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1 # The vasodilatory mechanism of nitric oxide and hydrogen sulfide in human
2 # mesenteric artery of colorectal cancer patients: role of potassium channels

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13 Short title: Vasodilatory mechanism of NO and H_2S in human mesenteric
14 artery of CRC patients

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25 # Abstract

26 Recent studies focused on the role of gasotransmitters in cancer
27 progression and prevention. Therefore, this study was designed to explore
28 the vasodilator activity of NO and H_2S in human mesenteric artery of CRC
29 patients via activation of K^+ channels. For this purpose, two sets of
30 experiments were established. The blood samples from CRC patients were
31 obtained to detect serum levels of Endocan and MDA. Moreover, the role of
32 K^+ channels were assessed in mediating vasodilation of human mesenteric
33 artery in response to SNP and Na_2S. The level of serum Endocan was
34 decreased in CRC patients compared to healthy individuals, while serum
35 MDA was not changed. The arterial rings precontracted with NE were first
36 relaxed by cumulative addition of increasing concentrations of either SNP
37 (30nM-30μM) or Na_2S (1-6mM). Then maximal relaxation rates were
38 calculated for four times at each 15min intervals. Preincubation of arterial
39 rings for 20min with individual K^+ channels blockers were significantly
40 reduced relaxation caused by SNP and Na_2S at different time intervals.
41 Furthermore, pretreatment of L-NAME did not change the vasodilation
42 induced by Na_2S. Vasodilation of CRC mesenteric unchanged by synergistic
43 application of SNP and Na_2S. While preincubation of arterial rings with PAG
44 significantly enhanced vasodilation induced by SNP. In conclusion, these
45 results indicate that endothelial dysfunction and oxidative stress do not take
46 part in the pathogenesis of CRC. The dilatory mechanisms of NO and H_2S
47 in mesenteric arteries of CRC patients are K^+ channels and time dependent,

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48 and the activity of CSE enzyme slows down the vasodilator ability of
49 exogenous NO.

50 **Keywords**: Nitric oxide, Hydrogen sulfide, Mesenteric arteries, Potassium
51 channels, Colorectal cancer.

52 Introduction

53 Colorectal cancer (CRC) is the third most frequently occurring
54 malignancy and the second leading mortality cause of cancer worldwide.
55 More than 1.8 million new CRC cases and 881,000 deaths was reported in
56 2018, accounting for about 1 in 10 cases and deaths [1]. Regardless of, the
57 low incidence of CRC in the Middle East [2], the Kurdistan region of Iraq
58 has a higher annual incidence rate of cancer, and it represents about 38 to
59 61.7 cases/100,000 population and it's the fourth cancer in both sexes
60 separately, and the cause of mortality of about 8.6% [3]. The most common
61 histopathological type of CRC in this region is adenocarcinoma [4].

62 Endogenous gasotransmitters mainly nitric oxide (NO), carbon
63 monoxide (CO), and hydrogen sulfide (H_2S) are generated endogenously by
64 specific enzymes [5]. NO is produced from L-arginine by the enzyme NO
65 synthase (NOS) [6, 7] while H_2S is synthesised from L-cysteine by either
66 cystathionine beta-synthase (CBS), cystathionine gamma-lyase (CSE) or
67 mercaptopyruvate sulfurtransferase (MST) [8, 9]. They can modulate
68 different biological pathways and functions at a physiologically relevant
69 concentration [10] by the opening of various kinds of membrane ion

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70 channels [11]. NO is an essential regulator of angiogenesis [12] and
71 vasorelaxation through activation of guanylate cyclase and production of
72 cyclic guanidine monophosphate (cGMP) [13]. cGMP, in turn, will increase
73 an endothelial Ca^{+2} concentration and finally the opening of localised Ca^{+2}-
74 dependent potassium (K_+) channels (K_{Ca}) [14]. In contrast, H_2S exerts its
75 effect on vasodilation [15] and angiogenesis [16] via direct activation of
76 K_{ATP} channels [17].

77 Beside their independent actions, NO and H_2S can act synergistically or
78 antagonistically to elicit their downstream effects, ranging from their
79 biosynthesis to the signalling cascade in a cell target. NO, and H_2S are
80 mutually dependent on regulating the vasodilation, angiogenesis [18, 19] and
81 endothelial homeostasis [20] at different levels. The role of these gases in
82 cancer is quite confusing, as both gases have tumour promotion and anti-
83 tumour properties [21] and they can modulate various cancer cell functions,
84 such as proliferation, invasion, metastasis, and tumour angiogenesis [22].
85 Moreover, the enzymes responsible for NO and H_2S production are
86 upregulated in CRC cells, endogenously produced low-to-mid
87 concentrations of H_2S or NO support cell proliferation, while exogenous
88 delivery of H_2S or NO can suppress the colon cancer cells division [23].

89 Intra-tumoral blood vessels are of paramount for tumour growth,
90 metastasis and therapy [24] and the acquisition of a differential reactivity by
91 functional, mature blood vessels in the tumour microenvironment represents
92 a proper target for antitumor therapy [25]. Because cancer cells are

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93 exhibiting an accelerated metabolism, therefore they are demanding high
94 reactive oxygen species (ROS) concentrations to maintain their high
95 proliferation rate [26]. Thus, the high levels of ROS can damage or kill cells
96 by oxidising proteins, lipids, and nucleic acids [27]. Overall, these
97 observations elucidate that oxidative stress and cancer are closely linked
98 [28]. A growing number of researches is investigating the consequences of
99 ROS and endothelial dysfunction in cancer [29]. Nonetheless, to the best of
100 our knowledge, no researcher has linked between oxidative stress,
101 endothelial function and vascular reactivity to NO and H_2S in CRC patients.
102 Therefore, the study aimed to evaluate endocan as an endothelial function
103 marker and MDA as an oxidative stress marker in CRC patients. Also we
104 were aimed to evaluate the mechanisms responsible for NO and H_2S induced
105 vasodilation in human mesenteric artery of CRC patients. To approach, these
106 questions, possible roles of different K^+ channels in the vasodilation response
107 produced by NO and H_2S has been assessed.

108 Subjects and Methods

109 Subjects

110 This study is a case-control study. The study was approved by the human
111 ethics committee belonging to College of Science, Salahaddin University-
112 Erbil, and the informed consent from all the patients was obtained. For the
113 first experiment, the subjects were recruited in two hospitals in Erbil city,
114 Rizgray hospital Oncology department and Nanakaly Hospital. Venous

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115 blood was taken from 44 CRC patients with different stages of colorectal
116 cancer (24 males and 20 females). Also, 40 healthy volunteers of similar age
117 were recruited in this study as healthy individuals. While for the second
118 experiment colorectal tumour specimen were obtained from colorectal
119 cancer patients undergoing partial colectomy at CMC and welfare private
120 hospitals in Erbil city.

121 Patients were selected according to histological evidence of colonic or
122 rectal adenocarcinoma when ages of patients greater than 18 years and they
123 were able to donate blood. The blood samples were obtained by phlebotomy
124 under aseptic technique. Blood was put into clot activator tube for serum
125 separation, and then the sera were separated under 2000rpm centrifugation
126 for 5min. In contrast, patients with underlying immunodeficiency disorder
127 or immunodeficiency state and individuals who had other co-morbid health
128 problems which could introduce heterogeneity to the sample, (e.g. arthritis,
129 asthma, diabetes mellitus, hypertension, and other inflammatory diseases)
130 were excluded.

