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The effect of tryptophan depletion on the action of haloperidol in MK-801-treated rats

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Abstract

We investigated the effect of tryptophan depletion (tryptophan-free mixture) on locomotor activity in an animal model of schizophrenia, induced by acute administration of 5*R*,10*S*-(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]-cyclohepten-5,10-imine hydrogen maleate (MK-801), and the influence of the tryptophan-free mixture on the action of the typical antipsychotic haloperidol. Male rats were pre-treated with haloperidol 60 min after receiving the tryptophan-free mixture (or water). We measured total distance travelled in an open field during a 90-min period. Administration of the tryptophan-free mixture resulted in decreased levels of tryptophan, serotonin and its metabolite 5-hydroxyindolacetic acid in the frontal cortex. Serotonin depletion increased the total distance travelled by MK-801-treated rats, modified the inhibitory effect of haloperidol and normalized the locomotor activity pattern in the model of schizophrenia-like behaviour. The effect of the tryptophan-free mixture combined with the classical antipsychotic haloperidol in MK-801-treated rats indicates the possibly important role of the serotonergic system in the action of antipsychotics.

Keywords: Tryptophan depletion; Serotonin; Haloperidol; (Rat); Schizophrenia; MK-801

1. Introduction

All antipsychotic drugs block dopamine D2 receptors (D2, 3, 4) but differ in their affinity for other types of receptors. In addition to antidopaminergic activity, clozapine-like antipsychotics compound have affinity for various groups of serotonin (5-HT) receptors (5-$HT_{1;2;3;6;7}$) and also influence serotonin uptake (Richelson, 1999; Schmidt et al., 2001; Roth et al., 2003). The interaction between the serotonin and dopamine systems could play an important role in the mechanism of action of these drugs (Horáček, 2000; Conley and Kelly, 2002; Buckley, 2003). Particularly, the antagonism of serotonin 5-HT_{2A} receptors together with a weaker blockade of dopamine D2 receptors is thought to be crucial for the action of clozapine-like antipsychotics (Meltzer and Gudelsky, 1992; Remington, 2003). We hypothesized that the interaction with the serotonergic system could indirectly influence the schizophrenia-like behaviour induced by 5*R*,10*S*-(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]-cyclohepten-5,10-imine hydrogen maleate (MK-801) itself or could modulate the effect of antipsychotic haloperidol (dopamine D2 receptor antagonist) in a model of schizophrenia.

The animal model of schizophrenia-like behaviour is based on the psychogenic effect of *N*-methyl-D-aspartate (NMDA) receptor antagonists (as MK-801). Both acute and chronic administrations of NMDA receptor antagonists produce schizophrenia-like behaviour with positive and negative symptoms in humans (Snyder, 1980; Anis et

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al., 1983; Rosse et al., 1994). In rats, the highly selective non-competitive NMDA receptor antagonist, MK-801, induces hyperlocomotion and other signs of disorganized behaviour (Ginski and Witkin, 1994; Andine et al., 1999).

We influenced the serotonergic system in the rat brain by means of tryptophan depletion (tryptophan-free mixture). Administration of a tryptophan-free diet is followed by a decrease in tryptophan concentration and consequently by a fall in brain levels of serotonin (Moja et al., 1989; Benedetti and Moja, 1993; Brown et al., 1998; Fadda, 2000; Fadda et al., 2000). The technique of tryptophan depletion is based on the fact that tryptophan is converted into serotonin through 5-hydroxytryptophan in a reaction catalysed by the enzyme tryptophan hydroxylase. The activity of tryptophan hydroxylase is a rate-limiting step in the synthesis of serotonin (Gessa et al., 1975). The tryptophan-free mixture consists of large neutral amino acids, with the exception of tryptophan (phenylalanine, leucine, isoleucine, threonine, lysine, valine, arginine, histidine, methionine and glycine). The large neutral amino acids compete with tryptophan for a single blood–brain barrier transporter. Therefore, administration of the tryptophan-free mixture leads to a decrease in brain tryptophan and consequently to a decrease in serotonin synthesis (Miller et al., 1985; Bell et al., 2001).

