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Effect of Gasotransmitter H_2S on Adrenergic and Cholinergic Modulated Spontaneous Uterine Contractions

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Keywords

- H_2S
- AOAA
- NaHS
- Uterine contractions

Abstract

Background: Hydrogen sulfide (H_2S) is recognized as a gaseous transmitter in mammalian tissues. Several physiological roles for H_2S including vasodilation, and smooth muscle relaxation have been documented. The exact mechanism explaining its role in uterine contractility remain to be elucidated. The proposed study was conducted to elucidate H_2S role in uterine contractility in relation to adrenergic and cholinergic systems. **Methods**: Isolated uterus horn of adult virgin female ***sprage Dawely*** rats were used in vitro in physiological saline solution, the effects of H_2S donor (NaHS), H_2S blocker aminooxy acetic acid (AOAA) with or without adrenergic and cholinergic drugs and glibenclamide were determined. **Results**: It was revealed that, NaHS inhibited myometrial contractions as regard the tone and amplitude, whereas AOAA increased the tone of uterine contractility. The relaxant effect of H_2S on uterine contractility was mediated by modulation of the endogenous adrenergic and cholinergic systems in the myometrium, which was demonstrated by dual interaction of NaHS or AOAA with Adrenaline, Propranolol, Prazosin, Acetylcholine and Atropine on the tone, peak, amplitude and frequency of uterine contractions. Glibencalmide (K_{ATP} channels blocker) after NaHS significantly increased the tone, peak and amplitude. However Glibencalmide after AOAA produced no significant changes in the tone, peak and amplitude of uterine contractions. **Conclusion**: H_2S has a relaxant action in smooth muscles of myometrium. This inhibitory effect of H_2S on uterine contractility could be through dual interaction and modulation of the endogenous adrenergic and cholinergic systems and activation of K_{ATP} channels.

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INTRODUCTION

Hydrogen sulfide (H_2S) is a colorless and rotten egg shell gas, now it is recognized as the third gaseous transmitter in mammalian tissues after carbon monoxide and nitric oxide (NO) (1). Two key enzymes, cystathionine-γ-lyase (CSE), cystathionine-β-synthase (CBS), are involved in the natural production of H_2S in the body utilizing L-cysteine and/or homocysteine as substrate (2 & 3). Endogenously H_2S is produced from the amino acid L-Cysteine (L-Cys) through the activation of cystathionine-β-synthase (CBS) and cysthationine-γ-lyase (CSE) (4). H_2S is involved in multiple physiological and pathological processes in various body systems. A growing body of literatures have documented several physiological roles for H_2S including vasodilation, neuromodulation, and smooth muscle relaxation (5). In the peripheral nervous system, H_2S induces contraction of the bladder wall (6) and acts on enteric neurons in the human and guinea pig colon to increase chloride secretion (7 & 8). Also, it was found that H_2S has a role in urogenital tract (9).

It has been reported that H_2S has a role in gastrointestinal tract secretions and motility. Additionally, exogenous H_2S has been shown to protect the gastric mucosa on different model of experimentally induced gastric ulcer in rats (10 & 11). Gastrointestinal smooth muscles have shown that H_2S produces both direct and indirect smooth muscle relaxation, inhibits spontaneous motility or prevents chemically or electrically induced contractile responses (12). Particularly, both CBS and CSE are expressed in rat and human myometrium suggesting an H_2S contribution in uterine functions (13). Also, it was found that H_2S inhibits the spontaneous and oxytocin-induced contractility of human pregnant myometrium, NaHS evokes relaxation of human pregnant myometrium, suggesting a possible role of H_2S during pregnancy (14). Little research about the effect of H_2S on uterine contractions were published, and the exact mechanism explaining the role of H_2S in uterine motility remain to be elucidated. The proposed study was conducted to elucidate the role of H_2S in uterine contractility in relation to adrenergic and cholinergic receptors.

Material and methods

Drugs and chemicals

NaHS was used as H_2S donor, Amino-oxy-acetic acid (AOAA) as H_2S blocker. Acetylcholine, atropine, adrenaline, propranolol, Glibenclamide were used. All were purchased from Sigma Aldrich Chemical Co (Egypt).

Animals.

24 adult virgin female Sprague Dawely rats (180 – 200 g) in oestrus state, were used in the study, and determined by daily vaginal smear examination. All experiments were performed in accordance with the guidelines and principles for the care and use of laboratory animals at Mansoura University.

Tissue Preparation and Organ Bath.

Animals were killed by cervical dislocation. Following a longitudinal midline incision, the uterus was carefully removed and immediately immersed in Krebs-Heinseleit physiological salt solution (PSS) with the following composition (mM/L: NaCl 118.4, KCl 4.7, MgSO4 1.2, glucose 11, CaCl2 2.5, NaHCO3 25, KH2PO4 1.2) (Sigma-Aldrich, St. Louis, MO), at pH 7.4 and gassed with 95% O2-5% CO2 (15).

The uterus was placed in dissecting dish containing PSS. The right and left uterine horns

were carefully separated and mounted vertically in 50 mL organ baths, one end was fixed to a stainless-steel gas bubbler and the other end was connected to a force displacement transducer (Grass FT03, Astro-Med, Slough, UK) using silk thread. The output from the transducer was amplified by a PowerLab 4/35 and the data was recorded on a personal computer running LabChart v7 software (ADInstruments Ltd., Oxford, UK).

All experiments were carried out at 37 C, and the PSS was continuously gassed with 95% O2 - 5% CO2. A resting tension of 0.5 g was applied, and the uteri were allowed to contract spontaneously during an initial equilibration period of 30–60 min before commencing any experimental protocol. Following the equilibration period and development of spontaneous contractile activity, control contractions were recorded for 10 min. and various concentrations of drug were added to the tissue bath cumulatively (16).

