Carbon monoxide stimulates Ca\(^{2+}\)-dependent big-conductance K channels in the cortical collecting duct

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Wang Z, Yue P, Lin DH, Wang WH. Carbon monoxide stimulates Ca\(^{2+}\)-dependent big-conductance K channels in the cortical collecting duct. Am J Physiol Renal Physiol 304: F543–F552, 2013. First published December 12, 2012; doi:10.1152/ajprenal.00530.2012.—We used the patch-clamp technique to examine the role of carbon monoxide (CO) in regulating Ca\(^{2+}\)-activated big-conductance K (BK) channels in the principal cell of the cortical collecting duct (CCD). Application of CORM3 or CORM2, a CO donor, activated BK channels in the CCD, whereas adding inactivated CORM2/3 had no effect. Supersolution of the CCD with CO-hubbled bath solution also activated the BK channels in the cell-attached patches. The effect of CO on BK channels was not dependent on nitric oxide synthase (NOS) because the effect of CORM3 was also observed in the CCD treated with l-NAME, an agent that inhibits the NOS. Adding a membrane-permeable cGMP analog, 8-bromo-cGMP, significantly increased the BK channel in the CCD. However, inhibition of soluble guanylate cyclase failed to abolish the stimulatory effect of CORM3 on BK channels. Moreover, inhibition of cGMP-dependent protein kinase G did not block the stimulatory effect of CORM3 on the BK channels, suggesting that the stimulatory effect of CO on the BK channels was, at least partially, induced by a cGMP-independent mechanism. Western blot demonstrated that heme oxygenase type 1 (HO-1) and HO-2 were expressed in the kidney. Moreover, a high-K (HK) intake increased the expression of HO-1 but not HO-2 in the kidney. A HK intake also increased renal HO activity defined by NADPH-dependent CO generation following addition of heme in the cell lysate from renal cortex and outer medulla. The role of HO in regulating BK channel activity in the CCD was also suggested by experiments in which application of hemin increased the BK channels. The stimulatory effect of hemin on the BK channels was blocked by SnMP, a HO inhibitor. But, adding CORM3 was still able to activate the BK channels in the presence of SnMP. We conclude that CO activates the BK channels, at least partially, through a NO-cGMP-independent pathway and that HO plays a role in mediating the effect of HK intake on the BK channels in the CCD.

heme oxygenase; cGMP; PKG; renal K channels

THE CA\(^{2+}\)-ACTIVATED big-conductance K (BK) channels are expressed in the apical membrane of the cortical collecting duct (CCD) in both principal cells (PC) and intercalated cells (IC) (10, 13, 20, 32). Previous studies demonstrated that the BK channel activity in IC was higher than those in PC (33). Also, a large body of evidence strongly suggests that the BK channel in IC may be involved in K secretion when tubule flow rate is high or during increasing dietary K intake (22–24, 52). However, it is still not known whether BK channels in PC are also participated in K secretion because they have a low activity. We speculate that BK channel activity in PC may be suppressed under normal dietary K intake and it could increase by some factors induced by high-K intake or high flow rate thereby participating in K secretion. Indeed, it has been shown that inhibition of protein kinase A (PKA) increased the BK channel activity in PC and augmented the BK-dependent K secretion in the rabbit CCD, suggesting that the BK channels were constitutively inhibited by PKA (25). We previously demonstrated that 11,12-epoxyeicosatrienoic acid stimulated BK channels in PC and that inhibition of Cyp-epoxygenase activity blunted the flow-stimulated and BK channel-dependent K secretion in the rabbit CCD (41). A large body of the studies demonstrated that carbon monoxide (CO) stimulated the Ca\(^{2+}\)-activated BK channels in a variety of tissues (9, 14–16, 51). Since heme oxygenase (HO) is highly expressed in the renal tubules (8), it is conceivable that HO-dependent CO generation may also play a role in stimulating the BK channels in the CCD. Thus, the aim of the present study is to examine whether CO generated by HO stimulates the BK channels in the PC of the CCD.

