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Vasopressin-induced contraction in the rat basilar artery in vitro

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Abstract

Vasopressin ([Arg^8]vasopressin)-induced contraction was characterized using receptor agonists and antagonists for vasopressin and channel blockers in the rat basilar artery ring preparations. Vasopressin induced rhythmic contractions superimposed on a contraction in endothelium-intact preparations but not in denuded ones. Endothelium removal shifted the concentration–response curve for vasopressin leftward and upward. In endothelium-denuded preparations, vasopressin V_1 receptor antagonist shifted the concentration–response curve for vasopressin downward and rightward. Vasopressin V_1 receptor agonist caused contraction but V_2 receptor agonist did not. The contractile response to vasopressin was partly inhibited by nifedipine, SK&F 96365 (1-[β-[3-(4-methoxyphenyl)propoxy]-4-methoxyphenethyl]-1*H*-imidazole) and niflumic acid. In the absence of extracellular Ca^{2+}, vasopressin produced a transient contraction. Charybdotoxin produced an upward and leftward shift of the concentration–response curve for vasopressin. These results suggest that vasopressin elicits contraction due to Ca^{2+} influx through voltage-dependent and receptor-operated Ca^{2+} channels and to Ca^{2+} release from Ca^{2+} stores by activating vasopressin V_1 receptors in the rat basilar artery.

Keywords: Vasopressin; Vasopressin V_1 receptor; Basilar artery, rat; Endothelium; Nifedipine

1. Introduction

Vasopressin ([Arg^8]vasopressin), a neuropeptide consisting of nine amino acids, is well known to cause vasoconstriction in a variety of vascular regions. There are two types of vasopressin receptors (V_1 and V_2 receptors). Vasopressin V_2 receptors present in renal collecting duct cells are mainly responsible for antidiuretic effects through cAMP formation, whereas vasopressin V_1 receptors, present in blood vessels, are responsible for vasoconstriction through inositol-trisphosphate (IP_3) formation and thus Ca^{2+} mobilization from Ca^{2+} stores (Doyle and Rüegg, 1985).

Increased vasopressin in brain circulation may play a pathophysiological role in producing spasms of the brain arteries and thus brain ischemia, because vasopressin concentrations in the cerebrospinal fluid and blood are increased by brain hemorrhage (Cameron et al., 1984) and subarachnoid hemorrhage (Mather et al., 1981). In the canine basilar artery, vasopressin has been reported to elicit endothelium-dependent relaxation through vasopressin V_1 receptor activation (Katušić et al., 1984; Suzuki et al., 1992), which releases nitric oxide from the endothelium (Cosentino et al., 1993; Oyama et al., 1993). This mechanism of relaxation has been shown to be abolished by subarachnoid hemorrhage (Kim et al., 1989; Katušić et al., 1993). It has been reported that vasopressin elicits contraction in the human middle cerebral artery (Onoue et al., 1994) through vasopressin V_1 receptors (Martín de Aguilera et al., 1990) and in rabbit basilar artery (García-Villalón et al., 1996).

Intracarotid administration of vasopressin or vasopressin V_2 agonist is shown to increase cerebral blood flow with decreased cerebral vascular resistance in the rat (Koźniewska and Szczepańska-Sadowska, 1990). However, it has been reported that topical suffusion of vasopressin decreases the diameter of the rat pial arterioles and basilar artery without vasodilation (Faraci, 1989), and that the contractile effect is mediated by vasopressin V_1 receptors in the basilar artery (Murray et al., 1992). In an in vitro study of isolated and cannulated middle cerebral arteries of the rat, it has been reported that increasing the concentration of vasopressin induces a triphasic response of vasodilation, vasoconstriction and return to the control diameter, and that these responses are mediated by vasopressin V_1 receptors (Takayasu et al., 1993). Moreover, vasopressin induces rhythmic contractions in the rat basilar

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artery in vivo (Fujii et al., 1990) and in vitro (Rusch and Hermsmeyer, 1985). It is, therefore, of interest to quantitatively determine the effects of vasopressin on tension in the endothelium-intact and denuded basilar artery of the rat.

In cultured smooth muscle cells from the aorta and aortic smooth muscle cell line A7r5, it has been reported that vasopressin causes both increases in Ca^{2+} influx and Ca^{2+} release from Ca^{2+} stores (Capponi et al., 1985; Doyle and Rüegg, 1985; Knot et al., 1991), and that Ca^{2+} influx is mediated through receptor-operated channels (Capponi et al., 1985; Wallnöfer et al., 1987). Vasopressin has been shown to activate Ca^{2+}-permeable non-selective cation channels (receptor-operated channels) in A7r5 cells (Nakajima et al., 1996). In the rat basilar artery, it remains obscure whether vasopressin exhibits Ca^{2+} mobilization from the stores and /or Ca^{2+} influx because of a lack of functional and pharmacological studies in vitro. If vasopressin activates receptor-operated channels like non-selective cation channels, it should produce membrane depolarization, activating voltage-dependent Ca^{2+} channels. it has also been reported that arterial smooth muscle cells have Ca^{2+}-activated Cl^- channels, the activation of which produces depolarization leading to the opening of voltage-dependent Ca^{2+} channels to produce contraction (Criddle et al., 1996). Thus, it is worth studying the mechanism of contraction induced by vasopressin using various channel blockers in the rat basilar artery.

