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Desmopressin-induced dog ciliary artery relaxation

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Abstract

In isolated dog posterior ciliary arteries contracted with prostaglandin $F_{2\alpha}$, desmopressin (10^{-10} to 10^{-8} M), a vasopressin V_2 receptor agonist, produced a concentration-related relaxation, which was reversed to a contraction by removal of the endothelium. Desmopressin was approximately 1/100 as potent as arginine vasopressin. Treatment with N^G-nitro-L-arginine, a nitric oxide (NO) synthase inhibitor, reversed the desmopressin-induced relaxation to a contraction and the addition of L-arginine restored the relaxation. SR49059 ((2*S*)1-[(2*R*3*S*)-(5-chloro-3-(2-chlorophenyl)-1-(3,4-methoxybenzene-sulfonyl)-3-hydroxy-2,3-dihydro-1H-indole-2-carbonyl]-pyrrolidine-2-carboxamide), a selective vasopressin V_1 receptor antagonist, suppressed the relaxation. In endothelium-denuded arteries, desmopressin-induced contractions were also inhibited by SR49059. It is concluded that desmopressin, although much less potent than vasopressin, relaxes ciliary arteries via a mediation of NO synthesized from L-arginine in the endothelium. Vasopressin V_1-receptor subtypes appear to be involved in the desmopressin-induced relaxation and contraction. © 1998 Elsevier Science B.V.

Keywords: Desmopressin; Ciliary artery; Vasopressin V_1 receptor; Endothelium; Nitric oxide (NO)

1. Introduction

Arginine vasopressin is an endogenous mediator leading to raised systemic blood pressure by an increment of peripheral vascular resistance and a decrease in urine output. Vascular effects of vasopressin are mediated by vasopressin V_1 and V_2 receptors and the antidiuretic action is mediated by vasopressin V_2 receptors (Laszlo et al., 1991). Desmopressin synthesized by Zaoral et al. (1967) has a quite selective antidiuretic action and is used as a preferred drug for the treatment of central diabetes insipidus (Robinson, 1976). The potency of desmopressin in inducing antidiuresis is 900–3000 times higher than that in eliciting vasoconstriction (Zaoral et al., 1967; Manning et al., 1973; Manning et al., 1976).

It is known that the major action of vasopressin on vasculatures is to increase vascular resistance by vasoconstriction that is mediated by vasopressin V_1 receptors (Ohlstein and Berkowitz, 1986; Edwards et al., 1989; Laszlo et al., 1991). However, this peptide produces vasodilatation in some blood vessels from mammals, including cerebral, ciliary, renal arteries and arterioles in vitro and in vivo (Katusic et al., 1984; Onoue et al., 1988; Suzuki et al., 1993; Aki et al., 1994, Okamura et al., 1997); nitric oxide (NO) from the endothelium being a mediator for the vasodilatation (Russ and Walker, 1992; Cosentino et al., 1993; Onoue et al., 1994; Rudichenko and Beierwaltes, 1995; Garcia-Villalon et al., 1996; Okamura et al., 1997). The involvement of vasopressin receptor subtypes in the vasodilator action of vasopressin is still controversial. The vasopressin V_1 receptor seems to be involved in relaxations of dog cerebral and ciliary arteries (Katusic et al., 1984; Okamura et al., 1997) and the vasopressin V_2 receptor might participate in human forearm and dog renal vasodilatation (Hirsch et al., 1989; Naitoh et al., 1993).

Our previous study has revealed that isolated dog ciliary arteries respond to vasopressin at low concentrations (as low as 10^{-11} M) with moderate relaxation that is endothelium-dependent and is mediated by vasopressin V_1 receptors (Okamura et al., 1997). Our preliminary study with the same arteries indicated that desmopressin, known as a selective vasopressin V_2 receptor agonist, also produced relaxation, although the potency was appreciably less than that of vasopressin. Therefore, the present study was undertaken to determine whether desmopressin has an ability to activate the vasopressin V_1 receptor in the dog ciliary artery or the V_2 receptor are also involved in the relaxation induced by this peptide.

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2. Materials and methods

The studies review board at our university approved the use of ciliary arteries isolated from dogs in this study.