The amplitude (in gm) and frequencies (number of contractions in 10 min) of contractions were evaluated at 10-min intervals before and after applications of each dose of the drug.

Experimental design

The record of basal uterine contractility (before application of the drug) acts as the control compared with the record of uterine contractility after application of the drug on the uterine tissue in the organ path.

To study the role of H_2S on uterine contractility and its relation to adrenergic and cholinergic neuron interaction through the following experiments (6 rats each):

1) **Group 1 experiments:** The effect of H_2S on uterine contractility by using NaHS (as H_2S donors) in a dose of (1 mM/L), and or Amino-oxy-acetic acid AOAA (H_2S synthesis blocker) in a dose of (1 mM/L) (17).
2) **Group 2 experiments:** Effect of H_2S on adrenergic stimulated uterine contractility by using AOAA (in a dose of (1 mM/L)) or NaHS (in a dose of (1 mM/L)) on adrenergic agonist (adrenaline, in a dose of (1 µM/L)) and antagonists alpha blocker (prazosin, in a dose of (1 µM/L)) and beta blockers (propranolol, in a dose of (1 µM/L)) (18).
3) **Group 3 experiments:** Effect of H_2S on cholinergic stimulated uterine contractility by using AOAA (in a dose of (1 mM/L)) or NaHS (in a dose of (1 mM/L)) on cholinergic agonist (acetylcholine, in a dose of (0.1 mM/L)) and antagonists (atropine, in a dose of (1 µM/L)) (18).
4) **Group 4 experiments:** Effect of Glibenclamide (K_{ATP} channels blocker, in a dose of (6 µM/L)) on H_2S modulated uterine contractility, by using AOAA (in a dose of (1 mM/L)) or NaHS (in a dose of (1 mM/L)) (19).

Data analysis and statistical procedures

The recorded figures were analyzed by Lab Chart Reader 7. Data analysis was done by the statistical package for social science (SPSS), version 20. Paired t test with Wilcoxon signed rank test was used for analysis of variance and data were expressed as Mean ± SD.

Results

The responses were quantified by calculating the tone of uterine segment was determined by the base of contraction, the amplitude of contractions was determined by the extent from the base to the peak of contraction and frequency of the contractions. The rate was determined by counting the number of contractions over a 10-minute period before the next addition of the drug.

1) Effect of H_2S donor (NaHS) or H_2S blocker (AOAA) on the uterine motility:

The effect of increased H_2S on uterine contractility

It was found that NaHS significantly decreased the base (tone), peak and amplitude of uterine contractions (P= 0.005, 0.028, 0.023) as compared with the basal uterine contractions, but NaHS significantly increased the frequency of uterine contractility from 8.8 ± 0.84 to 14.4 ± 0.547 (P < 0.001) (Fig. 1 A & Table1).

Effect of endogenous H_2S on uterine contractility

It was found that AOAA that block H_2S synthesis produced significant increase in the tone (P= 0.005), but insignificant decrease in amplitude of uterine contractions. Whereas AOOA significantly increased the rate of contractions (P < 0.001) as compared with basal uterine contractions.

Meanwhile NaHS after AOAA produced significant decrease in the tone (P= 0.005) as compared with that of AOAA and significant decrease in the rate of contraction (P= 0.009) as compared with that of AOAA, that was still significantly higher than the basal contractions (P < 0.001) (Fig. 1 B & Table 2).

2) Effect of H_2S donor (NaHS) or H_2S blocker (AOAA) on the adrenergic modulated uterine motility:

Adrenaline and H_2S on uterine contractions.

It was also found that adrenaline significantly decreased the base (tone), the peak and the amplitude of uterine contractions (P= 0.003), also significantly decreased the frequency of contractions from 11.2 ± 0.84 to 3.6 ± 0.547 (P < 0.001) as compared with the basal contractions. Whereas AOAA after adrenaline significantly increased the peak and amplitude (P= 0.003), also significantly increased the frequency of contractions from 3.6 ± 0.547 to 8.4 ± 1.67 (P < 0.001), that was still significantly lower than the basal contractions (Fig 2 A, Fig 5 & Table 2).

Beta blocker and H_2S on uterine contractions

The study revealed that NaHS significantly decreased the peak and amplitude (P= 0.006, 0.005) respectively, but significantly increased the frequency of contractions from 7 ± 0.63 to 9.3 ± 0.84 (P= 0.02), meanwhile the base (tone) insignificantly decreased as compared with the basal uterine contractions. Whereas propranolol after NaHS significantly increased the base (tone), peak and amplitude (P= 0.01, 0.003, 0.003) respectively as compared with that of NaHS, also the base (tone) became significantly higher than the basal uterine contractions (P= 0.005) (Fig 2 B, Table 1).

It was also found that propranolol significantly increased the base (tone) (P= 0.016), but significantly decreased the peak and amplitude of contractions (P= 0.004) meanwhile significantly increased the frequency (P= 0.001) as compared with the basal uterine contractions. Whereas NaHS after propranolol produced insignificant changes in

the tone, amplitude and the frequency of uterine contractions as compared with that of propranolol (Fig 2 C, Fig 5 & table 1).

