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Effects of Prostaglandin $F_{2\alpha}$ on Membrane Potentials and K^+ Currents in Rabbit Middle Cerebral Arterial Cells

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The purpose of our investigation was to examine the effects of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) on membrane potentials, Ca^{2+}-activated K^+ (K_{Ca}) channels, and delayed rectifier K^+ (K_V) channels using the patch-clamp technique in single rabbit middle cerebral arterial smooth muscle cells. $PGF_{2\alpha}$ significantly hyperpolarized membrane potentials and increased outward whole-cell K^+ currents. $PGF_{2\alpha}$ increased open-state probability of K_{Ca} channels without the change of the open and closed kinetics. $PGF_{2\alpha}$ increased the amplitudes of K_V currents with a leftward shift of activation and inactivation curves and a decrease of activation time constant. Our results suggest that the activation of K_{Ca} and K_V channels, at least in part, may lead to attenuate or counteract vasoconstriction by $PGF_{2\alpha}$ in middle cerebral artery.

Key Words: Prostaglandin $F_{2\alpha}$, Membrane potentials, Ca^{2+}-activated K^+ channels delayed rectifier K^+ channels, Rabbit middle cerebral arterial smooth muscle cells, Patch-clamp technique

INTRODUCTION

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is the product of cyclooxygenation of arachidonic acid, which is released from membrane phospholipids. It has complex effects on various tissues including heart, lung, trachea, uterine, and vascular smooth muscles.

$PGF_{2\alpha}$ increases cardiac contractility (Nutter & Ratts, 1973; Karmazyn et al, 1981; Otani et al, 1988), being the most potent of prostaglandins studied. Additionally, it depresses ventricular recovery of reperfused heart (Karmazyn, 1986), modulates cardiac rhythm (Forster et al, 1973), releases atrial natriuretic factor (Gardner & Schultz, 1990), and causes ventricular hypertrophy (Lai et al, 1996). The mechanisms underlying action of $PGF_{2\alpha}$ are not fully understood but it is known to be an agonist at FP prostaglandin receptors that are linked to phosphoinositide metabolism (Coleman et al, 1994). $PGF_{2\alpha}$ has been reported to increase inositol 1,4,5-trisphosphate levels in rat papillary muscle (Otani et al, 1988), to inhibit the Na^+/H^+ ATPase (Karmazyn et al, 1981), and to stimulate the calcium uptake by sarcoplasmic reticulum (Sabatini, 1970). These actions would directly or indirectly be expected to increase the amount of calcium available for contraction.

$PGF_{2\alpha}$ has been known to exert contractile effect on various vascular smooth muscles including coronary artery, cerebral artery, aorta, renal artery, and jugular vein (Horton & Main, 1983; Uski & Andersson, 1984; Suba & Roth, 1987; Zhang et al, 1991; Chen et al, 1995). $PGF_{2\alpha}$ mediated rat aortic contraction through phosphoinositide (PI) hydrolysis and this PI hydrolysis does not appear to depend on extracellular calcium flux (Suba & Roth, 1987). $PGF_{2\alpha}$ does affect free intracellular calcium levels or L-type calcium currents. But, incubation of the preparations in a calcium-free medium did not abolish contraction induced by $PGF_{2\alpha}$. Instead, 63 % of the response to $PGF_{2\alpha}$ remained after pretreatment of the arteries in a calcium-free solution (Uski & Andersson, 1984). $PGF_{2\alpha}$ constricted bovine middle cerebral arteries by two mechanisms: first, by promoting calcium uptake from low-affinity binding sites through receptor-operated channels sensitive to the calcium antagonists, and second, by releasing calcium from depletable internal stores. This constriction by $PGF_{2\alpha}$ was near-

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maximally inhibited in calcium-deficient solutions but only partially inhibited by calcium antagonists (Wendling & Harakal, 1991). On the other hand, a possible mechanism for vasoconstrictive action of $PGF_{2\alpha}$ is that it may occur partly by sensitization of the contractile filaments to calcium (Hori et al, 1993; Suematsu et al, 1991; Yew et al, 1998). In contrast to above observations, Chen et al (1995) showed that $PGF_{2\alpha}$ potently relaxed the jugular vein. This effect of relaxations elicited by $PGF_{2\alpha}$ appears to be mediated by nitric oxide and potassium channels. Therefore, it is difficult to reconcile these observations as these differences may relate to species variation.

