Difference in Pressor Responses to Ng-Monomethyl-L-Arginine between Conscious and Anesthetized Rats

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ABSTRACT — Ng-Monomethyl-L-arginine (L-NMMA; 0.1–10 mg/kg, i.v.), a selective inhibitor of nitric oxide (NO) synthesis derived from L-arginine, elicited a greater increase in blood pressure in urethane/α-chloralose- and pentobarbital-anesthetized rats than in conscious Wistar rats. The pressor response to phenylephrine was almost equivalent in both conscious and anesthetized rats. These findings suggest that the experimental conditions (anesthetized or conscious) modify the spontaneously released NO's contribution to blood pressure regulation in vivo.

One of the endothelium-derived relaxing factors (EDRFs), first described by Furchgott and Zawadzki (1), has been identified as nitric oxide (NO) or a labile nitroso-compound (2, 3). Recent lines of evidence suggest that the guanidino nitrogen of L-arginine is the endogenous substrate for endothelial NO synthesis, and Ng-monomethyl-L-arginine (L-NMMA) competitively inhibits the generation of NO from L-arginine (4–6). NO synthesis from L-arginine is not limited to vascular endothelium; it has also been shown to occur in vascular smooth muscle, vasodilator nerve and activated macrophages, and this is also inhibited by L-NMMA (6–8). Thus, L-NMMA can be utilized as a tool to selectively inhibit NO production. One of us and others have proposed that the basal and acetylcholine-induced NO production are sufficient to modulate peripheral vascular resistance; hence NO may play a role in arterial pressure homeostasis (9–11). In anesthetized guinea pigs (9, 12) and rabbits (10), L-NMMA, but not D-NMMA, elicits a large and sustained increase in arterial blood pressure (BP); this response is reversed by L-arginine. In conscious rats, L-NMMA also elicits an increase in BP (13). However, L-NMMA elicits a much greater BP increase in anesthetized guinea pigs (9) than in conscious rats (13). Then, we have examined if general anesthetic agents have any effect on the pressor response to L-NMMA using conscious and anesthetized rats.

Twelve to 15 week-old male Wistar rats were used. A group of rats was anesthetized with urethane plus α-chloralose (500 mg/kg and 100 mg/kg, i.p., respectively) and a second group of rats was anesthetized with pentobarbital (65 mg/kg i.p.); and then both groups were subjected to the subsequent experiments (see below). In a third group of rats, under sodium thiopental (40 mg/kg, i.p.) anesthesia, indwelling polyethylene catheters (PE 10) were inserted into the distal aorta via the femoral artery for BP measurement and into the jugular vein for drug administration according to the methods described before (13). The catheters were filled with heparin
sodium solution (500 U/ml) to prevent blood coagulation. More than 3 days after the surgical operation, the animals were subjected to the subsequent experiments (see below). BP was measured with the aid of a pressure transducer (MPU-0.5A, Nihon Kohden, Tokyo), and heart rate (HR) was determined with a heart rate counter (AT-600T, Nihon Kohden) triggered by the BP pulse according to the method described before (14). First, the effect of physiological saline solution (vehicle) given intravenously (i.v.) in a volume of 0.1 ml/100 g was studied, and each dose of drug was administered i.v., in an increasing order of dose. Changes in BP and HR were continuously recorded on a polygraph recorder (RM-6200, Nihon Kohden).

L-NMMA HCl was synthesized according to the modified method of Corbin and Reporter and diluted with saline solution as described before (14). Phenylephrine (Sigma, St. Louis, U.S.A.) was dissolved in saline solution. The results obtained were expressed as the mean ± S.E. The results were analyzed with Tukey’s multiple range test using Yukms Statistical Library Series (Yukms Corp., Tokyo).

After the stabilization of BP, the mean BP (MBP) values in conscious and anesthetized rats were 114 ± 2 mmHg (N = 9), 101 ± 8 mmHg (N = 9, urethane plus α-chloralose) and 113 ± 3 mmHg (N = 9, pentobarbital), and HR values were 355 ± 16 beats/min (N = 9), 388 ± 24 beats/min (N = 9, urethane plus α-chloralose) and 388 ± 17 beats/min (N = 9, pentobarbital), respectively. The administration of L-NMMA (0.1–10 mg/kg, i.v. bolus injection) into the conscious and anesthetized rats elicited a pressor response that increased in magnitude with increasing doses of L-NMMA. Both diastolic and systolic levels were increased to the same extent, and thus pulse pressure remained constant. The time to peak pressor response to L-NMMA was 2–4 min at the highest dose of 10 mg/kg in three groups of rats, while the time to peak response to phenylephrine was within 2 min.

The dose-dependent pressor responses to L-NMMA and phenylephrine are shown in Fig. 1. As can be seen in Fig. 1A, L-NMMA elicited a greater increase in MBP in both groups of anesthetized rats than in conscious rats. On the other hand, no difference was seen in the pressor response to phenylephrine (1, 10, 100 μg/kg, i.v.) among the 3 groups of rats (Fig. 1B). The pressor response to L-NMMA, but not phenylephrine, is due to an inhibition of the synthesis of endothelium-derived NO from L-arginine because the response can be abolished by treatment with L-arginine (9).

L-NMMA at a dose of 10 mg/kg tended to elicit a moderate bradycardia that was almost equal in both groups (Fig. 1A). Therefore, the differences in BP increase induced by L-NMMA treatment between conscious and anesthetized rats were not due to the differences of HR changes. As shown in Fig. 1B, phenylephrine elicited a greater decrease in HR in conscious rats than in both groups of anesthetized rats.

Accordingly, our present findings imply that the spontaneously released NO plays a significant role in the regulation of peripheral vascular resistance and hence of arterial blood pressure, and that in anesthetized rats, NO is released more markedly than in conscious rats. The fact that the pressor response to L-NMMA was more marked in the anesthetized animal suggests that NO synthesis is accelerated by anesthetics such as urethane, α-chloralose and pentobarbital. Although the mechanism of the potentiation of the pressor response to L-NMMA by these anesthetics remain to be studied, the possible mechanism is open to speculation. One possibility is that such anesthetics may modify the synthesis of NO in the vasculature. The other possibility is that NO synthesized in the brain regulates BP, and the general anesthetics change NO synthesis in the brain. However, our study is the first to show that L-NMMA has such a central nervous effect. Vargas et al. reported that the pressor response by L-NMMA was attenuated in pithed rats (14). They speculated that the vascular tone may physiologically regulate the release of nitric oxide in vivo, thus the attenuation in pithed rats was not due to the
changes of central nervous activity.

L-NMMA elicited a moderate bradycardia in conscious and anesthetized rats, and in anesthetized guinea pigs and rabbits as well (9, 10, 13). It has been already reported that the decrease in heart rate was mediated by the baroreceptor reflex because the decrease was abolished by pretreatment with atropine (9). The changes of HR mediated by the baroreceptor reflex were smaller in L-NMMA-treated rats than in phenylephrine-treated rats. Sakuma et al. reported that L-NMMA increased renal sympathetic nerve activity in the nerve-intact and denervated rats under anesthesia with urethane plus α-chloralose (15). Thus, HR decrease mediated by the reflex may be compensated by the effect of L-NMMA on the sympathetic nerve activity.

In conclusion, our findings suggest that the routinely used general anesthetic agents modify the contribution of NO to BP regulation.

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