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Involvement of inward rectifier and M-type currents in carbachol-induced epileptiform synchronization

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ABSTRACT

Exposure to cholinergic agonists is a widely used paradigm to induce epileptogenesis *in vivo* and synchronous activity in brain slices maintained *in vitro*. However, the mechanisms underlying these effects remain unclear. Here, we used field potential recordings from the lateral entorhinal cortex in horizontal rat brain slices to explore whether two different K^+ currents regulated by muscarinic receptor activation, the inward rectifier (K_{IR}) and the M-type (K_M) currents, have a role in carbachol (CCh)-induced field activity, a prototypical model of cholinergic-dependent epileptiform synchronization. To establish whether K_{IR} or K_M blockade could replicate CCh effects, we exposed slices to blockers of these currents in the absence of CCh. K_{IR} channel blockade with micromolar Ba^{2+} concentrations induced interictal-like events with duration and frequency that were lower than those observed with CCh; by contrast, the K_M blocker linopirdine was ineffective. Pre-treatment with Ba^{2+} or linopirdine increased the duration of epileptiform discharges induced by subsequent application of CCh. Baclofen, a $GABA_B$ receptor agonist that activates K_{IR}, abolished CCh-induced field oscillations, an effect that was abrogated by the $GABA_B$ receptor antagonist CGP 55845, and prevented by Ba^{2+}. Finally, when applied after CCh, the K_M activators flupirtine and retigabine shifted leftward the cumulative distribution of CCh-induced event duration; this effect was opposite to what seen during linopirdine application under similar experimental conditions. Overall, our findings suggest that K_{IR} rather than K_M plays a major regulatory role in controlling CCh-induced epileptiform synchronization.

1. Introduction

Cholinergic agonists like carbachol (CCh) or pilocarpine, by activating M1 muscarinic receptors (Cruickshank et al., 1994; Bymaster et al., 2003), induce seizure activity when administered *in vivo* (Turski et al., 1983), and epileptiform discharges in brain slices (Dickson and Alonso, 1997). It is still unclear which of the different transductional events activated upon M1 receptor stimulation is responsible for the appearance of this synchronous activity. A wealth of experimental evidence points to the activation of non-specific cation currents as the major factor involved even though the identity of these currents remains poorly defined. D'Antuono et al. (2001) provided evidence for a major role of the so called I_{CAN} current (Colino and Halliwell, 1993), a non-specific cationic current activated by $[Ca^{2+}]_i$ increases, which induces the appearance of depolarizing plateau potentials in limbic areas involved in temporal lobe epilepsy (TLE) (see Gloor, 1997) including the subiculum (D'Antuono et al., 2001), the entorhinal cortex (EC) (Klink and Alonso, 1997), and the hippocampal CA1 subfield (Fraser and MacVicar, 1996). By contrast, Egorov et al. (2003) provided evidence for a CCh-activated Ca^{2+}-independent non-specific cation channel.

In addition to non-specific cationic channels, CCh interferes with a number of additional targets, which may play a relevant role in CCh-induced epileptiform discharges. In particular, M1 receptors activate phospholipase C (PLC), which, in turn, leads to a depletion of phosphatidylinositol 4,5-bisphosphate (PIP2), a phospholipid crucially involved in the control of a wide variety of ionic currents

Abbreviations: ACSF, artificial cerebrospinal fluid; CCh, carbachol; EC, entorhinal cortex; K_{IR}, inward rectifier K^+ current; K_M, M-current; TLE, temporal lobe epilepsy.

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(Suh and Hille, 2005). Among PIP2-modulated currents are inward rectifiers (K_{IR}) (Huang et al., 1998; Sohn et al., 2007; Carr and Surmeier, 2007) and the M-current (K_M), which may therefore regulate the genesis or maintenance of CCh-induced epileptiform discharges. K_{IR}-M1receptor interaction may be particularly relevant to TLE, since inward rectifier K^+ channels are highly expressed in the hippocampus and in the EC (Karschin et al., 1996), where they participate in TLE pathogenesis (Young et al., 2009). Depending upon their subunit composition, K_{IR} may be open at resting conditions (*constitutively active* K_{IR}) and control resting membrane potential, or require the stimulation of G-protein-coupled receptors to become active (Nichols and Lopatin, 1997). The latter, also referred to as GIRK channels, largely mediate the responses to inhibitory neurotransmitters (Mark and Herlitze, 2000).

