Sulfhydryl Group Donors Potentiate the Hypotensive Effect of Acetylcholine in Rats

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Nitric oxide mediates the vasodilator and hypotensive responses of acetylcholine infusion. It has been reported that nitric oxide could be protected from free radical destruction by forming an S-nitrosothiol compound. Furthermore, sulfhydryl donors such as N-acetylcysteine or thiosalicylic acid enhance nitric oxide production from nitroglycerin. Consequently, the hypotensive effect of intravenous acetylcholine infusion might be potentiated during the simultaneous administration of sulfhydryl donors. The objective of the present study was to test in Okamoto spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats (1) whether the hypotensive effect of acetylcholine (10 μg/kg per minute) was affected by the simultaneous administration of N-acetylcysteine (10 μg/kg per minute) or thiosalicylic acid (10 μg/kg per minute), and (2) whether N⁵-nitro-L-arginine-methyl ester (100 μg/kg per minute) administration was able to reverse the changes induced by acetylcholine plus N-acetylcysteine or acetylcholine plus thiosalicylic acid. The administration of acetylcholine reduced (P<.05) mean arterial pressure in WKY rats (13±2%) and SHR (14±2%) without affecting urine flow rate, urinary sodium excretion, and glomerular filtration rate. In the presence of N-acetylcysteine, the acetylcholine-induced reduction in mean arterial pressure was potentiated (P<.05) in WKY rats (24±4%) and SHR (20±2%). These changes in mean arterial pressure were accompanied by significant reductions in urine flow rate and urinary sodium excretion in WKY rats, as well as in glomerular filtration rate in SHR. During the administration of thiosalicylic acid, the acetylcholine-induced hypotensive response was potentiated (P<.05) in both WKY rats (31±4%) and SHR (22±3%). These effects were also accompanied by significant reductions in urine flow rate, urinary sodium excretion, and glomerular filtration rate. The administration of N⁵-nitro-L-arginine-methyl ester reversed (P<.05) the changes induced by acetylcholine plus N-acetylcysteine and acetylcholine plus thiosalicylic acid in mean arterial pressure, glomerular filtration rate, urine flow rate, and urinary sodium excretion in both rat strains. Under these conditions, urine flow rate and urinary sodium excretion levels were significantly higher than those found during the infusions of acetylcholine and thiosalicylic acid, respectively. The administration of N-acetylcysteine or thiosalicylic acid, at the doses indicated, did not modify any of the measured arterial pressure or renal responses when compared with saline infusion. These results suggest that sulfhydryl donors effectively potentiated the hypotensive action of acetylcholine through a nitric oxide–dependent mechanism. (Hypertension 1993;22:156-160)

KEY WORDS • nitric oxide • acetylcholine • sulfhydryl compounds • hypotension • rats, inbred SHR • rats, inbred WKY
TSA in rats. The renal response in all rat groups was also evaluated. Because Okamoto spontaneously hypertensive rats (SHR) have been reported to have a reduced in vitro vascular relaxation in response to acetylcholine, which could cause the concomitant release of EDRF and endothelium-derived contracting factor (EDCF), this study compared the hypotensive effects of acetylcholine with the simultaneous administration of NAC or TSA in SHR and in normotensive control Wistar-Kyoto (WKY) rats.

