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Interaction of Dexanabinol (HU-211), a novel NMDA receptor antagonist, with the dopaminergic system

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Abstract

The interaction of 7-hydroxy-Δ^6-tetrahydrocannabinol 1,1-dimethylheptyl (Dexanabinol; HU-211), a novel NMDA receptor antagonist, with the dopaminergic system was examined using in vitro and in vivo systems. HU-211 (50 or 100 μM) inhibited the binding of [^{3}H]*R*(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepin-7-ol hydrochloride ([^{3}H] SCH-23390), a dopamine D_1 receptor antagonist, by 29.7 $\pm$ 1.8% and 52.7 $\pm$ 6.3%, respectively. HU-211 10 μM, like the dopamine D_1 receptor agonist *R*(+)-1-phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8-diol hydrochloride (SKF-38393), enhanced the conversion of [^{3}H]adenine to cyclic AMP (cAMP) (51.8 $\pm$ 29.7% and 35.6 $\pm$ 21.5% over control, respectively). The HU-211-induced increase was not inhibited by SCH-23390. HU-211 together with the dopamine D_1 receptor agonist caused a synergistic elevation (314.7 $\pm$ 14.3%). HU-211 reduced the catalepsy induced by dopamine receptor antagonists. At 10 mg/kg, HU-211 significantly ($P < 0.001$) reduced the catalepsy time induced by D_1, D_2 and non-selective dopamine receptor antagonists. Overall, the results of the present study demonstrate that HU-211 interacts with the dopaminergic system and enhances activity at the dopamine D_1 receptor level. This activity may have implications in diseases involving the dopaminergic system, such as Parkinson's disease. © 1997 Elsevier Science B.V.

Keywords: HU-211; NMDA receptor antagonist; Dopamine receptor antagonist; Catalepsy; Parkinson's disease

1. Introduction

High levels of excitatory amino acids are associated with many central nervous system (CNS) pathologies including brain ischemia, brain trauma, spinal cord ischemia, spinal cord trauma and possibly also Huntington's chorea disease, Alzheimer's disease and Parkinson's disease (Iversen, 1975; Sonsalla et al., 1989; Meldrum and Garthwaite, 1990; Moore et al., 1993). Overactivity of excitatory amino acids neurotransmission is involved in pathologies of the dopaminergic system (Sonsalla et al., 1989; Meldrum and Garthwaite, 1990; Baldwin et al., 1993). The interactions between the glutaminergic and dopaminergic system in the brain are not fully understood, but as reported by Singh et al. (1992), there are cortical glutaminergic projections to extrapyramidal and limbic structures and high levels of NMDA receptors in these regions. This suggests a possible role for glutamate in the regulation of the dopaminergic system. In Parkinson's disease, dopaminergic hypofunction leads to increased activity of the excitatory glutaminergic output (Moore et al., 1993). It has been suggested that dopaminergic and glutaminergic systems within the striatum may be reciprocally balanced. Several studies have demonstrated the effects of NMDA receptor antagonists on the dopaminergic system (Sonsalla et al., 1989; Löscher et al., 1991; Gandolfi and Dall'olio, 1992; Moore et al., 1993; Baldwin et al., 1993), both in vivo and in vitro. An increase in dopamine turnover and/or release was seen in several brain regions following treatment with NMDA receptors antagonists (Löscher et al., 1991). The NMDA receptor antagonists, phenylcyclidine, ketamine and 2-amino-5-phosphonopentanoic acid (AP-5), reduce dopamine receptor antagonist-induced catalepsy. (+)-5-methyl-10,11-dihydro-5H-dibenzo [a,d] cyclohepten-5,10-imine hydrogen maleate (MK-801) also produces a moderate reduction in dopamine levels in the substantia nigra following treatment with methamphetamine (Moore et al., 1993).

HU-211 (Dexanabinol) is a synthetic cannabinoid (7-hydroxy-Δ^6-tetrahydrocannabinol 1,1-dimethylheptyl) which does not bind to the cannabinoid receptor (Howlett et al., 1988, 1990; Devane et al., 1988, 1992). However,

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the compound binds with low affinity (Feigenbaum et al., 1989) but high selectivity (Eshhar et al., 1993) to the NMDA receptor channel. HU-211 induced neuroprotection in a closed head injury model (Shohami et al., 1993, 1995), in global ischemia in gerbils and rats (Bar-Joseph et al., 1994a; Belayev et al., 1995a), and in focal ischemia in rats (Biegon and Bar-Joseph, 1995; Belayev et al., 1995b,c). Animals treated with HU-211 did not exhibit any of the behavioral, physiological or neuropathological (morphological) side effects that are characteristic of other NMDA receptor antagonists (Bar-Joseph et al., 1995), supposedly because of the lower affinity and fast dissociation of the compound. In vitro studies showed that HU-211 protected cultured neurons from NMDA-induced toxicity (Eshhar et al., 1993; Nadler et al., 1993a), blocked NMDA-mediated $^{45}Ca^{2+}$ influx into neurons (Nadler et al., 1993b) and showed antioxidant activities (Eshhar et al., 1995).

Therefore, the purpose of the present study was to test the interaction of HU-211 with the dopaminergic system in vitro and in vivo and to compare it with MK-801, a representative non-competitive NMDA receptor antagonist. The in vivo study was based on the fact that dopamine receptor antagonists induce cataleptic behavior in animals, and that NMDA receptor antagonists can reduce this effect (Moore et al., 1993). The in vitro studies attempted to examine a more specific interaction of HU-211 with the dopamine D_1 receptor.

