Nitric oxide increases the activity of the apical 70-pS K⁺ channel in TAL of rat kidney

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Lu, Ming, Xiaohong Wang, and Wenhui Wang. Nitric oxide increases the activity of the apical 70-pS K⁺ channel in TAL of rat kidney. Am. J. Physiol. 274 (Renal Physiol. 43): F946–F950, 1998.—We have previously shown that nitric oxide (NO) mediates the stimulatory effect of angiotensin II on the apical 70-pS K⁺ channel in the thick ascending limb (TAL) of Henle's loop of the rat kidney (12). In the present study, we used the patch-clamp technique to examine the effects of NO on the 70-pS K⁺ channel. Addition of 10 µM S-nitroso-N-acetylpenicillamine (SNAP), a NO donor, increased the channel activity in cell-attached patches. In contrast, application of 100 µM N⁵-nitro-L-arginine methyl ester (L-NAME), an inhibitor of nitric oxide synthase (NOS), reduced the channel activity by 75 ± 7%. The effect of L-NAME was the result of inhibiting NOS, since N⁵-nitro-L-arginine methyl ester (L-NNAME), which does not block NOS activity, had no effect on the channel activity. In addition, the effect of L-NAME was abolished in the presence of 1 mM L-arginine or by addition of 10 µM SNAP, further supporting the role of NO. Finally, the L-NAME-induced inhibition was also reversed by adding 8-bromoguanosine 3',5'-cyclic monophosphate (8-BrcGMP). That the effect of NO is mediated by the cGMP-dependent pathway is also suggested by experiments in which inhibition of guanylate cyclase abolished the effect of SNAP. Finally, 10 µM SNAP significantly increased cGMP concentration of the cell-attached patches, as measured with ELISA. We conclude that NO is involved in regulating the activity of the apical 70-pS K⁺ channel in the TAL of the rat kidney.

THE THICK ASCENDING LIMB (TAL) reabsorbs 20–25% of the filtered Na⁺ load and plays a key role in the urinary concentrating mechanism (5, 7). Active NaCl reabsorption takes place by Na⁺ transport across the apical membrane by a Na-K-Cl cotransporter and subsequently by active Na⁺ movement via the Na-K-ATPase (5, 7). The function of the TAL is regulated by several hormones and autacoids (5, 7, 8, 26). In addition to the Na-K-Cl cotransporter, the apical K⁺ channels play a key role in K⁺ recycling across the apical membrane, a process which is essential for maintaining the function of the Na-K-Cl cotransporter and thus for the Na⁺ transport in the TAL. First, K⁺ recycling across the apical membrane maintains the transepithelial current flow, since K⁺ exit hyperpolarizes the cell membrane and provides the driving force for Cl⁻ diffusion across the basolateral membrane. Second, K⁺ recycling across the apical membrane potentiates the lumen-positive potential, the driving force for paracellular NaCl reabsorption (7). Third, K⁺ recycling provides an adequate K⁺ supply to the Na-K-Cl cotransporter in the cortical TAL, where the K⁺ concentration in the lumen is at least one order of magnitude lower than those of Cl⁻ and Na⁺.

Although three types of K⁺ channels (30 pS, 70 pS, and Ca²⁺-dependent “maxi” K⁺) have been found in the apical membrane of the TAL (4, 6, 24), the 30- and 70-pS K⁺ channels are mainly responsible for the apical K⁺ conductance (24). Furthermore, our previous study has further shown that the 70-pS K⁺ channel is predominant in the TAL (25). We have also demonstrated that angiotensin II (ANG II) has biphasic effects: low concentrations of ANG II reduced, whereas high concentrations of ANG II increased, the activity of the 70-pS K⁺ channel. The stimulatory effect of ANG II is abolished in the presence of N⁵-nitro-L-arginine methyl ester (L-NAME), an inhibitor of nitric oxide synthase (NOS), suggesting that NO may be involved in mediating the effect of ANG II. In the present study, we have used the patch-clamp technique to explore further the role of NO in regulating the apical 70-pS K⁺ channel.

