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HYDROGEN SULFIDE CAN BE AN ENDOGENOUS RELAXATION FACTOR IN THE RAT BLADDER AND PROSTATE

Hypothesis/aims of study

Hydrogen sulfide (H_2S) is the third endogenous gasotransmitter besides carbon monoxide and nitric oxide, and has a wide range of biological functions including neuromodulation, vasorelaxation and cytoprotection [1]. In the lower urinary tract, H_2S donors induce contraction of the rat detrusor [2] and relaxation of the pig bladder neck [3], suggesting a possibility that H_2S may have site-specific effects on the bladder. However, the detailed functions of H_2S in each part of the bladder are still unclear. In addition, there are no reports showing physiological roles of H_2S in the prostate.

Endogenous H_2S is produced from L-cysteine by enzymes: cystathionine ß-synthase (CBS), cystathionine□□-lyase (CSE), 3-mercaptopyruvate sulfurtransferase (MPST) and cysteine aminotransferase (CAT) [1]. CBS and CSE produce H_2S from L-cysteine directly, and MPST produces H_2S from 3-mercaptopyruvate (3MP), which is produced by CAT from L-cysteine [1]. Recently, a novel pathway for endogenous H_2S production from D-cysteine is also reported, namely, D-cysteine is metabolized by D-amino acid oxidase (DAO) to 3MP, which is a substrate for MPST to produce H_2S [1].

In the present study, therefore, we investigated (1) pharmacological profile of exogenous H_2S-induced relaxation of the rat bladder dome and trigone (BL-D and BL-T) and dorsolateral and ventral prostate (PR-D and PR-V), and (2) expression levels of CBS, CSE, MPST, CAT and DAO in each site of these tissues.

Study design, materials and methods

BL-D, BL-T, PR-D, PR-V, liver and cerebellum (L and C) were prepared from male Wistar rats (300-400 g) sacrificed with an overdose of sodium pentobarbital (80 mg/kg, i.p.).

1. Organ bath study: By using 1 mm x 5 mm strips of bladder and prostate tissues, effects of NaHS (H_2S donor: 1×10^{-9} to 3×10^{-4} M) were evaluated on carbachol (1×10^{-5} M)-induced contractions of bladder and on noradrenaline (1×10^{-5} M)-induced ones of prostate. Prostate strips were pretreated with propranolol (1×10^{-6} M) 30 min before the pre-contraction.

2. Detection of H_2S synthesizing enzymes: Gene and protein expression levels of CBS, CSE, MPST, CAT and DAO were investigated by quantitative real-time PCR and western blot analysis, respectively, in bladder and prostate tissues. L and C were used for positive controls.

Results

1. Exogenously administered NaHS dose-dependently induced relaxation on the BL-D and BL-T pre-contracted by carbachol and on the PR-D and PR-V pre-contracted by noradrenaline. There were no significant differences of the maximal relaxation rate against the pre-contractions among any of these tissues (Table 1). There were no significant differences of the EC_{50} values between the BL-D and BL-T, nor between the PR-D and PR-V, respectively (Table 1). The EC_{50} values in the bladder were more potent than those in the prostate (Table 1).

2. CBS was expressed in the PR-D and PR-V, but not in the BL-D or BL-T (Table 2 and 3). Gene and protein expression levels of CBS in the PR-V were higher than those in the PR-D (Table 2 and 3). MPST was expressed in all four tissues (Table 2 and 3). Gene and protein expression levels of MPST in the prostate were higher than those in the bladder, and those in the PR-D were higher than those in the PR-V (Table 2 and 3). CAT was expressed in all four tissues (Table 2 and 3), but there was no significant difference of gene and protein expression levels among each tissue (Table 2 and 3). CSE and DAO were not expressed in each bladder and prostate tissue, although they were expressed in both gene and protein levels in positive control samples prepared from the L (for CSE) and the C (for DAO) tissues (data not shown).