2. Materials and methods

2.1. In vitro studies

In this study we examined the interaction of HU-211 with the dopaminergic D_1 receptor system in vitro; in membranes and neuronal culture. The dopamine D_1 receptors are most abundant in the rat cortex, while the dopamine D_2 receptor is the main subtype in other brain regions. Striatal or cortical cultures do not contain dopaminergic projections. However, the cortex provides more tissue and is easier to work with. Therefore, both binding and culture studies utilized cortical tissue rather than striatal tissue.

2.1.1. Materials

R(+)-SKF-38393 hydrochloride [*R*(+)-1-phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8-diol hydrochloride], *R*(+)-SCH-23390 hydrochloride [*R*(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-benzazepin-7-ol hydro-chloride], (+)-butaclamol hydrochloride and *S*(−)-propanolol hydrochloride were purchased from Research Biochemicals International (RBI). The radioactive compound, SCH-23390 (*n*-methyl-^{3}H), 70 Ci/mmol, was purchased from New England Nuclear (NEN). Adenine-2-^{3}H, 10.3 Ci/mmol was purchased from Rotem, Israel. All other materials were purchased from Sigma.

2.1.2. Forebrain membrane preparation

Repeatedly washed rat forebrain membranes (from Sprague–Dawley rats) were prepared as described in detail elsewhere (Eshhar et al., 1989). Briefly, forebrains were homogenized in 30 vol of 0.32 M sucrose containing 5 mM EDTA and 1 mM phenylmethyl–sulfonylfluoride, and centrifuged for 10 min at $1400 \times g$. All procedures were performed at 4°C. The supernatant was centrifuged for 15 min at $50\,000 \times g$ and the resulting pellet was washed three times in 10 mM Tris–HCl buffer (pH 7.4) containing 1 mM phenylmethyl–sulfonylfluoride. The final pellet was suspended in buffer and kept at −70°C until use.

2.1.3. Preparation of cortical cultures

Cortical cells were derived from Sprague–Dawley fetal rats and cultured according to procedures described elsewhere (Eshhar et al., 1993). Briefly, cultures from 18 to 20-day-old embryonic rat brains were prepared by mechano–enzymatic dissociation. Dissociated tissue was suspended in medium consisting of 5% fetal calf serum and 5% heat-inactivated horse serum prepared in Eagle's minimum essential medium (MEM) enriched with 0.6% glucose, 2 mM glutamine and 15 μg/ml gentamycine. The cell suspension was plated on 24-well plates coated with poly-L-lysine (20 μl in 0.1 M borate buffer, pH 8.5), at a density of 10^6 cells per well. Plates were incubated in a humidified CO_2 (5%) incubator at 37°C. On days 6–7 after plating, the medium was replaced by a medium consisting of 10% horse serum in enriched MEM. To this was added a cell division blocker, consisting of a mixture of 5′-fluoro-2 deoxyuridine (20 μg/ml) and uridine (50 μg/ml). All the experiments were performed on cells at day 10 in culture.

2.1.4. Binding studies

Binding assays were performed as described elsewhere (Deary et al., 1990). Briefly, 0.7 nM [^{3}H]SCH-23390 was incubated with 0.3 mg membranes in 50 mM Tris buffer, pH 7.4, 0.9% NaCl and 0.025% ascorbic acid. Binding was carried out in triplicate samples of 0.5 ml at 30°C for 1 h. Non-specific binding was defined in the presence of 10 μM (+)-butaclamol. HU-211 was dissolved in ethanol to give 5 mM, and diluted to a stock solution of 200 μM with 5% bovine serum albumin, which was again diluted to the desired concentrations. All the assay samples contained the same bovine serum albumin/ethanol concentration (0.25% bovine serum albumin and 0.2% ethanol, final concentration). The reaction was stopped by filtration through glass fiber filters (GF/C), followed by three washings with 4 ml ice-cold 10 mM Tris buffer.

2.1.5. Adenylate cyclase assay

The assay was performed as described previously (Salomon, 1991). Briefly, the cultured cells were incubated for 2 h in a medium containing 5 μCi/ml of [^{3}H]adenine. This medium was replaced with 0.3 ml per well of en-

Table 1
Adenylate cyclase assay experimental design

Group	Drugs
1	Control (vehicle of HU-211)
2	10 μM SK-38393
3	10 μM SCH-23390
4	10 μM SKF-38393 + 10 μM SCH-23390
5	10 μM HU-211
6	10 μM SCH-23390 + 10 μM HU-211
7	10 μM SKF-38393 + 10 μM HU-211
8	10 μM MK-801
9	10 μM MK-801 + 10 μM SKF-38393
10	1 μM isoproterenol
11	1 μM propanolol
12	1 μM isoproterenol + 1 μM propranolol
13	1 μM isoproterenol + 10 μM HU-211
14	1 μM propanolol + 10 μM HU-211

riched MEM, containing 0.1 mM 3-isobutyl-1-methylxanthine (IBMX), 0.2 μM forskolin and the appropriate treatments. A stock of 200 μM of HU-211 was prepared, as described above, and the compound was further diluted into the medium to give a concentration of 10 μM. The experimental design is shown in Table 1.

