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Attenuation by prolonged nitric oxide synthase inhibition of the enhancement of fibrinolysis caused by environmental stress in the rat

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1 Nitric oxide (NO) suppresses platelet aggregation and plasminogen activator inhibitor (PAI) release from platelets, playing physiological and/or pathological roles in the haemostatic system. We investigated the effect of N^G-nitro-L-arginine methyl ester (L-NAME), an NO synthase inhibitor, on the disseminated intravascular coagulation (DIC)-like phenomena in rats under environmental stress, induced by prolonged fluctuation in air temperature, known as SART (specific alternation of rhythm in temperature) stress.

2 Exposure of rats to SART stress for 7 days caused mild DIC-like symptoms such as thrombocytopenia, hypofibrinogenemia, decreased factor VIII: coagulant activity and shortened euglobulin clot lysis time (ECLT). The enhanced fibrinolysis was accompanied by a marked decrease in the activity of plasma PAI.

3 L-NAME, but not its D-enantiomer, when administered orally at 0.3-10 mg kg^{-1}, twice a day for 7-day exposure to stress, inhibited the stress-induced decrease in fibrinogen levels in a dose-dependent manner, whereas it failed to alter platelet count, factor VIII:coagulant activity and plasma protein levels in stressed rats. All these parameters in unstressed rats were resistant to L-NAME at 10 mg kg^{-1}.

4 Repeated treatment with 10 mg kg^{-1} of L-NAME blocked the shortening of ECLT and the decrease in PAI activity following stress exposure, although it was without effect in unstressed rats.

5 The inhibitory effects of L-NAME at 10 mg kg^{-1} on the stress-induced alterations in fibrinogen levels and in ECLT were significantly reduced by coadministered L-arginine at 1000 mg kg^{-1}.

6 These findings demonstrate that repeated administration of L-NAME attenuates the enhanced fibrinolysis, without aggravating thrombocytopenia, in SART-stressed rats. Endogenous NO appears to contribute to the stress-induced development of fibrinolysis by suppressing plasma PAI activity, most probably as a result of inhibition of the PAI release from platelets.

Keywords: Nitric oxide (NO); N^G-nitro-L-arginine methyl ester; stress; disseminated intravascular coagulation (DIC); fibrinogen; fibrinolysis; euglobulin clot lysis time; plasminogen activator inhibitor; platelets

Introduction

Nitric oxide (NO) plays physiological roles in various biological events, and is also implicated in the pathogenesis of a number of diseases including circulatory failure (Hutcheson *et al.*, 1990; Wright *et al.*, 1992; Moncada & Higgs, 1993; Thiemermann, 1994). NO appears to play a complex dual role in the development of the circulatory failure in septic shock. An overproduction of NO by inducible NO synthase contributes to the severe hypotension and multiple organ dysfunction syndrome in endotoxaemia, implying the therapeutic significance of NO synthase inhibitors (Thiemermann & Vane, 1990; Nava *et al.*, 1991; Thiemermann *et al.*, 1995). Nevertheless, high doses of non-selective NO synthase inhibitors aggravate the injury of the liver (Harbrecht *et al.*, 1992), kidney (Shultz & Raij, 1992) and intestine (Hutcheson *et al.*, 1990) as well as the mortality (Wright *et al.*, 1992; Kawabata, 1995) in endotoxaemic rodents. Selective inhibition of the inducible isoform of NO synthase appears more beneficial, since the inhibition of endothelial NO synthase may be responsible for the reported adverse consequences (Szabo *et al.*, 1994; Thiemermann, 1994; Thiemermann *et al.*, 1995).

NO suppresses platelet aggregation and plasminogen activator inhibitor (PAI) release from platelets, playing physiological and/or pathological roles in the haemostatic system (Lidbury *et al.*, 1990; Korbut *et al.*, 1990; 1995; May *et al.*, 1991). It is of special interest that N^G-nitro-L-arginine methyl ester (L-NAME), an NO synthase inhibitor, improves some of the disseminated intravascular coagulation (DIC) phenomena in endotoxaemic rats, such as the decrease in fibrinogen levels and the shortening of euglobulin clot lysis time (ECLT), possibly by enhancing plasma PAI activity, although it does not alter the degree of the drop in platelet count (Korbut *et al.*, 1994). The effect of NO synthase inhibitors in other types of DIC models remains to be examined at present.

