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Contractile responses of human deferential artery and vas deferens to vasopressin

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Abstract

We studied the effects of vasopressin on isolated rings of human deferential artery and vas deferens (prostatic portion) obtained from patients undergoing radical cystectomy ($n = 11$) or prostatectomy ($n = 10$). Ring segments of artery or vas deferens were studied in organ bath experiments at optimal resting tension. In artery rings, vasopressin produced concentration-dependent, endothelium-independent contractions with an EC_{50} of 4.5×10^{-10} M. The presence of N^G-nitro-L-arginine methyl ester hydrochloride (10^{-4} M), an inhibitor of nitric oxide synthase, did not change significantly ($P > 0.05$) the vasopressin-induced contraction. In ring preparations of the prostatic part of the vas deferens, vasopressin induced phasic contractions with an EC_{50} of 7.0×10^{-9} M. The vasopressin V_1 receptor antagonist, $d(CH_2)_5Tyr(Me)AVP$ (10^{-8} and 10^{-6}), displaced to the right in parallel the control curve to vasopressin in artery and vas deferens rings. These results indicate that vasopressin exerts a powerful constrictor action on human deferential artery and vas deferens by direct stimulation of V_1 receptors. It is concluded that the deferential artery may dampen the passage of blood to the vas deferens in circumstances characterized by increased plasma vasopressin levels.

Keywords: Deferential artery, human; Vas deferens; Vasopressin; Endothelium; Nitric oxide (NO)

1. Introduction

To date no information is available on the functional properties and pharmacological receptors of the human deferential artery. Yet, such an assessment could be of wide interest because of the influence that changes in blood supply to the vas deferens might have on its motility and responsiveness. The deferential artery is a long, slender vessel which joins the vas deferens at its distal end, close to the ampulla, and runs within its adventitial sheath to provide blood to the entire length of the vas deferens and to the cauda and corpus epididymidis (De Kretser et al., 1982; Gray, 1989). Very recently we found that the human deferential artery has a marked ability to contract in response to adrenergic stimulation and to relax in response to acetylcholine. Involvement of endothelial nitric oxide in these responses has been postulated since endothelium removal or treatment with N^G-nitro-L-arginine methyl ester, an inhibitor of nitric oxide synthase, potentiates adrenergic stimulation and inhibits the acetylcholine-induced relaxation (Martínez et al., 1994b).

Vasopressin causes powerful constriction in a variety of human vascular preparations (Lluch et al., 1984; Ohlstein and Berkowitz, 1986; Martín de Aguilera et al., 1990; Vanner et al., 1990). In addition, vasopressin can stimulate contraction of the human vas deferens (Andersson et al., 1988). The vasopressin receptor designated V_1 seems to mediate the constrictor action of the peptide in vascular smooth muscle and vas deferens (Penit et al., 1983; Maggi et al., 1987; Andersson et al., 1988). The presence of high concentrations of vasopressin in the vas deferens (Andersson et al., 1988) together with the pharmacological characterization of specific V_1 receptors in this tissue (Maggi et al., 1987) raises the possibility that vasopressin could also play a role in regulating the caliber of the deferential artery. Therefore, we designed this study to determine the direct effects of vasopressin on isolated human deferential arteries, with special emphasis on endothelium-dependent responses. We also studied the effects of vasopressin on segments of the prostatic portion of the vas deferens to

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obtain a better understanding of humoral control of this region and to determine whether the pattern of responses is similar to that observed in the epididymal part of the vas deferens.

2. Materials and methods

Macroscopically normal segments of the prostatic part of the vas deferens with deferential artery attached were taken from eight men (aged 50–73 years) undergoing radical prostatectomy because of prostate cancer, and seven (aged 65–83 years) undergoing radical cystectomy because of bladder cancer. The study was approved by the Ethics Committee of our institution and informed consent was obtained from each patient before the study. There was no relationship between age or type of surgery and the ability of the preparations to develop tension in response to KCl. The specimens were placed into physiological salt solution (NaCl, 0.9%), kept on ice and were transported back to the laboratory for dissection and isolation of the deferential artery and vas deferens.