The mechanism underlying action of $PGF_{2\alpha}$ in vascular smooth muscles has yet not been fully investigated. Furthermore, the role of $PGF_{2\alpha}$ in modulating the membrane currents and membrane potentials has received comparatively little attention. Ca^{2+}-activated K^+ channels (K_{Ca} channels) have been characterized by their large single channel conductance and abundance in various excitable cells, including smooth muscles. The channels are known to play a major role in the regulation of smooth muscle excitability by control of resting membrane potential and termination of action potential (Benham et al, 1986). Furthermore, from a review of the current literature, it is apparent that delayed rectifier K^+ channels (K_V channels) may play an important role in the control of vascular membrane potential and myogenic reactivity (Hara et al, 1980; Knot & Nelson, 1995). Therefore, the purpose of our investigation was to examine the effects of $PGF_{2\alpha}$ on membrane potentials, K_{Ca} channels, and K_V channels using the patch-clamp technique in single rabbit middle cerebral arterial smooth muscle cells.

METHODS

Cell preparation

New Zealand White rabbits (n=50) of either sex weighing 0.8 to 1.2 kg, were anesthetized with an injection of pentobarbital sodium (1 mg/kg) and euthanatized by exsanguination. The middle cerebral arteries were removed and cleaned of extraneous connective tissues in a dissecting solution containing (mM): 137 NaCl, 5.6 KCl, 0.42 Na_2HPO_4, 0.44 NaH_2PO_4, 4.17 $NaHCO_3$, 1 $MgCl_2$, 2.6 $CaCl_2$, 10 *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), pH 7.3 with NaOH. The arteries were then transferred to an isolation solution (mM: 55 NaCl, 6 KCl, 88 L-glutamic acid, 10 HEPES, 10 glucose, pH 7.3 with NaOH) for 20 min. Cerebral arteries were digested for 10 min in an isolation solution containing (mg/ml): 1 albumin, 1 papain, and 1 dithioerythritol, then for 10 min in a isolation solution containing (mg/ml): 1 albumin, 1 collagenase F, and 1 hyaluronidase type I-S. Single arterial smooth muscle cells were obtained by gentle trituration with a wide-bore pipette in fresh isolation with albumin (1 mg/ml), stored at 4°C and used within 12 hours.

Data recordings

The whole-cell and excised patch configurations of the patch-clamp technique were utilized in this study. The data were recorded with a patch-clamp amplifier (Axopatch-1D, Axon Instruments, Foster City, CA, USA). Pipettes of 5~10 M resistance were pulled from borosilicate glass capillaries (Clark Electrochemical, Pangbourne, England) using a vertical puller (Narishige PP-83, Japan). Their tips were coated with Sylgard and fire polished. Membrane potentials and whole-cell currents were filtered at 5 kHz and stored in digitized format on digital audiotapes using a Biologic DTR-1200 recorder (Grenoble, France). Single-channel currents were digitized at a sampling rate of 48 kHz and stored in digitized format on digital audiotapes using a Biologic DTR-1200 recorder. For the analysis, the data were transferred to a computer with pCLAMP v 6.0 software (Axon Instruments, Burlingame, CA, USA) through an analogue-to-digital converter interface (Digidata-1200, Axon Instruments Inc.).

Data analysis and quantification of single channel activity

The threshold for judging the open state was set at half of the single-channel amplitude (Ogden & Colquhoun, 1983). The open time histogram was formed from continuous recordings of more than 60 sec. The open probability (P_O) was calculated using the formula:

$$P_O = (\sum_{j=1}^{N} t_j j)/(T_d N)$$

where t_j is the time spent at current levels corresponding to j=0,1,2, ... N channels in the open state,

T_d is the duration of the recording and N is the number of channels active in the patch. The number of channels in a patch was estimated by dividing the maximum current that observed by the mean unitary current amplitude. P_O was calculated over 30 sec records.

Solutions and drugs

The solutions used in whole-cell experiments for membrane potentials and K^+ currents were (in mM): 133 K-Aspartic acid, 7 KCl, 2.5 Mg-ATP, 2.5 Na-ATP, 2.5 tris-creatine phosphate, 2.5 Na-creatine phosphate, 5 HEPES and 0.05 or 10 ethylene glycol-bis (β-aminoethyl ether) *N, N, N', N'*-tetraacetic acid (EGTA), pH 7.3 for the pipette and 143 NaCl, 5.4 KCl, 5 HEPES, 0.33 NaH_2PO_4, 1 $MgCl_2$, 16.6 glucose, 0 or 1.8 $CaCl_2$, pH 7.4 for the bath. The solutions used in outside-out patch experiments were (in mM): 140 KCl, 1.2 KH_2PO_4, 2.5 $CaCl_2$, and 5 HEPES, pH 7.3 for the bath; the composition of pipette solution was similar, except that the Ca^{2+}, EGTA ratio was adjusted to give a pCa of 6×10^{-7} at which simultaneous opening of 1 to 3 channels could be typically observed.

