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Adenosine A_1 and A_2 receptor agonists significantly prevent the electroencephalographic effects induced by MK-801 in rats

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Abstract

Both N^6-cyclopentyladenosine (CPA, adenosine A_1 receptor agonist) and 2-[4-(2-carboxyethyl)phenethylamino]-5′-*N*-ethylcarboxamido-adenosine (CGS 21680, adenosine A_2 receptor agonist) inhibited the electroencephalographic (EEG) effects induced by the noncompetitive NMDA receptor antagonist (+)-5-methyl-10,11-dihydro-5H-dibenzo-(a,d)cyclohepten-5,10-imine maleate (MK-801) in rats. While the inhibitory effects of CPA were evident at doses (0.1 and 0.5 mg/kg i.p.) devoid of intrinsic behavioral effects, CGS 21680 was effective only when administered at depressant doses (2 mg/kg i.p.). Since the effects induced by NMDA receptor antagonists may be regarded as a model of psychosis, these results suggest a possible role of adenosine receptor agonists as antipsychotics. © 1997 Elsevier Science B.V.

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1. Introduction

Noncompetitive NMDA receptor antagonists, such as phencyclidine (PCP) and MK-801, induce marked behavioral and EEG effects in rodents (Popoli et al., 1995; Sagratella et al., 1992). Several studies have shown the involvement of central dopaminergic neurotransmission in the effects induced by NMDA antagonists. In particular, the hypermotility induced by MK-801 in rats is attenuated by reserpine and α-methyl-paratyrosine (Willins et al., 1993), as well as by dopamine receptor antagonists (Ouagazzal et al., 1993; Martin et al., 1994).

In the last years, increasing evidence has been provided for the modulatory role of adenosine in dopaminergic neurotransmission (Ferré et al., 1992 for review). In particular, adenosine A_1 and A_2 receptors have been reported to inhibit the activity of dopamine D_1 and D_2 receptors, respectively (Ferré et al., 1991, 1994), and it has been shown that adenosine receptor agonists exert the same effects as those elicited by dopamine receptor blockade in some experimental models (Heffner et al., 1989). It is thus conceivable that adenosine receptor agonists inhibit, as dopamine receptor antagonists have been reported to do, the effects induced by NMDA receptor antagonists. There are indeed some reports in the literature showing the inhibitory influence of adenosine A_1 and $A_1 > A_2$ receptor agonists towards PCP-induced behavioral effects in rats (Browne and Welch, 1982) and rabbits (Popoli et al., 1990).

The aim of the present paper was to verify whether selective adenosine A_1 and A_2 receptor agonists prevent MK-801-induced effects. Since electroencephalographic (EEG) recording is a suitable means for studying NMDA receptor antagonist-induced effects (Sagratella et al., 1992; Popoli et al., 1995), a computerized EEG study was undertaken in rats.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (250–280 g) were used. The animals were kept under standardized temperature, humidity and lighting conditions, with free access to water and food.

Screw cortical electrodes were implanted under Equitesin anaesthesia (3 ml/kg) and fixed with dental acrylic to the skull surface.

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Animal care and use followed the directives of the Council of the European Communities (1986).

2.2. Experimental procedure

The experiments were performed 5 to 6 days after implantation. Thirty min before the start of EEG recordings, the animals were placed in individual cylindrical Plexiglas containers (diameter 38.5 cm × 40 cm high). Groups of 6–8 animals each were randomly assigned to the following treatments: saline; MK-801 0.4 mg/kg; CGS 21680 1 or 2 mg/kg plus (10 min thereafter) MK 801 0.4; CPA 0.05, 0.1 or 0.5 mg/kg plus (10 min thereafter) MK-801 0.4. The effects induced by CGS 21680 (1 and 2 mg/kg) and by CPA (0.1 and 0.5 mg/kg) alone were tested in separate groups of 4 animals/dose.