131 Methods

132 Determination of Endocan

133 The concentration of Endocan was determined according to Sandwich-
134 ELISA method. The micro ELISA plate was pre-coated with an antibody
135 specific to Endocan. Standards or samples were added to the suitable micro
136 ELISA plate wells and combined with the specific antibody. Then a

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137 biotinylated detection antibody specific for Endocan and Avidin-
138 Horseradish Peroxidase conjugate and substrate were added to each
139 microplate well. Only those wells that contain Endocan, biotinylated
140 detection antibody and Avidin-HRP conjugate appeared blue in colour and
141 reaction was stopped with 1N H_2SO_4 solution, and the colour turns yellow.
142 The absorbance was measured spectrophotometrically at a wavelength of
143 450nm.

144 **Determination of Serum Malondialdehyde**

145 Malondialdehyde (MDA) was determined according to Ohkawa
146 method [30]. The procedure was started by TBA preparation; in which 0.66g
147 of Thiobarbituric acid (TBA) dissolved in 100 ml of 0.05M of NaOH with
148 simple heating. Then, Trichloroacetic acetic acid (TCA) was prepared by
149 dissolving 17.5g of TCA in 100 ml distilled water; while TCA2 was prepared
150 by dissolving 70 g of TCA in 100 ml distilled water. Finally, 150 ml of
151 serum was added 1ml of TCA1 mixed for 2 min and incubated in boiling
152 water bath for 15 min, then 1 ml of TCA2 was added and incubated for 20
153 min at 37C^o then centrifuged for 5 min at 2000 rpm the supernatant was read
154 at 532 nm.

155 Myographical recording

156 **Vessel Collection and preparation**

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157 Human mesenteric arteries from patients suffering from CRC and
158 underwent partial colectomy was collected. The arteries supplying blood to
159 the tumour were dissected out surgically and directly were added to a beaker
160 containing cold modified Krebs solution (Fig 1) and aerated with 95% O_2
161 and 5% CO_2. In the laboratory, the excess tissue and fat were removed, and
162 the arteries were cut into rings about 3-4 mm long.

163 **Fig 1.** Mesenteric artery feeding colon tumour.

164 Recording of Isometric tension

165 The procedure of [31] with some modifications was followed to study
166 the vasodilator activity of the isolated mesenteric arteries. The arterial rings
167 were held up by two stainless steel clamps. The first clamp was attached to
168 a hook at the underside of the organ bath jacket and the other was connected
169 to the force transducer through a thread to record the isometric tension of the
170 mesenteric arteries, and the data was recorded by LabChart data acquisition
171 software. The held up arterial rings were immersed in Krebs solution (NaCl
172 5.10gm/L, $NaHCO_3$ 1.94/L, $MgSO_4$ 0.686gm/L, KCl 2.24gm/L, KH_2PO4
173 0.15gm/L, $CaCl_2$ 0.277gm/L, C6H12O6 2gm /L), contained in a 10 ml organ
174 chamber. Krebs solution was maintained at pH 7.4 and was constantly
175 aerated with 95% O_2 5% CO_2 at 37°C (Panlab Harvard Apparatus, USA).

176 The mesenteric arterial rings were tensed to a stable basal strain of 4gm
177 before left to equilibrate for 2hrs. To remove cellular metabolites, Krebs
178 solution was replaced every 15-20min intervals in the bath chamber until it

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179 reaches the stability and the experimental substances acquaint with the bath
180 chambers according to the protocols, the arteries were incubated with drugs
181 for 20 minutes then norepinephrine (NE) 1μM until reaches maximum
182 contractility and plateau, then relaxation happened by bolus single dose
183 application of sodium nitroprusside (SNP) or sodium disulfide (Na_2S).

184 **Experimental protocol**

185 The arterial rings precontracted with NE were first relaxed by
186 cumulative addition of either SNP (30nM-30μM) or Na_2S (1-6 mM). Based
187 on these initial experiments, relative half-inhibitory concentration (IC_{50}) of
188 SNP (2.3μM), Na_2S (2.4mM) had retested for the ability to relax
189 precontracted rings in three separate sets of experiments. In the first
190 experiment, when the NE-induced contraction reached the uppermost value,
191 SNP (2.3μM) or Na_2S (2.4mM) was added and left for 60min, and the
192 maximal relaxation rate (%) was calculated four times at each 15min
193 intervals (n=8). Then the role of K^+ channels in the progress of SNP and
194 Na_2S mediated relaxation were tested via incubation of the arterial rings for
195 20min with the Tetraethylammonium (TEA;1mM), Glibenclamide
196 (GLIB;0.1μM), Barium Chloride ($BaCl_2$; 1mM) and 4-aminopyridine (4-
197 AP;1mM). While, in the second experiment, the role of endogenous NO and
198 H_2S were tested by preincubation of arterial rings with either L-NAME
199 ($3x10^{-4}$M) or D,L- propargylglycine (PAG) (10mM), antagonists of eNOS
200 and CSE for 20min before applying SNP (n=8). Finally, to examine whether

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201 the combination of H_2S and NO potentiate or inhibit the vasorelaxation when
202 the NE-induced contraction reached the highest value, SNP and Na_2S were
203 added simultaneously and left for 60 min, (n=8).

204 Statistical analysis

205 The statistical analysis of myographical data was performed using two-way
206 analysis of variance (ANOVA) followed by Bonferroni *post hoc* test.
207 Maximum relaxation responses were calculated as a percentage of the
208 contraction produced by NE and were expressed as the means±standard error
209 of the mean (SEM). The tension created by NE was defined as 0% relaxation,
210 and the baseline tension before the addition of NE was determined as 100%
211 relaxation. While, comparison between CRC and healthy individuals were
212 made by Mann-Whitney test and the values were represented as median and
213 quartiles. A p-value less than 0.05 ($P<0.05$) were considered statistically
214 significant. The graphs, calculation and statistical analysesp were performed
215 using GraphPad Prism software 6.0 (GraphPad Software, San Diego,
216 California, USA).

217 Results

218 Serum Endocan and Malondialdehyde concentration

219 Serum endocan concentration was markedly lower in the CRC patients
220 (67.56, 43.04-94.28) than the healthy individuals (88.68, 59-101.3). While,
221 there were no significant differences in MDA concentration between the

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222 CRC patients (3.62, 2.86-4.96) and the healthy individuals (3.78, 3.23-4.24),
223 Fig 2A and B, respectively.

224 **Fig 2. Comparison between Endocan (pg/mL), MDA (μmol/L) level in a**
225 **healthy group and CRC patients**. Endocan significantly (*P<0.05,)
226 decreased in CRC patients compared to the healthy control group, while
227 Non-significant change was observed in MDA level. The comparison was
228 performed using the unpaired Mann-Whitney test.

229 Measurement of IC_{50}

230 Sodium nitroprusside at concentrations from 30nM-30μM caused a
231 relaxant effect on mesenteric arteries of CRC when precontracted with NE
232 (1 μM) with IC_{50}'s of 2.42±0.16 μM (IC_{50} of CI 95% between 1.18 to 4.95
233 μM), and the percentage of relaxation was 80.74±7.256%. While Na_2S at
234 concentrations from 1-6 mM had a relaxant effect on mesenteric arteries of
235 CRC precontracted with NE, the calculated IC_{50}'s was 3.54±1.07mM (IC_{50}
236 of CI 95% between 1.4 to 5.68mM), and the percentage of relaxation was
237 84.43±22.05%. Concentration-response curve for the effect of SNP and Na_2S
238 against NE-mediated contractions are shown in Fig 3A and B, respectively.

239 **Fig 3**. Cumulative dose-response curve for the vasorelaxant effects of (A)
240 SNP (30nM-30μM); and (B) Na_2S (1-6mM) on NE (1μM) induced
241 contraction in mesenteric arteries of CRC patients.

