The Role of K and Ca Channels in Hydrogen Sulfide Induced Relaxation in Arteries Feeding Human Colorectal Cancer

Kamaran. H. Mohammed[1,3], Omar A. M. Al-Habib[2], Sardar H. Arif [4]

[1]Lecturer, Department of Veterinary, Shaqlawa Technical College, Erbil Polytechnic University, Erbil, [2]Professoer, College of Science, Nawroz University, Duhok, [3]Lecturer Department of Biology, Faculty of Science, Zakho University, [4]Assistant Professor, Department of Surgery, Medical College, University of Duhok, Kurdistan Region, Iraq

Abstract

This study was designed to find out the vasorelaxant effects of hydrogen sulfide (H_2S) on arteries feeding human colon cancer. In addition, it also included the study of the possible roles of potassium (K^+) and calcium (Ca^{2+}) channel types in H_2S-induced relaxation in the isolated arteries. Sodium sulfide (Na_2S) showed a potent dose-dependent relaxant effect on Norepinephrine ($1X10^{-5}$ M) precontracted arteries. The use of different specific K^+ channel blockers ($BaCl_2$, 4-AP, GLIB, and TEA) individually indicated H_2S-induced relaxation was affected by all K channel types participated to a various extent, except Kv channels. Both Kca^{2+} and Kir channels played a major role in the induced relaxation, while K_{ATP} played a minor and non-significant role. On the other hand, Kv channel played no direct role, and the induced response curve was very close to that of the control. Possible combinations of K channel blockers showed that some produced synergistic effect to different extent, whereas others produced mild and non-significant effect except at the highest doses used on dose-response curves. Thus, combinations of (GLIB+4-AP), (GLIB+$BaCl_2$) and ($BaCl_2$+TEA) caused a highly significant blocking in the induced response curve, while ($BaCl_2$ +4-AP) and (TEA+4-AP) produced a mild inhibition except at the highest doses used in which the inhibition was significant.

Keywords: *Human Artery, colon cancer, H_2S, K^+ and Ca^{2+} channels.*

Introduction

Cancer describes a range of diseases that can affect different organs and tissues of the body. Colorectal cancer (adenocarcinoma), represents the third foremost identified malignant tumour and the second most common cause of cancer death [1,2, 3].

Arteries can be mechanically described as a long-range elastic element (elastin) arranged in parallel with a system of continuous collagen fibers that set the limit of extension. Tunica intima, tunica media and tunica adventitia are three recognizable layers in an artery [4]. The adventitia is the outermost layer of the vessel and consists of connective tissue created from elastin, collagen, fibroblasts, mast cells, macrophages, and nerve axons [5]. Tunica media, consists mainly of vascular smooth muscle cells (VSMCs), are in charge of generation of vascular tone (vasoconstriction). Depolarization of VSMC membrane evoked contraction mainly by opening of voltage dependent calcium (Ca^{2+}) channel which leads to an increase in $[Ca^{2+}]_i$ [6]. The inner layer is the tunica intima, which includes a single layer of cells pointed as the vascular endothelium [7]. Endothelium

Corresponding author:
Omar A. M. Al-Habib
habbibomar@yahoo.com

is the active inner monolayer of the blood vessels which forms an interface between circulating blood and the vessel wall. It plays a crucial role in vascular homeostasis [8], involved in many pathological and physiological processes, including the regulation of smooth muscle tone, control of thrombosis, inhibition of leukocyte, platelet adhesion and promotion of intra-arterial permeability [9]. The endothelium regulates the activity of smooth muscle fibers by synthesizing several vasoactive substances that affect the contractility of the arterial wall in response to various stimuli [10]. The most essential vasodilators are nitric oxide (NO), prostaglandin I_2 (PGI_2), and endothelium-derived hyperpolarizing factor (EDHF) [11]. The smooth muscle has a variety of selective and nonselective ion channels. Among the selective channels, are those transporting Ca^{2+} or potassium (K^+) have been investigated most intensively, but chloride (Cl^-) and sodium (Na^+) channels are equally important [12].

Pathological angiogenesis is a hallmark of cancer and various ischemic, inflammatory diseases and the potent angiogenic factor vascular endothelial growth factor (VEGF) which is associated with metastasis in human colon cancer. Also, the proliferation rate of endothelial cells decreases when grown in conditioned media from human colon cancer cells with decreased VEGF expression [13].

