Kidney International, Vol. 64 (2003), pp. 199–207

Modulation of Ca^{2+}-activated K^+ channel in renal artery endothelium in situ by nitric oxide and reactive oxygen species

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Modulation of Ca^{2+}-activated K^+ channel in renal artery endothelium in situ by nitric oxide and reactive oxygen species.

Background. Endothelium-derived nitric oxide and reactive oxygen species (ROS) have been proposed to regulate vascular tone by complex mechanisms, including the modulation of ion channel function. In endothelial function itself, activation of Ca^{2+}-activated K^+ channels (K_{Ca}) plays a crucial role by inducing hyperpolarization, which promotes membrane potential-driven Ca^{2+} influx and Ca^{2+}-dependent synthesis of vasodilatory factors. In the present study, we tested whether nitric oxide and ROS modulate endothelial K_{Ca} function.

Methods. By employing the patch-clamp technique in endothelium of porcine renal arteries in situ, we identified a large-conductance Ca^{2+}-activated K^+ channel (big K^+ channel, BK_{Ca}) with a conductance of 297 ± 6 pS.

Results. Channel activity was strongly controlled by the membrane potential and the cytosolic Ca^{2+} concentration (EC_{50} 3.1 ± 0.5 μmol/L Ca^{2+} at 0 mV). Channel activity was inhibited by Ba^{2+} and iberiotoxin. At submicromolar $[Ca^{2+}]_i$, nitric oxide induced a dose-dependent stimulation of BK_{Ca} activity with a 10-fold increase at the highest dose tested (1 μmol/L). A similar stimulation was achieved by the nitric oxide donors, sodium nitroprusside (SNP), and diethylamine nitric oxide complex (DEA-NO). In contrast, ROS and, in particular, hydrogen peroxide (H_2O_2) led to dose-dependent inactivation of BK_{Ca} with an IC_{50} of 80 ± 6 nmol/L and 1.1 ± 0.4 μmol/L, respectively. In isolated porcine renal arteries, bradykinin-induced vasodilation was significantly reduced by either iberiotoxin or H_2O_2.

Conclusion. Direct stimulation of endothelial BK_{Ca} by nitric oxide might represent a novel mechanism of autocrine regulation of endothelial function and points to a positive feedback mechanism by promoting hyperpolarization and nitric oxide production itself. The ROS-induced inhibition of BK_{Ca} could be part of the cellular mechanisms by which ROS impairs endothelium-dependent vasodilation.

The endothelium plays a pivotal role in the regulation of vascular tone [1]. In response to humoral factors or hemodynamic forces endothelial cells release vasodilating factors, including nitric oxid [2], prostacyclin (PGI_2) [3], as well as an endothelium-derived hyperpolarizing factor (EDHF) [4]. In contrast, reactive oxygen species (ROS) generated as by-products of oxydative metabolism have been proposed to promote vasoconstriction and have been associated with cardiovascular diseases, such as hypertension [5–8].

In the control of endothelial function itself, activation of Ca^{2+}-activated K^+ channels (K_{Ca}) induces endothelial hyperpolarization, which provides the electrochemical driving force for Ca^{2+} influx that is required for an adequate Ca^{2+}-dependent synthesis of vasodilatory factors [9]. This is further underlined by the observation that blockade of K_{Ca} and especially the large-conductance K_{Ca} (BK_{Ca} or maxi K) completely attenuates flow-induced vasodilation [10, 11]. However, thus far, it is not clearly understood how activation of endothelial BK_{Ca} contributes to vasodilation as BK_{Ca} is strongly active only upon depolarization and requires a substantial increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), especially at negative resting membrane potentials. However, increases in endothelial $[Ca^{2+}]_i$ (<1 μmol/L) in response to hemodynamic or humoral stimulation [12] are presumably not sufficient to induce a considerable channel activation. Thus, it is likely that other intracellular second messengers in addition to $[Ca^{2+}]_i$ costimulate endothelial BK_{Ca} activity.

Several intracellular second messengers besides Ca^{2+} have been shown to regulate the BK_{Ca} in vascular smooth muscle cells (VSMC) [13, 14]. For instance, cyclic guanosine monophosphate (cGMP) stimulates channel activity presumably via BK_{Ca} phosphorylation through cGMP-dependent protein kinases [14]. Moreover, nitric oxide independently of nitric oxide–mediated cGMP generation has been reported to directly activate a BK_{Ca} in VSMC [15].

Also ROS have been proposed to be regulators of K^+ channel function in various tissues [16]. For instance, an ATP-sensitive K^+ channel (K_{ATP}) in the heart was activated by ROS challenging, whereas voltage-gated K^+ channels in blood cells are inhibited [17, 18]. Regarding

Key words: endothelium, renal artery, K^+ channels, nitric oxide, reactive oxygen species.

Received for publication June 24, 2002
and in revised form January 27, 2003
Accepted for publication February 24, 2003

vascular cells, inconsistent (i.e., inhibitory as well as stimulatory) effects of ROS on the function of K^+ channels and, in particular, on the function of endothelial K_{Ca} have been reported [19, 20]. Thus, it is still elusive how ROS modulate endothelial K^+ channel functions.

Therefore, we performed a study to identify and characterize BK_{Ca} in renal artery endothelium and tested the regulation of BK_{Ca} by nitric oxide and ROS. Especially, we thought it important to characterize BK_{Ca} channel function in intact endothelium in situ to avoid alterations of BK_{Ca} function due to cell isolation and culturing [21–23].

METHODS

Preparation of renal arteries

Porcine kidneys were obtained from the local abattoir. Renal arteries were excised and rinsed free of blood. Segments of 4 to 6 mm were cleaned of connective tissue and carefully cut open longitudinally to avoid any damage to the luminal surface. Tissue segments were immediately used for patch-clamp experiments.

