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Short communication

Vinpocetine is as potent as phenytoin to block voltage-gated Na^+ channels in rat cortical neurons

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Abstract

The effects of vinpocetine and phenytoin on voltage-gated Na^+ channels were examined on cultured cerebrocortical neurones of the rat using a conventional whole-cell patch-clamp method. Vinpocetine and phenytoin decreased Na^+ currents in a concentration-dependent manner, with IC_{50} values of 44.2 ± 14.6 and 50.5 ± 17.4 μM, respectively. Both compounds shifted the voltage dependence of the steady-state inactivation of the channel in the hyperpolarising direction. This pronounced Na^+ channel blocking activity may contribute to the neuroprotective and anticonvulsant effects of vinpocetine.

Keywords: Vinpocetine; Phenytoin; Na^+ channel, voltage-gated; Anticonvulsant; Neuroprotection; Cortical neuron; (Rat)

1. Introduction

Vinpocetine (14-ethoxycarbonyl-(3α,16α-ethyl)-14,15-eburnamine; Cavinton), first described as cerebral vasodilator (Kárpáti and Szporny, 1976), is a widely used nootropic drug exhibiting favourable clinical effects in a variety of dementias (Balestreri et al., 1987). Although vinpocetine has been classified as a nootropic agent (Fröstl and Maître, 1989), its neuroprotective effects have also been documented both in vitro and in vivo. In cortical cultures, vinpocetine reduced excitotoxic neuronal death (Erdő et al., 1990b) and potentiated the neuroprotective effect of adenosine against hypoxic damage (Krieglstein and Rischke, 1991). Moreover, in a rat model of cerebral ischemia, vinpocetine significantly reduced the loss of hippocampal neurones (Sauer et al., 1988).

The anticonvulsant activity of vinpocetine has been demonstrated in rodent models, such as pentylenetetrazole-induced convulsions, and amygdala or cortical kindling (Schmidt, 1990). However, this pharmacological activity has not been clinically utilised to our knowledge.

There is increasing evidence that anticonvulsants acting through the blockade of voltage-gated Na^+ channels exert pronounced neuroprotective effects (Rataud et al., 1994). This correspondence of pharmacological profiles prompted us to investigate whether vinpocetine, like the prototype anticonvulsant Na^+ channel blocker, phenytoin, also inhibits Na^+ currents. We report here on the actions of vinpocetine on voltage-gated Na^+ currents recorded from rat cerebrocortical neurones in primary culture.

2. Materials and methods

2.1. Primary cortical cultures

Primary cultures were prepared from embryonic rat cerebral cortex using a method described elsewhere (Erdő et al., 1990a). Briefly, cells were isolated from 17-day-old rat (Sprague-Dawley, Charles River) foetuses, plated onto poly-D-lysine-coated glass coverslips placed in 24-well plates at a density of 3×10^5 cells per well, in a volume of 0.5 ml. Cultures were maintained for up to 14 days in Dulbecco's modified Eagle medium supplemented with 10% foetal calf serum and in a humidified 5% CO_2 atmosphere.

2.2. Whole-cell patch-clamp

Voltage-clamp recordings were performed at room temperature on the stage of an inverted phase-contrast

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microscope (Olympus, IMT2). Coverslips with the cultured cells (7–14 days in culture) were transferred into the recording chamber, rinsed and continuously perfused by gravity with an extracellular solution containing (in mM) NaCl 140, KCl 5, $CaCl_2$ 2, $MgCl_2$ 2, Hepes 10, glucose 20, saccharose 10, pH = 7.34. Patch pipettes (5–10 MΩ) were prepared from filament containing standard-wall borosilicate glass capillaries (outer diameter = 1.2 mm, Clark Electromedicals) with a micropipette puller (P-87, Sutter). The intracellular solution contained (in mM) CsF 130, NaCl 15, TEA-Cl 10, CaC_2 0.1, $MgCl_2$ 2, ATP 2, Hepes 10, EGTA 1, pH = 7.25.

Whole-cell currents were recorded with an Axopatch 200A amplifier using the pClamp 5.5 software (Axon). Signals were filtered at 5 kHz and sampled at 20 kHz. Capacitive transients and series resistance were compensated for and linear leakage current was subtracted using the P/6 protocol (leakage current was calculated based on six small and short hyperpolarising pre-pulses before conditioning and test-pulses, see user manual of pClamp 5.5).

Effects on Na^+ currents were measured applying voltage steps (20 ms duration) to −10 mV from a holding potential of −60 mV (−70 mV in inactivation studies) in every 20 s. Drugs dissolved in bath solution were administered in single or in increasing concentrations 3 min apart with a seven-barrelled, gravity-driven, fast drug administration system directly to the neurones. Steady-state inactivation curves were obtained by clamping the membrane at one of a series of 15-s pre-pulse potentials, followed 1 ms later by a 20-ms test pulse to −10 mV.

