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Prostanoids regulate proliferation of vascular smooth muscle cells induced by arginine vasopressin

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Abstract

The aim of the present study was to investigate the effect of arginine [Arg^8]vasopressin (vasopressin) on proliferation of vascular smooth muscle cells and the mechanisms underlying the action of vasopressin. To clarify these issues, we used two different types of vascular smooth muscle cells, cultured adult rat aortic smooth muscle cells and A10 cells, a cell line derived from fetal rat aorta. Vasopressin (10^{-8} to 10^{-6} M) significantly stimulated the proliferation of rat aortic smooth muscle cells in a dose-dependent manner. In contrast, vasopressin significantly inhibited the proliferation of A10 cells. This inhibition was abolished when A10 cells were treated with indomethacin. Vasopressin stimulated the production of prostanoids several-fold in A10 cells but not in rat aortic smooth muscle cells. These effects were completely blocked by the vasopressin V_1 receptor antagonist, 1-{1-[4-(3-acetylamino-propoxy)benzoyl]4-piperidyl}-3,4-dihydro-2(1*H*)-quinolinone (OPC21268), but not by the vasopressin V_2 receptor antagonist, (±)-5-dimethylamino-1-[4-(2-methylbenzoylamino)benzol]-2,3,4,5-tetrahydro-1*H*-benzazepine hydrochloride (OPC31260). These results indicate that vasopressin has diverse effect on proliferation of vascular smooth muscle cells through the vasopressin V_1 receptor, depending on the production of growth regulatory prostanoids.

Keywords: [Arg^8]vasopressin; Prostanoid; Smooth muscle cell; Cell proliferation

1. Introduction

Proliferation of vascular smooth muscle cells is thought to play an important role and to be a fundamental step in the development of atherosclerosis (Schwartz, 1983; Ross, 1986). It has been shown that arginine [Arg^8]vasopressin (vasopressin), one of the most potent vasoconstrictors (Altura and Altura, 1977; Stallone et al., 1991), modulates the proliferation of vascular smooth muscle cells (Boswell and Robertson, 1981; Geisterfer and Owens, 1989; Hamada et al., 1990; Turla et al., 1991; Murase et al., 1992). In addition, we have recently reported that vasopressin mRNA is expressed in vascular smooth muscle cells (Nagano et al., 1997). In general, vasoconstrictors are known to stimulate the proliferation of vascular smooth muscle cells. It is, however, still controversial whether vasopressin has a stimulatory or an inhibitory effect on proliferation of vascular smooth muscle cells (Geisterfer and Owens, 1989; Hamada et al., 1990; Murase et al., 1992).

There are several candidates to explain the conflicting results. These include the type of cell (Boswell and Robertson, 1981; Turla et al., 1991), the phase of cultured cell cycle (Murase et al., 1992), and the cell culture medium which contains various growth-influencing factors, such as prostanoids, nitric oxide, cytokines, and other vasoactive substances (Boswell and Robertson, 1981; Geisterfer and Owens, 1989; Hamada et al., 1990). Therefore the aim of this study was to clarify the mechanism underlying the contradictory action of vasopressin on cell proliferation. In the present study, using two types of vascular

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smooth muscle cells, we demonstrated that vasopressin exhibits growth-promoting or -inhibitory effects on vascular smooth muscle cells depending on the production of prostanoids such as prostacyclin.

2. Materials and methods

2.1. Chemicals

Arginine vasopressin, prostaglandin E_2, a thromboxane A_2 stable analogue, 9,11-dideoxy-9α, 11α-methanoepoxy prostaglandin $F_{2\alpha}$ (U-46619) (Crichton et al., 1993), N^G-monomethyl-L-arginine, and kanamycin were purchased from Sigma Chemical (St. Louis, MO). Indomethacin was purchased from Funakoshi Pharmaceutical (Tokyo, Japan). A vasopressin V_1 receptor specific antagonist 1-{1-[4-(3-acetylamino-propoxy)benzoyl]4-piperidyl}-3,4-dihydro-2(1*H*)-quinolinone (OPC21268) and a V_2 receptor specific antagonist (±)-5-dimethylamino-1-[4-(2-methylbenzoylamino)benzol]-2,3,4,5-tetrahydro-1*H*-benzazepine hydrochloride (OPC31260), were obtained from Otsuka Pharmaceutical (Tokyo, Japan). A prostacyclin stable analogue, sodium (±)-(1*R**, 2*R**, 3a*S**, 8b*S**)-2,3,3a,8b-terahydro-2-hydroxy-1[(*E*)-(3*S**)-3-hydroxy-4-methyl-1-ocen-6-ynyl]-1*H*-cyclopenta[*b*]benzofuran-5-butyrate(beraprost sodium) (Hirasawa et al., 1995) was obtained from Yamanouchi Pharmaceutical (Tokyo, Japan). Dulbecco's modified Eagle's medium (DMEM) was purchased from GIBCO (Grand Island, NY), fetal bovine serum was from Immuno-Biological Laboratories (Gunma, Japan) and trichloroacetic acid was from Wako Pure