Alpha blocker and H_2S on uterine contractions

It was found that NaHS significantly decreased the base (tone), the peak and the amplitude (P= 0.016. 0.008, 0.008) respectively, but significantly increased the frequency of uterine contractions from 12.5 ± 0.577 to 15.5 ± 0.577 (P = 0.005) as compared with the basal one. Whereas prazosin after NaHS produced insignificant changes in the uterine contractility as regard the base (tone), peak, amplitude and the frequency as compared with that of NaHS, but the peak and the amplitude were still significantly lower than that of basal contractions (P= 0.037, 0.028) respectively meanwhile the frequency of contractions was still significantly higher than that of basal contractions (Fig 2 D, table 1).

Prazosin significantly decreased the base (tone), peak, amplitude and frequency of uterine contractions (P= 0.008, 0.001, 0.023, 0.015) as compared with the basal one. Whereas NaHS after prazosin significantly decreased the tone, peak and amplitude of contractions (P= 0.002, 0.003, 0.009) as compared with that of prazosin but these parameters were still significantly lower than that of basal contractions (Fig 2 E, Fig 6).

AOAA significantly increased the tone but decreased the peak and amplitude of uterine contraction (P= 0.05), meanwhile it significantly increased the frequency of as compared with that of basal uterine contractions. Whereas prazosin after AOAA significantly increased the peak (P= 0.05) but significantly decreased the frequency of contractions (P= 0.016), as compared with that of AOAA (Fig 2 F, table 2).

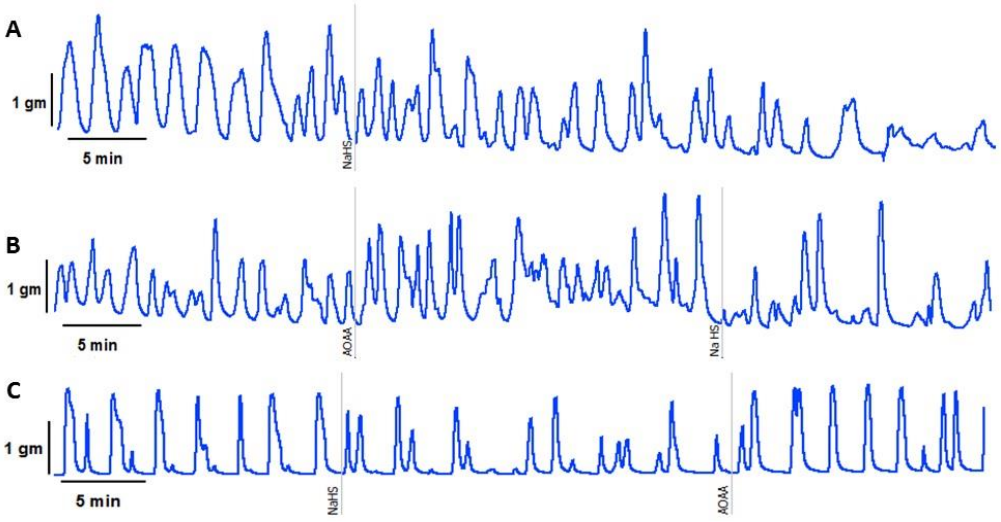

Fig 1: Effect of H_2S on basal spontaneous uterine contractions. A) NaHS (H_2S donor) on basal uterine contractions, B) AOAA (H_2S synthesis blocker) on basal uterine contractions followed by NaHS, C) NaHS on basal uterine contractions followed by AOAA.

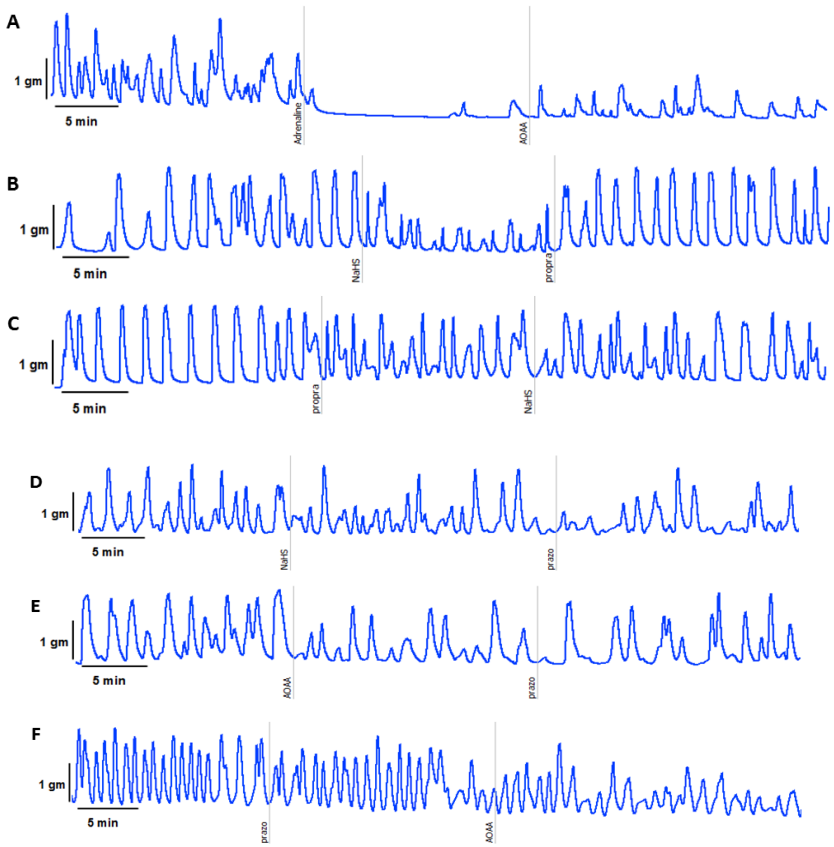

Fig 2: Effect of H_2S on adrenergic modulated uterine contractions. A) adrenaline on basal uterine contractions followed by AOAA (H_2S synthesis blocker), B) NaHS (H_2S donor) on basal uterine contractions followed by propranolol (non selective B blocker), C) propranolol on basal uterine contractions followed by NaHS, D) NaHS on basal uterine contractions followed by prazosin (alpha blocker), E) AOAA on basal uterine contractions followed by prazosin (alpha blocker), F) prazosin on basal uterine contractions followed by AOAA (H_2S synthesis blocker).

