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# Pharmacological investigation of hydrogen sulfide ($H_2S$) contractile activity in rat detrusor muscle

Riccardo Patacchini[1], Paolo Santicioli*, Sandro Giuliani, Carlo Alberto Maggi

*Department of Pharmacology, Menarini Ricerche SpA, via Rismondo 12/A, 50131, Florence, Italy*

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**Abstract**

We have investigated the mechanism through which hydrogen sulfide ($H_2S$) stimulates capsaicin-sensitive primary afferent neurons in the rat isolated urinary bladder. Sodium hydrogen sulfide (NaHS), a donor of $H_2S$, produced concentration-dependent contractile responses ($pEC_{50}$=3.5±0.1) that were unaffected by the transient receptor potential vanilloid receptor 1 (TRPV1) antagonist capsazepine (30 μM) and SB 366791 (10 μM) and by the N-type $Ca^{2+}$ channel blocker omega-conotoxin GVIA (ω-CTX; 100 nM). In contrast, the unselective transient receptor potential (TRP) cation channels blocker ruthenium red (30 μM) almost abolished NaHS-induced contractions. Ruthenium red (30 μM) greatly reduced capsaicin-induced contractions, whereas it did not attenuate the contractile response to neurokinin A. The putative TRPV1 receptor antagonist iodo-resiniferatoxin, from 100 nM upward, produced agonist responses per se, and could not be tested against NaHS. We conclude that $H_2S$ either acts at TRPV1 receptorial sites unblocked by capsazepine or SB 366791, or stimulates a still unidentified transient receptor potential-like channel co-expressed with TRPV1 on sensory neurons.


*Keywords:* Hydrogen sulfide ($H_2S$); Capsaicin; Sensory neuron; Urinary bladder

## 1. Introduction

Hydrogen sulfide ($H_2S$) is a smelly gas representing a chemical hazard in certain industrial manufacturings, whose exposure produces serious toxic effects that have widely been reported in literature (Guidotti, 1996, for review). The interest of pharmacologists on $H_2S$ has recently been renewed by demonstration that it can be generated endogenously in mammals from cysteine, via at least two different enzymatic pathways (Hosoki et al., 1997; Kimura, 2002; Wang, 2003; Chen et al., 2004). Various studies conducted on cardiovascular (Hosoki et al., 1997; Zhao et al., 2001; Cheng et al., 2004), reproductive (Hayden et al., 1989; Teague et al., 2002) and gastrointestinal (Hosoki et al., 1997; Teague et al., 2002) smooth muscles have shown that $H_2S$ produces both direct and indirect smooth muscle relaxant effects, inhibits spontaneous motility or prevents chemically or electrically induced contractile responses (e.g. Teague et al., 2002; Moore et al., 2003 for review). The opening of ATP-sensitive $K^+$ channels ($K_{ATP}$) has been shown to be a pathway through which $H_2S$ relaxes rat aorta (Zhao et al., 2001) and rat mesenteric artery bed (Cheng et al., 2004), but this mechanism does not account for the relaxant effect produced by $H_2S$ in the guinea-pig ileum (Teague et al., 2002).

Recently, we have described a previously unrecognized mechanism through which $H_2S$ affects smooth muscle tone in the rat isolated urinary bladder. We provided evidence that $H_2S$ stimulates capsaicin-sensitive primary afferent nerve terminals; an effect occurring at concentrations of $H_2S$ (partially) overlapping those present in body fluids under physiological conditions (Patacchini et al., 2004; see Chahl, 2004, for commentary). Our conclusion was based on the observation that $H_2S$-induced contraction of the rat

* Corresponding author. Tel.: +39 55 5680735; fax: +39 55 5680419.
*E-mail addresses:* r.patacchini@chiesigroup.com (R. Patacchini), psanticioli@menarini-ricerche.it (P. Santicioli).
[1] Present address: Department of Pharmacology, Chiesi Pharmaceuticals SpA, Via Palermo 26/A, 43100, Parma, Italy.