METHODS

Preparation of CCDs. Sprague-Dawley rats (5 wk old, either sex) were purchased from Taconic Farms (Germantown, NY). The animals were fed with either normal rat chow (containing 1% K) or a high-K diet (10% K; Harlan Lab, Madison, WI) for 7 days before experiments. To obtain the CCDs, the rats (<90 g) were killed by cervical dislocation and kidneys were removed immediately. Several thin slices of the kidney (<1 mm) were cut and placed on an ice-cold Ringer solution for further dissection of the CCD. The isolated CCD was placed on a 5 × 5-mm cover glass coated with polylysine (Sigma) and the cover glass was transferred to a chamber (1,000 μl) mounted on an inverted Nikon microscope. The tubule was superfused with HEPES-buffered NaCl solution containing 140 mM NaCl, 5 mM KCl, 1.8 mM CaCl\(_2\), 1.8 mM MgCl\(_2\), and 10 mM HEPES (pH 7.4). The CCD was cut open with a sharpened micropipette to expose the apical membrane. The protocol for using animals was approved by an independent animal user committee (Institutional Animal Care and Use Committee) at New York Medical College.

Patch-clamp technique. An Axon200A patch-clamp amplifier was used to record channel currents. The currents were low-pass filtered at 1 kHz by an eight pole Bessel filter (902LPF, Frequency Devices, Haverhill, MA) and digitized by an Axon interface (Digidata 1200). Data were analyzed using the pClamp software system 7 (Axon). Channel activity defined as NP/2 (a product of channel number and open probability) was calculated from data samples of 60-s duration in the steady state as follows: NP/2 = \(\sum(t_i + 2t_2 + \ldots + t_n)\), where \(t_i\) is the fractional open time spent at each of the observed current levels. The experiments were performed in the PC of the CCD. PC cells were recognized by their unique hexagonal shape while IC cells were identified by their irregular shape. In addition, we deliberately selected the patches without ROMK channel activity to avoid the difficulty in analyzing channel activity. The pipette solution was composed of (in mM) 140 KCl, 1.8 MgCl\(_2\), 10 HEPES (pH 7.4).

Tissue preparation. Western blot, and immunostaining. The renal cortex and outer medulla were separated under a dissecting micro-
scope and suspended in RIPA solution (1:8 ratio, wt/vol) containing 50 mM Tris·HCl (pH 7.4), 10 mM NaCl, 1% NP-40, 1% Triton X-100, 0.1% SDS, 1 mM sodium molybdate, 1 mM para-nitrophenyl-phosphate, and 1 mM EDTA. For every 125-mg tissue sample, we added a 25-µl cocktail of protease and phosphatase inhibitors containing aprotinin (1 µg/ml), leupeptin (1 µg/ml), pepstatin A (1 µg/ml), sodium vanadate (Na3VO4; 1.5 mM), and sodium fluoride (1 mM). The samples were left on ice for 15 min and homogenized with a mortar and pestle. The protein concentrations were measured twice using the Pierce BSA protein assay. The homogenized tissue sample was incubated in the presence of DNAse (5 µg/ml) and rabbit IgG serum at 4°C for 60 min. The mixture was centrifuged at 3,000 rpm for 10 min at 4°C and the resultant supernatant was collected. The proteins were separated by electrophoresis on 10% SDS-polyacrylamide gels and transferred to nitrocellulose membranes. The membranes were blocked with 5% nonfat dry milk in Tris-buffered saline (TBS), rinsed, and washed with 0.05% Tween 20-TBS buffer. The membranes were washed three times (10 min for each wash) with PBS and scanned by Odyssey infrared imaging system (LI-COR, Lincoln, NE) at wavelengths of 680 or 800 nM. We followed the methods published in a previous study for double immunostaining in the rat kidney (39).

Assessment of HO activity.

We followed the method published previously to measure CO-generating activity of HO (36). Briefly, kidneys were homogenized in ice-cold 100 mM potassium phosphate buffer, pH 7.4, containing 1,370 mM NaCl, 27 mM KCl, 0.1 mM butylated hydroxytoluene, and 10% protease inhibitor cocktail (Sigma). The homogenates were then centrifuged (10,000 for 10 min at 4°C) and the protein concentrations of the supernatant were determined. An aliquot (10 to 20 µl containing ~300 µg protein) was mixed with 40 µM heme in the presence of or in the absence of a NADPH-generating system containing 10 mM MgCl2, 6.8 mM glucose-6-phosphate, 3.3 U/ml glucose-6-phosphate dehydrogenase, and 2.6 mM NADPH. The reaction mixture (1 ml final volume) was incubated for 1 h at 37°C in 2-ml amber vials capped with rubberized Teflon liners. The vials were then placed on ice, and an internal standard made of isotopically-labeled CO (13C18O, Sigma) was injected into the vials, and the CO content of the headspace was determined by gas chromatography/mass spectroscopy, as previously described (16). CO-generating activity was defined as picomoles per milligram of protein per hour. Values obtained in the absence of NADPH (NADPH-independent CO generation) were subtracted from values in the presence of NADPH (total CO generation); the resulting values represent the NADPH-dependent generation of CO, which is an index of HO activity (45).

