Vol. 206, No. 1, 1995 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 5, 1995 Pages 77-81

THE ROLE OF NITRIC OXIDE IN THE CENTRAL CONTROL OF BLOOD PRESSURE

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Received November 21, 1994

Summary. In these studies blood pressure responses to intracerebroventricular (i.c.v.) infusions were recorded in anesthetized rats. NO donors caused a fall in blood pressure, whereas L-NAME, which blocks the enzyme (NOS) that produces NO, caused a rise in blood pressure. Calcium, i.c.v.,stimulates NOS to lower blood pressure. The depressor action of NO is reduced by blocking the action of cGMP. This central NO/cGMP system is tonically active to maintain blood pressure at a normal level.

Since its introduction to biologists in 1992 as "the molecule of the year" (1), nitric oxide (NO) has been recognized as a novel regulatory messenger with important physiological functions in many organs. It is released in varying amounts in several parts of the brain (2) where its many regulatory functions are under active investigation. Acting in the nucleus tractus solitarious (3) or the paraventricular nucleus (4), NO causes a fall in systemic blood pressure that reflects a decrease in sympathetic outflow (5). It is the aim of the current study to characterize this central depressor action of NO. The role of calcium in the production of this NO, and that of cGMP in the action of NO have been evaluated. These observations make it evident that a tonic release of NO in the cardiovascular regulatory center plays a physiological role in maintaining blood pressure at a normal level.

METHODS

Animals

Male Wistar Kyoto (WKY) rats, weighing 225.6 ± 2.3 g, with mean arterial pressures of 103 ± 3.3 mmHg, were from Harlan Industries, Indianapolis.

Surgical procedure

The rats were anesthetized with Ketamine, 0.85 mg/Kg, and Xylazine, 0.15 mg/Kg, both i.m. A heparin-filled catheter was inserted in the left femoral artery and advanced into the aorta for blood pressure recording via a Gould-Statham pressure transducer. The rat was mounted in a stereotaxic apparatus and a 22 gauge guide cannula was placed in the right lateral cerebral ventricle, using stereotaxic coordinates; anteroposterior, 5.6 mm; lateral, 1.7 mm; and dorsoventral, 3.0 mm. The cannula was fixed to the skull with Super Glue.

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0006-291X/95 $5.00

Drugs and statistical analysis

All agents administrated i.c.v. were infused over a 10 seconds period in 10 µl physiological salt solution (PSS) of the following composition (in mM): NaCl (130), KCl, (4.7), KH_2PO_4, (1.18), $MgSO_4$, (1.17), dextrose, (5.5), disodium, calcium versenate (EDTA; 0.026), hepes buffer, (30). The following agents were obtained from Sigma, St. Louis, MO: L-NAME and all of the components of the PSS; Rp-8-Br-cGMPS was from BioLog-Life Science Institute, La Jolla, CA, and DEA/NO was kindly given to us by Dr. Larry K. Keefer, Frederick, MD.

Responses were quantified as the maximum change in MAP from that recorded immediately before the agent was administrated. Means and SEMS were calculated for the blood pressure responses to each agent. For comparisons of data before and after treatment with L-NAME or with Rp-8-Br-cGMPS, the paired Student's t test was used. A p value of < 0.05 was considered to represent a statistically significant difference.

RESULTS

Nitric oxide content in the lateral ventricle was increased by administration of NO donors. Either sodium nitroprusside or DEA/NO consistently produced depressor responses. Figure 1A depicts the dose-dependence of this effect when DEA/NO was administered. A dose as small as 100 nmol produced a significant fall in MAP. Blockade of the endogenous production of NO by administration of L-NAME produced a dose-dependent pressor response (figure 1B).

Since neural nitric oxide synthase (NOS) is known to be a calcium-dependent enzyme (6), we determined whether its activity could be increased by central administration of calcium. In figure 2A it is evident that i.c.v. administration of calcium chloride (100 nmol) produced a depressor effect and that this depressor effect was eliminated after the NOS had been blocked by an i.c.v. infusion of L-NAME (1.0 µmol). In figure 2B, the control study demonstrates that the depressor response to calcium chloride persists if the NOS is not blocked.

Several of the central actions of NO have been shown to be mediated by the stimulation of guanylyl cyclase which produces cGMP (7). We determined whether the depressor effect of NO

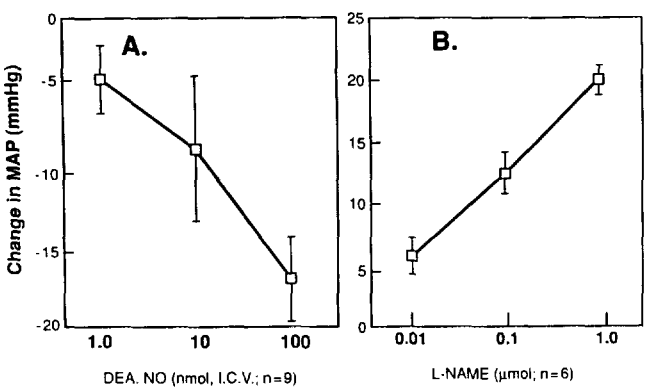

Figure 1. Changes in mean arterial pressure (MAP) resulting from manipulations of the concentration of NO in the brain. A. Dose-response relationship of the i.c.v. administration of the NO donor, DEA/NO. Mean responses with standard error bars. n= 9. B. Dose-response relationship of the i.c.v. administration of the NO synthase blocker, L-NAME. n = 6.