The tryptophan-free mixture influences various forms of behaviour, such as pain sensitivity, sexual behaviour, aggressiveness and sleep, in both rodents and humans (Fadda et al., 2000). Moreover, various effects of serotonin depletion in different psychiatric diseases have been reported (Sharma et al., 1997; Fadda, 2000; Bell et al., 2001; Golightly et al., 2001). There is evidence of an effect of a tryptophan-free mixture in schizophrenic patients. The tryptophan-free mixture impaired executive function, but had no effect on negative or positive symptoms (Sharma et al., 1997; Bell et al., 2001; Golightly et al., 2001). Interestingly, in healthy volunteers, the tryptophan-free mixture did not alter executive functions such as speed of information processing, divided attention or planning functions (Schmitt et al., 2000). It seems that the effect of the tryptophan-free mixture on cognitive function in humans depends on the individual vulnerability of the serotonergic system (Horáček et al., in press).

The goal of the study was to determine whether the tryptophan-free mixture influences the schizophrenia-like behaviour of rats and can change the effect of haloperidol on the behaviour. With respect to these aims, we tested the following specific hypotheses: the tryptophan-free mixture decreases serotonin level in the frontal brain; the tryptophan-free mixture alone changes the effect of MK-801 on behaviour; the administration of the tryptophan-free mixture increases the total distance travelled by the control and the haloperidol pre-treated groups; and the administration of the tryptophan-free mixture increases the total distance travelled by haloperidol/MK801 pre-treated rats.

2. Methods and materials

2.1. Animals

All experiments were carried out with adult (200–250 g b.w.; total 98 animals; each group containing 10 animals) male Wistar rats (specific pathogen-free animals; Hannover breed Konárovice, Czech Republic). Cages with two rat males were housed in a temperature-controlled room (21–22 °C), under a 12:12-h light/dark regimen (lights on at 6:00 a.m.) with free access to food (ST-1 diet) and water. Each rat was experimentally naive and was tested only once. All manipulations followed the Guidelines of the European Union Council (86/609/EU), and the instructions of the National Committee for the Care and Use of Laboratory Animals.

2.2. Drugs

MK-801 was dissolved in saline. The dose of MK-801, 0.3 mg/kg b.w., was dissolved in 5 ml of saline and injected intraperitoneally (i.p.). Haloperidol was dissolved in sterile isotonic glucose solution and was administered subcutaeously (s.c.) in the dose of 0.1 mg/kg b.w. (volume 5 ml/kg b. w.). The amino acids mixture without tryptophan (tryptophan-free mixture) contained L-phenylalanine 180 mg, L-leucine 80 mg, L-isoleucine 160 mg, L-lysine 400 mg, L-threonine 140 mg and L-valine 200 mg (Moja et al., 1989). These amino acids were dissolved in 10 ml of distilled water and this solution was administered by *gavage* in a volume of 10 ml/kg b.w.

2.3. Experimental protocol

On the day of the experiment (15 h after removal of food), the rats received the tryptophan-free mixture or water (10 ml/kg) per gavage. MK-801 (0.3 mg/kg b.w.; 5 ml/kg b.w.) was administered i.p 120 min thereafter. Behavioural observation started 5 min after the injection of MK-801 and continued for the next 90 min. Two groups of rats were pre-treated with haloperidol s.c. (0.1 mg/kg b. w.; 5 ml/kg b.w.) 60 min after the tryptophan-free mixture (or water) administration. The rats that received water per gavage and saline s.c. and i.p. in the same volume served as the control group.

Locomotor activity—expressed as a total distance travelled during 90 min in a box (68×68×30 cm) located in a sound proof room—was measured using a video tracking system for automation of the behavioural experiments (Noldus, EthoVision-Version 2.1.).

