Articles in PresS. Am J Physiol Heart Circ Physiol (June 12, 2015). doi:10.1152/ajpheart.00171.2015

ANALYSIS OF CARDIOVASCULAR RESPONSES TO THE H_2S DONORS NA_2S AND NAHS IN THE RAT

Daniel Yoo[1]*, Ryan C. Jupiter [1]*, Edward A. Pankey[1], Vishwaradh G. Reddy[1], Justin A. Edward[1], Kevin W. Swan[1], Taylor C. Peak[1], Ricardo Mostany[1], Philip J. Kadowitz[1]

[1]Department of Pharmacology, Tulane University School of Medicine, New Orleans, Louisiana

* Both authors contributed equally to this manuscript

Corresponding Author: Philip J. Kadowitz, Ph.D.
Department of Pharmacology, SL83
Tulane University School of Medicine
1430 Tulane Avenue
New Orleans, Louisiana 70112-2699 USA
Phone: 504 988-2634
Fax: 504 988-5283
Email: pkadowi@tulane.edu

Running title: Analysis of Cardiovascular Responses to H_2S Donors

Research Support: Supported in part by NIH Grant HL 77421

Key Words: H_2S Donors, K_{ATP} Channels, Hypotension, Bradycardia, Cardiac Output

ABSTRACT

Hydrogen sulfide (H_2S) is an endogenous gaseous molecule formed from L-cysteine in vascular tissue. In the present study, cardiovascular responses to the H_2S donors Na_2S and NaHS were investigated in the anesthetized rat. The intravenous injections of Na_2S and NaHS 0.03 – 0.5 mg/kg produced dose-related decreases in systemic arterial pressure and heart rate, and at higher doses decreases in cardiac output, pulmonary arterial pressure, and systemic vascular resistance. H_2S infusion studies show that decreases in systemic arterial pressure, heart rate, cardiac output, and systemic vascular resistance are well-maintained and responses to Na_2S are reversible. Decreases in heart rate were not blocked by atropine suggesting that the bradycardia was independent of parasympathetic activation and was mediated by an effect on the sinus node. The decreases in systemic arterial pressure were not attenuated by hexamethonium, glybenclamide, L-NAME, sodium meclofenamate, ODQ, miconazole, 5-hydroxydecanoate, or TEA suggesting that ATP-sensitive potassium channels, nitric oxide, arachidonic acid metabolites, cyclic GMP, p450 epoxygenase metabolites, or large conductance calcium-activated potassium channels are not involved in mediating hypotensive responses to the H_2S donors in the rat and that responses are not centrally mediated. The present data indicate that decreases in systemic arterial pressure in response to the H_2S donors can be mediated by decreases in vascular resistance and cardiac output and that the donors have an effect on the sinus node independent of the parasympathetic system. The present data indicate that the mechanism of the peripherally mediated hypotensive response to the H_2S donors is uncertain in the intact rat.

NEW AND NOTEWORTHY

H_2S is a gaseous mediator that produces decreases in arterial pressure, heart rate, and at higher doses cardiac output. Systemic vascular resistance was decreased and responses were not blocked by K_{ATP} channel, N.O., cyclooxygenase, lipoxygenase, or autonomic antagonists. H_2S donors may be useful in the treatment of cardiovascular diseases.

INTRODUCTION

Gaseous modulators such as nitric oxide (NO) and carbon monoxide (CO) have been reported to play an important role in the regulation of cardiovascular function (15, 20, 21, 30). Hydrogen sulfide (H_2S), which has long been known as a toxic gas, has recently been shown to have an important role in the regulation of cardiovascular function as a third endogenous gaseous transmitter (17, 18, 26). It has been hypothesized that H_2S is a major physiologic endothelial derived hyperpolarizing factor (17, 18, 26). H_2S is synthesized from L-cysteine by at least four enzymes with cystathionine γ-lyase (CSE) appearing to play a major role in vascular tissue (4, 7, 23, 36, 37, 39, 41, 46). Transgenic mice lacking CSE have been reported to develop hypertension similar to that observed in eNOS knockout mice (46). It has been reported that hypotensive and vasorelaxant responses to H_2S or H_2S donors are inhibited by the ATP-sensitive potassium (K_{ATP}) channel antagonist glybenclamide and are mediated at least in part by the opening of K_{ATP} channels (9, 14, 23, 48-50). It has also been reported that there are interactions between H_2S and the NO – cyclic guanylate monophosphate (cGMP) system and arachidonic acid pathways as well as effects on large conductance calcium-activated potassium channels (2, 9, 10, 18, 22, 27, 28, 42, 50).

The present study was undertaken to investigate cardiovascular responses to H_2S using the H_2S donors sodium sulfide (Na_2S) and sodium hydrosulfide (NaHS) in the anesthetized rat. The hypothesis that decreases in arterial pressure and heart rate in response to the H_2S donors are mediated by the activation of K_{ATP} channels was investigated. The results of these studies show that H_2S donors decrease systemic

arterial pressure and heart rate in a dose-dependent manner and at higher doses decrease cardiac output and pulmonary arterial pressure. These data indicate that decreases in systemic arterial pressures can be mediated by an effect on vascular tone and cardiac output and that systemic vasodepressor responses are not blocked by hexamethonium, glybenclamide, 5-hydroxydecanoate, L-NAME, sodium meclofenamate, ODQ, TEA, or miconazole suggesting that the mechanism of action in the intact rat is uncertain.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee of the Tulane University School of Medicine approved the experimental protocol employed in these studies and all procedures were conducted in accordance with institutional guidelines. For these experiments, male Sprague–Dawley rats (273 - 456 g) were anesthetized with an intraperitoneal (ip) injection of Inactin (thiobutabarbital; (Sigma-Aldrich, St. Louis, MO)) in a dose of 100 mg/kg. Supplemental doses of Inactin were given ip as needed to maintain a uniform level of anesthesia. Body temperature was maintained with a heating lamp. The trachea was cannulated with a short segment of PE-240 tubing to maintain a patent airway, and the animals breathed room air enriched with 100% O_2. The left carotid artery or left femoral artery was catheterized with PE-50 tubing for the measurement of systemic arterial pressure. Systemic arterial pressure was measured with a Namic Perceptor DT pressure transducer and a data acquisition system (Biopac MP 100A-CE, Santa Barbara, CA). Systemic arterial pressure and mean arterial pressure were obtained by electronic averaging of the pressure signal and heart rate signal. These data were continuously recorded, displayed, and stored on a Dell PC. The

left jugular vein was catheterized with PE-50 tubing for the systemic injection of drugs and fluids. The left femoral vein was also catheterized with PE-50 tubing for systemic infusion of drugs.

For the measurement of pulmonary arterial pressure, a specially designed 3-F single-lumen catheter with a curved tip and radio-opaque marker was passed from the right jugular vein into the main pulmonary artery under fluoroscopic guidance (Picker-Surveyor Fluoroscope) as described previously (19, 33-35). Pulmonary arterial pressure was measured with a Namic Perceptor DT pressure transducer and data acquisition system and stored on the PC. The cardiac output was measured by the thermodilution technique with a Cardiomax II computer (Columbus Instruments). A known volume (0.2 ml) of room temperature 0.9% saline solution was injected into the jugular vein catheter with its tip near the right atrium, and changes in blood temperature were detected by the 1.5-F thermistor microprobe catheter (Columbus Instruments) positioned in the aortic arch from the left carotid artery . The indicator dilution data was stored on the PC.

Drugs

Na_2S, NaHS, sodium nitroprusside (SNP), phentolamine, atropine sulfate, acetylcholine chloride, norepinephrine, cromakalim, N-w-Nitro-L-Arginine Methyl Ester hydrochloride (L-NAME), sodium meclofenamate, and tetraethylammonium (TEA), hexamethonium, and 5-hydroxydecanoate (Sigma-Aldrich, St. Louis, MO) were dissolved in 0.9% NaCl, and solutions were prepared on a frequent basis. Glibenclamide (Sigma-Aldrich, St. Louis, MO) was dissolved in 4 ml propylene glycol with 300 µg concentrated NaOH, 1.7 ml of 100% ethanol, and 4 ml of Tris (pH 8.4) and

sonicated. ODQ (SigmaAldrich, St. Louis, MO) was dissolved in a 10/10/80 ratio of transcretol, cremephor, and 0.9 % NaCl respectively. Miconazole (SigmaAldrich, St. Louis, MO) was dissolved in DMSO. The doses of the antagonists used in the present study were determined from doses used in previous studies in the literature and from pilot studies in our laboratory (11, 29, 33-35, 50). The vehicles for the drugs used in the study did not alter responses to Na_2S and NaHS.

Statistics

The data are expressed as mean ± SE and were analyzed using a one-way ANOVA and a student's t-test for paired and unpaired data. A p value of less than 0.05 was used as the criterion for statistical significance.

