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PUTATIVE DEPOLARISATION-INDUCED RETROGRADE SIGNALLING ACCELERATES THE REPEATED HYPOXIC DEPRESSION OF EXCITATORY SYNAPTIC TRANSMISSION IN AREA CA1 OF RAT HIPPOCAMPUS VIA GROUP I METABOTROPIC GLUTAMATE RECEPTORS

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Abstract—Excitatory synaptic transmission in area CA1 of the mammalian hippocampus is rapidly depressed during hypoxia. The depression is largely attributable to an increase in extracellular adenosine and activation of inhibitory adenosine A_1 receptors on presynaptic glutamatergic terminals. However, sequential exposure to hypoxia results in a slower subsequent hypoxic depression of excitatory synaptic transmission, a phenomenon we have previously ascribed to a reduction in the release of extracellular adenosine. In the present study we show that this delayed depression of excitatory postsynaptic currents (EPSCs) to repeated hypoxia can be reversed by a period of postsynaptic depolarisation delivered to an individual CA1 neuron, under whole-cell voltage clamp, between two periods of hypoxia. The depolarisation-induced acceleration of the hypoxic depression of the EPSC is dependent upon postsynaptic Ca^{2+} influx, the activation of PKC and is blocked by intracellular application of GDP-β-S and N-ethylmaleimide (NEM), inhibitors of membrane fusion events. In addition, the acceleration of the hypoxic depression of the EPSC was prevented by the GI mGluR antagonist AIDA, but not by the CB1 cannabinoid receptor antagonist AM251. Our results suggest a process initiated in the postsynaptic cell that can influence glutamate release during subsequent metabolic stress. This may reflect a novel neuroprotective strategy potentially involving retrograde release of adenosine and/or glutamate.

Key words: hypoxia, ischemia, adenosine, retrograde signalling, mGluR.

INTRODUCTION

The control of neurotransmitter release via the activation of presynaptic receptors is of fundamental importance to the functioning of the mammalian brain. This control can be exerted at different levels. For example, autoreceptors sense the local presence of neurotransmitter, to either inhibit further release, eg via $GABA_B$ receptors (Davies et al., 1990) or, as has been suggested for kainate receptors, to promote glutamate release and the induction of long-term potentiation (LTP) (Lauri et al., 2001; Schmitz et al., 2001). In addition, these orthograde transmitters can act at distant receptors to promote or inhibit the release of the same or a distinct transmitter, a process referred to as ''spillover'' (Kullmann, 2000; Szapiro and Barbour, 2007; Okubo and Iino, 2011). However, an additional facet to the control of transmitter release arises from the ability of presynaptic receptors to switch from a facilitatory to inhibitory mode, depending on agonist exposure (Herrero et al., 1998; Rodriguez-Moreno et al., 1998).

The concept of transmitters feeding back to influence subsequent release has been complemented by the description of a process initiated in the postsynaptic cell (Ovsepian and Dolly, 2011). Thus, retrograde signalling, instigated by postsynaptic depolarisation, has been shown to influence the release of GABA and glutamate at a variety of central synapses via the postsynaptic release of opioids, endocannabinoids, glutamate and GABA (Wagner et al., 1993; Zilberter et al., 1999; Zilberter, 2000; Kreitzer and Regehr, 2001; Wilson and Nicoll, 2001; Duguid et al., 2007; Regehr et al., 2009). This mechanism allows postsynaptic neurons considerable synapse-specific control over incoming signals.

In addition to these control mechanisms of known cellular origin, an additional influence over transmitter release is provided by the diffuse influence of neuromodulators, a prime example of which is the purine nucleoside adenosine (Dunwiddie and Masino, 2001).

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Abbreviations: AIDA, (RS)-1-Aminoindan-1,5-dicarboxylic acid; AM251, N-(Piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide; CsMeSO3, caesium methanesulfonate; D-600, methoxy-verapamil; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; EPSC, excitatory postsynaptic current; fEPSP, field excitatory postsynaptic potential; GI mGluR, group I metabotropic glutamate receptor; LTP, long-term potentiation; MW, Mann–Whitney *U*-test; NEM, N-ethylmaleimide; NSF, N-ethylmaleimide-sensitive factor; PKC, protein kinase C; PKCI, protein kinase C inhibitor; QX-314, lidocaine *N*-ethyl bromide; T_{50}, time to 50% depression of the EPSC by hypoxia for the first ($T_{50}1$) and second ($T_{50}2$) hypoxic episodes; ΔT_{50}, difference between the T_{50} for the second and first hypoxic episodes ($T_{50}2 - T_{50}1$); VSP, voltage step protocol; WIN 55,212-2, (R)-(+)-[2,3-Dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone mesylate.

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Adenosine, as well as providing a tonic inhibitory influence under normoxic conditions, is released during metabolic and traumatic stress to reduce glutamate release and hyperpolarize postsynaptic neurons (Pearson et al., 2003; Dale and Frenguelli, 2009). For these reasons, the release of adenosine is widely regarded as neuroprotective (Cunha, 2005). Although the direct release of endogenous adenosine from neurons to inhibit incoming glutamatergic excitation has been proposed for some time on theoretical and empirical grounds (Brundege and Dunwiddie, 1996, 1998), only recently have observations consistent with this prediction been made (Lovatt et al., 2012).