2.4. Concentration of tryptophan, 5-hydroxyindolacetic acid and serotonin in the frontal cortex

Rats received water (n=9) or the tryptophan-free mixture (n=9) from an independent experimenter and were decapitated under halothane anaesthesia, their brains were quickly removed, and both the right and left sides of the prefrontal cortex were isolated by using an ice-cooled plate and immediately frozen at −80 °C. For analysis, pieces of tissue (10–20 mg) were weighed in conical 1.5-ml test tubes and sonicated (at 200 W for 5 s while kept on ice) in the respective mobile phase; the pH had been adjusted to 3 with 42.5% orthophosphoric acid. The mobile phase consisted of an aqueous buffer (50 mM K_2HPO_4, 12 mM heptafluorobutyric acid, 0.1 mM EDTA-NA_2 and 2 mM NaCl; pH 3)–acetonitrile (92.5: 7.5, v/v). The flow rate of the mobile phase was 0.7 ml/min. Homogenates were centrifuged at 12,000×g for 10 min and the supernatants were used for high performance liquid chromatography (HPLC) analysis of tryptophan, serotonin and its metabolite 5-hydroxyindolacetic acid. We modified the method of Patthy and Gyenge (1988), using perfluorinated carboxylic acid as the pairing ion, separating serotonin, 5-hydroxyindolacetic acid and tryptophan as well as dopamine and its metabolites 3,4-dihydroxyphenylacetic acid and homovanillic acid in 12 min. Our HPLC system consisted of a Dionex P580A pump, a Rheodine 7125 injector with a 20-μl sample loop and a precolumn (15×3 mm C18) directly attached to a 150×3-mm analytical column of Separon SGX C18 with a 5-micron particle size ODS-silica (Tessek, Czech Republic). The electrochemical detector Antec Decade (Antec Leyden, The Netherlands) with a glassy carbon-working electrode operated at 0.75 V vs. an Ag/AgCl reference electrode at 10 nA full-scale. The detection limit for all compounds was approximately 200 pg/ml (depending on the compound). The linearity of standards was excellent (r>0.999 in all cases). Intraday and interday precision levels for samples were acceptable (intraday assay CV<5.5% and interday assay CV<7.0%). The average recovery of standard added to samples was >90%.

2.5. Sources of chemicals

Chemicals were purchased from Sigma-Aldrich; Prague, Czech Republic or from commercial sources and were of an analytical grade. Haloperidol was obtained from Gedeon Richter, Hungary; L-threonine from Flamma and other amino acids (L-phenylalanine, L-leucine, L-isoleucine, L-lysine, L-valine) came from SHS Liverpool.

2.6. Statistics

We used a non-parametric analogue of a one-way analysis of variance (ANOVA) Kruskal–Wallis test for three or more independent samples (the Monte Carlo estimate of the exact p value based on a sample estimate of size 10,000) for a priori postulated hypotheses. The Kruskal–Wallis test was followed by non-parametric multiple testing for trends and/or pair-wise contrasts (exact significance one-tailed for all analyses except a two-tailed evaluation for the hypothesis that the tryptophan-free mixture alone changed the effect of MK-801 on behaviour) to isolate sources of difference upon rejection of the null hypothesis (Meddis, 1984). For two independent samples, we used the Mann–Whitney U test with the Bonferroni inequality correction. The specific hypotheses were rejected on probability level 5%.

For qualitative analysis of the rat trajectories and locomotor distribution patterns in the arena, we used the sum of frequency in zones of the arena for rats of one experimental group. We suggested a scale from white to 90% black. The white colour was represented as 0–100 sum of frequency for 10 rats; the 10% black means 100–200 sum of frequency; 50% black means 200–300 frequency; 90% black means <300 frequency.

3. Results

3.1. The effect of the tryptophan-free mixture on brain serotonin level

The specific hypothesis was that the tryptophan-free mixture would decrease the serotonin level in the brain. The levels of tryptophan, serotonin and 5-hydroxyindolacetic acid (pg/mg) in the frontal cortex 215 min after administration of water or the tryptophan-free mixture are summarized in Table 1. Administration of the tryptophan-free mixture led to a marked decrease in tryptophan (U=0.00; P<0.05), serotonin (U=6.5; P<0.05) and 5-hydroxyindolacetic acid (U=1.5; P<0.05) levels in the rat frontal cortex.

Table 1
The concentration of tryptophan, 5-hydroxyindolacetic acid and serotonin in frontal rat cortex (median±99.2% Cl [pg/mg])

	Water (pg/mg)			Tryptophan-free mixture (pg/mg)		
	Median	99.2% Cl		Median	99.2% Cl	
		Lower bound	Upper bound		Lower bound	Upper bound
Tryptophan	4800 (9)	4300	5300	3150 (9)	2700	3600
5-Hydroxyindolacetic acid	660 (9)	620	760	540 (9)	510	580
Serotonin	284 (9)	228	348	186 (9)	144	252

