Microionophoretic study with milacemide, a glycine precursor, on mammalian central nervous system cells

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1 The effect of milacemide, a glycine precursor known to increase γ -aminobutyric acid (GABA) and glycine content in the brain, and to have anticonvulsant properties, was tested by ionophoresis on 247 neurones situated in the cerebral cortex and in deeper structures of cats and rats anaesthetized with urethane.

2 Virtually all the neurones, either firing spontaneously or exogenously driven by the excitatory amino acids, glutamate, N-methyl-D-aspartate (NMDA), kainate and quisqualate or by acetylcholine, were reversibly depressed in a dose-dependent fashion. The same depressant effect was observed in animals pretreated with the monoamine oxidase B inhibitor (IMAO-B) deprenyl which is known to reduce milacemide metabolism into glycinamide and glycine. Intravenous administration of milacemide (10 to 100 mg kg⁻¹) also depressed the firing induced by glutamate, NMDA and acetylcholine.

3 When compared to GABA, milacemide was a weaker depressant. However, its effect could still be observed in the presence of the reversible GABA_A antagonist, SR 95531, and thus milacemide is unlikely to act through GABA receptors. In addition, on cells unaffected by glycine, milacemide also had a depressant effect, and on cells inhibited by glycine, it was still capable of depressing cell firing during reversible blockade by strychnine of the glycine inhibitory action; thus milacemide is unlikely to act through glycine receptors. Simultaneous release of milacemide and GABA or of milacemide and glycine, did not show potentiation of the inhibitory amino acid action. However, the depressant effect of milacemide was additive with that of GABA and glycine.

4 No consistent depression of glutamate-induced firing was obtained by ionophoresis of glycinamide, the first metabolite of milacemide.

5 It is concluded that milacemide by itself is a depressant agent and that its depressant effect does not necessarily require its metabolism into glycine, or its stimulator effect on the production of GABA.

Introduction

Milacemide, 2-n-pentylaminoacetamide, is active against convulsions in various acute and chronic animal models of epilepsy; it has also been shown to improve epileptic patients without sedative effects (Roba *et al.*, 1986).

In early studies, milacemide appeared particularly effective against convulsions induced by bicuculline (Van Dorsser *et al.*, 1983). This prompted the suggestion that it could interfere with the metabolism of γ -aminobutyric acid (GABA), the dysfunction of which has been suspected in some types of epilepsy (Meldrum, 1975). Biochemical investigations revealed that acute or chronic administration of milacemide to rats, at a dose of 25 to 100 mg kg⁻¹, increases the GABA content in the brain, particularly in the substantia nigra (Janssens de Varebeke *et al.*, 1983). The substantia nigra is known to be a site of anticonvulsant activity mediated by GABA (Iadarola & Gale, 1982; Bonhaus *et al.*, 1986).

Milacemide is transformed, through a monoamine oxidase B inhibitor (IMAO-B)-sensitive process, into glycinamide and glycine (Janssens de Varebeke *et al.*, 1988). Following its administration, the glycine content of the brain is significantly increased (Christophe *et al.*, 1983). The action of milacemide is believed to be indirectly due, at least in part, to its degradation into glycine (Hunter *et al.*, 1986; Roba *et al.*, 1986; Janssens de Varebeke *et al.*, 1988; Youdim *et al.*, 1988). However, milacemide does not appear very potent against convulsions induced by strychnine (Van Dorsser *et al.*, 1983; Roba *et al.*, 1986), which is a known glycine antagonist (Curtis & Johnston, 1974). It has been suggested (Janssens de Varebeke *et al.*, 1988) that glycine produced from milacemide preferentially influences the strychnine-insensitive glycine system, recently

described by Johnson & Ascher (1987), which modulates N-methyl-D-aspartic (NMDA) acid-induced currents. However, such a hypothesis, if compatible with the enhancement of cognitive processes shown to occur in the course of milacemide treatment (Saletu *et al.*, 1986; Janssens de Varebeke *et al.*, 1988), would not account for the antiepileptic effect of milacemide.

The above studies thus suggest that anticonvulsant properties of milacemide are related to the increased concentrations of inhibitory amino acids which it induces in the nervous tissue, with emphasis on the GABA system. However, recent electrophysiological studies have pointed out that antiepileptic agents, supposed to act on the GABA inhibitory system, exert direct effects on nerve cells (Slater & Johnston, 1978; Schmutz *et al.*, 1979; Aickin *et al.*, 1981; Harrison & Simmonds, 1982; Carlen *et al.*, 1983; McLean & MacDonald, 1983; Morre *et al.*, 1984; Heinemann *et al.*, 1985; Willow *et al.*, 1985; Meldrum & Chapman, 1986; Van Dongen *et al.*, 1986; Willow, 1986). These direct effects are of major importance, because they could indeed account for some of the anticonvulsant properties of these drugs.

