Flupirtine: A Review of Its Neuroprotective and Behavioral Properties


University of Tübingen, Dept. Neuropharmacology, Tübingen, Germany; *RWTH Aachen, Dept. Neurology, Aachen, Germany; *Asta Medica AWD GmbH, Frankfurt am Main, Germany

Key Words: Ca\textsuperscript{2+} homeostasis—Flupirtine—Muscle relaxation—Neuroprotection—NMDA receptor—Parkinson’s disease.

INTRODUCTION

Flupirtine is a centrally acting non-opioid analgesic; it has been in clinical use since 1984. The pharmacological and therapeutic properties of flupirtine in pain states have been reviewed extensively (7). The present review addresses new and challenging data that have been published during recent years. In particular, it has been discovered that flupirtine, in a clinically relevant dosage range, has potent cyto- and neuroprotective potential as well as anticonvulsant, and myorelaxant effects. Flupirtine reverses akinesia and rigidity in dopamine-depleted animals. These findings, and the reported reversal of N-methyl-D-aspartate (NMDA)-receptor-mediated effects, point to a functional NMDA antagonism by flupirtine. No binding of this drug at any known receptor has yet been demonstrated, but there are hints about a possible mechanism of action.

Flupirtine maleate (hereafter referred to as flupirtine) was synthesized by Homburg, Degussa Pharma Group of Frankfurt, Germany in 1981 (1) and is available in Europe under the trademark of Katadolon® (ASTA Medica AG, Frankfurt, Germany). Flupirtine can be described as a pyridine derivative that is substituted in the 2-, 3- and 6-positions with different amino groups, (2-amino-3-ethoxy-carbonylamino-6-fluoro-benzylamino-pyridine maleate). It was originally selected for clinical evaluation as the most active compound in a group of analgesic amino-substituted pyridine derivatives. The molecular structure of flupirtine (Fig. 1) does not resemble that of any analgesic currently on the market. Its synthesis is a four-step process starting from 2,6-dichloro-3-nitro-pyridine (4).
Flupirtine is a centrally acting non-narcotic analgesic; it possesses also a muscle-relaxing effect. It has a unique spectrum of pharmacological activities (11,15,16,22,23,43) and is devoid of the typical side effects of natural or synthetic opioids, such as respiratory depression, constipation, tolerance, physical and/or psychological dependence, and liability to cause addiction. Up to now, more than 5000 patients have received flupirtine in open and controlled analgesia studies, and many more have done so by prescription of their general practitioners. Flupirtine is a well-tolerated and safe substance that can even be prescribed for children below the age of six years. Infrequent side effects are fatigue, drowsiness, dizziness, headache, nausea and vomiting.

**METABOLISM AND PHARMACOKINETICS**

The biotransformation of flupirtine takes place in the liver. It is converted to two primary metabolites, p-fluoro-hippuric acid and an acetylated metabolite that has 20% to 30% of the analgesic activity of the parent compound (10). The two metabolites are formed by oxidative degradation and acetylation of a hydrolysis product, respectively.

Flupirtine is almost completely absorbed from the gastrointestinal tract in experimental animals (24) and humans (10); up to 90% after oral intake and up to 70% after rectal administration. The kinetics of its plasma levels following intravenous administration show a short distribution phase followed by an elimination phase. The plasma half-life after intravenous or oral administration in humans is ~9.5 h while in rats and dogs the half-life ranges from 2.5 to 3.5 h. Peak plasma flupirtine levels of 2.7 µg/ml occur within 0.5 to 2.0 h after a single 200-mg oral dose. After rectal application (150 mg), peak plasma flupirtine levels of 1.2 µg/ml occur within 3.0 to 5.7 h. Single doses of 250 mg and daily dosages of up to 900 mg have been well tolerated with no significant toxicity. Renal excretion amounts to 20% (rat) and 36% (dog) after intravenous administration, and to 22% (rat) and 35% (dog) after oral administration. The major part of the dose is eliminated in feces (24).
RECEPTOR-BINDING STUDIES

The central site of pharmacological action of flupirtine is not fully understood. Flupirtine does not appear to interact directly with adrenoceptors or dopamine, nicotine, or 5-hydroxytryptamine receptors (7). Furthermore, no specific binding of flupirtine to opiate receptors in rat brain was found in concentrations up to $10^{-5}$ mol/l (11).

