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Hydrogen Sulfide as an Allosteric Modulator of ATP-Sensitive Potassium Channels in Colonic Inflammation

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ABSTRACT

The ATP-sensitive potassium channel (K_{ATP}) in mouse colonic smooth muscle cell is a complex containing a pore-forming subunit (Kir6.1) and a sulfonylurea receptor subunit (SUR2B). These channels contribute to the cellular excitability of smooth muscle cells and hence regulate the motility patterns in the colon. Whole-cell voltage-clamp techniques were used to study the alterations in K_{ATP} channels in smooth muscle cells in experimental colitis. Colonic inflammation was induced in BALB/C mice after intracolonic administration of trinitrobenzene sulfonic acid. K_{ATP} currents were measured at a holding potential of −60 mV in high K^+ external solution. The concentration response to levcromakalim (LEVC), a K_{ATP} channel opener, was significantly shifted to the left in the inflamed smooth-muscle cells. Both the potency and maximal currents induced by LEVC were enhanced in inflammation. The EC_{50} values in control were 6259 nM ($n = 10$) and 422 nM ($n = 8$) in inflamed colon, and the maximal currents were 9.9 ± 0.71 pA/pF (60 μM) in control and 39.7 ± 8.8 pA/pF (3 μM) after inflammation. As was seen with LEVC, the potency and efficacy of sodium hydrogen sulfide (NaHS) (10–1000 μM) on K_{ATP} currents were significantly greater in inflamed colon compared with controls. In control cells, pretreatment with 100 μM NaHS shifted the EC_{50} for LEV-induced currents from 2838 ($n = 6$) to 154 ($n = 8$) nM. Sulfhydration of sulfonylurea receptor 2B (SUR2B) was induced by NaHS and colonic inflammation. These data suggest that sulfhydration of SUR2B induces allosteric modulation of K_{ATP} currents in colonic inflammation.

Introduction

There is growing evidence that hydrogen sulfide (H_2S), similar to nitric oxide and carbon monoxide, is a cell-signaling molecule with an important role in basic physiology as well as pathophysiology of organ systems. A protective role of H_2S has been implicated in the gastrointestinal tract during inflammatory bowel disease. Although genetic, nutritional, and environmental factors are assumed to be involved in the incidence of inflammatory bowel disease that is characterized by tissue inflammation and degeneration (Podolsky, 1999; Pezzone and Wald, 2002), altered motility is a common symptom of colonic inflammation leading to bloody diarrhea or constipation (Snape and Kao, 1988; Reddy et al., 1991; Collins, 1996; Myers et al., 1997). Significant remodeling of ion channel activity of smooth-muscle cells accounts, in part, for altered motility in colonic inflammation (Akbarali et al., 2010). Several earlier studies reported decreased activity of the voltage-gated Ca^{+2} channels (L-type, $Ca_v1.2b$) of colonic smooth muscle cell during inflammation due to decreased expression and/or modification of the channel protein (Liu et al., 2001; Akbarali et al., 2010; Ross et al., 2010). We have also previously shown that colonic inflammation results in enhanced activity of ATP-sensitive potassium channels (K_{ATP}) of the colonic smooth muscle cell in an experimental model of colitis (Jin et al., 2004). The underlying mechanism of this alteration is not clearly understood, but these channels appear to be potential targets of H_2S.

K_{ATP} channel is a hetero-octamer consisting of two main subunits: a pore-forming Kir6.× (Kir6.1 and Kir6.2) and a sulfonylurea receptor (SUR1, SUR2A, and SUR2B). A functional channel is formed upon association of both these subunits (Babenko et al., 1998). The combination of the various subunits is tissue-dependent, with Kir6.1 and SUR2B being the predominant complex of the K_{ATP} channel in colonic smooth muscle (Jin et al., 2004). These channels are weakly inwardly rectifying, regulate the contractility of the smooth muscle cell, and contribute to the motility patterns in the colon (Koh et al., 1998).

Recent studies have shown that the K_{ATP} channel is one of the major targets of H_2S (Mustafa et al., 2009; Zhong et al., 2010; Mustafa et al., 2011). H_2S is produced by three main enzymes—cystathionine-β-synthase, cystathionine-γ-lyase, and 3-mercaptopyruvate sulfurtransferase—in enteric neurons, smooth muscle cells, and other cell types. It is also known to be produced by enteric bacteria. Although there still remains significant controversy as to the pro- or anti-inflammatory properties of H_2S (Whiteman and Winyard, 2011), several H_2S-releasing drugs have been developed, with

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ABBREVIATIONS: HENS, HEPES-NaOH; K_{ATP}, ATP-sensitive potassium channel; LEVC, levcromakalim; MPO, myeloperoxidase; NEM, *N*-ethylmaleimide; SUR2B, sulfonylurea receptor; TNBS, trinitrobenzene sulfonic acid.

TABLE 1
Solutions used for cell isolation and electrophysiological recordings

Low Ca^{+2} Tyrode	Whole-Cell Recording		
	Internal	5 K^+ External	140 K^+ External
		mM	
137 NaCl	100 K^+ aspartate	135 NaCl	140 KCl
2.7 KCl	30 KCl	5.4 KCl	10 HEPES
0.008 $CaCl_2$	5 HEPES	0.33 NaH_2PO_4	1 $MgCl_2$
0.88 $MgCl_2$	1 $MgCl_2$	5 HEPES	0.1 $CaCl_2$
0.36 NaH_2PO_4	10 EGTA	1 $MgCl_2$	1 TEA
12 $NaHCO_3$	0.1 ATP Na_2	2 $CaCl_2$	
5.5 glucose		5.5 glucose	

TEA, tetraethylammonium chloride.

promising results in the preclinical studies (Linden et al., 2010; Wallace et al., 2012). In an experimental model of colitis, Wallace et al. (2009) demonstrated an increased capacity of the colon tissue to synthesize H_2S and provide a protective role due to modulation of the K_{ATP} channels. However, the mechanism and the interaction between H_2S and the K_{ATP} channels in the setting of colonic inflammation are not known.

In the present study, we sought to determine the basis by which K_{ATP} channel is modulated by H_2S in colonic smooth muscle during inflammation. Our studies show that the potency and efficacy of the K_{ATP} channel opener, levcromakalim (LEVC), and H_2S are enhanced during colonic inflammation. The data also indicate that H_2S allosterically modulates the K_{ATP} channel through sulfhydration of the SUR2B subunit, resulting in enhanced activation of the channel and providing a basis for altered motility in inflammatory conditions.

Materials and Methods

Trinitrobenzene sulfonic acid (TNBS), glibenclamide, and trypsin (from bovine pancreas) were purchased from Sigma-Aldrich (St. Louis, MO). LEVC was purchased from Tocris Bioscience (Minneapolis, MN). H_2S was purchased from Cayman Chemicals (Ann Arbor, MI). Collagenase was purchased from Worthington (Lakewood, NJ). Bovine serum albumin was purchased from American Bioanalytical (Natick, MA). Sodium chloride (NaCl), magnesium chloride ($MgCl_2$), calcium chloride ($CaCl_2$), glucose, ATP disodium salt, HEPES, EGTA, and tetraethylammonium chloride were purchased from Sigma-Aldrich. Mouse Kir6.1 cDNA was purchased from Open Biosystems (Lafayette, CO).

Animals

Adult male BALB/C mice that weighed 25–30 g were housed in animal care quarters under a 12-hour/12-hour light/dark cycle with food and water. All animal procedures were approved by the Institutional Animal Care and Use committee at Virginia Commonwealth University. All studies were performed under the *Guidelines for the Care and Use of Laboratory Animals* as promulgated by the US National Institutes of Health.

Methods

Induction of Inflammation. Inflammation was induced in the colon of the BALB/C mice through intracolonic administration of TNBS (0.1 ml). TNBS solution was prepared by mixing equal proportions of 5% w/v picrylsulfonic acid with 50% ethanol. Weights of the mice were monitored on a daily basis, and myeloperoxidase (MPO) assay was performed on the colon tissue at different time points after the administration of TNBS to determine the severity of inflammation.

MPO Assay. Colon samples were collected from control and inflamed mice. Cell lysate was prepared from these samples and centrifuged. Supernatant collected from centrifuged sample was used for the assay. Chlorination activity assay was performed to determine the MPO activity of the sample. The assay was performed as directed by the protocol provided along with the MPO assay kit.