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bladder was totally prevented by desensitization of sensory fibres afforded by high capsaicin pretreatment of the tissue. $H_2S$-induced contractile response could also be prevented by the concomitant administration of tachykinin $NK_1$ and $NK_2$ receptor-selective antagonists, indicating that endogenous tachykinins released from sensory nerve terminals are the final mediators of $H_2S$-induced excitatory effect in the rat bladder (Patacchini et al., 2004). In the present study we have addressed the question of the mechanism(s) through which $H_2S$ stimulates sensory fibre terminals in the rat urinary bladder. To this aim, we have used the transient receptor potential vanilloid receptor 1- or TRPV1- (Caterina et al., 1997; Montell et al., 2002) receptor antagonist capsazepine (Bevan et al., 1992; Maggi et al., 1993), the noncompetitive and unselective transient receptor potential cation channels blocker ruthenium red (Maggi et al., 1988b; Amann and Maggi, 1991; Gunthorpe et al., 2002) and the recently identified TPRV1 receptor selective antagonists iodo-resiniferatoxin (Wahl et al., 2001; Rigoni et al., 2003) and SB 366791 (Gunthorpe et al., 2004) against contractile responses of the rat detrusor muscle produced by NaHS, a donor of $H_2S$ in buffered solutions (Hosoki et al., 1997). We have used also omega-conotoxin GVIA (ω-CTX), a N-type $Ca^{2+}$ channel blocker possessing the capability to prevent activation of sensory nerve fibres afforded by electrical stimuli (Maggi et al., 1988a) or high potassium (Kageyama et al., 1997) but unable to prevent direct activation of the sensory fibres obtained through stimulation of TRPV1 receptors (Maggi et al., 1988a,b). The use of ω-CTX was instrumental for discriminating between two independent modes of sensory neuropeptide release from capsaicin-sensitive sensory nerves (Maggi et al., 1988a).

## 2. Materials and methods

### 2.1. General

Male albino rats (Wistar strain, 275–350 g) were decapitated under ether anaesthesia. The whole urinary bladder, cleared of surrounding tissue, was excised and cut along its longitudinal axis to obtain 2–4 parallel strips, as described previously (Patacchini et al., 2004). Briefly, the strips were placed in 5-ml organ baths filled with oxygenated normal Krebs–Henseleit solution added of atropine (1 μM) and indomethacin (3 μM), except in the case of experiments conducted on carbachol-precontracted preparations. Motor activity of the strips was recorded isotonically (load 5 mN). After a 60-min equilibration period the preparations were challenged with KCl (80 mM) to obtain the maximal contractile response of the muscle. The preparations were then exposed to electrical field stimulation (single pulses of stimuli of 0.1 Hz, 0.5 ms pulse width, supramaximal voltage) for 15 min, by means of two platinum wire electrodes placed at the top and the bottom of the organ bath and connected to a Grass S88 stimulator. Concentration–response curves to NaHS or capsaicin were constructed cumulatively in the absence or in the presence of TRPV1 or other receptor antagonists or ion channel blockers. Due to the lack of reproducibility of NaHS-induced contractile effects, one strip obtained from a common rat bladder was pretreated with vehicle (distilled water or dimethyl sulfoxide, DMSO; 1–3 μl/ml each) and used to construct a control curve to NaHS, while the other strips were pretreated with various concentrations of the antagonists and, after a 30-min incubation period, received NaHS. Each value in text, tables and figures is mean±S.E.M. Statistical analysis was performed by means of Student's *t*-test for unpaired data or by means of analysis of variance, when applicable. Ethical approval of the experimental protocol with animals was obtained from the local Ethic Committee.

### 2.2. Chemicals

NaHS, capsaicin, carbachol, indomethacin, atropine sulphate and SB 366791 were from Sigma (St. Louis, MO, USA). Capsazepine and iodo-resiniferatoxin were from Tocris Cookson (Avonmouth, UK). Ruthenium red was from Aldrich-Chemie (Steinheim, Germany). ω-CTX was from Bachem (Bubendorf, Switzerland). Neurokinin A was from Espikem (Florence, Italy).

## 3. Results

### 3.1. Excitatory motor effect of NaHS as compared to capsaicin

After a 60-min equilibration period, the preparations were electrically stimulated and the contractile twitch response obtained used as an internal standard. As stated before (Patacchini et al., 2004), cumulative additions of NaHS or capsaicin to quiescent rat bladder strips produced contractile responses (Table 1) which underwent dramatic tachyphylaxis and could not be reproduced after washout of the preparations. NaHS was about 3000-fold weaker than capsaicin, and elicited a maximal effect averaging ~35% of that produced by capsaicin (cfr. Table 1). NaHS-induced contractions were totally and selectively prevented by in vitro desensitization of sensory neurons achieved by administration of a supramaximal concentration of capsaicin (10 μM for 15 min) at the beginning of the experiments (Patacchini et al., 2004).

### 3.2. Effect of TRPV1 receptor antagonists and ω-conotoxin GVIA on NaHS or capsaicin-induced contractile effects

The unselective transient receptor potential channels blocker ruthenium red (30 μM) produced an insurmountable and almost complete blockade of NaHS-induced contractions (Table 1; Figs. 1 and 2A), whereas it barely reduced

