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Antiparkinsonian and other motor effects of flupirtine alone and in combination with dopaminergic drugs

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Abstract

In this study we attempted to specify the behavioural profile of the analgesic flupirtine (1, 10 and 20 mg/kg p.o.) in the rat with respect to (i) its antiparkinsonian potential alone and as an adjunct to L-dihydroxyphenylalanine (L-DOPA) in the haloperidol-induced catalepsy (0.5 mg/kg haloperidol i.p.), (ii) locomotion and exploratory behaviour in the open field with holeboard, and (iii) possible psychomotor stimulating effects in the experimental chamber. In the two latter tests, behaviour was additionally challenged by D-amphetamine (2 mg/kg i.p.). In the catalepsy tests (horizontal bar, podium, grid) flupirtine alone was anticataleptic at doses of 10 and 20 mg/kg p.o., and the antiparkinsonian potential of a subthreshold dose of L-DOPA (50 mg/kg p.o.) was potentiated by 1 and 10 mg/kg p.o. flupirtine. On spontaneous forward locomotion in the open field with holeboard, flupirtine (1 and 10 mg/kg p.o.) had no marked effect but increased the frequency and duration of head dips, indicative for augmenting exploratory behaviour. Spontaneous rearing was reduced and D-amphetamine-induced rearing was enhanced by 1 mg/kg p.o. flupirtine. Grooming was reduced by 1 and 10 mg/kg p.o. flupirtine. In contrast, turning and grooming behaviour (spontaneous as well as D-amphetamine-induced) was not markedly influenced by flupirtine in the experimental chamber. Sniffing was increased in this test by 1 mg/kg p.o. flupirtine but not by the higher dose. Flupirtine is highly effective in antagonising neuroleptic-induced catalepsy as well as in potentiating L-DOPA treatment in the rat, suggesting it is a prospective new candidate for the therapy of Parkinson's disease.

Keywords: Flupirtine; NMDA receptor, antagonistic; Catalepsy; Locomotion; L-DOPA (L-dihydroxyphenylalanine); Parkinson's disease

1. Introduction

The centrally acting but non-opioid analgesic flupirtine maleate (2-amino-3-ethoxy-carbonylamino-6-(4-fluoro-benzylamino)-pyridine maleate) is available under the trademark of Katadolon (ASTA Medica, Frankfurt, Germany) and is frequently used in the clinic in a variety of pain disorders (Friedel and Fitton, 1993; Million et al., 1984; Moore et al., 1983). Beside its analgesic properties, flupirtine also exerts anticonvulsant/antiepileptic effects (Seaman et al., 1986), muscle relaxant activities in animals (Schwarz et al., 1994, 1995) and humans (Timmann et al., 1995), and has neuroprotective potency in several in vitro and in vivo assays (Block et al., 1994, 1997; Osborne et al., 1996; Perovic et al., 1994, 1995; Rupalla et al., 1995) in clinically relevant doses. Given the above range of indications in which flupirtine is active, the good long-term compliance (Herrmann et al., 1993) and the fact that it does not induce development of morphine-like dependence or tolerance and does not possess prominent toxic side effects (Friedel and Fitton, 1993) may render flupirtine an attractive and clinically safe drug.

The central site of pharmacological action of flupirtine is not fully understood. It does not appear to directly interact in receptor-binding studies with adrenoceptors, dopamine, nicotine, or 5-HT receptors (Friedel and Fitton, 1993). However, although flupirtine does not bind to any of the so far known NMDA receptor complex-associated binding sites (Osborne et al., 1996; Schwarz et al., 1996) it shows several functional NMDA receptor-antagonistic properties in in vivo and in vitro experiments. The first evidence for a similarity with NMDA receptor antagonists in the myorelaxant action of flupirtine was obtained by Schwarz et al. (1994) who showed that flupirtine depresses the spinal polysynaptic flexor reflex which is mediated via NMDA receptors, whereas the monosynaptic Hoffmann

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reflex which is mediated via non-NMDA receptors is not influenced by flupirtine. It was also shown in the same study that the depressant action of flupirtine on the polysynaptic reflex can be prevented by intrathecal coadministration of NMDA. Immunohistochemical evidence for flupirtine acting antagonistically on the NMDA-induced release of γ-aminobutyric acid (GABA) in the retina was reported by Osborne et al. (1994). Additionally, its neuroprotective action is supposed to be mediated by an NMDA-antagonistic effect since flupirtine displayed potent cytoprotection on rat cortical neurones treated with NMDA as well as treated with the human immunodeficiency virus (HIV-1) coat protein gp120, the prion protein or β-amyloid protein (Müller et al., 1996a,b; Perovic et al., 1994, 1995). Flupirtine also is able to prevent the glutamate-induced increase in cytosolic Ca^{2+} (Rupalla et al., 1995) which is one of the first events in the excitotoxic cascade leading to lethal swelling of the neurone and, hence, acts as a neuroprotectant agent.