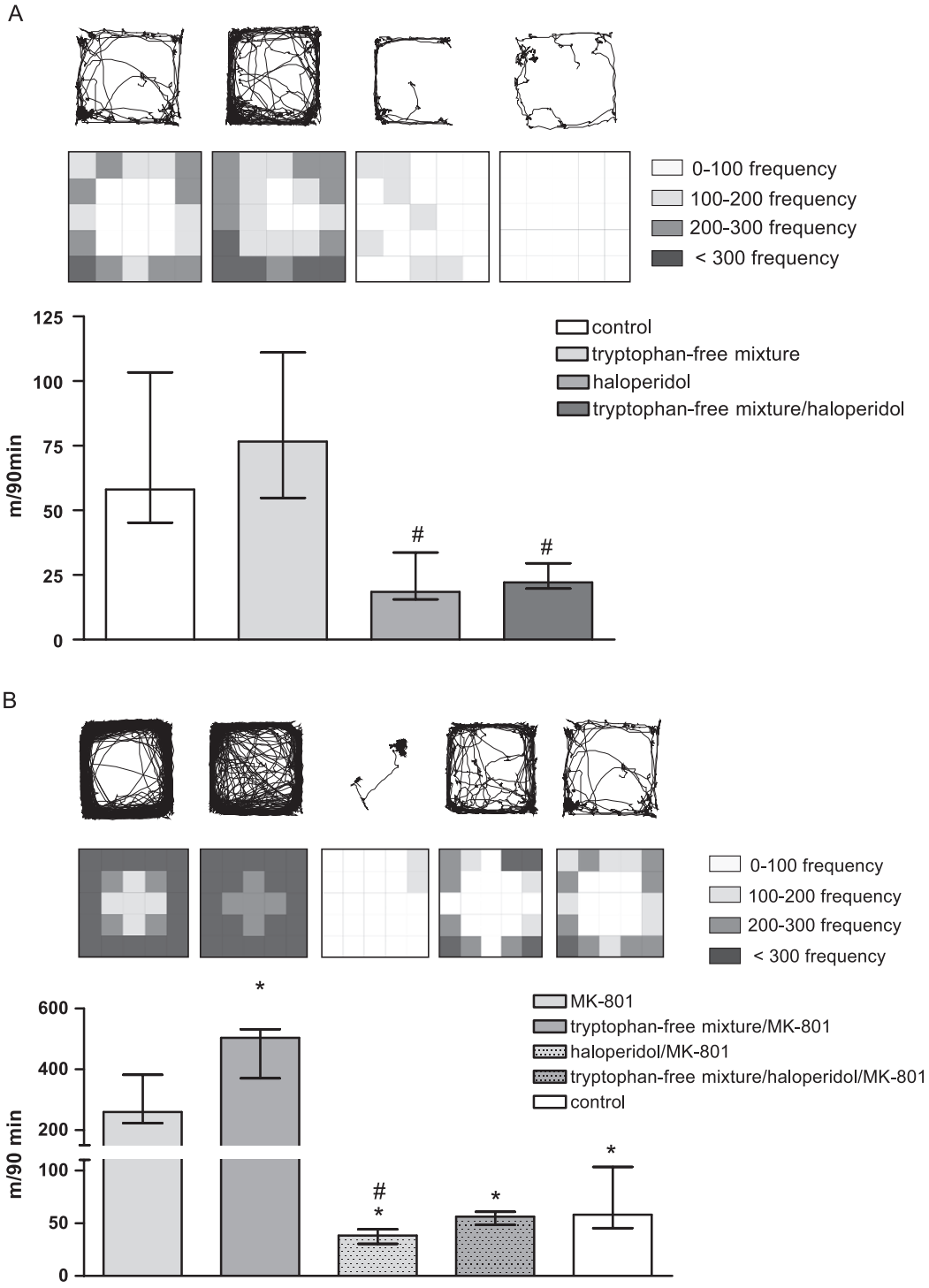

Fig. 1. The effect of the tryptophan-free mixture on the total distance travelled (m/90 min). The values represent the median±97.9%Cl. On the top of the figure, there are samples of each trajectory in the experimental arena and sum of frequency (n=10 animals per group) in each square of arena. Part A shows the total distance traveled after administration of haloperidol alone or with tryptophan-free mixture. Administration of haloperidol without MK-801 induced a marked inhibition of locomotor activity, which was not changed by the induced tryptophan depletion. The total distance traveled after the tryptophan-free mixture alone was slightly increased. The locomotor pattern of control rats is illustrated under the graph. The controls moved with a higher frequency around the walls of the arena than in the centre. Part B administration of MK-801 induced hyperlocomotion, which was increased when MK-801 was combined with the tryptophan-free mixture. Haloperidol decreased the total distance travelled by in the MK-801-treated rats. The total distance travelled by the haloperidol/MK801-treated rats was shorter than the total distance travelled by the control group. Tryptophan depletion (tryptophan-free mixture) increased the total distance travelled by the haloperidol/MK801-treated rats. The total distance travelled by the tryptophan-free mixture/haloperidol/MK801-treated rats did not differ from that of the controls. The locomotor pattern of the haloperidol/MK-801-treated group was not uniform, compared to the locomotor pattern of the tryptophan-free mixture/haloperidol/MK-801-treated group and the controls. *P<0.05 vs. the MK-801 group; $^{\#}P$<0.05 vs. the control group.

3.2. The effect of the tryptophan-free mixture on locomotor activity in MK-801-treated rats

We tested the influence of the tryptophan-free mixture in combination with MK-801 on locomotion. The administration of MK-801 increased the total distance travelled by the treated rats in comparison with that of the control group, see Fig. 1 (U=0.000, $P \leq 0.05$). Tryptophan depletion increased the total distance travelled by MK-801-treated rats in comparison with that of the MK-801 control group by about 42 %, (U=17; $P \leq 0.05$); Fig. 1, Part B; Table 2.

3.3. The effect of the tryptophan-free mixture on the haloperidol action in MK-801-treated rats