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242 **The role of K^+ channels in the NO-induced relaxation**

243 To identify the role K^+ channels on the time-dependent change of CRC
244 feeding mesenteric arteries relaxation responses to effect of SNP, arterial
245 rings were incubated with K^+ channels blockers for 20 min before the
246 addition of SNP and the net vasorelaxation effect of SNP in mesenteric
247 arteries was measured every 15min over the course of 60 min. Preincubation
248 of mesenteric arteries with either GLIB (10µM, n=6), TEA (1 mM, n=6) or
249 4-AP (1 mM, n=6) remarkably reduced the net vasorelaxation effect of SNP
250 in mesenteric arteries at all-time intervals. While, the reduction of
251 vasorelaxation responses of SNP was lasted for 45 min after preincubation
252 of the mesenteric arterial ring with $BaCl_2$ (1mM, n=6), as shown in Table 1
253 and Fig 4.

254 **Table 1. Involvement of K^+ channels in the mechanism of time-**
255 **dependent relaxation responses to effect of SNP in mesenteric arteries**
256 **of CRC patients**

	Time			
	15 min	**30 min**	**45 min**	**60 min**
Control	41.7±6.09	62.9±5.64	63.1±5.65	62.7±6.38
TEA	3.6±4.52	12.9±10.5	17.9±12.7	20.6±13.8
GLIB	0.1±3.14	0.3±9.04	4.4±12.2	7.4±13.4
$BaCl_2$	5.9±3.39	23.7±11.8	33.9±15.6	40.4±17.2
4-AP	5.1±2.39	23.5±5.67	35.5±6.77	39.4±8.15

257

258 **Fig 4. Time-dependent change of relaxation responses to effect of SNP**
259 **in mesenteric arteries preincubated with TEA (1mM), $BaCl_2$ (1mM), 4-**

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260 **AP (1mM), GLIB (10µM) respectively**. (A) SNP-induced vasorelaxation
261 significantly inhibited by TEA (■) pretreatment (**P<0.01, 5 min, ***
262 P<0.001, 30, 45, 60min). (B) SNP-induced vasorelaxation significantly
263 inhibited by GLIB (■) pretreatment (*** P<0.001, 15, 30, 45,60min). (C)
264 SNP -induced vasorelaxation significantly inhibited by $BaCl_2$ (■)
265 pretreatment (**P<0.01, 15 and 30min , (*P<0.05, 45 min). (D) SNP-
266 induced vasorelaxation significantly inhibited by 4-AP (■) pretreatment
267 (**P<0.01, 15 and 45 min, **P<0.01, 30min, *P<0.05, 60 min).

268 The role of K^+ channels in the H_2S-induced relaxation

269 The impairment of Na_2S induced mesenteric artery relaxation taken from
270 CRC patients was continued throughout the experiment when the arteries
271 were previously incubated with either GLIB (n=6) or 4-AP (n=6). While
272 $BaCl_2$ reduced vasorelaxation responses produced by Na_2S only in the
273 middle of the experiment. In contrast, TEA had failed to amend the
274 vasorelaxation responses of Na_2S, as shown in Table 2 and Fig 5.

275 **Table 2. Involvement of K^+ channels in the mechanism of time-**
276 **dependent relaxation responses to effect of Na_2S in mesenteric arteries**
277 **of CRC patients**

	Time			
	15 min	30 min	45 min	60 min
Control	37.1±6.26	73±10.1	68.8±9.85	64.7±9.21
TEA	13.9±4.52	81.3±10.5	105±12.7	116±13.8
GLIB	7.8±1.91	17.9±4.82	29.4±6.71	37.5±7.61
$BaCl_2$	17±13.2	29.5±21.7	37.4±24.1	40.5±25.4

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4-AP	2±1.34	2±2.6	-0.3±3.53	3.9±3.72

278

279 **Fig 5. Time-dependent change of relaxation responses to the effect of**
280 **Na_2S in mesenteric arteries preincubated with TEA (1mM), GLIB**
281 **(10µM.), $BaCl_2$ (1mM), 4-AP (1mM) respectively.** (A) TEA (■) had no
282 significant effect on Na_2S -induced time-dependent arterial relaxation. (B)
283 Na_2S -induced vasorelaxation significantly inhibited by GLIB (■)
284 pretreatment (*** P<0.001, 30, 45, min, * P<0.05, 60min). (C) Na_2S -
285 induced vasorelaxation significantly inhibited by $BaCl_2$ (■) pretreatment (*
286 P<0.05, 30min). (D) Na_2S -induced vasorelaxation significantly inhibited by
287 4-AP (■) pretreatment (** P<0.01, 15min, *** P<0.001, 30, 45, 60min).

288 Interaction effects of SNP and Na2S

289 Combination of SNP and Na_2S did not modify relaxation responses at
290 all time-intervals in comparison to the relaxation induced by individual
291 application of either SNP or Na_2S. Furthermore, preincubation of arterial
292 rings with L-NAME (n=6) did not change the extent of the Na_2S-induced
293 relaxation at all time-courses of the study. On the other hand, treating the
294 mesenteric arterial rings with PAG (n=6) significantly increased
295 vasorelaxation induced by SNP at all time-courses of the study, as shown in
296 Table 3 and Fig 6.

297 **Table 3. The combination effects of SNP and Na_2S, L-NAME and PAG**
298 **preincubation on the time-dependent relaxation responses of the**
299 **mesenteric arteries of CRC patients**

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	Time			
	15 min	**30 min**	**45 min**	**60 min**
SNP+Na_2S	34.7±8.96	48.2±10.4	47.9±8.09	48.7±8.67
L-NAME	35.8±5.73	56.8±6.23	61.2±8.87	57.1±8.3
PAG	89±5.87	-25.7±9.42	-36.5±11.3	-42.3±12.9

300

301 **Fig 6. The combination effects of SNP and Na_2S, L-NAME and PAG**
302 **preincubation on the time-dependent relaxation responses of the**
303 **mesenteric arteries precontracted with NE**. (A) The combination of SNP
304 and Na_2S (▲) did not change the time-dependent relaxation. (B) L-NAME
305 (0.3mM; ■) had no significant effect on Na_2S-induced time-dependent
306 relaxation. (C) SNP-induced relaxation significantly inhibited by PAG
307 (10mM; ■) pretreatment (*** P<0.001, 15, 30,45,60min).

308 Discussion

309 The first part of the present study ascertains that endothelium cells of
310 CRC patients are functioning normally, because the serum Endocan level
311 markedly decreased, which is an endothelial cell marker [32]. The reason of
312 endocan decrement was explained by [33-35], that is due to either
313 chemotherapy or VEGF receptor-2 kinase inhibitors treatment or the
314 downregulation of endocan expression, suggesting that the expression of
315 endocan is related to the development and differentiation of CRC [32]. While,
316 [36] stated that Endocan correlates with colon tumour size, depth of invasion,
317 lymph node metastasis, distant metastasis and Dukes' staging. Also, there has

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318 been a growing interest in studying MDA as a marker of oxidative stress in
319 cancer progression [37]. In the present, we showed that the serum MDA level
320 was not changed in CRC patients comparing to healthy individuals.
321 Although, previous studies had been observed that MDA is increased in CRC
322 patients [38], but [39] explained that chemotherapeutical treatment would
323 normalize the oxidative stress in CRC patients. Taken together, these results
324 indicate that CRC patients have intact arteries and their endothelium are
325 functioning normally.

326 In the second part of the present study, we found that SNP profoundly
327 relaxes mesenteric arteries in CRC patients. In this concern, the previous
328 study conducted by [40] recorded 69% relaxation of rat mesenteric arteries,
329 whilst the relaxation of human hand veins reached 103% [41]. Then, to
330 explore the exact mechanism of relaxation of SNP, the role of K^+ channels
331 were tested in the mesenteric arteries of CRC patients.

