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Protective effects of exogenous and endogenous hydrogen sulfide in mast cell-mediated pruritus and cutaneous acute inflammation in mice

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ABSTRACT

The recently described 'gasomediator' hydrogen sulfide (H_2S) has been involved in pain mechanisms, but its effect on pruritus, a sensory modality that similarly to pain acts as a protective mechanism, is poorly known and controversial. The effects of the slow-releasing (GYY4137) and spontaneous H_2S donors (Na_2S and Lawesson's reagent, LR) were evaluated in histamine and compound 48/80 (C48/80)-dependent dorsal skin pruritus and inflammation in male BALB/c mice. Animals were intradermally (i.d.) injected with C48/80 (3 μg/site) or histamine (1 μmol/site) alone or co-injected with Na_2S, LR or GYY4137 (within the 0.3–100 nmol range). The involvement of endogenous H_2S and K_{ATP} channel-dependent mechanism were also evaluated. Pruritus was assessed by the number of scratching bouts, whilst skin inflammation was evaluated by the extravascular accumulation of intravenously injected ^{125}I-albumin (plasma extravasation) and myeloperoxidase (MPO) activity (neutrophil recruitment). Histamine or C48/80 significantly evoked itching behavior paralleled by plasma extravasation and increased MPO activity. Na_2S and LR significantly ameliorated histamine or C48/80-induced pruritus and inflammation, although these effects were less pronounced or absent with GYY4137. Inhibition of endogenous H_2S synthesis increased both Tyrode and C48/80-induced responses in the skin, whereas the blockade of K_{ATP} channels by glibenclamide did not. H_2S-releasing donors significantly attenuate C48/80-induced mast cell degranulation either *in vivo* or *in vitro*. We provide first evidences that H_2S donors confer protective effect against histamine-mediated acute pruritus and cutaneous inflammation. These effects can be mediated, at least in part, by stabilizing mast cells, known to contain multiple mediators and to be primary initiators of allergic processes, thus making of H_2S donors a potential alternative/complementary therapy for treating inflammatory allergic skin diseases and related pruritus.

1. Introduction

Pruritus (itch) is an autonomous pain-independent sensation that, similarly to pain, acts as a distressing physiological self-protective mechanism in both humans and animals. This response greatly affects life quality and can be triggered by inflammatory skin diseases, systemic diseases, neuropathic conditions and psychogenic disorders. According to the etiology, it may be acute or chronic (duration longer than 6 weeks), localized or generalized [1,2]. A range of mediators, such as histamine [3], prostaglandins [4], serotonin [5], bradykinin [6], cytokines [7], endothelin-1 [8], leukotrienes [9], proteases [10,11], neuropeptides [12] and opioids [13] orchestrate this response by acting on their receptors located on the nerve terminals. Pruritus (scratching behavior) is also a common symptom that results from insect bites, and can be exper-

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imentally induced in animals by the intradermal (i.d.) injection of insect saliva or venom toxins [12,14,15].

The management of pruritus, either dependent or independent of histaminergic pathways, is always recommended when removal of the trigger factors does not control the itch or perhaps unknown. Topical (*e.g.* anaesthetics, antihistamines, steroids, calcineurin inhibitors and capsaicin cream) or systemic approach (*e.g.* antihistamines, antidepressants and immunossupressors) are normally prescribed according to the etiology [2].

Interestingly, over the last ten years, hydrogen sulfide (H_2S) a new mediator that belongs to the class of endogenous gases such as nitric oxide (NO) and carbon monoxide (CO), has emerged and brought about divergent findings regarding its role in acute/chronic inflammatory responses and nociception [16–19]. However, the use of slow-releasing H_2S donors (such as SG-1002, diallyl trisulfide and GYY4137) and hybrid H_2S-releasing non steroidal anti-inflammatory compounds (such as the naproxen derivative ATB-346) strengthens the beneficial therapeutical effects of H_2S in articular inflammation [20], colorectal cancer [21], periodontitis [22] and pain [23] with additional gastrointestinal safety [24]. More recently, low serum levels of H_2S has been associated with psoriasis [25], a disease often associated with pruritus [26]. In contrast, Wang and co-workers [27] showed that the i.d. injection of high doses of NaHS or Na_2S, but not GYY4137, evoked a dose-dependent scratching behavior in mice, which is possibly related to the H_2S releasing rate. Considering that the results on the effects of H_2S on itch behavior are rather limited and controversial, this study was carried out to evaluate whether slow and spontaneous-H_2S releasing donors, used at low doses, are able to reduce acute pruritus and the related cutaneous inflammation mediated by histamine.

2. Material and methods

2.1. Animals

Male BALB/c mice (20–30 g) and Wistar rats (180–200 g) were obtained from the local animal care facilities and housed in groups (up to five animals per cage) under standard controlled conditions (22 °C; 12/12 h light/dark cycle) with free access to commercial rodent chow and water. All the experimental protocols were approved by the local ethics committee (CEUA-ICB; protocol no. 33, pgs. 85, book no. 02/2010), in accordance with the guidelines from the Brazilian Council for Control of Animal Experimentation (CONCEA) and the Directive 2010/63/EU, comprising with the Animal Welfare Act.

