ELSEVIER

European Journal of Pharmacology 285 (1995) 199–202

ejp

Short communication

[Arg^8]Vasopressin-induced responses of the human isolated coronary artery: effects of non-peptide receptor antagonists

Willem A. Bax [a,*], Pieter H. Van der Graaf [a], Wiro B. Stam [a], Egbert Bos [b], Dino Nisato [c], Pramod R. Saxena [a]

[a] *Department of Pharmacology, Laboratory for Human Pharmacology, Faculty of Medicine and Health Sciences, Erasmus University Rotterdam, P.O. Box 1738, 3000 DR Rotterdam, Netherlands*

[b] *Department of Thoracic Surgery, Thorax Centre, Faculty of Medicine and Health Sciences, Erasmus University Rotterdam, P.O. Box 1738, 3000 DR Rotterdam, Netherlands*

[c] *Sanofi Recherche, Montpellier Cedex, France*

Received 19 June 1995; revised 8 August 1995; accepted 11 August 1995

Abstract

Contractions induced by [Arg^8]vasopressin (vasopressin) and the effect of nonpeptide vasopressin receptor antagonists were studied in the human isolated coronary artery. Vasopressin induced contraction of coronary artery segments with a high pD_2 (9.25) but a low E_{max} (11.8% of the response to 100 mM K^+). This response was not affected by removal of the endothelium. Contraction was antagonized by the vasopressin V_1 receptor antagonist SR 49059 ((2*S*) 1-[(2*R* 3*S*)-5-chloro-3-(2-chlorophenyl)-1-(3,4-dimethoxybenzene-sulfonyl)-3-hydroxy-2,3-dihydro-1*H*-indole-2-carbonyl]-pyrrolidine-2-carboxamide) (pA_2: 9.76). OPC-31260 ([5-dimethylamino-1-{4-(2-methylbenzoylamino)benzoyl}-2,3,4,5-tetrahydro-1*H*-benzazepine]: vasopressin V_2 receptor antagonist) and OPC-21268 (1-{1-[4-(3-acetylaminopropoxy) benzoyl]-4-piperidyl}-3,4-dihydro-2(1*H*)-quinolinone: reported vasopressin V_1 receptor antagonist) were less potent antagonists of vasopressin-induced contractions (pA_2: 7.31 and 5.6, respectively). The antagonist potency order (SR 49059 > OPC-31260 > OPC-21268) corresponds to the reported affinity order for the human cloned vasopressin V_1 receptor. Therefore, the vasopressin V_1 receptor antagonist SR 49059, but not OPC-21268, appears to be an appropriate tool to investigate further the role of vasopressin in pathological processes involving coronary vasoconstriction in humans.

Keywords: [Arg^8]Vasopressin; Coronary artery, human; Vasopressin V_1 receptor; OPC-21268; OPC-31260; SR 49059; (Human)

1. Introduction

[Arg^8]Vasopressin (vasopressin) contributes to cardiovascular homoeostasis by inducing vasoconstriction and by stimulating renal water absorption. These effects are mediated via vasopressin V_1 and V_2 receptors, respectively. In general, vasopressin V_1 receptors stimulate phospholipase C, resulting in the formation of inositol 1,4,5-triphosphate (IP_3) and 1,2-diacylglycerol, whereas vasopressin V_2 receptors activate adenylyl cyclase (Manning and Sawyer, 1989). The human vasopressin V_1 and V_2 receptors have both been cloned (Thibonnier et al., 1994; Hirasawa et al., 1994). The existence of an additional vasopressin receptor subtype was postulated in rat adenohypophysis (Jard et al., 1986). This receptor was cloned in humans and designated vasopressin V_3 (De Keyzer et al., 1994) or vasopressin V_{1b} receptor (Sugimoto et al., 1994).

Antagonists for vasopressin receptors may be beneficial in the treatment of ischemic heart disease, hypertension, and congestive heart failure (see László et al., 1991, for a review). Recently, two nonpeptide vasopressin antagonists were developed with apparent selectivity for vasopressin V_1 receptors: OPC-21268 (Yamamura et al., 1991), and SR 49059 (Serradeil-Le Gal et al., 1994). However, Burrell et al. (1994) showed that OPC-21268 did not antagonize vasopressin-induced contractions of the human isolated internal

* Corresponding author. Tel.: (+)31 10 408 7551; fax: (+)31 10 436 6839; e-mail: bax@farma.fgg.eur.nl.