Although flupirtine does not bind to any of the as-yet identified NMDA receptor-complex-associated binding sites (46,27,38), it shows several functional NMDA receptor-antagonistic properties both in vivo and in vitro. However, radioligand binding studies on rat brain synaptic membranes have demonstrated that flupirtine does not displace either the competitive NMDA antagonist $[^3$H]CGS-19755 or the non-competitive NMDA antagonist $[^3$H]MK-801 from their binding sites (29). Antagonists of the glycine- or polyamine-binding sites of the NMDA receptor have an inhibitory influence on the binding of $[^3$H]MK-801. Flupirtine lacks this inhibitory influence. The failure of flupirtine to decrease $[^3$H]MK-801 binding suggests that flupirtine does not act at these binding sites of the NMDA receptor complex. Moreover, flupirtine does not influence the effect of Mg$^{2+}$ on $[^3$H]MK-801 binding. Thus, there is no experimental evidence that flupirtine has any affinity for the various binding sites of the NMDA receptor complex. There is, however, some experimental evidence that flupirtine might suppress channel opening by acting as an oxidizing agent at the redox site of the NMDA receptor (29).

NEUROPROTECTIVE EFFECTS IN VIVO

Flupirtine counteracts the effects of both focal cerebral ischemia in mice (34) and global cerebral ischemia in rats (4) (Table 1). In the mouse, occlusion of the middle cerebral artery for a period of 48 h produces defined infarction areas in the brain. Previous but not subsequent treatment of mice with flupirtine significantly reduced the infarction volumes (34). Transient global ischemia in the rat induced by a 20-min interval of four-vessel occlusion leads morphologically to selective neuronal damage in the CA1 sector of the hippocampus and in the striatum (Fig. 2), functionally expressed as deficits in spatial learning and memory in the water maze. Pretreatment of animals with flupirtine attenuated both the morphological and behavioral consequences of global cerebral ischemia (4). In common with the findings for focal ischemia in the mouse, however, posttreatment with flupirtine had no protective effect against either the functional deficits or the neuronal damage induced by global cerebral ischemia (4). These data indicate that flupirtine is not a good candidate for clinical treatment of stroke or of global cerebral ischemia due to cardiac arrest. It may, however, find a use in situations that bear the risk of a transient cerebral ischemia, such as coronary artery bypass operations, or in the treatment of neurodegenerative diseases.

It is known from animal experiments that flupirtine can attenuate not only cerebral ischemia but also retinal ischemic dysfunction. The functional status of the retina can be monitored continuously by recording of the electroretinogram (ERG), i.e., the full-field light-evoked potential recorded on the surface of the cornea (5). The b-wave,
which is induced by K⁺ efflux shunted from ON-bipolar cells into the vitreous humor by the Müller cells in response to retinal illumination, is the ERG component most susceptible to ischemia (reviewed in 5). Incomplete retinal ischemia induced by transient occlusion of the common carotid arteries in normotensive rats leads to both a reduction in the amplitude and to a delay in the implicit time of the b-wave of the ERG (2), which recovers gradually to normal levels on reperfusion. In experiments utilizing a 24-min occlusion of the carotid arteries, in which rats were treated with flupirtine either before or after the onset of ischemia, the recovery of the b-wave was enhanced and the delay of implicit time was reduced (3,29). These experiments suggest that flupirtine mitigates the detrimental effects to the retina induced by a reduced blood flow.

A more severe ischemic insult, with almost complete ischemia, can be delivered to the retina by raising the intraocular pressure above the systolic blood pressure. When this is done over a 75-min period in the rabbit, a complete loss of the b-wave occurs (27). This subsequently recovers to only about 20% of its original value, even after 2 d of reperfusion. Intraocular injection of flupirtine just after ischemia facilitates the recovery of the b-wave to about 60% of its original value (27). This ischemic insult to the rabbit retina also causes a change in the nature of the γ-aminobutyric acid (GABA) immunoreactivity. The changes are presumed to be caused by release of glutamate, which activates ionotrophic glutamate receptors associated with subsets of GABAergic cells to cause a release of endogenous GABA (25). However, when flupirtine or the non-competitive NMDA-receptor antagonist MK-801 are injected into the vitreous humor at the onset of the ischemic insult, these changes in the GABA immunoreactivity are much reduced (27). In addition, elevation of the intraocular pressure above systolic blood pressure causes: 1) loss of choline acetyltransferase-containing neurons, 2) reduction of the retinal level of the NMDAR1 mRNA due to cell death of some neurons containing NMDA receptors; and 3) reduction in the thickness of the inner nuclear and plexiform layers. Pretreatment with flupirtine prevented all these consequences of retinal ischemia (29).
Exposure of primary cortical or hippocampal cultures to glutamate causes damage assessable either by trypan blue exclusion or release of lactate dehydrogenase. Flupirtine protected neurons against glutamate-induced cell damage (3,29). Short exposure of hippocampal neurons to glutamate also induces an increase in intracellular Ca\(^{2+}\) levels, revealed using the Ca\(^{2+}\) indicator fura-2, and this release is also significantly attenuated by flupirtine (34). The glutamate-induced Ca\(^{2+}\) overload is thought to be mediated mainly by the NMDA receptor. Indeed, exposure of cortical cultures to NMDA caused the cells to accumulate \(^{45}\)Ca\(^{2+}\) from the external medium (29). This NMDA-induced response was blocked completely by MK-801 and partially by flupirtine. The NMDA-induced accumulation is significantly enhanced by the disulfide-reducing agent dithiothreitol (DTT), showing that once the NMDA receptor is...

