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Short communication

Actions of sipatrigine, 202W92 and lamotrigine on R-type and T-type Ca^{2+} channel currents

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Abstract

Relatively little has been published on the pharmacology of R-type and T-type Ca^{2+} channels. Here, whole-cell Ca^{2+} channel currents were recorded from human embryonic kidney 293 cell-lines transfected with either α1E subunits (R-type currents) or α1G or α1I subunits (T-type currents). R-type currents were inhibited by sipatrigine and the related compound 202W92 (R-(−)-2,4-diamino-6-(fluromethyl)-5-(2,3,5-trichlorophenyl)pyrimidine) with IC_{50} 10 and 56 μM, respectively. A therapeutic concentration of lamotrigine (10 μM) inhibited R-type currents (30%) but was without effect on α1I-mediated T-type currents. Lamotrigine was also a weak inhibitor of T-type currents mediated by α1G subunits (<10% inhibition by 100 μM).

Keywords: Ca^{2+} channel, voltage-activated; Lamotrigine; Antiepileptic drug; Anticonvulsant

1. Introduction

R-type (resistant) Ca^{2+} channel currents are found in most cell types studied, and the α1E ($Ca_V2.3$) subunit, known to underlie some R-type currents (see Lee et al., 2002), is widely distributed in the nervous system both at somata and nerve endings (Williams et al., 1994; Wu et al., 1998; Yokoyama et al., 1995). Published R-type pharmacology is sparse, with no agents reported to inhibit R-type currents at therapeutic concentrations (Cai et al., 1997; Nakashima et al., 1998; Randall and Tsien, 1997; Tottene et al., 2000; Williams et al., 1994). Some low-voltage-activated T-type Ca^{2+} channel currents have similar electrophysiological properties to R-type currents (Randall and Tsien, 1997), and T-type currents were originally attributed to the α1E subunit (Soong et al., 1993), now known to produce R-type. T-type Ca^{2+} currents (mediated by α1G, α1H or α1I subunit-containing channels, Perez-Reyes et al., 1998) can potently influence neuronal excitability (Randall and Benham, 1999). Excessive activity of T-type currents in thalamo-cortical projection neurons has been proposed to underlie the slow wave discharges characteristic of typical absence seizures (Coulter et al., 1989), though this model is likely to be an oversimplification (Leresche et al., 1998; Crunelli and Leresche, 2002).

The chemically related series sipatrigine (previously BW619C89), 202W92 (R-(−)-2,4-diamino-6-(fluromethyl)-5-(2,3,5-trichlorophenyl)pyrimidine) and lamotrigine have shown varying degrees of inhibition in a variety of Na^+ and Ca^{2+} channel preparations (Caputi et al., 2001; Hainsworth et al., 2000; Xie and Hagan, 1998). Lamotrigine is a broad spectrum antiepileptic agent with efficacy in typical absence seizures and Lennox-Gastaut syndrome (Matsuo, 1999; McCabe, 2000). Though the principle mechanism of action is considered to be blockade of voltage-gated Na^+ and Ca^{2+} channels (Leach et al., 1995; Xie and Hagan, 1998), the utility of lamotrigine in absence epilepsy and Lennox-Gastaut syndrome is as yet incompletely explained. Here we report for the first time the actions of sipatrigine, 202W92 and lamotrigine on R-type current mediated by α1E channels and of lamotrigine on T-type currents mediated by α1G and α1I channels.

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2. Methods

Human embryonic kidney 293 cells stably expressing either rat α1G or rat α1I subunits ($Ca_V3.1$ and $Ca_V3.3$, respectively, McNaughton et al., 2000), or human α1E ($Ca_V2.3$) with β3 subunits (Mehrke et al., 1997), were grown in Dulbecco's modified Eagle medium supplemented with foetal bovine serum (10–15%). In standard whole-cell recordings made at room temperature, Ca^{2+} channel currents were recorded using test pulses to −25 mV (T-type currents) or +10 mV (R-type currents), duration of 20–100 ms, applied every 10 s from a holding potential of −90 mV (McNaughton et al., 2000). A cesium-based intracellular solution containing 4 mM ATP and a tetraethylammonium-based extracellular solution containing 2.0–5.0 mM Ca^{2+} or Ba^{2+} ions, with no added sodium salts, allowed Ca^{2+} channel currents to be isolated. Series resistance (<10 MΩ) was compensated 70–80%. Data were low pass filtered (−3 dB, 2 kHz, 8-pole Bessel response) and sampled at ≥5 kHz. Drugs were dissolved in extracellular solution on the day of use and applied via a multi-barrel fast perfusion system. Sipatrigine mesylate and 202W92 mesy-

