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The effects of tiazofurin on basal and amphetamine-induced motor activity in rats

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Abstract

The effects of tiazofurin (TR; 2-β-D-ribofuranosylthiazole-4-carboxamide), a purine nucleoside analogue on basal and amphetamine (AMPH)-induced locomotor and stereotypic activity of adult Wistar rat males were studied. The animals were injected with low (3.75, 7.5, and 15 mg/kg ip) and high (62.5, 125, and 250 mg/kg ip) TR doses. Neither low nor high TR doses influenced basal locomotor and stereotypic activity in comparison with the corresponding controls treated with saline only. However, pretreatment with TR at any dose applied, except for the lowest one, significantly decreased AMPH-induced (1.5 mg/kg ip) locomotor activity, while AMPH-induced stereotypic activity was inhibited with the two highest TR doses. In addition, TR was detected in the brain by HPLC already 15 min after the injection (125 mg/kg ip) to reach a maximum 2 h after the administration and was detectable in this tissue during the next 4 h. Our results indicate that TR modifies central regulation of the motor activity, possibly by influencing dopaminergic (DA-ergic) transmission.

Keywords: Tiazofurin; Amphetamine; Motor activity; Open field; HPLC; Rat brain

1. Introduction

Tiazofurin (TR; 2-β-D-ribofuranosylthiazole-4-carboxamide), a C-nucleoside acting as adenosine agonist that selectively binds to the adenosine A1 receptors in the central nervous system, has been identified as a potent inhibitor of inosine 5′-monophosphate dehydrogenase (IMPDH) (Robins, 1982). IMPDH is a rate-limiting enzyme of de novo purine biosynthesis. The activity of this enzyme was shown to be significantly increased in tumor cells and is therefore considered to be a potential target for cancer chemotherapy. The inhibition of IMPDH and subsequent reduction in adenine and guanine nucleotides interrupts DNA and RNA synthesis in rapidly dividing tumor cells. TR is a prodrug that requires metabolic activation by conversion to its active thiazole-4-carboxamide adenine dinucleotide (TAD) which inhibits IMPDH at the nicotinamide adenine dinucleotide (NAD/NADH) ligand site on the enzyme molecule (Weber et al., 1996).

TR as a novel chemotherapeutic agent is already in the Phase II/III of clinical studies in patients with various types of malignancies (Grifantini, 2000). Clinical studies showed that human recipients of intravenous TR frequently complained of headaches and displayed personality changes and obtundation (Grifantini, 2000). Different aspects of TR activity, such as induction of apoptosis and differentiation (Piperski et al., 1998; Drabek et al., 2000), inhibition of proliferation (Pesic et al., 2000), and immunomodulation (Stosic-Grujicic et al., 2002), have been extensively examined in our laboratories, but its effects on the central nervous system have not been studied in detail.

TR passes the blood–brain barrier via the adenosine transport system (Redzic et al., 1996) and has a moderate binding affinity for adenosine A1 receptors (Franchetti et al., 1995). Activation of these receptors, predominantly occurring in hippocampus, cerebral cortex, thalamic nuclei, basal ganglia, and cerebellum (Rivkees et al., 1995; Ochiishi

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et al., 1999), was shown to induce a decrease of adenylate cyclase activity and, consequently, production of cAMP (Dunwiddie and Fredholm, 1989), to stimulate the conductance of K^+ current (Trussell and Jackson, 1985) and to reduce Ca^{++} influx (Scholz and Miller, 1991; Wu and Saggau, 1994). In this manner, adenosine A1 receptors mediate reduction of presynaptic neurotransmitter release and modulate synaptic transmission (Fredholm and Dunwiddie, 1988; Prince and Stevens, 1992).

A psychostimulant amphetamine (AMPH) is a known activator of forebrain dopaminergic (DA-ergic) system, acting through the enhancement of DA release from presynaptic terminals by impulse- and Ca^{++}-independent and carrier-dependent mechanisms (Seiden et al., 1993). Previously published data indicates that high AMPH doses induce stereotypic behaviours as a consequence of DA release from striatal terminals, while low and moderate doses increase the locomotion, which is dependent on the integrity of the DA-ergic innervation to the limbic forebrain (Creese and Iversen, 1974; Fink and Smith, 1980).

