Effects of Fenamate on Inhibitory Postsynaptic Currents in Purkinje's Cells

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The effects of nonsteroid antiinflammatory drugs of the fenamate group (mefenamic and tolfenamic acids) on spontaneous miniature inhibitory postsynaptic currents in Purkinje's cells were studied in mouse cerebellar slices by the whole cell patch-clamp method. Both drugs in concentrations of 3-30 μM significantly prolonged miniature inhibitory postsynaptic currents and reduced their amplitude.

Key Words: *nonsteroid antiinflammatory drugs; mefenamic acid; tolfenamic acid; cerebellum; potential fixation method*

Nonsteroid anti-inflammatory drugs (NAID) are among the most frequently used drugs all over the world [5] and at the same time belong to the main sources of intoxications [10]. The effects of NAID are mainly due to inhibition of cycloxygenase isoforms, which underlies their antiinflammatory, analgesic, antipyretic, and cardioprotective effects [12].

Tolfenamic (TPA) and mefenamic (MPA) acids belong to the fenamate group NAID including anthranilic acid derivatives (Figs. 1, *c*; 2, *c*). On the Russian pharmacological market these substances are presented by Clotam (TPA), Lysalgo (MPA), *etc.* Analgesic activity of fenamates surpasses that of salicylates, while antipyretic activity is the same.

Fenamates easily penetrate through the blood-brain barrier [1], which explains their central effects. Overdosage of MPA leads to the development of convulsions and coma [11]. Highly effective suppression of migraine attacks with TPA was recently demonstrated [8]. Neuroprotective effects of MPA were demonstrated in neurodegenerative diseases [9,10] and experimentally on models with glutamate toxicity [2,3]. However, the mechanisms underlying these effects remain little studied.

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Both acids potentiate GABA-evoked currents in oocytes expressing $GABA_A$ receptors [6,13] and in cultured hippocampal neurons [4]. In addition, MPA and TPA in concentrations >10 μM induce chlorine currents, which can be blocked by bicuculline and potentiated by diazepam [4]. On the other hand, the effects of these drugs on the inhibitory synaptic transmission under conditions of endogenous GABA interactions with postsynaptic receptors were never studied. We studied the effects of TPA and MPA on inhibitory synaptic transmission in cerebellar slices.

MATERIALS AND METHODS

Experiments were carried out on 12-15-day-old C57Bl/6J mice. The animals were decapitated under deep ether narcosis, the brain was rapidly removed and placed in cold (4°C) solution containing (in mM): 125 NaCl, 4 KCl, 10 glucose, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 0.5 $CaCl_2$, and 2.5 $MgCl_2$ and constantly saturated with a gas mixture of 95% O_2 and 5% CO_2 (pH 7.3). The cerebellum was isolated and divided into two hemispheres. Sagittal slices of the cerebellum (200 μ) were made on a vibratome. The slices were then incubated in artificial cerebrospinal fluid (ACSF) containing (in mM): 125 NaCl, 4 KCl, 10 glucose, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 2 $CaCl_2$,

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and 1 $MgCl_2$ and constantly saturated with a gas mixture of 95% O_2 and 5% CO_2 (pH 7.3). ACSF osmolarity was 330 mOsm. The slices were left in the incubation cell for at least 1 h and then transferred into registration cell on a platform of an Axioscope FS microscope (Zeiss, Oberkochen). The slices were constantly perfused with ACSF containing 10 μM 6,7-dinitroquinoxaline-2,3-dion (DNQX; AMPA/cainate receptor antagonist), 50 μM DL-2-amino-5-phosphonopentaenic acid (NMDA receptor blocker), and 0.5 μM tetrodotoxin (TTC; potential-sensitive Na channel blocker). The rate of ACSF flow realized at the expense of gravitation force was 1 ml/min. Spontaneous postsynaptic currents (PSC) in Purkinje's cells were recorded by the whole cell patch-clamp method. Purkinje's cells were identified by size, location in the ganglionar layer, and orientation of dendritic stems. The solution in the pipette contained (mM): 100 K gluconate, 50 KCl, 5 NaCl, 0.5 $CaCl_2$, 5 EGTA, 20 HEPES, 3 ATP-Mg, and 0.3 GTP (guanosine triphosphate); pH was brought to 7.2 by adding KOH. The osmolarity of this solution was 320 mOsm, resistance of recording pipettes 2-4 MΩ. The currents were recorded using EPC-7 amplifier (List, Darmstadt), 16-bite ACP/CAP (ITC-16, HEKA Electronic, Lambrecht), and TIDA 4.11 software (HEKA Electronic). The signals were filtered at a frequency of 3 kHz and digitalized at a frequency of 10 kHz. The potential was clumped at -70 mV, chlorine reversion potential was -20 mV. The access resistance was regulated by applying a hyperpolarizing current pulse (10 mV amplitude). Only cells with the access resistance varying by no more than 20% during the experiment and less than 40 MΩ were taken for the analysis.