3) Effect of H_2S donor (NaHS) or H_2S blocker (AOAA) on the cholinergic modulated uterine motility:

It was found that acetylcholine significantly increased the base (tone), peak and amplitude (P= 0.006, 0.005, 0.002) respectively, but significantly increase the frequency of contractions from 7 ± 0.63 to 14 ± 0.84 (P= 0.002), as compares with the basal uterine contractions. Whereas NaHS after acetylcholine significantly decreased the base (tone), peak and amplitude of uterine contractions (P= 0.001, 0.003, 0.003) respectively as compared with that of Ach (Fig 3A).

NaHS significantly decreased the base (tone), peak and amplitude of uterine contractions (P= 0.005, 0.008, 0.023) as compared with the basal contractions, also it significantly decreased the frequency from 11.6 ±0.55 to 9.2 ±0.84 (P=0.009). Whereas atropine after NaHS insignificantly decreased the tone, peak and amplitude but significantly decreased the frequency of uterine contractions from 9.2 ±0.84 to 6.2 ±0.84 (P=0.001) as compared with that of NaHS, these reduction in the uterine contractility parameter became more significantly lower than the basal contractions (P= 0.005, 0.002, 0.002, <0.001) (Fig 3 B & Fig 6).

AOAA significantly increased the base (tone) (P= 0.017) and frequency of contractions from 10.8 ±0.84 to 13 ±0.71 (P= 0.011), but significantly decrease the peak (P= 0.041), as compared with

the basal contractions. Whereas atropine after AOAA significantly decrease the base (tone), peak of uterine contractions (P= 0.003, 0.004) as compared with that of AOAA, also became significantly lower than the basal contractions (P= 0.003). Atropine significantly decreased the frequency of uterine contractions from 13 ±0.71 to 10 ±0.71 (P= 0.011) as compared with that of AOAA (Fig 3 C & Table 2).

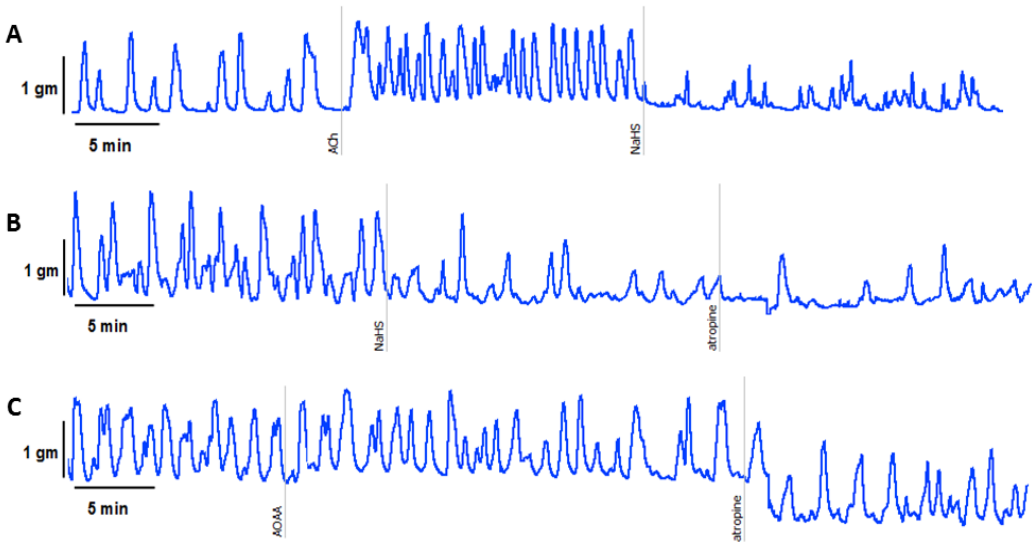

Fig 3: Effect of H_2S on cholinergic modulated uterine contractions. A) acetylcholine on basal uterine contractions followed by NaHS (H_2S donor), B) NaHS on basal uterine contractions followed by atropine (muscarinic blocker), C) AOAA (H_2S synthesis blocker) on basal uterine contractions followed by atropine (muscarinic blocker).

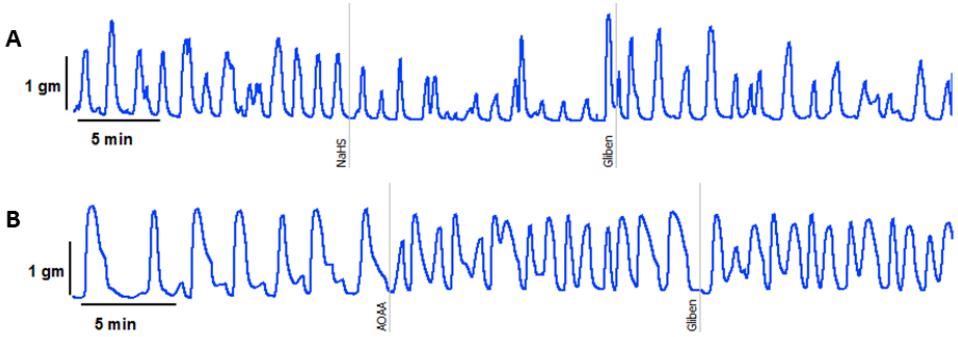

Fig 4: Role of Glibenclamide (K_{ATP} channel blocker) in H_2S modulated uterine contractions. A) NaHS (H_2S donor) on basal uterine contractions followed by Glibenclamide, B) AOAA (H_2S synthesis blocker) on basal uterine contractions followed by Glibenclamide.