Experimental materials and statistics.

Antibodies for HO-1, aquaporin-2 (AQP-2), and HO-2 were purchased from Santa Cruz Biotechnology. Stannous mesoporphrin (SnMP), a nonselective HO inhibitor (36), was purchased from Frontier Scientific and was dissolved in 50 mM Na2CO3. CORM2, CORM3 (CO-releasing molecule), inactivated CORM2/3, KT5823, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ), N(G)-nitro-L-arginine methyl ester (L-NAME), and 8-bromo-cGMP were obtained from Sigma (St. Louis, MO). KT5823 was dissolved in DMSO and the final concentration of DMSO in the bath was 0.1%. The data are presented as means ± SE. We used Student’s t-test or a one-way ANOVA test to determine the statistical significance. P < 0.05 was considered to be significant.

Fig. 1. Channel recording demonstrates the effect of CORM3 on Ca2+-activated big-conductance K (BK) channels in the cortical collecting duct (CCD) of the rat kidney. The experiments were performed in cell-attached patches and the holding potential was 0 mV. Top trace shows the time course of the experiment and 2 parts of the recording indicated by numbers were extended to show the fast time course. CORM3 (10 µM) was directly added to the bath. Bottom: bar graph summarizes the results. #P < 0.05 was considered to be a significant difference from the value of the rest of the group.
RESULTS

We confirmed the previous finding that BK channels were expressed in the apical membrane of both the PC and IC of the CCD (20). To explore the effect of CO on the BK channels in the CCD, we performed the patch-clamp experiments in the apical membrane of the PC. We first examined whether the BK channel activity was increased by CORM2 or CORM3, which has been used as a CO donor (11, 38). Figure 1 is a representative recording showing that adding 10 µM CORM3 stimulated the BK channels and increased $NP_\text{o}$ from 0.006 ± 0.002 to 0.35 ± 0.1 ($n = 18$) in the CCD. The effect of CORM3 on the BK channels was the result of CO release because adding inactivated CORM3, which could not release CO, had no effect (data not shown). The finding that CO stimulated the BK channels was also confirmed in experiments in which the effect of CORM2 and iCORM2 (inactivated CORM2) on the BK channels was examined. From inspection of Fig. 2, it is apparent that adding iCORM2 (10 µM) failed to activate the BK channels while adding CORM2 stimulated the BK channels in the same CCD. In four similar experiments, adding CORM2 (10 µM) significantly increased the $NP_\text{o}$ of the BK channels from 0.01 ± 0.01 to 0.63 ± 0.15. We next directly examined the effect of CO on the BK channels by superfusing the CCD with CO-bubbled bath solution ($\sim3$ µM). Figure 3 is a channel recording demonstrating that switching to a CO-containing bath solution activated the BK channels within 60 s and increased $NP_\text{o}$ to 0.35 ± 0.07 ($n = 5$). In contrast, switching to the air-bubbled bath solution had no effect on the BK channels (data not shown), suggesting that the BK channel was activated by CO rather than by nonspecific mechanic disturbance.

CO has been shown to stimulate nitric oxide synthase (NOS) thereby increasing the release of NO (44), which has been reported to activate BK channels in the smooth muscles (4). Thus, we examined whether the stimulatory effect of CO on the BK channels was the result of activating NOS. We performed the study in the CCD pretreated with 0.5 mM l-NAME for 30 min to inhibit NOS and examined the effect of CORM3 on BK channels in the presence of l-NAME. Figure 4 is a typical recording demonstrating that adding CORM3 was still able to stimulate the BK channels and it increased $NP_\text{o}$ from 0.01 ± 0.01 to 0.48 ± 0.12 ($n = 7$) in the CCD pretreated with l-NAME. This strongly suggests that CO stimulates the BK channels, at least in part, by a NO-independent mechanism.