The dynamic interplay of Ca^{2+} and K^+ channels on the plasma membrane of VSMCs plays a pivotal role in modulating the vascular tone of small arteries and arterioles [14]. Potassium channels form the most plentiful with various classes of ion channels. These are membrane-spanning proteins allows efflux of K^+ through a K^+ selective pore. The activity of K^+ channels may be modulated by voltage, Ca^{2+} and neurotransmitters [15]. These ion channels play an important role in regulating the membrane potential (Vm) of VSMCs [16]. Many types of K^+ channel have been recognized in endothelial and (SMCs); such as selective (ATP)-dependent K^+ channels (K_{ATP}), voltage-gated K^+ channels (K_V), inward rectifier K^+ channels (K_{IR}) and Ca^{2+} activated K^+ channels (K_{Ca}) [17]. In excitable cells, the role of all K^+ channels is related to stabilization of the resting Vm [18]. In contrary, Ca^{2+} channels in many alternative cell types activate membrane depolarization and mediate Ca^{2+} influx in response to action potentials and sub-threshold depolarizing signals. Calcium ions entering the cell through voltage gated calcium channels (VGCCs) initiate many different cellular events and serves as the second messenger for electrical signaling [19].

Hydrogen sulphide (H_2S) is a gaseous signaling molecule that mediate vasodilatory effects on the arterial tree. It is produced by the action of at least three enzymes, cystathionine beta synthase (CBS), cystathionine gamma lyase (CSE) and mercaptopyruvate-sulfur transferase (MST) [20,21]. Numerous physiological functions assigned to be exclusively or partly regulated by H_2S, some of which are vasodilation [22], and angiogenesis [23] via direct activation of K_{ATP} channels [24].

Materials and Methods

Colon tissue collection Colon tissues used in the current study were taken from patients with colon cancer. The 5 samples used were taken from colon cancer patients (including 3 female and 2 male) aged between 30 to 70 years in Duhok Province. Blood vessels and mesenteric artery branches were obtained from patients under-going colectomy that diagnosed as colon cancer. After operation, the mesenteric artery branches were isolated carefully from cancer tissue to avoid any damage in the blood vessels. The blood vessels with a part of removed tissue were kept in Krebs solution oxygenated with carbogen (95% O_2 and 5% CO_2), maintained at 4°C and transported within an hour from Vajeen Hospital (Duhok) to Advanced Physiology Research Lab, Department of Biology, Faculty of Science, University of Zakho. In the lab, the arteries were cleaned from connective and other unwanted tissues, cut into equal segments, each of about 2-3mm in length.

Tissue preparation: Blood vessel segments prepared for this study were immediately placed in a Petri jar with oxygenated Krebs physiological solution, maintained at 4°C, rinsed and freed from clotted blood. The mean time between harvesting and experimentation was about 60 minutes. The vessels were mounted to PanLab glass tissue chambers.

Experimental Protocol

Each isolated arterial ring was mounted between two stainless steel hooks, connected from one end to the tissue holder at the base of 10 ml capacity tissue glass chamber containing physiological solution at 37ºC. From the other end, it was connected to a force transducer coupled to transbridge amplifier and PowerLab Data Acquisition System and the tissue was set at 5 grams resting tension. The preparation was left to equilibrate for 3-5 hours at 37ºC with changing of physiological solution at 15-minute intervals with maintain the pH at 7.4 by continuous aeration with carbogen gas.

The arterial segments were initially exposed to ($1X10^{-5}$) NE to test their functional integrity. Later, the bath medium was changed several times until a stable resting tone was recorded. The dose-response curve for sodium disulfide (Na_2S; 1-6 mM) was constructed against NE-precontracted rings. To study the role of different K^+ channel types in relaxation induced by Na_2S, the arterial rings were pre-incubated for 20 minutes with the following K channel inhibitors, tetraethylammonium (TEA; 1mM), glibenclamide (GLIB; 10 μM), barium chloride ($BaCl_2$;1mM) and 4-aminopyridine (4-AP; 1mM), which are the inhibitors of K_{Ca}, K_{ATP}, K_{IR} and K_V channels, respectively. These inhibitors were used individually or in combinations.