Cell Isolation. Smooth muscle cells were isolated from the colon of male BALB/C mice (25–30 g) as described previously (Jin et al., 2004). Mice were euthanized and the colon was isolated. The colon was then cut open across the mesenteric border and the mucosa was scrapped off to isolate the muscle layer. This whole process was carried out in a low–calcium Tyrode's solution. The muscle layer was then cut into small pieces and transferred into Tyrode's solution (containing 1.5 mg of collagenase, 1 mg of trypsin, and 5 mg of bovine serum albumin in 5 ml) for 10–12 minutes at 37°C. Then the tissue was subjected to gradual trituration with a flame-polished glass bore. The partially digested tissue was then transferred into the enzyme-free solution, subjected to further trituration, and monitored under a microscope to check for the dispersed cells. The dispersed cells were stored in ice and could be used for 6 hours. All the electrophysiological recordings were done at room temperature (22–25°C).

Electrical Recordings. Standard whole-cell configuration was used for all recordings. The patch-clamp amplifier used was EPC 10 (HEKA, Bellmore, NY). The micropipettes were prepared on a Flaming-Brown horizontal puller (P-87; Sutter Instruments, Novato, CA) and fire polished. Resistance of the pipettes used was 5–10 MΩ. In a gap-free protocol, the cell was held at a voltage of −60 mV and currents were measured continuously for 15 minutes; in the I-V protocol, the cell was held at a voltage of –60 mV and the currents were elicited by depolarization from –120 to 0 mV in 10-mV steps.

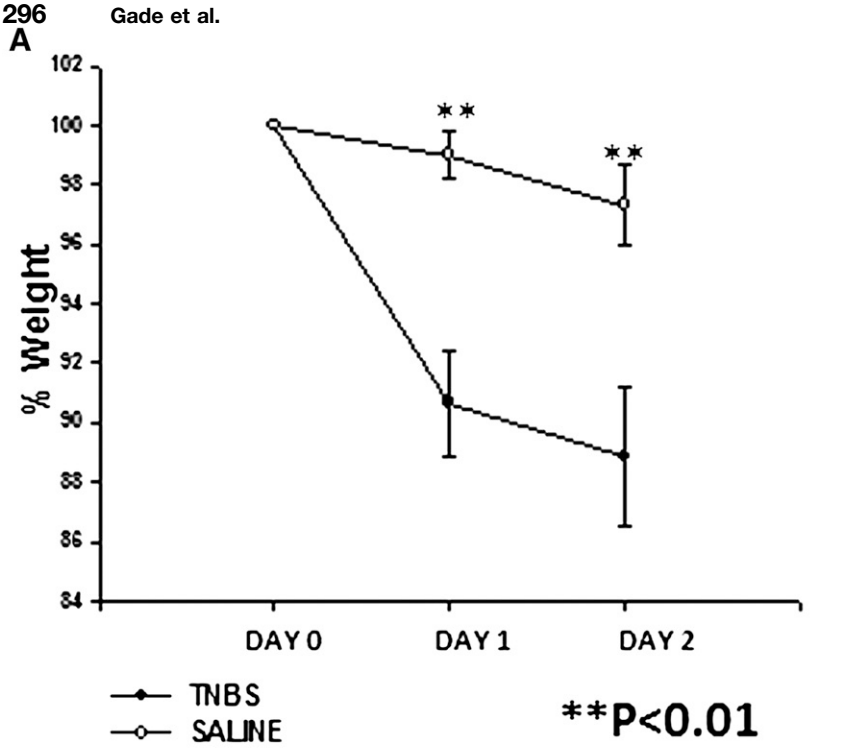
Solutions. Solutions used for recordings in the whole-cell configuration are listed in Table 1. The low-calcium Tyrode's solution was equilibrated with 95% O_2,5% CO_2. The pH of all bathing solutions was adjusted to 7.4 by using 3N KOH. The K_{ATP} currents were recorded in a high K^+ (140 K^+ external) external bath solution that specifically isolated and amplified the K_{ATP} currents.

Biotin-Switch Assay. The assay was carried out as described previously (Mustafa et al., 2009) with modification. In brief, mouse colon tissues or cells treated with or without 1 mM NaHS were homogenized in HEN buffer (250 mM HEPES-NaOH [pH, 7.7], 1 mM EDTA, 2.5% SDS, and 0.1 mM neocuproine) supplemented with 100 μM deferoxamine. Protein samples (250 μg) were added to blocking buffer (HEN buffer adjusted to 2.5% SDS and 20 mM methylmethane thiosulfonate) at 50°C for 20 minutes with frequent vortexing. After acetone precipitation, the proteins were resuspended in HENS buffer (adjusted to 1% SDS). A total of 4 mM biotin-HPDP (N-[6-(Biotinamido)hexyl]-3′-(2′-pyridyldithio)-propionamide) in dimethyl formamide was added to the suspension. After 3-hour incubation at 37°C, biotinylated proteins were precipitated by streptavidin-agarose beads, which were then washed with HENS buffer. The biotinylated proteins were eluted by SDS-PAGE sample buffer and subjected to Western blot analysis. For quantitation of protein sulfhydration, samples were run on blots alongside total lysates ("load"). Anti–goat-SUR2B and anti–goat-Kir6.1 were used at 1:200 to approximately 1:1000 dilution (Santa Cruz Biotechnology, Santa Cruz, CA).

Data Analysis. SigmaPlot 11.0 (Systat Software Inc., San Jose, CA) was used for the analysis of the data and to plot the graphs. EC_{50} values were calculated using a four-parameter logistic nonlinear regression model in SigmaPlot. Significance levels were determined using unpaired *t* tests. A P value ≤ 0.05 was considered to represent a statistically significant finding. All data are expressed and mean ± SEM.

Results

Mice treated with TNBS displayed a significant weight loss on day 1 and 2 after the treatment. MPO assay performed with the colon tissues also displayed a significant increase in MPO activity on day 1 and 2 after treatment with TNBS. This



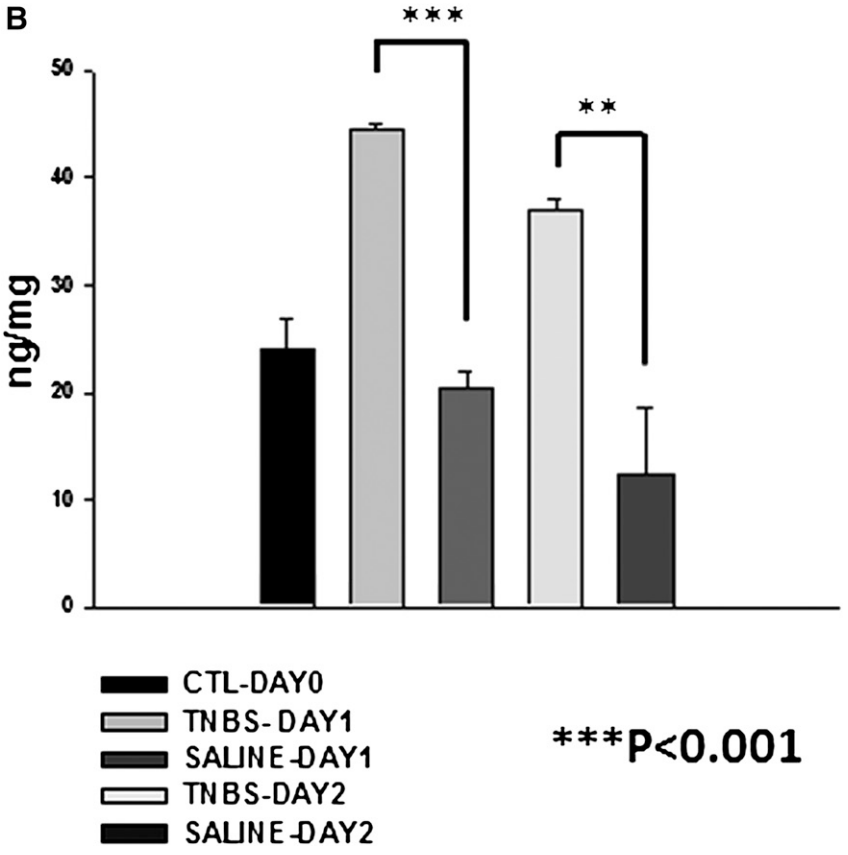


Fig. 1. Disease activity index. (A) percentage decrease in the weight of animals after treatment with TNBS (n = 13) and saline (n = 5). (B) MPO activity in mice in control and after treatment with TNBS (n = 4) and saline (n = 5).

