ANTIARRHYTHMIC ACTIVITY OF A NOVEL ANALOGUE OF AAP*

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Three novel analogues of antiarrhythmic peptide (AAP), [Sar^2, Pro^3]AAP (*I*), [Sar^3]AAP (*II*) and [Sar^2, Sar^3]AAP (*III*), have been synthesized in order to get peptides with enhanced antiarrhythmic activity. Their antiarhythmic activity has been evaluated against aconitine induced arrhythmia in rats. [Sar^2, Sar^3]AAP has been found to be more active than AAP. It is equipotent to the commonly used antiarrhythmic drug quinidine, so far as delay in the onset of ventricular tachycardia, ventricular fibrillation and cardiac arrest are concerned. Relationship of biological activities of these peptides with their CD is discussed. The results suggest that the spatial structure of *III* attributed to Sar^2–Sar^3 linkage might be contributing to its higher antiarrhythmic activity.

The antiarrhythmic peptide (Gly-Pro-Hyp-Gly-Ala-Gly; AAP) isolated from bovine atria has been reported to improve the rhythmicity of mycocardial cell clusters[1] and drug induced arrhythmia in vivo[2]. In the course of structure–activity studies of this peptide we found that [Pro^3]AAP was more active than AAP against aconitine induced arrhythmia in rats[3]. The prolyl-proline part of this synthetic congener seems to have a favourable influence on the biological activity as the conformational requirements inherent to pyrrolidine ring lead to well defined structure(s) in solution. To examine this point in detail, we have prepared analogues in which one or both Pro residues have been replaced by Sar, another imino acid having no side chain functionality. This sort of replacement will give an insight into the role of prolyl-proline segment in the expression of biological activity of the hexapeptide. This paper deals with the synthesis, CD studies and antiarrhythmic activity of three new analogues of AAP: [Sar^2, Pro^3]AAP (*I*), [Sar^3]AAP (*II*) and [Sar^2, Sar^3]AAP (*III*).

EXPERIMENTAL

Synthesis of peptides: All peptides were synthesized by the well established solution phase procedure. The method used for the synthesis of AAP and [Pro^3]AAP was the same as described

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earlier[4,5]. Analogues *I*, *II*, and *III* were synthesized by coupling their corresponding N-terminal and C-terminal tripeptide fragments by mixed anhydride method. Synthesis of C-terminal fragment Boc-Gly-Ala-Gly-OBzl was achieved by the procedure described earlier[4]. Various N-terminal fragments were synthesized in a stepwise manner using DCC/HOBt procedure[6]. Homogeneity of all the intermediates and analogues was checked on reverse phase HPLC using C_{18} Novapak column with MeOH–H_2O as mobile phase. The physico-chemical characteristics of final peptides are given in Table I.

TABLE I

Analytical data of analogues *I*, *II*, and *III*

Compound	$[\alpha]_D^{25}$ (c)[a]	R_F[b]	Retention time min[c]	Formula (M.w.)	Calculated/Found		
					% C	% H	% N
I	−33·3° (1·2)	0·32	6·7	$C_{17}H_{28}N_6O_7.CH_3COOH.H_2O$ (506·5)	45·05 44·71	6·71 6·98	16·60 16·52
II	−30·0° (1·0)	0·30	6·5	$C_{17}H_{28}N_6O_7.CH_3COOH.H_2O$ (506·5)	45·05 44·83	6·98 6·88	16·52 16·31
III	−23·5° (1·4)	0·25	5·3	$C_{16}H_{30}N_6O_7.HCOOH.H_2O$ (466·4)	41·20 40·93	6·43 6·64	18·02 18·21

[a] In methanol; [b] system butanol–acetic acid–water (4 : 1 : 5); [c] system methanol–water–trifluoroacetic acid (500 : 500 : 0·25), flow rate 0·5 ml/min.

