Modulation by endogenous ATP of the light-evoked release of ACh from retinal cholinergic neurones

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The retina is an area of the central nervous system that possesses intrinsic cholinergic neurones which release acetylcholine (ACh) in response to stimulation with flickering light. Using an eye-cup preparation in anaesthetized rabbits we found that when the retina was exposed to the P₂-purinoceptor antagonist, PPADS, the light-evoked release of ACh was strikingly increased (by over 40%). In contrast, ATP reduced the light-evoked release of ACh by 20%. The inhibitory effect of ATP was not due to its catabolism to adenosine because it was not affected by the A₁-adenosine receptor antagonist, DPCPX, in combination with adenosine deaminase. The actions of both ATP and PPADS were completely blocked by strychnine. We conclude that during physiological stimulation of the retina with light, ATP is co-released with ACh and partially inhibits ACh release by activating (with ACh) an inhibitory glycinergic feedback loop.

Keywords: ATP release; retina; cholinergic neurones; ACh release; purinergic transmission; P₂-purinoceptors

Introduction Adenosine is co-located with acetylcholine (ACh) in displaced amacrine cells in the retina (Blazynski, 1989) and since adenosine in the retina is rapidly phosphorylated to ATP (Perez *et al.*, 1986), it is highly probable that ACh and ATP are co-localized in these central neurones. In the present study we provide evidence that co-released ATP modulates the light-evoked release of ACh from cholinergic neurones by stimulating (together with ACh) a glycinergic inhibitory feedback loop.

Methods The methods have been described previously (Cunningham & Neal, 1985). Briefly, rabbits were anaesthetized with urethane (1.5g kg⁻¹, i.p.) and an eye-cup prepared which was filled, for 30 min, with Krebs Ringer bicarbonate containing [³H]-Ch (0.5 µM). After washing with fresh medium containing physostigmine (30 µM), a syringe was used to place 0.5 ml of medium in the eye-cup, and this was replaced at 5 min intervals. The total radioactivity in the resulting samples was measured by liquid scintillation counting. The retina was stimulated for 5 min periods by flickering light (3Hz). [³H]-ACh represented 95–100% of the increase in total radioactivity evoked by light flashes (Cunningham & Neal, 1985).

Pyridoxalphosphate -6- azophenyl -2', 4'- disulphonic acid (PPADS) was obtained from Cookson Chemicals.

Results When the retina was stimulated with flickering light in the absence of drugs (controls) the release of ACh was increased 2.1 ± 0.3 fold (mean $\pm$ s.e.mean, $n = 24$) compared with the spontaneous resting release. The effect of exposing the retina to medium containing ATP (1 mM) is illustrated in Figure 1a. On average ATP reduced the light-evoked release of ACh by $19.5 \pm 3.5\%$ ($n = 4$) (Figure 1b). The inhibitory effect of ATP was not due to its catabolism to adenosine because it produced the same effect on ACh release in the presence of the A₁-adenosine receptor antagonist, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) (0.1 µM) in combination with adenosine deaminase (1 u ml⁻¹) (Figure 1b). Either of these agents alone completely blocks the effects of adenosine on retinal ACh release (Blazynski *et al.*, 1992). In contrast, the P₂-purinoceptor antagonist, PPADS, (Lambrecht *et al.*, 1992) (100 µM) completely blocked the effects of ATP on the light-evoked release of ACh (Figure 1b). When

Figure 1 (a) Typical rabbit eye-cup experiment illustrating the effect of ATP (1 mM) (hatched horizontal bar) and PPADS (100 µM) (solid horizontal bar) on the light-evoked release of ACh. Each column is a 5 min collection period. Hatched columns indicate stimulation with flickering light (3Hz). Figures in parentheses indicate stimulated release/resting release and are taken as a measure of 'light-evoked release of ACh'. (b) Summary of effects of drugs on the light-evoked release of ACh. The results are expressed as (light-evoked release in presence of drug/control light-evoked release) $\times 100$: (A) control; (B) ATP 1 mM; (C) ATP + DPCPX 0.1 µM + adenosine deaminase 1 u ml⁻¹; (D) ATP + strychnine 20 µM; (E) ATP + PPADS 100 µM; (F) PPADS 100 µM; (G) PPADS + strychnine 20 µM. Each column is the mean $\pm$ s.e.mean of 3–6 separate rabbit experiments. *Indicates significantly different from control $P < 0.01$, Student's *t*-test. For abbreviations, see text.

