137P ARE β1- AND β2-ADRENOCEPTOR-MEDIATED FUNCTIONAL RESPONSES DIFFERENTIALLY DESENSITIZED? A ROLE FOR PHOSPHODIESTERASE?

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Prolonged or repeated exposure of humans, animals, cells and tissues to β-adrenoceptor (βAR) agonists results in the well known phenomenon of desensitization, whereby a subsequent agonist challenge produces an attenuated response (Harden, 1983). There is, however, remarkably little agreement whether β_1- and β_2-adrenoceptors undergo desensitization equally. For example, preferential down-regulation of β1AR was reported in patients with dilated cardiomyopathy (Michel *et al.*, 1991). Selective loss of vascular (Cohen & Schenck, 1987) and cardiac (Martin & Broadley, 1994) β_1AR function compared with vascular β_2AR has been reported after chronic isoprenaline infusions. In contrast, there are reports of loss of β_2AR sensitivity and numbers equal to or greater than for β_1AR, following isoprenaline infusions (Molenaar *et al.*, 1990).

In the airways, the situation is also confusing. It has long been believed that tolerance develops to the bronchodilator effects of β-agonists, and abundant studies have shown desensitization of βAR-mediated responses of isolated trachea after prolonged incubation with isoprenaline. The bronchodilator responses to β_2AR agonists are, however, now regarded as being relatively resistant to desensitization (Barnes, 1995; Giembycz, 1996). In contrast, the non-smooth muscle β_2AR responses, including antiinflammatory actions, do appear to undergo desensitization.

Examples of *in vitro* desensitization of β_1AR-mediated functional responses will be illustrated. Selective desensitization of cardiac β_1AR-mediated responses compared with vascular and airways β_2AR-mediated responses after prolonged (4-6h) *in vitro* incubation with isoprenaline (10-6M) will be demonstrated. Factors such as correction from time-matched controls, adequate washout of the isoprenaline and the methods of plotting dose-response curves are taken into account.

Notwithstanding the observed selectivity of β_2AR desensitization and discrepancies in the literature, there is ample evidence that β_2AR are capable of desensitization and uncoupling from G-protein when assessed from adenylyl cyclase activation (Summers *et al.*, 1997).

An early study showed that human β_2AR expressed in CHW cells desensitized after only 10min incubation with isoprenaline (2 μM). The maximum shift of the concentration-response curve for isoprenaline-stimulated adenylyl cyclase was twofold (Hausdorff *et al.*, 1989). Thus, a selective desensitization at the receptor level is unlikely and alternative explanations for the apparent differences in susceptibility to desensitization of βAR-mediated responses is required. Possible reasons include differences in receptor reserve between tissues and variable rates of resensitization during washout of the agonist. Thirdly, upregulation of phosphodiesterase (PDE) during agonist exposure has been suggested to occur during agonist exposure (Giembycz, 1996). The enhanced degradation of second messenger cAMP could reduce sensitivity and if this varied between tissues, could explain different degrees of desensitization.

Results showing that inhibition of PDE by IBMX or rolipram (PDE4-selective) does not interfere with *in vitro* βAR desensitization will be presented.

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138P ADENOSINE MODULATES PHENOMENA OF SYNAPTIC PLASTICITY IN THE HIPPOCAMPUS OF OLD RATS

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Adenosine, a neuromodulator with an important role in synaptic transmission and neuronal excitability, acts through adenosine A_1 receptors to modify phenomena of synaptic plasticity, like paired-pulse facilitation (PPF), long-term potentiation (LTP), long-term depression (LTD) and depotentiation (DP) in the hippocampus of young rats (de Mendonça & Ribeiro, 1997). In the present work we investigated whether the neuromodulatory role of adenosine on phenomena of synaptic plasticity would also be present in the hippocampus of old rats.