RESULTS

Cardiovascular responses to the H_2S donors, Na_2S and NaHS, were investigated in the rat, and these results are summarized in figure 1 and figure 2. The iv injections of Na_2S and NaHS in doses of 0.03-0.5 mg/kg produced dose-related decreases in systemic arterial pressure and in heart rate (Figs. 1 and 2). The decreases in systemic arterial pressure and heart rate were rapid in onset and short in duration. At the higher doses studied, the iv injections of Na_2S and NaHS produced significant decreases in pulmonary arterial pressure and in cardiac output (Figs. 1 and 2). Systemic vascular resistance was decreased significantly at the 0.1, 0.3, and 0.5 mg/kg doses of Na_2S and NaHS (Figs. 1 and 2). Input pulmonary vascular resistance was not decreased significantly at the 0.1 – 0.5 mg/kg iv doses of the H_2S donors (Figs. 1 and 2). The time course of the decreases in systemic arterial pressure, mean arterial pressure, and heart rate in response to iv injections of high doses of the H_2S donors and to iv injections of

the NO donor SNP are shown in figure 3 where it can be seen that a secondary increase in systemic arterial pressure is observed during the recovery phase of the response of the H_2S donors and that SNP does not produce bradycardia (Fig. 3). The secondary increase in systemic arterial pressure during the recovery phase of the pressor response to the H_2S donors was significantly attenuated after administration of the alpha receptor blocking agent phentolamine 0.5 mg/kg iv indicating that this overshoot in systemic arterial pressure was mediated by alpha adrenergic stimulation (Fig. 7C). The decreases in heart rate in response to iv injection of the H_2S donors were not attenuated after administration of atropine 0.5 mg/kg iv indicating that the bradycardia was not mediated by activation of the parasympathetic nervous system (Fig. 7A). After administration of the muscarinic receptor antagonist atropine, the decreases in systemic arterial pressure in response to iv injections of acetylcholine were decreased significantly; whereas, decreases in systemic arterial pressure in response to iv injections of the H_2S donors were not changed (Fig. 7A). The decreases in systemic arterial pressure and heart rate in response to iv injection of Na_2S were not attenuated by treatment with phentolamine or the ganglionic blocking agent hexamethonium (Fig. 7B, 7C). The data with atropine, phentolamine, and hexamethonium suggest that the decreases in arterial pressure and heart rate in response to the H_2S donors are mediated by peripheral actions and that effects on the central nervous system do not play a major role. After administration of phentolamine, the pressor response to norepinephrine was significantly attenuated (Fig. 7C).

In order to investigate steady state responses to an H_2S donor the effect of an infusion of Na_2S was studied, and these data are summarized in figure 4. The iv

infusion of Na_2S 0.3 mg/min at a rate of 0.1 mL/min produced a significant and well-maintained decrease in systemic arterial pressure and heart rate (Fig. 4). Cardiac output and systemic vascular resistance was decreased significantly during the infusion period, and arterial pressure, heart rate, cardiac output, and systemic vascular resistance returned to control value shortly after the Na_2S infusion was terminated (Fig. 4).The iv infusion of Na_2S produced a significant decrease in plasma glucose levels from 115±5 mg/dl to 83±13mg/dl.

The mechanism by which the H_2S donors decrease systemic arterial pressure and heart rate was investigated and these data are summarized in figure 5. The decreases in systemic arterial pressure and heart rate in response to the iv injections of Na_2S or NaHS were not significantly decreased after administration of L-NAME 50 mg/kg iv, glybenclamide 10 mg/kg iv, sodium meclofenamate 5 mg/kg iv, ODQ 2 mg/kg iv, miconazole 50 mg/kg iv, or TEA 60 mg/kg iv (Figs. 5A, 5B). The decreases in systemic arterial pressure and heart rate in response to iv injections of Na_2S and NaHS were significantly enhanced after administration of L-NAME, ODQ, TEA, and miconazole (Figs. 5A, 5B). The iv administration of the mitochondrial K_{ATP} channel antagonist 5-hydroxydecanoate in a dose of 10 mg/kg had no effect on the decrease in systemic arterial pressure in response to the H_2S donors (Fig. 5C). The effect of administration of a larger dose of glybenclamide on the decrease in systemic arterial pressure and heart rate in response to iv injection of Na_2S and NaHS 0.5 mg/kg was investigated in 7 rats and following administration of glybenclamide , in a total dose up to 20-30 mg/kg iv and/or ip decreases in systemic arterial pressure and heart rate in response to the H_2S donors were not attenuated.

The administration of L-NAME produced a significant well-maintained increase in systemic arterial pressure; whereas, glybenclamide, sodium meclofenamate, ODQ, miconazole, 5-hydroxydecanoate, and TEA had small inconsistent effects on systemic arterial pressure over the time course of these experiments.

The iv injections of K_{ATP} channel agonist cromakalim 30 μg/kg decreased systemic arterial pressure, and the decrease in pressure in response to cromakalim was attenuated after administration of glybenclamide 10 mg/kg iv (Fig. 6). The iv injection of cromakalim 30 μg/kg did not produce an increase in heart rate. The iv administration of glybenclamide produced a significant decrease in blood glucose levels (control 116±5 mg/dl; after glybenclamide 82±4 mg/dl).

DISCUSSION

The results of the present study show that iv injections of the H_2S donors Na_2S and NaHS decrease systemic arterial pressure in the rat and are consistent with previous results in the literature (18, 38, 46). New findings in the present study are that decreases in systemic arterial pressure in response to the H_2S donors are associated with dose-dependent decreases in heart rate and at higher doses significant decreases in cardiac output. These results indicate that the hypotensive response to the H_2S donors can be mediated by a decrease in systemic vascular resistance and a decrease in cardiac output or both. The decrease in heart rate in response to the H_2S donors can exceed 100 beats/min and was not attenuated by atropine indicating that the bradycardia is not mediated by an increase in parasympathetic tone. These data suggest that the effect of the H_2S donors is mediated by an action on the sinus node

and are consistent with studies showing that H_2S decreases action potential amplitude and has a suppressive effect on electrical activity in the isolated rat sinus node (1, 44). The decreases in cardiac output in response to the H_2S donor correlates with the decrease in heart rate and can probably be prevented or attenuated by cardiac pacing.

Decreases in systemic arterial pressure and heart rate in response to iv injections of the H_2S donors were rapid in onset and short in duration. The decrease in heart rate in response to the H_2S donors is different than the response to the NO donor SNP which does not induce bradycardia and can increase heart rate. The recovery phase of the arterial pressure response to high doses of the H_2S donors was associated with a secondary increase in systemic arterial pressure that was attenuated by the alpha receptor blocking agent phentolamine suggesting that the secondary increase in systemic arterial pressure was mediated by the activation of the adrenergic nervous system and may be a component of a reflex compensatory response. The decreases in arterial pressure and heart rate in response to iv injection of the H2S donors were not attenuated by treatment with atropine, phentolamine, or hexamethonium suggesting that they were not dependent on an effect on the central nervous system.

Because of the short duration of action and in order to investigate steady state responses to an H_2S donor the effect of an iv infusion of Na_2S was studied. The results of infusion studies show that the decreases in systemic arterial pressure, heart rate and cardiac output were maintained during the period of the infusion, and these parameters returned to control value several minutes after the infusion was terminated. These studies also show that the H_2S donor has metabolic effects in that blood glucose levels

were significantly reduced. The mechanism of this hypoglycemic effect is uncertain and will be addressed in future studies.

The effect of iv injections of the H_2S donors on the pulmonary vascular bed was investigated, and at higher doses pulmonary arterial pressure was decreased significantly. This was associated with a significant decrease in cardiac output. However, the H_2S donors did not decrease input pulmonary vascular resistance. The direct effect of the H_2S donors on the pulmonary vascular bed requires further study in experiments in which blood flow (cardiac output) is maintained constant and baseline tone in the pulmonary vascular bed is increased in the intact chest rat model.

The results of the present study are consistent with the observation that H_2S has hypotensive activity in the rat (50). However, the present data indicate that cardiovascular responses to the H_2S donors are complex. The observation that decreases in systemic and pulmonary arterial pressures can be associated with decreases in cardiac output suggests that hypotensive responses may be mediated by decreases in vascular tone, decreases in cardiac output, or a combination of effects on both determinants of systemic arterial pressure.

The mechanism by which H_2S decreases systemic arterial pressure in the rat has been investigated, and the results of an early study show that decreases in systemic arterial pressure in response to iv injection of an H_2S solution are attenuated by glybenclamide suggesting a role for K_{ATP} channels (9, 14, 23, 48-50). The role of K_{ATP} channels in mediating hypotensive responses to H_2S donors was investigated in the present study and following administration of glybenclamide in a dose that attenuated

the decrease in systemic arterial pressure in response to iv injection of the K_{ATP} agonist cromakalim, the decreases in systemic arterial pressure and heart rate in response to Na_2S and NaHS were not attenuated. The administration of glybenclamide decreased blood glucose levels which were restored in some experiments by an iv infusion of glucose, and the K_{ATP} channel antagonist decreased hypotensive responses to the K_{ATP} channel agonist cromakalim. The hypotensive responses to H_2S donors were not attenuated by 5-hydroxydecanoate which blocks mitochondrial K_{ATP} channels. These data obtained in a large number of experiments suggest that the decreases in systemic arterial pressure in response to the H_2S donors Na_2S and NaHS are not mediated by the activation of K_{ATP} channels in the membrane of vascular smooth muscle or endothelial cells or by activation of mitochondrial K_{ATP} channels in the cardiovascular system in the intact rat. In an attempt to provide more information about K_{ATP} channels, a second 10 mg/kg dose of glybenclamide was administered and decreases in systemic arterial pressure and heart rate in response to the H2S donors were not attenuated. The explanation for the different results in the present study and in the previous study is uncertain, and there are studies in the literature indicating that aortic vasorelaxant responses to H_2S are independent of K_{ATP} channel activation (8, 24).

It has been reported that the NO-cGMP pathway is involved in mediating or modulating vasorelaxant responses to H_2S and that H_2S is an endothelium dependent vasodilator agent (10, 12, 31). The role of the NO-cGMP pathway in mediating decreases in systemic arterial pressure in response to the H_2S donors was investigated in the rat. The administration of the NOS inhibitor L-NAME which increased systemic arterial pressure did not attenuate decreases in systemic arterial pressure in response

to iv injection of Na_2S and NaHS suggesting that NO is not involved in mediating the hypotensive response in the rat. The experiments with ODQ which inhibits the activation of soluble guanylyl cyclase (sGC) by NO indicate that increases in sGC activity and cGMP levels are not playing an important role in mediating or modulating hypotensive response to the H_2S donors in the rat. The observation that sodium meclofenamate or miconazole did not inhibit responses to Na_2S or NaHS suggests that decreases in systemic arterial pressure in the rat are not dependent on the formation of products in the arachidonic acid, cyclooxygenase, or P450 epoxygenase pathways.

