Nitric Oxide Signaling Pathway Regulates Potassium Chloride Cotransporter-1 mRNA Expression in Vascular Smooth Muscle Cells*

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Rat vascular smooth muscle cells (VSMCs) express at least two mRNAs for K-Cl cotransporters (KCC): KCC1 and KCC3. cGMP-dependent protein kinase I regulates KCC3 mRNA expression in these cells. Here, we show evidence implicating the nitric oxide (NO)/cGMP signaling pathway in the expression of KCC1 mRNA, considered to be the major cell volume regulator. VSMCs, expressing soluble guanylyl cyclase (sGC) and PKG-I isoforms showed a time- and concentration-dependent increase in KCC1 mRNA levels after treatment with sodium nitroprusside as demonstrated by semiquantitative RT-PCR. sGC-dependent regulation of KCC1 mRNA expression was confirmed using YC-1, a NO-independent sGC stimulator. The sGC inhibitor LY83583 blocked the effects of sodium nitroprusside and YC-1. Moreover, 8-Br-cGMP increased KCC1 mRNA expression in a concentration- and time-dependent fashion. The 8-Br-cGMP effect was partially blocked by KT8523 but not by actinomycin D. However, actinomycin D and cycloheximide increased basal KCC1 mRNA in an additive manner, suggesting different mechanisms of action for both drugs. These findings suggest that in VSMCs, the NO/cGMP-signaling pathway participates in KCC1 mRNA regulation at the post-transcriptional level.

Most cells are freely permeable to water, and the movement of water correlates with the osmotic pressure gradient between the intracellular and extracellular space. High intracellular osmolarity increases cellular volume because of a net movement of water into the cell. The activation of highly regulated efflux mechanisms for intracellular ions elicited by cell swelling contributes to cellular regulatory volume decrease. The electro-neutral K-Cl cotransporter (KCC),1 in particular its ubiquitous KCC1 isoform, is considered to be a transporter that maintains volume homeostasis in all cells (1, 2). KCC activity is regulated by dephosphorylation (3), intracellular Mg2+ concentration and pH, and cellular ATP levels (1). Additionally, a NO/cGMP signaling pathway appears to be an important modulator of the KCC activity in red blood cells, and VSMCs (4–6).

Four KCC isoforms have been characterized, KCC1 to KCC4, and their tissue distribution determined (7–12). Primary cultures of rat VSMCs express KCC1 and KCC3 mRNAs (13), and their physiological relevance awaits further investigation. In rat VSMCs, KCC3 mRNA is acutely (in 1 h) up-regulated at the post-transcriptional level by the PKG-I signaling pathway (13) and through unknown mechanisms by angiogenic factors and certain cytokines in human umbilical endothelial cells (9). These findings suggest that KCC mRNA expression is relevant for vascular physiology.

NO regulates VSMC relaxation through the sGC-mediated activation of PKG-I by cGMP. This enzyme is thought to be responsible for the phosphorylation of intracellular proteins and for the regulation of ion fluxes involved in smooth muscle relaxation (14–18). The active form of sGC is a heme-containing heterodimeric protein composed of α- and β-subunits. VSMCs express the α1ββ2 sGC at the mRNA and protein level (18, 19), and basal intracellular cGMP levels correlate with the sGC content in these cells (20).

The N terminus of PKG-I is encoded by two alternatively spliced exons that specify for the PKG-Iα and Iβ isoforms, and it supervises substrate specificity (21, 22). In VSMCs, almost all of the PKG immunoreactivity belongs to PKG-Iα, and only the type Iα mRNA can be detected by Northern blot (23). However, PKG-Iβ is immunodetected in aortic smooth muscle (24). The content of PKG-I enzymes, as well as sGC, in primary cultures of rat VSMCs is highly variable and depends on the tissue of origin and culture conditions (20).

NO regulates gene expression through cGMP-dependent mechanisms (25–27) and via cGMP activation of PKG-I in sGC-expressing VSMC (17, 28). The NO/cGMP signaling pathway has been associated with the cGMP-dependent control of gene expression via several promoter response elements (25, 29, 30) and through RNA-polymerase II-independent mechanisms (31–33) including KCC3 mRNA expression in VSMCs (13). The involvement of the NO/cGMP signaling pathway in the regulation of the expression of KCC1 mRNA, the most abundant KCC isoform expressed in VSMCs (13), has not yet been addressed.