EEG recording was started immediately after drug injection and lasted for 40 min. The EEG was recorded simultaneously on paper (polygraph OTE Biomedical, model E 10b) and in a computer (IBM PS/2, model 70 386). Sequential power spectra of 20 s EEG epochs (1 epoch every min) were analyzed by fast fourier transform (FFT) with a frequency resolution of 0.35 Hz (software by Enrico Staderini). Each 20 s epoch power spectrum was the mean spectrum resulting from every single 2 s power spectra of the epoch, overlapped by 50%. All the power spectra relevant to an EEG tracing were recorded on a optical disk (940 MB, RPS) and then analyzed to calculate the relevant power of each frequency band. Frequency bands similar to those proposed by Kropf and Kuschinsky, 1993 were considered: 1.2–4 Hz (δ), 4.35–7 Hz (Θ), 7.35–9.5 Hz (α_1), 9.85–12.5 Hz (α_2), 12.85–16 Hz (β_1), 16.35–30 Hz (β_2).

One-way analysis of variance (ANOVA) followed by Dunnett's test was used for the statistical analysis of the results.

2.3. Drugs

(+)-5-Methyl-10,11-dihydro-5H-dibenzo-(a,d)cyclohepten-5,10-imine maleate (MK-801), 2-[4-(2-carboxyethyl)phenethylamino]-5′-*N*-ethylcarboxamido-adenosine hydrochloride (CGS 21680, adenosine A_2 receptor agonist), and N^6-cyclopentyladenosine (CPA, adenosine A_1 receptor agonist) were purchased from Research Biochemicals International (licensed in Italy by Amersham Italia, Milano, Italy).

All drugs were dissolved in water and administered intraperitoneally (i.p.).

3. Results

As previously reported by our group (Sagratella et al., 1992), the administration of MK-801 to rats induced an EEG picture characterized by an increased amplitude of the background voltage and by the appearance of clustered slow waves. The dose of MK-801 used in this investigation was chosen on the basis of preliminary experiments showing the high reproducibility of the effects elicited by 0.4 mg/kg MK-801. In terms of quantitative EEG analysis (Figs. 1 and 2), MK-801 induces a statistically significant increase in relative power in the δ band, and a decrease in relative power in α and β_1 bands.

Behaviorally, MK-801-treated animals showed both excitatory (increased locomotion, head-weaving, circling) and depressant (ataxia) effects.

At the dose used in this investigation, neither CPA nor CGS 21680 significantly modified the EEG tracing per se (Fig. 3). Behavioral depression (reduced locomotion, hypotonicity) was observed after the administration of CGS 21680 at the dose of 2 mg/kg.

As shown in Figs. 1 and 2, both CPA and CGS 21680 prevented the EEG effects induced by MK-801 in a statistically significant and dose-dependent way. Even though behavior was not quantified, both CPA (0.5 mg/kg) and CGS 21680 (2 mg/kg) were noticed to reduce the excitatory effects induced by MK 801. Ataxia was, conversely, not reduced by CPA, and even seemed to be potentiated by CGS 21680 2 mg/kg.