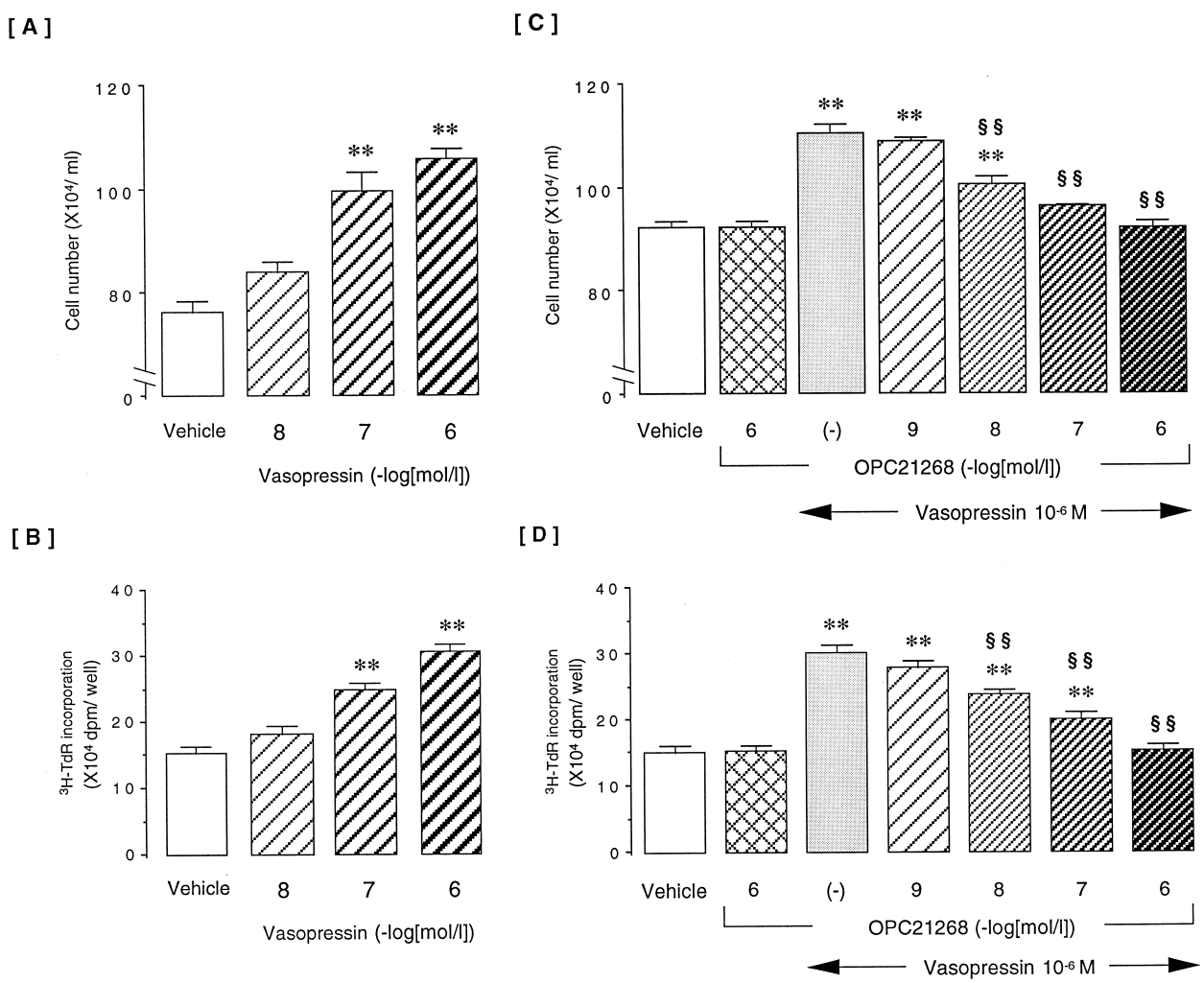

Fig. 1. Dose–response of cell number increase (A) and DNA synthesis (B) of rat aortic smooth muscle cells stimulated by arginine vasopressin, and effect of vasopressin V_1 receptor antagonist OPC21268 on cell proliferation (C) and on DNA synthesis (D) of rat aortic smooth muscle cells stimulated by vasopressin. Rat aortic smooth muscle cells were stimulated by vasopressin in the culture medium supplemented with 5% fetal bovine serum. Cell number (A) and DNA synthesis (B) of rat aortic smooth muscle cells were measured 72 and 24 h after stimulation by vasopressin (10^{-8} to 10^{-6} M) respectively. After pretreatment with vasopressin V_1 receptor antagonist OPC21268 (10^{-9} to 10^{-6} M) for 30 min, rat aortic smooth muscle cells were stimulated by vasopressin (10^{-6} M) in the culture medium supplemented with 5% fetal bovine serum for 72 h to measure cell number (C), or for 24 h to measure DNA synthesis (D). $n = 6$, $^{**}P < 0.01$ vs. vehicle. §§$P < 0.01$ vs. vasopressin (10^{-6} M) without OPC21268.