Table 1
Effect of various antagonist compounds on NaHS- or capsaicin-induced contractions of rat isolated detrusor muscle strips

| Compound | $pEC_{50}$[a] | $E_{max}$[b] (% of EFS) |
|---|---|---|
| *NaHS* | | |
| Control | 3.5±0.1 | 167±18 |
| RR (30 μM)[c] | 3.2±0.2 | 24.6±6** |
| Capsazepine (30 μM) | 3.4±0.1 | 124±14 |
| ω-CTX (100 nM)[d] | 3.6±0.1 | 119±17 |
| SB 366791 (10 μM) | 4.0±0.1 | 132±29 |
| *Capsaicin* | | |
| Control | 7.1±0.07 | 491±23 |
| RR (30 μM) | 7.3±0.7 | 177±67* |
| capsazepine (30 μM) | 6.1±0.2 | 341±40 |
| SB 366791 (10 μM) | 6.6±0.1 | 451±38 |

Significantly different from control response; (*) $P<0.05$ or (**) $P<0.01$. All data are mean±S.E.M. of 4–16 experiments.
[a] $pEC_{50}$; ($-\log EC_{50}$).
[b] % of EFS; percent of the twitch response induced by electrical field stimulation.
[c] RR; ruthenium red.
[d] ω-CTX; omega-conotoxin GVIA.

twitch contractions elicited by electrical field stimulation (−18±2%; $n$=12, n.s.) and did not modify the contractile effects afforded by neurokinin A ($pEC_{50}$=7.3±0.2; $E_{max}$=903±42% of electrical field stimulation in the absence, and $pEC_{50}$=7.2 ± 0.1; $E_{max}$=802 ± 97% of electrical field stimulation in the presence of ruthenium red 30 μM; $n$=4 each; Fig. 2C). As expected from previous investigations, ruthenium red (30 μM) significantly reduced capsaicin-induced contractions in this preparation (Table 1; Fig. 2B). In contrast, both capsazepine, up to 30 μM, and SB-366791, up to 10 μM, were unable to block NaHS-induced contractions, whereas the same compounds produced a consistent rightward shift of the concentration–response curve to capsaicin (Table 1; Fig. 3). The other TRPV1 receptor antagonist employed–iodo-resiniferatoxin–failed to reduce capsaicin-induced contractions up to 10 nM ($n$=4; not shown). A higher concentration of iodo-resiniferatoxin (100 nM) slowly but consistently increased smooth muscle tone of the bladder per se (150±40% of electrical field stimulation; $n$=4). Capsaicin pretreatment (10 μM for 15 min, 60 min before) totally prevented iodo-resiniferatoxin-induced contraction ($n$=4), thus providing indirect evidence that this effect depends on stimulation of sensory nerve terminals. Due to its residual agonist activity, iodo-resiniferatoxin could not be tested as an antagonist of NaHS-induced contractile effects under the present experimental conditions. ω-CTX (100 nM) failed to significantly

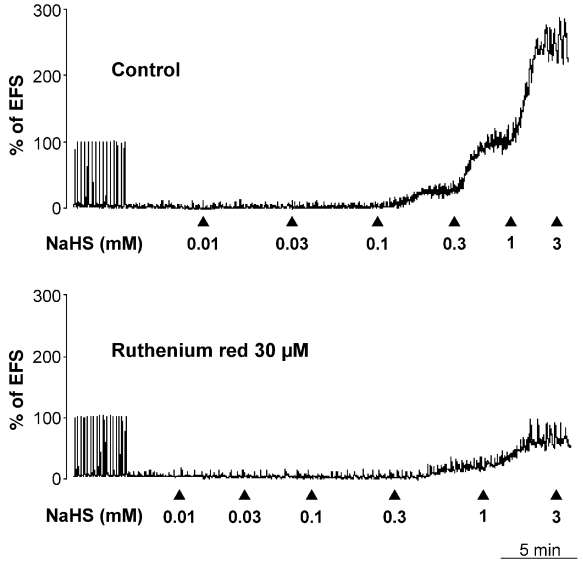


Fig. 1. Typical tracings showing NaHS-induced contractions of rat isolated urinary bladder in control (upper panel) or ruthenium red-pretreated (30 μM for 30 min; lower panel) strips.