The present experiments were designed to determine receptor subtypes responsible for vasopressin-induced constriction using specific receptor agonists and antagonists and the involvement of endothelium in the effect of vasopressin in rat basilar artery ring preparations. Furthermore, we also examined the effects of various blockers such as nifedipine (a voltage-dependent L-type Ca^{2+} channel blocker), SK&F 96365 (a receptor-operated channel blocker, 1-[β-[3-(4-methoxyphenyl)propoxy]-4-methoxyphenethyl]-1*H*-imidazole), niflumic acid (a Ca^{2+}-activated Cl^- channel blocker), apamin and charybdotoxin (Ca^{2+}-activated K^+ channel blockers) on vasopressin-induced contraction to determine the mechanism of contraction induced by vasopressin in the rat basilar artery.

2. Materials and methods

2.1. Tissue preparation and experimental setup

All experiments were carried out under the regulations of the Animal Research Committee of the Graduate School of Veterinary Medicine. The experiments were performed on isolated basilar arteries of male Wistar rats (250–300 g) that were killed by a blow to the head and exsanguination. The basilar artery was removed together with the brain and placed in modified Krebs solution gassed with 95%O_2 and 5%CO_2. The basilar artery was carefully freed from surrounding tissues with the help of a binocular stereomicroscope and then ring preparations 200 μm in length were prepared. The rat basilar artery was about 200 μm in diameter under the resting condition. The preparation was mounted in an organ bath (1.5 ml) maintained at 37°C with hot water circulated under the bath (Fig. 1). Two fine tungstic wires were passed through the lumen of the artery. The tip of the tungstic wires was 30 μm in diameter. The wire was fixed to a strain gauge (BG10, Kulite Semiconductor Products, USA), and the other was fixed to a micromanipulator. Great care was taken not to injure the endothelium. Isometric contraction was measured continuously with the strain gauge, the output of which was amplified (DSA-601B, Strain meter, Minebia, Japan) and displayed on a chart recorder (Recti-Hori-8K, Sanei, Japan) and a hard disk of a personal computer through an interface (MacLab/2e, AD Instruments, Australia).

2.2. Experimental protocol

Each preparation was allowed to equilibrate for a period of about 10 min under a resting tension of 30 mg, under which 80-mM KCl caused a maximal contraction. KCl (80 mM) was repeatedly applied to the ring preparation for 2 min until the contraction became constant. The amplitude of the contraction induced by 80-mM KCl varied from preparation to preparation, so that the amplitude of the contraction induced by vasopressin was expressed as a percentage of that of the maximal 80-mM KCl-induced contraction. In the endothelium-denuded preparations, a hair was passed into the arterial lumen to rub its inner wall before making ring preparations. To test whether the endothelium was intact, acetylcholine (1 μM) was applied to all preparations precontracted with 5-hydroxytryptamine (0.1 μM) before the start of the experiments. The ring preparations in which acetylcholine relaxed to less than 50% of the precontraction induced by 5-hydroxytryp-

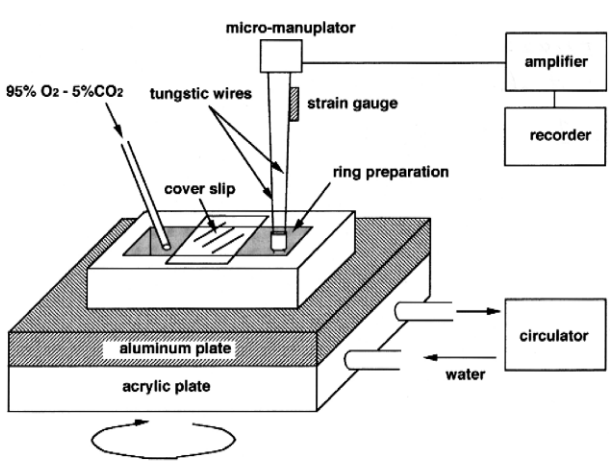

Fig. 1. Experimental setup for tension recording in a ring preparation of the rat basilar artery.

tamine were considered to be endothelium-intact arteries, and those in which acetylcholine failed to produce relaxation, to be endothelium-denuded ones.

Tissues were maintained in modified Krebs solution of the following composition (mM), NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11.1 and HEPES 10. The solution was gassed with 5% CO_2 in O_2 and had a pH of 7.4 at 37°C. In 80-mM KCl solution, NaCl was decreased to 42.7 mM. In Ca^{2+}-free solution, $CaCl_2$ was omitted and 2-mM EGTA was added. Five milliliters of Ca^{2+}-free solution or 80-mM KCl-solution were applied to the organ bath and the overflowing solution was aspirated with suction. For construction of concentration–response curves for vasopressin and its analogues, they were added to the organ bath cumulatively. Drugs at 100-fold concentration were directly applied to the bathing solution mixed with a small magnetic stirrer. The bathing solution containing drugs was washed out five times with 1.5 ml of modified Krebs solution. Receptor antagonists or various channel blockers were applied 5 min before application of vasopressin or its analogues. This time was enough to reach equilibrium condition in vascular preparations.