A series of our studies (Hata *et al.*, 1988; 1989; 1991; 1992; Kawabata & Hata, 1993a,b) have demonstrated that mild DIC-like phenomena develop in rats exposed to environmental stress induced by prolonged fluctuation in air temperature, known as SART (specific alternation of rhythm in temperature) stress (Kita *et al.*, 1975). This stress can be produced by repeated exposure of rodents to sudden changes in ambient temperature (from room temperature to low temperature) for a week according to a certain schedule (Hata *et al.*, 1984), resulting in a variety of adverse biological events including haemostatic (Hata *et al.*, 1988; 1989; 1991; 1992; Kawabata & Hata, 1993a,b) and haemodynamic (Hata *et al.*, 1985; 1986) dysfunctions, and also alterations in the immune systems (Hori *et al.*, 1993; Tagoh *et al.*, 1995).

To clarify the complex role of NO in various types of circulatory failure, the present study examined the effect of prolonged NO synthase inhibition by repeatedly administered L-NAME on the mild DIC-like symptoms caused by SART stress in rats. The results obtained imply the involvement of endogenous NO in the enhanced fibrinolysis under stress.

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Methods

Experimental animals

Male Wistar rats weighing 200–300 g (Japan SLC. Inc.) were maintained on a 12-h light-dark cycle on a standard laboratory diet and tap water *ad libitum* before experiments.

Exposure of animals to SART stress

The rats were stressed essentially according to the previously established protocol (Hata *et al.*, 1984; Kawabata & Hata, 1993b). Wire-grating cages (38 × 25 × 17 cm) for stress exposure were prepared in a room maintained at 24°C and in a cold room maintained at −3°C. Rats in groups of 3 or 4 were alternately transferred at 1-h intervals to each cage placed in the former room and in the latter between 09 h 00 min and 16 h 00 min, and were housed in the cage in the latter (−3°C) room between 16 h 00 min and 09 h 00 min overnight. These procedures were repeated for 7 days, and thereafter terminated at 09 h 00 min on the final day of stress.

Blood collection and haemostatic examination

Blood collection was conducted between 11 h 00 min and 12 h 00 min on the final day of stress. Each rat was anaesthetized by subcutaneous administration of pentobarbitone at 40 mg kg^{-1}, and 5 ml of citrated blood (containing 1/10 volume of 3.8% sodium citrate) was withdrawn from the abdominal aorta. Blood examinations were carried out as reported previously (Hata *et al.*, 1991; Kawabata & Hata, 1993b). Briefly, after the visual estimation of platelet count in whole blood, platelet-poor plasma was obtained by centrifuging the citrated blood at 1700 *g* and 4°C for 10 min, and plasma fibrinogen and protein levels were determined by the thrombin time method and by Lowry's method, respectively. Euglobulin clot lysis time (ECLT) was measured essentially according to Gallimore *et al.*, (1971), using the euglobulin fraction prepared by the method of Kluft *et al.* (1976), as described previously (Hata *et al.*, 1991). The activity of blood coagulation factor VIII:coagulant and of plasminogen activator inhibitor (PAI) in plasma was determined spectrophotometrically, with commercially available assay kits using chromogenic substrates (Chmielewska *et al.*, 1983; Kawabata & Hata, 1993b). The activity of factor VIII:coagulant in the samples was expressed as a percentage of the activity in dilutions of plasma pooled from 5 normal rats. In PAI assay, 40 u ml^{-1} of authentic tissue plasminogen activator (tPA) was incubated at 25°C for 20 min in the presence or absence of the citrated sample plasma. After destroying plasmin inhibitors, especially α_2-antiplasmin, by 20 min incubation at 37°C, the residual tPA activity was measured spectrophotometrically by adding Glu-plasminogen, chromogenic plasmin substrate and solubilized fibrin, in micro test plates.

Drug administration schedule

N^G-nitro-L-arginine methyl ester (L-NAME) or its D-enantiomer (D-NAME), in a dose-range of 0.3–10 mg kg^{-1}, were administered orally to rats twice a day, at 09 h 00 min and 16 h 00 min, for 7 consecutive days, 14 times in all, from the first day until the day preceding the termination of stress. Unstressed rats were also treated with L-NAME in the same manner. In the experiments to study the interaction between L-NAME and L-arginine, L-NAME at 10 mg kg^{-1} was coadministered orally with L-arginine at 1000 mg kg^{-1} according to the above schedule. Control animals received vehicle only.