The data were processed using PeakCount V3.2 (Henneberger, Institute of Neurophysiology) and Prism V4.03 (GraphPad Software Inc.) software. The results are presented as the mean±standard error of the mean. The normality of data distribution was verified by the Kolmogorov—Smirnov test. The significance of differences was evaluated using Student's *t* test.

RESULTS

In all neurons (data on activities registered in 12 cells are presented) we observed spontaneous PSC, which were completely and reversibly blocked with gabazine (specific $GABA_A$ receptor antagonist; 10 μM) and reversed at a potential of -20 mV (reversion potential for chlorine ions). Since perfusion solution contained DNQX, DL-APV, and TTC, we concluded that spontaneous PSC were miniature inhibitory PSC (mIPSC; Fig. 1, *a*, *b*; 2, *a*, *b*).

Registration of mIPSC in the control sample showed that their mean amplitude was 63.99±1.045 pA (standard deviation (SD) 51.86 pA), time of increment $T_{20\text{-}80\%}$=0.91±0.01 msec (SD=0.65 msec). A total of 2464 events in 12 cells were analyzed. Attenuation of mIPSC in the control was optimally approximated by a single-exponential curve with attenuation time constant (Tay) equal to 10.65± 0.12 msec (SD=3.58; 957 events in 5 cells).

In order to detect the effects of fenamates on mIPSC, MPA and TPA in concentrations of 3, 10, and 30 μM were added into the perfusion solution. The solution in the registration cell was completely changed within <2 min. mIPSC were recorded 4 min after the start of addition of the solution. Solutions were used in the order of ascending fenamate concentrations.

Application of MPA and TPA led to the development of at least three concentration-dependent effects. First, fenamates used in experiments reduced the amplitude of mIPSC (Fig. 1, *b*; 2, *b*). The mean amplitudes of mIPSC in the presence of 3, 10, and 30 μM MPA were 90.4±2.9, 84.7±4.3, and 64.6±12.0% of the mean control amplitude, respectively (the data on 5 cells; Fig. 1, *f*), while in the presence of 3, 10, and 30 μM TPA these values were 83.8±5.1, 70.9±6.7, and 66.6±14.5% of the control, respectively (data on 7 cells; Fig. 2, *f*). Second, MPA and TPA accelerated the increment of mIPSC (Fig. 1, *b*; 2, *b*). The mean time of mIPSC increment from 20 to 80% ($T_{20\text{-}80\%}$) in the presence of 3, 10, and 30 μM MPA in comparison with the mean $T_{20\text{-}80\%}$ in the control was 82.1±3.0, 71.6±6.6, and 62.3±7.7%, respectively (data on 5 cells; Fig. 1, *h*), while in the presence of 3, 10, and 30 μM TPA the values were 87.6±3.3, 73.9±4.8, and 65.7± 6.3%, respectively (data on 7 cells; Fig. 2, *h*).

Third, application of the studied fenamates caused splitting of the monoexponential mIPSC decay into two components, fast and slow (Fig. 1, *b*; 2, *b*). In the presence of just 3 μM MPA and TPA, the attenuation curves were approximated by a biphasic exponential curve better than by a monoexponential one. For evaluation of Tay values for different components, 100 events per point were estimated and averaged. The resultant mIPSC attenuation curve was approximated by a biphasic curve using Prism V4.03 software. Tay values for fast and slow components (Tay^f and Tay^s) were calculated. The following Tay^f values in the presence of 3, 10, and 30 μM MPA were obtained: 8.77±0.80, 8.00±0.81, and 7.18±0.47 msec, respectively (Fig. 1, *d*); for Tau^s: 222.87±80.57, 509.97±124.24, and 710.75±214.27 msec, respectively (Fig. 1, *d*; data on 5 cells). The Tau^f value in the presence of 3, 10, and 30 μM TPA