Patch-clamp experiments

For patch-clamp experiments, thin vessel slices of about 2 mm^2 were fixed on a holding glass capillary and placed in the experimental chamber mounted on the stage of an inverted microscope (Zeiss Axiovert 135, Deisenhofen, Germany) with the luminal surface facing the bath solution. This enabled a direct access to the endothelium with the patch-clamp pipette. After forming a gigaohm seal, single-channel currents in cell-attached and inside-out patches were recorded with an EPC-9 patch-clamp amplifier (HEKA, Lambrecht, Germany) using voltage steps from −100 to 100 mV and were low-pass filtered (−3 dB, 1000 Hz) at a sampling time of 0.5 msecond [24, 25]. Patch pipettes were pulled from borosilicate glass capillaries with 0.3 mm wall thickness and had a tip resistance of 5 to 7 mΩ in symmetric KCl solutions.

If not otherwise stated, the patch pipette solution contained (in mmol/L) 140 KCl, 1 $CaCl_2$, 1 $MgCl_2$, and 10 HEPES (pH 7.4). The standard bath solution contained (in mmol/L) 140 NaCl, 4.3 KCl, 1 $MgCl_2$, 1 $CaCl_2$, and 10 HEPES (pH 7.4). The KCl bath solution contained (in mmol/L) 140 KCl, 1 $CaCl_2$, 1 $MgCl_2$, and 10 HEPES (pH 7.4). In experiments using Ca^{2+}-buffered bath solutions, the free Ca^{2+} concentration was adjusted with appropriate amounts of $CaCl_2$ and ethyleneglycol tetraacetic acid (EGTA) or N-(2-hydroxyethyl)ethylenediamine-N,N,N-triacetic acid (HEDTA) according to Fabiato and Fabiato [26].

Nitric oxide solutions were prepared according to Bolotina et al [15]. The nitric oxide gas was bubbled into 8 mL of standard bath solution for 20 minutes to yield a saturated stock solution containing nominally 1 mmol/L nitric oxide. The pH was adjusted to pH 7.2. To yield nitric oxide concentrations from 10 nmol/L to 1 μmol/L appropriate amounts of this stock solution were added to the bath solution containing an additional 1 μmol/L methylene blue, a blocker of guanylate cyclase [15]. Diethylamine nitric oxide complex (DEA-NO), 10 nmol/L, 0.1 μmol/L, and 1 μmol/L and sodium nitroprusside (SNP), 10 μmol/L were freshly prepared and immediately used.

For preparation of bath solutions containing rose bengal, appropriate amounts of a 1 mmol/L stock solution were added and kept in darkness until use. ROS generation was elicited by light exposure [17]. H_2O_2 solutions (1 nmol/L to 1 mmol/L) were prepared by adding appropriate amounts of a 30% stock solution and adjusted to pH 7.2.

In an additional set of experiments, the endothelium was pretreated with the selective BK_{Ca} opener NS1619 (25 μmol/L) [27] followed by cell-attached patch-clamp experiments using patch pipettes that were backfilled with a pipette solution containing either 1 μmol/L nitric oxide or 100 μmol/L H_2O_2 to modulate BK_{Ca} activity at the extracellular face of the channel.

All experiments were performed at 35°C.

In the displayed current traces, single-channel currents carried by cations moving from the extracellular to the intracellular side are depicted as downward (negative) currents. The given potential value resembles the patch-clamp potential and the sign of the potential refers to the cytosolic side. Data analysis was performed as described previously [24].

Pressure myograph experiments

Renal interlobar arteries were freshly dissected from porcine kidney and segments of 4 to 5 mm in length were cannulated with micropipettes in an experimental chamber mounted on the stage of a Zeiss Axiovert 100. Vessel diameter was continuously monitored with a video camera. The bath and perfusion solution contained (in mmol/L) 145.0 NaCl, 1.2 NaH_2PO_4, 4.7 KCl, 1.2 $MgSO_4$, 2.0 $CaCl_2$, 5.0 glucose, 2.0 pyruvate, and 3.0 3-[N-morpholino] propane sulfonic acid (MOPS) buffer along with 1 g 100 mL^{-1} bovine serum albumin (BSA) (pH 7.4 at 37°C). Renal interlobar arteries were pressurized to 80 mm Hg with a pressure myograph system (J.P. Trading P100, Aarhus, Denmark) and continuously perfused at a flow rate of 0.6 mL/min^{-1} and a constant intraluminal pressure. After an initial equilibration period for 40 minutes, renal interlobar arteries were preconstricted with the thromboxane mimetic U46619 (1 μmol/L) in the bath solution. After development of a stable tone, vasodilatory responses were determined by perfusion with 1 nmol/L bradykinin alone or with 100 μmol/L H_2O_2 or with 50 nmol/L iberiotoxin, a selective BK_{Ca} blocker. In an additional subset of experiments, the K^+ channel opener NS1619 (25 μmol/L) was applied intraluminally alone