The experiments were performed *in vitro* on hippocampal slices taken from young adult (5-6 weeks old) and aged (2 years old) male Wistar rats. Two separate sets of the Schaffer pathway were alternately stimulated and the field excitatory postsynaptic potentials (fEPSP) extracellularly recorded in the *stratum radiatum* of CA1 area. To elicit PPF, one set of the Schaffer pathway was stimulated twice with a 50 ms interpulse interval. The synaptic facilitation was quantified as the ratio between the slopes of the second and the first stimulus (S2/S1). To obtain LTD, low frequency stimulation (1 Hz, during 15 min) was applied to the pathway. LTP was induced either by a high frequency stimulation pattern (2 trains of 100 Hz, 100 stimuli, separated by 30 s) or by a θ-burst stimulation pattern (3 trains of 100 Hz, 3 stimuli, separated by 200 ms). To obtain DP, LTP was first induced by high frequency stimulation, and after 1 h DP was elicited by low frequency stimulation. LTD, LTP and DP were quantified as the % change in the average slope of the potentials taken 50-60 min after the induction protocol, in relation to the average slope of the fEPSP considered during the 10 min that preceded the protocol.

The adenosine A_1 selective receptor antagonist, 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) (50 nM), attenuated PPF in young adult rats, ratios S2/S1=1.64±0.05% (n=6) being obtained in the presence of DPCPX (50 nM) and S2/S1=1.76±0.05% (n=6, P<0.05) in the control solution, as well as in old rats, in which ratios S2/S1=1.33±0.05% were observed in the presence of DPCPX (50 nM, n=6) and S2/S1=1.55±0.10% in the control solution (n=6, P<0.05). A larger DP was observed in the presence of DPCPX (50 nM), 27.6±4.4% (n=7), than in the control pathway, 16.8±4.7% (n=7, P<0.05) in young adult rats, as well as in old rats, in which DP was 41.3±5.1% (n=6) in the presence of DPCPX (50 nM) and 16.1±2.7% (n=6, P<0.05) in its absence. High frequency stimulation-induced LTP was not different in the control solution and in DPCPX (50 nM), either in young or old rats. θ-burst stimulation induced a very small LTP (6.4±1.1%, n=5) in control conditions, and a marked LTP 53.9±4.9% (n=5, P<0.05) in the presence of DPCPX (50 nM), in young rats. In contrast, in aged rats θ-burst stimulation was sufficient to elicit a marked LTP in control conditions, 81.8±17.9% (n=7), which was not significantly further enhanced in the presence of DPCPX (50 nM), a value of 98.5±24.2% (n=7) being obtained. Endogenous adenosine thus modulates phenomena of synaptic plasticity in the hippocampus of aged rats.

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139P STRIATAL ADENOSINE RECEPTORS AND PARKINSON'S DISEASE

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Adenosine controls the release of the major striatal neurotransmitters via A_1 (glutamate, acetylcholine) and A_{2A} receptors (GABA, acetylcholine), and there is a large body of behavioural evidence showing that adenosine receptor ligands affect motor behaviour.

In order to elucidate the mechanisms involved, we have examined purine release and adenosine receptor- (primarily A_{2A}) mediated control of neurotransmitter release from synaptosomes and slices *in vitro*.

The A_{2A} receptor agonist CGS21680 increases the release of acetylcholine from synaptosomes and striatal slices in superfusion experiments. This effect was blocked by A_{2A} receptor antagonists, but not by GABA and opioid receptors antagonists, and was inhibited by N or P type calcium channel blockers. mRNAs encoding all four known adenosine receptors, and N and P type calcium channel alpha subunits were shown to be expressed in striatal cholinergic interneurons. In contrast, A_{2A} receptor agonists inhibited the release of GABA, presumably from recurrent collaterals of striatopallidal neurones which express high levels of A_{2A} receptor mRNA.

This inhibition of GABA release onto striatal output neurons reduces the inhibition of these neurons and may contribute to the observed increase in activity of striatopallidal neurons in the dopamine-depleted striatum. A hypothesis will be proposed which suggests a mechanism by which the A_{2A} receptor antagonists exert their anti-Parkinsonian effects.