Since NMDA receptor antagonists have been shown to possess antiparkinsonian effects, the question was raised whether flupirtine shares this property. The common therapy of Parkinson's disease is the treatment with L-3,4-dihydroxyphenylalanine (L-DOPA), a precursor of dopamine. However, most patients receiving L-DOPA therapy for some years experience fluctuations in their motor functions (Greenamyre and O'Brien, 1991). Several studies have shown that the dopaminergic and glutamatergic systems in the striatum may be reciprocally balanced (Carlsson and Carlsson, 1990; Schmidt, 1986; Schmidt and Bury, 1988). Interference into the glutamatergic transmitter system would lead to a decrease of symptoms in Parkinson's disease and may provide a better therapy to probably slow down the progressing degeneration, provide a neuroprotective component, or at least make possible a well tolerated therapy without inducing prominent side effects.

Thus a depression of the glutamatergic system would lead to a decrease of symptoms in Parkinson's disease or in neuroleptic-induced catalepsy in rats. Among the glutamate receptor subtypes, especially the modulation of the NMDA receptor complex has been discussed to be a promising new target for the treatment of Parkinson's disease symptoms. Agents blocking the NMDA receptor complex function (being either competitive or non-competitive antagonists, or substances blocking the allosteric strychnine-insensitive glycine binding site) are anticataleptic in animal models (Kretschmer et al., 1994; Mehta and Ticku, 1990; Schmidt and Bubser, 1989; Schmidt et al., 1991) and may therefore exert antiparkinsonian effects in humans. On the other hand, non-NMDA receptor antagonists may not possess such an antiparkinsonian potential (Hauber and Andersen, 1993; Yoshida et al., 1991; Zadow and Schmidt, 1994). However, non-competitive NMDA receptor antagonists like dizocilpine (MK-801: (+)-5-methyl-10,11-dihydroxy-5*H*-dibenzo(*a*,*d*)cyclohepten-5,10-imine), albeit being assumed to exert strong beneficial symptomatic effects in Parkinson's disease, especially in combination with L-DOPA (Greenamyre and O'Brien, 1991), are not safe for clinical use due to their strong psychomotor stimulating and amnesic effects (Schmidt, 1995). In higher doses they even may cause neurodegeneration in the posterior cingular/retrosplenial cortex with lasting cognitive impairments (Olney et al., 1989; Wozniak et al., 1996).

Based on the above mentioned NMDA-antagonistic effect of flupirtine on neurotransmission mediated by NMDA and the clinical safety of this drug, we attempted to specify in the haloperidol-induced catalepsy in the rat, whether flupirtine exerts antiparkinsonian effects itself and whether it might be useful as an adjunctive treatment in the L-DOPA therapy of Parkinson's disease. The neuroleptic-induced catalepsy, a severe immobility of the rat, is considered to be an animal model for some aspects (rigor and akinesia) of Parkinson's disease and is attributed to blockade of central dopamine receptors (Hornykiewicz, 1979).

To delineate whether flupirtine acts more in a competitive or a non-competitive NMDA receptor-antagonistic manner, the behavioural profile of flupirtine was assessed in the open field with holeboard with special reference to locomotion and exploratory behaviour (spontaneous and D-amphetamine-challenged). Competitive NMDA receptor antagonists could be differentiated from non-competitive ones in that the latter but not the first exert an increase in forward locomotion while suppressing exploratory behaviour (Bubser et al., 1992; Kretschmer et al., 1992; Rückert and Schmidt, 1993). Finally, in order to reveal eventual psychomotor stimulating effects, the influence of flupirtine on the spontaneous and D-amphetamine-induced behaviour in the experimental chamber (Schmidt, 1986) was investigated.

2. Materials and methods

2.1. Animals

125 male Sprague-Dawley rats (Interfauna, Tuttlingen, Germany), weighing 250–270 g, were housed under constant conditions (light from 6.00 a.m. to 6.00 p.m., temperature 22 ± 3°C) in groups of four or five in standard Macrolon cages ($57 \times 35 \times 19$ cm). Each rat received 15 g laboratory chow each day in the afternoon, or after the experimental session and always had free access to water.

2.2. Drugs and application

Haloperidol (Janssen, Beerse, Belgium) was diluted with, and benserazide (Hoffmann-La Roche, Basel, Switzerland) was dissolved in physiological saline to the respective final concentrations. Flupirtine (ASTA Medica, Frankfurt, Germany) and L-DOPA (Sigma, Deisenhofen, Germnay) were suspended in 2% tylose. L-DOPA suspen-

sions additionally included 2 mg/ml of ascorbic acid. D-Amphetamine sulphate (Merck-Schuchart, Darmstadt, Germany) was dissolved in distilled water.