SSDI 0014-2999(95)00503-X

mammary artery. Another putative vasopressin V_1 receptor antagonist, [d(CH_2)$_5$sarcosine^7]vasopressin (SAVP), as well as the reported vasopressin V_2 receptor antagonist, OPC-31260 (Yamamura et al., 1992), acted as competitive antagonists of vasopressin-induced contraction of the human internal mammary artery (Burrell et al., 1994).

In the present study, we investigated vasopressin-induced contraction of the human isolated coronary artery. We studied the effect of the vasopressin V_1 receptor antagonist SR 49059, and compared it to the effect of OPC-21268 and OPC-31260.

2. Materials and methods

2.1. Preparation of the tissue and experimental protocol

Hearts were obtained from 14 organ donors, who had died of non-cardiac disorders (5 cerebrovascular accident, 7 polytrauma, 2 cerebral hypoxia; 9 male, 5 female; age 10–54 years). The hearts were provided by the Rotterdam Heart Valve Bank (Bio Implant Services Foundation) after removal of the valves for transplantation purposes. The study was approved by the Joint Medical Ethics Committee of the Erasmus University Rotterdam and the University Hospital Rotterdam 'Dijkzigt'. Ring segments (4 mm) of the right epicardial coronary artery were suspended in 15 ml organ baths containing Krebs bicarbonate solution (37°C; aerated with 95% O_2 and 5% CO_2; composition in mM: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_2$ 25, and glucose 8.3; pH: 7.4). The endothelium was left intact, as verified by observing relaxation to substance P (1 nM) after precontraction with prostaglandin $F_{2\alpha}$ (1 μM). In some ring segments the endothelium was removed using a cotton swab.

Changes in tension were recorded using a Harvard isometric transducer. Tissues were stretched to a stable tension of 20 mN and were subsequently exposed to 100 mM K^+. After a washout period of 30 min, segments were incubated with vehicle (controls) or an antagonist for 30 min, before a cumulative concentration-response curve was obtained.

2.2. Analysis of data

Curves were analyzed by means of a computerized curve fitting technique (De Lean et al., 1978) to obtain the pD_2 value ($-\log [EC_{50}]$, mol/l). All data are presented as means ± S.E.M. pD_2, and maximal effects (E_{max}) of agonist-induced contractions in the absence and presence of an antagonist were compared using a paired Student's t-test. A P-value less than 0.05 was assumed to denote a significant difference.

2.3. Compounds

Prostaglandin $F_{2\alpha}$ (tris salt), substance P acetate, and [Arg^8]vasopressin (acetate salt) (Sigma Chemical Co., USA) were dissolved in distilled water. OPC-21268 (1-{1-[4-(3-acetylaminopropoxy)benzoyl]-4-piperidyl}-3,4-dihydro-2(1*H*)-quinolinone), OPC-31260 ([5-dimethylamino-1-{4-(2-methylbenzoylamino)benzoyl}-2,3,4,5-tetrahydro-1*H*-benzazepine]), and SR 49059 ((2*S*) 1-[(2*R* 3*S*)-5-chloro-3-(2-chlorophenyl)-1-(3,4-dimethoxybenzene-sulfonyl)-3-hydroxy-2,3-dihydro-1*H*-indole-2-carbonyl]-pyrrolidine-2-carboxamide) were synthesized by Sanofi Recherche, France, and dissolved in dimethyl sulphoxide (DMSO) at a concentration of 1 mM, and subsequently diluted in distilled water.

3. Results

100 mM K^+ caused a mean contractile response of endothelium-intact coronary artery segments of 40 ± 4 mN (n = 14). In these preparations, 1 nM substance P caused 73 ± 7% relaxation after precontraction of the segments with 1 μM prostaglandin $F_{2\alpha}$. Removal of the endothelium reduced relaxation to 10 ± 7% of the response to prostaglandin $F_{2\alpha}$ (n = 8).

