ELSEVIER

European Journal of Pharmacology 276 (1995) 247–255

ejp

N-Methyl-D-aspartate and α_2-adrenergic mechanisms are involved in the depressent action of flupirtine on spinal reflexes in rats

Michael Schwarz [a,*], Thomas Schmitt [b], Gabriela Pergande [c], Frank Block [a,b]

[a] *Department of Neurology, RWTH Aachen, Pauwelsstr. 30, D-52057 Aachen, Germany*
[b] *Department of Neurology, University of Essen, Hufelandstr. 55, D-45122 Essen, Germany*
[c] *ASTA Medica AG, Abteilung Medizin Deutschland, Weismüllerstr. 45, D-60314 Frankfurt, Germany*

Received 20 October 1994; revised 28 December 1994; accepted 13 January 1995

Abstract

In urethane-chloralose anesthetised rats the muscle relaxant activity of flupirtine was investigated on the monosynaptic Hoffmann reflex recorded from plantar foot muscles and on the polysynaptic flexor reflex recorded from tibialis muscle. Intraperitoneal (i.p.; 2.5–25 μmol/kg) and intrathecal (i.t.; 33–330 nmol) administration of flupirtine depressed the polysynaptic flexor reflex in anesthetised rats in a dose-dependent manner without affecting the monosynaptic Hoffmann reflex. Flupirtine produced a similar pattern on spinal reflexes as NMDA receptor antagonists, such as (−)-2-amino-7-phosphonoheptanoic acid (500 nmol i.t.) and memantine (125 μmol/kg i.p.), the benzodiazepines diazepam (18 μmol/kg i.p.) and midazolam (80 nmol i.t.), and the α_2-adrenoceptor agonist tizanidine (2 μmol/kg). In contrast, the $GABA_A$ receptor agonist muscimol (21 μmol/kg i.p.; 20 nmol i.t.) and the $GABA_B$ receptor agonist baclofen (47 μmol/kg i.p.; 2 nmol i.t.) reduced the magnitude of both the flexor and the Hoffmann reflex, whereas the non-NMDA receptor antagonist 6,7-dinitroquinoxaline-2,3-dione (DNQX; 10 nmol i.t.) depressed the Hoffmann reflex without affecting the flexor reflex. The effect of i.t. injection of flupirtine was prevented by coadministration of the mixed α_1/α_2-adrenoceptor antagonist yohimbine (10 nmol) and the excitatory amino acid *N*-methyl-D-aspartate (NMDA; 0.1 nmol), but neither by coadministration of the α_1-adrenoceptor antagonist prazosine (10 nmol), the $GABA_A$ receptor antagonist bicuculline (1 nmol), the $GABA_B$ receptor antagonist phaclofen (100 nmol), the non-NMDA receptor agonist α-amino-3-hydroxy-5-tertbutyl-4-isoxazolepropionic acid (ATPA; 0.1 pmol) nor by pre-treatment with the benzodiazepine receptor antagonist flumazenil (16 μmol/kg). These observations suggest that α_2-adrenoceptors and NMDA receptors might be involved in the mediation of the muscle relaxant activity of flupirtine. The presumed NMDA receptor antagonistic effect of flupirtine would be of particular clinical relevance, since flupirtine is free of typical side effects of NMDA receptor antagonists.

Keywords: Excitatory amino acid; Flupirtine; Muscle relaxant; NMDA (*N*-methyl-D-aspartate); Spasticity; Spinal reflex; (Rat)

1. Introduction

Flupirtine is a novel analgesic agent used in humans suffering from pain of various origin (Million et al., 1984; Moore et al., 1983). After intrathecal and intraventricular injection in rodents flupirtine inhibited the nociceptive responses induced by chemical, thermal, mechanical and electrical stimuli (Jakovlev et al., 1985a,b; Nickel et al., 1985; Bleyer et al., 1988; Carlsson and Jurna, 1987). Whereas the results of these experiments strongly suggest a central site of action, the mechanism of analgesic action of flupirtine is yet not completely known.

Although the pharmacological profile of flupirtine is similar to that of the opioids pentazocine and tramadol, opioid mechanisms do not seem to be involved in its analgesic action. Firstly, the antinociceptive effect of flupirtine was not antagonized by the opioid receptor antagonist naloxone and was not associated with development of tolerance and physical dependence of the opioid type (Nickel and Aledter, 1987; Nickel et al., 1985; Sofia et al., 1987; Vaupel et al., 1989). Secondly, flupirtine showed no affinity for opioid receptors

* Corresponding author. Neurologische Klinik, RWTH Aachen, Pauwelsstr. 30, D-52057 Aachen, Germany. Tel. 0241-80-89625, fax 0241-8888444.

SSDI 0014-2999(95)00043-7

(Nickel et al., 1985). Other studies provide evidence that flupirtine modifies pain perception via activation of descending noradrenergic pathways, although it has no pharmacologically relevant affinity for α_1- and α_2-adrenoceptors (Swedberg et al., 1988; Szelenyi et al., 1989).

In addition to its potent analgesic action flupirtine has been shown to exert muscle relaxant properties. After intraperitoneal injection this drug reduced the muscle tone in rats at doses comparable with those exerting antinociceptive effects (Nickel et al., 1990b). Although a recent study provides some preliminary evidence that muscle relaxing activity of flupirtine may be due to stimulation of $GABA_A$ receptors (Weiser et al., 1992), the exact mode of this effect is not yet completely known. As the suppression of exaggerated spinal reflexes is supposed to represent a basic mechanism of action of centrally acting muscle relaxant drugs (Davidoff, 1985), it has been postulated that flupirtine is able to inhibit mono- and/or polysynaptic reflexes at the spinal level (Nickel et al., 1990a).

