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# ROLE OF HYDROGEN SULFIDE IN THE INHIBITORY NEUROTRANSMISSION TO THE PIG INTRAVESICAL URETER.

Hypothesis / aims of study

Hydrogen sulfide ($H_2S$) is considered as the third endogenous gaseous transmitter besides nitric oxide (NO) and carbon monoxide (CO) [1]. $H_2S$ is synthesized from L-cysteine by the action of two pyridoxal-5´-phosphate-dependent enzymes, cystathionine γ-lyase (CSE) or cystathionine β-synthase (CBS) [1]. The autonomic nervous system plays an essential role in the maintenance of ureteral motor activity. Thus, together with the regulation exerted by noradrenergic and cholinergic systems, a nitric oxide (NO)-dependent, as well as an unknown nature neurogenic component in non-adrenergic, non-cholinergic (NANC) inhibitory transmission of the intravesical ureter has also previously been elucidated [2]. $H_2S$ has been identified as a powerful inhibitory gaseous signaling molecule in the bladder outflow region [3], however, no data exists about of its possible role in the regulation of ureteral smooth muscle contractility. Therefore, the aim of the current study was to investigate the involvement of $H_2S$ in the inhibitory neurotransmission to the pig intravesical ureter.

Study design, materials and methods

For immunohistochemical studies, intravesical ureter segments were fixed in 4 % paraformaldehyde in 0.1 M phosphate buffer, pH 7.4 (PB), for 2 to 4 h at 4º C, and subsequently placed in 30 % sucrose in 0.1M PB for cryoprotection and stored at -80°C.Transversal sections 5μm thick were obtained by means of a cryostat and preincubated in 10 % normal goat serum in PB containing 0.3 % Triton-X-100, for 2-3 h. Then, sections were incubated with rabbit anti-cystathionine γ-lyase (anti-CSE) antibody at 4-8 μg/ml final concentration plus a mouse anti-protein gene product 9.5 (anti-PGP 9.5), as neuronal marker, diluted 1:50 during 48 h at 4º C, washed and reacted with the second antibodies Alexa Fluor 594 goat-antirabbit (1:200 dilution) to detect CSE and Alexa Fluor 488 goat-antimouse (1:200 dilution) to detect PGP 9.5 for 2 h at room temperature.

Concerning to functional studies, ureteral strips 4-6 mm long and 2-3 mm wide were suspended horizontally with one end connected to an isometric force transducer (Grass FT 03C) and the other one to a micrometer screw, in 5 ml organ baths containing physiological saline solution (PSS) at 37° C gassed with carbogen (95 % $O_2$ and 5 % $CO_2$) to obtain a final pH of 7.4. The signal was continuously recorded on a polygraph (Graphtec Multicorder MC 6621). Passive tension of 2 g was applied to the preparations and they were allowed to equilibrate for 60 min. On 0.1 μM U46619-induced tone, under NANC conditions, frequency- and/or concentration-response relaxation curves to electrical field stimulation (EFS, 1 ms duration, 0.5-16 Hz, 20 s trains, with constant current output adjusted to 75 mA) and to the $H_2S$ donor *P*-(4-methoxyphenyl)-*P*-4-morpholinylphosphinodithioic acid (GYY4137), respectively, were obtained in the absence or presence of CSE and NO synthase inhibitors.

Results

CSE expression was observed as nerve fibers distributed in the intravesical ureter smooth muscle layer. On U46619-precontracted ureteral strips, under NANC conditions, EFS (0.5-16 Hz) and the $H_2S$ donor *P*-(4-methoxyphenyl)-*P*-4-morpholinylphosphinodithioic acid (GYY4137, 0.1 nM-30 μM) evoked frequency- and concentration-dependent relaxations. EFS-elicited responses were reduced by inhibition of CSE and abolished by blockade of CSE and NO synthase.

Interpretation of results

The present results suggest that neuronal $H_2S$, synthesized by CSE, is involved in the NO-independent inhibitory transmission to the pig intravesical ureter.

Concluding message

Relaxation to $H_2S$ donors may be useful in the therapeutic management of obstructive uropathy produced by ureteric calculi.

References

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Disclosures

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