Published in final edited form as:

Neurochem Int. 2007 ; 51(6-7): 440–446.

Characterization of the Interaction between Fenamates and Hippocampal Neuron GABA_A Receptors.

Leanne Coyne,

TJ Long School of Pharmacy & Health Sciences, University of the Pacific, Stockton, CA, USA

Jiping Su,

Dept of Otolaryngology, First Affiliated Hospital, Guangxi Medical University, Guangxi, PR China

Debra Patten, and

Dept. of Biological & Biomedical Sciences, University of Durham, England, UK

Robert F. Halliwell*

TJ Long School of Pharmacy & Health Sciences, University of the Pacific, Stockton, CA, USA

Abstract

Fenamate NSAIDs have several *central* effects, including anti-epileptic and neuroprotective actions. The underlying mechanism(s) of these actions are not presently understood. In this study, the effects of five members of the fenamate NSAID group were investigated on native ligand-gated ion channels expressed in cultured rat hippocampal neurons. All fenamates tested, (1–100 μM) dose-dependently potentiated GABA-evoked currents; mefenamic acid (MFA) was the most potent and efficacious and was found to shift the GABA dose response curve to the left without effect on the maximum amplitude or the GABA Hill Slope. The modulation of GABA receptors by MFA was not reduced in the presence of the benzodiazepine antagonist, flumazenil (10 μM) and was moderately voltage-dependent. MFA at concentrations ≥10 μM evoked dose-dependent currents in the absence of GABA. These currents were potentiated by diazepam (1 μM) and blocked by bicuculline (10 μM). The MFA (50 μM) current-voltage relationship and reversal potential were similar to that evoked by GABA. MFA (1–100 μM) had no effects on sub-maximal glycine, glutamate or NMDA evoked currents. These data show that fenamate NSAIDs are a highly effective class of GABA_A receptor modulator and activators.

Keywords

Anticonvulsant; NSAID; electrophysiology; glutamate receptors; glycine receptors

1. Introduction

NSAIDs are the most widely consumed medicines world-wide with an estimated 100 million prescriptions written per year in the USA. They are also a common source of self-poisoning with 100,000 hospitalizations annually from their adverse effects (Smolinske, et al., 1990; Frolich, 1997; Pascucci, 2002). This chemically diverse group of drugs, prototypically represented by acetylsalicylic acid (*aspirin*), are used for their analgesic, anti-inflammatory,

Corresponding Author: Dr Robert F. Halliwell, Department of Physiology & Pharmacology, TJ Long School of Pharmacy & Health Sciences, University of the Pacific, Stockton, California, 95211, Tel: 209-946-2074., Fax: 209-946-2857, E-mail: rhalliwell@pacific.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

anti-pyretic and cardio-protective properties (Weissmann 1991; Pascucci 2002). The therapeutic properties of NSAIDs are thought to result, primarily, from their inhibition of cyclooxygenase (COX) isoenzymes and thereby inhibition of prostaglandin synthesis, although other mechanisms are now under investigation (Vane 1971; Vane & Botting 1996; Arber & DuBois, 1999).

The fenamate NSAID, mefenamic acid (MFA) prevents convulsions and protects rats from seizure-induced forebrain damage evoked by pilocarpine (Ikonomidou-Turski et al., 1988) and is *anti-epileptogenic* against pentylenetetrazol (PTZ)-induced seizure activity, but at high doses induces seizures (Wallenstein, 1991). In humans, MFA overdose can lead to convulsions and coma (Balali-Mood et al, 1981; Young et al., 1979; Smolinske et al., 1990).

More recent data by Chen and colleagues (1998) have shown that the fenamates, flufenamic, meclofenamic and mefenamic acid, protect chick embryo retinal neurons against ischaemic and excitotoxic (kainate and NMDA) induced neuronal cell death *in vitro* (Chen et al., 1998a; 1998b). MFA has also been reported to reduce neuronal damage induced by intraventricular amyloid beta peptide ($A\beta_{1-42}$) and improve learning in rats treated with $A\beta_{1-42}$ (Joo et al., 2006).

The mechanisms underlying these anti-epileptic and neuroprotective effects are not well understood but together suggest that fenamates may influence neuronal excitability through modulation of ligand and/or voltage-gated ion channels. In the present study, therefore, we have investigated this hypothesis by determining the actions of five representative fenamate NSAIDs at the major excitatory and inhibitory ligand-gated ion channels in cultured hippocampal neurons.

