Effects of Nitric Oxide-Related Compounds and Carperitide on Hemodynamics and Hematocrit in Anesthetized Rats

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ABSTRACT—Sodium nitroprusside, nitroglycerin and carperitide (α -human ANP) all reduced mean blood pressure, but only carperitide increased the hematocrit in rats with bilateral renal artery- and ureter-ligation. N^G -Monomethyl-L-arginine, a selective inhibitor of nitric oxide synthesis, elevated the mean blood pressure but did not change the hematocrit significantly. These findings suggest that ANP has a physiological role in regulating circulatory blood volume distinct from that of NO, although both increase intracellular cyclic GMP in the vasculature.

Keywords: Atrial natriuretic peptide, Nitric oxide, Hematocrit

Nitric oxide (NO) is produced in vascular endothelial cells, and atrial natriuretic peptide (ANP) is produced in and released from atrial cells. Both NO and ANP relax vascular smooth muscles (1, 2). ANP activates the particulate guanylate cyclase in smooth muscle (3), while NO activates soluble guanylate cyclase (4). Since ANP has a potent diuretic action (2), it has been suggested that ANP might have a role in regulating blood volume (5). Several investigators have reported that administered atriopeptin (rat ANP) increases the hematocrit in rats (6, 7). However, the increase in hematocrit has not been totally explained by fluid loss due to the diuretic action of ANP. Almedia et al. (6) have hypothesized that the rat ANP-induced increase in hematocrit may be due to an increase in the fluid efflux from capillaries, which thereby causes a decrease in plasma volume. On the other hand, kidney function has also been reported to be regulated by NO (8), but to our knowledge, the effect of NO on hematocrit is not known yet. To assess whether exogenous NO and ANP can modulate the hematocrit, we have examined the effect of NO-generating drugs and carperitide (α -human ANP) on the hematocrit by concomitantly monitoring arterial blood pressure and heart rate in anesthetized rats.

Male Wistar rats weighing 200–290 g were used (Shizuoka Agr. Coop., Hamamatsu, Japan). Each of the drug-treatment groups consisted of 5 rats. Rats

were anesthetized with urethane plus α -chloralose (500 mg/kg and 100 mg/kg, i.p., respectively). The bilateral renal arteries, veins and ureters exposed by a dorsal approach were ligated concomitantly. A tracheotomy was performed to keep good ventilation. Polyethylene catheters (PE 50) were inserted into the left juglar vein for infusion of drugs and into the right common carotid artery for measurement of systemic blood pressure and withdrawal of blood sample for determination of hematocrit. Blood pressure (BP) was measured by a pressure transducer (MPU-0.5A, Nihon Kohden, Tokyo), and heart rate (HR) was measured with a heart rate counter (AT-600T, Nihon Kohden) triggered by the BP pulse. Changes in BP and HR were continuously recorded on a polygraph recorder (RM-6200, Nihon Kohden).

At first, the effect of physiological saline solution (vehicle) given intravenously in a volume of 80 μ l/kg/min for 30 min was studied in all animals used, and then each of the test drugs was cumulatively infused by increasing the concentration in a stepwise fashion at the same infusion speed as saline alone.

Sodium nitroprusside (Nacalai Tesque, Kyoto), nitroglycerin (Nihon Kayaku, Tokyo), carperitide (recombinant α -human ANP; Suntory), and N^G -monomethyl-L-arginine acetate (Sigma, St. Louis) were used in the present study.

The results obtained were expressed as the mean $\pm$ S.E and analyzed with Dunnett's multiple range test us-

Fig. 1. Effects of saline ($\circ$, A; n = 5), sodium nitroprusside ($\blacktriangle$, B; n = 5), nitroglycerin ($\blacksquare$, B; n = 5), carperitide ($\bullet$, C; n = 5) and L-NMMA ($\square$, D; n = 5) on the blood pressure (MBP) and heart rate (HR) in anesthetized rats. Each drug was cumulatively infused at 80 $\mu\text{l/kg/min}$ (i.v.) for 30 min each. Asterisks denote significant differences: *P < 0.05, **P < 0.01, vs. pre-infusion of saline period.

ing Stat View II (Abacus Concepts, Inc., Berkeley, CA) for the Macintosh IIfx (Apple Computer, Inc., Cupertino, CA).