332 The results of the present study unveil that K^+ channels have a great role
333 in the SNP-induced relaxation because pretreatment of mesenteric arteries
334 with either TEA, GLIB, $BaCl_2$ or 4-AP markedly delayed vasodilation. NO
335 activates several K^+ channels of SMCs in rat, rabbit mesenteric artery and
336 cerebral arteries, including ATP-sensitive K^+ channels (Deka and Brading
337 2004; Koh et al. 1995; Murphy and Brayden 1995) and induce membrane
338 hyperpolarization by decreases in $[Ca^{2+}]_i$ levels through inhibition of Ca^{2+}
339 influx or Ca^{2+} release from intracellular stores [42]. Moreover, NO
340 hyperpolarize arterial smooth muscle cells via activation both K_V and K_{Ca}

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341 channels on VSMC in rat superior mesenteric artery, coronary and cerebral
342 artery and large artery [43-45], through a cGMP-dependent mechanism, in
343 that way inhibiting the evoked membrane depolarization and upsurge in
344 $[Ca^{2+}]_i$ [46]. Furthermore, this result is in accordance with the earlier finding
345 of Schubert, Krien (47), they were observed that VSMC K_{IR} currents are
346 regulated by NO. Meanwhile, Hempelmann, Seebeck (48) displayed that
347 neither 4-AP nor $BaCl_2$ modulate NO-induced relaxation in rat basilar artery.
348 This result proposes that NO might exert vasodilation possibly by the
349 opening of different K^+ channels. Hence, we can conclude that K^+ channels
350 play a central role in the vasodilation mechanism of NO.

351 Consistently, we were the first to observe that Na_2S potently relax
352 mesenteric arteries in CRC patients. Recently, Materazzi, Zagli (49)
353 exhibited that H_2S could relax precontracted human mesenteric arterial rings
354 in a concentration-dependent manner. Moreover, we found that the
355 relaxation of mesenteric arteries induced by Na_2S depends on the activation
356 of K_{ATP} and K_V channels. The importance of K_{ATP} channel activation
357 confirms in a human colonic mesenteric artery what has already been
358 observed in the rat arterial smooth muscle by [50] human mammary artery
359 by [51], either through hyperpolarization of SMCs membrane, in turn meight
360 close voltage-gated Ca^{2+}channels [52] or through channel protein
361 sulfhydration [15]. In the same way, in the rat aorta H_2S induces
362 vasorelaxation was diminished by KCNQ-type K_V channels blockage [53].
363 In contrast [54] had found that K_{ATP} channels did not mediate the relaxations

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364 caused by H_2S in the guinea-pig ileum and trout urinary bladder. Even
365 though, K_{IR} channels weakly participated in the relaxation of mesenteric
366 arteries in CRC patients, while previous studies conducted by [55-57] were
367 reported that the mechanism of relaxation in rat aorta is mainly mediated by
368 the stimulation of K_{IR} channels and subsequent K_{IR}-dependent
369 hyperpolarization from the endothelium to the smooth muscle cells

370 Although, H_2S was found to activate BK_{Ca} [58], IK_{Ca}, and SK_{Ca} channels
371 in endothelial cells [15]; and BK_{Ca} channels in SMCs of mesenteric arteries
372 [58] and cerebral arterioles [59]. The results of the present study disclosed
373 that TEA did not change the vasodilation of mesenteric arteries induced by
374 Na_2S, signifying that K_{Ca} might not be accountable for H_2S induced
375 vasorelaxation. More or less the same results were observed by [60], who
376 noted that different blockers for K_{Ca} channels ineffective in the vascular
377 effect of H_2S. Opposite to our results [55] found that the maximum relaxation
378 of VSMC of rat artery [15, 61] and human mammary artery [51] induced by
379 NaHS was significantly attenuated by K_{Ca} channels blockers.

380 Because both H_2S and NO are vasorelaxant factors, and they have
381 dissimilar mechanisms of action, one may predict an improver effect when
382 the two gases are applied together [50], whereas the results of the current
383 study showed that the combination of SNP and Na_2S donors were not
384 changed maximal relaxation during all time course of the study compared to
385 SNP and Na_2S respectively. One explanation is a combination of H_2S, and
386 NO generate a new molecule (perhaps nitrosothiol), which does not relax

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387 blood vessels either *in vitro* or *in vivo* [62]. Consequently, the formation of
388 this novel molecule most likely denotes a means for biological inactivation
389 or perhaps sequestration of released NO [63]. In contrast, rat aortic relaxation
390 was prolonged when both gas donors are added together [64]. This
391 synergistic action may be due to the production of HSNO and HNO as a
392 result of a chemical reaction between H_2S and nitrite [65], which releases
393 NO and polysulfides and relax VSMCs through soluble guanylyl cyclase
394 activation [66].

395 At the same time, preincubation of L-NAME did not change the
396 relaxation effects induced by Na_2S. Similar results have been reported by
397 Ohia, Opere (67); Monjok, Kulkarni (68) they were notified that L-NAME
398 did not modify the relaxation effects of Na_2S in isolated porcine irides, this
399 means that endogenous NO has no impact on the vasoactivity of H_2S donor.
400 In contrast, preincubation of CRC arterial rings with PAG increased relaxant
401 activity induced by NO donor. The proper explanation for this result is that
402 endogenous H_2S inhibits the action of NO. In the same manner, SNP-induced
403 vasorelaxation of rat aorta and human internal mammary artery was
404 diminished by low concentration of H_2S by suppressing NO action or
405 inhibition of NO synthase [52, 60].

406 Conclusion

407 In conclusion, low Endocan and normal MDA values reveal that
408 endothelial dysfunction and oxidative stress do not take part in the

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409 pathogenesis of CRC. Beside this, the mechanism of NO and H_2S-induced
410 mesenteric artery vasodilation is time and K^+ channels dependent, in which
411 NO dilates mesenteric arteries via activation of K_{ATP}, K_{Ca}, K_{IR} and K_V
412 channels, while vasodilation activity of H_2S is due to the modulation of K_{ATP}
413 and K_V channels. Moreover, NO and H_2S are interacting at the level of
414 enzymes, the activity of CSE enzyme slows down the vasodilator ability of
415 exogenous NO.

