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# Electrophysiological actions of felbamate on rat striatal neurones

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**1** We have investigated the effects of the anticonvulsant drug, felbamate (FBM), on striatal neurones, recorded *in vitro* by using both intracellular and extracellular conventional recordings in slices and whole-cell recordings in acutely isolated neurones.

**2** FBM, at therapeutically relevant concentrations (30-300 $\mu$M) showed multiple mechanisms of action. Like other antiepileptic drugs, FBM (30-300 $\mu$M) showed a direct inhibitory action on current-evoked firing discharge of striatal neurones. A patch-clamp analysis of this effect revealed a dose-related reduction of voltage-dependent sodium ($Na^+$) currents (10-100 $\mu$M), with a half inhibition dose ($IC_{50}$) value of 28 $\mu$M.

**3** We also tested whether FBM affected corticostriatal glutamatergic transmission. In control medium (1.2 mM external magnesium), both extracellularly recorded field potentials and intracellularly recorded excitatory postsynaptic potentials (e.p.s.ps) evoked by cortical stimulation were not affected by bath application of 30-300 $\mu$M FBM.

**4** When magnesium was removed from the perfusing solution, a procedure which reveals a N-methyl-D-aspartate (NMDA)-mediated component in the corticostriatal synaptic potential, FBM (30-300 $\mu$M) produced a dose-dependent reduction of the amplitude of both the field potential and the e.p.s.p.

**5** FBM reduced the inward currents produced either by bath or by focal applications of 30 $\mu$M NMDA, a finding consistent with the hypothesis that the observed reduction of the NMDA-mediated component of the synaptic potentials may be caused at postsynaptic level.

**6** The reduction of the NMDA-mediated component of the synaptic transmission by FBM and its depressant effect on the voltage-dependent $Na^+$ channels, may account for the antiepileptic action of this drug. Moreover, the pharmacological properties of FBM might render this drug interesting as a neuroprotectant agent.

**Keywords:** Felbamate; antiepileptic drugs; synaptic potentials; patch-clamp; striatum; excitatory amino acids; NMDA; sodium channels

## Introduction

FBM (2-phenyl-1,3-propanediol-dicarbamate), is a new antiepileptic drug, which has been shown to be effective in the treatment of partial onset seizures and in the Lennox-Gastaut syndrome (The felbamate study group in Lennox-Gastaut syndrome, 1993). FBM has been demonstrated to be efficacious in a large spectrum of *in vivo* epilepsy animal models. In rodents, FBM inhibits seizures induced by maximal electroshock, by picrotoxin, pentylentetrazol and 4-aminopyridine, but not by bicuculline or strychnine (Swinyard *et al.*, 1986; for review see Burdette & Sackellares, 1994). In Rhesus monkeys, FBM reduced seizures produced by aluminium hydroxide injections into cortical regions (Lockard *et al.*, 1987). Moreover FBM antagonizes seizures induced by N-methyl-D-aspartate (NMDA) and kainate-induced status epilepticus (White *et al.*, 1992; Chronopoulos *et al.*, 1993). Most common anticonvulsants affect $Na^+$ channels, calcium channels, and/or the $GABA_A$-benzodiazepine receptor complex. Investigations on the mechanism of action of FBM demonstrated that this drug has a unique pharmacological profile: in fact an action on voltage-dependent $Na^+$ channels has been described (White *et al.*, 1992). However, its primary antiepileptic effect appears to be at the NMDA receptor channel complex, where FBM displaces 5,7-[$^3$H]-dichlorokynurenate from the strychnine-insensitive glycine binding site (McCabe *et al.*, 1993). This effect has been demonstrated also in human postmortem brains (Wamsley *et al.*, 1994). In whole-cell recordings from cultured hippocampal neurones, FBM blocked NMDA responses. The same group reported a potentiation of $GABA_A$ responses by FBM (Rho *et al.*, 1994); however a clear action on GABA receptors is controversial (Ticku *et al.*, 1991; Domenici *et al.*, 1994). Furthermore, an action of FBM on non-NMDA glutamate receptors has been described (De Sarro *et al.*, 1994; Domenici *et al.*, 1994).

Experimental evidence emerged in favour of a neuroprotectant action of this drug. In *in vivo* models, FBM reduced the area of infarction following bilateral carotid ligation (Wasterlain *et al.*, 1992). When tested *in vitro* against hypoxic insults in the CA1 region of the hippocampus, FBM showed a marked neuroprotection (Wallis *et al.*, 1992).

In an attempt to elucidate further the mechanism of action of FBM, we studied the electrophysiological effects of this drug on striatal neurones, recorded either from a corticostriatal brain slice preparation or from acutely isolated cells. Corticostriatal fibres represent the major excitatory input to the neostriatum (Reubi & Cuenod, 1979). Overactivity of excitatory pathways within the basal ganglia has been thought to play an important role in the pathogenesis of neurodegenerative disorders such as Huntington's disease and Parkinson's disease (Turski & Turski, 1993). The pharmacological modulation of excitatory amino acid transmission in the basal ganglia might be of interest for the treatment of such disorders. Our findings suggest that FBM might deserve evaluation for its pharmacological properties as a neuroprotectant agent.

## Methods

### *Preparation and maintenance of the slices and of isolated cells*

Adult male Wistar rats (200-250 g) were used for all the experiments. Details of the preparation and maintenance of the

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slices have been previously described (Calabresi *et al.*, 1991). Briefly, rats were killed by a heavy blow to the chest under ether anaesthesia, which severed major blood vessels. The brain was quickly removed and coronal slices (200–300 μm thick) including cortex and neostriatum were prepared from tissue blocks with the use of vibratome. A single slice was then transferred to a recording chamber and continuously perfused with a solution (36°C, 2–3 ml $min^{-1}$) containing (in mM): sodium chloride (NaCl) 126, potassium chloride (KCl) 2.5, monosodium acid phosphate ($NaH_2PO_4$) 1.2, magnesium chloride ($MgCl_2$) 1.2; calcium chloride (CaCl) 2.4, glucose 10 and sodium carbonate ($NaHCO_3$) 26; the solution was gassed with a mixture of $O_2$ (95%) and $CO_2$, (5%). In some experiments $MgCl_2$ was omitted from the solution.