Can milacemide and/or its metabolite glycinamide also affect nerve cell excitability directly?

Brief accounts of the results in this paper have been presented at meetings of the European and American Neuroscience Societies (Godfraind, 1986a,b; 1988).

Animal preparation

Experiments were performed on 38 adult animals breathing spontaneously: 16 cats were anaesthetized with 1.5 g kg⁻¹ urethane administered through a catheter inserted into the

femoral vein under local anaesthesia; 22 rats were anaesthetized with the same drug injected intraperitoneally. When needed, supplementary doses of anaesthetic were given. After tracheotomy, animals were fixed in a stereotaxic frame and subjected to routine care (Godfraind, 1978).

Recording

Extracellular unit recordings were performed through the central barrel of five-barrelled pipettes filled with a 2 M NaCl solution, from cerebral cortex (most often in cats), or from deep structures e.g. hippocampus and thalamus (most often in rats). No attempt was made to characterize the neurones. The spikes were converted to standard pulses and fed into an integrator (Gould integrator amplifier; recycling time: 1 s) the output of which was connected to a small computer (CED 1401) and to a chart-recorder, in order to follow the firing frequency. Occasionally, spikes were also recorded on magnetic tape for analysis and photographic display.

Ionophoresis

The outer barrels of the five-barrelled pipettes were filled with the following substances: glutamate, 1 M, pH 7.4 (Fluka); N-methyl-D-aspartic acid (NMDA), 20 mM in 165 mM NaCl, pH 8 (Cambridge Research Chemicals); DL-2-amino-5-phosphono-valeric acid (APV), 20 mM in 165 mM NaCl, pH 8 (Sigma); kainic acid, 20 mM in 165 mM, pH 8 (Cambridge Research Chemicals); quisqualic acid, 20 mM in 165 mM NaCl, pH 8 (Cambridge Research Chemicals); acetylcholine, 1 M, pH 4.4 (BDH); GABA, 0.5 or 1 M, pH 4.3 (Fluka); the GABA_A antagonist SR 95531 (2-(carboxy-3'-amino-6-paramethoxy-phenylpyridazinium bromide)), 5 mM in 165 mM NaCl, pH 3.8 (Clin-Midy, Sanofi); glycine HCl, 500 mM, pH 4 (Aldrich Chemie); strychnine sulphate, 10 mM in 165 mM NaCl, pH 5.2 (Vel); NaCl, 165 mM, pH 3.6; glycylamide, 1 M, pH 3.6 (Janssen Chimica), and milacemide, 1 M, pH 4.3 for the first batch, and pH 4.4 for the second batch (Continental Pharma Laboratories). Retaining currents ranged from 10 to 20 nA. All drugs, except glutamate, glutamate agonists and APV, were delivered with positive currents. Cells were allowed to recover between successive and regular applications of the drugs. When comparing the effect of milacemide on the excitatory effects of the three glutamate agonists, these agonists were applied successively in a sequence determined at random for a given cell.

Intravenous administration of milacemide

In 6 experiments on the cat, milacemide was injected intravenously at doses ranging from 10 to 100 mg kg⁻¹. Milacemide was dissolved in 2 or 3 ml of 0.9% NaCl solution and slowly injected over 2 or 3 min. In these animals, the effect of milacemide applied by iontophoresis was also examined before and after the intravenous injection of milacemide.

Analysis

For each substance, the results are based on observations made on at least 3 different animals, except when the effects of APV and milacemide were compared, 2 animals were used. The test responses, obtained during application of the various drugs made with different iontophoretic currents, were measured and expressed as a percentage of the control responses. The values thus obtained were plotted against iontophoretic currents. A microcomputer programme (Linefit of Barlow, 1983), using the least squares method, calculated the regression lines corresponding to these X-Y values, and produced the resulting slope values.