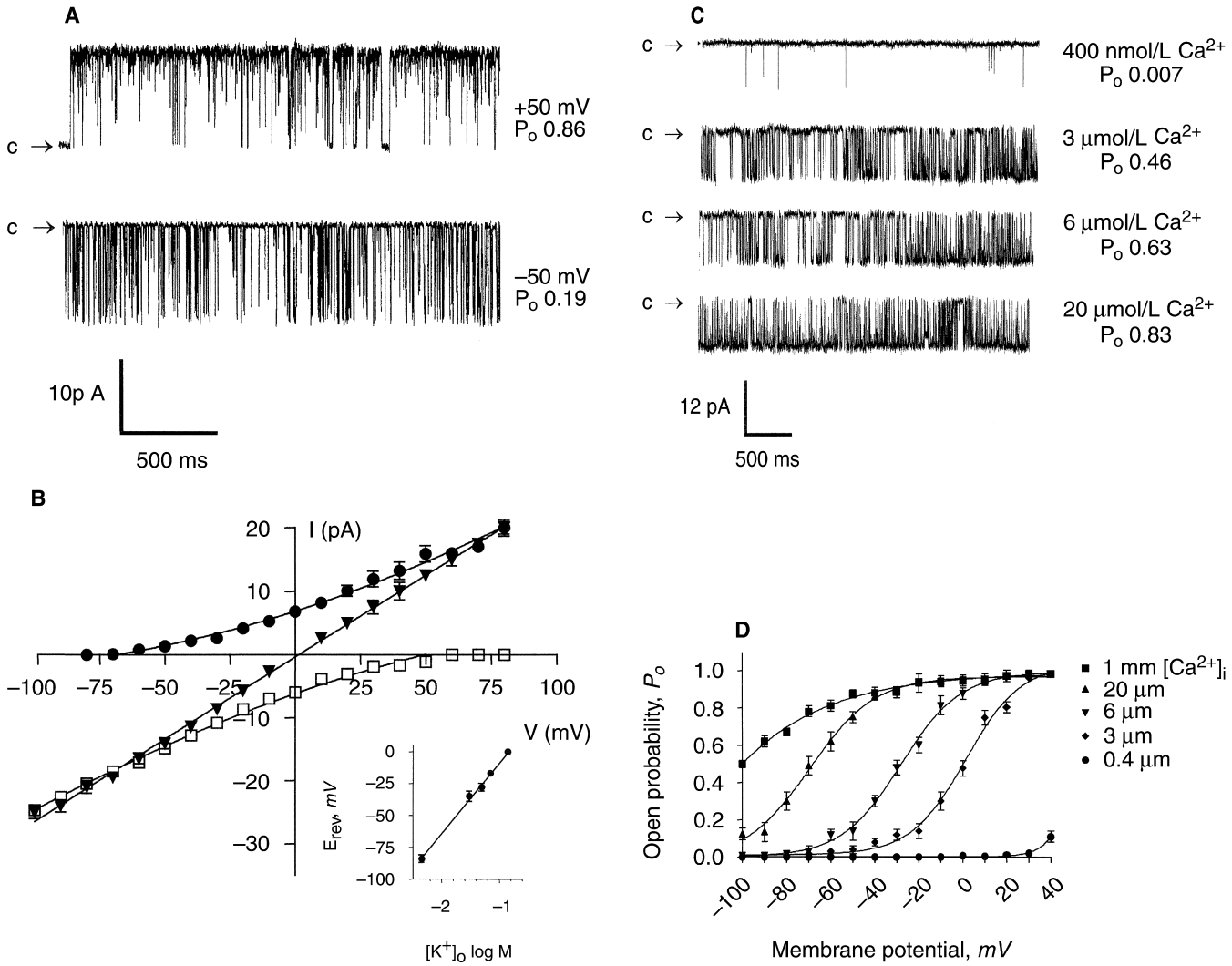

Fig. 1. Various readings of currents. (*A*) Inward (down) and outward (up) BK_{Ca} currents in an inside-out patch (symmetric 140 mmol/L K^+ and 6 µmol/L $[Ca^{2+}]_{free}$). c → denotes closed state of the channel. (*B*) Current voltage relationship of BK_{Ca} in symmetric 140 mmol/L K^+ (▼), 140 mmol/L K^+ pipette and 4.5 mmol/L K^+ bath (□), and 4.5 mmol/L K^+ pipette and 140 mmol/L K^+ bath (●) in inside-out patches. Inlet: K^+ selectivity of BK_{Ca}-currents. Each point represents reversal potentials (E_{rev}, $N = 3$ to 6) of BK_{Ca} currents at the designated $[K^+]_o$. Straight line indicates linear regression to the data (slope 56 mV). (*C*) Representative traces showing BK_{Ca} currents in inside-out patch-clamp experiments at holding potential of −40 mV and at cytosolic $[Ca^{2+}]_{free}$ as indicated. (*D*) Relationship between open probability (P_o) and cytosolic $[Ca^{2+}]_{free}$ at holding potentials ranging from −100 mV to +100 mV. Data are given as mean ± SE ($N = 5$). Data were fitted to the Boltzmann equation.

or in combination with 1 nmol/L bradykinin. Diameter changes were expressed as a percentage of the maximal dilation measured in response to 1 µmol/L SNP.

Statistical analysis

Statistical analysis was performed using the Wilcoxon rank-sum test. P values of <0.05 were considered significant. If not otherwise stated, data are given as mean ± SE.

Solutions and drugs

DEA-NO was obtained from Alexis Corp. (North Vancouver, British Columbia, Canada) and NS1619 from TOCRIS (Ballwin, MO, USA). All other chemicals and toxins were obtained from Sigma Chemical Co., Deisenhofen, Germany.