2.3. Chemicals

Materials used were: apamin, charybdotoxin, desmopressin ([deamino-Cys^1, D-Arg^8]vasopressin), [d(CH$_2$)$^1_5$, Tyr^2(Me)]vasopressin ([β-mercapto-β,β-cyclopentamethylene-propionyl^1, O-Me-Tyr^2, Arg^8]vasopressin), 5-hydroxytryptamine, niflumic acid and uridine 5′-triphosphate (UTP), all from Sigma (USA). Acetylcholine was purchased from Daiichi Pharmaceutical (Japan), vasopressin ([Arg^8]vasopressin) was from Peptide Ins., (Japan), [Phe^2, Orn^8]vasotocin ([Phe^2, Ile^3, Orn^8]vasopressin) from Peninsula Lab. (USA), [d(CH$_2$)$^1_5$, D-Ile^2, Ile^4]vasopressin ([β-mercapto-β,β-cyclopentamethylene-propionyl^1, D-Ile^2, Ile^4, Arg^8]vasopressin) from Bachem (Switzerland), nifedipine from Wako (Japan) and SK&F 96365 from Biomol Res. Lab. (USA).

2.4. Statistical analysis

All results are expressed as the means ± S.E.M. Statistical significance was estimated by paired and non-paired Student's *t*-tests or by the Tukey–Kramer test after analysis of variance (ANOVA). *P* values of less than 0.05 were considered to be statistically significant.

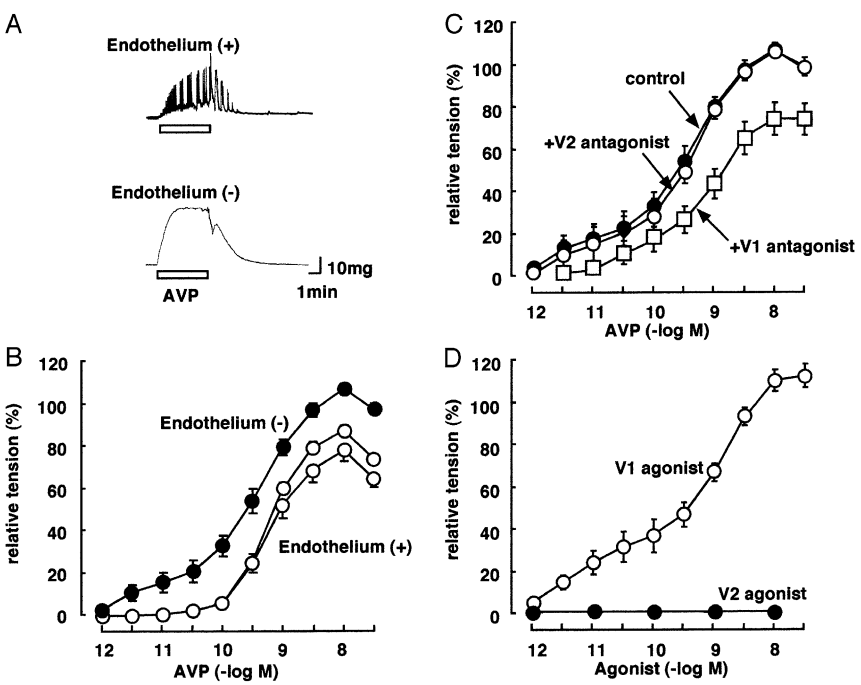

Fig. 2. Effects of vasopressin, vasopressin V_1 and V_2 receptor antagonists, and agonists in the basilar artery of the rat. (A) Representative trace showing vasopressin (AVP)-induced contraction in the endothelium-intact (+) and denuded (−) ring preparations. (B) Concentration–response curves for vasopressin were constructed by the cumulative application of vasopressin in the presence (○, $n = 6$) and absence (●, $n = 13$) of the endothelium. The ordinate scale shows the relative tension (%) of vasopressin-induced contraction to the peak amplitude of 80-mM KCl-induced contraction. The abscissa scale shows the vasopressin concentration on a log scale. In the endothelium-denuded preparation, upper and lower curves (○) indicate the peak and bottom tensions of rhythmic activities, respectively. (C) The effects of vasopressin V_1 receptor antagonist, [d(CH$_2$)$^1_5$, Tyr^2 (Me)]vasopressin (1 nM, □, $n = 7$), and V_2 receptor antagonist, [d(CH$_2$)$^1_5$, D-Ile^2, Ile^4]vasopressin (1 nM, ○, $n = 5$), on contraction induced by the cumulative application of vasopressin in the endothelium-denuded preparation. The control concentration–response curve (●) was transferred from (B). (D) Concentration–response curves for vasopressin V_1 receptor agonist, [Phe^2, Orn^8]vasotocin (○, $n = 5$), and V_2 receptor agonist, desmopressin (●, $n = 4$), were constructed by the cumulative application of these agonists. The ordinate scale shows the peak tension (%) of contraction. The abscissa scale shows vasopressin or receptor agonist concentration on a log scale.