We used primary cultures of freshly isolated rat VSMCs expressing sGC (α1/β1) mRNAs and the two type I versions of PKG (α/β) to determine whether the NO/cGMP signal transduction pathway is involved in the regulation of KCC1 mRNA expression, as well as the role of novo transcription and
translation. As recently reported for KCC3 mRNA expression in VSMCs (13), we now show that the NO/cGMP signaling mechanism is also involved in KCC1 mRNA regulation. In addition to SNP, we tested the effect of YC-1, a NO-independent activator of sGC. Activation and inhibition of PKG-I by 8-Br-cGMP and KT5823, respectively, were also assessed on KCC1 mRNA expression. Actinomycin D, an inhibitor of mRNA synthesis, and cycloheximide, a protein synthesis inhibitor, were used to determine the mechanism involved in KCC1 mRNA expression.

EXPERIMENTAL PROCEDURES

Materials—SNP, YC-1, LY385383, KT5823, 8-Br-cGMP, actinomycin D, and cycloheximide were from Calbiochem (La Jolla, CA). Dulbecco’s modified Eagle’s culture medium, TRIZol reagent for total RNA extraction, and all tissue culture grade or molecular biology reagents were purchased from Life Technologies, Inc. The access RT-PCR kit, specific rat actin primer set, and 100-bp DNA ladder were from Promega Corp.

Primary Culture of Rat VSMCs—Primary cultures were obtained according to the protocols described previously (5, 34) with some modifications as published in detail elsewhere (15). VSMCs, grown in 6-well culture flasks were maintained in Dulbecco’s modified Eagle’s culture medium, 10% fetal bovine serum/antibiotics as described elsewhere (13), were cultured in a controlled atmosphere of air–CO2 (5%) at 37 °C, 10% fetal bovine serum/antibiotics as described elsewhere (13), were cultured in a controlled atmosphere of air–CO2 (5%) at 37 °C until confluence was reached (6–7 days). Only 90–95% confluent rat VSMCs at passage 1 were used for our experiments after 24 h of serum deprivation.

Total RNA Extraction, RT-PCR, and Semiquantitative KCC1 mRNA Expression in VSMCs—Total RNA from rat VSMCs in primary culture was obtained by using the TRIZol reagent following the instructions of the manufacturer. Specific sets of primers for rat KCC1, sGC1 (a/b), and human PKG-I (α/β) mRNAs were synthesized according to the sequences published previously (7, 19, 21, 35), and these were as follows: KCC1 sense (exon 7), 5′-825GGT TTG CCA GAA CCT TGT ATC CAC C 1585-3′, and antisense (exon 11), 5′-1244TTG GCC ACC ACA TAC AG1223-3′; sGC1 sense, 5′-954ACCC ATG CTC TTC TCA GAT ATC G 1915-3′, and antisense, 5′-337GTC TTT GAA TAA CTT GTA TGT CGG 3313-3′; sGC1 sense, 5′-254GAG TCT GGG GAC ATG AGA CAC C 1820-3′, and antisense, 5′-1644TG TTT TCT GGG GAC ATG AGA CAC C 1820-3′; PKG-Iα sense (exon 1a), 5′-33AAA AGG ACC AGC CAT GTA GGA GAC 233-3′, and antisense (exon 1a), 5′-405GAC TGT GAG AAC TTA CGG AAT GCC 377-3′; PKG-Iβ sense (exon 1a), 5′-405GGG AGC ATG AGC ACC C 377-3′; PKG-Iα sense (exon 1a), 5′-405GGG AGC ATG AGC ACC C 377-3′; PKG-Iβ sense (exon 1a), 5′-405GGG AGC ATG AGC ACC C 377-3′; PKG-Iα sense (exon 1a), 5′-405GGG AGC ATG AGC ACC C 377-3′; PKG-Iβ sense (exon 1a), 5′-405GGG AGC ATG AGC ACC C 377-3′.

RT-PCR followed the same conditions as described above for KCC1 mRNA expression relative to actin mRNA as described elsewhere (13). Confluent cells were deprived of serum for 24 h. RT-PCR analysis was done using 0.5 μg of total RNA each. Representations of the PKG-Iα and PKG-Iβ mRNAs of the expected sizes. We also detected very low levels of the PKG-Iβ mRNA under our experimental conditions. These results indicate that the major components of the NO/cGMP signaling cascade are present at the mRNA level and show optimal conditions for studying the actions of NO donors and cGMP analogs in VSMCs.

