European Journal of Pharmacology 640 (2010) 55–62

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Neuropharmacology and Analgesia

α1-Adrenoceptor activation is involved in the central *N*-methyl-D-aspartate-induced adrenomedullary outflow in rats

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ARTICLE INFO

Article history:
Received 22 July 2009
Received in revised form 9 April 2010
Accepted 25 April 2010
Available online 5 May 2010

Keywords:
Adrenomedullary outflow
α1-Adrenoceptor
Hypothalamic paraventricular nucleus
N-methyl-D-aspartate
Plasma catecholamine
Thromboxane A_2

ABSTRACT

N-methyl-D-aspartate (NMDA) has been implicated in the regulation of several autonomic responses in the brain. The present study determined whether activation of α_1-adrenoceptors is involved in the centrally administered NMDA-induced adrenomedullary catecholamine outflow, using urethane-anesthetized rats. The NMDA (5.0 nmol/animal, i.c.v.)-induced elevation of plasma levels of noradrenaline and adrenaline was reduced by phentolamine (0.33 μmol/animal, i.c.v.), a non-selective α-adrenoceptor antagonist, and by 2-{[b-(4-hydroxyphenyl)ethyl]aminomethyl}-1tetralone (HEAT) (90.0 nmol/animal, i.c.v.), a selective α_1-adrenoceptor antagonist. In contrast, sotalol (0.8 μmol/animal, i.c.v.), a non-selective β-adrenoceptor antagonist, did not alter the responses. In addition, U-73122, a phospholipase C inhibitor (5.0 nmol/animal, i.c.v.), RHC-80267, a diacylglycerol lipase inhibitor (1.3 μmol/animal, i.c.v.) and URB 602, a monoacylglycerol lipase inhibitor (0.85 and 1.7 μmol/animal, i.c.v.), reduced the NMDA-induced plasma elevation of both catecholamines. Furthermore, perfusion of the hypothalamic paraventricular nucleus with NMDA (0.3 and 1.0 mM) dose-dependently elevated both noradrenaline levels in the hypothalamic paraventricular nucleus and plasma catecholamine levels. These responses were abolished by co-administration of dizocilpine malate (MK-801, 0.1 mM), a selective non-competitive antagonist of the NMDA receptor and by co-administration of (+)-S-145 (2.5 mM), a selective competitive antagonist of the thromboxane A_2 receptor. These results suggest that activation of central α_1-adrenoceptors is involved in the centrally administered NMDA-induced activation of adrenomedullary catecholamine outflow in rats. Furthermore, signaling cascades downstream of the α_1-adrenoceptor in the hypothalamic paraventricular nucleus may play an important role in the process.

1. Introduction

Noradrenaline has widespread distribution throughout the brain, and has been implicated as a primary neurotransmitter for the maintenance of circulatory control and the regulation of sympathetic responses (Lightman, et al., 1984; Woodruff, et al., 1986; McCall, 1988). Several lines of evidence have revealed an important link between central noradrenergic activity and sympathetic outflow (Isaac, 1980; Esler et al., 1995). It is also broadly accepted that the actions of noradrenaline are exerted by an activation of adrenoceptors, classified into three sub-categories: α_1, α_2 and β (Docherty, 1998). Among them, α_1-adrenoceptors have been thought to mediate the excitatory effects of noradrenaline in the central nervous system (Rogawski and Aghajanian, 1982). alpha$_1$-Adrenoceptors belong to the G-protein-coupled receptor superfamily of cell surface membrane proteins driving a phospholipase C–protein kinase C signaling pathway (Summers and McMartin, 1993). Interestingly, α_1-Adrenoceptors can also promote the release of arachidonic acid (a source of prostanoids such as thromboxane A_2) by phospholipase C through the formation of diacylglycerol and subsequent deacylation by di- or monoacylglycerol lipase (Bell et al., 1979; Prescott and Majerus, 1983).

Abundant glutamatergic nerve terminals have been detected in the hypothalamus (van den Pol, 1991), including the hypothalamic paraventricular nucleus, recognized as a key regulatory center of autonomic output (Sawchenko and Swanson, 1983). Recent anatomical studies have revealed that *N*-methyl-D-aspartate (NMDA) receptors are expressed in all subdivisions of the hypotalamic paraventricular nucleus, mediating glutamate-induced excitatory actions (Herman et al., 2000). The NMDA injected into the hypothalamic paraventricular nucleus produced dose-dependent increases in renal sympathetic nerve activity, blood pressure, and heart rate (Goren et al., 2000; Badoer, 2001). These observations suggest a role for NMDA receptors in the regulation of several autonomic responses in the brain.