All chemicals and drugs in this study were obtained from Sigma Chemical (St. Louis, MO, USA).

Statistical analysis

Data are presented as mean±SEM. Statistical comparison was made by the Student *t* test. The level of significance of all tests of comparison was $P<0.05$.

RESULTS

Membrane potentials are measured in current-clamp using the pipette solution containing 0.05 mM EGTA. Membrane potentials averaged -33.52 ± 2.10 mV (n=21). The application of $PGF_{2\alpha}$ (100 nM) did significantly alter membrane potentials ($-42.82\pm$ 4.46 mV, $\triangle=-8.18\pm1.97$, n=11, Fig. 1A). Change in membrane potentials in response to $PGF_{2\alpha}$ is summarized (Fig. 1A, *inset*).

In order to examine the effect of $PGF_{2\alpha}$ on the membrane currents, steps of voltages from -60 mV to $+50$ mV in 10 mV increments were applied from a holding potential of -60 mV (Fig. 1B-a). Control currents showed time- and voltage-dependent outward currents. Stepping up to positive potentials evoked much noisier outward current upon which spontaneous transient outward currents (STOCs) (Benham et al, 1986) were often superimposed. $PGF_{2\alpha}$ (100 nM) activated outward currents that exceeded the amplitudes of control currents and markedly increased STOCs, which were K_{Ca} currents (Benham et al, 1986). The current-voltage relationship is given in Fig. 1B-b. The analog signal was digitized at 5 kHz and every two points were averaged. The graph shows peak outward current plotted against test potential. Currents were normalized as a relative scale to the control after application with $PGF_{2\alpha}$.

To test the hypothesis that K_{Ca} channels are involved in the actions of $PGF_{2\alpha}$ (Fig. 1), the effects of $PGF_{2\alpha}$ on K_{Ca} channels were investigated in outside-out patch configuration. In the experiment shown in Fig. 2A, $PGF_{2\alpha}$ (100 nM) increased the open probability (P_o) from 0.25 to 0.41 at $+50$ mV (Fig. 2A-a and b). The mean increase in P_o to $PGF_{2\alpha}$ was 1.4 ± 0.8-fold (n=5; $p<0.05$; Fig. 2A-c). $PGF_{2\alpha}$ had no effect on unitary current amplitude (11.1 ± 0.1 pA before $PGF_{2\alpha}$ and 11.2 ± 0.2 pA after $PGF_{2\alpha}$; n=4; at $+50$ mV; Fig. 2B). To examine further the stimulatory action of $PGF_{2\alpha}$, the effects of $PGF_{2\alpha}$ on the open and close kinetics of K_{Ca} channel were analyzed by measuring the open- and closed-times before and after application of $PGF_{2\alpha}$ (Fig. 2C). Both open- and closed-time histograms were well fitted by a biexponential. The time constant of the fast component ($\tau_{o,f}$) was 1.0 ms (0.9 ± 0.3, n=5; mean±S.E.) and that of the slower component ($\tau_{o,s}$) was 15.3 ms (14.8 ± 0.8, n=5; mean±S.E.) in the open-time histogram (Fig. 2C-a). $PGF_{2\alpha}$ had little effect on $\tau_{o,f}$ (0.9 ± 0.2, n=5, $p>0.05$) and $\tau_{o,s}$ (15.1 ± 1.7, n=5, $p>0.05$); in this representative patch, $\tau_{o,f}$=1.1 ms and $\tau_{o,s}$=15.9 ms (Fig. 2C-b). The time constant of the fast component ($\tau_{c,f}$) was 0.5 ms (0.45 ± 0.03, n=5; mean±S.E.) and that of the slower component ($\tau_{c,s}$) was 2.0 ms (2.67 ± 0.65, n=5; mean±S.E.) in the closed-time histogram (Fig. 2C-a, *inset*). $PGF_{2\alpha}$ had little effect on $\tau_{c,f}$ (0.47 ± 0.03, n=5, $p>0.05$) and little effect on $\tau_{c,f}$ (0.47 ± 0.03, n=5, $p>0.05$) and $\tau_{c,s}$ (1.83 ± 0.17, n=5, $p>0.05$); in this representative patch, $\tau_{c,f}$=0.5 ms and $\tau_{c,s}$=2.0 ms (Fig. 2C-b, *inset*). Fig. 2C-a (*inset traces*) shows channel activity under control conditions. After addition of $PGF_{2\alpha}$ to the external side of the channel, long closings were disappeared between bursts of activity (Fig. 2C-b, *inset traces*). Note that the duration of the long

