145P SUPPRESSION OF THE PRESYNAPTIC EFFECTS OF ADENOSINE BY METABOTROPIC GLUTAMATE RECEPTORS IN RAT HIPPOCAMPAL SLICES

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Interactions between adenosine and metabotropic glutamate receptors have been reported (de Mendonca & Ribeiro, 1997). The aim of this work is to determine whether those interactions are specific for adenosine receptors and whether they occur primarily at presynaptic or postsynaptic sites.

Hippocampal slices from male Wistar rats (150-200g) were maintained in artificial cerebrospinal fluid (ACSF) gassed with 95% O2 and 5% CO2 and containing (in mM): KH_2PO_4 2.2, KCl 2, $NaHCO_3$ 25, NaCl 115, $CaCl_2$ 2.5, $MgSO_4$ 1.2, glucose 10. Stimulation was delivered to the Schaffer collaterals and evoked field excitatory postsynaptic potentials (EPSPs) were recorded from the CA1 area. Paired-pulse inhibition is expressed as the change in the second response of a pair compared with the first response. Significance was calculated with repeated measures ANOVA followed by the Student-Newman-Keuls test.

Before application of glutamate receptor agonists, adenosine depressed the EPSP size by 59.35% ± 3.39 (n=4; $p<0.001$). Following application of the non-selective mGluR receptor agonist (1S,3R)-1-amino-cyclopentane-dicarboxylic acid (ACPD), adenosine was less effective, reducing the EPSPs by 38.7% ± 5.59 (n=4; $p<0.01$) after 20 minutes. Responses obtained 40 and 60 minutes after ACPD were still significantly reduced ($p<0.05$). The effect of baclofen was also significantly reduced after ACPD. Baclofen 2μM depressed single EPSP slope by 59.86%±2.64 ($P<0.001$; n=4) whereas after ACPD, baclofen reduced the EPSP slope by only 41.66%±.3.57 (n=4, $p<0.01$), 43.61%±1.69 ($p<0.01$) and 47.57%±3.42 ($p<0.05$) after 20, 40 and 60 minutes. A depression of adenosine responses was also produced in the paired pulse experiments. All these effects of ACPD were reproduced by the mGluR1a receptor agonist (R,S)-3,5-dihydroxyphenylglycine (DHPG). (S)-(+)-α-amino-4-carboxy-2-methylbenzene acetic acid (LY367385 100μM), an antagonist at mGLU1a receptors, prevented the depression by DHPG of adenosine sensitivity but the mGLU5 receptor antagonist 2-methyl-6-(2-phenylethenyl) pyridine (SIB 1893) had no effect. Responses to the A_1 receptor agonist N6-cyclopentyladenosine (CPA) were reduced after perfusing DHPG and adenosine deaminase and this reduction was not altered by the adenosine A_{2A} receptor selective antagonist 4-(2-[7-amino-2-(2-furyl)-1,2,4]-triazolo[2,3a] [1,3,5]triazin-5-ylamino ethyl phenol (ZM241385).

In conclusion, activation of mGluR1a metabotropic glutamate receptors is responsible for the suppression of adenosine sensitivity, which is mediated selectively via adenosine A_1 receptors, but is also able to suppress responses mediated by $GABA_B$ receptors. The paired-pulse experiments indicate that the interactions occur at presynaptic sites.

Budd, D.C. and Nicholls, D.C. (1995). *J. Neurochem.* 65, 615-621.
De Mendonca, A. and Ribeiro, J.A (1997). *Brit. J. Pharmacol.* 121, 1541-1548.

146P WINFLUOR - AN INTEGRATED SYSTEM FOR THE SIMULTANEOUS RECORDING OF CELL FLUORESCENCE IMAGES AND ELECTROPHYSIOLOGICAL SIGNALS ON A SINGLE COMPUTER SYSTEM.

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An integrated computer system for recording fluorescence images simultaneously with membrane current and voltage signals from single patch-clamped cells will be demonstrated. Combining the data acquisition within one computer in this way greatly simplifies the experimental recording and subsequent data analysis, as well as ensuring accurate synchronisation of images and analogue signals.

The system is based around a Nikon TE 300DV inverted microscope (Nikon, UK), Princeton I-PentaMAX intensified CCD camera (Roper Scientific, Trenton, NJ, USA), Optoscan computer-controlled monochromator (Cairn Research, Faversham, UK) and National Instruments (Austin, TX, USA) data acquisition and timing hardware, running on a 1.6 GHz Pentium IV computer under Microsoft Windows 2000.

Using a mechanically masked section of the I-PentaMAX camera's CCD sensor and its specialised "virtual chip" operating mode, image capture rates as high as 140 frames per second at a 160x160 pixel spatial resolution can be achieved. Up to 8 analogue signal channels can be acquired simultaneously with images at a maximum rate of 5,000 samples per second per channel. Image/signal capture at these rates can be sustained for long periods limited only by computer storage disc capacity.

The wavelength of the fluorescence excitation light can be switched within the range 300-600 nm in less than 1 ms on a frame by frame basis, with the CCD camera being shuttered during wavelength changes by a gating pulse applied to the intensifier unit attached to the camera. This permits the acquisition of the image pairs required for ratiometric calcium imaging, using dual excitation fluorophores such as fura-2, at the frame rate of the camera (70 ratios per second max.). In addition, substitution of the microscope's standard tungsten transmission light source with a digitally controlled high intensity white light emitting diode (Lumileds, San Jose, CA, USA) permits rapidly acquired transmission light images to be interspersed with fluorescence images, for applications such as cell length measurement. Six TTL digital pulses can also be generated during recording to control perfusion flow valves or apply electrical stimuli.

The WinFluor software used to control the system displays live images and analogue signal traces within the same window during an experiment. Analysis features permit the plotting of the time course of the average intensity within multiple regions of interest within the images along with selected analogue signal channels. Ion concentration time courses can also be computed from fluorescence ratio images using the standard binding equations for ratiometric fluorophores.

The WinFluor software is available free of charge to academic and non-commercial users and can be downloaded from the web site www.strath.ac.uk/Departments/PhysPharm/ses.htm.