Our previous study demonstrated that perfusion of the hypothalamic paraventricular nucleus with NMDA via a microdialysis probe produces thromboxane A_2, one of the cyclooxygenase metabolites of arachidonic acid, and elevates plasma noradrenaline and adrenaline levels in rats. In addition, the responses were abolished by intracerebroventricular

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doi:10.1016/j.ejphar.2010.04.038

pretreatment with furegrelate, a thromboxane A_2 synthase inhibitor (Okada et al., 2000). Recently, we have further confirmed that increased plasma noradrenaline evoked by centrally applied NMDA is due to secretion from the adrenal gland and not due to release from sympathetic nerve terminals, in a brain prostanoid dependent manner (Okada et al., 2008). Collectively, these results suggest that thromboxane A_2 produced in the hypothalamic paraventricular nucleus might be involved in the NMDA-induced central regulation of adrenomedullary outflow in rats. Although several studies suggest the involvement of NMDA-induced increase of cyclic guanosine monophosphate in noradrenaline release (Wood et al., 1992; Puumala et al., 1998), there have been no detailed studies on the interrelationship between α_1-adrenoceptors, NMDA receptors mediating the release of noradrenaline from noradrenergic nerve terminals, and production of thromboxane A_2 in the hypothalamic paraventricular nucleus.

In the present study, we pharmacologically examined the possibility that centrally administered NMDA can activate α_1-adrenoceptors to induce adrenomedullary outflow. In addition, we also examined whether or not direct application for NMDA into the hypothalamic paraventricular nucleus can release noradrenaline by using brain microdialysis technique.

2. Materials and methods

2.1. Experimental procedures

Male Wistar rats weighing approximately 350 g were maintained in an air-conditioned room at 22–24 °C under a constant day–night rhythm for more than 2 weeks and given food (laboratory chow, CE-2; Clea Japan, Hamamatsu, Japan) and water ad libitum. Under urethane anesthesia (1.0 g/kg, intraperitoneally), a femoral venous line was inserted for infusion of saline (1.2 ml/h) and a femoral arteral line for collecting blood samples, as described previously (Okada et al., 2008). Next, the animal was placed in a stereotaxic apparatus, as described previously (Okada et al., 2008). A hole was drilled in the skull for intracerebroventricular administration of test substances through a stainless-steel cannula (0.3 mm outer diameter). The stereotaxic coordinates of the tip of the cannula were as follows (in mm): AP: −0.8, L:1.5, V: 4.0 (AP, anterior from the bregma; L, lateral from the midline; V, below the surface of the brain), according to the rat brain atlas of Paxinos and Watson (1997). Three hours were allowed to elapse before the application of NMDA or pharmacological blocking agents.

NMDA and other pharmacological blocking agents were dissolved in sterile saline and injected slowly into the right cerebral ventricle in a volume of 5 µl/animal, with a 10 µl Hamilton syringe. Phentolamine, a non-selective α_1-adrenoceptor antagonist, 2-{[b-(4-hydroxyphenyl) ethyl]aminomethyl}-1tetralone (HEAT), a selective α_1-adrenoceptor antagonist, and sotalol, a non-selective β-adrenoceptor antagonist, were intracerebroventricularly administered 30 min before NMDA . U-73122 [1-(6-((17β-3-methoxyestra-1,3,5 (10)-trien-17yl) amino) hexyl)-1H-pyrrole-2,5-dione], U-73343 [1-(6-((17β-3-methoxyestra-1,3,5(10)-trien-17-yl)amino)hexyl)-2,5-pyrrolidinedione], RHC-80267 [1,6-bis-(cyclohexyloximinocarbonylamino)-hexane], and URB 602 (biphenyl-3-yl carbamic acid cyclohexyl ester) were dissolved in N, N-dimethylformamide (DMF). The pharmacological blocking agents were intracerebroventricularly administered in a volume of 2.5 µl/animal 30 min before application of NMDA.

All experiments were conducted in compliance with the guidelines for the care and use of laboratory animals approved by the Graduate School of Medicine, Kochi University, Nankoku Kochi, Japan.