2. Methods

2.1. Hippocampal neuron cultures

Rat embryonic (e17–19 days gestation) hippocampal neurons were isolated and cultured using methods described previously (e.g. Halliwell et al 2002). Briefly the hippocampus was dissected free, chopped in to small fragments and enzymatically and mechanically dissociated in to a single cell suspension. Cells were plated onto 35mm *Primaria* culture dishes (Falcon, Becton Dickinson, New Jersey, USA) in minimal essential medium, supplemented with fetal calf serum, rat serum (Harlan, Sera-Lab, Indianapolis, USA), penicillin/streptomycin, glutamine (2mM, Gibco-Invitrogen, Carlsbad, CA, USA) and glucose (20mM). Cells were then maintained at 37°C, 95% air, 5% CO₂ and 100% relative humidity until required for electrophysiological study. Two thirds of the media was replaced every 5–7 days. Proliferation of non-neuronal cells was inhibited by adding 10 μ M cytosine arabinoside (Sigma-Aldrich, St Louis, MO, USA) to the culture media for 48 hours after 7 days *in vitro*.

2.2. Electrophysiology

Agonist-evoked currents were recorded from cultured hippocampal neurons using the whole-cell configuration of the patch-clamp technique as described previously (Halliwell et al., 2002). Briefly, patch electrodes were made from borosilicate glass pipettes (Harvard, Holliston, MA, USA) on a Narishige PB-7 electrode puller (East Meadow, NY, USA) and had tip resistances of 1–4 M Ω when filled with internal solution. Currents were recorded using an Axopatch 200B amplifier and headstage (Axon Instruments, Foster City, USA) and low pass filtered at 10kHz before digitization via a National Instruments DAQ card and a National Instruments BNC-2090 interface board (Austin, TX, USA) and storage on a PC running WinWCP software (University of Strathclyde, UK). Whole cell currents were monitored on the desktop PC. Series resistance and pipette and whole-cell capacitance were cancelled

electronically. Cells were perfused with a bath solution containing the following (in mM): NaCl (140.0), KCl (2.8), MgCl₂ (2.0), CaCl₂ (1.0), HEPES (10.0). Tetrodotoxin (0.5 μM) was also added to the bath solution to block spontaneous voltage-dependent sodium channel activity. When recording N-methyl-D-aspartate (NMDA)-activated currents, magnesium ions were omitted and glycine (1 μM) was added to the bath solution. In all experiments potassium currents were suppressed by dialyzing the cell interior with a CsCl -based internal solution containing the following (in mM): CsCl (140.0), MgCl₂ (2.0), CaCl₂ (0.1), EGTA (1.1), HEPES (10.0) MgATP (2.0). Bath and internal solutions were titrated to pH 7.2 and filtered before use (using 0.22 μm Whatman, UK). Neurons were normally voltage-clamped at a holding potential of -60 mV. For determination of agonist-evoked current-voltage relationships, the membrane potential was either stepped between -140 mV and 40 mV in 20 mV increments or a ramp protocol was used to clamp a cell between -140 and 40 mV over 500 ms. All experiments were carried out at ambient room temperature (23–25 °C).

2.3. Drugs and their application

All drugs were obtained from Sigma-Aldrich (St Louis, MO, USA) except TTX which was purchased from Tocris Bioscience (Ellisville, MO, USA). Agonists and drugs were applied directly to neurons under voltage-clamp from the tip of a 250 μm pipette connected to a Y-tube (fabricated in-house). Fresh bath solution was also perfused through the culture dish (at 2 ml/min) using a gravity-feed system manufactured in-house to ensure there was no build up of drug solutions in the bath. At least 3 control responses were recorded before application of drugs. When a clear asymptotic drug effect was observed, it was washed off. During the drug wash-out phase, currents were monitored until the control responses were re-established which usually occurred within 5 minutes.

2.4. Data analysis

Sub-maximal (approximating EC₁₀₋₁₅) agonist-evoked currents were measured at their peak amplitude. Agonist-evoked responses in the presence of drugs are expressed as a percentage of the control response ($\pm$ s.e.m. of *n* experiments). For concentration-response data, GABA-evoked currents were normalized to the maximal response evoked by a saturating concentration of agonist (1 mM GABA). These data were fitted, by a least squares, non-linear regression analysis (GraphPad Prism 4™ San Diego, CA, USA) to the logistic equation. The GABA reversal potential was determined for each cell from the data line intercepting the X axis at 0 current on the current to voltage (I-V) plot. For statistical comparisons, the Students t-test for repeated measures, or one-way ANOVA and Dunnett's multiple comparison tests were used where appropriate.

