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Circulatory consequences of reduced endogenous nitric oxide production during small-volume resuscitation

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Hypertonic small-volume resuscitation transiently restores the cardiovascular function during various circulatory disturbances. Nitric oxide (NO) is an important mediator of flow-induced peripheral and central hemodynamic changes, and therefore, we hypothesized that a decreased endogenous NO production could influence the consequences and the effectiveness of hypertonic fluid therapy. The main goal of this study was to outline and compare the circulatory effects small volume hypertonic saline-dextran (HSD, 7.5% NaCl-10% dextran; 4 ml/kg iv) infusion with (n=7) or without (n=7) artificially diminished NO production in normovolemic anesthetized dogs. HSD administration significantly increased cardiac index (CI), coronary flow (CF) and myocardial contractility, and elevated plasma nitrite/nitrate (NO_x) and endothelin-1 (ET-1) levels. However, the late (2 h) postinfusion period was characterized by significantly decreased myocardial NO synthase (NOS) and enhanced myeloperoxidase activities. Pre-treatment with the non-selective NOS inhibitor N-nitro-L-arginine (NNA, 4 mg/kg) immediately increased cardiac contractility, and the HSD-induced CI and CF elevations and the positive inotropy were absent. Additionally, plasma ET-1 levels increased and NO_x levels were significantly decreased. In conclusion, our results demonstrate that HSD infusion leads to preponderant vasoconstriction when endogenous NO synthesis is diminished, and this could explain the loss of effectiveness of HSD resuscitation in NO-deficient states.

Keywords: nitric oxide, hypertonic saline–dextran, endothelin-1, cardiac contractility

Nitric oxide (NO) is a broad-spectrum regulator of the cardiovascular homeostasis; it modulates the systemic blood pressure but participates in vascular remodelling and angiogenesis as well (23, 35, 36). A variable-level, but steady generation of NO is achieved by a family of NO synthase (NOS) isoenzymes, including neuronal NOS (nNOS), inducible NOS (iNOS) and endothelial NOS (eNOS) (35). NO is produced by many cells in the body; however, its production by the vascular endothelium is particularly important in the regulation of the blood flow, through the Ca^{2+}/calmodulin-dependent constitutive types of NOS (cNOS) activity (27, 35).

The integrity of the endothelium is essential for flow-mediated vasodilation (26), which is largely due to the release of NO (17, 25). Physiologically, the most important stimulus for the continuous formation of NO is the viscous drag (shear stress) generated by the streaming blood on the endothelial layer (27). Indeed, it has been demonstrated that endothelial dysfunction is closely associated with reduced NO bioavailability in the cardiovascular system (8, 26).

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Disturbances of flow-mediated vasodilation may have several pathophysiological consequences. Humans with atherosclerosis, diabetes or hypertension often exhibit impaired NO pathways (9, 33). In patients with coronary artery disease and impairment of the endothelial vasodilatory function, increases in blood flow through the diseased coronary artery cause paradoxical vasoconstriction, which can contribute to myocardial ischemia during exercise or mental stress (42). In hypercholesterolemic animals and man, endothelium-dependent relaxation is reduced and vasoconstriction is enhanced. Again, this is largely due to a reduction in NO activity, and not to the physical absence of the endothelium (9).

Small-volume resuscitation by means of hypertonic solutions has been proposed as an effective means for restoration of cardiovascular function in acute circulatory failure. Indeed, it has been shown that small-volume resuscitation with hypertonic saline-dextran (HSD) solutions improves the cardiovascular function much more efficiently than normotonic volume replacement (28, 29, 49). The primary target organ of fluid replacement is the vascular endothelium, a sensitive sensory and transmitter surface between the circulating blood and the wall of the vessels throughout the body, where several vasoactive mediators of endothelial origin are synthesized and released (14, 30). Our earlier study demonstrated that the HSD infusion-induced peripheral flow stimulus correlated with cardiac contractility changes in line with an enhanced peripheral NO and endothelin-1 (ET-1) output (11). Another study from our laboratory pointed out that the nonspecific inhibition of NOS by N-nitro-L-arginine (NNA) enhances the ET-A receptor-dependent myocardial contractile responses (7).

Several experimental and clinical studies have investigated the efficacy, dosages and infusion rates of different hyperosmotic solutions: primarily 7.5% hypertonic saline administered alone or in combination with dextran or hetastarch (3, 29). Although the clinical data do not indicate that the administration of hypertonic solutions increases mortality in the clinical setting (50, 52). The results of experimental studies suggest that the administration of HSD exacerbates bleeding from injured vessels and often leads to early death in anesthetized animals (30, 48).

As we hypothesized that the bioavailability of NO, or its amount relative to other inotropic agents is of crucial importance in vasoregulation, we sought to examine the consequences of artificially diminished NO production on the peripheral and central hemodynamics. Secondly, we assumed that a decreased endogenous NO production could play significant roles in the circulatory consequences of HSD infusion. With these objectives, we set out to identify the HSD-induced circulatory and myocardial contractility changes caused by NO-dependent and NO-independent mechanisms by using the non-selective NOS inhibitor (NNA) prior to HSD infusions.

Materials and Methods

The experiments were performed in adherence to the NIH guidelines for the use of experimental animals. The study was approved by the Ethical Committee for the Protection of Animals in Scientific Research at the University of Szeged.