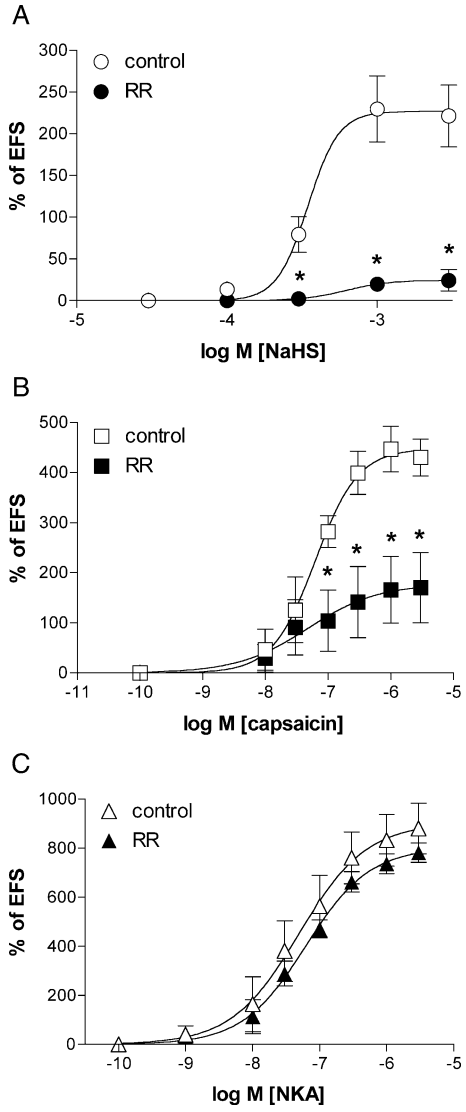


Fig. 2. Contractile response curves to NaHS (A) to capsaicin (B) or to neurokinin A (NKA) (C) in rat detrusor smooth muscle strips in the absence or in the presence of ruthenium red (RR; 30 μM). Responses are plotted as percentage of twitch contraction elicited by electrical field stimulation (EFS). (*) Significantly different from the matched control response: $P<0.05$. Each value is mean±S.E.M. of at least 4 experiments.

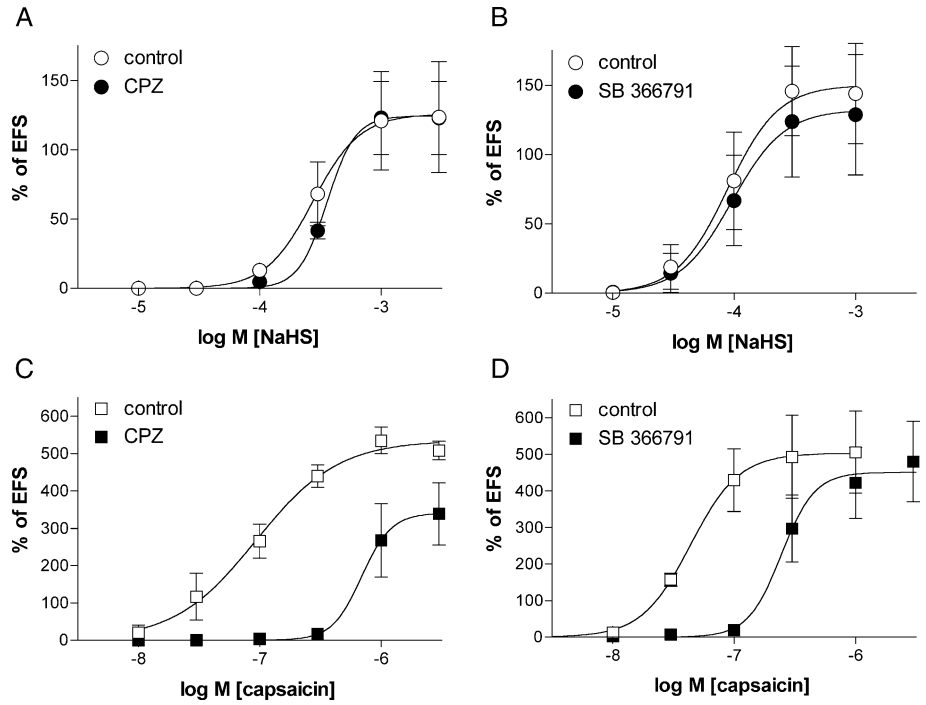


Fig. 3. Contractile response curves to NaHS (A,B) or to capsaicin (C,D) in rat detrusor smooth muscle strips in the absence or in the presence of capsazepine (CPZ; 30 μM) or SB 366791 (10 μM). Responses are plotted as percentage of twitch contraction elicited by electrical field stimulation (EFS). Each value is mean±S.E.M. of at least 4 experiments.

reduce NaHS-induced contractions (Table 1). In addition, at the concentration employed ω-CTX (100 nM) significantly decreased electrical field stimulation-induced twitch contractions (−33±7%; $P<0.05$; $n=6$).

### 3.3. Failure of ruthenium red to affect NaHS-induced inhibitory motor effects on precontracted preparations

NaHS produces smooth muscle relaxant effects in the rat urinary bladder that are independent from capsaicin-sensitive sensory nerve fibres activation (Patacchini et al., 2004). The present experiments with NaHS on precontracted preparations were performed on tissues that had been exposed to high capsaicin (10 μM for 15 min) pretreatment, in order to avoid any disturbing excitatory motor effect produced through activation of sensory nerve fibres. Under these conditions, a concentration-dependent inhibitory curve to NaHS could be obtained in carbachol (1 μM)-precontracted strips ($pIC_{50}=3.3\pm0.1$; $E_{max}=-84\pm8\%$; $n=3$). Ruthenium red (30 μM), which greatly reduced NaHS-induced excitatory motor effect in quiescent bladder strips, failed to modify NaHS-induced inhibition of smooth muscle tone ($pIC_{50}=3.4\pm1$; $E_{max}=-81\pm3\%$; $n=3$ not significantly different from control).