**Fig. 2.** Neuronal damage in the CA1 sector of the hippocampus (A) and in the striatum (B) of sham-operated controls (n = 11, white bars), four vessel occluded (4VO) rats injected with solvent (n = 10, solid bars), 4VO rats treated with flupirtine before (n = 8, hatched bars) or after ischemia (n = 11, crossed bars). Values are means ± S.E.M. *P < 0.05, **P < 0.01 vs. 4VO, Mann–Whitney U-test. Reprinted with permission from Elsevier Science from reference 4: Block F, Pergande G, Schwarz M. Flupirtine reduces functional and morphological consequences induced by global ischemia in rats. *Brain Res* 1997;754:279–284.

**NEUROPROTECTIVE EFFECTS IN VITRO**

Exposure of primary cortical or hippocampal cultures to glutamate causes damage assessable either by trypan blue exclusion or release of lactate dehydrogenase. Flupirtine protected neurons against glutamate-induced cell damage (3,29). Short exposure of hippocampal neurons to glutamate also induces an increase in intracellular Ca\(^{2+}\) levels, revealed using the Ca\(^{2+}\) indicator fura-2, and this release is also significantly attenuated by flupirtine (34). The glutamate-induced Ca\(^{2+}\) overload is thought to be mediated mainly by the NMDA receptor. Indeed, exposure of cortical cultures to NMDA caused the cells to accumulate \(^{45}\)Ca\(^{2+}\) from the external medium (29). This NMDA-induced response was blocked completely by MK-801 and partially by flupirtine. The NMDA-induced accumulation is significantly enhanced by the disulfide-reducing agent dithiothreitol (DTT), showing that once the NMDA receptor is...
activated a reducing agent potentiates the response. The reducing agent is thought to act on the redox site of the NMDA receptor (8). Flupirtine and the oxidizing agent 5,5-dithio-bis-2-nitrobenzoate (DTNB) appear to be more effective in antagonizing the influx of $^{45}$Ca$^{2+}$ in the presence of DTT than in the absence of the reducing agent (29). Moreover, the presence of N-ethyl-maleimide, which is thought to alkylate reduced sulphhydryl groups at the NMDA receptor redox site and thus to prevent their subsequent oxidation or reduction (8,13), prevents the effects of DTT, DTNB, and flupirtine on the NMDA-induced influx of $^{45}$Ca$^{2+}$. These data suggest that flupirtine may act as an oxidizing agent at the redox site of the NMDA receptor (29).

Incubation of primary cortical neurons from rat embryos with the excitatory amino acid NMDA or with the human immunodeficiency virus type I (HIV-I) coat protein gp120 results in $> 90\%$ reduction in cell viability and in apoptotic cell death, as determined by appearance of the characteristic step ladder-like DNA fragmentation pattern and by the TUNEL (terminal dUTP-biotin nick end-labeling) technique. The neurotoxic effect of gp120 is at least partly mediated by the activation of phospholipase A2; this results in an increased release of arachidonic acid, which in turn sensitizes the NMDA receptor (17). Preincubation with flupirtine almost completely protects cortical neurons against apoptotic cell death caused by NMDA or gp120 (30). Flupirtine has neuroprotective actions in the same cortical cell against apoptotic cell death caused by lead acetate, the prion agent PrPSc, or the toxic fragment 25-35 of the β-amyloid peptide (17,18,19,31,32,33). In addition, flupirtine protects CD3$^+$, CD4$^+$, and CD8$^+$ lymphocytes against apoptosis induced by the oxygen radical-forming hypoxanthine/xanthine oxidase system (19).