It has been reported that H_2S induced relaxation of vas deferens smooth muscle is attenuated by inhibition of large conductance calcium-activated potassium channels which can be inhibited by TEA, a nonspecific K^+ channel antagonist, and are not blocked by NOS inhibitors or by glybenclamide (29). In order to determine if there was a role for large conductance calcium-activated potassium channels in mediating decreases in systemic arterial pressure in response to the H_2S donors, the effect of TEA was investigated. The results of these studies show that TEA in a dose that attenuated erectile responses to the H_2S donors in the rat in previous experiments did not inhibit decreases in systemic arterial pressure in response to iv injections of Na_2S or NaHS suggesting that a TEA-sensitive mechanism is not involved in mediating hypotensive responses to the H_2S donors in the rat. However, it should be mentioned that TEA is a broad spectrum K^+ channel antagonist that has actions on many tissues.

The mechanism by which the H_2S donors decrease systemic arterial pressure in the rat is uncertain and subject to conjecture. Although hypotensive responses to the donors are not blocked by L-NAME or ODQ, it has been reported that H_2S can be

converted to polysulfides that can oxidize protein kinase G (PKG) and induce PKG dimerization which can induce vasodilation in a NO-cGMP independent manner (5, 6, 40, 43). However the role of the PKG dimerization in mediating hypotensive responses to the H_2S donors in the present study is uncertain.

The purpose of the present study was to investigate and characterize cardiovascular responses to the H_2S donors in the anesthetized intact-chest rat. The results of these studies show that Na_2S and NaHS decrease systemic arterial pressure in the rat and are in agreement with the results of an early study (50). The present study extends previous results by showing that decreases in systemic arterial pressure in response to the H_2S donors are associated with a dose-dependent decrease in heart rate that is not mediated by the parasympathetic system and at higher doses the H_2S donors decrease cardiac output and systemic vascular resistance. Although the mechanism of the hypotensive response to the H_2S donors is not explained by the present data, the results of the present study show that decreases in systemic arterial pressure can be mediated by a decrease in systemic vascular resistance, a decrease in cardiac output, which is not central in nature, or a combination of these actions and that they are mediated by peripheral actions of the H_2S donors. The observation that blood pressure responses to the H_2S donors in the intact chest rat are not attenuated by glybenclamide or L-NAME does not mean that K_{ATP} channels or NO are not involved in mediating responses in some local vascular segments or regions. The present data indicate that K_{ATP} channels or NO do not play a major role in mediating the overall decreases in systemic arterial pressure in response to iv injections of the H_2S donors in the rat and are similar to data in eNOS knockout mice where the hypotensive response

to acetylcholine is not impaired but that vasodilator responses in some regional vascular segments isolated from eNOS knockout mice are attenuated (16, 25, 33).

The present data also show that H_2S donor injections or infusion can produce hypoglycemia which is not consistent with a stimulatory effect on K_{ATP} channels in the pancreatic beta cells and this action requires further investigation (3, 47).

In summary, the results of the present study show that the H_2S donors have the ability to decrease systemic arterial pressure in the rat by an by a peripheral effect on systemic vascular resistance and on cardiac output. These data indicate that the mechanism of the overall decrease in systemic arterial pressure in the rat is uncertain and that H_2S donors decrease heart rate by a mechanism that is independent of the parasympathetic nervous system.

LIMITATIONS

In regard to limitations in the present study the responses to H_2S were investigated using the H_2S donors, Na_2S and NaHS and not H_2S solutions prepared using H_2S gas which may produce different responses in the anesthetized rat. In addition, the role of K_{ATP} channels in mediating responses to H_2S may depend on species and the vascular the preparation studied. Although Na2S and NaHS have a short duration of action, these H2S donors have been used as pharmacologic agents in the treatment of cardiac ischemia-reperfusion injury (13, 32, 45). The mechanism of the putative vascular effect of the H_2S donors promoting vasodilation is not clarified in the present study and needs more direct examination in better controlled experiments.

ACKNOWLEGEMENTS

The present study was supported in part by NIH Grant HL 77421.

DISCLOSURES

No financial or other conflicts of interest are declared by the authors.

FIGURE LEGENDS

Figure 1. Bar graphs showing dose related decreases in systemic arterial pressure, heart rate, pulmonary arterial pressure, cardiac output, systemic and input pulmonary vascular resistances in responses to iv injections of Na_2S in doses of 0.03 – 0.5 mg/kg iv. The decreases in systemic arterial pressure and heart rate in response to all doses of Na_2S with the exception of the two lowest doses are significantly different from each other. *n* = 9. * with bar indicates $p<0.05$. ANOVA and paired comparison, Bonferroni correction. Pulmonary vascular resistance is input pulmonary vascular resistance because left atrial pressure was not measured.

Figure 2. Bar graphs showing dose related decreases in systemic arterial pressure, heart rate, pulmonary arterial pressure, cardiac output, systemic and pulmonary vascular resistances in responses to iv injections of NaHS in doses of 0.03 – 0.5 mg/kg iv. The decreases in systemic arterial pressure and heart rate in response to all doses of Na_2S with the exception of the two lowest doses are significantly different from each other. *n* = 9. * with bar indicates $p<0.05$. ANOVA and paired comparison, Bonferroni

correction. Pulmonary vascular resistance is input pulmonary vascular resistance because left atrial pressure was not measured.

Figure 3. Records from an experiment illustrating decreases in systemic arterial pressure, mean arterial pressure, and heart rate in response to an iv injection of Na_2S (0.5 mg/kg), NaHS (0.5 mg/kg), and SNP (10 µg/kg).

Figure 4. Line graph illustrating the decreases in mean arterial pressure, and heart rate in response to an iv infusion of Na_2S (0.3 mg/min) *n=10*(A). Bar graphs showing decreases in cardiac output and systemic vascular resistance in response to iv infusion of Na_2S (0.3 mg/min) *n=8*(B). * indicates $p<0.05$. paired comparison

Figure 5. Bar graphs showing the effect of the NOS inhibitor L-NAME (50 mg/kg iv), the K_{ATP} channel antagonist glybenclamide (10 mg/kg iv), the cyclooxygenase inhibitor sodium meclofenamate (5 mg/kg iv), the soluble guanylate cyclase inhibitor 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (2 mg/kg iv), the Ca^{2+}-activated K^+ channel inhibitor tetraethyl ammonium (60 mg/k iv), the p450 epoxygenase inhibitor miconazole (50 mg/kg iv) on decreases in mean systemic arterial pressure and heart rate in response to iv injections of Na_2S (0.03 – 0.5 mg/kg) (A), iv injections of NaHS (0.03 – 0.5 mg/kg) (B). Bar graphs showing the effect of the mitochondrial K^+_{ATP} channel antagonist 5-hydroxydecanoate (10 mg/kg iv) on decreases in

mean system arterial pressure in response to iv injections of Na_2S (0.03 – 0.5 mg/kg) and NaHS (0.03 – 0.5 mg/kg) (C). *n* ()=6-12 all groups. * indicates p<0.05. paired comparison.

Figure 6. Bar graphs showing the effect of the K_{ATP} channel antagonist glybenclamide (10 µg/kg iv) on the decreases in mean arterial pressure and the change in heart rate in response to iv injection of the K_{ATP} channel agonist cromakalim (30 µg/kg) (A) and acetylcholine (0.1 µg/kg iv) (B). *n* = 6. * indicates p < 0.05, paired comparison.

Figure 7 Bar graphs showing decreases in mean arterial pressure and changes in heart rate in response to iv injections of Na_2S (0.3 mg/kg), NaHS (0.3 mg/kg), and acetylcholine (0.1 µg/kg) before and after treatment with atropine (0.5 mg/kg iv) *n=8-10* (A). Bar graphs showing secondary increases in arterial pressure, decreases in arterial pressure in response to iv injection of Na_2S (0.5 mg/kg) and NaHS (0.5 mg/kg) before and after treatment with phentolamine (0.5 mg/kg iv). Bar graph showing increases in arterial pressure in response to norepinephrine (0.1 µg/kg iv) before and after treatment with phentolamine (0.5 mg/kg iv). *n* = *11*(C). * indicates p<0.05. paired comparison