## 4. Discussion

The capsaicin receptor was cloned in 1997 by Caterina and coworkers from rat sensory neurons and named vanilloid receptor 1 or TRPV1, as it shows high sequence homology to the transient receptor potential family of ion channels (Clapham et al., 2003). The TRPV1 is a non-selective ligand-gated cation channel expressed by certain sensory neurons that can be gated by an heterogeneous series of chemical and physical stimuli, including capsaicin and other vanilloid compounds, noxious heat, protons, ethanol, anandamide, 12-hydroperoxyeicosatetraenoic acid (12-HPETE) and other lipid derivatives (Szallasi and Blumberg, 1999; Gunthorpe et al., 2002; Clapham et al., 2003). Our present results show that capsaicin effects, as expected, are blocked by all TRPV1 antagonists employed. In contrast, we found that capsazepine, the first potent and selective antagonist of the TRPV1 receptor (Bevan et al., 1992) failed to block NaHS-induced contractions. It is worth mentioning, however, that in rat preparations capsazepine has been shown to be less (or not) effective against activating stimuli (e.g. heat or protons) different from vanilloids (McIntyre et al., 2001; Walker et al., 2003). In addition to capsazepine, also the newly introduced and highly selective TRPV1 receptor antagonist SB 366791 (Gunthorpe et al., 2004) failed to block $H_2S$-induced contractions in the rat bladder. Another high-affinity antagonist of the TRPV1 receptor, iodo-resiniferatoxin (Wahl et al., 2001; Rigoni et al., 2003), could not be tested under the present experimental conditions, as it elicited a sustained muscular tonic response per se. This effect was due to stimulation of sensory nerve terminals, as shown by its sensitivity to high capsaicin pretreatment. The undesired motor effect produced by this putative antagonist was not

the only reason that prompted us to discard iodo-resiniferatoxin as a tool. We thought that the sensory nerve terminal desensitization afforded by iodo-resiniferatoxin–a phenomenon that invariably follows the excitatory action of vanilloids at TRPV1 receptors (Szallasi and Blumberg, 1999)–could add to the putative antagonism by this compound against NaHS-induced contraction. To this regard, it is worth noting that resiniferatoxin, the parent compound of iodo-resiniferatoxin, is by far more potent in producing desensitization of sensory nerve fibres in the rat urinary bladder than in producing excitatory motor responses (Maggi et al., 1990). In agreement with our previous results (Patacchini et al., 2004) showing that contractile responses to NaHS could only marginally be reduced by tetrodotoxin, our present data show also that ω-CTX does not affect NaHS-induced excitatory motor effects. Overall, our results indirectly show that the stimulatory activity of $H_2S$ at sensory neuron terminals is largerly independent from axonal conduction via fast sodium channels (Patacchini et al., 2004), nor it depends on the opening of N-type $Ca^{2+}$ channels (present data). Of the various TRPV1 receptor antagonists employed, only ruthenium red reduced NaHS-induced contractions. Ruthenium red is an inorganic dye bearing $Ca^{2+}$ antagonist properties that was reported to noncompetitively block capsaicin-induced acute effects and capsaicin-induced desensitization of sensory neurons (Maggi et al., 1988b, 1993; Amann and Maggi, 1991 for review) by acting at the capsaicin-sensitive cation channel (TRPV1). Our data provide evidence that the blockade produced by ruthenium red of NaHS-induced contractile effects is the result of a selective action of this antagonist on capsaicin-sensitive sensory nerve terminals. This is proven by the specific inhibition afforded by ruthenium red of the contractile response produced by capsaicin. In contrast, our results show that ruthenium red failed to inhibit contractions produced by exogenous neurokinin A and that it minimally reduces electrically evoked twitch contractions in this preparation. Moreover, in experiments performed on pre-contracted preparations ruthenium red failed to affect NaHS-induced inhibition of smooth muscle tone; this latter effect of $H_2S$ being totally independent from activation of sensory neurons (Patacchini et al., 2004). The failure of ruthenium red to prevent the inhibitory activity of NaHS allows to rule out the possibility that ruthenium red blockade of NaHS-induced contraction originates from a "chemical" antagonism: i.e. that it could be the result of a chemical reaction between the two inorganic compounds leading to $HS^-/H_2S$ depletion from the bath solution, instead of being a true receptorial antagonism.

