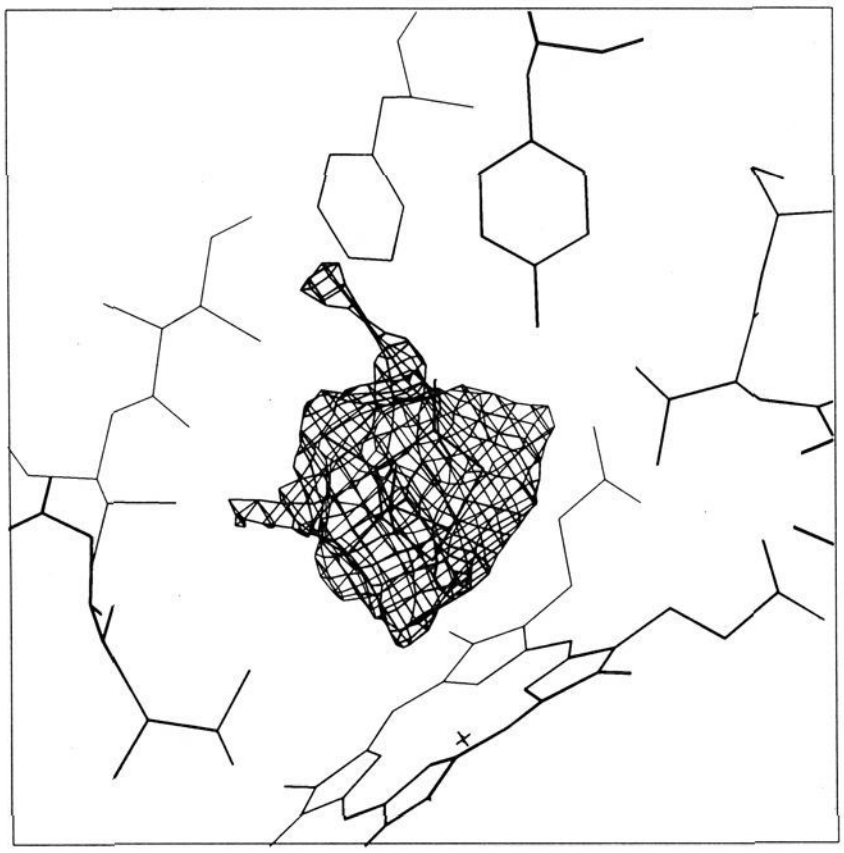

Figure 10. The energy contours at −2.0 kcal/mol for a methyl group probe interacting with cytochrome P450-cam. These energy contours surround the camphor substrate except for its polar carbonyl oxygen atom, and they define the position of the camphor molecule much more clearly than the van der Waals surface of the protein does.

protein itself. This shows the way in which program GRID can be used in sterically fitting a small molecule to a receptor site. It can be seen that the camphor oxygen atom alone is outside these methyl contours, showing that it is in a less hydrophobic region of the active site than the rest of the substrate molecule. The orientation of the camphor is apparently controlled by the precisely defined geometry of the hydrogen bond between the camphor oxygen and Tyr 96, while the position of the hydrophobic part of the substrate is determined by steric interactions with the surrounding protein.

Conclusion

An energy function has been developed for use in calculating the interaction between a probe group and a target molecule. The energy function consists of a Lennard-Jones, an electrostatic, and a hydrogen-bond term. There are sufficient experimental observations of hydrogen bonds in the literature to enable a set of hydrogen-bond functions to be modeled and fitted to experimental data. These hydrogen-bond functions are specific to different types of atom and model the variation in strength and geometry of the hydrogen bond according to the chemical nature of the donor and acceptor atoms as well as their position and orientation in the system under study. However, current experimental data for some types of atom are sparse and so functions to describe the hydrogen-bonding characteristics of certain atoms are still poorly defined. As more experimental data become available in the future, it should be possible to refine and improve such hydrogen-bond functions.

When appropriate, the hydrogen-bond term takes account of the mobility of the hydrogens and lone pairs analytically. Consideration of this property is vital in order that realistic predictions may be made. When a tautomeric change occurs on the approach of a ligand molecule, the functions allow for the appropriate charge redistribution in the target molecule. This treatment of molecular polarization is only applied to histidine residues in the present work, but it exemplfies an important principle which can be extended to other target systems.