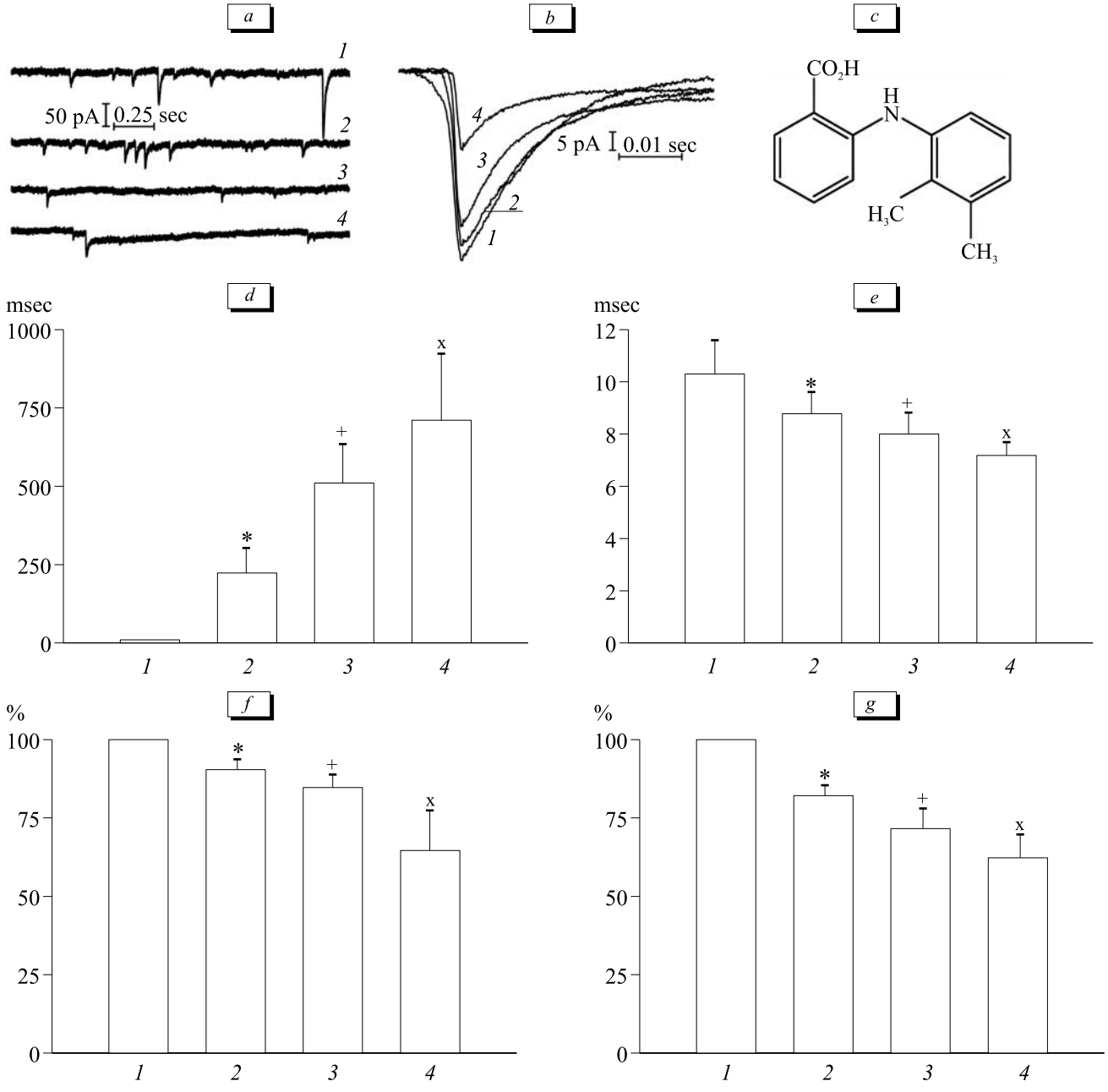

Fig. 1. Effects of MPA on the mIPSC parameters in Purkinje's cells. *a*) mIPSC in the control and with MPA. Here and in fragments *b*, *d-h*: *1*) control; *2*) 3 μM MPA; *3*) 10 μM MPA; *4*) 30 μM MPA. *b*) averaged mIPSC for 100 events in the control and in the presence of MPA; *c*) structural formula of MPA; *d*) slow component of mIPSC attenuation (Tay^s) in two-exponential approximation of averaged attenuation curves for mIPSC in the presence of MPA. $^*p$=0.037 compared to the control, ^+p=0.038 compared to 3 μM MPA, and ^xp=0.127 compared to 10 μM MPA. *e*) fast component of mIPSC attenuation (Tay^f) in two-exponential approximation of averaged curves of mIPSC attenuation in the presence of MPA. $^*p$=0.033 compared to the control, ^+p=0.072 compared to 3 μM MPA, ^xp<0.172 compared to 10 μM MPA. *f*) time course of mIPSC amplitude in the presence of MPA (% of the control). $^*p$=0.003 compared to the control, ^+p=0.027 compared to 3 μM MPA, ^xp=0.110 compared to 10 μM MPA. *h*) time of mIPSC increment in the presence of MPA, % of the control. $^*p$=0.004 compared to the control, ^+p=0.006 compared to 3 μM MPA, ^xp=0.007 compared to 10 μM MPA.

was 8.54±0.40, 7.55±0.45, and 6.59±0.56 msec, respectively (Fig. 2, *d*), for Tau^s 173.8±14.23, 311.3±62.6, and 583.95±160.48 msec, respectively (Fig. 2, *d*; data for 5 cells).

Hence, our experiments showed that MPA and TPA caused similar changes in the fast synaptic transmission parameters in GABAergic synapses in Purkinje's cells. Both drugs in concentration of just 3 μM reduced the amplitude, accelerated increment kinetics, and significantly inhibited attenuation kinetics of mIPSC. The fast and slow components of MIPSC attenuation appeared in the presence of fe-

