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SHORT COMMUNICATION

cAMP-Dependent protein kinase A activity modulates topiramate potentiation of $GABA_A$ receptors

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Summary Activation of cAMP-dependent protein kinase A (PKA) prevents inhibition of non-NMDA glutamate receptors by the anticonvulsant topiramate. Using two-electrode voltage-clamp techniques, we demonstrate that PKA activity also modulates topiramate potentiation of recombinant $GABA_A$ receptors expressed in *Xenpus laevis* oocytes. PKA activators, dibutyryl-cAMP and forskolin, attenuate topiramate potentiation, whereas the PKA inhibitor H-89 increases topiramate potentiation. Thus, endogenous PKA activity and receptor phosphorylation states may contribute to topiramate treatment efficacy.

Introduction

The broad spectrum anticonvulsant topiramate [2,3:4,5-bis-*O*-(1-methylethylidene)β-D-fructopyranose sulfamate] modulates several ion channels including voltage-gated Na^+, Ca^{2+} and K^+ channels and ligand-gated non-NMDA glutamate receptors and $GABA_A$ receptors (Simeone, 2010). Depending on the ion channel, phosphorylation of serine/threonine residues by cAMP-dependent protein kinase A can either increase or decrease channel function (Swope et al., 1999; Dai et al., 2009). It has been suggested that topiramate may simulate phosphate binding and prevent ion channel phosphorylation, thus providing a mechanism by which topiramate affects such a diverse array of ion channel families (Shank et al., 2000). Supporting this notion, increasing the activity of PKA prevents topiramate inhibition and dephosphorylation of non-NMDA glutamate receptors (Gibbs et al., 2000; Ängehagen et al., 2004, 2005). In the current study, we aimed to test the hypothesis that PKA activity modulates topiramate potentiation of $GABA_A$ receptors.

Methods

Oocyte isolation and injection

Oocytes were isolated from *Xenopus laevis* frogs and injected as previously described (Simeone et al., 2006a,b). All procedures involving animals were in accordance with the Declaration of Helsinki and National Institutes of Health guidelines and were approved by the University of Utah Institutional Animal Care and Use Committee. Recombinant human α_2 and γ_{2S} and rat β_3 receptor subunit cDNAs

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were obtained from the laboratory of the late Dr. Dolan Pritchett via Dr. Roy Twyman while at the University of Utah. These cDNA constructs (Pritchett et al., 1989; Hadingham et al., 1993) have previously expressed functional $GABA_A$ receptors in our hands (Simeone et al., 2006a,b).

Electrophysiology

Electrophysiological recordings were performed 1—4 days following cDNA injection and were conducted at room temperature (20—22 °C) in a 100 μl chamber continuously perfused (6 ml/min) with a Ringer's solution containing in mM: 115 NaCl, 2.5 KCl, 1.0 $BaCl_2$, and 10 HEPES (pH = 7.4). Two-electrode voltage-clamp recordings were obtained with a GeneClamp 500 amplifier (Axon Instruments, Union City, CA, U.S.A.) using 3 M KCl-filled microelectrodes (1—5 MΩ). Recordings were performed at a holding potential of −60 mV and acquired with pClamp6 Fetchex software (Axon Instruments). Consecutive applications of 1 mM topiramate, 0.3 μM GABA, 0.3 μM GABA + 100 μM topiramate, and 0.3 μM GABA + 1 mM topiramate were performed every 3 min (see Fig. 1). Five to eight applications established baseline responses, after which PKA modulators were bath applied and continuously perfused over the oocyte. In the presence of PKA modulators, application of the above sequence of GABA and topiramate continued every 3 min. Once the effects of the PKA modulators on the GABA-current and topiramate potentiation reached a plateau (dibutyryl-cAMP: 8 applications; forskolin: 9—20 applications; H-89: 8—20 applications), Ringer's solution was perfused to wash-out the PKA modulator (dibutyryl-cAMP: 8 applications; forskolin: 8—20 applications; H-89: 8—15 applications). For each oocyte, averages of the last three GABA and topiramate applications during PKA modulation was compared to the average of the five to eight baseline applications. Similarly, averages of the last three applications during washout was compared to the average of the five to eight baseline applications. Data are reported as the mean percent of either (i) the baseline GABA response or (ii) the baseline topiramate potentiation of the GABA response ± SEM. Data were analyzed using pClamp6 Fetchan software (Axon Instruments), and PRISM4 software (Graphpad Software Inc., San Diego, CA, U.S.A.). Statistical significance was determined with paired *t*-tests.