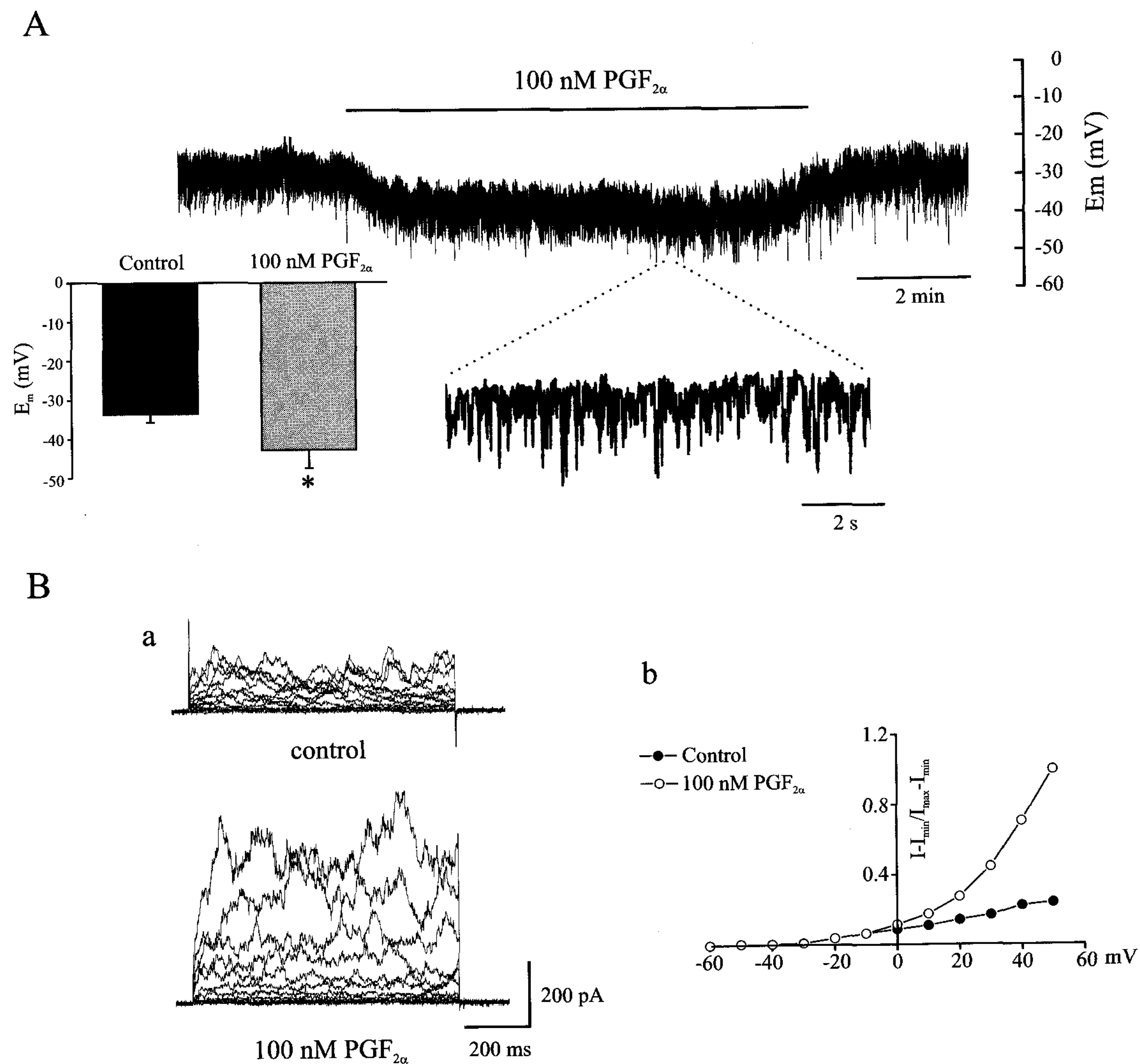

Fig. 1. Effects of $PGF_{2\alpha}$ on membrane potentials and membrane currents of cerebral vascular smooth muscle cells. A. Cerebral vascular smooth muscle cells were intracellularly perfused with a solution containing 0.05 mM EGTA. Upon extracellular application of 100 nM $PGF_{2\alpha}$, the membrane hyperpolarized. Inset: summary of membrane potential hyperpolarization by the application of $PGF_{2\alpha}$. * $P<0.05$ compared with controls. B-a, Comparison of currents observed in the normal solution (control) and in the presence of $PGF_{2\alpha}$ using step protocols. $PGF_{2\alpha}$ increased outward whole-cell K^+ currents. B-b, The current-voltage relationships for control and $PGF_{2\alpha}$.

closings in the control is decreased after application of $PGF_{2\alpha}$.