2.1. Preparation

Sixteen mongrel dogs of either sex, weighing 7 to 12 kg, were anesthetized with intravenous injections of sodium pentobarbital (30 mg/kg) and killed by bleeding from the carotid arteries. The eyeballs attached with optic nerves and extraocular tissues were rapidly removed from the orbital cavities. Branches of short posterior ciliary arteries (0.3 to 0.5 mm outside diameter) were isolated. The arteries were cut into helical strips of approximately 20 mm long, with special care being taken to avoid endothelial damage. The specimens were vertically fixed between hooks in a muscle bath (20 ml capacity) containing the modified Ringer–Locke solution maintained at 37 ± 0.3°C and aerated with a mixture of 95% O_2 and 5% CO_2. Constituents of the solution were as follows (in mM): NaCl, 120; KCl, 5.4; $CaCl_2$, 2.2; $MgCl_2$, 1.0; $NaHCO_3$, 25.0 and dextrose, 5.6. The pH of the solution was 7.36 to 7.43. The hook anchoring the upper end of the strip was connected to the lever of a force–displacement transducer. The resting tension was adjusted to 0.7 g which is optimal for inducing the maximal contraction (Kitamura et al., 1993). Before the start of experiments, all of the strips were allowed to equilibrate for 60 to 90 min in the bathing media during which time the fluid was replaced every 10 to 15 min.

2.2. Recording

Isometric contractions and relaxations were displayed on an ink-writing oscillograph. The contractile response to 30 mM K^+ was first obtained and the arterial strips were repeatedly rinsed with fresh media and equilibrated. The concentration–response curves for desmopressin or vasopressin were obtained by adding the drug directly to the bathing media. The strips were partially contracted with prostaglandin $F_{2\alpha}$ (5 to 20×10^{-7} M), the contraction being in a range between 25 and 40% of the contraction induced by 30 mM K^+. In order to minimize tachyphylaxis, high concentrations (10^{-8} M or higher) of desmopressin were not applied under control conditions in the strips with the intact endothelium. At the end of each series of experiment, papaverine (10^{-4} M) was applied to attain the maximal relaxation. The relaxation and contraction induced by the test drugs were presented as values relative to the responses induced by 10^{-4} M papaverine and 30 mM K^+, respectively. Preparations were treated for at least 20 min with blocking agents, before the response to desmopressin was obtained. In some experiments ($n = 6$), responses to desmopressin were compared in pairs of arterial strips with and without the endothelium obtained from the same dogs. The arterial endothelium was removed by gently rubbing the intimal surface with a cotton ball. The endothelial function was verified by the relaxant response to substance P (10^{-8} M).

2.3. Statistics and drugs used

The results shown in the text and captions are expressed as mean values ± S.E. Statistical analyses were made using the Student's paired and unpaired *t*-test for two groups and the Tukey's method after one-way analysis of variance for three or more groups. Drugs used were desmopressin (deamino-Cys[1], D-Arg[8]-vasopressin; Sigma Chemical Co., St. Louis, MO), arginine vasopressin, [Pmp[1], Tyr(Me)[2]]-Arg[8]-vasopressin, substance P, N^G-nitro-L-arginine (L-NA), N^G-nitro-D-arginine (D-NA) (Peptide Institute, Minoh, Japan), SR49059 ((2*S*)1-[(2*R*3*S*)-(5-chloro-3-(2-chlorophenyl)-1-(3,4-dimethoxybenzene-sulfonyl)-3-hydroxy-2,3-dihydro-1H-indole-2-carbonyl]-pyrrolidine-2-carbozamide; Sanofi Recherche, Cedex, France), L- and D-arginine (Nacalai Tesque, Kyoto, Japan), prostaglandin $F_{2\alpha}$ (Pharmacia-Upjohn, Tokyo, Japan) and papaverine hydrochloride (Dainippon Co., Osaka, Japan).

3. Results

3.1. Relaxant response

The addition of desmopressin in concentrations of 10^{-10} to 10^{-8} M produced a dose-related relaxation in the canine ciliary arterial strips with the endothelium, the effect being approximately 1/100 less than that of arginine vasopressin when compared in the same preparations (Fig. 1, left). The endothelial integrity was determined by the relaxation induced by 10^{-8} M substance P; the mean value was $85.4 \pm 2.1\%$ ($n = 8$). Endothelium denudation abolished the response to substance P and reversed the desmopressin-induced relaxation to a contraction (Fig. 1, right). The typical tracings are illustrated in Fig. 2.

