]. Zero points were determined prior to administration of peptides by injection of saline vehicle alone.

Data Analysis. In all cases data points were calculated as ΔMAP or ΔHR by subtraction of mean pretreatment zero values ($n = 4$) from posttreatment values for each animal at each dose and time. These data were then grouped for each dose and time, and means ± SEM were calculated. Dose–response curves were fitted to the 1-min data by computer iteration (Bolt, Beranek and Newman Research Systems RS/1 Biocomputing Software) and ED_{50}'s (the effective dose required to elicit 50% of the maximal response) were calculated. Relative potencies were then calculated as ED_{50} (compound)/ED_{50} (NPY) for those compounds for which the experimentally determined maximum (max) was within 20% of that determined for NPY.

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Synthesis and in Vitro Activity of 1β-Methyl C-2 Quaternary Heterocyclic Alkylthio Carbapenems

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New 1β-methylcarbapenems having various (substituted) quaternary heterocyclic alkythio groups at the C-2 position were synthesized and tested for antibacterial activity and renal dipeptidase susceptibility. Compounds having the 1β-methyl substituent were found to possess an increased stability to the enzyme. In addition, combination of the 1β-methyl substituent and the C-2 quaternary heterocyclic alkylthio side chain generated compounds with excellent antipseudomonal activity and improved stability toward hydrolysis by renal dipeptidase.

Carbapenems, as exemplified by the natural product thienamycin, have the widest spectrum of antibacterial activity of all β-lactams.[1] However, the discovery that they are susceptible to hydrolysis by a mammalian enzyme, renal dipeptidase, has hampered development in this class of compounds.[2] Since the disclosure of the first practical carbapenem synthesis by the Merck group, many research institutes have prepared various substituted carbapenems to address this deficiency.[3] The vast majority of these

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