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Short communication

Vasopressin V2 (SR121463A) and V1a (SR49059) receptor antagonists both inhibit desmopressin vasorelaxing activity

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Abstract

Although [Arg^8]vasopressin is a potent vasoconstrictor, it possesses vasorelaxant properties manifested either after vasopressin V1 receptor blockade or directly in some vascular beds. The nature of the receptor involved in the vasorelaxant effect of [deamino-Cys^1 D-Arg^8]vasopressin (desmopressin), a vasopressin V2 receptor agonist, was studied on rat precontracted aortic rings by the use of highly selective new non-peptide vasopressin receptor antagonists. The present study demonstrates for the first time that desmopressin relaxant effect is antagonized by the vasopressin V2 receptor antagonist SR121463A, but also by the vasopressin V1A receptor antagonist SR49059, suggesting that desmopressin-induced relaxation is mediated by a receptor subtype sharing both V1A and V2 pharmacological profiles.

Keywords: Vasopressin; Desmopressin; Vasodilation; Aorta; (Rat)

1. Introduction

[Arg^8]vasopressin induces antidiuresis mediated by renal vasopressin V2 receptor activation. It is also a potent vasoconstrictor through stimulation of vascular vasopressin V1A receptors. However, arginine vasopressin possesses vasorelaxant properties manifested either in precontracted isolated vascular preparations after vasopressin V1 receptor blockade (Martinez et al., 1994a,b; Medina et al., 1996) or directly in some vascular beds (Cosentino et al., 1993).

The type of receptors involved in vasodilation is still a matter of debate. Vasodilation could be reproduced by [deamino-Cys^1 D-Arg^8]vasopressin (desmopressin), a synthetic vasopressin V2 receptor agonist and be antagonized in some cases by vasopressin V1 (Yamada et al., 1993) or mixed V1–V2 (Martinez et al., 1994a,b), but not by peptidic V2 (Johns, 1990; Yamada et al., 1993) receptor antagonists. The recent development of highly specific non-peptide antagonists of vasopressin V1A (SR49059; Serradeil-Le Gal et al., 1993) or V2 (SR121463A; Serradeil-Le Gal et al., 1996) receptors led us to reassess the nature of receptor involved in the relaxing effect of desmopressin, using rat isolated precontracted aorta. The effects of arginine vasopressin were also determined in the same experimental conditions.

2. Materials and methods

2.1. Measurement of contraction or relaxation on isolated aorta segments

The effects of desmopressin or [Arg^8]vasopressin were measured as previously described (Cadène et al., 1997) on aorta segments with functional endothelium. Briefly, male Wistar rats (250–300 g) were sacrificed by a blow on the head and exsanguination. Thoracic aorta was rapidly removed and aortic rings (3–4 mm long) mounted on organ baths (2.5 ml) for isometric force recording under 1 g resting tension in Krebs'–Henseleit buffer of the following composition (mM): NaCl, 119; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25; glucose, 11. Buffer was continuously gassed (95% O_2, 5% CO_2)and maintained at 37°C.

After a 60-min equilibration period, the preparation was contracted by noradrenaline (10^{-8} M) and the presence of a functional endothelium checked by the occurrence of a relaxation in response to acetylcholine (10^{-5} M). The preparations were then rinsed thoroughly and allowed to

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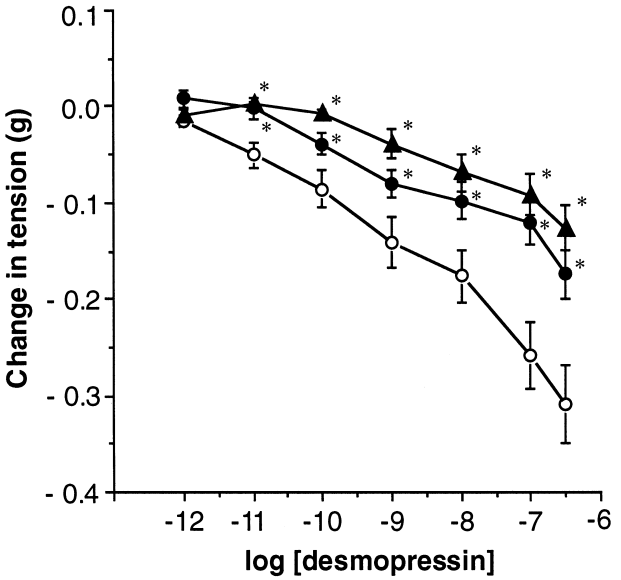