Flupirtine also attenuates apoptotic cell death induced by glutamate or NMDA in human Ntera/D1 (hNT) neurons (32). During glutamate/NMDA-mediated apoptosis the levels of the intracellular antiapoptotic agents Bcl-2 and glutathione drop by more than 50%. Bcl-2 and glutathione are believed to inhibit apoptosis by decreasing the production or increasing the scavenging of reactive oxygen species (6). Flupirtine completely abolished reduction of Bcl-2 and glutathione levels induced by glutamate or NMDA. In light of these data, it has been proposed that flupirtine achieves its antiapoptotic action by increasing the levels of Bcl-2 and glutathione in neurons, thus preventing the oxidative stress caused by glutamate or NMDA (32,33).

Additional evidence for an antiapoptotic action of flupirtine mediated by changes in various anti- or proapoptotic proteins comes from experiments on cultured retinal pigment epithelium (RPE) cells. When RPE cells were subjected to experimental ischemia in a medium free of serum, glucose, and oxygen for 72 h, ~65% of the cells were apoptotic as determined by TUNEL technique and agarose gel electrophoresis. Flupirtine, but not NMDA-receptor antagonists, prevented ischemia-induced apoptosis and caused the level of Bcl-2 protein to rise (28). In contrast, the oncogene proteins for TIAR and ICH-1t were lower in flupirtine-treated cells than in control cells. In addition, flupirtine prevented the induction of reactive oxygen species by iron ascorbate in retinal cells. In conclusion, these results suggest that flupirtine reduces the formation of reactive oxygen species in retinal dissociates and causes changes in various oncogene products in RPE cultures, which may explain its action in preventing ischemia-induced apoptosis (28).
These experimental data (Table 2) suggest that flupirtine might be useful in the treatment of infections of the nervous system, such as AIDS, and prion diseases, or neurodegenerative diseases such as Alzheimer’s disease.

MODE OF NEUROPROTECTIVE ACTION

A large body of studies has suggested that flupirtine, *in vitro* and *in vivo*, has an antagonistic action on neurotransmission and neurotoxicity mediated by NMDA receptors.

Electrophysiological experiments on spinal reflexes in both rats and humans have shown that flupirtine weakens spinal polysynaptic reflexes (37,39,45). These reflexes are mediated by the stimulation of NMDA receptors. In contrast, the same experiments have shown that flupirtine had no influence on monosynaptic spinal reflexes, which are mediated by the stimulation of non-NMDA receptors. Co-administration of NMDA, but not of the non-NMDA-receptor agonist α-amino-3-hydroxy-5-tertbutyl-4-isoxazolepropionic acid (ATPA), abolished the depressant action of flupirtine on polysynaptic spinal reflexes (37,39). This argues in favor of the involvement of NMDA-mediated mechanisms in the observed myorelaxant effects of flupirtine.

Flupirtine can protect *in vitro* primary neuronal cultures against neurotoxicity induced by glutamate (34), as well as that induced by NMDA, prions, or the HIV-1 coat protein gp120 (30,31). Prions and gp120 exert pleiotropic effects on neurons; these effects include activation of NMDA receptors. Moreover, in primary cortical and hippocampal cell cultures, flupirtine reduced significantly the glutamate-induced intracellular Ca\(^{2+}\) overload, which is thought to be mediated mainly by the NMDA receptor (29,34). Finally, changes in GABA immunoreactivity in the rat retina have been used to investigate possible antagonistic actions of flupirtine against excitatory amino acids *in vitro* (25). In these experiments, flupirtine antagonized NMDA-induced changes in GABA immunoreactivity, but not changes induced by kainate (26). All these experiments suggest an antagonistic action of flupirtine on NMDA-mediated mechanisms.

Radioligand-binding studies with rat brain synaptic membranes have demonstrated that flupirtine displaces neither the competitive NMDA antagonist \[^3\text{H}\]CGS-19755 nor the non-competitive NMDA-antagonist \[^3\text{H}\]-MK-801 from their binding sites (see Receptor-Binding Studies).

An alternative possibility is that flupirtine acts downstream of the NMDA receptor complex, modulating intracellular effects of the cascade of NMDA receptor activation and Ca\(^{2+}\) overload in the cell. Indeed, flupirtine is capable of protecting cortical and hippocampal primary neuronal cultures against glutamate-induced neurotoxicity by inducing a dose-dependent reduction of glutamate-induced rise in cytosolic Ca\(^{2+}\) concentration (29,34). Flupirtine inhibits swelling in isolated heart mitochondria, increases Ca\(^{2+}\) uptake, maintains membrane potential, and increases ATP synthesis (49). All these effects provide important evidence for a cytoprotective action of this drug at the mitochondrial level.

