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MUSCARINIC ACETYLCHOLINE RECEPTOR-MEDIATED EFFECTS IN SLICES FROM HUMAN EPILEPTOGENIC CORTEX

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Abstract—Acetylcholine has been implicated in higher cortical functions such as learning, memory and cognition, yet the cellular effects of muscarinic acetylcholine receptor (mAChR) activation are poorly understood in the human cortex. Here we investigated the effect of the mAChR agonist carbachol (CCh) and various mAChR antagonists in human cortical slices (from tissue removed during neurosurgical treatment of epilepsy) by intracellular and extracellular recordings. CCh increased neuronal firing, which was antagonised by atropine (non-selective mAChR antagonist) and pirenzepine (M_1/M_4 mAChRs antagonist) when applied before or after CCh application. AF-DX 116 (M_2/M_4 mAChRs antagonist) had no effect on CCh-induced increase of firing. CCh also reduced evoked excitatory postsynaptic potentials (EPSP), and the CCh-induced depression of EPSP was fully reversed by atropine. Pirenzepine reversed the depression of CCh on EPSP, but failed to prevent the depression when applied before CCh. AF-DX 116 prevented the CCh-induced depression of evoked EPSP when applied before CCh. CCh also depressed GABAergic transmission and this effect was antagonised by AF-DX 116. Xanomeline (M_1/M_4 mAChR agonist) increased neuronal firing and decreased EPSP, but had no effect on GABAergic transmission. Reduction (with linopirdine) and enhancement (with retigabine) of the M-current (mediated by K_V7 channels), increased and decreased neuronal firing, respectively, but had marginal effects on the evoked EPSP. Our results indicate that three pharmacologically distinct mAChRs modulate neuronal firing, glutamatergic and GABAergic transmissions in the human epileptogenic neocortex. The data are discussed towards possible implications of altered mAChR signalling in hyperexcitability and cognitive functions in the human neocortex. **© 2012 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: carbachol, M-current, muscarinic acetylcholine receptors, human epilepsy.

INTRODUCTION

Alteration of muscarinic acetylcholine receptor (mAChR) function has been implicated in several neurological disorders including schizophrenia, Alzheimer's Disease and epilepsy (Bymaster et al., 2003a; Langmead et al., 2008). A plausible reason for the diverse functions of these receptors is provided by distinct localisations in the central nervous system (CNS) and the diversity in molecular subtypes. The mAChRs belong to the family of heptahelical G-protein-coupled receptors (Langmead et al., 2008) and comprise five members (M_1–M_5) with highly conserved transmembrane domains (Caulfield and Birdsall, 1998). Upon activation of the mAChR, the variable intracellular domain (Peralta et al., 1987) activates G_i/G_o-proteins modulating second messengers and ion channels (Wess, 2004; Delmas and Brown, 2005; Langmead et al., 2008). The M_1 mAChR seems to play a key role in epileptogenesis, since pilocarpine fails to induce seizures in mice lacking M_1 mAChRs (Hamilton et al., 1997). This crucial role of the M_1 mAChR may relate to the M-current (mediated by K_V7 channels), which normally dampens neuronal excitability (Delmas and Brown, 2005). A further link between M-current and epilepsy is provided by mutations of $K_V7.2$ and $K_V7.3$ encoding genes (Jentsch, 2000) detected in patients with benign familial neonatal convulsions (BFNC).