The arteries were immediately placed in chilled Krebs-Henseleit solution, and rings 3–4 mm long were cut for isometric recording of tension. The outside diameter of the rings was measured using an ocular micrometer within a Wild M8 zoom microscope (Heerbrugg, Switzerland) and ranged from 0.5 to 1 mm. In approximately 50% of the artery rings the endothelium was removed mechanically by inserting a roughened stainless-steel wire into the lumen and gently rolling the vessel ring on a wet filter paper. The contractile response to 60 mM KCl was similar in rubbed and unrubbed arterial rings (900 ± 150 versus 1025 ± 110 mg; $P > 0.05$). Functional integrity of the endothelium was confirmed routinely by the presence of relaxation induced by acetylcholine (10^{-7}–10^{-6} M) during contraction obtained with norepinephrine (10^{-6}–3×10^{-6} M). After each experiment the arteries were carefully opened flat and stained with $AgNO_3$ to visualize the endothelium. Only results from vessels with more than 70% of the endothelium were considered as control rings. Vessels in which the endothelium had been rubbed never showed more than 5% of their intima covered with endothelium either before or after the experiment.

Segments of the prostatic part of the vas deferens were obtained from the same patients. Fat and connective tissue were removed and the segments were divided into ring preparations 3–4 mm long.

Ring preparations of the artery or the vas deferens were suspended between two L-shaped stainless steel pins. One pin was fixed to the organ bath wall while the other was connected to a strain gauge (model Grass FT03). Changes in isometric force were recorded on a Grass recorder (model 7). Each segment was set up in a 4-ml bath containing modified Krebs-Henseleit solution of the following millimolar composition: NaCl, 115; KCl, 4.6; $MgCl_2 \cdot 6H_2O$, 1.2; $CaCl_2$, 2.5; $NaHCO_3$, 25; glucose, 11.1; and disodium EDTA, 0.01. The solution was equilibrated with 95% oxygen and 5% carbon dioxide to give a pH of 7.3–7.4. The temperature was held at 37°C. The preparations were allowed to equilibrate for 2 h and during this time the tension was adjusted to a final value of 1 g for artery rings or 2 g for vas deferens rings.

Concentration-response curves for vasopressin were obtained with paired segments in the absence and presence of the V_1 receptor antagonist, $d(CH_2)_5Tyr(Me)AVP$. EC_{50} values (concentration of vasopressin producing half maximal contractions) were determined from individual concentration-response curves by non-linear regression analysis, and the geometric means with 95% confidence intervals were calculated from these values (Fleming et al., 1972). Concentration-response curves to vasopressin were also obtained in paired arterial rings in the absence and presence of the inhibitor of nitric oxide synthase N^G-nitro-L-arginine methyl ester hydrochloride (L-NAME) (10^{-4} M).

Antagonists were added to the organ bath chambers 15 min before the initiation of cumulative concentration-response curves to vasopressin. Only one concentration-response curve to vasopressin was made in each preparation.

The following drugs were used: norepinephrine hydrochloride, acetylcholine chloride, N^G-nitro-L-arginine methyl ester hydrochloride (L-NAME), arginine vasopressin acetate salt and $d(CH_2)_5Tyr(Me)AVP$ [(1-(β-mercapto-β,β-cyclopentamethylenepropionic acid)-2-(*O*-methyl)-[Tyr,Arg^8]vasopressin] (Sigma Chemical Co, St. Louis, MO, USA). All drugs were dissolved in Krebs' solution to the proper final concentration. Drugs were added to the organ bath in volumes of less than 70 μl. Stock solutions of the drugs were freshly prepared every day, and kept on ice throughout the experiment.

The data are expressed as means ± S.E.M. For each experimental group n indicates the number of human subjects. Differences between agonist- and antagonist-treated groups were evaluated by two-way analysis of variance (ANOVA). The significance of differences between groups was evaluated by t-test. Statistically significance was accepted at $P < 0.05$.