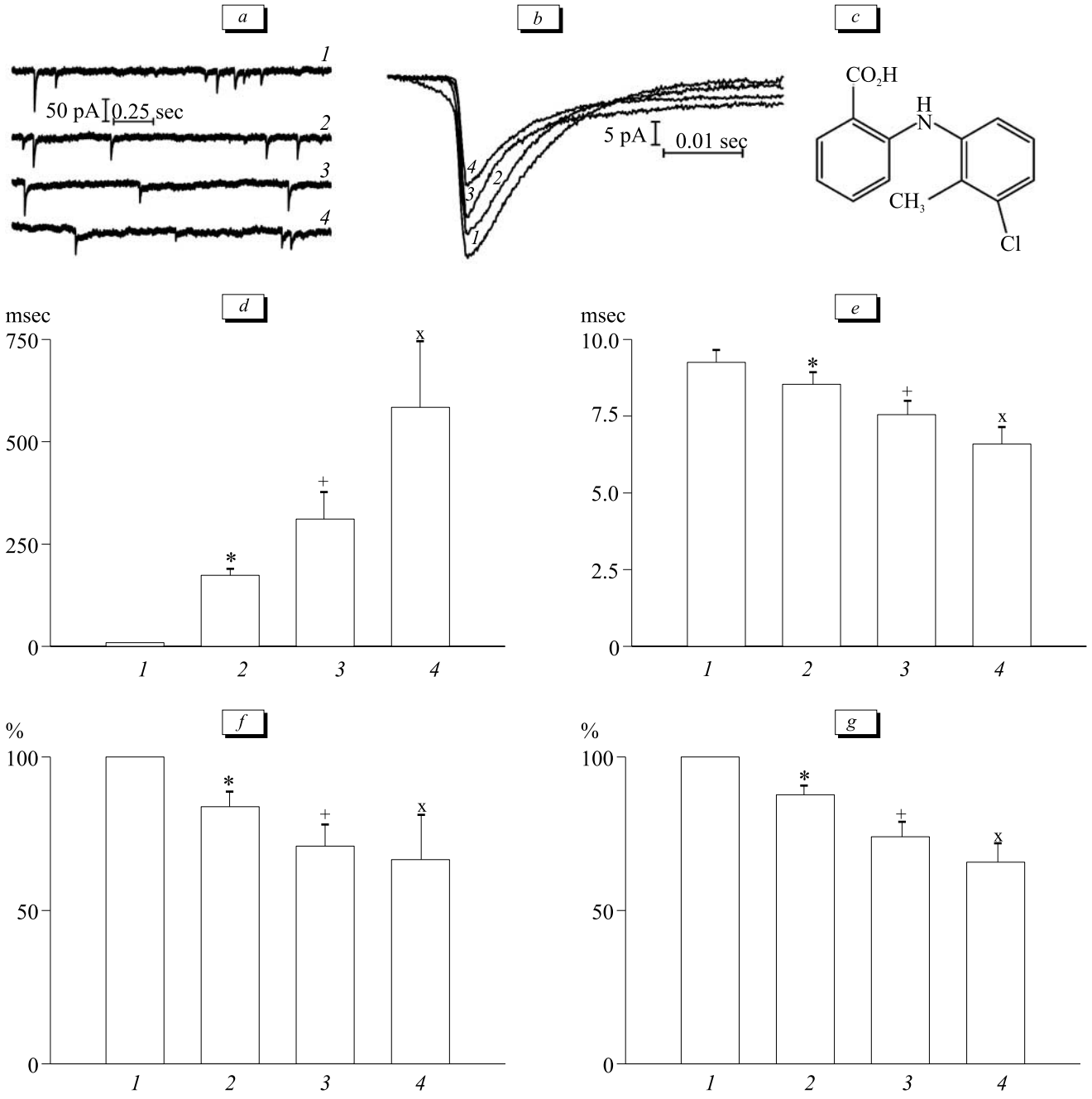

Fig. 2. Effects of TPA on the mIPSC parameters in Purkinje's cells. *a*) mIPSC in the control and with TPA. Here and in fragments *b*, *d*-*h*: *1*) control; *2*) 3 μM TPA; *3*) 10 μM TPA; *4*) 30 μM TPA. *b*) averaged mIPSC for 100 events in the control and in the presence of TPA; *c*) structural formula of TPA; *d*) slow component of mIPSC attenuation (Tay^s) in two-exponential approximation of averaged attenuation curves for mIPSC in the presence of TPA. $^*p$=0.002 compared to the control, ^+p=0.024 compared to 3 μM TPA, and ^xp=0.133 compared to 10 μM TPA. *e*) fast component of mIPSC attenuation (Tay^f) in two-exponential approximation of averaged curves of mIPSC attenuation in the presence of TPA. $^*p$=0.012 compared to the control, ^+p=0.148 compared to 3 μM TPA, ^xp<0.139 compared to 10 μM TPA. *f*) time course of mIPSC amplitude in the presence of TPA (% of the control). $^*p$=0.024 compared to the control, ^+p=0.021 compared to 3 μM TPA, ^xp=0.228 compared to 10 μM TPA. *h*) time of mIPSC increment in the presence of TPA, % of the control. $^*p$=0.013 compared to the control, ^+p=0.039 compared to 3 μM TPA, ^xp=0.004 compared to 10 μM TPA.

namates. The time of the fast component attenuation in the presence of fenamates was somewhat shorter than in the control, while the time of the slow component attenuation was by tens times longer than in the control.

Our data are in good agreement with the results obtained on cultured hippocampal neurons [4], indicating that fenamates potentiate currents induced by low concentrations of GABA, which explains more rapid kinetics of postsynaptic current incre-

ment and appearance of the mIPSC attenuation slow component, observed in our experiments. However, in the presence of high concentrations of GABA, fenamates produce a blocking effect, which can explain the appearance of the slow component of attenuation and reduced amplitude of mIPSC.

The appearance of slow attenuation component of mIPSC in the presence of MPA and TPA suggests that these drugs due to temporary summation of individual events can lead to creation of a frequency-dependent tonic inhibition. Reduced amplitude of mIPSC in the presence of high MPA and TPA concentrations suggests that these drugs can suppress rapid inhibitory signaling. Plasma concentrations of these drugs in therapeutic doses can reach tens of μM; for example, for MPA this value reaches 40-80 μM [11]. Hence, the effects of MPA and TPA on mIPSC parameters and possible aftereffects can occur *in vivo* during therapy with these drugs.

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