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Vasopressin receptors involved in adrenergic neurotransmission in the circular muscle of the human vas deferens

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Abstract

We studied the effects of vasopressin on the adrenergic responses of in vitro preparations of circular muscle from the vas deferens obtained from 28 men undergoing elective vasectomy. Vasopressin (3×10^{-9}–3×10^{-8} M) enhanced the phasic contractions elicited by electrical field stimulation and noradrenaline. This potentiation was blocked by the vasopressin V_1 receptor antagonist d(CH_2)$_5$Tyr(Me)vasopressin (10^{-6} M) but not by the vasopressin V_2 receptor antagonist [d(CH_2)$_5$, D-Ile^2,Ile^4,Arg^8]vasopressin (10^{-6} M). The Ca^{2+} antagonist nifedipine (10^{-6} M) did not affect the potentiation of electrical field stimulation induced by vasopressin and noradrenaline but reduced KCl-induced contractions and abolished the induction of phasic activity by vasopressin in the presence of KCl. The results demonstrate that vasopressin, in addition to its direct contractile effects, strongly potentiates contractions of human vas deferens elicited by adrenergic stimulation. Both the direct and indirect effects of vasopressin appear to be mediated by vasopressin V_1 receptor stimulation and are independent of Ca^{2+} entry through dihydropyridine Ca^{2+} channels.

Keywords: Vas deferens; (Human); Vasopressin; Electric stimulation; Noradrenaline

1. Introduction

Vasopressin is a neuropeptide which promotes the reabsorption of water in renal tubular cells, an action mediated by vasopressin V_2 receptors, and produces constriction of smooth muscle, an action mediated by vasopressin V_1 receptors (Michell et al., 1979; Penit et al., 1983; Thibonnier, 1988). Upon binding to its specific V_1 receptor, vasopressin initiates a series of biochemical events that results in the accumulation of inositol 1,4,5-triphosphate and diacylglycerol, leading to mobilization of Ca^{2+} from intracellular stores and the stimulation of protein kinase C (for review see Thibonnier, 1992). Activated protein kinase C and intracellular Ca^{2+} may act synergistically to contract smooth muscle (Rasmussen et al., 1987). Vasopressin may also have indirect effects that result from its influence on the vasoconstrictor activity of other vasoactive agents that are found in plasma or which are released from nerve fibers (Bartelstone and Nasmyth, 1965; Guc et al., 1992). It has been reported that a potentiating interaction between the sympathetic nervous system and vasopressin is apparent in human vas deferens (Andersson et al., 1988) and in human and rat mesenteric artery (Medina et al., 1997; Noguera et al., 1997). The mechanisms underlying this potentiation have only been studied in isolated arteries and they appear to be diverse. In human mesenteric arteries the binding of vasopressin to its V_1 receptor potentiates the contractions induced by sympathetic stimulation and noradrenaline through a mechanism insensitive to the Ca^{2+} channel blocker nifedipine whereas in rat mesenteric arteries the potentiating effects of vasopressin result from an increase in Ca^{2+} entry through voltage-dependent Ca^{2+} channels (Medina et al., 1997; Noguera et al., 1997).

The presence of high concentrations of vasopressin in the vas deferens (Andersson et al., 1988) together with the pharmacological characterization of specific vasopressin V_1 receptors in this tissue (Maggi et al., 1987; Medina et

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al., 1996) raises the possibility that vasopressin could also play a role in regulating the contractile responses to adrenergic stimulation and influence vas deferens motility. In order to elucidate further the role of this neuropeptide in vas deferens motility, the present work was designed to investigate whether low concentrations of vasopressin could modify the responses elicited by noradrenaline, KCl, and stimulation of nerves supplying the human vas deferens. Others have also investigated this (Andersson et al., 1988), but in that study a relatively high concentration of vasopressin was used which by itself induced smooth muscle contraction (Andersson et al., 1988). Our interest was focused on the influence of concentrations of vasopressin within a range which, while having no direct action, could amplify sympathetically mediated contractions. We also determined whether the modulating effect of vasopressin on vas deferens responsiveness depends on the activation of vasopressin V_1 or V_2 receptors. To examine the possibility that stimulation of vasopressin receptors may facilitate Ca^{2+} entry through dihydropyridine Ca^{2+} channels, experiments were performed in the presence of the Ca^{2+} channel blocker nifedipine.

2. Materials and methods

2.1. Preparations

Segments (10–20 mm long) of the epididymal part of the vas deferens were taken from 28 men (aged 31–43 years) who were sterilized by elective vasectomy. The study was approved by the Ethics Committee of our institution and informed consent was obtained from each subject before the study. The specimens were placed into physiological salt solution (NaCl, 0.9%), kept on ice and transported back to the laboratory. Fat and connective tissue were removed and the segments were divided into ring preparations 3–4 mm long.

Ring preparations 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 Macintosh computer by use of Chart version 3.4/s software and a MacLab/8e data acquisition system (ADInstruments). Each preparation 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. Ca^{2+}-free solution containing 1 mM EGTA was made by omitting $CaCl_2$ and EDTA from the control solution. The tissues were washed out with this solution ($3\times$) and Ca^{2+}-free K^+-depolarizing solution was prepared by omitting $CaCl_2$ and replacing 30 mM NaCl by 30 mM KCl.