Table 1: The dual effect of H2S donor (NaHS), α & β adrenergic receptors blockers and K_{ATP} channel blocker on spontaneous uterine contraction parameters base (tone), amplitude (gm) and frequency.

Experimental group		Base			Amp			Frequency
		Med	Min	Max	**Med**	Min	Max	**Mean** ±SD
G1	**Basal**	**-0.26**	-0.31	-0.18	**0.49**	0.32	1.01	**8.83** ±0.84
	NaHS	**-0.36** [a]	-0.38	-0.26	**0.29** [a]	0.17	0.64	**14.4** ±0.55 [a]
G2	**Basal**	**0.03**	-0.06	0.11	**1.81**	0.47	2.03	**7.0** ±0.63
	NaHS	**-0.01**	-0.05	0.14	**0.53** [a]	0.20	0.76	**9.20** ±0.84 [a]
	Propra	**0.11** [a, b]	0.03	0.15	**1.82** [b]	1.11	1.90	**8.20** ±0.45
G3	**Basal**	**-0.35**	-0.41	-0.27	**1.39**	0.84	1.97	**12.5** ±0.56
	NaHS	**-0.40** [a]	-0.43	-0.35	**0.72** [a]	0.22	1.85	**15.5** ±0.56 [a]
	Prazo	**-0.32**	-0.43	-0.25	**0.65** [a, b]	0.14	1.84	**14.4** ±0.55 [a]
G4	**Basal**	**-0.15**	-0.18	-0.07	**1.03**	0.54	1.55	**8.4** ±0.55
	NaHS	**-0.22** [a]	-0.23	-0.17	**0.42** [a]	0.12	1.36	**10.0** ±0.71 [a]
	Gliben	**-0.2** [a, b]	-0.23	-0.12	**0.88** [b]	0.51	1.70	**8.8** ±0.84

G = group, Propra = propranolol, Prazo = prazosin, Gliben = glibenclamide. Amp = amplitude, Med = median, Min = minimum, Max = maximum, SD = standard deviation, a significant as compared with the basal uterine contractions, b significant as compared with the effect of NaHS on uterine contractions.

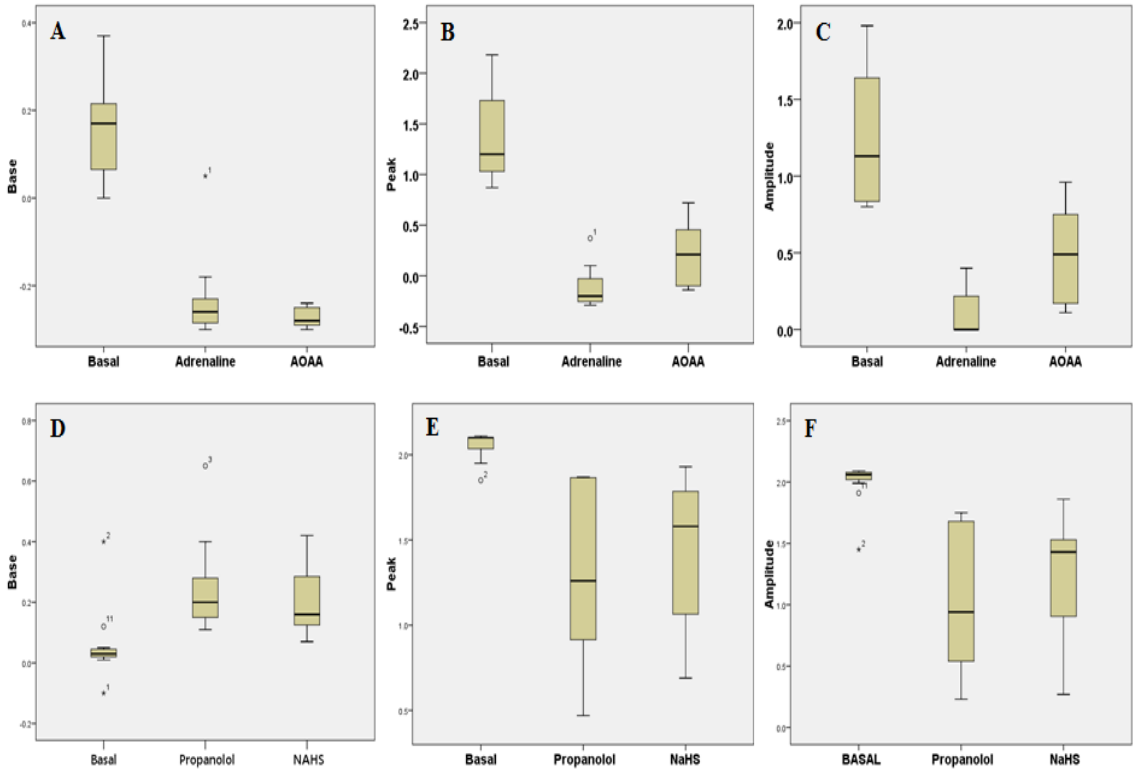

Fig 5: Effect of H_2S on adrenaline and propranolol modulated uterine contractility: A) adrenaline and AOAA on the base (tone), B) adrenaline and AOAA on the peak, C) adrenaline and AOAA on the amplitude of uterine contractions. D) Propranolol and AOAA on the base (tone), E) propranolol and AOAA on the peak, F) propranolol and AOAA on the amplitude of uterine contractions.