2.2. Measurement of plasma catecholamines

Arterial blood samples (250 µl) were collected in a heparinized tube through an arterial catheter and were preserved on ice during experiments. Immediately after the final sampling, plasma was prepared by centrifugation (3500 *g* for 10 min at 4°C). Catecholamines in the plasma were extracted by the method of Anton and Sayre (1962) with a slight modification and were assayed by high-performance liquid chromatography (HPLC) with electrochemical detection (Okada et al., 2008). Briefly, plasma (100 µl) was transferred to a sample tube containing 30 mg of activated alumina (grade for catecholamine determination and acidic, Wako Pure Chemical Industries, Ltd, Osaka, Japan), 100 µl of 10 ng/ml solution of 3,4-dihydroxybenzylamine as an internal standard and 3 ml of 0.5 M Tris Buffer (pH 8.6) containing 0.1 M disodium EDTA. The tube was shaken for 10 min and aspirated off the supernatant. Then the alumina was washed three times with 4 ml of ice-cold double deionized water. Then catecholamines adsorbed onto the alumina were eluted with 300 µl of 4% acetic acid containing 0.1 mM disodium EDTA. A pump (EP-300: Eicom, Kyoto, Japan), a sample injector (Model-231XL; Gilson, Villiers-le-Bel, France), an electrochemical detector (ECD-300: Eicom) equipped with a graphite electrode were used for the HPLC analyses. Analytical conditions were as follows: detector + 450 mV potential against a Ag/AgCl reference electrode; column, Eicompack CA-50DS, 150 × 2.1 mm i.d. (Eicom); mobile phase, 85% 0.1 M NaH_2PO_4-Na_2HPO_4 buffer (pH 6.0), 15% methanol, 750 mg/l 1-octane sulfate sodium and 50 mg/l EDTA dihydrate, at a flow rate of 0.18 ml/min. The retention times for each of the catecholamines as follows; noradrenaline (6.1 min), adrenaline (7.8 min), 3,4-dihydroxybenzylamine (DHBA) (14.2 min). The amount of catecholamines in each sample was calculated using the peak height ratio relative to that of DHBA, an internal standard. This assay could determine 0.5 pg of adrenaline and noradrenaline accurately.

2.3. Microdialysis

In the microdialysis experiment, a stainless steel guide cannula held on the tip of L-shaped stainless steel cannula was implanted stereotaxically just above the right hypothalamic paraventricular nucleus, as previously reported (Okada et al., 2000; 2002). The stereotaxic coordinates of the sites of implantation were as follows: AP: −1.7 mm, L: 0.3 mm, DV: 7.0 mm. The microdialysis probe (220 µm outer diameter, 1 mm of membrane length; Eicom, Kyoto, Japan) was inserted into the guide cannula, which, in turn, was inserted into the hypothalamic paraventricular nucleus. The hypothalamic paraventricular nucleus was perfused with Ringer's solution (147 mM NaCl, 4 mM KCl and 2.3 mM $CaCl_2$) at a flow rate of 2 µl/min using a microinfusion pump (EP-60, Eicom). Three hours were allowed to elapse for stabilization of the basal release of noradrenaline, and dialysate was collected every 20 min in a collection tube containing 20 µl of 0.1 N perchloric acid. Three consecutive dialysates were used to measure the baseline release of noradrenaline. The NMDA, dizocilpine maleate (MK-801) and (+) S-145 were dissolved in Ringer's solution. These parmacological agents were applied into the hypothalamic paraventricular nucleus through a microdialysis probe. Samples were frozen (−20 °C) until analysis. Noradrenaline released in the samples (40 µl) was directly measured using HPLC as shown in the section of measurement of plasma catecholamines. According to the manufacturer's guide, the *in vitro* probe recovery determined was approximately a 15% for noradrenaline, when the probe was placed in Ringer's solution at 37 °C containing 10 ng/ml of noradrenaline and perfused with Ringer's solution without noradrenaline for 30 min at a rate of 2 µl/min. At the termination of the experiment, the rats were sacrificed by deep anesthesia, and their brains removed and fixed with 10% formalin. Serial coronal sections sliced at 20 µm were stained with cresyl violet to verify the location of the tip of the dialysis probe. All subjects tested with probe tips lying right on the border of the hypothalamic paraventricular nucleus were included for analysis.