140P TONIC ADENOSINE A_1 AND A_{2a} RECEPTORS ACTIVATION MODULATE FACILITATORY ACTIONS OF NEUROPEPTIDES ON TRANSISSION IN THE RAT HIPPOCAMPUS

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Tonic activation of adenosine A_{2A} receptors facilitates the action of the neuropeptide calcitonin gene-related peptide (CGRP) at motor nerve terminals (Correia-de-Sá and Ribeiro, 1994). We now investigated how manipulation of the degree of activation of adenosine A_1 and A_{2A} receptors influences the action of the neuropeptides, CGRP and vasoactive intestinal peptide (VIP), on synaptic transmission and excitability in the hippocampus.

Hippocampal slices (400μm) from the male Wistar rats (5-6 weeks old) were kept at 32°C under continuous perfusion (3 ml/min) with gassed (95% O_2/5% CO_2) Krebs solution (mM: NaCl 124, KCl 3, NaH_2PO_4 1.25, $NaHCO_3$ 26, $MgSO_4$ 1, $CACl_2$ 2, glucose 10). Stimulation (once every 10s or 15s) was through a concentric bipolar electrode placed at the Schaffer collateral/commissural fibres and recording was through a microelectrode (4 M NaCl, 3-5 MΩ resistance) placed in the CA1 *stratum radiatum* to record field excitatory postsynaptic potentials (fEPSPs) or in the CA1 *stratum pyramidale* to record population spikes (PS). Averages of 8 consecutive responses were continuously monitored. The neuropeptides were applied to each slice only once and either alone or in the presence of adenosine receptor agonists and/or antagonists, which were perfused for at least 45 min before addition of the neuropeptides.

When applied alone to hippocampal slices, CGRP (1-30 nM) was virtually devoid of effect on fEPSPs (fEPSP slope: 0.36 ± 0.07 mV/ms in the absence and 0.35 ± 0.07 mV/ms in the presence of 30 nM CGRP, n=4). However, CGRP (10-30 nM) significantly ($P<0.05$) increased (18 ± 2.6%, n=8, for 30 nM CGRP), in a concentration-dependent manner, the fEPSP slope when applied to hippocampal slices in the presence of the adenosine A_1 receptor antagonist, 1,3-dipropyl-8-cyclopenthylxanthine (DPCPX, 10 nM). The adenosine A_{2A} receptor antagonist, ZM 241385 (10 nM) prevented the enhancement of the fEPSP slope caused by CGRP (30 nM) in the presence of DPCPX (10 nM) (EPSP slope: 0.58 ± 0.03 mV/ms in the absence and 0.60 ± 0.04 mV/ms in the presence of CGRP, n=3). In the presence CGS 21680 (10 nM), an adenosine A_{2A} receptor agonist, CGRP (10-30 nM) also increased (36 ± 8.2%, $P<0.05$, n=5, for 30 nM CGRP), in a concentration-dependent manner, the slope of the fEPSPs. In the presence of 10 nM CGS 21680, VIP (10nM) increased PS amplitude by 17 ± 2.1% (n=4, $P<0.05$), an effect significantly larger ($P<0.05$) than that observed (7 ± 1.6% increase, n=4) when VIP (10 nM) was applied in the absence of CGS 21680.

It is concluded that adenosine A_{2A} receptor activation 'triggers' the facilitatory action of CGRP on synaptic transmission and enhances the facilitatory action of VIP on excitability in the hippocampus. This A_{2A} receptor mediated action might be counteracted by tonic adenosine A_1 receptor activation by endogenous adenosine.

