Nitric Oxide and Central Antihypertensive Drugs
One More Difference Between Catecholamines and Imidazolines

Guata Yoro Sy, Véronique Bruban, Pascal Bousquet, Josiane Feldman

Abstract—NO is known to be involved in the peripheral and central regulation of the cardiovascular function. It plays a neuromodulatory role via a direct action on presynaptic nerve terminals, stimulating the release of γ-aminobutyric acid, glutamate, and norepinephrine. Our aim was to study the possible role of NO in the cardiovascular effects of the central antihypertensive drugs clonidine, rilmenidine, and α-methyl-norepinephrine (α-MNA). Sites and mechanisms of the hypotensive action of these drugs were different; clonidine and rilmenidine acted on imidazole receptors in the nucleus reticularis lateralis, whereas α-MNA acted upon α2-adrenoceptors in the nucleus tractus solitarius. The influence of N0-nitro-L-arginine, an NO synthase inhibitor, on the central hypotensive effects of these drugs was investigated in pentobarbital-anesthetized rabbits. The intracisternal (IC) administration of α-MNA (30 μg/kg) induced hypotension (79±2 versus 103±4 mm Hg) and bradycardia (222±8 versus 278±4 bpm) (P<0.05) (n=5). Clonidine (0.07 μg/kg IC) also induced hypotension (69±5 versus 99±4 mm Hg) and bradycardia (266±7 versus 306±10 bpm) (P<0.05) (n=5). In addition to clonidine, rilmenidine (1 μg/kg IC) induced hypotension (64±4 versus 97±4 mm Hg) and bradycardia (264±11 versus 310±4 bpm) (P<0.05) (n=5). Pretreatment with N0-nitro-L-arginine (900 μg/kg IC) completely prevented the hypotensive effect of α-MNA but influenced the cardiovascular effects of neither clonidine nor rilmenidine. These results confirm that imidazole drugs, such as clonidine, rilmenidine, and the catecholamine α2-adrenoceptor agonist α-MNA, have distinct mechanisms of action. (Hypertension. 2001;37:246-249.)

Key Words: nitric oxide ■ blood pressure ■ clonidine ■ norepinephrine ■ central nervous system

The free radical NO is known to be involved in the central and peripheral regulation of the cardiovascular function (for a review, see Krukoff1). In the central nervous system, NO has a neuromodulatory role, influencing the peripheral autonomic function.2,3 In neurons, the synthesis of NO from L-arginine is catalyzed by the NO synthase enzyme (nNOS), which is calcium/calmodulin dependent. This enzyme is stimulated by glutamate-induced activation of N-methyl-D-aspartate receptors, which increases the intracellular influx of calcium; in turn, the diffusible NO activates soluble guanylyl cyclase, producing cGMP in neighboring neurons and astrocytes to trigger its effect.1,4 Immunohistochemical studies have shown the existence of cellular groups containing nNOS that are constitutive of many important autonomic structures of the hypothalamus, brain stem, and spinal cord.5-9

We are particularly interested in the brain stem structures of the baroreflex arch, including the nucleus tractus solitarius (NTS) and the rostroventrolateral medulla/nucleus reticularis lateralis (RVLM/NRL). The NTS is the site of the hypotensive action of α-methyl-norepinephrine (α-MNA), the active metabolite of α-methyl-dopa, which is a catecholamine, full agonist at α2-adrenoceptors.10,11 The RVLM/NRL is the major site of the hypotensive effect of imidazole drugs such as clonidine and rilmenidine, which requires specific imidazole receptors, insensitive to catecholamines.12-17

Ma et al18 showed that N0-nitro-L-arginine methyl ester, an NO inhibitor applied directly into the NTS, inhibited the electrical activity of the cardiovascular neurons. According to Chan and Sawchenko,19 there is a large amount of NOS mRNA in the NTS. These authors also reported that NOS expression was enhanced in the RVLM region during the baroreflex activation. They described a direct NOergic pathway from the NTS to the RVLM, which does not relay in the caudal ventrolateral medulla. Microinjections of L-arginine into the RVLM/NRL induced an inhibition of the sympathetic tone, which was prevented by NOS blockers.20,21 Agmatine, which was described as an endogenous ligand of the imidazoline receptors, as well as NO, derives from L-arginine.22 Agmatine is also a competitive inhibitor of NOS and thus could be an endogenous regulator of the production of NO.23 A large body of evidence supports that NO plays a neuromodulatory role and influences the release of several neurotransmitters, such as glutamate, γ-aminobutyric acid (GABA), and norepinephrine.24-29

Our aim in the present work was to study the possible role of NO in the cardiovascular effects of antihypertensive imidazoline drugs, such as clonidine, rilmenidine, and the catecholamine α-MNA. The central cardiovascular effects of α-MNA are due to stimulation of the α2-adrenoceptors of the
NTS, whereas those of clonidine and rilmenidine involve the specific imidazoline receptors of the NRL.\textsuperscript{12–17} To study whether NOergic pathways are involved in the cardiovascular effects of clonidine, rilmenidine, and/or α-MNA, we used N\textsuperscript{ω}-nitro-L-arginine (L-NNA) as an NOS inhibitor.