The aim of the present study was to characterize the pharmacological mechanisms involved in the central muscle relaxant action of flupirtine. For this purpose we have investigated whether intraperitoneal or intrathecal injection of flupirtine suppresses monosynaptic Hoffmann reflexes and polysynaptic flexor reflexes in rats. Since previous data (Schwarz et al., 1988a,b, 1992; Klockgether et al., 1989) indicated that central relaxant drugs like diazepam, baclofen, tizanidine or memantine exert their effects on spinal reflexes through changes in synaptic transmission of GABA, noradrenaline or excitatory amino acids, respectively, the possible involvement of these mechanisms in the muscle relaxant effects of flupirtine was also addressed. Therefore, we compared the effect of flupirtine on spinal reflexes in rats with the effect of drugs acting as agonists on the benzodiazepine, GABA and α-adrenoceptors or as antagonists on excitatory amino acid receptors. In addition, we examined whether coadministration of either antagonists at the benzodiazepine, GABA and α-adrenoceptors or of agonists at the excitatory amino acid receptors modifies the effects induced by flupirtine. Some of the present findings have been briefly reported elsewhere (Schwarz et al., 1994).

2. Materials and methods

Male Wistar rats (250–280 g) were anesthetised with urethane (400 mg/kg i.p.) and α-chloralose (80 mg/kg i.p.). For stimulation of the Hoffmann reflex a pair of needle electrodes was transcutaneously inserted into the surrounding of the tibial nerve (single square-wave shocks, 0.2 ms duration at 2.0 reflex threshold). Electromyographic (EMG) recordings were made with a pair of skin clip surface electrodes from the plantar foot muscle. Low intensity electrical stimulation of the tibial nerve elicits a reflex response similar to the human Hoffmann reflex, which has been attributed to monosynaptic excitation of spinal α-motoneurons predominantly by primary muscle spindle afferent fibres (Meinck, 1976). With increasing stimulus strength it is preceded by an EMG wave of shorter latency, the M wave, which is due to direct excitation of axons of α-motoneurons. EMG signals were amplified and band-pass filtered (8 Hz–10 kHz), collected at a sample rate of 10 kHz, averaged and evaluated using Signal Averager (CED, Cambridge, UK) on an IBM-compatible 286 personal computer. Ten consecutive responses were averaged both before (control) and either 20 min after intraperitoneal or 10 min after intrathecal injection of solvent or drug. The magnitude of M wave and Hoffmann reflex were evaluated by measuring the peak-to-peak amplitude. For the flexor reflex a hindpaw was stimulated with a pair of fine subcutaneous needle electrodes (5 square-wave shocks at 500 Hz, 0.2 ms duration at 3.0 reflex threshold). EMG recordings were made with a pair of fine needle electrodes inserted into the ipsilateral tibialis muscle. Seven consecutive EMG responses were rectified and processed as desribed for the Hoffmann reflex. The magnitude of flexor reflexes was evaluated by measuring the area bounded by the averaged responses and the baseline. In all reflex experiments, values measured after solvent or drug application were expressed as a percentage of the corresponding pre-injection value. Statistical evaluation of group differences was performed using the Mann-Whitney *U*-test. Statistical analysis for dose dependency of the efffects of flupirtine was carried out by means of the Kruskal-Wallis test.

For intrathecal injection the rats were fitted with intrathecal polyethylene catheters (PE 10). The atlanto-occipital membrane was exposed and carefully slit along its midline. The catheter was then inserted and its tip advanced to the lumbar enlargement. Solvent or drugs were delivered in a volume of 5 μl at 1 μl/min. Injections were followed by 10 μl of vehicle to clear the catheter of the drug. After performance of the experiments laminectomy was carried out and correct location of the catheter tip was verified by inspection of the spinal cord during injection of 2% Evans blue dye through the catheter.

Flupirtine (ASTA Medica, Germany), muscimol (Sigma Chemicals, USA), bicuculline methiodide (Sigma), yohimbine (Sigma), prazosine (RBI, USA), tizanidine (Ciba-Geigy, Switzerland) and memantine (Merz, Germany) were dissolved in saline. NMDA (Sigma), 6,7-dinitroquinoxaline-2,3-dione (DNQX; RBI), (−)-2 amino-7-phosphonoheptanoic acid (AP7;

RBI) and α-amino-3-hydroxy-5-tertbutyl-4-isoxazolepropionic acid (ATPA, kindly supplied by Prof. Dr. L. Turski, Schering, Germany) were solubilized by a small quantity of 1 M NaOH and the final volume was made up with saline. Baclofen (Ciba-Geigy) and phaclofen (Tocris, UK) was brought into solution by adding 0.2 N HCl and the final volume was made up with saline. Flumazenil (kindly supplied by Prof. Dr. W. Haefely, Hoffmann-La Roche, Switzerland) was suspended in Tween 80 and destilled water. The pH of all solutions was adjusted to 7.2–7.4.

3. Results

3.1. Intraperitoneal injection

Intraperitoneal application of solvent affected neither the Hoffmann reflex nor the flexor reflex (Fig. 2, Table 1). Intraperitoneal injection of the $GABA_A$ receptor agonist muscimol (21 μmol/kg) or the $GABA_B$ receptor agonist baclofen (47 μmol/kg) reduced the magnitude of both the flexor and the Hoffmann reflex (Table 1). Systemic administration of the benzodiazepine diazepam (18 μmol/kg), the α_2-adrenoceptor agonist tizanidine (2 μmol/kg) or the non-competetive NMDA receptor antagonist memantine (125 μmol/kg) reduced the magnitude of the flexor reflex and did not alter the Hoffmann reflex (Table 1).

