SYNTHESIS OF FOUR NEW V_1 ANTAGONISTS OF ARGININE--VASOPRESSIN (AVP) CONTAINING NEW THIOACIDS AT POSITION 1 AND THEIR VASOCONSTRICTOR ACTIVITY TOWARDS ISOLATED MESENTERIC ARTERIAL VESSELS OF RATS

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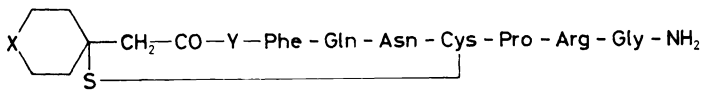
In an attempt to determine some of the structural features in position 1 that account for V_1 antagonism, four new analogues of arginine-vasopressin were synthesized and the effect of the modifications on the vasoconstrictor activity was checked using isolated mesenteric arterial vessels of rats. The protected precursors required for these analogues were synthesized by a solid phase method of peptide synthesis. One of the reported analogues, namely [1-(4-mercapto-4--tetrahydrothiopyraneacetic acid), 2-O-methyltyrosine, 8-arginine]vasopressin appears to be a potent competitive antagonist of the vasoconstrictor effect caused by AVP.

In the design of antagonists of the vasopressor responses to arginine-vasopressin (V_1 antagonists of AVP*) the replacement of Cys^1 residue in this hormone by a variety of β,β-dialkyl-β-mercaptopropionic acid residues alone or in combination with alkylation of the tyrosine at position 2 has been shown to be of particular value[2,3]. The effects of tyrosine O-alkylation on the antivasopressor potency of AVP antagonists, however, are not consistent and depend heavily on the substitutions at other positions (i.e. positions 1, 4, 8) (refs[2,3]). $d(CH_2)_5Tyr(Me)AVP$ is one of the most potent antagonists of pressor response to AVP known to date[4].

We previously reported the synthesis and some pharmacological properties of a number of potent V_1 antagonists of AVP which have larger and more lipophilic substituents than 1-mercaptocyclohexaneacetic acid [$d(CH_2)_5$] in position 1. They include some of the most potent antagonist of the vasopressor response to AVP (ref.[5]). Our results once more emphasized the importance of position 1 for V_1 antagonism and prompted us to synthesize new analogues which have two new thioacids[6]

* Unless stated otherwise, all chiral amino acids belong to the L-series. The nomenclature and symbols of the amino acids, their derivatives and peptides obey the published IUPAC recommendations[1]. SCA and OCA denote 4-mercapto-4-tetrahydrothiopyraneacetic acid and 4-mercapto-4-tetrahydropyraneacetic acid, respectively.

recently obtained in our laboratory at position 1. The four new analogues are as follows: [1-(4-mercapto-4-tetrahydropyraneacetic acid), 8-arginine]vasopressin (*I*), [1-(4-mercapto-4-tetrahydropyraneacetic acid), 2-O-methyltyrosine, 8-arginine]vasopressin (*II*), [1-(4-mercapto-4-tetrahydrothiopyraneacetic acid, 8-arginine]vasopressin (*III*) and [1-(4-mercapto-4-terahydrothiopyraneacetic acid), 2-O-methyltyrosine, 8-arginine]vasopressin (*IV*).



I, X = O ; Y = Tyr
II, X = O ; Y = Tyr(Me)
III, X = S ; Y = Tyr
VI, X = S ; Y = Tyr(Me)

We believe that the presence of a heteroatom in the ring of thioacid residue occupying position 1 may influence the pharmacological properties of analogues due to changes in the stereochemical structure of the region which is known to be important for V_1 antagonism of the peptides.

In our study we used isolated mesenteric arterial vessels in order to check how analogues *I*–*IV* and d$(CH_2)_5$Tyr(Me)AVP which served as a reference peptide would affect the vasoconstrictor effect of AVP and thus evaluate their antivasoconstrictor potency.