3. Results

3.1. Contractile response to vasopressin

Vasopressin (1 nM) elicited contraction regardless of the presence or absence of the endothelium (Fig. 2A). Excess KCl also caused contraction in the presence and absence of the endothelium. Vasopressin produced a sustained contraction, on which regular and rhythmic contractions were superimposed in 35 out of 49 ring preparations with endothelium. The peak and bottom tensions of the oscillatory contractile response to vasopressin were 29.4 ± 2.9 mg and 15.5 ± 2.6 mg ($n = 35$), respectively. The remainder elicited a sustained contraction without rhythmic contractions. The amplitude of the maximal contraction was 38.2 ± 6.2 mg ($n = 14$), which was not significantly different from the peak amplitude of the oscillatory response. In the endothelium-denuded ring artery, the maximal amplitude of vasopressin-induced sustained contraction was 39.7 ± 3.8 mg ($n = 30$). In some cases, spontaneous and transient relaxations, which returned to the original tone, occurred during the contraction.

Excess KCl (80 mM) for 2 min elicited a biphasic contraction with a transient peak followed by a sustained contraction. The peak amplitude of contractile responses to 80-mM KCl in ring preparations with endothelium (67.9 ± 4.2 mg, $n = 49$, $p < 0.05$) was significantly greater than that without endothelium (51.0 ± 4.9 mg, $n = 30$). The removal of the endothelium appeared to decrease the contractile activity of basilar arterial smooth muscles. In the following experiments, therefore, contractile responses to vasopressin were expressed as percentages of the peak tension induced by 80-mM KCl, which was obtained at the beginning of all experiments. Vasopressin was cumulatively applied to the basilar artery at 3-min intervals by increasing concentrations from 1 pM to 30 nM. Vasopressin elicited a concentration-dependent increase in contraction with regular and oscillatory contractions in six out of nine preparations with endothelium. The concentration–response curves for vasopressin in the basilar artery with and without endothelium are shown in Fig. 2B. Although vasopressin elicited contraction at lower concentrations in the endothelium-denuded arterial ring than in the endothelium-intact one, the contraction reached a maximum at 10 nM in both preparations. However, the maximal tension was larger in the endothelium-denuded ring than in the endothelium-intact one. The mean EC_{50} value for vasopressin was smaller in the endothelium-denuded preparation (0.30 ± 0.08 nM, $n = 13$, $p < 0.05$) than in the endothelium-intact one (0.54 ± 0.09 nM, $n = 9$).

3.2. The effects of vasopressin receptor antagonists and agonists

The effects of a selective vasopressin V_1 receptor antagonist, [d(CH_2)$^1_5$, Tyr^2(Me)]vasopressin (1 nM) and V_2 receptor antagonist, [d(CH_2)$^1_5$, D-Ile^2, Ile^4]vasopressin (1 nM) on vasopressin-induced contraction were examined in the endothelium-denuded arterial ring (Fig. 2C). These antagonists had no effect on resting tension. Vasopressin V_1 receptor antagonist reduced the maximal contraction induced by vasopressin to $73.6 \pm 7.7\%$ of the maximal 80-mM KCl-induced contraction ($n = 7$, $p < 0.01$) and increased mean EC_{50} value for vasopressin to 0.84 ± 0.15 nM ($p < 0.05$). Vasopressin V_2 receptor antagonist did not have any effect on either parameter ($104.9 \pm 2.1\%$ of the 80-mM KCl-induced contraction, 0.32 ± 0.10 nM, $n = 5$).

The effects of a selective vasopressin V_1 receptor agonist, [Phe^2, Orn^8]vasotocin, and V_2 receptor agonist, desmopressin, on tension were also examined in endothelium-denuded basilar arteries. The V_1 receptor agonist caused a concentration-dependent contraction (Fig. 2D). The maximal contraction induced by the V_1 receptor agonist was similar in magnitude ($112.9 \pm 2.1\%$ of the 80 mM KCl-induced contraction, $n = 5$) to that by vasopressin.