Statistics

The median and interquartile range of the slopes, obtained during the applications of milacemide, glycylamide, GABA,

APV or acetylcholine, were determined. The Mann-Whitney U test was used to compare the slope values corresponding to the responses obtained during the applications of the three pairs of drugs: GABA-milacemide, milacemide-glycylamide and GABA-glycylamide (NP-Scat and U test (Siegel, 1956; Barlow, 1983)). Data based on glutamate, glutamate agonists and acetylcholine-induced responses obtained during the release of milacemide or APV, were submitted to an analysis of variance (T & ANOVA, Barlow, 1983). For comparing the potency of milacemide, GABA or glycine in the absence and presence of the antagonists of GABA and glycine, the responses obtained with the appropriate excitants in the control and test situations were paired and submitted to a Wilcoxon matched-pairs signed-ranks test (Conover, 1980; Barlow, 1983). The Wilcoxon test was also used to compare the effect obtained during release of milacemide and GABA or glycine to those obtained during the simultaneous release of milacemide and GABA or glycine.

Results

Two hundred and forty seven neurones were obtained, 93 from 22 rats, and the remaining 154 from 16 cats.

Effect of milacemide on the spontaneous activity

Ionophoretic applications of milacemide induced a reversible reduction of the spontaneous firing, on 9 neurones in 5 experiments. This effect was dose-dependent. At doses of 30 to 60 nA of milacemide, the firing rate slowed down within some 60 s after the beginning of the release. At the end of application, the firing returned gradually to its previous level within about 40 s.

Effect of milacemide on the responses evoked by glutamate and its agonists

In the course of 38 experiments, milacemide was applied by iontophoresis on 190 neurones driven by glutamate or glutamate agonists.

Effect of milacemide on glutamate-evoked responses and comparison with the effect of glycylamide For virtually all the cells (150 cells, 26 experiments), the glutamate-evoked responses were depressed, sometimes up to complete suppression, with iontophoretic currents as low as 5 nA. Of course, the depressant effect and the recovery occurred within a time course depending on the amount of iontophoretic current used (Figures 1, 3 and 5).

The depressant effect of milacemide was clearly dose-dependent (Figures 1, 2 and 3). The potency of milacemide was estimated using the median of the slope values of the regression lines (dose-response) for 24 neurones. The corresponding iontophoretic current needed to produce 50% inhibition was thus determined to be 36 nA.

The depressant effect of milacemide was not due to a direct current effect: indeed, the depression often outlasted the milacemide application (Figure 1), and, on several cells, a positive current of the same intensity applied through a NaCl-filled barrel never mimicked the milacemide effect (Figure 5). In addition, the action potential amplitude was not affected during milacemide release (Figure 2).

The milacemide effect was compared to that of its first metabolite, glycylamide. Out of 22 cells examined in 3 experiments, 9 were either little affected or slightly excited following iontophoresis of glycylamide (Figure 1). The firing of the thirteen other cells was depressed during glycylamide ejection. However, except in one case, the depressant effect of glycylamide

Figure 1 A comparison of the effects of milacemide, GABA and glycine on glutamate-induced discharges. The glutamate-firing (G) was reduced respectively during 60, 80 and 100 nA milacemide (Mi) applications, to 47%, 23% and 16% of the control (mean of three consecutive responses; b, c and f). During glycine (Gly) application with 60 and 80 nA, the glutamate-firing was little affected: firing rate reached 85% and 100% of the control (mean of three responses; a and d). GABA was the most potent depressant substance: 40 nA reduce the firing to 16% of the control (e). Between the upper and middle traces, and the middle and lower traces, no interruption occurred; upper and lower traces were interrupted respectively for 152 and 120 s. In the middle trace, the two halves are quasi continuous traces. In this and subsequent rate-meter records: dark lines below the records indicate the time of drug application; the ordinate scales: spikes per s (Sp s^{-1}); the abscissa scales: time in s.

mide was smaller than that of milacemide. The slope values of the dose-response curves clearly indicated that glycineamide was a poor depressant agent. It was calculated by extrapolation that an ionophoretic current of about 190 nA should be applied to reach 50% inhibition. The Mann-Whitney U test applied to the slope values of the pairs GABA-glycinamide and milacemide-glycinamide, showed that the populations were significantly different ($1 \times 10^{-6} < P < 1 \times 10^{-7}$ for GABA-glycinamide, and $1 \times 10^{-5} < P < 1 \times 10^{-6}$ for milacemide-glycinamide; one-tail test).

Effect of milacemide on NMDA-, kainate- and quisqualate-evoked responses To test whether or not milacemide acts preferentially on one of the glutamate receptor subtypes, it was ejected during regular cycles composed of four responses induced by successive applications of the glutamate agonists. The order of amino acid presentation was determined at random for each cell, and only the three last responses of a given sequence were retained for statistics.