Due to the observed antagonistic relationship between adenosine receptors and DA-ergic function (for a review, see Fuxe et al., 1998), behavioural effects of the compounds acting on the specific adenosine receptor subtype may be the consequence of this complex interaction. The present study was designed to examine the influence of TR, a specific agonist of the adenosine A1 receptors, in order to on basal and AMPH-induced locomotor and stereotypic activity, as well as to determine its brain content, in order to understand better the effects of this nucleoside analogue on the central nervous system and its neurotoxicity.

2. Material and methods

2.1. Animals and drugs

Adult Wistar rat males (250–350 g) were housed in groups of four to five animals per cage under standard conditions (room temperature of 23 ± 2 °C, relative humidity of 60–70%, 12-h light–dark cycle, and food and water ad libitum) in the vivarium of the Institute for Biological Research, Belgrade. The animals were maintained in accordance to the principles enunciated in the Guide for Care and Use of Laboratory Animals, NIH publication No 85-23.

TR and D-amphetamine sulfate were obtained from ICN Pharmaceuticals, Costa Mesa, USA. Both substances were dissolved in saline (1.0 ml 0.9% NaCl).

2.2. Motor activity measurement system

The motor activity of the animals was monitored in open field by an automatic device Columbus Auto-Track System (Version 3.0 A, Columbus Institute, OH, USA). Each monitoring instrument (Opto-Varimex) consisted of a Plexiglas cage ($44.2 \times 43.2 \times 20$ cm) connected to the Auto-Track interface and intercrossed by horizontal and vertical infrared beams. Interruption of a beam generated an electrical impulse, which was subsequently processed and sent to a computer linked to the Auto-Track interface. The Auto-Track system detected 11 behavioural parameters, including locomotor and stereotypic activity. The type of activity, characterized by the animal's movements, was determined by a user-defined box size (in this experiment, set to five beams). Thus, the rat had to break five infrared beams for the activity to be classified as locomotor activity. Activity within the space defined by outer borders of the quadrant formed by five intercrossing beams was rendered as a stereotypic movement. The described parameters were defined in accordance with Auto-Track system for IBM-PC/XT/AT version 3.0A Instruction Manual 0113-005L (1990).

To eliminate any interaction of animals with the environment during the experimental sessions, Opto-Varimexes were placed into the light- and sound-attenuated chambers, with artificially regulated ventilation and illumination (100 lx).

2.3. Behavioural test

Each rat was experimentally naive and tested only once. The experiments were performed between 9:00 a.m. and 3:00 p.m. All animals, divided into 14 experimental groups (five to seven animals per group), received two intraperitoneal injections to maintain the same experimental design.

For evaluation of the changes in basal motor activity, the first injection contained low (3.75, 7.5, and 15 mg/kg) or high (62.5, 125, and 250 mg/kg) TR dose, followed by the second injection of saline (1 ml/kg), and the response was compared with double saline control.

For evaluation of the changes in AMPH-induced motor activity, the first injection contained low (3.75, 7.5, and 15 mg/kg) or high (62.5, 125, and 250 mg/kg) TR dose, followed by the second injection of AMPH (1.5 mg/kg). The results were compared with AMPH-treated control animals, which received saline (1 ml/kg) as the first injection, followed by AMPH (1.5 mg/kg).

All rats were habituated in experimental cages for 30 min prior to the first injection. The time elapsed between the first and the second injection was 30 min. Motor activity was monitored between the two injections and continued up to 120 min after the second injection.

2.4. HPLC analysis

For determination of TR content in the whole brain, the animals were injected with TR (125 mg/kg ip) and were sacrificed 15, 30, 60, 120, 240, and 360 min later. The brains ($n = 5$/per time point) were dissected out and prepared for HPLC analysis, as previously described (Tasic et al., 2002).

Concentrations of TR were also determined in selected brain regions (hippocampus, cortex, and striatum). For that purpose, five animals were injected with TR (125 mg/kg ip) and were sacrificed 2 h later. Brain regions were carefully separated according to the atlas of Paxinos and Watson (1986), then pooled, and analyzed using the same procedure as above.

The analyses were performed on HPLC system Hewlett-Packard 1100 with the binary pump and diode–array detector (chromatographic conditions: column—Zorbax SB-C18, 4.6 × 250 mm, 5 μm; guard-column—Pelliguard LC-18, 2 cm × 4.6 mm, 5μm; temperature—35 °C; flow—2 ml/min; injection volume—100 μl; detection—254 nm; mobile phase—Na acetate 0.05 M, pH 4.6).