For whole-cell recordings, isolated striatal cells were used. Preparation and maintenance of acutely isolated striatal cells have been described in detail by Stefani *et al.* (1994). In brief, coronal slices, 450 μm thick, were incubated in a HEPES-buffered Hank's balanced salt solution (HBSS), bubbled with 100% $O_2$ and warmed at 34°C. One hour later one slice was transferred to HBSS medium supplemented with 1.5 mg $ml^{-1}$ protease XIV. After enzymatic treatment, the tissue was rinsed and mechanically triturated. The cell suspension was finally placed in a Petri dish mounted on a stage of an inverted microscope (Nikon).

### *Electrophysiological studies*

Extracellular recording electrodes were filled with 2 M NaCl; intracellular recording electrodes were filled with either 2 M KCl or 2 M K-acetate (30–60 MΩ). For synaptic stimulation, bipolar electrodes were used. These were placed in the cortex, close to the recording electrode, or in the white matter between the cortex and the striatum. The field potential amplitude was defined as the average of the amplitude from the peak of the early positivity to the peak negativity, and the amplitude from the negativity to the peak late positivity (Alger & Teyler, 1976). During voltage-clamp experiments headstage voltage was monitored continuously. An Axoclamp 2A amplifier was used for recordings. Traces were displaced on an oscilloscope and

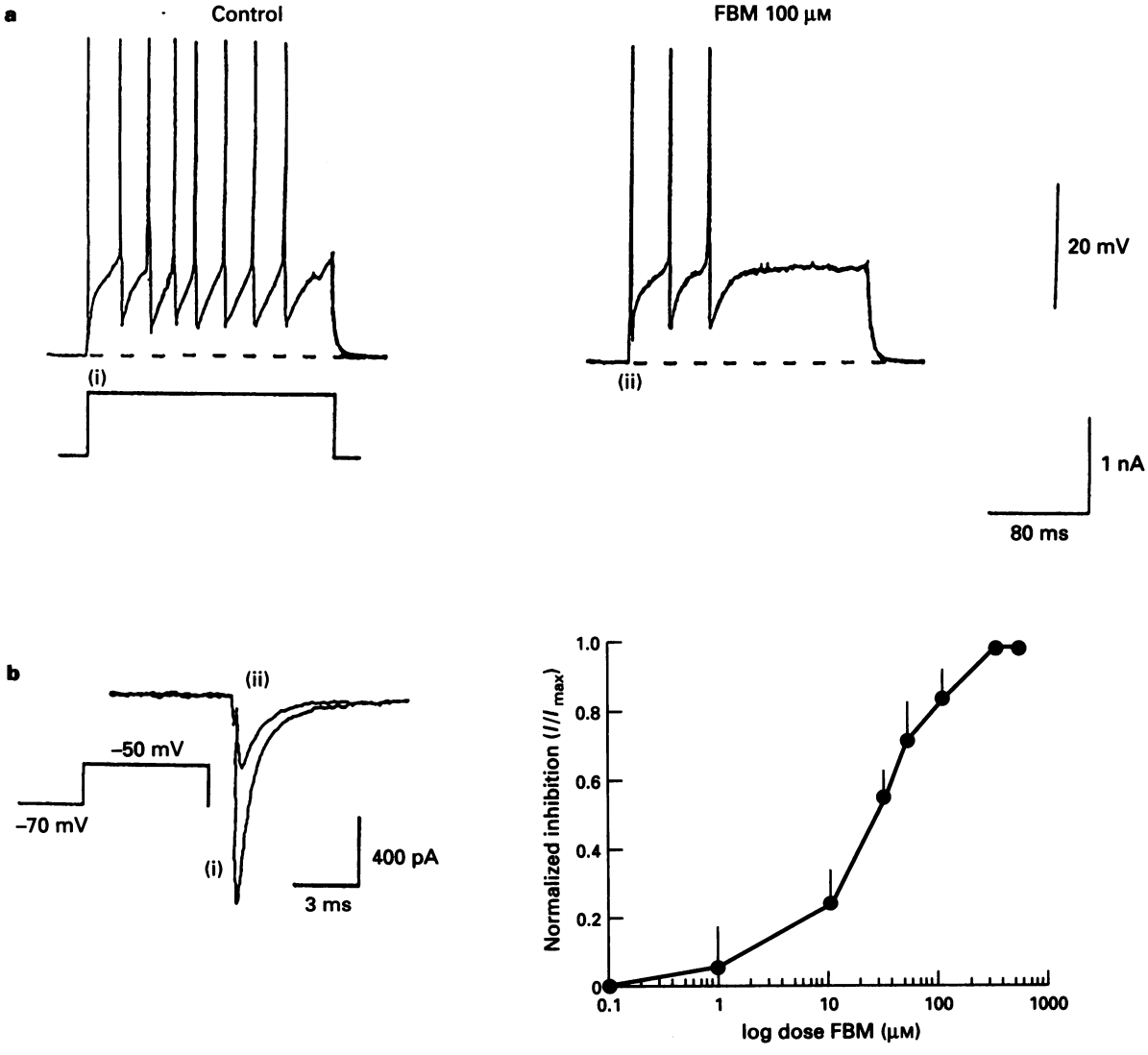



**Figure 1** Felbamate (FBM) reduces repetitive firing discharge and inhibits $Na^+$ currents in striatal neurones (a(i)). A depolarizing current-pulse (+1 nA intensity) induced a tonic firing discharge in a striatal neurone recorded from a corticostriatal slice preparation (RMP −80 mV) (a(ii)). In the presence of FBM 100 μM, the firing frequency evoked by the depolarizing step was reduced. Note that the action potentials were partially truncated by digital plotting (a). Effect of FBM on the $I_{Na}$ of a striatal neurone. On the left, the currents were activated by depolarizing step pulses to −20 mV (from a holding potential of −70 mV): control (b(i)) and FBM 50 μM (b(ii)). The FBM-mediated inhibition was 60%. On the right, dose-response curve for the inhibitory action of FBM on the peak amplitude of $I_{Na}$. Each point represents the mean ± s.e.mean relative amplitude of 3 experiments. The $IC_{50}$ for FBM action was 28 μM.

stored on a digital system. Values given in the text and in the figures are expressed as mean ± s.e.mean. The statistical significance of the experiments was evaluated with the use of Student's $t$ test. Drugs were bath-applied by switching the superfusing solution to one containing known concentrations of drugs. In some experiments NMDA was applied by ejecting a few nanolitres of a 500 $\mu$M solution from the tip of a blunt pipette beneath the surface of the superfusing solution and just above the tissue slice.