3. Results

3.1. Effects of vasopressin in artery rings

Cumulative application of vasopressin (10^{-11}–10^{-8} M) produced a constrictor response that was concentration-dependent. The maximal tension developed as well as the concentration of vasopressin producing half-maximal contractions (EC_{50}) were similar in arteries with and without endothelium ($P > 0.05$) (Fig. 1A and Table 1). The presence of L-NAME (10^{-4} M) did not change significantly ($P > 0.05$) the concentration-response curve for vasopressin (Fig. 1A and Table 1). However, this compound

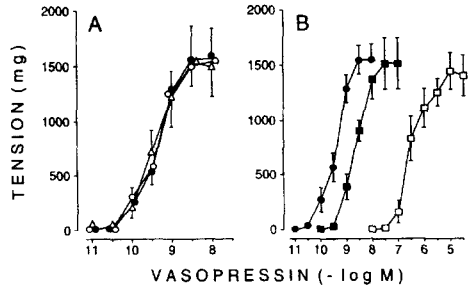

Fig. 1. Concentration-response curves for vasopressin determined in human deferential artery rings. (A) Contractile effects of vasopressin in arterial rings with (●) ($n = 7$) and without (○) ($n = 6$) endothelium and in the presence of 10^{-4} M N^G-nitro-L-arginine methyl ester (△) ($n = 7$). (B) Responses to vasopressin in the absence (●) ($n = 10$) and in the presence of the V_1 receptor antagonist, $d(CH_2)_5Tyr(Me)AVP$ (■, 10^{-8} M; □, 10^{-6} M) ($n = 6$). Values are means ± S.E.M.

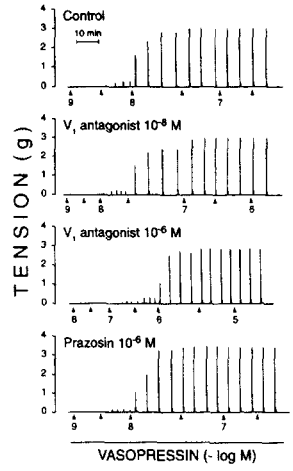

Fig. 2. Sections of experimental records showing effects of vasopressin on human vas deferens rings in the absence (control) and in the presence of the V_1 receptor antagonist, $d(CH_2)_5Tyr(Me)AVP$, or prazosin.

prevented acetylcholine-induced relaxation in artery rings precontracted with noradrenaline (10^{-6} M), showing the ability of these vessels to display nitric oxide-mediated relaxation (results not shown). The presence of the V_1 receptor antagonist, $d(CH_2)_5Tyr(Me)AVP$, (10^{-8} M) in the organ bath displaced the control curve for vasopressin 6-fold to the right in a parallel manner, but differences in the maximal tensions developed were not significant ($P > 0.05$). Increasing the concentration of $d(CH_2)_5Tyr(Me)$-AVP to 10^{-6} M further displaced (680-fold) the control curve for vasopressin (Fig. 1B and Table 1).

3.2. *Effects of vasopressin in vas deferens rings*

Cumulative addition of vasopressin (10^{-9}–10^{-7} M) to the preparations induced repetitive phasic contractions with

Table 1
Geometric mean EC_{50} values and maximal responses to vasopressin in human deferential arteries

Artery rings	EC_{50} (M) (95% confidence interval)	Maximal response (mg ± S.E.M.)
With endothelium (control) ($n = 7$)	4.5×10^{-10} (3.1×10^{-10}–6.5×10^{-10})	1570 ± 270
With endothelium + L-NAME ($n = 7$)	6.6×10^{-10} (2.5×10^{-10}–1.7×10^{-9})	1490 ± 260
Without endothelium ($n = 6$)	2.2×10^{-10} (1.3×10^{-10}–3.6×10^{-10})	1560 ± 180
With $d(CH_2)_5Tyr(Me)AVP$		
10^{-8} M ($n = 6$)	2.7×10^{-9}* (1.6×10^{-9}–4.3×10^{-9})	1520 ± 230
10^{-6} M ($n = 6$)	3.2×10^{-7}* (1.4×10^{-7}–7.2×10^{-7})	1580 ± 180

n indicates the number of patients. * $P < 0.05$ compared with control rings.