A few examples of the energy maps have been shown in order to demonstrate the general shape of the predicted interaction between hydrogen-bonding groups. Predictions of substrate binding in cytochrome P450-cam demonstrate that GRID predictions can be accurate and specific even for a complicated system containing protein, a prosthetic group, a transition metal and water. The new energy function produces better results than the function originally used in program GRID, which only had a very simple and general hydrogen-bond term. It should be of value in the assessment of many different types of intermolecular interactions including those between drugs and their receptors.

Acknowledgment. Support from the Science and Engineering Research Council and the Medical Research Council is gratefully acknowledged.

Registry No. Tyrosine, 60-18-4; water, 7732-18-5; histidine, 71-00-1; L-noradrenaline, 51-41-2; D-noradrenaline, 149-95-1; L-adrenaline, 51-43-4; digitoxigenin, 143-62-4; gitoxigenin, 545-26-6; glucose, 50-99-7; cytochrome P450, 9035-51-2.

Analogues of Atriopeptin(103–125)amide Having High Binding Selectivity

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Analogues of atriopeptin(103–125)amide were prepared having a disulfide bridge at positions different from that found in the natural product. Most of these conformationally perturbed peptides were found to bind selectively to one subclass of binding sites. Binding affinity to a class of specific binding sites that is not associated with any known biological activity (nonvasorelaxant or NVR binding sites) is unaffected or even modestly improved. Affinity for the receptor subclass that is associated with vasorelaxation (VR subclass) decreases in most examples. In several cases, binding to the VR subclass is below the limits of detection for the assay used here. The data demonstrate that binding of atrial peptides to VR receptors requires rigidly defined receptor/ligand interactions. In contrast, the NVR subclass of binding sites appears to tolerate changes in peptide structure quite well.

The atrial peptides, a related group of cyclic hormones, are known to be potent vasorelaxants, natriuretics, and diuretics. These effects indicate a role in the regulation of fluid volume and blood pressure as well as electrolyte balance. As a result, this group of hormones, variously known as atriopeptin (AP), atrial natriuretic factor (ANF), and atrial natriuretic peptide (ANP), has been the subject

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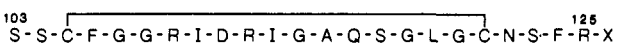
0022-2623/89/1832-1094$01.50/0

of intense study.[2] Recent investigations[3-6] in this area led to the discovery of at least two different specific high-affinity binding sites of AP. One subclass of binding sites is linked to guanosine 3′,5′-monophosphate (cGMP) production and correlates with smooth muscle relaxant activity. For convenience, they are referred to here as vasorelaxant (VR) receptors. A second class of high-affinity binding sites is not associated with guanylate cyclase or vasorelaxation and is termed the nonvasorelaxant (NVR) subclass of binding sites. The function of these sites is not currently understood. They have not been shown to be associated with any biological function and so have been called "biologically silent receptors".[7] From studies involving an analogue of ANF having high affinity for only one receptor subtype, it was hypothesized by Maack and co-workers[7] that these binding sites may serve as clearance and/or storage receptors that function to modulate plasma levels of the hormone. In the absence of any demonstrated biological function, we refer to them here as "binding sites" rather than "receptors".

As part of ongoing efforts to elucidate those conformational requirements of AP that permit effective interactions with its receptor(s), we recently reported[8] the synthesis and structure/activity relationship (SAR) studies of a series of conformationally restricted analogues of atriopeptin(103–125)amide (**1a**). The novel bicyclic



1a: X = NH_2
b: X = CO_2H
c: X = Tyr

peptides and several of the monocyclic precursors described were found to have a preference for one subclass of binding sites. In each case, binding to the NVR sites was about equipotent to that of **1a**. Affinity to the VR sites decreased, often by 2 orders of magnitude, although good potencies were still maintained in some cases (apparent K_i's of about 25 nM). The results suggested that the atrial peptides have considerably more specific conformational requirements for effective interactions with the VR subclass of receptors than they do for the NVR binding sites.