Intraperitoneal injection of flupirtine (2.5–25 μmol/kg) reduced the magnitude of the flexor reflex in a dose-dependent manner ($P < 0.045$, Kruskal-Wallis test) (Figs. 1 and 2A, Table 1). This effect of flupirtine appeared within 10 min, was maximal 10–30 min after injection and lasted for 20 min, 50 min and more than 60 min after a dose of 2.5 μmol/kg, 12.5 μmol/kg and 25 μmol/kg, respectively. In contrast, the Hoffmann reflex was unaffected by flupirtine (Figs. 1 and 2B; Table 1). Even the highest dose of flupirtine used in the present study (25 μmol/kg), which reduced

Table 1
Effect of intraperitoneal injection of solvent, muscimol (21 μmol/kg), baclofen (47 μmol/kg), diazepam (18 μmol/kg), tizanidine (2 μmol/kg), memantine (125 μmol/kg), and flupirtine (25 μmol/kg) on the monosynaptic Hoffmann reflex and on the polysynaptic flexor reflex in anesthetised rats

Substance	Receptor	Hoffmann reflex (%)	Flexor reflex (%)
Solvent		96 ± 4	95 ± 5
Muscimol	$GABA_A$	49 ± 11 [a]	53 ± 5 [a]
Baclofen	$GABA_B$	25 ± 16 [b]	17 ± 14 [b]
Diazepam	Benzodiazepine	101 ± 4	20 ± 6 [b]
Tizanidine	α_2	83 ± 9	30 ± 6 [b]
Memantine	NMDA	98 ± 6	36 ± 3 [b]
Flupirtine		104 ± 7	50 ± 5 [b]

Values of the Hoffmann reflex and the flexor reflex are given as a percentage of the respective pre-injection (control) values. Significance: [a] $P < 0.01$, [b] $P < 0.001$ versus solvent, Mann-Whitney U-test.

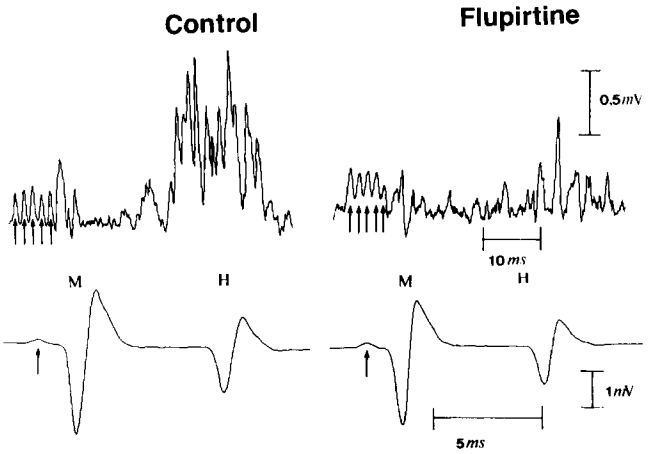

Fig. 1. Flexor reflex (upper trace) recorded from tibial muscle evoked by 5 electrical shocks (3.0×reflex threshold) applied to the hindpaw and Hoffmann reflex and M wave (lower trace) recorded from plantar foot muscles evoked by electrical stimulation (2.0×reflex threshold) of the tibial nerve before (control) and 20 min after (flupirtine) intraperitoneal injection of flupirtine, 25 μmol/kg. Stimulus artefacts are indicated by arrows.

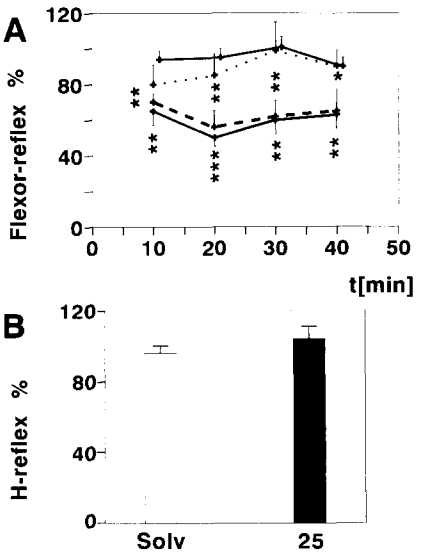

Fig. 2. A: Time course of action of intraperitoneal injection of solvent ($n = 17$) or different doses of flupirtine (2.5–25 μmol/kg; $n = 6$–8) on the magnitude of the flexor reflex. Abscissa: time (min); ordinate: magnitude of the flexor reflex expressed as a percentage of the respective pre-injection value (means ± S.E.M.). Significances versus injection of solvent: * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, Mann-Whitney U-test. Significances for dose dependency: flupirtine $P < 0.045$, Kruskal-Wallis test. B: Effect of intraperitoneal injection of solvent (Solv) or flupirtine (25 μmol/kg) on the amplitude of the Hoffmann reflex, expressed as a percentage of the respective pre-injection value (means ± S.E.M.) for 8–10 rats.

Table 2
Effect of intrathecal injection of solvent, flupirtine (165 nmol), muscimol (20 nmol), bicuculline (1 nmol), baclofen (2 nmol) and phaclofen (100 nmol) on the monosynaptic Hoffmann reflex and on the polysynaptic flexor reflex in anesthetised rats

Substance	Receptor	Hoffmann reflex (%)	Flexor reflex (%)
Solvent		105 ± 3	92 ± 8
Muscimol	$GABA_A$	29 ± 11 [a]	19 ± 8 [a]
Bicuculline		93 ± 6	104 ± 5
Muscimol + bicuculline		102 ± 13 [b]	74 ± 13 [b]
Baclofen	$GABA_B$	28 ± 11 [a]	27 ± 6 [a]
Phaclofen		96 ± 6	97 ± 9
Baclofen + phaclofen		99 ± 9 [c]	78 ± 11 [c]
Flupirtine		–	64 ± 3 [d]
Flupirtine + bicuculline		–	71 ± 8
Flupirtine + phaclofen		–	69 ± 7

Values of the Hoffmann reflex and the flexor reflex are given as a percentage of the respective pre-injection (control) values. Significance: [a] $P < 0.001$ versus solvent, [b] $P < 0.01$ versus muscimol, [c] $P < 0.05$ versus baclofen, [d] $P < 0.01$ versus solvent, Mann-Whitney U-test.

the flexor reflex by about 50%, failed to affect the Hoffmann reflex (Fig. 2B, Table 1).