The dose-response curves were fitted with a Hill equation, from which the half maximal inhibitory concentration (IC_{50}) values were obtained as geometric mean. Maximum contractile responses to Na_2S were calculated as a percentage of the contraction produced by NE and were expressed as the means ± standard error of the mean (SEM). The tension produced by NE was defined as 0% relaxation, and the baseline tension before addition of vasoconstrictors were defined as 100% relaxation.

Statistical Analysis

The statistical analysis of the data was performed using two-way analysis of variance (ANOVA) supported by Bonferroni test when carrying out pair wise comparison between the same doses of different groups using GraphPad Prism software version 6.0 for Windows. P-values less than 0.05 ($P<0.05$) were considered as statistically significant. In all figures, the symbols (*, ** and ***) representing mean differences are significant at the 0.05, 0.01 and 0.001 levels, respectively.

Results

The role of K^+ channels in arterial Na_2S induced relaxation

In arteries feeding colon cancer precontracted with NE, pre-incubation with TEA did not decrease the relaxant effect induced by Na_2S significantly at low doses (1mM and 2mM), whereas at higher doses (3 to 6 mM), it reduced the relaxant effect of Na_2S significantly ($P<0.05$ to 0.001). Thus, the E_{max} value was significantly reduced from (108.00±7.89%) in the control to (40.94± 7.11%) in arteries feeding colon cancer pre-incubated with TEA (Figure 1A).

In arteries feeding colon cancer precontracted with NE, pre-incubation with GLIB caused a mild and non- significant reduction in the relaxation induced by Na_2S. Thus, the E_{max} was also reduced from (108.00±7.89%) in the control to (91.35±18.16%) in arteries feeding colon cancer treated with GLIB (Figure 1B).

In arteries feeding Colon cancer precontracted with NE, pre-incubation with $BaCl_2$ did not decrease the relaxant effect induced by Na_2S at low doses (1 to 3 mM), whereas, at higher doses (4 to 6 mM), it reduced the relaxant effect induced by Na_2S at a

highly significant (P<0.001) level. Thus, the E_{max} value was reduced at a highly significant level from (108.00±7.89%) in the control to only (41.97± 6.36%) in arteries pre-incubated with $BaCl_2$ as (Figure 1C).

In arteries feeding colon cancer precontracted with NE, pre-incubation with 4-AP did not affect vasorelaxation induce by Na_2S. Accordingly, the E_{max} remain unchanged; it was (108.00±7.89%) in the control and (108.10±1.08%) in arteries treated with 4-AP (Figure 1D).