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141P PURINE RECEPTOR INTERACTIONS AND NUCLEOTIDE EFFECTS IN RAT HIPPOCAMPUS

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We have recently studied the neuroprotective properties of adenosine analogues against kainic acid-induced neurotoxicity in the rat hippocampus. The results indicated that A_1 receptor agonists could afford protection by acting at a central site, and that A_{2A} receptor agonists could protect via a peripheral site. However, A_{2A} antagonists were also able to protect, but by a central site of action (MacGregor & Stone, 1993; MacGregor *et al.*, 1993, 1996; Jones et al. 1998a, b). One explanation of this phenomenon lies in possible interactions between A_1 and A_{2A} receptors, and we have examined this using electrophysiological techniques.

Using extracellular recordings A_1 receptor agonists suppress EPSP-spike coupling. Intracellular recordings confirm that A_1 receptor agonists increase spike threshold at concentrations which do not change membrane potential or input resistance. The application of the A_{2A} agonist CSG21680 prevents all these effects.

ATP receptors are involved in the modulation of spontaneous epileptiform bursts of action potentials, indicating a role for P2 receptors in the control of neuronal excitability (Ross *et al.*, 1998a,b). We have therefore examined the effects on evoked potentials and single neurone activity in more detail. On CA1 evoked potentials, ATP initially suppresses potential size but after its removal there is a long-lasting potentiation of the potentials which persists for over 30 minutes. On single pyramidal cells, ATP consistently depresses amino acid induced firing and produces hyperpolarisation. However, about 50% of interneurones respond with an initial depression followed by rebound excitation. This may underlie the long-lasting potentiation observed on evoked potentials.

Overall the results indicate that both P1 and P2 receptors can modify the excitability of single neurones in a manner which can explain effects on evoked potentials and the modulation of excitotoxicity.

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142P ADENOSINE MODULATES DIFFERENTIALLY THE RELEASE OF ACh AND GABA IN CULTURES ENRICHED IN AMACRINE-LIKE NEURONS

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The adenosine A_1 receptors are present in the retina of several vertebrate species, being the highest density found in the inner plexiform and ganglion-cell layers (Blazynski & Perez, 1991), where the amacrine cells are located.

In this work, we have investigated the effect of endogenous adenosine on the release of radiolabelled ACh and GABA in cultures enriched in amacrine-like neurons, isolated form 8-day-old chick embryos (White Lerghorn). We have found that the KCl-evoked release of [^{3}H]ACh was increased, up to 132.1±8.7% or 137.7±5.8% of control in the presence of 2U/ml ADA, or in the presence of 50 nM DPCPX, respectively. On the contrary, the KCl-evoked [^{3}H]GABA release was not significantly affected by ADA or DPCPX (99.4±4.2% and 105.8±3.2% of the control, respectively). The inhibition of [^{3}H]ACh release induced by 300 nM ω-CgTx GVIA (22.0±5.5%), was not significantly enhanced by A_1 receptor activation with CPA (33.1±6.3%), which suggests that the effect of adenosine A_1 receptor of ACh release was due to a selective inhibition of N-type Ca^{2+} channels. Fura-2 imaging experiments confirmed that adenosine A_1 receptors inhibits elevation in the $[Ca^{2+}]_i$ in the neurites, induced by KCl depolarization. Depolarization of retina cells with 50 mM KCl, for 1 min, also induced extracellular accumulation of adenosine, from 48.9±7.3 nmol/mg protein, in basal conditions, to 91.0±12.1 nmol/mg protein This increase was inhibited by 200 μM AOPCP, or by 10 μM NBTI, to 14.3±1.9 and to 30.4±5.2 nmol/mg prot, respectively. These results suggest that extracellular accumulation of adenosine, in response to KCl depolarization, was due to release of endogenous adenosine *per se*, and to release of nucleotides. Depolarization of cultured retina cells with 50 mM KCl, for one min, induced a small, but not significant, increase of [^{3}H]inositol phosphates accumulation ([^{3}H]IP), which was potentiated when the cells were depolarized in the presence of 2U/ml ADA, or in the presence of 50 nM DPCPX (136.9±6.8% or 136.1±11.3% of the control). On the contrary, when the depolarization was induced in the presence of 2U/ml ADA together with the CPA (100 nM), the [^{3}H]IP accumulation was decreased to basal levels (107.7±4.3% of the control).