The endothelium-dependent relaxation by desmopressin was reproducible when the concentrations were not raised to 10^{-8} M or higher. Therefore, effects of blocking agents were evaluated with the agonist concentrations of 10^{-10} and 10^{-9} M, unless the response was suppressed by the blockers. The relaxation in the endothelium-intact strips was reversed to a contraction by treatment with 10^{-5} M L-NA and restored by the addition of L-arginine (10^{-3} M) (Fig. 3, left). D-NA did not change the response to desmopressin and D-arginine did not restore the relaxation abolished by L-NA ($n = 3$).

Relaxations associated with desmopressin were markedly inhibited by treatment with 10^{-9} M SR49059, a selective vasopressin V_1 receptor antagonist (Fig. 3, right), which also suppressed the relaxant response to vasopressin in dog ciliary arteries (Okamura et al., 1997). Repeated rinsing of the strips with drug-free media reversed the

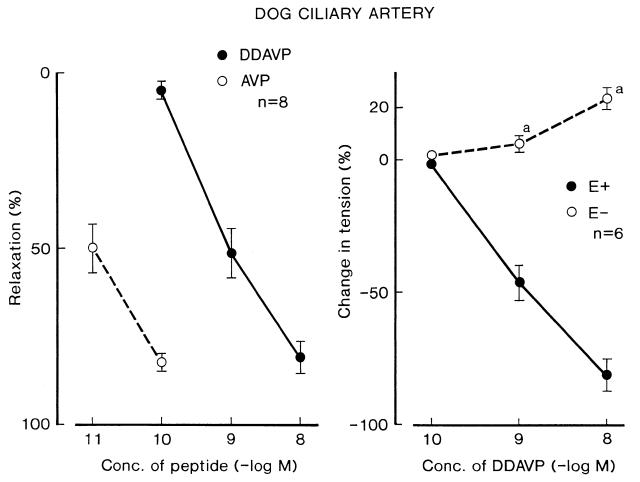

Fig. 1. Concentration–response curves of desmopressin (DDAVP) and arginine vasopressin (AVP) in endothelium-intact ciliary arterial strips (left figure) and of desmopressin in the strips with (E+) and without the endothelium (E−). The strips were partially contracted with prostaglandin $F_{2\alpha}$. Relaxations induced by 10^{-4} M papaverine were taken as 100% relaxation and contractions caused by 30 mM K^+ were taken as 100% contraction. Significantly different from the value in endothelium-intact strips, $^aP < 0.001$ (unpaired *t*-test). '*n*' denotes the number of strips from separate dogs. Responses to desmopressin and vasopressin were compared in the same strips with the endothelium (left panel) and those to desmopressin of endothelium-intact and -denuded strips from the same dogs were compared (right). Vertical bars indicate S.E.

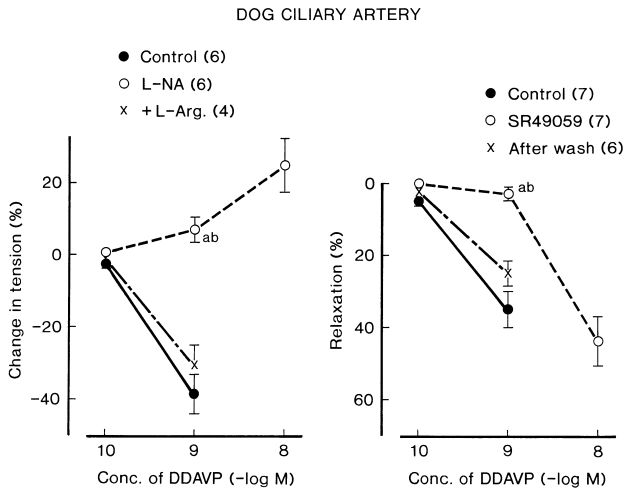

Fig. 3. Modifications by L-NA (10^{-5} M) and L-arginine (10^{-3} M) (left figure) and SR49059 (10^{-9} M) (right) of the relaxant response to desmopressin (DDAVP) in ciliary arterial strips with the intact endothelium, partially contracted with prostaglandin $F_{2\alpha}$. Relaxations induced by 10^{-4} M papaverine were taken as 100% relaxation and contractions caused by 30 mM K^+ were taken as 100% contraction. In the left figure, L-arginine (+ L-Arg.) was added to the strips treated with L-NA. Significantly different from control, $^aP < 0.01$; significantly different from the value with L-arginine (for left figure) or after wash (right), $^bP < 0.01$ (Tukey's method). Numbers in parentheses indicate the number of strips from separate dogs. Vertical bars indicate S.E.