We also tested the hypothesis that K_V currents are involved in the actions of $PGF_{2\alpha}$. In order to isolate K_V currents, we used the pipette solution containing 10 mM EGTA and bathing solution containing 1 mM TEA and 10 μM nicardipine in Ca^{2+}-free Tyrode solution. The bath application of PGF_2 (100 nM) hyperpolarized membrane potentials about $\triangle = -5.67 \pm 0.33$ (n=3, Fig. 3A). K_V currents were evoked by step depolarization to 0 mV and +50 mV from a holding potential of −60 mV under control conditions and after application of $PGF_{2\alpha}$. $PGF_{2\alpha}$ increased the amplitudes of the K_V currents. We summarized the effect of $PGF_{2\alpha}$ on K_V currents at 0 and +50 mV (Fig. 3B, n=4). To further assess the effect of $PGF_{2\alpha}$, effects of $PGF_{2\alpha}$ on the activation and inactivation kinetics of K_V currents were analyzed before and after application of $PGF_{2\alpha}$. The activation and inactivation curves were well fitted by Boltzman

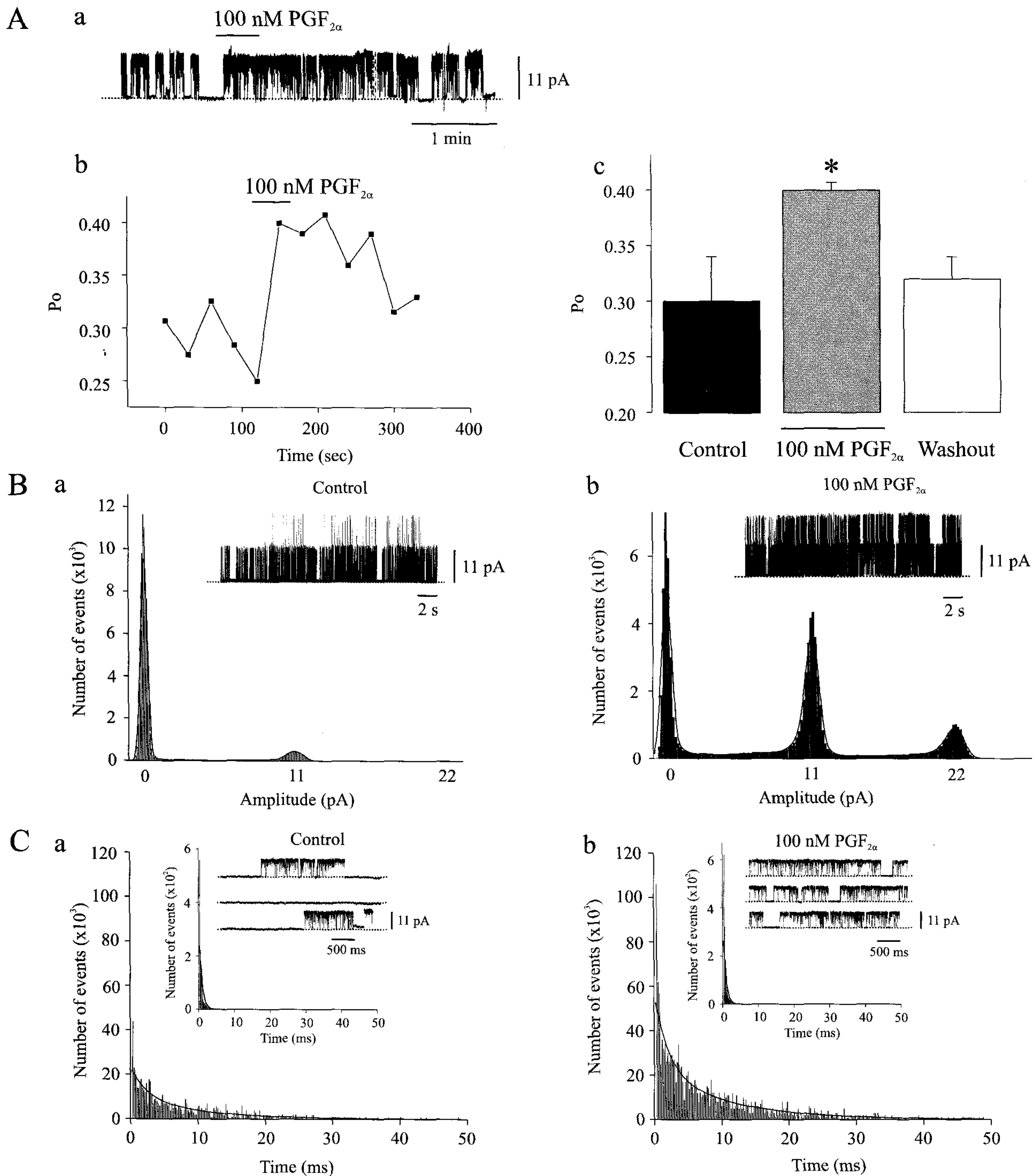

Fig. 2. Effect of PGF_2 on Ca^{2+}-activated K^+ (K_{Ca}) channel activity. A-a, Channel activity was recorded and the dotted line represented the zero current level. A-b, Time dependent change of open probability (P_O) from the same patch as A-a, calculated over 30-sec-long intervals. A-c, summarized data (n=6); before (closed column) and after addition of 100 nM $PGF_{2\alpha}$ (gray column) and washout of 100 nM $PGF_{2\alpha}$ (open column). B. Single-channel current traces and accompanying all-points histograms obtained from an outside-out membrane patch are shown before (a) and after addition (b) of 100 nM $PGF_{2\alpha}$. Note that the amplitude of the channel was not affected by 100 nM $PGF_{2\alpha}$. C. The effect of 100 nM $PGF_{2\alpha}$ on the single-channel kinetics. Histograms of the open- and closed-times (insets) below 50 ms were obtained before (a) and after addition of 100 nM $PGF_{2\alpha}$ (b) from an outside-out patch at +50 mV. Each histogram was fitted to the sum of two exponentials. The insets also show the examples of the current records in each condition. The dotted line indicates the closed level.