METHODS
Preparation of rat TAL. Cortical TAL (CTAL) and medullary TAL (MTAL) were isolated from kidneys of pathogen-free Sprague-Dawley rats (Taconic Farms, Germantown, NY), as described previously (24). The tubules were placed onto a 5-mm × 5-mm cover glass coated with Cell-Tak (Collaborative Research, Bedford, MA) to immobilize the tubules and superfused with HEPES-buffered NaCl solution. The TAL was cut open with a sharpened micropipette to expose the apical membrane. The temperature of the chamber (1,000 µl) was maintained at 37 ± 1°C by circulating warm water surrounding the chamber.

Patch-clamp technique. We used an Axon 200A patch-clamp amplifier to record channel current. The current was low-pass filtered at 1 kHz, using an eight-pole Bessel filter (902 LPF; Frequency Devices, Haverhill, MA), digitized at a sampling rate of 44 kHz using a modified Sony PCM-501ES pulse-code modulator, and stored on videotape (JVC-HR-J 400U). For analysis, data stored on the tape were collected to an IBM-compatible 486 computer (Gateway 2000) at a rate of 4 kHz and analyzed using the pCLAMP software system 6.03 (Axon Instruments, Burlingame, CA). Channel activity was defined as Nₚ₀, a product of channel number (N) and channel open probability (Pₒ). The Nₚ₀ was calculated from data samples of 60-s duration in the steady state as follows:

\[ N_{p_0} = \sum (t_1 + t_2 + \ldots + t_n) \]  

where tᵢ is the fractional open time spent at each of the observed current levels.

cGMP and protein concentration assay. TALs of total length of 10 mm were collected and incubated in a Ringer solution (300 µl) in the presence of 1 mM 3-isobutyl-1-methylxanthine at 37°C for 15 min. After adding either 10 µM S-nitroso-N-acetylpenicillamine (SNAP) or vehicle solution (DMSO) to the tubule suspension for 2 min at 37°C, experiments were terminated by addition of 0.7 ml ice-cold ethanol. The sample was frozen in liquid nitrogen and dried in a speed-vacuum.
concentrator. The residues were resuspended in 100 µl of phosphate buffer and acetylated. cGMP content was measured with a specific enzyme-linked immunosorbent assays (ELISA) (Cayman Chemical, Ann Arbor, MI).

Experimental solution and statistics. The pipette solution contained (in mM) 140 KCl, 1.8 MgCl₂, 1 EGTA, and 10 HEPES (pH 7.40). The bath solution for cell-attached patches was composed (in mM) of 140 NaCl, 5 KCl, 1.8 CaCl₂, 1.8 MgCl₂, 5 glucose, and 10 HEPES (pH 7.40), and the composition of the solution used for excised patches was the same as that in cell-attached patches, except that Ca²⁺ was 100 nM. L-NAME, D-NAME, and 8-BrcGMP were purchased from Sigma Chemical (St Louis, MO). SNAP, L-arginine, and 1H-(1,2,4)-oxadiazole(4,3-a)quinoxalin-1-one (ODQ) were obtained from Calbiochem (La Jolla, CA). The chemicals were added directly to the bath to reach the final concentration.

Data are shown as means ± SE, and the paired Student’s t-test was used to determine the significance of differences between control and experimental periods. Statistical significance was taken as P < 0.05.

RESULTS

Figure 1A is a representative recording showing the effect of SNAP, a NO donor, on the activity of the apical 70-pS K⁺ channel in a cell-attached patch. It is apparent that addition of 10 µM SNAP significantly increased the channel activity. Figure 1B is a representative fit of all-points amplitude histograms obtained under control conditions and in the presence of 10 µM SNAP, showing that SNAP increased NPo from the control value of 0.48 ± 0.05 to 1.2 ± 0.1 (n = 20). To exclude the possibility that NO donors may directly stimulate the 70-pS K⁺ channel, we examined the effect of SNAP on channel activity also in inside-out patches and found that, in inside-out patches, 10 µM SNAP had no significant effect on channel activity (data not shown).

