Leukocyte trafficking and pain behavioral responses to a hydrogen sulfide donor in acute monoarthritis

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Andruski B, McCafferty D-M, Ignacy T, Millen B, McDougall JJ. Leukocyte trafficking and pain behavioral responses to a hydrogen sulfide donor in acute monoarthritis. Am J Physiol Regul Integr Comp Physiol 295: R814–R820, 2008. First published July 30, 2008; doi:10.1152/ajpregu.90524.2008.—Hydrogen sulfide (H₂S) is an endogenous gaseous mediator with the ability to modulate tissue inflammation and pain. The aim of this study was to determine the effect of an H₂S donor (Na₂S) on leukocyte-endothelium interactions, blood flow, and pain sensation in acutely inflamed knee joints. Acute arthritis was induced in urethane anesthetized C57bl/6 mice by intra-articular injection of kaolin/carrageenan (24-h recovery), and the arthritis was induced in urethane anesthetized C57bl/6 mice by intra-articular injection of Na₂S on leukocyte trafficking was measured by intravital microscopy. Synovial blood flow was measured in inflamed knees by laser Doppler perfusion imaging. Finally, the effect of an intra-articular injection of Na₂S on joint pain in control and inflamed rats was determined by hindlimb incapacitance and von Frey hair algesiometry. Local administration of an H₂S donor to inflamed knees caused a dose-dependent reduction in leukocyte adherence and an increase in leukocyte velocity. These effects could be inhibited by coadministration of the ATP-sensitive potassium (Kₐ₅₆) channel blocker glibenclamide. Local administration of Na₂S to inflamed joints caused a pronounced vasoconstrictor response; however, there was no observable effect of Na₂S on joint pain. These findings establish H₂S as a novel signaling molecule in rodent knee joints. H₂S exhibits potent anti-inflammatory properties, but with no detectable effect on joint pain.

INFLAMMATORY JOINT DISEASE and its associated clinical syndromes represent a major health care burden worldwide, and the prevalence of these diseases is rising as the population of the developed world ages (28a, 22). Key features of joint inflammation are alterations in tissue blood flow, increased vascular permeability, pain, and the recruitment of leukocytes into joint tissues. The infiltration of leukocytes into the joint occurs through a multistep process of leukocyte interactions with activated endothelium and leads to the exacerbation of the inflammatory response (7).

Recently, interest has grown in the role of the gaseous transmitter hydrogen sulfide (H₂S) in inflammatory processes in various tissue organs, including the gastrointestinal tract, liver, and lungs (3, 5, 6, 29, 30). H₂S is synthesized endogenously from t-cysteinyne by two enzymes: cystathionine-β-synthetase and cystathionine-γ-lyase (CSE). CSE is the predominant source of H₂S in the circulatory system, where it can act to relax (27) or contract (21) vascular smooth muscle. Studies have demonstrated that H₂S alters vasomotor tone and can influence leukocyte-endothelial interactions (3, 5, 29). The ability of H₂S to induce these effects appears to be due to its ability to activate ATP-sensitive K⁺ (Kₐ₅₆) channels (2, 27).

The action of H₂S during inflammation and its effect on leukocyte-endothelium interactions is not clear cut and may depend on the inflammatory stimulus or the organ that is inflamed. For example, H₂S and CSE are both upregulated in rodent models of acute pancreatitis, and inhibition of CSE by N-proparglyglycine (PAG) reduced the severity of pancreatitis (24). Similarly, PAG administration in a carrageenan-induced model of inflammation inhibited edema formation and neutrophil infiltration in a rat hindpaw (1). Furthermore, leukocyte recruitment to the lung during sepsis was inhibited by prophylactic or therapeutic administration of PAG (30). In the same study, an H₂S donor was shown to increase the expression of adhesion molecules in the lungs of septic mice. Contrary to these studies demonstrating a proinflammatory role for H₂S, Zanardo et al. (29) showed that H₂S donors could suppress leukocyte adherence induced by aspirin in mesenteric venules, reduce leukocyte infiltration in the air pouch model, and reduced edema formation to carrageenan in the hindpaw in rats; inhibition of endogenous H₂S could reverse these effects. In addition, other studies have suggested that H₂S can have anti-inflammatory properties through inducing neutrophil apoptosis (16) and interfering with granulocyte killing of cells and microbes (28).