The preparations were allowed to equilibrate for 2 h and during this time tension was adjusted to a final tension of 2 g.

Electrical field stimulation was provided by a Grass S88 stimulator via two platinum electrodes positioned on each side and parallel to the axis of the ring. Single square wave pulses at supramaximum voltage (20 V), 0.25 ms pulse duration, at a frequency of 20 Hz were used. The train duration was 5 s and the stimulation interval was 180 s. To assess the nature of the contractile responses a group of rings was stimulated before and after treatment with tetrodotoxin (10^{-6} M) or prazosin (10^{-6} M) following procedures previously described (Hedlund et al., 1985; Medina et al., 1997).

When the preparations were stable, agonists and antagonists were added cumulatively to the preparation and the effects of electrical field stimulation were recorded. The drugs tested included vasopressin (10^{-9} to 3×10^{-8} M), the selective vasopressin V_1 receptor agonist [Phe^2,Orn^8]vasotocin (10^{-9} to 3×10^{-8} M), the vasopressin V_2 receptor agonist desmopressin (10^{-8} to 10^{-6} M), the vasopressin V_1 receptor antagonist d(CH_2)$_5$Tyr(Me)vasopressin (10^{-6} M), the vasopressin V_2 receptor antagonist [d(CH_2)$_5$, D-Ile^2,Ile^4,Arg^8]vasopressin (10^{-6} M), the reuptake blocker cocaine (10^{-6} M) and the Ca^{2+} channel blocker nifedipine (10^{-6} M).

Concentration–response curves for noradrenaline and KCl were determined in a cumulative manner and control (in the absence of vasopressin) and experimental (in the presence of vasopressin or vasopressin together with the vasopressin V_1 receptor antagonist) curves were recorded from separate preparations. When KCl was used, phentolamine (10^{-6} M) was added to the organ bath in order to prevent activation of α-adrenoceptors by noradrenaline released by neuronal depolarization.

To study the effects of vasopressin on Ca^{2+}-induced contractile responses, a group of rings was incubated in Ca^{2+}-free solution containing 30 mM KCl. After a 30-min washout period, concentration–response curves for $CaCl_2$ (10^{-5} to 10^{-2} M) were determined in paired rings in the absence and presence of either vasopressin (10^{-9} M) or vasopressin together with the vasopressin V_1 receptor antagonist (10^{-6} M).

In another group of experiments, the preparations were preincubated with the Ca^{2+} channel blocker nifedipine (10^{-6} M) for 20 min before the addition of vasopressin.

2.2. Drugs

The following drugs were used: tetrodotoxin, nifedipine, prazosin, noradrenaline hydrochloride, arginine vasopressin acetate salt, [(1-(β-mercapto-β,β-cyclopentamethylenepropionic acid)-2-(*O*-methyl)-tyrosine, 8-arginine) vasopressin] (d(CH_2)$_5$Tyr(Me)vasopressin), deamino-8-D-arginine vasopressin (desmopressin), phentolamine hydrochloride (Sigma, St. Louis, MO, USA),

[Phe^2,Orn^8]vasotocin, [d(CH$_2$)$_5$, D-Ile^2,Ile^4,Arg^8]-vasopressin (Peninsula Laboratories Europe, Merseyside, UK) and cocaine chlorhydrate (Abelló, Madrid, Spain). All drugs were dissolved in Krebs solution except nifedipine and prazosin, which were dissolved initially in ethanol and further diluted in Krebs solution to the appropriate 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.

2.3. Data analysis

The contractile responses were measured as the maximum of repetitive phasic contractions or as a sustained change in basal tension (i.e., tonic tension). All values are expressed as means ± S.E.M. Contractions are reported as absolute values (g) or as percentages of control responses. EC_{50} values (concentration of agonist 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). The number of rings taken from each subject varied from four to seven. The responses obtained in each subject were averaged to yield a single value. Therefore, all *n* values are presented as the number of subjects. Differences between agonist- and antagonist-treated groups were assessed by two-way analysis of variance (ANOVA). For electrical stimulation experiments in which the same rings were stimulated in the absence and presence of vasopressin, a paired *t*-test was used. Statistical significance was accepted at $P < 0.05$.

3. Results

3.1. Effects of vasopressin

Vasopressin (10^{-8}–10^{-6} M) caused concentration-dependent, repetitive phasic contractions with an EC_{50} of 4.9×10^{-8} M (Fig. 1A and G). The presence of the vasopressin V_1 receptor antagonist d(CH$_2$)$_5$Tyr(Me)vasopressin (10^{-6} M) in the organ bath displaced the control curve for vasopressin 316-fold to the right in a parallel manner, but differences in the maximal tension

