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SPECIAL REPORT

Evidence that endogenous nitric oxide modulates plasma fibrinogen levels in the rat

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This study investigated the effect of prolonged inhibition of nitric oxide (NO) synthase on plasma fibrinogen levels and platelet count in the rats. N^G-nitro-L-arginine methyl ester (L-NAME), when administered orally twice a day, at 10–100 mg kg^{-1}, for 7 consecutive days, 14 times in all, significantly elevated fibrinogen levels and systolic blood pressure in a dose-dependent manner. The same dose range of L-NAME failed to alter platelet count and plasma protein concentrations. The increase in fibrinogen levels produced by chronic treatment with L-NAME at 30 mg kg^{-1} was reversed by L-arginine at 500–1500 mg kg^{-1} in a dose-dependent manner. These findings suggest that endogenous NO tonically acts to reduce plasma fibrinogen levels in rats under physiological conditions.

Keywords: Nitric oxide (NO); N^G-nitro-L-arginine methyl ester (L-NAME); L-arginine; fibrinogen; platelet count; fibrinolysis; haemostasis

Introduction Nitric oxide (NO), which plays a complex dual role in various biological events (Wright *et al.*, 1992; Kawabata *et al.*, 1994), appears involved in haemostatic modulation. NO suppresses platelet aggregation and/or adhesion, and may act as an endogenous regulator of platelet activation within the pulmonary circulation (May *et al.*, 1991). NO is also involved in the fibrinolytic system. Sodium nitroprusside, a NO donor, enhances fibrinolytic activity through increased tissue-type plasminogen activator (t-PA) activity as a result of inhibition of plasminogen activator inhibitor (PAI) release from platelets in rats (Lidbury *et al.*, 1990). By contrast, this compound reduces the bradykinin- or platelet-activating factor-induced release of t-PA in a rat isolated hindleg perfusion system in a cyclic GMP-independent manner (Tranquille & Emeis, 1993). A recent study found that N^G-nitro-L-arginine methyl ester (L-NAME), a NO synthase inhibitor, when preadministered twice at 30 mg kg^{-1}, improves some of DIC phenomena in rats challenged with *E. coli* lipopolysaccharide, such as the decrease in fibrinogen levels and the shortening of euglobulin clot lysis time, possibly by an increased generation of PAI, although it does not affect the drop in platelet count (Korbut *et al.*, 1994). Taken together with their potent antihypotensive properties, NO synthase inhibitors may serve for the treatment of septic shock, although some reservations still remain (Wright *et al.*, 1992; Kawabata, 1995). On the other hand, the above haemostatic parameters in intact rats are resistant to L-NAME administered twice at 30 mg kg^{-1} (Korbut *et al.*, 1994). To the best of my knowledge, it is unclear whether long-term administration of NO synthase inhibitors modifies haemostatic parameters in intact rats. Here this study reveals that prolonged inhibition of nitric oxide synthase by L-NAME produces hyperfibrinogenemia but does not alter platelet count in the rat.

Methods Male Wistar rats (Japan SLC. Inc.) weighing 200–300 g were used throughout the experiments. L-NAME (Sigma, U.S.A.) or its D-enantiomer (Bachem, Switzerland), in a dose-range of 10–100 mg kg^{-1}, was administered orally twice a day, at 09 h 00 min and 16 h 00 min, for 7 consecutive days, 14 times in all. In the experiments to study the interaction between L-NAME and L-arginine, L-NAME at 30 mg kg^{-1} was co-administered orally with L-arginine (Nacalai Tesque, Japan) at 500–1500 mg kg^{-1}, according to the above schedules. On the day following the last administration, each rat was anaesthetized with pentobarbitone at 11 h 00 min (19 h after the 14th dose), and 5 ml of citrated blood (containing 1/10 volume of 3.8% sodium citrate) was obtained from the abdominal aorta. Blood examinations were carried out essentially as described previously (Kawabata & Hata, 1993). Briefly, the platelet count was visually estimated, and plasma fibrinogen and protein levels were determined by the thrombin time method and by Lowry's method, respectively. Systolic blood pressure was measured by the tail-cuff method using an automatic blood pressure meter (UR-5000, Ueda Co. Ltd., Japan), before anaesthetization with pentobarbitone injection. The results are expressed as the mean with s.e.mean, and statistical significance was analysed by ANOVA followed by Newman-Keuls' multiple comparison test, and was set at a $P<0.05$ level.

Results Repeated administration of L-NAME at 10–100 mg kg^{-1} dose-dependently elevated systolic blood pressure in conscious rats, although its D-enantiomer was without effect. L-NAME, but not its D-enantiomer, in the same dose-range, significantly increased plasma fibrinogen levels in a dose-dependent manner. In contrast, platelet count and plasma protein levels were resistant to chronic administration of L-NAME at 10–100 mg kg^{-1} (Figure 1). The increase in plasma fibrinogen levels produced by repeated treatment with L-NAME at 30 mg kg^{-1} was reversed by coadministration of L-arginine at 500–1500 mg kg^{-1} in a dose-dependent manner (Figure 2).

Discussion To my knowledge, this study is the first to demonstrate that prolonged inhibition of NO synthase results in hyperfibrinogenemia in intact rats. The increase in plasma fibrinogen levels by repeated L-NAME treatment is not due to haemoconcentration, since plasma protein concentrations did not change following L-NAME. Considering the findings that the D-enantiomer was without effect, and that the L-NAME-induced increase in fibrinogen levels was reversed by L-arginine, the present study suggests that endogenous NO modulates plasma fibrinogen levels, i.e. it may tonically act to reduce plasma fibrinogen levels.