Vasopressin induced concentration-dependent contractions (pD_2: 9.25 ± 0.15; E_{max}: 11.8 ± 1.8% of the response to 100 mM K^+; n = 14). Removal of the endothelium did not result in a significantly altered response to vasopressin (pD_2: 9.30 ± 0.32; E_{max}: 14.8 ± 4.3%; n = 8). Furthermore, we did not observe relaxation after adding 0.1–1 nM vasopressin to coronary artery segments (n = 3) precontracted with 0.1 μM prostaglandin $F_{2\alpha}$ (precontraction: 15.4 ± 1.1% of the

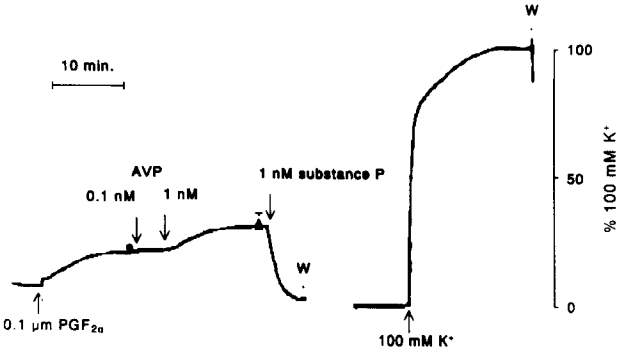

Fig. 1. Original tracing of the response of an endothelium-intact coronary artery segment. No relaxation was observed after adding 0.1–1 nM vasopressin to the coronary artery segment precontracted with 0.1 μM prostaglandin $F_{2\alpha}$. The segments subsequently relaxed completely in response to 1 nM substance P. The mean response (±S.E.M.) of 3 segments is also indicated (●, ▲; expressed as percentage of the reference contractile response to potassium, 100 mM). W, wash with Krebs bicarbonate solution.

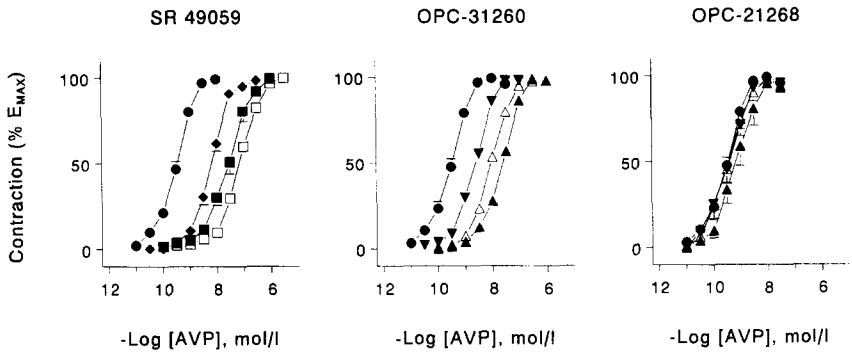

Fig. 2. Contractions of the human isolated coronary artery to vasopressin in the absence (control: •) or presence of the vasopressin receptor antagonists SR 49059, OPC-31260 and OPC-21268 ($n = 5$–7). Concentrations of receptor antagonists were 3 nM (♦), 0.01 μM (■), 0.03 μM (□), 0.1 μM (▽), 0.3 μM (▼), 1 μM (△) or 3 μM (▲).

response to 100 mM K^+). By contrast, addition of 1 nM vasopressin induced an additional contractile response of 6.7 ± 2.9% of the response to 100 mM K^+. All three segments subsequently relaxed completely after addition of 1 nM substance P (Fig. 1).

In endothelium-intact coronary artery segments, SR 49059 (3–30 nM) and OPC-31260 (0.3–3 μM) induced a concentration-dependent, parallel rightward shift of the vasopressin concentration-response curve, with associated pA_2 values of 9.76 ± 0.16 and 7.31 ± 0.18, respectively ($n = 7$, Fig. 2). Schild analysis revealed that the slopes for SR 49059 and OPC-31260 (1.08 ± 0.10 and 1.07 ± 0.07, respectively) were not significantly different from unity, indicating that these compounds antagonized the response to vasopressin in a simple competitive manner. OPC-21268 caused a significant rightward shift only at the highest concentration used (3 μM). An apparent pA_2 of 5.6 ± 0.3 ($n = 6$) was calculated, assuming a Schild plot slope of unity.