Methods

Experiments were conducted in male SHR or WKY rats (Harlan Sprague Dawley, Inc, Indianapolis, Ind) weighing 270 to 300 g. The animals were fed a normal Purina rat chow containing 0.1 mEq of sodium per gram and had free access to water. All experimental procedures conformed to National Institutes of Health regulations and were approved by the Institutional Animal Care and Use Committee. Rats were anesthetized with an intraperitoneal injection of 100 mg/kg thiobutabarbital (BYK-Gulden, Konstanz, Germany) and were placed on a heated table to maintain body temperature at 37°C. A tracheostomy was performed, and a PE-240 catheter was placed in the trachea. A PE-90 catheter with a flared tip was placed in the bladder for urine collection. PE-50 catheters were placed in the left carotid artery for mean arterial pressure (MAP) measurement and blood collection and in the left jugular vein for intravenous infusions. An intravenous infusion of 1 mL/100 g body wt per hour of a solution of 6.25% albumin and 3% inulin was started and lasted for the duration of the experiment. Saline or drugs were infused simultaneously at a rate of 1 mL/100 g body wt per hour. Rats were allowed to recover and equilibrate for 60 minutes after surgical procedures were completed. Then, a 15-minute control period with saline infusion was undertaken. At this time, acetylcholine (10 μg/kg per minute) was infused for 30 minutes, after which saline was infused again for 45 minutes. NAC or TSA was infused in SHR and WKY rats at a rate of 10 μg/kg per minute for the rest of the experiment. Forty-five minutes after the infusions of NAC or TSA were started, acetylcholine (10 μg/kg per minute) infusion was started and continued until the end of the study; 30 minutes later L-NAME (100 μg/kg per minute) was infused for 30 additional minutes.

Urine samples were collected during the last 15 minutes of each infusion period. Blood samples (0.5 mL) from the carotid artery were withdrawn at the middle of each 15-minute clearance period. Hematocrit (ranging from 45% to 39%) was monitored during the experiment. No replacement of plasma was given to rats, because a continuous volume supplementation of 1 mL/100 g body wt of a solution of 6.25% albumin in saline was infused during the duration of the experiment. At the end of the experiments, all rats were killed by an intravenous infusion of potassium chloride while still under deep anesthesia. Sodium and potassium concentrations in plasma and urine were measured with an electrolyte analyzer (model E2A, Beckman Instruments, Brea, Calif). Inulin concentrations in plasma and urine were measured according to a colorimetric method previously described.15 Glomerular filtration rate (GFR) was calculated from inulin clearances. All chemicals, analytical grade, were purchased from Sigma Chemical Co, St Louis, Mo.

Data within groups were analyzed with randomized block analysis of variance followed by Newman-Keuls Multiple Range Test.

Results

Effects of Intravenous Acetylcholine in Rats With and Without Simultaneous Infusions of N-Acetylcysteine or Thiosalicylic Acid

The administration of acetylcholine induced a similar decrease (P<.05) in MAP in WKY rats (119±4 to 103±3 mm Hg) and SHR (163±5 to 139±6 mm Hg) (Fig 1, top panel) as well as in the second group of WKY rats (121±3 to 104±4 mm Hg) and SHR (167±6 to 144±5 mm Hg) (Fig 1, bottom panel) without producing changes in urine flow rate (V), urinary sodium excretion (UNaV), or GFR (Fig 2). The infusion of NAC plus acetylcholine produced a significant (P<.05) potentiation of the acetylcholine-induced reduction of MAP in WKY rats (123±3 to 93±4 mm Hg) and SHR (155±7 to 125±7 mm Hg) (Fig 1). These

![Graph showing percent changes in MAP](http://hyper.ahajournals.org/lookup/doi/10.1161/01.HYP.84.3.157)
changes were accompanied by significant reductions in V and U\textsubscript{NaV} in both rat groups and in GFR in the SHR group (Fig 2).

The administration of TSA plus acetylcholine potentiated (P<.05) the acetylcholine-induced hypotension in both WKY rats (120±4 to 83±6 mm Hg) and SHR (163±7 to 127±8 mm Hg) (Fig 1). These effects were also accompanied by significant reductions in V and U\textsubscript{NaV} (Fig 3). GFR values decreased significantly during the infusion of acetylcholine plus TSA in WKY rats and SHR when compared with values during TSA infusion alone (Fig 3).

