Sildenafil attenuates mouse uterus contractility through L-Cys/H_2S pathway activation

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Background: Sildenafil, a selective phosphodiesterase type 5 (PDE5) inhibitor that prevents cGMP degradation and promotes smooth muscle relaxation [1], is commonly used in the oral treatment for erectile dysfunction [2]. Nonetheless, it has been reported that sildenafil relaxes human bladder through the activation of hydrogen sulfide (H_2S) signaling [3]. H_2S is an endogenous gaseous transmitter produced from the aminoacid L-Cysteine (L-Cys) principally through the activation of two pyridoxal-5-phosphate-dependent enzymes i.e. cystathionine-β-synthase (CBS) and cysthationine-γ-lyase (CSE) [4,5]. H_2S is involved in several physiological and pathological processes, and a role in urogenital tract has been shown [6]. Particularly, both CBS and CSE are expressed in rat and human myometrium tissues suggesting an H_2S contribution in uterus functioning [7].
Aim: To investigate the effect of sildenafil and the role of L-Cys/H_2S pathway in spontaneous uterus contractility in mice.
Methods: Virgin female mice (CTR), and CSE-ablated mice ($CSE^{-/-}$) were used. During the estrus period, uteri were cleaned and processed. Western blot analysis was performed to evaluate the presence of both CBS and CSE. Spontaneous mouse uterus motility was measured and tissues were challenged with L-Cys, sodium hydrogen sulfide (NaHS) or D-Cys (100nM–300μM) as well as sildenafil (0.1nM-3μM), in presence or in absence of DL-propargilglycine (PAG, 10mM, CSE inhibitor) or aminooxiacetic acid (AOAA, 1mM, CBS inhibitor). L-Cys and sildenafil-induced effect were also evaluated on uterine harvested form $CSE^{-/-}$ mice. In addition H_2S generation and cGMP content was measured in homogenates of mouse uteri.
Results: CBS and CSE are expressed and able to convert L-Cys into H_2S in mouse uterus. Sildenafil significantly increases H_2S production in mouse uterus and this effect is notably reduced by CBS or CSE inhibition. In parallel, L-Cys, NaHS or sildenafil but not D-Cys decreases spontaneous uterus contractility. The blockage of CBS and CSE reduces this latter effect even if a major role for CSE than CBS has been observed. This data has been strongly confirmed by using $CSE^{-/-}$ mice. Indeed, neither L-Cys nor sildenafil affects H_2S production in $CSE^{-/-}$ mice. In parallel, the effect of H_2S or sildenafil on spontaneous contractility is markedly lower in $CSE^{-/-}$ mice compared to CTR mice. A decisive proof for the involvement of H_2S/CSE signaling in sildenafil-induced effect has been confirmed by the measurement of cGMP .Indeed sildenafil-induced increase in cGMP level is significantly reduced by CSE inhibition.
Conclusions: L-Cys/CSE/H_2S signaling modulates the mouse uterus motility and the sildenafil effect .Therefore the study may suggest new therapeutical approaches for the management of the uterus abnormal contractility disorders.

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