Patch-clamp recordings in the whole-cell configuration were performed with fire-polished pipettes (Corning 7052), with impedance from 3 to 8 MΩ. Bath as well as dialysing solutions were designed to separate clearly the voltage-dependent tetrodotoxin (TTX)-sensitive $Na^+$ current ($I_{Na}$). Pipettes were filled with an internal solution consisting of (in mM): EGTA 10, $TrisPO_4$ 72, Tris base 41, magnesium 4, phosphocreatine 20, ATP 2–4, GTP 0–0.2 , leupeptin 0.2; pH was adjusted to 7.3 with phosphoric acid; the osmolarity was 275–285 mOsm $l^{-1}$. After obtaining the whole-cell configuration, cells were bathed in a medium composed of (in mM): HEPES 10, NaCl 30–60, TEA 140–100, calcium 2, magnesium 1, cadmium 0.4. Control as well as drug solutions were applied with a linear array of six, gravity-fed capillaries positioned within 500 $\mu$m of the patched neurone. Recordings were made with an Axopatch 1D at room temperature. Series resistance compensation (70–80%) was employed. Data were low-pass filtered (corner frequency = 5 kHz). For data aquisition and analysis pClamp 55.1 running on PC486 was used.

### *Sources and handling of compounds*

Compounds were obtained from Sigma (St. Louis, U.S.A.) with the exception of FBM (2-phenyl-1,3-propaneidol-dicarbamate) which was a gift from Schering-Plough (Milano,