An *in vitro* enhancement of basal levels of ATP has also been demonstrated in retinal cells (27). This indicates another possible mechanism whereby the substance could
exert neuroprotective action. In this context, it is relevant to recall that there is evidence that the rundown or sustained opening of the pore of the NMDA receptor can be prevented by ATP (14), suggesting that when intracellular levels of ATP are increased, the opening of the NMDA channel pore caused by an agonist will be counteracted. Thus, a substance that raises the intracellular ATP levels would function not unlike an NMDA antagonist.

Finally, flupirtine prevents glutamate- or NMDA-induced apoptotic cell death of hNT neurons and retinal cells by potently increasing Bcl-2 and glutathione levels and decreasing the levels of oncogene proteins for TIAR and ICH-1t (29,32,33). Since it has been suggested that Bcl-2 inhibits neuronal cell death at a point distal to the in-

<table>
<thead>
<tr>
<th>TABLE 2. In vitro neuroprotective mechanisms of action of flupirtine</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Model</strong></td>
</tr>
<tr>
<td>------------</td>
</tr>
<tr>
<td>Induced apoptosis</td>
</tr>
<tr>
<td>NMDA and HIV gp120 (cortical neurons)</td>
</tr>
<tr>
<td>Prp 105-126 and lead acetate (cortical neurons)</td>
</tr>
<tr>
<td>Glu/NMDA (hNT neurons)</td>
</tr>
<tr>
<td>β-AP 25-35 (cortical neurons)</td>
</tr>
<tr>
<td>Ionomycine and ROS (lymphocytes)</td>
</tr>
<tr>
<td>Bcl-2</td>
</tr>
<tr>
<td>Flupirtine alone</td>
</tr>
<tr>
<td>Flupirtine 2 h post</td>
</tr>
<tr>
<td>Prp 105-126 (cortical neurons)</td>
</tr>
<tr>
<td>Glu/NMDA-induced Bcl-2 decrease (hNT-neurons)</td>
</tr>
<tr>
<td>(hRP-epithelial cells)</td>
</tr>
<tr>
<td>ATP</td>
</tr>
<tr>
<td>(isolated mitochondria from myocardial cells)</td>
</tr>
<tr>
<td>ATP</td>
</tr>
<tr>
<td>ATP</td>
</tr>
<tr>
<td>GSH/antioxidation</td>
</tr>
<tr>
<td>Prp 106-126-induced GSH decrease (cortical neurons)</td>
</tr>
<tr>
<td>Glu/NMDA-induced GSH decrease (hNT-neurons)</td>
</tr>
<tr>
<td>β-AP 25-35-induced GSH decrease (cortical neurons)</td>
</tr>
<tr>
<td>Ascorbate/iron-induced ROS-generation (retinal cells)</td>
</tr>
<tr>
<td>Ca(^{2+}) metabolism (intracellular)</td>
</tr>
<tr>
<td>Glu-induced Ca(^{2+}) increase in cytosol (hippocampal neurons)</td>
</tr>
<tr>
<td>(isolated mitochondria from myocardial cells)</td>
</tr>
<tr>
<td>mitochondrial Ca(^{2+}) uptake ↑</td>
</tr>
</tbody>
</table>
crease in intracellular Ca$^{2+}$ levels (50), the effect of flupirtine on oncogenes might represent another mechanism of action, that is additional to those described above.

BEHAVIORAL PHARMACOLOGY

Tolerance, Dependency and Drug Discrimination

Studies designed to assess the liability of rats administered regular doses of flupirtine to develop dependence have found that flupirtine does not induce physical dependence or possess abuse potential of the opiate type (23,41). This was determined by observing the jumping behavior of mice or by measuring body weight reduction in rats following repeated oral administration. Flupirtine doses equal to or greater than its analgesic dose were given for up to five weeks in these studies. No tolerance to the analgesic activity of flupirtine developed in mice or rats dosed for up to 19 consecutive days. Furthermore, flupirtine did not appear to possess the reinforcing effects commonly reported for opiates, inasmuch as morphine-dependent rhesus monkeys did not self-administer (i.v.) flupirtine at rates exceeding those of placebo (41).

