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Research report

Iptakalim hydrochloride protects cells against neurotoxin-induced glutamate transporter dysfunction in in vitro and in vivo models

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Abstract

Iptakalim hydrochloride (Ipt), a novel antihypertensive drug, exhibits K_{ATP} channel activation. Here, we report that Ipt remarkably protects cells against neurotoxin-induced glutamate transporter dysfunction in in vitro and in vivo models. Chronic exposure of cultured PC12 cells to neurotoxins, such as 6-OHDA, MPP^+, or rotenone, decreased overall [^{3}H]-glutamate uptake in a concentration-dependent manner. Pre-treatment using 10 μM Ipt significantly protected cells against neurotoxin-induced glutamate uptake diminishment, and this protection was abolished by the K_{ATP} channel blocker glibenclamide (20 μM), suggesting that the protective mechanisms may involve the opening of K_{ATP} channels. In 6-OHDA-treated rats (as an in vivo Parkinson's disease model), [^{3}H]-glutamate uptake was significantly lower in synaptosomes isolated from the striatum and cerebral cortex, but not the hippocampus. Pre-conditioning using 10, 50, and 100 μM Ipt significantly restored glutamate uptake impairment and these protections were abolished by blockade of K_{ATP} channels. It is concluded that Ipt exhibits substantial protection of cells against neurotoxicity in in vitro and in vivo models. The cellular mechanisms of this protective effect may involve the opening of K_{ATP} channels. Collectively, Ipt may serve as a novel and effective drug for PD therapy.

Theme: Excitable membranes and synaptic transmission
Topic: Uptake and transporters

Keywords: Iptakalim hydrochloride; ATP-sensitive potassium channel; Glutamate transporter; Glutamate uptake; Parkinson's disease; Neuroprotection

1. Introduction

Parkinson's disease (PD) is a widespread neurodegenerative disorder. The major pathological change in PD is degeneration of dopamine-containing neurons of the substantia nigra pars compacta (SNpc) and the appearance of Lewy bodies [12,32,40,52]. Though the neurochemical defects and neuropathological characteristics of this disease are well defined, its etiology remains unclear. Accumulating lines of evidence indicate that one of the major mechanisms responsible for onset of PD results from neurotoxicity of excitatory amino acids (EAAs), especially glutamate [43]. Glutamate acts as an excitatory neurotransmitter in the mammalian central nervous system (CNS), as well as a potent neurotoxin [39]. Overstimulation of postsynaptic glutamate receptors, especially NMDA receptors, is thought to be a common mechanism for cell degeneration [38] in ischemia, epilepsy, PD, or Alzheimer's disease (AD). Therefore, the application of glutamate receptor antagonists (e.g., MK 801—an NMDA receptor/channel antagonist) to prevent

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neuron degeneration has been extensively employed. On the other hand, glutamate transporters play an important role in maintaining extracellular glutamate concentrations below neurotoxic levels, thereby contributing to the prevention of neuronal damage from excessive activation of glutamate receptors [33]. Five different isoforms of glutamate transporters have now been identified: GLAST (EAAT1), GLT1 (EAAT2), EAAC1 (EAAT3), EAAT4, and EAAT5 [9]. Immunolocalization studies have revealed that GLAST and GLT1 are primarily expressed in glial cells, whereas EAAC1 and EAAT4 are primarily present in neurons [51]. EAAT5 is expressed specifically in the human retina [1]. Some serious neuronal diseases, such as epilepsy [31], amyotrophic lateral sclerosis (ALS) [59], AD [14], and cellular damage from stroke [3] may be linked to the dysfunction of glutamate transporters. It has been reported that disruption of the ionic gradients during inhibition of metabolism can lead to glutamate release, impairment of glutamate transport, and activation of NMDA receptors [35]. Therefore, the protection or enhancement of glutamate transporter function provides a new therapeutic strategy for neural degeneration diseases, such as PD and AD.