On the basis of these results, two main hypotheses can be put forward on the molecular target activated by $H_2S$ on sensory neuron terminals of the rat urinary bladder, as follows:

(1) $H_2S$ stimulates TRPV1 receptor by acting at a receptor site distinct from that operated by vanilloids and possibly by other known activators of the channel. In favour of this hypothesis is the sensitivity of $H_2S$-induced contractile effects to ruthenium red, a known blocker of TRPV1 channel. The molecular site of action of ruthenium red on TRPV1 has long been debated, since this compound was found unable to displace the specific binding of [$^3$H]resiniferatoxin–an ultrapotent vanilloid TRPV1 receptor agonist (Szallasi and Blumberg, 1989)–from TRPV1 receptors present on rat dorsal root ganglia neuronal membranes (Szallasi and Blumberg, 1990). The inability of ruthenium red to interact with the same binding sites shared by vanilloid compounds has more recently been confirmed at recombinant TRPV1 rat receptors (Wahl et al., 2001). Nevertheless, it has recently been demonstrated that ruthenium red interacts with the TRPV1 cation channel protein by site-directed mutagenesis experiments in which substitution of $Asp^{646}$–a key aminoacid present in the pore region of the receptor–by asparagine, rendered the TRPV1 receptor 10-fold less sensitive to ruthenium red-blocking activity (Garcia-Martinez et al., 2000). In support of our first hypothesis, is the observation that other physiological activators of the TRPV1 receptor such as heat and protons promote the opening of the TRPV1 channel through pathways distinct from those of vanilloids, as shown by studies using point-mutated receptors (Welch et al., 2000; Jordt et al., 2000). Finally other examples have been described in the literature of chemical compounds, like certain products present in cigarette smoke (Geppetti et al., 1993) that, alike $H_2S$, activate capsaicin-sensitive sensory nerve fibres in a ruthenium red-sensitive but capsazepine-insensitive manner.

(2) A second possibility is that $H_2S$ stimulates other receptor(s) present on terminals of capsaicin-sensitive sensory neurons. This receptor(s) might belong to the transient receptor potential cation channels family, as several members of this family–in addition to TRPV1–are sensitive to ruthenium red. Actually TRPM6, TRPA1 (or ANKTM1) and all six TRPV channels since now identified (i.e. TRPV1–6) have been shown to be blocked, with different affinities, by ruthenium red (Patapoutian et al., 2003; Gunthorpe et al., 2002; Clapham et al., 2003). Among transient receptor potential cation channels sensitive to ruthenium red that are present on sensory neurons, the only one which is expressed within a subset of neurons also bearing the TRPV1 is TRPA1, a receptor activated by noxious cold and other pungent compounds (Story et al., 2003; Jordt et al., 2004). As the contractile effect of $H_2S$ in the rat urinary bladder is absent in rat detrusor muscle preparations in which sensory neurons were rendered unresponsive by high capsaicin pretreatment (Patacchini et al., 2004) and capsaicin being a selective activator of TRPV1 (Gunthorpe et al., 2002) it can be deduced that TRPA1 channels have high probability to be the (a) molecular target of $H_2S$ in the rat urinary bladder.

Finally, our present and previous (Patacchini et al., 2004) results support the hypothesis that endogenous $H_2S$ might

be capable of exerting a basal stimulatory activity on capsaicin-sensitive primary afferent neurons innervating the urinary bladder and, possibly, other organs. This hypothesis originates from the observation that the reported levels of $H_2S$ detected in blood (10–100 μM) and brain (50–160 μM) (Warenycia et al., 1989; Zhao et al., 2001; Wang, 2003 for review) overlap the first effective concentrations of NaHS in the detrusor smooth muscle. The physiological significance of such possible activity of $H_2S$, as well as the possible consequences linked to pathological variation of $H_2S$ production, are speculative issues deserving further investigations for being clarified.

In conclusion, our present study confirms and extends our previous finding that $H_2S$ stimulates capsaicin-sensitive primary afferent neurons in the rat detrusor muscle. We have provided functional evidence that $H_2S$ activates sensory neurons in a ruthenium red-sensitive but capsazepine- and SB 366791-insensitive manner. Although the molecular target(s) at which $H_2S$ acts on rat sensory neurons remain to be further clarified, our data suggest that this could be either a receptorial domain on the TRPV1 cation channel independent from those bound by vanilloids and other known TRPV1 activators, or another ruthenium red-sensitive transient receptor potential cation channel co-expressed with TRPV1 on primary afferent neuron terminals.

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## References

Amann, R., Maggi, C.A., 1991. Ruthenium red as a capsaicin antagonist. Life Sci. 49, 849–856.

Bevan, S., Hothi, S., Hughes, G., James, I.F., Rang, H.P., Shah, K., Walpole, C.S., Yeats, J.C., 1992. Capsazepine: a competitive antagonist of the sensory neurone excitant capsaicin. Br. J. Pharmacol. 107, 544–552.

Caterina, M.J., Schumacher, M.A., Tominaga, M., Rosen, T.A., Levine, J.D., Julius, D., 1997. The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 389, 816–824.