In humans, repeated administration of flupirtine for 18 d did not induce any development of tolerance; in this respect its analgesic activity was comparable to that of codeine (11,23). In a long-term flupirtine administration study in humans (9), patients were treated in a single-blinded manner with placebo for 14 d after a 1-y treatment with flupirtine. No overall tendency of flupirtine toward an increase in the subjective morphine-like dependency symptoms was found in this study. In addition, there were no indications during the study of an euphoria-inducing effect of flupirtine, development of tolerance, or an increase in the daily intake of flupirtine.

Studies in which rats were trained to discriminate have been shown to be very selective behavioral methods for investigating pharmacological properties of drugs that act upon the central nervous system. The study of Swedberg and coworkers (42) demonstrated that flupirtine (10 mg/kg) can function as a discriminative stimulus in rats, and that the duration of action of flupirtine is short inasmuch as a pretreatment interval of 10 min was more effective than one of 30 min in maintaining discriminative control of behavior. It was also shown in the same study that the opioid analgesics pentazocine, codeine, and tramadol all failed to produce flupirtine-appropriate responses, and that the opioid antagonist naltrexone failed to reverse the discriminative effects of flupirtine. The mixed $\alpha_1/\alpha_2$-adrenoceptor agonist clonidine and the highly selective $\alpha_2$-adrenoceptor agonist UK-14304 partially substituted for flupirtine in a dose-dependent manner. On the other hand, the mixed $\alpha_1/\alpha_2$-adrenoceptor antagonist yohimbine partially decreased the amount of flupirtine appropriate responding in a dose-dependent manner. Neither of the $\alpha_1$-adrenoceptor agonists, phenylephrine and ST 587, produced flupirtine-appropriate responses, nor did the $\alpha_1$-adrenoceptor antagonist prazosin antagonize flupirtine responding.

Thus, it seems that the discriminatory effects of flupirtine are mediated primarily by an unknown mechanism and partially by $\alpha_2$ adrenoceptors, not by $\alpha_1$ adrenoceptors or by opioid mechanisms.
Muscle Relaxant Activities

Flupirtine exerts muscle relaxant activities in the awake rat at doses comparable to those exerting analgesic effects (22). Flupirtine reduces the skeletal muscle tone with an ED$_{50}$ = 3.5 mg/kg i.p. and 18 mg/kg p.o. Flupirtine, 5 mg/kg i.p., causes a strong tonus reduction similar to that caused by baclofen (10 mg/kg i.p.), diazepam (2 mg/kg i.p.), or tetrazepam (10 mg/kg i.p.) without inducing benzodiazepine-like dependence. As flupirtine’s myorelaxant action cannot be abolished by the benzodiazepine-receptor antagonist flumazenil, but by the GABA$_A$, antagonist bicuculline, the GABAergic system and not the benzodiazepine binding site has been considered to play a role in the muscle-relaxant property of flupirtine. It has also been suggested that this myorelaxant action is due to an inhibition of both mono- as well as polysynaptic reflexes. With the support of results from patch-clamp experiments (47), it was speculated that GABA$_A$ receptors might be involved by the strengthening of the presynaptic GABAergic inhibition of the motoneurons.

More recent preclinical studies have revealed the first signs of an NMDA-receptor antagonist-like action of flupirtine, however, probably together with an indirect $\alpha_2$-adrenoceptor mechanism for the muscle-relaxant effects. The first evidence for a similarity of flupirtine with NMDA-receptor antagonists in the myorelaxant action was obtained by Schwarz et al. (37,39) in anesthetized rats. They showed that by intraperitoneal or intrathecal (i.th.) administration flupirtine depressed the spinal polysynaptic flexor reflex, which is mediated by NMDA receptors, whereas the monosynaptic Hoffmann reflex (H-reflex), which is mediated by non-NMDA receptors, was not influenced.

Flupirtine (2.5 to 25 $\mu$mol/kg i.p.) reduced the magnitude of the flexor reflex in a dose-dependent manner. This effect appeared within 10 min, reached its maximum 10 to 30 min after injection and lasted for 20 min, 50 min, and > 60 min after doses of 2.5 $\mu$mol/kg, 12.5 $\mu$mol/kg, and 25 $\mu$mol/kg, respectively. Neither dose altered the H-reflex. A comparison of the potency of the muscle-relaxant action of flupirtine with that of antispastic drugs revealed that, after intraperitoneal administration, flupirtine was less potent than the GABAB receptor agonist baclofen, the benzodiazepine diazepam, or the $\alpha_2$-adrenoceptor agonist tizanide, but was as potent as the GABA$_A$ receptor agonist muscimol and the non-competitive NMDA-receptor antagonist memantine (39).