Although the classical role of K_{ATP} channels in regulation of insulin secretion in pancreatic β-cells has been extensively studied, their neuronal function remains to be fully evaluated. It has been demonstrated in a variety of brain regions that metabolic inhibition of glycolysis or of the mitochondrial respiratory chain acts as a potent activator of neuronal K_{ATP} channels [4,24,67]. These channels are not only relevant to acute metabolic challenges, but also to chronic genesis of neurodegenerative disorders like AD [22] or PD [26]. Recent works support the idea of K_{ATP} channel activation as a neuroprotective strategy. K_{ATP} channel openers (KCOs) have been shown to exert strong neuroprotective effects when injected shortly prior to severe hypoxia/ischemia or epileptic insult [6,15,49]. Compound 33, a novel anti-ischemic compound, shows good protective activity in neuronal cells against oxidative stress and may have therapeutic potential in neuroprotection mediated by K_{ATP} channel opening [66]. K_{ATP} channels are also involved in neuroprotection afforded by anoxic pre-conditioning in hippocampal slices [46]. Initiation and execution of hypoxia/ischemia-induced neuronal cell death is believed to critically depend on excessive glutamate release and subsequent excitotoxicity [11]. Activation of plasma membrane or mitochondrial K_{ATP} channels produces neuroprotective effects, perhaps through different mechanisms, thereby increasing the likelihood of cell survival. On the other hand, the blockade of K_{ATP} channels has been shown to potentiate cyanide-induced neurotoxicity [44].

Iptakalim hydrochloride (Ipt), a novel compound, was initially designed and synthesized in Wang's laboratory [60]. The molecular mechanisms underlying its antihypertensive action include K_{ATP} channel activation and endothelin antagonism. The pre-clinical investigation of Ipt, according to technical requirements for novel anti-hypertensive drug approval, has been completed and clinical trials are currently being planned [61]. However, it is unclear whether Ipt exhibits neuroprotective effects in PD models. In the present study, we examined the protective effects of Ipt on neurotoxin-induced glutamate transporter dysfunction in both in vitro and in vivo models to determine whether: (1) Ipt protects PC12 cells against neurotoxin-induced glutamate transport dysfunction, (2) glutamate transport protection is mediated via the opening of K_{ATP} channels, and (3) Ipt protects cells against glutamate transport dysfunction in an in vivo PD rat model.

2. Materials and methods

2.1. Materials

L-[^{3}H]-glutamate (1 mCi/ml) was obtained from the National Institute of Atomic Energy (Beijing, China). Dulbecco's modified Eagle's medium (DMEM) was obtained from Gibco RBL (Grand Island, NY). Ipt was a gift from the Institute of Pharmacology and Toxicology, Academy of Military Medical Sciences (Beijing, China). The chemical structure of Ipt is shown in Fig. 1. Pinacidil (a K_{ATP} channel opener), glibenclamide (a K_{ATP} channel blocker), 6-hydroxydopamine (6-OHDA), rotenone, and 1-methyl-4-phenylpridinium (MPP^+) were purchased from Sigma Chemical (St. Louis, MO). Pinacidil, glibenclamide, and rotenone were dissolved in dimethyl sulfoxide (DMSO). The final concentration of DMSO in synaptosomes and cell cultures did not exceed 0.1%, and at this concentration DMSO has no harmful effect. All other chemicals were dissolved in tissue culture grade water.

2.2. Cell culture

PC12 cells were obtained from American Type Culture Collection and cultured in DMEM supplemented with 15% (v/v) fetal bovine serum (FBS), 50 U/ml penicillin, and 50 μg/ml streptomycin in a 200-ml vented culture flask. Cultures were maintained at 37 °C in a humidified incubator with 5% CO_2 and 95% atmosphere. Cells were harvested and seeded onto a 16-mm-diameter, 24-well plate at a cell density of 100,000 cells/ml [55].

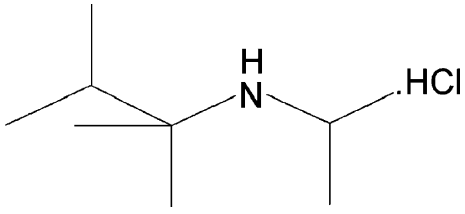

Fig. 1. Chemical structure of iptakalim hydrochloride.