3. Results

3.1. Fenamate NSAIDs Potentiate Neuronal GABA_A receptors

We first investigated 5 fenamate NSAIDs on native GABA_A receptors. Addition of either flufenamic acid (FFA), meclofenamic acid (MCFA), mefenamic acid (MFA), tolafenamic acid (TFA) or niflumic acid (NFA) (all at 10 μM, 30 μM and 100 μM) potentiated sub-maximal GABA (3 μM)-activated currents in a concentration-dependent manner recorded from hippocampal neurons. The order of efficacy, on the basis of maximal potentiation of I_{GABA}, was MFA > TFA > MCFA = FFA > NFA. The effects of all fenamates were rapid in onset and readily reversible upon drug washout (see fig 1).

Further investigation showed that MFA (1–100 μM) potentiated sub-maximal GABA (3 μM)-evoked currents in a concentration-dependent manner with a maximum potentiation to 698 $\pm$ 135% at 100 μM MFA (figure 2). The EC₅₀ for modulation was 37 μM (*n*=4). MFA alone at $\geq$ 10 μM evoked concentration-dependent inward currents which we subtracted from its efficacy

to potentiate the GABA response (figure 2b). We next determined that potentiation of GABA-activated currents by MFA (10 μ M and 100 μ M) was not reduced by the benzodiazepine antagonist, flumazenil (10 μ M) whereas potentiation by chlordiazepoxide (10 μ M) was blocked by 10 μ M flumazenil (figure 2a and 2c).

3.2. Mefenamic Acid Activates Neuronal GABA_A Receptors

Application of MFA (10–1000 μ M, 30s, at approx 2 mins intervals) to voltage-clamped hippocampal neurons evoked inward currents in the absence of exogenous GABA (figure 3) with an EC₅₀ of 138 μ M [95% CI = 79–239] and a Hill Slope of 1.7 $\pm$ 0.7 (n = 4). Sub-maximal MFA (50 μ M) activated currents were blocked by bicuculline (10 μ M) to 16 $\pm$ 4% (n = 5) of control and potentiated by diazepam (1 μ M) to 189 $\pm$ 14% (n = 5) of control (see figure 3c). These effects were fully reversible upon washout of bicuculline and diazepam. The current to voltage relationship for MFA (50 μ M) evoked currents between –120 and 60mV holding potential was similar to that of GABA and reversed at 0mV, the equilibrium potential for chloride ions under our recording conditions (figure 3d).

We also determined if the potentiation of the GABA-evoked response by MFA was voltage-dependent. Our experiments showed that potentiation of sub-maximal GABA (3 μ M) -evoked currents by mefenamic acid (10 μ M) was greatest at negative holding potentials of –80mV to –120mV (see figure 4). The control GABA reversible potential (E_{GABA}) was –1.75mV ($\pm$ 1.2, n = 4) and –1.6mV ($\pm$ 0.1, n = 4) in presence of mefenamic acid (10 μ M). These values are not significantly different to the expected chloride equilibrium potential (E_{Cl}) of 0mV under our recording conditions.

To determine if modulation of the GABA response by MFA was associated with an increase in receptor affinity for the agonist or, alternatively, by an increase in agonist efficacy, concentration-response curves to GABA (0.1–3000 μ M) were determined in the absence and presence of MFA (3 μ M, 10 μ M, and 30 μ M). GABA concentration-response curves were shifted to the left by mefenamic acid with no effect on the maximum GABA response. The control GABA EC₅₀ was 27 μ M which was reduced to 26 μ M, 12 μ M and 5 μ M in the presence of MFA at 3 μ M, 10 μ M and 30 μ M, respectively (see figure 5). The control GABA Hill slope (mean $\pm$ s.e.m, n = 8 neurons) was 1.1 $\pm$ 0.1 and 1.0 $\pm$ 0.1, 0.98 $\pm$ 0.1 and 0.8 $\pm$ 0.1 in the presence of 3 μ M, 10 μ M and 30 μ M MFA. These values were not significantly different.