Chahl, L.A., 2004. Hydrogen sulphide: an endogenous stimulant of capsaicin-sensitive primary afferent neurons? Br. J. Pharmacol. 142, 1–2.

Chen, X., Jhee, K.H., Kruger, W.D., 2004. Production of the neuromodulator $H_2S$ by cystathionine {beta}-synthase via the condensation of cysteine and homocysteine. J. Biol. Chem. 10 (279), 52082–52086.

Cheng, Y., Ndisang, J.F., Tang, G., Cao, K., Wang, R., 2004. Hydrogen sulfide-induced relaxation of resistance mesenteric artery beds of rats. Am. J. Physiol., Heart Circ. Physiol. 287, H2316–H2323.

Clapham, D.E., Montell, C., Schultz, G., Julius, D., 2003. International Union of Pharmacology. XLIII. Compendium of voltage-gated ion channels: transient receptor potential channels. Pharmacol. Rev. 55, 591–596.

Garcia-Martinez, C., Morenilla-Palao, C., Planells-Cases, R., Merino, J.M., Ferrer-Montiel, A., 2000. Identification of an aspartic residue in the P-loop of the vanilloid receptor that modulates pore properties. J. Biol. Chem. 275, 32552–32558.

Geppetti, P., Bertrand, C., Baker, J., Yamawaki, I., Piedimonte, G., Nadel, J.A., 1993. Ruthenium red, but not capsazepine reduces plasma extravasation by cigarette smoke in rat airways. Br. J. Pharmacol. 108, 646–650.

Guidotti, T.L., 1996. Hydrogen sulphide. Occup. Med. 46, 367–371.

Gunthorpe, M.J., Benham, C.D., Randall, A., Davis, J.B., 2002. The diversity in the vanilloid (TRPV) receptor family of ion channels. Trends Pharmacol. Sci. 23, 183–191.

Gunthorpe, M.J., Rami, H.K., Jerman, J.C., Smart, D., Gill, C.H., Soffin, E.M., Luis Hannan, S., Lappin, S.C., Egerton, J., Smith, G.D., Worby, A., Howett, L., Owen, D., Nasir, S., Davies, C.H., Thompson, M., Wyman, P.A., Randall, A.D., Davis, J.B., 2004. Identification and characterisation of SB-366791, a potent and selective vanilloid receptor (VR1/TRPV1) antagonist. Neuropharmacology 46, 133–149.

Hayden, L.J., Franklin, K.J., Roth, S.H., Moore, G.J., 1989. Inhibition of oxytocin-induced but not angiotensin-induced rat uterine contractions following exposure to sodium sulfide. Life Sci. 45, 2557–2560.

Hosoki, R., Matsuki, N., Kimura, H., 1997. The possible role of hydrogen sulfide as an endogenous smooth muscle relaxant in synergy with nitric oxide. Biochem. Biophys. Res. Commun. 237, 527–531.

Jordt, S.E., Tominaga, M., Julius, D., 2000. Acid potentiation of the capsaicin receptor determined by a key extracellular site. Proc. Natl. Acad. Sci. U. S. A. 97, 8134–8139.

Jordt, S.E., Bautista, D.M., Chuang, H.H., McKemy, D.D., Zygmunt, P.M., Hogestatt, E.D., Meng, I.D., Julius, D., 2004. Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKTM1. Nature 427, 260–265.

Kageyama, M., Fujita, H., Nakata, K., Shirasawa, E., 1997. Involvement of both L- and N-type voltage-dependent $Ca^{2+}$ channels in KCl- and veratridine-evoked transmitter release from non-adrenergic, non-cholinergic nerves in the rabbit iris sphincter muscle. Naunyn-Schmiedeberg's Arch. Pharmacol. 355, 638–644.

Kimura, H., 2002. Hydrogen sulfide as a neuromodulator. Mol. Neurobiol. 26, 13–19.

Maggi, C.A., Patacchini, R., Giuliani, S., Santicioli, P., Meli, A., 1988a. Evidence for two independent modes of activation of the 'efferent' function of capsaicin sensitive nerves. Eur. J. Pharmacol. 156, 367–373.

Maggi, C.A., Patacchini, R., Santicioli, P., Giuliani, S., Geppetti, P., Meli, A., 1988b. Protective action of ruthenium red toward capsaicin desensitization of sensory fibers. Neurosci. Lett. 88, 201–205.

Maggi, C.A., Patacchini, R., Tramontana, M., Amann, R., Giuliani, S., Santicioli, P., 1990. Similarities and differences in the action of resiniferatoxin and capsaicin on central and peripheral endings of primary sensory neurons. Neuroscience 37, 531–539.

Maggi, C.A., Bevan, S., Walpole, C.S., Rang, H.P., Giuliani, S., 1993. Comparison of capsazepine and ruthenium red as capsaicin antagonists in the rat isolated urinary bladder and vas deferens. Br. J. Pharmacol. 108, 801–805.

