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BRAIN RESEARCH

Research Report

A spontaneous tonic chloride conductance in solitary glutamatergic hippocampal neurons

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ABSTRACT

GABA-A receptors mediate both phasic synaptic inhibition and more recently appreciated tonic currents in the vertebrate central nervous system. We addressed discrepancies in the literature regarding the pharmacology of tonic currents by examining tonic currents in a controlled environment of dissociated, solitary glutamatergic neurons. We describe a novel tonically active, bicuculline-sensitive chloride conductance that is insensitive to gabazine and to picrotoxin and thus not mediated by conventional GABA receptors. We exclude a significant contribution from small conductance calcium-gated potassium (SK) channels. We also pharmacologically exclude calcium-gated chloride channels, glycine receptors and the chloride current associated with glutamate transport. Finally, we demonstrate that, although small, this current modulates neuronal excitability. We speculate that this tonic current may provide a complementary mechanism for the regulation of neuronal excitability, particularly in regions with low ambient GABA concentrations. We conclude that this bicuculline-sensitive conductance needs to be accounted for in studies of GABA tonic currents, lest it be confused with currents associated with GABA overflow.

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Abbreviations:
17-PA, (3α,5α)-17-phenylandrost-16-en-3-ol
3α5αP (allopregnanolone), (3α,5α)-3-Hydroxypregnan-20-one
D-APV, D(–)-2-Amino-5-phosphonopentanoic acid
EGTA, Ethylene glycol-bis (β-aminoethyl ether)-*N*,*N*,*N*,*N*-tetraacetic acid
GABA, γ-aminobutyric acid
HEPES, *N*-(2-Hydroxyethyl) piperazine-*N*′-(2-ethanesulfonic acid) hemisodium salt
NBQX, 1,2,3,4-tetrahydro-6-nitro-2,3-dioxobenzo[*f*]quinoxaline-7-sulfonamide
TBOA, DL-*threo*-β-benzyloxyaspartate
TPMPA, (1,2,5,6-tetrahydropyridin-4-yl)methylphosphinic acid

1. Introduction

Tonic, resting membrane conductances, such as those mediated by leak K^+, Cl^- and transmitter-gated channels, strongly influence neuronal excitability. There is recent particular interest in tonic conductances mediated by GABA-A receptors, often defined pharmacologically (Farrant and Nusser, 2005). GABA's actions at GABA-A receptors underlie well-known phasic synaptic actions (Mehta and Ticku, 1999; Sieghart, 1995) as well as more recently described tonic conductances (Farrant and Nusser, 2005). GABA-A mediated tonic chloride conductances, although small in amplitude, can have a profound influence on neuronal excitability because the tonic current provides an ongoing shunt to voltage-gated currents (Semyanov et al., 2004). Tonic currents are defined by the ability of GABA site antagonists, like bicuculline or gabazine, to block a resting conductance. A tonic conductance has been reported in many neuronal types and is currently thought to arise primarily from GABA that accumulates at extrasynaptic sites during synaptic activity (Rossi and Hamann, 1998; Wei et al., 2003). Nevertheless, there is controversy over whether the antagonist pharmacology of tonic conductances is distinct from those of phasic synaptic conductances (Bai et al., 2001; Stell and Mody, 2002). In some studies, tonic, but not phasic, currents have exhibited resistance to gabazine but sensitivity to bicuculline, two GABA site antagonists (Bai et al., 2001). It is possible that the pharmacology of antagonists is not always selective enough to truly identify GABA-receptor-mediated conductances.

Here we use a reduced, single-cell preparation to re-examine the pharmacology of tonic chloride conductances in hippocampal neurons. Like others (Bai et al., 2001), we find evidence for a bicuculline-sensitive, gabazine-insensitive tonic current. However, the tonic current exhibits a different pharmacology than the tonic current gated by low GABA concentrations or by neurosteroids, potential endogenous regulators of GABA-receptor-mediated tonic currents. Notably, the current is insensitive to the non-competitive GABA-A receptor antagonist picrotoxin. Therefore, this current is not mediated by ambient GABA or by conventional GABA receptors. We conclude that this novel, bicuculline-sensitive chloride conductance could contribute to tonic inhibition under some conditions. Future studies of tonic currents should account for this conductance since it can easily be confused with currents mediated by GABA overflow.

2. Results

2.1. *Tonic chloride conductance in solitary glutamatergic neurons*

Fig. 1A illustrates representative autaptic currents from solitary excitatory hippocampal neurons. Shown is the response in the absence of antagonist (baseline), in the presence of NBQX (1 μM) and in the presence of 1 μM NBQX plus 25 μM bicuculline. Despite the glutamate phenotype of this solitary neuron, addition of bicuculline decreased the baseline noise level and produced an outward shift in the holding current level (Fig. 1B) with no effect on the evoked autaptic current. Similar results were obtained in 5 other neurons. Although GABA release from glutamate neurons is reported from dentate granule neurons under some conditions (reviewed in Gutierrez, 2005) and from cultured hippocampal neurons (Bekkers, 2005), the lack of effect of bicuculline on evoked responses argues against GABA release in our system and our subsequent pharmacology (see Section 2.3) further excludes an important role for GABA in the bicuculline-sensitive current.

A KCl-containing pipette solution was used for these experiments to promote the ability to detect any synaptically released GABA from glutamatergic cells (Bekkers, 2005; Gutierrez, 2005) (negligible in Fig. 1A). For subsequent experiments, we set the chloride equilibrium potential to more