Progress in Neuro-Psychopharmacology & Biological Psychiatry 57 (2015) 146–154

Contents lists available at ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

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

Withdrawal from repeated administration of a low dose of reserpine induced opposing adaptive changes in the noradrenaline and serotonin system function: A behavioral and neurochemical *ex vivo* and *in vivo* studies in the rat

Lucyna Antkiewicz-Michaluk *, Agnieszka Wąsik, Edyta Możdżeń, Irena Romańska, Jerzy Michaluk

Department of Neurochemistry, Institute of Pharmacology Polish Academy of Sciences, 31-343 Krakow, Poland

ARTICLE INFO

Article history:
Received 14 July 2014
Received in revised form 15 October 2014
Accepted 21 October 2014
Available online 8 November 2014

Keywords:
Brain structures
Dopamine D1 and D2 receptors
Forced swim test
In vivo release of monoamines
Metabolism of monoamines
Reserpine model of depression

ABSTRACT

Reserpine is an inhibitor of the vesicular monoamine transporter 2 (VMAT2) and monoamine releaser, so it can be used as a pharmacological model of depression. In the present paper, we investigated the behavioral and neurochemical effects of withdrawal from acute and repeated administration of a low dose of reserpine (0.2 mg/kg) in Wistar Han rats. We demonstrated the behavioral and receptor oversensitivity (postsynaptic dopamine D1) during withdrawal from chronic reserpine. It was accompanied by a significant increase in motility in the locomotor activity test and climbing behavior in the forced swim test (FST). Neurochemical studies revealed that repeated but not acute administration the a low dose of reserpine triggered opposing adaptive changes in the noradrenergic and serotonin system function analyzed during reserpine withdrawal, *i.e.* 48 h after the last injection. The tissue concentration of noradrenaline was significantly decreased in the hypothalamus and nucleus accumbens only after repeated drug administration (by about 20% and 35% *vs.* control; $p < 0.05$, respectively). On the other hand, the concentration of its extraneuronal metabolite, normetanephrine (NM) increased significantly in the VTA during withdrawal both from acute and chronic reserpine. The serotonin concentration was significantly reduced in the VTA after chronic reserpine (by about 40% *vs.* the control group, $p < 0.05$) as well as its main metabolite, 5-HIAA (by about 30% *vs.* control; $p < 0.05$) in the VTA and hypothalamus. Dopamine and its metabolites were not changed after acute or chronic reserpine administration. *In vivo* microdialysis studies clearly evidenced the lack of the effect of a single dose of reserpine, and its distinct effects after chronic treatment on the release of noradrenaline and serotonin in the rat striatum. In fact, the withdrawal from repeated administration of reserpine significantly increased an extraneuronal concentration of noradrenaline in the rat striatum but at the same time produced a distinct fall in the extraneuronal serotonin in this brain structure. On the basis of the presented behavioral and neurochemical experiments, we suggest that chronic administration of reserpine even in such low dose which not yet acted on the release of monoamines but produced an inhibition of VMAT2 caused a long-lasting disadvantageous effect of plasticity in the brain resembling depressive disorders.

1. Introduction

It is well known that monoamine neurotransmitters, such as dopamine (DA), noradrenaline (NA) and serotonin (5-HT) in the central nervous system play a key role in the pathophysiology of depression (Cantello et al., 1989 Chan-Palay and Asan, 1989; Colpaert, 1987; Elhwuegi, 2004; Mayeux et al., 1984). However, abnormalities in monoaminergic neurotransmission are associated with a number of neurological disorders including Parkinson's disease (PD) and schizophrenia. Understanding how to prevent and treat depression is, therefore, an urgent issue. The monoamines, particularly dopamine (DA) and noradrenaline (NA) have the ability to undergo spontaneous oxidation in the cytosol, which is potentially damaging to cellular structures (Antkiewicz-Michaluk et al., 2006; Graham, 1978; Wąsik et al., 2009).