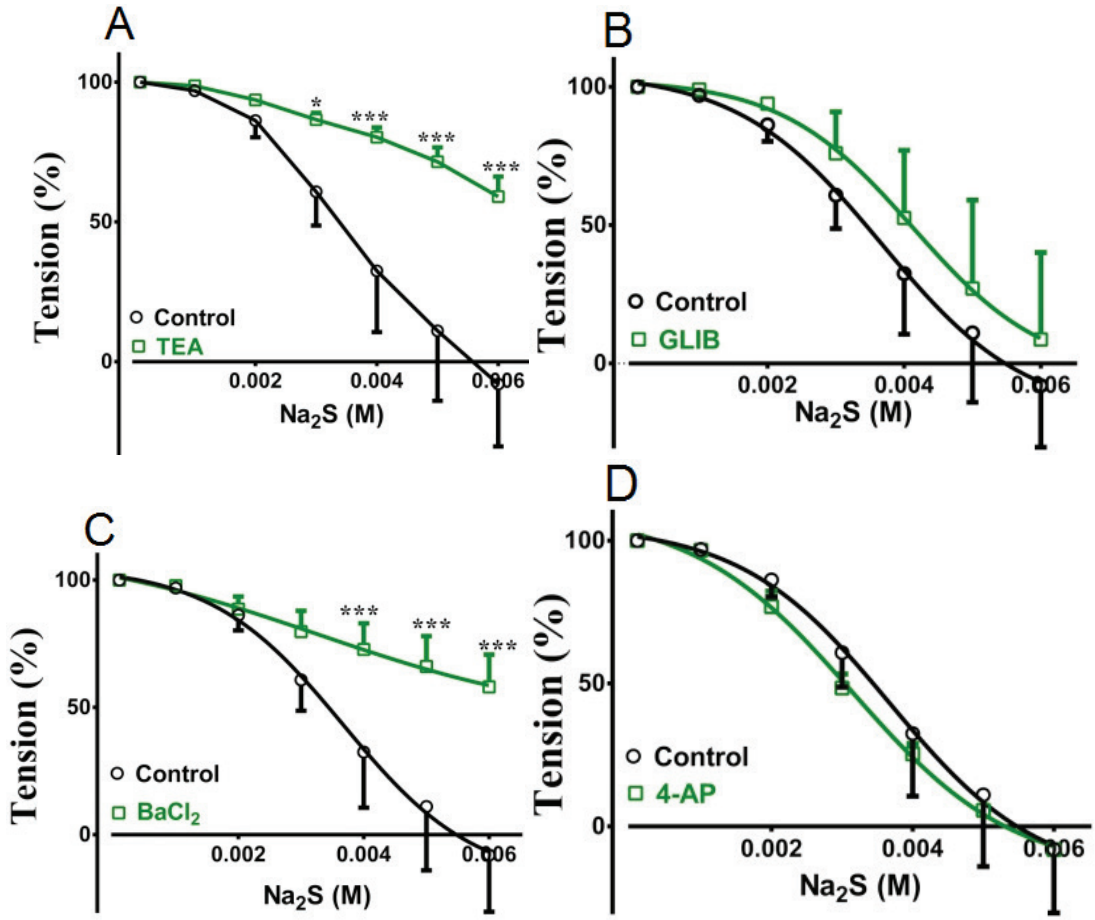


Figure 1. Role of K^+ channels in the vasodilator effects of Na_2S (1 to 6 mM) on NE-precontracted artery feeding colon cancer incubated in physiological solution containing (A) 1mM TEA, (B) 10µM GLIB, (C) 1mM $BaCl_2$ and (D) 1mM 4-AP for 20 min. All data are expressed as % of relaxation of NE-induced artery tone and are represented as the Mean±SEM. * P<0.05 versus control; *** P<0.001 versus control.

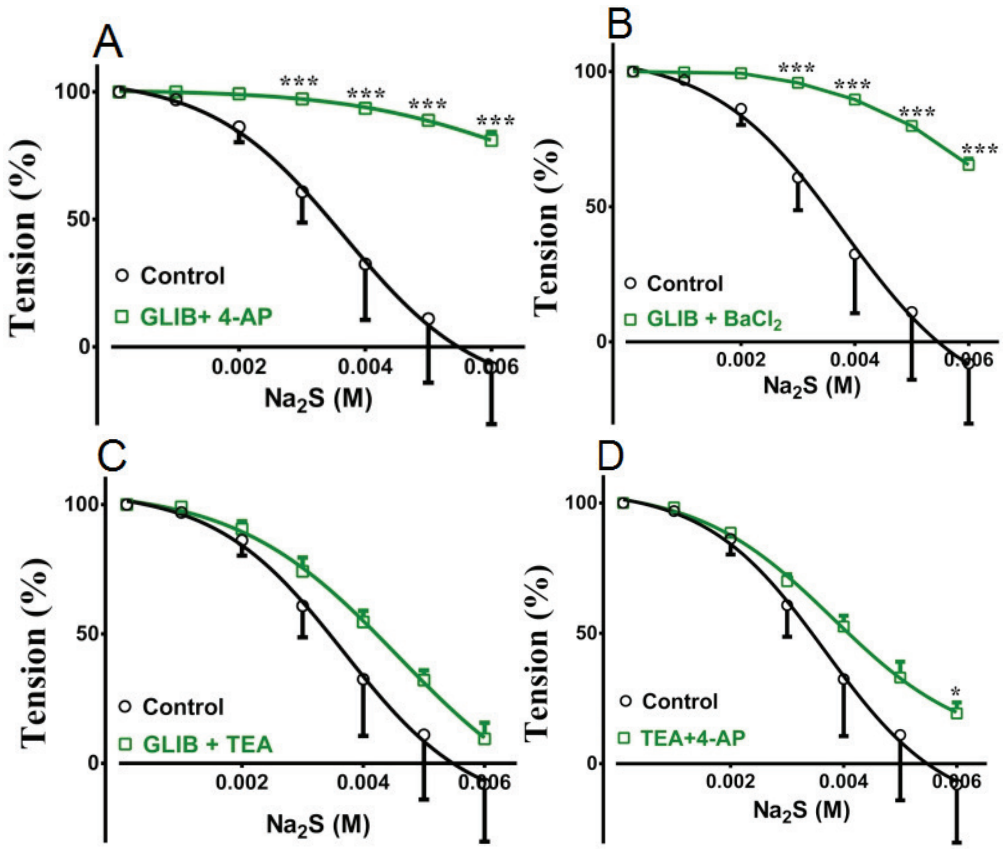
In arteries feeding colon cancer precontracted with NE, pre-incubation with a mixture of (GLIB+4-AP) produced a highly significant (p< 0.001) decrease in the relaxation induced by Na_2S at high doses (3 to 6 mM), whereas at low doses produced non-significant reduction. Therefore, the E_{max} was reduced significantly from (108.00±7.89%) in the control to only (19.01± 3.20%) in arteries pre-incubated with a mixture of (GLIB+ 4-AP) (Figure 2-A).