Because of the good selectivity that was observed for several of the monocyclic analogues, we became interested in exploring the effect of changes in the location of the disulfide bond on binding selectivity. Cystine groups certainly decrease flexibility of proteins and peptides and it seems possible that the conformational constraints enforced by the presence of a disulfide bridge between residues 105 and 121 of AP could stabilize the conformation required for productive association with the VR subclass of receptors to a greater extent than for the NVR binding sites. Altering the conformational restrictions already inherent in the natural product by moving the location of the cystine moiety might then have a relatively greater effect on binding to the VR receptors. We report here the synthesis and receptor binding studies of several AP analogues in which the location of the cystine group has been varied from the position found in the native hormone (105/121).

Table I. Binding Affinities of AP Analogues Relative to AP(103–125)NH_2

analogues of AP(103–125)NH_2[a]	binding affinity: relative potency NVR[b]	VR[c]	selectivity factor[d]
1c, AP(103–126)	0.31[e,f]		1
1a, AP(103–125)NH_2	1.0[f]		1
2a, Cys120 Gly121	0.91[f]		1
2b, Cys120 Ala121	0.94	0.16	6
2c, Cys120 Nva121	0.63	0.047	13
2d, Cys120 Ser121	1.3	0.056	23
2e, Cys120 Cys(*S*-Me)121	1.3	0.041	32
3a, Cys104 Gly105	1.8	0.056	32
3b, Cys104 Ala105	2.2	0.088	25
3c, Cys104 Abu105	2.1	0.053	40
3d, Cys104 Nva105	0.75	0.0084	89
3e, Cys104 Ser105	3.8	0.24	16
3f, Cys104 Cys(*S*-Me)105	6.6	0.28	24
4, Cys122 Cys(*S*-Me)121	3.1	0.10	31
5a, Cys104,122 Gly105,121	1.5	0.0069	217
5b, Cys104,122 Ala105,121	1.3	0.0028	464
5c, Cys104,122 Nva105,121	0.47	0.0010	470
5d, Cys104,122 Ser105,121	2.0	0.0050	400
6a, Cys108,117 Gly105,121	1.3	0.011	118
6b, Cys108,117 Ala105,121	0.63	0.0013	485
6c, Cys108,117 Nva105,121	0.26	0.00091	286
6d, Cys108,117 Ser105,121	0.44	0.0015	293
6e, Cys108,117 Cys(*S*-Me)105,121	0.59	0.0016	369
7a, Cys108,118 Gly105,121	0.81	0.00072	1125
7b, Cys108,118 Ala105,121	0.44	<0.00059	>746
7c, Cys108,118 Nva105,121	0.27	<0.00059	>458
7d, Cys108,118 Ser105,121	0.41	<0.00059	>695

[a] All structures are analogues of AP(103–125)NH_2 unless otherwise specified. Norvaline (Nva) and α-amino butyric acid (Abu) are commercially available nonprotein amino acids. [b] Nonvasorelaxant subclass. [c] Vasorelaxant subclass. [d] NVR/VR. [e] Apparent K_i = 0.25 ± 0.16 nM (n = 6). [f] Multiple binding sites could not be detected.

Chemistry

The AP analogues discussed here are all numbered as fragments of the 126 amino acid prohormone. The peptides were prepared by solid-phase peptide synthesis methodology and no difficulties were encountered either during synthesis or purification. In order to allow correlation with other ongoing SAR studies, analogues of **1a**, the C-terminal carboxamide derivative of AP(103–125) (**1b**), were synthesized. Oxidation of the pair of deblocked cysteine residues in each peptide to the corresponding cystine moiety was accomplished either through the use of atmospheric oxygen or potassium ferricyanide. While yields were not optimized, satisfactory quantities of pure cyclic peptide were typically obtained.

