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Research report

Pharmacological characterization of antiepileptic drugs and experimental analgesics on low magnesium-induced hyperexcitability in rat hippocampal slices

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Abstract

Perfusion of acute hippocampal slices with stimulatory buffers has long been known to induce rhythmic, large amplitude, synchronized spontaneous neuronal bursting in areas CA1 and CA3. The characteristics of this model of neuronal hyperexcitability were investigated in this study, particularly with respect to the activity of antiepileptic drugs and compounds representing novel mechanisms of analgesic action. Toward that end, low Mg^{2+}/high K^{+}-induced spontaneous activity was quantified by a virtual instrument designed for the digitization and analysis of bursting activity. Uninterrupted streams of extracellular field potentials were digitized and analyzed in 10-s sweeps, yielding four quantified parameters of neuronal hyperexcitability. Following characterization of the temporal stability of low Mg^{2+}/high K^{+}-induced hyperexcitability, compounds representing a diversity of functional mechanisms were tested for their effectiveness in reversing this activity. Of the four antiepileptic drugs tested in this model, only phenytoin proved ineffective, while valproate, gabapentin and carbamazepine varied in their potencies, with only the latter drug proving to be completely efficacious. In addition, three investigational compounds having analgesic potential were examined: ZD-7288, a blocker of HCN channels; EAA-090, an NMDA antagonist; and WAY-132983, a muscarinic agonist. Each of these compounds showed strong efficacy by completely blocking spontaneous bursting activity, along with potency greater than that of the antiepileptic drugs. These data indicate that pharmacological agents with varying mechanisms of action are able to block low Mg^{2+}/high K^{+}-induced hyperexcitability, and thus this model may represent a useful tool for identifying novel agents and mechanisms involved in epilepsy and neuropathic pain.

Theme: Disorders of the nervous system
Topic: Epilepsy: anti-convulsant drugs

Keywords: Epilepsy; Hippocampus; Antiepileptic drugs; Pain

1. Introduction

Neuropathic pain is a chronic condition characterized by intense pain that is independent of discernable nociceptive input to the nervous system. Thought to result from maladaptive changes occurring within damaged sensory nerves, neuropathic pain is intimately associated with the so-called ectopic discharges observed in damaged peripheral nerves [17,23,42]. Indeed, a number of established [32] as well as experimental [14] therapies aimed at neuropathic pain have been shown to be effective in both blocking ectopic discharges and producing behavioral analgesia in animal models of neuropathic pain. Ectopic discharges resemble epileptiform neuronal hyperexcitability. That is, discharges of both the ectopic and epileptic variety are spontaneous, large amplitude, synchronized neuronal bursts. Not surprisingly, therefore, antiepileptic drugs have been used in the treatment of neuropathic pain [6,25,50]. This

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therapeutic approach has proven modestly effective, as antiepileptic drugs provide partial pain relief to a portion of patients suffering from neuropathic pain. Nonetheless, antiepileptic therapy is an interesting and promising field of investigation, as ectopic discharges may represent a treatment-resistant form of neuronal hyperexcitability with implications for both epilepsy and neuropathic pain.

Epileptiform, spontaneous bursting activity in hippocampal slices has been studied by a number of groups in the past [16,30,31,41,49,51]. These studies have noted and described in detail the epileptiform bursting activity induced by pharmacological blockers of $GABA_A$ receptors or K^+ channels (among others). The systematic examination of the mechanisms capable of blocking these bursts has been lacking, however.

In the present study, we sought to characterize hippocampal slice bursting using a non-pharmacological means of stimulating spontaneous activity. Toward this end, low Mg^{2+}/high K^+-induced spontaneous activity recorded in acute hippocampal slices represents a particularly interesting model of neuronal hyperexcitability [22,55]. Typified by spontaneous, large amplitude, synchronized neuronal bursts, low Mg^{2+}/high K^+-induced spontaneous activity shows some pharmacological specificities that may prove to be predictive of the inhibition of ectopic discharges and, by extension, of neuropathic pain. An in vitro model of treatment-resistant hyperexcitability that could serve as a proxy to true ectopic discharges would prove to be a valuable model.