Interpretation of results

Our results indicate that; (1) H_2S induces relaxation of bladder and prostate smooth muscle as evidenced by exogenous H_2S-induced relaxation response to pre-contracted bladder and prostate strips, and (2) some H_2S synthesizing enzymes are expressed in both gene and protein levels in the bladder and prostate. Expression patterns of these enzymes are different between the bladder and prostate, and differences of site-specific expression patterns are also observed. In the bladder, the MPST/CAT pathway is major for H_2S biosynthesis, while in the prostate, CBS and MPST/CAT pathways are involved in the biosynthesis. In the PR-D, the MPST/CAT pathway seems to be major for the biosynthesis, while in the PR-V, CBS and MPST/CAT pathways are involved in the biosynthesis.

Concluding message

H_2S could function as an endogenous relaxation factor in both bladder and prostate. Endogenous H_2S might open new avenues of therapeutic interventions for lower urinary tract dysfunction such as overactive bladder and benign prostatic hyperplasia.

Table 1. Data from our organ bath study in the rat bladder and prostate tissues in response to NaHS

Group	Relaxation rate (%)	EC_{50} (M)
BL-D	43.8 ± 3.4 (n=12)	9.64 ± 3.78 (× 10^{-8}) (n=14)
BL-T	38.5 ± 4.1 (n=14)	4.33 ± 1.19 (× 10^{-8}) (n=19)
PR-D	39.1 ± 2.9 (n=9)	10.13 ± 10.09 (× 10^{-6}) (n=18)
PR-V	42.7 ± 4.2 (n=14)	1.35 ± 1.29 (× 10^{-6}) (n=22)

BL-D: bladder dome; BL-T: bladder trigone; PR-D: dorsolateral prostate; PR-V: ventral prostate.
Values present as means ± SEM. The number of animals per group is indicated in parentheses.

Table 2. Quantitative real-time PCR data from the rat bladder and prostate tissues

Group	CBS/β-actin (fold)	MPST/β-actin (fold)	CAT/β-actin (fold)
BL-D	ND (n=5)	0.23 ± 0.03# (n=5)	0.58 ± 0.13 (n=5)
BL-T	ND (n=5)	0.37 ± 0.08# (n=5)	0.55 ± 0.10 (n=5)
PR-D	0.19 ± 0.03* (n=5)	3.11 ± 0.61* (n=5)	1.32 ± 0.35 (n=5)
PR-V	1 ± 0 (n=5)	1 ± 0 (n=5)	1 ± 0 (n=5)

BL-D: bladder dome; BL-T: bladder trigone; PR-D: dorsolateral prostate; PR-V: ventral prostate;
CBS: cystathionine β-synthase; MPST: 3-mercaptopyruvate sulfurtransferase;
CAT: cysteine aminotransferase.
The amount of mRNA for each enzyme was normalized to the amount of mRNA for β-actin.
Values present as means ± SEM. *P<0.05, vs PR-V; #P<0.05, vs PR-D.
The number of animals per group is indicated in parentheses. ND: not detected.

Table 3. Western blot analysis data from the rat bladder and prostate tissues

Group	CBS/β-actin (fold)	MPST/β-actin (fold)	CAT/GAPDH (fold)
BL-D	ND (n=6)	0.42 ± 0.08*# (n=5)	1.56 ± 0.36 (n=4)
BL-T	ND (n=6)	0.37 ± 0.06*# (n=5)	0.86 ± 0.08 (n=4)
PR-D	0.08 ± 0.03* (n=8)	1.69 ± 0.20* (n=7)	1.39 ± 0.31 (n=4)
PR-V	1 ± 0 (n=9)	1 ± 0 (n=8)	1 ± 0 (n=4)

BL-D: bladder dome; BL-T: bladder trigone; PR-D: dorsolateral prostate; PR-V: ventral prostate;
CBS: cystathionine β-synthase; MPST: 3-mercaptopyruvate sulfurtransferase;
CAT: cysteine aminotransferase; GAPDH; glyceraldehyde-3-phosphate dehydrogenase.
Each band intensity relative to the inner control (β-actin or GAPDH) is presented as means ± SEM.
*P<0.05, vs PR-V; #P<0.05, vs PR-D. The number of animals per group is indicated in parentheses.
ND: not detected.

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Disclosures

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