For the catalepsy tests, all rats were injected intraperitoneally (i.p.) with haloperidol (0.5 mg/kg) 60 min before testing. Rats further received either oral (p.o.) vehicle applications (2% tylose) as control (two groups with $n = 8$, each), or flupirtine (1, 10 or 20 mg/kg p.o.; $n = 9$, $n = 10$, $n = 10$, respectively) 20 min before testing, L-DOPA (50 mg/kg p.o.; two groups with $n = 10$, each) 90 min before testing, or a combination of flupirtine and L-DOPA (group sizes $n = 10$, each). L-DOPA-treated rats additionally received benserazide (25 mg/kg i.p.) 105 min before testing.

Behavioural testing in the open field with holeboard took place 30 min after D-amphetamine (2 mg/kg i.p.) and 20 min after flupirtine (1 and 10 mg/kg p.o.) administration. Investigation in the experimental chamber was carried out directly after the 5 min in the open field for a further 5 min. Rats received either vehicle, or D-amphetamine, or flupirtine, or a combination of the two latter drugs.

2.3. Behavioural testing

2.3.1. Experiment 1: catalepsy

Catalepsy was measured in three established tests in the following order of succession: (1) Horizontal bar: placing both forepaws on a horizontal bar 9 cm above a table surface. (2) Podium: placing the right forepaw on a 2.5 cm high podium. (3) Grid: hanging with all four paws on a vertical grid.

The degree of catalepsy was assessed by measuring the time from placement of the rat until removal of one of its paws (descent latency), with a cut-off time of 180 s.

2.3.2. Experiment 2: open field with holeboard

The open-field/holeboard apparatus consisted of a grey plastic open-field arena ($70 \times 70 \times 29$ cm) with a plastic holeboard of the same colour positioned 3 cm above the floor inside. The holeboard was divided into 4×4 squares with 16 centred holes 4 cm in diameter. In order to eliminate external disturbances the apparatus was placed in a sound-attenuating wooden box ($75 \times 75 \times 107$ cm) with an opening of 6 cm in diameter in the centre of the top for video recording. The inside of the box was illuminated from the top with dim red light (4×25 W). Continuous ventilation supplied fresh air and also reduced background noise.

The behaviour of a rat was recorded for 5 min with the help of videotaping. After the experiment the following parameters (frequency and duration) were assessed by manually typing the different behaviours into a personal computer (program by Bader, Tübingen, 1990): (1) Crossings: entering of a new square with all four paws. (2) Head dips: dipping of the head into one hole (exploration). (3) Rearing: rearing with and without wall contact (standing only on the hind legs). (4) Grooming: sitting and grooming.

2.3.3. Experiment 3: experimental chamber

In this experiment a Plexiglas box ($10 \times 10 \times 30$ cm) as described by Schmidt (1986) was used. The experimental chamber allows quantification of drug-induced changes in spontaneous behaviour, especially stereotyped sniffing, while impeding forward locomotion. Each rat was put directly after the open-field investigation into the experimental chamber for 5 min. The behaviour of the rats was videotape-recorded to be analysed later as described above.

The following behavioural parameters (frequency and duration) were examined: (1) Turns: turns of 180° around the dorsoventral body axis. (2) Sniffing head up: wall contact in the upper half of the chamber with the rat's snout, accompanied by movement of the vibrissae. (3) Sniffing head down: wall contact in the lower half of the chamber with the rat's snout, accompanied by movement of the vibrissae. (4) Grooming: sitting and grooming.

2.4. Statistics

The results of the catalepsy tests were submitted to a Kruskal-Wallis one-way analysis of variance (ANOVA) and when there was an effect of Treatment ($P \leq 0.05$) the significances were localised by a Fisher's least significant difference (LSD) protected *t*-test. Significances were calculated between single drug session and control, between combined drug session and control, between single drug sessions, between combined drug sessions, and between single drug session and corresponding combined drug session.

The results of the open field with holeboard and the experimental chamber were made by one-way ANOVA (completely randomised) and after an effect of Treatment ($P \leq 0.05$) the significances again were localised by the Fisher's LSD protected *t*-test. For spontaneous behaviour significances were calculated between single drug session and control and between single drug sessions. For D-amphetamine-challenged behaviour, significances were calculated between combined drug session and D-amphetamine-control and between combined drug sessions.

Absence of asterisks in all cases implies absence of significance. $P \leq 0.05$ was set as indicating statistically significant differences. All statistical analyses were made with the help of the GB-STAT program, version 5.3. Data are presented as means ± S.E.M.

3. Results

3.1. Experiment 1: catalepsy

Fig. 1 shows the results of 1 mg/kg flupirtine tested in the catalepsy tests. 1 mg/kg flupirtine alone was not able

to antagonise the haloperidol-induced catalepsy at neither of the three tests (Fig. 1A: horizontal bar, B: podium, C: grid). However, the combination of L-DOPA + 1 mg/kg flupirtine had a stronger anticataleptic effect in the horizontal bar and the grid test than L-DOPA alone: at the horizontal bar L-DOPA alone at a threshold dose was statistically not distinguishable from control, but the combination of L-DOPA + 1 mg/kg flupirtine antagonised the haloperidol effect ($P \leq 0.05$). In the grid test, L-DOPA ($P \leq 0.05$) as well as L-DOPA + 1 mg/kg flupirtine ($P \leq 0.01$) showed a significant anticataleptic effect, and again the combination of the two drugs was more effective.