Fig. 1. Effect of SR49059 and SR121463A on desmopressin-induced relaxation in noradrenaline-precontracted aortic rings. Cumulative CRC for desmopressin were performed on aorta precontracted by 10^{-6} M noradrenaline in the absence (○) or in the presence of the vasopressin V1A receptor antagonist SR49059 (▲) or the vasopressin V2 receptor antagonist SR121463A (●), both used at the concentration of 10^{-8} M. Changes in tension (in grams) are plotted as a function of desmopressin concentration (M). Values are mean ± S.E.M. of 11–20 determinations in each case. * Indicates a significant difference (Newmann–Keuls' test) as compared to the effect of desmopressin measured in the absence of antagonist.

stabilize for an additional 60 min period before aorta were again contracted with noradrenaline (10^{-6} M). Tension was maximally developed within 20 min and remained constant for at least 60 min. After noradrenaline-induced contraction had reached a plateau (2.43 ± 0.8 g above basal tension, $n = 103$), [Arg^8]vasopressin or desmopressin cumulative concentration–response curves (CRC) were performed in the absence or the presence of selective vasopressin receptor antagonists. Vasopressin V1 (SR49059) and V2 (SR121463A) receptor antagonists were added to the bath fluid 10 min before the second noradrenaline-induced contraction. SR49059 or SR121463A were both used at the same concentration of 10^{-8} M, chosen as not susceptible to have any significant interaction with V2 or V1B receptors for SR49059 or with V1A or V1B receptors for SR121463A (Serradeil-Le Gal et al., 1993, 1996). Preliminary experiments had shown that SR49059 or SR121463A (10^{-8} M) had no significant effect on basal or noradrenaline-induced developed tension.

2.2. Chemicals

Acetylcholine chloride, [Arg^8]vasopressin, [deamino-Cys^1 D-Arg^8]vasopressin (desmopressin) and [−]-noradrenaline bitartrate were from Sigma (St Louis, MO, USA). SR49059 and (1-[4-(*N*-tert-butylcarbamoyl)-2-methoxybenzene sulfonyl]-5-ethoxy-3-spiro-[4-(2-morpholinoethoxy)cyclohexane]indol-2-one, fumarate (SR121463A) were generously supplied by Sanofi Recherche (Toulouse, France).

Solutions of all compounds were made up in twice-distilled water. In the case of noradrenaline, ascorbic acid ($0.57\ 10^{-3}$ M) was added as an antioxidant. SR49059 was first dissolved in dimethyl sulphoxide at the concentration of 10^{-2} M, then subsequently diluted in twice-distilled water.

2.3. Expression of results and statistics

Results were expressed as grams of developed tension or relaxation as compared to the level of precontraction induced by noradrenaline. Statistical comparison were performed by analysis of variance followed by the Newmann–Keuls multiple comparison test. A *P*-value less than 0.05 was assumed to denote a significant difference.

3. Results

Figs. 1 and 2 illustrate the effect of cumulative concentrations of desmopressin or [Arg^8]vasopressin, respec-