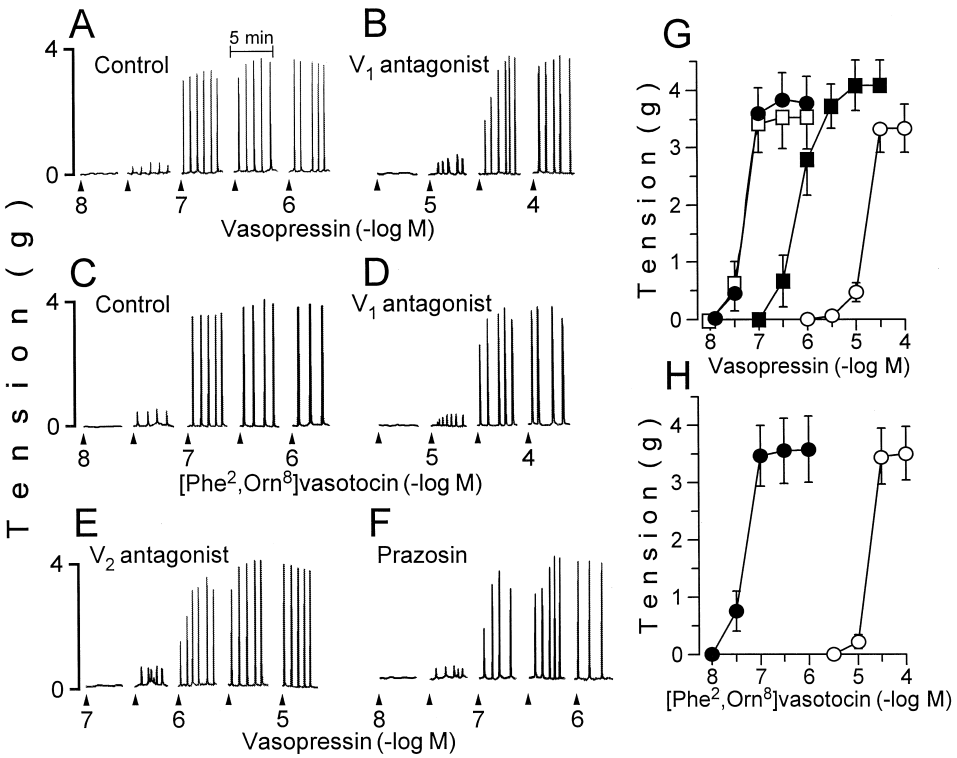

Fig. 1. Responses of human vas deferens rings to vasopressin. Records from A to F show responses from six different preparations. (A) and (B) Responses to vasopressin in the absence and in the presence of the vasopressin V_1 receptor antagonist d(CH$_2$)$_5$Tyr(Me)vasopressin (10^{-6} M). (C) and (D) Responses to the vasopressin V_1 receptor agonist [Phe^2,Orn^8]vasotocin in the absence and in the presence of the vasopressin V_1 receptor antagonist d(CH$_2$)$_5$Tyr(Me)vasopressin (10^{-6} M). (E) and (F) Responses to vasopressin in the presence of the vasopressin V_2 receptor antagonist [d(CH$_2$)$_5$, D-Ile^2,Ile^4,Arg^8]vasopressin (10^{-6} M) or prazosin (10^{-6} M). (G) Concentration–response curves for vasopressin in the absence (●, $n = 7$) and in the presence of the vasopressin V_1 receptor antagonist d(CH$_2$)$_5$Tyr(Me)vasopressin (○, 10^{-6} M, $n = 7$), the vasopressin V_2 receptor antagonist [d(CH$_2$)$_5$, D-Ile^2,Ile^4,Arg^8]vasopressin (■, 10^{-6} M, $n = 6$) or the α_1-adrenoceptor antagonist prazosin (□, 10^{-7} M, $n = 6$). (H) Concentration–response curves for the vasopressin V_1 receptor agonist [Phe^2,Orn^8]vasotocin in the absence (●, $n = 7$) and in the presence of the vasopressin V_1 receptor antagonist d(CH$_2$)$_5$Tyr(Me)vasopressin (○, 10^{-6} M, $n = 7$). Values in G and H are shown as means ± S.E.M.

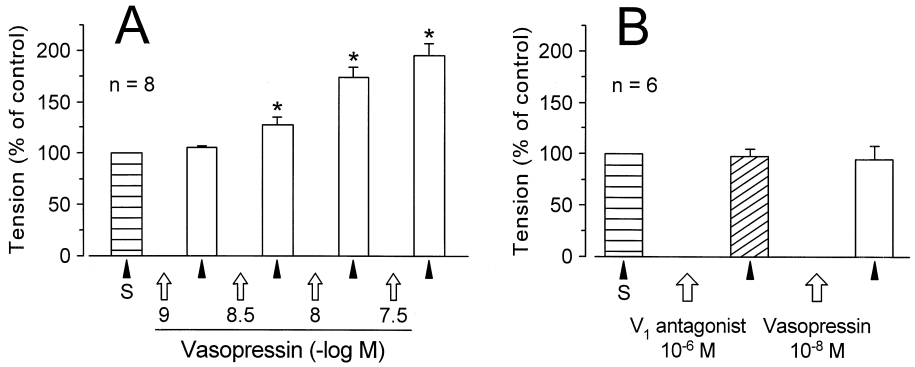

Fig. 2. (A) Bar graph of contractile responses to electrical stimulation (S) in the absence and in the presence of vasopressin (10^{-9} to 3×10^{-8} M). (B) Bar graph showing the lack of potentiation by vasopressin of neurogenic contractile responses after treatment with the vasopressin V_1 receptor antagonist $d(CH_2)_5$Tyr(Me)vasopressin. Values are shown as the means ± S.E.M. *n*, number of subjects. $* P < 0.05$ vs. control.

developed were not significant ($P > 0.05$) (Fig. 1B and G).