2.3. Data analysis

The peak amplitudes of the Na^+ currents evoked by the voltage steps were measured using the pClamp 5.5 program. IC_{50} values were calculated for each experiment by direct curve fitting (SigmaPlot for Windows). Shifts in steady-state inactivation curves were calculated using two-state Boltzmann fitting of the data (see legend to Fig. 2).

2.4. Chemicals and drug solutions

All chemicals were obtained from Sigma and were of analytical grade. Phenytoin (Sigma) was dissolved in the extracellular solution. Vinpocetine (G. Richter, Budapest) was first dissolved in 1 N HCl at a concentration of 20 mM, and thereafter was diluted further with extracellular solution. The pH was adjusted to 7.0 with 1 N NaOH. The amount of NaOH used for the adjustment of pH resulted in less than 1% elevation of the Na concentration in the extracellular solution. In pilot experiments, the pH 7.0 vehicle failed to influence Na^+ currents.

3. Results

In pilot experiments vinpocetine (100 μM) and phenytoin (80 μM) caused a rapidly developing, reversible inhibition of the Na^+ currents evoked by the

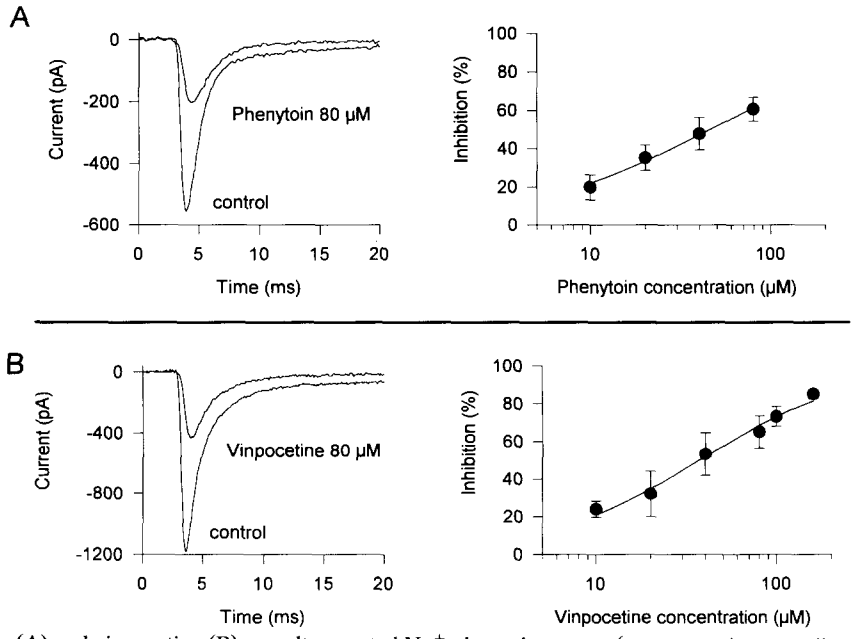

Fig. 1. Effect of phenytoin (A) and vinpocetine (B) on voltage-gated Na^+ channel currents (representative recordings: left panel; concentration-inhibition curves, averaged from four independent experiments ± S.E.M.: right panel). Na^+ channel currents were evoked applying voltage steps to −10 mV from a holding potential of −60 mV in every 20 s. Vinpocetine and phenytoin, administered in increasing concentrations, decreased Na^+ currents in a concentration-dependent manner (for IC_{50} values, see text).