Fig. 2 shows the results of 10 and 20 mg/kg flupirtine tested in the catalepsy tests. 10 mg/kg flupirtine antagonised the neuroleptic-induced catalepsy in the horizontal bar and podium test ($P \leq 0.05$ each). While L-DOPA alone was anticataleptic only in the grid test ($P \leq 0.05$), the combination of L-DOPA + 10 mg/kg flupirtine had an antagonistic effect in all three catalepsy tests ($P \leq 0.05$ each). 20 mg/kg flupirtine alone was anticataleptic in either of the three tests ($P \leq 0.05$ each). However, the combination of L-DOPA + 20 mg/kg flupirtine failed to have an antagonistic effect on the haloperidol-induced catalepsy.

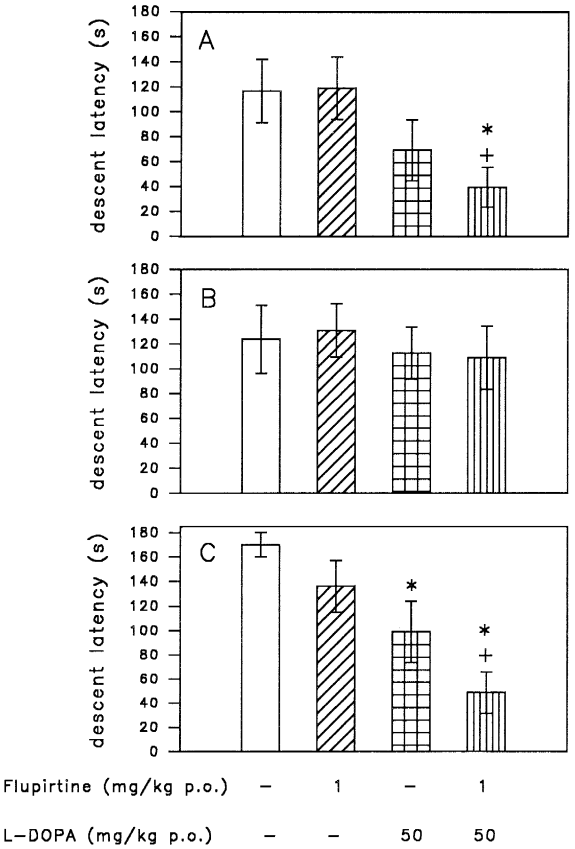

Fig. 1. Descent latencies in s (means ± S.E.M.) in three catalepsy tests (A, horizontal bar; B, podium; C, grid), tested 60 min after 0.5 mg/kg i.p. haloperidol, 20 min after 1 mg/kg p.o. flupirtine, 90 min after L-DOPA 50 mg/kg p.o., or a combination of both. Group sizes were between 8 and 10. * $P \leq 0.05$ versus control; + $P \leq 0.05$ versus flupirtine 1 mg/kg p.o. Kruskal-Wallis one-way ANOVA followed where appropriate by Fisher's LSD (protected t) test.

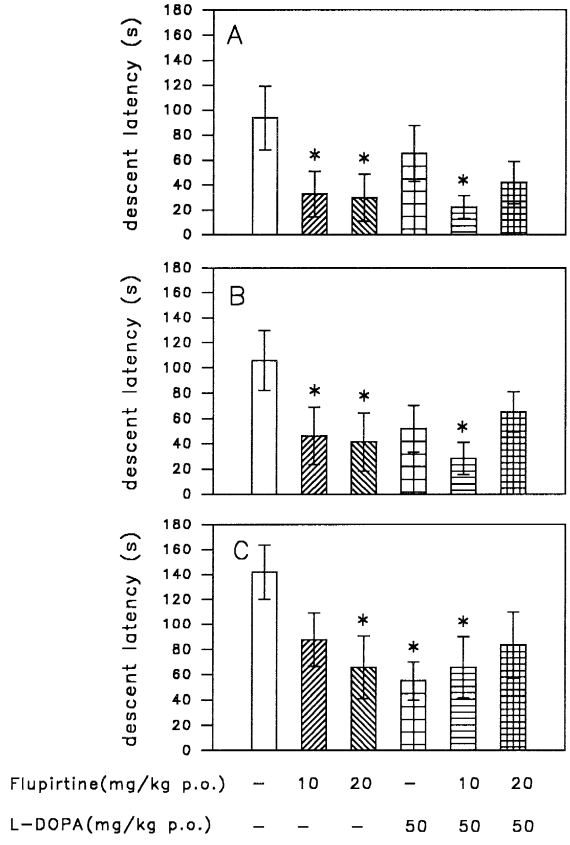

Fig. 2. Descent latencies in s (means ± S.E.M.) in three catalepsy tests (A, horizontal bar; B, podium; C, grid), tested 60 min after 0.5 mg/kg i.p. haloperidol, 20 min after 10 or 20 mg/kg p.o. flupirtine, 90 min after L-DOPA 50 mg/kg p.o., or a combination of both. Group sizes were between 8 and 10. * $P \leq 0.05$ versus control. Kruskal-Wallis one-way ANOVA followed where appropriate by Fisher's LSD (protected t) test.