The selective vasopressin V_1 receptor agonist [Phe^2,Orn^8]vasotocin (10^{-8}–10^{-6} M) induced concentration-dependent contractions in all rings tested (Fig. 1C and H). Maximal responses and EC_{50} values were equivalent to those obtained with vasopressin. The vasopressin V_1 receptor antagonist $d(CH_2)_5$Tyr(Me)vasopressin (10^{-6} M)

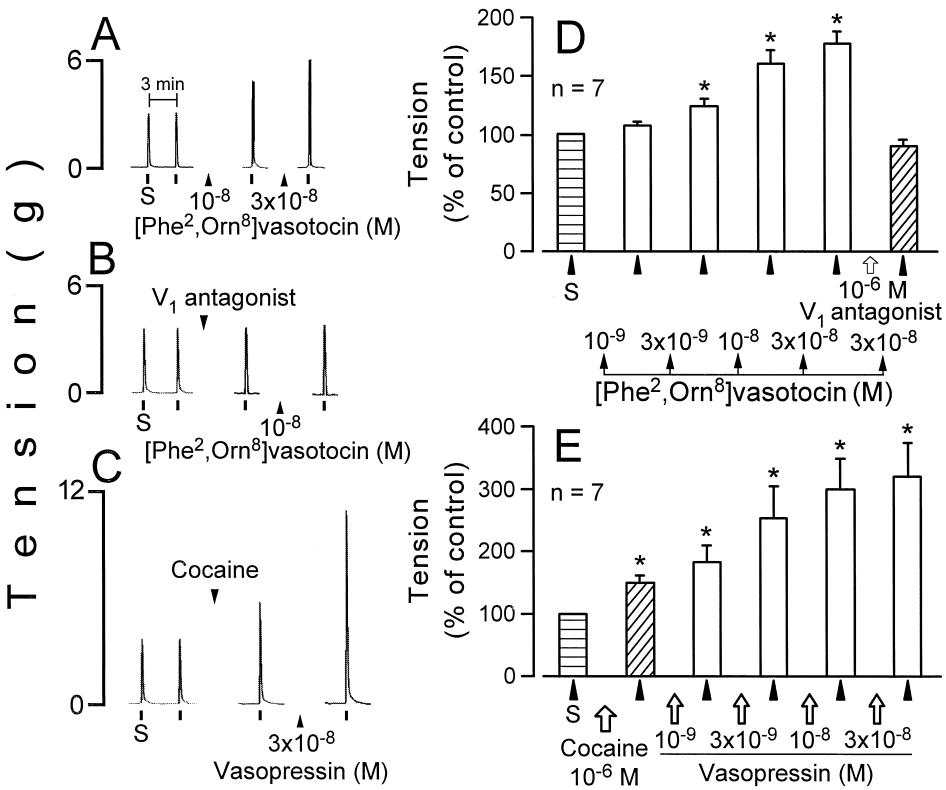

Fig. 3. Contractile responses of human vas deferens rings to electrical stimulation (20 V, 0.25 ms pulse duration, 20 Hz, train duration 5 s, stimulation interval 180 s). Records from A, B and C correspond to three different preparations. (A) Contractile responses to electrical stimulation (S) in the absence and in the presence of the vasopressin V_1 receptor agonist [Phe^2,Orn^8]vasotocin (10^{-8} and 3×10^{-8} M). (B) The presence of the vasopressin V_1 receptor antagonist $d(CH_2)_5$Tyr(Me)vasopressin (10^{-6} M) abolished the augmentation of the contraction to electrical stimulation produced by the vasopressin V_1 receptor agonist. (C) Responses to electrical stimulation before and after treatment with cocaine (10^{-6} M) and after addition of 3×10^{-8} M vasopressin. (D) Bar graph of contractile responses to electrical stimulation in the presence of the vasopressin V_1 receptor agonist (10^{-9} to 3×10^{-8} M) and inhibition of the potentiation by the vasopressin V_1 receptor antagonist (10^{-6} M). (E) Bar graph illustrating the effects of vasopressin (10^{-9} to 3×10^{-8} M) on electrical stimulation-induced contractions in the presence of cocaine (10^{-6} M). Values in D and E are shown as the means ± S.E.M. *n*, number of subjects. $* P < 0.05$ vs. control.

produced a parallel, rightward shift of the control curve (275-fold) which did not differ from that obtained when vasopressin was the agonist. The vasopressin V_2 receptor antagonist [d(CH_2)$_5$, D-Ile^2,Ile^4,Arg^8]vasopressin (10^{-6} M) was also evaluated for its ability to antagonize vasopressin-induced contractions. This compound displaced the control curve for vasopressin 18-fold to the right ($P < 0.05$) in a parallel manner without causing differences in the maximal response (Fig. 1E and G). However, the selective vasopressin V_2 receptor agonist desmopressin (10^{-8}–3×10^{-6} M) did not produce significant changes in the resting tension ($n = 6$) (results not shown). The α_1-adrenoceptor antagonist prazosin (10^{-6} M) did not affect the concentration–response curve for vasopressin (EC_{50} 4.6×10^{-8} M) (Fig. 1F and G).