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the retina was exposed to PPADS alone, there was no significant effect on the spontaneous resting release of ACh (mean release of 2 collection periods in presence or absence of drug: mean release of preceding 2 collection periods = 0.64 ± 0.04 and 0.54 ± 0.03 respectively, $n = 5$). However, PPADS strikingly increased the light-evoked release of ACh (Figure 1a) and on average, PPADS increased the light-evoked release of ACh by $45.1 \pm 5.6\%$ ($n = 6$) (Figure 1b) suggesting that endogenous ATP modulates the light-evoked release of ACh.

PPADS ($100 \mu\text{M}$) did not affect ACh release by activating GABA_B receptors (Figure 2) because CGP 35348 (3-aminopropyl(diethoxymethyl) phosphinic acid) (0.5 mM) did not alter the enhancing effect of PPADS on the light-evoked release of ACh (light-evoked release as % of control: PPADS alone $142 \pm 8.2\%$ ($n = 4$); PPADS + CGP 35348 $148.1 \pm 8.2\%$ ($n = 4$)). Also PPADS did not antagonize the inhibitory effect of muscarine ($10 \mu\text{M}$) on the light-evoked release of ACh from the retina (light-evoked release as % of control: muscarine $61.3 \pm 6.3\%$, muscarine + PPADS $60 \pm 7.1\%$ ($n = 4$)), confirming its lack of antimuscarinic activity (Lambrecht *et al.*, 1992).

We tested the possibility that ATP co-released with ACh might be stimulating the release of glycine from inhibitory neurones by examining the effect of strychnine on the actions of ATP and PPADS. Both drugs were completely blocked by strychnine (Figure 1b) at a concentration ($20 \mu\text{M}$) that itself

did not affect ACh release and that specifically blocks glycine receptors in the retina (Neal & Cunningham, 1994).

Discussion The present study shows that ATP decreases and PPADS increases the light-evoked release of ACh from the retina. The finding that PPADS enhances the light-evoked release of ACh suggests that endogenous ATP modulates ACh release in the rabbit retina. Our concentration of PPADS was higher than that usually reported but high concentrations of drugs are sometimes required in the eye-cup preparation, perhaps because of the glial barrier formed by the end-feet of the Müller cells (see Neal & Cunningham, 1994). Since we ruled out effects of PPADS at muscarinic receptors and GABA_B receptors that might have enhanced the light-evoked release of ACh (Figure 2), the effects of PPADS on ACh release seem most likely to be due to antagonism of ATP at P_{2X}-purinoceptors.

We do not yet know the cellular location of the P₂-purinoceptors in the retina. However, the actions of both ATP and PPADS were blocked by strychnine suggesting that P₂-purinoceptors are present on glycinergic amacrine cells. Our experiments suggest that when the retina is stimulated physiologically with flickering light, ACh and ATP are released and together stimulate glycinergic neurones which provide inhibitory feedback to the cholinergic amacrine (Figure 2).

Figure 2 Diagram showing possible sites of action of ATP and PPADS in the retina. The depolarizing (ON) bipolar cell (DBPC) releases glutamate which activates kainate receptors (Cunningham & Neal, 1985) on the cholinergic amacrine cells (ACh). ACh and ATP from these cells activates muscarinic (M) and purinoceptors (P₂) respectively on the glycinergic amacrine cells (shaded) which in turn releases glycine (Gly) onto the cholinergic amacrine cells. Activation of strychnine-sensitive glycine receptors (G) partially inhibits ACh release. Drugs that interrupt the feedback loop (Neal & Cunningham, 1994) (atropine, baclofen, strychnine, PPADS?) enhance the light-evoked release of ACh, whilst drugs that stimulate the glycinergic neurones (muscarine, ATP) reduce the evoked-ACh release. The present results are most simply explained if ATP is co-released with ACh from cholinergic neurones but we cannot rule out the possibility that ATP is released from other neurones (dashed).

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