RESULTS AND DISCUSSION

The antivasoconstrictor properties of four new analogues of AVP together with those of d$(CH_2)_5$Tyr(Me)AVP are presented in Figs 1–5. In all preparations, the AVP evoked a dose-dependent vasoconstriction. None of the tested analogues induced any change of perfusion pressure by itself. During administration of the tested compounds, however, the pressure responses to AVP were decreased depending on the dose of AVP and the analogue tested. The comparative analysis of the dose-response curves of AVP obtained during and before infusion of the tested analogues, particularly comparison of their slopes and the values of the maximal responses to AVP shows that there is an essential difference between the mode of action of the d$(CH_2)_5$Tyr(Me)AVP (reference peptide) and analogue *I*, and analogues *II*, *III*, *IV*. Namely, the nonparallel shift of the dose-response curves of AVP caused by reference peptide and analogue *I*, as well as the significant diminution of the maximal response to AVP, suggest that both compounds are noncompetitive antagonists of V_1 receptors. On the contrary, peptides *II*, *III* and *IV* due to parallel shift of the dose-response curves and the lack of significant attenuation of the

maximal response to AVP appear to be competitive V_1 antagonists. However, no clearly consistent pattern of antivasoconstrictor potency with the type of substituent in position 1 and methylation of Tyr^2 emerged. In our assay only peptide *IV* seems to be a potent and competitive antagonist of the vasoconstrictor effect caused by AVP. Thus, it may have potential for physiological studies of the role of AVP in vascular regulation of different regions. The reference peptide which was known as a potent pressor antagonist turned out to be also a potent noncompetitive vasoconstrictor inhibitor.

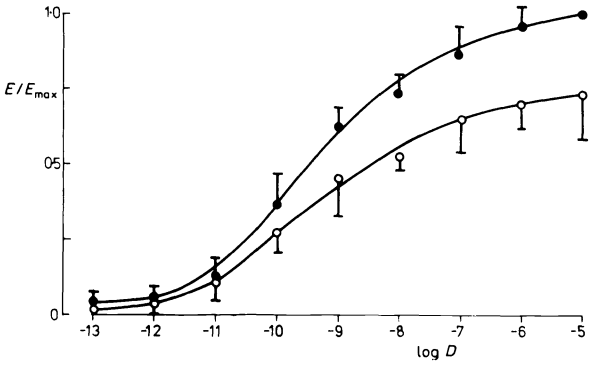


FIG. 1

Dose-effect relationship of AVP in the mesenteric arterial vessels of a rat before (●) and during the infusion of OCAAVP (*I*) (○) at a concentration of 10^{-8} mol/l ($n = 4$), *D* in mol

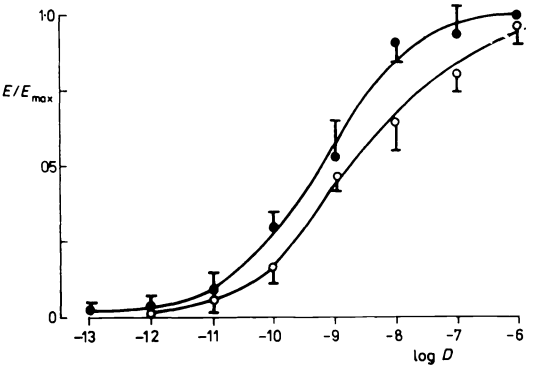


FIG. 2

Dose-effect relationship of AVP in the mesenteric arterial vessels of a rat before (●) and during the infusion of OCATyr(Me)AVP (*II*) (○) at a concentration of 10^{-8} mol/l ($n = 8$), *D* in mol

