Brief Rapid Communication

Central Hypotensive Action of Clonidine Requires Nitric Oxide

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Background—Clonidine has an antihypertensive effect by its action in the brain and, because we observed that the tonic production of nitric oxide (NO) in the brain is required to maintain blood pressure at its low, normotensive level, the current study was designed to determine whether the hypotensive action of clonidine resulted from its stimulation of excess NO in the brain.

Methods and Results—Porphyritic microsensors were used to quantify NO concentration in the nucleus tractus solitarius (NTS) in vitro in brain slices and in vivo in the anesthetized rat. In both preparations, the basal production of NO in the NTS was 15±3 nmol/L. In vitro stimulation of the NTS with clonidine (50 nmol/L) resulted in an increase in the NO concentration to 84±7 nmol/L. In vivo, the intracerebroventricular (ICV) infusion of clonidine (0.03 μg) caused an increase in NO concentration in the NTS to 128±17 nmol/L. This ICV injection of clonidine caused a fall in mean arterial pressure of −22±1 mm Hg and a decrease of heart rate of −18±2%. The blockade of NO production with Nω-nitro-L-arginine-methyl ester (2 μmol; delivered ICV, 30 minutes before the clonidine) reduced responses to clonidine for both mean arterial pressure and heart rate (−3±1 mm Hg and −2±1% change, respectively).

Conclusion—The stimulation of the release of NO in the brain by clonidine contributes to its central antihypertensive action. (Circulation. 2001;104:1884-1886.)

Key Words: nitric oxide ■ brain ■ hypertension ■ clonidine ■ solitary nucleus

The central antihypertensive action of clonidine was established by Sattler and van Swieten1 in 1967. In their report, they described how this α2-adrenergic agonist produced a depressor effect when it was injected into the vertebral artery but not when it was injected intravenously. In 1995, we observed that nitric oxide (NO), acting centrally, had a physiologically important blood pressure lowering action.2 Other investigators3–5 have reported that the nucleus tractus solitarius (NTS) is a blood pressure regulating center in which NO causes a hypotensive response.

The current studies were undertaken to determine whether the hypotensive action of clonidine, administered centrally, was mediated by its stimulation of the release of NO in the brain. In vitro observations were made on brain slices and were supported by measurements of NO release and blood pressure and heart rate changes in anesthetized rats.

Methods
Male Sprague-Dawley rats (Harlan Industries, Indianapolis, Ind) weighing between 250 and 300 g were used. All procedures were approved by the University Committee on the Use and Care of Animals.

In Vitro Measurements
For NO measurements, rats were decapitated and brains were removed and cut into sagittal slices that were 1 mm thick. The slices were placed in Hanks’ balanced salt solution at 37°C. NO measurements were performed in this bath using a 3-electrode system that consisted of a porphyritic microsensor,6,7 a platinum counter electrode, and a silver-silver chloride reference electrode. The system was coupled with a FAS1 femtostat and an IBM-compatible computer with electrochemical software (Gamry Instruments). With the aid of a micromanipulator, the NO sensor was carefully placed on the NTS of the brain slice, and the baseline was recorded. To determine if clonidine stimulates NO release from the NTS, various concentrations of clonidine (0 to 150 nmol/L) were infused directly on the surface of the brain slices, and the responses were recorded. To confirm that the response was due to α2-adrenergic receptor activation, 50 nmol/L of α-methyl-norepinephrine (α-MNA), another α2-adrenoceptor agonist, was infused on the NTS and NO release was recorded.

In Vivo Measurements
Rats were anesthetized with 50 mg/kg pentobarbital IP. The rat was then mounted in a stereotaxic apparatus, and a 22-gauge guide cannula was placed in the right lateral cerebral ventricle using the following coordinates from bregma: posterior 0.6 mm, lateral 1.4 mm, and deep from the dura 4.0 mm. The cannula was fixed to the skull with super glue (Loctite) All agents administered intracerebroventricularly (ICV) were infused over a 10-second period in 10 μL of physiological salt solution. All chemicals and pharmacological agents were obtained from Sigma.

For recording in vivo NO concentration, a catheter-protected microsensor was advanced into the NTS using the following stereotaxic coordinates from bregma: posterior −12.7 mm, lateral 1.4 mm,
and deep from the dura 8.0 mm. The exact position of the microsensor was determined post mortem. In vivo measurements of NO release were made continuously before (baseline recording), during, and after the ICV infusion of clonidine (0.03 μg).

For recording mean arterial pressure (MAP), a catheter containing heparinized saline was inserted in the left femoral artery before implanting the cannula in the lateral cerebral ventricle. Heart rate was also continuously monitored from the pressure signal.

Statistical evaluation was done using ANOVA with the help of Microcal Origin software. Values are expressed as means ± SEM, with $P<0.05$ considered statistically significant.

### Results

#### In Vitro NO Measurement

Figure 1A shows a representative amperogram of NO release from the NTS stimulated with clonidine (50 nmol/L) or α-MNA (50 nmol/L). Immediately after infusion, an increase in NO concentration was observed from its basal level of 15±3 nmol/L; this concentration reached 84±7 nmol/L by 9±2 seconds after stimulation with clonidine and 49±5 nmol/L by 10±3 seconds after stimulation with α-MNA. A dose-response curve is shown in Figure 1B (left) for the release of NO by clonidine at the NTS (Figure 1B, right).