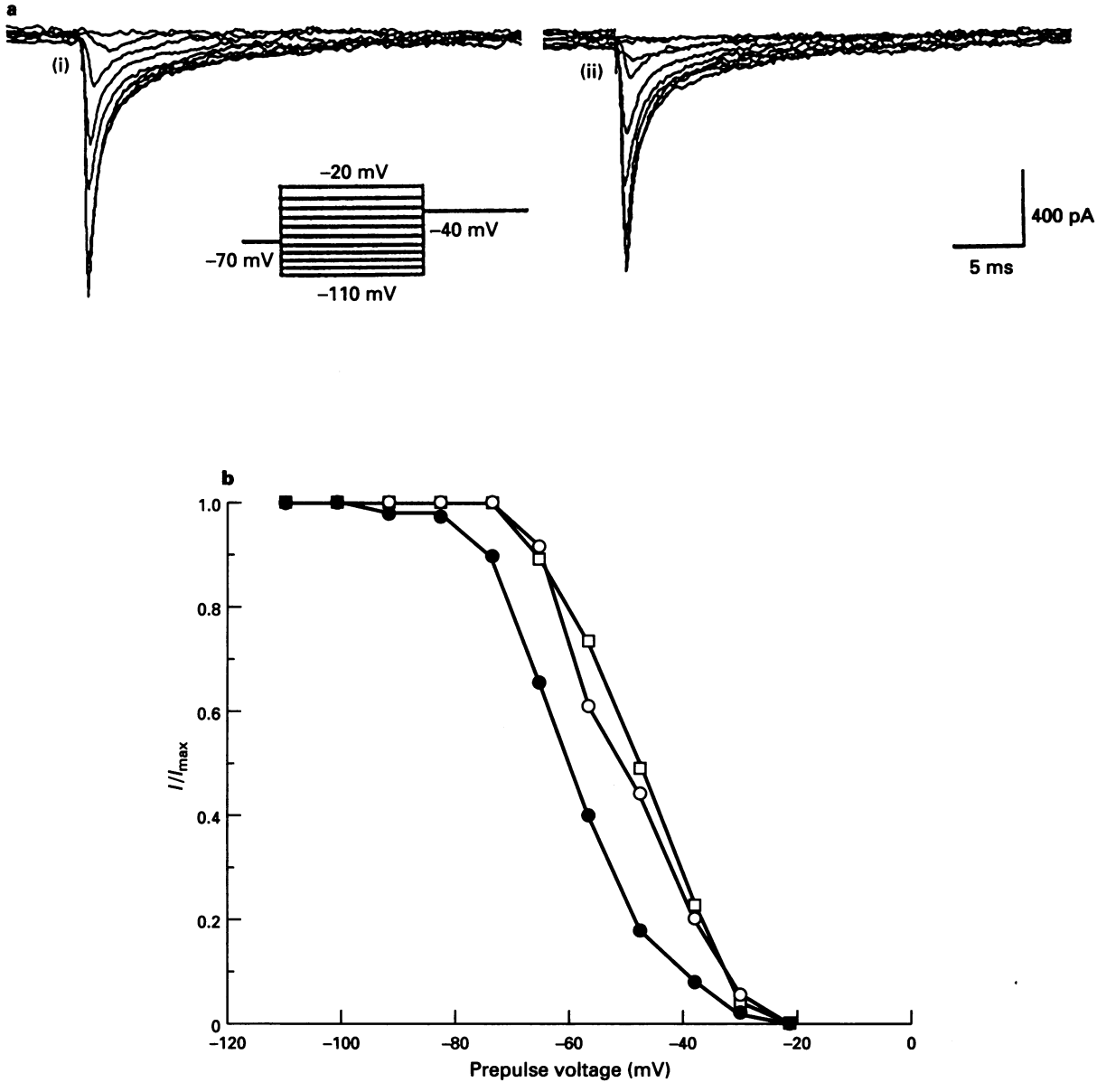



**Figure 2** Effect of felbamate (FBM) on the steady-state inactivation curve of $Na^+$ channels. (a) Traces represent currents activated by step depolarizations to −40 mV from progressively less polarized potentials (from −110 to −20 mV), both in control condition (a(ii)) and in the presence of 30 $\mu$M M FBM (a(ii)). (b) The data points were collected as normalized current amplitude (vertical axis) at −20 mV following the corresponding conditioning voltage step (abscissa scale): (○) Control; (●) FBM 30 $\mu$M; (□) wash. The curves were well fitted according to the Boltzmann equation $I = 1/1 + \exp [V \text{ conditioning} - V_{1/2}/V_h]$ where $V_{1/2}$ is the conditioning voltage at which $I_{Na}$ is half maximal. The slope factor was 7.29 in control condition, 7.22 in the presence of FBM, 7.4 after FBM wash.

Italy). FBM was dissolved in dimethylsulphoxide (DMSO) in a 100 mM stock solution, and diluted in perfusion medium to the final concentration immediated prior to use. The highest concentration of DMSO in final working solution was about 0.3%. Experiments with this concentration of the vehicle were performed in all the configurations described. In none of these did DMSO produce significant effects on intrinsic membrane properties or on synaptic potentials amplitude (data not shown). The limited solubility of FBM precluded the possibility of testing higher concentrations of this drug. In fact, bath-application of 1 mM FBM, or the corresponding volume of vehicle, induced an irreversible membrane depolarizing. During intracellular recordings, performed in the voltage-clamp mode, TTX 1 μM was applied throughout the recording in order to avoid contamination of the inward current by voltage-activated $Na^+$ conductances.

## Results

### *Electrophysiological characteristics of the recorded cells and action of FBM on firing activity and $Na^+$ current*

Conventional current- and voltage-clamp recordings were obtained from 56 electrophysiologically identified 'principal' striatal neurones. The characteristics of these cells have been described in detail previously (Calabresi *et al.*, 1990). These neurones showed high resting membrane potential (RMP,

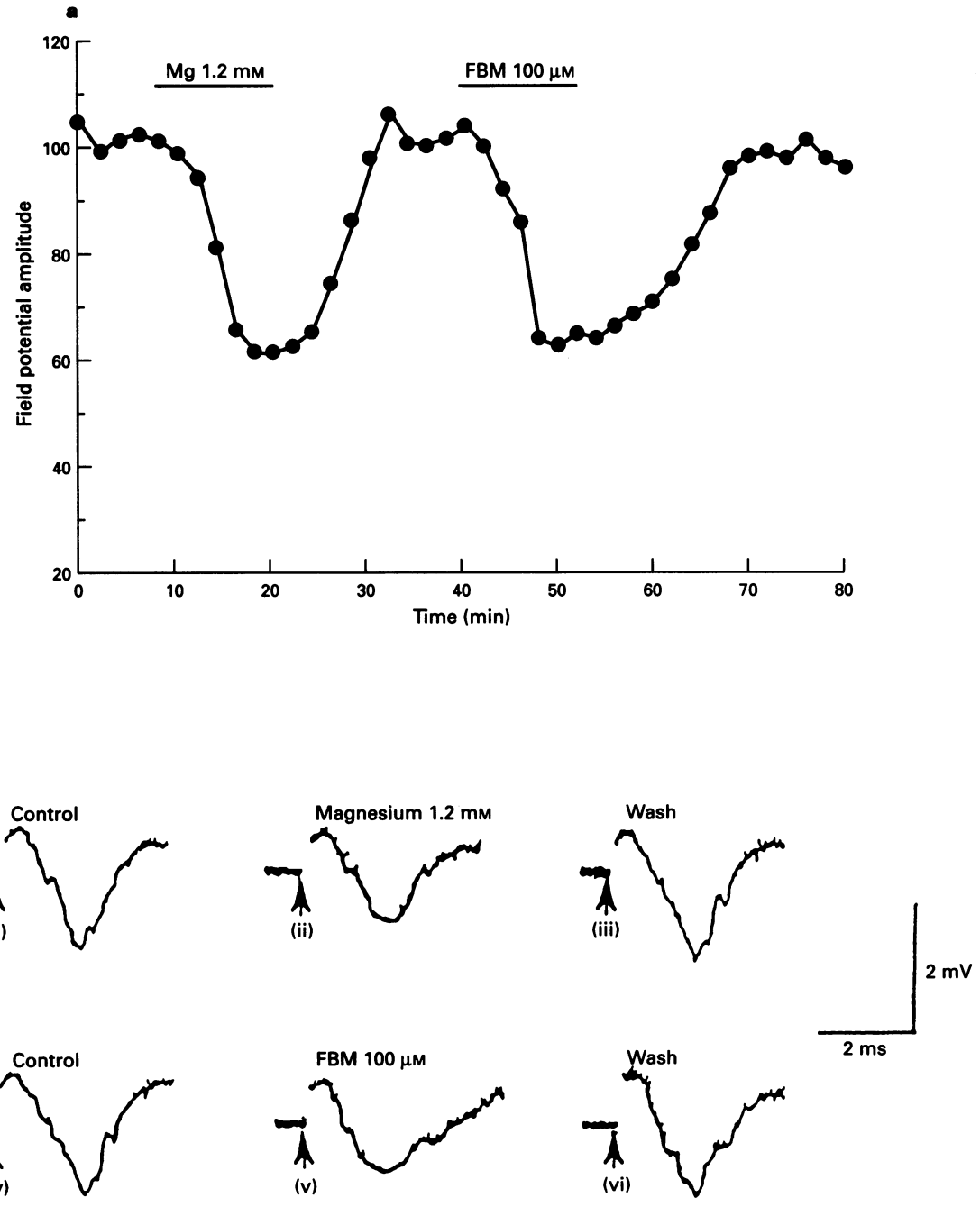



**Figure 3** In a magnesium-free solution the reduction in the field potential amplitude produced by adding 1.2 mM magnesium to the bathing solution is mimicked by 100 μM felbamate (FBM). In (a) the graph shows the time-course of a single experiment. Bath-application of a 1.2 mM magnesium containing solution produced a reduction of the field potential amplitude. After wash-out of magnesium, application of 100 μM FBM induced an inhibition which mimicked the effect of magnesium. In (b) the traces represent the different phases of the experiment: control in a magnesium-free solution (i), in the presence of 1.2 mM magnesium (ii), wash in the absence of magnesium (iii), (iv), 100 μM FBM (v), wash FBM (vi).