The vesicular monoamine transporter 2 (VMAT2) is one of such custodians that function to regulate the cytosolic environment of neurons, protecting them from endogenous and exogenous toxins (Uhl, 1998; Miller et al., 1999). Localized on vesicular membranes in neurons, VMAT2 acts to accumulate cytosolic monoamines in synaptic vesicles after they have been synthesized from their precursors for regulated exocytotic release as well as after their re-uptake from the synaptic cleft into the neuron (Surratt et al., 1993).

Abbreviations: FST, forced swimming test; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; NA, noradrenaline; NM, normetanephrine; 5-HT, serotonin; 5-HIAA, 5-hydroxyindolacetic acid; HPLC, high-performance liquid chromatography; ED, electrochemical detection; VTA, ventral tegmental area.

* Corresponding author at: Neurochemistry Department, Institute of Pharmacology PAS, 31-343 Krakow, Poland.
E-mail address: antkiew@if-pan.krakow.pl (L. Antkiewicz-Michaluk).

http://dx.doi.org/10.1016/j.pnpbp.2014.10.009
0278-5846/

Reserpine is a vesicular monoamine re-uptake blocker (Naudon et al., 1996) which depletes monoamines in the brain (Vergo et al., 2007; Antkiewicz-Michaluk et al., 2014), and produces depression-like syndrome in animals (Fleckenstein et al., 2009; Kandel, 2000; Nagakura et al., 2009; Rojas-Corrales, 2004). Reserpine as the inhibitor of VMAT2 interferes with the storage of monoamines in intracellular vesicles, causing monoamine depletion in nerve terminals, muscular rigidity and hypolocomotion depending on the dose (Colpaert, 1987; Gerlach and Riederer, 1996; Rung et al., 2011; Skalisz et al., 2002). The dose range that usually induces monoamine depletion and motor alterations in rodents is 2–10 mg/kg (Rung et al., 2011; Shiozaki et al., 1999; Skalisz et al., 2002)

In the present paper, we examined behavioral and neurochemical effects of 48 h withdrawal from acute and repeated treatment with a low dose of reserpine (0.2 mg/kg i.p.). We used the locomotor activity test to check the motor function and the forced swim test (FST) to examine potential "pro-depressive" behavior in rats. Recently, a behavioral sampling technique was developed that scores individual response categories, including immobility, swimming and climbing (Detke et al., 1995). Although all antidepressant drugs reduce immobility time in the FST, at least two distinct active behavioral patterns are produced by antidepressant drugs differing in pharmacological selectivity (Borsini and Meli, 1988). Serotonin selective re-uptake inhibitors increase swimming behavior while drugs acting primarily to elevate extracellular levels of NA or DA increase climbing behavior (Borsini, 1995; Detke and Lucki, 1996; Detke et al., 1995).

In the second part of the study, in addition to the behavioral tests we carried out also receptor binding studies and neurochemical *ex vivo* studies in the rat brain structures (ventral tegmental area VTA, nucleus accumbens, and hypothalamus) to determine: the levels of monoamines, metabolites and the rate of their metabolism.

Moreover, the microdialysis studies carried out in freely moving rat allowed for an *in vivo* analysis of monoamine release in the rat striatum. It was noted, the interaction between noradrenergic and serotonergic system might be involved in common physiological aspects of their function in the brain.

2. Materials and methods

2.1. Animals

Behavioral tests were carried out on male Wistar rats (Charles River), of initial body weight 230–240 g (about 8 weeks old). The animals were kept under standard laboratory conditions with free access to laboratory food and tap water, at room temperature of 22 °C with an artificial day-night cycle (12/12 h, light on at 7 a.m.). All the procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were granted an approval from the Bioethics Commission as compliant with Polish Law. The experimental protocols were approved by the Local Bioethics Commission of the Institute of Pharmacology, Polish Academy of Sciences in Kraków.

2.2. Drugs

Reserpine (Sigma-Aldrich, USA) was suspended in 1% Tween 80. Reserpine was administrated once (acute treatment) and repeatedly for 14 days (chronic treatment) intraperitoneally in a low dose (0.2 mg/kg i.p.). Control group was administered 1% Tween 80 for 14 days. All behavioral tests and biochemical analysis were performed 48 h. after the last dose of reserpine.