4. Discussion

We showed that vasopressin induced contractions of the human isolated coronary artery with a high pD_2 but with a low maximal response. Contraction was not affected by removal of the endothelium. In contrast to OPC-21268, SR 49059 was a potent antagonist of contractions induced by vasopressin.

The vasopressin V_1 receptor antagonist OPC-21268 was previously found to have a pK_i value of 7.24 for rat vasopressin V_1 receptors expressed in cell lines (Hirasawa et al., 1994), which would appear to be inconsistent with the presently observed pA_2 value in human isolated coronary arteries (pA_2: 5.6). The latter pA_2 value should perhaps be regarded with some caution, since it was calculated on the basis of a single antagonist concentration, which induced only a small rightward shift. However, it is obvious that the affinity of OPC-21268 for the vasopressin receptor inducing contraction in this blood vessel is relatively low. Similarly, OPC-21268 has relatively low affinity for the human cloned vasopressin V_1 receptor (pK_i: 4.3; Thibonnier et al., 1994). SR 49059 was shown not to discriminate between rat and human vasopressin V_1 receptors (pK_i: 9.10 and 8.49–8.89, respectively; Serradeil-Le Gal et al., 1994; Hirasawa et al., 1994). Therefore, the pA_2 values obtained for OPC-21268 (5.6) and SR 49059 (9.76) in our human coronary artery bioassay are in agreement with affinity estimates for the human, but not rat, cloned vasopressin V_1 receptor. Apparently, rat and human vasopressin V_1 receptors have a distinct pharmacological profile, which raises the question whether these receptors are species homologues or separate receptor entities. The effect of OPC-31260 may also be explained by its affinity for the human vasopressin V_1 receptor (pK_i: 6.47–6.60; Hirasawa et al., 1994; Serradeil-Le Gal et al., 1994). The observed pA_2 value of 7.31 for OPC-31260 would rule out involvement of the human vasopressin V_3 receptor, for which OPC-31260 has low affinity ($K_i > 1000$ nM; De Keyzer et al., 1994).

It was observed that the maximal effect of vasopressin reached only 11.8% of the contractile response to 100 mM K^+. This is less than in the human internal mammary artery (approx. 50% of the maximum response to noradrenaline; Burrell et al., 1994) or the human middle cerebral artery (approx. 120% of the response to 30 mM K^+; Onoue et al., 1994). In the human basilar artery, a maximal response of approx. 15% of the response to 30 mM K^+ was reached (Onoue et al., 1994). In dogs, it was shown that vasopressin caused myocardial ischemia by constricting especially small coronary arteries (Maturi et al., 1991). In accordance, the present data suggest that a major proportion of a yet hypothetical role of vasopressin in pathological processes involving coronary vasoconstriction is likely to occur in small coronary artery segments. We

are not aware of endogenous hormones which contract human isolated epicardial coronary artery with similar or higher potency (pD_2) than vasopressin.

We also investigated whether the response to vasopressin was modulated by the presence of endothelium. Although removal of the endothelium resulted in a slightly, but non-significantly enhanced contractile response to vasopressin, we observed no relaxation in response to vasopressin in endothelium-intact vessel segments precontracted with prostaglandin $F_{2\alpha}$ (Fig. 1). The present data therefore provide no evidence for modulation by endothelial vasopressin receptors of vasopressin-induced contraction, as was also concluded in recent studies with human cerebral and omental arteries (Onoue et al., 1994; Martínez et al., 1994).

In summary, vasopressin induced endothelium-independent contraction of the human isolated coronary artery, mediated via a receptor sensitive to the vasopressin V_1 receptor antagonist SR 49059 but not to OPC-21268. This is consistent with the binding characteristics of the recently cloned human vasopressin V_1 receptor. Therefore, SR 49059 appears to be an appropriate tool to investigate further the role of vasopressin in pathological processes involving coronary vasoconstriction in humans.

Acknowledgements

W.A.B. was supported by the Netherlands Heart Foundation, grant 89.252. The authors would like to express their sincere gratitude to the Rotterdam Heart Valve Bank (Bio Implant Services Foundation) for their help in supplying us with the human heart tissue.