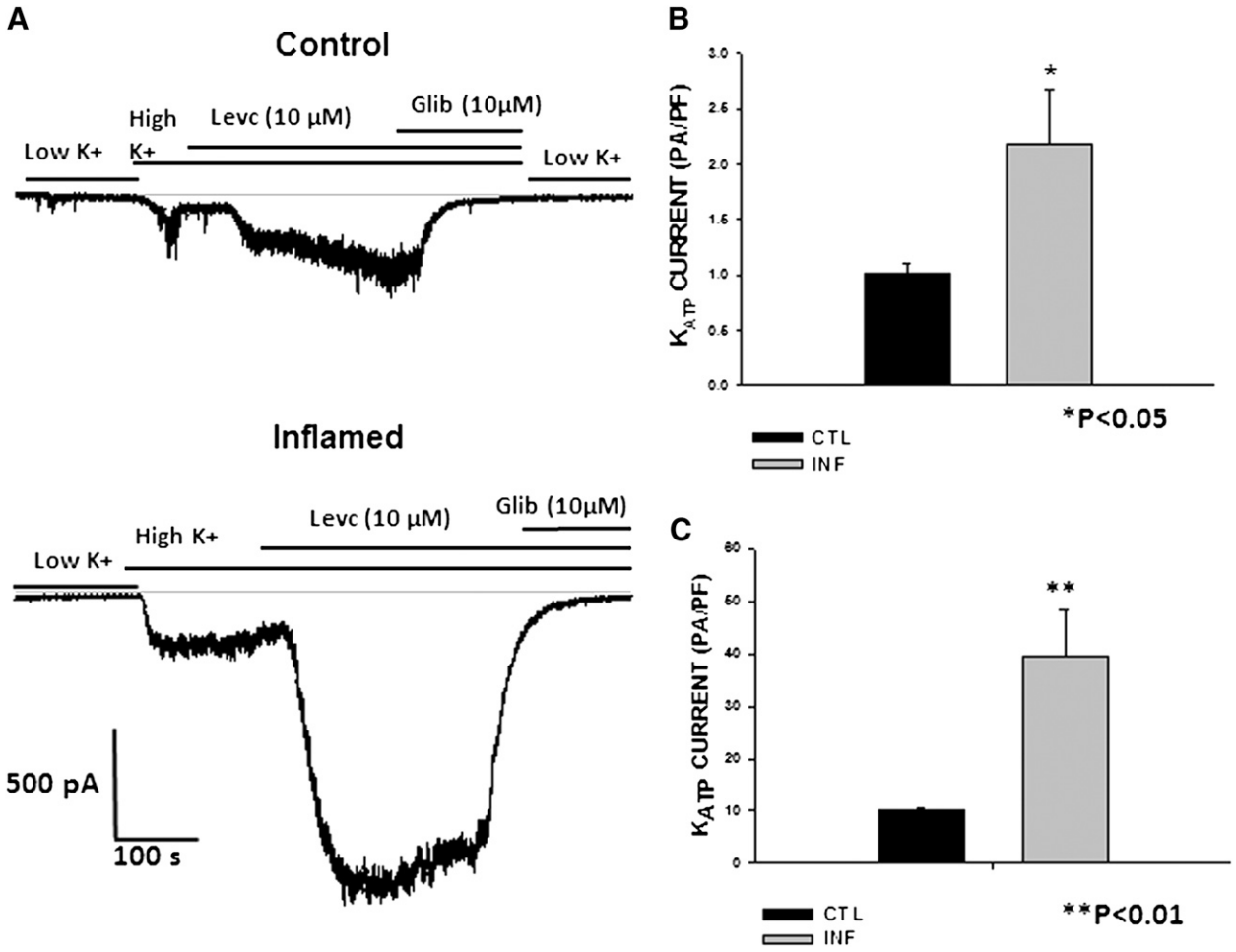


Fig. 2. LEVC-induced currents in control and inflamed cells. (A) raw traces showing the inward current induced by the channel opener LEVC in a whole-cell recording of gap-free protocol at −60 mV in the control and inflamed cells. (B) normalized amplitude of basal K_{ATP} currents in control and inflamed cells (control, 0.9 ± 0.12 pA/pF [n = 14]); inflamed, 5.5 ± 1.4 pA/pF [n = 12]). (C) normalized amplitude of LEVC (10 μM)-induced currents in control and inflamed cells (control, 9.9 ± 0.71 pA/pF [n = 12]; inflamed, 39.6 ± 8.8 pA/pF [n = 10]). Glib, glibenclamide. CTL, control; INF, inflamed.

increase in MPO activity also showed significant differences compared with mice treated with control vehicle (Fig. 1).

Enhancement of the K_{ATP} Channel Opener Induced Currents in Inflammation. To study the alterations in K_{ATP} channel activity in inflammation, currents were recorded from freshly dispersed smooth muscle cells of distal colon using the whole cell configuration of the voltage clamp technique. To identify the K_{ATP} channel currents, cells were bathed in a high K^+ (140 mM) external bath solution, held at a holding potential of −60 mV and dialyzed with low $_{ATP}$ (0.1 mM) in the pipette solution as previously described (Jin et al. 2004). Perfusion from a low- (5.4 mM) to high-K^+ solution resulted in inward currents. The basal currents recorded in the high-K^+ solution were 0.9 ± 0.12 pA/pF (n = 14) in controls and 2.17 ± 0.4 pA/pF (n = 10) in colonic smooth muscle cells from TNBS-treated mice, henceforth referred to as inflamed cells. The average capacitance was 58.93 ± 2.05 pF (n = 39) in control and 45.40 ± 2.28 pF (n = 20) in inflamed cells (P < 0.001). Although the average cell size was significantly decreased in inflamed cells, the average current amplitude normalized to cell capacitance was significantly enhanced. The high K^+-induced currents were abolished by glibenclamide, suggestive of increased basal activity of K_{ATP} in inflamed cells. The K_{ATP} channel opener, LEVC, further enhanced inward currents at −60 mV. The channel opener-induced currents measured after subtraction of baseline currents in high K^+ showed a remarkable increase from 9.9 ± 0.71 pA/pF in control cells to 39.7 ± 8.8 pA/pF in cells from inflamed colon, demonstrating an enhancement of almost sevenfold in inflammation (Fig. 2).

Test depolarizations from −120 to 0 mV in 10-mV increments (holding potential, −60 mV) resulted in time-independent and weakly voltage-dependent currents. Figure 3 shows current-voltage relationships for LEVC-induced currents in control and inflamed cells in the presence of various concentrations of LEVC. Compared with control cells, inflamed cells induced significantly larger currents at each potential and were more sensitive to the channel opener. A concentration-response curve for LEVC-induced currents was plotted at each voltage. Figure 4 shows the concentration response at −60 mV for control (open circles) and inflamed (closed circles). There was both a leftward shift in the concentration response and an enhancement of the maximal current in inflamed cells. The significant shift in potency was evident when current amplitudes at each concentration were plotted as a fraction of the maxima (Fig. 3B). The EC_{50} values calculated for