3.2. Experiment 2: open field with holeboard

Fig. 3 summarises the results of experiment 2.

3.2.1. Line crossings

Flupirtine (1 and 10 mg/kg) did not influence the number of line crossings in the open field with holeboard. Only the time spent with spontaneous locomotion (duration crossings) was reduced by 1 mg/kg flupirtine compared to controls ($P \leq 0.05$) as well as compared to rats receiving 10 mg/kg flupirtine ($P \leq 0.01$). The D-amphetamine-challenged locomotor activity was not affected by either dose of flupirtine.

3.2.2. Head dips

Both doses of flupirtine (1 and 10 mg/kg) increased frequency and duration of spontaneous head dips ($P \leq 0.05$, respectively), compared to controls. The D-

amphetamine-challenged increase in head dipping was decreased only by 1 mg/kg flupirtine referring to number (compared to 10 mg/kg flupirtine; $P \leq 0.01$) and duration (compared to D-amphetamine alone or the combination of D-amphetamine + 10 mg/kg flupirtine; $P \leq 0.01$, respectively).

3.2.3. Rearing

Flupirtine affected rearing in the open field with hole-board only at the dose of 1 mg/kg. While spontaneous rearing was reduced (compared to controls; $P \leq 0.05$), the same dose of flupirtine potentiated D-amphetamine-challenged rearing (compared to D-amphetamine alone; $P \leq 0.05$). The latter was also seen in the duration of rearing under D-amphetamine ($P \leq 0.05$).

3.2.4. Grooming

Both doses of flupirtine (1 and 10 mg/kg) reduced the number as well as the duration of grooming, compared to controls ($P \leq 0.01$ and $P \leq 0.05$, respectively). The reduction after 1 mg/kg flupirtine was more prominent than that seen after 10 mg/kg flupirtine. D-Amphetamine-inhibited grooming was not further reduced by flupirtine, perhaps because of a ceiling effect.

3.3. Experiment 3: experimental chamber

Fig. 4 summarises the results of experiment 3.

3.3.1. Turns

Neither frequency nor duration of the spontaneous turns in the experimental chamber was significantly affected by