Haloperidol decreased the total distance travelled by the MK801-treated rats [L=169, (λ_j=1,0,0,1; n_j=7,10,10,10), Z=5.09; P<0.01], see Fig. 1, Part B. The total distance travelled by the haloperidol/MK801-treated rats was shorter than the total distance travelled by the control group, [L=139, (λ_j=0,1,0,−1; n_j=7,10,10,10), Z=2.87; P<0.05], see Fig. 1, Part B. Tryptophan depletion increased the total distance travelled by the haloperidol/MK801-treated rats by about 43 % [L=119 (λ_j=0,0,1,−1; n_j=7,10,10,10) Z=2.46; P<0.05], see Fig. 1, Part B; Table 2. The total distance travelled by he tryptophan-free mixture/haloperidol/MK801-treated rats did not differ from that of the controls, [L=20, (λ_j=0,1,−1,0; n_j=7,10,10,10), Z=0.41; P>0.05] and was shorter than the total distance travelled by the tryptophan-free mixture/MK-801- and MK-801-treated groups see Fig. 1, Part B. The critical value of the normal distribution is Z=2.24 (5%); we reached Z=3.1 for four comparisons.

To evaluate the role of the antiserotonergic action in a MK-801 model of schizophrenia-like behaviour, we compared the MK-801 group with the tryptophan-free mixture/haloperidol/MK-801 and tryptophan-free mixture/MK-801 groups. The critical value of normal distribution is Z=2.13 (5%); we reached Z=3.78 (P<0.05) for three comparisons. In this case, we used a two-tailed comparison between groups (Mann–Whitney test). The total distance travelled by the tryptophan-free mixture/MK-801 group vs. that of the tryptophan-free mixture/haloperidol/MK-801 group was statistically different (U=0.0, P<0.05). The total distance travelled by the MK-801 group and the tryptophan-free mixture/MK-801 group was also statistically different (U=0.0, P<0.05). All of the experimental data are summarized in Table 2.

The H_0 hypothesis, namely that the total distance travelled by the groups MK-801, control, the tryptophan-free mixture/haloperidol/MK-801 and haloperidol/MK-801 would be the same, was rejected in favour of the specific hypothesis that the total distance travelled by the group MK-801 was greater than that travelled by the control. The total distance travelled by the tryptophan-free mixture/haloperidol/MK-801-treated group of rats did not differ from that of the controls and was longer than the total distance travelled by the haloperidol/MK-801-treated group [L=1575, (λ_j=3,2,2,1; n_j=7,10,10,10), Z=4.177; P<0.01].