These studies were performed on two groups of five rats, control animals and animals pretreated with deprenyl (to

Figure 2 Effect of milacemide on glutamate agonists. A cell was fired by successive applications of 10 nA kainate (Kain), 91 nA N-methyl-D-aspartate (NMDA) and 2 nA quisqualate (Quis) during, respectively, control (upper horizontal row), milacemide release (second and lowest rows), and recovery (third row). During the 35 nA milacemide application, responses induced by the glutamate agonists are clearly depressed, and much less affected by 20 nA milacemide.

Figure 3 Effect of milacemide on acetylcholine-induced firing. A thalamic cell was fired by alternate applications of 40 nA glutamate (G) and 15 nA acetylcholine (ACh). Milacemide applied with 15, 5 and 55 nA (respectively upper, middle and lower row), depressed the firing induced by both substances in a dose-dependent manner. Upper and middle traces are continuous recordings; middle and lower traces were interrupted for about 6 min.

reduce milacemide catabolism). Deprenyl was injected intraperitoneally at a dose of 1 mg kg^{-1} late in the afternoon preceding the experiment, some 19 h before recording. In the first group, dose-response curves were based on data obtained from 9 out of 12 cells. The responses to all glutamate agonists were depressed (Figure 2). The slope values corresponding to the dose-response curves of the three agonists were submitted to an analysis of variance (T & ANOVA from Barlow, 1983). The F test value was below 1, which indicates that milacemide was about equally potent in depressing the NMDA, kainate and quisqualate responses. In the second series of experiments, data were obtained from 15 cells but only 11 were suitable for analysis. Variance analysis of the slope values yielded a F value below 1. Thus, whether or not the milacemide degradation was impaired, milacemide appeared to depress the responses to the glutamate agonists similarly.

For 10 cells (2 rats), APV, a specific NMDA antagonist, was found to be more powerful than milacemide in depressing NMDA responses. Variance analysis of the slope values obtained for APV and milacemide, made on 6 cells out of these 10, yielded an F value above 15 ($P = 0.01$), confirming that APV was more potent than milacemide.

Effect of milacemide on acetylcholine-induced firing

Milacemide was also tested on 30 cholinergic cells in the cerebral cortex and deeper structures (hippocampus, thalamus) of 8 animals (6 cats and 2 rats). Milacemide did reversibly depress the acetylcholine-induced excitations, whether these were of the prolonged, muscarinic type, or of the shorter duration, so-called mixed nicotinic-muscarinic responses (Figure 3). This effect was also dose-dependent. Further, this depressant effect was clearly non-specific, in that it affected both glutamatergic and cholinergic responses (Figure 3), in all neurones. Data collected from 11 cells in 4 experiments, enabled dose-response lines to be drawn. Variance analysis of the slope values produced an F value below 1, i.e. the two populations, glutamate and acetylcholine, could not be statistically differentiated, thus showing signifi-

cantly that the effect of milacemide was non-specific. On two other cells, NMDA and acetylcholine responses were compared: in contrast to the effect of APV, milacemide depressed both responses.

Comparison of the milacemide- and GABA- or glycine-evoked depressant effects

GABA GABA was clearly more potent than milacemide in depressing cell firing, as observed on 68 cells (Figures 1 and 4). Data collected from 24 neurones on which both GABA and milacemide were applied alternately, were used to draw dose-responses regression lines. The medians of the slope values were used to calculate the amount of ionophoretic current needed to produce 50% inhibition with milacemide and GABA, 36 nA and 10 nA respectively. A Mann-Whitney U test applied to these slope values, showed that the GABA and milacemide populations were significantly different ($1 \times 10^{-6} < P < 1 \times 10^{-7}$; one-tail test). To check for a possible interference between the two substances, the effect of milacemide was tested on the inhibition (sub-maximal or near-threshold) evoked by GABA, on 39 neurones driven by regular pulses, or by continuous release of glutamate. The effect of GABA was never enhanced nor prolonged during or just after the application of milacemide. However, the glutamate-evoked responses were significantly more depressed by the simultaneous release of GABA and milacemide than by GABA alone (Wilcoxon test: $0.021 < P < 0.022$; $n = 5$; one-tail test); this indicates that the effects of GABA and milacemide are additive.

We further examined whether milacemide could still depress glutamate-evoked discharges while GABA inhibition was specifically suppressed by applying the very potent GABA_A-antagonist, SR 95531, (Michaud *et al.*, 1986; Wermuth & Bizire, 1986). Sequences such as that shown in Figure 4, are easy to obtain provided that the dose of SR 95531 is minimal, to avoid the occurrence of paroxysmal discharges against which milacemide is ineffective. When SR 95531 was ejected with currents sufficient to reversibly abolish (6 cells) or significantly reduce (3 cells) the depressant effects of GABA, it had no significant effect on the depressant action of milacemide (same 9 neurones in 4 experiments; Wilcoxon test).