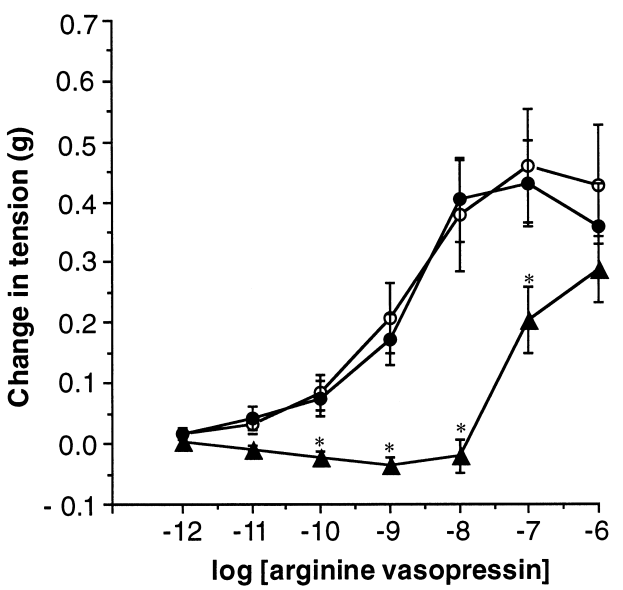

Fig. 2. Effect of SR49059 and SR121463A on [Arg^8]vasopressin-induced relaxation in noradrenaline-precontracted aortic rings. Cumulative CRC for [Arg^8]vasopressin were performed on aorta precontracted by 10^{-6} M noradrenaline in the absence (○) or in the presence of the vasopressin V1A receptor antagonist SR49059 (▲) or the vasopressin V2 receptor antagonist SR121463A (●), both used at the concentration of 10^{-8} M. Changes in tension (in grams) are plotted as a function of arginine vasopressin concentration (M). Values are mean ± S.E.M. of 17–20 determinations in each case. * Indicates a significant difference (Newmann–Keuls' test) as compared to the effect of [Arg^8]vasopressin measured in the absence of antagonist.

tively, in the absence or in the presence of SR49059 or SR121463A.

Desmopressin concentration-dependently relaxed the precontracted aorta (Fig. 1). In the presence of 10^{-8} M SR121463A, desmopressin CRC was shifted to the right, confirming the vasopressin V2 receptor antagonistic activity of SR121463A in this model. In addition, the same concentration of the V1A antagonist SR49059 also induced a rightward shift of desmopressin CRC. Concentrations of 10^{-9} M of either antagonists did not induce any significant change of desmopressin-induced relaxation (not illustrated).

Arginine vasopressin induced a concentration-dependent contraction on noradrenaline-precontracted aorta (Fig. 2). Arginine vasopressin CRC was significantly shifted to the right in the presence of SR49059. In the presence of SR121463A, no significant change in the arginine vasopressin-induced contraction was observed. In the latter case, a slight, albeit significant, decrease in tension was observed in the presence of low concentrations of arginine vasopressin.

4. Discussion

The present study confirmed that desmopressin, a vasopressin V2 receptor agonist, was able to relax precontracted rat isolated aorta and demonstrated for the first time that this effect could be antagonized by a highly specific non-peptidic vasopressin V2 receptor antagonist, SR121463A.

Rat isolated aorta has largely been used for studying the contractile effects of vasopressin and the influence of vasopressin V1A peptidic receptor antagonists, although, to our knowledge, the effect of a non-peptidic vasopressin receptor antagonist such as SR49059 had not been described on this experimental model. Our own (non-illustrated) preliminary studies performed on non-precontracted preparations have indicated that while SR49059 or 121463A had no effect on basal tension, SR49059 — but not SR121463A — induced a shift to the right of the arginine vasopressin concentration–contraction response curve. We also checked that desmopressin had no significant effect in the same non-precontracted preparation.

Although a vasopressin-induced relaxation could be observed on some vascular beds (Hirsch et al., 1989; Cosentino et al., 1993), the presence of a contracting agent was required in order to visualize a vasodilation in most isolated vascular models, such as human cerebral (Martinez et al., 1994a) or mesenteric (Martinez et al., 1994b) arteries or rat aorta (Johns, 1990; Yamada et al., 1993). Noradrenaline was often used for this purpose as the pre-contracting agent (Martinez et al., 1994a). However, in these experimental conditions, the issue of a possible change of the responsiveness to vasopressin receptor agonists by noradrenaline should be addressed. In our hands, aorta precontracted with noradrenaline were still responsive to the contractile effect of arginine vasopressin, an effect which was antagonized by SR49059, but not by SR121463A. This result indicated the functionality of contracting vasopressin V1A receptors and the lack of effect of the vasopressin V2 receptor antagonist SR121463A on those receptors in our experimental conditions.