**Methods**

**Animals and Hemodynamic Measurements**

Normotensive male rabbits (Zika strain) weighing 2.5 to 3.5 kg were anesthetized with 40 mg/kg sodium pentobarbinate injected through the marginal vein of the ear. Rectal temperature was maintained at 38±0.5°C with the aid of a warming blanket as soon as anesthesia was established (Harvard Apparatus Ltd). The animals were tracheotomized, immobilized with pancuronium bromide (1 mg/kg IV), and artificially ventilated with room air (model 6025; Hugo Sachs Elektronik). The ventilation parameters were adjusted to maintain Pa\textsubscript{O\textsubscript{2}} at 100 mm Hg and Pa\textsubscript{CO\textsubscript{2}} at <40 mm Hg. The right femoral vein was catheterized to allow intravenous injections, and the instantaneous arterial pressure was measured through a catheter inserted in the abdominal aorta via the right femoral artery and connected to a pressure processor and recorder (model BS-272; Gould Electronics). Mean arterial pressure (MAP) was calculated as diastolic pressure plus one third of the differential pressure. The heart rate (HR) was also continuously monitored from the pressure signal with a Gould Biostach amplifier (model 13-4615-66).

**Drugs**

The following drugs were used: sodium pentobarbinate (Sanofi), pancuronium bromide (Pavulon), L-NNA (Sigma), clonidine (Catapressan; Boehringer-Ingelheim), α-MNA (Sigma), and rilmenidine (Servier).

**Intracerebral Injections**

The animal’s head was placed in a stereotaxic frame (La Précision Cinématographique Française). At the beginning of each experiment, before any drug injection, an equal volume of cerebrospinal fluid was withdrawn. Single doses of clonidine (0.07 μg/kg), rilmenidine (1 μg/kg), or α-MNA (30 μg/kg) were injected intracisternally (100 μL) directly within the cisterna magna after a pretreatment with vehicle or L-NNA. In pretreated animals, L-NNA was injected 15 minutes before the single dose of clonidine, rilmenidine, or α-MNA. The effects of the drugs were measured only when the steady-state effect was achieved (ie, 5 to 10 minutes after central injection). Repeated injections of vehicle never produced any significant change of the hemodynamic parameters (n=5).

**Statistics and Calculations**

Data are given as mean±SEM. Effects of drugs in the presence or absence of L-NNA were compared. The homogeneity of the initial cardiovascular parameters in all groups was checked with a 1-way ANOVA. Results were then compared with an intergroup ANOVA with repeated measures followed by a post hoc test. A value of \(P<0.05\) was used as the criterion of significance, and \(n\) represented the number of experiments. The calculations were made with computer-assisted analyses with the StatView software (Abacus Concepts).

**Results**

In all groups of animals, the initial cardiovascular parameters were checked for their homogeneity and were not significantly different (\(P>0.05\)).

**Central Cardiovascular Effects of α-MNA in the Presence or Absence of L-NNA**

The intracisternal (IC) administration of a single dose of α-MNA (30 μg/kg) induced hypotension and bradycardia; MAP varied from 103±4 to 79±2 mm Hg (\(P<0.05\)), and HR varied from 278±4 to 222±8 bpm (\(P<0.05\)) (n=5).

L-NNA (900 μg/kg IC) had no significant effect on the cardiovascular parameters by its own: MAP varied from 96±4 to 106±2 mm Hg (NS) and HR varied from 290±7 to 294±4 bpm after 15 minutes of observation (NS, n=5). Pretreatment with L-NNA (900 μg/kg IC) completely prevented the hypotensive effect of α-MNA but only partially its bradycardic effect. MAP varied from 96±4 to 97±3 mm Hg (NS), and HR varied from 290±7 to 256±10 bpm (\(P<0.05\)). Compared with the maximal bradycardic effect observed with α-MNA alone (ΔHR 56 bpm), α-MNA–induced bradycardia was significantly weakest in animals pretreated with L-NNA (ΔHR 34 bpm) (\(P<0.05\)) (Figure).