EXPERIMENTAL

Apparatus and methods. The m.p. values are uncorrected. The elemental analyses were determined on a Carlo Erba Model 1106 analyzer. The optical rotations were measured with a Hilger–Watts polarimeter with an accuracy of 0·01°. For amino acid analysis the peptides were hydrolyzed with constantly boiling hydrochloric acid (400 μl), containing phenol (20 μl), in evacuated, sealed ampoules placed for 18 h at 110°C. The analyses were performed on a Mikrotechna typ AAA 881 analyser. TLC was carried out on silica plates (Merck), and the spots were visualized by iodine. The following solvent systems were used: A chloroform–methanol (7 : 3, v/v); B

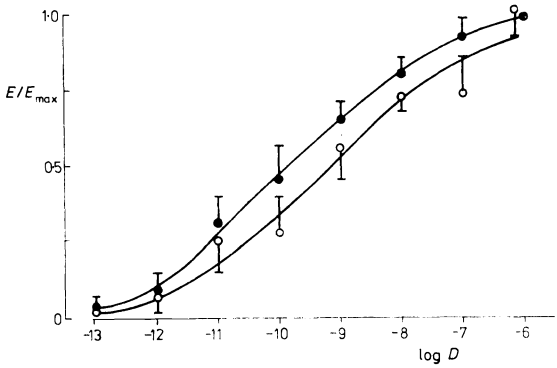


FIG. 3

Dose-effect relationship of AVP in the mesenteric arterial vessels of a rat before (●) and during the infusion of SCAAVP (*III*) (○) at a concentration of 10^{-8} mol/l ($n = 5$), D in mol

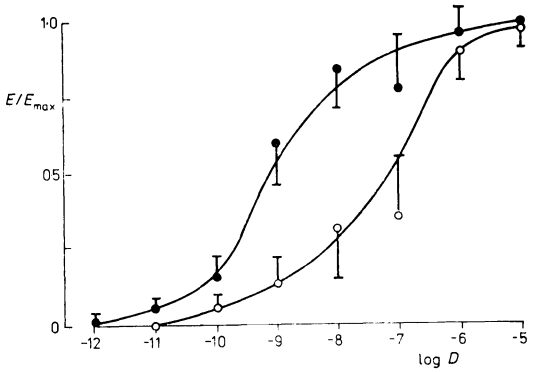


FIG. 4

Dose-effect relationship of AVP in the mesenteric arterial vessels of a rat before (●) and during the infusion of SCATyr(Me)AVP (*IV*) (○) at a concentration of 10^{-8} mol/l ($n = 9$), D in mol

1-butanol–acetic acid–water (4 : 1 : 5, v/v, upper phase); C 1-butanol–acetic acid–water–pyridine (15 : 3 : 3 : 10, v/v). N,N-Dimethylformamide (DMF) was distilled under reduced pressure; triethylamine (NEt_3) was distilled from ninhydrine. Other solvents and reagents were of analytical grade.

Synthesis of the Peptides

The protected peptide precursors required for the synthesis of analogues *I*—*IV* were prepared by the Merrifield method of solidphase synthesis entirely on the resin. First chloromethylated resin (Bio-Rad, Bio-Beads S × 1, 0·75 mmol Cl/g) was esterified with Boc-Gly to a load of 0·42 mmol/g (ref.[7]). Then SCA($BzlOCH_3$)-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly--resin, SCA($BzlOCH_3$)-Tyr(Me)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-resin, OCA($BzlOCH_3$)--Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-resin, and OCA($BzlOCH_3$)-Tyr(Me)-Phe--Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-resin were prepared using solid phase methodology as previously described[8,9]. Coupling reactions were mediated by either the DCC or DCC-HOBt method[10].