Therefore, modulators of mAChRs may have therapeutic benefits in epilepsy, similar to retigabine which facilitates opening of K_V7 channels (Rundfeldt, 1997; Otto et al., 2002). Yet, side effects of non-selective mAChR antagonists have been reported (Bymaster et al., 2003b; Langmead et al., 2008). In particular, these compounds may alter cholinergic signalling, which is usually associated with cognitive deficits in animals (Wess, 2004), and schizophrenia and Alzheimer's Disease in humans (Harbaugh et al., 1984; Minzenberg et al., 2004). Development of subtype-selective ligands with promising therapeutic benefits (Felder et al., 2000), however, is hampered by the orthosteric binding site

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Abbreviations: BFNC, benign familial neonatal convulsions; CCh, carbachol; CNS, central nervous system; DMSO, dimethylsulfoxide; EGTA, ethylene glycol tetraacetic acid; E_m, membrane potential; EPSP, excitatory postsynaptic potential; GABA, gamma aminobutyric acid; GM, grand mal seizures; IPSP, inhibitory postsynaptic potential; mAChR, muscarinic acetylcholine receptor; R_m, neuronal input resistance; TLE, temporal lobe epilepsy.

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which is highly conserved among the mAChRs. A further obstacle impeding the development and functional testing of selective ligands is the lack of established protocols to screen different mAChR agonists/antagonists at native receptors in mammalian CNS neurons. This poses a major problem since the pharmacology of native rat receptors or recombinant human receptors does not necessarily reflect the properties of native human receptors (e.g. Thomas et al., 2010).

We investigated the effects of the mAChR agonist carbachol (CCh) and several relevant compounds on neuronal firing and synaptic transmission in human epileptogenic cortical tissue (cortical layers II/III), where expression of M_1, M_2 and M_4 mAChRs predominate (Levey et al., 1991; Caulfield, 1993; Mrzljak et al., 1993, 1998; Disney et al., 2006).

EXPERIMENTAL PROCEDURES

Tissue handling and preparation

Human neocortical tissues were obtained during neurosurgical treatment of pharmacoresistant patients with temporal ($N = 38$), parietal ($N = 1$) or frontal ($N = 3$) lobe epilepsy. The patients (24 male, 18 female) were on average 33.3 ± 1.8 years old. All experiments on human tissues were conducted in accordance with the Declaration of Helsinki. The patients were informed about the scientific use of the tissues and provided written consent with adequate understanding. Formal approval to use the tissue was obtained from the Local Ethics Committee and could be provided upon request.

The methods for tissue slice preparation have previously been described (see Deisz, 1999; Teichgräber et al., 2009; Deisz et al., 2011). In brief, the tissue was collected in the operating theatre and transported to the laboratory in cold modified artificial cerebrospinal fluid (mACSF; see below). Smaller tissue blocks were cut into slices (400 μm) with a vibratome (HM 650 V, MICROM International, Walldorf, Germany) in ACSF (5 ± 1 °C, see below). The slices were stored submerged in ACSF (room temperature, equilibrated with 95% O_2/5% CO_2).

Solutions and substances

Standard ACSF contained (in mM): 124 NaCl, 5 KCl, 2 $MgSO_4$, 2 $CaCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, and 10 D(+)-glucose (pH 7.4 after equilibration with 95% O_2 and 5% CO_2). mACSF contained (in mM): 70 NaCl, 2.5 KCl, 7 $MgSO_4$, 0.5 $CaCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 25 D(+)-glucose, and 75 sucrose. All substances were of analytical grade (Merck, Darmstadt, Germany).

The compounds atropine (Sigma, Taufkirchen, Germany), carbamylcholine-chloride (CCh; gift from GlaxoSmithKline), linopirdine dihydrochloride (Tocris, Bristol, UK) and pirenzepine (Sigma) were prepared as aqueous stock solutions. AF-DX 116 (AFDX; Tocris), retigabine and xanomeline (both a gift from GlaxoSmithKline) were dissolved in dimethylsulfoxide (DMSO). The final concentration of DMSO was always below 0.1%. All stock solutions were stored at −20 °C until used. The compounds were added immediately before experiments to the ACSF.