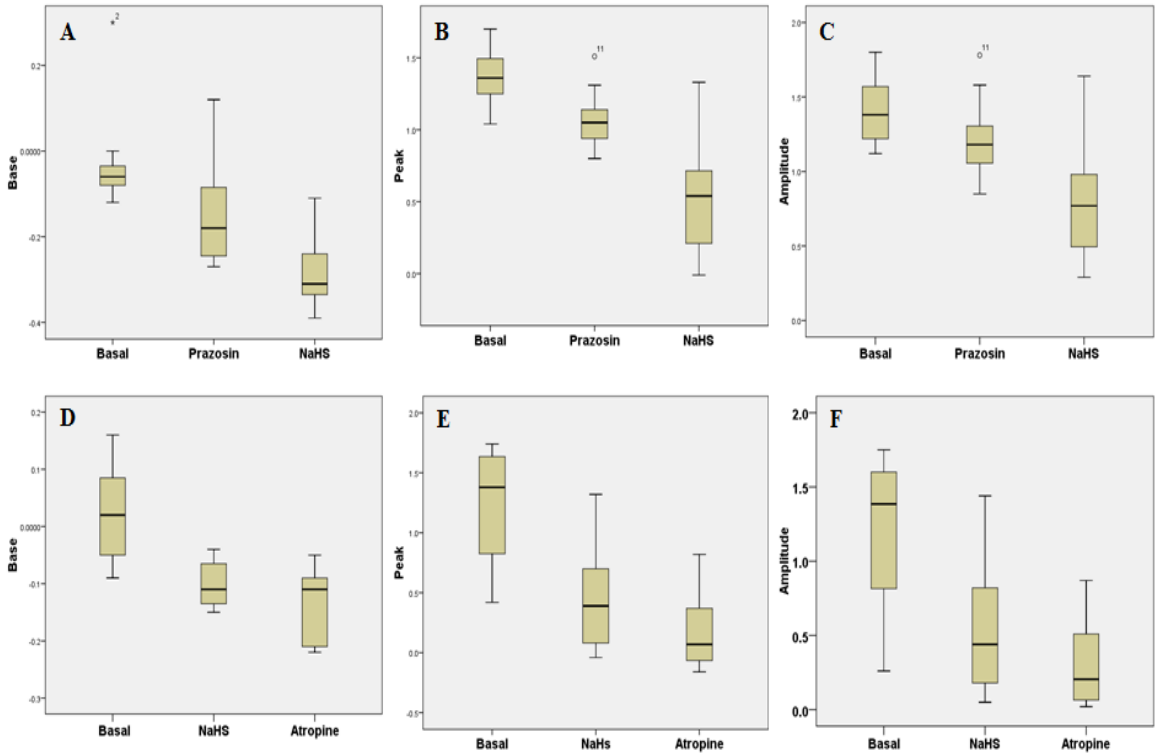

Fig 6: Effect of H_2S on prazosin and Atropine modulated uterine contractility: A) prazosin and AOAA on the base (tone), B) prazosin and AOAA on the peak, C) prazosin and AOAA on the amplitude of uterine contractions. D) Atropine and AOAA on the base (tone), E) Atropine and AOAA on the peak, F) Atropine and AOAA on the amplitude of uterine contractions.

Table 2: The dual effect of H2S synthesis blocker (AOAA), α adrenergic and muscarinic receptors blockers and K_{ATP} channel blocker on spontaneous uterine contraction parameters base (tone), amplitude (gm) and frequency.

Experimental group		Base			Amp			Frequency
		Med	Min	Max	**Med**	Min	Max	**Mean** ±SD
G5	**Basal**	**-0.26**	-0.26	-0.18	**1.55**	1.0	2.0	**5.83** ±0.75
	AOAA	**-0.14** [a]	-0.21	-0.06	**1.31**	0.56	1.78	**12.0** ±0.89 [a]
	NaHS	**-0.25** [b]	-0.26	-0.15	**1.5**	1.22	1.85	**10.4** ±0.54 [a, b]
G6	**Basal**	**-0.09**	-0.28	0.19	**1.68**	0.40	2.04	**11.2** ±0.84
	AOAA	**-0.18** [a]	-0.24	0.27	**0.78** [a]	0.32	1.83	**13.0** ±0.71 [a]
	Prazo	**-0.18**	-0.29	0.58	**1.39**	0.47	1.90	**11.4** ±0.55 [b]
G7	**Basal**	**-0.17**	-0.32	-0.02	**1.39**	0.48	1.79	**10.8** ±0.84
	AOAA	**-0.38** [a]	-0.51	0.01	**1.11**	0.40	1.86	**13.0** ±0.71 [a]
	Atropine	**-1.48** [a, b]	-1.56	-1.37	**1.14**	0.41	1.67	**10.0** ±0.71 [b]
G8	**Basal**	**-0.02**	-0.05	0.01	**1.48**	1.39	1.57	**7.0** ±0.71
	AOAA	**0.11** [a]	0.05	0.36	**1.08** [a]	0.63	1.30	**15.0** ±0.71 [a]
	Gliben	**0.12** [a]	0.02	0.37	**1.08** [a]	0.57	1.34	**18.0** ±0.71 [a, b]

G = group, AOAA = amino-oxy acetic acid, Prazo = prazosin, Gliben = glibenclamide. Amp = amplitude, Med = median, Min = minimum, Max = maximum, SD = standard deviation, a significant as compared with the basal uterine contractions, b significant as compared with the effect of AOAA on uterine contractions.