2.5. Statistical analysis

Statistical analysis of HPLC results was carried out using Friedman analysis of variance (ANOVA) and Kendall's concordance, followed by Wilcoxon matched pairs test.

Kruskal–Wallis one-way ANOVA by ranks was applied to establish the motor activity differences between the groups, and subsequent statistical comparisons were performed with the Mann–Whitney *U* test.

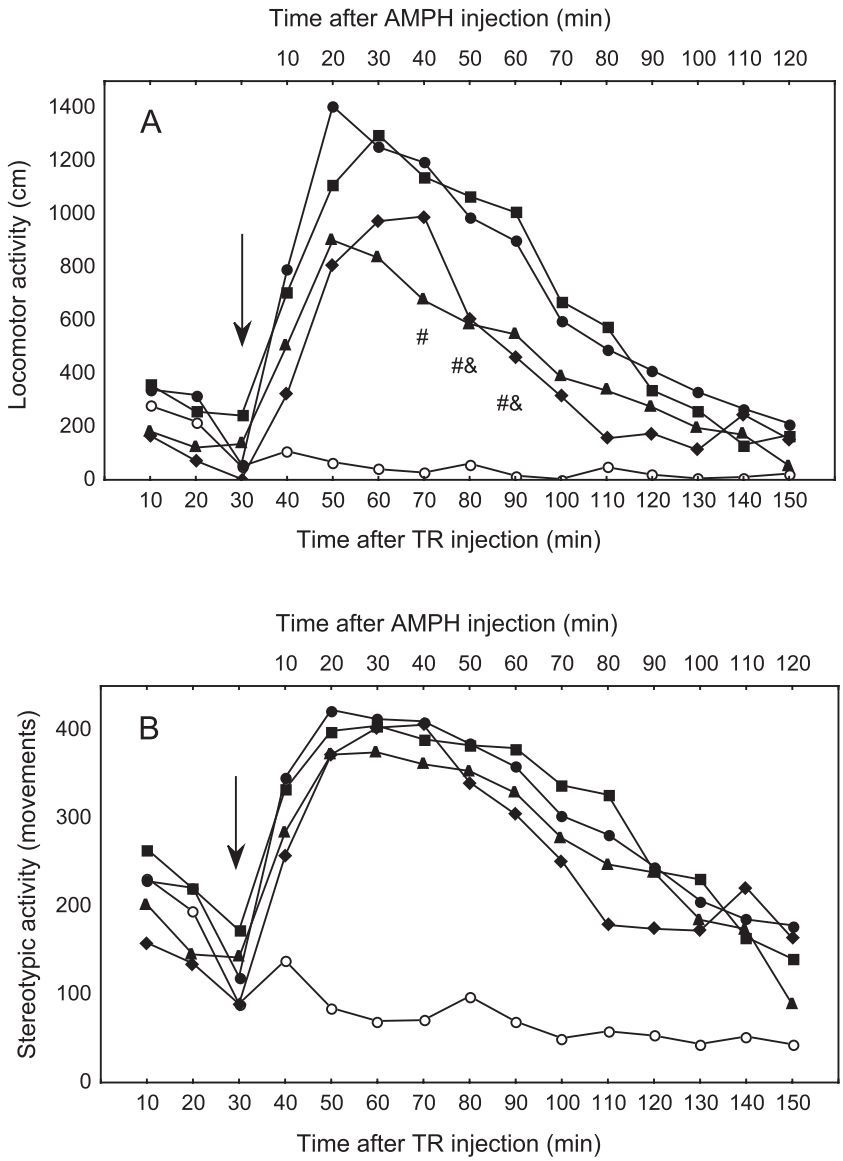

Fig. 1. The effects of pretreatment with low TR doses (3.75, 7.5, and 15 mg/kg ip) on AMPH-induced (1.5 mg/kg ip) rat locomotor (A) and stereotypic (B) activity. Motor activity was monitored 30 min after TR injection and continued up to 120 min after AMPH injection. The arrow indicates the time of AMPH injection. The saline controls received two saline injections by the same schedule, while AMPH controls received saline before AMPH. —○—saline control; —●—AMPH control; —■—TR 3.75 mg/kg + AMPH; —▲—TR 7.5 mg/kg + AMPH; —◆—TR 15 mg/kg + AMPH. Each point represents a mean value for the specific experimental group (n = 5–7). #$P < .05$: AMPH control vs. TR 7.5 mg/kg + AMPH; &$P < .05$: AMPH control versus TR 15 mg/kg + AMPH (*U* test).