Surgical preparation

The experiments were performed on f 24 inbred mongrel dogs (average weight 17 ± 2.1 kg). Anesthesia was induced with sodium pentobarbital (30 mg kg^{-1} iv) and sustained with 0.2 mg kg^{-1} h^{-1} supplementary doses. After intubation of the trachea, the animals were mechanically ventilated with room air (Harvard Apparatus, South Natick, MA, U.S.A.). The left femoral

artery and vein were cannulated for the measurement of mean arterial pressure (MAP) and the administration of drugs and fluids, respectively. Blood gas parameters were regularly controlled throughout the experiments. The animals received 15 ml kg^{-1} h^{-1} Ringer's lactate infusion during surgical procedure, until the start of HSD treatment. A Swan-Ganz thermodilution catheter (Corodyn TD-E-N, 5011-110-7Fr; Braun Melsungen AG, Melsungen, Germany) was positioned into the pulmonary artery via the right femoral vein to measure the cardiac otput (CO) and calculate the cardiac index (CI). To measure coronary blood flow, an ultrasonic flow probe (Transonic Systems, Ithaca, NY, USA) was placed around the left circumflex coronary artery supplying the left ventricle.

An inflatable balloon-catheter (Foley catheter, 14Fr, Kendall Company Ltd., Basingstoke, U.K.) was introduced into the inferior caval vein via the left jugular vein. The filling volume was 10 ml. A catheter tip micromanometer (Millar Instruments Inc., Houston, TX, U.S.A.) was introduced into the left ventricle through the left internal carotid artery to monitor the LV pressure (LVP). A left thoracotomy was performed at the sixth intercostal space and the pericardium was opened. A pair of ultrasonic dimension crystals (3 MHz, ID-4, Custom Transducers, Poway, CA, U.S.A.) were sutured onto the anterior and posterior walls of the left ventricle, opposite each other, using an atraumatic surgical technique for measurement of the LV diameter (LVD). The thoracic cavity was revised and the chest wall was closed in four layers. The air was removed from the thorax; the animals were then breathing spontaneously. Their body temperature was maintained at 38 ^{o}C with a homeothermic blanket. At the end of the experiments, a myocardial tissue biopsy sample was taken from the left ventricle and the animals were killed with an overdose of pentobarbital.

Macrohemodynamic measurements

All hemodynamic signals (pressures, LVP, LVD and coronary flow) were registered with a computerized data-acquisition system (SPEL Advanced Haemosys 2.72, Experimetria Ltd., Budapest, Hungary). The MAP and central venous pressure (CVP) were monitored with Statham P23Db transducers. The heart rate (HR) was calculated from the MAP curve. The CO was determined by thermodilution, using a Cardiostar CO-100 computer (Experimetria Ltd., Budapest, Hungary), normalized for body weight and expressed as CI (ml kg^{-1} min^{-1}). The total peripheral vascular resistance (TPR) was calculated via the standard formula.

The ultrasonic dimension crystals were connected to a sonomicrometer (Triton Technology, Inc., San Diego, CA, U.S.A.). Via the LVP and LVD signals, the end-systolic elastance, as a parameter of the LV myocardial contractility, was estimated from the slope of the end-systolic pressure *vs* diameter relationship (20) with a computer program developed by our group. The inferior caval vein was briefly occluded by a balloon catheter, and the pressure *vs* diameter loops were registered for 8 s. The end-systolic points of the loops (which can be fitted to a sigmoid curve) were recorded. The linear part of the curve was selected on the basis of the lowest variance, and a straight line was fitted to the selected points. The computer program calculated contractility as the slope of the end-systolic pressure *vs* diameter relationship, and the variance of fitting was determined. The calculation was based on a minimum of 8 cardiac cycles.

Biochemical measurements

Plasma ET-1 measurements

Two-ml blood samples were drawn from the jugular vein into chilled polypropylene tubes containing EDTA (1 mg ml^{-1}) and aprotinin (Trasylol, Bayer, Leverkusen, Germany) (500

KIU/mL) before and after NNA infusions, and at the end of the observation period. The blood samples were centrifuged at 1200 g for 10 min at 4 °C. The plasma samples were then collected and stored at –70 °C until assay. Plasma samples were analyzed for ET-1 with an ELISA kit (Biomedica, Vienna, Austria). According to the manufacturer, the cross-reactivity with ET-1 and ET-2 was 100%.

Plasma nitrite/nitrate level measurements

The levels of plasma nitrite/nitrate (NO_x), stable end-products of NO, were measured by the Griess reaction. The assay depends on the enzymatic reduction of nitrate to nitrite, which was then converted into a colored azo compound detected spectrophotometrically at 540 nm (37).

NOS activity measurements

NO formation in cardiac tissues was measured via the conversion of [^{3}H]L-citrulline from [^{3}H]L-arginine (11). Briefly, heart biopsies kept on ice were homogenized in phosphate buffer (pH 7.4) containing 50 mM Tris-HCl, 0.1 mM EDTA, 0.5 mM dithiotreitol, 1 mM phenylmethylsulfonyl fluoride, 10 μg ml^{-1} soybean trypsin inhibitor and 10 μg ml^{-1} leupeptin. The homogenate was centrifuged at 4 °C for 20 min at 24,000 *g* and the supernatant was loaded into centrifugal concentrator tubes (Amicon Centricon-100; 100,000 MW cut-off ultrafilter). The tubes were centrifuged at 1000 *g* for 150 min and the concentrated supernatant was washed out from the ultrafilter with 250 μl homogenizing buffer. The samples were incubated with a cation-exchange resin (Dowex AG 50W-X8, Na^+ form) for 5 min to deplete endogenous L-arginine. The resin was separated by centrifugation (1500 *g* for 10 min) and the supernatant containing the enzyme was assayed for NOS activity.

