The aim of the study was to compare hemodynamic effects of intravenously (i.v.) applied nitric oxide (NO) donor S-nitroso N-acetyl-DL-Penicillamine (SNAP) in conscious spontaneously hypertensive (SHR) to those observed in normotensive Wistar Kyoto (WKY) rats. The study was performed on 7 SHR and 8 WKY instrumented with polyethylene catheters inserted to the abdominal aorta and vena cava for blood pressure (MAP) and heart rate period (HRp) monitoring, and for i.v. administration of SNAP (0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 40.0 and 75.0 μM/kg of body weight). The following differences were found between SHR and WKY rats: 1) the threshold dose of SNAP, eliciting significant decrease of MAP was markedly higher in SHR (1.0 μM/kg b.w.) than in WKY (0.2 μM/kg b.w.), 2) SHR responded with significantly smaller maximum decreases of MAP to administration of 1.0, 2.0, 5.0 and 10.0 μM/kg b.w. of SNAP and with smaller heart rate acceleration to administration of 10.0, 40.0 and 75.0 μM/kg b.w. of SNAP, 3) in SHR MAP decreased progressively, the greatest decline being observed after administration of the highest dose (75 μM/kg b.w.) of SNAP while in WKY the log dose/ΔMAP response curve reached plateau beginning with 2 μM/kg b.w. of SNAP, 4) the slopes and intercepts of the regression lines describing relationship between MAP and HRp after administration of SNAP were significantly different in SHR and WKY rats (P<0.01). The results indicate that SHR are significantly less sensitive to hypotensive effects of NO generated from moderate doses of SNAP.

Key words: Nitric oxide, SNAP, WKY, SHR, hypertension, hypotension

INTRODUCTION

A number of studies have demonstrated significant vasodilatory effect of endothelium derived vasodilatory factor identified with nitric oxide (1, 2). Several lines of evidence indicate that the role of NO in regulation of the cardiovascular functions may be significantly altered in hypertension. Studies
on isolated vessels support evidence that the endothelium-dependent vasorelaxation is impaired in spontaneously hypertensive rats (2—4), however the role of NO in these alterations is not clear. Recently, Kelm et al. (5) have reported that the local generation of nitric oxide in the coronary circulation is significantly greater in spontaneously hypertensive (SHR) rats than in their parent normotensive Wistar Kyoto (WKY) strain. The authors suggested that enhanced generation of NO may play an important compensatory role in hypertensive animals by preventing excessive elevation of the regional resistance. In our in vivo experiments on conscious rats blockade of nitric oxide synthase by i.v. administration of L-nitro N\textsuperscript{G}-L-arginine caused similar elevation of MAP in SHR and WKY (6) while systemic i.v. administration of sodium nitroprusside, a donor of NO, elicited comparable decreases of MAP in conscious SHR and WKY (7, 8). These findings might indicate that under \textit{in vivo} conditions regulation of blood pressure by systemically released NO may be not altered in SHR. The present experiments were performed to further explore the role of NO in blood pressure regulation in SHR and WKY by comparing cardiovascular effects of another NO donor S-nitroso N-acetylpenicillamine (SNAP) applied systemically in conscious SHR and WKY rats.

\section*{MATERIAL AND METHODS}

\subsection*{Animals and surgical preparation}

The study was performed on 7 conscious SHR and 8 WKY rats. The animals were obtained from the Department of Animal Breeding of the Medical School of Warsaw where they were bred from WKY and SHR rats generously donated in 1986 by the Small Animal Section Veterinary Resources Branch Division of Research Services of the National Institutes of Health (Bethesda, MD, USA). Before the experiment the rats had free access to water and a rodent pellet food containing 0.5% NaCl. The experimental protocols were reviewed and approved by the Ethical Committee on the Animal Research of the Medical School of Warsaw.