In addition, qualitative evaluation of the trajectories showed that the control rats had a typical locomotor pattern with more frequent movement near the wall and less frequent occupation of the centre of the experimental arena. The locomotor pattern of the control rats was regular. The MK-801-treated rats had a disorganized random pattern of movement, with a high frequency of movement in the periphery and in the centre of the arena. The haloperidol-treated rats were inhibited and typically moved around one place in the arena. The rats treated with the tryptophan-free mixture/haloperidol/MK-801 showed a similar locomotor pattern as the control rats. The typical samples and the sum of frequency in the zones of the arena of 10 rats in each group are shown in Fig. 1, Part B.

3.4. The effect of haloperidol combined with the tryptophan-free mixture without MK-801 administration

The hypothesis was that the total distance travelled by the tryptophan-free mixture/haloperidol-treated group would be greater than the total distance travelled by the water/haloperidol-treated group and the control group. The critical value of normal distribution is Z=2.13 (5%); we reached Z=3.4 for three comparisons. All the experimental data are summarized in Table 2.

The total distance travelled by the tryptophan-free mixture/haloperidol-treated group was not significantly

Table 2
Total distance travelled (median [m/90 min]±97.9% Cl) in an open field during 90 min

	Saline			MK-801		
	Median	97.9% Cl		Median	97.9% Cl	
		Lower bound	Upper bound		Lower bound	Upper bound
Saline (n)	58.1 (10)	42.87	113.53	264.02 (10)	220.89	403.61
Haloperidol (n)	18.53 (10)	14.8	34.7	38.38 (10)	29.29	44.31
Tryptophan-free mixture (n)	76.59 (10)	53.13	112.28	503.45(10)	346.71	544.32
Tryptophan-free mixture /haloperidol (n)	22.13 (10)	19.23	34.53	56.2 (10)	45.16	61.34

The tryptophan-free mixture was administered 120 min per gavage, haloperidol (0.1 mg/kg b.w., s.c.), 60 min and MK-801 (0.3 mg/kg b.w., i. p.) 5 min before the start of observation. The control group obtained an equal volume of water per gavage and saline (s.c. and i.p.). The percentages represent the decrease or the increase in total distance traveled compared with the control (saline/saline) or MK-801-treated groups.

different from that of the haloperidol-treated group [L=22, (λ_j=1,−1,0); n_j=10,10,10), Z=0.56; P>0.05], Fig. 1, Part A. The total distance travelled by the rats given haloperidol with the tryptophan-free mixture [L=−133, (λ_j=1,0,−1); n_j=10,10,10), Z=3.38; P<0.01] or without the tryptophan-free mixture was statistically shorter than that of the control by about 63% [L=−155, (λ_j=0,1,−1); n_j=10,10,10), Z=3.93; P<0.05] see Table 2.

4. Discussion

In our study we investigated the effect of tryptophan depletion (tryptophan-free mixture) on locomotor activity in an animal model of schizophrenia, induced by the acute administration of MK-801, and the influence of the tryptophan-free mixture on the action of haloperidol, with the aim to clarify the role of serotonin in the antipsychotic activity. Our hypothesis was that changes in the serotonergic system could influence the schizophrenia-like behaviour induced by MK-801 itself or could modulate the effect of the antipsychotic haloperidol (dopamine D2 receptor antagonist) in this model of schizophrenia.

Firstly, we found that administration of a tryptophan-free mixture of amino acids induced serotonin depletion in the rat frontal cortex. We admit that this technique has some limitations. First, the mixture includes many amino acids which could have interfered with the catecholaminergic system. Nevertheless, the concentrations of tyrosine and the catecholamine metabolites remain unchanged (Brown et al., 1998; Fadda, 2000). Secondly, our control group had baseline levels of tryptophan and large neutral amino acids in plasma. Most other authors, however, used a balanced mixture with a standard amount of large neutral amino acids plus tryptophan (Brown et al., 1998; Fadda, 2000; Shansis et al., 2000). In our pilot experiment (Bubeníková et al., 2002), we used the balanced mixture with 54 mg of tryptophan (Moja et al., 1989), but we found a significant increase in tryptophan plasma levels (an increase in tryptophan level by 15% compared to control group).

Despite these limitations of the tryptophan depletion technique, the method can be used as a suitable tool for studying brain serotonergic systems both in humans and in animals (Bel and Artigas, 1996; Fadda, 2000; Klaassen et al., 2002). Under our experimental conditions, the tryptophan-free mixture produced a significant decrease in tryptophan by 33.3%, serotonin by 37.2% and its metabolite 5-hydroxyindolacetic acid by 21.5% in the frontal cortex. Previous data also showed a decrease in the total plasma tryptophan level by 8% (Bubeníková et al., 2002).

