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European Journal of Pharmacology 419 (2001) 65–72

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Novel selective hypotensive vasopressin peptides: cardiovascular and structure–activity-relationship studies

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Received 19 October 2000; received in revised form 30 March 2001; accepted 3 April 2001

Abstract

Recently, we discovered a series of peripheral acting selective hypotensive vasopressin peptides. Whether these peptides may interact with receptors outside the vasopressin receptor family and affect cardiac function could not be excluded. Accordingly, we tested the effects of these hypotensive vasopressin peptides on blood pressure and heart rate in intact rats and on the heart rate, ventricular contractile force and coronary flow of isolated perfused rat hearts. We found that the hypotensive vasopressin peptides did not modify cardiac function, either in vivo or in vitro. The vasodepressor potency was reduced when assayed in rats with vasopressin-maintained baseline blood pressure, suggesting that vasopressin and the hypotensive peptide compete for a common vasodilating vasopressin receptor in the vasculature. We have now synthesized more potent and radioiodinatable hypotensive peptides that could serve as lead compounds for the development of a radiomarker for the putative vasodilating vasopressin receptor. © 2001 Published by Elsevier Science B.V.

Keywords: Vasopressin peptide, selective hypotensive; Vasopressin receptor, vasodilating; Vasopressin receptor subtype, new; Structure–activity–relationship

1. Introduction

Recently, we reported the discovery of a series of novel hypotensive vasopressin peptides (Chan et al., 1998a,b; Manning et al., 1999a). In contrast to vasopressin, which causes vasoconstriction and a rise in blood pressure, these novel vasopressin peptides cause a fall in blood pressure. The hypotensive (vasodepressor) response was not antagonized by the classical vasopressin V_{1A} receptor, vasopressin V_2 receptor and oxytocin receptor antagonists. Neither blockade of autonomic receptors and bradykinin B_2-receptors, nor inhibition of cyclooxygenase and nitric oxide synthase, had any significant effect on the hypotensive response (Chan et al., 1998b). These findings suggest that the novel hypotensive vasopressin peptides probably interact with a new unknown vasodilating vasopressin receptor subtype. If confirmed, the finding would have broad physiological and pharmacological implications. It would suggest that, contrary to what is commonly believed, the vasodilating action of arginine-vasopressin and vasopressin V_2 receptor agonist observed in regional vascular beds may not be mediated by vasopressinV_2 receptors (Naitoh et al., 1993; Nakanishi et al., 1995; Tagawa et al., 1995) and not be nitric oxide-dependent (Yamada et al., 1993; Liard, 1994). Accordingly, a new vasodilating vasopressin receptor subtype may exist in the vasculature.

Yet, the possibility that these hypotensive vasopressin peptides may interact with receptors outside the vasopressin receptor family cannot be excluded. Also, the vasodepressor response of the hypotensive vasopressin peptides may be due to a decrease in cardiac output rather than a direct vascular effect. In this paper, we report the results of our investigations designed to address these two possibilities. We investigated whether arginine-vasopressin and the hypotensive vasopressin peptides compete for a common vasodilating vasopressin receptor. Furthermore, to ascertain whether a cardiac action is a component of the hypotensive response, we determined the effects of the

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0014-2999/01/$ - see front matter © 2001 Published by Elsevier Science B.V.
PII: S0014-2999(01)00959-1

hypotensive vasopressin peptides on the heart rate of anesthetized rats and on heart rate, left ventricular contractile force and coronary flow rate of isolated rat hearts. From our ongoing studies, we also present the structure–activity–relationship data of eight selected hypotensive peptides. These improved hypotensive vasopressin peptides may provide the lead compounds and foster the development of specific markers for the identification of the putative new vasopressin receptor subtype.

2. Materials and methods

2.1. Animals used in experiments

Adult Osborne–Mendel rats (210–250 g) obtained from our institution's Research Animal Resource Center were used in this study. The use of animals in these experiments and the experimental protocols had been approved by the Institutional Animal Care and Use Committee.