\subsection*{Implantation of intraarterial and intravenous catheters}

Twenty four hours before the experiment the rats were subjected to light ether anesthesia and implanted with the arterial catheter for blood pressure monitoring and with intravenous catheter for administration of SNAP. The intravascular parts of arterial and venous catheters made from 35—40 mm long tubes (Dural Plastics and Engineering, Auburn, Australia; I.D.—0.5 mm, O.D.—0.8 mm) were introduced to the femoral artery and vein so that their tips were located 20 mm below the renal arteries. The extravascular parts made from the polyvinyl tubing were tunneled under the skin and exteriorized on the neck. The catheters were filled with saline containing heparin (500 U/ml). After the surgery the rats were placed in individual experimental cages for recovery.
Experimental design

The experiments were performed 24—48 hours after completion of the surgical procedures on conscious freely moving animals. Each experiment consisted of two 2 h experimental sessions performed at 24 h intervals. At the beginning of experiment the arterial catheter was connected to the blood pressure unit for blood pressure measurements. Thirty minutes were allowed for stabilization of blood pressure (MAP) and heart rate (HR). The following doses of SNAP were tested: 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0 (day 1) and 10.0, 20.0, 40.0 and 75.0 (day 2) μM per kg of body weight. In each individual rat the consecutive doses were administered in an ascending fashion at 15—30 min intervals when the blood pressure returned to the baseline level. SNAP was dissolved in 100 μl of 0.9% NaCl and injected i.v. during 10 sec. MAP and HR were monitored throughout the whole experiment.

Measurements and statistical analysis

The blood pressure unit for MAP and HR measurements consisted of a transducer, amplifier (Statham Gould P23D) and analog to digital converter connected to PC 386 computer. The following parameters were calculated on line: systolic, diastolic and mean arterial pressure and heart rate period (HRp). The HRp corresponded to the interval between the consecutive systolic pressure peaks.

Statistical analysis

Means together with their standard errors are presented in the text and Figures. Single factor analysis of variance (ANOVA) for repeated measurements was applied to evaluate effect of individual doses of SNAP on changes of MAP and HRp in time. The differences between responses to SNAP in WKY and SHR rats were evaluated by factorial ANOVA on differences from the baseline. The individual significant differences were isolated by multiple comparisons test. Sigmoid curve analysis was performed to determine ED$_{50}$. Linear regression analysis was applied to compare the slopes of relationships between MAP and HRp after administration of various doses of SNAP when the blood pressure decrease (ΔMAP$_{max}$) attained the lowest value (usually 1 min after administration of SNAP). The results were considered significant if P<0.05.

RESULTS

Resting values of MAP in WKY and SHR rats amounted to 108±8 and 172±26 mmHg, respectively. Resting heart rate period in WKY was equal to 161±7 msec (HR = 373±16 beats/min) and in SHR to 141±10 msec (HR = 423±30 beats/min).

Effect of increasing doses of SNAP on blood pressure

Both strains responded with significant dose dependent decreases of blood pressure to i.v. administration of SNAP; the maximum effect being observed 1—2 min after injection. As shown in Fig. 1 SHR were less sensitive to the hypotensive effect of SNAP than the WKY strain. A significant decrease of
Fig. 1A—D. Changes of mean arterial pressure (MAP) in time after administration of various doses of SNAP in SHR and WKY rats. Means and standard errors are shown. * significant difference from baseline.
MAP in the former group was observed after injection of 1.0 μM/kg of SNAP (F(10,50)=2.75; P<0.01) whereas the threshold hypotensive dose in WKY amounted to 0.2 μM/kg (F(10,70)=4.59; P<0.001) (Fig. 1). In addition the maximum decreases of blood pressure (ΔMAP_max) were smaller in SHR than in WKY rats after administration of 1.0 (P<0.01), 2.0 (P<0.001) 5.0 (P<0.001) and 20.0 (P<0.05) μM of SNAP/kg body weight. Significant differences between the two strains were confirmed by analysis of sigmoid curves describing log dose/ΔMAP_max relationships (Fig. 2). The data collected in WKY plateaued within the higher range of doses (between 5—75 μM/kg), with ED_{50} being equal to 0.85 μM/kg. In SHR the shape of the dose response curve was significantly different. Within the range of 0.05—1.0 μM/kg there was no apparent relationship between the dose and the effect. Subsequently, MAP continued to decrease progressively with the increasing dose of SNAP reaching no plateau. As shown in Fig. 2 the log dose/ΔMAP_max curves fitted for SHR and WKY crossed each other at the point corresponding to 40 μM of SNAP; the hypotensive effects observed after administration of this dose being identical in both strains. After administration of 75 μM/kg of SNAP MAP decrease appeared even greater in SHR than in WKY however the difference between the two strains did not reach a level of significance. Because the log dose/ΔMAP_max effect did not achieve plateau in SHR, the results did not allow for reliable determination of ED_{50} in this strain. However, inspection of Fig. 2 strongly suggests that ED_{50} must have been significantly greater in SHR than
WKY
SNAP(µmol/kg)

