ELSEVIER

Autonomic Neuroscience: Basic and Clinical 128 (2006) 19 – 24

AUTONOMIC NEUROSCIENCE: Basic & Clinical

www.elsevier.com/locate/autneu

Opposite to α_2-adrenergic agonists, an imidazoline I_1 selective compound does not influence reflex bradycardia in rabbits

Guata Yoro Sy, Pascal Bousquet *, Josiane Feldman

Laboratoire de Neurobiologie et Pharmacologie Cardiovasculaire, INSERM U 715, Faculté de Médecine, Université Louis Pasteur, 11 rue Humann, 67000 Strasbourg, France

Received 23 September 2005; received in revised form 18 November 2005; accepted 4 December 2005

Abstract

This work aimed to study the respective effects of central α_2-adrenergic receptors (α_2-AR_S) and I_1 imidazoline receptors (I_1Rs) in the facilitatory effects of imidazoline-like drugs on the reflex bradycardia (RB). Experiments were performed in anaesthetized rabbits. The reflex bradycardic response was induced by phenylephrine injected i.v. LNP 509, rilmenidine and dexmedetomidine were administered intracisternally (i.c.).

LNP509 (1 mg/kg, i.c.), a ligand highly selective for I_1Rs, induced hypotension (54 ± 3 vs. 93 ± 2 mm Hg) and bradycardia (260 ± 13 vs. 322 ± 13 beats/min) ($p < 0.05$, $n = 5$) but did not affect RB.

Rilmenidine (1 μg/kg, i.c.), a hybrid ligand which binds to both I_1 and α_2-AR_S, also decreased arterial pressure (61 ± 2 vs. 101 ± 2 mm Hg) and heart rate (260 ± 4 vs. 308 ± 8) ($p < 0.01$, $n = 5$); it potentiated the RB (maximum R–R interval: 284 ± 17 vs. 196 ± 6 ms) ($p < 0.05$, $n = 5$).

Dexmedetomidine (1 μg/kg, i.c.), a ligand selective for α_2-ARs, reduced blood pressure (53 ± 3 vs. 104 ± 2 mm Hg) and heart rate (246 ± 4 vs. 312 ± 8 beats/min) ($p < 0.05$, $n = 5$) and potentiated the RB (maximum R–R interval: 518 ± 38 vs. 194 ± 4 ms) ($p < 0.05$, $n = 5$). The potentiation of RB was much greater than that observed with rilmenidine and was significantly prevented by L-NNA injected centrally.

This study shows that: (i) an exclusive action on I_1Rs which decreases arterial pressure, does not potentiate the RB ii) activation of α_2-ARs potentiates the RB (iii) the R–R prolongation caused by α_2-ARs stimulation is prevented by central NOS inhibition.

Keywords: Imidazoline receptors; α_2-adrenergic receptors; Nucleus tractus solitarii; Rostroventrolateral medulla/nucleus reticularis lateralis; Nitric oxide; Phenylephrine induced bradycardia

1. Introduction

Brain stem structures, including the nucleus tractus solitarii (NTS) and the nucleus reticularis lateralis of the rostral ventrolateral medulla region (NRL/RVLM), are the sites of action of centrally acting anti-hypertensive drugs such as catecholamines and imidazolines. The NRL/RVLM is the major site of the hypotensive effect of imidazoline-like drugs such as clonidine, moxonidine, rilmenidine. This effect was shown to require specific imidazoline receptors (I_1Rs) insensitive to catecholamines (Bousquet et al., 1981, 1984; Ernsberger et al., 1990; Feldman et al., 1990, 1998; Bousquet, 1997). Imidazoline-like compounds are also able to decrease blood pressure when injected into the NTS. However, the doses of imidazoline-like drugs such brimonidine and clonidine required to reduce blood pressure when injected into the NTS are much higher than the doses that are efficient when injected into the NRL/RVLM (Zandberg et al., 1979; Head and Burke, 1998). Sy et al. (2002) have recently shown that brimonidine, which binds with high affinity to both α_2-adrenoceptors (α_2-AR_S) and non-adrenergic I_1Rs (Pazoz et al., 1988; Angel et al., 1995) is about 10 times more active to reduce blood pressure when injected into the NRL than into the NTS (Sy et al., 2002).

* Corresponding author. Tel.: +33 3 90 24 33 90; fax: +33 3 90 24 33 88.
E-mail address: Pascal.Bousquet@medecine.u-strasbg.fr (P. Bousquet).