For the Ca^{2+}-dependent NOS (cNOS) activity, 50 μl enzyme extract and 100 μl reaction mixture (pH 7.4, containing 50 mM Tris-HCl buffer, 1 mM NADPH, 10 μM tetrahydrobiopterine, 1.5 mM $CaCl_2$, 100 U ml^{-1} calmodulin and 0.5 μCi [^{3}H]L-arginine (Amersham U.K., specific activity 63 Ci mmol^{-1})) were incubated together for 60 min at 37 °C. The reaction was stopped by the addition of 1 ml ice-cold HEPES buffer (pH 5.5) containing 2 mM EGTA and 2 mM EDTA. Measurements were performed with the NOS inhibitor NNA (3.2 mM) to determine the extent of [^{3}H]L-citrulline formation independent of the NOS activity. Ca^{2+}-independent NOS activity (iNOS) was measured without Ca-calmodulin and with EGTA (8 mM). One ml reaction mixture was applied to Dowex cation-exchange resin (AG 50W-X8, Na^+ form) and eluted with 2 ml distilled water. The eluted [^{3}H] L-citrulline activity was measured with a scintillation counter (Tri-Carb Liquid Scintillation Analyzer 2100TR/2300TR, Packard Instrument Co, Meriden, CT, U.S.A.). Protein contents of samples were determined by the Lowry method.

Myocardial myeloperoxidase (MPO) activity measurement

The MPO activity, as a marker of tissue polymorphonuclear leukocyte infiltration, was measured via cardiac muscle biopsies (32). Briefly, the sample was homogenized with Tris-HCl buffer (0.1 M, pH 7.4) containing 0.1 mM phenylmethylsulfonyl fluoride to block tissue proteases, and then centrifuged at 4 °C for 20 min at 2000 *g*. The MPO activities of the samples were measured at 450 nm (UV-1601 spectrophotometer, Shimadzu, Japan) and the data were referred to the protein content.

Experimental protocols

Surgery was followed by a recovery period for cardiovascular stabilization, and baseline variables were then determined during a 30-min control period. The animals were randomly allocated to one or other of three groups. Group 1 (n = 10), which as control, was treated with 0.9% saline (4 ml kg^{-1}), while Groups 2 (n = 7) and 3 (n = 7) were infused iv with 4 ml kg^{-1} HSD during 15 min. The solution was prepared from isotonic 10% dextran-40 (Baxter, Munich, Germany) and 7.2% NaCl solution. The animals in Group 3 were additionally treated with 4 mg kg^{-1} NNA (Sigma Chem., U.S.A.) in 2 ml kg^{-1} saline during a 5-min iv infusion 15 min before HSD treatment. The beginning of HSD infusion served as the zero point of the experiments, and hemodynamic measurements were performed every 30 min. The animals were observed for a further 120 min in all groups.

Statistical analysis

Data analysis was performed with a statistical software package (SigmaStat for Windows, Jandel Scientific, Erkrath, Germany). Nonparametric methods were used. Friedman repeated measures analysis of variance on ranks was applied within the groups. Time-dependent differences from the baseline (time 0) for each group were assessed by Dunn's method, and differences between groups were analyzed with Kruskal-Wallis one-way analysis of variance on ranks, followed by Dunn's method for pairwise multiple comparison. In the Figures and Table 1, median values and 75th and 25th percentiles are given. P values < 0.05 were considered significant.

Results

The concentration of the HSD solution and the optimal conditions for the volume expander protocol were determined in pilot studies (data not shown). In the control group (Group 1), there were no significant hemodynamic changes as compared with the baseline values, and the mediator level did not change significantly during the 120-min observation period.

Hemodynamic effects of HSD infusion with or without NNA treatment

The HSD-induced peripheral circulatory reaction was characterized by transient MAP (Fig. 1A) and CVP increases (after HSD infusion: 3.8±0.26 mmHg *vs* control: 1.7±0.45 mmHg). TPR showed a biphasic change: an initial decrease was followed by a return to the baseline during the early phase of the postinfusion period, and the TPR was significantly elevated at 120 min in the postinfusion period (Fig. 1B). NNA pretreatment significantly elevated the MAP before HSD infusion, but there was no significant difference between the two HSD-infused groups (Fig. 1A). This pretreatment caused significant, long-lasting elevations in CVP (after NNA+HSD infusion: 5.9±0.65 mmHg) and TPR relative to the HSD and control groups (Fig. 1B).

The cardiac consequences of the HSD-induced volume loading included a significant increase in CI (Fig. 2A), together with a gradually elevated HR (Fig. 1C). HSD caused a marked and significant elevation in coronary blood flow, too (Fig. 2B). HSD infusion significantly increased the LVD and myocardial contractility until 60 min of the postinfusion period (Fig. 3A and B).