2.2. Bioassays of hypotensive vasopressin peptides

Standard oxytocic, vasopressor and antidiuretic bioassays for neurohypophysial peptides (Sawyer, 1961) were performed to characterize the pharmacological properties of the hypotensive vasopressin peptides. Bioassay procedures were published in detail in a previous paper (Chan et al., 1998b). In brief, peptides were measured for the three biological activities characteristic of vasopressin and oxytocin peptides. (1) Vasopressor activity (a vasopressin V_{1A} receptor response) was determined in urethane-anesthetized rats, 1.0 g kg^{-1} i.p. followed by 0.75 g kg^{-1} s.c. Blood pressure was monitored by a transducer via a carotid arterial cannula. (2) Antidiuretic activity (a vasopressin V_2 receptor response) was determined in ethanol-anesthetized rats under a constant water-load as described by Sawyer (1961). Urine was collected via a bladder cannula and the flow rate measured by an electronic drop counter. (3) Uterotonic activity (an oxytocin receptor response) was determined in vitro on isolated uteri from rats that had been treated the previous afternoon with 50 μg diethylstilbestrol in oil per rat injected subcutaneously. The uterine horn was suspended in an organ chamber for isotonic contraction recording. In bioassays, agonistic potencies of the peptides were determined by the four-point (2×2 parallel line) assay design (Holton, 1948). Antagonistic potencies were measured by the pA_2 method (Schild, 1947). Arginine-vasopressin and oxytocin, standardized against the US Pharmacopeia Posterior Pituitary Standard for vasopressor and oxytocic activities, were used as the working standards in the bioassays of all test samples. At least four independent assays ($n = 4$), each in a different animal preparation, were performed for each bioassay. The bioassay value is expressed as mean ± S.E.M.

2.3. Determination of vasodepressor and heart rate responses to hypotensive peptides in rats

The effects of the hypotensive vasopressin peptides on the mean arterial blood pressure and heart rate were determined in urethane-anesthetized (1.0 mg kg^{-1} i.p. followed by 0.75 g kg^{-1} s.c.) male rats. Tracheostomy was performed. One or both jugular veins were cannulated for drug administrations. A carotid artery was cannulated for blood pressure monitoring. Electrocardiogram (ECG) was recorded through limb surface needle electrode leads. Heart rate was determined with an ECG-tachograph. Under our experimental conditions, the initial blood pressure of urethane-anesthetized normotensive rats varied from rat to rat in a range of 80–100 mm Hg. Since the magnitude of the hypotensive response depends on the initial blood pressure level, the response being greater at a higher baseline blood pressure (Chan et al., 1998b), vasodepressor potencies were determined in rats having baseline blood pressure elevated and maintained at 110–120 mm Hg (mean blood pressure) by the infusion of phenylephrine. Phenylephrine, 25 μg ml^{-1}, was infused at a rate 0.01–0.05 ml min^{-1} as needed, to maintain the blood pressure at the required range for the 5-min period before the injection of the test peptide. The infusion was continued for another 5-min period following the peptide injection and then ceased. Upon recovery of the vasodepressor response, phenylephrine infusion was re-instituted for the next peptide injection. The vasodepressor response was measured by the area under the hypotensive response curve (AUC), determined by a polar planimeter, for the 5-min period following the injection of the hypotensive peptide. Vasodepressor potency was determined by the i.v. dose required to produce an arbitrarily chosen hypotensive response endpoint of 5 cm^2 AUC in 5 min. This dose was designated as the "Effective Dose" (ED). A low and a high dose of the test peptide were injected to produce a response smaller than 5 cm^2 AUC and a response greater than 5 cm^2 AUC, respectively. The "Effective Dose" in μg 100 g^{-1} was extrapolated from the two-point dose–response curve.

In experiments in which effects on blood pressure and heart rate were determined over the course of the vasodepressor response, normotensive anesthetized rats (no phenylephrine infusion or other drug treatment) were used. A dose of the hypotensive peptide was chosen that would produce a fall in blood pressure of 30–40 mm Hg, so that the effect on heart rate in different rats was determined within a similar blood pressure response range.