Glycine On 29 cells in 6 experiments, observations made with glycine and milacemide paralleled those obtained for GABA and milacemide, though with some differences. In the cerebral cortex, glycine was without effect on 7 cells and had only a weak effect on another 10 neurones; this was, however, less marked than that of milacemide. On the other cells situated in deep nuclei, a few nA glycine did suffice to bring complete silence. These cases were the most favourable to perform the tests with strychnine.

On cells unaffected by glycine, milacemide nevertheless proved to be an active depressant agent. To check for a possible interference between glycine and milacemide, a 'window' test was performed in 4 cells. At near-threshold dose, milacemide did progressively induce a slight depression, followed by slow recovery. During continuous release of glycine, a similar depression was obtained by a re-application of milacemide.

In cells where both glycine and milacemide depressed the firing rate, an additive effect was also observed when both drugs were released simultaneously (3 cells).

When strychnine was applied with currents sufficient to reversibly abolish (9 cells) or significantly reduce (2 cells) the glycine inhibition, it did not alter significantly the depressant effect of milacemide (11 cells, 4 experiments; Wilcoxon test).

Effect of intravenous injections of milacemide

Milacemide was injected intravenously in 6 cats at doses ranging from 10 to 100 mg kg^{-1} . Some animals received more

Figure 4 Effect of milacemide in the presence of SR 95531, a GABA_A-antagonist. In the upper row, 11 nA GABA and 40 nA milacemide depressed the glutamate firing respectively to 32% and 41% of the control. In the ejection of the GABA_A-antagonist (downwards arrow SR 95531 at the beginning of second row), the inhibitory effect of GABA was completely abolished (second and third row), while milacemide still depressed the firing. The GABA-induced inhibition recovered rapidly after SR 95531 release (third row). Lower trace right, SR 95531 was reapplied, and the sequence replayed. Note the excitatory effect of SR 95531. The four traces were continuous records.

than one injection, and the time elapsed between two injections varied from 30 min to several hours. Hence the cumulative dose could eventually exceed 100 mg kg^{-1} . In four experiments, control intravenous injections of saline did

not alter the amplitude of the glutamate-evoked responses (Figure 5).

About 10 min after the intravenous injection of a small dose of milacemide (10 to 30 mg kg^{-1}), the amplitude of the

Figure 5 Effect of milacemide injected intravenously. (a) Ionophoretic ejection of 50 nA milacemide reduced progressively the glutamate-induced firing to almost complete silence. Na^+ current application failed to mimic the effect of milacemide. (b) the firing rate was not affected following intravenous injection of saline, whereas, intravenous injection of milacemide at a dose of 80 mg kg^{-1} , was quickly followed by a complete disappearance of the glutamate-response. (c) After increasing the glutamate ionophoretic current, the cell was slightly reactivated. The traces are continuous records.

glutamate-evoked responses was usually reduced by 10 to 50% (4 cells). Such an effect could be overcome by increasing the ionophoretic current of glutamate but ionophoretic application of milacemide was still able to depress the remaining glutamate responses. This suggests that the sites acted upon by milacemide were not saturated.

The depressant effect of milacemide was more clear-cut after injection of larger doses (80 mg kg^{-1}) (first injection for this preparation) (Figure 5b). The discharges induced by glutamate, NMDA or acetylcholine were then totally suppressed for several minutes and recovered progressively after about 9–10 min. It should be mentioned that at the end of intravenous injections of such large doses of milacemide, a clear and transitory change in the respiratory rhythm could be observed. As previous investigations had not described any effect of milacemide on the cardiovascular system (Roba *et al.*, 1986), blood pressure and heart rate were not monitored during these experiments.

Discussion

It has been shown here that milacemide, applied by ionophoresis or by the intravenous route, reversibly depresses neuronal excitation, i.e. spontaneous activity, glutamate-, glutamate agonists- and acetylcholine-evoked firing, without interfering with the inhibitory mechanisms of GABA and glycine. These observations were made on unidentified cells recorded in the cerebral cortex and in deep structures from two different animal species; this covers a wide range of neuronal types, and thus the milacemide effects described here are unlikely to be linked either to a particular cell type, to a particular neuronal microcircuit, or to a defined neurotransmitter system. Since glycine is much less effective than milacemide in reducing cell firing, it is proposed that milacemide is the active agent. This proposition is further strengthened by the experiments made with animals pretreated with deprenyl, which reduces milacemide metabolism into glycine and glycine (Janssens de Varebeke *et al.*, 1988): the firing was as depressed in untreated as in treated animals.

