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European Journal of Pharmacology 332 (1997) 9–14

ejp

Ca^{2+} channel blockade prevents lysergic acid diethylamide-induced changes in dopamine and serotonin metabolism

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Received 27 December 1996; revised 14 May 1997; accepted 21 May 1997

Abstract

To investigate the effect of a single and multiple administration of lysergic acid diethylamide (LSD) on cerebral metabolism of dopamine and serotonin, male Wistar rats were treated with low and high doses (0.1 and 2.0 mg/kg i.p.) of LSD and the levels of dopamine, 3,4-dihydroxyphenylacetic acid, homovanillic acid, 3-methoxytyramine, serotonin and 5-hydroxyindoleacetic acid were assayed by HPLC in the nucleus accumbens, striatum and frontal cortex. Some rats received nifedipine, 5 mg/kg i.p., before each injection of LSD to assess the effect of a Ca^{2+} channel blockade. High-dose LSD treatment (8×2 mg/kg per day) caused a strong stimulation of dopamine metabolism in the nucleus accumbens and striatum, and serotonin metabolism in the nucleus accumbens: the changes were observed 24 (but not 1 h) after the last dose. The changes induced by the low-dose treatment (8×0.1 mg/kg per day) had a different pattern, suggesting the release of dopamine from vesicles to cytoplasm. Co-administration of nifedipine completely prevented the LSD-induced biochemical changes. The results suggest that Ca^{2+} channel blocking agents may prevent development of some behavioral consequences of chronically used LSD. © 1997 Elsevier Science B.V.

Keywords: LSD (lysergic acid diethylamide); Chronic treatment; Biogenic amines; Ca^{2+} channel; Nifedipine; Brain

1. Introduction

Lysergic acid diethylamide (LSD) is a classic hallucinogenic agent that produces a combination of hallucinations, pseudohallucinations and illusions. Patients with the LSD psychosis may require an antipsychotic and those exhibiting prolonged drug-induced psychoses may require a variety of treatments including electroconvulsive treatment, lithium and L-5-hydroxytryptophan (Leikin et al., 1989). Unfortunately, the illegal use of LSD, that declined in the 80's, is now growing (Schwartz, 1995).

A body of evidence suggests that hallucinations are related to abnormalities in dopaminergic (White, 1986; Watts et al., 1995) and serotonergic transmissions (Glennon et al., 1984; Krebs and Geyer, 1994; Penington and Fox, 1994). Thought disorders and hallucinations, not unlike those observed in LSD abusers, seem to be preferentially associated with central dopamine hyperactivity and are a frequent side-effect of L-3.4-dihydroxyphenylalanine (L-dopa) therapy (Moskovitz et al., 1978, Zemlan et al., 1986). The L-dopa-induced hallucinations are an important problem as they appear in more than 22% of the patients with Parkinson's disease maintained on long-term treatment with L-dopa (Friedman and Sienkiewicz, 1991). The release of dopamine in such patients is accelerated, as evidenced by an increase in the level of 3,4-dihydroxyphenylacetic acid (DOPAC) and DOPAC/dopamine ratio in the blood plasma (Nakamura et al., 1988). Changes in serotonin metabolism were also described after administration of hallucinogenic drugs: they cause an increase in the content and decrease in turnover of serotonin in the rat brain structures (Pieri et al., 1978, Romano et al., 1994).

There is a paucity of data concerning the prolonged administration of LSD. In this study we investigated the effect of prolonged administration of LSD on dopamine and serotonin metabolism in discrete areas of rat brain. As anecdotal reports indicate that calcium channel blockers may interrupt hallucinations in the acute phase of schizophrenia, and as it was suggested that cerebral artery spasms produced by several hallucinogens may be related to hallucinations (Altura and Altura, 1981), we investigated also whether co-administration of a calcium channel

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PII S0014-2999(97)01025-X

blocker vasodilator, nifedipine, would affect the biochemical effects of LSD treatment.

We have shown that chronic administration of high doses of LSD accelerates dopamine (and to a lesser extent, serotonin) metabolism. The pattern of changes induced by a low-dose treatment was complicated: a fall in dopamine and 3-methoxytyramine levels with the concomitant increase in DOPAC suggest the release of dopamine from the vesicles into the cytoplasm in the nerve ending. Co-administration of nifedipine prevented the appearance of all those LSD-induced changes.