After 1 h at 37°C, the medium was transferred and the reaction was terminated by addition of 1 ml of 2.5% perchloric acid containing 0.1 mM unlabeled cAMP. After 30 min at 4°C, 0.8 ml volumes were removed into Eppendorf tubes, neutralized with a solution of 4.2 M KOH supplemented with water to a volume of 1.3 ml. Aliquots of 0.1 ml were sampled into scintillation vials in order to calculate the total uptake of [^{3}H]adenine into the cells. Aliquots (1.2 ml) of the supernatants, after pelleting of the potassium perchlorate, were applied to a two-step column separation procedure for cAMP (Salomon, 1991). The [^{3}H]cAMP was eluted directly into scintillation vials and counted. cAMP accumulation was calculated as % conversion of [^{3}H]adenine to [^{3}H]cAMP from the total uptake.

2.2. In vivo study

2.2.1. Animals

Male BALB/c mice, weighing 25–30 g, (Anilab, Hulda), were used. Animals were kept under a 12 h dark–light cycle, in an environmentally controlled facility: temperature 21 ± 4°C and relative humidity of 55 ± 15%. Animals were fed ad lib. with a standard mouse diet (Kopulk, Petach-Tikva) and filtered tap water.

2.2.2. Drugs

MK-801 (RBI, USA) and haloperidol (Halidol, Abic) were dissolved in saline (Travenol, Israel). HU-211 (Pharmos, Israel) was dissolved in co-solvent (70% emulphor, 30% ethanol, Pharmos Israel) and diluted with saline before injection. Clebopride (Sigma, USA) was dissolved in 35% transcutal in water (Gattefose, France). All drugs were freshly prepared prior to experimental sessions, and administered IP in a constant volume of 10 ml/kg body weight.

2.2.3. Study procedure

Each treatment group ($n = 6$) was given the appropriate test drug combination: saline 10 ml/kg, MK-801 1 mg/kg, co-solvent (70% emulphor, 30% ethanol) 10 ml/kg and HU-211 10 mg/kg immediately after (less then 1 min) mice were treated with the chosen dopamine receptor antagonist. Three sets of experiments were performed: (1) with haloperidol 1 mg/kg; (2) with clebopride 20 mg/kg; and (3) with SCH-23390 5 mg/kg. Catalepsy duration was then recorded at hourly intervals, commencing 1 h after dosing. The recording of catalepsy for SCH-23390 was performed every 30 min, commencing 30 min post-drug administration. Catalepsy was determined by placing the animals' front paws over a rod raised 6 cm above the experimental platform and recording the time the animal stayed in this position. The maximum time per measurement was 120 s.

2.2.4. Statistical analysis

The data (expressed as mean ± S.E.M.) were analyzed by one-way analysis of variance (ANOVA), followed by Duncan's post hoc test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. In vitro study

3.1.1. Binding study

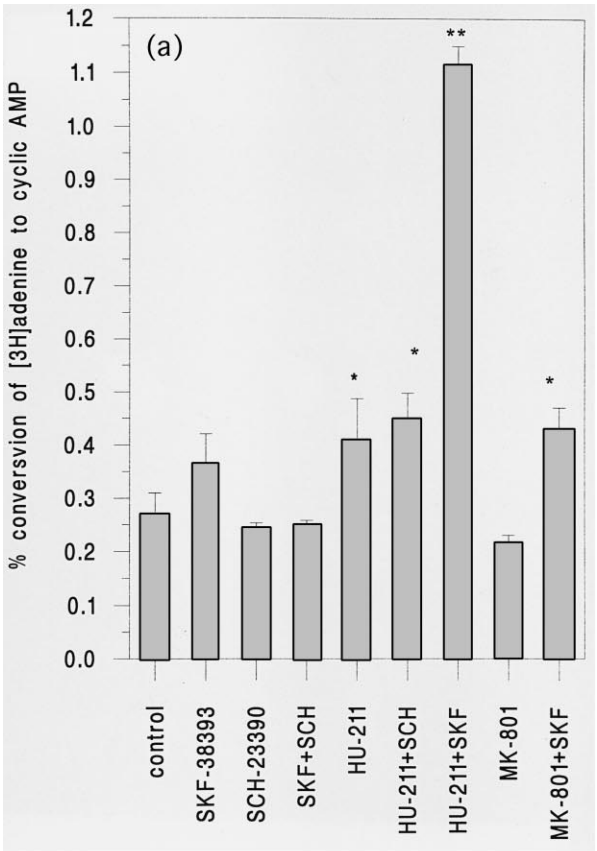
HU-211 inhibited the binding of the radioactive dopamine D_1 receptor antagonist only at a very high concentration (Table 2): 50 μM HU-211 inhibited 29.7 ± 1.8% of the specific binding of [^{3}H]SCH-23390 to the forebrain membranes, while 100 μM inhibited 52.7 ± 6.3%.