SNP and the Regulation of KCC1 mRNA Expression in VSMCs—KCC1 mRNA is expressed at higher levels than KCC3 mRNA in VSMCs, and the cGMP/PKG signaling pathway is involved in the acute regulation of KCC3 gene expression (13). Thus, we tested the mechanisms involved in KCC1 mRNA regulation by using SNP as a NO donor, because most of the actions of nitrovasodilators are mediated through stimulation of the cGMP-generating enzyme, sGC (18, 20). Fig. 2, A and C, show the time course and concentration-dependent effects, respectively, of 2 h of SNP treatment on KCC1 mRNA expression levels. The respective semiquantitative actin-normalized densitometric analyses are also shown (Fig. 2, B and D). These results clearly show that SNP was able to induce KCC1 mRNA expression in sGC/PKG-I expressing rat VSMCs in primary culture in a time- and concentration-dependent fashion.

Soluble Guanylyl Cyclase Involvement in SNP-induced KCC1 mRNA Expression in VSMCs—The involvement of sGC in SNP-induced KCC1 mRNA expression was studied by incubating VSMCs with SNP in the presence or absence of the well characterized sGC inhibitor, LY83583. As shown in Fig. 3, SNP-induced KCC1 mRNA expression in VSMCs was effectively blocked by LY83583, suggesting an sGC-dependent mechanism for SNP. However, we corroborated the direct participation of sGC in the regulation of KCC1 mRNA expression by incubating VSMCs with YC-1, a NO-independent stimulator of sGC (37). As shown in Fig. 4, A and B, YC-1 increased the KCC1 mRNA expression levels up to a concentration of 50 μM. Higher YC-1 concentrations resulted in a visible crystallization of the drug in the culture medium. As anticipated, YC-1-stimulated KCC1 mRNA expression in VSMCs was abolished by coincubation with 50 μM LY83583 (Fig. 4, C and D), validating the role of sGC on KCC1 mRNA expression in VSMCs.

![Figure 1. Expression of sGCα1β1 and PKG-Iαβ mRNAs in rat VSMCs](http://www.jbc.org/doi-figures/44535)
PKG Involvement in the Regulation of KCC1 mRNA Expression in VSMCs

Several lines of evidence suggest that NO signals through PKG-I in smooth muscle and other cells by increasing the intracellular levels of cGMP (16, 17). Thus, to test whether PKG activity is involved in the regulation of KCC1 mRNA expression, we incubated freshly isolated rat VSMCs with 8-Br-cGMP at concentrations known to stimulate PKG-I (38), protein phosphorylation (27), and KCC3 mRNA expression (13) in VSMCs. As shown in Fig. 5, A and B, KCC1 mRNA expression was acutely stimulated by the cGMP analog at the indicated exposure time and drug concentrations. Because not all the effects of cGMP are mediated by activation of PKG-I (39), the above results suggest, but do not prove, that PKG-I activity participates in the acute regulation of KCC1 mRNA expression in VSMCs. In fact, 8-Br-cGMP-induced KCC1 mRNA expression partially disappeared in the presence of KT5823, a known inhibitor of PKG-I (23), as shown in Fig. 6, A and B. KT5823 per se had no effect on basal KCC1 mRNA expression (Fig. 6, C and D). Higher KT5823 concentrations (>5.0 μM) resulted in visible cellular detachment from the culture plates and were not investigated further.

Post-transcriptional Mechanisms Involved in 8-Br-cGMP-induced KCC1 mRNA Expression—The NO/cGMP signaling pathway regulates the expression of several genes via RNA-polymerase II-independent mechanisms (31–33). For example, KCC3 mRNA expression is acutely up-regulated by 8-Br-cGMP in the presence of actinomycin D in VSMCs (13). Therefore, we examined whether de novo mRNA synthesis participates in the changes observed in 8-Br-cGMP-induced KCC1 mRNA expression. To this end, we incubated VSMCs with 8-Br-cGMP in the presence or absence of the transcriptional inhibitor actinomycin D and with actinomycin D alone for 2 h. As shown in Fig. 7, A and B, 8-Br-cGMP stimulated KCC1 mRNA expression even in the presence of actinomycin D, suggesting that 8-Br-cGMP increases KCC1 mRNA expression at the post-transcriptional level.
Control) of three independent experiments. All of the results were normalized with respect to the actin signal.