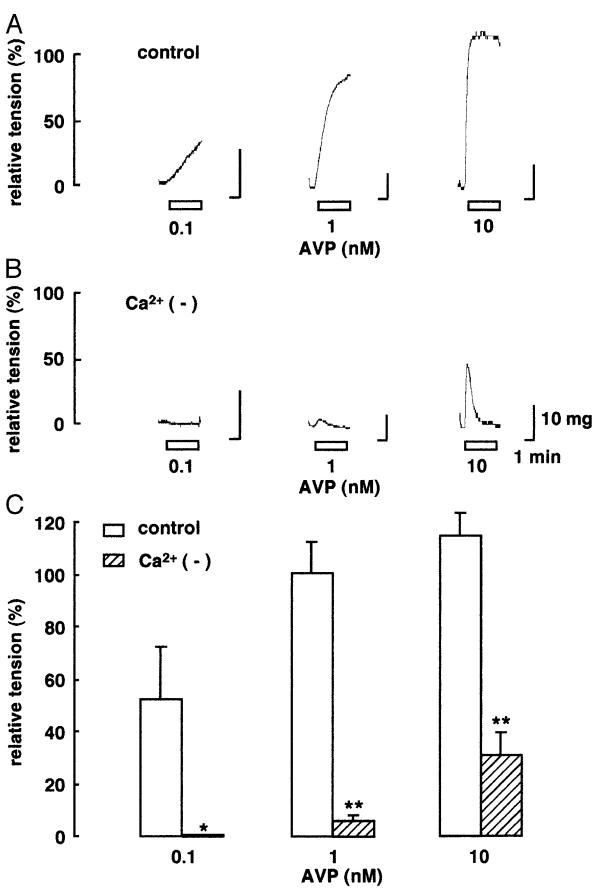

Fig. 3. Effect of extracellular Ca^{2+} removal on AVP-induced contraction. Typical traces showing contractions induced by AVP at concentrations of 0.1 ($n = 3$), 1 ($n = 6$) and 10 nM ($n = 4$) in the presence (A) and absence (B) of extracellular Ca^{2+}. (C) Columns show the relative peak tension induced by vasopressin (% of 80-mM KCl-induced contraction) at various concentrations (0.1 nM, $n = 3$; 1 nM, $n = 6$; 10 nM, $n = 4$) in the presence (open) and absence (hatched) of extracellular Ca^{2+}. $^{*}P < 0.05$; $^{**}P < 0.01$ compared with control responses.

The mean EC_{50} value for the V_1 receptor agonist was 0.95 ± 0.32 nM, $n = 5$). The V_2 receptor agonist had no effect on tension development ($n = 4$).

3.3. The effects of Ca^{2+} removal and nifedipine on vasopressin-induced contraction

The effect of Ca^{2+} removal on contraction induced by vasopressin was examined in the endothelium-denuded arterial ring. Vasopressin at 0.1 nM caused a contraction that was abolished by removal of extracellular Ca^{2+}. However, increasing the concentration of vasopressin to 10 nM elicited a transient contraction 30 s after removal of extracellular Ca^{2+} (Fig. 3).

The effect of an L-type Ca^{2+} channel blocker, nifedipine, on vasopressin-induced contraction was studied in the denuded basilar artery of the endothelium. Nifedipine caused a concentration-dependent inhibition of contractions induced by 1 nM vasopressin (Fig. 4A,B). Maximal inhibition was obtained with 0.1-μM nifedipine. In the presence of this concentration of nifedipine, vasopressin elicited a concentration-dependent contraction but nifedipine caused a downward and rightward shift of the concentration–response curve for vasopressin (Fig. 4C). Therefore, the mean EC_{50} value was increased to 1.14 ± 0.06 nM ($n = 6$, $P < 0.05$) and the maximal contraction was significantly decreased to $81.2 \pm 9.0\%$ of the 80-mM KCl-induced contraction. The percentage of inhibition by nifedipine was estimated at each concentration of vasopressin and plotted against a log concentration of vasopressin (Fig. 4D). The contractile responses to low concentrations of vasopressin were abolished by nifedipine. However, increasing the concentrations of vasopressin resulted in decreases in the inhibitory effect of nifedipine.

3.4. The effects of SK&F 96365 and niflumic acid on vasopressin-induced contraction

SK&F 96365, an inhibitor of receptor-operated channels, has been reported to inhibit voltage-dependent Ca^{2+} channels (Merritt et al., 1990). In the present experiments, we first tested the specificity of this compound using 80-mM KCl-induced contraction in the endothelium-denuded basilar artery. SK&F 96365 inhibited the 80-mM KCl-induced contraction at concentrations ranging from 10 to 100 μM. Therefore, we examined the effect of SK&F 96365 on vasopressin-induced contraction in the presence of nifedipine, by which L-type Ca^{2+} channels had already been blocked. Vasopressin-induced contraction was suppressed to about 40% of 80-mM K-induced contraction by