**Central Cardiovascular Effects of Clonidine in the Presence or Absence of L-NNA**

The IC administration of a single dose of clonidine (0.07 μg/kg) reduced MAP from 99±4 to 69±5 mm Hg and HR from 306±10 to 266±7 bpm (\(P<0.05\)) (n=5). In another series of 5 experiments, L-NNA (900 μg/kg IC) had no significant effect on the cardiovascular parameters by itself: MAP varied from 101±2 to 107±2 mm Hg (NS) and HR varied from 302±10 to 308±10 bpm (NS) within 15 minutes of injection. In these animals, L-NNA (900 μg/kg IC) prevented neither the hypotensive nor the bradycardic effects of clonidine subsequently administered intracisternally: MAP still varied from 100±2 to 64±3 mm Hg, and HR varied from 302±10 to 248±17 bpm (\(P<0.05\)) (n=5). These values were not significantly different from control values observed with clonidine alone (Figure).

**Central Cardiovascular Effects of Rilmenidine in the Presence or Absence of L-NNA**

The IC administration of a single dose of rilmenidine (1 μg/kg) induced hypotension and bradycardia; MAP varied from 97±4 to 64±4 mm Hg, and HR varied from 310±4 to 264±11 bpm (\(P<0.05\)) (n=5). L-NNA (900 μg/kg IC) did not significantly change the cardiovascular parameters: MAP varied from 96±2 to 106±1 mm Hg and HR varied from 318±9 to 334±10 bpm after 15 minutes of observation (NS, n=5). As observed with clonidine, the pretreatment with L-NNA did not prevent the hypotensive and bradycardic effects of rilmenidine. MAP varied from 96±2 to 70±3 mm Hg, and HR varied from 318±9 to 270±4 bpm (\(P<0.05\), n=5).

**Discussion**

Based on the data from preliminary experiments, we selected the dose of each drug (clonidine, rilmenidine, α-MNA) that reduced blood pressure by ~30% when injected intracisternally.

This study shows that a central pretreatment with the NOS inhibitor L-NNA completely prevented the hypotensive effect of α-MNA, whereas under the same experimental conditions, the cardiovascular effects of clonidine and rilmenidine were not affected by this pretreatment. This observation provides further evidence that the pharmacological mechanisms of action of α-MNA on the one hand and of clonidine and
rilmenidine on the other hand and/or the central structures that they target are different. α-MNA is a catecholamine with high affinity and selectivity for α₂-adrenoceptors, whereas clonidine and rilmenidine require nonadrenergic imidazoline-specific receptors to reduce blood pressure. It is established that the site of the hypotensive action of the α-MNA is the NTS, where imidazoline-like drugs are inactive, whereas the NRL is the site of the hypotensive action of imidazoline-like drugs but not of catecholamines. Rilmenidine is a centrally acting antihypertensive drug that is more selective for imidazoline receptors than clonidine. Like clonidine, the cardiovascular effects of rilmenidine were insensitive to the NOS blocker L-NNA. The present data provide additional evidence that show clonidine and rilmenidine injected intracisternally do not act on the α₂-adrenoceptors of the NTS, which are normally targeted by α-MNA injected in the same manner. They also indicate that the central hypotensive effect of imidazoline-like drugs does not involve NO.

The most attractive hypothesis concerning the mechanisms beyond the present data are that an interaction may occur at the presynaptic level on neuronal pathways that project into the NRL. Stimulation of the NTS by α-MNA is known to elicit a presynaptic GABA release within the NRL. This GABA release might be modulated by NO. In vitro studies carried out in isolated brain nerve endings demonstrated that the depolarizing effect of NO was due to a direct action on the presynaptic membrane; this depolarization was mediated by a decrease in potassium permeability and an inhibition of the sodium pump. In addition, electrophysiological experiments performed on rat nodose ganglia nerve endings have shown that NO donors such as the diethylamine-NO and S-nitroso-N-acetylpenicillamine induced a dose-dependent depolarization; a pretreatment with LY83583, an inhibitor of guanylate cyclase, prevented the depolarizing effect of these drugs, suggesting that the depolarizing effect of NO was mediated by cGMP. The NOS inhibitor could therefore block the NO-stimulated GABA release in the NRL and consequently prevent the hypotensive effect of α-MNA. Further experimental studies are needed to test these hypotheses, which are not exclusive.

The hypotensive effect of clonidine, which is known to originate in the NRL, where α-MNA is inactive, is insensitive to NOS blockers. Therefore, any direct interaction among clonidine, rilmenidine, and NO in the NRL can be ruled out. We also observed a difference between the bradycardic effects of α-MNA and clonidine in the presence of L-NNA. The bradycardic effect of clonidine and rilmenidine was not prevented by the pretreatment with L-NNA, whereas that of α-MNA was diminished by L-NNA.

In conclusion, the present study provides further evidence that the central sites and/or mechanisms of the cardiovascular effects of clonidine, rilmenidine, and α-MNA are different. We also show for the first time that the central hypotensive effect of the α₂-adrenoceptor full agonist α-MNA is NO dependent.

References


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