3.2. Intrathecal injection

Intrathecal application of solvent affected neither the Hoffmann reflex nor the flexor reflex (Fig. 3). Injection of either the $GABA_A$ receptor agonist muscimol (20 nmol), or the $GABA_B$ receptor agonist baclofen (2 nmol) into the subarachnoid space reduced the magnitude of both the flexor and the Hoffmann reflex (Table 2). The effect of muscimol and baclofen was prevented by coadministration of the specific $GABA_A$ receptor antagonist bicuculline (1 nmol) and the $GABA_B$ receptor antagonist phaclofen (100 nmol), respectively (Table 2). Intrathecal injection of DNQX (10 nmol), a specific antagonist at the non-NMDA receptor, reduced the Hoffmann reflex but did not affect the flexor reflex (Table 3). The depressant action of DNQX on the Hoffmann reflex was not affected by coadministration of NMDA (0.1 nmol) (Table 3). In contrast, intrathecal coadministration of the non-NMDA receptor agonist ATPA (0.1 pmol) prevented the depressant action of DNQX on the Hoffmann

Table 3
Effect of intrathecal injection of solvent, flupirtine (165 nmol, 330 nmol), DNQX (10 nmol), ATPA (0.1 pmol), AP7 (500 nmol) and NMDA (0.1 nmol) on the monosynaptic Hoffmann reflex and on the polysynaptic flexor reflex in anesthetised rats

Substance	Receptor	Hoffmann reflex (%)	Flexor reflex (%)
Solvent		105 ± 3	92 ± 8
DNQX	Non-NMDA	26 ± 9 [b]	84 ± 16
ATPA		101 ± 4	94 ± 7
DNQX + ATPA		81 ± 18 [c]	–
DNQX + NMDA		38 ± 5	–
AP7	NMDA	86 ± 13	18 ± 5 [b]
NMDA		102 ± 13	106 ± 16
AP7 + NMDA		–	69 ± 12 [d]
AP7 + ATPA		–	24 ± 7
Flupirtine (165 nmol)		–	64 ± 3 [a]
Flupirtine + NMDA		–	86 ± 7 [e]
Flupirtine + ATPA		–	51 ± 10
Flupirtine (330 nmol)		–	27 ± 4 [b]
Flupirtine + NMDA		–	57 ± 10 [f]

Values of the Hoffmann reflex and the flexor reflex are given as a percentage of the respective pre-injection (control) values. Significance: [a] $P < 0.01$, [b] $P < 0.001$ versus solvent, [c] $P < 0.01$ versus DNQX, [d] $P < 0.05$ versus AP7, [e] $P < 0.05$ versus flupirtine (165 nmol), [f] $P < 0.01$ versus flupirtine (330 nmol), Mann-Whitney U-test.

Table 4
Effect of intrathecal injection of solvent, flupirtine (165 nmol, 330 nmol), tizanidine (100 nmol), yohimbine (10 nmol), and prazosine (10 nmol) on the monosynaptic Hoffmann reflex and on the polysynaptic flexor reflex in anesthetised rats

Substance	Receptor	Hoffmann reflex (%)	Flexor reflex (%)
Solvent		105 ± 3	92 ± 8
Tizanidine	α_2	93 ± 7	98 ± 10
Yohimbine	α_2/α_1	–	87 ± 4
Prazosine	α_1	–	92 ± 10
Flupirtine (165 nmol)		–	64 ± 3 [a]
Flupirtine + yohimbine		–	75 ± 4 [c]
Flupirtine + prazosine		–	60 ± 9
Flupirtine (330 nmol)		–	27 ± 4 [b]
Flupirtine + yohimbine		–	60 ± 6 [d]

Values of the Hoffmann reflex and the flexor reflex are given as a percentage of the respective pre-injection (control) values. Significance: [a] $P < 0.01$, [b] $P < 0.001$ versus solvent, [c] $P < 0.05$ versus flupirtine (165 nmol), [d] $P < 0.01$ versus flupirtine (330 nmol), Mann-Whithey U-test.

reflex (Table 3). Intrathecal injection of the α_2-adrenoceptor agonist tizanidine (100 nmol) failed to alter the flexor and the Hoffmann reflex (Table 4). After intracerebroventricular injection this dose of tizanidine strikingly depressed the flexor reflex to about 20% without affecting the Hoffmann reflex (Schwarz et al., unpublished result). Injection of the benzodiazepine midazolam (80 nmol) or the competetive NMDA receptor antagonist AP7 (500 nmol) into the subarachnoid space reduced the magnitude of the flexor reflex without affecting the Hoffmann reflex (Tables 3 and 5). Coadministration of NMDA (0.1 nmol) reversed the depressant effect of AP7 (500 nmol, Table 3), but did not influence the depressant action of midazolam on the flexor reflex (Table 5). Intraperitoneal pretreatment with the specific benzodiazepine receptor antagonist flumazenil (16 μmol/kg) prevented the effect of midazolam on the flexor reflex (Table 5). Flumazenil was given intraperitoneally due to its insolubility to water.