2. Materials and methods

The experiments were carried out according to the Polish governmental regulations concerning experiments on animals (decree No. 71, art. 492 of December 28, 1959) with the appropriate permission granted.

2.1. Animals

The subjects were male Wistar rats, of initial weight 220–240 g, kept under standard laboratory conditions, 8 to a large animal cage (55 × 35 × 25 cm), with free access to standard laboratory food and tap water, at room temperature (approx. 22°C), on the natural day–night cycle (November–January). The experiments were carried out between 9.00 h and 16.00 h. The animals were killed by decapitation at the end of experiment, 60 min after a single injection and 1 or 24 h after the last of 8 daily injections.

2.2. Drugs

Nifedipine (Polfa), 5 mg/kg, was administered intraperitoneally (i.p.) as a suspension in 1% Tween 80. The suspension was prepared and kept in darkness. LSD (0.1 or 2.0 mg/kg i.p.), was dissolved in water; when drugs were given in combination, nifedipine was given always 20 min before LSD. The controls received the vehicle in a volume of 4 ml/kg.

2.3. Preparation of samples and chromatographic assay of dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA)

Immediately after decapitation with a guillotine the brains were excised and the following structures were dissected on an ice-chilled plate: frontal cortex, nucleus accumbens and striatum. The tissues were kept in dry ice until used.

The tissue samples were weighed and homogenized in ice-cold 0.1 M trichloroacetic acid containing 0.05 mM ascorbic acid. After centrifugation (10 000 × g, 5 min), the supernatants were filtered through RC 58 0.2 μm cellulose membranes (Bioanalytical Systems, West Lafayette, IN) and dopamine, DOPAC, HVA, 3-methoxytyramine, serotonin and 5-hydroxyindoleacetic acid (5-HIAA) were determined by high-performance liquid chromatography (HPLC) with electrochemical detection. A BAS-400 liquid chromatograph was equipped with a 7 μm ODS guard column and a 3 μm Phase-2 ODS cartridge column (100 × 3.2 mm). The mobile phase consisted of 0.05 M citrate-phosphate buffer, pH 3.5, 0.1 mM EDTA, 1 mM sodium octyl sulfonate and 3.5% methanol. The flow rate was maintained at 0.8 ml/min. The amines and their metabolites were quantified by peak height comparisons with standards run on the day of analysis with a sensitivity of 10–100 pg.

2.4. Presentation and statistics

The levels of neurotransmitters and their metabolites are presented at mean ± S.E.M. The results were analyzed by means of one-way analysis of variance followed, when appropriate, with Fisher's least significant difference test. In addition the ratio of metabolite to neurotransmitter, an index of metabolic activation, was calculated.

3. Results

3.1. LSD-induced changes

Single injections of LSD, 0.1, 0.5 or 2.0 mg/kg, did not affect the levels nor metabolism of biogenic amines investigated (results not shown). In contrast, chronic administration of LSD produced biochemical changes that were more pronounced 24 than 1 h after the last injection. In fact, the dose of 2 mg/kg produced significant changes only 24 h after the last injection. They consisted in significant alteration of dopamine metabolism in the nucleus accumbens and the striatum. The changes produced by the low dose were more expressed 24 than 1 h after the last injection. The treatment with a high dose of LSD produced also a significant acceleration of the dopamine metabolism in the frontal cortex.

In the nucleus accumbens the treatment with a low dose (0.1 mg/kg) resulted in a fall in 3-methoxytyramine level by 30%, while the level of amines and other dopamine and serotonin metabolites did not change significantly. 24 h after the last dose there appeared a dramatic fall in dopamine level (by 60%) with a concomitant sharp, 2.9-fold rise in DOPAC, and a 50% fall in 3-MT level. Owing to that, 24 h after the end of the treatment the DOPAC/dopamine ratio increased from 0.14 to 0.98 (7-fold) and HVA/dopamine ratio increased from 0.06 to 0.18 (3-fold). After the high dose of LSD (2.0 mg/kg), no biochemical changes were observed 1 h after the last dose and 24 h later the changes in dopamine and DOPAC levels still did not reach the level of significance, but the rise in HVA level (by 40%) was significant. The

DOPAC/dopamine and HVA/dopamine ratios were elevated at this time by 70 and 59%, respectively (Fig. 1A and B).