The results were considered to be significantly different at $P < .05$. Parametric tests were not used inasmuch as most of the data obtained did not have a normal distribution (Zar, 1984).

3. Results

Monitoring of basal locomotor and stereotypic activity showed no effect of intraperitoneal injections of either low or high TR doses in comparison with saline control (data not shown).

As expected, AMPH administration (1.5 mg/kg ip) resulted in a statistically significant increase of both examined parameters of motor activity through the entire registration period in comparison with saline control (Figs. 1 and 2; $P < .05$, U test). This motor hyperactivity was referred to as AMPH control and was used for the evaluation of TR pretreatment effects.

Pretreatment with TR in the dose of 3.75 mg/kg expressed no effect on either parameter of AMPH-induced motor activity (Fig. 1A,B). In contrast, pretreatment with TR in the dose of 7.5 mg/kg significantly decreased AMPH-induced locomotor activity during the time interval

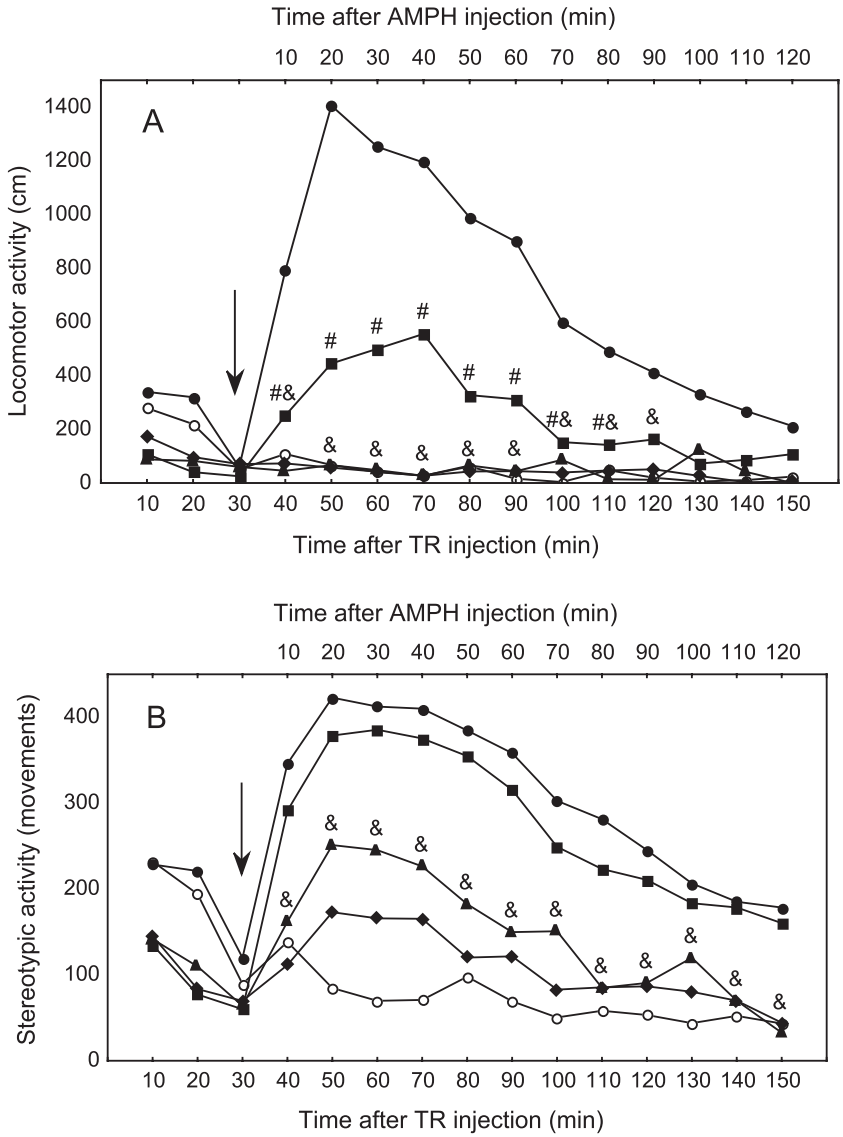

Fig. 2. The effects of pretreatment with high TR doses (62.5, 125, and 250 mg/kg ip) on AMPH-induced (1.5 mg/kg ip) rat locomotor (A) and stereotypic (B) activity. Motor activity was monitored 30 min after TR injection and continued up to 