CO has been shown to stimulate soluble guanylate cyclase (sGC) thereby increasing cGMP generation (7, 12), which in turn activates BK channels (1, 40). We thus examined the effect of 8-brome-cGMP on the BK channels in the CCD. Figure 5 is a recording showing that adding membrane-permeable cGMP analog (100 µM 8-brome-cGMP) stimulated the BK channels in the CCD and increased $NP_\text{o}$ from 0.01 ± 0.005 to 0.34 ± 0.08 ($n = 4$), suggesting that the BK channels are...
Fig. 3. Channel recording demonstrates the effect of carbon monoxide (CO) on Ca^{2+}-activated BK channels in the CCD of the rat kidney. The experiments were performed in cell-attached patches and the holding potential was 0 mV. Top trace shows the time course of the experiment and 2 parts of the recording indicated by numbers were extended to show the fast time course. The arrow indicates the solution switching from the control to CO-bubbled media. Pure CO gas was used to bubble the bath solution in a sealed bottle for 30 min before experiments and the final concentration in the media was 3 µM. Bottom: bar graph summarizes the results. #P < 0.05 was considered to be a significant difference from the value of the rest of the group.

Fig. 4. Channel recording demonstrates the effect of CORM3 on Ca^{2+}-activated BK channels in the CCD of the rat kidney in the presence of 0.5 mM N^\text{G}-nitro-L-arginine methyl ester (L-NAME). The experiments were performed in cell-attached patches and the holding potential was 0 mV. Top trace shows the time course of the experiment and 2 parts of the recording indicated by numbers were extended to show the fast time course. Bottom: bar graph summarizes the results. #P < 0.05 was considered to be a significant difference from the value of the rest of the group.
activated by cGMP. Moreover, the stimulatory effect of 8-bromo-cGMP on the BK channels was absent in the CCD treated with 10 \( \mu \)M KT5823 (Fig. 5). If stimulatory effect of CO was due to the stimulation of sGC thereby increasing cGMP-dependent pathway, inhibition of sGC should abolish the effect of CO on the BK channels. Thus, we examined the effect of CO on the BK channels in the CCD pretreated with ODQ, an agent that inhibits sGC inhibitor (50). Not only did ODQ (10 \( \mu \)M) not have a significant effect on the BK channels (data not shown), but it also failed to block the stimulatory effect of CO on the BK channels. Figure 6 is a channel recording demonstrating that adding CORM3 increase \( NP_0 \) from 0.01 ± 0.005 to 0.30 ± 0.08 in the CCD pretreated with ODQ (\( n = 7 \)), suggesting that CO stimulated BK channels, at least in part, by a cGMP-independent mechanism. This notion was further supported by the experiments in which the effect of CORM3 on the BK channels was tested in the CCD pretreated with KT5823, an inhibitor of cGMP-dependent PKG (46). Figure 7 is a recording showing that CORM3 activated the BK channels and increased \( NP_0 \) to 0.31 ± 0.08 (\( n = 5 \)) in the CCD pretreated with 10 \( \mu \)M KT5823. Therefore, the results strongly suggest that CO stimulates the BK channels in the CCD, at least in part, by a NO- and cGMP-independent mechanism.

Since CO is a HO-dependent metabolic product of heme-containing protein, we next examined the role of endogenous HO in regulating the BK channel activity in the CCD. First, we used Western blot to examine the expression of HO-1 and HO-2 in the CCD of the rat kidney. From inspection of Fig. 8A, it is apparent that both HO-1 and HO-2 are expressed in the kidney. Also, immunostaining shows that HO-1 was expressed in the AQP-2-positive CD (Fig. 8B). Moreover, the expression of HO-1 in the kidney was upregulated in animals on a high-K diet while a high-K intake did not affect the expression of HO-2. To determine whether a high expression of HO-1 increased the HO activity in the kidney, we measured CO generation as an index of the HO activity using tissue lysates isolated from renal cortex and outer medulla. The results are summarized in Fig. 8B demonstrating that a high-K intake increased NADPH-dependent or HO-dependent CO generation from control value 451 ± 130 to 1,100 ± 205 pmol-mg protein\(^{-1}\)-h\(^{-1}\) in renal cortex and from 225 ± 30 to 950 ± 100 pmol-mg protein\(^{-1}\)-h\(^{-1}\) in the outer medulla (\( n = 5 \) rats). Thus, results demonstrated that HO was expressed in the kidney and a high-K intake increased HO-1 expression and HO-dependent CO generation.