In the striatum of the rats receiving the low dose of LSD, 1 h after the treatment the dopamine level was depressed by 20%, and the level of DOPAC was elevated by 23%. After 24 h the only significant change was a sharp increase in the DOPAC level (290% of control). No changes were observed in rats receiving the high dose of LSD 1 h after the treatment, whereas after 24 h the levels of all three dopamine metabolites were elevated. The DOPAC/dopamine ratio was increased from 0.10 to 0.24 (2.4 fold) after the low and to 0.14 (1.5 fold) after the high dose of LSD. After the high dose the HVA/dopamine and 3-methoxytyramine/dopamine ratios were elevated by 48% and 35% (Fig. 2A and B).

In the frontal cortex the levels of dopamine, DOPAC and HVA were lower than in the two other brain areas by an order of magnitude, and the 3-methoxytyramine concentrations were below the detection level. The only significant change induced by chronic LSD administration was a 2-fold elevation of HVA level after treatment with a high

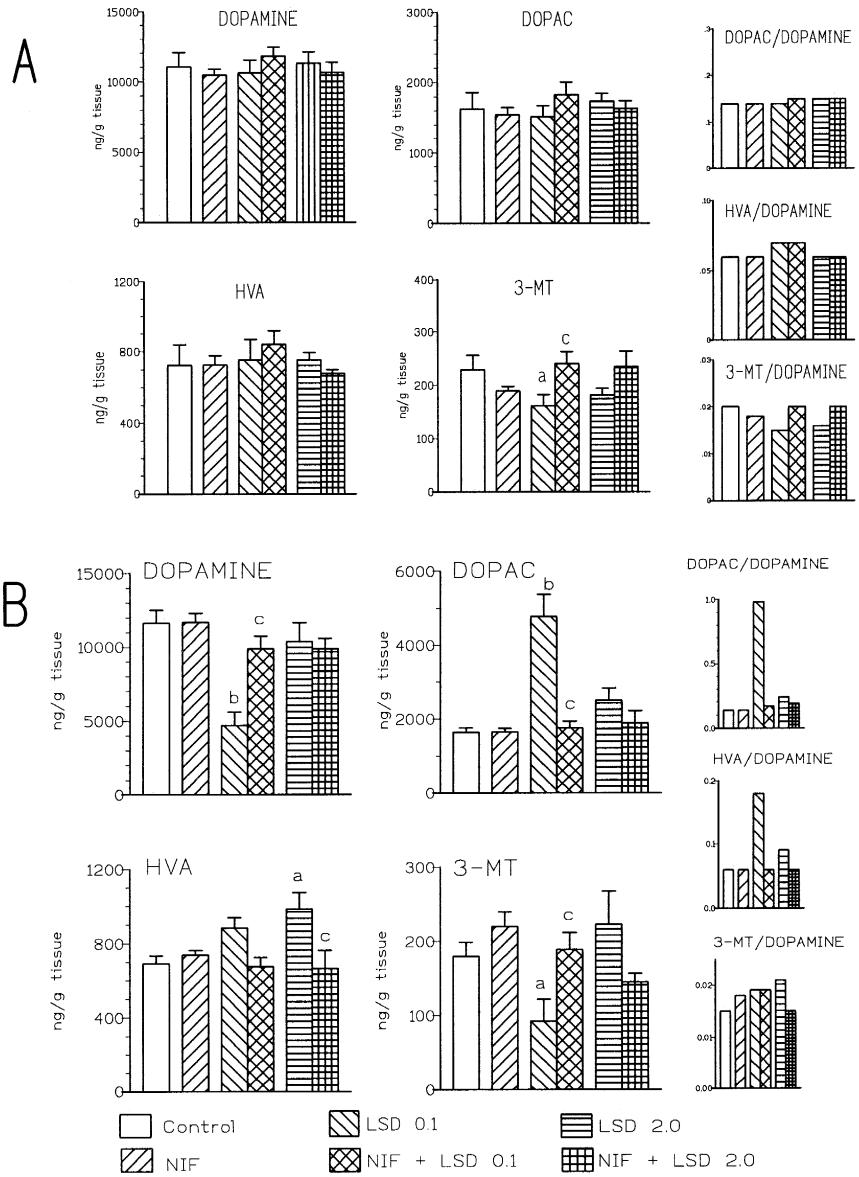

Fig. 1. The effect of chronic treatment with LSD alone or together with nifedipine on dopamine metabolism in the nucleus accumbens of the rat. The bars represent the mean + S.E.M. concentration of dopamine or its metabolites from 5–8 samples or the mean ratio of metabolite/dopamine concentration. [a] $P < 0.05$, [b] $P < 0.01$ in comparison with control, [c] $P < 0.05$ in comparison with the group receiving no nifedipine (Fisher's least significant difference). (A) The changes 1 h after the last LSD injection. The one-way analysis of variance showed the following F values for each assay: dopamine, $F(5,29) = 0.46$; DOPAC, $F(5,29) = 2.53$; HVA, $F(5,29) = 0.54$; 3-methoxytyramine (3-MT), $F(5,29) = 2.38$. (B) The changes 24 h after the last LSD injection. The one-way analysis of variance showed the following F values for each assay: dopamine, $F(5,40) = 9.01$; DOPAC, $F(5,41) = 12.87$; HVA, $F(5,41) = 3.92$; 3-methoxytyramine (3-MT), $F(5,35) = 3.82$.

dose of LSD (from 74 ± 6 to 153 ± 39 ng/g tissue) 24 h after the last injection; the tendencies to depress dopamine and elevate that of HVA did not reach significance. The DOPAC/dopamine and HVA/dopamine ratios were increased from 0.23 to 0.51 (2.1 fold) and from 0.12 to 0.38 (3.1 fold).

The significant change in the serotonin system was observed only in the nucleus accumbens, where both low and high doses of LSD caused a significant depression in the serotonin level (by 65 and 30% respectively) 24, but not 1 h after the last dose; because of that, in spite of no changes in 5-HIAA level, the 5HIAA/serotonin ratios were elevated 2.8 and 1.4-fold, respectively (Fig. 3).

3.2. The effect of nifedipine on action of LSD

Nifedipine given either once, or chronically, did not produce any change in the level of dopamine, serotonin