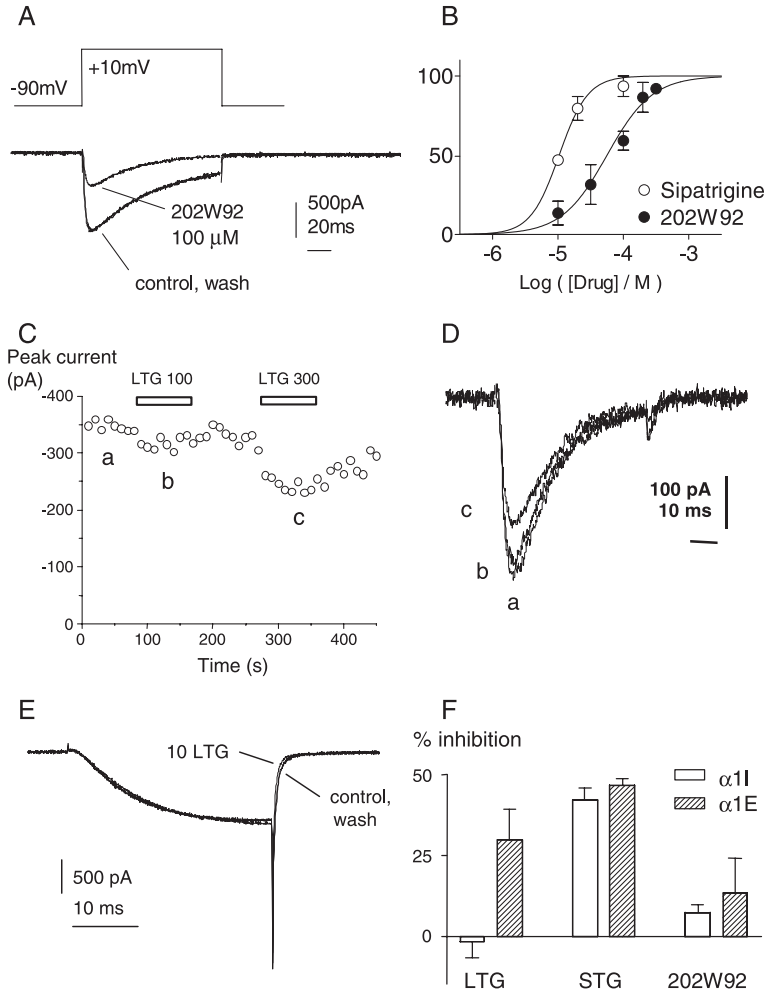

Fig. 1. Inhibition of R-type and T-type Ca^{2+} channel currents. (A) R-type whole-cell Ca^{2+} channel currents recorded from a human embryonic kidney 293 cell transfected with human α1E and β3 subunits in response to a square pulse command to +10 mV from a holding voltage of −90 mV (100 ms duration, applied every 10 s). Current traces are superimposed before (control) and during application of 202W92 (100 μM), then 1 min after removal of the drug (wash). (B) Concentration–inhibition relations for inhibition of R-type currents by sipatrigine (open symbols) and 202W92 (filled symbols). Points show mean % inhibition of peak current and error bars show 1 S.E.M. ($n=4$ in each case). Solid lines show the Langmuir–Hill curves best fit with IC_{50} values of 10 and 56 μM for sipatrigine and 202W92, respectively, and Hill coefficients of 2.0 and 1.3 for sipatrigine and 202W92, respectively. (C) Time-course of peak amplitude of T-type whole-cell currents recorded from a human embryonic kidney 293 cell transfected with rat α1G subunit in response to a square pulse command to −25 mV from a holding voltage of −90 mV (100 ms every 10 s). Horizontal bars indicate application of lamotrigine (LTG, 100, 300 μM). (D) Example traces of α1G subunit-mediated T-type whole-cell current at time points marked a, b and c in panel C. (E) T-type whole-cell currents recorded from human embryonic kidney 293 cells transfected with rat α1I subunits in response to a square pulse command of +65 mV from a holding voltage of −90 mV (100 ms every 10 s). Example traces are shown before (control) and during application of lamotrigine (LTG, 10 μM), then 1 min after removal of the drug (wash). (F) Comparison of the actions of lamotrigine (LTG), sipatrigine (STG) and 202W92 (all 10 μM) on rat α1I-mediated T-type and human α1E-mediated R-type currents. Bars show % inhibition of peak current, mean ± 1 S.E.M., $n=3–7$. Bars for inhibition of α1I-mediated currents by sipatrigine and 202W92 are derived from previously published data (Caputi et al., 2001; McNaughton et al., 2000).