Table 2
Geometric mean EC_{50} values and maximal responses to vasopressin in human vas deferens

Vas deferens rings	EC_{50} (M) (95% confidence interval)	Maximal response (mg ± S.E.M.)
Control ($n = 7$)	7.0×10^{-9} (5.1×10^{-9}–9.7×10^{-9})	3288 ± 511
With $d(CH_2)_5Tyr(Me)AVP$		
10^{-8} M ($n = 5$)	2.6×10^{-8}* (1.6×10^{-8}–4.0×10^{-8})	3390 ± 520
10^{-6} M ($n = 5$)	1.9×10^{-6}* (1.1×10^{-6}–3.5×10^{-6})	3065 ± 665

n indicates the number of patients. * $P < 0.05$ compared with control rings.

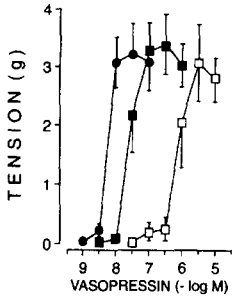

Fig. 3. Concentration-response curves for vasopressin determined in human vas deferens rings in the absence (●) ($n = 7$) and in the presence of the V_1 receptor antagonist, $d(CH_2)_5Tyr(Me)AVP$ (■, 10^{-8} M; □, 10^{-6} M) ($n = 5$). Values are means ± S.E.M.

an EC_{50} of 7.0×10^{-9} M (Fig. 2 and Table 2). The threshold concentration of vasopressin ranged from 10^{-9} to 3×10^{-9} M and the maximal tensions amounted to 256% of the KCl-induced contractions. The presence of the V_1 receptor antagonist, $d(CH_2)_5Tyr(Me)AVP$, (10^{-8} and 10^{-6} M) in the organ bath displaced the control curve for vasopressin 4- and 270-fold to the right, respectively, but differences in the maximal tensions developed were not significant ($P > 0.05$) (Fig. 3). Pretreatment with the α_1-adrenoceptor antagonist, prazosin (10^{-6} M, $n = 4$), did not affect contractile responses to vasopressin (Fig. 2).

4. Discussion

In the present study the contractile responses of the human deferential artery to vasopressin were characterized and compared to those elicited in the prostatic part of the vas deferens. This could be of relevance since vasopressin is most probably supplied by this artery and stored in the vas deferens. The possibility of local synthesis of vasopressin in the vas deferens has also been postulated (Maggi et al., 1987). The deferential artery, because of its anatomical location and reactivity, could play an important role in regulating flow or pressure of blood that arrives to the vas deferens. We recently reported that adrenergic constriction and cholinergic relaxation of this artery follow the pattern observed in other peripheral arteries (Martínez et al., 1994b). These responses are modulated by nitric oxide, a potent relaxant of vascular smooth muscle synthetized from endogenous L-arginine by the nitric oxide synthase system in the vascular endothelium (Ignarro et al., 1987; Palmer et al., 1987).

Our results indicate that vasopressin is a potent agonist for the contraction of smooth muscle of both deferential artery and vas deferens through activation of specific V_1 vasopressin receptors. The maximal tensions attained in deferential arteries with vasopressin are higher than those induced with KCl, and the EC_{50} values for vasopressin are lower than those for noradrenaline in the same vascular preparations (Martínez et al., 1994b). Interestingly, the sensitivity of these arteries to vasopressin (in terms of EC_{50} values) are similar to those exhibited by other human arteries (Martín de Aguilera et al., 1990; Martínez et al., 1994a, Martínez et al., 1994c). Accordingly, the deferential artery has a marked responsiveness to vasopressin, and it is suggested that this vessel could contribute to the regulation of vas deferens motility in pathophysiological states characterized by an increased plasma vasopressin level. Under these circumstances the deferential artery may damp the passage of blood to the vas deferens by increasing the resistance to blood flow.