Chemicals used

Drugs used were N^G-nitro-L-arginine methyl ester hydrochloride (Sigma, U.S.A.), N^G-nitro-D-arginine methyl ester hydrochloride (Bachem, Switzerland) and L-arginine hydrochloride (Kishida Chem., Japan). All these drugs were dissolved in saline. Assay kits for fibrinogen (FibrinogenB-Test Wako), factor VIII:coagulant (Testzym FVIII) and plasminogen activator inhibitor (Spectrolyse-fibrin-tPA/PAI) were purchased from Wako Pure Chemicals (Japan), Chromogenix AB (Sweden) and Biopool AB (Sweden), respectively.

Statistics

The results are expressed as the mean with s.e.mean. Statistical analysis was performed by Student's unpaired *t* test or by Newman-Keuls' multiple comparison test, and significance was set at a $P<0.05$ level.

Results

Haemostatic alterations in rats exposed to SART stress

Exposure of rats to SART stress for 7 days resulted in significant decreases in platelet count, fibrinogen levels and factor VIII:coagulant activity, and significant shortening of euglobulin clot lysis time (ECLT). In addition, SART-stressed rats exhibited a marked drop in the activity of plasminogen activator inhibitor (PAI) (Figure 1).

Effect of repeatedly administered N^G-nitro-L-arginine methyl ester on platelet count, coagulation factors and plasma protein levels in stressed rats

Repeated administration of N^G-nitro-L-arginine methyl ester (L-NAME) or its D-enantiomer (D-NAME) at 0.3–10 mg kg^{-1} did not alter platelet count in unstressed and stressed rats. In contrast, L-NAME, but not D-NAME, given in the same dose-range, drastically augmented the decreased plasma fibrinogen levels in stressed rats, although it produced

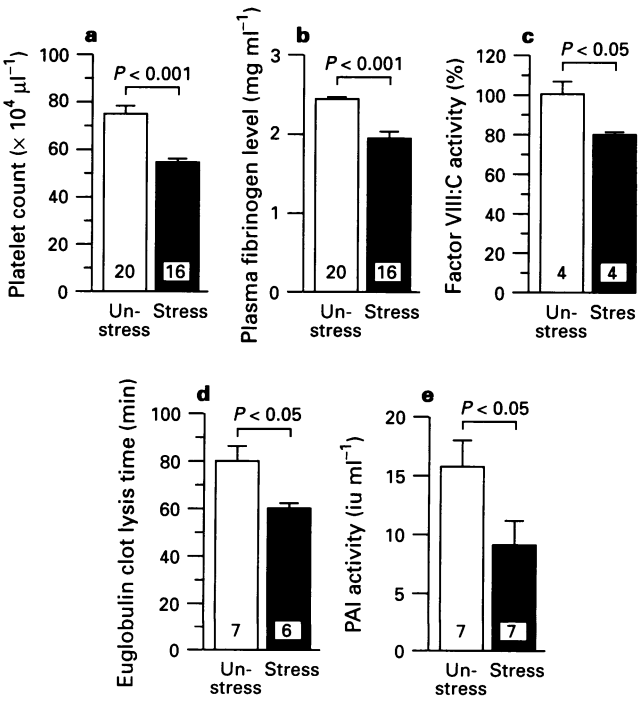

Figure 1 Haemostatic alterations in rats under SART stress: (a) platelet count; (b) plasma fibrinogen; (c) factor VIII: C activity; (d) euglobulin clot lysis time and (e) PAI activity. Rats were exposed to SART stress for 7 days. Data indicate the mean with s.e.mean. The figures in columns show the number of rats used. PAI, plasminogen activator inhibitor.

no effect in unstressed rats. Plasma protein levels were resistant to stress exposure and to repeated treatment with L-NAME as well as D-NAME at 0.3–10 mg kg^{-1} (Figure 2). On the other hand, repeated L-NAME at 10 mg kg^{-1} had no effect on the decreased factor VIII:coagulant activity in stressed rats; the activity of factor VIII:coagulant in unstressed rats treated with vehicle, and in stressed rats treated with vehicle and with L-NAME was 100.5±5.5, 79.4±2.1 and 81.0±3.3% (not significantly different from the vehicle-treated, stressed group), respectively ($n=4$).

Inhibitory effect of repeated N^G-nitro-L-arginine methyl ester on the stress-induced alterations in the fibrinolytic systems in the rat

L-NAME, when administered repeatedly at 10 mg kg^{-1}, inhibited the shortening of ECLT due to stress, while it produced no effect on ECLT in unstressed rats. The same dose of L-NAME also completely blocked the stress-induced drop in the activity of PAI, without altering PAI activity in unstressed rats (Figure 3).