Chemical Industries (Osaka, Japan). [^{3}H]thymidine was purchased from Amersham LIFE SCIENCE (Buckinghamshire, UK).

2.2. Cell culture

Rat aortic smooth muscle cells were obtained by enzymatic digestion of the medial layer of the thoracic aorta of 6-week-old male Wistar rats (Nippon Bio-Supply Center, Tokyo, Japan) according to the method of Chamley et al. (1977). Rat aortic smooth muscle cells were passaged using 0.25% trypsin-EDTA for subsequent subculture. Subcultured aortic smooth muscle cells (fourth to eighth passage from the primary culture) were used in this study. Rat aortic smooth muscle cells and A10 cells, a cloned cell-line of vascular smooth muscle cells derived from fetal rat aorta (Kimes and Brandt, 1976), were maintained in DMEM supplemented with 10% fetal bovine serum at 37°C in a humidified atmosphere of 95% air and 5% CO_2. The concentration of vasopressin in DMEM supplemented with 10% fetal bovine serum was not detectable by radioimmunoassay, the sensitivity of which was 10^{-12} M.

2.3. Measurement of cell number

Vascular smooth muscle cells were plated in 24-well flat-bottom polystyrene microplate dishes at an initial concentration of 5×10^4 cells/ml in 0.5 ml DMEM supplemented with 10% fetal bovine serum in each well, and incubated until they became confluent. The medium was then switched to DMEM supplemented with 0.1% fetal bovine serum to keep the cells quiescent. After 48 h, the medium was replaced with 0.5 ml DMEM containing chemical agents such as vasopressin, indomethacin and prostanoids.

Preliminarily, the time-course of the effect of vasopressin (5×10^{-7} M) on cell proliferation of rat aortic smooth muscle cells and A10 cells was examined. In both cell lines, a significant effect was found after 48 and 72 h of incubation. Therefore, the cell number was counted after 72 h of incubation in the present study.

The vasopressin V_1 receptor antagonist, OPC21268, was dissolved in ethanol. The final concentration of ethanol in the culture medium was 0.001%, which had no effect on proliferation of rat aortic smooth muscle cells or A10 cells (data is not shown). The vasopressin V_2 receptor antagonist, OPC31260, was dissolved directly in the culture medium. Cells were pretreated with OPC21268 or OPC31260 for 30 min before stimulation with chemical agents such as vasopressin, indomethacin and prostanoids.

After incubation, vascular smooth muscle cells were trypsinized and suspended. The cell number was measured by using a Coulter Counter ZM (Coulter, Hialeah, FL). Each sample was measured at least three times.

2.4. Measurement of DNA synthesis

DNA synthesis was evaluated as the incorporation of [^{3}H]thymidine into DNA. Vascular smooth muscle cells were seeded in 24-well culture dishes described before, and incubated until they became confluent. The medium was then switched to DMEM supplemented with 0.1% fetal bovine serum to keep the cells quiescent for 48 h. The medium was replaced with DMEM containing [^{3}H]thymidine (1 μCi/ml) and aforementioned agents. After incubation for another 24 h, vascular smooth muscle cells were rinsed twice with ice-cold phosphate-buffered saline, and subsequently incubated with ice-cold 5% trichloroacetic acid for 20 min at 4°C. After incubation, vascular smooth muscle cells were washed twice with ice-cold 5% trichloroacetic acid, and then with ice-cold phosphate-buffered saline again. The cells were lysed with 1 N NaOH. The radioactivity of the cells was counted using a liquid scintillation counter LSC-1000 (Aloka, Tokyo, Japan).

2.5. Measurement of prostanoids

Vascular smooth muscle cells were seeded in 24-well culture dishes described before, and incubated until they became confluent. The medium was then switched to