1566-0702/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.autneu.2005.12.003

Previous studies performed in conscious rabbits have shown that clonidine (0.5–1.5 μg/kg) injected intracerebroventricularly (i.c.v.), not only reduced blood pressure but also enhanced the heart period variations (HPV) in response to blood pressure increases through vagal excitation and sympathetic inhibition (Korner et al., 1974). In anaesthetized animals also, we have observed that hybrid drugs which bind to both α_2-AR_S and I_1Rs such as clonidine and brimonidine markedly facilitated the PE-induced HPV when administered intracisternally [unpublished data]. Taking advantage of the latter observation, our purpose was to investigate further on the pharmacological mechanism of the facilitatory action of some centrally acting drugs on RB. Thanks to the specific involvement of NO in the sympathetic pathways originating in the NTS (Sy et al., 2001) and also to new ligands, highly selective for I_1 receptors or α_2-AR_S, we investigated on: (i) the respective effects of centrally acting α_2-AR and I_1R agonists on the RB in pentobarbitone anaesthetized rabbits, (ii) the role of NO in this facilitation. We used LNP 509 as a hypotensive drug highly selective for I_1Rs, rilmenidine as a ligand with some selectivity for I_1Rs over α_2-AR_S but still a hybrid drug and dexmedetomidine as a "pure" α_2-adrenergic agonist (Feldman et al., 1990; Ernsberger, 2000; Schann et al., 2001).

2. Materials and methods

2.1. Animals and haemodynamic measurements

Normotensive male rabbits (Zika strain) weighing 2.5 to 3.5 kg were anaesthetized with sodium pentobarbitone 40 mg/kg administered into 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 the anaesthesia was established (Harvard Apparatus Ltd., Millis MA). The animals were tracheotomized, immobilized with pancuronium bromide (1 mg/kg, i.v.), and artificially ventilated with room air (Hugo Sachs elektronic model 6025, March-Hugstetten, Germany). Ventilation parameters were adjusted to maintain paO_2 at approximately 100 mm Hg and $paCO_2$ below 40 mm Hg. The right femoral vein was catheterized to allow i.v. injections and the instantaneous arterial pressure was continuously monitored through a catheter placed in the abdominal aorta via the right femoral artery, with a pressure transducer (Gould P23D) and recorder (Gould Electronics model BS-272, Longjumeau, France). Mean arterial pressure (MAP) was calculated as diastolic pressure plus one third of the differential pulse pressure. Values of MAP and heart rate (HR) recorded 10 min after pretreatment with vehicle or antagonists were used as baseline values to calculate MAP and HR changes. RR interval (interval between successive R waves on an ECG) is more accurate than HR to quantify the sensitivity of the bradycardic response during short lasting rises of arterial pressure. The cardiovascular parameters were recorded for 30 min after administration of, LNP509, rilmenidine or dexmedetomidine, and were used as baseline values to calculate RR variations after i.v. injection of PE. The maximum RR interval was measured at the peak of arterial pressure after PE i.v.

All the experiments were carried out in accordance with the EEC directive of 24 November 1986 related to the use of laboratory animals (86/609/EEC).

2.2. Intracisternal injections

The animal's head was placed in a stereotaxic frame (Unimécanique, Epinay sur Seine, France). A needle with an internal diameter of 0.45 mm was placed into the cisterna magna, across the atlanto-occipital membrane. A 100 μl volume of cerebrospinal fluid was withdrawn prior to any i.c. injection. Dexmedetomidine was injected i.c. (100 μl) in single doses 10 min after pretreatment with vehicle, rauwolscine or L-NNA. In other series of experiments, single doses of rilmenidine or LNP509 were injected i.c (100 μl) 10 min after vehicle i.c. The MAP and RR interval were continuously measured during 30 min after administration of the above-mentioned drugs. PE (10 μg/kg, i.v.) was then administered, the maximal RR interval was measured at the peak of arterial pressure.

A 10 min time interval between pre-treatment with antagonists and the central delivery of agonists was necessary to reach stabilization of the cardiovascular parameters. We chose a delay of 30 min between the agonist injection and the PE test because the maximal effects of the agonists are always reached at that time.

2.3. Bilateral microinjections into the NTS

The head of the rabbit was placed in a stereotaxic frame. An incision was made between the ears, and muscles were dissected to expose the cisterna magna. The atlanto-occipital membrane was cut and removed. The dura was incised and the obex was visualised. A glass micropipette (30–50 μm), connected to a Hamilton syringe (10 μl) was then placed horizontally in the injection site (1 mm laterally from the median line, 0.5 mm above the obex) and inserted 1 mm deep into the medulla oblongata. In preliminary experiments, L-glutamate (0.5 μg/kg) has been injected into the NTS; this dose decreased the arterial pressure and allowed us to determine the stereotaxic coordinates of the site of injection within the NTS. The drug or vehicle (0.5 μl) was delivered over a period of 15 s. After injection, the pipette was left in situ for 30 s and then removed. To check the sites of injection, Evans blue dye was administered at the end of each experiment, and the brain was removed for postmortem examination.

Control experiments were performed to check the possible spread of dexmedetomidine around the sites of injection. The drug was then injected 1 mm rostrally, caudally and on each side of the NTS ($n=2–3$ for each site). Injected into these sites, dexmedetomidine changed neither blood pressure nor RR interval responses to PE.