Biology

The receptor binding properties of the AP analogues discussed here were evaluated in a test involving competitive displacement of ^{125}I-AP(103–126) from a rabbit lung membrane preparation as described elsewhere.[8,9] Both

(2) For recent reviews, see: *Atrial Hormones and Other Natriuretic Factors*; Mulrow, P. J., Schrier, R., Eds.; American Physiological Society; Bethesda, MD, 1987. *Endocrinology and Metabolism Clinics of North America*; Rosenblatt, M., Jacobs, J. W., Eds.; W. B. Saunders Co.: Philadelphia, PA, 1987; Vol. 16. Trapani, A. J.; Olins, G. M.; Blaine, E. H. In *Annual Reports in Medicinal Chemistry*; Allen, R. C., Ed.; Academic Press, Inc.; San Diego, CA, 1988; Vol. 23, pp 101–109.

(3) Leitman, D. C.; Andresen, J. W.; Kuno T.; Kamisaki, Y.; Chang, J-K.; Murad, F. *J. Biol. Chem.* **1986**, *261*, 11650.

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of the known classes of binding sites are present in this tissue. The VR receptors constitute the smaller portion (approximately 27%) while the larger fraction (approximately 73%) consists of the NVR class of binding sites. Multiple classes of AP binding sites have been reported for several different tissues. Their relative proportions vary widely with the tissue.

Results and Discussion

The native AP hormones all contain a cyclic 17 amino acid core having a high degree of homology and a disulfide bond connecting residues 105 and 121. Each of these peptides, including **1b** and AP(103–126) (**1c**), have about the same apparent inhibitor constant values for both the VR and the NVR class of binding sites. Previous SAR studies[10] suggested that this lack of selectivity is typical of analogues having a 17-amino acid ring connected by a single disulfide bond that bridges residues 105 and 121.

In each of the examples shown in Table I, moving the cystine residue from the native position required the simultaneous introduction of several changes in the peptide. It was necessary to replace one or two residues with cysteine moieties in order to shift the location of the disulfide bridge. To accomplish this, residues were chosen that appeared from other SAR studies to tolerate a wide variety of substituents without a dramatic loss of binding potency. Substitutions of residues that were judged to be important for the expression of high binding affinities were avoided. Furthermore, having moved the cysteine residues from positions 105 and/or 121, it was necessary to replace these sites with other amino acids in order to maintain the 23 amino acid length of **1a**. Since glycine is the structurally least complex amino acid and alanine is the least complex amino acid having optical activity, substitutions with these amino acids were chosen. In addition, an approximation of the electronic properties of the sulfhydryl group of cysteine was made by substituting residues 105/121 with serine or *S*-methylcysteine. Finally, the nonprotein amino acid norvaline was chosen as a rough mimic of the steric bulk of the R group of cysteine which has none of the polarizability of a thiol or a disulfide.

Within each series, substitutions of the residues at positions 105 or 121 afforded relatively small changes in affinity. Also, differences in the selectivity factors (defined here as the relative binding affinity to the NVR sites divided by the relative binding affinity to the VR sites) were typically less than 1 order of magnitude. In contrast, the location of the cystine moiety and/or ring size could have a dramatic effect on receptor selectivity. Moving the cysteine from residue 121 to position 120 afforded analogues (**2a–e**) having a ring size of 16 amino acids. While binding to the NVR sites was largely unaffected by this change, a small (up to 24-fold) decrease in binding affinity to the VR sites was observed. There is a trend for reduced binding to the VR receptors as the size of the R group is increased, although this is not pronounced. Moving Cys^{105} to position 104 increased the ring size by one amino acid to 18 residues. Within this series, binding to the NVR sites actually improved in most cases. These analogues (**3a–f**) were also found to have modestly lower binding affinities to the VR subclass of receptors relative to **1a**. It is interesting to note that **3d** has about 1 order of magnitude lower affinity for the VR receptors than do the other analogues in this series. This effect cannot be attributed solely to the added steric bulk of the *n*-propyl side chain of norvaline since the (methylthio)methyl R group present in **3f** is even somewhat bulkier. At the same time, less bulky R groups such as a proton, a methyl group, or an ethyl group (**3a**, **3b**, or **3c**, respectively) have higher affinities than **3d** for the VR sites. These data suggest that interactions of residue 105 with the VR subclass of receptors are favored by a relatively nonbulky R group. However, additional steric bulk is tolerated well if it includes a polarizable moiety such as a hydroxyl group (e.g., serine) or a sulfide (e.g., *S*-methylcysteine). We conclude that VR receptor/ligand interactions are destabilized by a sterically bulky hydrophobic R group at residue 105 of the ligand and stabilized by interactions with a polar functional group at this position. Similar results were obtained by moving the cysteine from position 121 to 122. This analogue (**4**) also contained an 18 amino acid ring and exhibited increased affinity to the NVR class of binding sites as well as a modest decrease in affinity to the VR receptors.