**Effect of L-NAME on the Actions Exerted by Infusions of Acetylcholine Plus N-Acetylcysteine or Plus Thiosalicylic Acid**

The administration of L-NAME during the infusions of acetylcholine plus NAC (Fig 2) and acetylcholine plus TSA (Fig 3) reversed (P<.05) the changes induced by acetylcholine plus NAC and acetylcholine plus TSA in MAP, GFR, V, and U\textsubscript{NaV} in both SHR and WKY rats. Under these conditions, V and U\textsubscript{NaV} levels were higher than those found during the infusions of NAC and TSA, respectively (Figs 2 and 3).

The administration of NAC or TSA did not modify any of the parameters measured when compared with a previous control period when saline solution was infused (Figs 2 and 3).

**Discussion**

The present study shows that the hypotensive effect of a systemic infusion of acetylcholine in normotensive and hypertensive rats is potentiated by the sulfhydryl donors NAC and TSA. This effect appears to be synergistic because the infusions of NAC or TSA, at doses prevalently indicated, did not alter MAP levels significantly. Furthermore, because the specific inhibitor of NO synthesis L-NAME reversed that effect, a mediatory role of NO could be proposed.

In 1973, Needleman et al7 showed that tissue sulfhydryl groups were required to demonstrate relaxation of precontracted aortic strips by nitroprusside and other nitrogen oxide–containing vasodilators. This suggested that the vascular smooth muscle relaxation induced by organic nitrates and NO may be attributed to the formation of S-nitrosothiols,15 which may be responsible for guanylate cyclase activation.17 Repeated administration of large concentrations of organic nitrates was believed to oxidize these sulfhydryl groups to the disulfide form (which had a much lower affinity than sulfhydryl for organic nitrates), thus explaining the development of tolerance to organic nitrates. It has been demonstrated that nitrate tolerance can be prevented and reversed by the administration of the sulfhydryl-containing compound NAC.19 Furthermore, several investigators11,12 haveshown that NAC potentiated the hypotensive effect of nitroglycerin. According to the previous hypothesis, the synergistic action of NAC and nitroglycerin might be mediated by the formation of S-nitrosothiol in the systemic circulation.18 A subsequent report by Münnzel et al19 also suggested the possibility of extracellular interaction between nitroglycerin and NAC enhancing the production of NO. In a recent study, Myers et al9 compared the vasodilator potencies of NO, S-nitrosocysteine, and EDRF, reaching the conclusion that EDRF is much more likely to be a nitrosylated compound such as S-nitrosothiol than authentic NO. These investigators suggested that the incorporation of NO into the nitrosothiol compound might enhance its absolute stability and its potency.
Furthermore, the transmembrane transport of NO in the vascular smooth muscle cells relies on the association of NO with a carrier molecule. Although S-nitrosothiol uptake into the vascular smooth muscle may also depend on a transport mechanism, it might degrade at the cell membrane to yield NO. This process may more efficiently deliver NO to the cytoplasm of the vascular smooth muscle. According to these notions, our results might suggest that the sulfhydryl donors would enhance the hypotensive effect of acetylcholine by forming S-nitrosothiol compounds that will protect NO against degradation. This compound would be directly or indirectly responsible for NO-induced relaxation of vascular smooth muscle. According to these notions, our results might suggest that the sulfhydryl donors would enhance the hypotensive effect of acetylcholine by forming S-nitrosothiol compounds that will protect NO against degradation. This compound would be directly or indirectly responsible for NO-induced relaxation of vascular smooth muscle.