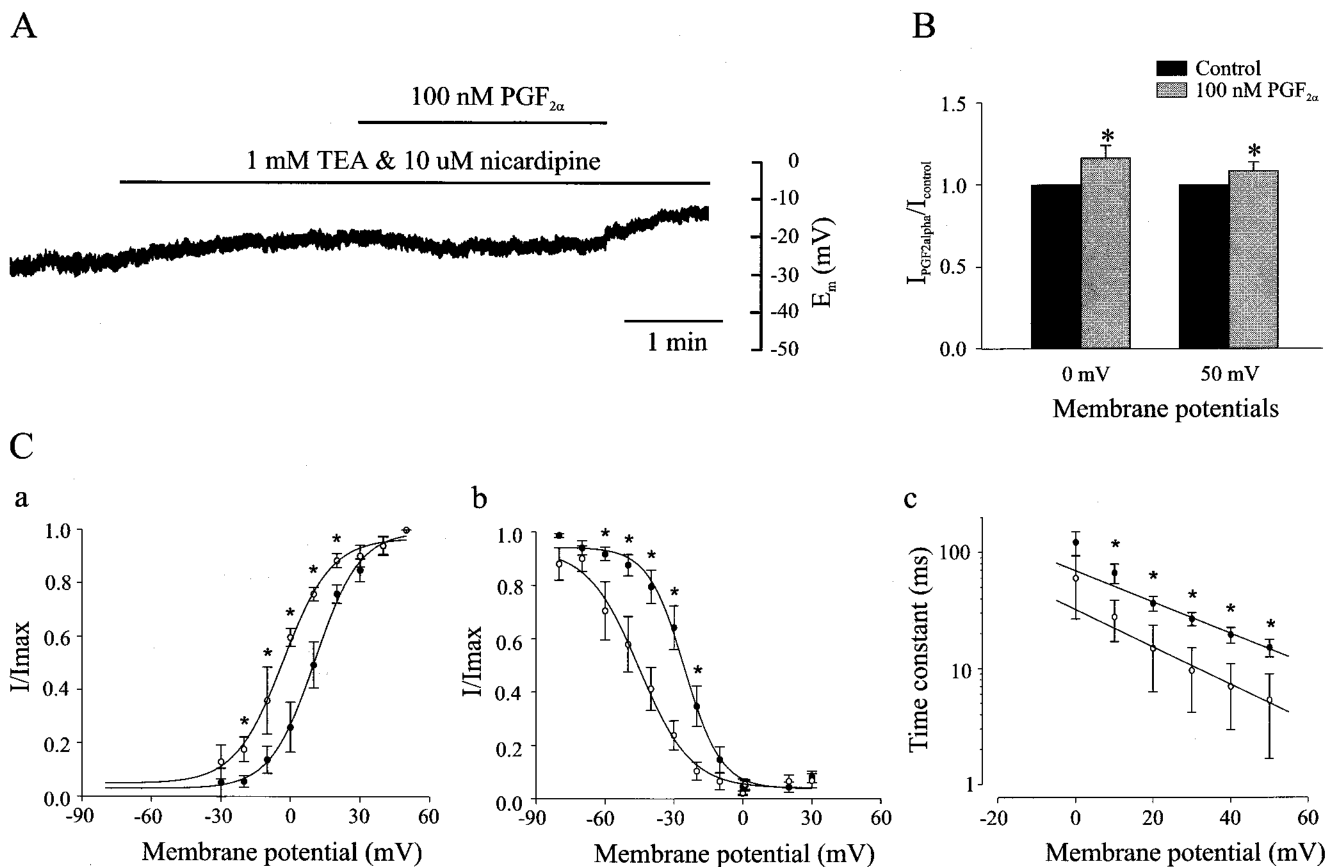

Fig. 3. Effects of $PGF_{2\alpha}$ on membrane potentials and membrane currents in the presence of 1 mM TEA and 10 μM nicardipine. A. Cerebral vascular smooth muscle cells were intracellularly perfused with a solution containing 10 mM EGTA and extracellular perfused with a solution containing 1 mM TEA and 10 μM nicardipine. The application of 100 nM $PGF_{2\alpha}$ hyperpolarized the membrane. B. Summary of K_V currents activation to $PGF_{2\alpha}$. K_V currents traces were elicited by voltage step to 0 and +50 mV from holding potential of −60 mV. Current in this experiment was increased after application of 100 nM $PGF_{2\alpha}$. * $P<0.05$ compared with controls. C. Effect of $PGF_{2\alpha}$ on the kinetics of K_V currents. C-a, The activation points are means±S.E. (n=4) and were determined by fitting deactivation tail at −35 mV after voltage steps to various potentials. The voltage steps that preceded deactivation varied in length so as to return to −35 mV at the peak of the current. The amplitude of each tail normalized using the largest tail. These points were fitted with Boltzmann distribution equation. The activation curve was shifted to the left by $PGF_{2\alpha}$. C-b, The inactivation points are means±S.E. (n=4) and represent normalized peak outward current at +40 mV after holding at various potentials until this current stabilized. The smooth curve through these points is the best fit to the Boltzmann distribution equation. The inactivation curve was shifted to the left by $PGF_{2\alpha}$. C-c, The time constants were decreased by $PGF_{2\alpha}$ (n=5). The time constants plotted on a natural logarithmic scale against test potential. The fitted straight lines have correlation coefficients of −0.987 for control and −0.979 for $PGF_{2\alpha}$.

distribution equation. In activation curve (Fig. 3C-a), half-maximal activation voltage and slope factor were 10.57±1.03 mV and 9.54±1.02 for control (n=4) and −3.16±1.52 mV and 10.35±1.34 for $PGF_{2\alpha}$ (n=4). In inactivation curve (Fig. 3C-b), half-maximal inactivation voltage and slope factor were −25.37±0.99 mV and 7.93±0.88 for control (n=4) and −45.01±1.68 mV and 11.18±1.47 for $PGF_{2\alpha}$ (n=4). Analysis of the voltage dependence of activation time constants is represented in Fig. 3C-c. $PGF_{2\alpha}$ decreased activation time constants (n=5).