$-80 \pm 5$ mV), absence of spontaneous action potential discharge, tonic firing activity during current-induced membrane depolarization. Morphological studies suggest that these cells are medium-sized spiny neurones which are the large majority of neuronal population in the striatum (Groves, 1983) and represent the GABAergic output drive towards globus pallidus and substantia nigra.

As shown in Figure 1a, depolarizing current pulses (+1 nA intensity, 200 ms duration) evoked a sustained action potential discharge with no detectable accomodation (Figure 1a(i)). As shown in Figure 1a(ii), bath application of 100 $\mu$M FBM reduced the current-evoked firing activity of striatal neurones ($n=13$). Interestingly, in most of the recorded neurones, the first action potentials occurring in the initial phase of the current-induced membrane depolarization were not inhibited. In 3 out of 13 cells only a partial recovery was observed. These findings suggested that FBM, as already described for other anticonvulsants, modulates the voltage-dependent $I_{Na}$. In order to test this possibility, we isolated $I_{Na}$ in whole-cell recordings from acutely dissociated medium-sized spiny neurones. This current was completely blocked by nanomolar concentrations (0.3–0.6 $\mu$M) of TTX ($n=10$, data not shown). Figure 1b shows that FBM modulates the leak-subtracted $I_{Na}$ (−60%, Figure 1b(ii)). In all the neurones tested ($n=30$), FBM (0.1–100 $\mu$M) reduced the peak $I_{Na}$. As revealed by the dose-response curve (1b), the $IC_{50}$ for this effect was 28 $\mu$M.

The action of FBM on $I_{Na}$ was further investigated by steady-state inactivation protocols (Figure 2a). The neurones was clamped at progressively less polarized potential (from −110 to −20 mV, as indicated in Figure 2b) and finally tested with the test pulse at −40 mV. $I_{Na}$ was normalized to the current induced by the prepulse to −110 mV. As shown in Figure 2b, 30 $\mu$M FBM shifted the inactivation curve towards more negative values by 10.4 mV, without clear modification of the slope. A full recovery of the FBM response was obtained (Figure 2b). Analogous findings were observed in 5 neurones ($-11 \pm 3$ mV).

### *Effects of FMB on cortically-evoked synaptic potentials*

In control medium, synaptic potentials evoked by cortical stimulation have been shown to be completely blocked by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonists (Calabresi *et al.*, 1992). However, when magnesium is omitted from the external solution, a NMDA-mediated component is unmasked by the removal of the voltage-dependent blockade of this ion on the NMDA receptor-channel complex (Calabresi *et al.*, 1992). In this condition, re-application of a 1.2 mM magnesium in the medium significantly reduced ($-39 \pm 3\%$, $n=4$; $P<0.005$) the amplitude of extracellularly recorded field potential (Figure 3 compare b(i) with b(ii)). This effect was fully reversible (Figure 3b(iii)) and was mimicked by the NMDA antagonist, APV (50 $\mu$M, $n=4$; data not shown).

In control conditions (1.2 mM magnesium), FBM (30–300 $\mu$M) did not affect the amplitude of both field potentials (Figure 4a) and e.p.s.ps (Figure 5a). However, in the absence of magnesium, FBM decreased the amplitude of field potential (Figure 4) and e.p.s.p. (Figure 5) in a dose-dependent fashion. This inhibition was fully reversible in most of the experiments after a period of wash lasting at least 15 min (Figure 4c(iii)). In some experiments only a partial recovery was observed after 30 min wash (Figure 5c(iii)). The maximal inhibition of the synaptic potentials was reached with 100 $\mu$M FBM. These concentrations of FMB caused neither changes of the membrane potential, nor modifications of the current/voltage relationship at subthreshold levels (data not shown). As shown in Figure 3, a saturating concentration of FBM (100 $\mu$M) produced the same percentage inhibition in the field potential amplitude as was caused by 1.2 mM magnesium. In the absence of magnesium an increase in the synaptic potential amplitude was observed. For this reason in most of the experiments, the intensity of the stimulation was reduced, in order to have a subthreshold e.p.s.p. before the application of FBM. In a magnesium-free solution, 'epileptic' discharge activity was not detected from striatal slices. This may be partially explained by the effectiveness of hyperpolarizing potassium conductances in striatal neurones (Calabresi *et al.*, 1987).

### *Effects of FBM on NMDA-induced responses in voltage-clamped striatal neurones*

In order to characterize the effect of FBM at the postsynaptic level, we tested whether FBM affected the postsynaptic sensitivity to NMDA of voltage-clamped striatal neurones recorded from slices. These experiments were performed in the presence of magnesium and of 1 $\mu$M TTX in the bathing solution. NMDA was either bath-applied or locally administered by local pressure ejection. As shown in Figure 6a, in the presence of magnesium, in all the tested neurones (6 out of 6), 100 $\mu$M FBM caused a significant reduction ($-24 \pm 3\%$; $P<0.001$) of