In arteries feeding colon cancer precontracted with NE, pre-incubation with a mixture of (GLIB+$BaCl_2$) decrease the relaxation induced by Na_2S at a highly significant (p<0.001) level at high doses (3 to 6 mM), while, at lower doses, it produced a mild and non-significant reduction in the relaxant response. Thus, the E_{max} was significantly reduced from (108.00±7.89%) in the control to (34.50±2.30%) in arteries pre-incubated with a mixture of GLIB+$BaCl_2$ (Figure 2-B).

In arteries feeding colon cancer precontracted with NE, pre-incubation with a mixture of (GLIB+TEA) showed a mild and non-significant reduction in Na_2S induced relaxation. The E_{max} value was reduced non-significantly from (108.00±7.89%) in the control to (90.54±6.20%) in artery pre-incubated with a mixture of (GLIB+TEA) (Figure 2-C).

In arteries feeding colon cancer precontracted with NE, pre-incubation separately with a mixture of (4-AP+TEA) and (4-AP+$BaCl_2$) produced non-significant inhibitory effects at all doses used except the highest dose of each mixture in which the Na_2S induced relaxation was significantly ($P<0.05$) reduced at a dose of (6 mM). The E_{max} values reduced considerably from (108.00±7.89%) in the control to (80.72±4.20%) and (80.70±4.55%), respectively in arteries treated with (4-AP+TEA) and (4-AP+ BaCl2) (Figures 2-D and E).

In arteries feeding colon cancer precontracted with NE, pre-incubation with a mixture of ($BaCl_2$+TEA) decreased the relaxant effect induced by Na_2S non-significantly at low doses (1 and 2 mM), whereas at higher doses (3 to 6 mM), it reduced the relaxant effect induced by Na_2S significantly ($P<0.05$ to 0.001). Thus, the E_{max} was reduced significantly from (108.00±7.89%) in the control to only (35.32± 4.51%) in arteries pre-incubated with a mixture of $BaCl_2$+TEA (Figure 2-F).



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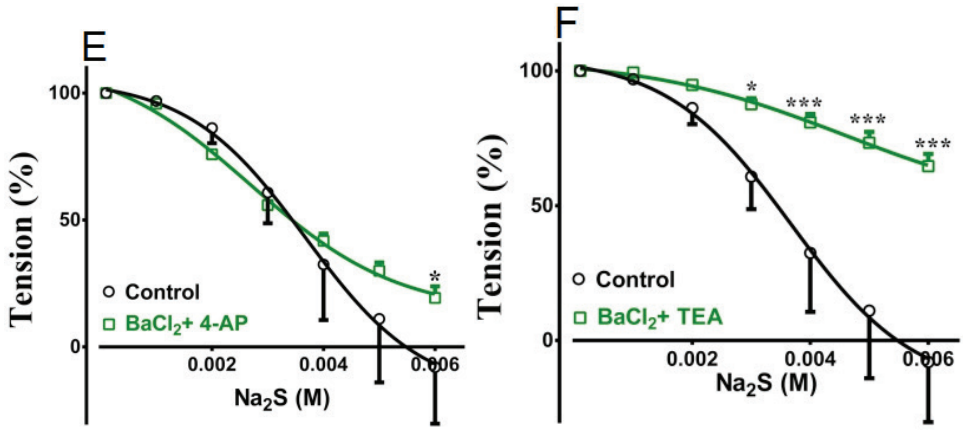