The differential action of flupirtine on flexor reflex and Hoffmann reflex seen after intraperitoneal administration was also observed after local injection into the subarachnoid space. Flupirtine (33–330 nmol) injected intrathecally reduced the flexor reflex in a dose-dependent manner ($P < 0.0001$, Kruskal-Wallis test) without affecting the Hoffmann reflex (Fig. 3). The effect was maximal 10 min after injection and lasted for 40 min and more than 60 min after injection of 165 nmol and 330 nmol, respectively.

In order to get insight whether changes in transmission of GABA, noradrenaline or excitatory amino acids are involved in the muscle relaxant activity of flupirtine, we investigated the influence of different drugs acting as agonists and antagonists at these receptors on the effect of flupirtine. The depressant action of flupirtine (165 nmol) on the flexor reflex was not affected by coadministration of the $GABA_A$ receptor antagonist bicuculline (1.0 nmol; Table 2), the $GABA_B$ receptor antagonist phaclofen (100 nmol; Table 2), the non-NMDA receptor agonist ATPA (0.1 pmol; Table 3) or the α_1-adrenoceptor antagonist prazosine (10 nmol; Table 4). Intraperitoneal injection of the benzodiazepine receptor flumazenil (16 μmol/kg; Table 5) did not influence the effect of flupirtine either. In contrast, intrathecal coadministration of the excitatory amino

Table 5
Effect of intrathecal injection of solvent, flupirtine (165 nmol), NMDA (0.1 nmol), midazolam (80 nmol), and of intraperitoneal injection of flumazenil (16 μmol/kg) on the monosynaptic Hoffmann reflex and on the polysynaptic flexor reflex in anesthetised rats

Substance	Receptor	Hoffmann reflex (%)	Flexor reflex (%)
Solvent		105 ± 3	92 ± 8
Midazolam	Benzodiazepine	100 ± 2	43 ± 12 [b]
Flumazenil		98 ± 5	92 ± 4
NMDA		102 ± 13	106 ± 16
Midazolam + flumazenil		–	81 ± 10 [c]
Midazolam + NMDA		–	48 ± 9
Flupirtine		–	64 ± 3 [a]
Flupirtine + flumazenil		–	51 ± 4

Values of the Hoffmann reflex and the flexor reflex are given as a percentage of the respective pre-injection (control) values. Significance: [a] $P < 0.01$, [b] $P < 0.001$ versus solvent, [c] $P < 0.01$ versus midazolam, Mann-Whitney U-test.

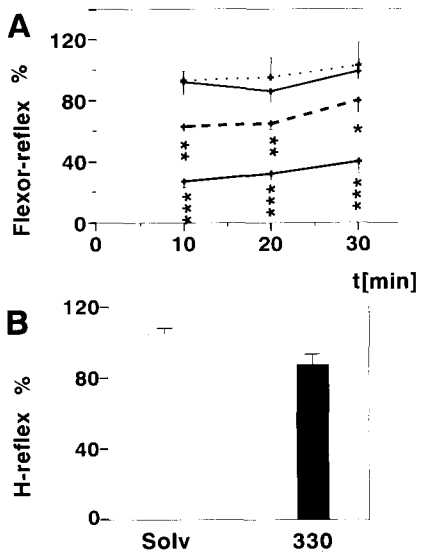

Fig. 3. A: Time course of action of intrathecal injection of solvent ($n = 8$) or different doses of flupirtine (33–330 nmol; $n = 9$–10) on the magnitude of the flexor reflex. Abscissa: time (min); ordinate: magnitude of the flexor reflex expressed as a percentage of the respective pre-injection value (means ± S.E.M.). Significance versus injection of solvent: $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$, Mann-Whitney U-test. Significance for dose dependency: flupirtine $P < 0.0001$, Kruskal-Wallis test. B: Effect of intrathecal injection of solvent (Solv) or flupirtine (330 nmol) on the amplitude of the Hoffmann reflex, expressed as a percentage of the respective pre-injection value (means ± S.E.M.) for 8–10 rats.

acid NMDA (0.1 nmol; Table 3) or the mixed α_1/α_2-adrenoceptor antagonist yohimbine (10 nmol; Table 4) prevented the depressant action of flupirtine on the flexor reflex. Doses of bicuculline, phaclofen, ATPA, prazosine, flumazenil, NMDA and yohimbine were chosen which did not affect reflexes, when given alone (Tables 2–5), but have been shown to be sufficient to prevent the depressant action of the $GABA_A$ receptor agonist muscimol, the $GABA_B$ receptor agonist baclofen, the non-NMDA receptor antagonist DNQX, the α_2-adrenoceptor agonist tizanidine, the benzodiazepine midazolam, and the NMDA receptor antagonists AP7 and memantine, respectively, on spinal reflexes in rats (Tables 2–5) (Block and Schwarz, 1993; Block et al., 1993; Klockgether et al., 1989; Schwarz et al., 1988a,b, 1992; Wüllner et al., 1989).

Neither injection of solvent nor injection of drugs had any influence on the magnitude of M waves. This indicates the stability of the preparation used and contradicts any anesthetic activity of the drugs at the concentration tested.