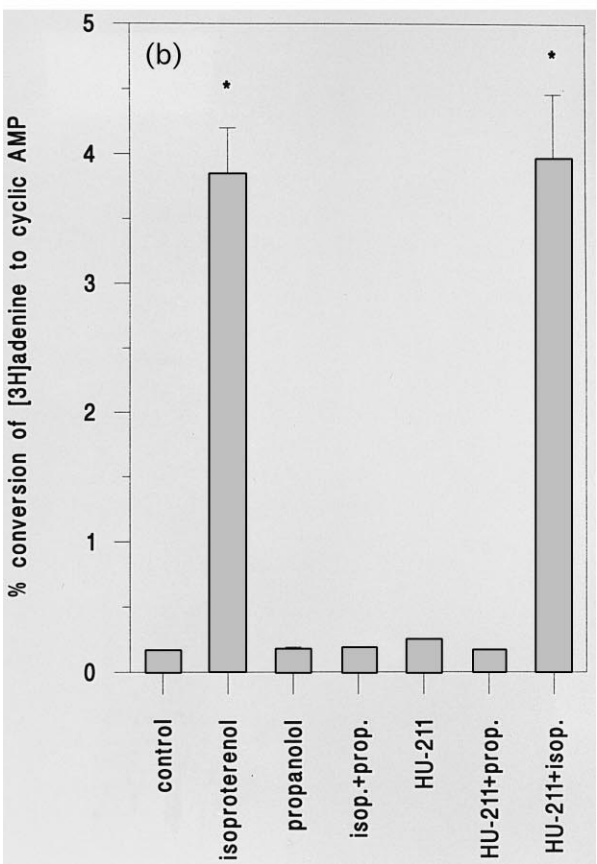
HU-211 was screened for binding to various neurotrans-

Table 2
Inhibition of the binding of [^{3}H]SCH-23390 to rat forebrain membranes by HU-211

HU-211	% Inhibition of [^{3}H]SCH-23390 specific binding
25	2.1 ± 2.2
50	29.7 ± 1.8
100	52.7 ± 6.3

The binding of [^{3}H]SCH-23390 to 0.3 mg rat forebrain membranes was measured with or without HU-211 prepared in 0.25% bovine serum albumin and 0.2% ethanol. Non-specific binding was determined using 10 μM (+)-butaclamol and was found to be 20.9 ± 2.9% of the total binding. The results are means ± S.E.M. of three different experiments performed in triplicate.





mitter receptors and was found to be unable to compete for binding to the serotonin receptors even at extremely high concentrations (>100 μM) (unpublished data). For this reason, we exclude the possibility that HU-211 competes with [^{3}H]SCH-23390 in binding to the cortical serotonin receptors.

3.1.2. cAMP study

HU-211 (10 μM) caused a small, statistically significant increase in cAMP formation in the rat primary cortical cultured neurons, as compared to the control group ($51.8 \pm 29.7\%$ over control), and this increase was not reduced by 10 μM of the dopamine D_1 receptor antagonist, SCH-23390 ($66.6 \pm 18.7\%$ over control, Fig. 1a). The dopamine D_1 receptor agonist, SKF-38393 (10 μM), also caused a small elevation in cAMP formation, as compared to the control group ($35.6 \pm 21.5\%$ over control), but 10 μM of SCH-23390 totally inhibited this increase ($-7.4 \pm 3.7\%$ over control, Fig. 1a). The combination of HU-211 with SKF-38393 produced a marked elevation in the activation of adenylate cyclase, which was much greater than the sum of the contributions of each agent separately ($314.7 \pm 14.3\%$ over control) and seemed to be synergistic. The selective NMDA receptor antagonist, MK-801, did not enhance the SKF-38393-induced increase in cAMP accumulation ($59.2 \pm 15.5\%$ over control, Fig. 1b), and did not elevate cAMP formation on its own.

Elevated cAMP accumulation in cells might be a non-specific phenomenon rather than one selective for the dopamine receptor system. In order to examine this possibility, we checked if HU-211 had a similar effect on the β-adrenergic system, which is known to be a cAMP-elevating system (Van Calker et al., 1978). We used isoproterenol as an agonist, and propanolol as an antagonist, of the β-adrenoreceptor. Isoproterenol (1 μM) increased the forskolin-induced stimulation of cAMP accumulation ($2250 \pm 207\%$ over control, as compared to $45 \pm 10\%$ conversion in the HU-211 group) and propanolol completely inhibited the isoprotrenolol-induced elevation (Fig. 1b). However, HU-211 failed to further elevate the isoproterenol-induced stimulation (Fig. 1b).

These findings suggest an interaction of HU-211 with

Fig. 1. HU-211 synergistically enhances the SKF-38393-induced elevation in cAMP formation (A), while failing to do so in the isoproterenol-stimulated cells (B). (A) Cells were incubated for 1 h with 0.2 μM forskolin only (control), or in the presence of the indicated treatments (10 μM of each). % conversion of [^{3}H]adenine to cAMP for the control group was 0.268 ± 0.042 $^{*}P \leq 0.05$ compared to vehicle. $^{**}P \leq 0.05$ compared to SKF-38393. (B) Cells were incubated for 1 h with 0.2 μM forskolin only (control), or in the presence of the indicated treatments (1 μM of isoproterenol, 1 μM of propanolol or 10 μM HU-211). $^{*}P \leq 0.05$ compared to control. (One-way ANOVA followed by Duncan's post hoc test.) All other details were as described in Section 2. Values are means $\pm$ S.E.M. of triplicate determinations. Data are representative of 3 similar experiments with comparable results.