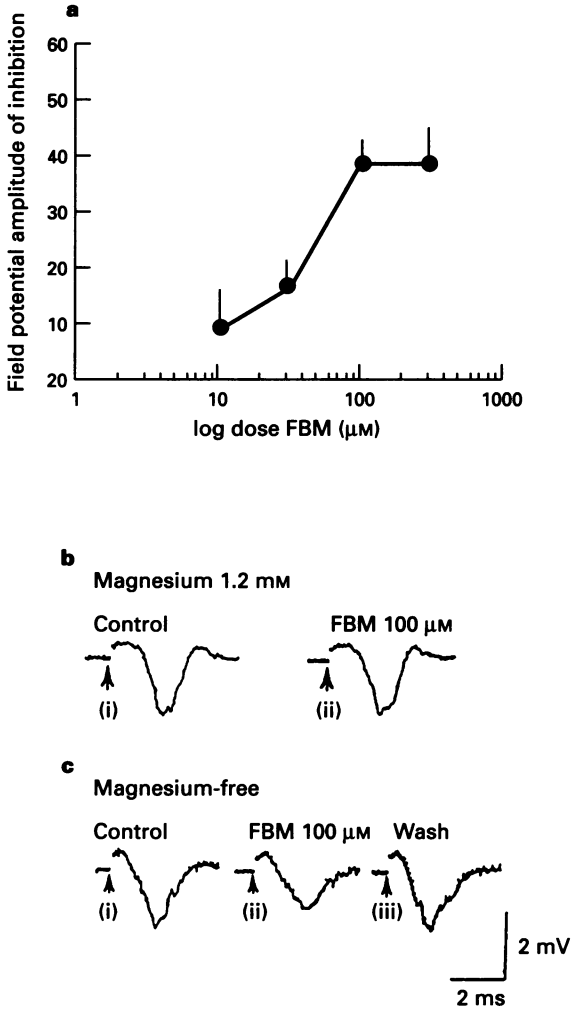
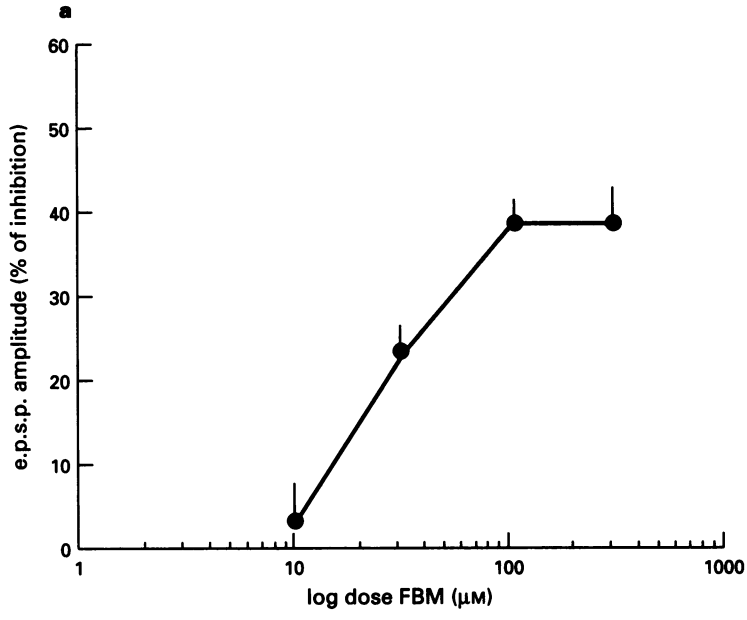



**Figure 4** Effects of felbamate (FBM) on the amplitude of extracellulary recorded field potentials. The graph in (a) shows that, in the absence of magnesium, increasing doses (10–300 $\mu$M) of FBM produced a dose-dependent decrease in the amplitude of field potentials, reaching a plateau at 100 $\mu$M. Each point represents the mean (± s.e.mean) of several experiments (10 $\mu$M, $n=4$; 30 $\mu$M, $n=5$; 100 $\mu$M, $n=7$; 300 $\mu$M, $n=4$). In (b) and (c) the traces represent the field potentials (each trace is the average of four sweeps) recorded in the presence of magnesium (b) and when magnesium was omitted (c). Note that 100 $\mu$M FBM had no effect on field potential amplitude in a magnesium-containing solution (b(i),(ii)), while when magnesium was omitted a clear decrease in the field potential amplitude was observed (b(i)–(iii)). Calibrations apply for both (b) and (c).

the inward current caused by pressure ejection of NMDA (500 $\mu$M, Figure 6a(ii)). This effect was completely reversed after 15 min FBM wash (Figure 6a(iii)). Figure 6b shows that, in the presence of magnesium in the perfusing medium, the inward current induced by bath application of NMDA (30 $\mu$M, $n=8$) was also reduced by 100 $\mu$M FBM ($-29\pm4\%$, $n=5$; $P<0.001$) (Figure 6b(ii)). In some of the recorded neurones (4 out of 8) only a partial recovery was observed after 15 min wash (Figure 6b(iii)). Experiments were performed in order to evaluate the effect of FBM (100 $\mu$M) on the current-voltage relationship of the recorded neurones. In none of the tested cells ($n=4$), did FBM significantly affect the current-voltage plot (data not shown).

## Discussion

The present study provides evidence that FBM limits the firing activity of striatal neurones, by acting directly on voltage-dependent $Na^+$ currents. In addition, FBM blocks the NMDA