RESULTS

BK_{Ca} in endothelium of porcine renal arteries in situ

In in situ patch-clamp experiments of the unstimulated endothelium, cell-attached patches usually showed no spontaneous channel activity. After excising the patch and exposing the cytosolic face of the membrane patch to the high $[Ca^{2+}]$ bath solution, a K_{Ca} channel activity was observed in 156 of 565 inside-out patches (28%). This K_{Ca} exhibited the characteristics of BK_{Ca}. Single channel currents exhibited no distinct inward rectification and showed voltage sensitivity with increasing activity at depolarizing membrane potentials (Fig. 1A). Mean channel conductance was 297 ± 6 pS ($N = 30$) in symmetric KCl solution and the channel was highly selective for

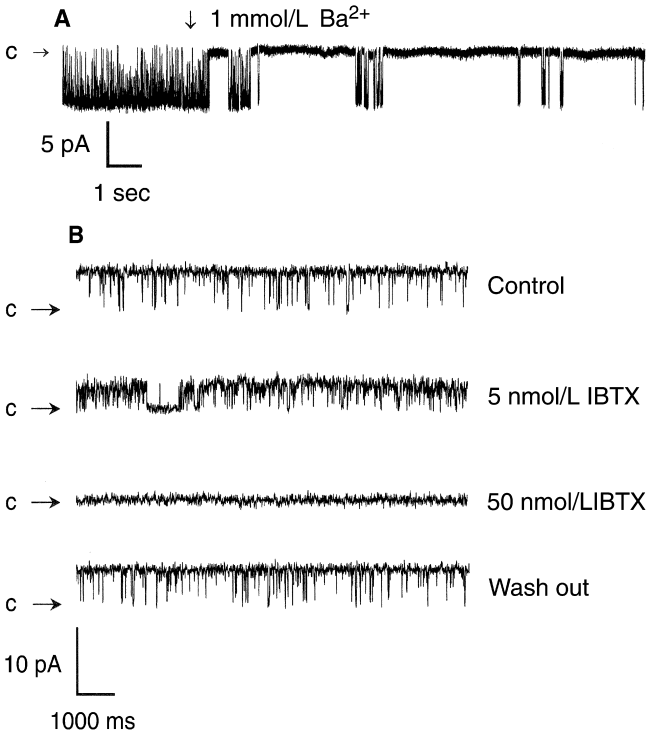

Fig. 2. Inhibition of BK_{Ca} channel activity by 1 mmol/L barium (*A*) at a holding potential of 0 mV in inside-out patch-clamp experiments, and by 5 nmol/L and 50 nM iberiotoxin (*B*) at a holding potential of +10 mV in outside-out patch-clamp experiments. c → denotes closed state of the channel.

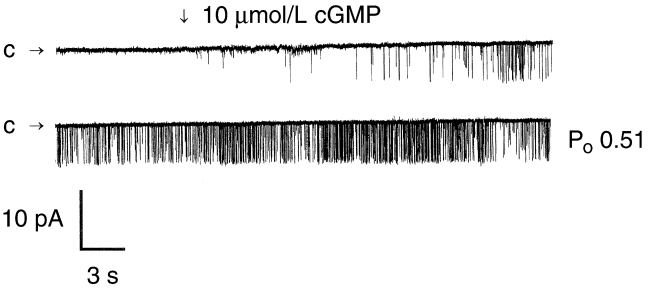

Fig. 3. BK_{Ca} activation by 10 μmol/L cyclic guanosine monophosphate (cGMP) at a holding potential of 0 mVin inside-out patch-clamp experiments. The $[Ca^{2+}]_{free}$ at the cytosolic face of the membrane was 400 nmol/L. c → denotes closed state of the channel.

K^+ (Fig. 1B). BK_{Ca} activity strongly depended on the $[Ca^{2+}]_i$ at the cytosolic face of the channel with EC_{50} values of 3.1 ± 0.5 μmol/L, 6.3 ± 1.3 μmol/L, and 23.7 ± 3 μmol/L cytosolic Ca^{2+} at 0 mV, −30 mV, and −70 mV, respectively ($N = 6$) (Fig. 1 C and D).

In inside-out patches, channel activity was completely blocked by Ba^{2+} (1 mmol/L) at positive membrane potentials and showed a potential-dependent blocking characteristic of Ba^{2+} at negative membrane potentials (Fig. 2A). The highly selective BK_{Ca} inhibitor iberiotoxin (50 nmol/L) as well as charybdotoxin (data not shown) completely blocked the channel activity in outside-out patches (Fig. 2B).

Stimulation of BK_{Ca} by cGMP, nitric oxide, and nitric oxide donors

In inside-out patches at a membrane potential of −40 mV and in the presence of submicromolar $[Ca^{2+}]_i$ (400 nmol/L) channel activity of BK_{Ca} was low (P_o <0.05). The addition of 10 μmol/L cGMP to the cytosolic face of the membrane resulted in a marked increase in channel activity reaching a maximal P_o of 0.42 ± 0.10 after about 50 seconds at 0 mV ($N = 6$) (Fig. 3). Nitric oxide stimulated dose-dependently channel activity to a maximal P_o of 0.32 ± 0.08 at 0 mV ($N = 5$) within 30 seconds at the highest dose tested (1 μmol/L) (Fig. 4 A and B). After 90 seconds, BK_{Ca} channel activity returned to basal levels even when no washout was performed. Similar to the stimulatory effect of nitric oxide, the nitric oxide donor DEA-NO was also effective in stimulating BK_{Ca} activity in a dose-dependent manner. An increase in channel activity occurred within 15 seconds and reached a maximal P_o of 0.85 ± 0.10 ($N = 9$) after 60 seconds at the highest dose tested (1 μmol/L). After 3 minutes, channel activity returned to basal level again. A substantial stimulation of channel activity occurred already at a low concentration of DEA-NO (10 nmol/L) and a maximal stimulation of BK_{Ca} was achieved by 1 μmol/L DEA-NO (Fig. 4C). The calculated EC_{50} for DEA-NO stimulation of channel activity was 90 ± 10 nmol/L. In experiments using 10 μmol/L SNP as nitric oxide donor, stimulation of channel activity occurred in a comparable fashion with an increase in channel activity to a P_o of 0.85 ± 0.10 ($N = 6$) after about 60 seconds (Fig. 4D).