DISCUSSION

The main finding of the present study is that $PGF_{2\alpha}$ causes membrane hyperpolarization and increase of K^+ currents of quiescent or depolarized vascular

smooth muscle cells of the rabbit middle cerebral artery.

$PGF_{2\alpha}$ has been known to be an extremely potent and fast-acting vasoconstrictor. Most studies have focused on association of $PGF_{2\alpha}$ with constriction of cerebral arteries *in vivo* and *in vitro* (Brandt et al, 1981; White et al, 1982; Uski & Andersson, 1984). However, the precise mechanisms of $PGF_{2\alpha}$ induced vasospasm that are responsible for change in contractility have not yet been clarified.

It is possible that cerebral vasospasm by $PGF_{2\alpha}$ is associated with the alterations of the electrophysiological properties of cerebral arterial smooth muscle cell. These alterations may lead to Ca^{2+} influx and contractile tone including an increase in Ca^{2+} currents, a decrease in K^+ currents, or a decrease in the resting membrane potential (Nelson & Quayle, 1995). It has been shown that $PGF_{2\alpha}$ induced contractions are not completely relaxed by addition of Ca^{2+} channel antagonists (Uski & Andersson, 1984; Wendling & Harakal, 1991). Thus, it appears that other mechanisms are also related with $PGF_{2\alpha}$ induced contractions in addition to an increase in Ca^{2+} currents.

Recently, it has been reported that regulation of arterial smooth muscle membrane potential through activation or inhibition of K^+ channel activity provides an important mechanism to dilate or constrict arteries. Relatively little is known about the role of K^+ channel activation in the action of $PGF_{2\alpha}$. This study is the first report showing that $PGF_{2\alpha}$ regulates membrane potentials, K_{Ca} channels, and K_V channels in rabbit middle cerebral arterial smooth muscle cells.