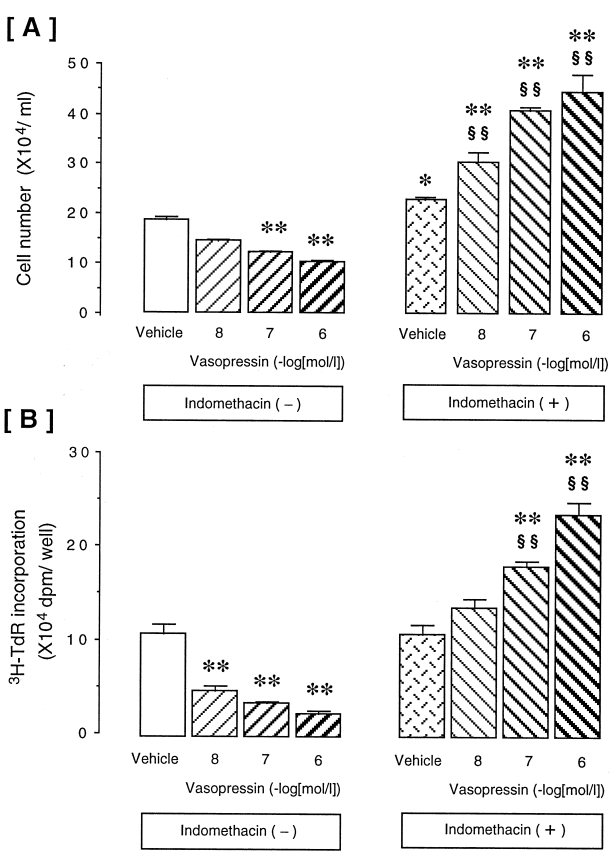

Fig. 2. Effect of arginine vasopressin with or without indomethacin on cell number (A) and on DNA synthesis (B) of A10 cells. The cell number and DNA synthesis of A10 cells were measured after 72 and 24 h, respectively, of stimulation by vasopressin with or without indomethacin (10^{-5} M) in the culture medium supplemented with 10% fetal bovine serum. $n = 6$; $^*P < 0.05$, $^{**}P < 0.01$ vs. vehicle (indomethacin (–)); §§$P < 0.01$ vs. vehicle (indomethacin (+)).

DMEM supplemented with 0.1% fetal bovine serum to keep the cells quiescent. Rat aortic smooth muscle cells and A10 cells were stimulated by vasopressin (10^{-7} to 10^{-6} M) in the culture medium supplemented with 5% fetal bovine serum for 72 h. The concentrations of prostaglandin E_2, 6-keto-prostaglandin $F_{1\alpha}$, and thromboxane B_2 in the culture medium were measured by radioimmunoassay.

2.6. Statistical analysis

All of the data in the figures are presented as mean ± S.E.M. The data were evaluated by analysis of variance (ANOVA) followed by the Student's Newman–Keuls multicomparison test. Probabilities less than 0.05 were considered statistically significant.

3. Results

3.1. Effect of arginine vasopressin on proliferation of rat aortic smooth muscle cells and A10 cells

The proliferation of rat aortic smooth muscle cells was augmented by vasopressin (10^{-8} to 10^{-6} M) in a dose-dependent manner. A pharmacological dose of vasopressin significantly increased the cell number and DNA synthesis of rat aortic smooth muscle cells ($P < 0.01$ at 10^{-7} to 10^{-6} M; Fig. 1A and B). OPC21268 (10^{-6} M) alone had no effect on the cell number and DNA synthesis of rat aortic smooth muscle cells compared to vehicle (Fig. 1C and D). The increase in a cell number and DNA synthesis stimulated by vasopressin (10^{-6} M) was completely blocked by a V_1 receptor antagonist, OPC21268 in a dose-dependent manner (10^{-9} to 10^{-6} M; Fig. 1C and D), whereas a V_2 receptor antagonist, OPC31260 (10^{-7} M) had no effect. (data is not shown). Neither indomethacin (10^{-5} M) nor N^G-monomethyl-L-arginine (10^{-3} M) affected the proliferation stimulated by vasopressin (10^{-6} M) in rat aortic smooth muscle cells (data not shown).

In contrast to rat aortic smooth muscle cells, in A10 cells, vasopressin significantly decreased cell number ($P < 0.01$ at 10^{-7} to 10^{-6} M) and DNA synthesis ($P < 0.01$ at 10^{-8} to 10^{-6} M) in a dose-dependent manner (Fig. 2A and B, left panels). To evaluate the role of prostanoids, A10 cells were treated with a cyclooxygenase inhibitor, indomethacin (Fig. 2A and B, right panels). Interestingly, the effect of vasopressin was reversed. Vasopressin, when applied with indomethacin (10^{-5} M), dose-dependently increased the cell number and DNA synthesis of A10 cells.