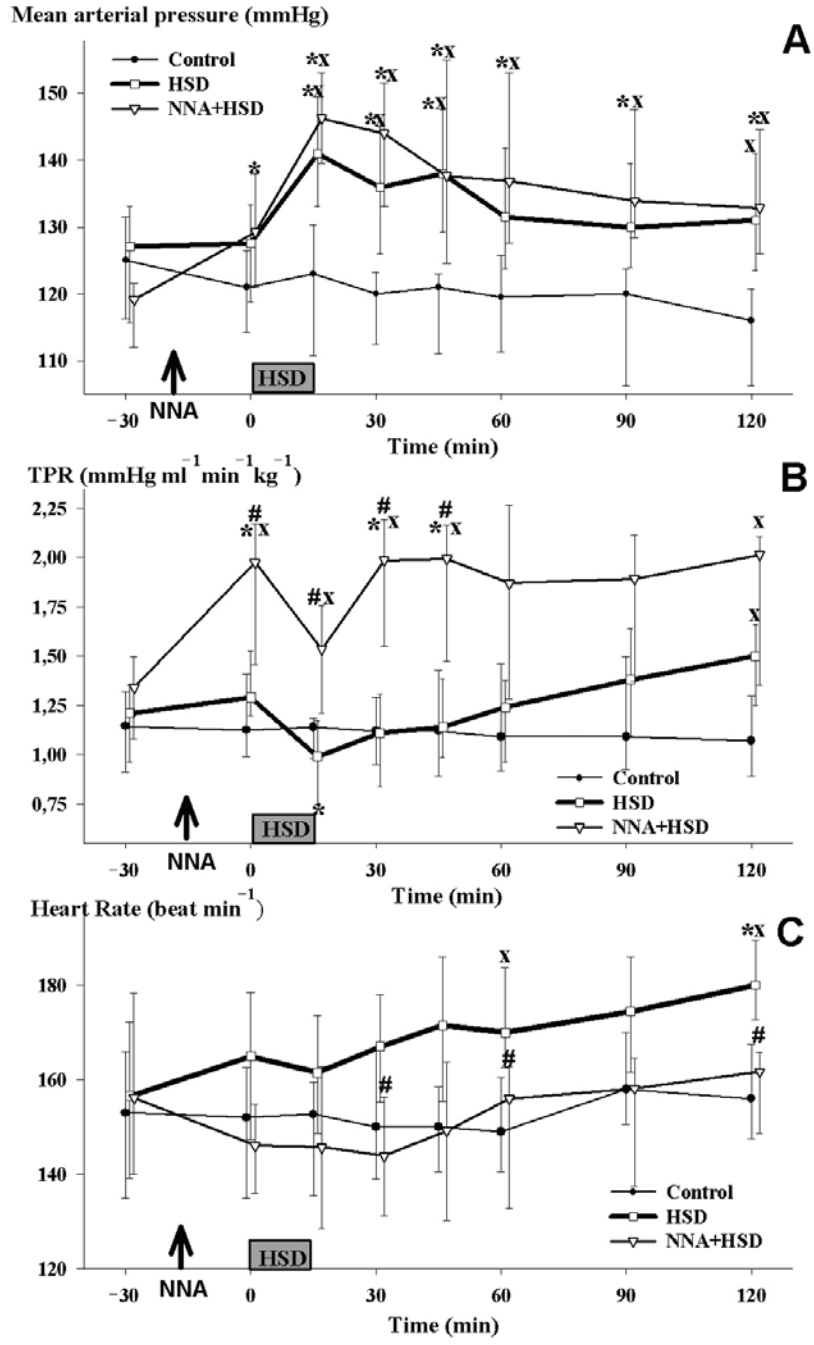

Fig. 1. Changes in MAP (A), TPR (B) and in HR in the saline-treated control group (circles), the HSD-treated group (squares) and the NNA+HSD treated group (triangles). * $P < 0.05$ within group; ^{X} $P < 0.05$ between groups *vs* saline-treated control group values; $^{\#}$ $P < 0.05$ between groups *vs* HSD-treated group values