In the present study, we show that the depression of excitatory synaptic transmission by hypoxia, a process largely dependent upon the activation of presynaptic adenosine A_1 receptors (Lipton and Robacker, 1982; Fowler, 1989; Gervitz et al., 2001; Johansson et al., 2001; Pearson et al., 2001; Arrigoni et al., 2005), can be accelerated by postsynaptic depolarisation. This process is Ca^{2+}- and protein kinase C (PKC)-dependent and can be inhibited by intracellular application of N-ethylmaleimide (NEM) and GDP-β-S, both of which inhibit membrane trafficking/fusion events. In addition, this process requires the activation of GI mGlu receptors, but not CB1 cannabinoid receptors. We propose the existence of a form of retrograde signalling, potentially involving glutamate and/or adenosine and activated during times of metabolic stress, which allows vulnerable postsynaptic neurons to reduce incoming glutamatergic excitation. As such this may represent a novel neuroprotective or retaliatory strategy in the mammalian brain.

EXPERIMENTAL PROCEDURES

Slice preparation

Sprague Dawley rats of 12–24 days of age and of either sex, were killed by cervical dislocation in accordance with Schedule 1 of the UK Government Animals (Scientific Procedures) Act 1986 and in accordance with Local Ethical Review procedures. After decapitation, the brain was rapidly removed and placed in ice-cold artificial CSF (aCSF) containing 11 mM Mg^{2+} wherein 400 μm sagittal hippocampal slices were cut with a Vibratome (IntraCel, Royston, Herts, UK) as described previously (Dale et al., 2000; Pearson et al., 2001, 2006). Slices were placed in an incubation chamber comprising a nylon mesh within a beaker of continuously circulating, oxygenated (95% O_2/5% CO_2) standard aCSF (1 mM Mg^{2+}) and kept at room temperature for at least 1 h before use, after which slices were transferred to a recording chamber and submerged in aCSF flowing at 6 ml min^{-1} at 33–34 °C. The composition of the standard aCSF solution was (in mM): NaCl 124, KCl 3, $CaCl_2$ 2, $NaHCO_3$ 26, NaH_2PO_4 1.25, D-glucose 10, $MgSO_4$ 1, pH 7.4 with 95% O_2/5% CO_2.

Whole-cell patch clamp

Recordings from CA1 pyramidal neurons were made under visual guidance with a Carl Zeiss Axioskop FS upright microscope (Carl Zeiss, Welwyn Garden City, UK). Patch electrodes (4–8 MΩ) were filled routinely with a standard intracellular solution comprising (in mM): $CsMeSO_3$ (100); HEPES (40); NaATP (2); NaGTP (0.3); $MgCl_2$ (5); glutathione (5); EGTA (0.2), and QX314 (5), which was included to block voltage-gated Na^+ channels. However, when the experiment dictated, the standard solution included one of the following: methoxy-verapamil (D-600) (150 μM), $PKC_{19–36}$ (protein kinase C inhibitor (PKCI); 10 μM), N-ethylmaleimide (NEM, 5 mM) or GDP-β-S (0.6 or 1 mM). An aCSF-filled glass stimulating electrode was placed in stratum radiatum ~50 μm from the cell body layer and 50–100 μm from the patched cell. Excitatory postsynaptic currents (EPSCs) recorded at −60 mV (in the presence of 100 μM picrotoxin) were sampled at 10 kHz, filtered at 1 kHz, and recorded with an Axopatch-1D amplifier (Molecular Devices, Sunnyvale, CA). Data acquisition was under the control of the DOS version of LTP software (www.winltp.com) (Anderson and Collingridge, 2001). EPSCs, approximately 50% of maximum response, were evoked at 15 s intervals, and four events (1 min of data) were averaged. Small voltage steps (±5 mV) were evoked before an EPSC to monitor membrane and series resistance.

The depolarising voltage step protocol (VSP) consisted of a series of 40 voltage steps each stepping from −80 to +20 mV for 3 s, with 5 s in between each step (Wyllie and Nicoll, 1994). The VSP was assigned randomly to cells and delivered ~2–5 min after the return to normoxia. EPSCs were not evoked during the VSP.

Induction of hypoxia

In all experiments, hypoxia was induced by the substitution of standard aCSF with identical aCSF pre-equilibrated with 95% N_2/5% CO_2 as described previously (Frenguelli, 1997; Dale et al., 2000). This manipulation reduced bath oxygen tension from ~80–90% saturation to <10%, as measured by a Diamond General oxygen microelectrode (IntraCel). The duration of hypoxia was fixed at 10 min, and up to two hypoxic episodes, separated by approximately 10–15 min, which varied to allow sufficient time for recovery of the EPSC, were given in any one experiment. Tissue was exposed to a drug for at least 30 min before the induction of hypoxia. Control, voltage step, and drugs experiments were interleaved throughout the study to avoid the potential for any systematic experimental bias.

Chemicals

Chemicals used in the aCSF were supplied by BDH (Lutterworth, Leics, UK). Caesium methanesulfonate ($CsMeSO_3$), HEPES, EGTA, NaATP, NaGTP, $MgCl_2$, picrotoxin, D-600, $PKC_{19–36}$, NEM, GDP-β-S and glutathione were obtained from Sigma–Aldrich (Poole, Dorset, UK). 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) and lidocaine *N*-ethyl bromide (QX-314) were supplied by RBI (Poole, Dorset, UK). N-(Piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (AM251), (RS)-1-Aminoindan-1,5-dicarboxylic acid (AIDA) and (R)-(+)-[2,3-Dihydro-5-methyl-3-(4-morpholinylmethyl) pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanon-emesylate (WIN 55,212-2) were supplied by Tocris-Cookson (Avonmouth, Bristol, UK). DPCPX was dissolved in ethanol; the final concentration of vehicle was 0.001–0.02%.

Data analysis

The effect of hypoxia on excitatory synaptic transmission was quantified in terms of the time to the 50% depression (T_{50}) of the EPSC during a 10 min period of hypoxia by fitting a Gompertz function. This value: (a) allowed objective comparisons of the effects of experimental manipulations on the depression of the EPSC by hypoxia, (b) was consistent across all experimental manipulations and (c) was independent of assumptions regarding the time-course of decay.