References

Burrell, L.M., P.A. Phillips, K.A. Rolls, B.F. Buxton, C.I. Johnston and J.J. Liu, 1994, Vascular responses to vasopressin antagonists in man and rat, Clin. Sci. 87, 389.

De Lean, A., P.J. Munson and D. Rodbard, 1978, Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay and physiological dose-response curves, Am. J. Physiol. 235, E97.

De Keyzer, Y., C. Auzan, F. Lenne, C. Beldjord, M. Thibonnier, X. Bertagna and E. Clauser, 1994, Cloning and characterization of the human V_3 pituitary vasopressin receptor, FEBS Lett. 356, 215.

Hirasawa, A., K. Shibata, K. Kotosai and G. Tsujimoto, 1994, Cloning, functional expression and tissue distribution of human cDNA for the vascular-type vasopressin receptor, Biochem. Biophys. Res. Commun. 203, 72.

Jard, S., R.C. Gaillard, G. Guillon, J. Marie, P. Schoenenberg, A.F. Muller, M. Manning and W.H. Sawyer, 1986, Vasopressin antagonists allow demonstration of a novel type of vasopressin receptor in the rat adenohypophysis, Mol. Pharmacol. 30, 171.

Lászlo, F.A., F. Lászlo and D. De Wied, 1991, Pharmacology and clinical perspectives of vasopressin antagonists, Pharmacol. Rev. 43, 73.

Manning, M. and W.H. Sawyer, 1989, Discovery, development, and some uses of vasopressin and oxytocin antagonists, J. Lab. Clin. Med. 114, 617.

Martínez, M.C., J.M. Vila, M. Aldasoro, P. Medina, B. Flor and S. Lluch, 1994, Relaxation of human isolated mesenteric arteries by vasopressin and desmopressin, Br. J. Pharmacol. 113, 419.

Maturi, M.F., S.E. Martin, D. Markle, M. Maxwell, C.R. Burruss, E. Speir, R. Greene, Y. Moo Ro, D. Vitale, M.V. Green, S.R. Goldstein, S.L. Bacharach and R.E. Patterson, 1991, Coronary vasoconstriction induced by vasopressin; production of myocardial ischemia in dogs by constriction of nondiseased small vessels, Circulation 83, 2111.

Onoue, H., N. Kaito, M. Tomii, S. Tokudome, M. Nakajima and T. Abe, 1994, Human basilar and middle cerebral arteries exhibit endothelium-dependent responses to peptides, Am. J. Physiol. 267, H880.

Serradeil-Le Gal, C., D. Raufaste, E. Marty, C. Garcia, J.-P. Maffrand and G. Le Fur, 1994, Binding of [^{3}H]SR 49059, a potent nonpeptide vasopressin V_{1a} antagonist, to rat and human liver membranes, Biochem. Biophys. Res. Commun. 199, 353.

Sugimoto, T., M. Saito, S. Mochizuki, Y. Watanabe, S. Hashimoto and H. Kawashima, 1994, Molecular cloning and functonal expression of a cDNA encoding the human V_{1b} vasopressin receptor, J. Biol. Chem. 43, 27088.

Thibonnier, M., C. Auzan, Z. Madhun, P. Wilkins, L. Berti-Mattera and E. Clauser, 1994, Molecular cloning, sequencing, and functional expression of a cDNA encoding the human V_{1a} vasopressin receptor, J. Biol. Chem. 269, 3304.

Yamamura, Y., H. Ogawa, T. Chihara, K. Kondo, T. Onogawa, S. Nakamura, T. Mori, M. Tominaga and Y. Yabuuchi, 1991, OPC-21268, an orally effective, nonpeptide vasopressin V_1 receptor antagonist, Science 252, 572.

Yamamura, Y., H. Ogawa, H. Yamashita, T. Chihara, H. Miyamoto, S. Nakamura, T. Onogawa, T. Yamashita, T. Hosokawa, T. Mori, M. Tominaga and Y. Yabuuchi, 1992, Characterization of a novel aquaretic agent, OPC-31260, as an orally effective, nonpeptide vasopressin V_2 receptor antagonist, Br. J. Pharmacol. 105, 787.