late salts were synthesized in the University of Greenwich, London, UK.

3. Results

Whole-cell R-type currents were routinely recorded from cells transfected with α1E and β3 subunits (see control traces in Fig. 1A) and were abolished by external application of 100 μM Ni^{2+} (not shown). Sipatrigine (10–100 μM), 202W92 (10–300 μM) and lamotrigine (10 μM) inhibited α1E-mediated R-type currents (see example traces for 202W92, Fig. 1A). Pooled concentration–inhibition data for α1E-mediated R-type currents by sipatrigine and 202W92 is shown in Fig. 1B (IC_{50}, 10 μM for sipatrigine, 56 μM for 202W92).

T-type currents have been previously reported in the α1G-transfected and α1I-transfected cell-lines used here (Caputi et al., 2001; McNaughton et al., 2000) and were routinely observed in the present experiments (see control traces in Fig. 1D,E). α1G-mediated and α1I-mediated T-type currents showed little sensitivity to lamotrigine under the conditions used here (Fig. 1C–F). At high concentrations, lamotrigine inhibited α1G-mediated currents (< 10% inhibition by 100 μM, 30 ± 4% inhibition by 300 μM, mean, S.E.M., $n = 4$, see Fig. 1C–D). Pooled data comparing peak current inhibition of α1E-mediated R-type with that of α1I-mediated T-type currents by all three drugs at 10 μM concentration is shown in Fig. 1F.

4. Discussion

R-type currents mediated by α1E subunits were inhibited by sipatrigine and 202W92, both of which are neuroprotective agents in animal models of ischemia (Caputi et al., 2001; Hainsworth et al., 2000). Neuroprotective doses of sipatrigine produced brain concentrations in monkeys of 20–100 μM (Hainsworth et al., 2000). Sipatrigine has shown anticonvulsant activity in the seizure models where it has been tested—the genetically epilepsy-prone rat and DBA/2 audiogenic mouse (Meldrum et al., 1994; Reddy et al., 1998)—though these models have little specific relation to absence or Lennox-Gastaut syndrome. α1E-mediated currents were also inhibited by lamotrigine (10 μM), a concentration within the estimated range of therapeutic brain concentrations (4–40 μM, Leach et al., 1995). α1E protein is widely distributed in the central nervous system, including the somata and dendrites of thalamic neurons (Yokoyama et al., 1995). Thus, it is possible that inhibition of R-type currents may contribute to the anti-absence action of lamotrigine. This is the first report of the actions of sipatrigine, 202W92 and lamotrigine on R-type currents.

α1G-mediated and α1I-mediated T-type currents were previously shown to be inhibited by sipatrigine and 202W92 (Caputi et al., 2001; McNaughton et al., 2000). Ca^{2+} channel currents mediated by α1G subunits or α1I subunits were relatively insensitive to lamotrigine under the conditions used here. These two channel subtypes are highly expressed in brain tissue, α1G most likely being the form expressed in thalamic neurons (Randall and Benham, 1999).

In conclusion, α1E-mediated R-type currents were inhibited by sipatrigine and less potently by 202W92. These R-type currents—much more than α1G-mediated or α1I-mediated T-type currents—were sensitive to a therapeutic concentration of lamotrigine.

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