McIntyre, P., McLatchie, L.M., Chambers, A., Phillips, E., Clarke, M., Savidge, J., Toms, C., Peacock, M., Shah, K., Winter, J., Weerasakera, N., Webb, M., Rang, H.P., Bevan, S., James, I.F., 2001. Pharmacological differences between the human and rat vanilloid receptor 1 (VR1). Br. J. Pharmacol. 32, 1084–1094.

Montell, C., Birnbaumer, L., Flockerzi, V., Bindels, R.J., Bruford, E.A., Caterina, M.J., Clapham, D.E., Harteneck, C., Heller, S., Julius, D., Kojima, I., Mori, Y., Penner, R., Prawitt, D., Scharenberg, A.M., Schultz, G., Shimizu, N., Zhu, M.X., 2002. A unified nomenclature for the superfamily of TRP cation channels. Mol. Cell 9, 229–231.

Moore, P.K., Bhatia, M., Moochhala, S., 2003. Hydrogen sulphide: from the smell of the past to the mediator of the future? Trends Pharmacol. Sci. 24, 609–611.

Patacchini, R., Santicioli, P., Giuliani, S., Maggi, C.A., 2004. Hydrogen sulfide ($H_2S$) stimulates capsaicin-sensitive primary afferent neurons in the rat urinary bladder. Br. J. Pharmacol. 142, 31–34.

Patapoutian, A., Peier, A.M., Story, G.M., Viswanath, V., 2003. Thermo TRP channels and beyond: mechanisms of temperature sensation. Nat. Rev., Neurosci. 4, 529–539.

Rigoni, M., Trevisani, M., Gazzieri, D., Nadaletto, R., Tognetto, M., Creminon, C., Davis, J.B., Campi, B., Amadesi, S., Geppetti, P., Harrison, S., 2003. Neurogenic responses mediated by vanilloid receptor-1 (TRPV1) are blocked by the high affinity antagonist, iodo-resiniferatoxin. Br. J. Pharmacol. 138, 977–985.

Story, G.M., Peier, A.M., Reeve, A.J., Eid, S.R., Mosbacher, J., Hricik, T.R., Earley, T.J., Hergarden, A.C., Andersson, D.A., Hwang, S.W., McIntyre, P., Jegla, T., Bevan, S., Patapoutian, A., 2003. ANKTM1, a TRP-like channel expressed in nociceptive neurons, is activated by cold temperatures. Cell 112, 819–829.

Szallasi, A., Blumberg, P.M., 1989. Resiniferatoxin, a phorbol-related diterpene, acts as an ultrapotent analog of capsaicin, the irritant constituent in red pepper. Neuroscience 30, 515–520.

Szallasi, A., Blumberg, P.M., 1990. Specific binding of resiniferatoxin, an ultrapotent capsaicin analog, by dorsal root ganglion membranes. Brain Res. 524, 106–111.

Szallasi, A., Blumberg, P.M., 1999. Vanilloid (Capsaicin) receptors and mechanisms. Pharmacol. Rev. 51, 159–212.

Teague, B., Asiedu, S., Moore, P.K., 2002. The smooth muscle relaxant effect of hydrogen sulphide in vitro: evidence for a physiological role to control intestinal contractility. Br. J. Pharmacol. 137, 139–145.

Wahl, P., Foged, C., Tullin, S., Thomsen, C., 2001. Iodo-resiniferatoxin, a new potent vanilloid receptor antagonist. Mol. Pharmacol. 59, 9–15.

Walker, K.M., Urban, L., Medhurst, S.J., Patel, S., Panesar, M., Fox, A.J., McIntyre, P., 2003. The VR1 antagonist capsazepine reverses mechanical hyperalgesia in models of inflammatory and neuropathic pain. J. Pharmacol. Exp. Ther. 304, 56–62.

Wang, R., 2003. The gasotransmitter role of hydrogen sulfide. Antioxid. Redox Signal. 4, 493–501.

Warenycia, M.W., Goodwin, L.R., Benishin, C.G., Reiffenstein, R.J., Francom, D.M., Taylor, J.D., Dieken, F.P., 1989. Acute hydrogen sulfide poisoning. Demonstration of selective uptake of sulfide by the brainstem by measurement of brain sulfide levels. Biochem. Pharmacol. 38, 973–981.

Welch, J.M., Simon, S.A., Reinhart, P.H., 2000. The activation mechanism of rat vanilloid receptor 1 by capsaicin involves the pore domain and differs from the activation by either acid or heat. Proc. Natl. Acad. Sci. U. S. A. 97, 13889–13894.

Zhao, W., Zhang, J., Lu, Y., Wang, R., 2001. The vasorelaxant effect of $H_2S$ as a novel endogenous gaseous K(ATP) channel opener. EMBO J. 20, 6008–6016.