Shifting the positions of both of the cysteine residues substantially affected the selectivity factor. Introduction of a disulfide bridge between residues 104 and 122 afforded AP analogues (**5a–d**) having a 19 amino acid ring. In these examples, a 2 order of magnitude decrease in binding affinity to the VR receptor subclass was observed. Since binding of these analogues to the NVR sites remained equipotent to that of the standard, selectivity factors ranging from over 200 to almost 500 were observed for these peptides.

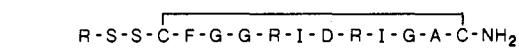
Conformational constraints within the cyclic portion of the peptide were increased by moving the cysteine moieties to positions 108 and 117. Although a high degree of selectivity was found for these cyclic analogues (**6a–e**) having only 10 amino acids in the ring, the selectivity factors did not differ greatly from those obtained for **5a–d**. The potency of **6a** is particularly noteworthy since it still binds to the VR subclass of receptors with high affinity (app K_i = 7.4 nM) and is also about 1 order of magnitude more potent than any of the other analogues in this series. This reinforces the suggestion that the nature of the R group(s) at residues 105 and/or 121 does affect binding to the VR receptor. Effective VR receptor/ligand interactions appear to be favored by a small substituent at one or both of these residues. Within this series, these interactions do not seem to be improved by polarizable functional groups.

Analogues (**7a–d**) having 11 amino acids within the cyclic core were synthesized by substituting residues 108 and 118 with cystine. Binding affinities to the NVR sites were similar to those of the other peptides discussed here. However, affinities to the VR binding sites were weak and, in some cases (**7b–d**), were below the limits of detection (apparent K_i's > 132 nM). These peptides may represent examples of AP analogues that bind exclusively to the NVR sites.

It is clear from the data summarized in Table I that selectivity in these analogues invariably favors binding to the NVR sites. In fact, this subclass of binding sites appears to tolerate the changes in the location of the disulfide bond described here very well indeed. In most cases, binding affinity to the NVR sites is either little changed or even somewhat improved when compared to **1a**. In contrast, in every example but one (**2a**), binding affinity to the VR subclass of receptors decreases upon shifting the position of the disulfide bridge. Small (i.e., one amino acid)

(10) See for example: Nutt, R. F.; Veber, D. F. in ref 2b, p 19. Nutt, R. F.; Ciccarone, T. M.; Brady, S. F.; Colton, C. D.; Paleveda, W. J.; Lyle, T. A.; Williams, T. M.; Veber, D. F.; Wallace, A.; Winquist, R. J. In *Peptides: Chemistry and Biology*; Marshall, G. R., Ed.; ESCOM: Leiden, The Netherlands, 1988; p 444. Konishi, Y.; Frazier, R. B.; Olins, G. M.; Blehm, D. J.; Tjoeng, F. S.; Zupec, M. E.; Whipple, D. E. *Ibid.* p 479.

changes in the ring size appear to result in modest decreases in relative affinity to the VR binding sites (**2a–e**, **3a–f**, or **4**) while more dramatic changes afford a sharp drop in affinity to this receptor subclass (**5a–d**, **6a–e**, or **7a–d**). The peptides described here are still highly flexible. However, even AP analogues that are more conformationally restricted exhibited similar binding selectivity.[8] The ability of the NVR sites to productively associate with such a disparate group of analogues suggests that only a (relatively) small fragment of the native hormone is actually involved in interactions with this subclass of binding sites. It is possible that the conformational perturbations described here all permit an acceptable degree of conformational flexibility of this putative "active fragment". Interestingly, **8**, the selective peptide reported by Maack and co-workers,[7] is a 15 amino acid ring-deleted analogue of ANF. Other reported analogues that are selective for the NVR sites are a 21 amino acid linear analogue[11] (**9**) and an eight amino acid AP fragment[12] (**10**). The existence of truncated analogues having dissimilar affinities for different classes of specific AP binding proteins has also been observed by Inagami and co-workers.[5] In addition Budzik et al.[13] have reported the separation of vasorelaxant and cGMP activities both in vivo and in vitro by synthetic AP analogues.