response to desmopressin. The upper tracings of Fig. 2 demonstrates the responses in the absence and presence of the antagonist. Desmopressin-induced relaxations were also attenuated by 10^{-9} M [Pmp^1,$Tyr(Me)^2$]-Arg^8-vasopressin; mean values of the response before and after the antagonist were 27.0 ± 4.4 and $1.5 \pm 1.5\%$ ($n = 4$, $P < 0.01$, unpaired *t*-test), respectively, at 10^{-9} M desmopressin.

3.2. Contractile response

As shown in Fig. 1, right and lower tracings of Fig. 2, endothelium-denuded strips responded to desmopressin with dose-related contractions, which were reproducible by repeated trials when the concentrations were not raised to 10^{-7} M or higher. The contraction was depressed by

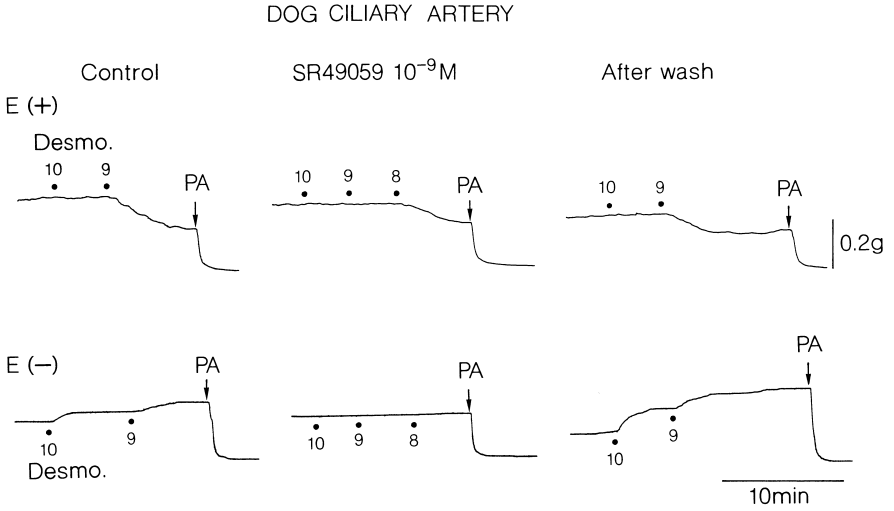

Fig. 2. Typical tracings of the response to desmopressin before and after treatment with SR49059 in ciliary arterial strips with (E+; upper tracings) and without the endothelium (E−; lower) obtained from the same dog. The strips were partially contracted with prostaglandin $F_{2\alpha}$. Concentrations of desmopressin from 10 to 8: 10^{-10}, 10^{-9} and 10^{-8} M, respectively. After wash: after repeated washing with drug-free media. PA: 10^{-4} M papaverine that produced the maximal relaxation.

treatment with 10^{-9} M SR49059; mean values before and after the treatment were 8.0 ± 2.4 and 0% ($n = 5$, $P < 0.02$, unpaired *t*-test), respectively, at 10^{-9} M of the agonist and 25.4 ± 3.6 and 4.0 ± 1.9% ($n = 5$, $P < 0.001$, unpaired *t*-test), respectively, at 10^{-8} M. The inhibitory effect was reversed by repeated rinsing of the strips.

4. Discussion

We have concluded on the basis of pharmacological study with selective vasopressin receptor antagonists, such as SR49059 and [Pmp[1], Try(Me)[2]]Arg[8]-vasopressin, that vasopressin produces relaxation of isolated dog ciliary arteries which is mediated by vasopressin V_1 receptors (Okamura et al., 1997). On the other hand, the present study clearly demonstrated that desmopressin, known as a selective vasopressin V_2 receptor agonist, relaxed the ciliary artery. The potency of desmopressin was about 1/100 of that of vasopressin, although the effective concentration was as low as 10^{-9} M. The desmopressin-induced relaxation was revered to a contraction by endothelium denudation and also by treatment with L-NA, a NO synthase inhibitor. D-NA was without effect and the relaxation depressed by L-NA was restored by L-arginine but not by D-arginine. These findings strongly suggest that the response is mediated by NO liberated from the endothelium. A selective vasopressin V_2 receptor agonist, [Val[4], D-Arg[8]]-vasopressin, increases systemic vascular conductance in unanesthetized dogs, which is suggested to be mediated by NO (Liard, 1994). Desmopressin-induced relaxation of human cerebral arterial rings is seen only when treated with vasopressin V_1 receptor antagonist (Martinez et al., 1994). The relaxation is dependent partially on the endothelium and is inhibited by indomethacin but not by N^G-monomethyl-L-arginine, another NO synthase inhibitor.