The completeness of each coupling reaction was monitored by the Kaiser test[11]. 4-[(*p*-Methoxyphenyl)methyl]thio-4-tetrahydropyraneacetic acid[6] and 4-[(*p*-methoxyphenyl)methyl]thio-4--tetrahydrothiopyraneacetic acid were each used in the final coupling steps. The protected peptides were cleaved from the resin by ammonolysis[8]. Following evaporation of the solvent, the products were extracted into hot DMF, precipitated with boiling water, and left overnight at room temperature. The peptides were collected by filtration, washed with water, and dried in vacuo over P_2O_5. The product was further purified by dissolving in DMF and reprecipitating with $EtOH/Et_2O$ (1 : 4). The physico-chemical properties of protected peptides (*V*—*VIII*) are summarized in Table I.

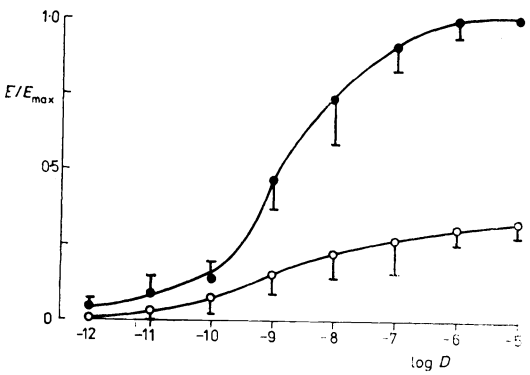


FIG. 5

Dose-effect relationship of AVP in the mesenteric arterial vessels of a rat before (●) and during the infusion of $d(CH_2)_5Tyr(Me)AVP$ (○) (reference peptide) at a concentration of 10^{-8} mol/l ($n = 9$), *D* in mol

[1-(4-Mercapto-4-tetrahydropyraneacetic acid),8-arginine]vasopressin (*I*)

A solution of the protected acyloctapeptide amide (*V*) (166 mg, 0·104 mmol) in sodium dried and redistilled ammonia (400 ml) was treated at boiling point and with stirring with sodium

TABLE I

Physico-chemical data for protected intermediates

Compound	No.	R_F A / B / M.p., °C	$[\alpha]_D$ (*c* 1, DMF)	Formula (M.w.)	Calculated/Found % C	% H	% N
OCA(BzlOCH$_3$)-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg-(Tos)-Gly-NH$_2$	*V*	0·44 / 0·51 / 175–178	−39·4	$C_{79}H_{98}N_{14}O_{16}S_3$ (1 595·93)	59·46 / 59·21	6·19 / 6·28	12·29 / 12·42
OCA(BzlOCH$_3$)-Tyr(Me)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH$_2$	*VI*	0·39 / 0·50 / 179–181	−37·8	$C_{73}H_{94}N_{14}O_{16}S_3$ (1 519·83)	57·69 / 57·39	6·23 / 6·18	12·90 / 12·98
SCA(BzlOCH$_3$)-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH$_2$	*VII*	0·29 / 0·55 / 140–142	−36·7	$C_{79}H_{98}N_{14}O_{15}S_4$ (1 611·99)	58·86 / 58·49	6·13 / 6·28	12·16 / 12·35
SCA(BzlOCH$_3$)-Tyr(Me)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH$_2$	*VIII*	0·25 / 0·42 / 145–148	−37·3	$C_{73}H_{94}N_{14}O_{15}S_4$ (1 535·89)	57·09 / 56·82	6·17 / 5·92	12·77 / 12·48

TABLE II

Physico-chemical characteristics of AVP analogues

Peptide	R_F B / C	$[\alpha]_D$ (*c* 0·5, 1M-AcOH)	Amino acid analysis Tyr Pro	Phe Arg	Glu Gly	Asp NH_3	Cys
I	0·23 / 0·45	−58·5	1·02 / 1·01	1·01 / 1·04	1·04 / 1·00	0·98 / 3·06	0·95
II	0·29 / 0·47	−57·5	1·01 / 1·03	1·03 / 1·04	1·03 / 1·00	0·99 / 3·07	0·94
III	0·21 / 0·28	−56·7	1·03 / 1·02	1·02 / 1·03	1·04 / 1·00	1·00 / 3·07	0·94
IV	0·30 / 0·50	−57·1	1·03 / 1·04	1·02 / 1·03	1·01 / 1·00	1·01 / 3·06	0·97