These results show that activation of A_1 receptors inhibit accumulation of inositol phosphates. As for, the effect of adenosine on ACh release may be due to a direct inhibition of N-type Ca^{2+} channels and/or secondary to inhibition of PLC. We have observed previously that the release of ACh and the release of GABA have different sensitivities to voltage sensitive calcium channel blockers (Santos *et al.*, 1998). This differential modulation of ACh and GABA release by adenosine further supports the hypothesis that synaptic vesicles containing the two neurotransmitters may be located in different cellular sites. The differential distribution of active zones containing GABA and ACh may be of physiological significance related the direction selectivity (DS) mechanism in retina.

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P2Y receptors are heptahelical receptors linked to activation of phospholipase C, generation of inositol phosphates and release of Ca^{2+} stores. Five human P2Y (hP2Y) receptors have been cloned to date (hP2Y1,2,4,6,11) and each has a distinct pharmacological profile towards the natural agonists adenosine 5'-triphosphate (ATP), adenosine 5'-diphosphate (ADP), uridine 5'-triphosphate (UTP) and uridine 5'-diphosphate (UDP).

The hP2Y1 receptor is highly selective for ADP and ATP. It has been suggested that ATP is an hP2Y1-antagonist, but a recent study showed that ATP is in fact a partial agonist at the hP2Y1 receptor. Consequently, in conditions where there is low receptor expression or receptor desensitisation, then ATP can inhibit the action of the full agonist ADP, at the hP2Y1 receptor.

The hP2Y2 receptor is a triphosphate-preferring receptor. UTP and ATP are approximately equipotent full agonists, whilst ADP and UDP have little or no effect. This receptor corresponds to what was formerly known as the P2U receptor.

At the hP2Y4 receptor UTP is a potent, full agonist, but ATP has been reported to be a full agonist, a partial agonist or inactive. ADP and UDP have little or no effect. Recently, we showed that ATP is in fact an antagonist at hP2Y4 receptors and that its apparent agonist actions depends upon production of UTP in the bathing media, which then acts as an agonist.

The hP2Y6 receptor is a pyrimidine-preferring receptor, where UDP is more potent than UTP. ATP and ADP have little or no effect. Thus, this receptor may represent the pyrimidine receptor that was proposed 10 years ago.

The hP2Y11 receptor is highly selective for ATP over ADP, whilst UTP and UDP are inactive. The hP2Y11 receptor is unusual, as it is the only one of the five hP2Y receptor subtypes that is also linked to activation of adenylyl cyclase.

Several non-mammalian sequences have also been cloned. The chick and turkey p2y1 receptors are considered to be avian orthologues of the hP2Y1 receptor. Likewise, the chick p2y3 receptor may correspond to the mammalian P2Y6 receptor. At present, no mammalian orthologue of the Xenopus p2y8 receptor has been identified.

The hp2y5 & 7 receptors display <30% sequence homology with hP2Y receptors, but no purine or pyrimidine has been found to act as an agonist at either site. In fact, the p2y7 receptor was subsequently revealed to be the leukotriene B4 receptor. Two further sequences, hp2y9 & 10 are deposited in Genbank, but no functional studies have been published to confirm that they are indeed activated by purine and pyrimidine nucleotides.

Finally, it is likely that at least one further P2Y receptor remains to be isolated, as the receptor in platelets which is activated by ADP and coupled to platelet aggregation and inhibition of adenylyl cyclase has not been cloned.

The cloning of multiple P2Y receptor subtypes represents an enormous step forward in this field. Now, one of the main aims has to be to correlate the cloned receptors with the native P2Y receptors that mediate many of the actions of purine and pyrimidine nucleotides throughout the body.