The desmopressin-induced ciliary arterial relaxation was clearly reduced by treatment with a low concentration (10^{-9} M) of SR49059, a selective vasopressin V_{1a} receptor antagonist (Serradeil-Le Gal et al., 1993), or a peptide type vasopressin V_1 receptor antagonist, [Pmp[1], Tyr(Me)[2]]-Arg[8]-vasopressin. Similar inhibition by these antagonists was obtained in the relaxant response to vasopressin in dog ciliary arteries (Okamura et al., 1997). The confusing results on receptor subtypes involved may be explained either by a blockade by the vasopressin V_1 receptor antagonists of V_2 receptors or by an activation by desmopressin of vasopressin V_1 receptors in the arteries used. The concentrations of the antagonists (10^{-9} M) and the agonist (10^{-9} M) used do not seem to be quite high in producing non-selective actions on various receptor subtypes. According to a review article by Hruby and Chow (1990), the potency ratio (antidiuretic vasopressin V_2 receptor-mediated action/vasopressor V_1 receptor-mediated action) of vasopressin is 1.03 (Meienhofer et al., 1970) and the ratio of desmopressin is 906 (Zaoral et al., 1967, 1978), 1910 (Manning et al., 1973) or 3077 (Manning et al., 1976). The antidiuretic action of desmopressin is 1.5–2 times higher than that of vasopressin, whereas vasopressor potencies of desmopressin and vasopressin are 1 and 500–1000 (Hruby and Chow, 1990). The ability of vasopressin to displace [^{3}H]vasopressin bound to membranes of COS-7 cells transfected with the cloned rat vasopressin V_{1a} receptors is 753 times higher than that of desmopressin, whereas the ability of vasopressin in the cells transfected with V_2 receptors is 0.76 of that of desmopressin (Lolait et al., 1995). The present study showed that vasopressin was approximately 100 times as potent as desmopressin in producing ciliary arterial relaxation. It would be reasonable to conclude that desmopressin in a selective dose range (less than 10^{-9} M) does not act on the ciliary artery, possibly because of a paucity or lack of vasopressin V_2 receptors in the endothelium, but the peptide would bind to the V_{1a} receptors when the concentration is raised to 100 times higher than the effective concentration of vasopressin (10^{-11} M). In contrast to this finding, vasodilatation by vasopressin V_2 receptor agonists in isolated human cerebral arteries (Martinez et al., 1994) is suggested to be mediated by V_2 receptors. In the present study using the arteries treated with the vasopressin V_1 receptors antagonist, desmopressin at 10^{-8} M produced significant relaxation which may be due to a mediation of the V_2 receptor.

Desmopressin elicited contractions of dog ciliary arteries when the endothelium was removed or the NO synthase was inactivated, as was observed with vasopressin (Okamura et al., 1997). This contraction was also suppressed by SR49059, suggesting the involvement of vasopressin V_{1a} receptors. Contractions induced by desmopressin at 10^{-9} and 10^{-8} M averaged 8 and 25% of the contraction induced by 30 mM K^+, whereas those by vasopressin at 10^{-11} M were 10–20% (Okamura et al., 1997). Again, desmopressin is 100–500 times less potent than vasopressin. Martinez et al. (1994) have concluded that desmopressin and vasopressin are primarily a constrictor of human cerebral arteries by vasopressin V_1 receptor stimulation.

In conclusion, desmopressin relaxes dog ciliary arteries, possibly by acting on vasopressin V_{1a} receptors in the endothelium, resulting in a release of NO synthesized from L-arginine. The V_{1a} receptors in smooth muscle seem to be involved in the desmopressin-induced contraction.

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