from a stick of the metal contained in a small-bore glass tube until a light-blue colour persisted in the solution for 30 s. Dry acetic acid (0·4 ml) was added to discharge the colour. The solution was evaporated, the residue dissolved in aqueous acetic acid (0·5%, 900 ml) and this solution was treated with 2M ammonium hydroxide solution to give a solution of pH 6·5. An excess of a solution of potassium ferricyanide (0·01 mol l^{-1}, 21 ml) was added gradually with stirring. The yellow solution was stirred for an additional 2 min and then for 10 min with anion exchange resin (Amberlite IR-45, acetic form, 10 g damp weight). The reaction mixture was filtered through a bed of resin (10 g damp weight). The bed was washed with aqueous acetic acid (0·2%, 100 ml) and the combined filtrate and washings were lyophilized. The resulting material was dissolved in 7 ml of aqueous acetic acid (50%) and desalted on a Sephadex G-15 column (120 × 2·9 cm) eluted with aqueous acetic acid (50%) with a flow rate of 6·5 ml/h. The eluate was fractioned and monitored for absorbance at 254 nm. The fractions comprising the major peak were pooled and lyophilized and the residue (113 mg) was further subjected to gel filtration on a Sephadex LH-20 column (120 × 1·4 cm) eluted with aqueous acetic acid (30%) with a flow rate of 4 ml/h. The peptide was eluted as a single peak. Lyophilization of the pertinent fractions gave vasopressin analogue *I*: yield 68·3 mg (60%) based on the amount of protected peptide used in the reduction-reoxidation procedure.

The physico-chemical properties of this and the remaining free peptides *II*—*IV*, which were prepared in the same way as for *I*, are given in Table II.

Pharmacological Methods

The experiments were carried out on 35 preparations of isolated mesenteric arterial vessels obtained under ethylurethane anaesthesia (1 g/kg i.p.) from adult male Wistar rats weighing 250 to 300 g, according to McGregor's method[12] with our own modifications[13].

Aerated and heated to 37°C modified Kreb's solution (composition in mmol/l, as following: NaCl 112, KCl 5·0, $MgCl_2$ 1·1, NaH_2PO_4 1·6, $CaCl_2$ 25·0, $NaHCO_3$ 24·0, dextrose 11·2) was delivered by a roller pump ("Elmed" Type 304, Warsaw, Poland), at a constant rate (12 ml/min) to the preparation through a polyethylene cannula (Venflon — 14, "Viggo", Sweden) inserted into the trunk of the superior mesenteric artery. Perfusion pressure, expressed in mm of mercury was measured with an inductive sensor (Institute of Fluid Flow Machinery the Polish Academy of Sciences, Gdańsk, Poland) and recorded continuously (recorder TZ-21S, "Laboratorní přístroje" Prague, Czechoslovakia). Having stabilized the baseline perfusion pressure, AVP dissolved in 0·1 ml of Kreb's solution was serially injected into a preparation at subsequent doses (in mol) of: 10^{-15}, 10^{-14}, 10^{-13}, 10^{-12}, 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6} and 10^{-5}. The preparations were then washed with modified Krebs's solution for 1 h. Next, the preparations were continouously perfused with a 10^{-8} mol/l solution of a tested antagonist. During this, injections of AVP were repeated following the pattern of its administrations as before the use of the antagonist. In order to avoid tachyphylaxis evoked by AVP its subsequent doses were accordingly injected: up to 10^{-10} mole — every 20 min and starting from 10^{-10} to 10^{-5} every 1 h. The values of increments of perfusion pressure during the vasoconstrictive responses to AVP have been analyzed. In order to analyse parallelness and positions of dose-response curves of AVP during and before infusion of the tested analogues the commonly known methods were used[14].

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