TABLE II

^{13}C Chemical shifts of analogues *I*, *II*, and *III*

Analogue	Gly^1	Pro^2	Sar^2	Pro^3	Sar^3	Ala^5	Gly^4, Gly^6
I	C^α 42·82		C^α 39·95 CH_3 29·57	C^α 61·14 C^β 24·67 C^γ 20·95 C^γ 47·34		C^α 49·74 C^β 17·05	C^α 44·18 C^α 44·54
II	C^α 42·78	C^α 58·08 C^β 24·41 C^γ 20·29 C^δ 47·26			C^α 40·69 CH_3 28·52	C^α 49·75 C^β 17·02	C^α 44·20 C^α 44·46
III	C^α 42·59		C^α 39·78 CH_3 35·76		C^α 40·18 CH_3 36·09	C^α 49·65 C^β 16·87	C^α 42·92 C^α 42·75

Spectral measurements: Enantiomeric purity and amino acid composition were determined by ^{13}C NMR at 100 MHz on Bruker 400 FT NMR spectrometer (Table II), optical rotations were determined with Perkin–Elmer 241 polarimeter. CD spectra were recorded on Jobin Yvon Mark III dichrograph using 0·5 mg/ml solutions. The band intensities are expressed as molar ellipticities $[\Theta]$ in deg cm^2 dmol^{-1} (Fig. 1).

Aconitine induced arrhythmia in rats: Experiments were carried out in male rats (100–200 g) anaesthetized with urethene (1 mg/kg, i.p.). The jugular vein was cannulated for infusion of aconitine nitrate in saline at the rate of 3·9 μg/min. The ECG changes (Lead II) were monitored and recorded on a polygraph before and after administration of drugs during the infusion. Synthetic peptides and quinidine (reference drug) were administered slowly 5 min before the infusion at doses of 10 mg/kg (i.v.). Control group received saline only. Results were expressed in terms of aconitine required for the onset of early arrhythmia (appearance of ectopic beat EA), ventricular tachycardia (VT), ventricular fibrillation (VF) and cardiac arrest (CA).

RESULTS

CD *Studies*

Proline has a unique place among naturally occurring amino acids as its introduction limits the number of possible conformations in solution. However, its replacement with Sar residue will have adverse effect as it will increase the number of possible conformations. These factors, in turn, should give rise to different CD characteristics for peptides having Pro and/or Sar.

The CD spectra of analogues *I*, *II*, and *III* are dominated by a large band around 205 nm. Analogue *II*, however, exhibits an additional positive band at 212 nm. In comparison with the CD spectrum of [Pro^3]AAP (ref.[5]), it was observed that the negative band around 200 nm for all the three analogues exhibits ellipticity values

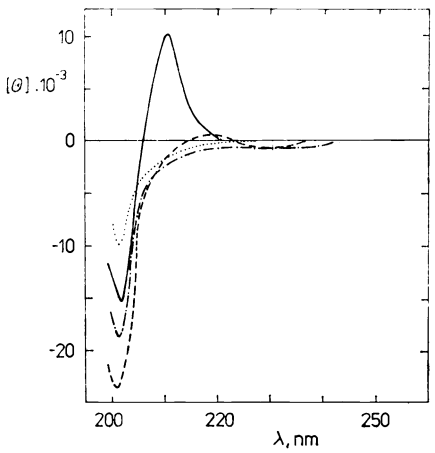

FIG. 1
CD spectra of [Pro^3]AAP (– – –), [Sar^2, Pro^3]AAP (–x–x–), [Sar^3]AAP (———) and [Sar^2, Sar^3]AAP (·····)

smaller than [Pro^3]AAP. The ellipticity values were found to be in the following order: [Pro^3]AAP > *I* > *II* > *III*. This difference in the ellipticity values may be attributed to flexibility induced by Sar residue(s). It is therefore quite apparent that the analogue *III* having Sar^2-Sar^3 segment will have maximum flexibility at position 2 and 3 among all the analogues reported here.