L-Arginine antagonism of the effect of repeated N^G-nitro-L-arginine methyl ester treatment on the decreased fibrinogen levels and shortened euglobulin clot lysis time in stressed rats

In SART-stressed rats, L-arginine, when administered at 1000 mg kg^{-1} in the same schedule as that for L-NAME, did not modify fibrinogen levels by itself, whereas, when coadministered with L-NAME at 10 mg kg^{-1}, it significantly attenuated the L-NAME-induced elevation in fibrinogen levels. Similarly, L-arginine at 1000 mg kg^{-1}, coadministered with L-NAME at 10 mg kg^{-1}, significantly reduced the L-NAME-induced prolongation of ECLT, without altering ECLT by itself, in stressed rats (Figure 4).

Discussion

This study demonstrates that repeated administration of L-NAME, an NO synthase inhibitor, abolishes the decrease in fibrinogen levels, the shortening of ECLT and the decrease in

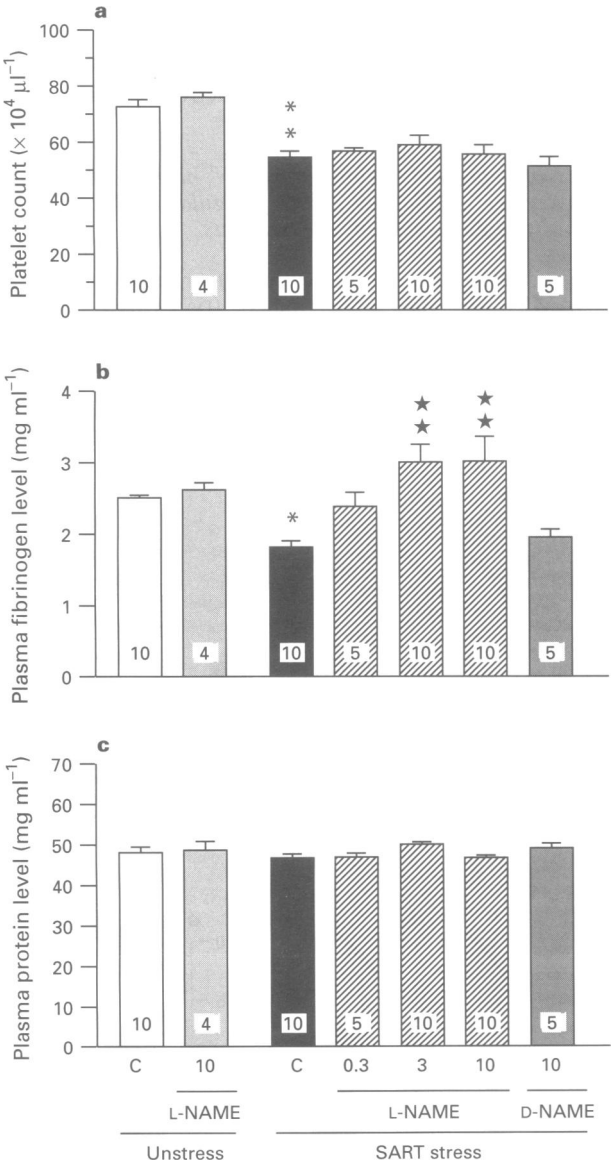

Figure 2 Effect of repeated administration of N^G-nitro-L-arginine methyl ester (L-NAME) on (a) platelet count, (b) plasma fibrinogen and (c) plasma protein levels in unstressed and SART-stressed rats. L-NAME or its D-enantiomer (D-NAME), at 0.3, 3 or 10 mg kg^{-1}, was administered orally twice a day, at 09 h 00 min and 16 h 00 min, for 7 days (14 times in all) during exposure to stress. Data indicate the mean with s.e.mean. The figures in columns show the number of rats used. C, control. *$P<0.05$, **$P<0.01$ vs. the unstressed control; ★★$P<0.01$ vs. the stressed control.