3.3. Mefenamic Acid has little or no effect at Glutamate or Glycine Receptors

To determine the selectivity of action of fenamates at ligand-gated receptors, we determined the effects of MFA (10 and 100 μ M) on the strychnine-sensitive glycine receptor and the NMDA and non-NMDA ionotropic glutamate receptors in hippocampal neurons. Since MFA directly activates the GABA_A receptor at concentrations of 10 μ M and above, we tested its effects on the glycine and glutamate receptors in the presence of the GABA_A receptor antagonist, bicuculline (10 μ M) to prevent confounding interactions. Sub-maximal currents evoked by glycine (100 μ M), glutamate (10 μ M) or NMDA (30 μ M) were little or not affected by either a low (10 μ M) or high (100 μ M) concentration of MFA (see figure 6). In contrast, control experiments showed that NMDA-evoked currents were blocked by MK-801 (10 μ M), glutamate currents were reduced in the presence of gamma-D-glutamylglycine (10 μ M) or kynurenic acid (10 μ M) and glycine-evoked currents were abolished in the presence of strychnine (10 μ M) consistent with many other studies in this and other laboratories (not shown).

4. Discussion

This study demonstrates for the first time that mefenamic acid and 4 other representatives of the fenamate NSAIDs are highly effective and potent modulators of native hippocampal neuron GABA_A receptors. MFA was the most potent and at concentrations equal to or greater than 10 μ M was also able to directly activate the GABA_A gated chloride channel. A previous study from this laboratory reported that mefenamic acid potentiated *recombinant* GABA_A receptors expressed in HEK-293 cells and in *Xenopus laevis* oocytes (Halliwell et al., 1999). Together these studies lead to the conclusion that fenamate NSAIDs should now also be considered a robust class of GABA_A receptor modulators.

Also demonstrated for the first time here is the direct activation of neuronal GABA_A receptors by mefenamic acid. Other allosteric potentiators, including the neuroactive steroids and the depressant barbiturates share this property, with MFA at least equipotent to neurosteroids and significantly more potent than the barbiturates. The mechanism(s) of the direct gating of GABA_A receptor chloride channels by MFA requires further investigation using ultra-fast perfusion techniques but may be distinct from that reported for neurosteroids (see, Hosie et al., 2006).

Mefenamic acid induced a leftward shift in the GABA dose-response curve consistent with an increase in receptor affinity for the agonist. This is an action observed with other positive allosteric GABA_A receptor modulators, including the benzodiazepine agonist, diazepam, the neuroactive steroid, allopregnanolone, and the intravenous anesthetics, pentobarbitone and propofol (e.g. Johnston, 2005). To our knowledge, a unique property of MFA was that it was significantly ($F = 10.35$; $p \leq 0.001$) more effective potentiating GABA currents at hyperpolarized holding potentials (especially greater than -60 mV). Further experiments are required however to determine the underlying mechanism(s).

Although only a limited number of fenamates were tested here, these data also show that those that are predicted to assume conformations where the two rings have non-planar orientations such as mefenamic acid (Dhanaraj et al., 1988) are most able to enhance GABA_A receptor function. In contrast, niflumic acid, in which its two rings are near planar, was the weakest modulator of GABA currents. The present results also indicate that substitutions on the R2 group of the B ring (which = CH₃ in MFA, see figure 1) influence activity at GABA receptors (see also Woodward et al., 1994). The structure-activity relationship of the fenamates to modulate GABA responses is therefore consistent with a binding site (or sites) on the receptor.

This study also reports for the first time that mefenamic acid has little or no effect at the native strychnine-sensitive glycine receptor or the non-NMDA or NMDA type glutamate receptors over the concentrations that potentiates and directly activates the GABA_A receptor in cultured hippocampal neurons. One previous study reported that niflumic acid and flufenamic acid non-competitively inhibited NMDA-activated currents recorded from spinal neurons with IC₅₀s of approximately 350 μ M (Lerma & Del Rio, 1992). Differences in the neuron types and the fenamates investigated, together with potential confounding actions of fenamates at GABA_A receptors not reported by Lerma & Del Rio might account for this discrepancy. Indeed, an important practical implication of our data showing direct activation of GABA_A receptors by low concentrations of mefenamic acid is that this action must now be taken in to consideration when using such agents as chloride channel blockers (Lerma & Del Rio *op cit.* and references within).