Having demonstrated that addition of exogenous NO donors stimulates channel activity, we investigated the role of endogenous NOS in the regulation of the apical 70-pS K⁺ channel. Figure 2A shows the effect of L-NAME, an inhibitor of NOS, on channel activity in a cell-attached patch. Pipette holding potential was 0 mV, and “C” indicates channel closed level. Top, time course of experiment; 4 parts of trace are extended with a fast time scale. B: All-points amplitude histograms of channel current were made under control conditions in the presence of 100 µM L-NAME and in the presence of 10 µM SNAP + L-NAME. “C” indicates closed channel current.

Fig. 1. A: recording showing effect of 10 µM S-nitroso-N-acetylpenicillamine (SNAP) on activity of 70-pS K⁺ channel in a cell-attached patch. Channel closed level is indicated by “C,” and holding potential was 0 mV. Time course of experiment is shown at top, and 2 parts, indicated by 1 and 2, are extended to show channel activity at fast time resolution. B: All-points amplitude histograms of channel currents were obtained under control conditions and in the presence of 10 µM SNAP. Closed channel current is indicated by “C.”

Fig. 2. A: channel recording showing effect of 100 µM Nω-nitro-L-arginine methyl ester (L-NAME) on 70-pS K⁺ channel in a cell-attached patch. Pipette holding potential was 0 mV, and “C” indicates channel closed level. Top, time course of experiment; 4 parts of trace are extended with a fast time scale. B: All-points amplitude histograms of channel current were made under control conditions in the presence of 100 µM L-NAME and in the presence of 10 µM SNAP + L-NAME. “C” indicates closed channel current.
Fig. 3 summarizes the results of experiments in which the effect of L-NAME was abolished either by addition of 10 µM SNAP or in the presence of 1 mM L-arginine.

To explore whether the effect of NO is mediated by a cGMP-dependent pathway, we examined the effect of cGMP on channel activity. Figure 4A is a recording showing the effects of L-NAME and cGMP on channel activity in a cell-attached patch. Figure 4B is representative fit of all-points amplitude histograms obtained under control and experimental conditions. Application of 100 µM L-NAME reduced the initial control $P_0$ (0.7 ± 0.1) by 70 ± 7%; however, adding 100 µM 8-BrcGMP reversed the inhibitory effect of L-NAME and restored the $P_0$ to 0.65 ± 0.2 ($n = 5$). The notion that the effect of NO is mediated by a cGMP-dependent pathway is confirmed by experiments in which addition of ODQ, an inhibitor of guanylate cyclase, abolished the effect of SNAP (Fig. 5). Application of 1 µM ODQ decreased channel activity to 55 ± 15% of the control value ($n = 3$). Furthermore, in the presence of ODQ, 10 µM SNAP has no significant effect on channel activity, suggesting that the effect of SNAP is mediated by a cGMP-dependent pathway. This notion is also supported by the observation that 10 µM SNAP significantly increased the measured concentration of cGMP of MTALs from a control value 12.4 ± 2.5 fM/µg to 38.9 ± 9 fM/µg ($n = 8$) (Fig. 6).
Recently, we have shown that NO plays an important role in the regulation of the small-conductance K⁺ channel in the isolated perfused CCDs and cultured CCD cells. Stoos et al. (17, 18) have found that NO reduces amiloride-sensitive Na⁺/H⁺ exchange in the rabbit proximal tubule (16). Three types of NOS, neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS), are present in rat kidneys (1, 2). It is generally believed that the constitutive NOS, nNOS or eNOS, is responsible for the regulation of cell functions under physiological conditions. With the use of the RT-PCR technique, several studies have shown that constitutive NOS (2, 22) and soluble guanylate cyclase (20) are expressed in the TAL. McKee et al. (13) used the NOS isozyme-independent marker NADPH-d to determine the cellular distribution of NOS in the rat and human kidney and observed NADPH-d-positive tubules with particularly intense staining in the TAL of Henle's loop (13), suggesting the possible presence of NOS. Recently, we have used the immunocytochemical staining to show the presence of nNOS in both the CTAL and MTAL (27). Therefore, the presence of constitutive NOS suggests a role of NO in the regulation of cell functions in the TAL.