Inhibition of BK_{Ca} by ROS

In the presence of a higher 6 μmol/L $[Ca^{2+}]_i$ at the cytosolic face of the membrane to yield considerable channel activation, photo-activated rose bengal dose dependently decreased BK_{Ca} channel activity in a membrane potential range of −80 mV to +40 mV (IC_{50}, 80 ± 6 nmol/L at −20 mV, $N = 5$) (Fig. 5 A and B). Inhibition started within 2 to 5 seconds after light exposure and was complete after about 60 seconds. Channel inhibition persisted after washout of rose bengal.

In another series of experiments, H_2O_2 in concentrations ranging from 1 nmol/L to 1 mmol/L led to comparable inactivation of BK_{Ca} with an IC_{50} of 1.1 ± 0.4 μmol/L at −20 mV, $N = 5$) Fig. 5 C and D). In the outside-out patch-clamp configuration BK_{Ca} was similarly inactivated when H_2O_2 was applied to the extracellular side of the membrane ($N = 3$, data not shown). Similar to BK_{Ca} inactivation by rose bengal, H_2O_2-induced inhibition of channel activity persisted after washout of H_2O_2. In both sets of experiments, channel inactivation was apparently not

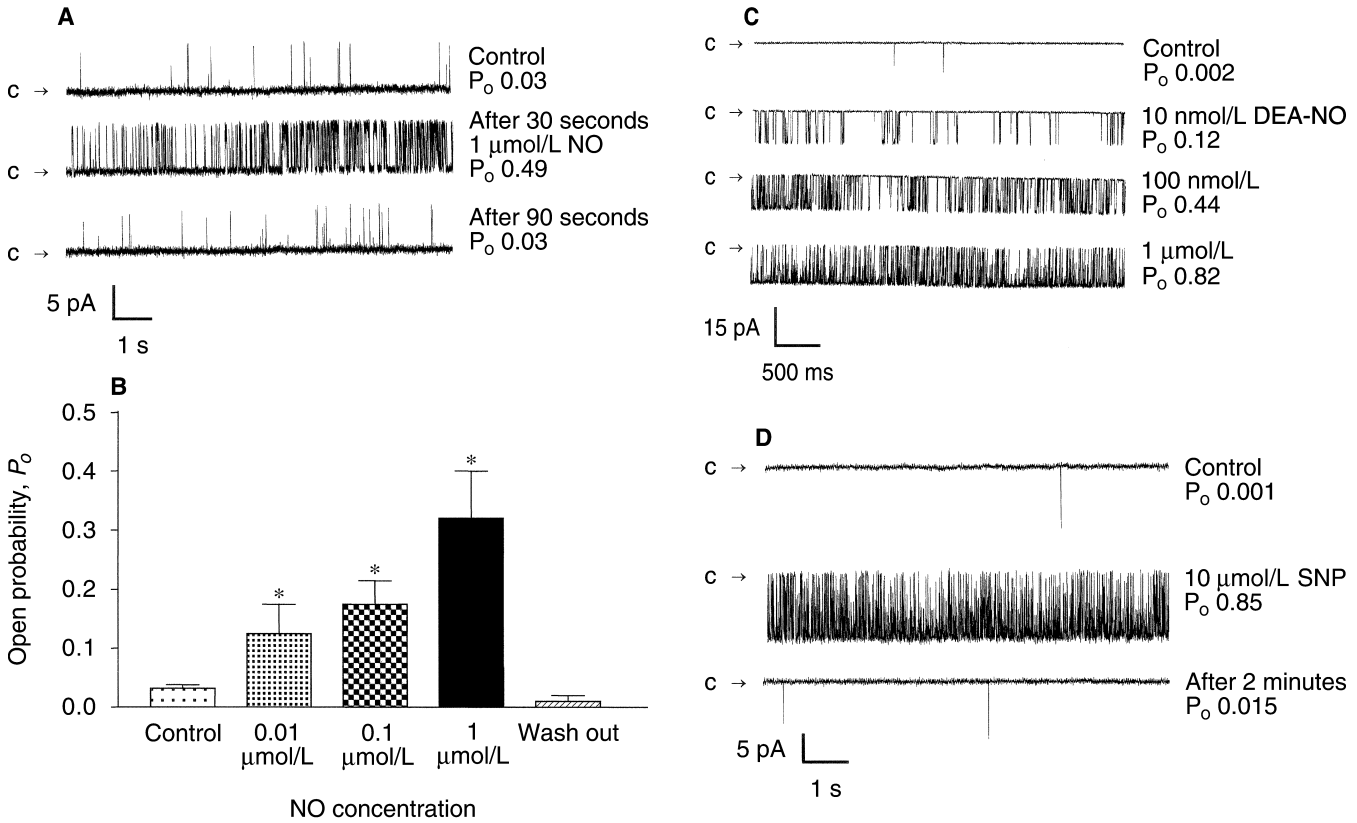

Fig. 4. BK_{Ca} activation. (*A*) BK_{Ca} activation by 1 μmol/L nitric oxide (NO) at a holding potential of +20 mV in inside-out patch-clamp experiments. (*B*) Concentration-dependent activation of BK_{Ca} by nitric oxide ($N = 4$). *$P < 0.05$. (*C*) BK_{Ca} activation by 10 μmol/L sodium nitroprusside (SNP) at a holding potential of −40 mV. (*D*) BK_{Ca} activation by 10 nmol/L to 1 mmol/L diethylamine nitric oxide complex (DEA-NO) at a holding potential of −20 mV. c → denotes closed state of the channel.

due to nonspecific rundown as channel activity remained stable over 5 minutes in the absence of ROS or H_2O_2.