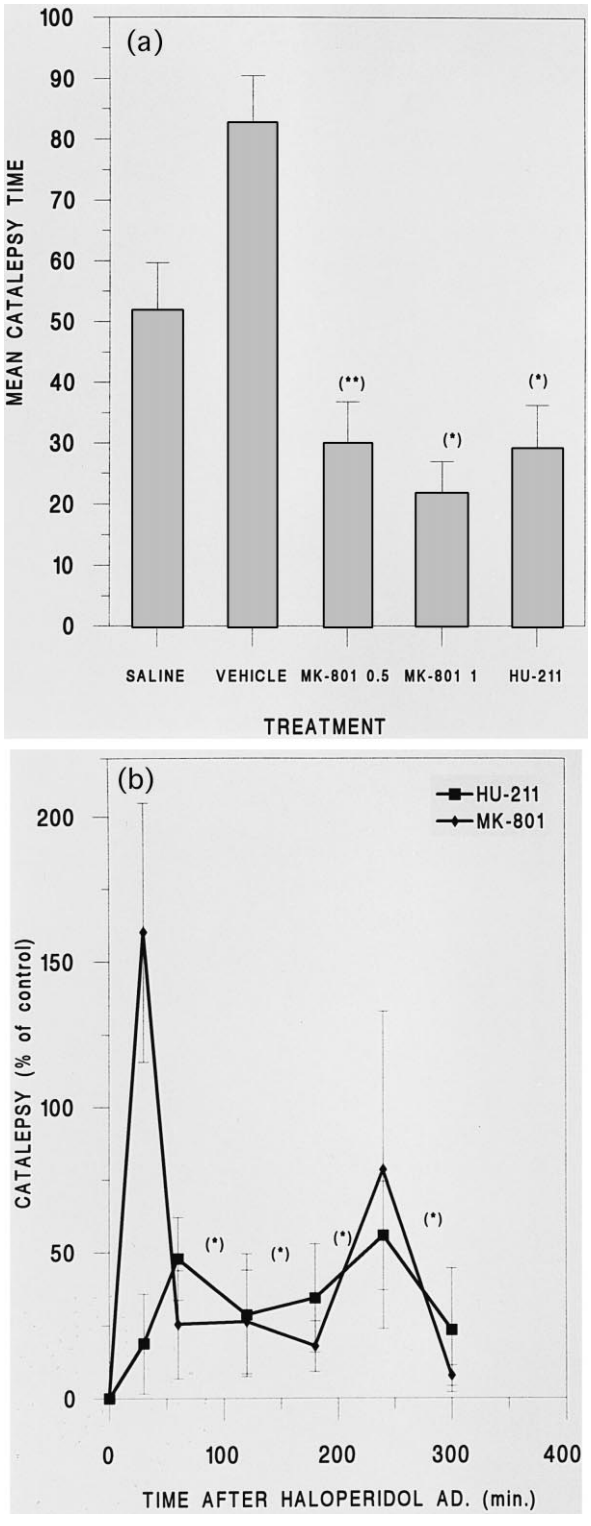


Fig. 2. Effect of HU-211 (10 mg/kg) on haloperidol-induced catalepsy. (A) Mean catalepsy time. The maximal catalepsy time is 120 s. $^{*}P < 0.001$ compared to the vehicle of HU-211 (co-solvent). $^{**}P < 0.003$ compared to the vehicle of MK-801 (saline). (B) Time course of catalepsy and drug effects $^{*}P < 0.001$, each drug compared to its own vehicle. The bars in (A) represent the mean catalepsy time ± S.E.M. for groups of 6 mice and in (B) the catalepsy time at each time point expressed as % of the relevant vehicle. (One way ANOVA followed by Duncan's post hoc test.)