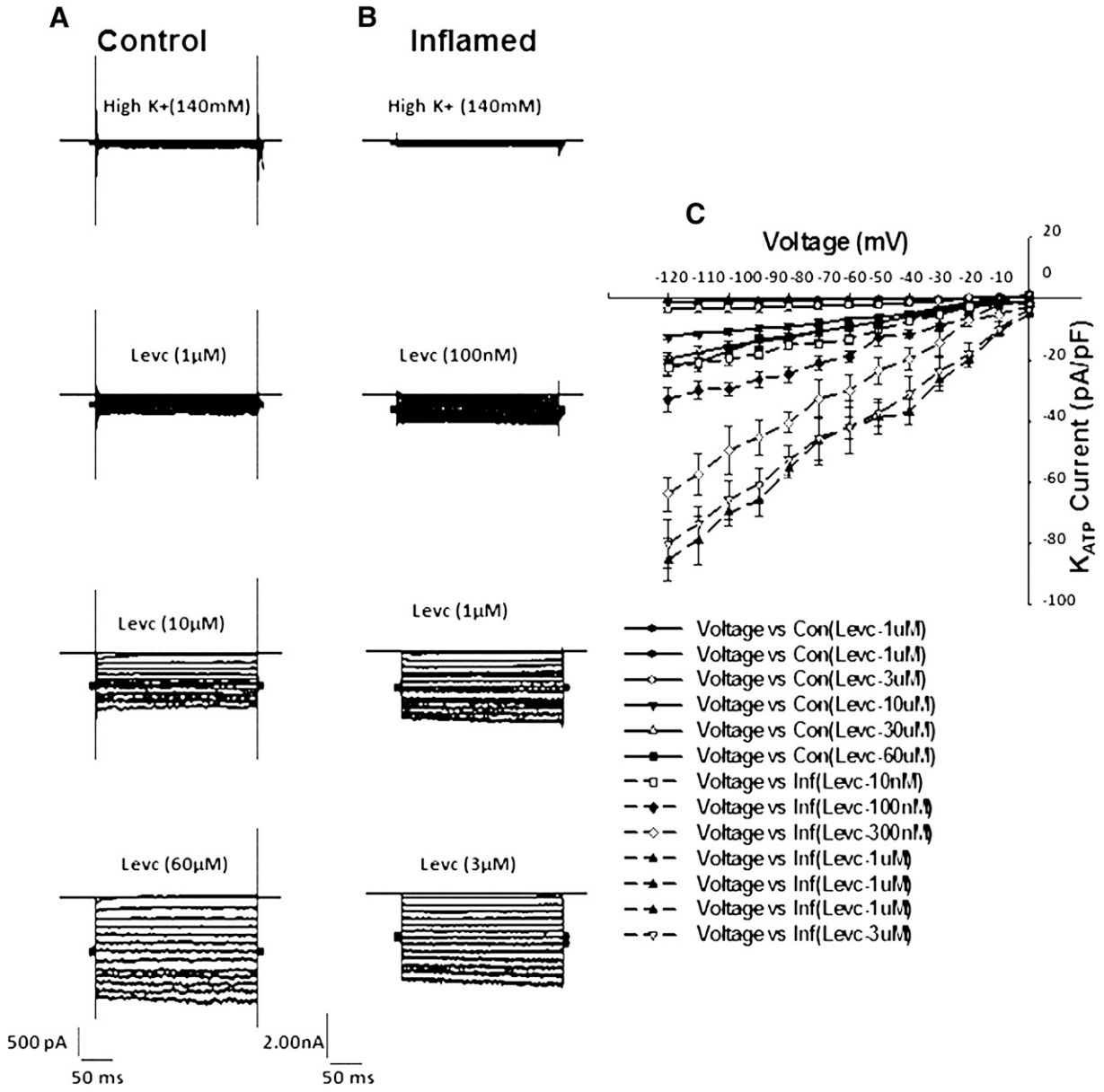


Fig. 3. Voltage and dose dependence of LEV-induced currents. Currents were recorded in a series of step voltages applied from −120 to 0 mV in 10-mV increments from a holding potential of −60 mV in high K^+ solution. (A and B) current traces in high K^+ and in different concentrations of LEVC in control and inflamed cells. Note the difference in scale bars. (C) current-voltage relationship with different concentrations of LEVC in control and inflamed cells. Con, control; Inf, inflamed.

LEVC shifted from 6259 nM (95% confidence limits [CL], 4909–7625 nM) (n = 10) in control cells to 422 nM (95% CL, 273–522 nM) (n = 8) in cells from the inflamed colon showing a 10-fold difference. This finding suggested that inflammation results in an increase in affinity and efficacy for the K_{ATP} channel opener. To further examine whether there was a voltage dependency to the affinity for LEVC, the EC_{50} values were plotted for each potential. The EC_{50} values were not different at any of the different potentials, with inflamed cells being more sensitive to LEVC (Fig. 4).

Effect of K_{ATP} Channel Blocker in Inflammation. We next tested whether the K_{ATP} channel blocker glibenclamide demonstrated any difference in the potency toward inhibition of LEVC-induced currents during inflammation. A cumulative concentration response for glibenclamide-induced inhibition of the K_{ATP} currents was conducted in the presence of 10 μM LEVC (Fig. 5). Although there were significantly larger LEVC-induced currents in inflamed cells, the concentration-response relationship showed no difference in the potency of glibenclamide to inhibit K_{ATP} currents in control or inflamed cells. The IC_{50} values were 183 nM (95% CL 154–217 nM) (n = 6) in control and144 nM (95% CL 128–162 nM) (n = 5) in the cells from inflamed colon (Fig. 5).

Effect of H_2S on K_{ATP} Channels of Colonic Smooth Muscle Cell. We next examined the effect of H_2S, an endogenous signaling molecule whose levels have been shown to be increased in colonic inflammation (Wallace et al., 2009). Exogenous NaHS (1 mM) when added to the external bath solution induced inward currents at −60 mV in a gap-free protocol. The currents were abolished by glibenclamide (10 μM).

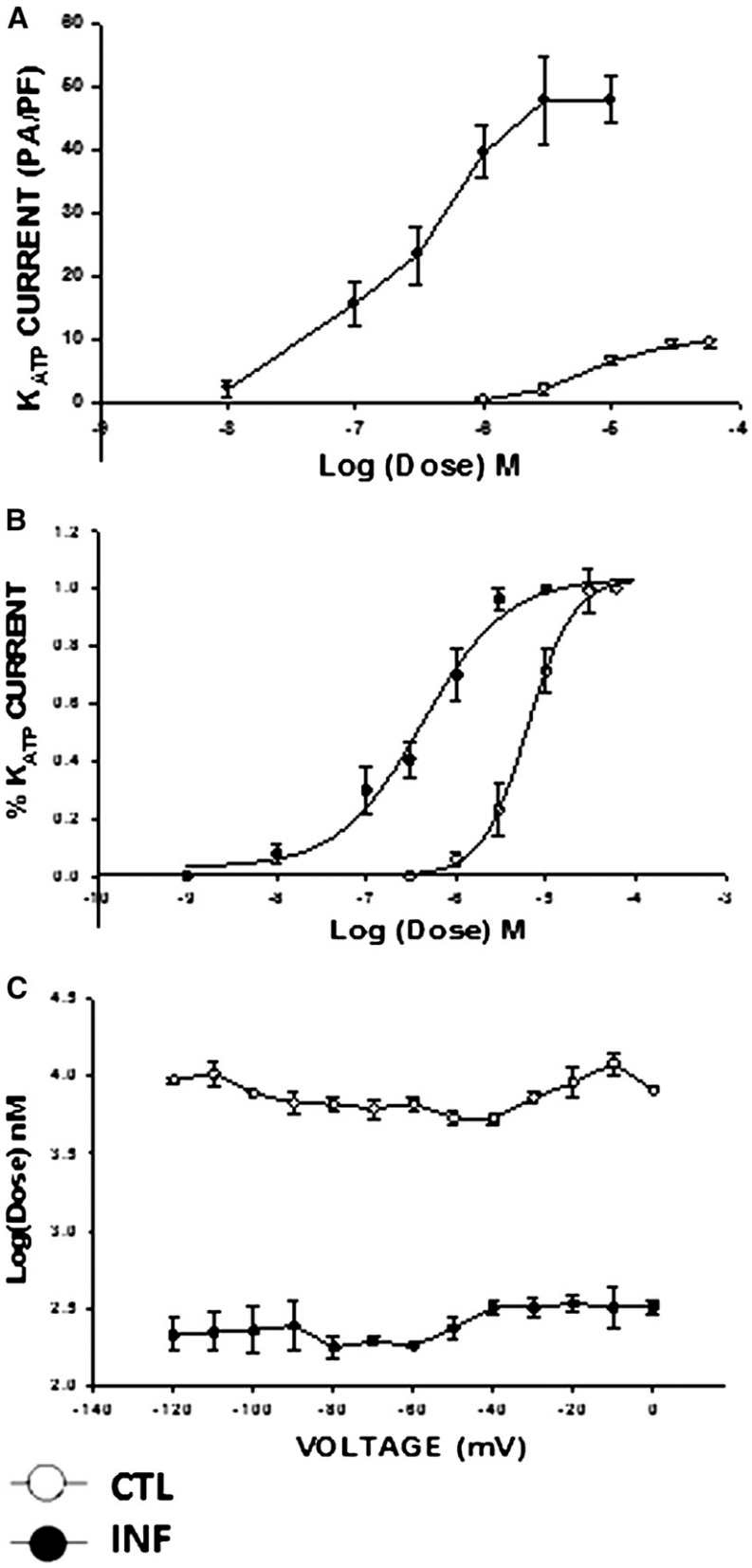


Fig. 4. Concentration-response relation of LEVC. (A) the normalized amplitude of current induced at each dose of LEVC measured at a holding voltage of −60 mV in the smooth muscle cells from control and inflamed colon. (B) the percentage of current induced at each dose of LEVC as a function of the maximum current induced at a holding voltage of −60 mV in the smooth muscle cells of the control and inflamed colon cells. The affinity and efficacy of the channel opener are enhanced in colonic inflammation. The EC_{50} values calculated for the drug shifted from 6259 nM (95% CL 4909–7625 nM) (n = 10) in controls to 422.1 nM (95% CL 273–522 nM) (n = 8) in cells from the inflamed colon. (C) voltage dependence of calculated EC_{50} values in control and inflamed cells. CTL, control; INF, inflamed.

