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Inhibition of hyperpolarization-activated cation currents by phencyclidine and some sigma ligands in rat hippocampal CA1 pyramidal neurons in vitro

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Abstract

Using whole-cell voltage-clamp recordings, hyperpolarization-activated cation currents (I_h) were elicited with hyperpolarizing voltage jumps in CA1 pyramidal neurons of rat hippocampal slices, and the effects of phencyclidine (PCP) and some sigma ligands on I_h were studied. PCP concentration-dependently (0.1−100 μM) suppressed I_h and shifted the activation curve of I_h to the negative direction. D-3-(2-Carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP, 20 μM) and MK-801 (30 μM), competitive and non-competitive NMDA blockers, respectively, failed to mimic the inhibitory effect of PCP on I_h, and suppression of I_h by PCP was unaffected in the presence of these blockers. To explore the involvement of sigma$_1$ receptors in the reduction of I_h, the effects of representative sigma$_1$ ligands were studied. SKF10047 (100 μM), a sigma$_1$ agonist, attenuated the maximal I_h and shifted the half-activation potential of I_h to the hyperpolarized direction. In the presence of the sigma$_1$ antagonist NE-100 (1 μM), which alone did not affect I_h, the effect of SKF10047 on I_h was unaltered. By contrast, a higher concentration of NE-100 (10 μM) mimicked the effect of SKF10047. Again, no antagonism of I_h suppression by SKF10047 was obtained with rimcazole (100 μM), a sigma$_1$ receptor antagonist that is structurally distinct from NE-100. This concentration of rimcazole alone resulted in a slight but significant reduction of I_h. Thus these major sigama$_1$ ligands appear to suppress I_h independently of their agonistic or antagonistic properties. The results of this study suggest that PCP and some sigma ligands could modulate cell excitability partly through their action on I_h.

Keywords: I_h channels; PCP; Sigma receptors; NMDA; Excitability

1. Introduction

Hyperpolarization-activated cation channels (I_h channels) are activated in response to hyperpolarization, resulting in the generation of an inward current (I_h; Pape, 1996). I_h channels, distributed widely in the central nervous system (Monteggia et al., 2000), contribute to the determination of resting membrane potential (Maccaferri et al., 1993; Gasparini and DiFrancesco, 1997) and normalization of temporal integration at proximal dendrites (Magee, 1999), with the result that small changes in the properties of I_h channels can have large influences upon neuronal excitability (Chen et al., 2001). I_h channels are also expressed at presynaptic terminals in the central (Southan et al., 2000; Mellor et al., 2002) and peripheral (Beaumont and Zucker, 2000) nervous system, where there are suggested to regulate synaptic transmission (but see Chevaleyre and Castillo, 2002). In line with this physiological significance of I_h channels, recent evidence has

Abbreviations: I_h, hyperpolarization-activated cation currents; PCP, phencyclidine; CPP, D-3-(2-Carboxypiperazin-4-yl)-propyl-1-phosphonic acid; NE-100, *N*,*N*-dipropyl-2-[4-methoxy-3-(2-phenylethoxy)-phenyl]-ethylamine monohydrochloride; TEA, tetraethylammonium; 4-AP, 4-aminopyridine; HCN channels, hyperpolarization-activated, cyclic nucleotide-gated cation channels.

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suggested that changes in I_h channel activity are involved in various pathological conditions related to altered neuronal excitability (Chen et al., 2002).

Phencyclidine (PCP) is a dissociative anesthetic, and produces behavioral and/or pharmacological effects, including psychotomimetic, neuroprotective and anticonvulsive actions. Most of these effects are mediated by PCP binding sites in the *N*-methyl-D-aspartate (NMDA) receptor-ion channel complex, as demonstrated by a number of previous studies (Anis et al., 1983; ffrench-Mullen and Rogawski, 1989). However, PCP interacts with various sites independent of PCP binding sites in the NMDA receptor-ion channel complex. For instance, PCP acts as a dopamine transporter inhibitor (Dersch et al., 1994), and as a non-competitive inhibitor of the nicotinic acetylcholine receptor (Aguayo et al., 1986). Moreover, PCP blocks voltage-dependent K^+ channels (Bartschat and Blaustein, 1986; Rothman, 1988; ffrench-Mullen and Rogawski, 1989) and Ca^{2+} channels (Ffrench-Mullen and Rogawski, 1992), as shown by studies using mammalian brain. Some of these actions are also considered to contribute to the behavioral and pharmacological effects of PCP. In addition, PCP has affinity for sigma receptors (Largent et al., 1984; Sonders et al., 1988), which directly and/or indirectly mediate some of the effects of PCP (Ault and Werling, 1999; Takahashi et al., 2001). Indeed, PCP and some sigma ligands block the same presynaptic K^+ channels (Bartschat and Blaustein, 1988). Sigma receptors, initially proposed as a new category of opioid receptors (Martin et al., 1976), are distinguished from opioid receptors and PCP binding sites in the NMDA receptor-ion channel complex.

Given the profound participation of I_h channels in the regulation of seizure threshold (Chen et al., 2001) and neuronal resistance to ischemic insult (Ray et al., 2003), it can be postulated that PCP affects I_h channel activity. Although Malouf et al. (1988) have suggested that PCP and some sigma ligands appear to affect this cation conductance, no detailed studies have investigated their modulation of I_h channels. Here, I showed that PCP suppressed I_h with a negative shift of its activation. Furthermore, some sigma ligands mimicked the action of PCP on I_h channels. This action on I_h channels, which appears to contribute to the reduction of neuronal hyperexcitability, may explain some of the pharmacological effects shared by PCP and sigma ligands.