120 min after AMPH injection. The arrow indicates the time of AMPH injection. Saline controls were injected at both times with saline, while AMPH controls received saline as the first injection. —○—saline control; —●—AMPH control; —■—TR 62.5 mg/kg + AMPH; —▲—TR 125 mg/kg + AMPH; —◆—TR 250 mg/kg + AMPH. Each point represents a mean value for the specific experimental group ($n = 5–7$). #$P < .05$: AMPH control versus TR 62.5 mg/kg + AMPH; &$P < .05$: AMPH control versus TR 125 mg/kg or TR 250 mg/kg + AMPH (U test).

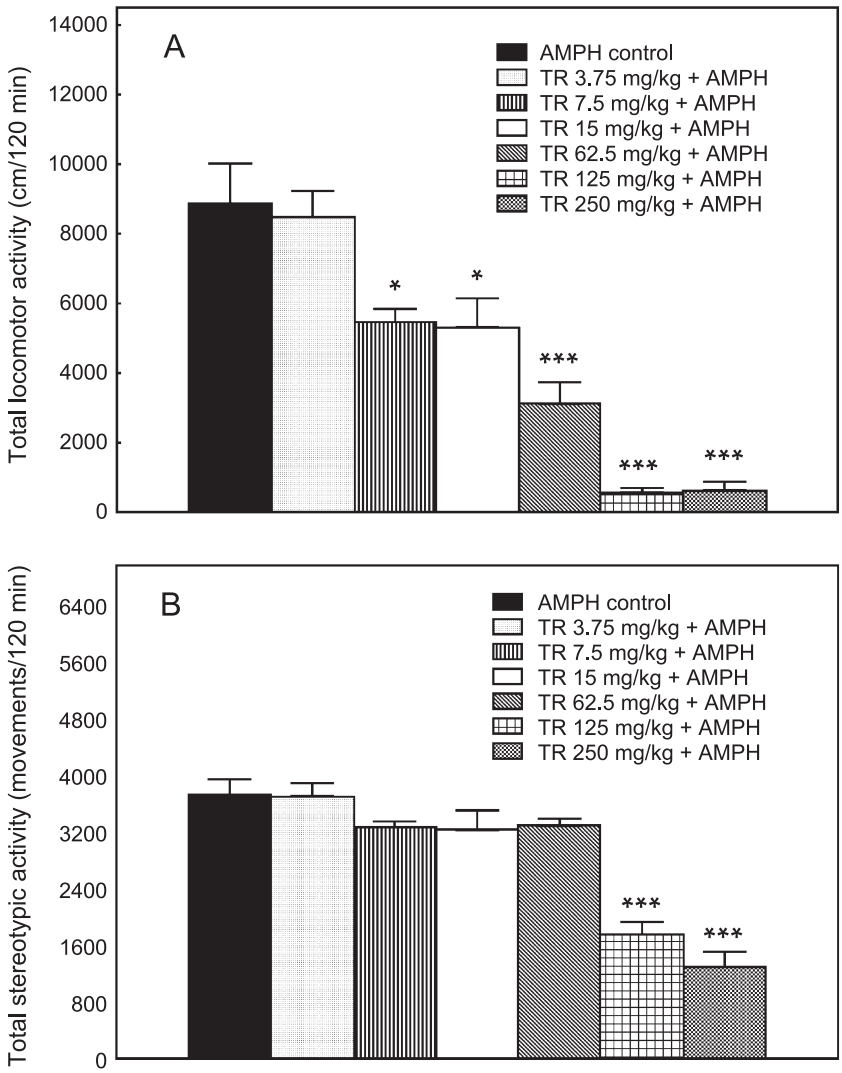

Fig. 3. The effects of pretreatment with low (3.75, 7.5, and 15 mg/kg ip) and high (62.5, 125, and 250 mg/kg ip) TR doses on total locomotor (A) and total stereotypic (B) activity during the registration period of 120 min after administration of AMPH (1.5 mg/kg ip). AMPH controls were first injected with saline. The results are presented as means ± S.E.M. ($n = 5–7$). * $P < .05$ versus AMPH control; *** $P < .005$ versus AMPH control (U test).

from 40 to 60 min ($P < .05$, U test), while the dose of 15 mg/kg had the same effect during the time period from 50 to 60 min ($P < .05$, U test) after AMPH injection (Fig. 1A). However, AMPH-induced stereotypic activity of the same animals was only slightly changed upon these TR doses (Fig. 1B).