SHR
SNAP (µmol/kg)

Fig. 3A—D. Changes of heart rate period (HRp) in time after administration of various doses of SNAP in SHR and WKY rats. *significant difference from baseline.
in WKY rats. No consistent differences in duration of the hypotensive effect of SNAP were found in the two strains.

**Effect of increasing doses of SNAP on heart rate**

Administration of SNAP elicited significant HR acceleration as shown by reduction of the heart rate period (Fig. 3). In both strains the threshold dose for significant heart rate acceleration corresponded to 1 μM/kg of SNAP (SHR: F(10,50) = 3.11; P < 0.01, WKY: F(10,70) = 4.76; P < 0.001) (Fig. 3). The increases of HR were smaller in SHR than in WKY rats after administration of 10.0 (F(1,12) = 5.21, P < 0.05), 40.0 (F(1,12) = 11.58; P < 0.01) and 75.0 (F(1,11) = 5.67, P < 0.05) μM/kg of SNAP (Fig. 3).

The slopes of regression lines describing relationship between MAP and HRp before and after administration of increasing doses of SNAP were significantly different (P < 0.05) in SHR and WKY rats (Fig. 4).

![Fig. 4](image)

**DISCUSSION**

Nitric oxide is well recognized as a powerful vasodilator and an impaired vascular smooth muscle relaxation due to inadequate buffering by endogenous nitric oxide has been postulated to be an important factor in pathogenesis of hypertension (2). The present study supports evidence that under in vivo conditions conscious SHR rats are significantly less sensitive to hypotensive
effect of systemically applied SNAP — the NO donor, than their parent normotensive WKY strain. This is indicated by the finding showing that the threshold dose of SNAP necessary to induce significant decrease of blood pressure had to be five times higher (1 \mu M) in SHR than in WKY (0.2 M) rats. Moreover, the hypotensive responses to several corresponding suprathreshold doses of SNAP were found to be smaller in SHR than in WKY rats. With this regard inspection of Fig. 2 reveals that the shapes of curves describing log dose/ΔMAP relationships in SHR and WKY are significantly different. In WKY the hypotensive responses to administration of 10, 20, 40 and 75 \mu M/kg of SNAP did not differ significantly from each other, reaching a plateau. On the other hand, in SHR the hypotensive effects produced by the same doses of SNAP were still in the descending range of the dose response curve. Although in SHR the log dose/ΔMAP did not attain plateau and ED\textsubscript{50} could not be reliably determined it is clear that it must have been several times greater than in WKY, possibly exceeding 75 \mu M/kg. We did not attempt to apply higher doses of SNAP in SHR to determine the full range of the dose response curve, because of restlessness of severely hypotensive SHR which interfered with reliable blood pressure measurements.