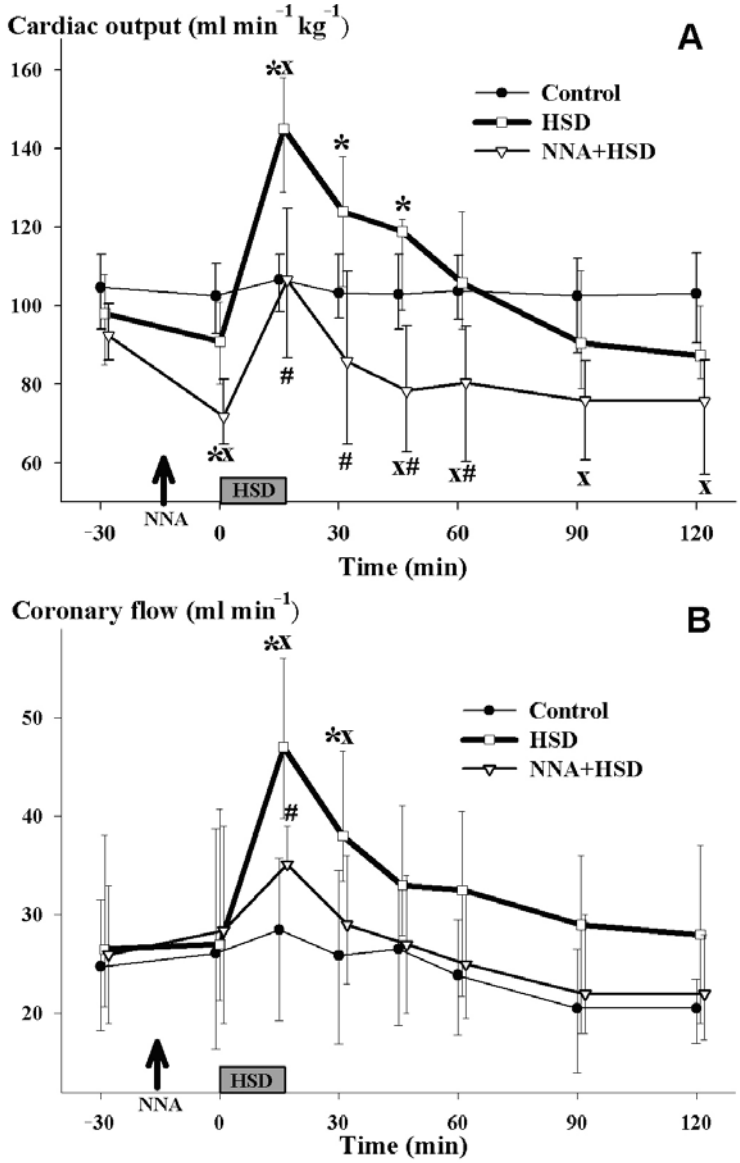

Fig. 2. Changes in CI (A) and in coronary flow (B) in the saline-treated control group (circles), the HSD-treated group (squares) and the NNA+HSD treated group (triangles). * $P < 0.05$ within group; ^{x} $P < 0.05$ between groups *vs* saline-treated control group values; $^{\#}$ $P < 0.05$ between groups *vs* HSD-treated group values

The NNA pretreatment immediately caused a 22% decrease in CI before HSD infusion and significantly inhibited the HSD-induced CI elevation for 60 min (Fig. 2A). This treatment resulted in a lower HR as compared with the HSD infusion, especially in the postinfusion phase (Fig. 1C). Nonselective NOS inhibition decreased the HSD-induced coronary flow elevation (Fig. 2B). The preload index LVD did not differ significantly from the result in the HSD-only group, but the late effect of the NNA treatment was a noteworthy decrease as a percentage of LVD (Fig. 3A).

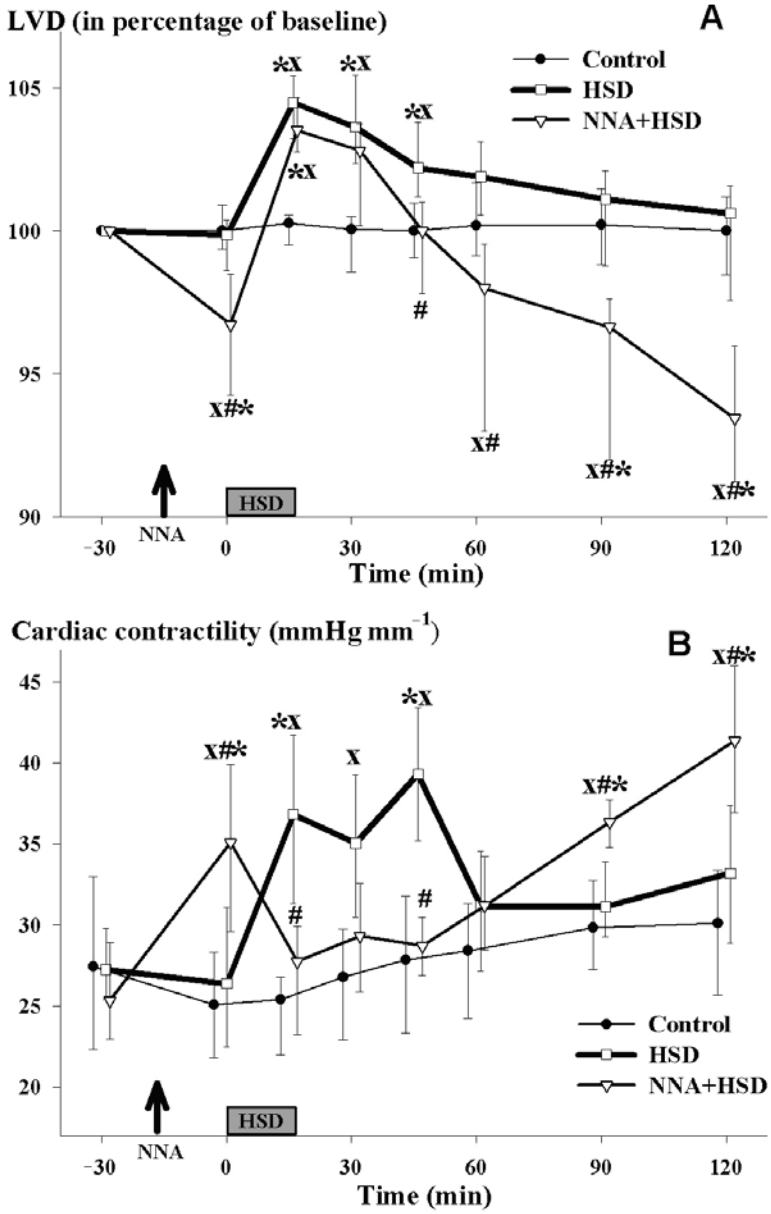

Fig. 3. Changes in diastolic LVD (A) and in myocardial contractility (B) in the saline-treated control group (circles), the HSD-treated group (squares) and the NNA+HSD-treated group (open triangles). * $P < 0.05$ within group; ^{X} $P < 0.05$ between groups *vs* saline-treated control group values; $^{\#}$ $P < 0.05$ between groups *vs* HSD-treated group values

The cardiac contractility showed a biphasic reaction under this experimental protocol: NNA treatment immediately increased the end-systolic pressure-diameter relationship before volume loading, but the HSD-induced positive inotropy was decreased significantly until 60 min after HSD infusion and gradually increased again in the late phase of the postinfusion period (Fig. 3B).