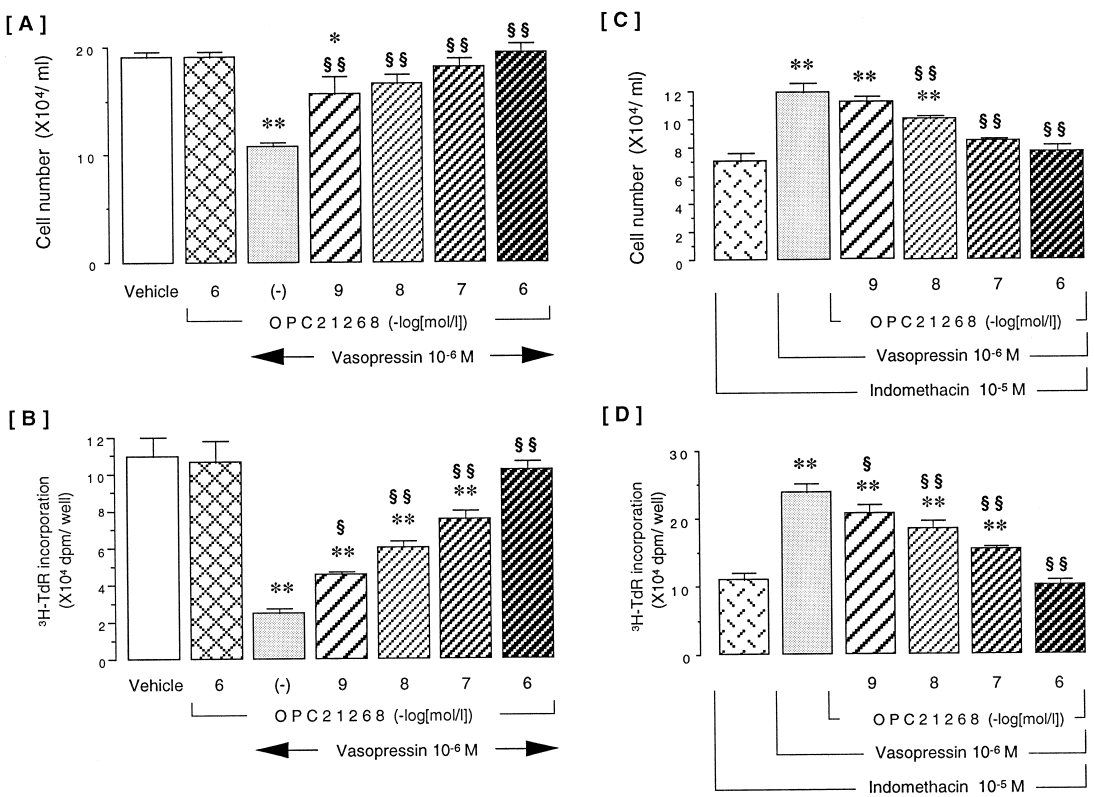

Fig. 3. Effect of vasopressin V_1 receptor antagonist OPC21268 without indomethacin on cell number (A) and on DNA synthesis (B) of A10 cells stimulated by vasopressin, and effect of OPC21268 with indomethacin on cell number (C) and on DNA synthesis (D) of A10 cells stimulated by vasopressin. After pretreatment with vasopressin V_1 receptor antagonist OPC21268 (10^{-9} to 10^{-6} M) for 30 min, A10 cells were stimulated by vasopressin (10^{-6} M) with or without indomethacin for 72 h to measure cell number (A, C), or for 24 h to measure DNA synthesis (B, D). $n = 6$; $^{*}P < 0.05$, $^{**}P < 0.01$ vs. vehicle; §$P < 0.05$, §§$P < 0.01$ vs. vasopressin (10^{-6} M) without OPC21268.

To investigate another possible mechanism for the inhibitory effect of vasopressin, A10 cells were treated with a nitric oxide synthase inhibitor, N^G-monomethyl-L-arginine. However, N^G-monomethyl-L-arginine (10^{-3} M) had no effect on proliferation of A10 cells induced by vasopressin (10^{-6} M) (data not shown). Then, the responsible vasopressin receptor was examined (Fig. 3A and B). OPC21268 (10^{-6} M) alone had no effect on proliferation of A10 cells. OPC21268 abolished the growth inhibitory effect of vasopressin in a dose-dependent manner (10^{-9} to 10^{-6} M; Fig. 3A and B). Furthermore, the growth-promoting effect of vasopressin with indomethacin was also eliminated by OPC21268 in a dose-dependent manner (10^{-9} to 10^{-6} M; Fig. 3C and D). In contrast, OPC31260 (10^{-7} M) showed no influence on the growth promoting or inhibitory effect of vasopressin in A10 cells (data not shown).

3.2. Effect of arginine vasopressin on prostanoid production of vascular smooth muscle cells

To confirm the involvement of prostanoids in the growth inhibitory effect of vasopressin, the concentrations of vasoactive prostanoids in the culture medium were measured by radioimmunoassay. Rat aortic smooth muscle cells and A10 cells were stimulated by vasopressin (10^{-7} to 10^{-6} M) in the culture medium supplemented with 5% fetal bovine serum for 72 h.

The basal levels of prostanoids such as prostaglandin E_2, 6-keto-prostaglandin $F_{1\alpha}$, and thromboxane B_2 in the culture medium of A10 cells were higher than those in the medium of rat aortic smooth muscle cells. Furthermore, the production of these prostanoids in A10 cells was significantly enhanced by vasopressin in a dose-dependent manner ($P < 0.01$ at 10^{-7} to 10^{-6} M; Fig. 4B, left panel). This enhancement was abolished when A10 cells were exposed to indomethacin (10^{-5} M). 6-Keto-prostaglandin $F_{1\alpha}$, a prostacyclin metabolite, was the predominant prostanoid in the culture medium. In contrast, the production of these prostanoids was very low in the culture medium of rat aortic smooth muscle cells stimulated by vasopressin (10^{-7} to 10^{-6} M) with or without indomethacin (Fig. 4B, right panel). To investigate whether prostacyclin is the dominant growth inhibitory prostanoid in A10 cells, we examined the effect of exogenous prostanoids on cell proliferation. A10 cells were pretreated with indomethacin (10^{-5} M), then exogenous prostanoids or their analogues were added. Based on the concentrations of endogenous prostanoids stimulated by vasopressin (Fig. 4B), we examined 10^{-8} M of the prostanoids. Prostaglandin E_2 and beraprost sodium, a prostacyclin analogue, inhibited proliferation whereas U-46619, a