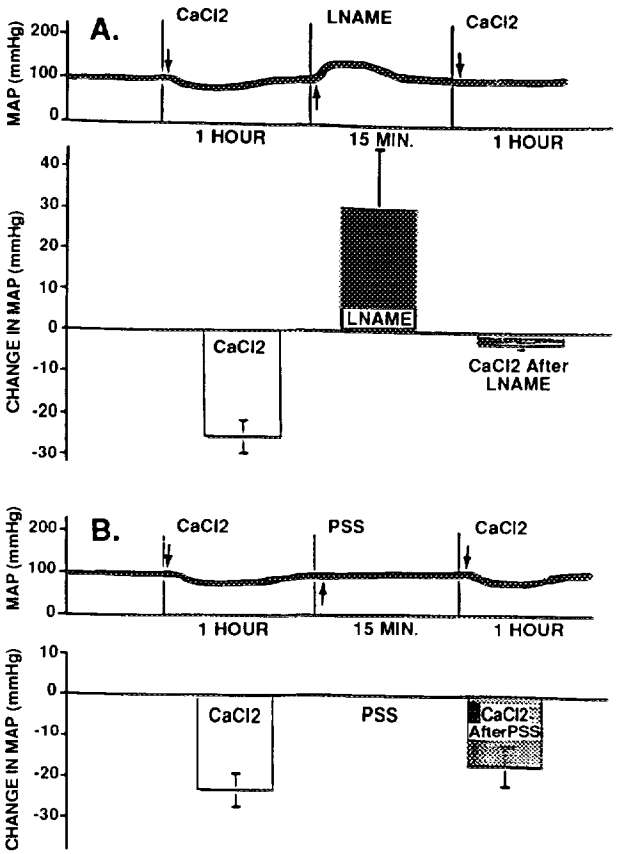

Figure 2. Effect of L-NAME on the depressor response caused by calcium chloride. In 2A the tracing represents the mean arterial pressure (MAP) responses of a representative experiment in which the arrows indicate the times of i.c.v. infusions of $CaCl_2$ (100 nmol) and L-NAME (1.0 μmol). Note the difference in the time axis, indicating that the depressor response to calcium is longer in duration than the pressor response to L-NAME. The bar graphs indicates that the depressor response to the i.c.v. administration of $CaCl_2$ was reduced from -24.1 ± 2.5 mmHg to -2.3 ± 1.9 mmHg following i.c.v. infusion of L-NAME ($p < 0.01$; $n = 8$). In the control study, summarized in 2B, the i.c.v. infusion of 10 μl PSS did not significantly change the response to $CaCl_2$.

was cGMP-mediated by blocking the action of this cyclic nucleotide with Rp-8-Br-cGMPS (8; figure 3). This blocking agent itself caused a pressor response and greatly diminished the depressor response to subsequently administered DEA/NO.

DISCUSSION

These observations have confirmed reports that NO, acting centrally, causes a depressor response. Prior studies have indicated that the site of this action is in nucleus tractus solitarious (3), the paraventricular nucleus (4), or the ventral medulla (5). Our study, making infusions into the lateral cerebral ventricle, did not permit a localization of the site of action of the agents that we

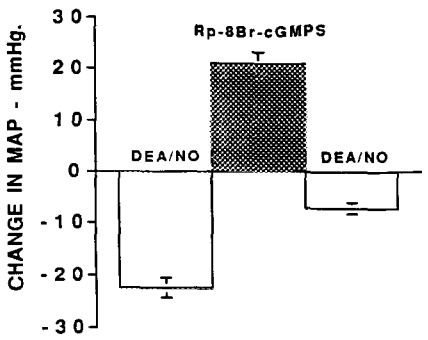

Figure 3. Effect of blocking the action of cGMP on the depressor response to NO. Rp-8-Br-cGMP (which blocks the action of cGMP on cGMP-dependent protein kinase), administered i.c.v. (20 nmol) caused a pressor response and decreased the depressor response caused by DEA/NO (25 nmol, i.c.v.), demonstrating that cGMP is physiologically acting to hold the blood pressure down to its normal level and that this second messenger mediates the action of NO.

administered, however we have observed that the blood pressure effects of i.c.v. infusion of L-NAME were not altered in rats with AV3V lesions (unpublished observation).

Our observations have presented evidence for the first time indicating that the central depressor action of calcium is mediated by its stimulation of NOS and that the depressor action of NO is dependent on its stimulation of cGMP.

An increase in extracellular calcium has been shown to increase the release of EDRF (NO) by endothelial cells (9). This NO is produced by a constitutive form of NOS as is the NO in the brain. Itoh et al. (10) reported that calcium injected i.c.v. consistently produced a dose-dependent decrease in mean arterial pressure in the rat. Pretreatment with the calcium channel blocker, diltiazem, attenuated the response. Although these investigators did not consider the cellular mechanism by which calcium produced this effect in the brain, they presented evidence that the depressor action was associated with an inhibition of the sympathetic nervous system. Based on our observation that the depressor effect can be eliminated by blockade of NOS with L-NAME (figure 2) we propose that, in the unblocked system, calcium stimulates the release of NO which, acting in a cardiovascular regulatory center, depresses sympathetic outflow.

An important mechanism by which NO mediates its physiological effects is the stimulation of guanylyl cyclase to produce cGMP (7). Our observation that the depressor action of i.c.v. DEA/NO is greatly diminished by blockade of the action of cGMP (figure 3) indicates this second messenger plays an important role in the depression of the sympathetic nervous system by NO.

ACKNOWLEDGMENTS

The authors are indebted to Ms. Leslie Rolston for her skillful preparation of this manuscript. The studies were supported by National Heart, Lung and Blood Institute grants HL-46402 and HL-18575.

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