In light of these contradictory findings, the present study investigated the effects of an H₂S donor [disodium sulfide (Na₂S)] on leukocyte-endothelial interactions in vivo in the synovial microvasculature of the acutely inflamed mouse knee using intravital microscopy. By using the Kₐ₅₆ channel blocker glibenclamide, the effects of H₂S are shown to be mediated via these cationic channels. H₂S appears to alter pain perception with both pronociceptive (13) and antinociceptive (2) responses having been described. Thus the effect of H₂S on knee joint nociception was also examined by joint incapacitance and von Frey hair algesiometry.

MATERIALS AND METHODS

Animals. Male C57Bl/6 mice (weighing 19–26 g) and male Wistar rats (weighing 206–313 g) were housed in standard animal care facilities on a 12:12-h light-dark cycle at 22°C, with free access to standard laboratory chow and water. The experimental protocols were approved by the University of Calgary Animal Care Committee, in accordance with standards set by the Canadian Council for Animal Care.

Acute knee inflammation model. To assess acute inflammatory changes in the joint, the kaolin-carrageenan monoarthritis model was chosen. Unlike other more chronic arthritis models (e.g., Freund’s complete adjuvant monoarthritis model), kaolin-carrageenan causes a...
gradual inflammatory reaction that peaks ~24 h after treatment. Animals were deeply anesthetized (2–4% isoflurane; 100% O2 at 1 l/min), and adequate anesthesia was confirmed by absence of the hindpaw withdrawal reflex. The right knee was shaved and swabbed with 100% ethanol. A 10-μl intra-articular injection of 2% kaolin was administered and manual extension/flexion of the knee joint was performed for 10 min. Subsequently, 10 μl of 2% carrageenan were similarly injected, and the joint was moved for 30 s to disperse the carrageenan throughout the joint. Animals were allowed to recover for 24 h, and a positive inflammatory reaction was confirmed by an observable increase in knee joint diameter, as measured by a digital micrometer (Mitutoyo Instruments, Tokyo, Japan) oriented across the joint line in a mediolateral plane.

Production of H2S by a donor. To test the role of H2S on joint inflammation and pain, the H2S donor Na2S was used at doses of 10, 30, and 50 μM. In solution, Na2S dissociates into H2S and NaOH. The use of a donor is preferred over bubbling H2S gas directly onto the tissue, since it permits us to define more accurately the concentration of H2S being administered to the joint.

Intravital microscopy of the synovial microcirculation. Mice were anesthetized by intraperitoneal injection of 10 mg/kg xylazine (MTC Pharmaceuticals, Cambridge, Ontario) and 200 mg/kg ketamine hydrochloride (Rogar/STB, Montreal, Quebec), and depth of anesthesia was confirmed by abolition of the hindpaw withdrawal reflex. Venous access was obtained by surgical cannulation of the left jugular vein. The skin and connective tissue over the right knee were surgically removed to expose the anteriomedial aspect of the knee joint, and animals were placed in dorsal recumbency on a homeothermic heat blanket. The exposed knee joint was immediately and continuously perfused with warmed buffer (135 mmol/l NaCl, 20 mmol/l NaHCO3, 5 mmol/l KC1, 1 mmol/l MgSO4·7H2O, pH = 7.4) at a rate of 12 ml/h using a peristaltic pump (Gilion, Guelph, Ontario). The mouse was placed on a specially designed stage, which allowed the knee joint to be secured in a presentation suitable for microscopy. A glass coverslip was gently placed over the medial aspect of the joint and secured to the stage with vacuum grease.

Leukocytes were stained in vivo by intravenous injection of 0.05% Rhodamine 6G (Sigma-Aldrich). The microcirculation was examined under incident fluorescent light microscopy using a Mikron IV 500 microscope (Mikron Instruments, San Marcos, CA) with a ×40 objective lens (Zeiss Achromat 40X/0.75W) and a Periplan eyepiece (final magnification ×400). Straight, unbranched, postcapillary venules (diameter 20–50 μm), located directly on the knee joint capsule, were selected for analysis. Leukocyte kinetics were recorded using a XR/MEGA-10 video camera (Stanford Photonics Palo Alto, CA). To determine whether H2S could alter leukocyte kinetics, the knee joint was perfused with buffer (37°C) containing 0, 10, 30, or 50 μM Na2S continuously for 1 h, and 1-min recordings were made at 5, 15, 30, and 60 min. A 1-min control recording was acquired before Na2S perfusion. Recordings were subsequently analyzed offline to determine leukocyte trafficking within the microvasculature.