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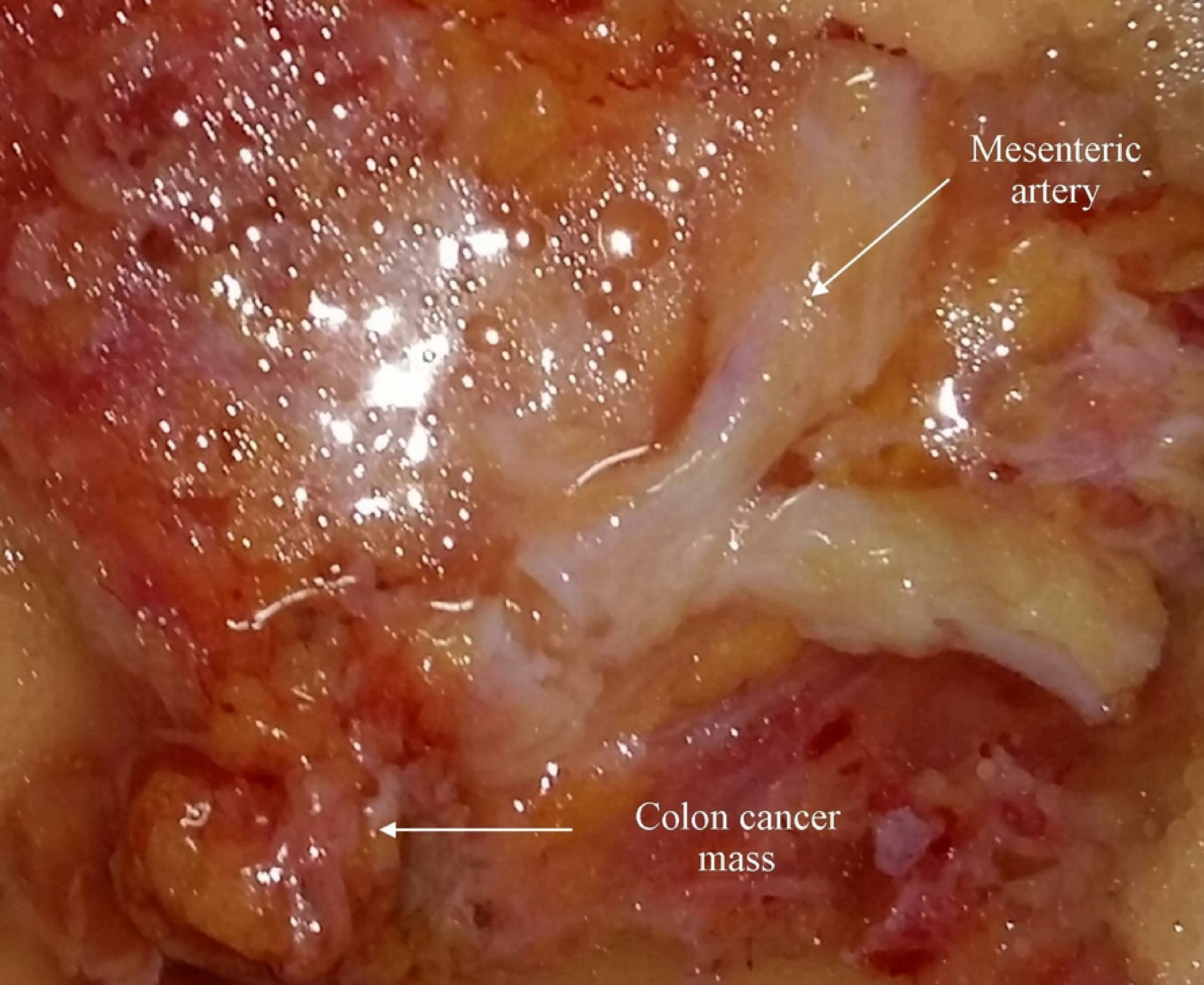

Mesenteric artery
Colon cancer mass

A

Endocan (pg/mL)

300

200

100

0

*

Control

Colorectal Cancer

B

MDA (μmol/L)

8

6

4

2

0

Control

Colorectal Cancer

A

Tension (%)

120
100
80
60
40
20
0

-8 -7 -6 -5 -4

SNP (log-M)

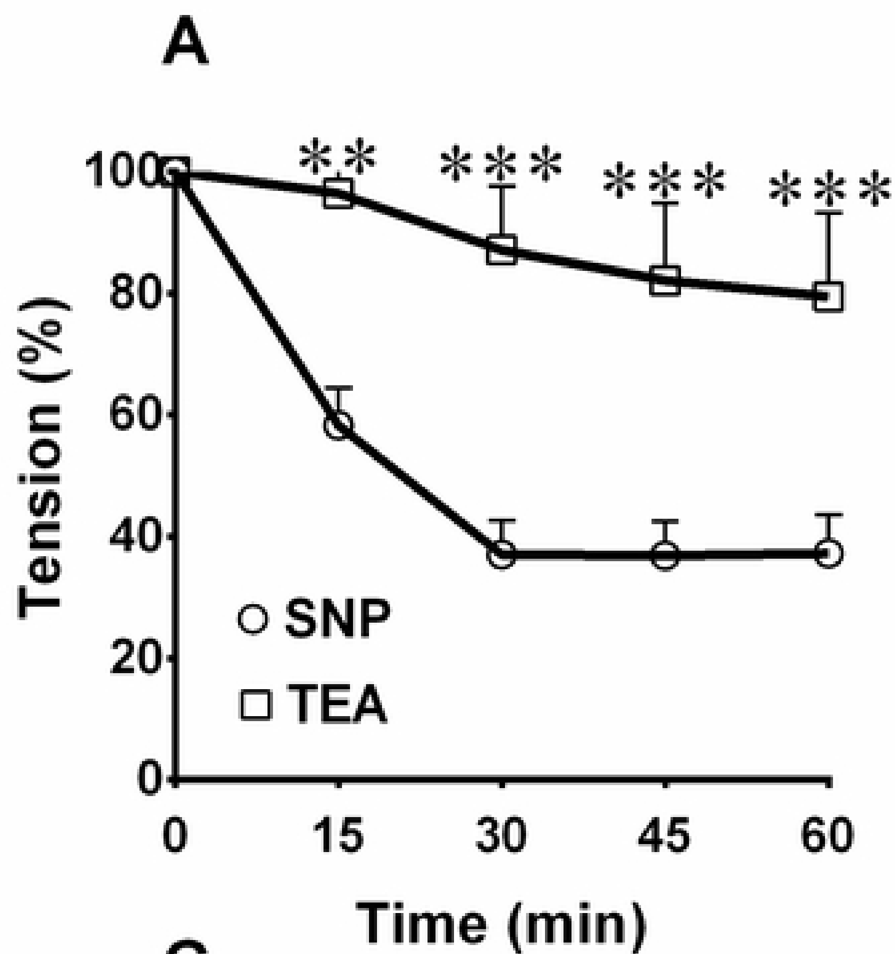
B

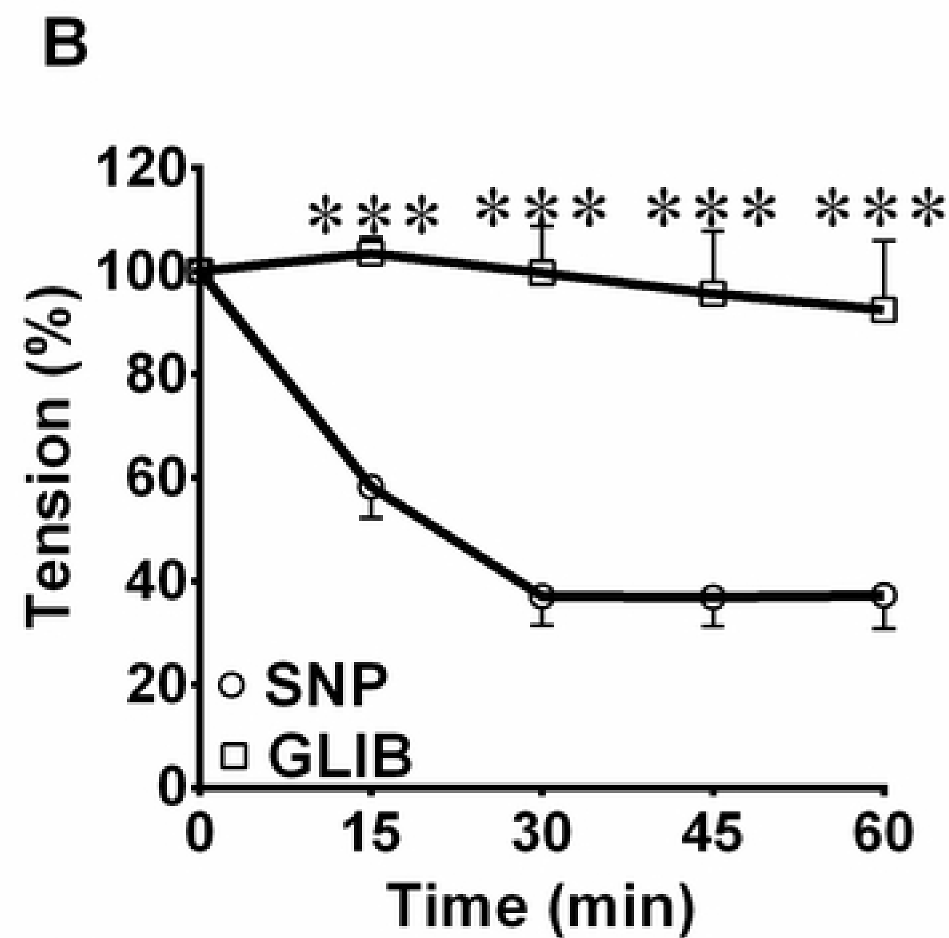
Tension (%)