2. Methods

All of the experimental protocols used in this study were approved by the Sankyo Code of Ethical Research, and were carried out according to the guidelines of the National Institutes of Health and the Japanese Pharmacological Society, and also the Animal Care and Use Committee of Nagoya City University.

2.1. Slice preparation

Wistar rats (Charles River Japan, Inc., Tokyo, Japan) aged 11–18 days were killed by cervical dislocation under ether anesthesia. The brains were then quickly removed and placed in ice-cold artificial CSF (ACSF), pH 7.4 (after bubbling with 95% O_2 and 5% CO_2) containing the following (in mM): NaCl 113, KCl 3, NaH_2PO_4 1, $NaHCO_3$ 25, D-glucose 11, $CaCl_2$ 2, $MgCl_2$ 1. The hippocampus was dissected out, and slices 200 μm thick were prepared using a vibratome (DSK-1000, Dosaka, Kyoto, Japan). The slices were equilibrated for at least 60 min at room temperature (22–24 °C), then transferred to a recording chamber mounted on the stage of a microscope (Axioskop; Zeiss, Jena, Germany) and superfused with ACSF at a rate of 3 ml/min at 32 °C.

2.2. Patch-clamp electrophysiology

Whole-cell voltage-clamp recordings were made from visually identified CA1 pyramidal cells, whose surfaces were cleaned by a stream of perfusion solution applied through a large-bore pipette (10 μm), under observation using an upright microscope with a water-immersion objective lens (40×; Zeiss) with Nomarski optics. The patch electrodes (2.5–3 μm tip diameter) were pulled from borosilicate glass capillary tubes (Harvard Apparatus, Edenbridge, UK) and had a resistance of 3–5 MΩ when filled with the internal solution containing the following (in mM): K-gluconate 140, HEPES 10, EGTA 1.1, $MgCl_2$ 2, MgATP 3, TrisGTP 0.3, pH 7.2 adjusted with KOH. Whole-cell currents were amplified (EPC-7; List Medical, Darmstadt, Germany), stored on a PCM tape recorder (Sony PC204A, Tokyo, Japan), low-pass filtered at 4 kHz, and digitized at 2–4 kHz for computer analysis with pClamp7 software (Molecular Devices, Union City, CA). The access resistance was monitored by measuring capacitative transients obtained in response to a hyperpolarizing voltage step (10 mV, 500 ms), and any experiments showing major changes in the access resistance (>20%) were discarded. All experiments were performed in the presence of tetrodotoxin (TTX, 0.5 μM).

2.3. Protocols and analysis

In order to evaluate the effect of drugs on I_h with respect to time, I_h was induced with hyperpolarizing voltage steps (1 s duration) from a holding potential of −60 to −120 mV at 0.05 Hz. In some experiments, the currents were evaluated in response to hyperpolarizing voltage steps (1 s duration) within the range −130 to −60 mV with 10-mV increments from a holding potential of −60 mV. The I_h amplitude activated at a given voltage step was taken as the difference between the instantaneous current, measured at a point immediately after the capacitative transient, and the current at the end of the voltage step. When tail currents were recorded, cells were hyperpolarized from a holding potential of −50 mV to between −50 and −130 mV for 1.5 s, followed by a voltage jump to −130 mV for 500 ms.

The concentration-inhibition curve of I_h obtained with PCP was fitted, and the IC_{50} value was determined using non-linear regression analysis with the embedded logistic function in Origin (Microcal Software, Northampton, MA): $\%I = E_{max}/\{1 + (IC_{50}/[D])^n\}$, where $\%I$ is the percentage inhibition of I_h, $[D]$ is the concentration of PCP, E_{max} is the maximal percentage inhibition of I_h, and n is the Hill slope. The time constant of I_h activation was fitted by exponential functions also embedded in Origin.

In the analysis of the tail current, the half-activation voltage ($V_{1/2}$) of I_h was determined by using non-linear regression analysis with the embedded Boltzmann function in Origin: $(I_{(tail,\ max)} - I_{tail})/I_{(tail,\ max)} = \{1 + \exp([V_m - V_{1/2}]/k)\}^{-1}$, where I_{tail} is the measured tail current amplitude recorded at −130 mV following a step to membrane potential V_m, $I_{(tail,\ max)}$ is the maximum measured tail current amplitude, $V_{1/2}$ is the calculated half-activation voltage of I_h, and k is the slope factor.

All data are expressed as means ± SEM. Student's *t*-test (two-tailed) was used to compare the data between two groups. Differences at $P < 0.05$ were considered to be significant.

2.4. Drugs

Drugs were applied directly via the superfusion solution. PCP and *N*, *N*-dipropyl-2-[4-methoxy-3-(2-phenylethoxy)-phenyl]-ethylamine monohydrochloride (NE-100) were synthesized by Sankyo. (+)-MK-801 and D-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP) were purchased from RBI (Natick, MA). ZD7288, (+)-SKF10047 hydrochloride and TTX citrate were from Tocris Cookson (Bristol, UK). Cesium chloride (CsCl) and tetraethylammonium chloride (TEA-Cl) were from Nakarai (Kyoto, Japan), and 4-aminopyridine (4-AP) was from Sigma (St. Louis, MO).