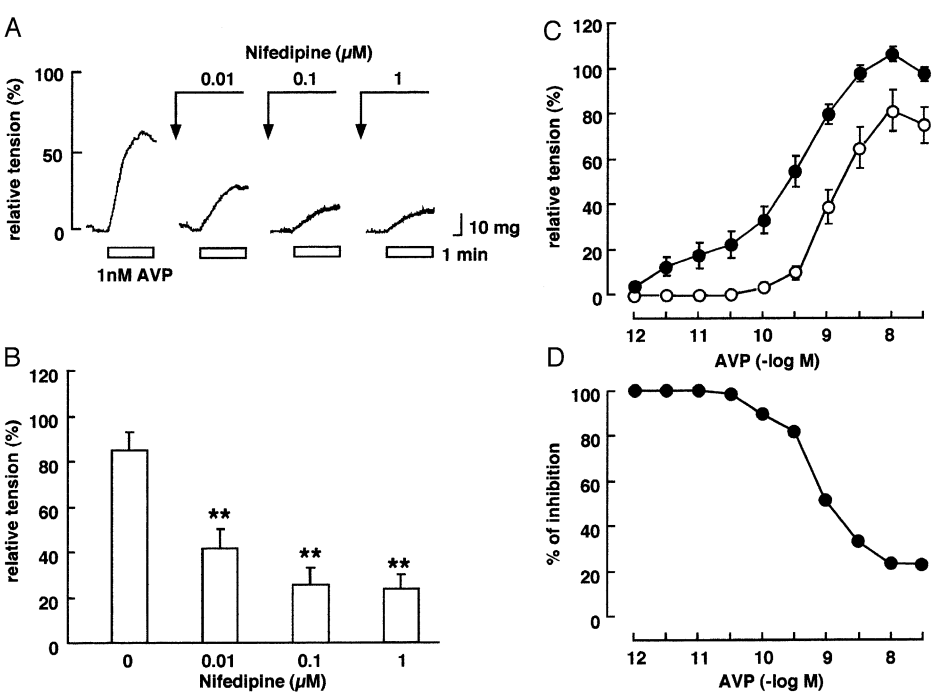

Fig. 4. Effects of nifedipine on AVP-evoked contraction in the endothelium-denuded preparation. (A) Representative traces showing the inhibitory effects of nifedipine (0.01, 0.1 and 1 μM) on the contraction of rat basilar artery induced by 1-nM vasopressin (AVP). (B) Columns indicate the relative peak contractile responses to 1-nM vasopressin (% of 80-mM KCl-induced contraction) in the presence of nifedipine (0.01, 0.1 and 1 μM) and its absence (control) ($n = 6$). (C) Concentration–responses curves for vasopressin were constructed by the cumulative application of vasopressin in the presence of 0.1 μM nifedipine (○, $n = 6$). The control concentration–response curve (●) was transferred from Fig. 2B. The ordinate scale shows the relative peak tension of vasopressin-induced contraction (% of 80-mM KCl-induced contraction). The abscissa scale shows vasopressin concentration on a log scale. (D) The percent inhibition of contraction induced by various concentrations of vasopressin by 0.1-μM nifedipine. $^{**}P < 0.01$ compared with the absence of nifedipine.

0.1-μM nifedipine. Further application of SK&F 96365 produced a concentration-dependent inhibition of the contractile response to vasopressin (Fig. 5A,B).

In the rat aorta, activation of Ca^{2+}-activated Cl^- channels has been reported to lead to the opening of voltage-dependent Ca^{2+} channels to produce contraction (Criddle et al., 1996). The effects of niflumic acid, a Ca^{2+}-activated Cl^- channel blocker, on vasopressin-induced contractions were examined in the endothelium-denuded arterial ring (Fig. 5C,D). Niflumic acid partly inhibited contractile responses to vasopressin. The rate of tension development, at which contraction attained a maximum, and maximal tension were decreased by niflumic acid in a concentration-dependent manner ($n = 4$). However, niflumic acid, even at 10 μM, failed to abolish vasopressin-induced contraction.

3.5. The effects of apamin and charybdotoxin on vasopressin-induced contraction

The effects of apamin, an SK^+ channel inhibitor, and charybdotoxin, a BK^+ channel inhibitor, on vasopressin-induced contraction were examined in the endothelium-denuded basilar artery (Fig. 6). Charybdotoxin at 10 nM for 5 min slightly increased tension, being less than 5% of 80-mM KCl-induced contraction, and it increased the maximal amplitude of contraction induced by vasopressin to 132.6 ± 9.4% ($n = 9$) of 80-mM KCl-induced contraction (110.0 ± 3.3% for the control, $n = 13$). Spontaneous and transient relaxations superimposed on the sustained contraction induced by vasopressin disappeared with this toxin. An EC_{50} value was decreased to 0.2 ± 0.11 nM by charybdotoxin (0.27 ± 0.06 nM for the control). Apamin at 0.1 μM had neither effect on the resting tension nor the concentration–response curve for vasopressin ($n = 4$). Apamin failed to affect spontaneous and transient relaxations superimposed on the sustained contraction induced by vasopressin.

3.6. Endothelium-dependent relaxation by vasopressin

In endothelium-intact preparations, vasopressin produced regular and rhythmic contractions superimposed on a sustained contraction. In the endothelium-denuded ring, however, vasopressin elicited a sustained contraction without regular and rhythmic contractions. In some cases, transient relaxations, which returned to the original tone, sometimes occurred during the sustained contraction in response to vasopressin. Therefore, endothelium-dependent relaxation by vasopressin was investigated in the basilar artery precontracted by UTP at 10 μM. Acetylcholine at 1 μM elicited rapid relaxation, but vasopressin, vasopressin