Pretreatment with TR in the dose of 62.5 mg/kg significantly decreased AMPH-induced locomotor activity during the time interval from 10 to 80 min after AMPH administration (Fig. 2A; $P < .05$, U test), while stereotypic activity was similar to that of AMPH-treated rats (Fig. 2B). Applied in the doses of 125 and 250 mg/kg, TR induced a significant decrease of AMPH-induced locomotor activity during the time period from 10 to 90 min after AMPH injection (Fig. 2A; $P < .05$, U test), as well as of AMPH-induced stereotypic activity (Fig. 2B; $P < .05$, U test) during the entire registration period.

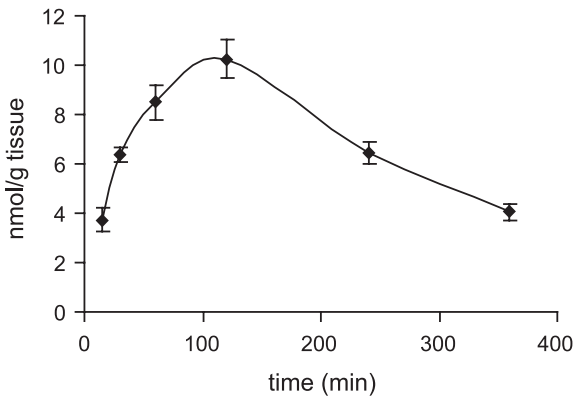

Fig. 4. TR concentration in the brain at different time intervals after its administration (125 mg/kg ip) determined by HPLC. Mean values ± S.D. of five samples are presented.

Table 1
TR concentrations in selected brain regions 2 h after drug administration (125 mg/kg ip)

Brain region	Concentration (nmol/g tissue)
Cortex	1.59 ± 0.13
Hippocampus	1.14 ± 0.09
Striatum	0.67 ± 0.07

The results represent the mean values ± S.D. of five independent samples.

Calculation of total AMPH-induced locomotor activity showed a dose-dependent inhibitory effect of TR pretreatment (Fig. 3A), while total AMPH-induced stereotypic activity was decreased only upon pretreatment with the TR doses of 125 and 250 mg/kg (Fig. 3B).

Quantification of TR in rat brains showed that this compound (125 mg/kg ip) was detectable 15 min postinjection, reaching the maximum 2-h period after the administration and was still present in this tissue after 6 h (Fig. 4). Inasmuch as TR concentration reached its maximum 2 h after the injection, this period was chosen for the determination of this drug in individual brain structures. The results obtained showed the presence of nanomolar TR concentrations in all three examined brain regions (cortex, hippocampus, and striatum). The highest concentration of this compound was found in the cortex (Table 1).

4. Discussion

The present study was aimed at investigating the effects of TR on basal and AMPH-induced motor activity in rats. This C-nucleoside analogue is a novel antitumor agent expressing, however, certain neurotoxic effects (Grifantini, 2000), which have not been fully examined so far. It has been shown that TR acts as an adenosine agonist, which binds selectively with a moderate affinity to adenosine A1 receptors of the central nervous system (Franchetti et al., 1995). As to our knowledge, this work represents the first report on behavioural effects of TR.

Our results showed that TR administration had no influence on basal motor activity, while TR pretreatment inhibited both locomotor and stereotypic AMPH-induced hyperactivities. In addition, HPLC analysis of TR brain content confirmed its presence in certain brain regions, including striatum, the structure playing a very important role in the regulation of motor activity.

Available literature data show that adenosine agonists exert their behavioural effects acting predominantly on the central adenosine A1 and A2a receptors (Ferre et al., 1993, 1997; Marston et al., 1998). Adenosine A1 receptors are widely distributed in the brain, including basal ganglia (Rivkees et al., 1995; Ochiishi et al., 1999). In the striatum, these receptors are localized on both pre- and postsynaptic terminals (Alexander and Reddington, 1989), participating in neurotransmitter release and modulation of behaviour mediated by the dopamine D1 receptors, respectively (Ferre et al., 1994; Okada et al., 1996). It was hypothesized that the activation of the A1 adenosine receptors changes the binding characteristics of the dopamine D1 receptors (for a review, see Fuxe et al., 1998) and that A1 and D1 receptors are physically associated, forming heteromeric complexes (Gines et al., 2000).