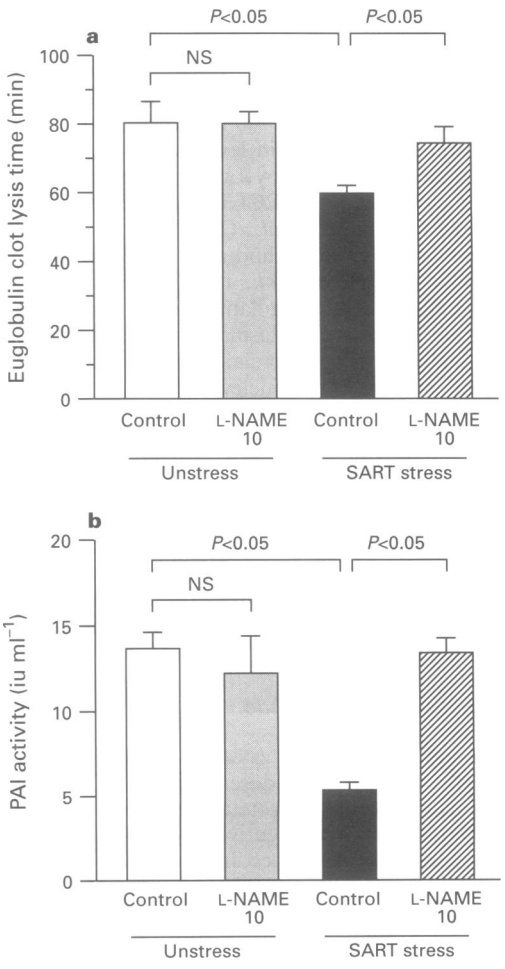

Figure 3 Effect of repeatedly administered N^G-nitro-L-arginine methyl ester (L-NAME) on (a) the shortened euglobulin clot lysis time and (b) the decreased activity of plasminogen activator inhibitor (PAI) in SART-stressed rats. L-NAME at 10 mg kg^{-1} was administered orally twice a day, at 09 h 00 min and 16 h 00 min, for 7 days (14 times in all) during exposure to stress. Data indicate the mean with s.e.mean from 5–8 rats. NS, not significant.

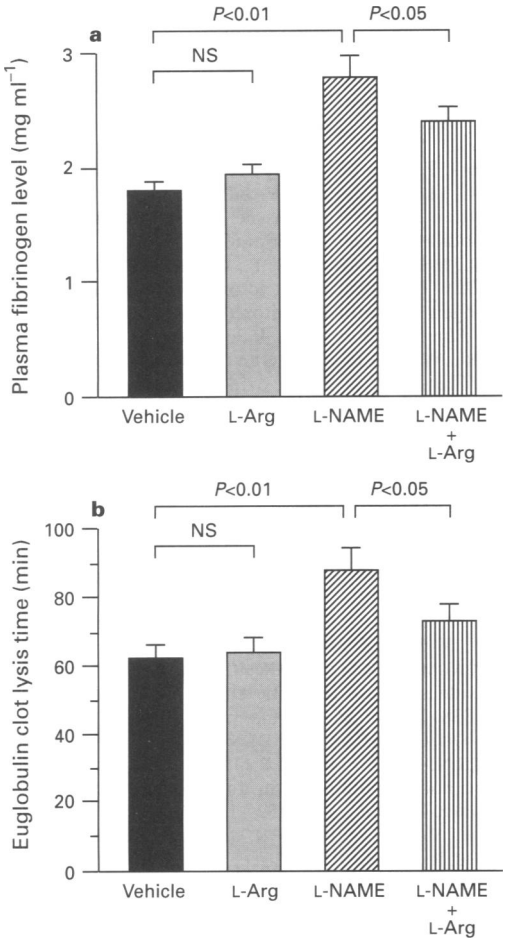

Figure 4 L-Arginine antagonism of the suppressive effect of N^G-nitro-L-arginine methyl ester (L-NAME) on the stress-induced alterations in (a) fibrinogen levels and (b) euglobulin clot lysis time in rats. L-NAME at 10 mg kg^{-1} and L-arginine (L-Arg) at 1000 mg kg^{-1} were coadministered orally twice a day, at 09 h 00 min and 16 h 00 min, for 7 days (14 times in all) during exposure to stress. Data indicate the mean with s.e.mean from 6–10 rats. NS, not significant.

PAI activity in SART-stressed rats, but does not alter the magnitude of the stress-induced decreases in platelet count and factor VIII:coagulant activity. Neither haemoconcentration nor haemodilution are responsible for the alterations in the haemostatic parameters produced by stress exposure and by L-NAME, since plasma protein levels remained constant following either treatment. Thus, repeated L-NAME appears to block the enhanced fibrinolysis among the DIC-like symptoms caused by stress in rats. The findings that the effect of L-NAME was stereospecific, and was attenuated by coadministered L-arginine, a substrate for NO synthase, indicate that the anti-fibrinolytic effect of L-NAME in stressed rats results from the inhibition of NO synthase. Collectively, the present study suggests that L-NAME suppresses the enhanced fibrinolysis by increasing PAI activity as a result of inhibition of NO synthase in stressed rats. In other words, endogenous NO appears to contribute to the stress-induced development of fibrinolysis by suppressing PAI activity.