4) Effect of K channel blocker (glibenclamide) on H_2S donor (NaHS) or H_2S blocker (AOAA) on the uterine motility:

NaHS significantly decreased the base (tone), peak and amplitude of uterine contractions (P= 0.004, 0.021, 0.026), whereas significantly increased the frequency of uterine contractions (P= 0.016) as compared with the basal uterine contractions. However, glibenclamide after NaHS significantly increased the base (tone), peak and amplitude (P= 0.049, 0.016, 0.021), but insignificantly lower the frequency of contractions as compared with that of NaHS (Fig 4 A & Table 1).

AOAA significantly increased the base (tone) (P= 0.018), but significantly decreased peak and amplitude of uterine contractions (P= 0.018, 0.018), whereas significantly increased the frequency from 7 ±0.71 to 15 ±0.71 (P < 0.001) as compared with the basal contractions. However, glibenclamide after AOAA produce no significant changes in the base (tone), peak and amplitude of uterine contractions, but significantly increased the frequency of contractions as compared with that of AOAA (Fig 4 B & Table 2).

Discussion

The numerous physiological mechanisms that control myometrial contractility involving the adrenergic system, cholinergic system, oxytocin, sex steroids and prostaglandins have led to the elaboration and investigation of various therapeutic methods. Uterine innervation has a unique plasticity governed by the endocrine milieu (20). During pregnancy, the uterus undergoes profound remodeling involving the nerve fibers supplying both the myometrium and its vasculature. This remodeling of uterine neurons affects all types of nerves, i.e. adrenergic, cholinergic and peptidergic neurons that play great physiological functions during pregnancy (21; 22). The present study was conducted to evaluate the role of Hydrogen sulfide (H_2S) in uterine contractility and its relation to the adrenergic and cholinergic autonomic neuronal system.

Hydrogen sulfide is recognized as a new endogenous gaseous transmitter in mammalian tissues (1). H_2S is produced from the amino acid 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) (2; 3). The presence of CBS and CSE has been reported in smooth muscle tissues. CSE is the main H_2S producing enzyme in vasculature smooth muscle (23), whereas both CBS and CSE are found in gastrointestinal smooth muscle (24). Particularly, both CBS and CSE are expressed in rat and human myometrium tissues; the rat uterus homogenate is capable of producing H_2S from its precursor L-cysteine in vitro suggesting the contribution of H_2S in uterus function (13). Also, both CBS and CSE were identified in human pregnant myometrium tissues and mainly localized to smooth muscle cells (25).

The present study demonstrated that, H_2S has a relaxant effect on uterine smooth muscle, this effect was confirmed by the effect of NaHS (H_2S donor) on spontaneous uterine contractions; NaHS significantly decreases the myometrial tone and the amplitude of contractions, but significantly increases the frequency of contractions. These findings are in agreement with that of Sidhu et al., (17) who demonstrated that H_2S donor L-Cystein or NaHS inhibits spontaneous uterine contractility

in vitro. These was confirmed by that AOAA the blocker of endogenous H_2S synthesis that significantly increases the tone of uterine contractility that means increased spasm of uterine smooth muscle. These findings are in agreement with previous reports demonstrated that the blockage of CBS and CSE reduced the relaxant effect of H_2S on uterine smooth muscle, even if a major role for CSE than CBS has been observed (26). Whereas in other group of experiments it was demonstrated that NaHS after AOAA reverse the effect of AOAA on uterine contractility.

It is known that H_2S is toxic at high concentrations (27), and that H_2S bind to metal-containing proteins, thus potentially interfering with the contraction mechanism of smooth muscle by uncoupling the electron transport chain (28). It is possible that low levels of H_2S could inhibit ATP production and therefore reduce contractility in smooth muscle cells (29). It has been shown that physiological concentrations of H_2S increase the production of cyclic AMP in neurons and oocytes in culture (30). Increased intracellular levels of cAMP are known to relax smooth muscles.

It has been shown in previous animal studies that, in parallel with the β2-adrenoceptors (β2-AR), the α1-adrenoceptors (α 1-AR) also play a major role in the regulation of myometrium contractility (31). The density of α1-AR in the rat myometrium was found to be increased by the end of gestation, which suggests that these receptors are involved in the increase of uterine contractility (32). However, blockade of α1A-AR results in relaxation of smooth muscle in rat uterus, also antagonism of the α1D-AR subtype results in modest relaxation of the pregnant rat uterine smooth muscle (33).

It was found that adrenaline significantly decreases the base (tone), the peak and the amplitude of uterine contractions and significantly decreases the frequency of contractions. These findings are consistent with previous reports found that stimulating adrenergic receptors with selective agonists blocks uterine contractility in rats in vitro (34). Whereas AOAA after adrenaline significantly increases the peak and amplitude of uterine contractions, also significantly increases the frequency of contractions. These findings indicate the dual relation and interaction of endogenous H_2S production and adrenergic system, the relaxant effect of adrenaline on myometrium contractility may be mediated through the increased production of endogenous H_2S. Also the relaxant effect of H_2S on uterine contractility may be mediated through the increased endogenous catecholamine in the myometrium. As it was well known that the myometrium contractility is controlled by myogenic, neurogenic and hormonal mechanisms (35).

Our explanation is evidenced by the present results that demonstrated that propranolol after NaHS abolishes the effect of NaHS on uterine contractility, as it significantly increases the base (tone), peak and amplitude of uterine contractions as compared with the effect of NaHS. Meanwhile in other group of experiments propranolol significantly increases the base (tone), but significantly decreases the peak and amplitude of uterine contractions meanwhile significantly increases the frequency as compared with the basal uterine contractions. Whereas NaHS after propranolol produces insignificant changes in the tone, amplitude and frequency of uterine

contractions as compared with that of propranolol. These findings could be explained by that the relaxant effect of H_2S on uterine contractility could be through modulation of the endogenous catecholamine in myometrium. Similarly, Estan et al., (36) also obtained a blocking effect of catecholamine on uterus contractility carried out through β-adrenergic receptors.