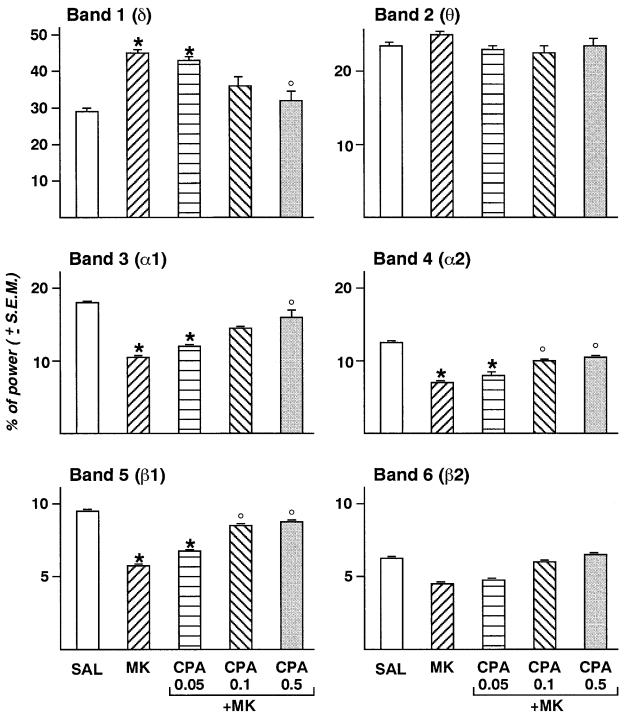

Fig. 1. Influence of CPA 0.05, 0.1 and 0.5 mg/kg on the EEG effects (relative power distribution) induced by MK-801 (0.4 mg/kg) in rats. Each bar represents the mean ± S.E.M. from 6 to 8 experiments. Doses of CPA are expressed as mg/kg i.p. For definition of frequency bands see Section 2. (*) $P < 0.05$ vs. saline; (°) $P < 0.05$ vs. MK 801, according to one-way ANOVA followed by Dunnett's test.

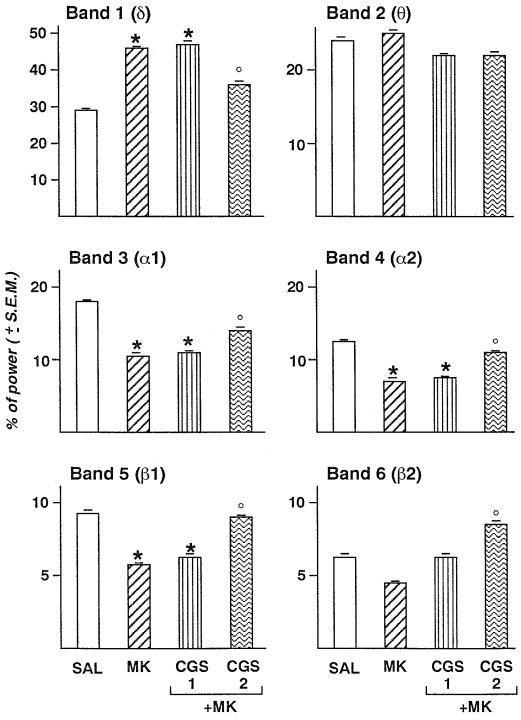

Fig. 2. Influence of CGS 21680 (1 and 2 mg/kg) on the EEG effects (relative power distribution) induced by MK-801 (0.4 mg/kg) in rats. Each bar represents the mean ± S.E.M. from 6 to 8 experiments. Doses of CGS 21680 are expressed as mg/kg i.p. For definition of frequency bands see Section 2. (*) $P < 0.05$ vs. saline; (°) $P < 0.05$ vs. MK 801, according to one-way ANOVA followed by Dunnett's test.

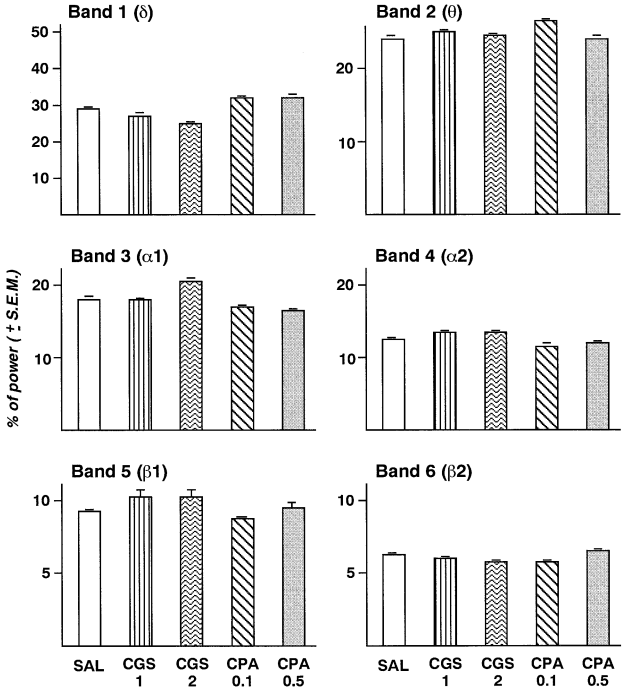

Fig. 3. Influence of CPA (0.1 and 0.5 mg/kg) and CGS 21680 (1 and 2 mg/kg), administered alone, on relative EEG power distribution in rats. Each bar represents the mean ± S.E.M. from 6 (saline) or 4 (CPA and CGS) experiments. Doses are expressed in mg/kg i.p.