References

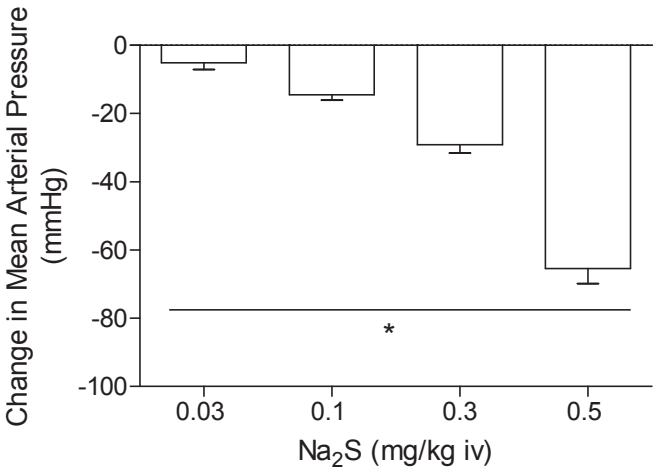
1. **Abramochkin DV, Moiseenko LS, and Kuzmin VS.** The effect of hydrogen sulfide on electrical activity of rat atrial myocardium. *Bulletin of experimental biology and medicine* 147: 683-686, 2009.
2. **Ali MY, Ping CY, Mok YY, Ling L, Whiteman M, Bhatia M, and Moore PK.** Regulation of vascular nitric oxide in vitro and in vivo; a new role for endogenous hydrogen sulphide? *British journal of pharmacology* 149: 625-634, 2006.
3. **Ali MY, Whiteman M, Low CM, and Moore PK.** Hydrogen sulphide reduces insulin secretion from HIT-T15 cells by a KATP channel-dependent pathway. *The Journal of endocrinology* 195: 105-112, 2007.
4. **Bian JS, Yong QC, Pan TT, Feng ZN, Ali MY, Zhou S, and Moore PK.** Role of hydrogen sulfide in the cardioprotection caused by ischemic preconditioning in the rat heart and cardiac myocytes. *The Journal of pharmacology and experimental therapeutics* 316: 670-678, 2006.
5. **Burgoyne JR and Eaton P.** Detecting disulfide-bound complexes and the oxidative regulation of cyclic nucleotide-dependent protein kinases by H2O2. *Methods in enzymology* 528: 111-128, 2013.
6. **Burgoyne JR, Prysyazhna O, Rudyk O, and Eaton P.** cGMP-dependent activation of protein kinase G precludes disulfide activation: implications for blood pressure control. *Hypertension* 60: 1301-1308, 2012.
7. **Calvert JW, Jha S, Gundewar S, Elrod JW, Ramachandran A, Pattillo CB, Kevil CG, and Lefer DJ.** Hydrogen sulfide mediates cardioprotection through Nrf2 signaling. *Circulation research* 105: 365-374, 2009.
8. **Cheang WS, Wong WT, Shen B, Lau CW, Tian XY, Tsang SY, Yao X, Chen ZY, and Huang Y.** 4-aminopyridine-sensitive K+ channels contributes to NaHS-induced membrane hyperpolarization and relaxation in the rat coronary artery. *Vascular pharmacology* 53: 94-98, 2010.
9. **Cheng Y, Ndisang JF, Tang G, Cao K, and Wang R.** Hydrogen sulfide-induced relaxation of resistance mesenteric artery beds of rats. *American journal of physiology Heart and circulatory physiology* 287: H2316-2323, 2004.
10. **Cortese-Krott MM, Fernandez BO, Kelm M, Butler AR, and Feelisch M.** On the chemical biology of the nitrite/sulfide interaction. *Nitric oxide : biology and chemistry / official journal of the Nitric Oxide Society*, 2014.
11. **Dabisch PA, Liles JT, Baber SR, Golwala NH, Murthy SN, and Kadowitz PJ.** Analysis of L-NAME-dependent and -resistant responses to acetylcholine in the rat. *American journal of physiology Heart and circulatory physiology* 294: H688-698, 2008.
12. **Edwards G, Feletou M, and Weston AH.** Hydrogen sulfide as an endothelium-derived hyperpolarizing factor in rodent mesenteric arteries. *Circulation research* 110: e13-14; author reply e15-16, 2012.
13. **Elrod JW, Calvert JW, Morrison J, Doeller JE, Kraus DW, Tao L, Jiao X, Scalia R, Kiss L, Szabo C, Kimura H, Chow CW, and Lefer DJ.** Hydrogen sulfide attenuates myocardial ischemia-reperfusion injury by preservation of mitochondrial function. *Proceedings of the National Academy of Sciences of the United States of America* 104: 15560-15565, 2007.

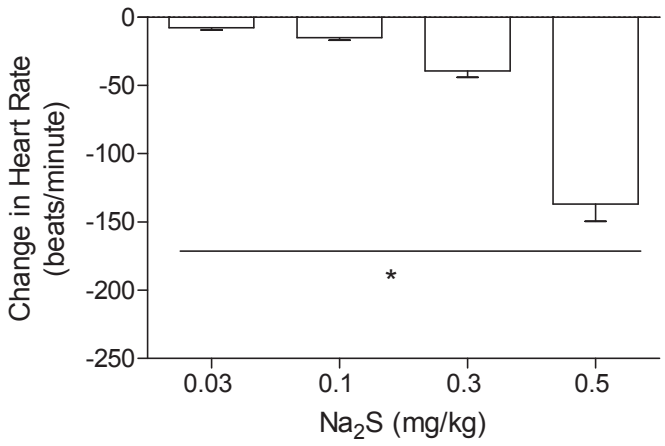
14. **Fiorucci S, Antonelli E, Mencarelli A, Orlandi S, Renga B, Rizzo G, Distrutti E, Shah V, and Morelli A.** The third gas: H2S regulates perfusion pressure in both the isolated and perfused normal rat liver and in cirrhosis. *Hepatology* 42: 539-548, 2005.
15. **Furchgott RF and Jothianandan D.** Endothelium-dependent and -independent vasodilation involving cyclic GMP: relaxation induced by nitric oxide, carbon monoxide and light. *Blood vessels* 28: 52-61, 1991.
16. **Garland CJ and McPherson GA.** Evidence that nitric oxide does not mediate the hyperpolarization and relaxation to acetylcholine in the rat small mesenteric artery. *British journal of pharmacology* 105: 429-435, 1992.
17. **Geng B, Yang J, Qi Y, Zhao J, Pang Y, Du J, and Tang C.** H2S generated by heart in rat and its effects on cardiac function. *Biochemical and biophysical research communications* 313: 362-368, 2004.
18. **Hosoki R, Matsuki N, and Kimura H.** The possible role of hydrogen sulfide as an endogenous smooth muscle relaxant in synergy with nitric oxide. *Biochemical and biophysical research communications* 237: 527-531, 1997.
19. **Hyman AL, Hao Q, Tower A, Kadowitz PJ, Champion HC, Gumusel B, and Lippton H.** Novel catheterization technique for the in vivo measurement of pulmonary vascular responses in rats. *The American journal of physiology* 274: H1218-1229, 1998.
20. **Ignarro LJ.** Biological actions and properties of endothelium-derived nitric oxide formed and released from artery and vein. *Circulation research* 65: 1-21, 1989.
21. **Ignarro LJ, Buga GM, Wood KS, Byrns RE, and Chaudhuri G.** Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proceedings of the National Academy of Sciences of the United States of America* 84: 9265-9269, 1987.
22. **Jackson-Weaver O, Paredes DA, Gonzalez Bosc LV, Walker BR, and Kanagy NL.** Intermittent hypoxia in rats increases myogenic tone through loss of hydrogen sulfide activation of large-conductance Ca(2+)-activated potassium channels. *Circulation research* 108: 1439-1447, 2011.
23. **Johansen D, Ytrehus K, and Baxter GF.** Exogenous hydrogen sulfide (H2S) protects against regional myocardial ischemia-reperfusion injury--Evidence for a role of K ATP channels. *Basic research in cardiology* 101: 53-60, 2006.
24. **Kiss L, Deitch EA, and Szabo C.** Hydrogen sulfide decreases adenosine triphosphate levels in aortic rings and leads to vasorelaxation via metabolic inhibition. *Life sciences* 83: 589-594, 2008.
25. **Koeppen M, Feil R, Siegl D, Feil S, Hofmann F, Pohl U, and de Wit C.** cGMP-dependent protein kinase mediates NO- but not acetylcholine-induced dilations in resistance vessels in vivo. *Hypertension* 44: 952-955, 2004.
26. **Kubo S, Doe I, Kurokawa Y, and Kawabata A.** Hydrogen sulfide causes relaxation in mouse bronchial smooth muscle. *Journal of pharmacological sciences* 104: 392-396, 2007.
27. **Li L, Hsu A, and Moore PK.** Actions and interactions of nitric oxide, carbon monoxide and hydrogen sulphide in the cardiovascular system and in inflammation--a tale of three gases! *Pharmacology & therapeutics* 123: 386-400, 2009.
28. **Li L, Rose P, and Moore PK.** Hydrogen sulfide and cell signaling. *Annual review of pharmacology and toxicology* 51: 169-187, 2011.