Biochemical changes in blood and myocardial tissue

The plasma ET-1 concentration was significantly increased (approximately 1.5-fold) by the end of the infusion (HSD group: 3.09±0.21 *vs* control: 1.72±0.11 fmol ml^{-1}), and remained significantly higher than in the control group up to the end of the 120-min observation period (Fig. 4A). Concomitantly, the HSD infusion caused a transient and significant, 45-min elevation in plasma NO_x level (Fig. 4B). The NNA pretreatment significantly enhanced the HSD-induced increase in plasma ET-1 level throughout the whole observation period as compared with the HSD-only group (Fig. 4A), while a lowered level of the plasma NO_x was detected in the course of the postinfusion period (Fig. 4B).

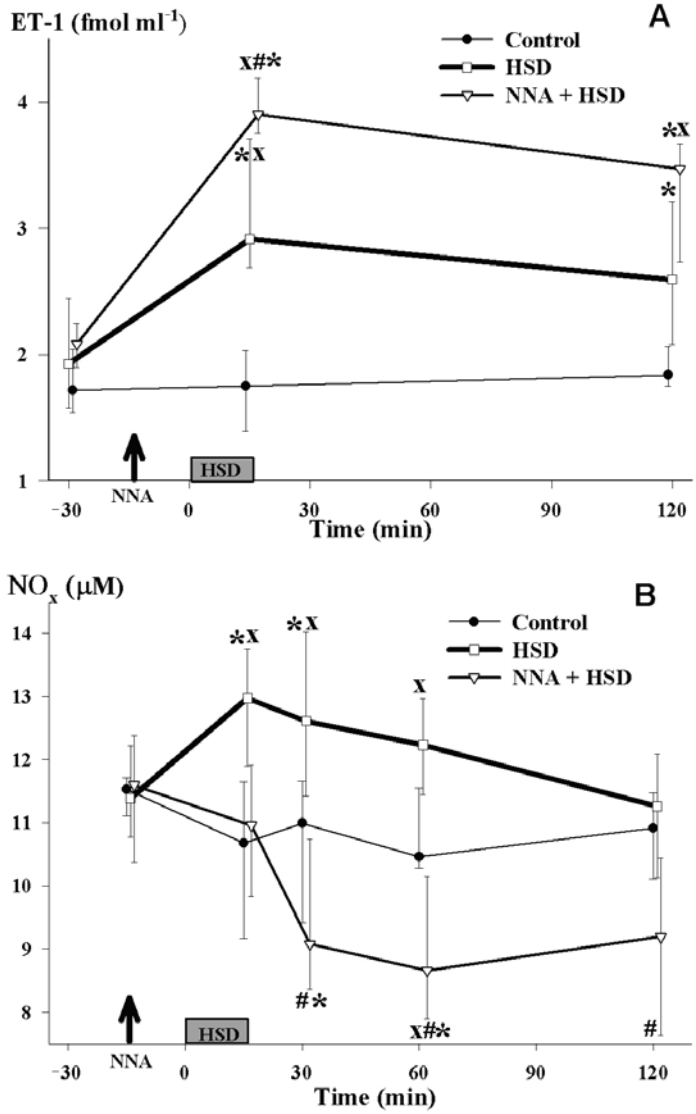

Fig. 4. Changes in plasma ET-1 level (A) and in plasma NO_x level (B) in the saline-treated control group (circles), the HSD-treated group (squares) and the NNA+HSD treated group (triangles). * $P < 0.05$ within group; ^{X} $P < 0.05$ between groups *vs* saline-treated control group values; $^{\#}$ $P < 0.05$ between groups *vs* HSD-treated group values

However, 120 min after the HSD infusion, the myocardial cNOS activity was significantly lower as compared with that in the control group (Fig. 5A). In these biopsies, the tissue MPO activity was significantly increased relative to the control group (Fig. 5B). The nonselective NOS inhibitor pretreatment lowered the HSD-induced decrease in myocardial cNOS activity more appreciable by the end of the postinfusion period, but the NOS values were not significantly different from those in the HSD group (Fig. 5A). The administration of NNA increased the myocardial MPO activity, but the differences were not statisticaly significant in MPO activity between the HSD-only group and the NNA-pretreated groups.

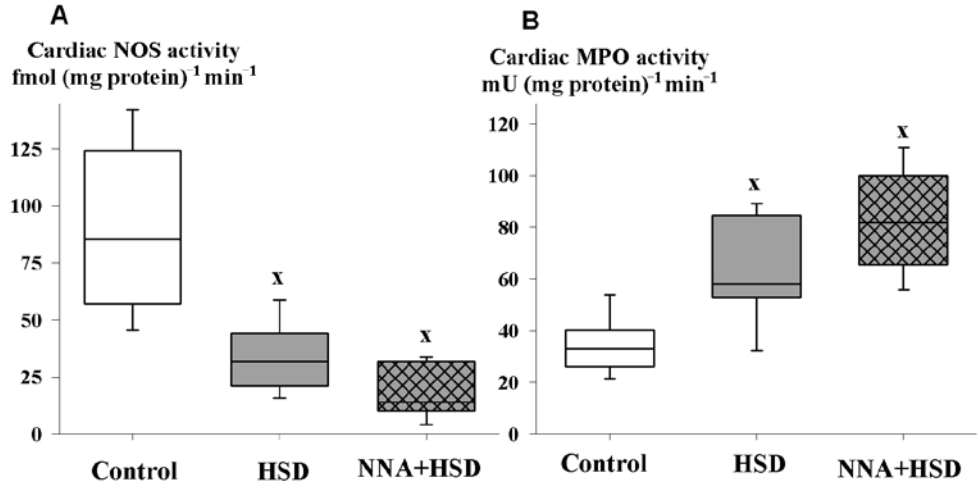

Fig. 5. cNOS activities (A) and myocardial MPO activities (B) in myocardial tissue 120 min after treatment in the saline-treated (empty box), the HSD-treated (gray box) and the NNA-treated (checked box) animals. ^{X} $P < 0.05$ between groups *vs* saline-treated control group values, $^{\#}$ $P < 0.05$ between groups *vs* HSD-treated group values

Discussion

Our study was designed to outline the role of NO in the HSD-evoked global hemodynamic changes and to investigate the feasibility of small-volume resuscitation in conditions associated with diminished NO production. The results revealed that HSD is less effective in NO-deficient state, and HSD-induced alterations of cardiac contractility have NO-dependent and independent components. Indirectly, the data suggest that an extreme upset of the NO – ET-1 balance significantly decreases the coronary perfusion which has predominant role to maintain cardiac contractility.