Figure 2. Role of K^+ channel types in the vasodilator effects of Na_2S (1 to 6 mM) on NE-constricted artery feeding colon cancer in Human incubated in physiological solution containing (A) GLIB+4-AP, (B) GLIB+$BaCl_2$, (C) GLIB+TEA, (D) 4-AP+TEA, (E) 4-AP+$BaCl_2$ and (F) $BaCl_2$+TEA for 20 min and then contracted with 10µM NE. All data are expressed as % of relaxation of NE-induced artery tone and are represented as the mean ±SE. * P<0.05 versus control; *** P<0.001 versus control.

The role of L-type Ca channels in Na_2S induced arterial rings relaxation.

In arteries feeding colon cancer precontracted with NE, pre-incubation with Nifedipine, produced a non-significant reduction in the relaxant effects of Na_2S. Thus, the E_{max} values were very close (108.00±7.89%) in the control and (107.44±26.46%) in arteries treated with Nifedipine (Figure 3).

Discussion

Relaxant Responses of Arteries Feeding Colon Cancer to Na_2S

The Role of K^+ Channels

The relaxation induced by Na_2S- in vascular smooth muscle is predominantly induced through the activation of potassium channels, efflux of K^+, hyperpolarization of SMCs and subsequent relaxation. The participation of several additional signaling pathways and mechanisms confirmed also including changes in intracellular pH or ATP levels as well as endothelium-derived hyperpolar-izing factors [25]. Several types of potassium channels have been reported to be the major molecular targets for H_2S which resulted vasorelaxant effects. A rise in K^+ permeability normally hyperpolarizes cell membrane; and thus, inhibits Ca^{2+} influx through voltage gated L-type Ca^{2+}channels resulting in muscle relaxation. The first clear connection between H_2S and K^+ channels was indicated by [26] through a series of *in vivo* and *in vitro* studies. They showed that H_2S induced relaxation of VSMCs occurs via the opening of K_{ATP} channels.

The data of the current study demonstrate that H_2S produced a dose dependent relaxant effect in human NE pre-contracted arteries feeding colon cancers. This effect is in line with that of [27] who showed that H_2S donor (NaHS) caused relaxation in pre-contracted non-cancer human mesenteric arterial rings in a concentration-dependent manner.

The inhibitory effect of $BaCl_2$ on H_2S induced relaxation, indicates that K_{ir} channels play an important role in H_2S-induced dilation in arteries feeding Human colon cancer, is supported those of [28] who showed that $BaCl_2$ (a specific inhibitor of K_{ir} channel) decreased the sensitivity of Na_2S-induced vasorelaxation in mouse aorta without reducing the maximum response. Also, it has been observed that K_{ir} channels located

on the endothelium and gap junctions may mediate a conduction of K_{ir} dependent hyperpolarization from endothelium to the smooth muscle cells [29]. Using the same vascular bed, [30] showed that K^+-mediated relaxations were endothelium-dependent. It has been reported that the mechanism of relaxation is mainly mediated by the activation of K_{ir} channels and inhibition of K_{Ca} channels [31]. Therefore, K^+ efflux through K_{Ca} channel could act upon endothelium K_{ir} channel which could amplify the endothelial cell hyperpolarization and thereby increase the magnitude of the electrical signal passing electroto-nically to the smooth muscle layer [32].

The present study showed that Na_2S induced relaxation in human arteries feeding colon cancer reduced by K_{Ca} channel blocker. This result suggests that a part of the relaxation of the arteries feeding colon cancer in human produced by H_2S is due to the activation of K_{Ca} channels. It is demonstrated that the activation of small and intermediate conductance K_{Ca} channels by H_2S cause relaxant effect [22]. Furthermore, both [33,34] reported that co-application of intermediate (IK_{Ca} blocker) and small conductance (SK_{Ca} blocker) reduced the extent of the H_2S-induced vasorelaxation. It has been demonstrated that H_2S increased the frequency of Ca^{2+} sparks in piglet cerebral arteriole smooth muscle cells causing an increase in the frequency of transient K_{Ca} current; and thus, vasorelaxation [35]. In contrast, Tang *et al*., (2010) observed that different K_{Ca} channels blockers failed to affect the vascular effect of H_2S [36].