In another set of cell-attached patches experiments, we tested whether application of nitric oxide or H_2O_2 to the extracellular face of the channel similarly modulates channel activity. To induce channel activation prior to nitric oxide or H_2O_2 challenge, the endothelium was pretreated with the selective BK_{Ca} opener NS1619 (25 μmol/L). As shown in Figure 6, this pretreatment yielded a substantial and durable increase of BK_{Ca} activity when compared to low channel activity in the absence of NS1619. When 1 μmol/L nitric oxide was applied to the extracellular face of channel via a backfilled patch pipette, the slow diffusion of nitric oxide to the cell-attached patch resulted in further increase of channel activity within 1 to 2 minutes (Fig. 6). In contrast, application of 100 μmol/L H_2O_2 to the extracellular face of the channel resulted in a considerable decrease of channel activity within 1 minute (Fig. 6).

Effect of BK_{Ca} modulators on endothelium-dependent vasodilation

Guided by the results from our patch-clamp studies, we then tested to which extent activation of endothelial BK_{Ca} contributes to endothelium-dependent vasodilation and whether modulation of BK_{Ca} by channel inhibitors or activators alters vasodilatory responses. As shown in Figure 7, intraluminal application of bradykinin (1 nmol/L) to preconstricted porcine renal interlobar arteries induced endothelium-dependent vasodilation ($N = 4$). This vasodilatory response was almost eliminated when bradykinin was applied together with 100 nmol/L iberiotoxin ($N = 4$). Importantly, this inhibitory effect elicited by selective BK_{Ca} inhibition was also mimicked by a combination of bradykinin and 100 μmol/L H_2O_2 ($N = 4$) (Fig. 7), suggesting that inhibition of BK_{Ca} by H_2O_2 is equally effective to block the BK_{Ca}-dependent component of endothelium-dependent vasodilation.

We then tested whether stimulation of endothelial BK_{Ca} activity by the selective BK_{Ca} opener NS1619 induces opposite effects (i.e., vasodilation). Intraluminal application of NS1619 (25 μmol/L, $N = 4$) alone did not induce considerable vasodilation (Fig. 7). However, a combined stimulation with bradykinin (1 nmol/L) and NS1619 (25 μmol/L, $N = 4$) resulted in a steep increase in the vasodilatory response (Fig. 7). This effectiveness of NS1619 in combination with bradykinin to induce vasodilation indicates that a bradykinin-mediated mobi-