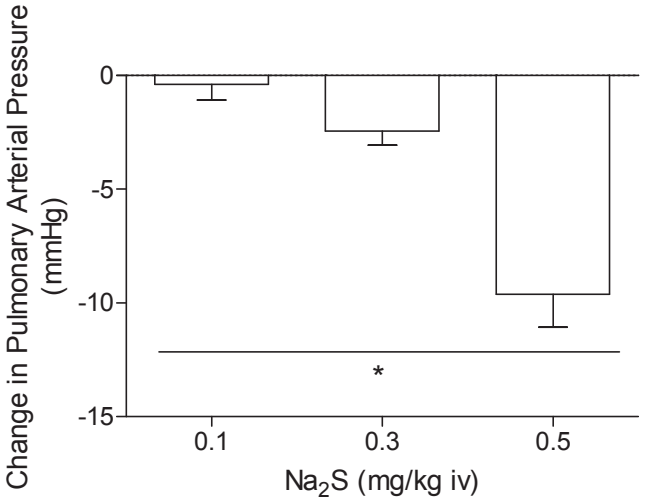
29. **Li Y, Zang Y, Fu S, Zhang H, Gao L, and Li J.** H2S relaxes vas deferens smooth muscle by modulating the large conductance Ca2+ -activated K+ (BKCa) channels via a redox mechanism. *The journal of sexual medicine* 9: 2806-2813, 2012.
30. **Murad F, Forstermann U, Nakane M, Schmidt H, Pollock J, Sheng H, Matsumoto T, Warner T, Mitchell J, Tracey R, and et al.** The nitric oxide-cyclic GMP signal transduction pathway in vascular smooth muscle preparations and other tissues. *Japanese journal of pharmacology* 58 Suppl 2: 150P-157P, 1992.
31. **Mustafa AK, Sikka G, Gazi SK, Steppan J, Jung SM, Bhunia AK, Barodka VM, Gazi FK, Barrow RK, Wang R, Amzel LM, Berkowitz DE, and Snyder SH.** Hydrogen sulfide as endothelium-derived hyperpolarizing factor sulfhydrates potassium channels. *Circulation research* 109: 1259-1268, 2011.
32. **Pan H, Xie X, Chen D, Zhang J, Zhou Y, and Yang G.** Protective and biogenesis effects of sodium hydrosulfide on brain mitochondria after cardiac arrest and resuscitation. *European journal of pharmacology* 741: 74-82, 2014.
33. **Pankey EA, Kassan M, Choi SK, Matrougui K, Nossaman BD, Hyman AL, and Kadowitz PJ.** Vasodilator responses to acetylcholine are not mediated by the activation of soluble guanylate cyclase or TRPV4 channels in the rat. *American journal of physiology Heart and circulatory physiology* 306: H1495-1506, 2014.
34. **Pankey EA, Thammasiboon S, Lasker GF, Baber S, Lasky JA, and Kadowitz PJ.** Imatinib attenuates monocrotaline pulmonary hypertension and has potent vasodilator activity in pulmonary and systemic vascular beds in the rat. *American journal of physiology Heart and circulatory physiology* 305: H1288-1296, 2013.
35. **Pankey EA, Zsombok A, Lasker GF, and Kadowitz PJ.** Analysis of responses to the TRPV4 agonist GSK1016790A in the pulmonary vascular bed of the intact-chest rat. *American journal of physiology Heart and circulatory physiology* 306: H33-40, 2014.
36. **Porter PN, Grishaver MS, and Jones OW.** Characterization of human cystathionine beta-synthase. Evidence for the identity of human L-serine dehydratase and cystathionine beta-synthase. *Biochimica et biophysica acta* 364: 128-139, 1974.
37. **Shibuya N, Tanaka M, Yoshida M, Ogasawara Y, Togawa T, Ishii K, and Kimura H.** 3-Mercaptopyruvate sulfurtransferase produces hydrogen sulfide and bound sulfane sulfur in the brain. *Antioxidants & redox signaling* 11: 703-714, 2009.
38. **Srilatha B, Adaikan PG, Li L, and Moore PK.** Hydrogen sulphide: a novel endogenous gasotransmitter facilitates erectile function. *The journal of sexual medicine* 4: 1304-1311, 2007.
39. **Stipanuk MH and Beck PW.** Characterization of the enzymic capacity for cysteine desulphhydration in liver and kidney of the rat. *The Biochemical journal* 206: 267-277, 1982.
40. **Stubbert D, Prysyazhna O, Rudyk O, Scotcher J, Burgoyne JR, and Eaton P.** Protein kinase g ialpha oxidation paradoxically underlies blood pressure lowering by the reductant hydrogen sulfide. *Hypertension* 64: 1344-1351, 2014.
41. **Uren JR, Ragin R, and Chaykovsky M.** Modulation of cysteine metabolism in mice--effects of propargylglycine and L-cyst(e)ine-degrading enzymes. *Biochemical pharmacology* 27: 2807-2814, 1978.
42. **Whiteman M, Li L, Kostetski I, Chu SH, Siau JL, Bhatia M, and Moore PK.** Evidence for the formation of a novel nitrosothiol from the gaseous mediators nitric oxide and hydrogen sulphide. *Biochemical and biophysical research communications* 343: 303-310, 2006.

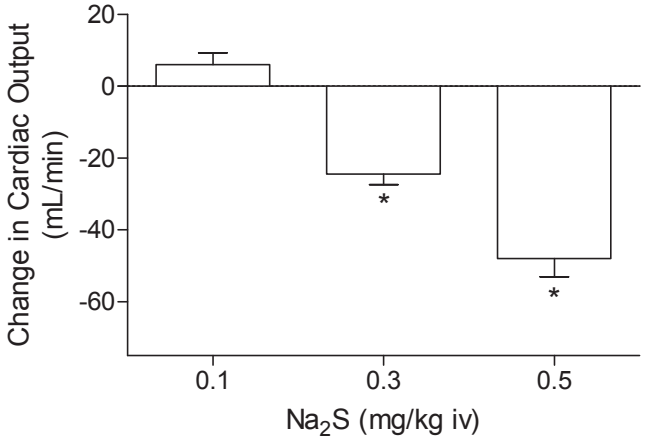
43. **Wolin MS.** Novel role for protein kinase g oxidative activation in the vasodilator and antihypertensive actions of hydrogen sulfide. *Hypertension* 64: 1196-1197, 2014.
44. **Xiao L, Wu YM, Zhang H, Liu YX, and He RR.** Hydrogen sulfide facilitates carotid sinus baroreflex in anesthetized rats. *Acta pharmacologica Sinica* 27: 294-298, 2006.
45. **Xie YH, Zhang N, Li LF, Zhang QZ, Xie LJ, Jiang H, Li LP, Hao N, and Zhang JX.** Hydrogen sulfide reduces regional myocardial ischemia injury through protection of mitochondrial function. *Molecular medicine reports* 10: 1907-1914, 2014.
46. **Yang G, Wu L, Jiang B, Yang W, Qi J, Cao K, Meng Q, Mustafa AK, Mu W, Zhang S, Snyder SH, and Wang R.** H2S as a physiologic vasorelaxant: hypertension in mice with deletion of cystathionine gamma-lyase. *Science* 322: 587-590, 2008.
47. **Yang W, Yang G, Jia X, Wu L, and Wang R.** Activation of KATP channels by H2S in rat insulin-secreting cells and the underlying mechanisms. *The Journal of physiology* 569: 519-531, 2005.
48. **Zhang Z, Huang H, Liu P, Tang C, and Wang J.** Hydrogen sulfide contributes to cardioprotection during ischemia-reperfusion injury by opening K ATP channels. *Canadian journal of physiology and pharmacology* 85: 1248-1253, 2007.
49. **Zhao W and Wang R.** H(2)S-induced vasorelaxation and underlying cellular and molecular mechanisms. *American journal of physiology Heart and circulatory physiology* 283: H474-480, 2002.
50. **Zhao W, Zhang J, Lu Y, and Wang R.** The vasorelaxant effect of H(2)S as a novel endogenous gaseous K(ATP) channel opener. *The EMBO journal* 20: 6008-6016, 2001.

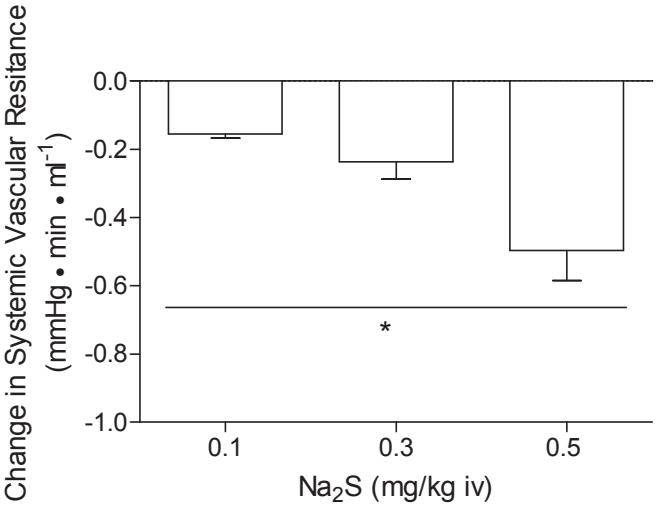
Figure 1


Change in Mean Arterial Pressure (mmHg)
0
-20
-40
-60
-80
-100
*
0.03
0.1
0.3
0.5
Na2S (mg/kg iv)


Change in Heart Rate (beats/minute)
0
-50
-100
-150
-200
-250
*
0.03
0.1
0.3
0.5
Na2S (mg/kg)


Change in Pulmonary Arterial Pressure (mmHg)
0
-5
-10
-15
*
0.1
0.3
0.5
Na2S (mg/kg iv)


Change in Cardiac Output (mL/min)
20
0
-20
-40
-60
*
*
0.1
0.3
0.5
Na2S (mg/kg iv)


Change in Systemic Vascular Resitance (mmHg • min • ml-1)
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
*
0.1
0.3
0.5
Na2S (mg/kg iv)