The present investigation describes the low Mg^{2+}/high K^+-induced hyperexcitability phenomenon under our conditions, with the goal of establishing a quantification and analysis methodology. Subsequent experiments apply this methodology to the pharmacological evaluation of a variety of compounds, representing both traditional antiepileptic agents as well as other investigational compounds.

2. Materials and methods

2.1. Hippocampal slice preparation

Animal protocols were performed in accordance with NIH *Guide for the Care and Use of Laboratory Animals*. Male Sprague–Dawley rats (250g BW) were anesthetized with pentobarbital (Nembutal® Sodium Solution, 50 mg/kg i.p.) then transcardially perfused with 50 ml of ice-cold modified (calcium-free) artificial cerebrospinal fluid (mACSF: NaCl 125.0 mM, KCl 5.0 mM, NaH_2PO_4 1.2 mM, $MgSO_4$ 2.0 mM, $NaHCO_3$ 25.7 mM, dextrose 10 mM). Rats were then decapitated, and their brains rapidly removed into ice-cold, oxygenated mACSF, where they remained for 1 min. Hippocampi were dissected free and chopped into 400 μm thick slices, which were placed in ice-cold, oxygenated mACSF. Slices were then moved with a camel hair paint brush to a Haas-type interface recording chamber, where they were superfused (100 ml/h) with 35 °C normal artificial cerebrospinal fluid (ACSF: NaCl 125.0 mM, KCl 5.0 mM, NaH_2PO_4 1.2 mM, $MgSO_4$ 2.0 mM, $NaHCO_3$ 25.7 mM, $CaCl_2$ 2.0 mM, dextrose 10 mM) and oxygenated by bubbling O_2/CO_2 gas (95:5) through the chamber. Slices were then allowed to recover for at least 60 min before commencement of the recording session.

2.2. Testing for slice viability

Before attempting to record spontaneous neuronal firing, slices were first tested for viability by checking their field potentials, thereby determining the best candidates for further recording. Micropipettes filled with 2.0 M sodium chloride (2–5 MΩ impedance) were lowered into the pyramidal cell layer of hippocampal area CA1 (stratum pyramidale). Platinum concentric bipolar stimulating electrodes were lowered onto the surface of the Schaffer collateral area of the stratum radiatum in CA3. Then, constant current (100–300 μAmp) stimulating pulses of 100 μs duration were delivered. Slice viability was determined by the presence of a population spike of at least 2 mV in amplitude.

2.3. Recording spontaneous neuronal firing

Hippocampal slice field recording and online analysis was achieved with instruNet data acquisition hardware interfaced with custom virtual instruments created in the SuperScope II software environment (GW Instruments, Somerville, MA) running on a Macintosh G4 computer (Apple Computer, Cupertino, CA). Perfusion of hippocampal slices with low Mg^{2+}/high K^+ ACSF (LMACSF: NaCl 125.0 mM, KCl 7.0 mM, NaH_2PO_4 1.2 mM, $MgSO_4$ 0 mM, $NaHCO_3$ 25.7 mM, $CaCl_2$ 2.0 mM, dextrose 10 mM) leads to the appearance of spontaneous neuronal firing in area CA1 within approximately 20 to 30 min. Low Mg^{2+}/high K^+ ACSF was the same as normal ACSF, except with elevated KCl (7mM) and no added $MgSO_4$. Recorded with the same micropipette in the same area as described above, this spontaneous neuronal activity was digitized and analyzed in real time, in 10-s sweeps, continuously throughout the duration of an experiment. An adjustable threshold setting was used to discriminate bursting activity from nonspecific background noise. Typically, up to four hippocampal slices from the same rat were recorded simultaneously.

2.4. Compound evaluation

Delivery of compound solutions, made up in LMACSF, to the hippocampal slices was controlled through a manifold system. Changes of solution took approximately 2 min to reach the recording chamber. Following observation of stable baseline activity of at least 10 min duration, as determined by inspection of the real-time analysis data (see below), a solution of test compound was perfused through the recording chamber for a period of 20 min, or 40 min in