Interestingly, a slight, albeit significant, relaxation was induced on noradrenaline-precontracted preparations by low concentrations of [Arg^8]vasopressin in the presence of SR49059. As previously stated, [Arg^8]vasopressin was shown to possess vasorelaxant properties manifested either on precontracted isolated vascular preparations after vasopressin V1-receptor blockade (Martinez et al., 1994b; Medina et al., 1996) or directly in some vascular beds (Cosentino et al., 1993; Hirsch et al., 1989), although an arginine vasopressin-induced vasorelaxation had never been shown on rat isolated aorta. A variable amount of V1A receptors (Gopalakrishnan et al., 1991), responsible for arginine vasopressin-induced contraction may explain the variability of response to [Arg^8]vasopressin in different vascular models and probably the difficulties to visualize an [Arg^8]vasopressin-induced relaxation in some of them. In any case, our result suggests the presence of relaxing vasopressinergic (non-V1A) receptors in our experimental conditions.

Desmopressin, which, as opposed to [Arg^8]vasopressin, had no contractile effect, was indeed able to relax precontracted aorta, as previously shown by Johns (1990) or Yamada et al. (1993), although these authors were unable to antagonize relaxation by a vasopressin V2 receptor antagonist. In the present work, we showed for the first time that the relaxing effect of desmopressin was antagonized by a specific vasopressin V2 receptor antagonist (SR121463A), after having checked that SR121463A (and also SR49059) did not antagonize the vasorelaxing activities of other vasorelaxants, such as sodium nitroprussiate or aminophylline (not illustrated).

Our results also indicated that the relaxing effect of desmopressin could also be antagonized by the vasopressin V1 receptor antagonist, SR49059. Those results confirm previous studies showing that it could be antagonized in some cases by peptidic (Yamada et al., 1993) or non-peptidic (i.e., SR49059, Toda et al., 1998) V1, or peptidic mixed V1–V2 (Martinez et al., 1994b) receptor antagonists and raise the issue of the nature of the receptor involved in its vasorelaxant activity.

As desmopressin has strong antidiuretic but no significant vasoconstricting activity, it was considered as a ''pure'' vasopressin V2 receptor agonist although the affinity of desmopressin for human vasopressin V2 receptor or human vascular type vasopressin V1A receptors were found similar in competitive binding (Pettitbone et al., 1992; Chini et al., 1995).

Recently, Saito et al. (1997) showed that desmopressin could act as an agonist of human or rat vasopressin V1B

receptors. This result raises the issue of a possible mediation of the relaxing effect of desmopressin through vasopressin V1B receptors. However, our data argue against a vasopressin V1B-mediated desmopressin-induced relaxation, as the relaxant effect of desmopressin could be antagonized by both SR121463A or SR49059, which do not significantly bind to vasopressin V1B receptors for the concentration used (10^{-8} M, Serradeil-Le Gal et al., 1993, 1996). Rather, our results further suggest the existence of a vasopressin receptor sharing both V2 and V1A pharmacological profiles.

In summary, the present study confirmed that desmopressin, a vasopressin V2 receptor agonist, was able to relax precontracted rat isolated aorta and demonstrated for the first time that this effect could be antagonized by a highly specific vasopressin V2 receptor antagonist, the non-peptidic SR121463A. However, the antagonistic effect of SR49059, a specific vasopressin V1A receptor antagonist, further suggests that desmopressin-induced relaxation is mediated by a receptor subtype sharing both V1A and V2 pharmacological profiles. Future studies will determine the nature and role of receptors involved in the vasodilation induced by vasopressin receptor agonists.

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References

Cadène, A., Grigorescu, F., Serrano, J.J., Cros, G., 1997. Characterization of vanoradrenalineyl sulfate effect on vascular contraction: roles of calcium and tyrosine phosphorylation. J. Pharmacol. Exp. Ther. 281, 491–498.