K_M, as well, is highly expressed in the hippocampus (Cooper et al., 2001), where it controls neuronal excitability by regulating action potential frequency adaptation (Yue and Yaari, 2004), after-depolarization/after-hyperpolarization amplitude and duration (Yue and Yaari, 2004) and, in some instances, resting membrane potential (Shah et al., 2008). Moreover, K_M modulates intrinsic firing properties and subthreshold membrane oscillations in EC cells (Yoshida and Alonso, 2007), and the epileptiform activity induced by perfusion with low Mg^{2+}-containing medium in the hippocampus (Qiu et al., 2007).

Despite these evidences, the possible involvement of K_{IR} and K_M in epileptiform synchronization consequent to cholinergic activation remains unexplored. Therefore, we employed field potential recordings from the EC, a limbic area having a major role in TLE (Du et al., 1995; de Guzman et al., 2008) and in generating the epileptiform activity induced by muscarinic agonists in brain slices (Nagao et al., 1996), along with pharmacological manipulations aimed at modulating K_{IR} and K_M, to investigate the roles of these two K^+ currents in CCh-elicited synchronous activity. Our data suggest that K_{IR} exerts a major regulation of CCh-induced epileptiform synchronization, whereas K_M appears to play a complementary modulatory role.

2. Methods

Brain slices were obtained from adult male Sprague–Dawley rats (Charles River, St. Constant, QC, Canada), 2–3 months of age (250–400 g). Animal housing and experimental procedures were performed according to the recommendations of the Canadian Council on Animal Care following a research protocol approved by the McGill Animal Care Committee. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Combined hippocampus-EC slices were obtained as described by de Guzman et al. (2008). Slices were transferred to a custom-made interface recording chamber and let equilibrate for 1–1.5 h while continuously superfused (1 ml/min) with artificial cerebrospinal fluid (ACSF) at ~32 °C, equilibrated at pH = 7.4 with gas mixture (95% O_2, 5% CO_2), and containing in mM: 124 NaCl, 2 KCl, 1.25 KH_2PO_4, 2 $MgSO_4$, 2 $CaCl_2$, 26 $NaHCO_3$ and 10 D-glucose. Extracellular field potentials were recorded using ACSF-filled borosilicate electrodes (5–10 MΩ) (World Precision Instruments, Sarasota, FL, USA) that were placed under visual control in the deep layers of the lateral EC. Field potentials were recorded with preamplifier probes connected to a Cyberamp 380 amplifier (Molecular Devices, Sunnyvale, CA) driven by the pClamp 8.2 software (Molecular Devices). Data were digitized at 5 kHz with a Digidata 1322A A/D converter (Molecular Devices) and stored on the hard drive for offline analysis performed using the Clampfit 9 software (Molecular Devices).

Unless otherwise stated, data are expressed as mean ± SEM. Statistical analysis was performed using the Sigma Plot 10 software with Sigma Stat 3.5 module. Normal distribution was assessed prior to statistical comparison, which was performed with one way or repeated measure ANOVA as appropriate, followed by Newman–Keuls post-hoc test. Statistical analysis of non-normally distributed data was performed with the Kruskall–Wallis or Friedman analysis, as appropriate, followed by the Dunn post-hoc test. Two-group comparisons were performed using the Student's *t* test for paired data (normally distributed datasets) or the Mann–Whitney Rank Sum Test (non-normally distributed datasets). Significance was set at $p < 0.05$.