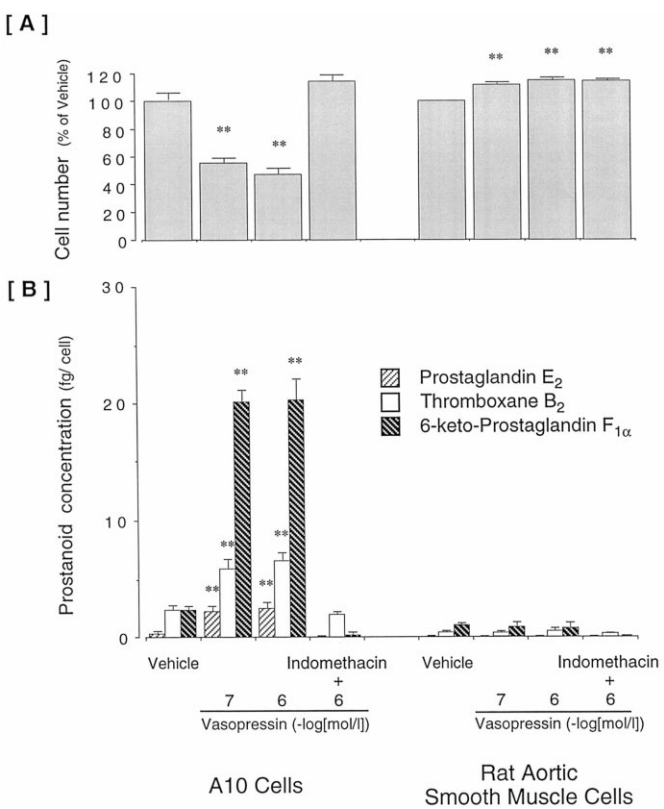

Fig. 4. Effects of arginine vasopressin on cell proliferation (A) and prostanoid production (B) in rat aortic smooth muscle cells and A10 cells. Rat aortic smooth muscle cells and A10 cells were stimulated by vasopressin (10^{-7} to 10^{-6} M) in the culture medium supplemented with 5% fetal bovine serum for 72 h. The cell number was counted and presented as percent of vehicle. The concentrations of prostaglandin E_2, thromboxane B_2, and 6-keto-prostaglandin $F_{1\alpha}$ in the culture medium were measured by radioimmunoassay, and normalized by cell number. $n = 6$; $^{**}P < 0.01$ vs. vehicle; indomethacin, 10^{-5} M.

thromboxane A_2 analogue, stimulated it (Fig. 5A). Although the directions of the effects were different, the magnitudes of the effects at the same dosage (10^{-8} M) were comparable. Furthermore, the growth inhibition was observed when a similar proportion of three prostanoids to Fig. 4B were added to A10 cells (data not shown). Taken together, prostacyclin may be the dominant prostanoid controlling cell proliferation in A10 cells.

Finally, to clarify the vasopressin receptor responsible for prostanoid production, A10 cells were pretreated with OPC21268 (10^{-6} M), and the concentration of prostanoids in the culture medium as well as cell number were measured. The effect of vasopressin (10^{-6} M) on prostanoid production as well as cell proliferation was completely blocked by OPC21268 (10^{-6} M) (Fig. 5B).