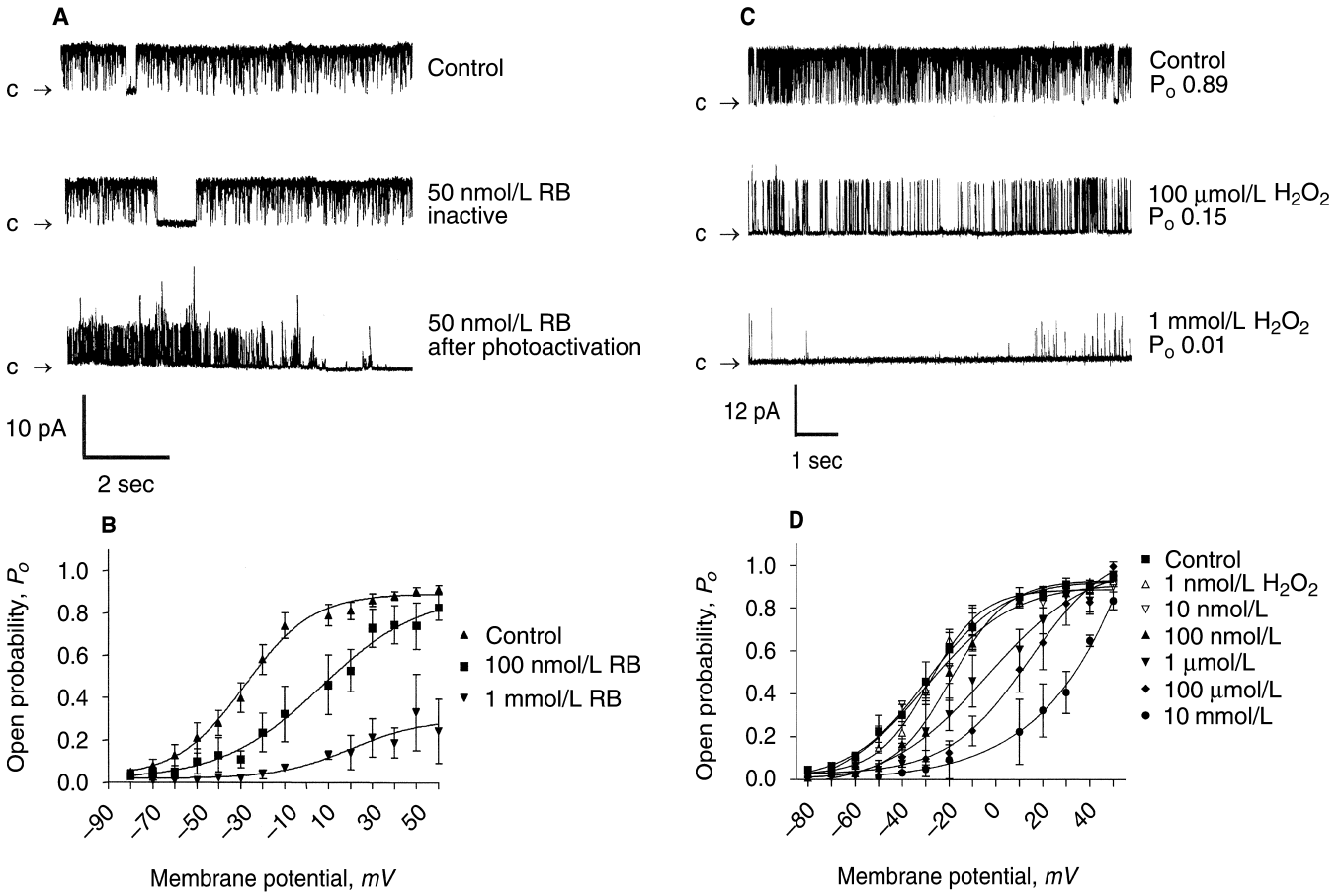

Fig. 5. Various readings of inactivation currents and relationship correlations. (*A*) BK_{Ca} inactivation by reactive oxygen species (ROS) generated by photo-activated rose bengal (RB) at a holding potential of +20 mV. (*B*) Relationship between open probability of BK_{Ca} and concentration of RB at holding potentials ranging from −80 to +60 mV. Data are given as mean ± SE ($N = 5$). (*C*) BK_{Ca} inactivation by H_2O_2 at a holding potential of +20 mV. (*D*) Relationship between open probability of BK_{Ca} and concentration of H_2O_2. Data are given as mean ± SE ($N = 5$). Data were fitted to the Boltzmann equation. All experiments were performed in the inside-out patch-clamp configuration. c → denotes closed state of the channel.

lization of intracellular Ca^{2+} and subsequent increase of BK_{Ca} channel activity above basal levels is required for efficient channel activation by NS1619. Such a requirement of a costimulation by Ca^{2+} for an effective channel activation by NS1619 has also been described for BK_{Ca} in neuronal cells and vascular smooth muscle [27, 28].

Taken together, these results from our pressure myograph experiments demonstrate that activation of endothelial BK_{Ca} is an important signaling mechansim in agonist-induced endothelium-dependent vasodilation and that H_2O_2 impairs vasodilation presumably by blocking endothelial BK_{Ca}.

DISCUSSION

In the present study, we characterized a large-conductance K_{Ca} (BK_{Ca}) in porcine renal artery endothelium in situ. With respect to electrophysiologic properties, such as single-channel conductance, Ca^{2+}-dependent activation, voltage sensitivity, and toxin sensitivity, the endothelial BK_{Ca} in porcine renal arteries resembled the characteristics of endothelial BK_{Ca} previously identified in porcine endocardial endothelium [29], in human umbilical vein endothelial cells (HUVEC) [23], as well as in a HUVEC line (EA.hy926) [30].

In the present study, we demonstrated the stimulation of endothelial BK_{Ca} activation by cGMP, which was similar to the cGMP-induced stimulation of BK_{Ca} in VSMC [14, 31, 32]. This indicates that cGMP, presumably generated by nitric oxide–induced stimulation of guanylate cyclase [13], is also a major intracellular regulator of endothelial BK_{Ca}.

In addition to BK_{Ca} regulation by cGMP, BK_{Ca} has also been reported to be activated by nitric oxide in a more direct fashion in VSMC [15, 33–36]. However, a more recent study failed to demonstrate such a direct stimulatory effect of nitric oxide on BK_{Ca} in cultured endothelial cells [23]. In the present study investigating BK_{Ca}

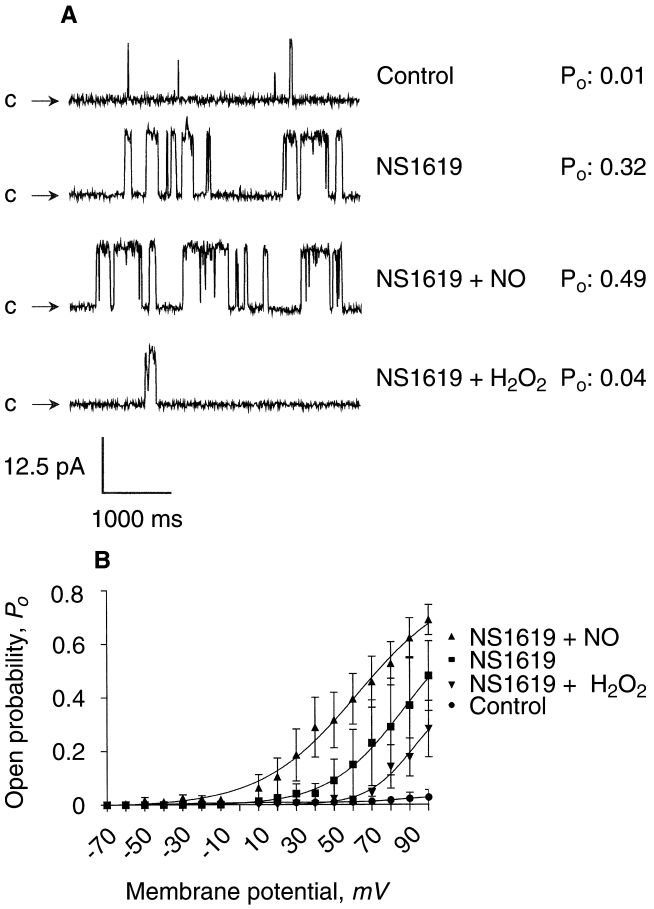

Fig. 6. Activation of BK_{Ca} with various agents. (*A*) BK_{Ca} activation by 25 μmol/L NS1619 alone or in combination with 1 μmol/L nitric oxide (NO) or 100 μmol/L H_2O_2 at a holding potential of +80 mV in the cell-attached patch-clamp configuration. (*B*) Open probability (P_o) of BK_{Ca} in the presence of 25 μmol/L NS1619, 25 μmol/L NS1619 and 1 μmol/L nitric oxide, or 25 μmol/L NS1619 and 100 μmol/L H_2O_2. Data are given as mean ± SE ($N = 5$).