3.2. Effects of electrical stimulation

Electrical stimulation increased tension in all the experiments and this increase was abolished by tetrodotoxin (10^{-6} M) and prazosin (10^{-6} M), thus indicating that the effect was due to the release of noradrenaline from adrenergic nerves acting on α_1-adrenoceptors.

Vasopressin (3×10^{-9}–10^{-8} M) did not induce contractions itself but significantly augmented the neurogenic contractions (Fig. 2A). At 3×10^{-8} M, vasopressin induced a small contraction (10–15% of maximal vasopressin-induced contraction) and further potentiated the contractions induced by electrical field stimulation (Fig. 2A). The vasopressin V_1 receptor antagonist d(CH_2)$_5$Tyr(Me)vasopressin (10^{-6} M) did not change the control response to electrical field stimulation but prevented the amplifying effect of vasopressin (Fig. 2B).

The selective vasopressin V_1 receptor agonist [Phe^2,Orn^8]vasotocin induced a potentiation of the electrical stimulation-evoked response of a magnitude similar to that observed in the presence of vasopressin (Fig. 3A and D). This potentiation was also inhibited in the presence of the V_1 receptor antagonist d(CH_2)$_5$Tyr(Me)vasopressin (10^{-6} M) (Fig. 3B and D).

Blockade of neuronal catecholamine reuptake by cocaine (10^{-6} M) increased the contractile response to electrical field stimulation ($P < 0.05$). In the presence of cocaine the contractile responses to electrical field stimulation were significantly enhanced by vasopressin to an extent similar (percentage-wise) to that observed in the absence of cocaine (Fig. 3C and E).

To determine whether vasopressin V_2 receptors are involved in the effects of vasopressin on electrical field stimulation, responses were obtained in the absence and in the presence of the vasopressin V_2 receptor antagonist [d(CH_2)$_5$, D-Ile^2,Ile^4,Arg^8]vasopressin. The results showed that the potentiation induced by vasopressin was not modified ($P > 0.05$; $n = 5$) in the presence of the vasopressin V_2 antagonist (10^{-6} M). In addition, the selective vasopressin V_2 agonist desmopressin (10^{-8}–10^{-7} M) did not

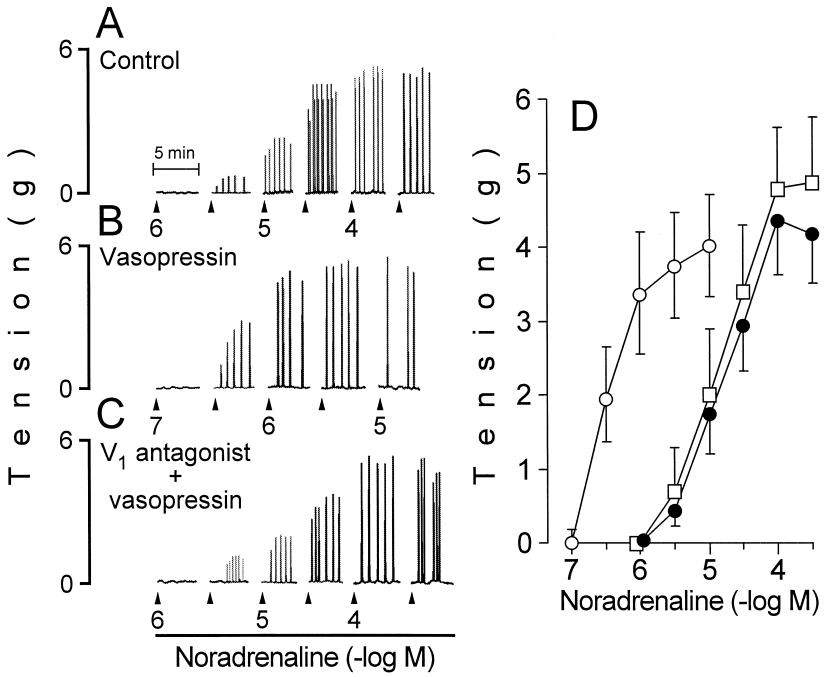

Fig. 4. Contractile responses of human vas deferens rings to noradrenaline in the absence (A) and in the presence of vasopressin (B, 3×10^{-8} M) or the vasopressin V_1 receptor antagonist d(CH_2)$_5$Tyr(Me)vasopressin (10^{-6} M) together with vasopressin (3×10^{-8} M) (C). Records from A, B and C correspond to three different preparations. (D) Concentration–response curve for noradrenaline in the absence (●, $n = 7$) and in the presence of either 3×10^{-8} M vasopressin (○, $n = 6$) or vasopressin together with the vasopressin V_1 receptor antagonist (□, 10^{-6} M, $n = 6$). Values in D are shown as means ± S.E.M.