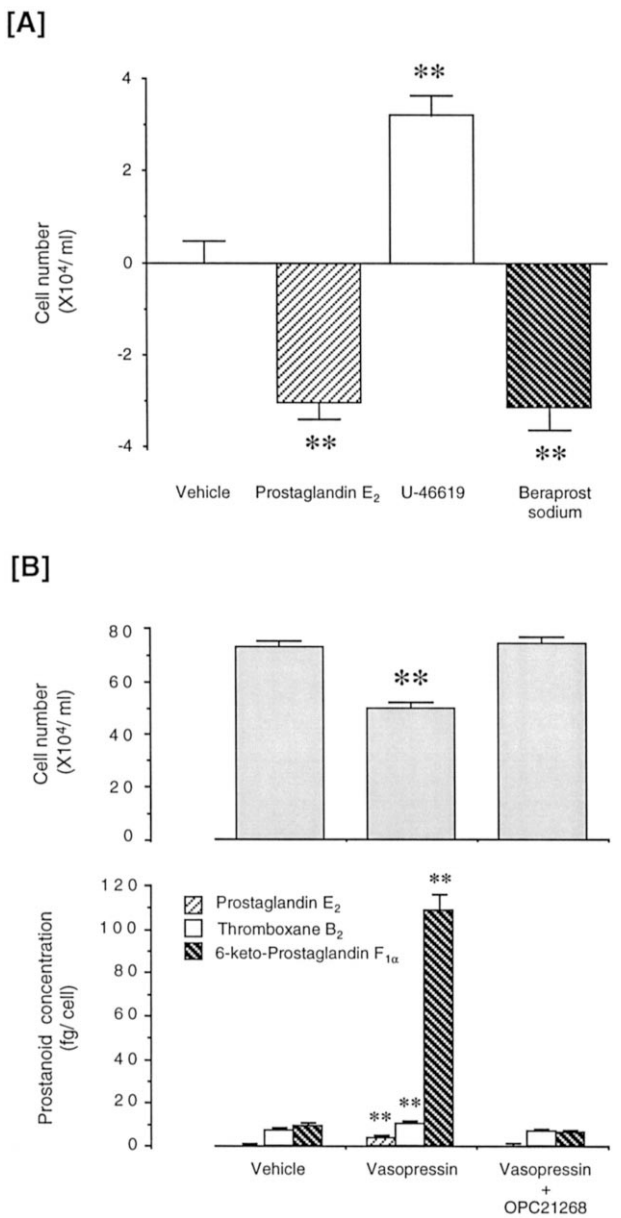

Fig. 5. Effect of exogenous prostanoids including prostaglandin E_2, beraprost sodium, and U-46619 on cell proliferation of A10 cells (A), and effect of vasopressin V_1 receptor antagonist OPC21268 on cell proliferation and on prostanoid production of A10 cells stimulated by vasopressin (B). A10 cells were pretreated with indomethacin (10^{-5} M) to block endogenous prostanoid and stimulated by prostaglandin E_2 (10^{-8} M), beraprost sodium (10^{-8} M) and U-46619 (10^{-8} M). The increase in cell number compared to that in vehicle-treated cells is shown (A). After pretreatment with vasopressin V_1 receptor antagonist OPC21268 (10^{-6} M) for 30 min, A10 cells were stimulated by vasopressin (10^{-6} M) for 72 h to measure cell number and the concentrations of prostaglandin E_2, thromboxane B_2, and 6-keto-prostaglandin $F_{1\alpha}$ in the culture medium (B). $n = 6$; $^{**}P < 0.01$ vs. vehicle.

4. Discussion

According to previous studies, it is still controversial whether arginine vasopressin stimulates or inhibits the proliferation of vascular smooth muscle cells. For example, vasopressin stimulates DNA synthesis of vascular smooth muscle cells derived from Wistar–Kyoto rats (Hamada et al., 1990). In contrast, vascular smooth muscle cells derived from Wistar rat in the later G_1 phase of cell cycle were inhibited by vasopressin in association with the synthesis of prostaglandin E series (Murase et al., 1992). Also, it has been shown that cell number per se remained unchanged when vascular smooth muscle cells were cultured in medium without serum, although the contents of many cellular proteins were increased (Geisterfer and Owens, 1989; Turla et al., 1991). Therefore, in the present study, we studied two different types of vascular smooth muscle cells, cultured adult rat aortic smooth muscle cells and A10 cells, a vascular smooth muscle cell line derived from fetal rat aorta. Vasopressin stimulated the proliferation of rat aortic smooth muscle cells in a dose-dependent manner. In contrast, vasopressin significantly inhibited the proliferation of A10 cells, while vasopressin stimulated cell growth when A10 cells were treated with a cyclooxygenase inhibitor, indomethacin. It has been shown that prostanoid production is stimulated by vasopressin in vascular smooth muscle cells, cerebromicrovascular endothelium, bladder epithelial cells, glomeruli, and myometrium (Burch and Halushka, 1982; Hassid and Williams, 1983; Yanagisawa et al., 1990; Spatz et al., 1994; Lobaccaro-Henri et al., 1996). It is also reported that proliferation of vascular smooth muscle cells is inhibited by prostaglandin E_2 and prostacyclin, and stimulated by thromboxane A_2 (Owen, 1986; Morisaki et al., 1988; Uehara et al., 1988; Shirotani et al., 1991; Koh et al., 1993; Asada et al., 1994; Sachinidis et al., 1995). Consequently, we hypothesized that growth regulatory prostanoids may be involved in the diverse actions of vasopressin on proliferation of vascular smooth muscle cells.

To prove this hypothesis, we measured three kinds of prostanoids; prostaglandin E_2, 6-keto-prostaglandin $F_{1\alpha}$ as a prostacyclin metabolite, and thromboxane B_2 as a thromboxane A_2 metabolite. The production of these prostanoids in the culture medium of A10 cells was much higher than that in rat aortic smooth muscle cells, and was increased

by vasopressin. In contrast, such an increase in prostanoid production by vasopressin was not found in the culture medium of rat aortic smooth muscle cells. Importantly, the production of 6-keto-prostaglandin $F_{1\alpha}$ in the culture medium of A10 cells was several-fold higher than that of other prostanoids. By administering exogenous prostanoids, we confirmed that prostaglandin E_2 and a prostacyclin analogue, beraprost sodium, inhibited proliferation whereas a thromboxane A_2 analogue, U-46619, stimulated it. Taken together with the magnitude of their effects and the proportions of prostanoid production, prostacyclin may be the predominant growth regulatory prostanoid in A10 cells.