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 considered to be involved in the activation of fibrinolysis, since various NO donors, including sodium nitroprusside, inhibit the release of PAI from platelets, leading to the apparent increase in tissue plasminogen activator (tPA) activity (Lidbury *et al.*, 1990; Korbut *et al.*, 1990; 1995). Endogenous NO may act tonically to reduce fibrinogen levels by activating fibrinolysis even under physiological conditions (Kawabata, 1996). In the rat challenged with endotoxin, an experimental DIC model, the NO synthase inhibitor L-NAME, preadministered twice at 30 mg kg^{-1}, blocks the decrease in fibrinogen levels and the shortening of ECLT, but does not aggravate the thrombocytopenia (Korbut *et al.*, 1994), suggesting that endogenous NO facilitates fibrinolysis, but does not effectively act to suppress intravascular platelet activation in endotoxaemia. Similarly, in the present study, repeated administration of L-NAME at 10 mg kg^{-1}, a dose which produced no effect in unstressed rats, abolished the stress-induced alterations in fibrinogen levels and in ECLT without aggravating the thrombocytopenia. Thus, L-NAME appears to block the enhanced fibrinolysis in the two distinct DIC models.

SART-stressed animals exhibit a variety of DIC-like symptoms, such as prolonged bleeding time, abnormal platelet functions and prolonged activated partial thromboplastin time, in addition to thrombocytopenia, hypofibrinogenemia, decreased factor VIII:coagulant activity and shortened ECLT (Hata *et al.*, 1988; 1989; 1991; 1992; Kawabata & Hata, 1993a,b). In the present study, we confirmed some of the above profile, and showed the marked decrease in plasma PAI activity for the first time. This decreased PAI activity that is considered responsible for the enhanced fibrinolysis, is a notable character of the stress-induced DIC-like phenomena, differing from the endotoxin-induced DIC (Korbut *et al.*, 1994). In the endotoxaemic model, plasminogen activators are induced in various organs, leading to the enhanced fibrinolysis, whereas, concurrently, PAI is also induced and then its activity in plasma drastically rises (Quax *et al.*, 1990; Padro *et al.*, 1994; Korbut *et al.*, 1994). L-NAME, given twice at 30 mg kg^{-1}, further enhances the increased activity of PAI in endotoxaemia, resulting in its anti-fibrinolytic effect (Korbut *et al.*, 1994). In contrast, we found that repeated treatment with L-NAME at 10 mg kg^{-1}, a dose which produced no effect in unstressed rats, reversed the decreased activity of PAI in stressed rats, to a level equivalent to that in unstressed rats, thereby suggesting that endogenous NO acts tonically to reduce plasma PAI activity and then enhances fibrinolysis under stress, possibly by suppressing the release of PAI from platelets (Lidbury *et al.*, 1990; Korbut *et al.*, 1990; 1995). Whether stress and L-NAME alter plasma levels of the PAI molecule itself is still not clear, because the levels of tPA/PAI complex and plasminogen activators in plasma were not determined in the present study. The effects of SART stress on the activity of constitutive and inducible NO synthase and on plasma nitrite/nitrate levels remain to be investigated.

Radical scavengers such as superoxide dismutase and catalase, when repeatedly administered s.c. according to the same schedule as that in the present study, prevent the decreases in platelet count, fibrinogen levels and factor VIII:coagulant activity in stressed mice, suggesting that oxygen-derived free radicals including superoxide anion participate in the pathogenesis of the DIC-like phenomena caused by SART stress (Kawabata & Hata, 1993b). However, it seems unlikely that NO reacts with superoxide anion, and the resulting product, peroxynitrite anion, participates in the pathogenesis of the DIC-like phenomena, because the prolonged inhibition of NO synthase by L-NAME reduced the hypofibrinogenemia but failed to improve the thrombocytopenia and decreased factor VIII:coagulant activity in stressed rats in the present study.

In conclusion, the prolonged inhibition of NO synthase by repeated administration of L-NAME blocks the enhanced fibrinolysis among the stress-induced DIC-like symptoms in the rat, providing novel evidence for a role of endogenous NO in the development of circulatory failure.