It is worth to note that the curves describing the log dose/ΔMAP relationships intersect in the higher range of doses which indicates interaction of effects and may suggest that application of still higher doses of SNAP in SHR could possibly result in significantly greater hypotensive response in SHR than in WKY rats. Thus, at present the statement about smaller hypotensive effectiveness of NO donors in SHR rats must be limited only to a lower range of concentrations. Presence of plateau effect in WKY rats indicates existence in this normotensive strain of some mechanisms effectively protecting against excessive NO-induced blood pressure decreases. The finding that WKY rats respond with greater acceleration of heart rate than SHR suggests that greater hypotensive sensitivity of the former strain is not caused by greater fall in cardiac output but rather by greater vasodilation. The present observations indicate significant deterioration of relationship between decreases of MAP and corresponding increases of heart rate in SHR. Lack of significant correlation between decrease of blood pressure and acceleration of heart rate in SHR may reflect impairment of cardiac component of the baroreflex which causes that unloading of baroreceptors does not produce adequate acceleration of heart rate. Similar deterioration of relationship between decreases of MAP and corresponding increases of heart rate in SHR has been observed in our previous studies (7,9).

Theoretically, the following factors may underly lower effectiveness of SNAP in SHR: 1) less efficient release of NO from SNAP, 2) reduced sensitivity of guanylate cyclase to NO, 3) enhanced activity of cGMP metabolizing phosphodiesterases, and 4) more efficient removal of NO by natural scavengers.
in SHR. Unfortunately, the mechanisms regulating the rate of metabolism of SNAP are only poorly recognized. The available evidence suggests that NO is released by spontaneous homolytic cleavage of SNAP (10, 11). In contrast to nitrates SNAP does not appear to elicit hemodynamic tolerance (12). Recent studies indicate that decomposition of SNAP is accelerated by copper and iron and that ferrohemoglobin effectively reduces vasodilator effect of this compound (10). With this regard it is interesting that spontaneously hypertensive rats have been shown to exhibit lower concentration of plasma iron and higher rate of Fe\(^{59}\) incorporation into the red cells than the normotensive Sprague Dawley rats (13). Moreover, it has been demonstrated that SHR have higher hemoglobin concentration than normotensive Wistar rats (14). Thus, it is possible that in SHR NO is less effective hemodynamically because it is released from SNAP at a lower rate, being at the same time more effectively buffered by hemoglobin. To our knowledge there is no information whether or not there are any differences in sensitivity of guanylate cyclase to NO, or in effectiveness of cGMP metabolism by specific phosphodiesterases among WKY and SHR. In our previous investigation (8), i.v. application of sodium nitroprusside (SNP) elicited comparable decreases of MAP in conscious WKY and SHR, the decrease of blood pressure in SHR being only slightly greater (56 mmHg) than in WKY (44 mm Hg) rats. However, only one dose of SNP was used in this study. Taking into account the present results (magnitude of MAP decreases) one can hypothesize that the amount of NO generated from SNP in our previous study was within the range of concentrations in which it produces similar decreases of MAP in both strains. However, in the study of Akiba et al. (7) on anaesthetized SHR and WKY rats no differences were observed between dose-dependent decreases of MAP produced in both strains by sodium nitroprusside. Among factors which may account for the discrepancies between responsiveness to SNAP and sodium nitroprusside in WKY and SHR in the present study and in that of Akiba et al. (7) are anesthesia and surgical trauma which could influence neurogenic tone and release of vasoactive substances. In addition, cardiovascular effects of different NO donors should be compared cautiously because various NO donors show different distribution and metabolism (15). Furthermore, SNP may also exert some effects which are not specifically related to release of NO. For instance it is known that its decomposition may result in generation of cyanide and in lowering of pH (16).

Recent studies indicate that some of the cardiovascular effects exerted by NO may be of central neurogenic origin (11, 17, 18, 19, 20). Our recent data indicate that administration of SNAP to the lateral cerebral ventricle in conscious rats, in a dose which is not effective when applied systemically, produces significant decrease of blood pressure in WKY but not in SHR (19).
Thus it is possible that greater hypotensive effect of SNAP observed in the present study in WKY may partly result from its central action.

In conclusion, the present study indicates that within the range of moderate blood pressure decreases SHR are significantly less sensitive to hypotensive effects of NO generated from SNAP than normotensive WKY rats.

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