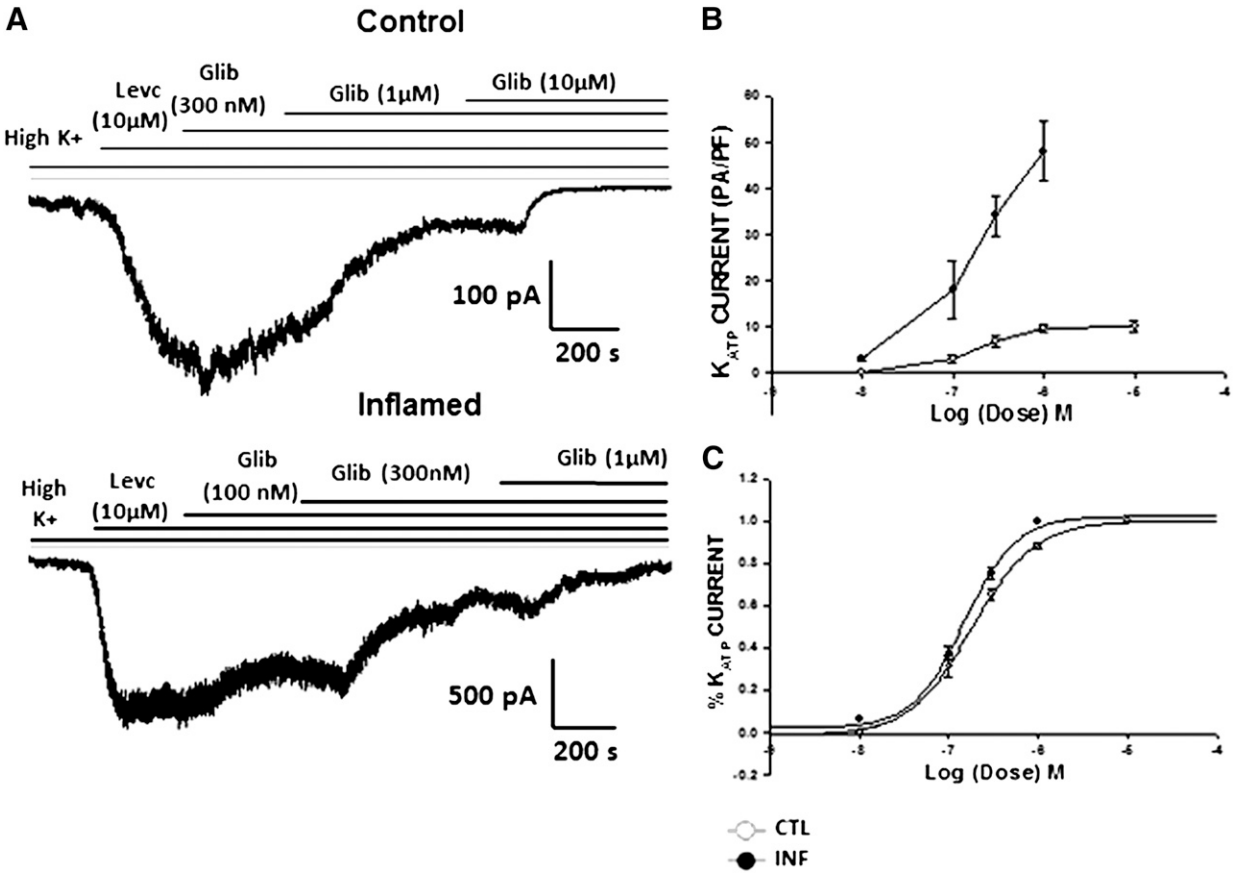


Fig. 5. Effect of glibenclamide (Glib) on K_{ATP} channels in inflammation. (A) raw traces showing the inhibition of the LEVC-induced currents by different concentrations of glibenclamide in a whole-cell recording through the gap-free protocol at −60 mV in control and inflamed cells. (B) concentration-response curve plotted with the normalized currents inhibited by different concentrations of glibenclamide. (C) concentration-response curve plotted with the percentage of current inhibited at different concentrations of glibenclamide as a function of maximum current inhibited. IC_{50} values shifted from 183 nM (95% CL 154–217nM) (n = 6) in controls to 144 nM (95% CL 128–162 nM) (n = 5) in the cells from inflamed colon. CTL, control; INF, inflamed.

Similar to the effects of LEVC, the inward currents activated by 1 mM NaHS were significantly larger in inflamed cells (8.6 ± 1.4 pA/pF; n = 6) than control cells (2.47 ± 0.1; n = 7)

We also tested the concentration dependence of NaHS in control and inflamed cells. There was a significant shift in the concentration-response curve to the left in inflamed cells with an increase in the maximal currents. When plotted as the fraction of maximal currents, the EC_{50} values shifted from 461 μM (95% CL 376–564 μM) (n = 7) in control cells to 199 μM (95% CL 140–283 μM) (n = 6) in inflamed cells (Fig. 6).

Effect of H_2S on K_{ATP} Opener Induced Currents. To examine whether NaHS acts as an allosteric modulator of LEVC-induced K_{ATP} currents, a low dose of H_2S (100 μM) was bath applied before LEVC concentration response was conducted. In the presence of 100 μM, the currents activated were 0.47 ± 0.04 pA/pF. In the presence of this concentration of H_2S, the channel opener showed an increased affinity toward the channel and induced currents at lower doses. The curve plotted shifted to the left and the EC_{50} values calculated shifted from 2838 nM (95% CL 954–4625 nM) (n = 6) to 154.9 nM (95% CL 94–251 nM) (n = 8) in the presence of 100 μM NaHS, demonstrating an increase in affinity of the drug similar to what was seen in the case of inflammation. At this concentration of NaHS, there was no increase in the maximal amplitude of current induced by LEVC (Fig. 7).

This study was repeated in the presence of a higher concentration of NaHS (1 mM) in which a maximal concentration of the channel opener (10 μM) was used to induce K_{ATP} currents. The maximal amplitude of the inward currents induced by the opener increased from 10.5 ± 1.6 pA/pF (n = 6) in the presence of 100 μM H_2S to 22 ± 5.4 pA/pF (n = 4) in the presence of 1 mM of H_2S, demonstrating an increase in the efficacy of the drug in the presence of higher concentration of H_2S (Fig. 8).

Effect of *N*-Ethylmaleimide on Opener and NaHS-Induced Current. To examine the involvement of cysteine residues in the action of H_2S, effect of *N*-ethylmaleimide (NEM, an alkylating agent of free cysteine residues) was tested on NaHS- and LEVC- induced currents. In the presence of 2 mM NEM, the responses produced by NaHS and LEVC were significantly decreased, indicating a strong involvement of cysteine residues on their action (Fig. 9). NaHS-induced currents decreased from 2.47 ± 0.56 pA/pF in control to 0.0397 ± 0.001 (n = 4) in the presence of NEM. LEVC-induced currents were reduced from 9.9 ± 0.71 in control to 0.45 ± 0.3 in the presence of NEM (n = 4).