The highly effective modulation of GABA_A receptors in cultured hippocampal neurons suggests the fenamates may have central actions. Consistent with this hypothesis, mefenamic acid concentrations are 40–80 μ M in plasma with therapeutic doses (Cryer & Feldman, 1998); fenamates can also cross the blood brain barrier (Houin et al., 1983; Bannwarth et al., 1989)

and in overdose in humans are associated with coma and convulsions (Smolinske et al., 1990). In animal studies, mefenamic acid is anticonvulsant and neuroprotective against seizure-induced forebrain damage in rodents (Ikonomidou-Turski et al., 1988). The present study would suggest that the anticonvulsant effects of fenamates may be related, in part, to their efficacy to potentiate native GABA_A receptors in the brain, although a recent study has suggested that activation of M-type K⁺ channels may contribute to this action (Peretz et al., 2005).

Finally, Joo and co-workers (2006) have recently reported that mefenamic acid provided neuroprotection against β -amyloid (A β ₁₋₄₂) induced neurodegeneration and attenuated cognitive impairments in this animal model of Alzheimer's disease. The authors proposed that neuroprotection may have resulted from inhibition of cytochrome c release from mitochondria and reduced caspase-3 activation by mefenamic acid. Clearly it would also be of interest to evaluate the role of GABA receptor modulation in this *in vivo* model of Alzheimer's disease. Moreover, considerable evidence has emerged in the last few years indicating that GABA receptor subtypes are involved in distinct neuronal functions and subtype modulators may provide novel pharmacological therapies (Rudolf & Mohler, 2006). Our present data showing that fenamates are highly effective modulators of native GABA_A receptors and that mefenamic acid is highly subtype-selective (Halliwell et al., 1999) suggests that further studies of its cognitive and behavioral effects would be of value.

Acknowledgements

This work was supported by a grant to RFH from the National Institute of Neurological Disorders and Stroke (NINDS, Grant No. 047187-01).

References

- Arber N, DuBois RN. Nonsteroidal anti-inflammatory drugs and prevention of colorectal cancer. *Curr Gastroenterol Rep* 1999;1:441–8. [PubMed: 10980984]
- Balali-Mood M, Critchley JA, Proudfoot AT, Prescott LF. Mefenamic acid overdosage. *Lancet* 1981;1 (8234):1354–6. [PubMed: 6113321]
- Bannwarth B, Netter P, Pourel J, Royer RJ, Gaucher A. Clinical pharmacokinetics of nonsteroidal anti-inflammatory drugs in the cerebrospinal fluid. *Biomed Pharmacother* 1989;43:121–6. [PubMed: 2660917]
- Chen Q, Olney JW, Lukasiewicz PD, Almlı T, Romano C. Fenamates protect neurons against ischemic and excitotoxic injury in chick embryo retina. *Neurosci Lett* 1998a;242:163–166. [PubMed: 9530931]
- Chen Q, Olney JW, Lukasiewicz PD, Almlı T, Romano C. Ca²⁺-independent excitotoxic neurodegeneration in isolated retina, an intact neural net: a role for Cl[−] and inhibitory transmitters. *Mol Pharmacol* 1998b;53:564–572. [PubMed: 9495825]
- Cryer B, Feldman M. Cyclooxygenase-1 and cyclooxygenase-2 selectivity of widely used nonsteroidal anti-inflammatory drugs. *American J Medicine* 1998;104:413–421.
- Dhanaraj V, Vijayan M. Structural studies of analgesics and their interactions. XII. Structure and interactions of fenamates: a concerted crystallographic and theoretical conformational study. *Acta Cryst* 1988;B44:406–412.
- Frolich JC. A classification of NSAIDs according to the relative inhibition of cyclooxygenase isoenzymes. *Trends Pharmacol Sci* 1997;18:30–34. [PubMed: 9114728]
- Halliwell RF, Thomas P, Patten D, James CH, Martinez-Torres A, Miledi R, Smart TG. Subunit-selective modulation of GABA_A receptors by the non-steroidal anti-inflammatory agent, mefenamic acid. *Eur J Neurosci* 1999;11:2897–2905. [PubMed: 10457186]
- Halliwell RF, Su J, Demuro A, Martinez-Torres A, Miledi R. Characterization of the interaction between a novel convulsant agent, norbiphen, and GABA(A) and other ligand-gated ion channels. *Neuropharmacology* 2002;43:778–787. [PubMed: 12367622]