2.4. Determination of a common vasodilating vasopressin receptor

To determine whether arginine-vasopressin and the hypotensive vasopressin peptide compete for a common vasodilating vasopressin receptor, the vasodepressor potency

Table 1
Pharmacological profiles of selective hypotensive vasopressin peptides (hypotensive peptides I–VIII) compared to arginine-vasopressin (AVP) and vasopressin/oxytocin antagonist d(CH_2)$_5$[D-Tyr(Et)^2,Val^4]AVP

No.	Peptide	Agonistic Activity, U mg^{-1}			Antagonistic Activity, pA_2		
		Antidiuretic (V_2)	Vasopressor (V_{1A})	Oxytocic (OT)	Anti-V_2	Anti-V_{1A}	Anti-OT
A	AVP[a]	323	382	14	–	–	–
B	d(CH_2)$_5$[D-Tyr(Et)^2,Val^4]AVP[b] (Parent molecule of peptides I–VIII)	–	–	–	7.81	8.22	8.32
	Hypotensive peptides I–VIII[c]	< 0.005	Vasodepressor	< 0.05	ND[d]	ND	ND

[a] Previously reported (Sawyer and Manning, 1973).
[b] Previously reported (Manning et al., 1982).
[c] See Table 3 for structures of peptides I–VIII.
[d] ND = Non-detectable. In anti-V_{1A} assays, up to 20 μg/rat i.v.; higher doses were vasodepressor. In anti-V_2 and anti-OT assays, up to 100 μg no detectable antagonism; estimated pA_2 ≪ 5.

(expressed by the "Effective Dose") of hypotensive peptide I was measured in one group of rats with baseline blood pressure maintained by phenylephrine (no common receptor) and in another group of rats with baseline blood pressure maintained by arginine-vasopressin (potential common receptor). Phenylephrine, 25 μg ml^{-1}, or arginine-vasopressin, 100 mU ml^{-1}, was infused at a rate of 0.01–0.05 ml min^{-1} as required to maintain the baseline blood pressure at 110–120 mm Hg for the bioassay of vasodepressor potency as described above (Section 2.3). The "Effective Doses" and the dose–response curves obtained from the two experimental conditions were compared and analyzed.

2.5. Determination of cardiac responses in the isolated perfused rat heart

Male rats were killed by cervical dislocation under light anesthesia with CO_2 vapor. The heart was rapidly excised, freed from fat and connective tissues, and transferred to a Langendorff apparatus (Park et al., 1992). The spontaneously beating heart was perfused through the aorta at constant pressure (80 cm H_2O) with Ringer's solution at 37°C saturated with 100% O_2 (pH 7.5). The composition of the Ringer's solution was 154.0 mM NaCl, 5.61 mM KCl, 5.55 mM $NaHCO_3$, 2.16 mM $CaCl_2$ and 5.95 mM dextrose. The ECG was recorded with surface electrodes from the right atrium and the left ventricle. Left ventricular contractile force was measured isometrically, by connecting the tip of the left ventricle to a force transducer, and recorded on a polygraph. The heart was perfused for 15 min before the experiment was begun to allow heart rate and ventricular contractile force to stabilize. Isolated hearts were challenged with acetylcholine and norepinephrine to ascertain that a negative and positive chronotropic response would be obtained, respectively. The coronary effluent was then collected into tubes at 2-min intervals and the coronary flow rate determined. Control parameters,