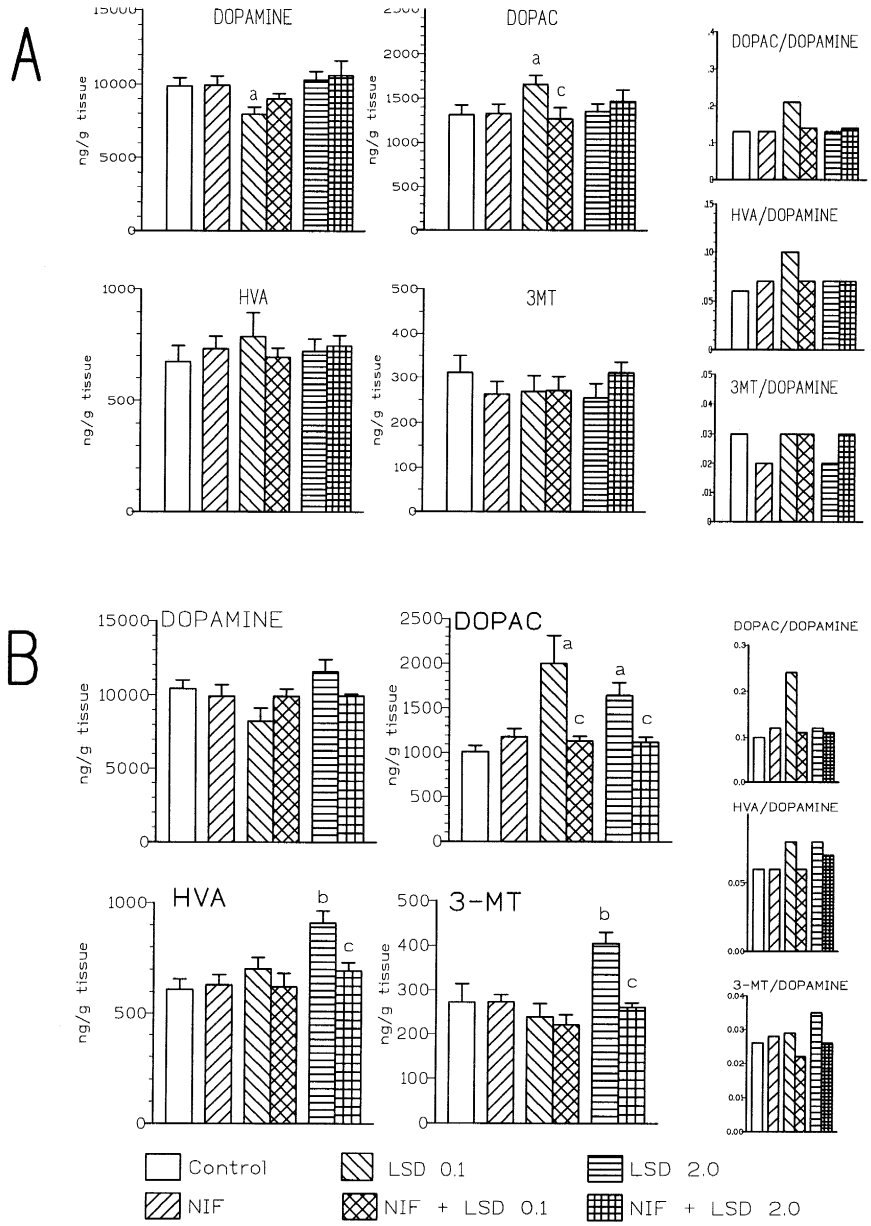

Fig. 2. The effect of chronic treatment with LSD alone or together with nifedipine on dopamine metabolism in the striatum of the rat. The bars represent the mean + S.E.M. concentration of dopamine or its metabolites from 5–8 samples or the mean ratio of metabolite/dopamine concentration. [a] $P < 0.05$, [b] $P < 0.01$ in comparison with control, [c] $P < 0.05$ in comparison with the group receiving no nifedipine (Fisher's least significant difference). (A) The changes 1 h after the last LSD injection. The one-way analysis of variance showed the following F values for each assay: dopamine, $F(5,29) = 2.20$; DOPAC, $F(5,29) = 1.37$; HVA, $F(5,30) = 0.34$; 3-methoxytyramine (3-MT), $F(5,30) = 0.59$. (B) The changes 24 h after the last LSD injection. The one-way analysis of variance showed the following F values for each assay: dopamine, $F(5,45) = 1.20$; DOPAC, $F(5,40) = 3.45$; HVA, $F(5,40) = 7.90$; 3-methoxytyramine (3-MT), $F(5,39) = 5.83$.