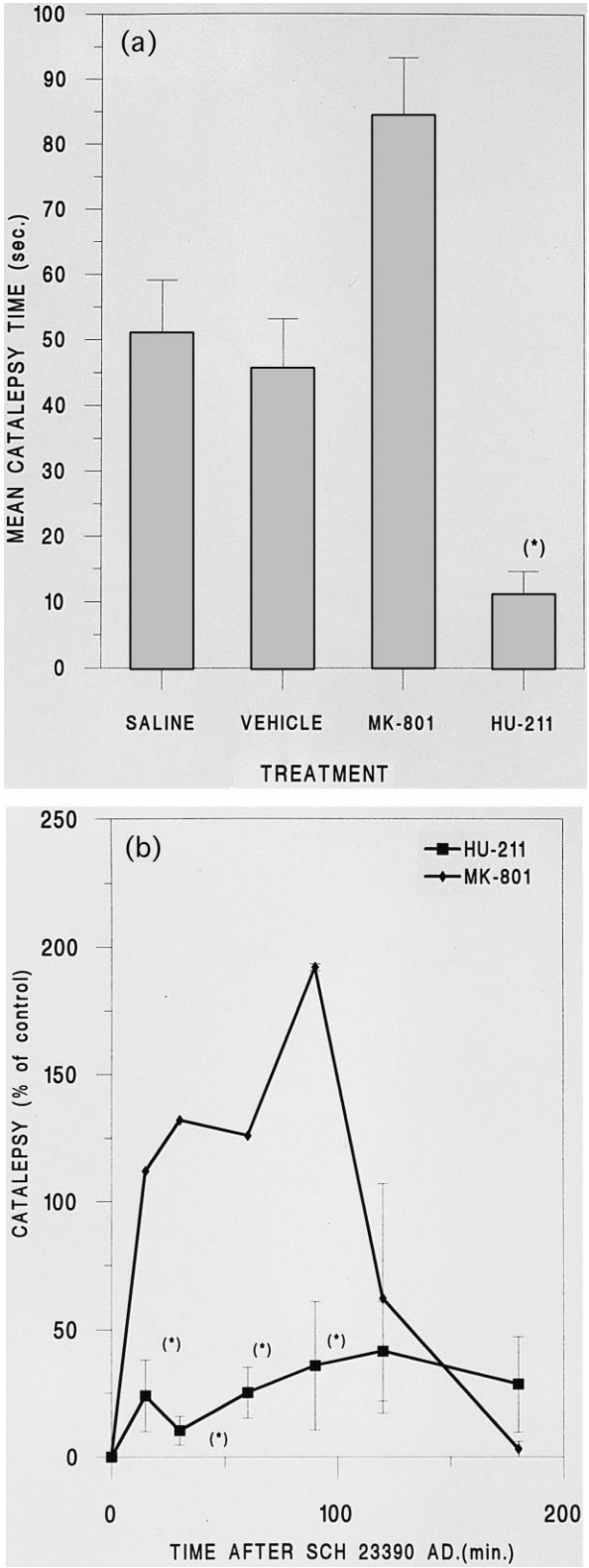
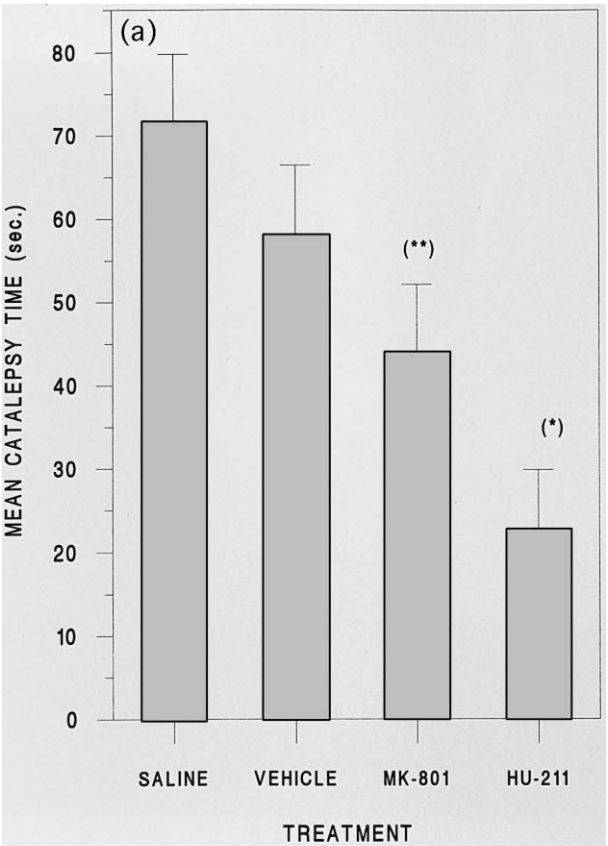
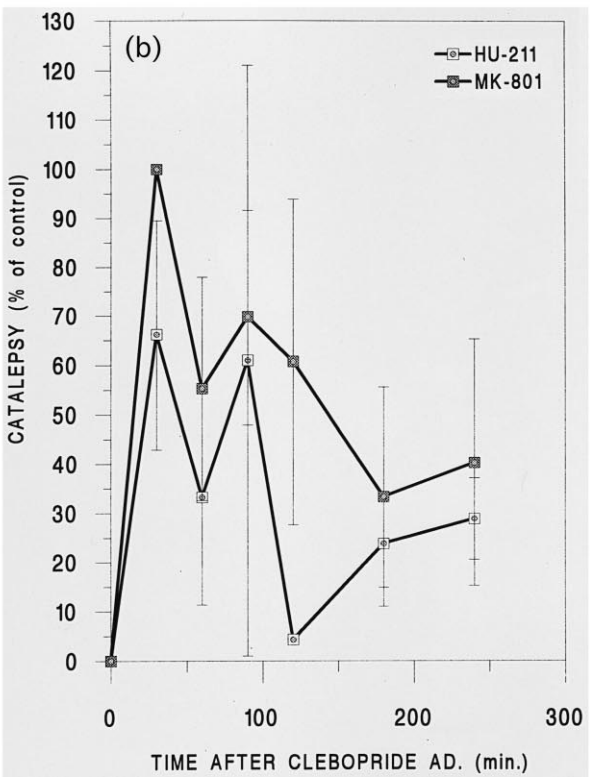


Fig. 3. Effect of HU-211 (10 mg/kg) on SCH-23390-induced catalepsy. (A) Mean total catalepsy time. The maximal catalepsy time is 120 s. $^{*}P < 0.001$, each drug compared to its own vehicle. (B) Time course of the catalepsy and drug effects. $^{*}P < 0.001$ compared to vehicle (co-solvent). The bars in (A) represent the mean catalepsy time ± S.E.M. for groups of 6 mice and in (B) the catalepsy time at each time point expressed as % of the relevant vehicle. (One-way ANOVA followed by Duncan's post hoc test.)

the dopamine D_1 receptor, which is expressed as a synergistic effect on the activation of the signal transduction pathway by dopamine or dopamine receptor agonists. This activity of HU-211 seems to be unrelated to its ability to antagonize the NMDA receptor.