Flupirtine (33 to 330 nmol i.th.) reduced the flexor reflex in a dose-dependent manner without affecting the H-reflex (Fig. 3). This effect was highest 10 min after injection and lasted for 40 min and more than 60 min after injection of 165 nmol and 330 nmol, respectively. After intrathecal injection, the central muscle-relaxant potency of flupirtine is weaker than that of the GABA-receptor agonists baclofen or muscimol, the non-NMDA-receptor antagonist DNQX, and the benzodiazepine midazolam, but it is comparable in potency with the NMDA antagonists AP7, memantine, and dextromethorphan. The depressant action of flupirtine on the polysynaptic flexor reflex can be prevented by intrathecal co-administration of the excitatory amino acid NMDA (0.1 nmol) or the mixed $\alpha_1/\alpha_2$-adrenoceptor antagonist yohimbine (10 nmol), but not by co-application of bicuculline or the AMPA-antagonist ATPA (39).
Fig. 3. Effect of intrathecal injection of solvent or different doses of flupirtine (33, 165, 330 nmol) on the monosynaptic Hoffmann-reflex and on the polysynaptic flexor reflex \( n = 6 \) to 10) in anaesthetized rats. Magnitude of the reflexes are expressed as a percentage of a respective preinjection value (mean ± S.E.M.). Significances versus injection of solvent: \(^* P < 0.01 \) \(^{**} P < 0.001\), Mann–Whitney U-test. Reprinted with permission from Elsevier Science from reference 3: Block F, Pergande G, Schwarz M. Flupirtine protects against ischemic retinal dysfunction in rats. *NeuroReport* 1994;5:2630–2632.
α₂-Adrenoceptor antagonists, such as yohimbine, can diminish the action of flupirtine on the flexor reflex. As flupirtine has no pharmacologically relevant affinity for α₁ and α₂ adrenoceptors, however, the α₂-adrenergic system seems to be only indirectly involved (44).

The results in rats presented above have been corroborated by clinical-neurophysiological investigations in humans (45). Two hours after intake of a single oral dose (400 mg) of flupirtine, healthy human subjects responded with a significant reduction of both the early phase of the electrically elicited polysynaptic flexor reflex (F1) of pretibial muscles and the medium latency response (ML) in dynamic posturography of the toe-up paradigm. Once again, no effect on the H-reflex of the soleus muscle was found in humans.

As flupirtine possesses analgesic as well as muscle-relaxing effects in similar dose ranges, this drug might be useful in the treatment of painful diseases of the motor system that involve increased muscle tone, such as spasticity and chronic musculoskeletal back pain (48).

**Antiparkinsonian Effects**

On the basis of the above-mentioned antagonistic effect of flupirtine on neurotransmission mediated by NMDA, and also of the clinical safety of this drug, the question has been raised whether flupirtine shares the antiparkinsonian effect of NMDA-receptor antagonists, making it of possible value as an adjunct to L-DOPA in Parkinson’s disease. As most patients receiving L-DOPA therapy for some years experience fluctuations in their motor functions, the development of new therapeutic strategies which reduce the requirement for dopamine-replacing drugs was an important research aim.

Rats were pretreated with reserpine and α-methyl-p-tyrosine methylester hydrochloride (α-MT), which produce akinesia and muscular rigidity by depletion of monoamine pools and blockage of dopamine synthesis. In this model, flupirtine (1 to 20 mg/kg i.p.) given alone strongly reduced muscle rigidity (38). This effect was greatest after 10 min and lasted for 2 h without any effect on akinesia. In combination with L-DOPA, flupirtine increased the ability of L-DOPA to reverse akinesia and to alleviate muscular hind-limb rigidity. This latter effect of flupirtine agrees well with the ML response and F1 effect (see Muscle Relaxant Activities). Rigor was decreased under the influence of the drug (45), indicating anti-rigor activity of flupirtine in dopamine-deficiency states such as Parkinson’s disease. Only a very small dose of flupirtine (~10% of the ED₅₀ of the muscle-relaxant or analgesic action) is sufficient to potentiate the effect of L-DOPA against hind-limb rigidity in monoamine-depleted rats (38).