Studies by Lüscher and Vanhoucke demonstrated that SHR produce an arachidonic acid-derived endoperoxide, a thromboxane EDCF, or both that attenuate the NO-mediated response to endothelium-derived vasodilators. However, in the present study, acetylcholine showed similar reduction of MAP in both SHR and WKY rats. The reason for the apparent disparity in the results can be found in the acetylcholine doses used in the two experiments. In the experiment by Lüscher and Vanhoucke, elevated acetylcholine doses causing release of EDCF were used. Thus, in our study when moderate doses of acetylcholine were used, the hypotensive effect of acetylcholine is comparable in both rat strains, suggesting that EDCF does not play an important role under the present experimental conditions. However, the fact that the potentiation of the hypotensive effect of acetylcholine with NAC or TSA is higher in WKY rats than in SHR suggests some impairment of endothelium-dependent vasodilation in SHR. It could be speculated that NO or S-nitrosothiol is more rapidly destroyed in SHR than in WKY rats because of a higher production of free radicals in the hypertensive rat strain. This hypothesis is supported by observations indicating that several pathological processes such as hypertension, diabetes, ischemia with subsequent reperfusion, and atherosclerosis are associated with abnormalities of endothelium-dependent relaxations. Many of these processes are associated with the generation of oxygen-free radicals within endothelial cells that may oxidize free sulfhydryl groups to inactive forms. This may result in an impaired ability of the endothelium to synthesize S-nitrosothiols and hence the observed reduced potentiation of the hypotensive effect of acetylcholine during the administration of NAC or TSA in SHR.

In the present study, we did not measure changes in heart rate during the administration of acetylcholine. However, it is highly unlikely that the potentiation of the acetylcholine effect by thiol donors is due to bradycardia, because the administration of 10 μg/kg per minute of both NAC and TSA did not produce hypotension. Furthermore, these doses of NAC and TSA were chosen in pilot studies and showed no effect on MAP over an infusion period of 120 minutes. Consequently, it could be postulated that the potentiation of the hypotensive effect of acetylcholine is not merely a summation of two vasodilator actions but is a real synergistic effect between acetylcholine and these sulfhydryl donors.
The demonstration that the hypotension induced by the coadministration of acetylcholine and NAC or acetylcholine and TSA is reversed by the specific inhibitor of NO synthesis L-NAME suggests that the observed potentiation of the hypotensive effect of acetylcholine might be a result of an enhanced production of NO or an S-nitrosothiol. Supporting the mediatory role of NO in the observed results is our study showing that the intrarenal administration of L-NMMA was able to blunt the renal response to intrarenal acetylcholine infusion in dogs. In a subsequent study, we also demonstrated that the renal response to other endothelium-dependent vasodilators such as bradykinin was prevented by L-NMMA, and this was reversed by the infusion of L-arginine. Note that in this experiment the administration of L-NAME prevents the hypotensive response to acetylcholine plus NAC or TSA, and no further pressor response was observed. This might be an apparent contradiction with our previous study showing that the NO synthesis inhibitor L-NAME increases MAP in anesthetized normotensive rats. In the present experiment, we intentionally used a dose of L-NAME that in the time infused was able to equilibrate the reduced MAP without producing further systemic effects.

In the present study, the modifications in renal excretory functions appear to follow the changes in MAP. The administration of acetylcholine induced no significant modification in V and U\textsubscript{Na}V. Furthermore, the administration of acetylcholine plus NAC or acetylcholine plus TSA, which produced a marked hypotension, was followed by significant reductions in GFR, V, and U\textsubscript{Na}V. Although the natriuretic and diuretic effects of acetylcholine have been clearly demonstrated, the reduction in MAP observed in the present study could result in no modification in the renal excretory response. It is well known that a reduction in MAP and as a consequence in renal perfusion pressure induces a reduction in sodium excretion. However, it should be noted that after the administration of acetylcholine, mean values of V and U\textsubscript{Na}V were higher, although not statistically significant, than those observed under basal conditions. This might be interpreted as a stimulatory effect of acetylcholine on V and U\textsubscript{Na}V that is counterbalanced by the decrease in MAP.

In conclusion, the present results show that an increased availability of sulfhydryl groups can enhance the hypotensive effect of acetylcholine in normotensive rats and SHR, thus supporting the importance of thiols in endothelium-dependent relaxation.

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