Three lines of evidence indicate that NO is involved in regulating the apical 70-pS K⁺ channels. First, application of NO donors such as SNAP increased the channel activity in cell-attached patches. Second, L-NAME, but not D-NAME, reduced the channel activity. Finally, the inhibitory effect of L-NAME can be reversed by SNAP or L-arginine. NO has been shown to regulate a variety of K⁺ channels, including the basolateral small-conductance K⁺ channel in the CCD (11). The effects of NO can be mediated by either a cGMP-dependent or a cGMP-independent pathway (10). Our experimental results suggest that the effect of NO on the apical 70-pS K⁺ channel is mediated by a cGMP-dependent pathway, because cGMP not only mimics the effect of SNAP but also reverses the L-NAME-induced channel inhibition. That the effect of SNAP is the result of stimulating the cGMP-dependent pathway is further suggested by the observations that inhibition of guanylyl cyclase abolished the effect of SNAP and that SNAP increased the cGMP formation in the MTAL. Although we did not measure cGMP concentration in the CTAL, since we were unable to collect a large amount of CTAL to carry out a study, it is conceivable that SNAP may also increase the cGMP concentration in the CTAL, because the effect of SNAP on channel activity can be mimicked by cGMP.

Although we have shown that NO stimulates the apical 70-pS K⁺ channel via a cGMP-dependent pathway in the TAL, the mechanism by which cGMP stimulates the channel activity is not known. Because cGMP has no effect on the channel activity in inside-out patches (unpublished observations), the possibility that the 70-pS K⁺ channel is a cGMP-gated channel can be excluded. However, there are at least two possibilities to explain the effects of cGMP: the 70-pS K⁺ channel or its closely associated proteins might be phosphorylated by a cGMP-dependent protein kinase, or, alternatively, cGMP might inhibit phosphodiesterase and accordingly increase the cAMP concentration (10). Although cGMP could also stimulate phosphodiesterase, it is unlikely that the effect of cGMP is mediated by a cGMP-activated phosphodiesterase, since it would result in decrease in cAMP production and, accordingly, channel activity. In addition, we cannot exclude the possibility that the effect of NO/cGMP is indirect and the result of altering activity of other transporters. We need further experiments to explore the mechanism by which cGMP regulates channel activity.

In addition to the 70-pS K⁺ channel, a 30-pS K⁺ channel has been found in the TAL under physiological conditions. Although the effect of NO on the 30-pS K⁺ channel was not explored, NO should have a significant effect on the apical K⁺ conductance, since the 70-pS K⁺
channel is predominant in the TAL. In the present study, we have shown that NO stimulates the apical 70-pS K⁺ channels; however, it is not known whether the effect of NO on the apical K⁺ channels could have a significant effects on net NaCl transport. It has recently shown that stimulation of iNOS decreased the net Cl⁻ reabsorption in the MTAL obtained from spontaneous hypertensive rats (19). Also, it has been reported that luminal application of cGMP reduced the net Cl⁻ reabsorption in the MTAL (14). Thus it is possible that the net effect of NO on NaCl transport in the TAL depends on NO concentrations, the site of cGMP actions, and isoform of NOS expressed in a given tubule.

The NO/cGMP-dependent pathway may play an important role in linking the activity of apical K⁺ channels to the Na-K-Cl cotransporter, since it has been expected to increase intracellular Na^⁺, at least partially regulated by Na⁺/Ca²⁺ exchange, which is driven by a favorable electrochemical gradient of Na⁺. Stimulation of Na-K-Cl cotransporter is expected to increase intracellular Na⁺ concentration and, accordingly, intracellular Ca²⁺, which activates the activity of NOS. As consequence, NO formation increases and stimulates the apical K⁺ recycling to cope with the turnover rate of the Na-K-Cl cotransporter. Further experiments are needed to test this hypothesis. We conclude that NO stimulates the apical 70-pS K⁺ channel by a cGMP-dependent pathways.

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