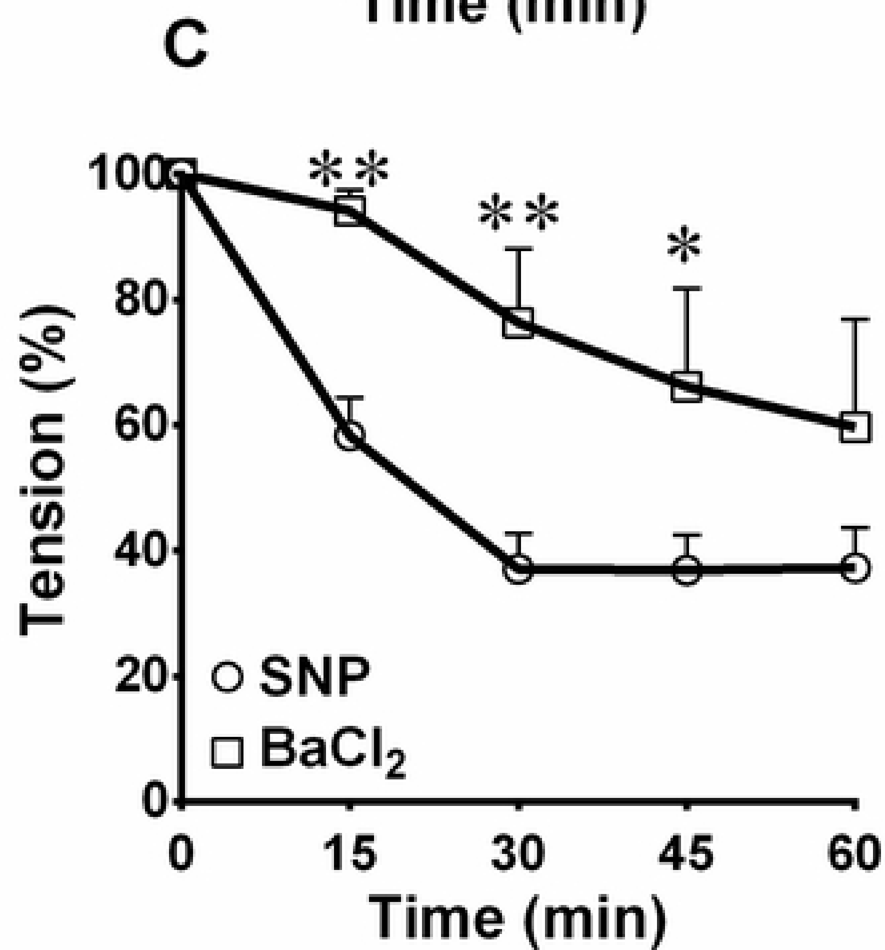
120
100
80
60
40
20
0

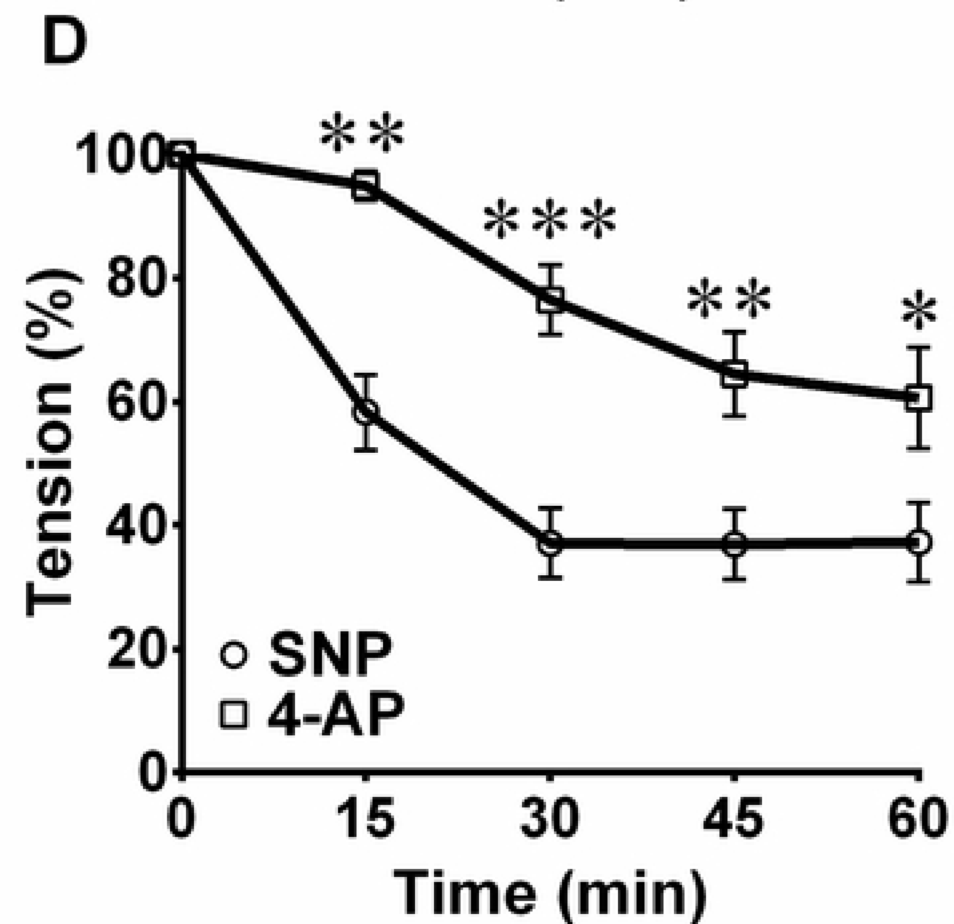
0 1 2 3 4 5 6

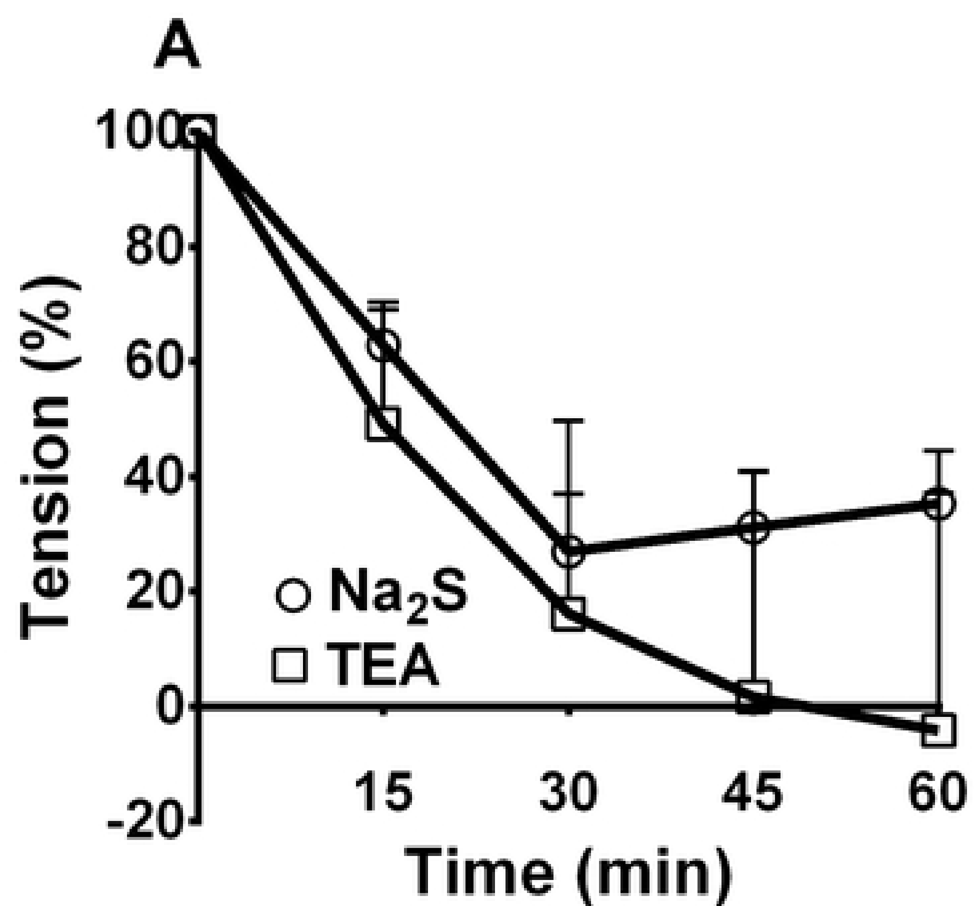