Table 1
EC_{50} values and maximal contractions elicited by noradrenaline alone (control), in the presence of either vasopressin or the vasopressin V_1 receptor antagonist d$(CH_2)_5$Tyr(Me)vasopressin together with vasopressin

Noradrenaline	EC_{50} (M) (95% confidence interval)	Maximal responses (mg ± S.E.M.)
Control ($n = 7$)	1.7×10^{-5} ($1.1–2.6 \times 10^{-5}$)	4987 ± 786
With vasopressin		
3×10^{-9} M ($n = 5$)	4.7×10^{-6} [a] ($2.1–8.1 \times 10^{-6}$)	4743 ± 960
10^{-8} M ($n = 5$)	4.1×10^{-7} [a] ($2.4–7.2 \times 10^{-7}$)	4975 ± 710
3×10^{-8} M[b] ($n = 6$)	1.7×10^{-7} [a] ($0.9–3.2 \times 10^{-7}$)	4897 ± 575
With d$(CH_2)_5$Tyr(Me)vasopressin (10^{-6} M) + vasopressin (3×10^{-8} M) ($n = 6$)	1.8×10^{-5} ($0.7–5.1 \times 10^{-5}$)	5100 ± 850

Values are means ± S.E.M.
n, number of subjects.
[a] $P < 0.05$, vs. control rings.
[b] When 3×10^{-8} M vasopressin elicited contraction, this effect was subtracted from the subsequent response to noradrenaline.

modify the contractile response to electrical field stimulation ($P > 0.05$; $n = 5$) (results not shown).

3.3. Effect of vasopressin on noradrenaline-induced contractions

Cumulative addition of noradrenaline (10^{-6}–3×10^{-4} M) induced repetitive phasic contractions with an EC_{50} of 1.7×10^{-5} M (Fig. 4A and D; Table 1). Vasopressin 3×10^{-8} M potentiated contractions elicited by low concentrations of noradrenaline but maximal contractions were unchanged (Fig. 4B and D). The vasopressin V_1 receptor antagonist d$(CH_2)_5$Tyr(Me)vasopressin (10^{-6} M) inhibited the potentiating effects of 3×10^{-8} M vasopressin and brought the EC_{50} to values similar to those obtained for the control curve (Fig. 4C and D; Table 1).

3.4. Effects of vasopressin on KCl-induced contractions

Fig. 5 is a typical recording illustrating the effects of increasing concentrations of KCl (10–120 mM) in the absence and in the presence of 10^{-9} M vasopressin or in the presence of vasopressin together with the vasopressin V_1 receptor antagonist (10^{-6} M). Under control conditions (without vasopressin) KCl induced concentration-dependent tonic contractions. The threshold concentration was approximately 30 mM and maximal contractions were reached at 100 mM KCl ($n = 5$). In the presence of subcontractile concentrations of vasopressin (10^{-9} M), KCl produced repetitive phasic contractions at a threshold concentration of 10 mM. At 30 mM KCl the frequency of spikes was increased and a tonic component, similar to that under control conditions, was observed. At 60 and 100 mM KCl, the phasic contractions disappeared and only the tonic component was evident. The maximal responses to KCl were not significantly different (3312 ± 289 mg vs. 3123 ± 270 mg; $P < 0.05$, $n = 5$). Pretreatment with the vasopressin V_1 receptor antagonist (10^{-6} M) prevented the phasic contractions induced by KCl in the presence of vasopressin (10^{-9} M).

3.5. Vasopressin and Ca^{2+}

The dihydropyridine Ca^{2+} antagonist nifedipine (10^{-6} M) did not change significantly the contraction induced by vasopressin (Fig. 6A). The presence of nifedipine diminished by approximately 30% the contractile response to electrical field stimulation (Fig. 6B). Nifedipine decreased the maximal response to noradrenaline and increased significantly the EC_{50} value (1.5×10^{-5} vs. 4.2×10^{-5} M, $P < 0.05$) (Fig. 6C). However, in the presence of nifedip-

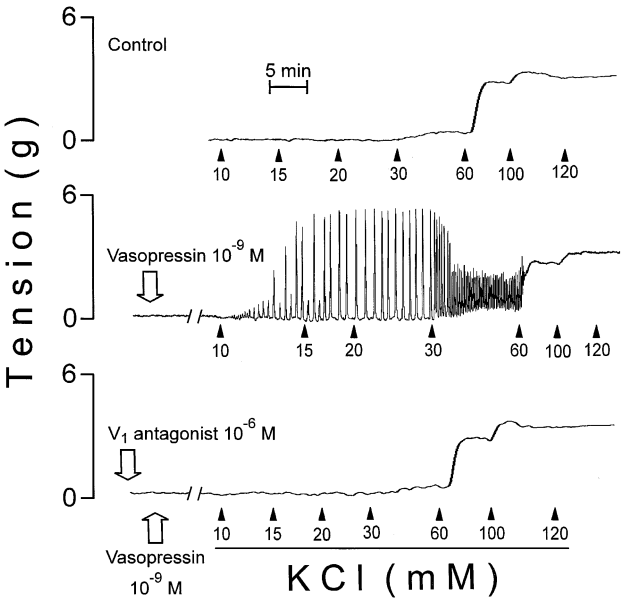

Fig. 5. Recordings illustrating the contractile effects of increasing concentrations of potassium chloride under control conditions, in the presence of 10^{-9} M vasopressin and in the presence of the vasopressin V_1 receptor antagonist d$(CH_2)_5$Tyr(Me)vasopressin (10^{-6} M) plus 10^{-9} M vasopressin.