H_2S Sulfhydrates SUR2B but Not Kir6.1 Subunit of K_{ATP} Channel. Because sulfhydration is known as a primary mechanism through which H_2S signals, we examined the sulfhydration levels in K_{ATP} channels of colonic smooth muscle cell after treatment with 1 mM NaHS using a Biotin Switch

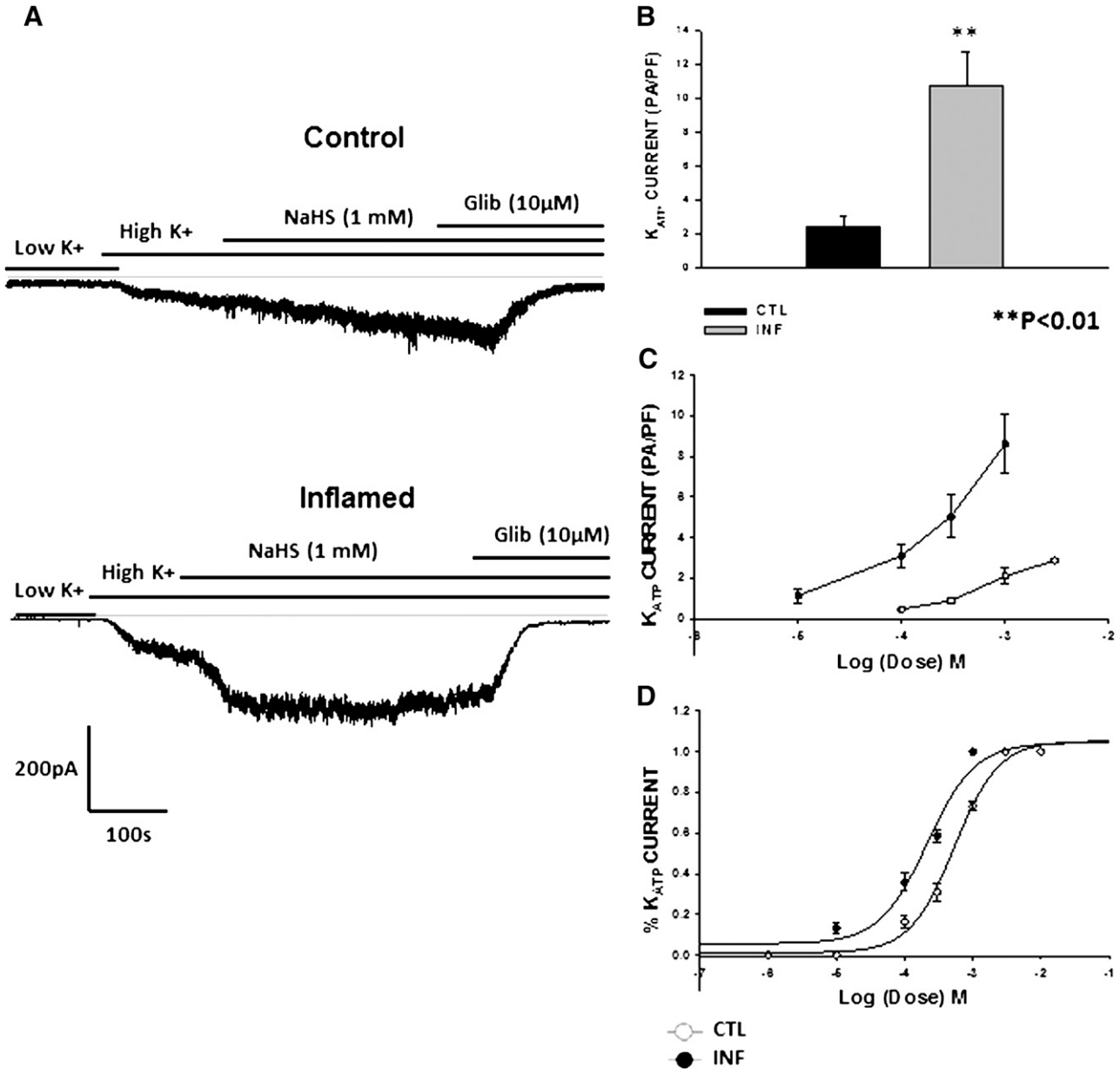


Fig. 6. Effect of H_2S on K_{ATP} channels of colonic smooth muscle cell. (A) raw traces showing the NaHS (H_2S donor)-induced currents in a whole cell recording at −60 mV voltage through a gap-free protocol in control and inflamed cells. (B) normalized amplitude of inward currents induced by NaHS in control and inflamed cells (control, 2.4 ± 0.5 pA/pF [n = 6]; inflamed, 10.7 ± 1.9 pA/pF [n = 7]) (C) concentration-response curve plotted using the amplitudes of currents induced with different concentrations of NaHS in control and inflamed cells. (D) the percentage of current induced at each dose of NaHS as a function of the maximum current induced at a holding voltage of −60 mV in the smooth muscle cells of the control and inflamed colon cells. The affinity and efficacy of the channel opener are enhanced in colonic inflammation. The EC_{50} values calculated for NaHS shifted from 461 μM (95% CL 376–564 μM) (n = 7) in controls to 199 μM (95% CL 140–283 μM) (n = 6) in cells from the inflamed colon. CTL, control; Glib, glibenclamide; INF, inflamed.

Assay. There was some basal sulfhydration of the SUR2B subunit of the K_{ATP} channel that was enhanced upon treatment with 1 mM NaHS (Fig. 10, A and B). The enhanced sulfhydration of SUR2B was also seen in inflamed colon without any treatment with NaHS (Fig. 10C). In Chinese hamster ovary cells heterologously expressing Kir6.1 and SUR2B, sulfhydration was evident for SUR2B but not the Kir6.1 subunit (Fig. 11).

Discussion

The importance of H_2S as a gaseous signaling molecule has been recognized in various physiologic and pathophysiologic conditions (Mustafa et al., 2009). In colonic inflammation, the protective role of H_2S has been, in part, attributed to modulation of the ATP-sensitive potassium channels (Wallace et al., 2009). In the present study, we have found that 1) the potency and efficacy of the K_{ATP} channel opener, LEVC, is enhanced during colonic inflammation; 2) similarly, H_2S-induced activation of the channel is also enhanced in inflamed cells; 3) H_2S modifies the activation of LEVC via an allosteric effect; and 4) H_2S *S*-sulfhydrates the SUR2B subunit but not Kir6.1.

Previously, Jin et al. (2004) demonstrated, in a mouse colitis model, an increase in both the amplitude of whole cell