We chose established mAChR antagonists pirenzepine (M_1/M_4 mAChRs antagonist), AFDX (M_2/M_4 mAChR antagonist) and atropine (non-selective mAChR antagonist, for reviews see Buckley et al., 1989; Moriya et al., 1999); and a M_1/M_4 preferring mAChR agonist xanomeline (Shannon et al., 2000). These compounds were used to delineate the mAChRs involved in the cellular effects. In addition, we used the M-current activator retigabine (Rundfeldt, 1997; Otto et al., 2002) and the M-current blocker linopirdine (Aiken et al., 1995), also known to modulate epileptiform activities *in vitro* (Cataldi et al., 2011). We selected established concentrations of agonist and antagonist based on a literature survey, a few pilot experiments and a preceding study in the rat neocortex (Gigout et al., 2012). The concentrations employed were CCh: 10 μM, atropine: 1 μM, AFDX: 2 μM: pirenzepine: 1 μM, xanomeline: usually 3 μM, retigabine and linopirdine: 10 μM.

Despite a moderate selectivity of the antagonists, the comparison of effectiveness might allow the delineation of receptors involved in a particular effect. Accordingly, (1) M_1 mAChR-mediated effects should be antagonised by pirenzepine but not by AFDX; (2) M_2 mAChR-mediated effects should be antagonised by AFDX but not by pirenzepine; (3) M_4 mAChR-mediated effects should be fully antagonised by pirenzepine or AFDX.

Intracellular and field potential recordings

Neocortical neurons (layers II/III) were recorded with sharp microelectrodes in submerged-type chambers, perfused with carbogenated ACSF (31 ± 1 °C; 4–5 ml/min). Electrodes were pulled on a Flaming/Brown P87 Puller (Sutter Instruments, Novato, USA) to resistances of 70–120 MΩ when filled with a solution containing 1 M K-acetate (plus 1 mM KCl and 5 mM EGTA; pH 7.2 adjusted with KOH). The electrodes were connected to an appropriate amplifier (SEC-05L, npi electronic, Tamm, Germany). The electrodes for field potential recordings were pulled on a DMZ universal puller (Zeitz, München, Germany) and had resistances of 2–8 MΩ when filled with ACSF. The recorded signals were fed to a high-impedance preamplifier (EXT-01C, npi electronic, Tamm, Germany) and processed through second-stage amplifiers with filtering capabilities (DPA 2F; npi electronic, Tamm, Germany).

We evaluated the current–voltage relationship from families of current injections (between −0.5 and +1 nA, increment 0.05 nA, duration 600 ms), to estimate neuronal input resistance (R_m) and firing behaviour. The number of APs increased approximately linearly with the magnitude of the injected current up to ~0.6 nA. Linear regression of the number of APs *vs.* injected current in the initial linear range was used to calculate the slope of neuronal firing (firing slope) and the intercept. The former parameter provides a quantitative index describing the AP firing behaviour, the latter represents the current just below the rheobase and was termed sub-rheobase.

Synaptic responses (intracellular and field potential recordings) were elicited by electrical stimuli (0–20 V, increment 2 V, 100 μs duration at 0.1 Hz, in triplicate) applied *via* a bipolar tungsten electrode placed in the deeper cortical layers (V/VI). The peak amplitude of the initial component of synaptic responses represents the excitatory postsynaptic potential (EPSP), although it might be slightly affected by the inhibitory postsynaptic potential (IPSP) with a slightly later time to peak. The input–output curves of EPSP were fitted by the Boltzmann equation:

$$Y = (\mathrm{A1} - \mathrm{EPSP}_{max})/(1 + e(x - x_0)/dx) + \mathrm{EPSP}_{max}$$

yielding the maximal EPSP amplitude ($EPSP_{max}$), the half maximal stimulus (x_0 representing the stimulus intensity yielding half-maximal responses, I_{50}) and the slope factor (dx).

From the on-line inspection of the input–output relation a stimulus intensity yielding about 60% amplitude (typically 12 or 14 V) was chosen and a second series of current injections were carried out to estimate synaptic conductance and reversal potential of $GABA_A$ and $GABA_B$ responses (see Deisz and Prince, 1989; Teichgräber et al., 2009; Deisz et al., 2011).

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