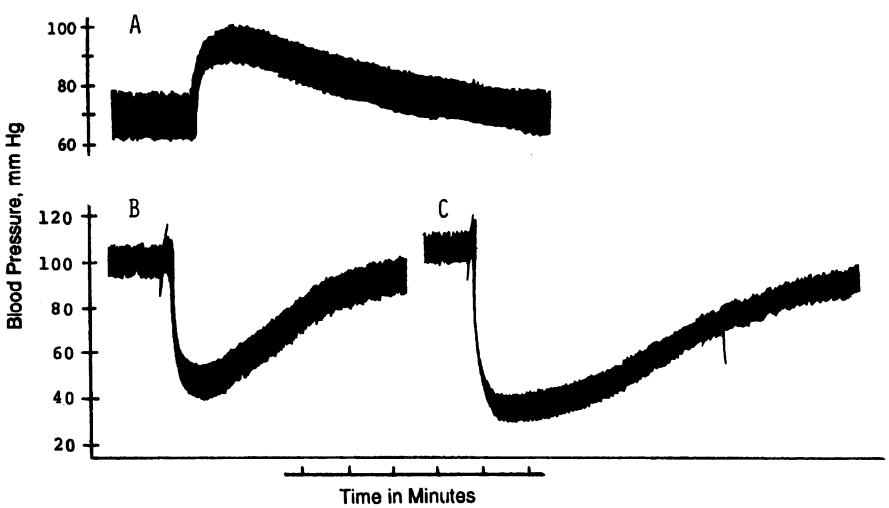

Fig. 1. Blood pressure responses to arginine-vasopressin and hypotensive vasopressin peptide in anesthetized rats. Tracing A shows the typical vasopressor response to 2 mU i.v. of arginine-vasopressin in a standard vasopressor bioassay (rats pretreated with atropine and phenoxybenzamine to lower the baseline blood pressure and maximize the vasopressor response as required by the bioassay protocol). In contrast, the hypotensive vasopressin peptides always produced a vasodepressor response in a same vasopressor bioassay protocol (not shown). Vasodepressor responses were greater in normotensive rats with a higher baseline blood pressure. Tracings B and C show the typical dose-dependent vasodepressor responses to 20 and 40 μg i.v. of hypotensive peptide I, d(CH_2)$_5$[D-Tyr(Et)^2,Arg^3,Val^4]AVP, in a normotensive rat (no pretreatment). Male rats, 215 and 220 g, respectively.

Table 2
Effects of hypotensive vasopressin peptide I on mean arterial blood pressure (BP) and heart rate (HR) in the rat

	Responses to hypotensive vasopressin Peptide I				
	Vasodepressor phase			Recovery phase	
	Control	Falling phase	At nadir	Half recovery	Full recovery
BP (mm Hg)	89 ± 3.2	–	67 ± 4.2	77 ± 4.0	83 ± 2.8
HR (beats min^{-1})	379 ± 9	379 ± 6	385 ± 10	389 ± 12	387 ± 14

Mean arterial blood pressure and heart rate of normotensive anesthetized male rats (210–240 g).
Values are means ± S.E.M.; $n = 6$ rats.
Hypotensive vasopressin peptide I = d$(CH_2)_5$[D-Tyr(Et)^2,Arg^3,Val^4]-AVP.
Dose: 5–10 μg 100 g^{-1} i.v.
For typical time–action of the vasodepressor response, see Fig. 1.

heart rate, ventricular contractile force and coronary flow rate were measured for 10 min. The test peptide was then added to the perfusing fluid and the heart was perfused with the peptide for 20 min. Heart rate, ventricular contractile force and coronary flow rate were again measured at 2 min intervals during the period of peptide perfusion.

2.6. Synthesis of hypotensive vasopressin peptides

All the hypotensive vasopressin peptides described in this study were synthesized in our laboratories by standard solid phase methods (Merrifield, 1964; Stewart and Young, 1984) according to procedures previously described (Manning et al., 1989, 1997, 1999b).

2.7. Drugs

Acetylcholine, arginine-vasopressin, norepinephrine, oxytocin, phenylephrine and urethane were purchased from Sigma (St. Louis, MO). All compounds, except urethane, were dissolved in 0.9% NaCl. Urethane was dissolved in deionized H_2O.

2.8. Statistical analysis

Bioassay values and sample means were analyzed for variance (ANOVA) and expressed as mean ± S.E.M. In experiments where the responses measured after the injection or perfusion of the hypotensive Vasopressin peptide were compared to the baseline values before the injection or perfusion, the baseline and post-injection values were from the same animal or isolated heart preparation (paired comparisons). The two sample means were analyzed by the paired Student's *t*-test. Differences were considered significant at $P < 0.05$ level.

3. Results

3.1. Pharmacological profile of hypotensive vasopressin peptides

d$(CH_2)_5$[D-Tyr(Et)^2,Arg^3,Val^4]AVP ([1-β-mercapto-β-β-cyclopentamethylenepropionic,2-*O*-ethyl-D-tyrosine,3-arginine,4-valine]arginine-vasopressin) (peptide I), the prototype hypotensive vasopressin peptide, and seven of its structural analogues (peptides II-VIII) were included in this study. All eight peptides were vasodepressor vasopressin peptides. Standard bioassays for V_{1A} (vasopressor), V_2 (antidiuretic) and in vitro oxytocic (uterotonic) activities showed that the eight peptides had no significant