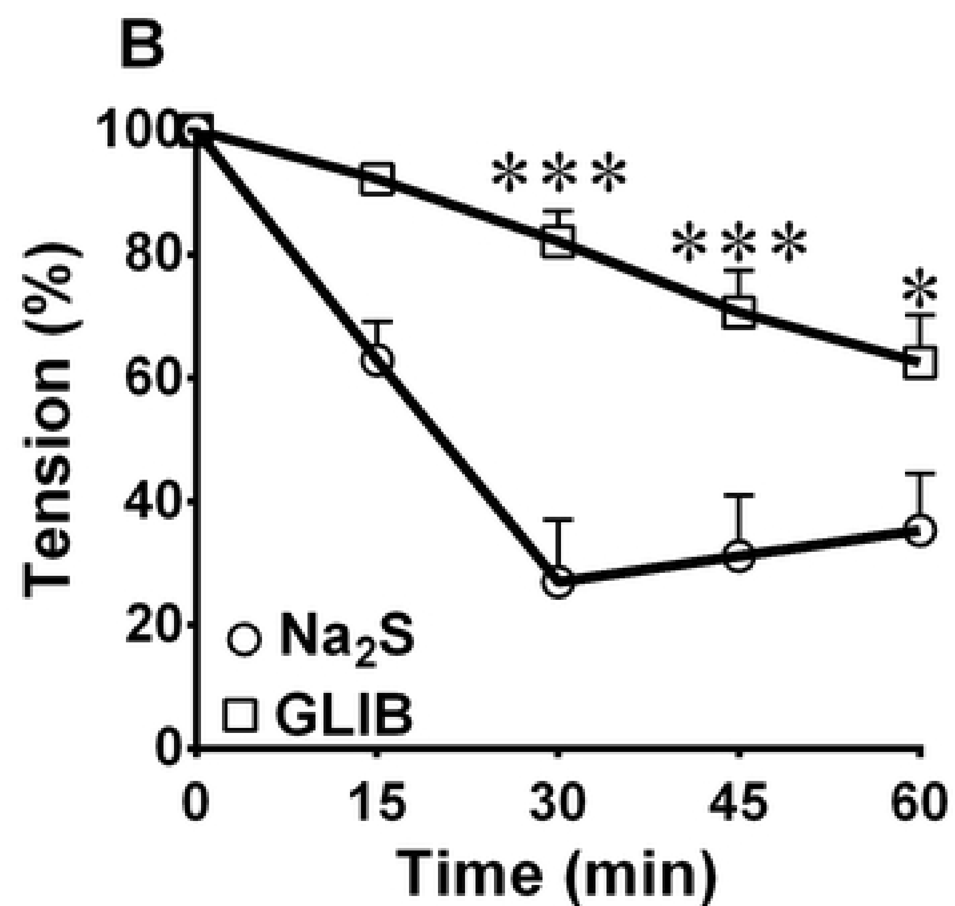
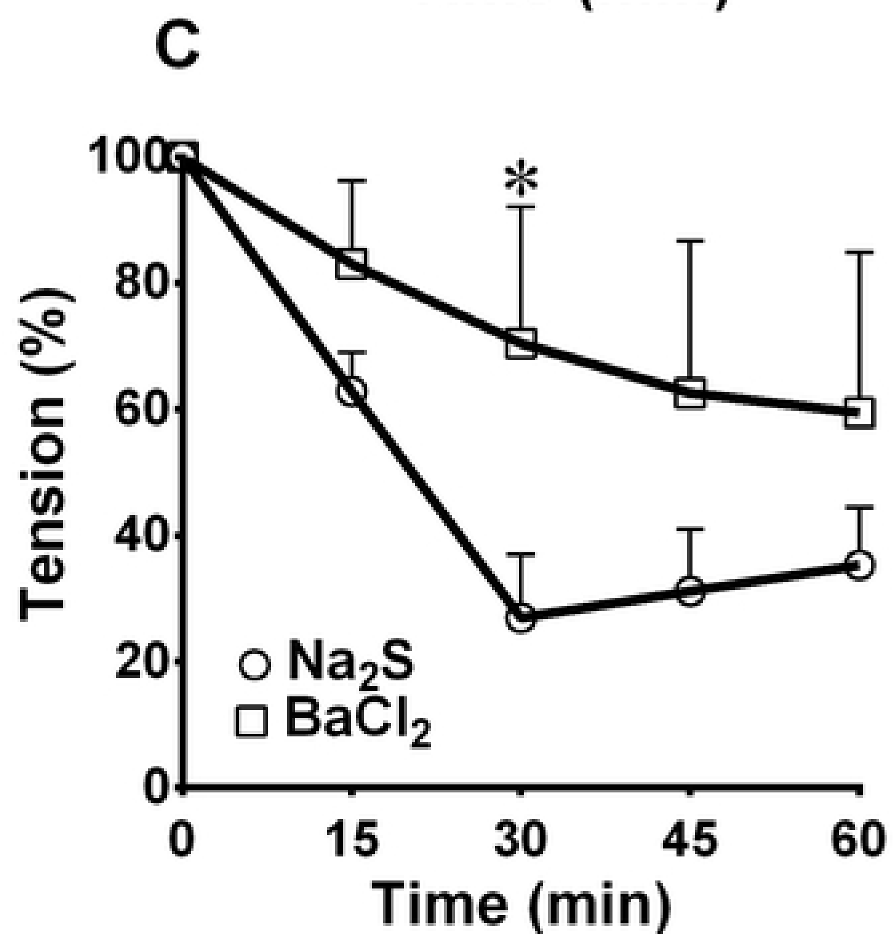
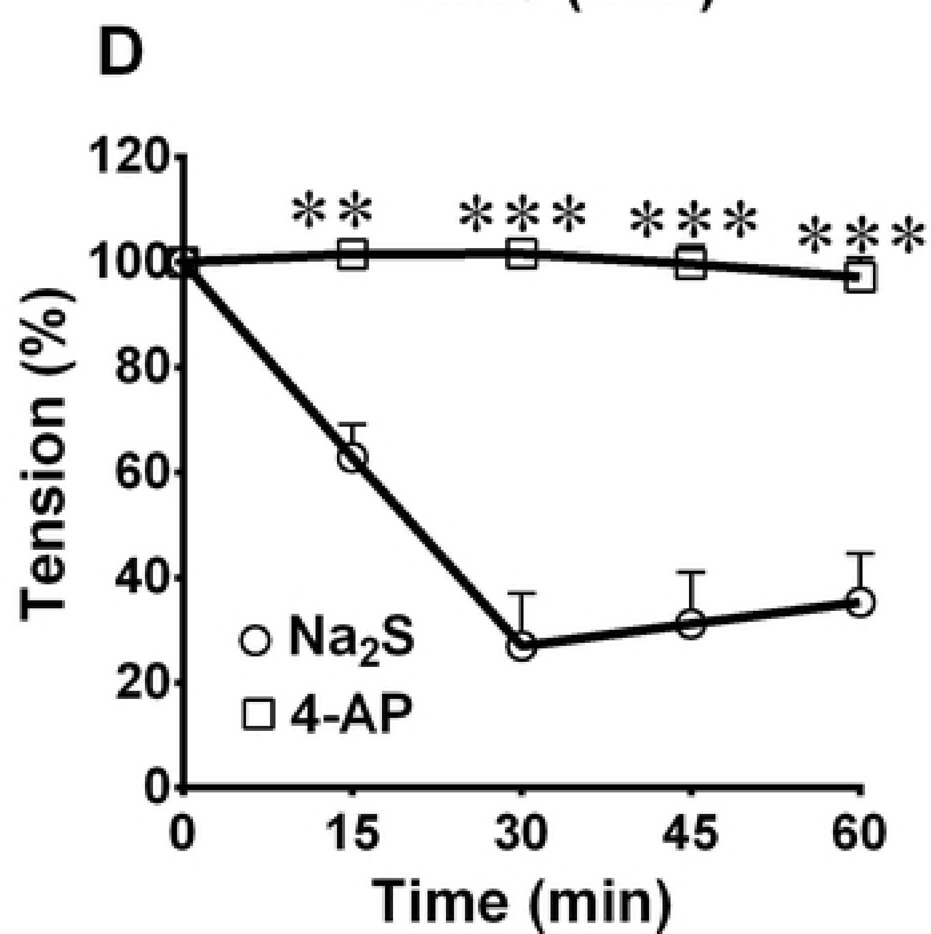
Na_2S (mM)