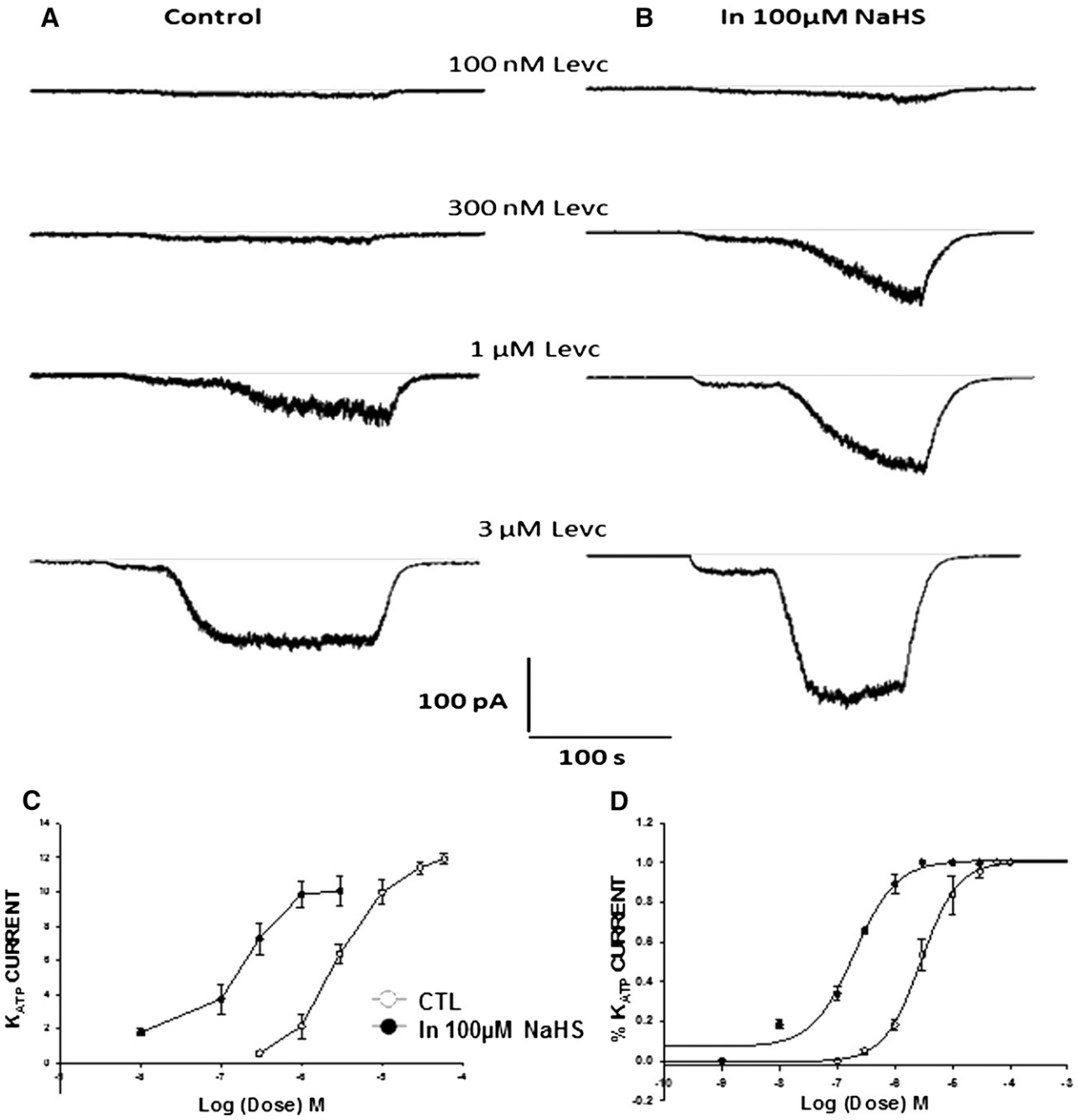


Fig. 7. Effect of H_2S on LEVC-induced currents. (A) current traces in the presence of different concentrations of LEVC in control. (B) current traces in the presence of different concentrations of LEVC in the presence of 100 μM NaHS. (C) the amplitude of current induced at each dose of LEVC measured at a holding voltage of −60 mV in the smooth muscle cell under control conditions and in the presence of 100 μM NaHS (n = 8). (D) the percentage of current induced at each dose of LEVC as a function of the maximum current induced at a holding voltage of −60 mV in the smooth muscle cell under control conditions and in the presence of 100 μM NaHS. In the presence of NaHS, the affinity of LEVC is enhanced. The EC_{50} values showed a leftward shift (control, 2838 nM [95% CL 1254–3625 nM]; inflamed, 100 μM NaHS:154.9 nM [95% CL 94–251 nM]). CTL, control; IN, inflamed.

K_{ATP} currents and in the bursting activity of single channel currents in colonic smooth muscle in the presence of LEVC. We compared the concentration-response relationship for LEVC in inflamed cells and identified that in addition to increase in maximal currents (efficacy), the potency for LEVC is significantly shifted after inflammation. Of note, the potency of glibenclamide-induced inhibition of the K_{ATP} channel complex was not altered with inflammation, although the potential binding sites for the channel opener and blocker are on the same subunit (i.e., the sulfonylurea receptor) (Mikhailov et al., 2001; Moreau et al., 2005). Similarly, the potency of H_2S toward activation of the K_{ATP} channel is also enhanced after inflammation. Although the activation of K_{ATP} channel by hydrogen sulfide has been demonstrated in several studies (Cheng et al., 2004; Spiller et al., 2010; Zhong et al., 2010; Liang et al., 2011; Liu et al., 2011;), the specific subunit that is affected is not entirely clear. In addition to its effects on K_{ATP} channels, H_2S also modulates other ion channels,

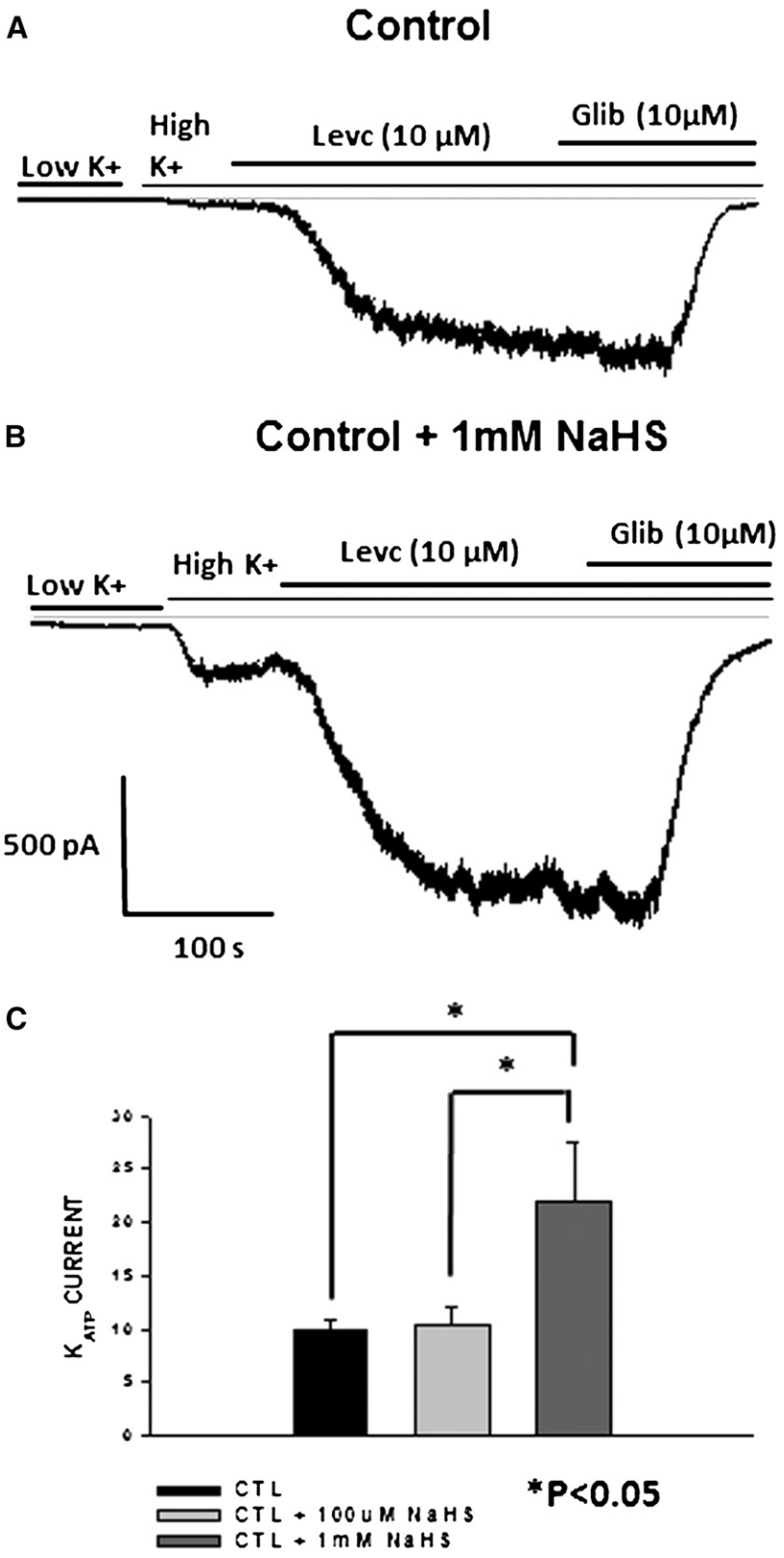


Fig. 8. (A) current trace showing the response to LEVC in controls. (B) current trace showing the response to LEVC in the presence of 1 mM NaHS. (C) amplitude of current induced by 10 μM LEVC in the control cells and in the presence of different doses of NaHS (H_2S donor). Amplitude of LEVC-induced currents is enhanced in the presence of high NaHS demonstrating an increase in efficacy of LEVC (control, 9.9 $\pm$ 0.71 pA/pF [n = 12]; control plus 100 μM NaHS, 10.5 $\pm$ 1.6 pA/pF [n = 8]; control plus 1 mM NaHS, 22 $\pm$ 5.4 pA/pF [n = 5]). CTL, control; Glib, glibenclamide.