Mean blood pressure (MBP) before infusion of saline in each group receiving saline, sodium nitroprusside (SNP), nitroglycerin (NTG), carperitide and N^G -monomethyl-L-arginine acetate (L-NMMA) was 69 ± 4 , 75 ± 6 , 77 ± 2 , 69 ± 5 and 84 ± 5 mmHg, respectively; and HR was 365 ± 17 , 384 ± 18 , 407 ± 28 , 386 ± 4 and 400 ± 15 beats/min, respectively. Figure 1 shows the time course of the effects of drugs used on MBP (left) and HR (right). Infusion of saline (control) caused no appreciable changes in MBP, but tended to decrease HR (Fig. 1A). SNP at 1 and 3 $\mu\text{g/kg/min}$ also significantly decreased MBP and tended to decrease HR (Fig. 1B), while NTG has no such clear effects on MBP or HR. Carperitide elicited a significant decrease in MBP at 1 and 3 $\mu\text{g/kg/min}$ and HR at 3 $\mu\text{g/kg/min}$ (Fig. 1C). Intravenous administration of 150 and 500 $\mu\text{g/kg/min}$ L-NMMA, a NO synthase inhibitor (9, 10), elicited a significant increase in BP, but no change in HR (Fig. 1D).

The hematocrit at 30 min after infusion of saline in each group for saline (control), SNP, NTG, carperitide and L-NMMA was 49 ± 1 , 51 ± 1 , 46 ± 2 , 45 ± 2 and $47 \pm 1\%$, respectively (Fig. 2). There were no significant differences in the hematocrit among the groups. Saline infusion elicited an appreciable decrease in hematocrit. SNP and NTG (0.1–3 $\mu\text{g/kg}$) tended to decrease the hematocrit in a manner similar to the control (Fig. 2A). Carperitide (0.1–3 $\mu\text{g/kg/min}$) elicited an increase in hematocrit, and the values at 30 min after infusion of 0.3, 1 and 3 $\mu\text{g/kg/min}$ were significantly ($P < 0.05$ or $P < 0.01$) greater than the corresponding value in the control group (Fig. 2B). L-NMMA (15–500 $\mu\text{g/kg/min}$) also tended to decrease the hematocrit to a lesser extent than the NO-producing compounds, but there was no significant difference as compared with the control (Fig. 2C).

In previous studies, rat ANP increased the hematocrit value due to an increase in capillary permeability accompanied by a decrease in plasma volume (6, 7). In the present study, carperitide (α -human ANP) induced an increase in hematocrit in the bilateral renal artery- and ureter-ligated rats, indicating that exogenous ANP including carperitide alters extracellular fluid partition.

Like carperitide, organic nitrocompounds such as SNP and NTG decreased MBP; however, they did not alter the hematocrit. These results suggest that cyclic GMP production in the vasculature may not always lead to hematocrit alterations and that the increase in hematocrit is unlikely to be due to arterial vasodilation alone, because SNP did not increase the hematocrit.

A. Sodium nitroprusside, nitroglycerin

B. Carperitide

C. L-NMMA

Fig. 2. Effects of saline ($\circ$, $n = 5$), sodium nitroprusside ($\blacktriangle$, A; $n = 5$), nitroglycerin ($\blacksquare$, A; $n = 5$), carperitide ($\bullet$, B; $n = 5$) and L-NMMA ($\square$, C; $n = 5$) on the hematocrit in anesthetized rats. Each drug was infused intravenously after pre-infusion of saline alone. * $P < 0.05$ vs. pre-infusion of saline period. $^{\dagger}P < 0.05$; $^{\dagger\dagger}P < 0.01$ vs. value of each period in the control.

Activation of particulate and soluble guanylate cyclases may have different functions in controlling fluid permeability.

It has been reported that ANP increases the hematocrit in nephrectomized rats. Trippodo and Barbee (7) have proposed that exogenous ANP alters the average whole-body capillary hydrostatic pressure in favor of facilitated filtration. Thus, it is plausible that reduction of the intravascular volume is involved in the carperitide-induced hematocrit increase.

The i.v.-bolus injection of L-NMMA, but not of D-NMMA, elicited an increase in arterial blood pressure; this response was reversed by L-arginine in anesthetized guinea pigs and in both anesthetized and conscious rats (11–13). These results indicate that basal NO production is sufficient to modulate peripheral vascular resistance. In the present study, intravenous infusion of L-NMMA elicited a large and sustained increase in arterial blood pressure, but did not cause a significant change in hematocrit. This finding implies that basal NO synthase activity may not be involved in regulating the fluid permeability.

It has been reported that plasma ANP levels are increased in congestive heart failure and renal failure, and also increased in hypertensive rats such as spontaneous hypertensive rat (SHR) and Dahl salt-sensitive rat (2, 14). NO synthesis may be promoted in stroke prone SHR (15). On the other hand, ANP has a strong diuretic effect (2, 14), but NO does not (8). Taken together, ANP might be homeostatically released to regulate hemodynamics and plasma volume, and NO might be released to regulate hemodynamics without changing plasma volume.

In conclusion, exogenous human ANP (carperitide), like rat ANP, modulates plasma volume, but either endogenous or exogenous NO does not. NO may have a different physiological role from ANP, although both are endogenous vasorelaxants which act by increasing the cellular cyclic GMP level.

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