The mechanisms by which L-NAME augments fibrinogen levels are unclear at present. In our previous study (Kawabata & Hata, 1993), superoxide dismutase, when repeatedly administered s.c. at 30,000 u kg^{-1} according to the same schedule as that in the present study, elevated plasma fibrinogen levels in both stressed and unstressed mice, suggesting that the

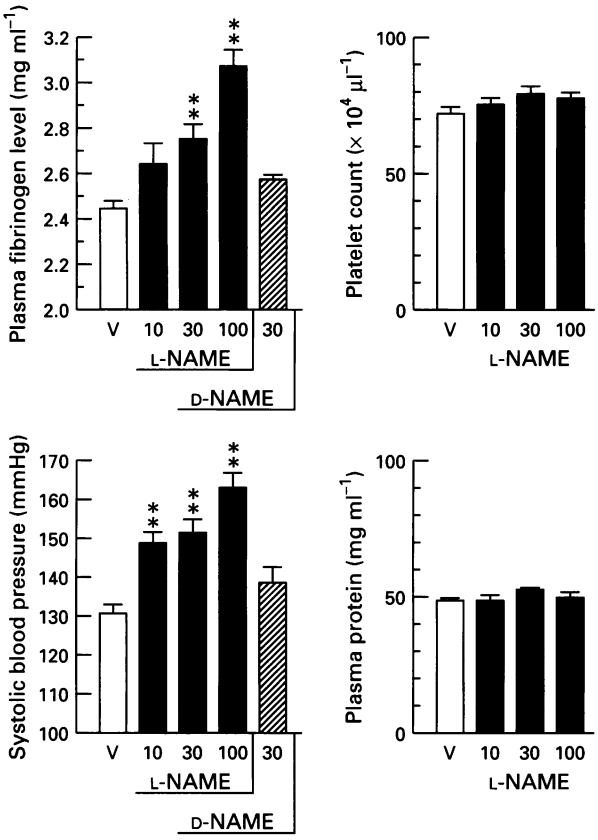

Figure 1 N^G-nitro-L-arginine methyl ester (L-NAME) or its D-enantiomer (D-NAME), at 10, 30 or 100 mg kg^{-1}, was administered orally twice a day, at 09 h 00 min and 16 h 00 min, for 7 days (14 times in all). Data indicate the mean with s.e.mean from 10 (vehicle) or 4–5 (L-NAME and D-NAME) rats. $^{**}P<0.01$ vs. vehicle (V).

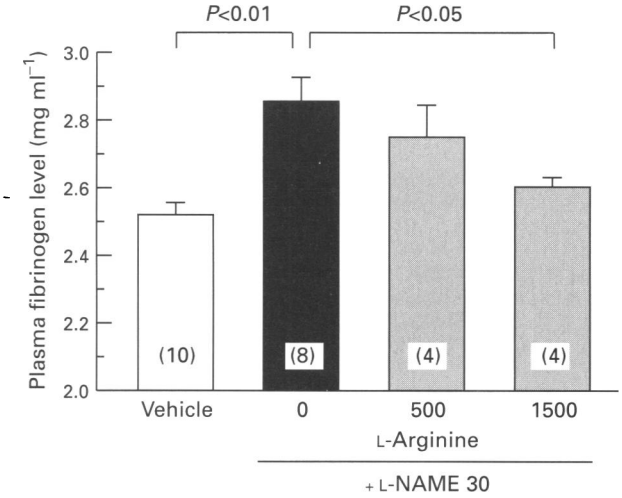

Figure 2 L-Arginine at 500 or 1500 mg kg^{-1} was coadministered orally with N^G-nitro-L-arginine methyl ester (L-NAME) at 30 mg kg^{-1}, twice a day, at 09 h 00 min and 16 h 00 min, for 7 days (14 times in all). Data indicate the mean with s.e.mean, and the figures in parentheses show the number of rats used.

haemostatic system is affected by superoxide anion even under unstressed conditions. Therefore, it is likely that NO reacts with superoxide anion, and the resulting product, peroxynitrite anion, may act to reduce plasma fibrinogen levels in intact animals. NO enhances fibrinolytic activity via an apparent increase in t-PA activity by inhibiting PAI release from platelets in rats (Lidbury *et al.*, 1990). NO synthase inhibitors can reduce enhanced fibrinolytic activity, the effect contributing to the improvement of the DIC phenomena induced by endotoxin (Korbut *et al.*, 1994). The chronic effect of L-NAME in the present study may reflect its anti-fibrinolytic potency through the enhancement of PAI release, if NO is tonically active and acts to enhance fibrinolysis in rodents even under physiological conditions.

The finding that chronic L-NAME failed to alter platelet count in intact rats, implies that endogenous NO does not play a role in the modulation of platelet aggregation or adhesion under physiological conditions. In contrast, Molnár *et al.* (1994) found that continuous infusion of L-NAME at 5 mg kg^{-1} h^{-1} for 3 days reduced platelet count by 58% and 50%, in gravid and virgin rats, respectively. The differences in the doses and administration schedules of L-NAME and in the sex of the animals employed may be responsible for the discrepancy.

In conclusion, for the first time, this study proposes the possibility that endogenous NO is tonically active and modulates plasma fibrinogen levels in rats under physiological conditions.

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