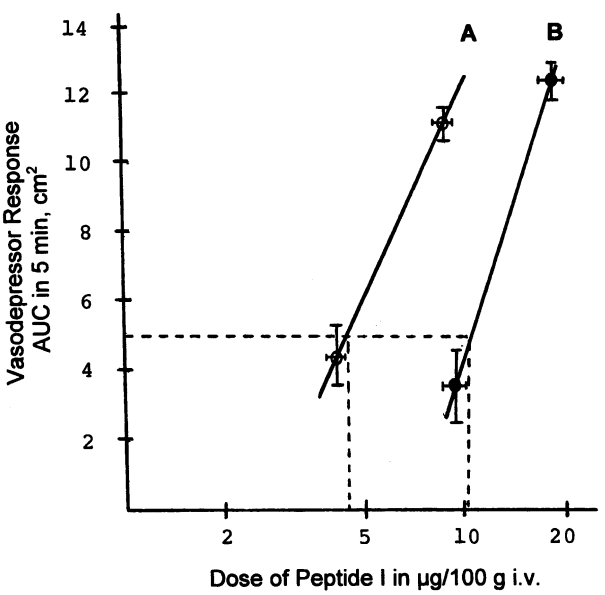

Fig. 2. Bioassays of vasodepressor potency of peptide I, d$(CH_2)_5$[D-Tyr(Et)^2,Arg^3,Val^4]AVP in anesthetized rats under phenylephrine-maintained baseline blood pressure and under arginine-vasopressin-maintained baseline blood pressure. The two dose–response curves show the vasodepressor response to peptide I under phenylephrine-maintained baseline blood pressure (A) and under arginine-vasopressin-maintained baseline blood pressure (B). Baseline mean arterial blood pressure was elevated and maintained at 110–120 mm Hg by infusion of either phenylephrine or arginine-vasopressin. The vasodepressor potencies were measured by the "Effective Dose", extrapolated from the dose–response curve, that would produce a response of 5 cm^2 area under the response curve (AUC) (see text for detail). The vasodepressor potency of peptide I (dotted lines) was higher under phenylephrine infusion, "Effective Dose" = 4.66 ± 0.24 μg 100 g^{-1} i.v. (Group A, $n = 6$ rats), than under arginine-vasopressin infusion, "Effective Dose" = 10.1 ± 1.6 μg 100 g^{-1} (Group B, $n = 6$ rats). The difference between the two groups was statistically significant, $P < 0.05$. The dose–response curves obtained under arginine-vasopressin were shifted to the right compared to the curves obtained under phenylephrine, suggesting that arginine-vasopressin and peptide I competed for a common vasodilating vasopressin receptor. Curves A and B each represents the mean ± S.E.M. of six experiments. Male rats, 210–250 g.

agonistic or antagonistic V_{1A}, V_2 and oxytocic activity. Table 1 compares the pharmacological activities of the hypotensive vasopressin peptides with those of the natural peptide hormone arginine-vasopressin (peptide A) and the parent peptide of the hypotensive vasopressin peptides, the V_{1A}/V_2/oxytocin antagonist d(CH_2)$_5$[D-Tyr(Et)^2,Val^4]-AVP ([1-β-mercapto-β-β-cyclopentamethylenepropionic, 2-*O*-ethyl-D-tyrosine,4-valine]arginine-vasopressin)(peptide B).

3.2. Hypotensive response and effects on heart rate

Intravenous injections of the selective hypotensive vasopressin peptides produced an immediate dose-dependent fall in blood pressure. Fig. 1A shows the typical vasopressor response to arginine-vasopressin in a standard vasopressor bioassay (rat pretreated with atropine and phenoxybenzamine to lower baseline blood pressure and maximize the pressor response as required by the bioassay protocol). In contrast, hypotensive vasopressin peptides always produced a vasodepressor response. Hypotensive responses were greater in normotensive rats exhibiting a higher baseline blood pressure. Fig. 1B and C shows the typical dose-dependent vasodepressor response to the hypotensive peptides in a normotensive rat (no pretreatment).

The hypotensive response was not associated with significant changes in heart rate. Table 2 shows the effects of peptide I on the blood pressure and the heart rate measured simultaneously over the course of the hypotensive response in six normotensive anesthetized rats. The dose range used was 5–10 μg 100 g^{-1} i.v., chosen to produce a fall in blood pressure of 30–40 mm Hg, so that the effect on heart rate in different rats was all determined within a similar blood pressure response range. The effects of peptides II–VIII on the blood pressure and heart rate were qualitatively similar to peptide I (data not shown). Therefore, like arginine-vasopressin, these peptides act on the vasculature but, unlike arginine-vasopressin, they produce a fall in blood pressure.