C, densitometric analysis (optical density in arbitrary units as

285 (KCC1) were electrophoresed in 2% agarose gel and stained with ethidium bromide to show bands of the expected sizes, 419 (Actin) bp.

in the presence or absence of KT5823 (1
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of time. Total RNA from rat VSMCs was obtained, and 0.5 µg of each was subjected to semiquantitative RT-PCR analysis. A, semiquantitative RT-PCR products were electrophoresed in 2% agarose gel and stained with ethidium bromide to show bands of the expected sizes, 419 (KCC1) and 285 (Actin) bp. B, densitometric analysis (optical density in arbitrary units as a % of Control) representing the mean ± S.E. (*, p < 0.01 versus control) of three independent experiments. All of the results were normalized with respect to the actin signal.

Note that actinomycin D per se increased basal KCC1 mRNA expression by about 2-fold, probably by blocking transcription of a factor(s) that negatively regulates KCC1 mRNA levels or by directly stabilizing KCC1 mRNA. Both possibilities appear to be plausible because prolonged incubation of VSMCs with actinomycin D alone increase KCC1 mRNA expression (Fig. 7, C, bottom, and D, filled circles). Additionally, the 2-h effect of 8-Br-cGMP on KCC1 mRNA expression persisted after the removal of the analog from the culture medium even in the presence of actinomycin D (Fig. 7, C, top, and D, open circles). The latter result resembles the time course effect of 8-Br-cGMP alone on KCC1 mRNA expression (Fig. 5, A and B). The post-transcriptional up-regulation of KCC1 mRNA expression in response to 8-Br-cGMP and actinomycin D appears to operate through a similar mechanism(s), as suggested by the nonadditive effect of both drugs (Fig. 7, A and B). See “Discussion” for a further interpretation of Fig. 7D.

Effect of Translation and 8-Br-cGMP on KCC1 mRNA Expression—Post-transcriptional regulation of mRNAs may or may not require ongoing protein synthesis. Hence, the role of translation in 8-Br-cGMP-induced KCC1 mRNA expression in VSMCs was investigated using cycloheximide as a protein synthesis inhibitor. VSMCs in primary culture were incubated with 8-Br-cGMP in the presence or absence of cycloheximide and with cycloheximide alone for 2 h. As shown in Fig. 8, 8-Br-cGMP, and inhibition of protein synthesis by cycloheximide apparently via different mechanism(s).
specific antibodies against PKG-I\(^{24}\). This finding may reflect the fact that transcripts at very low expression levels are usually not detected by Northern blot. Taken together, these results indicate that the main components of the NO/cGMP signaling pathway were present in our primary cultures of rat VSMCs. Furthermore, the potential of VSMCs to respond to...

**Fig. 7.** Effect of actinomycin D on 8-Br-cGMP-induced KCC1 mRNA in rat VSMCs. Rat VSMCs cultured as described under “Experimental Procedures” were treated with 10 \(\mu\)g/ml actinomycin D (ActD) alone or in combination with 8-Br-cGMP (1.0 mM). A, after 2 h of treatment, total RNA was extracted, and KCC1 (419 bp) and actin (285 bp) mRNAs expression levels were analyzed by semiquantitative RT-PCR. The RT-PCR products were electrophoresed in 2% agarose gel and stained with ethidium bromide to show bands of the expected sizes. B, actin-normalized densitometric analysis (optical density in arbitrary units as % of Control) representing the mean ± S.E. (\(*, p < 0.001\) versus control; **, \(p < 0.05\) versus control; \(n = 9\)) of three independent experiments.

**Fig. 8.** Effect of cycloheximide on 8-Br-cGMP-induced KCC1 mRNA in rat VSMCs. Rat VSMCs cultured as described under “Experimental Procedures” were treated with or without 10 \(\mu\)g/ml cycloheximide (Cx) or in combination with 8-Br-cGMP (1.0 mM). A, after 2 h of treatment, total RNA was extracted, and KCC1 (419 bp) and actin (285 bp) mRNA expression levels were analyzed by semiquantitative RT-PCR. The products were electrophoresed in 2% agarose gel and stained with ethidium bromide to show bands of the expected sizes. B, actin-normalized densitometric analysis (optical density in arbitrary units as % of Control) representing the mean ± range \((*, p < 0.001\) versus control; \(n = 9\)) of three independent experiments.