In addition, just at the end of an intravenous injection of a high dose of milacemide (80 mg kg^{-1}) in cats, a transient modification of the respiratory rhythm was noticed. Excitatory amino acids have been shown to play a role in respiration, and therefore it should not be surprising to find that an intravenous injection of milacemide alters the respiratory rhythm as do other specific and unspecific glutamate antagonists (McCrimmon *et al.*, 1986; Foutz *et al.*, 1988). The amounts of milacemide used here were low compared to those used in other studies (Albertson *et al.*, 1984; Youdim *et al.*, 1988).

The effect of milacemide on the three main classes of glutamate receptors (Watkins & Olverman, 1987) was also investigated: the NMDA-, kainate- and quisqualate-induced responses were all equally significantly depressed by milacemide, in control as well as in deprenyl-treated animals. A comparison of milacemide with APV, a specific NMDA antagonist (Davies *et al.*, 1981), on NMDA responses, showed that APV was significantly more potent as a depressant agent than milacemide. Milacemide appeared to be rather non-specific: it depressed the glutamatergic responses as well as those induced by acetylcholine, whether of the muscarinic type (cortical neurones) or of the mixed nicotinic-muscarinic type (deep cells).

The non-specific depressant effect of milacemide on excitation induced through a variety of receptors, together with the observation that $100 \mu\text{M}$ milacemide does not influence

directly ionic channels in patch-clamp experiments (Johnson, personal communication), and does not affect membrane input resistance (our unpublished data), all suggest that it could be acting via an intracellular biochemical mechanism.

Milacemide is significantly weaker than GABA in depressing cell firing. It does not affect the GABA inhibitory action. However, both drugs have additive effects. Results with the GABA_A-antagonist SR 95531, showing that the milacemide-induced depression was still present when the GABA inhibitory action was abolished, indicate that milacemide is unlikely to be acting through the GABA system. Binding studies did not reveal any interaction between milacemide and GABA receptors (Roba *et al.*, 1986).

Although milacemide is a precursor of glycine and is able to induce a widespread and significant increase of glycine in the brain (Christophe *et al.*, 1983), its depressant action is unlikely to be mediated by the glycine system. Its depressant effect persists in the presence of strychnine and in deprenyl-treated animals. In addition, milacemide is an active depressant agent on neurones unaffected by glycine. Glycine plays only a minor role in the inhibitory mechanisms observed in the cerebral cortex (Kelly & Krnjevic, 1969; Bernardi *et al.*, 1979). The 'window type' experiments conducted on the cells unaffected by glycine, showing that milacemide still depressed the firing during continuous glycine release, suggest that glycine and milacemide do not directly interact or share common sites of action. Also, binding studies did not reveal an interaction with glycine (Roba *et al.*, 1986), and milacemide is not very effective against strychnine-induced convulsions (Van Dorsser *et al.*, 1983). In addition, patch-clamp experiments made with milacemide do not show an interaction with glycine (Johnson, personal communication).

It was observed, on glycine-sensitive cells, that simultaneous release of glycine and milacemide does produce a more intense depression than that evoked by separate applications of the drugs. GABA and glycine share a common ionic mechanism to induce inhibition (Curtis & Johnston, 1974; Krnjevic, 1974). However, such a mechanism of action is unlikely to be the one through which milacemide is acting: intracellular recording in granule cells ($n = 3$) of the hippocampus maintained *in vitro*, showed that milacemide, at a concentration up to 1 mM in the bath, induced no change in membrane potential or in membrane input resistance (our unpublished data).

In conclusion, these experiments have shown that milacemide has a direct depressant effect on cell firing whether spontaneous or exogenously driven, and indicate that its site of action is likely to be postsynaptic. Without excluding the biochemical hypothesis (based mainly on increased levels of GABA and glycine), the present results call for further experiments to be done in order to identify the precise physiological mechanisms by which milacemide alters cell excitability.