3.3. Structure–activity–relationship and vasodepressor potencies

Peptide I, (d(CH_2)$_5$[D-Tyr(Et)^2,Arg^3,Val^4]AVP), the first selective hypotensive vasopressin peptide discovered, was chosen as the reference standard for vasodepressor potency comparisons. Baseline blood pressure maintained at 110–120 mm Hg by phenylephrine infusion was the standard condition for the bioassay of vasodepressor potency "Effective Dose" (the i.v. dose that would produce a vasodepressor response endpoint of 5 cm^2 AUC in 5 min). Fig. 2A shows that the "Effective Dose" for peptide I was 4.66 ± 0.46 μg 100 g^{-1}.

Table 3 shows the chemical structures and the vasodepressor potencies of the eight selected hypotensive vasopressin peptides. Peptides II–VIII are position-9 modified analogues of peptide I. With the exception of peptides II and III, all were found more potent than peptide I. Peptides VII and VIII were nearly five times more potent than peptide I. Peptide VIII carries a retro-Eda-linked Tyr in position-10. Thus, it has a radioiodinatable site.

3.4. Competition between arginine-vasopressin and hypotensive vasopressin peptide for a common vasodilating vasopressin receptor

In a separate series of experiments, the vasodepressor potency of peptide I was determined in rats whose baseline blood pressure was maintained by arginine-vasopressin. The vasodepressor potency of peptide I was reduced when arginine-vasopressin was used to maintain baseline blood pressure, with "Effective Dose" = 10.1 ± 1.6 μg 100 g^{-1}, significantly different from the "Effective Dose" obtained under phenylephrine, $P < 0.05$. Fig. 2B shows that the

Table 3
Structure–activity–relationship and relative potencies of some hypotensive vasopressin peptides[a]

No.	Peptide	Vasodepressor Potency ED, μg 100 g^{-1} i.v.[b]
I	d(CH_2)$_5$[D-Tyr(Et)^2,Arg^3,Val^4]AVP	4.66 ± 0.46
II	**desGly**,d(CH_2)$_5$[D-Tyr(Et)^2,Arg^3,Val^4]AVP	4.81 ± 0.99
III	**desGly-NH$_2$**,d(CH_2)$_5$[D-Tyr(Et)^2,Arg^3,Val^4]AVP	ND[c]
IV	d(CH_2)$_5$[D-Tyr(Et)^2,Arg^3,Val^4,**Arg-NH$_2^9$**]AVP	3.54 ± 0.67
V	d(CH_2)$_5$[D-Tyr(Et)^2,Arg^3,Val^4,**Eda**^{9}]AVP	2.43 ± 0.39
VI	**desGly**,d(CH_2)$_5$[D-Tyr(Et)^2,Arg^3,Val^4,**Arg**^{7}]AVP	3.01 ± 0.48
VII	d(CH_2)$_5$[D-Tyr(Et)^2,Arg^3,Val^4,**Arg**^{7},**Eda**^{9}]AVP	1.10 ± 0.25
VIII	d(CH_2)$_5$[D-Tyr(Et)^2,Arg^3,Val^4,**Arg**^{7},**Eda**^{9} ← **Tyr**^{10}]AVP	1.05 ± 0.22

Bold prints show substitutional changes from peptide I. desGly = desglycine; desGly-NH_2 = desglycinamide; Arg-NH_2 = arginylamide; Eda = ethylenediamine; Eda^9 ← Tyr^{10} = position-10 retro-Eda-linked tyrosine.

[a] Synthesis and preliminary potency data have been reported in Manning et al. (1999b).

[b] ED = Effective dose for relative potency comparison. See text for details. Values are means ± S.E.M.; n = 5–6 rats.

[c] ND = No effects on blood pressure up to 100 μg/rat i.v.

Table 4
Effects of hypotensive vasopressin peptide VIII on heart rate, ventricular contractility and coronary flow rate in isolated perfused rat hearts

Treatment	Heart rate (beats min^{-1})	Ventricular contractility (g)	Coronary flow rate (ml min^{-1} g^{-1})
Peptide VIII, 0.15 μg ml^{-1}			
Control before peptide perfusion	291 ± 5.9	13.3 ± 0.26	8.8 ± 0.29
During peptide perfusion	293 ± 6.7	13.3 ± 0.29	8.1 ± 0.16[a]
Peptide VIII, 0.75 μg ml^{-1}			
Control before peptide perfusion	274 ± 6.2	13.6 ± 0.47	8.4 ± 0.38
During peptide perfusion	289 ± 12.6	12.9 ± 0.34	8.8 ± 0.73

Control perfusion period was 10 min; peptide perfusion period was 20 min.
Parameter measurements were taken at 2 min interval.
Values are means ± S.E.M.; $n = 5$ perfused hearts.
[a]Significantly different from control values; paired *t*-test, $P < 0.02$ two-tailed.

dose–response curve obtained under arginine-vasopressin infusion was shifted to the right to a lower potency compared to the dose–response curve obtained under phenylephrine infusion.