4. Discussion

In addition to antinociceptive properties muscle relaxant effects of flupirtine have previously been reported in animal experiments (Nickel et al., 1990b). This muscle relaxant activity has been attributed to a potential effect of flupirtine on mono- and/or polysynaptic spinal reflexes (Nickel et al., 1990b). In support of these studies we found that systemic and intrathecal injections of flupirtine in rats depress the magnitude of spinal polysynaptic reflexes without affecting the monosynaptic Hoffmann reflex. Comparing the *potency* of the muscle relaxant action of flupirtine with that of antispastic drugs reveals that after intraperitoneal administration flupirtine was less potent than the $GABA_B$ receptor agonist baclofen, the benzodiazepine diazepam and the α_2-adrenoceptor agonist tizanidine, but was as potent as the $GABA_A$ receptor agonist muscimol and the non-competetive NMDA receptor antagonist memantine (Table 1) (Block and Schwarz, 1994). After intrathecal administration the central muscle relaxant potency of flupirtine is weaker than that of the GABA receptor agonists baclofen and muscimol, the non-NMDA receptor antagonist DNQX and the benzodiazepine midazolam, but it is comparable to the potency of the competitive NMDA receptor antagonists 3-((±)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid and AP7 and the non-competitive NMDA receptor antagonists memantine and dextromethorphane (Block and Schwarz, 1993, 1994; Klockgether et al., 1989; Schwarz et al., 1988b; Wüllner et al., 1989; Turski et al., 1990). The maximum effect of flupirtine on the flexor reflex was reached 10 min after intrathecal injection and 10–20 min after intraperitoneal injection. A similar time course of action was demonstrated for the antinociceptive action of flupirtine (Carlsson and Jurna, 1987). These observations suggest that the drug rapidly penetrates from the blood or cerebrospinal fluid into the central nervous system.

Comparing the *profile* of the effect on spinal reflexes of flupirtine with that of other centrally acting muscle relaxant substances reveals that both benzodiazepines, such as diazepam or midazolam, and NMDA receptor antagonists, such as AP7 and memantine, selectively depress polysynaptic reflexes without affecting the monosynaptic Hoffmann reflex. In contrast, the non-NMDA receptor antagonist DNQX selectively depressed monosynaptic reflexes without affecting polysynaptic reflexes. The α_2-adrenoceptor agonist tizanidine selectively influenced the flexor reflex after systemic application. After intrathecal injection, however, tizanidine was without any effect on either mono- or polysynaptic reflexes (Table 4; Klockgether et al., 1989; Schwarz et al., 1988a). The $GABA_A$ receptor agonist muscimol and the $GABA_B$ receptor agonist baclofen reduce both the Hoffmann reflex and the flexor reflex (Klockgether et al., 1989; Schwarz et al., 1988a,b; Wüllner et al., 1989). Therefore, the profile of action of flupirtine on spinal reflexes is similar to that of benzodiazepines and NMDA receptor antagonists, and,

in part, to that of α_2-adrenoceptor agonists. It differs, however, from that of GABA receptor agonists and non-NMDA receptor antagonists.

In addition to comparing the profile of the effect on spinal reflexes the present study tries to get insight into the mechanisms of the muscle relaxant action of flupirtine by coadministration of agonists and antagonists at the GABA, benzodiazepine, NMDA and non-NMDA receptors and α-adrenoceptors.

Although preliminary evidence from patch-clamp studies suggests that flupirtine enhances GABAergic responses (Weiser et al., 1992), in the present experiments GABA receptors do not seem to be involved in the muscle relaxant action of flupirtine. Coadministration of the $GABA_A$ receptor antagonist bicuculline and the $GABA_B$ receptor antagonist phaclofen did not influence the action of flupirtine. Doses of bicuculline and phaclofen were chosen which were devoid of action on reflexes by themselves but were sufficient to antagonize the depressant effect of the $GABA_A$ receptor agonist muscimol and of the $GABA_B$ receptor agonist baclofen on flexor reflexes (Table 2; Schwarz et al., 1988b; Wüllner et al., 1989).

Flupirtine does not act through benzodiazepine receptors either. In doses sufficient to antagonize the effect of diazepam or midazolam (Block and Schwarz, 1994; Klockgether et al., 1989; Schwarz et al., 1988a) the specific benzodiazepine receptor antagonist flumazenil failed to affect the depressant action of flupirtine on the flexor reflex. This result fits well with preliminary observations (Nickel et al., personal communication), that the flupirtine-induced decrease of muscle tone in rats is not influenced by flumazenil. In addition, flupirtine does not possess affinity to benzodiazepine receptors and does not induce drug dependency typical for benzodiazepines (Nickel et al., 1990a).

In contrast, α_2-adrenergic mechanisms seem to be involved in mediating the muscle relaxant effect of flupirtine. Thus, in the present experiments the mixed α_2/α_1-adrenoceptor antagonist yohimbine prevents the action of flupirtine on the flexor reflex. Since the α_1-adrenoceptor antagonist prazosine failed to influence the action of flupirtine, α_2-adrenergic mechanisms seem to be involved in the mediation of the muscle relaxant action of flupirtine. However, intrathecal injection of the α_2-adrenoceptor agonist tizanidine in doses which were sufficient to potently depress the flexor reflex after intracerebroventricular injection (Schwarz et al., unpublished results), did not influence the flexor reflex (Schwarz et al., 1988a; Klockgether et al., 1989). The latter result might indicate that spinal α_2-adrenergic mechanisms are not involved in the mediation of the flexor reflex.

Other studies support the assumption that flupirtine exerts its pharmacological actions at least in part through α_2-adrenergic mechanisms. Thus, the antinociceptive action induced by flupirtine depends on an intact descending noradrenergic pain-modulating system (Swedberg et al., 1988; Szelenyi et al., 1989). This assumption is substantiated by the capacity of α_2-adrenoceptor antagonists, such as yohimbine and idazoxane, to diminish the antinociceptive activity of flupirtine (Szelenyi et al., 1989). However, the adrenergic system seems only indirectly involved, since flupirtine has no pharmacologically relevant affinity for α_1- and α_2-adrenoceptors (Szelenyi et al., 1989). Furthermore, drug discrimination procedures have shown that α_2-adrenergic mechanisms are only partially responsible for mediating the discriminative stimulus properties of flupirtine (Swedberg et al., 1988). The latter study stressed that further research is needed to delineate the component of action of flupirtine, which is not mediated by α_2-adrenergic mechanisms.