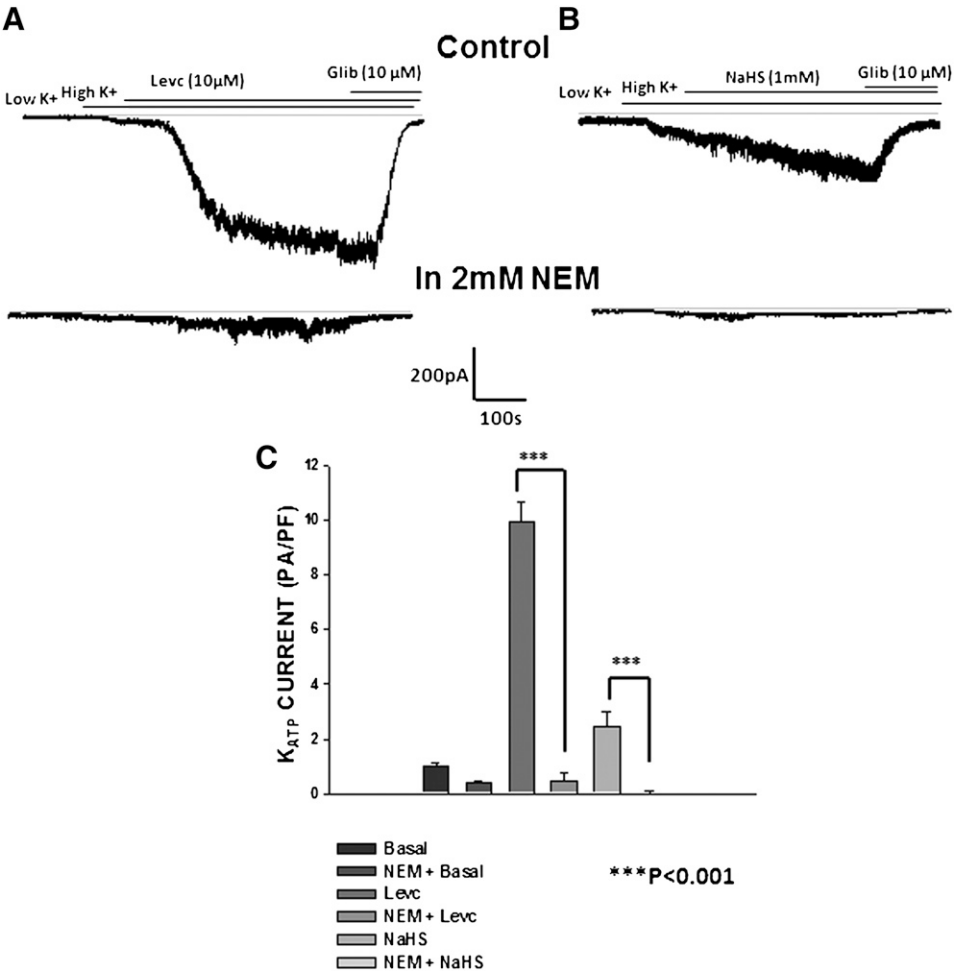


Fig. 9. (A) current traces showing the response to LEVC in controls and in the presence of 2 mM NEM. (B) current traces showing the response to 1 mM NaHS in controls and in the presence of 2 mM NEM. (C) bar graph showing the quantified differences in the amplitude of drug-induced currents blocked by NEM. Glib, glibenclamide.

notably L- and T-type calcium channels, as well as Na^+ channels. L-type Ca^{2+} channels are inhibited, whereas T-type channels are sensitized (Sun et al., 2008; Matsunami et al., 2012). In the human jejenum smooth muscle cells, H_2S enhanced Na^+ influx through $Na_v1.5$ via a redox-independent mechanism (Strege et al., 2011). *S*-sulfhydration of cysteine residues by H_2S has been established as a post-translational modification altering protein function. Mustafa et al. (2011) demonstrated sulfhydration of Cys43 of Kir6.1 as the potential site for H_2S-induced enhancement of K_{ATP} channel activity. On the other hand, Jiang et al. (2010) found that in HEK cells, expression of rvSUR1 subunit was necessary for H_2S-induced activation of K^+ currents and replacement of extracellular Cys6 and Cys26 abolished channel sensitivity to H_2S. We found that in colonic smooth muscle and in heterologously transfected cells, SUR2B was sulfhydrated by exogenous H_2S, alluding to the possibility that posttranslational modification of the sulfonylurea receptor during inflammation alters the sensitivity to potassium channel opener. *S*-sulfhdyration appears to induce an allosteric effect on the activation of K_{ATP} channels. This was evident when in the presence of low concentrations of H_2S the potency of LEVC is enhanced, an effect that is similar to inflammation. *S*-sulfhydration of the SUR2B subunit was also enhanced after colonic inflammation. Allosteric modulation of ion channels by endogenous signaling molecules including ATP, H_2O_2, glycine have been well described (Cui and Fan, 2002; Hogg et al., 2005; Chuang and Lin, 2009). Cui et al. suggested the modulation of K_{ATP} channel activity through sulfhydration of the cysteine residue of the Kir6.2 in heterologously expressed K_{ATP} channel (Cui and Fan, 2002). These studies demonstrated an allosteric block due to sulfhydration of extracellular cysteine residue. Our findings indicate that sulfhydration of SUR2B during colonic inflammation accounts for the enhanced sensitivity to K_{ATP} channel opener *S*-sulfhydration may result from enhanced H_2S production during inflammation both from sources within the lumen (i.e., enteric bacteria) and from endogenous production due to the enhanced activity of cystathionine-β-synthase and cystathionine-γ-lyase. It is noteworthy that breakdown of mucosal barrier in colitis could further exaggerate the exposure of smooth muscle to the levels of H_2S. In summary, the present study provides evidence of allosteric modulation through s-sulfhydration as a mechanism by which K_{ATP} channel activity is enhanced during colonic inflammation.

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Authorship Contributions

Participated in research design: Gade, Kang, Akbarali.
Conducted experiments: Gade, Kang, Akbarali.

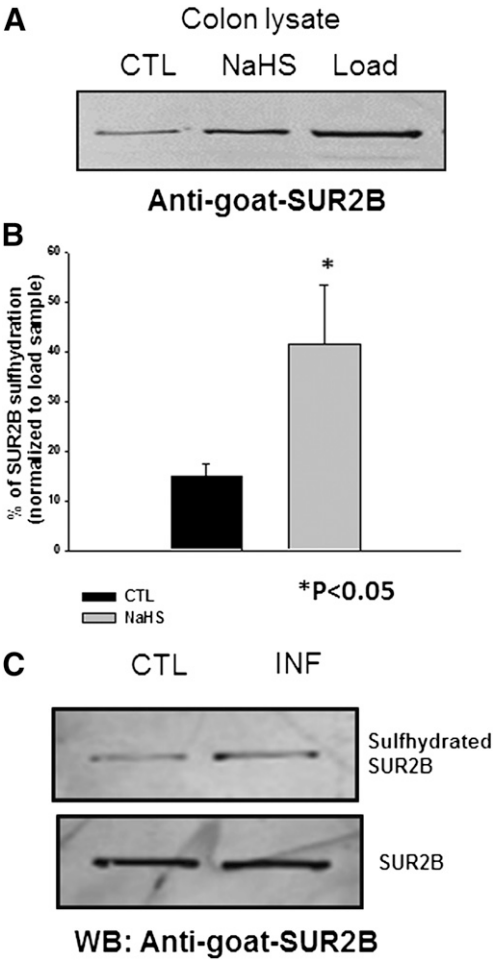

Fig. 10. (A) Western blot showing the difference in the sulfhydration levels in the controls and after treatment with 1 mM of NaHS in mouse colon tissue. (B) quantified data demonstrating the difference in the amount of sulfhydration of the SUR2B subunit of the K_{ATP} channel in control and after treatment with 1 mM NaHS in mouse colon tissue. (C) Western blot showing the difference in the sulfhydration levels in the control and in inflamed mouse colon tissue. CTL, control; INF, inflamed; WB, Western blot.

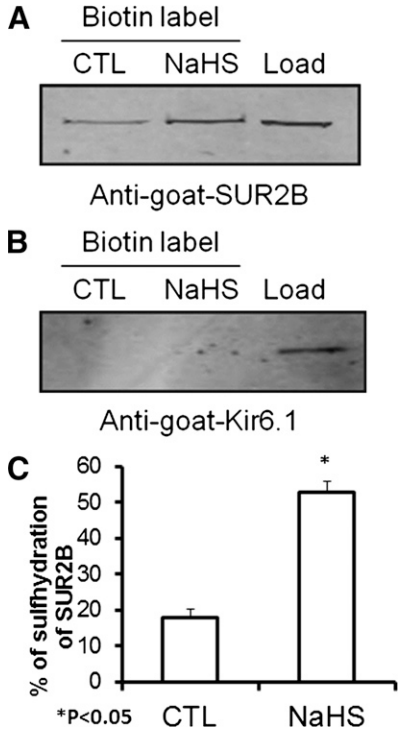

Fig. 11. (A) Western blot showing the difference in the sulfhydration levels in the controls and after treatment with 1 mM of NaHS in SUR2B but not Kir6.2 subunit–transfected K_{ATP} channel. (B) quantified data demonstrating the difference in the amount of sulfhydration of the SUR2B subunit of the K_{ATP} channel in controls and after treatment with 1 mM NaHS in transfected K_{ATP} channel (data normalized to the load value). CTL, control.

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Wrote or contributed to the writing of the manuscript: Gade, Kang, Akbarali.

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