3.5. Effects on the isolated rat heart

The direct cardiac effects of peptide VIII were determined in 10 Langendorff rat heart preparations. Two concentrations of peptide VIII, 0.15 and 0.75 μg ml^{-1}, each in five hearts, were investigated. These two concentrations are equivalent to the "Effective Dose", and five times the "Effective Dose", respectively, used in the in vivo vasodepressor assays (Table 3). Table 4 shows that peptide VIII had no significant effect on the isolated rat heart. There were no changes in heart rate and ventricular contractile force measured before and during peptide perfusion. The effects on coronary flow were inconsistent. A small inhibitory effect was observed at the lower but not at the higher concentration.

4. Discussion

We recently reported the synthesis of a series of novel selective hypotensive vasopressin peptides (Chan et al., 1998a,b; Manning et al., 1999a). These vasopressin peptides have no significant agonistic or antagonistic activities characteristic of oxytocin/vasopressin peptides but cause an immediate and marked fall in blood pressure in rats. Therefore, they can be considered as vasopressin agonists with a vasodepressor action. Although these findings suggested that the hypotensive vasopressin peptides act at the peripheral vasculature via an unknown new vasopressin receptor subtype, the possibility that the hypotensive vasopressin peptide may have a direct effect on cardiac output and interact with a new receptor that is outside the vasopressin receptor family could not be excluded. In this paper, we investigated specifically the cardiac effects to ascertain whether changes in cardiac function may contribute to the vasodepressor action of these hypotensive vasopressin peptides. We also determined, by dose–response curve displacement analysis, whether arginine-vasopressin and the hypotensive vasopressin peptide compete for a common vasodilating vasopressin receptor.

We monitored heart rate during the course of the hypotensive response and found that the fall in blood pressure was not accompanied by a decrease in heart rate (Table 2). A small, but not statistically significant, increase in heart rate occurred at the nadir and during recovery of the hypotensive response. Furthermore, in the isolated rat heart, peptide VIII, the most potent hypotensive vasopressin peptide we have synthesized to date, had no effects on the heart rate and ventricular contractile force (Table 4). The isolated rat hearts were perfused with two concentrations of peptide VIII, 0.15 or 0.75 μg ml^{-1}, for 20 min. These two concentrations are equivalent to the "Effective Dose" and five times the "Effective Dose" of peptide VIII (Table 3) based on the assumed volume of distribution of 6.7 ml 100 g^{-1} for oxytocin/vasopressin peptides used in in vivo bioassays (Dykes et al., 1974). Thus, it is evident that the vasodepressor response to the hypotensive vasopressin peptides is a direct vascular effect and not a result of decreased cardiac output.

A possible central neural system action for the hypotensive vasopressin peptides remains to be investigated, as it cannot be excluded by our present experiments. Vasopressin is known to sensitize the baroreflex. Rats anesthetized with urethane (standard condition for rat vasopressor bioassay), however, have greatly blunted baroreflex response. In vasopressor assays, the marked transient pressor responses to vasopressin peptides were not accompanied by a significant fall in heart rate. The lack of a reflex tachycardia in response to hypotension induced by the hypotensive vasopressin peptides, as shown in Table 2, could be explained by the blunted baroreflex in urethane-anesthetized rats. Therefore, it could not be ascertained from our present experiments whether the novel hypotensive vasopressin peptides also affect baroreflex response as arginine-vasopressin does. However, it can be reasonably

concluded that a central neural system mechanism for the hypotensive response is highly unlikely, since our earlier studies had shown that the hypotensive response was not abolished by complete peripheral autonomic blockade (Chan et al., 1998b).

In the isolated rat heart, coronary flow rate was slightly decreased at the lower concentration of peptide VIII but not at the higher concentration. We have no explanation for this inconsistent effect of peptide VIII on the coronary flow rate. The decrease in flow was small and hemodynamically insignificant, since there was no change in heart rate or ventricular contractile force compared with control baseline values (Table 4).