8

S-F-G-G-R-I-D-R-I-G-A-Q-S-G-L-G-N-S-F-R-Y

9

F-G-G-R-I-D-R-I-NH_2

10

None of the selective AP analogues described here or elsewhere maintain full binding affinity to the VR subclass of receptors. Accordingly, this suggests that highly specific requirements must be satisfied in order for the peptide to associate with this receptor type. It is not yet known whether this association involves simultaneous interactions with a large portion of the ligand, interactions with multiple (possibly distal) smaller fragments, and/or rigidly defined conformational requirements. Given these binding requirements, several of the analogues in this series have surprisingly high affinity to the VR receptor. Changes in ring size of one amino acid do not seem to afford dramatic changes in affinity to the VR site. Analogues such as **2a–e**, **3a–f**, and **4** still bind well to this receptor (app K_i's of 0.09–9.3 nM vs 0.08 nM for **1a**). While, in general, a larger change in ring size results in a greater decrease in binding to the VR receptor, several of these analogues (e.g., **5a**, **6a**) have affinities to this class of receptors that are still quite good (app K_i's of ~10 nM). Here, despite probable substantial conformational perturbations, these analogues can still effectively interact with the VR receptor subtype, although not as well as **1a**. While the data presented here do not permit identification of the specific nature of these interactions, they do encourage us to believe that conformationally restrained AP analogues having full potency to the VR receptor subtype can be prepared.

Conclusions

We have shown that shifting the location of the disulfide bridge in **1a** can result in the appearance of high binding selectivity. In every case, alterations of conformational restrictions present in **1a** decrease binding affinity to the VR sites without greatly affecting affinity to the NVR subclass. This preference is in agreement with our previous results[8] for other conformationally constrained AP analogues and supports the hypothesis that atrial peptides must satisfy highly specific requirements in order to be recognized by the VR sites. The analogues described here exhibit binding potencies to this subclass of receptors that vary over at least 3 orders of magnitude. Furthermore, these data suggest that the NVR binding sites tolerate a wide range of conformational and/or structural variations with little change in binding efficacy. The affinity for the NVR subclass of binding sites varies by only a factor of 25 in these examples. Since the biological function (if any) of this subclass is not known, the ability of these analogues to elicit a biological response cannot be estimated. However, AP analogues such as **7b–d** having very high receptor specificity could be useful probes for helping to determine the biological function of the NVR binding sites.

Experimental Section

Compositional analysis data were collected from 6 M HCl hydrosylates (vapor phase, 110 °C, 24 h) using ninhydrin-based analysis performed on a Beckman 6300 high-performance analyzer. Positive ion fast atom bombardment mass spectroscopy (FABMS) was carried out with a VG-ZAB SE double-focusing mass spectrometer. The FAB beam consisted of xenon neutrals at an acceleration of 9 kV (1 mA). The mass spectrometer scanned at a rate of 10 s/decade over a mass range of 100–3000 daltons at a resolution of 1000. The FAB matrix used was a mixture of glycerol/thioglycerol/HCl/dimethyl sulfoxide. HPLC was carried out on a Waters system consisting of two Model 510 pumps connected to a Model 680 automated gradient controller. Additional solvent mixing was accomplished by an Axxiom Model 400 mixer. Column effluent was monitored at 220 nm by a Waters Model 481 spectrophotometer and peak area was measured using a Waters Model 740 integrator. All eluents for both high-pressure and medium-pressure liquid chromatography were HPLC grade and 0.05% trifluoroacetic acid (spectrograde) was added.