To test whether the stimulation of endogenous HO increased the BK channel activity, the effect of hemin, a membrane-permeable HO substrate (29), on the BK channel activity was examined. Figure 9 is a channel recording showing that application of 10 \( \mu \)M hemin stimulated the BK channels and increased \( NP_0 \) from 0.01 ± 0.005 to 0.34 ± 0.08 (\( n = 5 \)). To test whether the effect of hemin on the BK channel was the result of stimulating HO-dependent CO production, we repeated the experiments in the presence of SnMP (10 \( \mu \)M), a HO inhibitor (36). Inhibition of HO had no significant effect on the BK channels (data not shown). However, pretreatment of the CCD with SnMP for 30 min blocked the stimulatory effect of hemin. Figure 10 is a channel recording showing that hemin failed to stimulate the BK channels in the CCD pretreated with SnMP. However, adding CORM3 was able to activate the BK chan-
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Fig. 6. Channel recording demonstrates the effect of CORM3 on Ca\(^{2+}\)-activated BK channels in the CCD of the rat kidney in the presence of 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ; 10 \(\mu\)M). The experiments were performed in cell-attached patches and the holding potential was 0 mV. Top trace shows the time course of the experiment and 2 parts of the recording indicated by numbers were extended to show the fast time course. ODQ was present throughout the experiments. Bottom bar graph summarizes the results. *\(P < 0.05\) was considered to be a significant difference from the value of the rest of the group.

DISCUSSION

The main finding of the present study is that CO stimulates the BK channels in the CCD. This conclusion is supported by two lines of evidence: 1) application of CO donor, CORM2/CORM3, but not inactivated CORM2/3 increased BK channel activity; and 2) superfusion of the CCD with CO-bubbled bath solution mimicked the effect of CORM2/3 and activated the BK channels in the CCD. It is well-established that like NO, CO plays a role in regulating cardiovascular, renal, and cerebrovascular circulation (16, 19). CO is the HO-1/HO-2-dependent metabolic product of heme-containing protein (28). In addition to CO, HO-1/2-dependent heme metabolites include iron and biliverdin (28). Biliverdin is a strong antioxidant that affects redox signaling thereby modulating a variety of physiological functions (37). A large body of evidence suggests that the HO-dependent CO generation plays a role in regulating vascular tone by stimulating the Ca\(^{2+}\)-activated BK channels (16, 18, 19). In the present study, we demonstrated that adding hemin stimulated the BK channel in the CCD. The stimulatory effect of hemin was the result of increasing HO-dependent CO release because the effect was completely blocked by suppression of HO activity while adding CORM3 was able to activate the BK channels in the presence of HO inhibitor. This strongly suggests that HO-dependent CO generation was also involved in the regulation of the BK channels in the CCD.

A large body of evidence has demonstrated that CO plays an important role in the regulation of K channels. For instance, CO activates BK activity in smooth muscle cells, carotid body, and endothelial cells (14, 47, 49, 51, 53). In the kidney, we previously demonstrated that CO stimulates the apical 70-pS K channels in the TAL (21). There are several mechanisms by which CO may activate K channels: 1) CO could increase NO release which, in turn, activates K channels; 2) CO could activate K channel by stimulating sGC and increasing the generation of cGMP; and 3) CO activates K channels by directly modulating K channel protein (15). Relevant to the first possibility is the report that CO increases NO release in endothelial cells (44) and smooth muscle cells (11, 17). Indeed, we observed that CO stimulates BK channels in endothelial cells partially by increasing NO release (9). However, the observation that application of CORM3 was still able to activate the BK channels in the CCD treated with L-NAME suggests that CO stimulates the BK channel, at least in part, by a NO-dependent mechanism, although we could not completely exclude the role of NO in mediating the effect of CO on BK. For the second possibility, CO has been shown to increase the generation of reactive oxygen species (43), which is a potent stimulator of sGC thereby activating BK channels by a cGMP-dependent mechanism (3, 5, 6, 26, 27). Also, CO could modulate phosphodiesterase activity thereby changing cGMP level (42). Inhibition of sGC has been demonstrated to attenuate the stimulatory effect of CO on BK channels in smooth muscle cells (47). We also showed that application of membrane-permeable cGMP analog increased the BK channel ac-

nels in the same CCD \((N_{Po}, 0.40 \pm 0.1, n = 5)\). This suggests that the stimulatory effect of hemin on the BK channels was induced by increasing HO-dependent CO production.
Fig. 7. Channel recording demonstrates the effect of CORM3 on Ca$^{2+}$-activated BK channels in the CCD of the rat kidney in the presence of KT5823 (10 µM). The experiments were performed in cell-attached patches and the holding potential was 0 mV. Top trace shows the time course of the experiment and 3 parts of the recording indicated by numbers were extended to show the fast time course. Bottom: bar graph summarizes the results. #P < 0.05 was considered to be a significant difference from the value of the rest of the group.