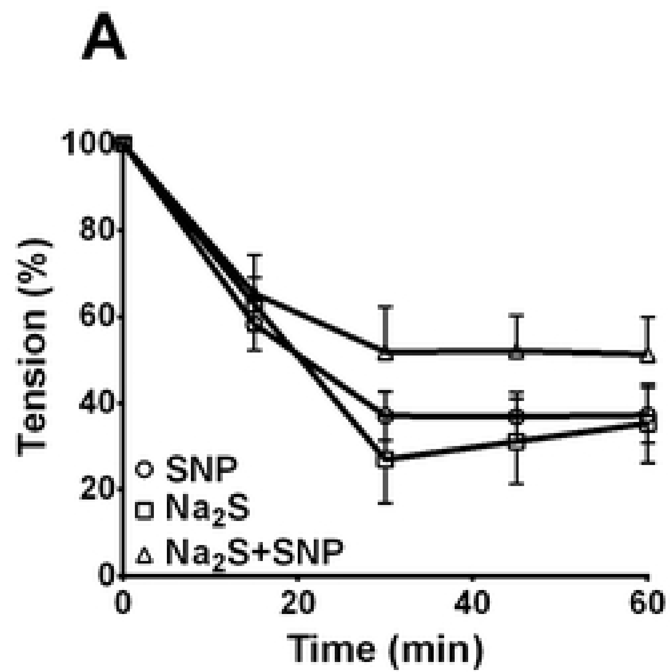

A
Tension (%)
Time (min)
SNP
TEA

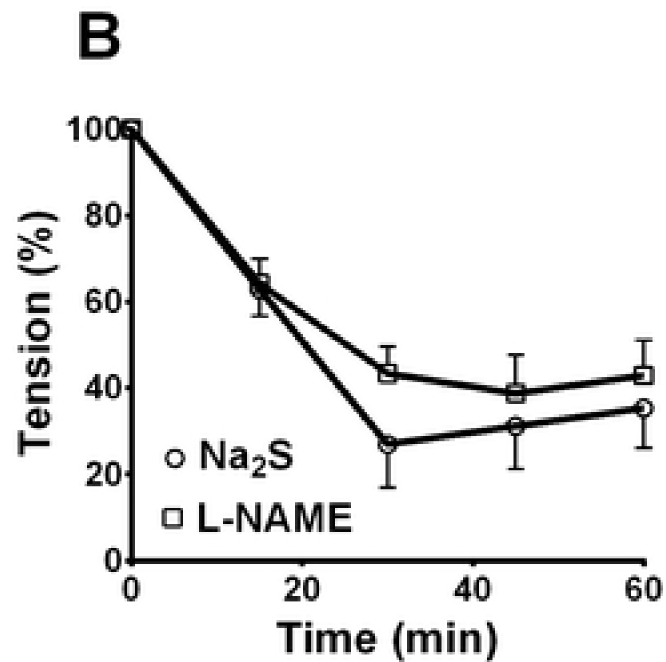

B
Tension (%)
Time (min)
SNP
GLIB

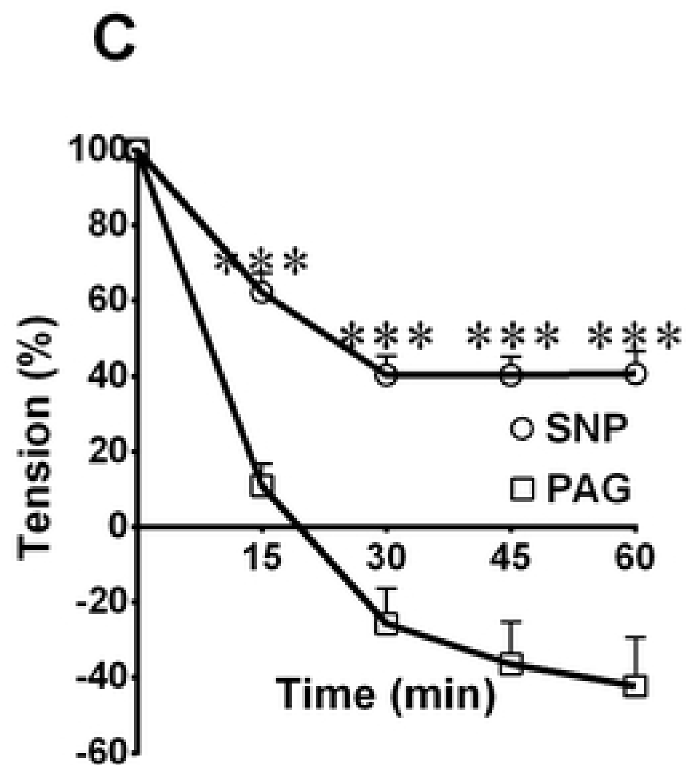

C
Tension (%)
Time (min)
SNP
$BaCl_2$


D
Tension (%)
Time (min)
SNP
4-AP


A
100
80
60
40
20
0
-20
Tension (%)
15
30
45
60
Time (min)
Na$_2$S
TEA

B
100
80
60
40
20
0
Tension (%)
0
15
30
45
60
Time (min)
***
***
*
Na$_2$S
GLIB

C
100
80
60
40
20
0
Tension (%)
0
15
30
45
60
Time (min)
*
Na$_2$S
BaCl$_2$

D
120
100
80
60
40
20
0
Tension (%)
0
15
30
45
60
Time (min)
**
***
***
***
Na$_2$S
4-AP


A
Tension (%)
Time (min)
SNP
Na$_2$S
Na$_2$S+SNP


B
Tension (%)
Time (min)
Na$_2$S
L-NAME


C
Tension (%)
Time (min)
SNP
PAG
***