Drug preparation

Drugs were prepared fresh before each recording session. Stock solutions of 100 mM dibutyryl-adenosine-3′,5′-cyclicmonophosphate, N^6,O^2 (dibutyryl-cAMP) (Calbiochem, San Diego, CA, U.S.A.), 10 mM H-89 (Calbiochem), and 10 mM forskolin (Calbiochem) were prepared in DMSO (Sigma). Topiramate (10 mM) was prepared in frog ringer's solution. All drugs were serially diluted down to the lowest experimental concentration and bath-perfused.

Results

Dibutyryl-cAMP and forskolin inhibit GABA-evoked currents, whereas H-89 enhances GABA-evoked currents

Dibutyryl-cAMP is a membrane permeable form of cAMP and forskolin activates adenylate cyclase stimulating the production of cAMP; both compounds increase the activity of PKA. We found that application of either dibutyryl-cAMP (100 μM) or forskolin (10 μM) inhibited GABA-currents by 22% and 16%, respectively (Fig. 1A and B; $p < 0.001$ and $p < 0.05$). These effects were reversible upon washout. In contrast, application of the PKA inhibitor N-[2-bromocinnamyl(amino)ethyl]-5-isoquinolinesulfonamide (H-89; 1 μM) enhanced GABA-currents by 27% ($p < 0.05$); however, H-89 displayed variable reversibility (Fig. 1C) suggesting either the necessity of longer washout durations or possible GABA-current ''run-up''. The former explanation is favored because H-89 effects on topiramate potentiation was reversible (see next section) and in control studies with no kinase activator or inhibitor GABA and topiramate responses remained stable over a 90-min period ($n = 4$, not shown).

Dibutyryl-cAMP and forskolin inhibit topiramate potentiation, whereas H-89 increases topiramate potentiation

Topiramate potentiated GABA-currents by 23.2 ± 6.7% (100 μM) and 84.5 ± 11.7% (1 mM) ($n = 16$; $p < 0.001$). Dibutyryl-cAMP (100 μM) significantly attenuated 100 μM topiramate potentiation by 45%, but had no effect on 1 mM topiramate potentiation (Fig. 1C and D; $n = 9$, $p < 0.001$ and $p = 0.124$). Forskolin (10 μM) decreased 100 μM and 1 mM topiramate potentiation by 42% and 32%, respectively ($n = 3$, $p < 0.01$ and $p < 0.05$). In contrast, H-89 (1 μM) increased 100 μM and 1 mM topiramate potentiation by 89% and 39%, respectively ($n = 4$, $p < 0.001$ and $p < 0.05$). All PKA modulator effects reversed upon washout.

Discussion

We previously reported that the anti-convulsant topiramate is a subunit-dependent modulator of $GABA_A$ receptors (Simeone et al., 2006b). Here, we present evidence that topiramate modulation of $GABA_A$ receptors is also sensitive to the activity of PKA. Specifically, we found that PKA activators attenuate the potentiation of $GABA_A$ receptors by topiramate, whereas inhibiting PKA increased topiramate potentiation.

Topiramate has proven to have effects on widely diverse ion channels, both voltage-gated and ligand gated. Although topiramate binding sites have yet to be identified, Shank et al. (2000) have put forth an interesting hypothesis that potentially unifies the promiscuity of topiramate actions. This hypothesis is derived from the original intent for topiramate to prevent phosphorylation by simulating phosphate binding. Each of the ion channels responsive to topiramate is regulated by phosphorylation and contains the PKA consensus sequence RRXS (Swope et al., 1999; Dai et al., 2009). These tetrapeptide sequences contain several proton donor groups that have the potential to form hydrogen bonds with proton accepting oxygen atoms found in topiramate. If the ion channels exist in populations of mixed phosphorylation states, topiramate could bind to dephosphorylated channels, elicit immediate effects, and prevent further phosphorylation, thereby shifting the whole population to the dephosphorylated state and exerting long-term effects (Shank et al., 2000). To date the only evidence for this unifying hypothesis has been obtained from experiments on non-NMDA glutamate receptors where PKA activation reduces topiramate inhibition and prevents the dephosphorylation of AMPA/kainate receptors (Gibbs et al., 2000;