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DIMINAZENE ACTION ON ACID-SENSING ION CHANNELS (ASICS) IN RAT AMYGDALA NEURONS

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Abstract

Diminazene inhibit ASIC currents in amygdala neurons with relatively high affinity.The purpose of this research is is to examine mechanisms of diminazene action on native ASIC receptors in amygdala. In this work at first time was shown that the action of diminazene slightly depends on pH .

Keywords: diminazene, ASIC blocker

Introduction

Acid-sensing ion channels (ASICs) are proton-gated cation channels that are predominantly expressed in the nervous system. ASICs are involved in a number of neurological diseases such as pain, ischemic stroke and multiple sclerosis but limited tools are available to target these channels and provide probes for their physiological functions. ASIC inhibitors attenuate peripheral acid-induced hyperalgesia in rats as well as pain in human subjects. However, the normal physiological functions of ASIC remain not fully understood, partly due to the lack of good pharmacological tools for their study. (ASIC)-1a supposed to be the major ASIC subunit in the neurons of CNS. Activation of these channels plays a critical role in normal synaptic plasticity, learning/memory, and in acidosis-mediated glutamate receptor-independent neuronal injury. The ASIC1a is widely expressed in the brain, with the highest expression levels found in the amygdala. The amygdala is an almond-shaped structure deep within the temporal lobe. This structure has attracted continued interest because of its central role in emotional processing. The word emotion is a difficult concept that describes subjective experiences and feelings such as pain, fear, desire, and hope as well as aspects of the behavior of individuals both public and private. A class of anti-protozoan diarylamidine such as diminazene, inhibit ASIC currents in amygdala neurons with relatively high affinity. Moreover, diminazene dramatically enhances the rate of desensitization of ASIC currents. This novel class of ASIC blockers might be useful for ASIC functional study and be of therapeutic significance for intervention in ASIC-related disorders such as chronic pain. The effect caused by diminazene on ASIC receptors and the site of its action on the receptor complex was not studied in great depth till now. Herein, the main goal of this study is to examine mechanisms of diminazene action on native ASIC receptors in amygdala.

1. Methods

Wistar rats 14 to 16 days old were prepared and decapitated. The brain was quickly removed and transversely sliced at a thickness of 400 μm. Slices were kept in the incubation solution (in mM: 124 NaCl, 5 KCl, 2.4 $CaCl_2$, 1.1 $MgSO_4$, 24 $NaHCO_3$, 10 glucose, 1.2 KH_2PO_4) saturated with 95% O_2 and 5% CO_2 at room temperature ($20 - 24$°C) for at least 1 h. Then we put slices in the ACSF (artificial cerebrospinal fluid) standard solution (in mM: 130 NaCl, 5KCl, 2$CaCl_2$, 2 $MgCl_2$, 10 HEPES, 10 glucose; and pH 7.4 adjusted by NaOH 1M.) and got isolated neurons by dissociation method. Slices were removed and the single neurons allowed to settle and adhere to the bottom of the dish for about 20 min. Such neurons undergoing dissociation retained their original morphological features including short portions of their proximal dendrites. The data was obtained by standard patch clamp method in configuration "whole cell". In all extracellular solutions that were used in experiments with pH ≥ 6 HEPES was applied as the buffering agent, whereas for all solutions with pH < 6 MES was applied.

2. Results

For initial experiments we used boundary pH means for ASIC currents activation (pH 6.7 and pH 5.0) with lowest concentration of diminazene applied as 100nM. The idea of such experiments was to evaluate the possible pH-dependence of diminazene effect on native receptors. At 100 nM concentration diminazene has negligible effect both on 6.7- and 5.0-activated currents (Fig. 1).

Next we used diminazene at concentration 1 μM, that is shown to be IC_{50} mean for previous experiments by other labs. In concentration 1 μM diminazene has the moderate blocking action on ASIC-activated currents (for 6.7 activated currents I/I_0 was calculated as 52.86% ($n = 6$) whereas for 5.0 activated currents I/I_0 was calculated as 68.44% ($n = 8$) (Fig. 2). The analysis of

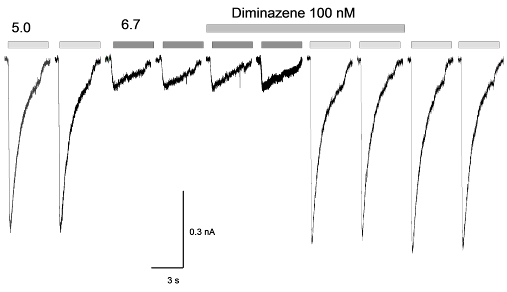

Fig. 1. Original traces of ASIC currents blocked by diminazene recorded in pyramid-like neurons of amygdala. The currents were activated by testing pH drop solutions 6.7 and 5.0 Diminazene was applied at 100 nM concentration.