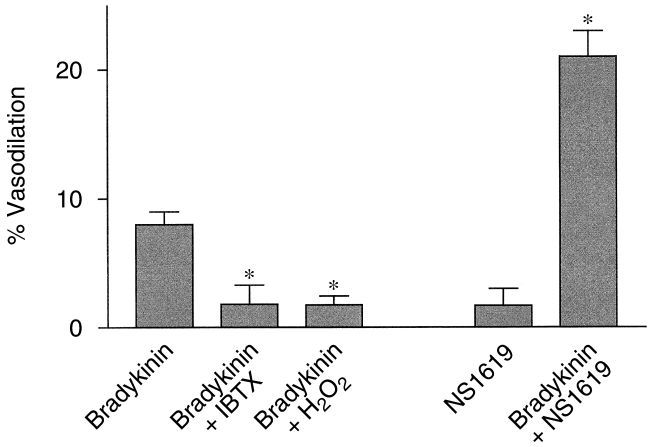

Fig. 7. Pressure myograph experiments showing vasodilatory responses to 1 nmol/L bradykinin alone or in combination with 25 μmol/L NS1619, 50 nmol/L iberiotoxin (IBTX), or 100 μmol/L H_2O_2. Vasodilatory responses are given as percentage of the maximal dilation measured in response to 1 μmol/L sodium nitroprusside (SNP). Data are given as mean ± SE ($N = 4$). *$P < 0.05$.

in endothelium of porcine renal artery in situ, we observed a direct stimulatory action of nitric oxide and nitric oxide donors (DEA-NO and SNP) on BK_{Ca}-channel activity. In our hands, this stimulatory effect was not due to alterations of BK_{Ca} function as a result of changes in pH [37] since the pH of the nitric oxide–containing solutions was strictly controlled. It is also unlikely that channel stimulation was mediated by a residual activity of soluble guanylate cyclase and protein kinase G (PKG) in the isolated membrane patch as a blocker of guanylate cyclase failed to prevent channel stimulation.

As nitric oxide–mediated stimulation of the BK_{Ca} occurred at either low cytosolic Ca^{2+} concentrations, which are inefficient to considerably stimulate BK_{Ca}, or after pharmacologic preactivation of the channel, it is tempting to speculate that nitric oxide might represent an additional and important cofactor for BK_{Ca} activation in the endothelium. In keeping with this idea, generation of nitric oxide and concomitant increase in BK_{Ca} channel function upon humoral or hemodynamic stimulation may represent a positive feedback mechanism in endothelial autoregulation. This is based on the assumption that BK_{Ca}-mediated hyperpolarization promotes membrane-potential driven Ca^{2+} influx and subsequent Ca^{2+}-dependent nitric oxide synthesis [9]. Moreover, endothelial hyperpolarization induced by nitric oxide–mediated BK_{Ca} activation may also stimulate EDHF-dependent vasodilation given that endothelial hyperpolarization is directly propagated to the underlying VSMCs via myoendothelial coupling leading to closure of voltage-gated Ca^{2+} channels in the vascular smooth muscle and subsequent vasodilation [38, 39]. Taking together this autocrine stimulatory action of nitric oxide mediated via cyclic nucleotides or even directly via nitric oxide itself might be especially important when humoral or flow-induced intracellular Ca^{2+} signals [12] do not reach micromolar concentrations, which are required to efficiently activate BK_{Ca}.

One major finding of the present study was that, in contrast to nitric oxide, intracellular as well as extracellular challenge of endothelial BK_{Ca} with H_2O_2 and ROS resulted in a dose-dependent and irreversible channel inactivation. Such an inhibition of K_{Ca} single-channel function by H_2O_2 has also been reported for another type of K_{Ca}, the intermediate-conductance K_{Ca} (iK), in bovine aortic endothelial cells [19]. However, our finding is in contrast to a more recent report that described an activation of BK_{Ca} whole-cell currents through H_2O_2 in cultured endothelial cells [10]. This inconsistency might be a consequence of distinct experimental approaches. In particular, it has been demonstrated, that extraluminally applied H_2O_2 induces leak conductances for Ca^{2+} due to membrane damage, which leads to a nonspecific and

substantial rise in $[Ca^{2+}]_i$ [40–42]. Therefore, such a stimulatory effect on whole-cell currents through BK_{Ca} might be a result of such H_2O_2-induced intrusion of Ca^{2+}, which presumably overrules the direct inhibitory effects of H_2O_2 on channel activity.

Regarding the mechanisms by which ROS promote vasoconstriction, it has been proposed that scavanging of nitric oxide by ROS and the formation of peroxynitrite may be one major mechanism [43, 44]. Our present finding of ROS-induced inhibition of BK_{Ca} indicates that ROS impair endothelial hyperpolarization and thereby disturb endothelium-dependent vasodilation. This conclusion is further supported by the present results showing that H_2O_2 impaired endothelium-dependent vasodilation in a similar extent as a selective BK_{Ca} blocker.

Therefore, inhibition of endothelial K_{Ca} by ROS might be an important additional mechanism by which ROS exert their vasoconstrictory actions. Furthermore, this mechanism might be especially important in disease states such as hypertension which are associated, at least in part, with an excessive generation of ROS [5, 6, 8].

CONCLUSION

BK_{Ca} activation by nitric oxide in a cGMP-dependent or independent fashion might represent an autocrine regulation of endothelial cell function and points to a positive feedback mechanism of the nitric oxide production itself. On the other hand, inhibition of BK_{Ca} by ROS could be part of the cellular mechanism by which ROS impair endothelium-dependent vasodilation.

ACKNOWLEDGMENTS

This work was supported by the Deutsche Forschungsgemeinschaft (FOR 341/1/5/7, HO 1103/2-4, GRK 276/2).

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