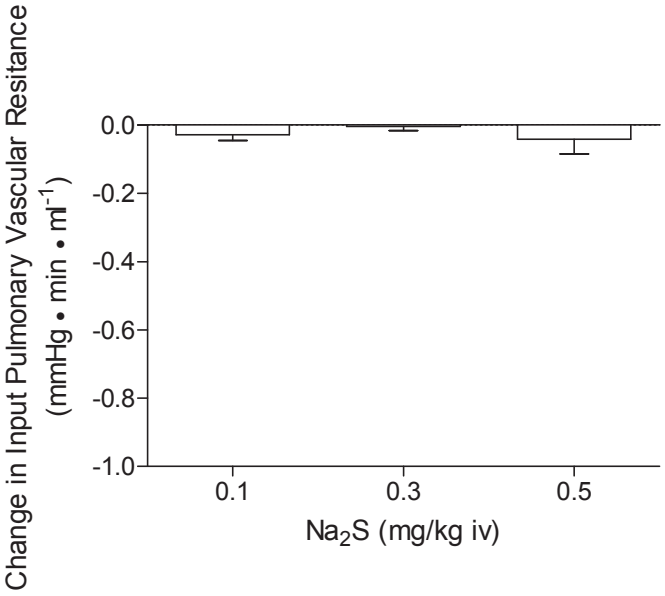
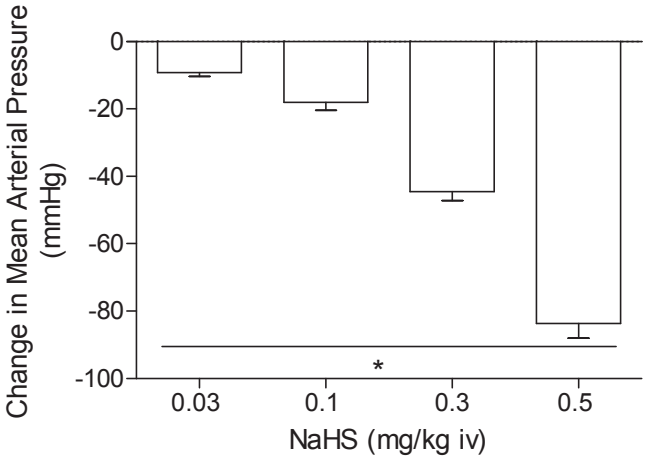
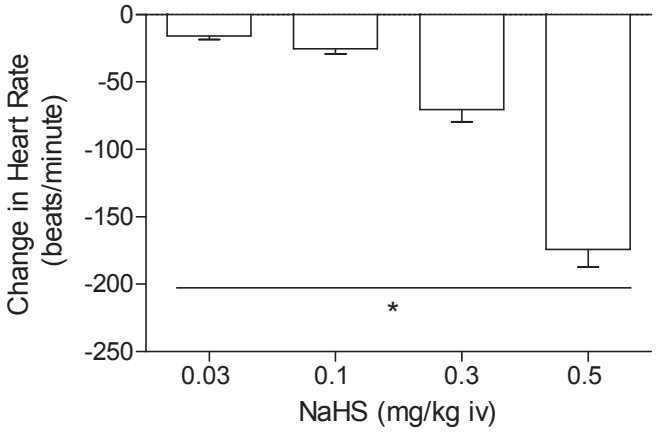
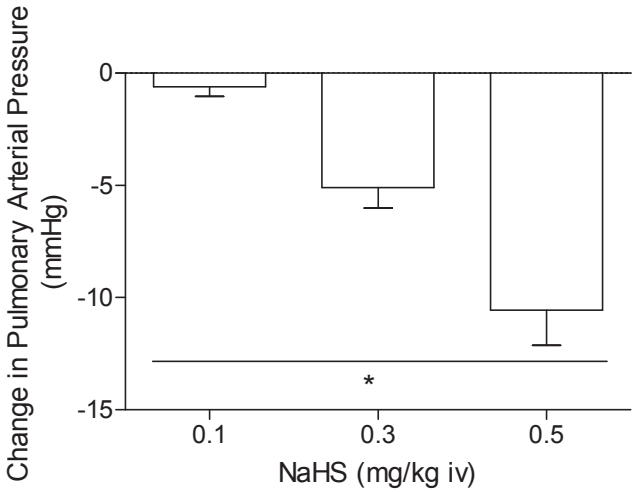
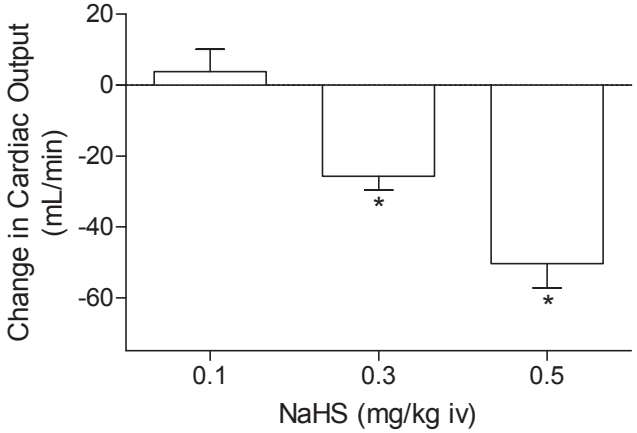

Change in Input Pulmonary Vascular Resitance (mmHg • min • ml-1)
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
0.1
0.3
0.5
Na2S (mg/kg iv)

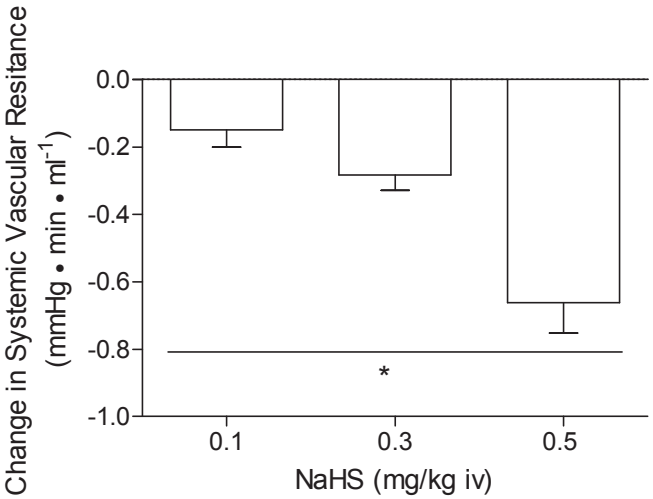
Figure 2


Change in Mean Arterial Pressure (mmHg)
0
-20
-40
-60
-80
-100
*
0.03
0.1
0.3
0.5
NaHS (mg/kg iv)


Change in Heart Rate (beats/minute)
0
-50
-100
-150
-200
-250
*
0.03
0.1
0.3
0.5
NaHS (mg/kg iv)


Change in Pulmonary Arterial Pressure (mmHg)
0
-5
-10
-15
*
0.1
0.3
0.5
NaHS (mg/kg iv)


Change in Cardiac Output (mL/min)
20
0
-20
-40
-60
*
*
0.1
0.3
0.5
NaHS (mg/kg iv)


Change in Systemic Vascular Resitance (mmHg • min • ml-1)
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
*
0.1
0.3
0.5
NaHS (mg/kg iv)

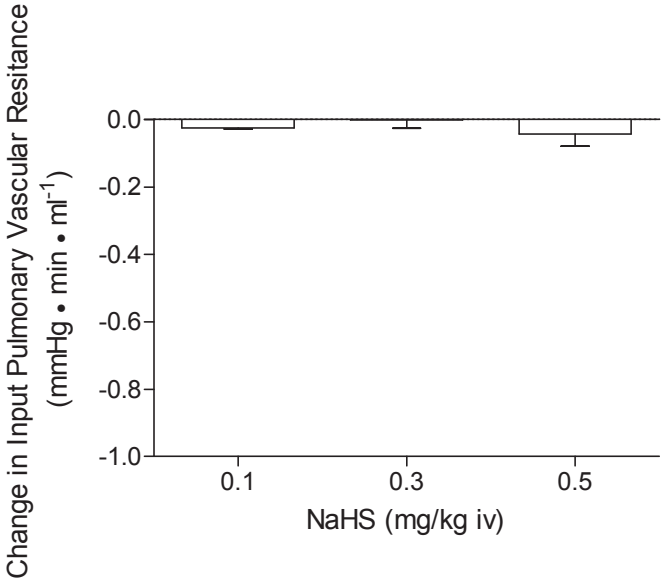

Change in Input Pulmonary Vascular Resitance (mmHg • min • ml-1)
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
0.1
0.3
0.5
NaHS (mg/kg iv)

Figure 3

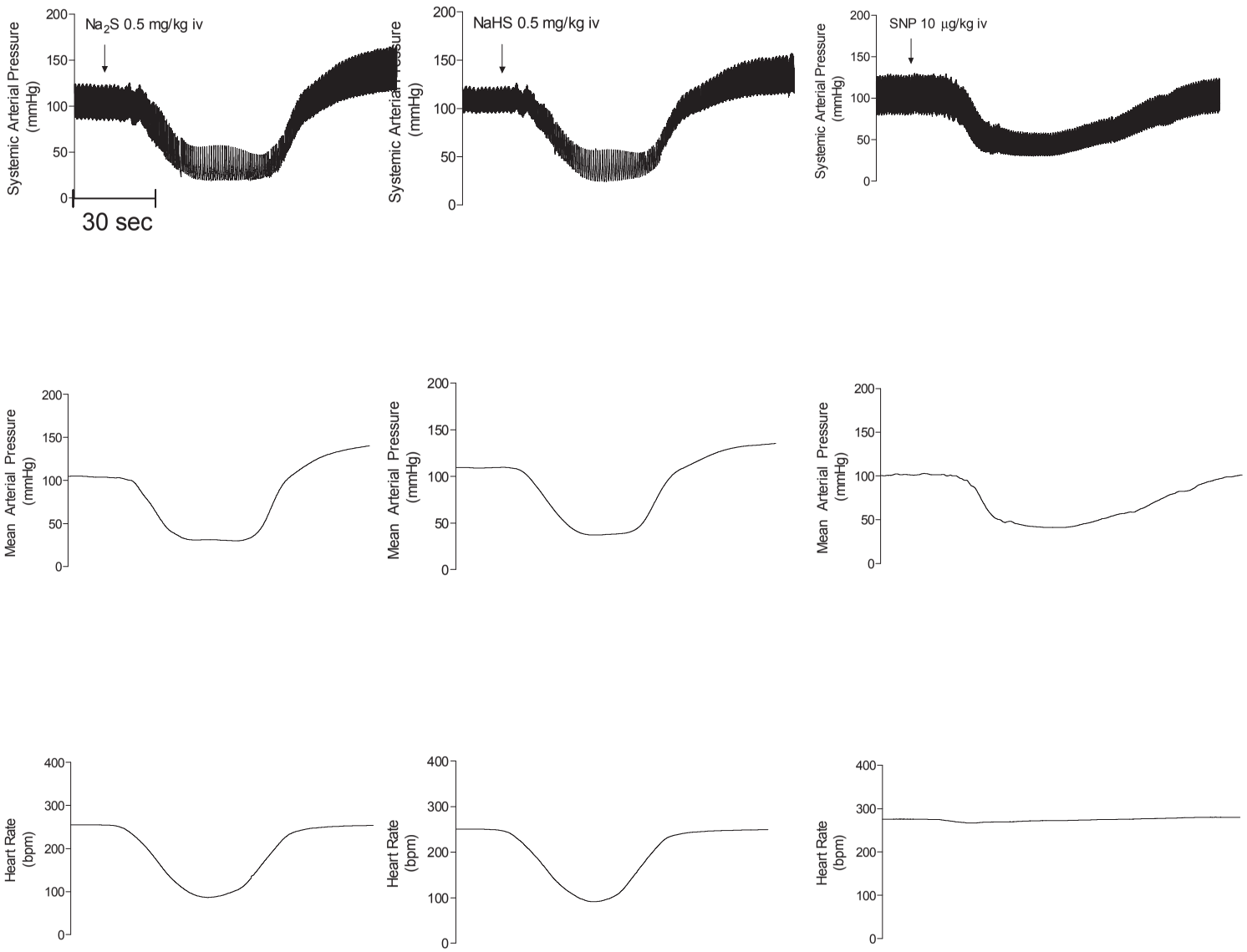

Na2S 0.5 mg/kg iv
NaHS 0.5 mg/kg iv
SNP 10 μg/kg iv
Systemic Arterial Pressure (mmHg)
Mean Arterial Pressure (mmHg)
Heart Rate (bpm)
30 sec
200
150
100
50
0
400
300