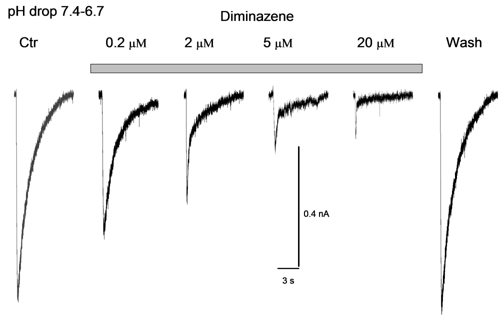

Fig. 3. Representative original traces of ASIC currents recorded from amygdala neurons affected by diminazene used in different concentrations.

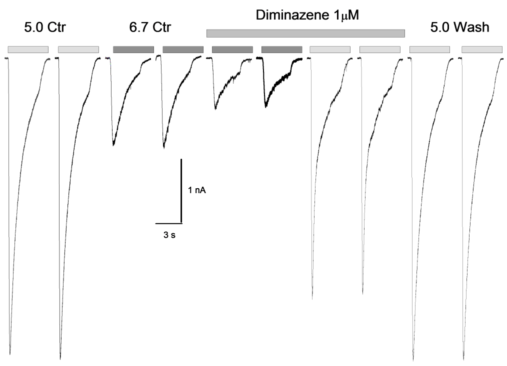

Fig. 2. Original traces of ASIC currents blocked by diminazene recorded in native amygdala neurons. The currents were activated by testing pH drop solutions 6.7 and 5.0 Diminazene was applied at $1\,\mu$M concentration.

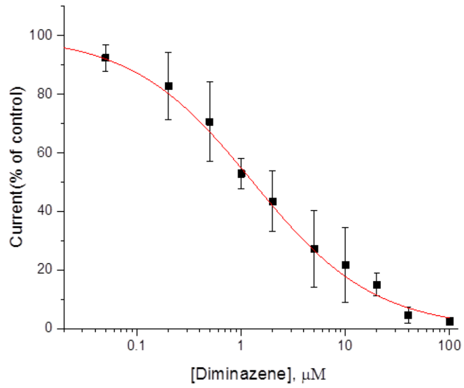

Fig. 4. Dose–response curve for diminazene effect on ASIC currents activated by 6.7 pH drop in pyramid-like amygdala neurons.

shown results indicate the effect of diminazane might have a slight dependence on pH and thus requires the further study.

In next series of experiments we examined the effect of diminazene on ASIC-activated currents (pH =6.7) applied in variety in concentration and the concentration dose-response curve was constructed upon the results (Fig 3 and 4).

This traces show the dependence of ASIC currents from diminazene concentration at pH drop to $7.4 - 6.7$. The efficiency of diminazene grows with the increase of its concentration (Fig. 3). The IC_{50} for diminazene effect was calculated as 1.29 μM, whereas in most experiments ASIC-activated currents were completely and reversibly inhibited by 100 μM. The recovery of the ASIC current after the diminazene action was fast ($2 - 5$ application of control solutions) and complete ($95 - 100\%$). An important characteristic that we took from the dose-response curve for diminazene action are IC_{50} and the Hill coefficient. IC_{50} is the half maximal inhibitory concentration (From Figure 4. $IC_{50} = 1.29\mu$M) and it is a measure of the effectiveness of a substance in inhibition (Fig. 4). The Hill coefficient is used to describes the fraction of the macromolecule saturated by ligand as a function of the ligand concentration. In our experiment it is $h = 0.75 \pm 0.05$ that is close to 1 and means the $1:1$ receptor-agent interaction.

Discussion

Diminazene was shown to reversibly inhibit ASIC currents in amygdala neurons with relatively high affinity ($IC_{50} = 1.29\mu$M for 6.7–induced ASIC currents). At first time was shown that the action of diminazene slightly depends on pH . The further investigation is required to compare the dose-response curve of diminazene effect on ASIC activated by 5.0 pH drop. Althogh preliminary data shown that diminazene has accelerate the kinetics of desensitization of ASIC currents and well as shown some dependence on the holding potential that is common for channel-blockers. Next series of experiments are devoted to investigate the clear mechanisms of diminazene binding to ASIC receptor-channel.

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