Hemodynamic effects of HSD

Hypertonic saline (HS) solutions were originally developed for prehospital use to replace larger volumes of isotonic solutions. The reasons for using a standard dose of 4 ml kg^{-1} HS or HSD seem to be based more on practicality rather than on any true physiologic concept (28, 29, 49). HSD solutions bring about rapid changes in the macrohemodynamics in various circulatory beds causing plasma volume expansion by withdrawing water from the extravascular space, reducing the extravascular pressure, increasing the circulating volume and creating favorable histological pressure conditions at the level of the capillaries (29). As

a result, HSD effectively improves the cardiovascular function and this could be related to a number of mechanisms, including a direct relaxing action on the vascular smooth muscle, a blood fluidity improvement by hemodilution, and a stretch-induced ventricular dilatation caused by a pressure or volume overload (18, 29). Indeed, in our study the HSD infusion resulted in prompt increases in MAP and CI and a decrease in TPR. The HR and coronary flow increased concomitantly, and a significant rise in myocardial contractility was noted in the early phase of the postinfusion period. The increased LV preload might contributed to the positive inotropy, but the mechanism of the HSD-induced myocardial contractility elevation is rather complex, and this change could not be a simple consequence of an increased intravascular volume. This hemodynamic pattern could increase the coronary blood flow, too (Gregg effect), which itself could lead to positive inotropy (13, 16). The rationale of our approach was to achieve a rapid increase in flow velocity. Lower molecular weight of the dextran component of the HSD solution (e.g. 40 kD) was preferable, since a significant improvement in blood flow was the primary goal. Higher molecular weight dextran (e.g. 70 kD) is advantageous when a longer circulation time is specifically required (15).

An expansion of the blood volume has been shown to increase the HR and blood pressure via the sympathetic nerves in a number of species, and this effect is based primarily on the activation of volume receptors at the venous-atrial junctions of the heart (24). Stimulation of volume receptors by acute volume expansion results in an elevation of the plasma atrial natriuretic factor (ANF) level, which correlates significantly with the right atrial pressure. An increase in ANF level contributes to the elevation in preload, which could indirectly influence the LV contractility (38). Indeed, blockade of the autonomic nervous system (by sinoaortic denervation, vagotomy or hexamethonium treatment) did not influence the increase in plasma ANF level during volume expansion by 20 ml/kg dextran in lactated Ringer's solution (24, 40).

Several studies suggest the increased activity of the humoral positive inotropic agents in the case of volume expansion. Wade et al. and Elgjo et al. demonstrated elevations in plasma renin and norepinephrine following HSD or hypertonic saline infusion (10, 51). Angiotensin II activation through AT1 receptors potentiates Na^+/H^+ exchange activity, and an increase in intraneuronal Na^+ will lead to the excessive release of norepinephrine from the sympathetic neurons (34). It has been shown that an acute volume expansion caused by a rapid infusion of hypertonic colloid solution results in an increase in plasma ET-1 (3, 11). The plasma level of ET-1 increased significantly in this setup, too, and the plasma ET-1 level subsequently remained elevated until the end of the experiment. Our earlier results revealed that mast cells could have a significant role in this process, since HSD-induced mechanical stimuli result in the mast cell-derived protease output in the plasma (11). An elevated flux of proteases could be increase level of ET-1 from pre–pro ET or from big-ET (34). The ET-1 induced positive inotropy can be prevented by ET-A receptor antagonist pretreatment (7, 11).

The postinfusion period was characterized by significant rises in the plasma NO and ET-1 concentrations. Various mechanical stimuli, such as fluid shear stress on the endothelium (14, 31) and the physical stretching of smooth muscle vascular cells or cardiomyocytes (5, 46) trigger release or syntheses of both vasoactive mediators. In our experiments, the HSD infusion induced moderate elevation of the plasma level of NO_x, an end-product of NO, could be a result of the enhanced plasma viscosity by dextran component. Tsai et al. demonstrated that elevation of the plasma viscosity increases shear stress in the microcirculation leading to the increase of NO release from the endothelium (45).

This shear stress dependent NO release could contribute positively to the LV contractility in this concentration. Although previous studies have indicated that excessive NO delivery from inflammatory cells (or cytokine-stimulated cardiomyocytes themselves) may result in profound cellular disturbances leading to attenuated cardiac contractility (35), others have reported that the stimulation of myocardial NO production can offset the increase in contraction in response to a rise in intracellular Ca^{2+}. Cardiac NO production is also activated by stretching and, under these conditions, NO has been shown to facilitate the Frank-Starling response and to contribute to the increase in intracellular Ca^{2+} transients that mediates the slow increase in contraction in response to stretching (5).