Figure 4

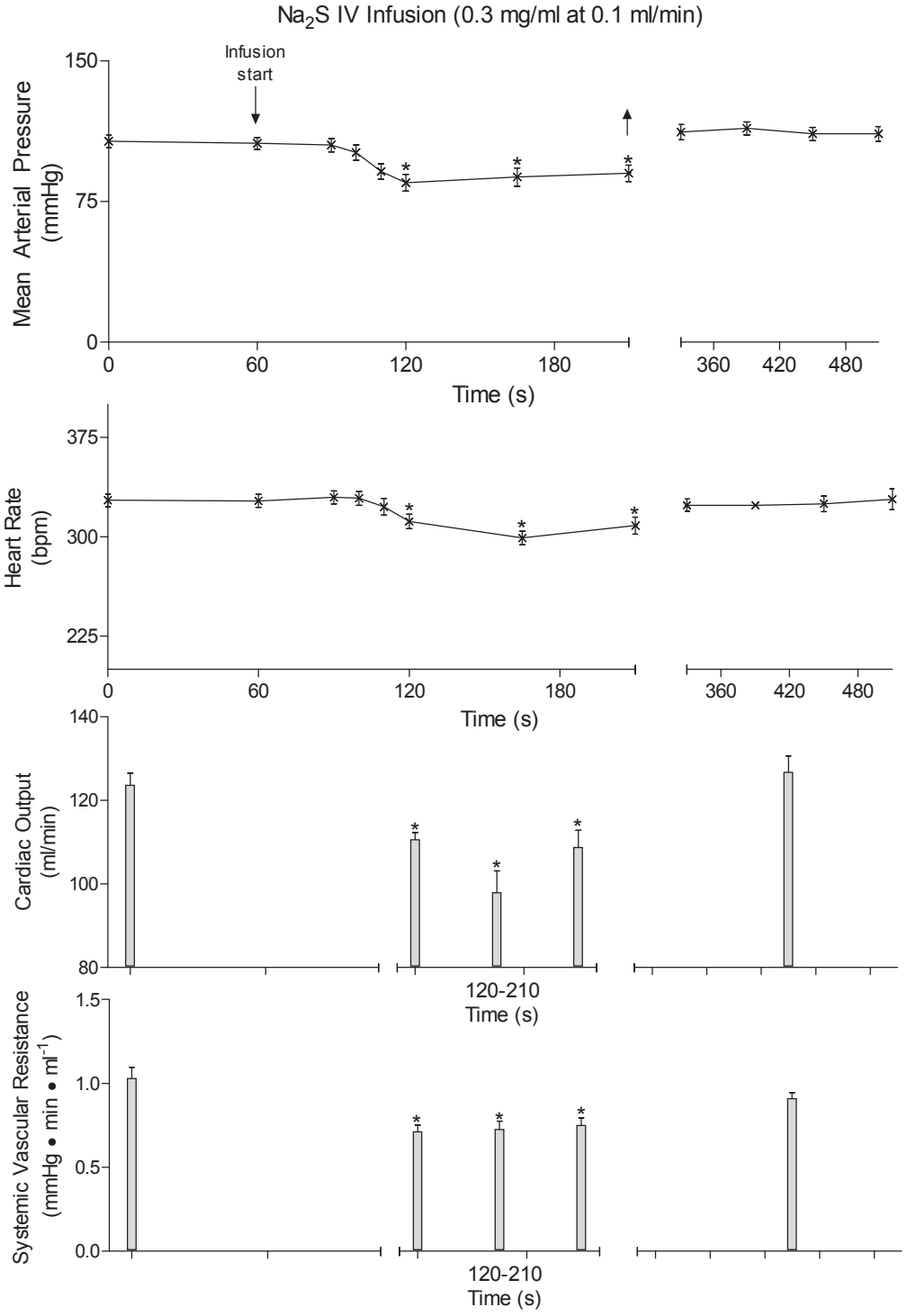

Na2S IV Infusion (0.3 mg/ml at 0.1 ml/min)
Infusion start
Mean Arterial Pressure (mmHg)
150
75
0
0
60
120
180
360
420
480
Time (s)
Heart Rate (bpm)
375
300
225
0
60
120
180
360
420
480
Time (s)
Cardiac Output (ml/min)
140
120
100
80
120-210
Time (s)
Systemic Vascular Resistance (mmHg • min • ml-1)
1.5
1.0
0.5
0.0
120-210
Time (s)

Figure 5

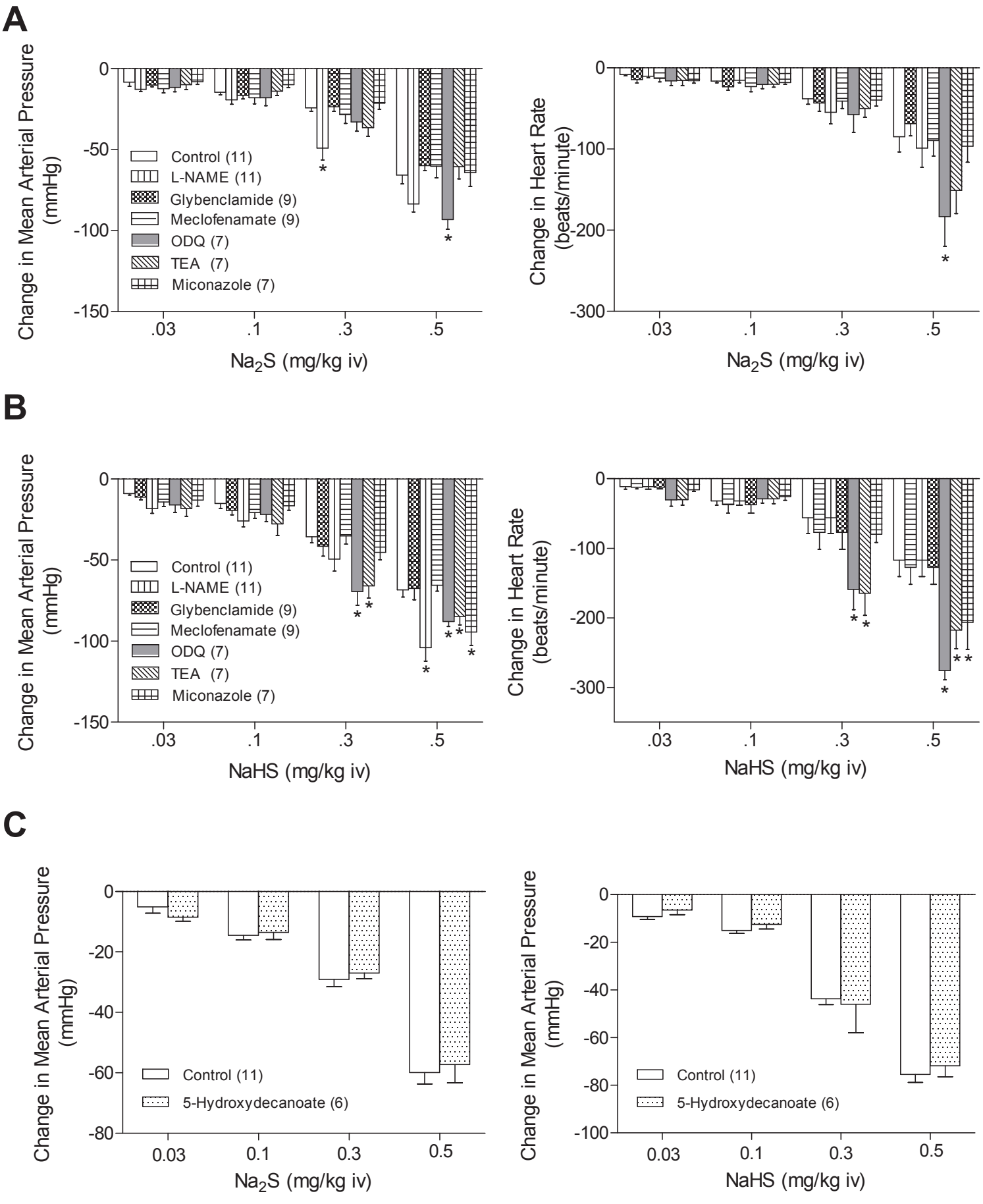

A
Change in Mean Arterial Pressure (mmHg)
0
-50
-100
-150
Control (11)
L-NAME (11)
Glybenclamide (9)
Meclofenamate (9)
ODQ (7)
TEA (7)
Miconazole (7)
.03
.1
.3
.5
Na2S (mg/kg iv)
Change in Heart Rate (beats/minute)
0
-100
-200
-300
.03
.1
.3
.5
Na2S (mg/kg iv)
B
Change in Mean Arterial Pressure (mmHg)
0
-50
-100
-150
Control (11)
L-NAME (11)
Glybenclamide (9)
Meclofenamate (9)
ODQ (7)
TEA (7)
Miconazole (7)
.03
.1
.3
.5
NaHS (mg/kg iv)
Change in Heart Rate (beats/minute)
0
-100
-200
-300
.03
.1
.3
.5
NaHS (mg/kg iv)
C
Change in Mean Arterial Pressure (mmHg)
0
-20
-40
-60
-80
Control (11)
5-Hydroxydecanoate (6)
0.03
0.1
0.3
0.5
Na2S (mg/kg iv)
Change in Mean Arterial Pressure (mmHg)
0
-20
-40
-60
-80
-100
Control (11)
5-Hydroxydecanoate (6)
0.03
0.1
0.3
0.5
NaHS (mg/kg iv)