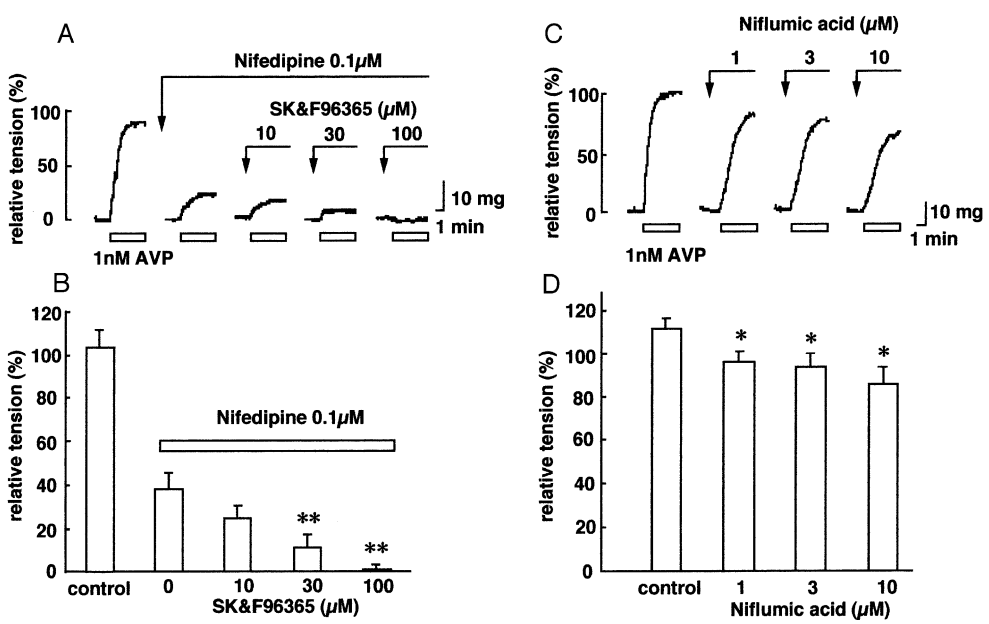

Fig. 5. Effects of SK&F 96365 and niflumic acid on vasopressin-evoked contraction in the endothelium-denuded preparation. (A) Representative traces showing the inhibitory effect of SK&F 96365 (10, 30 and 100 μM) on the contraction of rat basilar artery induced by 1-nM vasopressin in the presence of nifedipine (0.1 μM). (B) Columns represent the relative peak contractile responses to 1-nM vasopressin (% of 80-mM KCl-induced contraction) in the presence of SK&F 96365 (10, 30 and 100 μM) and nifedipine (0.1 μM), in the presence of nifedipine (0.1 μM) but not SK&F 96365 in the absence of both drugs (control) ($n = 3$). (C) Representative traces showing the inhibitory effects of niflumic acid (1, 3 and 10 μM) on the contraction of rat basilar artery induced by 1-nM vasopressin. (D) Columns indicate the relative peak contractile responses to 1-nM vasopressin (% 80-mM KCl-induced contraction) in the presence of niflumic acid (1, 3 and 10 μM) and its absence (control) ($n = 4$). $^*P < 0.05$ compared with the absence of niflumic acid. $^{**}P < 0.01$ compared with the absence of SK&F 96365 (in the presence of nifedipine).

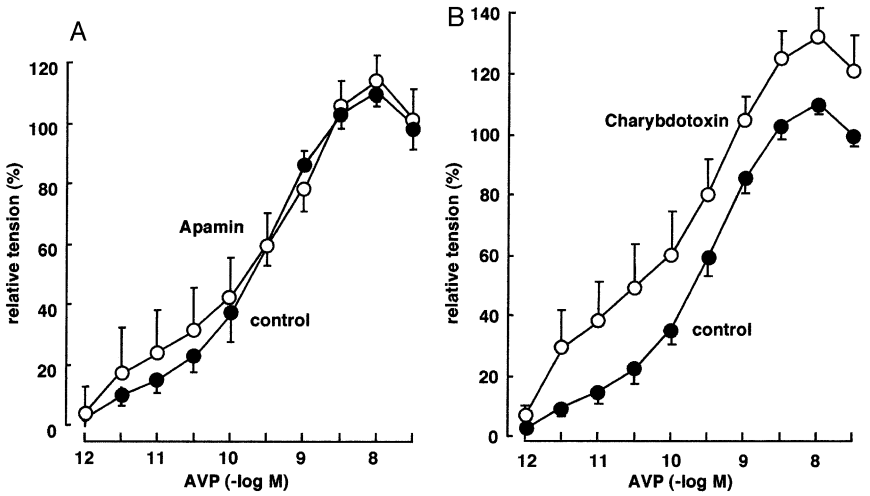

Fig. 6. Effects of apamin and charybdotoxin on vasopressin-evoked contraction in the endothelium-denuded preparation. Concentration–response curves for vasopressin were constructed by the cumulative application of vasopressin in the presence of 0.1-μM apamin (A, $n = 4$) and 0.01 μM charybdotoxin (B, $n = 9$). The control concentration–response curves (●) were transferred from Fig. 2B. The ordinate scale shows the relative peak tension of vasopressin-induced contraction (% of 80-mM KCl-induced contraction). The abscissa scale shows the vasopressin concentration on a log scale.

V_1 and V_2 receptor agonists at concentrations up to 1 nM did not. Vasopressin and V_1 receptor agonist produced contraction under this condition.