It is unknown why production of prostanoids is greater in A10 cells than in rat aortic smooth muscle cells. Although vasopressin has different effects on proliferation of vascular smooth muscle cells in different species (Boswell and Robertson, 1981; Geisterfer and Owens, 1989; Turla et al., 1991), in the present study, both rat aortic smooth muscle cells and A10 cells were derived from rats. The significant difference between the two types of vascular smooth muscle cells may be due to the developmental stages at which they are isolated. Rat aortic smooth muscle cells are derived from the adult rat and A10 cells are from the fetal rat. The vasculature derived from the fetus is reported to produce much greater amount of prostanoids than the vasculature of the adult (Terragno and Terragno, 1979; Matsumoto et al., 1984). The production of 6-keto-prostaglandin $F_{1\alpha}$ in the umbilical vessels is much higher than that of other prostanoids (Mitchell et al., 1980). Plasma concentration of 6-keto-prostaglandin $F_{1\alpha}$ in the umbilical veins is much higher than that of the mother (Matsumoto et al., 1984). These data indicate that fetal vessels produce more prostacyclin than adult vessels. Accordingly, A10 cells may represent the characteristics of fetal vessels, at least in terms of prostacyclin production. The cellular mechanism responsible for the difference between the two vascular smooth muscle cells is unknown. Both the effect of vasopressin on proliferation and prostanoid production in A10 cells and the effect of vasopressin on proliferation in rat aortic smooth muscle cells were abolished by a V_1 antagonist, indicating that all these effects are mediated via the V_1 receptor. We hypothesize that intracellular signaling through the V_1 receptor may split into two parts at some step, one for growth promotion and the other for prostanoid production. Rat aortic smooth muscle cells may lack some component essential to prostanoid synthesis. Further studies are necessary to address the intracellular events that diverge from V_1 receptor activation.

It has been reported that vasopressin stimulated the production of nitric oxide, a potent vasodilator and growth inhibitor, mainly by the endothelium of the vasculature through the V_2 receptor (Hirsch et al., 1989; Suzuki et al., 1989; Tagawa et al., 1993). Thus, we examined the possible involvement of nitric oxide, and demonstrated that N^G-monomethyl-L-arginine, a nitric oxide synthase inhibitor, had no influence on cell proliferation stimulated by vasopressin in both types of vascular smooth muscle cells. Therefore it is unlikely that nitric oxide produced by vascular smooth muscle cells is implicated in the effects of vasopressin on proliferation of vascular smooth muscle cells.

In the present study, a pharmacologically high concentration of vasopressin (10^{-8} to 10^{-6} M) was required to stimulate proliferation of vascular smooth muscle cells in comparison to the physiological circulating concentration (10^{-11} M). In a preliminary study, vasopressin at the normal circulating concentration did not change the number of vascular smooth muscle cells (data not shown). In general, arginine vasopressin is well known to be synthesized in the magnocellular neurons of the supraoptic and paraventricular nuclei of the hypothalamus and then transported to the posterior pituitary gland, and then secreted into the systemic circulation (Ivell and Richter, 1984; Meeker et al., 1991). In several species, extrahypothalamic production of vasopressin is demonstrated in a variety of organs and tissues including the adrenal medulla, ovary, testis, and endothelial cells of pulmonary, renal, and mesenteric arteries, demonstrated by immunohistochemical method, radioimmunoassay, high-performance liquid chromatography, or Northern blot analysis (Ang and Jenkins, 1984; Lim et al., 1984; Nussey et al., 1984; Lincoln et al., 1990; Foo et al., 1991; Loesch et al., 1991).

Recently, a number of vasoactive substances, including angiotensin II and endothelin, have been shown to be locally synthesized and stored within the vascular wall (Oliver and Sciacca, 1984; Yanagisawa et al., 1988; Mizuno et al., 1991). Furthermore, it is reported that by RNase protection assay, arginine vasopressin mRNA was identified in rat aorta, although its cellular localization remained unknown (Simon and Kasson, 1995). We have demonstrated that arginine vasopressin mRNA is expressed in rat aortic smooth muscle cells and A10 cells using reverse transcription polymerase chain reaction and Northern blot analysis, indicating that arginine vasopressin is locally produced in vascular smooth muscle cells (Nagano et al., 1997). This finding provides new insights into the role of vasopressin in pathophysiological conditions including hypertension and atherosclerosis. Vasopressin may play an important role in the modulation of vascular functions in an autocrine or paracrine fashion as a local factor, through the proliferation of vascular smooth muscle cells.

In summary, arginine vasopressin stimulated both growth promoting signals and prostanoid production through the V_1 receptor in vascular smooth muscle cells. The balance between them may determine the final growth state of vascular smooth muscle cells.

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