Leukocyte kinetics. A rolling leukocyte was defined as a white blood cell moving slower than the normal flow of blood in a given vessel (15). Leukocyte flux was calculated as the number of rolling cells to pass an arbitrarily defined line perpendicular to the axis of the venule per minute. Leukocyte velocity (μm/s) was calculated as the time required for a rolling leukocyte to travel 100 μm of vessel length and is presented as the average velocity of the first 10 leukocytes to pass an arbitrarily defined line. A leukocyte was considered to be adherent if it remained stationary for at least 30 s, and total leukocyte adhesion was quantified as the number of adherent cells within a 100-μm length of venule.

Laser Doppler imaging of synovial blood flow. Animals were anesthetized by intraperitoneal injection of ketamine (10 mg/kg) and xylazine (0.5 mg/kg) and placed in dorsal recumbency on a homeothermic heating blanket. The skin and connective tissue over the right knee joint were surgically removed, and the exposed knee was immediately and continuously perfused onto the surface of the tissue with warm buffer, which was delivered by a Gilson peristaltic pump set at a rate of 12 ml/h, as described above. Perfusion was temporarily suspended between blood flow measurements.

Changes in synovial blood flow to the medial aspect of the knee joint were measured using a Moor laser Doppler imager (LDI; Moor Instruments, Axminster, UK), as previously described (12, 17). This technique is based on the principal that laser photons of a known wavelength undergo Doppler shifting when encountering circulating erythrocytes. This “flux” component of the measurement is proportional to the velocity of moving blood cells. A “concentration” element is also recorded that relates to the number of erythrocytes detected in the microvasculature. The image is then normalized to the background illumination of nonvascular tissue and assigned a DC component. Mice were placed 30 cm under the scanner head of the laser, and the exposed knees were scanned, yielding a two-dimensional, color-coded map of joint blood flow. Scan resolution was set at 42 × 55 pixels, with a scan speed of 4 ms/pixel. LDI gain settings were DC = 0, flux = 0, and concentration = 4. A control scan of the knee joint was taken 5 min before the application of new perfusion buffer containing 50 μM Na2S, which was continuously perfused over the exposed knee joint for 1 h. Image scanning tended to last ~10 s. Additional scans were taken at 5, 15, 30, and 60 min after the initial application of the Na2S. At the conclusion of the experiment, the animal was killed by anesthetic overdose (pentobarbital sodium, 80 mg intracardiac), and a scan of the dead animal was taken. This “biological zero” (which accounts for tissue optical noise and Brownian motion) was subtracted from all captured images before data analysis.

The two-dimensional maps of blood flow generated by the laser scans were analyzed with MoorLDI Image Processing software (Moor Instruments, Axminster, UK). Images consist of multiple pixel points, each with an assigned perfusion value, where perfusion = (LDI flux × LDI concentration)/DC2. A region of interest approximating the joint capsule with underlying synovium was identified (see Fig. 5), and the mean perfusion within this area was calculated. Changes in joint blood flow were expressed as a percentage of the control scan captured before drug or vehicle superfusion. In a subgroup of animals, mean arterial blood pressure was continuously measured via an indwelling carotid cannula. Mean arterial pressure was unaffected by Na2S superfusion (data not shown), and as such all blood flow changes were a result of an alteration in vasomotor tone and, therefore, independent of any potential changes in systemic blood pressure.

Joint pain assessment. Behavioral responses to joint pain were carried out on male Wistar rats (206–313 g) and consisted of hindlimb weight distribution measurements and von Frey hair algesiometry, as previously described (18, 19). These techniques were chosen because in rats they produce robust, repeatable measures of joint pain and secondary allodynia, respectively. Hindlimb weight distribution was evaluated by an incapacitance meter (Linton Instrumentation, Norfolk, UK), which measures the weight born by each hindlimb while the animal is standing on dual-force plates. Over a 3-day period, the rat was trained to rear on its hindlimbs and remain in this upright position for 5 s, while the average weight placed on each hindlimb was measured by the incapacitance meter. Care was taken to ensure that the animal’s weight was borne by the paws and that no weight was dissipated via the tail. Each time point is the mean of three consecutive weight distribution measurements. The percent weight placed onto the treated (ipsilateral) hindlimb was calculated by the following equation:

\[
\text{[weight on ipsilateral hindlimb/ (weight on ipsilateral + weight on contralateral)]} \times 100
\]

von Frey hair algesiometry is a measure of tactile allodynia and involves the placement of a fine plastic filament onto the plantar surface of the rat hindpaw. The algesiometer (Ugo-Basile, Milan,
Italy) gradually increases the level of mechanical force being exerted by the filament, and the point at which the animal senses this tactile stimulus the hindpaw is withdrawn. The force required to elicit this voluntary withdrawal response is termed the nociceptive threshold and is measured in grams. A maximum force of 50 g and a ramp speed of 4.5 g/s were chosen for all of the algesiometry trials.

The animal groups used in the pain studies consisted of rats with an acute knee joint monoarthritis (2% kaolin, 2% carrageenan with a 24-h recovery period), which were subsequently treated with either an intra-articular injection of 50 µM Na2S or sterile 0.9% saline (control group). Two further control groups were tested, namely, noninflamed, nontreated naive controls and a group of rats that were given an intra-articular injection of sterile 0.9% saline and 24 h later were injected with a further intra-articular injection of the saline vehicle. All intra-articular injections were given under light isoflurane anesthesia, and treatment injections were administered as a 100-µl bolus. All animals were habituated to the test equipment over the 3 days preceding experimentation. Pain measurements were made before treatment (time 0) and then over the succeeding 2-h time period.

**Study groups.** Acutely inflamed mice were randomly assigned to one of three groups. The first group of mice received the H2S donor Na2S. In addition to Na2S administration, the second group also received a 25 mg/kg intraperitoneal injection of glibenclamide, a nonspecific KATP channel blocker, 45 min before the commencement of the experiment. The third group of mice were given an intraperitoneal injection of saline 45 min before the commencement of the experiment, and their joints were perfused with vehicle.

For the pain assessment experiments, the rats were randomly assigned to one of three groups. Group one animals were acutely inflamed and received an intra-articular injection of Na2S. Group two animals were also acutely inflamed, but received an intra-articular injection of vehicle. The final cohort of rats was given an intra-articular injection of the saline vehicle. All intra-articular injections were given under light isoflurane anesthesia, and treatment injections were administered as a 100-µl bolus. All animals were habituated to the test equipment over the 3 days preceding experimentation. Pain measurements were made before treatment (time 0) and then over the succeeding 2-h time period.

By 60 min, the adhesion was 4.5 g/s were chosen for all of the algesiometry trials. Since the slow leukocyte rolling flux did not change significantly within the microvasculature in noninflamed mice (56.7 ± 3.6 µm/s), the slow leukocyte rolling velocity observed in inflamed animals was maintained for 60 min in tissues perfused with vehicle; however, a significant increase in leukocyte rolling velocity was induced within 15 min in tissues perfused with 50 µM Na2S, and after 60 min the average leukocyte rolling velocity was approximately doubled (59.7 ± 3.6 µm/s). Figure **2B** illustrates leukocyte rolling velocity 60 min after superfusion with 10, 30, and 50 µM Na2S, with a significant increase in leukocyte velocity observed using the latter concentration. The effect of Na2S on leukocyte rolling velocity was completely reversed by an intraperitoneal injection of 25 mg/kg glibenclamide (Fig. **2B**).

Basal leukocyte rolling flux (cells/min) in inflamed knee microvasculature was in the range of 45–50 cells/min and was not statistically significantly different between the vehicle or Na2S-perfused groups (Fig. **3A**). This rolling flux was not significantly different from the number of rolling leukocytes in uninflamed microvasculature (45 ± 3 cells/min). Leukocyte rolling flux did not change significantly within the microvasculature superfused with vehicle (46 ± 2 cells/min at 60 min). After 60 min, the leukocyte flux was significantly lower than in the saline-treated group (36 ± 1 cells/min). Figure **3B** illustrates leukocyte rolling flux observed 60 min after superfusion with 10, 30, and 50 µM Na2S. No dose-dependent effect was observed in this parameter. Leukocyte rolling flux was returned to vehicle-treated levels in mice pretreated with 25 mg/kg glibenclamide (Fig. **3B**).