3.2. In vivo study

3.2.1. Haloperidol (dopamine $D_1 + D_2$ receptor antagonist)-induced catalepsy

Haloperidol 1 mg/kg induced a catalepsy that was evident from one hour post treatment and thereafter for 5 h. The maximal time of catalepsy, 100–120 s, was seen 3–4 h after haloperidol administration. HU-211 10 mg/kg reduced the mean catalepsy time induced by haloperidol, as compared to its vehicle ($P < 0.001$) and saline ($P < 0.03$) (Fig. 2a). MK-801 had a similar effect. HU-211 exhibited its effects 60 min post-administration and thereafter for 4 h. MK-801 was effective starting 2 h post-administration (Fig. 2b).

3.2.2. SCH-23390 (dopamine D_1 receptor antagonist)-induced catalepsy

SCH-23390 5 mg/kg induced a catalepsy shorter than that induced by haloperidol. Catalepsy (90 s) was already evident 30 min after SCH-23390 treatment and persisted for 90 min. By two hours post injection, no catalepsy was evident. HU-211 10 mg/kg significantly reduced ($P < 0.001$) the mean catalepsy time, as compared to its vehicle, saline and MK-801 (Fig. 3a). HU-211 was active starting 30 min after drug administration and thereafter for 120 min (Fig. 3b). MK-801 did not reduce the SCH-23390-induced catalepsy. Maximal catalepsy was induced by MK-801 (120 s), starting 15 min after drug treatment and lasting 2 h.

3.2.3. Clebopride (dopamine D_2 receptor antagonist)-induced catalepsy

Clebopride 20 mg/kg induced a prominent catalepsy (120 s) starting 30 min after drug administration. This lasted for 3 h and then started declining. HU-211 10 mg/kg significantly reduced the mean catalepsy time, as compared to its vehicle, saline ($P < 0.001$) and MK-801 ($P < 0.02$). MK-801 also significantly reduced the mean total catalepsy time, as compared to saline ($P < 0.02$) (Fig. 4a). HU-211 was effective commencing 60 min after drug administration and thereafter for 3 h. MK-801 was effective commencing 120 min after drug administration and thereafter for 4 h (Fig. 4b).

Fig. 4. Effect of HU-211 (10 mg/kg) on clebopride-induced catalepsy. (A) Mean catalepsy time. The maximal catalepsy time is 120 s. $^{*}P < 0.001$ compared to vehicle (co-solvent), $^{**}P < 0.001$ compared to saline. (B) Time course of the catalepsy and drug effects. $^{*}P < 0.001$ compared to vehicle (cosolvent). The bars in (A) represent the mean catalepsy time ± S.E.M. for groups of 6 mice and in (B) the catalepsy time at each time point expressed as % of the relevant vehicle. (One-way ANOVA followed by Duncan's post hoc test.)

4. Discussion

The results of the present study demonstrated that HU-211 blocks dopamine receptor antagonist-induced catalepsy in a manner similar to other NMDA receptor antagonists. In vitro, HU-211 inhibited the binding of SCH-23390, a dopamine D_1 receptor antagonist, only at extremely high concentrations. In primary neuronal culture, HU-211 enhanced the conversion of [^{3}H]adenine to cAMP. This effect was synergistic with the effect of a dopamine D_1 receptor agonist and the synergism was specific to the dopaminergic system.

HU-211 is a non-competitive NMDA receptor antagonist with a broad range of activities (Bar-Joseph et al., 1993, 1994a; Eshhar et al., 1993; Nadler et al., 1993a,b). Binding studies revealed that HU-211 blocks [^{3}H]MK-801 binding to the NMDA receptor channel in a stereospecific manner with a K_i in the micromolar range (Eshhar et al., 1993). Kinetic studies, utilizing [^{3}H]*N*-[1-(2-thienyl)-cyclohexyl]piperidine ([^{3}H]TCP) as a ligand, suggested that HU-211 interacts with the channel at a site close to, but not identical with, the TCP-MK-801 binding site (Feigenbaum et al., 1989). HU-211 also rescues neuronal cells from NMDA and glutamate toxicity in culture (Eshhar et al., 1993). HU-211 induced neuroprotection in global ischemia models in gerbils (Bar-Joseph et al., 1994a) and rats (Bar-Joseph et al., 1994b; Belayev et al., 1995a), in focal ischemia in rats (Biegon and Bar-Joseph, 1995; Belayev et al., 1995b), and in a brain trauma model in rats (Shohami et al., 1993, 1995).

This study showed that, like other NMDA receptor antagonists (Moore et al., 1993), HU-211 reduces the catalepsy induced by either dopamine D_1 or D_2 receptor antagonists. HU-211 reduced the dopamine receptor antagonist-induced catalepsy in a similar manner to MK-801 in the haloperidol- and clebopride-treated animals. A similar effect of HU-211 was detected on the SCH-23390-induced catalepsy. MK-801 did not reduce this catalepsy. This difference between HU-211 and MK-801 is probably related to their different behavioral effects. The catalepsy induced by SCH-23390 in the present study was relatively short and lasted 120–180 min, compared to 300–360 min with clebopride and haloperidol. With the latter drug, the effect of MK-801 became evident around 2 h after treatment, by which time the SCH-23390 induced-catalepsy was over. MK-801 1 mg/kg causes severe behavioral side-effects (Leander et al., 1988; Koek et al., 1990; Löscher and Honack, 1990) including ataxia and catalepsy. These behavioral side-effects were observed in the present study, and probably masked the anti-cataleptic activity of MK-801. In the dopamine D_2 receptor antagonist-induced catalepsy, these side-effects of MK-801 did not mask the anti-cataleptic effects since the catalepsy lasted longer (6 h), and MK-801 could exhibit its anti-cataleptic activity after its own behavioral side-effects had subsided.