References

CHMIELEWSKA, J., RÅNBY, M. & WIMAN, B. (1983). Evidence for a rapid inhibitor to tissue plasminogen activator in plasma. *Thromb. Res.*, **31,** 427–436.

GALLIMORE, M.J., TYLER, H.M. & SHAW, J.T.B. (1971). The measurement of fibrinolysis in the rat. *Thromb. Diath. Haemorrh.*, **26,** 295–310.

HARBRECHT, B.G., BILLIAR, T.R., STADLER, J., DEMETRIS, A.J., OCHOA, J.B., CURRAN, R.C. & SIMMONS, R.L. (1992). Inhibition of nitric oxide synthesis during endotoxemia promotes intrahepatic thrombosis and an oxygen radical-mediated hepatic injury. *J. Leukoc. Biol.*, **52,** 390–394.

HATA, T., KAWABATA, A. & ITOH, E. (1991). Blood coagulation and fibrinolysis in SART-stressed (repeated cold-stressed) rats and drug effects on the altered hemostatic parameters. *Jpn. J. Pharmacol.*, **56,** 403–412.

HATA, T., KAWABATA, A. & ITOH, E. (1992). Platelet hypoaggregability in rats exposed to SART stress (repeated cold stress). *Thromb. Res.*, **65,** 617–629.

HATA, T., KAWABATA, A., ITOH, E. & KITA, T. (1989). Thrombocytopenia in SART-stressed animals–Comparison to other types of stress. *Jpn. J. Psychosom. Med.*, **29,** 652–658.

HATA, T., KAWABATA, A., KITA, T., ITOH, E. & NISHIMURA, Y. (1988). Changes in platelet count and related parameters in SART-stressed mice and the action of administered Neurotropin. *Jpn. J. Pharmacol.*, **47,** 349–356.

HATA, T., KITA, T., ITOH, E. & HARADA, N. (1984). Experimental studies on optimal conditions of loading SART stress (repeated cold stress) upon animals. *Jpn. J. Psychosom. Med.*, **24,** 257–266.

HATA, T., KITA, T., KAWABATA, A., ITOH, E. & KAWASHIMA, Y. (1985). Effect of ginseng-20S-prosapogenin on tissue blood flow measured by the hydrogen clearance method in sympathicotonic- or parasympathicotonic-type stressed mice. *J. Pharmacobio-Dyn.*, **8,** 1068–1072.

HATA, T., KITA, T., KAWABATA, A., ITOH, E. & NISHIMURA, Y. (1986). Changes of tissue blood flow in mice loaded with SART (repeated cold) stress or restraint and water immersion stress and the effect of administered Neurotropin. *Jpn. J. Pharmacol.*, **41,** 69–79.

HORI, T., FUKUDA, M., SUZUKI, H., YANO, S. & ONO, T. (1993). SART stress effects on lymphocytes in the thymus and spleen of normal, adrenalectomized, and sympathectomized mice. *Clin. Immunol. Immunopathol.*, **68,** 243–255.

HUTCHESON, I.R., WHITTLE, B.J.R. & BOUGHTON-SMITH, N.K. (1990). Role of nitric oxide in maintaining vascular integrity in endotoxin-induced acute intestinal damage in the rat. *Br. J. Pharmacol.*, **101,** 815–820.

KAWABATA, A. (1995). Effect of aminoguanidine on the survival in mice treated with lipopolysaccharide. *Pharmaceut. Sci.*, **1,** 455–457.

KAWABATA, A. (1996). Evidence that endogenous nitric oxide modulates plasma fibrinogen levels in the rat. *Br. J. Pharmacol.*, **117,** 236–237.

KAWABATA, A. & HATA, T. (1993a). Characterization of platelet hypofunctions in rats under SART stress (repeated cold stress). *Thromb. Res.*, **69,** 197–207.

KAWABATA, A. & HATA, T. (1993b). Possible involvement of oxygen-derived free radicals in abnormal hemostasis induced by SART stress (repeated cold stress) in laboratory animals. *Thromb. Res.*, **72,** 321–331.

KITA, T., HATA, T., YONEDA, R. & OKAGE, T. (1975). Stress state caused by alteration of rhythm in environmental temperature, and the functional disorders in mice and rats. *Folia Pharmacol. Jpn.*, **71,** 195–210.