^{13}C NMR *Studies*

Further, in order to obtain information regarding the orientation of Pro residues in analogues *I* and *II*, we have calculated the dihedral angle ψ about the Pro C$^\alpha$—CO bond using ^{13}C chemical shift data obtained for proline. Siemion et al.[7] proposed a correlation between the dihedral angle ψ about the Pro C$^\alpha$—CO bond and C$^\beta$-C$^\gamma$ chemical shift difference. The $\Delta\delta_{\beta\gamma}$ value for Pro residue in analogue *I* was 3·7 ppm whereas in the analogue *II* it was 4·1 ppm (see Table II). These values correspond to ψ values of 145° and 154°, respectively. However, the ψ values for Pro^2 and Pro^3 in [Pro^3]AAP as reported earlier[5] were found to be 150° and 158°. Thus, the ψ values for Pro residues in analogues *I* and *II* are different from the ψ values for Pro residues in [Pro^3]AAP.

These observations suggest further that the spatial structure of *I* and *II* arising from Sar^2-Pro^3 and Pro^2-Sar^3 segment will be different from the spatial structure of [Pro^3]AAP arising from Pro^2-Pro^3 segment.

Antiarrhythmic Activity

The antiarrhythmic activities of analogues *I*, *II*, and *III* are given in Table III. Our results indicate that the analogues *I* and *II* are completely inactive against aconitine induced arrhythmia in rats. Analogue *III*, although ineffective in delaying onset of EA, showed significant protection against VT, VF, and CA. The protective effect of the analogue *III* against VT, VF, and CA was found to be higher than [Pro^3]AAP and equivalent to antiarrhythmic drug quinidine against aconitine induced arrhythmia in rats. Among the analogues reported in this paper, only [Pro^3]AAP showed significant protection against EA and was found to be comparable to quinidine.

DISCUSSION

Although sarcosine and proline are both imino acids and induce conformational constraints in the peptide backbone[8], the extent of rigidity induced by Pro will be significantly higher due to the conformationally restricted pyrrolidine ring. Thus, Sar-Sar, Sar-Pro, Pro-Sar and Pro-Pro segments may lead to different spatial structures resulting in different molecular arrangements in the peptide backbone. This is evident from our CD studies, which exhibit different CD characteristics for ana-

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TABLE III

Protective effect of peptides on aconitine induced arrhythmia in the rat

Compound	Dose (mg/kg i.v.)	No. of observation	Aconitine required[a] (mg/kg) for onset of			
			EA	VT	VF	CA
Control	—	30	90·83 ± 5·91	131·12 ± 7·08	212·74 ± 10·72	293·85 ± 13·92
Quinidine sulphate	10	35	149·71 ± 6·58[b]	203·59 ± 8·12[b]	337·66 ± 13·38[b]	563·79 ± 36·20[b]
AAP	10	4	89·38 ± 7·75	125·00 ± 5·52	331·33 ± 36·47[b]	347·50 ± 46·47
[Pro^3]AAP[c]	10	5	126·63 ± 23·44	180·38 ± 24·99[b]	331·25 ± 60·24[b]	463·00 ± 56·99[b]
I	10	5	58·29 ± 7·40[d]	101·20 ± 5·29	178·02 ± 17·96	222·86 ± 21·47
II	10	5	68·03 ± 13·25	99·22 ± 13·27	151·94 ± 16·75[d]	194·03 ± 22·66[d]
III	10	5	92·28 ± 13·94	213·41 ± 10·60[b]	355·79 ± 27·05[b]	560·00 ± 54·52[b]

[a] Values are mean ± S.E.; [b] $P < 0{\cdot}01$; [c] data taken from ref.[5]; [d] $P < 0{\cdot}05$.

logues *I*, *II*, *III* and [Pro^3]AAP. Further, the dihedral angle ψ for Pro residues obtained from ^{13}C NMR of the analogues *I* and *II* agree with our CD studies, exhibiting ψ values different from that of the ψ values for Pro residues in [Pro^3]AAP. Furthermore, the analogue *III* should have a different conformational behaviour from [Pro^3]AAP as one of us (BK) has reported earlier[5]. [Pro^3]AAP exists predominantly in PP_{II} like structure whereas the analogue *III* having Sar^2-Sar^3 segment exists predominantly in an unordered structure, as evidenced by CD. Thus, differences in the activities of analogues *I*, *II*, and *III* may be attributed to differences in their spatial structures arising from segments Sar-Pro, Pro-Sar, Sar-Sar, and Pro-Pro.

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