The effect of different compounds on the duration of CCh- or Ba^{2+}-induced events was quantified by applying the Kolmogorov–Smirnov test to compare the cumulative distributions of these events (bin = 250 ms) before and after application of the test drug. Significance was set at $p < 0.05$. The concentration-dependence curve of baclofen-induced decrease of the frequency of CCh-induced events was obtained by sequentially exposing each slice to 1, 3, 10, 30 and 100 μM baclofen, and by fitting the data to the four parameter logistic equation

$$y = \min + (\max - \min)/(1 + (x/EC_{50})^n)$$

where min and max were the minimal and maximal normalized values of the frequency of CCh-evoked events, respectively, y was the average frequency of CCh-induced events normalized to the values obtained in the absence of baclofen, x was the baclofen concentration and n the Hill slope.

All chemicals were obtained from Sigma–Aldrich (Oakville, Ontario, Canada) with the exception of flupirtine and CGP 55485 (Tocris, Bristol, UK). Retigabine was obtained from Valeant Pharmaceuticals (Aliso Viejo, CA, USA). CGP 55845, flupirtine and retigabine were dissolved in DMSO, and linopirdine was dissolved in ethanol. To rule-out any possible interference of the solvents on field activity, control experiments were performed by adding the appropriate vehicle to the ACSF.

3. Results

3.1. CCh-induced epileptiform discharges in the EC

Bath-application of CCh (100 μM) readily (10–30 min) induced the generation of synchronized oscillatory field potentials in the lateral EC in all slices ($n = 32$; Fig. 1A). Two types of epileptiform activity could be distinguished according to their duration (*cf.* de Guzman et al., 2004): (i) events shorter than 4 s (Fig. 1A, arrows), thereafter defined as 'interictal-like' and (ii) events longer than 4 s, termed as 'ictal-like' (Fig. 1A, dashed line). As indicated by the frequency distribution in Fig. 1B, interictal-like discharges represented the majority ($n = 3090/3116$ events, 99%) of CCh-induced events, and had a mean duration of 1.2 ± 0.4 s, whereas ictal-like discharges had a mean duration of 10.1 ± 1.2 s. Given the minor contribution of ictal-like discharges on average CCh-induced excitability, subsequent analysis was performed on all events pooled together, regardless of their classification as ictal-like or interictal-like discharges. Using this approach, the mean frequency of CCh-induced events was 0.33 ± 0.02 Hz whereas their mean duration was 1.4 ± 0.08 s. CCh-induced events were abolished by bath-application of 3 μM pirenzepine ($n = 6$; Fig. 1C), confirming that activation of muscarinic receptors is primarily involved in CCh excitatory actions within the EC.

3.2. K_{IR} and K_M regulate CCh-induced oscillations

Since muscarinic stimulation is known to decrease the activity of K_{IR} and K_M (Carr and Surmeier, 2007; Delmas and Brown, 2005; Sohn et al., 2007), we tested the hypothesis that epileptiform synchronous events similar to those triggered by CCh could be induced upon blockade of either of these two potassium currents. To this aim, we used micromolar concentrations of Ba^{2+} that should selectively block K_{IR} without affecting K_M (Kubo et al., 1993; Cloues and Marrion, 1996; Schoots et al., 1996), and linopirdine, a selective K_M blocker (Miceli et al., 2008). Bath-application of Ba^{2+} (200 μM; $n = 5$) caused the appearance of field discharges (Fig. 2A, top) with a frequency (0.06 ± 0.01 Hz) and duration (0.8 ± 0.2 s) significantly lower than those triggered by CCh (frequency = 0.33 ± 0.02 Hz and duration = 1.4 ± 0.08 s; $n = 32$, $p < 0.01$). As shown in Fig. 2B, the cumulative distribution curve of Ba^{2+}-induced events was shifted leftward when compared to that obtained for CCh-induced events ($p < 0.01$).

Subsequent application of CCh to Ba^{2+}-perfused slices (Fig. 2A, bottom) caused the appearance of field discharges that were longer in duration (7.3 ± 1.5 s, $p < 0.001$) and occurred less frequently (0.09 ± 0.01 Hz, $p < 0.001$) than those observed in the presence of CCh alone. These field events were also significantly longer ($p < 0.001$) and less frequent ($p < 0.001$) than those seen with Ba^{2+} alone. As shown in Fig. 2B, the cumulative distribution of the duration of CCh-induced events recorded in the presence of Ba^{2+} was shifted rightward compared to that describing the events