Fig. 8. A: Western blot shows the effect of dietary K intake on the expression of heme oxygenase type 1 (HO-1) and HO-2 in tissue lysate obtained from renal cortex and outer medulla (OM). B: immunostaining in the region between cortex and OM shows that HO-1 (indicated by an arrow) is expressed in the aquaporin-2 (AQP-2)-positive collecting duct (green, HO-1, red, AQP-2) in rats on a high-K (HK) diet (10%). C: effect of K intake on the NADPH-dependent CO production in renal cortex and OM. *Significant difference between HK and normal-K diet (NK; 1% K) groups.
tivity in the CCD. Therefore, it is conceivable that the stimulatory effect of CO is partially mediated by activating cGMP-PKG pathway. However, two lines of evidence suggested that the stimulatory effect of CO on BK channels in the CCD was, at least in part, by a mechanism other than stimulating sGC and PKG: 1) CORM3 was able to stimulate the BK channels in the presence of sGC inhibitor; and 2) inhibition of PKG suppressed the effect of cGMP but failed to abolish the stimulatory effect of CORM3 on the BK channels in the CCD. Therefore, our results suggest that CO may have a direct stimulatory effect on the BK channels in the CCD. This notion was also suggested by our observation that CO could modestly stimulate the BK channels in inside-out patch in a Ca^{2+}-free bath media (Wang W, unpublished observations). It has been reported that CO activated the BK channels by regulating the interaction between CO and heme molecule, which inhibited the BK channels by binding directly to histidine residue of the alpha subunit of BK channels (15). CO is able to replace heme on histidine residue thereby releasing the heme-induced inhibition of BK channels. Also, it has been reported that CO stimulates BK channels through CO binding to histidine residue of BK channel proteins in smooth muscle cells (48).

Fig. 9. Channel recording demonstrates the effect of hemin on Ca^{2+}-activated BK channels in the CCD of the rat kidney. The experiments were performed in cell-attached patches and the holding potential was 0 mV. Top trace shows the time course of the experiment and 2 parts of the recording indicated by numbers were extended to show the fast time course. The arrow indicates the time point where hemin-containing media were switched on. #P < 0.05 was considered to be a significant difference from the value of the rest of the group.

Fig. 10. Channel recording demonstrates the effect of hemin on Ca^{2+}-activated BK channels in the CCD of the rat kidney in the presence of stannous mesoporphrin (SnMP; 10 µM). The experiments were performed in cell-attached patches and the holding potential was 0 mV. Top trace shows the time course of the experiment and 3 parts of the recording indicated by numbers were extended to show the fast time course. Arrows indicate the ROMK channel activity in the patch.
It is well-established that BK channels are involved in K secretion when dietary K intake is increased (2, 34). CO-induced stimulation of BK channels may play a role in mediating K secretion during increasing dietary K intake. This speculation was also supported by the observation that HO activity defined by CO generation was higher in the renal tissue from animals on a high-K diet than those on a control diet. HO system is composed of HO-1 and HO-2 (28, 37). HO-2 is a 36-kDa protein that is constitutively expressed and located in mitochondria. HO-1 is a 32-kDa protein located in microsome and its expression is inducible by stimuli such as hypoxia (30). Both HO-1 and HO-2 are expressed in the kidney (8). Although it has been reported that HO has a higher expression in the medulla than in the cortex (54), we observed that HO-2 mRNA was expressed in the cortex (21). Moreover, immunostaining demonstrated that HO-1 was expressed in the CCD (Fig. 8f). Finally, HO activity measurement demonstrated that HO activity in the renal cortex was as high as those in the renal medulla.

Expression of HO-1 has been shown to provide a cytoprotective pathway in the kidney in response to stimuli such as ischemia and oxidative stress (31, 35). Although we could not determine whether HO-1 or HO-2 was responsible for the stimulation of the BK channels in the CCD, we speculate that HO-1 may be responsible for stimulating the BK channels in the CCD during increasing dietary K intake. This speculation was supported by the observation that a high-K intake stimulated HO-1 expression and HO activity in the kidney. Although the present study is mainly focused in studying the effect of CO on BK channels in the PC, it is conceivable that CO also increased BK channel activity in the IC. We speculate that an increase in HO activity induced by a high-K intake might play a role in mediating BK channel-dependent K secretion in the CCD during increasing K intake or when the tubule flow rate is high (24, 34). We conclude that CO stimulates the BK channels in the CCD and the stimulatory effect was partially mediated by a NOS- and PKG-independent mechanism. We speculate that CO-induced stimulation of BK channels may play a role in mediating K secretion in the CCD.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