2.3. Forced swim test (FST) procedure

The studies were carried out on rats and were based on the method of Porsolt et al. (1978). All the animals were individually tested in the FST on two consecutive days with one session per day. On the first day, the rats were individually placed in non-transparent plastic cylinders (diameter: 23 cm, height: 50 cm) containing 30 cm of water, maintained at 25–26 °C. They were let to swim for 15 min before being removed (pre-test session). After that the animals were dried and returned to their home cages. The procedure was repeated 24 h later, and the time of the escape-oriented behavior of the rats was recorded (5 min test session). The observed behavioral parameters, in the order of priority, included time spent floating in water (*immobility*), *swimming* and struggling (*climbing*). According to Detke et al. (1995), the immobility is described as behavior of the rat when it makes only the movements necessary to keep its head above the water. In this case, animals can make certain, slight swimming movements in order to remain afloat. Climbing is defined as vigorous movements of four limbs, with the forepaws breaking against the wall of the cylinder. During swimming rats make coordinated and sustained movements (more than necessary) with all four limbs, usually traveling around the interior of the cylinder, but without breaking the surface of the water with forelimbs. Water was changed between subjects. The FST was conducted 48 h after acute and chronic (14-day) administration of reserpine (0.2 mg/kg i.p.).

2.4. Locomotor activity test

The locomotor activity was measured in actometers (Opto-Varimex activity monitors, Columbus Instruments, USA) linked on-line to an IBM-compatible PC. Each cage (43 × 44 × 25 cm) was surrounded with a 15 × 15 array of photocell beams located 3 cm from the floor surface. Interruptions of these photocell beams were counted as a measure of horizontal and vertical locomotor activity. Horizontal locomotor activity was defined as the traveled distance (in cm), and the vertical activity as rearing time (in sec.). Locomotor activity was analyzed using Auto-Track Software Program (Columbus Instruments, USA) and recorded in 15 min intervals for 60 min (counting commenced immediately after introduction of the animals into experimental cages). Locomotor activity was measured during 60 min at 48 h after acute and chronic (14-day) administration of reserpine (0.2 mg/kg i.p.)

2.5. Dopamine D_1 and D_2 receptor binding studies in the rat striatum

The animals were treated acutely or chronically (14 days) with reserpine (0.2 mg/kg i.p.). Control group received chronically 1% Tween 80. All rats were killed by decapitation 48 h after the last dose of reserpine (during withdrawal after acute and chronic treatment). The brains were rapidly removed and dissected on an ice-cold glass plate. The brain structure (striatum) was dissected out and placed in dry ice until the binding assay was performed.

The tissue (striatum) was homogenized in 40 vol. of an ice-cold 50 mM Tris–HCl buffer, pH 7.4 using a polytron disintegrator. The homogenate was centrifuged at 25 000 ×*g* for 30 min, and the resulting pellet was resuspended in the buffer and recentrifuged in the same conditions. The final pellet (fraction P1 + P2) was used for binding studies. For incubation it was reconstituted in the Tris–HCl buffer pH 7.4, to obtain a final protein concentration (measured according to Lowry et al., 1951) of approximately 0.3 mg/ml.

The specific radioligand for dopamine D_1 receptor, [^{3}H]SCH-23390 ([^{3}H](R+)7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride, NEN, specific activity 82.9 Ci/mmol) was prepared in six concentrations from 0.05 to 2 nM. The incubation mixture (final volume 550 μl) consisted of 450 μl of membrane suspension, 50 μl of [^{3}H]SCH-23390 solution and 50 μl of Tris–HCl bufferwithout (total binding) or with (unspecific binding) cold SCH-23390 (at a final concentration of 10 μM). All assays were performed in duplicate and incubation proceeded in a shaking water bath at 30 °C for 60 min.

The procedure for the estimation the parameters of dopamine D_2 receptor was very similar as above but the incubation medium was Tris–HCl buffer pH 7.1 supplemented with 120 mM NaCl, 5 mM KCl,