Leukocyte adherence (cells·100 µm−1·min−1) within the synovial microvasculature is illustrated in Fig. **4**. Basal leukocyte adherence in carrageenan-treated mice knee microvasculature was 15 ± 1 cells·100 µm−1·min−1, which is significantly elevated compared with noninflamed control vessels (1 ± 0.3 cells·100 µm−1·min−1), indicating an inflamed phenotype. This level of leukocyte adherence was maintained for 60 min in tissues perfused with vehicle (Fig. **4A**). However, a significant decrease in leukocyte adherence was observed over 60 min of superfusion with 50 µM Na2S so that, by 60 min, the adhesion was 4 ± 0.8 cells·100 µm−1·min−1. Figure **4B** illustrates the dose-response effect of Na2S (10–50 µM) on
the leukocyte adhesion observed 60 min after superfusion. The action of 50 \( \mu M \) Na\(_2\)S was attenuated by pretreatment with 25 mg/kg glibenclamide (Fig. 4B).

**Knee joint blood flow.** Continuous superfusion of exposed mice knee joints with 50 \( \mu M \) Na\(_2\)S caused a gradual fall in synovial blood flow, which was maximal at 60 min (Fig. 5). In acutely inflamed knees, 50 \( \mu M \) Na\(_2\)S caused articular blood flow to decrease to 53.4 ± 4.7% of control levels. The hypoemic effect of Na\(_2\)S administration was more pronounced than vehicle-superfused inflamed knees (\( P < 0.05, \text{two-way ANOVA, } n = 8–40 \text{ animals/group} \)).

**Pain behavior.** Twenty-four hours after intra-articular injection of kaolin/carrageenan, animals tended to favor their non-inflamed hindlimb for weight bearing, such that only ∼10% of the animal’s weight was placed on the ipsilateral leg (Fig. 7A). In contrast, saline-injected rats distribute their body weight 50:50 between both hindlimbs and, in this respect, are no different from naive control animals (\( P > 0.05, \text{two-way ANOVA, } n = 8–40 \text{ animals/group} \)). Treatment of acutely inflamed rats with 50 \( \mu M \) Na\(_2\)S had no significant effect on hindlimb incapacitance over the 2-h evaluation period (\( P > 0.05 \)). High-dose Na\(_2\)S (100 \( \mu M \) intra-articular) also had no effect on joint pain (data not shown).

Secondary allodynia, as measured by von Frey hair algometry, showed a similar response pattern (Fig. 7B). Acute knee joint inflammation caused secondary allodynia in the ipsilateral hindpaw, as evidenced by a significant reduction in the force required to elicit hindpaw withdrawal (\( P < 0.0001, \text{two-way ANOVA between saline-injected control and saline-injected inflamed knees, } n = 8–40 \text{ animals/group} \)). Intra-articular injection of 50 \( \mu M \) Na\(_2\)S had no significant effect on secondary allodynia in the acutely inflamed group (\( P > 0.05 \)).

**DISCUSSION**

Gaseous molecules, such as nitric oxide and carbon monoxide, have proven to be potent regulators of tissue inflammation and neuromodulation. More recently, H\(_2\)S has been identified as an endogenous mediator whose physiological actions control vascular tone (10), neutrophil activity (1), nociception (2), and intestinal motility (5). To date, the majority of studies...
examining H₂S activity have been carried out in the gut, while the present study examined the role of H₂S in joint inflammation and pain control. Specifically, in the acutely inflamed mouse knee joint, H₂S appeared to have an inhibitory effect on leukocyte trafficking, but had no observable effect on knee joint pain.

A key event in inflammation is the recruitment of circulating leukocytes into the damaged tissue. Use of intravital fluorescence microscopy to visualize leukocyte-endothelial cell interactions in vivo has revealed a complex series of stages in which engaged leukocytes undergo rolling, adhesion, and finally emigration through microvascular fenestrations (8, 15). A few studies have reported these phenomena in joint tissues (7, 9, 26); however, the surgical approaches employed in these experiments were fairly invasive, involving the exposure of the intra-articular environment. The present study used a minimally invasive procedure, where only the overlying skin was removed, thereby leaving the joint microvasculature completely intact. By focusing on a region of the joint capsule with its underlying synovium, leukocytes could clearly be seen to roll and adhere to the endothelium of kaolin/carrageenan-