In the later phase of the postinfusion period**,** the HR gradually increased, the CI decreased and the myocardial contractility returned to a near-baseline level, while the TPR increased significantly. Further, these hemodynamic and biochemical changes were accompanied by enhanced myocardial MPO activity and decreases in myocardial cNOS activity. It has been proved in number of diseases involving circulatory failure (myocardial infarction, cardiogenic shock, atherosclerosis and congestive heart failure), also in surgical interventions that an increased ET-1 level is associated with decreased NO production (41), and increased MPO activity (21). This marked decrease in NOS activity may be an indirect consequence of the elevation in ET-1 level. ET-1 *per se* could inhibit endogenous NO synthesis through enhanced asymmetric dimethylarginine synthesis (39) or superoxide radical production, which simultaneously with instantaneous NO production, can lead to the formation of peroxynitrite, a known inhibitor of NOS activity (41, 44). On the other hand, peroxynitrite-mediated myocardial protein nitration has been associated with a depressed cardiac pump function. Borbely et al. proposed that alpha-actinin is a target for peroxynitrite in the human myocardium; and its nitration can induce a contractile dysfunction (4). Additionally, an *in vivo* interaction might occur between the increased nitrite level and myocardial MPO, affording reactive nitrosyl derivatives (6) and leading to protein nitration, too.

Effects of NNA pretreatment

NNA pretreatment definitely inhibited the HSD-evoked favorable hemodynamic features, the CI declined below the control level, and TPR increased significantly during the observation period. It has been demonstrated that NOS inhibition-induced elevation of pulmonary vascular resistance reduces CI, because of the increase in right ventricular after-load leading to impaired left ventricular function *in vivo* (2, 20). HSD-induced NO_x release was blocked by NNA pretreatment at 15 min of the postinfusion period. Moreover, the plasma level of ET-1 was significantly higher in the NNA+HSD group than in the HSD-only group. The extreme elevation of the ET-1 level could explain the increased TPR and venoconstriction, but ET-1-related positive chronotropy was not detected. As a result of NNA pretreatment, the HSD-induced HR elevation was abrogated; it remained permanently at the control level. Our results are consistent with findings of the acute study by Pontieri et al., in which microinjections of a nonselective NOS inhibitor into the nucleus tractus solitarius increased the baroreceptor reflex gain with decreased HR in conscious normotensive rats (43). In another study, the oral intake of an NOS inhibitor enhanced the baroreceptor reflex gain due to a potentiation of reflex bradycardia (47). Overall, it seems that HR regulation under volume expansion is an NO-dependent rather than an ET-1-dependent process.

NNA pretreatment did not influence the HSD-induced increase in LVD during the acute phase of volume expansion. Though NO largely accounts for the myocardial diastolic properties, the HSD-induced LVD increase seems to be NO-independent. This could be

explained by the mechanical atrial stretch due to volume expansion, or by the high sodium concentration of the HSD infusion, which stimulates ANF release with an enhanced ventricular diastole (1). However, in the late phase of the postinfusion period, the effect of NNA pretreatment was characterized by a gradually decreased LVD with a lowered filling volume, because of the ET-1-induced venoconstriction.

Flow-induced dilation of small coronary vessels is one of the most important mechanisms contributing to the local regulation of myocardial blood flow. This response is mediated by NO in most species (27). The impairment of flow-induced NO release likely leads to ischemic episodes and contributes to the development of coronary heart disease (33). Cardiac contractility is essentially dependent on oxygen supply and is characterized by high oxygen consumption (19). HSD-induced volume expansion demands a higher energy supply, which is covered by an increased coronary flow. It has been evidenced that positive inotropy related to the increased utilization of ATP leading to the release of adenine nucleosides. Moreover, it has been demonstrated that exogenous ET-1 causes a significant elevation in the purine metabolism and stimulates adenosine release (53). However, the inhibition of NO production reversed the cardiac contractility changes, with an extreme increase in plasma ET-1 level, which could be responsible for the decreased coronary perfusion (12, 19). Hence, the volume per cardiac cycle decreased to a level which was not able to maintain sufficient tissue perfusion. Accordingly, the diminished NO production with enhanced ET-1 release as an unfavorable side-effect of HSD fluid therapy could have a significant role in a cardiac power deficit. In the later phase of the postinfusion period, the coronary perfusion did not decrease under the control level, despite the decreased cardiac NO production. This could be explained by the enhanced level of adenosine originating from ATP breakdown, which is the main mediator of coronary metabolic adaptation in the heart (22). It could be assumed that the gradual increase in cardiac contractility at the end of the observation period was due to the adapted cardiac perfusion with the energy supply. This late positive inotropy means that volume expansion can stimulate the release of other vasoconstrictors, and not only ET-1. In this regard, the roles of the renin-angiotensin system and catecholamines could be of interest (34). Overall, the HSD-induced positive inotropy proved an indirect NO-dependent process, which predominates through the coronary perfusion rather than through direct effects on cardiac contractility.

The data reported here demonstrate that HSD-induced mechanical stimuli cause significant peripheral NO and ET-1 release in the heart. As a result of NNA pretreatment, the extreme upset of the NO – ET-1 balance significantly decrease the coronary perfusion, which has predominate role to maintain cardiac contractility. The maintained NO production is absolutely necessary for normal peripheral and cardiac hemodynamic functions. Our results suggest that medication via HSD volume therapy could exert a negative influence on the outcome in numerous diseases associated with a decreased NO production (myocardial infarction, congestive heart failure, atherosclerosis and diabetes), since the HSD-induced circulatory changes and in alterations of cardiac contractility have mainly NO-dependent components.

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