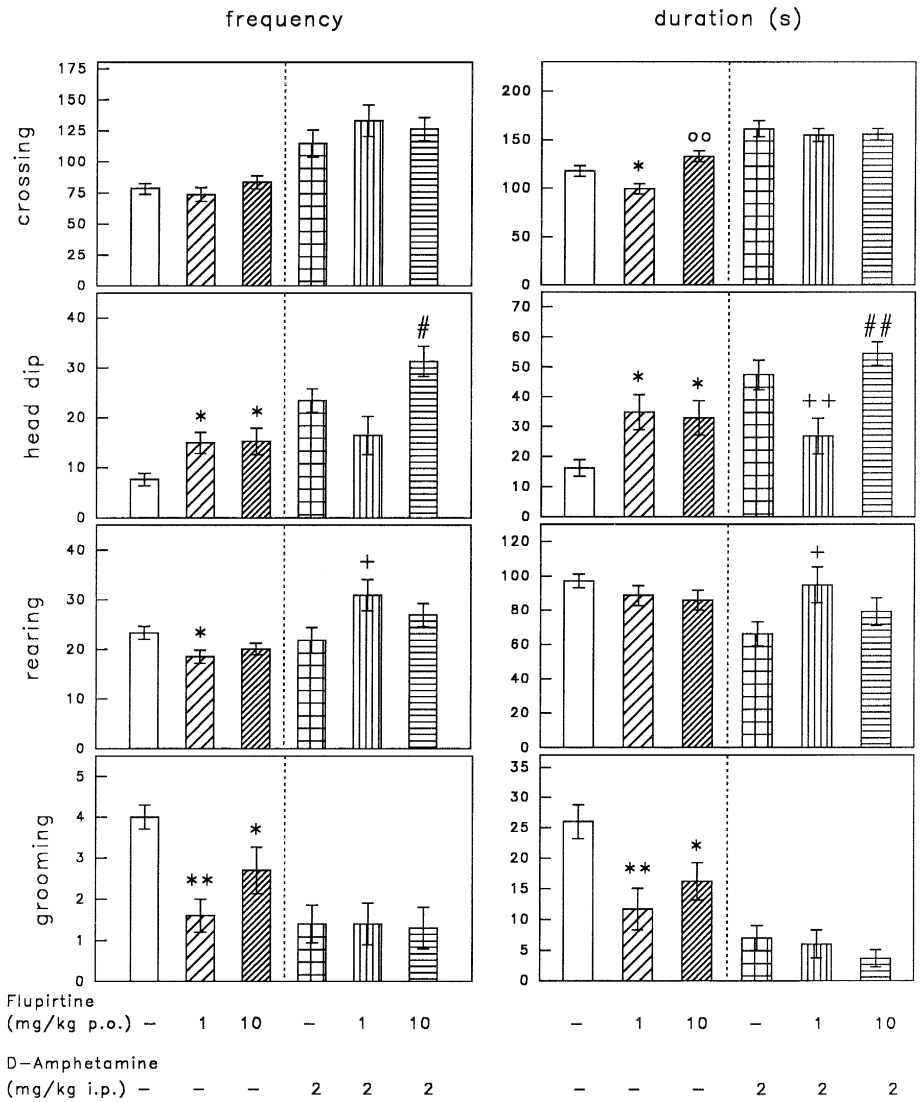

Fig. 3. Behavioural results in the experimental chamber. Values are presented as means ± S.E.M. $^{*,o,+,\#}$ $P \leq 0.05$; $^{**,oo,++,\#\#}$ $P \leq 0.01$. * Versus vehicle-control, ^{o}versus flupirtine 10 mg/kg p.o., $^{+}$ versus D-amphetamine-control, $^{\#}$ versus D-amphetamine + flupirtine 10 mg/kg p.o. One-way ANOVA (completely randomised) followed by Fisher's LSD (protected t) test.

flupirtine. 1 mg/kg flupirtine diminished the D-amphetamine-challenged increase in frequency and duration of turning behaviour. This was significant in the duration compared to D-amphetamine controls and to rats receiving D-amphetamine + 10 mg/kg flupirtine ($P \leq 0.05$ each).

3.3.2. Sniffing head up

1 mg/kg flupirtine potentiated the number of spontaneous as well as D-amphetamine-augmented sniffing head up (compared to controls and 10 mg/kg flupirtine; $P \leq 0.01$ each, and compared to D-amphetamine and D-amphetamine + 10 mg/kg flupirtine; $P \leq 0.01$ each). The duration of sniffing head up was not affected by flupirtine.

3.3.3. Sniffing head down

As in the parameter described above, 1 mg/kg flupirtine also potentiated the number of spontaneous sniffing head down (compared to controls and 10 mg/kg flupirtine; $P \leq 0.01$ each). Also, the D-amphetamine-augmented frequency of sniffing head down was potentiated by 1 mg/kg flupirtine (compared to D-amphetamine; $P \leq 0.05$, and compared to D-amphetamine + 10 mg/kg flupirtine; $P \leq 0.01$). The duration of the sniffing head down was not affected.

3.3.4. Grooming

None of both tested doses of flupirtine (1 and 10 mg/kg) was able to change grooming behaviour, although there was a dose-dependent trend to reduce the duration.