**Fig. 4.** Leukocyte adhesion (cells·100 μm⁻¹·min⁻¹) observed in postcapillary venules of inflamed mouse knee joints. Inflammation was induced by an intra-articular injection of kaolin and carrageenan (both 2%) 24 h before recordings. **A:** recordings were made before and for 60 min after superfusion of the microvasculature with vehicle (0.9% saline) or Na₂S (50 μM). **B:** data represent values obtained at 60 min following superfusion with vehicle (open bar), 10, 30, or 50 μM Na₂S (solid bars), or 50 μM Na₂S in mice treated with glibenclamide (25 mg/kg ip; hatched bar). Values are means ± SE; n = 4–8. *P < 0.05, **P < 0.01, ***P < 0.001.

**Fig. 5.** Representative laser Doppler perfusion images of an acutely inflamed mouse knee before (control image) and 60 min after continuous perfusion with 50 μM Na₂S. Images are color coded with blue/purple representing low blood flow, yellow/green representing medium blood flow, and orange/red indicating areas of highest blood flow. Na₂S clearly has a vasoconstrictor effect on knee joint blood vessels. Figure also shows a typical region of interest used in image analysis, which approximates to the joint capsule. High blood flow in the saphenous artery can also be detected in these images.

**Fig. 6.** Vasomotor response to local administration of a hydrogen sulfide donor in acutely inflamed mouse knee joints. Superfusion of exposed knee joints with 50 μM Na₂S caused a significant decrease in synovial blood flow in acutely monoarthritic knees. Values are means ± SE of %change in blood flow taken at the 60-min time point; n = 6–12. *P < 0.05.
Na$_2$S treatment, although this was not statistically significant. In adherence; leukocyte rolling appeared to be suppressed by a dose-dependent increase in leukocyte velocity and a decrease by the channel blocker glibenclamide. Glibenclamide itself has ciception. Values are means mediated by the KATP channel as responses could be attenuated in an intact rodent knee joint.

The inhibitory actions of H$_2$S on leukocyte trafficking were robust and reproducible means of assessing leukocyte behavior (data not shown). Thus intravital fluorescence microscopy is a discernible in this preparation. Saline-injected control joints in acutely inflamed knee joints with the H$_2$S donor Na$_2$S caused a profound decrease in ipsilateral weight bearing and paw withdrawal threshold ($P < 0.0001$, two-way ANOVA, $n = 8–40$). Treatment of inflamed joints with 50 $\mu$M Na$_2$S had no effect on joint incapacitation or mechanonociception. Values are means ± SE.

Fig. 7. Effect of Na$_2$S on pain behavior as measured by hindlimb incapacitance (A) and von Frey hair algesiometry (B). In control animals, an intra-articular injection of saline had no effect on hindlimb weight distribution nor nociceptive threshold compared with naive controls. Acute inflammation caused a profound decrease in ipsilateral weight bearing and paw withdrawal threshold ($P < 0.0001$, two-way ANOVA, $n = 8–40$). Treatment of inflamed joints with 50 $\mu$M Na$_2$S had no effect on joint incapacitation or mechanonociception. Values are means ± SE.

inflamed knees, although cellular extravasation was not readily discernible in this preparation. Saline-injected control joints did not show any of the typical signs of leukocyte activation (data not shown). Thus intravital fluorescence microscopy is a robust and reproducible means of assessing leukocyte behavior in an intact rodent knee joint.