The goal of the study was to investigate the effect of administration of a tryptophan-free mixture to MK-801-treated rats as a model of schizophrenia-like behaviour. We found the behaviour of rat (hyperlocomotion) induced by MK-801 was exacerbated after administration of the tryptophan-free mixture. A similar finding was reported by Plaanik et al., 1997 1 week after a serotonin lesion induced by 5,7-DHT lesion. There is evidence that an acute dose of MK-801 elevates the number of serotonin 5-HT_{1A} receptors in the prefrontal cortex and hippocampus. Simultaneously, the administration of MK-801 decreased the level of serotonin in the dorsal raphe nucleus (Wędzony et al., 1997). It is accepted that serotonin 5-$HT_{2A/2C}$ receptors play an important role in the behavioural action of MK-801 (Martin et al., 1997; Ninan and Kulkarni, 1998). Finally, MK-801 interacts with the serotonin transporter (Iravani et al., 1999) and can change serotonin-dependent behaviour in rats (Krebs-Thomson et al., 1998). We suggest that the increase in the total distance travelled observed after the tryptophan-free mixture combined with MK-801 could be the result of a different activation of pre- and postsynaptic serotonin receptors and a cross-influence of the tryptophan-free mixture and MK-801 on serotonin metabolism.

In the next step, we tested the hypothesis that the tryptophan-free mixture could increase and normalize the total distance travelled by haloperidol pre-treated rats in the animal model of schizophrenia-like behaviour. Haloperidol in therapeutic doses preferentially blocks the dopamine D_2 receptor (Horáček, 2000; Schmidt et al., 2001). The dose used in our experiment, 0.1 mg/kg, is supposed to produce optimal D_2 occupancy in rats, up to 70% (Kapur et al., 2000; Kapur and Seeman, 2001). This receptor occupancy is sufficient for antipsychotic-like activity (modelled as conditioned avoidance response) but does not reach the threshold for extrapyramidal side effects or catalepsy in animals. In our study, haloperidol statistically significantly inhibited the hyperlocomotion induced by MK-801 in the model of schizophrenia-like behaviour. Additionally, the locomotor pattern of rats after haloperidol/MK-801 treatment was very different to that of control rats (Fig. 1, Part B), and these data support the expectation that haloperidol pre-treatment would not normalize of MK-801-induced behaviour.

In the next step, we found that the tryptophan-free mixture increased the locomotor activity of haloperidol/MK-801-pre-treated rats and that the total distance travelled did not differ from that in controls. Furthermore, we found that the tryptophan-free mixture/haloperidol/MK-801-treated rats expressed a similar locomotor pattern as in the controls. With respect to these findings, we suggest that the administration of the tryptophan-free mixture optimizes the locomotor activity of haloperidol/MK-801-pre-treated rats and improves cognitive functions, as evidenced by the normalization of the locomotor pattern (Fig. 1A and B).

However, the tryptophan-free mixture combined only with haloperidol without MK-801 administration did not change the inhibitory effect of haloperidol alone on locomotor activity. The finding that the tryptophan-free mixture influenced the locomotor activity of rats only when the MK-801 was administered could be explained by a simultaneous interaction of the serotonergic (tryptophan free mixture induced serotonin depletion) and glutamatergic systems (MK-801). The idea that the tryptophan-free

mixture modifies the behaviour of haloperidol/MK-801-pre-treated rats through an interaction with the glutamatergic system is quite interesting. It is in contrast with the opinion that the better antipsychotic-like properties can be explained by the serotonergic/dopaminergic interaction of the drugs (Meltzer et al., 2003). However, the effect of atypical antipsychotic drugs on the glutamatergic system has been also discussed (Heresco-Levy, 2003).

In conclusion, the most interesting result was that schizophrenia-like behaviour was exacerbated by the tryptophan-free mixture. A tryptophan-free mixture combined with haloperidol (dopamine D2 receptor antagonist) pre-treatment induces locomotion only in rats with schizophrenia-like behaviour induced by MK-801 but not in rats without prior MK-801 pre-treatment. It seems that the effect of the tryptophan-free mixture on haloperidol pre-treatment could be explained by an interaction with the glutamatergic system rather than by an interaction with the dopaminergic system.

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