4. Discussion

Systemic administration of the NMDA receptor antagonist MK-801 induced significant modifications of the EEG tracing with respect to that of saline-treated animals, thus confirming that the EEG may be a suitable means to study the pharmacology of NMDA receptor antagonists. In particular, an increase in the relative EEG power of band δ, and a decrease in the relative EEG power of bands α_1, α_2 and β_1 were observed. These quantitative EEG findings fully agree with those of a previous report by Marquis et al. (1989).

Both adenosine A_1 and A_2 receptor agonists exerted a dose-dependent inhibition of MK-801-induced EEG effects. The adenosine A_1 receptor agonist CPA reduced the effects of MK-801, starting from doses low enough to be devoid of intrinsic behavioral effects. Conversely, the adenosine A_2 receptor agonist, CGS 21680, was effective only when administered at doses inducing behavioral depression per se.

In previous studies, it was reported that dopamine D_2 receptor antagonists prevented NMDA receptor antagonist-induced effects only at cataleptogenic doses, while the dopamine D_1 receptor antagonist SCH 23390 was active when administered at low, nonsedative doses (Popoli et al., 1990; Ouagazzal et al., 1993; Martin et al., 1994). Since specific inhibitory interactions between A_1 and D_1, and between A_2 and D_2 receptors have been reported (see Introduction), it is conceivable that the inhibitory effects exerted by adenosine A_1 and A_2 receptor agonists depend on the inhibition of dopamine D_1 and D_2 receptors, respectively. If this is the case, the present findings are in line with the above mentioned studies (Popoli et al., 1990; Ouagazzal et al., 1993; Martin et al., 1994), suggesting that dopamine D_1 receptor antagonists exert a greater inhibitory activity than dopamine D_2 receptor antagonists. It should be noted, however, that the present study does not allow us to ascertain that dopamine receptor inhibition is responsible for the antagonistic effects of CPA and CGS 21680 towards MK-801. First of all, the possibility that adenosine receptor agonists may counteract the effects of MK-801 through more direct mechanisms (e.g., by acting on the NMDA receptor) can not be ruled out. Moreover, some findings have suggested that the interactions between adenosine and dopamine subtypes (namely, A_1-D_1 and A_2-D_2 interactions) may be less specific than we claim (Morelli et al., 1994).

Whatever the mechanisms may be, the present results clearly show the inhibitory influence of adenosine receptor agonists on the EEG effects induced by MK-801.

On the basis of the findings that, in schizophrenic patients, [^{3}H]MK-801 and [^{3}H]PCP binding are increased in some areas (see Moghaddam, 1994), while glutamatergic neurotransmission seems to be reduced (Sherman et al., 1991), a major role has been proposed for excitatory amino acid-mediated neurotransmission in the pathogenesis of

schizophrenia (Carlsson and Carlsson, 1990; Moghaddam, 1994). Based on this hypothesis, as well as on the finding that PCP induces psychotomimetic symptoms in humans (Fauman et al., 1976), the effects induced by NMDA receptor antagonists in rodents may be considered animal models of schizophrenia (Javitt and Zukin, 1991). Interestingly, some of the EEG effects induced by MK-801 in rats are superimposable on the computerized EEG pattern of schizophrenic patients, as described by Itil et al. (1972).

Although animal models of psychosis should be regarded with great caution as far as their clinical predictivity is concerned (and this may be particularly true for an 'EEG' model), the present data confirm the possible role of adenosine receptor agonists as antipsychotics. In this respect, adenosine A_1 receptor agonists (although their possible clinical use is seriously limited by the cardiovascular effects they induce) seem to be better candidates than adenosine A_2 receptor agonists. This hypothesis is strengthened by the recent finding of an interaction between adenosine A_1 and dopamine D_1 receptors in the rat limbic system, a brain area highly involved in neuropsychiatric disorders (Ferré et al., 1996).

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