Prazosin significantly decreases the base (tone), peak, amplitude and the frequency of uterine contractions as compared with the basal contractions. These findings are in agreement with previous animal studies demonstrated that blockade of α1-AR results in relaxation of rat uterine smooth muscle (33). Whereas NaHS after prazosin produced more significant decrease in the tone, peak and amplitude of contractions as compared with that of prazosin. These findings suggest a synergistic relaxant effect of H_2S with that of α adrenoreceptors blocker on uterine contractility.

Another group of experiments confirmed that NaHS significantly decreases the base (tone), peak and amplitude of uterine contractions, but significantly increases the frequency of uterine contractions as compared with the basal contractions. While prazosin after NaHS produces insignificant changes in the uterine contractility as regard the base (tone), peak, amplitude and frequency as compared with that of NaHS, these findings could be explained by that the relaxant effect of H_2S was strong enough to mask the effect of alpha-adrenergic blockade of prazosin. Whereas other group of experiments showed that prazosin after AOAA significantly increases the peak of uterine contractions but significantly decreases the frequency of contractions, as compared with that of AOAA. These findings confirmed the synergistic effect of H_2S and alpha-adrenergic blocker.

To our knowledge no previous studies examine the interaction between endogenous H_2S and cholinergic system in the myometrium. Histochemical and functional studies have indicated the presence of cholinergic nerves in the uterus that their numbers decrease during pregnancy, possibly to protect the uterus from autonomic excitation (37 and 38). Other studies suggested the presence of muscarinic M2 subtypes in rat uterus. In functional studies indicated the additional presence of muscarinic M3 receptor or, possibly an atypical receptor subtype (39).

It was found that acetylcholine significantly increases the base (tone), peak, amplitude, and frequency of contractions, as compared with the basal uterine contractions. Whereas NaHS after acetylcholine significantly decreases the base (tone), peak and amplitude of uterine contractions as compared with that of Ach. Our findings suggested that the relaxant effect of H_2S on spontaneous uterine contractions could be mediated through modulation of endogenous cholinergic neurons and or its muscarinic receptors, as H_2S may decreases the endogenous release of Acetylcholine in the myometrium.

The muscarinic receptors blocker, atropine after NaHS produces insignificant changes in the tone, the peak and amplitude, but significantly decreases the frequency of uterine contractions as compared with that of NaHS, these reductions in the uterine contractility parameters become more significantly lower than the basal contractions.

The modulatory effect of H_2S on endogenous cholinergic system supported by the

finding that the muscarinic receptors antagonist, atropine after AOAA that block the endogenous H_2S, significantly decreases the base (tone), peak and frequency of uterine contractions as compared with that of AOAA. These means that the blocked release of H_2S allow endogenous ACh to act that action blocked by muscarinic antagonist atropine. (15).

Adenosine triphosphate (ATP)-sensitive potassium channels (K_{ATP} channels) are involved in β-AR agonists-induced smooth muscle relaxation in pulmonary vasorelaxation in the rat (40), vasorelaxation in the rat mesenteric artery (41), detrusor muscle relaxation in the rat (42), and myometrial relaxation in non-pregnant buffaloes (43). K_{ATP} channels are formed by a combination of two types of subunits, the pore-forming inwardly rectifying subunit and the sulphonylurea-binding regulatory subunit (SUR) (44). Since open K_{ATP} channels draw the cell membrane potential closer to the K^+ equilibrium potential, K_{ATP} channels are closely involved in reducing cellular excitability and contractility.

Of note, K_{ATP} channels were identified to mediate the vasodilatory effects of H_2S (45). Previous studies have shown that H_2S suppresses the spontaneous contraction of human myometrial strips via K_{ATP} channels (25). Our results showed that glibenclamide (K_{ATP} channels blocker) after NaHS significantly increase the base (tone), the peak and amplitude of uterine contractions, but insignificantly lowers the frequency of contractions as compared with that of NaHS. However, glibenclamide after AOAA produces no significant changes in the base (tone), peak and amplitude of uterine contractions, but significantly increases the frequency of contractions as compared with that of AOAA. These findings indicate that the relaxant effect of H_2S on the contractility of rat myometrium is mediated by K_{ATP} channels. Our results are in consistent with many previous studies on smooth muscles and K_{ATP} channels. It was demonstrated that H_2S activates K_{ATP} channel, thereby causing relaxation of smooth muscle (46). For instance, it was suggested that vasorelaxation induced by H_2S in rat aorta and mesenteric beds is mainly caused by K_{ATP} channel opening (23 and 47). The study by Zhao et al has also has shown that K_{ATP} channels mediated NaHS-induced relaxation of spontaneous contractions of gastric smooth muscle (48).

Also, it was demonstrated that blockage of K_{ATP} channels reversed H_2S suppression of proinflammatory cytokines. A number of studies have implicated that H_2S actually exerts its function by modifying l-cysteine in a large number of proteins by S-sulfhydration (49 and 50). Moreover, it has been reported that H_2S regulates K_{ATP} channels via sulfhydration of l-cysteine in these proteins (49 and 51).

In conclusion, the myometrium smooth muscle cells express CBS and CSE. Endogenous H_2S generated through CSE and CBS locally modulates the contractility of myometrium. The inhibitory effect of H_2S on uterine contractility is through modulation of the endogenous adrenergic and cholinergic systems and activation of K_{ATP} channels.

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