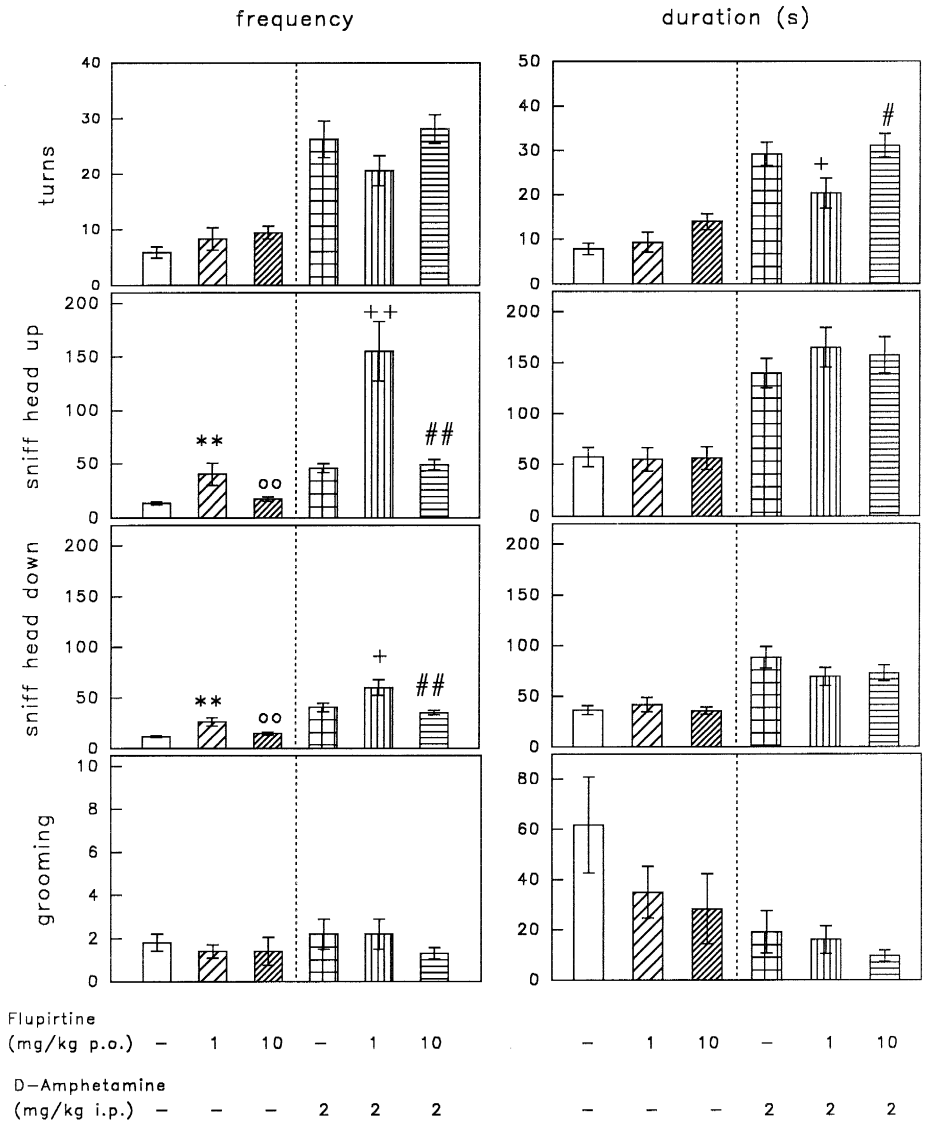

Fig. 4. Behavioural results in the open field with holeboard. Values are presented as means ± S.E.M.. $^{*,o,+,\#}$ $P \leq 0.05$; $^{**,oo,++,\#\#}$ $P \leq 0.01$. * Versus vehicle-control, ^{o}versus flupirtine 10 mg/kg p.o., $^{+}$ versus D-amphetamine-control, $^{\#}$ versus D-amphetamine + flupirtine 10 mg/kg p.o. One-way ANOVA (completely randomised) followed by Fisher's LSD (protected t) test.

4. Discussion

The centrally acting, non-opioid analgesic flupirtine maleate has been shown to possess several functional NMDA receptor-antagonistic properties in in vitro and in vivo experiments, as amplified in Section 1. Hence, as not only dopaminergic agents, but also NMDA receptor antagonists reveal an antiparkinsonian potential (Carlsson and Carlsson, 1989; Schmidt and Bubser, 1989), we tried to evaluate the antiparkinsonian potential of flupirtine in the neuroleptic-induced catalepsy in rats and further investigated its behavioural profile.

Flupirtine given alone showed a dose-response profile, similar to what is found with NMDA receptor function blockers (e.g., Klockgether and Turski, 1990). The small dose was ineffective, whereas the higher doses exerted a prominent anticataleptic effect: 10 mg/kg p.o. flupirtine counteracted catalepsy in the bar and podium test, and 20 mg/kg p.o. flupirtine showed an anticataleptic effect at all three catalepsy tests (bar, podium and grid). This is in good accordance with flupirtine's potent and dose-dependent reversal of rigidity in hypodopaminergic animals (Szelenyi and Nickel, 1991; Schwarz et al., 1996).

In combination with a subthreshold dose of L-DOPA (50 mg/kg p.o.), however, the effective dose-response profile of flupirtine was shifted to the left, i.e., to the lower doses of flupirtine. The per se ineffective dose of flupirtine (1 mg/kg) synergistically interacted with L-DOPA, especially in the bar and grid test (most sensitive for rigidity) and a small increase in this effectivity was found at 10 mg/kg. But 20 mg/kg flupirtine abolished the slight anticataleptic effect revealed by L-DOPA. Reversal of catalepsy by flupirtine may be considered to be mainly due to a reduction of limb rigidity since flupirtine did not induce marked locomotor activity on its own (see below). At the highest dose used in our catalepsy tests, flupirtine was reported to even suppress horizontal locomotor activity in normal rats due to its muscle relaxing properties (Schwarz et al., 1996). In monoamine-depleted rats, however, flupirtine in combination with an ineffective dose of L-DOPA increased locomotor activity at 10 mg/kg whereas lower and higher doses were without efficacy on this parameter, suggesting an additional antiakinetic property of flupirtine (Schwarz et al., 1996). The above reported findings imply a U-shaped dose-response profile of flupirtine, and hence, a limited therapeutic window.

Flupirtine had no marked effect on horizontal locomotor activity in intact rats. It did not change spontaneous or D-amphetamine-induced locomotion in the open field with holeboard; however, head dips were even enhanced by 1 and 10 mg/kg p.o. flupirtine, indicative for stimulation of explorative behaviour. This is in good accordance with the study of Schwarz et al. (1996) who reported that flupirtine up to 10 mg/kg has no locomotor stimulating effect and a high dose of flupirtine (20 mg/kg) even suppressed locomotor behaviour in normal rats.