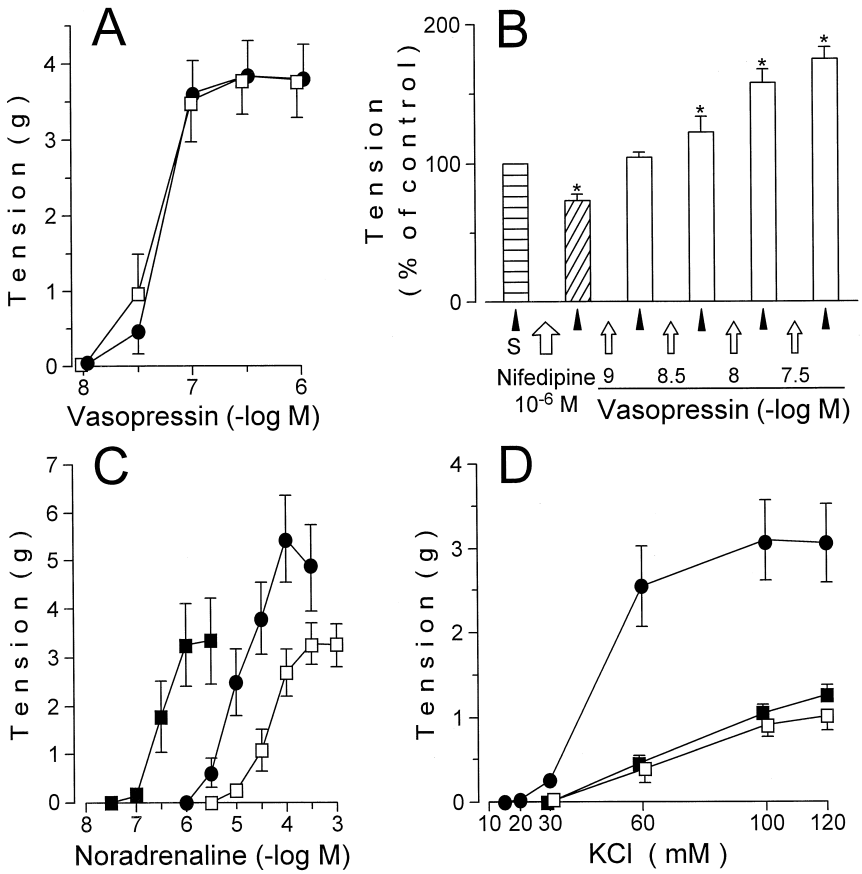

Fig. 6. (A) Concentration–response curves for vasopressin in the absence (●, $n = 7$) and in the presence of 10^{-6} M nifedipine (□, $n = 5$). (B) Effects of vasopressin (10^{-9}–3×10^{-8} M) on electrical stimulation (S)-induced responses in the presence of 10^{-6} M nifedipine ($n = 6$). (C) Concentration–response curves for noradrenaline in the absence (●, $n = 7$) and in the presence of either 10^{-6} M nifedipine (□, $n = 6$) or nifedipine together with 10^{-8} M vasopressin (■, $n = 6$). (D) Concentration–response curves for KCl in the absence (●) and in the presence of 10^{-6} M nifedipine (□) or nifedipine together with 10^{-8} M vasopressin (■) ($n = 6$). Values are shown as means ± S.E.M. $* P < 0.05$ vs. control.

ine the enhancement by vasopressin of the contractile responses to electrical field stimulation and noradrenaline was identical to that observed in the absence of nifedipine (Fig. 6B and C). Nifedipine reduced significantly the KCl-induced tonic contractions and abolished the induction of phasic activity by vasopressin in the presence of KCl (Fig. 6D).

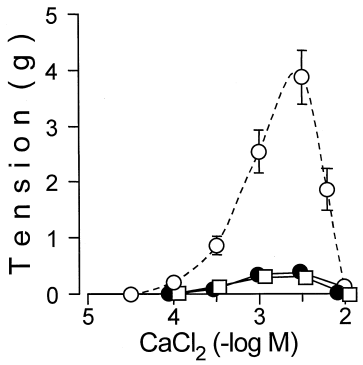

Fig. 7. Contractions of human vas deferens induced by the addition of $CaCl_2$ to Ca^{2+}-free depolarizing solution containing 30 mM KCl in the absence (●) and in the presence of 3×10^{-9} M vasopressin (○) or vasopressin plus the vasopressin V_1 receptor antagonist $d(CH_2)_5Tyr(Me)$vasopressin (□, 10^{-6} M) ($n = 5$). Contractions elicited by $CaCl_2$ in the presence of vasopressin (○) are given as the maximum height of repetitive phasic contractions whereas other measurements (●, □) are of tonic tension. Values are shown as means ± S.E.M.

In rings incubated in Ca^{2+}-free solution containing 30 mM KCl, addition of $CaCl_2$ (10^{-5}–3×10^{-3} M) elicited concentration-dependent tonic contractions of low magnitude (Fig. 7). In the presence of subcontractile concentrations of vasopressin (10^{-9} M), $CaCl_2$ induced repetitive phasic contractions which were maximal at 3×10^{-3} M; the addition of higher concentrations of $CaCl_2$ (6×10^{-3} and 10^{-2} M) induced concentration-dependent inhibition of the contraction. The vasopressin V_1 receptor antagonist (10^{-6} M) abolished the contractions induced by $CaCl_2$ in the presence of vasopressin.