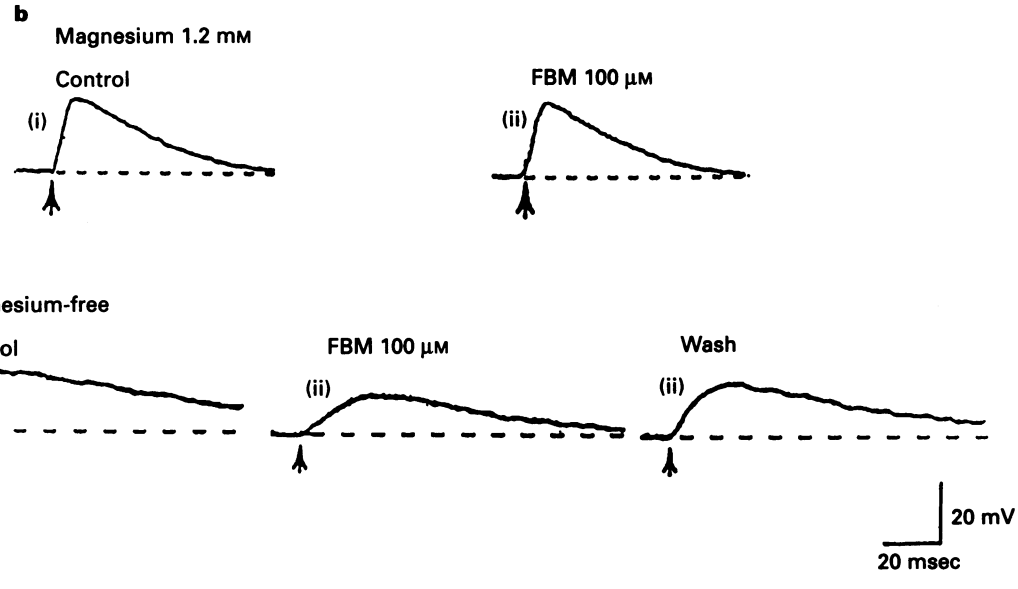




**Figure 5** Actions of felbamate (FBM) on the amplitude of cortically-evoked excitatory postsynaptic potentials (e.p.s.ps). The graph in (a) shows the dose-dependent reduction of the amplitude of intracellulary recorded e.p.s.p. in a magnesium-free bathing medium. The effect increased in a dose-dependent fashion, reaching a plateau at 100 $\mu$M. Each point represents the mean ($\pm$s.e.mean) of several experiments (10 $\mu$M, $n=4$; 30 $\mu$M, $n=4$; 100 $\mu$M, $n=7$; 300 $\mu$M, $n=3$). The traces in the lower part represent the e.p.s.ps (each trace is the average of four sweeps) recorded in the presence of 1.2 mM magnesium (b) and when magnesium was omitted (c). In control conditions no effect of FBM was observed on e.p.s.p. amplitude (b(i),(ii)). When magnesium was removed from the bathing solution, 100 $\mu$M FBM reduced the e.p.s.p. amplitude, and only a partial wash-out was observed (c(i)–(iii)). Calibrations apply for both (b) and (c).

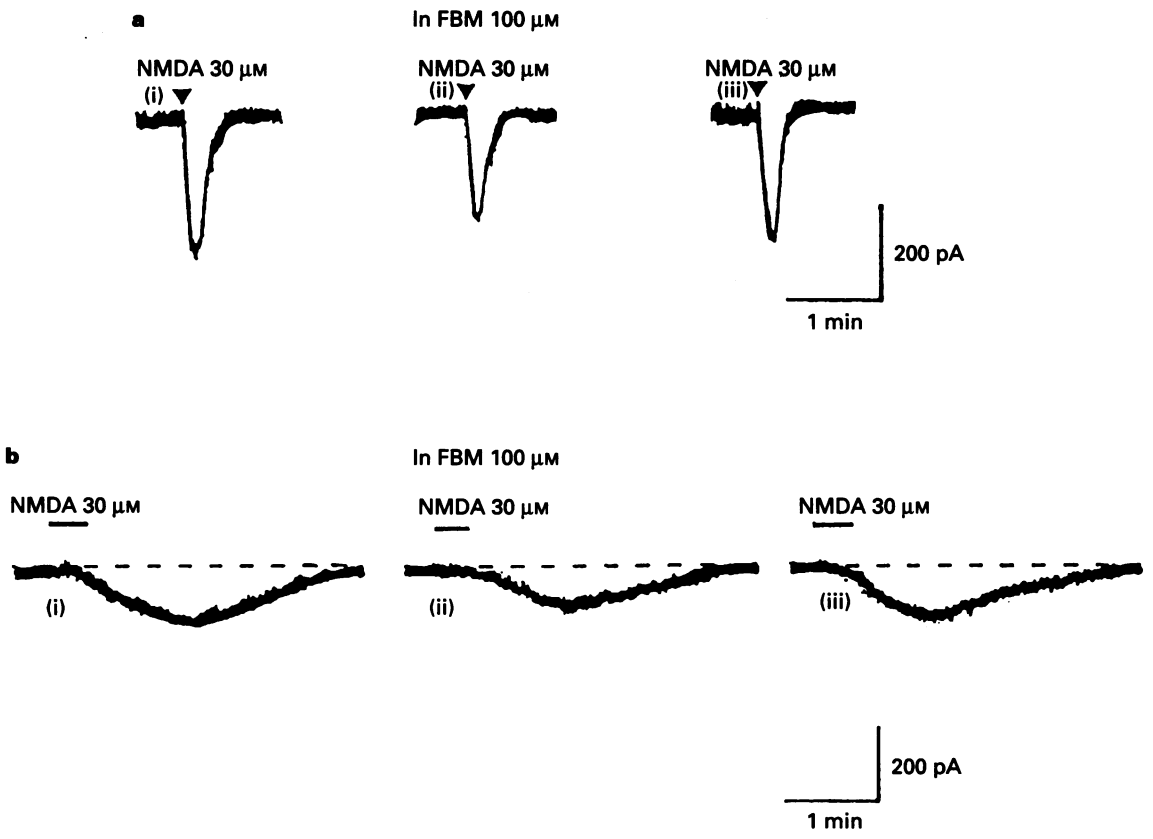



**Figure 6** Effects of felbamate (FBM) on NMDA-induced inward current in voltage-clamped striatal neurones recorded from a slice preparation in the presence of magnesium in the medium. (a) Focal application of 30 μM NMDA induced a rapid and transient inward current (a(i)), which was reduced in the presence of 100 μM FBM (a(ii)). After FBM wash-out, a recovery of the NMDA-induced inward current was observed (a(iii), RMP −85 mV) (b). Bath-application (30 s) of 30 μM NMDA induced a slow inward current. In the presence of 100 μM FBM, the NMDA-induced inward current was reduced. After wash-out of FBM, a partial recovery of the NMDA-induced inward current was recorded.

component of both extracellularly and intracellularly recorded synaptic potentials evoked by cortical stimulation and reduces NMDA-induced inward current measured in voltage-clamped striatal neurones. Although an interaction with the NMDA receptor channel complex has been already suggested for FBM (McCabe *et al.*, 1993; Rho *et al.*, 1994; Domenici *et al.*, 1994), to our knowledge this study represents the first investigation of the electrophysiological actions of FBM on an identified glutamatergic pathway in the mammalian brain. It is worth noting that both limitation of firing activity of striatal neurones and modulation of corticostriatal synaptic potentials occurred at concentrations very similar to those achieved either in the brain and plasma of FBM-treated animals and in plasma of human subjects during clinical trials (McCabe *et al.*, 1993; Wagner *et al.*, 1990), or in surgical samples from patients receiving therapeutic dosages of this drug (Adusumalli *et al.*, 1994; Taylor *et al.*, 1995).