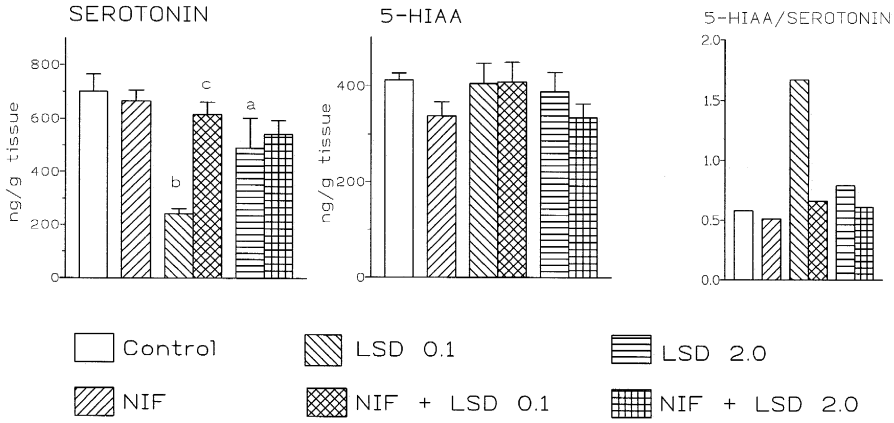

Fig. 3. The effect of chronic treatment with LSD alone or together with nifedipine on serotonin metabolism in the nucleus accumbens of the rat. The bars represent the mean + S.E.M. concentration of serotonin and 5-HIAA from 7–8 samples or the mean ratio of 5-HIAA/serotonin concentrations. The one-way analysis of variance showed the following F values for each assay: serotonin, $F(5,35) = 6.13$; 5-HIAA, $F(5,32) = 1.18$. [a] $P < 0.05$, [b] $P < 0.01$ in comparison with control, [c] $P < 0.05$ in comparison with the group receiving no nifedipine (Fisher's least significant difference).

and their metabolites but given concomitantly with LSD prevented completely all the biochemical changes induced by the chronic administration of the hallucinogen (Figs. 1–3).