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References

- AICKIN, C.C., DIESZ, R.A. & LUX, H.D. (1981). On the action of the anticonvulsant 5,5-diphenylhydantoin and the convulsant picrotoxin in crayfish stretch receptor. *J. Physiol.*, **315**, 157–173.
- ALBERTSON, T.E., STARK, L.G. & JOY, R.M. (1984). The effect of a glycine derivative (CP 1552-S) on kindled seizures in rats. *Neuropharmacol.*, **23**, 967–970.
- BARLOW, R.B. (1983). *Biodata Handling with Microcomputers*. pp. 1–261. Cambridge: Elsevier Biosoft.
- BERNARDI, G., CHERUBINI, E., MARCIANI, M.G. & STANZIONE, P. (1979). The inhibitory action of glycine on rat cortical neurons. *Neurosci. Lett.*, **12**, 335–338.
- BONHAUS, D.W., WALTERS, J.R. & McNAMARA, J.O. (1986). Activation of substantia nigra neurons: role in the propagation of seizures in kindled rats. *J. Neurosci.*, **6**, 3024–3030.
- CARLEN, P.L., GUREVICH, N. & POLC, P. (1983). Low-dose benzodiazepine neuronal inhibition: enhanced Ca^{++} -mediated K^{+} -conductance. *Brain Res.*, **271**, 358–364.
- CHRISTOPHE, J., KUTZNER, R., NGUYEN-BUI, N.D., DAMIEN, C., CHATELAIN, P. & GILLET, L. (1983). Conversion of orally administered 2-n. pentylaminoacetamide into glycinamide and glycine in the rat brain. *Life Sci.*, **33**, 533–541.
- CONOVER, W.J. (1980). *Practical Nonparametric Statistics*. Second edition. pp. 1–493. New York: John Wiley and Sons.
- CURTIS, D.R. & JOHNSTON, G.A.R. (1974). Amino acid transmitters in the mammalian nervous system. *Rev. Physiol.*, **69**, 97–188.
- DAVIES, J., FRANCIS, A.A., JONES, A.W. & WATKINS, J.C. (1981). 2-Amino-5-phosphonovalerate (2APV), a potent and selective antagonist of amino-acid-induced and synaptic excitation. *Neurosci. Lett.*, **21**, 77–81.
- FOUTZ, A.B., CHAMPAGNAT, J. & DENAVIT-SAUBIE, M. (1988). NMDA receptors control the termination of inspiration in cat. *Eur. J. Neurosci.*, Suppl. p. 265, abstr. 72.14.
- GODFRAIND, J.M. (1978). Acetylcholine and somatically evoked inhibition on perigeniculate neurons in the cat. *Br. J. Pharmacol.*, **63**, 295–302.
- GODFRAIND, J.M. (1986a). Iontophoretic study with milacemide, an antiepileptic agent, on cerebral cortical cells in the cat. *Neurosci. Lett.*, **26**, S479.
- GODFRAIND, J.M. (1986b). Effect of milacemide, an antiepileptic agent, released iontophoretically on rat and cat CNS cells *in vivo*. *Abstracts Soc. Neurosci.*, **12**, p. 1081, 295.11.
- GODFRAIND, J.M. (1988). Iontophoresis of milacemide, a glycine precursor, on neuronal responses evoked by iontophoresis of excitatory and inhibitory aminoacids in rat and cat. *J. Neurosci.*, Suppl., p. 64, abstract 16.26.
- HARRISON, N.L. & SIMMONDS, M.A. (1982). Sodium valproate enhances responses to Gaba receptor activation only at high concentrations. *Brain Res.*, **250**, 201–204.
- HEINEMANN, U., FRANSCETTI, S., HAMON, B., KONNERTH, A. & YAARI, Y. (1985). Effects of anticonvulsants on spontaneous epileptiform activity which develops in the absence of chemical synaptic transmission in hippocampal slices. *Brain Res.*, **325**, 349–352.
- HUNTER, C., CHUNG, E. & VAN WOERT, M.H. (1986). Antimyoclonic action of milacemide in p,p'-DDT-induced myoclonus in mice. *Abstr. Soc. Neurosci.*, **12**, p. 1243, 338.6.
- IADAROLA, M.J. & GALE, K. (1982). Substantia Nigra: site of anticonvulsant activity mediated by gamma-aminobutyric acid. *Science*, **218**, 1237–1240.
- JANSSENS DE VAREBEKE, P., CAVALIER, R., DAVID-REMACLE, M. & YODIM, M.B. (1988). Formation of the neurotransmitter glycine from the anticonvulsant milacemide is mediated by brain monoamine oxidase B. *J. Neurochem.*, **50**, 1011–1016.