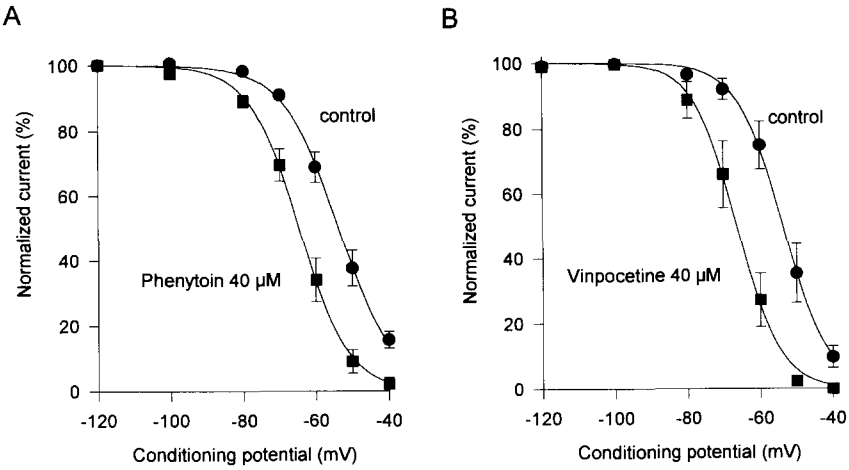

Fig. 2. Effect of phenytoin (A) and vinpocetine (B) on the steady-state inactivation of the voltage-gated Na^+ channels. Points and bars represent data averaged from four independent experiments ± S.E.M. Inactivation curves were generated by a two-pulse protocol consisting of 15-s conditioning pulses to different membrane potentials and a test pulse to −10 mV. The holding potential was −70 mV. Peak Na^+ currents were measured and expressed as percentages of the current obtained at a −120 mV conditioning pulse. Solid lines: two-state Boltzman fitting of the data to the equation $I/I_{max} = 1/[1 + \exp(V - V_{0.5})/A_h]$, where $V_{0.5}$ is the voltage for half-maximal inactivation and A_h is the number of millivolts required to produce an e-fold change in steady-state inactivation. At a concentration of 40 μM both compounds shifted the steady-state inactivation curves to the hyperpolarizing direction. $V_{0.5}$ changed from -55.6 ± 1.7 to -65.3 ± 1.5 and from -53.4 ± 2.2 to -66.2 ± 2.6 for phenytoin and vinpocetine, respectively. All changes were statistically significant (two-tailed Student's t-test): drugs did not change the slope of the curves significantly. Controls: $A_h = 6.9 \pm 0.8$ and 5.7 ± 0.4; shifted curves: $A_h = 6.1 \pm 0.4$ and 5.1 ± 0.2 for phenytoin and vinpocetine, respectively.

voltage steps to −10 mV (not illustrated). More than 90% of the maximal inhibition was reached within 3 min ($n = 4$). The drugs did not change the time course of the evoked currents. (Ascending and descending peak half-times – *ascending*, controls: 0.77 ± 0.09 and 0.46 ± 0.1 ms for phenytoin and vinpocetine, respectively; phenytoin (80 μM) 0.95 ± 0.2 ms, vinpocetine (100 μM) 0.51 ± 0.1 ms; *descending*, controls: 1.58 ± 0.2 and 0.95 ± 0.1 ms for phenytoin and vinpocetine, respectively; phenytoin (80 μM) 1.78 ± 0.2 ms, vinpocetine (100 μM) 1.1 ± 0.3 ms, none of the differences were statistically significant.) Similarly, the voltage dependence of Na^+ channel activation was not affected by phenytoin and vinpocetine, as measured by application of voltage steps to different membrane potentials (not illustrated).

Increasing concentrations of both compounds concentration dependently decreased the amplitudes of the Na^+ currents evoked by voltage steps to −10 mV from a holding potential of −60 mV (Fig. 1A and B). Vinpocetine and phenytoin inhibited Na^+ currents with IC_{50} values of 44.2 ± 14.6 and 50.5 ± 17.4 μM ($n = 4$), respectively (Fig. 1A and B). At a concentration of 40 μM, both compounds affected steady-state inactivation in a similar manner, i.e., they shifted its voltage dependence to the hyperpolarising direction (Fig. 2A and B).

4. Discussion

In our experiments vinpocetine showed a pronounced, concentration-dependent inhibitory effect on Na^+ currents. The Na^+ channel blocking potency of vinpocetine proved to be in the same range as that of phenytoin, a prototype anticonvulsant Na^+ channel blocker.

Qualitatively, the Na^+ channel blocking action of vinpocetine was similar to that of phenytoin in terms of shifting the steady-state inactivation curves to the hyperpolarising direction. This finding supports the view that both compounds bind preferably to the inactivated state of the channel (Ragsdale et al., 1991).

Attempts have already been made to elucidate the mechanism(s) by which vinpocetine may exert its neuroprotective and anticonvulsant effects. The previously reported, weak inhibition of NMDA (Kaneko et al., 1991) and non-NMDA receptors (Kiss et al., 1991), as well as the Ca^{2+} antagonistic effect demonstrated by Kaneko et al. (1990) occurred at very high vinpocetine concentrations which may have limited pharmacological relevance. Furthermore, the slight potentiation of the neuroprotective effect of adenosine, in vitro (Krieglstein and Rischke, 1991), is unlikely to be responsible for the in vivo efficacy of vinpocetine as neuroprotectant. In addition, the specific inhibition of Ca^{2+}-dependent cyclic GMP phosphodiesterase (Hidaka et al., 1984), although observed at vinpocetine concentrations in the lower micromolar range, does not seem to be directly related to the neuroprotective effects, since other potent inhibitors of the enzyme do not possess vinpocetine-like pharmacological activities (for references, see Hidaka et al., 1984). However, the specific blockade of voltage-gated Na^+ channels by pharmacologically relevant vinpocetine concentrations

demonstrated in our experiments may provide a reasonable explanation for both the neuroprotective and anticonvulsant effects of the drug.

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