The vasodepressor potency of peptide I, the prototype hypotensive vasopressin peptide, was determined in rats with phenylephrine- or arginine-vasopressin-maintained baseline blood pressure. The vasodepressor potency assayed under arginine-vasopressin was reduced significantly. The "Effective Dose" value was increased from 4.66 ± 0.46 μg 100 g^{-1} under phenylephrine to 10.1 ± 1.6 μg 100 g^{-1} under arginine-vasopressin. A plausible explanation is that arginine-vasopressin interacted with V_{1A} receptors, causing vasoconstriction that maintained the elevated baseline blood pressure, but it also interacted with the vasodilating vasopressin receptors causing vasodilation that was masked by the V_{1A} receptor-induced vasopressor response. Indeed, it has been shown that in the conscious rat the vasopressor response to arginine-vasopressin infusion was converted to a vasodepressor response when V_{1A} receptors were blocked (Walker, 1986). Thus, functionally arginine-vasopressin behaves like an "antagonist" of the hypotensive vasopressin peptide at the vasodilating vasopressin receptor. Therefore, when baseline blood pressure was maintained with arginine-vasopressin infusion, arginine-vasopressin competed with peptide I for the common vasodilating vasopressin receptors, resulting in a reduction of the vasodepressor potency of peptide I. The vasodepressor dose–response curve of peptide I was therefore displaced to the right to a lower potency (Fig. 2). In contrast, phenylephrine did not compete with peptide I for the vasodilating vasopressin receptor and thus yielded a higher vasodepressor potency in the bioassay.

The effects of arginine-vasopressin observed in the experiments reported here, together with our earlier finding that the vasodepressor response is not blocked by the classical V_{1A}, V_2 and oxytocin receptor antagonists (Chan et al., 1998b), provide the strongest evidence we have to date for the existence of a new vasodilating vasopressin receptor subtype. In this regard, it is of interest to note that a recent investigation, also in a rat model, using non-peptide antagonists to delineate the receptor subtype for the vasodilating action of desmopressin, a V_2 agonist, concluded that "desmopressin-induced relaxation is mediated by a receptor subtype sharing both V_{1A} and V_2 pharmacological profiles" (Mechaly et al., 1999). However, the possibility that the hypotensive vasopressin peptides may interact with receptors outside the vasopressin receptor family, although highly unlikely, cannot be excluded. Definitive identification of the putative new receptor will require molecular cloning of the receptor or successful specific radioligand labeling of the receptor. These procedures are not yet feasible.

The novel hypotensive vasopressin peptides described here may provide the lead compounds for the development of the sought-after ligand. In this paper, we present data on seven selected structural analogues of peptide I (peptides II–VIII, Table 3) that possess promising pharmacological properties relevant to the design of specific radioligand and/or potential new antihypertensive agents. These seven peptides were chosen from more than 25 hypotensive vasopressin peptides that we have synthesized in our ongoing structure–activity–relationship studies. Preliminary structure–activity–relationship data of these peptides have been published in papers describing their chemical synthesis (Manning et al., 1999a,b). With the exception of peptides II and III, all were more potent than peptide I. Of particular interest is peptide VIII. Peptide VIII is five times more potent than the prototype peptide I. It also has a retro-Eda-linked Tyr in position-10, providing a radioiodinatable site. Thus, peptide VIII is a good lead compound for the development of radio-markers for the putative new vasodilating vasopressin receptor. The development of these selective hypotensive vasopressin peptides, by providing new experimental tools, could significantly advance our knowledge of the cardiovascular physiology and pharmacology of arginine-vasopressin. Equally important, these new hypotensive vasopressin peptides may lead to the development of a novel class of antihypertensive agents. It should be noted that the hypotensive vasopressin peptides have been studied so far only in the rat model. Species differences in response to vasopressin and oxytocin analogues are well known. However, the vasodilating action of arginine-vasopressin and V_2 agonists has been demonstrated in rats (Yamada et al., 1993; Mechaly et al., 1999), dogs (Naitoh et al., 1993; Liard, 1994; Nakanishi et al., 1995) and in humans (Bichet et al., 1988; Tagawa et al., 1995; Van Lieburg et al., 1995).

Acknowledgements

This work was supported by National Institute of Health Grants GM-25280, HL-34215 and HL-46403.

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