**Fig. 9.** Effect of actinomycin D on cycloheximide-induced KCC1 mRNA in rat VSMCs. Rat VSMCs cultured as described under “Experimental Procedures” were treated with or without 10 \(\mu\)g/ml cycloheximide (Cx), 10 \(\mu\)g/ml actinomycin D (ActD), or in combination for 2 h. A, after treatment, total RNA was extracted, and KCC1 (419 bp) and actin (285 bp) mRNAs expression levels were analyzed by semi-quantitative RT-PCR. The products were electrophoresed in 2% agarose gel and stained with ethidium bromide to show bands of the expected sizes. B, actin-normalized densitometric analysis (optical density in arbitrary units as % of Control) representing the mean ± range \((*, p < 0.01\) versus control) from two independent experiments.
NO/cGMP signaling was demonstrated under our experimental conditions. Commensurate with and extending our previous reports on KCC activity and KCC3 mRNA expression in VSMCs (5, 6, 13), we show here that the NO donor SNP is able to induce KCC1 mRNA expression in VSMCs (Fig. 2). Additionally, and because many of the biological actions of NO are mediated through the activation of sGC, we investigated the actions of LY83583, a known sGC inhibitor, on the NO donor-induced KCC1 mRNA expression. The inhibitor blocked the SNP-induced KCC1 mRNA expression (Fig. 3), commensurate with a sGC-mediated effect of SNP in KCC1 mRNA regulation. Although our data are consistent with a sGC-mediated increase in KCC1 mRNA expression in VSMCs, actions of NO donor byproducts cannot be ruled out.

Independently of the mechanism(s) utilized by SNP or its derivatives in the induction of KCC1 mRNA expression in VSMCs, a direct role of sGC was validated using YC-1, a NO-independent stimulator of sGC (37). YC-1 increased KCC1 mRNA expression in a concentration-dependent manner (Fig. 4). However, high concentrations (>50 μM) of YC-1 were unable to preserve the effect, most likely because of a visible crystallization of the drug in the culture medium. Taken together, and because LY83583 also inhibited the YC-1-stimulated KCC1 mRNA expression under our experimental conditions (Fig. 4, C and D), these results suggest that sGC participates in the induction of KCC1 mRNA expression, probably by increasing the intracellular levels of cGMP.

Activation of sGC by NO donors produces cGMP (18, 20), a well known activator of PKG-I in VSMCs (16, 17, 38). The hypothesis of a PKG-I-mediated effect on KCC1 mRNA expression was addressed in our study by using 8-Br-cGMP as a cGMP analog. We showed that 8-Br-cGMP increased KCC1 mRNA expression in a concentration- and time-dependent manner (Fig. 5). The maximal effect of 0.1 mM 8-Br-cGMP on KCC1 mRNA expression was seen beyond 2 h of stimulus, whereas it was reported at 1 h for KCC3 mRNA (13), indicating the differential sensitivity of both closely related genes to the same stimulus. Furthermore, the maximal effect of the analog was reported to be at 0.1 mM for KCC3 mRNA (13) as compared with 1.0 mM for KCC1 mRNA expression in the present study. The physiological relevance of the differential response of these two closely related volume regulator genes remains to be further investigated.

We have shown previously that the maximal 8-Br-cGMP-induced KCC3 mRNA expression in VSMCs was completely blocked by 5 μM KT5823 (13), a selective inhibitor of PKG with little or no effect at this concentration on other potential cGMP-activated kinases, such as cAMP-dependent protein kinases (34). Nevertheless, KT5823, under identical experimental conditions as reported previously (13), was unable to completely reduce 8-Br-cGMP-induced KCC1 mRNA expression levels to control values (Fig. 6). These results imply that, in VSMCs, the effects of 8-Br-cGMP on KCC1 mRNA up-regulation are mediated, at least in part, through activation of PKG itself. However, a differential sensitivity of KCC1 mRNA to the inhibitory actions of KT5823 with respect to KCC3 mRNA cannot be excluded. The absence of an effect of KT5823 per se on basal KCC1 mRNA levels (Fig. 6, B and C) is in line with the observation that PKG-I is not required for basal KCC1 mRNA expression in VSMCs (41). The involvement of an alternative signaling transduction cascade cannot be ruled out because cGMP may target cAMP-dependent protein kinases, directly or indirectly, by increasing cAMP levels through inhibition of cAMP-phosphodiesterases (16, 36). Taken together, these observations suggest that the NO/sGC/PKG signaling transduction pathway is an up-regulator of KCC1 mRNA expression in VSMCs.

