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Albumin Is an Important Vascular Tonus Regulator as a Reservoir of Nitric Oxide

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Plasma albumin reacts with nitric oxide (NO) to form S-nitroso-albumin (albumin-S-NO). The present study was designed to elucidate whether NO-induced response on blood pressure may differ between analbuminemic rats (NAR) and normal rats. When NOC7, a NO donor, was intravenously injected in rats, blood pressure decreased and the initial depressor response was the same in both the NAR and the normal rats. However, the duration of NO-induced hypotension was 0.5 times longer in NAR than in the normal rats. After NOC7 injection, plasma thiol levels decreased and S-nitrosothiols levels increased in the normal rats, whereas these levels slightly changed in NAR. These results suggested that NO reacts with albumin-SH, resulting in albumin-S-NO formation, which produces a continuous hypotensive response in blood pressure as a slow releaser of NO.

It has been speculated that the endothelium-derived relaxing factor (EDRF) may not be accounted for by NO alone, but may be evinced by a closely related NO adduct (1-3). EDRF has the relative long half-life in contrast to the half-life of authentic NO in vivo. It suggests that NO is stabilized by the reaction with a carrier molecule(s) in vivo that prolongs its half-life and preserves its biologic activity. It has been reported that the sulfhydryl groups of plasma proteins can combine with NO under physiological conditions to form stable, biologically active NO adducts (4-7). Plasma albumin has a free thiol (Cys 34) and its levels are about 0.5mM in rat plasma. Such protein-associated NO has been albumin-S-NO which possesses EDRF-like properties in vivo (5,7,8). Furthermore, the proteins-S-NO has a much longer NO bioactivity than low molecular weight thiols-S-NO (9). To confirm the role of albumin-S-NO in the systemic hemodynamics, this study was performed using mutant rats with analbuminemia (10).

MATERIALS AND METHODS

Chemicals. [1-Hydroxy-2-oxo-3-(N-methyl-3-aminopropyl)-3-methyl-3-aminopropyl]-3-methyl-1-triazene (NOC7) was obtained from Dojin Co. (Kumamoto). Other reagents used were of analytical grade. S-nitroso-glutathione (GSNO) was prepared according to the method of Hart (11) by incubating equimolar amounts of glutathione and sodium nitrite in acidified water at 0°C. GSNO solutions were freshly prepared before each experiment from frozen GSNO powder. The concentration of GS-NO was spectrophotometrically determined at 335 nm ($e = 992\ dm^3\ mol^{-1}\ cm^{-1}$) and at 545 nm ($e = 15.9\ dm^3\ mol^{-1}\ cm^{-1}$).

Measurements of plasma thiols and S-nitrosothiols (RSNO). The total levels of thiols in plasma were determined using Ellman's reagent (12). Under light ether anesthesia, blood was collected into syringes containing EDTA to achieve a final concentration of 0.5 mM. Plasma samples was obtained by centrifugation at 3000 r.p.m. for 10 min. Either blood or plasma samples (100 μl) were mixed with 100 μl of ice-cold Ellman's reagent (20 mM). The mixtures were homogenized using a Polytron homogenizer for 30 sec and then sonicated for 10 sec. To the homogenates were

Abbreviations: NO, nitric oxide; GSNO, S-nitrosoglutathione; RSNO, S-nitrosothiols; albumin-S-NO, S-nitroso-albumin; NAR, analbuminemic rats; EDRF, endothelium-derived relaxing factor.

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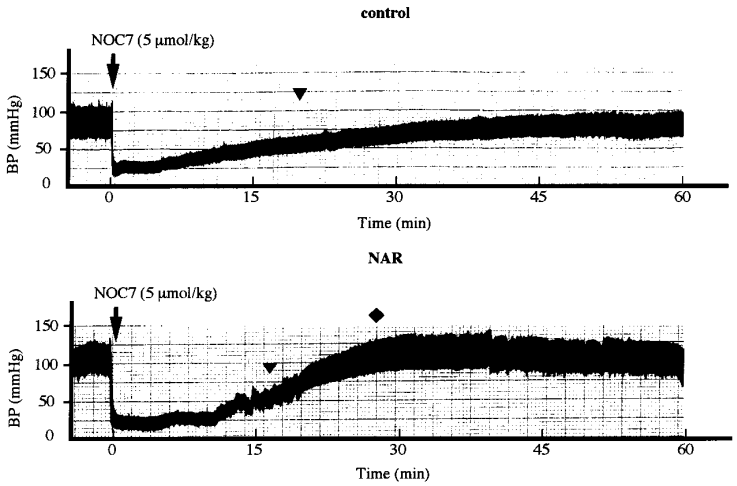

FIG. 1. Typical tracing of the hemodynamic effect of NOC7 injection in normal rats and NAR. Five μmol/kg NOC7 produced a rapid drop in blood pressure followed by a gradual asymptotic return to the baseline. The rapid fall in blood pressure was not different between normal rats and NAR. However, NAR recovered to the baseline faster than normal rats. Symbols indicated follows: ▼, 50% of recovery time; ◆, 100% of recovery time.

added ethanol to give a final concentration of 90%. After centrifugation at 12,000 × g for 5 min, the reaction product of Ellman's reagent (total thiols) in the ethanol-soluble fraction was spectrophotometrically determined at 412 nm. Nitrosothiols in plasma were measured by the method of Saville (13). GSNO was used as a standard.