In the present study, 4-AP did not decrease the vasodilation of arteries feeding colon cancer induced by Na_2S which evident that Na_2S cannot activate K_V channels.

The K_{ATP} channel blocker (GLIB) did not decrease vasodilation of artery feeding colon cancer induced by Na_2S suggesting that K_{ATP} might not be responsible for H_2S induced vasorelaxation. [37] showed that K_{ATP} channels were not involved in mediating effects of H_2S in rat coronary arteries. Other investigators have found that the relaxations caused by H_2S in the guinea-pig ileum were not mediated by K_{ATP} channels [38].

The results of the current study indicated that H_2S induced relaxation in smooth muscle cells of the arteries feeding colon cancer varies with the types of blocker combination and arteries feeding tissues when preincubated with specific K^+ channels blockers. Thus, a combination of (K_{ATP} and K_{ir}) channel blockers produced a synergistic effect on Na_2S induced relaxation in AFCC since it produced a significant inhibitory effect as compared with the effects of the individual blockers included in the combination since showed no inhibitory effects on their dose response curves. The same thing is true for the effect of (K_{ATP} and K_V channel blockers) which also produced a synergistic effect on Na_2S induced dose response curved.

However, a combination of (K_{ir} and $K_{Ca)}$ channels blockers produced significant inhibition in Na_2S dependent relaxation in the arteries feeding colon cancers. This result goes in parallel with the inhibitory effects produced by (K_{ir} and $K_{Ca)}$ channels blockers when used individually.

The remaining combinations of K^+ channels blockers which included (K_{ATP}+K_{Ca}), (K_V+K_{ir}) and (K_V+ K_{Ca}) blockers produced antagonistic effects since they produced no significant inhibitory effects on the dose response curved as compared with the effects produced when using the blockers included in the above combinations individually.

These results suggest that H_2S may have more than one target in vascular smooth muscle cells since the activation of these K^+ channels by H_2S would leads smooth muscle cells hyperpolarization and subsequent vasorelaxation. Since no data are available to compare these novel results. Any way it has been found that co-application of the K_{ATP} blocker with IK_{Ca}/SK_{Ca} blockers abolished all H_2S-mediated vasorelaxation in rat mesenteric arteries [22]. Conversely, in the current study, (K_{ATP} +K_{Ca}) channels blockers did not produce any effect on H_2S induced vasorelaxation in

artery feeding colon cancer in human.

Role of Ca^{2+} Channel

Nifedipine (L-type Ca^{2+} channel blocker) neither reduced nor enhanced H_2S induced vasorelaxation in arteries feeding colon cancer. Therefore, L-type Ca^{2+} channel plays a secondary role in H_2S induced relaxation in rings of arteries. This implies that vasorelaxation might result from a direct action of H_2S on K^+ channels followed by lowering of smooth muscle calcium and independent on the blockage of Ca^{2+} channel. Other proposed mechanisms of H_2S-induced relaxation include binding of the Ca^{2+}-calmodulin complex, its interaction with NO and/or endothelium-derived hyperpolarizing factor (EDHF), inhibition of phosphodiesterase (PDE), and elevation of intracellular Ca^{2+} in endothelial cells.

Conclusion

In arteries feeding colon cancer both K_{ir} and K_{Ca} played major roles in induced relaxation, whereas K_{ATP} and K_V played no significant role in H_2S dependent relaxation. In AFCC, K channel blocker combinations including (GLIB+ $BaCl_2$), (GLIB+4-AP) and ($BaCl_2$+TEA) produced significant inhibitory effects on H_2S dependent relaxation, whereas the remaining three combinations produced mild and non-significant inhibitions in the induced relaxation. L-type Ca channel blockers caused a mild enhancement. However, H_2S induced relaxation at a high dose, L-type Ca channels were showed neither inhibitory nor enhancing effect.

Conflict of Interest

The authors declare that there is no conflicts of interest regarding the publication of this manuscript.

Ethics Approval was Obtained from Ethics Committee at the University of Zakho- Duhok-Kurdistan Region, Iraq

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