Peptide Synthesis. Solid-phase peptide syntheses were carried out on *p*-methylbenzhydrylamine hydrochloride resin (~0.45 mequiv/g) with an ABI Model 430A peptide synthesizer. Unless otherwise specified, all protected amino acids, reagents, and solvents were purchased from ABI and used directly with no further purification. Boc-*S*-methylcysteine was purchased from Bachem and used directly with no further purification. Boc-α-aminobutyric acid and Boc-norvaline were purchased from Sigma and used directly with no further purification. The peptides were removed from resin and deprotected with anhydrous hydrogen fluoride (HF; 10 mL/g resin-bound peptide) containing anisole (1 mL/g) and 2-mercaptopyridine (0.1 g/g) at 0 °C for 1.5 h on a Peptide Institute Type I HF-Reaction Apparatus. After evaporation of the HF, the residue was triturated successively with ether (5 × 50 mL) and ethyl acetate (4 × 50 mL). The residue was then washed with aqueous acetic acid (50 mL of 30% AcOH followed by 2 × 50 mL of 5% AcOH). The aqueous fractions were combined and lyophilized. The crude peptide was dissolved in 0.1 M tris(hydroxymethyl)aminomethane (Tris) buffer (pH 8; 25 mL/g). An equal weight of dithiothreitol (DTT) was added and the resulting solution was stirred at room temperature for 1 h before being filtered and loaded directly onto a glass column (350 mm × 40 mm i.d.) packed with C_{18} silica gel (Vydac; 15–20 μm, 300 A) and equilibrated with 5% acetonitrile in water. Eluent flow (5% acetonitrile; ~6 mL/min) was initiated and isocratic conditions were maintained until DTT and Tris eluted. At this time, a gradient was initiated by adding 50% acetonitrile (1000 mL) dropwise to the solvent reservoir at such a rate that the reservoir level was maintained at 1000 mL. Fractions (~8 mL)

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(12) Scarborough, R. M.; Arfsten, A.; Kang, L. L.; Schwartz, K.; McEnroe, G. A.; Porter, J. G.; Suzuki, M.; Maack, T.; Lewicki, J. A. *J. Cellular Biochem.* 1988, Suppl. 12A, 20.
(13) Budzik, G. P.; Firestone, S. L.; Bush, E. N.; Connolly, P. J.; Rockway, T. W.; Sarin, V. K.; Holleman, W. H. *Biochem. Biophys. Res. Commun.* 1987, *144*, 422.
(14) Deakin, H.; Ord, M. G.; Stocken, L. A. *Biochem. J.* 1963, *89*, 296.

were collected and like fractions were combined and lyophilized to afford a solid, which was generally >80% pure.

Cyclization. A. Via Air Oxidation. The partially purified acyclic peptide was dissolved in 0.1 M ammonium bicarbonate (1.5 mg/mL) and stirred open to the air. The course of the reaction was monitored via HPLC. After cyclization was complete (several hours to several days), the solution was acidified (30% AcOH) and lyophilized. The resulting solid was purified via HPLC on a C_{18} silica gel column (Vydac; 22 mm i.d. × 250 mm, 15–20 μm, 300 A) eluting with a linear gradient of 15–35% acetonitrile over 25 min at a flow rate of 9 mL/min.

B. Via Potassium Ferricyanide. To a magnetically stirred solution of 0.1 M tris(hydroxymethyl)aminomethane (Tris) buffer (50 mL, pH 8–8.5) was added a solution of 0.1 N potassium ferricyanide (10 mL), followed by those pooled fractions collected from medium-pressure liquid chromatography (typically 36–40 mL) that have a purity >80%. The course of the reaction, as monitored by HPLC and Ellman's colorimetric assay,[12] was complete within 30 min. The solution was adjusted to a pH of 2.5–3.5 with acetic acid. Anion-exchange resin (Dowex SBR; nuclear grade, hydroxide form, strongly basic, 8% cross-linked, 20–50 dry mesh) was added until almost complete decoloration occurred. After filtration, lyophilization afforded a white solid, which was purified via HPLC as described in section A.