4. Discussion

The present results indicate that single administration of LSD does not produce any changes in dopamine and serotonin metabolism in the investigated brain structures. In contrast, multiple administration of LSD affects evidently the cerebral dopamine metabolism, while its effect on the serotonin metabolism is much more limited. As the changes in monoamine metabolism observed 24 h after the last of multiple doses of LSD are much more profound than in a short time after the last dose, it seems that the changes represent the adaptation to drug withdrawal. This agrees with the data showing a low half-life of LSD: in humans the LSD seems to be present in the body no longer than for 15 h (Grilly, 1989) and its half-time in plasma of a volunteer was found to be 5.1 h (Papac and Foltz, 1990). There are great species differences in the metabolism of LSD and in mice its half-life is approximately 7 min (cf. Jarvic, 1975). However, to the best of our knowledge, all these data were obtained after a single administration of LSD and no studies were carried out on pharmacokinetics of chronically administered LSD. In view of the assumption that the clearance of the drug is related to the period of acute effects (Freedman, 1984) and the rapid development of tolerance to acute effects of LSD, it might be speculated that the drug was absent in the brain of rats 24 h after the last dose.

The pattern of changes in dopamine metabolism produced by low and high doses of LSD is different. This is particularly marked in the nucleus accumbens, in which the low dose produces very marked decrease in the level of dopamine while the high dose does not change it. The low doses produce a complicated pattern of changes in dopamine metabolites, while the high dose produces a consistent increase in the levels of all metabolites. This indicates that high doses of LSD increase the dopamine turnover. The results observed after the low dose are difficult to interpret: the depression of dopamine level with a high increase in DOPAC may suggest a potent dopamine release, but as 3-methoxytyramine declines and the level of HVA is only slightly elevated, this might suggest that dopamine released from the vesicles does not leave the neuron where it is metabolized mainly by monoamine oxidase B. Similarly, multiple treatment with the low dose of LSD is more effective than the high dose in depressing the serotonin level in the nucleus accumbens. Regardless of the details of the mechanism of action of chronic LSD administration on dopamine and serotonin metabolism, their development critically depends on the functional activity of voltage dependent Ca^{2+} channel, as shown by its complete blockade by coadministration of the Ca^{2+} channel blocking agent nifedipine.

The LSD-induced activation of the dopaminergic system may be related either to hallucinogenic or to psychotomimetic effect of the compound. The tolerance to hallucinations induced by LSD in humans develops rapidly (Isbell et al., 1956). Although LSD is known primarily as a hallucinogenic agent, its chronic use may lead to development of psychoses (Bowers, 1977). It should be underlined, that hallucinations cannot be equated with schizophrenia (Asaad and Shapiro, 1986; Geyer and Brief, 1987; Seeman, 1987). As the effects of LSD observed in the present experiment appear only after multiple treatment, they seem rather to be related to psychomimetic properties, as hallucinations are fully developed after a single administration of LSD. In the light of present exper-

iments it might be supposed that LSD-induced psychoses may be regarded as adaptive changes to the direct, hallucination-related, effects of LSD.

All the biochemical effects of chronic administration of LSD were effectively prevented by L-type Ca^{2+} channel blockade. The reasons for such an effect of nifedipine are not clear yet, but there are two possibilities, that are not mutually exclusive. The first possibility is that the hallucinations produced by LSD and other hallucinogens may be caused by constriction of cerebral arterioles that were inhibited by a Ca^{2+} channel blocking agent verapamil (Altura and Altura, 1981). It might be supposed that nifedipine can also prevent the vasoconstrictory action of LSD.

The second possibility is that the change in dopaminergic system is an adaptive response to chronic LSD administration and may be considered as a part of the withdrawal syndrome. Nifedipine was described to prevent development of adaptive changes to several psychotropic drugs, and to prevent biochemical and behavioral changes observed upon withdrawal from chronic treatments with drugs such as morphine (Antkiewicz-Michaluk et al., 1993) and neuroleptics (Antkiewicz-Michaluk et al., 1995). The present results corroborate our previous notion that the functional state of voltage-dependent Ca^{2+} channels determines whether adaptive changes to drugs develop or not.

The present results, showing that in the rat nifedipine treatment prevents dopamine hyperactivity induced by LSD, suggest that clinical studies on Ca^{2+} channel blocking agents for the treatment of chronic LSD abusers are warranted.

Acknowledgements

This paper was partially supported by the Polish Committee for Scientific Research grant No. 6P 207 060 04.

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