The present study presents the first experimental evidence that blockade of transmission of excitatory amino acids might also be involved in the action of flupirtine. The results presented here suggest an antagonistic action of this drug on transmission mediated by NMDA receptors. This assumption rests on the observations that (1) flupirtine similar to NMDA receptor antagonists selectively depresses the flexor reflex without affecting the Hoffmann reflex (Block and Schwarz, 1993, 1994; Block et al., 1993; Schwarz et al., 1992; Turski et al., 1990, 1992) and (2) coadministration of NMDA prevents the depressant action of flupirtine on the flexor reflex.

It could be argued that several types of drug including benzodiazepines and α_2-adrenoceptor agonists (Siarey et al., 1992) have been shown to depress NMDA receptor-mediated spinal synaptic activity in vitro without any direct antagonism of the excitatory actions of NMDA. However, the block of the effect of flupirtine by coadministration of NMDA does not seem to be due to such a functional interaction but rather to a specific pharmacological antagonism, since the chosen dose of NMDA (1) is devoid of an intrinsic effect on the flexor reflex, (2) failed to prevent the depressant action of the benzodiazepine midazolam on the flexor reflex, and (3) failed to prevent the depressant action of the non-NMDA receptor antagonist DNQX on the Hoffmann reflex. Further evidence for an action of flupirtine through NMDA mechanisms derives from very recent in vitro experiments on rabbit retinas (Osborne et al., 1995). In this system flupirtine counteracted the GABA release of amacrine cells induced by NMDA and homocystic acid but had no effect on the responses induced by kainate and domoic acid.

However, the non-NMDA receptor-mediated transmission does not seem to be involved, since (1) in contrast to flupirtine non-NMDA receptor antagonists selectively depress the Hoffmann reflex without affecting the flexor reflex (Turski et al., 1990, 1992; Block

and Schwarz, 1993, 1994; Block et al., 1993) and (2) the non-NMDA receptor agonist ATPA failed to prevent the muscle relaxant effect of flupirtine in doses sufficient to antagonize the depressant effect of the non-NMDA receptor antagonist DNQX on the Hoffmann reflex. At present, it is unclear, whether flupirtine exerts its muscle relaxant action postsynaptically through an interaction with one of the binding sites of the NMDA receptor complex (Young and Fagg, 1990) or presynaptically through an impairment of release of excitatory amino acids. The protective effect of flupirtine on NMDA-induced cell death of rat cortical neurons (Perovic et al., 1994) suggests a postsynaptic site of action. Independent of the exact site of action a presumed NMDA receptor antagonistic effect of flupirtine would be of particular clinical relevance. NMDA-mediated neurotoxicity seems to be a final common pathway for many neurological disorders like cerebral ischaemia or degenerative diseases such as Parkinson's disease and Huntington's disease (Choi, 1988). Since flupirtine (1) is free of typical side effects of NMDA receptor antagonists such as psychomimetic properties and impairment of learning (Morris et al., 1986) and (2) has been shown to be protective against ischaemic retinal dysfunction (Block et al., 1994), the application of this drug in clinical pharmacology may be extended to the treatment of stroke and neurodegenerative disorders.

References

Bleyer, H., K.H. Carlsson, H.J. Erkel and I. Jurna, 1988, Flupirtine depresses nociceptive activity evoked in rat thalamus, Eur. J. Pharmacol. 151, 259.

Block, F. and M. Schwarz, 1993, *N*-Methyl-D-aspartate (NMDA)-mediated muscle relaxant action of dextromethorphan in rats, NeuroReport 4, 941.

Block, F. and M. Schwarz, 1994, The depressant effect of GYKI 52466 on spinal reflex transmission in rats is mediated via non-NMDA and benzodiazepine receptors, Eur. J. Pharmacol. 256, 149.

Block, F., T. Schmitt and M. Schwarz, 1993, Pentamidine, an inhibitor of rat's spinal flexor reflexes, is a potent *N*-methyl-D-aspartate (NMDA) antagonist 'in vivo', Neurosci. Lett. 155, 208.

Block, F., G. Pergande and M. Schwarz, 1994, Flupirtine protects against ischaemic retinal dysfunction in rats, NeuroReport 5, 2630.

Carlsson, K.-H. and I. Jurna, 1987, Depression by flupirtine, a novel analgesic agent, of motor and sensory responses of the nociceptive system in the rat spinal cord, Eur. J. Pharmacol. 143, 89.

Choi, D.W., 1988, Glutamate neurotoxicity and diseases of the nervous system, Neuron 1, 623.

Davidoff, R.A., 1985, Antispasticity drugs: mechanisms of action, Ann. Neurol. 17, 107.

Jakovlev, V., U. Achterrath-Tuckermann, A. Schlichtegroll, F. Stroman and K. Thiemer, 1985a, General pharmacological investigations with the analgesic flupirtine, Arzneim.-Forsch./Drug Res. 35, 44.

Jakovlev, V., R.D. Sofia, U. Achterrath-Tuckermann, A. Schlichtegroll and K. Thiemer, 1985b, Investigations on the pharmacological activity of flupirtine, a structurally new analgesic, Arzneim.-Forsch./Drug Res. 35, 5.

Klockgether, T., M. Schwarz, U. Wüllner, L. Turski and K.-H. Sontag, 1989, Myorelaxant effect after intrathecal injection of antispastic drugs in rats, Neurosci. Lett. 97, 221.