Our data also show that the vasopressin-induced contraction is not linked to the presence of an intact endothelium, thus indicating that vasodilator substances secreted by endothelial cells do not counteract the potent contractile effects of vasopressin on vascular smooth muscle cells. The evidence for this conclusion is that the contraction was similar in intact and endothelium-denuded arteries. Furthermore, the contraction is not modulated by the intervention of the L-arginine-nitric oxide pathway because L-NAME, a selective inhibitor of nitric oxide synthase, did not modify the vasopressin-induced contraction. The concentration of 10^{-4} M L-NAME was chosen for these experiments because our previous experiments have shown that this concentration inhibited acetylcholine-mediated vasodilatation and potentiated contractile responses to adrenergic stimulation (Martínez et al., 1994b). The present results are consistent with earlier results showing that vasopressin causes endothelium-independent contractions in human cerebral (Martín de Aguilera et al., 1990) and mesenteric arteries (Martínez et al., 1994c). Therefore, it is most likely that the contractile effects of vasopressin on human deferential arteries are due to direct stimulation of specific receptors located on smooth muscle cells.

Antagonists of vasopressin have been used to study the cardiovascular effects of vasopressin and to characterize the receptors involved (Sawyer et al., 1981). $d(CH_2)_5Tyr(Me)AVP$ has been reported to be a potent inhibitor of the pressor responses to vasopressin in anesthetized rats (Kruszynsky et al., 1980). Furthermore, this compound does not interfere with the vasoconstrictor responses to angiotensin II, noradrenaline or prostaglandin $F_{2\alpha}$ (Ohlstein and Berkowitz, 1986; Martínez et al., 1994c). We demonstrated that $d(CH_2)_5Tyr(Me)AVP$ is an effective antagonist of the responses to arginine vasopressin in human deferential arteries. At 10^{-6} M, the antagonist produced a 680-fold shift to the right of the control concentration-response curve for vasopressin, presumably due to a competitive agonist-antagonist interaction. Our results parallel those previously observed in human cerebral and mesenteric arteries using the same V_1 vasopressin receptor antagonist (Martín de Aguilera et al., 1990; Martínez et al., 1994c)

Several studies indicate that vasopressin plays a role in the regulation of contractility of the male genital tract (Maggi et al., 1987; Andersson et al., 1988). Our present

results show that vasopressin also exerts powerful contractile effects in the prostatic part of the vas deferens which are higher than those in the epididymal part of the vas deferens in terms of EC_{50} values (unpublished observations). We found that the V_1 receptor antagonist, $d(CH_2)_5Tyr(Me)AVP$, produced a shift to the right of the control concentration-response curve for vasopressin, a finding similar to that observed with the deferential artery. These data reinforce the hypothesis that the V_1 receptor (eliciting contraction) localized in the human vas deferens and in human seminal vesicles (Maggi et al., 1989) may be similar to V_1 receptors in the deferential artery and in other human arteries (Martín de Aguilera et al., 1990; Martínez et al., 1994c).

In conclusion, the results presented herein show that vasopressin elicits smooth muscle contraction of human deferential artery and vas deferens due to V_1 receptor stimulation. These V_1 receptors are probably similar to those present in the human male genital tract including the tunica albuginea, vas deferens and seminal vesicles (Andersson et al., 1988; Maggi et al., 1989). Our study was not focused on other mechanisms by which vasopressin may interact with smooth muscle. For instance, vasopressin may also dilate some human vascular beds both in vivo (Hirsch et al., 1989) and in vitro (Martínez et al., 1994b, Martínez et al., 1994c) due to V_2 receptor stimulation (c-AMP-dependent) or as a consequence of the release of dilating prostaglandins. Therefore, from a hypothetical point of view, the overall effects of vasopressin may result both from a V_1 receptor-mediated contraction and from V_2 receptor- or prostaglandin-mediated blunting of the contraction. These mechanisms should be clarified before a functional link can be shown between the effects of vasopressin in the vas deferens and its accompanying artery.

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