C. Via Iodine. The cysteine residues of **6a** were protected with acetamidomethyl (ACM) groups during synthesis. Lyophilization of the aqueous fraction obtained from hydrogen fluoride induced cleavage of the resin-bound peptide afforded the crude acyclic peptide (57 mg, ~37% by HPLC integration). This material was dissolved in 80% acetic acid (20 mL). Solid iodine (40 mg) was added in one portion and the resulting dark brown solution was stirred at room temperature for 3 h. The reaction mixture was diluted with water (40 mL) and extracted with chloroform (3 × 40 mL). The organic layer was washed once with water (40 mL), and the aqueous layers were combined and concentrated in vacuo at room temperature to about half of the original volume. Lyophilization left a white solid, which was purified via HPLC as described in section A to afford **6a** as a white solid (3.7 mg; 97% purity). Additional, less pure material was also obtained.

Yields were unoptimized. Greater emphasis was placed on peptide purity, which resulted in decreased yields. Moreover, only a sufficient quantity of peptide was purified to complete the necessary analyses/assays. All peptides were purified to greater than 97% purity. Amino acid analyses and FABMS were in agreement with the expected results.

Receptor Binding Assay. Atrial peptide analogues were studied in a competitive binding assay using rabbit lung membranes as described previously.[8,9]

Acknowledgment. We thank E. W. Kolodziej, E. J. Reinhard, P. C. Toren, D. E. Whipple, and J. F. Zobel for excellent technical assistance.

Registry No. **2a**, 119414-80-1; **2b**, 119414-81-2; **2c**, 119435-36-8; **2d**, 119435-37-9; **2e**, 119435-62-0; **3a**, 119414-82-3; **3b**, 119414-83-4; **3c**, 119414-84-5; **3d**, 119414-85-6; **3e**, 119414-86-7; **3f**, 119435-63-1; **4**, 119435-64-2; **5a**, 119414-87-8; **5b**, 119414-88-9; **5c**, 119414-89-0; **5d**, 119414-90-3; **6a**, 119435-65-3; **6b**, 119435-66-4; **6c**, 119414-91-4; **6d**, 119414-92-5; **6e**, 119435-67-5; **7a**, 119414-93-6; **7b**, 119414-94-7; **7c**, 119414-95-8; **7d**, 119435-68-6.

Retinobenzoic Acids. 3. Structure–Activity Relationships of Retinoidal Azobenzene-4-carboxylic Acids and Stilbene-4-carboxylic Acids

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Received August 23, 1988

Alkyl-substituted azobenzene-4-carboxylic acids are potent differentiation inducers of human promyelocytic leukemia cell line HL-60 to mature granulocytes. Their structure–activity relationships are very similar to those of other retinoidal benzoic acids which are generally represented by **4** and named retinobenzoic acids. The structure–activity relationships of azobenzenecarboxylic acids can also be applied to the known retinoid TTNPB (**3**). Thus, (*E*)-4-[2-(3,4-diisopropylphenyl)-1-propenyl]benzoic acid (St30 (**28**)) and (*E*)-4-[2-(3-*tert*-butylphenyl)ethenyl]benzoic acid (St40 (**29**)), the acyclic alkyl analogues of TTNPB, are nearly as active as retinoic acid. Among the oxidatively derived compounds (Az90, Ep series and Ox series) of azobenzene- or stilbenecarboxylic acids, Az90 (**71**) and Ep80 (**61**) have strong activities. However, all the bishydroxylated derivatives of TTNPB are inactive, while a diketo analogue Ox580 (**69**) has only weak potency. The activities of conformationally restricted compounds of TTNPB offer some information on the stereochemistry of the active form of these retinoidal compounds.

Retinoids, retinoic acid (RA, **1**; Chart I) and its analogues, have a fundamental and essential role in various processes of life, that is, in the maintenance of growth and as morphogens, etc.[1-3] One of the most important activities is the control of cellular differentiation and proliferation.[2] Retinoic acid acts as a specific modulator in many types of cells, both normal and neoplastic. Mechanistic studies of the retinoidal actions on cellular modulation have been reported. Retinoids control several gene expressions, including the suppression of the expression of *c-myc*[4,5] and the gene for collagenase,[6] and the enhancement of the expression of the genes of epidermal growth factor receptor (EGFR).[7] Thus, retinoids are considered to affect directly the expression of genes which control cellular differentiation and proliferation. Only recently, some hypotheses were proposed based on gene technology studies[8-10] or direct attempts to isolate specific receptor(s).[11] Now, the term "retinoids", originally defined in

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