Chini, B., Mouillac, B., Ala, Y., Balestre, M.N., Trumpp-Kallmeyer, S., Hoflac, J., Elands, J., Hibert, M., Manning, M., Jard, S., Barberis, C., 1995. Tyr115 is the key residue for determining agonist selectivity in the V1a vasopressin receptor. EMBO J. 14, 2176–2182.

Cosentino, F., Sill, J.C., Katsusic, Z.S., 1993. Endothelial L-arginine pathway and relaxations to vasopressin in canine basilar artery. Am. J. Physiol. 264, H413–H418.

Gopalakrishnan, V., Xu, Y., Sulakhe, P.V., Triggle, C.R., McNeill, C.R., 1991. Vasopresin V1 receptor characteristics in rat aortic smooth muscle cells. Am. J. Physiol.: Heart Circ. Physiol. 261 (30), H1907–H1936.

Hirsch, A.T., Dzau, V.J., Majzoub, J.A., Creager, M.A., 1989. Vasopressin-mediated forearm vasolidation in normal humans. J. Clin. Invest. 84, 418–426.

Johns, R.A., 1990. Desmopressin is a potent vasorelaxant of aorta and pulmonary artery isolated from rabbit and rat. Anesthesiology 72, 858–864.

Martinez, M.C., Aldasoro, M., Vila, J.M., Medina, P., Lluch, S., 1994a. Responses to vasopressin and desmopressin of human cerebral arteries. J. Pharmacol. Exp. Ther. 270, 622–627.

Martinez, M.C., Vila, J.M., Aldasoro, M., Medina, P., Flor, B., Lluch, S., 1994b. Relaxation of human isolated mesenteric arteries by vasopressin and desmopressin. Br. J. Pharmacol. 113, 419–424.

Medina, P., Vila, J.M., Martinez, M.C., Aldasoro, M., Chuan, P., Lluch, S., 1996. Effects of vasopressin on human renal arteries. Eur. J. Clin. Invest. 26, 966–972.

Pettitbone, D.J., Kishel, M.T., Woyden, C.J., Clineschmidt, B.V., Bock, M.G., Freidinger, R.M., Veber, D.F., Williams, P.D., 1992. Radioligand binding studies reveal marked species differences in the vasopressin V1 receptor of rat rhesus and human tissues. Life Sci. 50, 1953–1958.

Saito, M., Tahara, A., Sugimoto, T., 1997. 1-Desamino-8-D-arginine vasopressin (DDAVP) as an agonist on V1b vasopressin receptor. Biochem. Pharmacol. 53, 1711–1717.

Serradeil-Le Gal, C., Wagnon, J., Garcia, C., Lacour, C., Guiraudou, P., Christophe, B., Villanova, G., Nisato, D., Maffrand, J.P., Le Fur, G., Guillon, G., Cantau, B., Barberis, C., Trueba, M., Ala, Y., Jard, S., 1993. Biochemical and pharmacological properties of SR 49059, a new potent, nonpeptide antagonist of rat and human vasopressin V1A receptors. J. Clin. Invest. 92, 224–231.

Serradeil-Le Gal, C., Lacour, C., Valette, G., Garcia, G., Foulon, L., Galindo, G., Bankir, L., Pouzet, B., Guillon, G., Barberis, C., Chicot, D., Jard, S., Vilain, P., Garcia, C., Marty, E., Raufaste, D., Brossard, G., Nisato, D., Maffrand, J.P., Le Fur, G., 1996. Characterization of SR 121463 A, a highly potent selective, orally active vasopressin V2 receptor antagonist. J. Clin. Invest. 98, 2729–2738.

Toda, M., Ayajiki, K., Okamura, T., Azuma, I., Toda, N., 1998. Desmopressin-induced dog ciliary artery relaxation. Eur. J. Pharmacol. 344, 197–201.

Yamada, K., Nakayama, M., Nakano, H., Mimura, N., Yoshida, S., 1993. Endothelium-dependent vasorelaxation evoked by desmopressin and involvement of nitric oxide in rat aorta. Am. J. Physiol. 264, E203–E207.