In agreement with our previous results (13), and with the concept that NO is able to promote stabilization of different mRNAs in the absence of transcription (31–33), is the fact that inhibition of transcription by actinomycin D did not lower the 8-Br-cGMP-induced KCC1 mRNA expression to control values (Fig. 7, A and B). Additionally, the short-term effect (2 h) of 8-Br-cGMP still persisted for 24 h after removal of the analog even in the presence of actinomycin D (Fig. 7, C and D), resembling the time course effect of 8-Br-cGMP alone on KCC1 mRNA expression (Fig. 5). Therefore, the 8-Br-cGMP-mediated increase in KCC1 mRNA expression appears to occur at the post-transcriptional level rather than at the KCC1 gene transcription.

On the other hand, actinomycin D alone increased basal KCC1 mRNA expression in a time-dependent manner (Fig. 7, C and D), pointing to the existence of a short-lived mRNA(s) that either by itself or by its protein products negatively regulates KCC1 mRNA turnover. However, the presence of a putative short-lived protein(s) involved in KCC1 mRNA turnover is contradicted by the observation that actinomycin D-induced KCC1 mRNA expression was additive when coincubated with cycloheximide (Fig. 9) or with puromycin, another protein synthesis inhibitor (results not shown). These data indicate that transcriptional inhibition by actinomycin D may induce KCC1 mRNA expression independently of translation (and vice versa). Several lines of evidence support the view that transcriptional blockage increases stability of certain mRNAs in the absence of protein synthesis through the redistribution from nucleus to cytoplasm of pre-existing mRNA-stabilizing proteins (42–45). Whether the increase in KCC1 mRNA expression by actinomycin D correlates with redistribution of mRNA-stabilizing proteins in VSMCs remains to be determined.

The role of active translation on KCC1 mRNA expression in VSMCs appears to be complex. We found that cycloheximide alone (as well as puromycin and anisomycin in a concentration- and time-dependent manner; results not shown), increased basal KCC1 mRNA expression (Fig. 8). At least two mechanisms (not mutually exclusive) may contribute to the induction of gene expression in response to cycloheximide: direct induction of gene transcription or stabilization of mRNAs independently of transcription (31, 46–49). Although the reason for the increase in KCC1 mRNA expression in response to cycloheximide is not clear at this point, the mechanism(s) appear to be independent of transcription, as it still occurs in the presence of actinomycin D. This idea is further in line with the concept that different mechanisms are utilized by both inhibitors because both effects were additive (Fig. 9) and with the concept that inhibition of elongation by cycloheximide and mRNA stability are intimately linked in eukaryotic cells (46, 49). Whether cycloheximide increases KCC1 mRNA stability in addition to, or independently of, its ability to block protein synthesis needs to be established.

Most importantly, the fact that 8-Br-cGMP increased KCC1 mRNA expression to a similar extent as cycloheximide but in a nonadditive manner (as with puromycin; results not shown) suggests that protein synthesis inhibition and 8-Br-cGMP increase KCC1 mRNA expression via similar mechanism(s), probably by increasing the stability of KCC1 mRNA in a transcriptionally independent manner, because both effects were still observed in the presence of actinomycin D. Additionally, and because the effects of cycloheximide and actinomycin D on KCC1 mRNA expression were additive, the results suggest not only that transcription and translation blockage may increase, independently, KCC1 mRNA stability in VSMCs, but they also...
imply that 8-Br-cGMP might regulate KCC1 mRNA turnover through at least two different mechanisms.

In conclusion, this study shows that SNP and direct stimulation of sGC by YC-1, as well as PKG-I stimulation by 8-Br-cGMP, increased KCC1 mRNA levels in a concentration- and time-dependent manner. SNP and YC-1 effects were abolished by LY83583, a sGC inhibitor, whereas PKG-I inhibition by KT5823 partially blocked KCC1 mRNA expression in response to 8-Br-cGMP. On the other hand, 8-Br-cGMP increased KCC1 mRNA expression by actinomycin D and cycloheximide, respectively, appear to determine KCC1 mRNA transcription and translation by actinomycin D and cycloheximide, respectively, appear to determine KCC1 mRNA expression.

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