- JANSSENS DE VAREBEKE, P., NIEBES, P., PAUWELS, G., ROBA, J. & KORF, J. (1983). Effect of milacemide, a glycinamide derivative, on the rat brain gamma-aminobutyric acid system. *Biochem. Pharmacol.*, **32**, 2751–2755.
- JOHNSON, J.W. & ASCHER, P. (1987). Glycine potentiates the NMDA response in cultured mouse brain neurons. *Nature*, **325**, 529–531.
- KELLY, J.S. & KRNEVIC, K. (1969). The action of glycine on cortical neurones. *Exp. Brain Res.*, **9**, 155–163.
- KRNEVIC, K. (1974). Chemical nature of synaptic transmission in vertebrates. *Physiol. Rev.*, **54**, 418–540.
- MCCRIMMON, D.R., FELDMAN, J.L. & SPECK, D.F. (1986). Respiratory motoneuronal activity is altered by injections of picomoles of glutamate into cat brain stem. *J. Neurosci.*, **6**, 2384–2392.
- MCLEAN, J.M. & MACDONALD, R.L. (1983). Multiple actions of phenytoin on mouse spinal cord neurons in cell culture. *J. Pharmacol. Exp. Ther.*, **227**, 779–789.
- MELDRUM, B.S. (1975). Epilepsy and gamma-aminobutyric acid-mediated inhibition. *Int. Rev. Neurobiol.*, **17**, 1–36.
- MELDRUM, B.S. & CHAPMAN, A.G. (1986). Benzodiazepines receptors and their relationship to the treatment of epilepsy. *Epilepsia*, **27**, S3–S13.
- MICHAUD, J.C., MIENVILLE, J.M., CHAMBON, J.P. & BIZIÈRE, K. (1986). Interactions between three pyridazinyl-Gaba derivatives and central Gaba and glycine receptors in the rat, an *in vivo* microiontophoretic study. *Neuropharmacol.*, **25**, 1197–1203.
- MOORE, M., KEANE, P.E., VERNIÈRES, J.C., SIMIAND, J. & RONCUCCI, R. (1984). Valproate: recent findings and perspectives. *Epilepsia*, **25**, S5–S9.
- ROBA, J., CAVALIER, R., CORDI, A., GORISSEN, H., HERIN, M., JANSSENS DE VAREBEKE, P., ONKELINX, C., REMACLE, M. & VAN DORSSER, W. (1986). Milacemide. In *New Anticonvulsant Drugs*. ed. Meldrum, B.S. & Porter, R.J. pp. 179–190. London: John Libbey.
- SALETU, B., GRUNBERGER, J. & LINZMAYER, L. (1986). Acute and subacute CNS effects of milacemide in elderly people: double-blind, placebo-controlled quantitative EEG and psychometric investigations. *Arch. Gerontol. Geriatr.*, **5**, 165–181.
- SCHMUTZ, M., OLPE, H.R. & KOELLA, W.P. (1979). Central actions of valproate sodium. *J. Pharm. Pharmacol.*, **31**, 413–414.
- SIEGEL, S. (1956). *Nonparametric Statistics for the Behavioral Sciences*. pp. 1–312. London: McGraw-Hill Book Company.
- SLATER, G.E. & JOHNSTON, D. (1978). Sodium valproate increases potassium conductance in aplysia neurons. *Epilepsia*, **19**, 379–384.
- VAN DORSSER, W., BARRIS, D., CORDI, A. & ROBA, J. (1983). Anticonvulsant activity of milacemide. *Arch. Int. Pharmacodyn. Ther.*, **266**, 239–249.
- VANDONGEN, A.M.J., VANERP, M.G. & VOSKUYL, R.A. (1986). Valproate reduces excitability by blockage of sodium and potassium conductance. *Epilepsia*, **27**, 177–182.
- WATKINS, J.C. & OLVERMAN, H.J. (1987). Agonists and antagonists for excitatory amino acids receptors. *Trends Neurosci.*, **10**, 265–272.
- WERMUTH, C.G. & BIZIÈRE, K. (1986). Pyridazinyl-Gaba derivatives: a new class of synthetic GabaA antagonists. *Trends Pharmacol. Sci.*, **7**, 421–424.
- WILLOW, M. (1986). Pharmacology of diphenylhydantoin and carbamazepine action on voltage-sensitive sodium channels. *Trends Neurosci.*, **9**, 147–149.
- WILLOW, M., GONOI, T. & CATTERALL, W.A. (1985). Voltage clamp analysis of the inhibitory actions of diphenylhydantoin and carbamazepine on voltage-sensitive sodium channels in neuroblastoma cells. *Mol. Pharmacol.*, **27**, 549–558.
- YODIM, M.B.H., KEREM, D. & DUVDEVANI, Y. (1988). The glycine-prodrug, milacemide, increases seizure threshold due to hyperbaric oxygen: prevention by L-deprenyl. *Eur. J. Pharmacol.*, **150**, 381–384.

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