KLUFT, C., BRAKMAN, P. & VELDHUYZEN-STOLK, E.C. (1976). Screening of fibrinolytic activity in plasma euglobulin fractions on the fibrin plate. In *Progress in Chemical Fibrinolysis and Thrombosis* (Vol.2). ed. Davidson, J.F., Samana, M.M. & Desnoyers, P.C. pp. 57–65. New York: Raven Press.

KORBUT, R., LIDBURY, P.S. & VANE, J.R. (1990). Prolongation of fibrinolytic activity of tissue plasminogen activator by nitrovasodilators. *Lancet*, **335,** 669.

KORBUT, R., MARCINKIEWICZ, E., CIESLIK, K. & GRYGLEWSKI, R.J. (1995). The effect of nitric oxide donors on the release of plasminogen activator inhibitor (PAI) from rabbit platelets *in vitro*. *J. Physiol. Pharmacol.*, **46,** 37–44.

KORBUT, R., WARNER, T.D., GRYGLEWSKI, R.J. & VANE, J.R. (1994). The effect of nitric oxide synthase inhibition on the plasma fibrinolytic system in septic shock in rats. *Br. J. Pharmacol.*, **112,** 289–291.

LIDBURY, P.S., KORBUT, R. & VANE, J.R. (1990). Sodium nitroprusside modulates the fibrinolytic system in the rabbit. *Br. J. Pharmacol.*, **101,** 527–530.

MAY, G.R., CROOK, P., MOORE, P.K. & PAGE, C.P. (1991). The role of nitric oxide as an endogenous regulator of platelet and neutrophil activation within the pulmonary circulation of the rabbit. *Br. J. Pharmacol.*, **102,** 759–763.

MONCADA, S. & HIGGS, A. (1993). The L-arginine-nitric oxide pathway. *N. Engl. J. Med.*, **329,** 2002–2012.

NAVA, E., PALMER, R.M.J. & MONCADA, S. (1991). Inhibition of nitric oxide synthase in septic shock: how much is beneficial. *Lancet*, **338,** 1555–1556.

PADRO, T., QUAX, P.H.A., VAN DEN HOOGEN, C.M., ROHOLL, P., VERHEIJEN, J.H. & EMEIS, J.J. (1994). Tissue-type plasminogen activator and its inhibitor in rat aorta–effect of endotoxin. *Arterioscler. Thromb.*, **14,** 1459–1465.

QUAX, P.H.A., VAN DEN HOOGEN, C.M., VERHEIJEN, J.H., PADRO, T., ZEHEB, R., GELEHRTER, T.D., VAN BERKEL, T.J.C., KUIPER, J. & EMEIS, J.J. (1990). Endotoxin induction of plasminogen activator and plasminogen activator inhibitor type 1 mRNA in rat tissues *in vivo*. *J. Biol. Chem.*, **265,** 15560–15563.

SHULTZ, P.J. & RAIJ, I. (1992). Endogenously synthesized nitric oxide prevents endotoxin-induced glomerular thrombosis. *J. Clin. Invest.*, **90,** 1718–1725.

SZABO, C., SOUTHAN, G.J. & THIEMERMANN, C. (1994). Beneficial effects and improved survival in rodent models of septic shock with S-methylisothiourea sulphate, a potent and selective inhibitor of inducible nitric oxide synthase. *Proc. Natl. Acad. Sci. U.S.A.*, **91,** 12472–12476.

TAGOH, H., NISHIJO, H., UWANO, T., KISHI, H., ONO, T. & MURAGUCHI, A. (1995). Reciprocal IL-1β gene expression in medial and lateral hypothalamic areas in SART-stressed mice. *Neurosci. Lett.*, **184,** 17–20.

THIEMERMANN, C. (1994). The role of the L-arginine:nitric oxide pathway in circulatory shock. *Adv. Pharmacol.*, **28,** 45–79.

THIEMERMANN, C., RUETTEN, H., WU, C. & VANE, J.R. (1995). The multiple organ dysfunction syndrome caused by endotoxin in the rat: attenuation of liver dysfunction by inhibitors of nitric oxide synthase. *Br. J. Pharmacol.*, **116,** 2845–2851.

THIEMERMANN, C. & VANE, J.R. (1990). Inhibition of nitric oxide synthesis reduces the hypotension induced by bacterial lipopolysaccharides in the rat *in vivo*. *Eur. J. Pharmacol.*, **182,** 591–595.

WRIGHT, C.E., REES, D.D. & MONCADA, S. (1992). Protective and pathological roles of nitric oxide in endotoxin shock. *Cardiovasc. Res.*, **26,** 48–57.

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