2.2. Induction of pruritus (itching) in the mouse dorsal skin

Mice were transitorily anaesthetized with inhaled isoflurane (3% v/v in O_2) and the rostral part of the back (≅2 cm) near to the neck was shaved. Histamine (1 μmol/site), C48/80 (3 μg/site) or its corresponding vehicle Tyrode were i.d. injected, in a volume of 50 μl, alone or in combination with 0.3–10 nmol/site of Na_2S, Lawesson's reagent (LR; both, spontaneous H_2S donors) or the slow-release H_2S donor GYY4137. Mice were individually placed into a perspex transparent box (12 × 20 × 17 cm; Insight, Brazil) in a quiet room adapted with video camera (Sony HDR-PJ230), where the mice were daily acclimatized for 40 min during the two days previous to the experiments. A maximum of four mice were simultaneously recorded during the same period and the number of scratching bouts were counted as detailed in [12]. The number of scratching bouts was expressed either as absolute countings or as percentage values determined in 40 min. In all the experiments, the investigator who quantified the scratching behavior was unaware of the experimental group identities.

2.3. Assessment of dorsal cutaneous plasma extravasation

Mice were anaesthetized with urethane (2.5 g/kg; i.p.), the rostral back shaved, and 100 μl of ^{125}I–bovine serum albumin (^{125}I-BSA, 0.037 MBq) was intravenously (i.v.) injected via the tail vein. Histamine (30 nmol/site), C48/80 (3 μg/site) or Tyrode were i.d. injected alone or co-injected with Na_2S, LR or GYY4137 (1–100 nmol/site) throughout six randomized skin sites as previously described [28]. The result were expressed as μl of plasma per g of tissue or percentage based on the control values (obtained with either histamine or C48/80 alone).

2.4. Pharmacological treatments

To investigate the involvement of K_{ATP} channel in H_2S donors-mediated protective effects, a set of mice was pretreated (−30 min), via intraperitoneal (i.p.), with the K_{ATP} channel blocker, glibenclamide (10 or 30 mg/kg, i.p. [29]) or its corresponding vehicle carboxymethylcellulose (CMC; 0.1 ml, i.p.). In order to establish the effective dose of glibenclamide, another group of mice was pretreated (−30 min) with glibenclamide 10 or 30 mg/kg and then i.d. injected with the K_{ATP} channel opener, pinacidil (10–30 nmol/site; i.d.). In order to assess the role of endogenous H_2S in histamine-induced skin pruritus and skin inflammation, two independent groups of mice were pretreated (−60 min; i.p.) with the CSE and CBS inhibitors β-cyanoalanine (BCA, 50 mg/kg) and aminooxyacetic acid (AOAA, 20 mg/kg), a CSE and CBS inhibitors, respectively.

2.5. Biochemical analysis

2.5.1. Measurement of myeloperoxidase (MPO) activity

Mice were anaesthetized with isoflurane and i.d. injected with the test agents, as described above (item 2.3), and four hour later they were killed via an overdose of urethane followed by cervical dislocation. The injected skin sites were removed, and the myeloperoxidase (MPO) activity was measured as previously described [30]. The results were expressed as units of MPO per mg of protein (or percentage).

2.5.2. Production of H_2S by mouse dorsal skin

The endogenous H_2S production in the naïve and i.d. injected (Tyrode and C48/80 3 μg/site) mouse dorsal skin was carried out based on the formation of lead sulfide after 30 min and 4 h post injection, accordingly [31]. Briefly, naïve and i.d. injected skin, brain and liver were excised and homogenized. After centrifugation (10,000*g*, 10 min, 4 °C), the obtained supernatants (400 μg protein) was incubated with substrates (L-cysteine 10 mM and pyridoxal-5′-phosphate 2 mM) for 2 h 30 min at 37 °C. The dark dots densities on the lead acetate white paper strips (12 × 8 cm) placed over the 96-wells microplate were analyzed from the digitalized images using the software ImageJ (NIH, USA). Hydrogen sulfide concentrations were extrapolated from a calibration curve generated with NaHS (7.8–500 μM).

2.6. Assessment of rat mast cell degranulation

2.6.1. Intravital microscopy assay

Under a mixture of ketamine and xylazine anaesthesia (100 and 10 mg/kg, respectively, i.p.), the rat was placed on a homeothermic blanket system (37 °C), and the mesentery was exposed for microscopic observation as described previously [32]. The exteriorized mesentery was superfused with Ringer–Locke solution (154 mM NaCl, 5.6 mM KCl, 2 mM $CaCl_2{\cdot}2H_2O$, 6 mM $NaHCO_3$, 5 mM glucose, 0.025 mM ascorbic acid pH 7.2–7.4, 37 °C) and a volume of 30 μl of Tyrode or C48/80 (10 μg) was applied to the mesentery. Alternatively, 30 μl of the test agents Na_2S, LR or GYY4137 (30 nmol)