#### ICV Clonidine In Vivo

Immediately after the ICV infusion of clonidine (0.03 μg), a rapid increase in NO occurred, reaching a peak of 128±17 nmol/L (Figure 2A). The effect of clonidine infusion on MAP is depicted in Figure 2B. Shortly after the infusion of clonidine, a drop in MAP was observed; this decrease reached $-22±1$ mm Hg after 15.4±1.4 minutes and returned to normal values after 30±3 minutes. A similar fall in heart rate (−18±2% change) was observed 22±4 minutes after clonidine administration (Figure 2C).

In a control group of rats (n=6), clonidine (0.03 μg) was infused ICV 30 minutes after a 10 μL infusion of physiological salt solution. The effect of central N⁵-nitro-L-arginine-methyl ester (L-NAME), a blocker of NO synthase (NOS), on the response to clonidine was then studied in a different group of rats because the prolonged action of clonidine precluded administering the agent twice to the same rat. In this test group of rats (n=5), L-NAME (2 μmol) was infused ICV 30 minutes before the clonidine. The L-NAME caused a pressor response (26±0.8 mm Hg), which had returned to control levels in 12±3 minutes. Therefore, the MAP at the time that clonidine was administered in this group was not different from that in the control group of rats.

Pretreatment with L-NAME greatly reduced the concentration of NO release (from 128±17 to 21±4 nmol/L) and the duration and magnitude of the MAP and heart rate responses to clonidine (Figure 2). The time required to reach the nadir of the MAP response was reduced from 15.4±1.4 minutes to 6.5±1.4 minutes; time required to return to the basal MAP was reduced from 30±3 minutes to 11±2 minutes. The magnitude of the depressor response was reduced from $-22±1$ mm Hg to $-3±1$ mm Hg. There was no difference...
between the secondary pressor responses to clonidine of the control group (16.6±1.6 mm Hg) and the group that received L-NAME (14.4±1.1 mm Hg). The change of the heart rate was also reduced (from −18±2% after 22±4 minutes to −2±1% after 8±3 minutes).

Discussion
These observations confirm and quantify the central depressor action of clonidine and, for the first time, establish that clonidine stimulates the release of NO, which contributes to this depressor action.

The central depressor action of NO is well established. A definitive study by Shapoval et al. demonstrated the important finding that NOS is active tonically, is a continuous regulator of the activity of the sympathetic nervous system and, hence, of blood pressure. Shapoval et al. studied the influence of NO on the activities of the cardiovascular regulatory centers of the ventrolateral medulla in the anesthetized cat. They monitored the effects of NO on blood pressure and on renal sympathetic nerve traffic. Increases in NO concentration produced by the microinjection of either sodium nitroprusside or L-arginine into the rostral ventrolateral medulla, which contains sympathoexcitatory neurons, resulted in a fall in the frequency of sympathetic nerve firing and in blood pressure. Injections of Nω-monomethyl-L-arginine (L-NMMA) to block the endogenous production of NO by NOS resulted in an increase in the frequency of nerve activity and an increase in blood pressure. This observation demonstrated the important finding that NO is active tonically, producing a level of NO that suppresses the activity of the sympathetic nervous system. Decreasing the rate of NO production permits an increase in the rate of firing of the sympathoexcitatory neurons and a rise in blood pressure. Other investigators have observed a similar depressor action of NO in the paraventricular nucleus and in the NTS. Although in the current studies direct observations were made only in the NTS, we assume that the pharmacological responses that we report with the ICV administration of clonidine or L-NAME were mediated by their actions on all of these cardiovascular regulatory centers.

We hypothesize that clonidine may stimulate NO synthase activity by increasing intracellular calcium. Recently, extensive evidence by McCann and Rettori indicated that adrenergic activity causes an increase in intraneuronal calcium concentration, which stimulates NO production. Luo and Vincent demonstrated that adrenergic stimulation causes NO release in the hippocampus of the anesthetized rat. These observations give strong support to the hypothesis that adrenergic activity stimulates NOS activity by increasing the intracellular calcium concentration.

Our current study demonstrates that with NOS blockade using a dose of 2 μmol of L-NAME ICV, the depressor response to clonidine was reduced to one-seventh of the control depressor response. This observation, along with data obtained from direct in vivo and in vitro NO measurements, demonstrates that the major component of the central depressor action of clonidine is mediated by its stimulation of NO release.

The stimulation of NO production that we demonstrated in response to clonidine has special relevance to the use of this agent in the treatment of hypertension. In a recent study, we reported that the genetically hypertensive rat (SHRSP) is much more sensitive to the central hypotensive action of NO than is the normotensive reference strain of rats. We compared the hypotensive response produced by the ICV administration of DEA/NO (an NO donor kindly given to us by Dr Larry Keefer, Frederick, Md) to SHRSP rats with the responses of normotensive rats to this NO donor. The SHRSP rat was much more sensitive to this central NO action than was the normotensive rat. These observations suggest that the effect of this major hypotensive mechanism of clonidine may be exaggerated in hypertension.

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References
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