Anti-inflammatory effects of H$_2$S. Local superfusion of acutely inflamed knee joints with the H$_2$S donor Na$_2$S caused a dose-dependent increase in leukocyte velocity and a decrease in adherence; leukocyte rolling appeared to be suppressed by Na$_2$S treatment, although this was not statistically significant. The inhibitory actions of H$_2$S on leukocyte trafficking were mediated by the K$_{ATP}$ channel as responses could be attenuated by the channel blocker glibenclamide. Glibenclamide itself has been found to have no physiological effect on pain and inflammation (2, 29). Elsewhere, it has been confirmed that H$_2$S promotes K$^+$ conductance via the K$_{ATP}$ channel, while other types of K$^+$ channels, such as calcium-activated K$^+$ channels and voltage-gated K$^+$ channels, are thought to be not involved in H$_2$S effects (23, 32). The abrogation of leukocyte trafficking reported here may be due to an alteration in the expression of adhesion molecules and their associated ligands in the synovial microvasculature. In other tissues, a drop in H$_2$S production leads to an increase in expression of intracellular adhesion molecule-1 (ICAM-1), as well its integrin ligand lymphocyte function-associated antigen-1 (3), suggesting that H$_2$S has the propensity to diminish the generation of mediators necessary for leukocyte recruitment. Since lymphocyte function-associated antigen-1 is found on the surface of leukocytes and ICAM-1 is expressed by endothelial cells and leukocytes, then it is feasible that H$_2$S exerts its anti-inflammatory effects on both sides of the leukocyte-endothelial interface. It should be mentioned that contrasting data have been reported by Zhang et al. (31), who found that treatment of septic mice with PAG decreased the expression of the adhesion molecules ICAM-1, P-selectin, and E-selectin; however, this severe systemic form of inflammation may not be indicative of what occurs at the organ level in models of peripheral inflammation, as described here.

Synovial hyperemia is a common characteristic of acute joint inflammation and is analogous to the intermittent flare responses seen in chronic inflammatory joint disease. Continuous topical application of Na$_2$S caused a gradual decrease in joint blood flow, with maximal vasoconstriction occurring 60 min after the start of drug administration. Glibenclamide treatment was not carried out in the blood flow experiments, since K$_{ATP}$ channels are generally associated with vasodilator responses. The role of H$_2$S in vasoregulation is controversial, with reports of both vasorelaxation (5, 10, 27) and vasoconstriction (14, 20). To date, the majority of studies investigating the vasomotor effects of H$_2$S have been carried out on large systemic blood vessels, such as the aorta and carotid artery (27), and, as such, may not be representative of responses occurring in the microvasculature of a peripheral organ such as the joint. An interesting observation is that the vasoconstrictor effect of H$_2$S only occurs when the local O$_2$ tension is relatively high, whereas H$_2$S-mediated vascular smooth muscle relaxation occurs at lower O$_2$ concentrations (14). In the context of the present study, the hyperemia associated with kaolin/carrageenan-induced acute synovitis likely results in higher than normal articular O$_2$ levels, thereby creating a hyperoxic tissue microenvironment that is suited for H$_2$S vasoconstriction. It has been suggested that the vasoconstrictor effect of H$_2$S in high O$_2$ states may be due to the generation of oxidation products rather than a direct effect of H$_2$S on the vasculature (14). If this is the case, future analysis would be required to identify the nature of these oxidation products.

Effect of H$_2$S on joint pain. Very little is known about the role of H$_2$S in modulating pain. Anecdotal evidence suggests that H$_2$S may have a pronociceptive effect by causing irritation of mucosal surfaces, such as the eye and airways (11, 25). Here, it was found that local administration of Na$_2$S had no discernible effect on knee joint pain, as measured by hindlimb incapacitation and von Frey hair algesiometry. Vehicle-treated inflamed animals showed a weight-bearing deficit in the ipsilateral hindlimb, as well as a reduced nociceptive threshold to
a tactile mechanical stimulus, confirming increased pain sensitivity in the animal model. Elsewhere, it has been reported that H$_2$S reduces pain in the gastrointestinal tract and that this effect is mediated by K$_{ATP}$ channels (2). In this latter study, however, the H$_2$S donor was given systemically and could, therefore, be acting at higher pain centers. Future studies examining the effect of H$_2$S on neuronal excitability in the peripheral vs. central nervous system, as well as testing H$_2$S donors in other organs, will help elucidate the role of this gaseous transmitter in modulating pain transmission.

**Perspectives and significance.** The current series of experiments determined that local treatment of acutely inflamed knee joints with an H$_2$S donor reduced leukocyte recruitment and trafficking, as well as decreased synovial blood flow. These anti-inflammatory effects of H$_2$S were mediated via the K$_{ATP}$ channel, since responses could be blocked by glibenclamide treatment. Intra-articular administration of Na$_2$S had no effect on joint pain sensation nor secondary allodynia in the rat, although this observation needs to be corroborated in other animal species. These findings implicate H$_2$S as an endogenous regulator of joint function and whose action is distinctly anti-inflammatory. Future studies testing the effects of an inhibitor of H$_2$S synthesis would be useful in determining whether endogenous H$_2$S is able to attenuate the development of joint inflammation in vivo.

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