4. Discussion

The results of the present study show that low concentrations of vasopressin enhance the contractile effects of electrical stimulation, noradrenaline and KCl depolariza-

tion. The potentiating effects occur at vasopressin concentrations substantially lower than those required to produce a clear direct contractile response. In the presence of 10^{-8} M vasopressin, a concentration which did not elicit contraction, the response to electrical stimulation increased 75% and there was a 55-fold leftward shift of the control concentration–response curve for noradrenaline at the EC_{50} level.

Previous studies have shown that vasopressin may induce relaxation of vascular smooth muscle due to vasopressin V_2 receptor stimulation (Hirsch et al., 1989; Martínez et al., 1994; Tagawa et al., 1995). Thus we examined the potential role of vasopressin V_2 receptor stimulation in the enhancing effects of vasopressin. The results do not support the involvement of vasopressin V_2 receptors in these responses. First, the selective vasopressin V_2 agonist desmopressin did not modify the responses to electrical field stimulation. However, the vasopressin V_2 receptor antagonist [d(CH_2)$_5$, D-Ile^2,Ile^4,Arg^8]vasopressin did not affect the potentiation induced by vasopressin but attenuated the contractile effects of vasopressin, thus confirming previous results indicating that all vasopressin V_2 receptor antagonists have a vasopressin V_1 receptor antagonistic effect as well (Sawyer and Manning, 1985; Kinter et al., 1993; Burrell et al., 1994). Our results show that the selective vasopressin V_1 receptor antagonist d(CH_2)$_5$Tyr(Me)vasopressin inhibited the potentiating effects of vasopressin on electrical field stimulation and noradrenaline-induced contractions in a concentration-dependent manner. In addition, the selective vasopressin V_1 receptor agonist [Phe^2,Orn^8]vasotocin induced potentiating effects similar to those observed in the presence of vasopressin. Therefore the results exclude a role for vasopressin V_2 receptors in the potentiating effects of vasopressin and are consistent with the hypothesis that V_1 receptor stimulation by vasopressin in the absence of direct contraction is followed by enhancement of responses to both endogenous and exogenous noradrenaline.

The mechanism of the increased responses by vasopressin is not readily apparent. Because noradrenaline release was not measured in this study, a contribution of presynaptic facilitating effects induced by vasopressin cannot be excluded. In agreement with previous findings (Andersson et al., 1988), the concentration–response curve for vasopressin was not modified by prazosin, thus suggesting that the action of this peptide does not involve the release of noradrenaline. The possibility that vasopressin could block the reuptake of noradrenaline and therefore enhance the contractile response is unlikely since the potentiating effects were still evident in the presence of cocaine. Alternatively, vasopressin-induced potentiation could be due to alterations at the receptor level leading to an increased affinity of noradrenaline for its receptor. This is a likely explanation, because vasopressin increased the contractions elicited by exogenously applied noradrenaline. However, this mechanism cannot explain the potentiation by vasopressin of KCl-induced contractions. Thus the potentiating effects of vasopressin are not restricted to events triggered by one specific receptor, but seem to reflect a general modification of the contractile function of smooth muscle.

We considered the possibility that stimulation of vasopressin V_1 receptors may facilitate Ca^{2+} entry through dihydropyridine Ca^{2+} channels. Our results show that nifedipine did not affect the direct effect of vasopressin or prevent the potentiating action of vasopressin on noradrenaline- and electrical field stimulation-induced contractions. This indicates that the influx of extracellular Ca^{2+} through dihydropyridine-sensitive Ca^{2+} channels does not contribute in a substantial way to the direct contractile effects of vasopressin or participate in the potentiating effect of vasopressin on adrenergic contractions. The potentiating effect could be mediated by an increase in inositol phosphate metabolism and intracellular Ca^{2+} (Michell et al., 1979; Thibonnier et al., 1991). Vasopressin V_1 receptor stimulation also induces rapid activation of protein kinase C (Thibonnier, 1992; Kribben et al., 1993), which has been reported to be involved in smooth muscle contraction (for review see Horowitz et al., 1996). It is important to note that our observations are for circular muscle. There is evidence suggesting differences in the role of extracellular and intracellular Ca^{2+} mechanisms during activation of longitudinal or circular muscle of the human vas deferens (Amobi and Smith, 1993).

In addition, our results show that low concentrations of vasopressin induced phasic responses in the presence of increasing concentrations of KCl, an effect completely reversed by vasopressin V_1 receptor blockade. This suggests that Ca^{2+} entry through voltage-dependent channels during KCl depolarization enhances the phasic contractile effects of vasopressin. Confirming previous observations for arterial smooth muscle (Godfraind, 1983), we observed that high Ca^{2+} concentrations inhibited contractions of vas deferens depolarized by 30 mM KCl. This inhibitory effect of Ca^{2+} has been interpreted as being due to a decrease in Ca^{2+} entry at high Ca^{2+} concentrations (Godfraind and Kaba, 1969; Van Breemen et al., 1981).

In conclusion, the results of the present study demonstrate that vasopressin, in addition to its direct contractile effect, strongly potentiates the contractions of human vas deferens produced by noradrenaline, stimulation of sympathetic nerves and KCl. Both the direct and indirect effects of vasopressin appear to be mediated by vasopressin V_1 receptor stimulation.

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