- Hosie AM, Wilkins ME, da Silva HM, Smart TG. Endogenous neurosteroids regulate GABAA receptors through two discrete transmembrane sites. *Nature* 2006;444:486–9. [PubMed: 17108970]
- Houin G, Tremblay D, Bree F, Dufour A, Ledudal P, Tillement JP. The pharmacokinetics and availability of niflumic acid in humans. *Int J Clin Pharmacol Ther Toxicol* 1983;21:130–134. [PubMed: 6852999]
- Ikonomidou-Turski C, Cavaleiro EA, Turski L, Bortolotto ZA, Kleinrok Z, Calderazzo-Filho LS, Turski WA. Differential effects of non-steroidal anti-inflammatory drugs on seizures produced by pilocarpine in rats. *Brain Res* 1988;462:275–285. [PubMed: 3191389]
- Johnston GA. GABA(A) receptor channel pharmacology. *Curr Pharm Des* 2005;11:1867–1885. [PubMed: 15974965]
- Joo Y, Kim HS, Woo RS, Park CH, Shin KY, Lee JP, Chang KA, Kim S, Suh YH. Mefenamic acid shows neuroprotective effects and improves cognitive impairment in in vitro and in vivo Alzheimer's disease models. *Mol Pharmacol* 2006;69:76–84. [PubMed: 16223958]
- Jerma JM, del Rio R. Chloride transport blockers prevent N-methyl-D-aspartate receptor-channel complex activation. *Mol Pharmacol* 1992;41:217–222. [PubMed: 1371581]
- Pascucci RA. Use of nonsteroidal anti-inflammatory drugs and cyclooxygenase-2 (COX-2) inhibitors: indications and complications. *J Am Osteopath Assoc* 2002;102:487–489. [PubMed: 12361181]
- Peretz A, Degani N, Nachman R, Uziyel Y, Gibor G, Shabat D, Attali B. Meclofenamic acid and diclofenac, novel templates of KCNQ2/Q3 potassium channel openers, depress cortical neuron activity and exhibit anticonvulsant properties. *Mol Pharmacol* 2005;67:1053–1066. [PubMed: 15598972]
- Rudolf U, Mohler H. GABA-based therapeutic approaches: GABA_A receptor subtype functions. *Curr Opin Pharmacol* 2006;6:18–23. [PubMed: 16376150]
- Smolinske SC, Hall AH, Vandenberg SA, Spoerke DG, McBride PV. Toxic effects of nonsteroidal anti-inflammatory drugs in overdose. An overview of recent evidence on clinical effects and dose-response relationships. *Drug Saf* 1990;5:252–274. [PubMed: 2198051]
- Vane JR. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nat New Biol* 1971;231:232–5. [PubMed: 5284360]
- Vane JR, Botting RM. Mechanism of action of anti-inflammatory drugs. *Scand J Rheumatol Suppl* 1996;102:9–21. [PubMed: 8628981]
- Wallenstein MC. Attenuation of epileptogenesis by nonsteroidal anti-inflammatory drugs in the rat. *Neuropharmacology* 1991;30:657–63. [PubMed: 1922684]
- Weissmann G. Aspirin. *Sci Am* 1991;264:84–90. [PubMed: 1899486]
- Woodward RM, Polenzani L, Miledi R. Effects of fenamates and other nonsteroidal anti-inflammatory drugs on rat brain GABAA receptors expressed in *Xenopus* oocytes. *J Pharmacol Exp Ther* 1994;268:806–817. [PubMed: 8113993]
- Young RJ. Mefenamic acid poisoning and epilepsy. *Br Med J* 1979;2(6191):672. [PubMed: 497787]

Figure 1. Fenamates potentiate GABA-evoked currents in hippocampal neurons

A. shows sub-maximal GABA (G; 3 μ M) evoked whole-cell membrane currents recorded from a hippocampal neuron in the absence and presence of mefenamic acid (MFA), tolfenamic acid (TFA), flufenamic acid (FFA), meclofenamic acid (MCFA) and niflumic acid (NFA) all at 100 μ M. The cell was voltage-clamped at -60mV. B. shows a histogram summary (n = 4–14; mean $\pm$ s.e.m) of the effects of the five fenamates at 10 μ M, 30 μ M and 100 μ M on the sub-maximal GABA currents. C. shows the generic structure of fenamates and D. shows the list of fenamates tested, with abbreviations and substitutions of the R1-3 and X groups.