In another model of Parkinson’s disease, haloperidol-induced catalepsy of the rat (36), the antiparkinsonian and motor effects of flupirtine were tested. Flupirtine, alone and in combination with a sub-threshold dose of L-DOPA, exerted potent anticataleptic effects in the haloperidol-induced catalepsy. When given alone, it showed a dose-response profile similar to that found with NMDA-receptor blockers: at 1 mg/kg p.o. flupirtine was ineffective, whereas at higher doses (10 and 20 mg/kg p.o.) it exerted a strong anticataleptic effect in the podium, bar, and grid tests. In combination with a sub-threshold 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 p.o.) synergistically interacted with L-DOPA and a small increase in this effect was found at 10 mg/kg p.o. Flupirtine 20 mg/kg p.o. abolished the slight antica taleptic effect of L-DOPA. This reversal of catalepsy by flupirtine may be mainly due to a reduction in muscle tone, and not to psychomotor stimulant effects, since flupirtine did not induce marked locomotor activity on its own (36,38).

In order to reveal eventual psychomotor stimulating effects, the influence of flupirtine on spontaneous and D-amphetamine-induced behavior in the open field with holeboard and in sniffing behavior was investigated. Flupirtine had no marked effect on horizontal locomotor activity in intact rats. It did not change spontaneous locomotion in the open field with a holeboard. Head dips into the holes were even enhanced by flupirtine 1 and 10 mg/kg p.o., indicating stimulation of explorative behavior (36). This result is in good accordance with the findings of Schwarz et al. (38), who reported that flupirtine at doses up to 10 mg/kg i.p. has no locomotor-stimulating effect, while a high dose of flupirtine (20 mg/kg i.p.) even suppressed locomotion because of its muscle-relaxant effect. In combination with D-amphetamine locomotion was not changed; the lower dose of flupirtine attenuated head dipping whereas the higher dose enhanced it (36). Rearing was influenced only slightly, suggesting that no gross motor disability was induced. Sniffing behavior was assessed in the experimental chamber designed to prevent rats from forward locomotion (35). Sniffing increased only at the lower dose of flupirtine, showing a synergistic effect, especially in potentiating the D-amphetamine-induced sniffing-head up component (36).

Although flupirtine stimulates sniffing behavior at the lower dose, its behavioral profile is completely different from those of the non-competitive NMDA-receptor antagonists such as dizocilpine, and flupirtine is free of the prominent side effects of non-competitive NMDA-receptor antagonists (e.g., ataxia and psychomotor stimulation). The reported behavioral effects of flupirtine (36) share some similarities with those of competitive NMDA-receptor antagonists or strychnine-insensitive glycine-site NMDA-receptor antagonists, which also failed to produce a pronounced stimulation of locomotion and stereotypy (12). Since flupirtine did not cause locomotor stimulation in normal rats in either study, the possibility that anti-akinetic actions of this drug can be ascribed to a nonspecific hypermotility can be excluded. Accordingly, in humans, flupirtine revealed a sedative rather than a psychomotor stimulant effect (21).

Summarizing the behavioral data, flupirtine alone and in combination with a subthreshold dose of L-DOPA exerted potent antica taleptic effects in the hypodopaminergic rat. A bell-shaped dose-response profile was seen, especially in combination with dopaminomimetic drugs such as L-DOPA and D-amphetamine. From its profile in the animal experiments described, it may tentatively 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.
CONCLUSIONS

Although there are striking similarities between flupirtine and NMDA-receptor antagonists, flupirtine is devoid of the typical side effects of these drugs. It does not induce ataxia or psychomotor stimulation on its own, but it reverses rigidity and akinesia in dopamine-deficient animals. It also synergizes with L-DOPA, and thus may have some antiparkinsonian potential in humans. In addition, flupirtine exerts potent cyto- and neuroprotective actions in different \textit{in vivo} and \textit{in vitro} models: it counteracts the effects of focal cerebral ischemia in mice, global cerebral ischemia in rats, and retinal ischemia in rats and rabbits. In \textit{in vitro} preparations, flupirtine protects against neurotoxicity induced by NMDA or gp120, as well as against NMDA-induced changes in GABA immunoreactivity in the rabbit retina.

While NMDA-receptor antagonists reduce Ca$^{2+}$ influx by blocking the channel or the receptor, flupirtine neither blocks NMDA receptors nor shows significant binding to any other receptor tested so far. Very recent data indicate that flupirtine counteracts Ca$^{2+}$ increase in challenged cells, possibly by facilitation of Ca$^{2+}$ transport into mitochondria (49). Other mechanisms, such as stimulation of ATP formation and/or upregulation of Bcl-2 expression, may contribute to the pharmacological effects of flupirtine. However, the data available indicate that flupirtine may act by a previously unknown mechanism.

REFERENCES