4. Discussion

Vasopressin has been reported to cause contraction via the activation of vasopressin V_1 receptors in the basilar artery of the rat (Rusch and Hermsmeyer, 1985; Faraci, 1989), rabbit (García-Villalón et al., 1996) and human (Martín de Aguilera et al., 1990). This was confirmed in the present experiments, in which vasopressin V_1, but not V_2 receptor antagonist inhibited the vasopressin-induced contraction, and vasopressin V_1, but not V_2 receptor agonist elicited contraction regardless of the presence or absence of the endothelium, indicating that the contractile effect of vasopressin was attributable to vasopressin V_1 receptor activation on the smooth muscle cells of the rat basilar artery.

In the A7r5 smooth muscle cell line, vasopressin has been reported to produce IP_3 formation resulting in an increase in intracellular Ca^{2+} by Ca^{2+} release from intracellular Ca^{2+} stores (Doyle and Rüegg, 1985). In the present study, high concentrations of vasopressin elicited a transient contraction in the absence of extracellular Ca^{2+}, suggesting that vasopressin elicits Ca^{2+} release from the stores. It may be possible that contraction observed in the absence of extracellular Ca^{2+} is due to Ca^{2+}-independent mechanism such as G protein-dependent (Somlyo and Somlyo, 2000) or protein kinase C-dependent Ca^{2+} sensitization (Morgan and Leinweber, 1998). It is reported that vasopressin increases intracellular Ca^{2+} via Ca^{2+} influx insensitive to dihydropyridine derivatives in the A7r5 cell line and cultured rat arterial smooth muscle cells and suggested that receptor-operated Ca^{2+} channels play an important role in vasopressin-induced contraction but not voltage-dependent Ca^{2+} channels (Capponi et al., 1985; Wallnöfer et al., 1987; Nakajima et al., 1996). In the rat basilar artery, nifedipine abolished contractions induced by low concentrations of vasopressin but not contractions induced by high concentrations. The nifedipine-resistant contraction induced by vasopressin was completely abolished by SK&F 96365, a receptor-operated Ca^{2+} channel inhibitor. These results suggest the involvement of receptor-operated Ca^{2+} channels in vasopressin V_1 receptor stimulation in the rat basilar artery. The receptor-operated Ca^{2+} channels are considered to be non-selective cation channels associated with inward currents in smooth muscle cells (Zholos et al., 2000). Therefore, the activation of this channel may cause Ca^{2+} influx and depolarization resulting in further increases in Ca^{2+} influx through L-type voltage-dependent Ca^{2+} channels.

It has been reported that there are Ca^{2+}-activated Cl^- channels on arterial smooth muscles that are activated by noradrenaline and that the activation of Ca^{2+}-activated Cl^- channels leads to the opening of voltage-dependent Ca^{2+} channels (Criddle et al., 1996). Vasopressin has been reported to activate Ca^{2+}-activated Cl^- channels in the A7r5 smooth muscle cell line (Van Renterghem and Lazdunski, 1993). In the rat basilar artery, vasopressin-induced contraction was partly inhibited by niflumic acid, a Ca^{2+}-activated Cl^- channel inhibitor. If vasopressin-induced depolarization was only due to the activation of Ca^{2+}-activated Cl^- channels, niflumic acid would inhibit vasopressin-induced contraction to the same extent as nifedipine did. However, the inhibitory effect of nifedipine (about 60%) on contraction induced by vasopressin at 1 nM was much greater than that of nifulmic acid (about 20%). These results suggest that the activation of Ca^{2+}-activated Cl^-

channels producing depolarization is only partly involved in the contractile effect of vasopressin.

In some preparations, spontaneous and transient relaxation occurred during sustained contraction induced by vasopressin in endothelium-denuded arterial rings. Vasopressin has been reported to activate Ca^{2+}-activated K^+ channels because of an increase in intracellular Ca^{2+} in the A7r5 smooth muscle cell line (Knot et al., 1991) and porcine coronary artery (Wakatsuki et al., 1992). Charybdotoxin caused an upward and leftward shift of the concentration–response curve for vasopressin and inhibited spontaneous and transient relaxations during sustained contraction. Although charybdotoxin blocks KV1.3 channels in addition to high-conductance Ca^{2+}-activated K^+ channels (Garcia et al., 1995), it seems likely that vasopressin-induced depolarization is reduced by the activation of Ca^{2+}-activated K^+ channels.

Vasopressin has been reported to elicit endothelium-dependent relaxation in the basilar artery of the dog (Cosentino et al., 1993; Oyama et al., 1993) and rat (Takayasu et al., 1993). In the rat isolated basilar artery with endothelium precontracted by UTP, however, vasopressin and its V_1 receptor agonist failed to produce relaxation. In the endothelium-intact arterial ring, vasopressin has been reported to elicit spontaneous and rhythmic contractions in the rat basilar artery in vivo (Fujii et al., 1990) and in vitro (Rusch and Hermsmeyer, 1985). This was the case in the present experiments, and the spontaneous and rhythmic contractions disappeared in the endothelium-denuded preparation. It seems likely that the endothelium may play an important role in generating rhythmic activity.

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