In combination with D-amphetamine, the lower dose of flupirtine attenuated head dipping whereas the higher dose potentiated this parameter. Rearing was reduced slightly only by 1 mg/kg and not by 10 mg/kg flupirtine, suggesting that no gross motor disability was induced (D-amphetamine-induced rearing was even further enhanced by flupirtine 1 mg/kg p.o.). The slight decrease in spontaneous rearing under 1 mg/kg flupirtine might be considered in conjunction with the increase of head dips, which are favoured under flupirtine. A similar effect was seen in the D-amphetamine-challenged behaviour, when 1 mg/kg flupirtine tended to reduce head dips in favour of increased rearing behaviour. However, at the moment we have no explanation for the contradictory effects of flupirtine on spontaneous and D-amphetamine-challenged rearing. Grooming was strongly suppressed by flupirtine reminiscent of the effects of psychostimulant drugs such as amphetamine or apomorphine (e.g., Robinson and Becker, 1986).

Sniffing behaviour in the experimental chamber was increased only by the lower dose of flupirtine, showing a synergistic effect, especially in potentiating the D-amphetamine-induced sniffing head up component. The occurrence of sniffing at 1 mg/kg flupirtine shows some similarities to the effects of the non-competitive NMDA receptor antagonist dizocilpine which preferably increases the sniffing head up component (Tzschentke and Schmidt, 1996). But the higher dose (10 mg/kg), which may be more interesting for treatment of parkinsonian symptoms by flupirtine alone, had no psychomotor stimulating effect. Accordingly, in humans, flupirtine revealed a more sedative effect on psychomotor state (Müller-Limmroth, 1985).

The central site of the pharmacological action of flupirtine is not fully understood as it does not appear to directly interact with adrenoceptors, dopamine, nicotine, or 5-HT receptors (Friedel and Fitton, 1993). Although flupirtine does not bind to any of the known binding sites of the NMDA receptor complex (Osborne et al., 1996; Schwarz et al., 1996), it behaves as a functional NMDA receptor antagonist (Müller et al., 1996a; Osborne et al., 1994; Perovic et al., 1994; Schwarz et al., 1994). Taking into account that flupirtine is able to reduce the glutamate-induced rise in intracellular Ca^{2+} concentration (Rupalla et al., 1995) without interacting with extracellularly located receptors, we may assume that flupirtine's effects are mediated by an intracellular mechanism, which still has to be elucidated.

Compared to ligands at the NMDA receptor, flupirtine in our experiments shares some similarities with non-competitive NMDA receptor-, as well as competitive NMDA receptor- and strychnine-insensitive glycine receptor antagonists: non-competitive NMDA receptor antagonists (e.g., dizocilpine), which directly block the NMDA receptor-associated channel, produce forward locomotion as well as a suppression of explorative behaviour in the open field with holeboard (Rückert and Schmidt, 1993). Additionally, they

induce stereotyped sniffing (especially head up sniffing) in the experimental chamber (Tiedtke et al., 1990; Tzschentke and Schmidt, 1996) and produce learning deficits over the whole dose range tested (Bischoff and Tiedtke, 1992). Although flupirtine is able to increase sniffing behaviour at the lower dose, its behavioural profile is completely different from those of the non-competitive NMDA receptor antagonists like dizocilpine, and flupirtine is free of the prominent side effects of non-competitive NMDA receptor antagonists. The herein reported behavioural effects of flupirtine are more in line with what is revealed by competitive or strychnine-insensitive glycine-site NMDA receptor antagonists which did not produce a pronounced stimulation of locomotion and stereotypy (Kretschmer et al., 1995). However, competitive and strychnine-insensitive glycine-site NMDA receptor antagonists attenuate the marked hyperlocomotor response induced by systemic injection of amphetamine (Bristow et al., 1994) whereas flupirtine had no effect on this parameter.

In conclusion, the present study shows that flupirtine maleate alone and in combination with a subthreshold dose of L-DOPA exerted potent anticataleptic effects in the haloperidol-induced catalepsy in the rat. A U-shaped dose-response profile was seen especially in combination with dopaminomimetic drugs like L-DOPA and D-amphetamine. A U-shaped dose response of flupirtine has also been reported by Schwarz et al. (1996) in tests of muscular rigidity and locomotor activity in hypodopaminergic rats, and by Müller et al. (1997) in oxidant- and ionomycine-induced apoptosis in HIV-infected and uninfected individuals' lymphocytes.

The behavioural profile revealed in the open field with holeboard and in the experimental chamber is unique and does not resemble any of the yet known NMDA receptor antagonists. Tentatively, from its profile in animal experiments it may be predicted that flupirtine, in a given therapeutic window, may be advantageous as an adjunct to L-DOPA in the treatment of Parkinson's disease since (i) it synergises with dopaminomimetics, (ii) it may prevent development of L-DOPA-induced fluctuations as NMDA receptor antagonists do (Chase et al., 1996), (iii) it possesses a neuroprotective potential, and (iv) it is devoid of the side effects of NMDA receptor antagonists.

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