Figure 2. The actions of mefenamic acid are not dependent on the benzodiazepine site

A shows control GABA (G; $3 \mu\text{M}$) evoked whole cell currents in the absence and presence of chlordiazepoxide ($10 \mu\text{M}$) plus or minus flumazenil ($10 \mu\text{M}$) or MFA (10 and $100 \mu\text{M}$) plus or minus flumazenil ($10 \mu\text{M}$). Note that flumazenil blocks the potentiation of the GABA current by CDZ but has no effect on the potentiation by MFA. The holding potential was -60 mV . B shows a histogram summary ($n = 4$, mean $\pm$ s.e.m) of similar experiments shown in A. C. shows the concentration-response plot for potentiation of GABA (G; $3 \mu\text{M}$) currents by MFA ($\blacksquare$, $1 \mu\text{M} - 100 \mu\text{M}$). Note that the graph also shows the potentiation minus the direct MFA current ($\blacktriangledown$) and also the currents activated by MFA alone ($\bullet$) relative to the control GABA response ($3 \mu\text{M}$) ($n = 4$ cells);

Figure 3. Mefenamic acid activates the GABA_A gated chloride channel

A, shows whole-cell membrane currents evoked by application of mefenamic acid (MFA, 10–1000 μM , 30s duration at intervals of approximately 2 minutes) to a hippocampal neuron; B, the MFA (10–1000 μM) dose-response curve (mean $\pm$ s.e.m, $n = 4$); C, a histogram summary ($n=5$) of MFA (50 μM) -evoked responses, normalized to control, in the presence of diazepam (1 μM) or bicuculline (10 μM). D, the current-voltage (–120mV to +60mV) plot for MFA (50 μM)-evoked currents (mean $\pm$ s.e.m, $n= 3$). For panels A, B and C the cells were held at –60mV.

Figure 4. Potentiation of the GABA response by mefenamic acid is sensitive to holding potential
 A, shows GABA ($3\mu\text{M}$)-evoked whole cell membrane currents in a neuron held at -60mV and $+60\text{mV}$ in the absence and presence of mefenamic acid (MFA, $10\mu\text{M}$). B, a histogram summary of MFA ($10\mu\text{M}$)-induced potentiation of GABA-evoked recorded from neurons voltage-clamped between -120mV and $+60\text{mV}$. The bars represent the mean $\pm$ s.e.m of data from 4 neurons. C. the current to voltage (I-V) plot for the control GABA ($3\mu\text{M}$) evoked response (●) and the GABA response in the presence of MFA (○, $10\mu\text{M}$). The control GABA reversal potential was -1.75mV (± 1.2 , $n = 4$) and -1.6mV (± 0.1 , $n = 4$) in presence of MFA ($10\mu\text{M}$).

Figure 5. Mefenamic acid shifts the GABA dose-response curve leftwards

The graph shows the GABA (●) concentration (x-axis) response (y-axis) plot with responses normalized to 1mM GABA for each cell. Also plotted is the GABA concentration-response relationship in the presence of 3μM (○), 10μM (■) and 30μM (□) mefenamic acid. The graph represents the mean ± s.e.m of 7–8 neurons and curves are fitted by a least squares, non-linear regression analysis to the logistic equation. The holding potential was –60mV. The inset is a histogram of the GABA EC₅₀ (± s.e.m) in the presence of MFA (at 10μM, 30μM and 100μM).

Figure 6. Mefenamic acid has little or no effect at glutamate or glycine receptors

The top half shows discontinuous records of sub-maximal glycine (Gly, 100μM), glutamate (Glut, 10μM) and N-methyl-D-aspartate (NMDA, 30μM) evoked whole-cell membrane currents from hippocampal neurons in the absence and presence of mefenamic acid (MFA, 100μM). To prevent direct activation of the GABA_A receptor, bicuculline (10μM) was also included in the bath solution. The lower half shows a histogram representing the mean ± s.e.m. of 6–7 similar experiments in which MFA was tested at 10μM and 100μM. The holding potential for these experiments was –60mV. The scale bar represents 1500pA, 500pA and 308pA for Glycine, glutamate and NMDA, respectively.