Figure 6

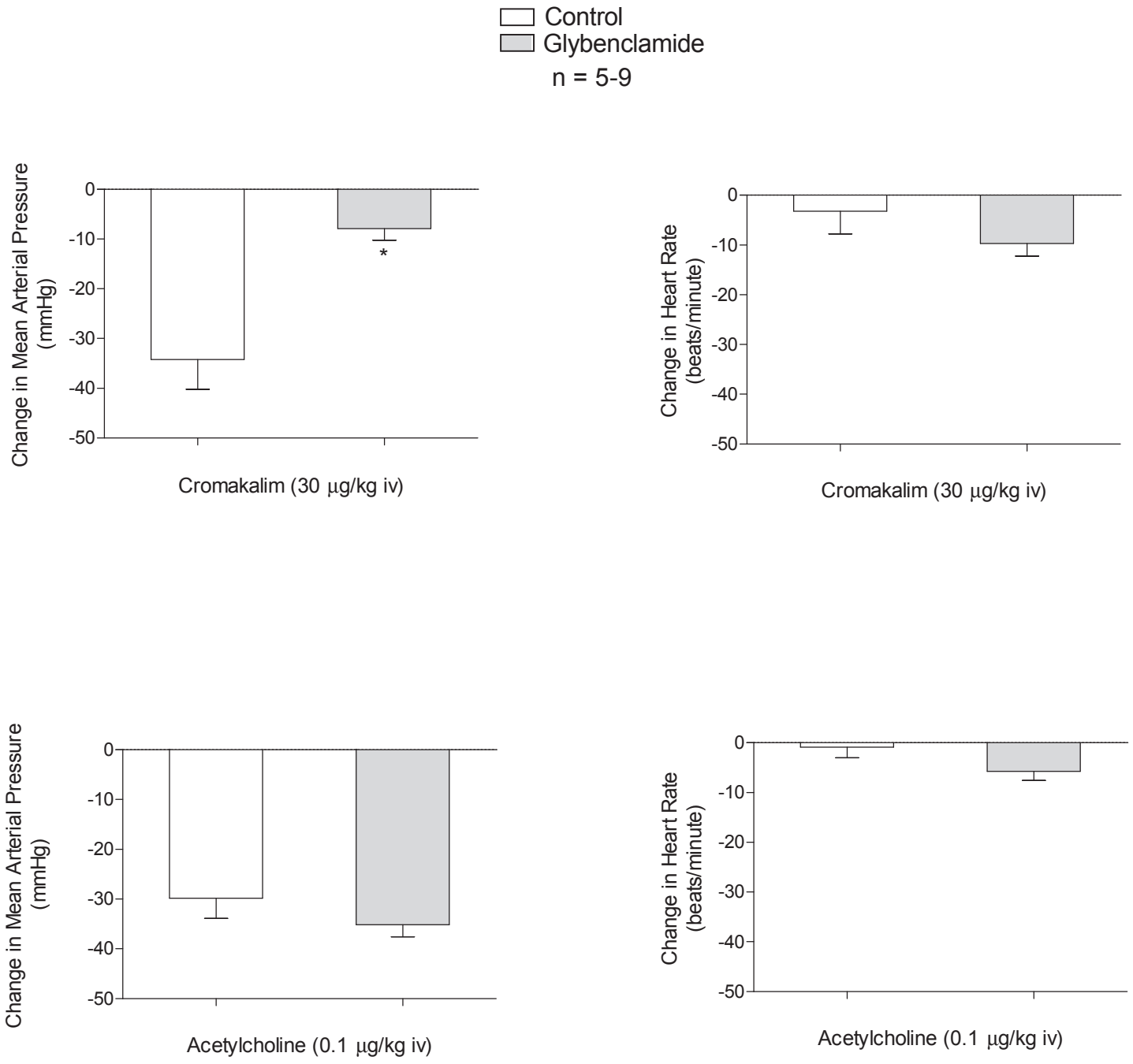
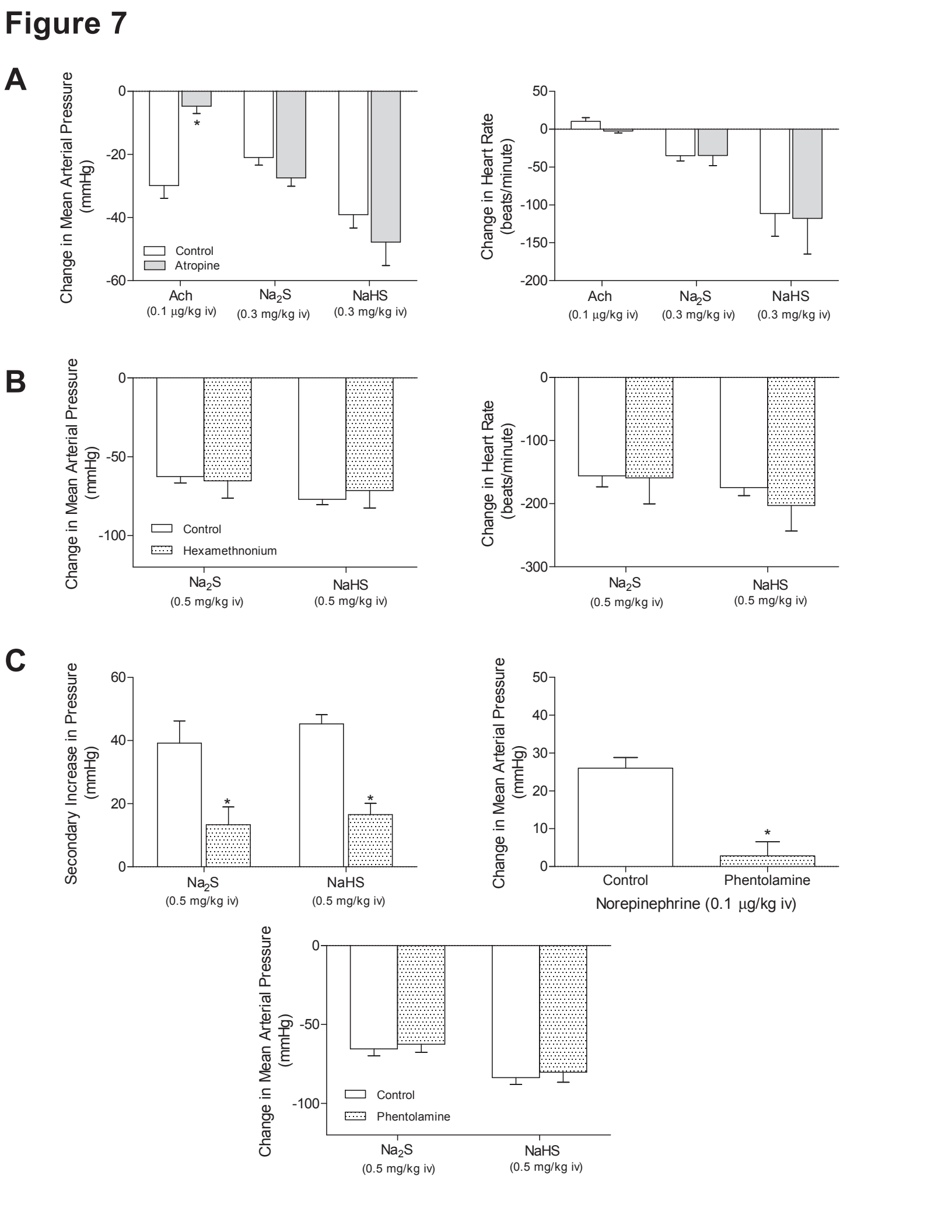

Control
Glybenclamide
n = 5-9
A
Change in Mean Arterial Pressure (mmHg)
0
-10
-20
-30
-40
-50
*
Cromakalim (30 µg/kg iv)
Change in Heart Rate (beats/minute)
0
-10
-20
-30
-40
-50
Cromakalim (30 µg/kg iv)
B
Change in Mean Arterial Pressure (mmHg)
0
-10
-20
-30
-40
-50
Acetylcholine (0.1 µg/kg iv)
Change in Heart Rate (beats/minute)
0
-10
-20
-30
-40
-50
Acetylcholine (0.1 µg/kg iv)

Figure 7


A
Change in Mean Arterial Pressure (mmHg)
0
-20
-40
-60
*
Control
Atropine
Ach
(0.1 μg/kg iv)
Na2S
(0.3 mg/kg iv)
NaHS
(0.3 mg/kg iv)
Change in Heart Rate (beats/minute)
50
0
-50
-100
-150
-200
Ach
(0.1 μg/kg iv)
Na2S
(0.3 mg/kg iv)
NaHS
(0.3 mg/kg iv)
B
Change in Mean Arterial Pressure (mmHg)
0
-50
-100
Control
Hexamethnonium
Na2S
(0.5 mg/kg iv)
NaHS
(0.5 mg/kg iv)
Change in Heart Rate (beats/minute)
0
-100
-200
-300
Na2S
(0.5 mg/kg iv)
NaHS
(0.5 mg/kg iv)
C
Secondary Increase in Pressure (mmHg)
60
40
20
0
*
*
Na2S
(0.5 mg/kg iv)
NaHS
(0.5 mg/kg iv)
Change in Mean Arterial Pressure (mmHg)
50
40
30
20
10
0
*
Control
Phentolamine
Norepinephrine (0.1 μg/kg iv)
Change in Mean Arterial Pressure (mmHg)
0
-50
-100
Control
Phentolamine
Na2S
(0.5 mg/kg iv)
NaHS
(0.5 mg/kg iv)