Meinck, H.M., 1976, Occurrence of the Hoffmann reflex and the F-wave in the rat, Electroenceph. Clin. Neurophysiol. 41, 530.

Million, R., B.R. Finlay and J.R. Whittington, 1984, Clinical trial of flupirtine in patients with migraine. Curr. Med. Res. Opin. 9, 204.

Moore, R., R.E.S. Bullingham, S. Simpson, G. O'Sullivan, P.J.D. Evans and J. McQuay, 1983, Comparison of flupirtine maleate and dihydrocodeine in patients following surgery, Br. J. Anaesth. 55, 429.

Morris, R.G.M., E. Anderson, G. Lynch and M. Baudry, 1986, Selective impairment of learning and blockade of long-term potentiation by an *N*-methyl-D-aspartate receptor antagonist, Nature 319, 774.

Nickel, B. and A. Aledter, 1987, Comparative physical dependence studies in rats with flupirtine and opiate receptor stimulating analgetics, Postgrad. Med. J. Suppl. 3, 41.

Nickel, B., A. Herz, V. Jakovlev and U. Tibes, 1985, Studies of the mode of action of the analgesic agent flupirtine, Arzneim.-Forsch./Drug Res. 35, 1402.

Nickel, B., H.O. Borbe and I. Szelenyi, 1990a, Investigations with the novel non-opioid analgesic flupirtine in regard to possible benzodiazepine-like abuse inducing potential, Arzneim.-Forsch./Drug Res. 40, 905.

Nickel, B., V. Jakovlev and I. Szelenyi, 1990b, Effects of flupirtine, some analgesics and of muscle relaxants on sceletal muscle tone in conscious rats, Arzneim.-Forsch./Drug Res. 40, 909.

Osborne, N.N., G. Pergande, F. Block and M. Schwarz, 1994, Immunohistochemical evidence for flupirtine acting as an antagonist on the *N*-methyl-D-aspartate (NMDA) and homocysteic acid-induced release of GABA in the rabbit retina, Brain Res. 667, 291.

Perovic, S., C. Schleger, G. Pergande, S. Iskric, H. Ushijima, P. Rytik and W.E.G. Müller, 1994, The triaminopyridine flupirtine prevents cell death in rat cortical cells induced by *N*-methyl-D-aspartate and gp120 of HIV-1, Eur. J. Pharmacol. Mol. Pharmacol. 288, 27.

Schwarz, M., T. Klockgether, L. Turski and K.-H. Sontag, 1988a, Intrathecal injection of antispastic drugs in rats: muscle relaxant action of midazolam, baclofen, 2-aminoheptanoic acid (AP7) and tizanidine, in: Local-Spinal Therapy of Spasticity, eds. H. Müller, J. Zierski and R.D. Penn (Springer, Berlin, Heidelberg, New York) p. 65.

Schwarz, M., T. Klockgether, U. Wüllner, L. Turski and K.-H. Sontag, 1988b, Deltaaminovaleric acid antagonizes the pharmacological actions of baclofen in the central nervous system, Exp. Brain Res. 70, 618.

Schwarz, M., F. Block and K.-H. Sontag, 1992, *N*-Methyl-D-aspartate (NMDA)-mediated muscle relaxant action of memantine in rats, Neurosci. Lett. 143, 105.

Schwarz, M., F. Block, G. Pergande and J. Noth, 1994, Muscle relaxant action of flupirtine: evidence for an involvement of α_2 adrenergic and *N*-methyl-D-aspartate (NMDA) receptors?, Soc. Neurosci. Abstr. 1722.

Siarey, R.J., S.K. Long and R.H. Evans, 1992, The effect of centrally acting myorelaxants on NMDA receptor-mediated synaptic transmission in the immature rat spinal cord in vitro, Br. J. Pharmacol. 107, 628.

Sofia, R.D., W. Diamantis and R. Gordon, 1987, Abuse potential and physical dependence liability studies with flupirtine maleate in laboratory animals, Postgrad. Med. J. 63 (Suppl. 3), 35.

Swedberg, M.D.B., H.E. Shannon, B. Nickel and S.R. Goldberg, 1988, Pharmacological mechanisms of action of flupirtine: a novel,

centrally acting, nonopioid analgesic evaluated by its discriminative effects in the rat, J. Pharmacol. Exp. Ther. 246, 1067.

Szelenyi, I., B. Nickel, H.O. Borbe and K. Brune, 1989, Mode of antinociceptive action of flupirtine in the rat, Br. J. Pharmacol. 97, 835.

Turski, L., K. Bressler, T. Klockgether and D.N. Stephens, 1990, Differential effects of the excitatory amino acid antagonists, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and 3-((±)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP), on spinal reflex activity in mice, Neurosci. Lett. 113, 66.

Turski, L., P. Jacobsen, T. Honore and D.N. Stephens, 1992, Relief of experimental spasticity and anxiolytic/anticonvulsant actions of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate antagonist 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(*F*)quinoxaline, J. Pharmacol. Exp. Ther. 260, 742.

Vaupel, D.B., B. Nickel and K. Becketts, 1989, Flupirtine antinociception in the dog is primarily mediated by nonopioid supraspinal mechanisms, Eur. J. Pharmacol. 162, 447.

Weiser, T., B. Nickel, I. Szelenyi and M. Wienrich, 1992, In vivo and in vitro findings about the muscle relaxing properties of flupirtine, Naunyn-Schmied. Arch. Pharmacol. 346 (Suppl. 1), R22.

Wüllner, U., T. Klockgether and K.-H. Sontag, 1989, Phaclofen antagonizes the depressant effect of baclofen on spinal reflex transmission in rats, Brain Res. 496, 341.

Young, A.B. and G.E. Fagg, 1990, Trends Pharmacol. Sci. 11, 126.