Four distinct types of K^+ channels have been identified in arterial smooth muscle: 1) Ca^{2+}-activated K^+ (K_{Ca}) channels (Brayden & Nelson, 1992), 2) delayed rectifier K^+ (K_V) channels (Robertson & Nelson, 1994), 3) inward rectifier K^+ (K_{IR}) channels (Bonev et al, 1994), and 4) ATP-sensitive K^+ (K_{ATP}) channels (Bonev & Nelson, 1993). Since the estimates of channel number per cell range from $-100\sim$ 500 per cell for K_{IR} and K_{ATP} channels to $1,000\sim$ 10,000 for K_{Ca} and K_V channels per cell in arterial smooth muscle cells, the regulation of arterial smooth muscle membrane potential through activation or inhibition of K_{Ca} and K_V channels provides major role to dilate or constrict arteries (Nelson & Quayle, 1995). For this reason, we tested K_{Ca} channels, K_V channels, and membrane potentials to the action by $PGF_{2\alpha}$ in rabbit middle cerebral artery.

The values of membrane potentials that we report are similar to those measured in other types of isolated vascular smooth muscle cell using the patch-clamp technique with an intracellular solution containing 0.1 mM EGTA, i.e. rabbit coronary artery cells (-32.2 mV; Leblanc et al, 1994), rabbit portal vein myocytes (-48.7 mV; Miller et al, 1993), human coronary artery cells in culture (-32 mV; StrØbaek et al, 1996), and human saphenous vein (-41 mV; Milesi et al, 1999). However, using intracellular microelectrodes, a membrane potential of -62 ± 5 mV has been measured in rabbit cerebral arterial smooth muscle cells of intact vessels (Knot & Nelson, 1995). It must be taken into account that in intact vessels, many factors may modify steady-state membrane potentials, such as the release of endothelial factors or endogenous neurotransmitters, which may hyperpolarize vascular smooth muscle cells. In the present study, $PGF_{2\alpha}$ significantly caused membrane hyperpolarization of quiescent (in 0.05 mM EGTA) or depolarized (in 10 mM EGTA) vascular smooth muscle cells of rabbit middle cerebral artery, although the depolarization of membrane potential after washout of $PGF_{2\alpha}$ in 10 mM EGTA cannot be understood. This result indicates that the membrane hyperpolarization by $PGF_{2\alpha}$ attenuates $PGF_{2\alpha}$ induced contractions.

In the present study, $PGF_{2\alpha}$ increased open-state probability, but had little effect on the open and closed kinetics of K_{Ca} channels. K_{Ca} channels had large unitary conductance; thus opening a few K_{Ca} channels has a significant impact on membrane potentials (Nelson & Quale, 1995; Peng et al, 1996). Gokina et al (1996) demonstrated that blockade of K_{Ca} channels by charybdotoxin and TEA resulted in membrane depolarization, an increase of the amplitude and duration of action potentials, and marked contraction of smooth muscle cells in human pial arteries. Asano et al (1993) suggested that the resting tone of cerebral arteries is determined by at least two opposite components: contraction due to the increased basal Ca^{2+} influx (probably through activation of myosin light chain kinase) and relaxation due to the activation of K_{Ca} channels and other Ca^{2+} extraction systems. The net balance of the two components resulted in the contraction. The increased basal Ca^{2+} influx in resting state produces contraction and activates K_{Ca} channels or other Ca^{2+} extraction systems, bring about hyperpolarization and direct Ca^{2+} extraction in dog cerebral arteries. Therefore, the acti-

vation of K_{Ca} channels by $PGF_{2\alpha}$ could counteract the $PGF_{2\alpha}$-induced vasoconstriction. Moreover, this may be direct effect of $PGF_{2\alpha}$ on K_{Ca} channels, because $PGF_{2\alpha}$ increased P_O of K_{Ca} channels in outside-out patch that we found here.

In the present study, $PGF_{2\alpha}$ increased the amplitudes of K_V currents with a leftward shift of activation and inactivation curves and a decrease of activation time constants. K_V currents have been postulated to play a role in determining the level of resting membrane potential in a number of different vascular smooth muscle preparations (Gelband & Hume, 1995; Post et al, 1995; Yuan, 1995). Therefore, the activation of K_V currents also could counteract the $PGF_{2\alpha}$-induced vasoconstriction.

Our results suggest that the mechanism of vasoconstriction by $PGF_{2\alpha}$ may not be involved via the depolarization of membrane potentials and the inhibition of K_{Ca} currents or K_V currents in rabbit middle cerebral arterial smooth muscle cells and support the idea that these alterations by $PGF_{2\alpha}$, at least in part, may lead to attenuate or counteract vasoconstriction by $PGF_{2\alpha}$ in middle cerebral artery.

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