Another difference between MK-801 and HU-211 was observed in the adenylate cyclase experiments. HU-211 was shown to act synergistically with SKF-38398 to increase cAMP accumulation in the cultured neurons, while MK-801 failed to do so. Elevated cAMP accumulation might have been a non-specific phenomenon (such as activation of the adenylate cyclase) rather than selective to a certain receptor system. In order to eliminate this possibility we checked if HU-211 had a similar synergistic effect on the isoproterenol-induced increase in cAMP accumulation. Activation of the β-adrenergic system is known to be positively coupled with the cAMP signal transduction pathway. The synergistic effect of HU-211 was shown to be specific to the dopamine D_1 receptor, as HU-211 failed to further elevate the isoproterenol-induced increase in cAMP accumulation. However, the interaction of HU-211 with the dopamine D_1 receptor does not seem to be direct, as the dopamine D_1 receptor antagonist (SCH-23390) failed to reduce the small elevation in cAMP induced by HU-211. Binding experiments showed that HU-211 reduced [^{3}H]SCH-23390 binding to rat forebrain membranes only at concentrations considerably higher than those required for adenylate cyclase activation. These data do not support the possibility of a direct interaction at the receptor binding site as the relevant mechanism, but suggest a post-receptor (post synaptic) interaction of HU-211 with the dopamine D_1 system.

However, a direct interaction at lower concentrations in vivo cannot be excluded completely: That high concentrations of HU-211 were required to inhibit the binding of [^{3}H]SCH-23390 to membranes may have been a consequence of the difficulty of dissolving HU-211 in hydrophilic media. HU-211 is a very hydrophobic compound, which makes it hard to use in in vitro experiments with membrane preparations or tissue culture. For binding studies of the dopamine D_1 receptor we tried to dissolve HU-211 in ethanol (final concentration of 5%), or a mixture of 0.077% ethanol, 0.077% emulphor 620 and 1.38% water (emulphor/ethanol, final concentrations), 0.25% bovine serum albumin and 0.2% ethanol (bovine serum albumin/ethanol, final concentrations). However, the ethanol and the mixture of emulphor/ethanol described above, which were used successfully in other receptor binding assays (Eshhar et al., 1993), caused a marked reduction in the specific binding of [^{3}H]SCH-23390 to the membranes, and therefore could not be used in this system. The bovine serum albumin/ethanol, which was chosen to introduce HU-211 into the system, produced a marked decrease in the ability of HU-211 to compete with [^{3}H]quinuclidinyl benzilate for binding to the muscarinic receptors, as compared to 10% ethanol (data not shown). This could have resulted from a complex that bovine serum albumin might create with HU-211, making HU-211 less available to the membrane receptors. Further experiments are required for investigation of the exact mechanism of HU-211.

Previous work has demonstrated that some of the ad-

verse effects of NMDA receptor antagonists are mediated through the dopaminergic system (Löscher et al., 1991). The exact mode of interaction between the glutaminergic and dopaminergic systems is controversial. Some reports showed that blockade of NMDA receptors potentiates dopamine D_1 responses in experimental models of dopamine deficiency (Löscher et al., 1991; Weihmuller et al., 1992; Di Chiara et al., 1994), while others claim that NMDA causes dopamine to be released from neostriatal slices (Sonsalla et al., 1989). A number of studies suggested that NMDA receptor antagonists may differentially affect behavior mediated via dopamine D_1 and D_2 receptors (Morelli et al., 1993). The potential interaction between glutaminergic and dopaminergic systems has attracted much interest because of its possible implications in diseases such as schizophrenia and Parkinson's (Carlsson and Carlsson, 1990; Klockgether and Turski, 1990; Ulas et al., 1994). HU-211 has an advantage over the known NMDA receptor antagonists, as it does not exhibit behavioral side-effects. In addition, HU-211 causes no adverse physiological changes, such as alterations in blood pressure or heart rate (Bar-Joseph et al., 1994b, 1995), nor does it cause morphological changes in the cingulate cortex (Bar-Joseph et al., 1995). It is also possible that HU-211 does not act exclusively through the NMDA receptor and its activity is probably different from that of MK-801, supposedly because of its lower affinity, fast dissociation, large molecular size, lipophilic nature, and existence of double bonds in the molecule that allows it to act like an antioxidant. The antioxidant activity of HU-211 was recently confirmed by in vitro work performed both in our and other laboratories (Eshhar et al., 1995). A major current hypothesis suggests that the etiology of the selective degeneration of nigrostriatal dopaminergic neurons in Parkinson's disease is linked to dopamine-induced excessive oxidant stress. It is assumed that the generation of toxic free radical species during dopamine oxidation leads to lipid peroxidation, dysfunction and rupture of cellular membranes, resulting in neuronal disintegration (Olanow, 1993; Ziv et al., 1994). HU-211, as an antioxidant agent, may interfere with such pathologic processes. Indeed, preliminary results from our laboratory showed that HU-211 partially protected neurons from dopamine-induced toxicity (data not shown).

Taken together, the results of the present study suggest that HU-211 may be of beneficial therapeutic use in diseases involving disorders of the dopaminergic system, such as Parkinson's disease.

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