Animal preparations. Male NAR and Wistar rats (200-220g) were obtained from SLC, Co. (Shizuoka), fed laboratory chows and water *ad libitum*, and used for the experiments without prior fasting. Under urethane anesthesia (1 g/kg, i.p.), the femoral vein and artery were cannulated through an inguinal incision using a polyethylene catheter (22G; Termo Co. Ltd., Tokyo) and the femoral artery was connected with a pressure transducer (Baxter, Tokyo). Blood pressure was recorded on a polygraph (model RM-6200; Nihon Koden, Tokyo). NOC7 (5 μmol/kg) was injected into the rats through the femoral vein. Blood samples for thiols and RSNO were collected from the femoral vein into EDTA-containing tubes. Papaverine (6 μmol/kg) was used as an NO-independent relaxant.

Statistical analysis. Unless otherwise stated, data are presented as means ± SEM. Statistical significance of difference was calculated according to Dunnet t-test; the levels of significance were $p<0.05$.

RESULTS AND DISCUSSION

The effect of an intravenous injection of NOC7 (5 μmol/kg) on blood pressure was studied in both normal rats and NAR. A representative tracing is shown in Fig. 1. NOC7 injection produced a significant decrease in the mean arterial pressure of both NAR and normal rats. Changes in blood pressure were 60 ± 6.1 and 63 ± 3.5 mmHg in normal rats and NAR, respectively (Table 1). However, blood pressure recovery times were significantly different between the normal rats and NAR. To quantify the biologic life of the NO adducts studied, recovery times were determined in both groups. These times were 61.3 ± 10.4 and 35.3 ± 6.7 min in the normal rats and NAR, respectively. The recovery time of blood pressure of NAR was significantly shortened by about 0.5-fold compared to that of normal rats. To examine the effect of the NO-independent pressure response, papaverine (6 μmol/kg) was injected into the normal rats and NAR. Papaverine showed the same responses (the initial response and the recovery time) in both groups. Therefore, it was suggested that the S-nitrosation of albumin at its single free thiol, cysteine 34, might form the bioactive NO adduct albumin-S-NO. Free thiols levels in plasma were measured after NOC7 injection (Fig. 2). The basal level of NAR plasma was 25% that of a normal rat's plasma. Thiols level in plasma markedly decreased by

TABLE 1
Effect of NOC7 and Papaverine on Blood Pressure Response in Normal Rats and NAR

	Normal	NAR
NOC7 (5 μmol/kg, iv)		
Pressure response (mm Hg)	60.1 ± 6.1	63.0 ± 3.5
Recovery time (min)	61.3 ± 10.4	35.3 ± 6.7**
Papaverine (6 μmol/kg, iv)		
Pressure response (mm Hg)	60.0 ± 6.1	65.0 ± 7.0
Recovery time (min)	5.1 ± 0.16	5.0 ± 0.03

Note. NOC7 (5 μmol/kg) or papaverine (6 μmol/kg) was intravenously injected in normal rats and NAR. Values are mean ± SE (n = 3–4). Recovery time indicates a recovery time to baseline after NOC7 administration.

**; $p < 0.01$ compared with normal rats.

about 35% after NOC7 injection (~60 min) in normal rats, whereas the level did not change in NAR. Furthermore, RSNO levels in plasma significantly increased by about 300% thirty min after NOC7 injection in normal rats, whereas these were only slightly increased in NAR (Fig. 3). These data provide direct evidence that the S-nitrosation of albumin and NO release from albumin-S-NO occur in vivo. Albumin-S-NO relaxes the vascular smooth muscle, and inhibits platelet aggregation (4, 7, 8). With prolonged in vivo hemodynamic effects in normal rats, it is suggested that albumin would be an important reservoir of NO under physiological conditions.

Although hypoalbuminemia has been known to associate with some human diseases, such as nephrotic syndrome and liver cirrhosis (14), the lack of formation of long active albumin-S-NO may inhibit further hyperdynamic circulation following such diseases as endotoxemia. Furthermore, it must be considered in case of the administration of NO donors such as nitroglycerine that the responsibility of the NO donor may be affected under these pathological conditions. These clinical implications should be further studied.

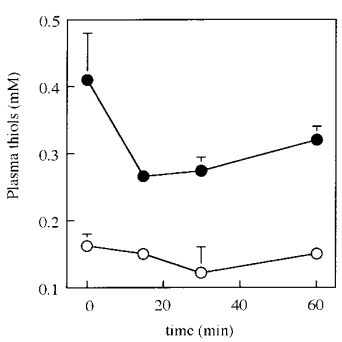

FIG. 2. Plasma thiol levels in normal rats and NAR after NOC7 administration. At indicated times, thiol levels of plasma were measured. NOC7 (5 μmol/kg) was intravenously injected. Thiol levels of normal rats (●) significantly decreased after NOC7 injection (~60 min) ($p<0.01$ as compared with basal levels). Thiol levels of NAR (○) had no significant changes at the indicated times.

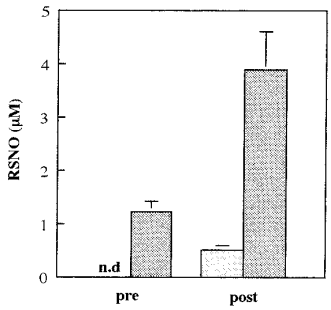

FIG. 3. Plasma S-nitrosothiols (RSNO) levels in normal rats and NAR after NOC7 administration. RSNO levels were measured at 30 min after NOC7 injection as described in the legend to Fig. 2. Administration of NOC7 produced a 3-fold significant increase in RSNO levels in normal rats ($p<0.01$ as compared with basal levels). In NAR, RSNO levels were not significantly different from basal levels. ▥, control rats; ▨, NAR.

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