The results obtained throughout the present study showed that none of the applied TR doses induced the changes of either basal locomotor or stereotypic activity. Inasmuch as HPLC data confirmed the TR presence in the striatum, it could be assumed that possible changes in basal motor activity induced by TR were below the limits of detection. Data about the influence of adenosine A1 receptor agonists on basal motor activity are contradictory (Nikodijevic et al., 1991; Barraco et al., 1993, 1994; Marston et al., 1998; Schwienbacher et al., 2002). These disagreements are probably a result of applied experimental procedure and administered agonists that, at different rates, pass blood–brain barrier and have different affinity for adenosine receptors.

Investigation of TR effects on AMPH-induced (1.5 mg/kg ip) motor hyperactivity showed that TR pretreatment at all tested doses, with the exception of the lowest one, significantly decreased AMPH-induced locomotor activity, while AMPH-induced stereotypic activity was inhibited only by the two highest TR doses. The obtained results may be attributed to several possible causes, such as uneven distribution of TR in basal ganglia subregions, heterogeneous expression of the A1 receptors in the basal ganglia, or different strength of the A1–D1 receptor interactions.

The excitatory behavioural effects of AMPH are generally considered to be closely associated with an increased DA release in the brain. Different regions of basal ganglia are responsible for the control of the two examined parameters of motor activity, as reported by Staton and Solomon (1984). According to the current theory, the locomotor stimulatory effects of AMPH are mediated by the DA release in the nucleus accumbens, while AMPH-induced stereotypy results from an increased DA release in the striatum (Sharp et al., 1987). The fact that in our study, administration of a moderate AMPH dose (1.5 mg/kg) induced an increase of both locomotor and stereotypic activity (Figs. 1 and 2) suggests that DA release was increased in both basal ganglia regions.

The recorded decrease of AMPH-induced motor activity by TR may be the consequence of the adenosine A1 receptor activation in the basal ganglia, resulting in an inhibition of the DA release. This is in accordance with the data of several authors, who observed inhibitory effects of some other adenosine agonists (Heffner et al., 1989; Turgeon et al., 1996). Another possibility is that TR, activating postsynaptic striatal A1 receptors, changes the binding characteristics of the D1 receptors, thus inhibiting motor activity. Other behavioural studies demonstrated that A1 receptor activation attenuates D1-induced motor activity (Ferre et al., 1994),

which is, on the other hand, stimulated by selective A1 receptor antagonists (Popoli et al., 1996). However, it should be kept in mind that TR is an A1 receptor agonist with a moderate binding affinity at these receptors (Franchetti et al., 1995) and that the abovementioned studies regarding the A1–D1 interaction were performed with high-affinity A1 receptor ligands. In addition, it was shown that the activation of the adenosine A1 receptors leads to the inhibition of the striatal haloperidol-induced DA release but not of K^+- and AMPH-induced release of this neurotransmitter (Ballarin et al., 1995). These findings also indicate that the inhibitory TR effects on AMPH-induced hyperactivity could be based on postsynaptic A1–D1 receptor interactions. However, to confirm this hypothesis, additional experiments are needed which will evaluate, on one hand, the influence of TR on binding, signal transduction, and functional characteristics of dopamine D1 receptors and, on the other hand, the effect of TR pretreatment on D1 receptor agonist-induced release of DA and hyperlocomotion.

Depressant effects of TR on both AMPH-induced parameters of motor activity are supported by our results on determination of TR content in the rat brain. The highest concentration of TR was detected during the time period from 60 to 120 min after the treatment, which corresponds to the period of the strongest inhibition of AMPH-induced motor activity.

In conclusion, the results obtained in this study demonstrate that TR inhibits AMPH-induced motor activity in the rat, probably by influencing the functioning of the DA-ergic system. Further studies, which are necessary to fully elucidate the mechanism(s) underlying the action of this antitumor agent on the central nervous system, i.e., its undesirable neurotoxic effects, are in progress.

Acknowledgements

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