The suppression of $I_{Na}$ has been indicated as a common mechanism of action of many anticonvulsants: the interaction with the $Na^+$ channels has in fact been demonstrated already for different antiepileptic drugs, like phenytoin (Yaari *et al.*, 1986), carbamazepine (McLean & MacDonald, 1986), oxcarbazepine (Calabresi *et al.*, 1995), gabapentin (Wamil & McLean, 1994), lamotrigine (Cheung *et al.*, 1992). In line with those evidences, FBM was also found to interfere with the voltage-dependent TTX-sensitive $I_{Na}$ of striatal neurones. In particular, we have shown a clear effect on the steady-state inactivation properties of $I_{Na}$. This action may well account for the FBM-mediated decrease of the current-induced action potential discharge of striatal neurones, intracellulary recorded from corticostriatal slices. Limitation of high frequency repetitive firing activity by FBM was use-dependent, as revealed by the evidence that the action potentials occurring in the late phase of the current-induced depolarization were preferentially inhibited. The apparent discrepancy between the FBM-induced reduction of the $Na^+$ observed in the whole-cell recordings and the lack of effect of this drug on the action potentials evoked at the beginning of the long-lasting depolarizing pulse may have different explanations. First, the recording conditions were different: in the whole-cell mode, the extracellular $Na^+$ concentration was maintained rather low (<60 mM, in comparison with the $Na^+$ concentration during conventional recordings from slices, which was 153.2 mM) thus allowing us to avoid the activation of very large currents and the generation of space-clamp problems. Moreover, it should be noted that, in the isolated cell preparation, $Na^+$ currents mainly depend on the activation of somatic channels since most of the dendritic arborization is lost. On the other hand, in slice preparations the firing discharge evoked by a depolarizing pulse represents the integration between somatic and dendritic active conductances. Thus, it is conceivable that the action of FBM on $Na^+$-dependent action potentials is less evident in the latter experimental condition which reflects both proximal and distal neuronal activity. However, in some experiments, was observed that FBM was also able to prevent the generation of the first action potential when it was induced by the minimal suprathreshold current (data not shown).

A reduction in the efficacy of the glutamatergic transmission has been claimed as part of the mechanism of action of some anticonvulsants (MacDonald & Kelly, 1993; Walker & Sander, 1994). Besides the 'local anaesthetic'-like properties, shared with most common anticonvulsants, here we have shown that FBM affects corticostriatal glutamatergic transmission. In particular, in the absence of magnesium from the external medium, a clear reduction of the synaptic potential amplitude induced by FBM was observed, suggesting a rather selective

action on the NMDA-mediated component of the glutamatergic potentials. We found that FBM alters the postsynaptic sensitivity to NMDA, in fact it reduces the NMDA-induced responses in voltage-clamped striatal neurones. This evidence suggests that the FBM-induced decrease in the NMDA-mediated component of synaptic potentials may also be due to an interaction at a postsynaptic site.

Recently, it has been hypothesized that some 'new' anticonvulsant drugs act presynaptically affecting glutamate release, presumably through a modulatory action on voltage-dependent calcium channels regulating transmitter release (Cheung *et al.*, 1992; Calabresi *et al.*, 1995; Stefani *et al.*, 1995). The evidence that FBM does not alter the amplitude of synaptic potentials recorded under physiological conditions (plus 1.2 mM magnesium), seems to suggest that the presynaptic mechanisms regulating release from corticostriatal terminals are not affected by this drug. A further indication for a lack of effect on presynaptic terminals comes from a recent report which suggests that conotoxin-sensitive, N-type voltage-dependent calcium channels, which have been shown to play a major role in the control of the corticostriatal transmission (Calabresi *et al.*, 1994) are not affected by FBM (Stefani *et al.*, in preparation). However, our experimental model does not allow us to exclude an effect at the presynaptic level.

Increased glutamatergic transmission within the basal ganglia has been implicated in the pathophysiology of epileptogenesis and of neurodegeneration disorders, such as Huntington's disease and Parkinson's disease (Neafsey *et al.*, 1979; Turski *et al.*, 1986; Beal, 1992). Many groups reported that antagonists of NMDA and non-NMDA receptors as well as calcium antagonists were effective as neuroprotective agents in a wide variety of *in vitro* and *in vivo* models (Simon *et al.*, 1986; Choi *et al.*, 1988).

Recent evidence suggests that FBM may have an anti-parkinsonian potential, since it reversed dopamine $D_2$ receptor antagonist-induced akinesia (Kretschmer, 1994). Yet, competitive and non-competitive NMDA receptor antagonists have been found efficacious in experimental models of Huntington's disease (Beal *et al.*,1993; Greene *et al.*, 1993). Unfortunately, most of the competitive antagonists acting on the glutamate recognition site of the NMDA receptor appear to be associated with severe cognitive side effects, similar to those caused by the noncompetitive NMDA channel blockers (Sveinbjornsdottir *et al.*, 1993). FBM competes with glycine or D-serine at the co-agonists recognition site of the NMDA receptor channel complex (McCabe *et al.*, 1993). Its antiepileptic efficacy may well be explained by the multiple effects described. Recent clinical experience suggests that the use of FBM may be associated with severe adverse effects, such as aplastic anaemia, (Nightingale, 1994). An interesting further pharmacological target would be the development of 'FBM-like' drugs, without such adverse effects.

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