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Actions of ADP, but not ATP, on cytosolic free Ca^{2+} in single rat hepatocytes mimicked by 2-methylthioATP

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1 Aequorin-injected, single rat hepatocytes generate series of repetitive transients in cytosolic free calcium concentration ($[Ca^{2+}]_i$) when stimulated with agonists acting through the phosphoinositide signalling pathway, including ADP and ATP. We have previously described differences in the $[Ca^{2+}]_i$ responses of aequorin-injected hepatocytes to ADP and ATP.

2 The effects of the phosphorothioate analogue of ATP, 2-methylthioATP (2-meSATP), have been examined on single rat hepatocytes. This analogue is believed to be the most potent agonist at the P_{2Y1} subclass of purinoceptor.

3 The $[Ca^{2+}]_i$ transients induced by 2-meSATP were indistinguishable from those induced by ADP, and in contrast to those induced by ATP.

4 At high concentrations, 2-meSATP and ADP both induced transients at high frequency. In contrast, hepatocytes responded to high concentrations of ATP with an initial rapid rise in $[Ca^{2+}]_i$, followed by a slowly decaying fall.

5 The modulatory effects of elevated intracellular cyclic AMP concentration were the same on both 2-meSATP- and ADP-induced $[Ca^{2+}]_i$ transients; the peak height and frequency of transients were enhanced. ATP-induced transients, however, underwent either an increase in duration or conversion into a sustained rise in $[Ca^{2+}]_i$.

6 ATP-induced transients were specifically potentiated by the co-addition of α,β-methyleneATP, whereas 2-meSATP- and ADP-induced transients were unaffected by this treatment.

7 We conclude that 2-meSATP acts at the same receptor as ADP on rat hepatocytes, and that this is distinct from the receptor(s) mediating the effects of ATP.

Keywords: P_{2Y} purinoceptors; cytosolic free calcium concentration; rat hepatocytes; calcium transients; 2-methylthioATP; ADP; ATP

Introduction

According to the classification system for purinoceptors devised by Burnstock (1978), ADP and ATP act at the same extracellular site to bring about their physiological response. Our understanding of P_2-purinoceptors has been hampered by a lack of specific antagonists, necessitating a classification based primarily on rank order of potency series of a range of ATP analogues (Burnstock & Kennedy, 1985). The classification system has recently been expanded and the P_{2X} and P_{2Y} subclasses themselves sub-divided (Abbracchio & Burnstock, 1994), following the first reports of cloning and expression of ATP receptors (Webb *et al.*, 1993; Lustig *et al.*, 1993; Barnard *et al.*, 1994a,b). However, the classification of these cloned receptors still depends on the potency series of ATP analogues, or of the pyrimidine nucleotide, UTP. The phosphorothioate analogue of ATP, 2-methylthioATP, has played a central role in the development of the purinoceptor classification system and is regarded as the most potent analogue at P_{2Y} purinoceptors (new designation P_{2Y1}), whilst being ineffective at P_{2U} purinoceptors (new designation P_{2Y2}; Cusack, 1993; Abbracchio & Burnstock, 1994; Fredholm *et al.*, 1994).

In common with many other cell types (Berridge, 1990), single rat hepatocytes generate repetitive transients in cytosolic free calcium concentration ($[Ca^{2+}]_i$) when stimulated with agonists acting through the phosphoinositide signalling pathway (Woods *et al.*, 1986), including ADP and ATP (Cobbold *et al.*, 1988). The duration of the $[Ca^{2+}]_i$ transients has been shown to be dependent on the receptor species being activated, so that transients of very different duration can be induced in the same individual hepatocyte by different agonists (Woods *et al.*, 1987). Increasing the agonist concentration increases the frequency of transients without altering the duration of individual transients (Woods *et al.*, 1986). Activation of a given receptor species evokes transients of consistent duration within the same cell and also between cells (Woods *et al.*, 1987). The receptor believed to mediate the effects of both ADP and ATP in rat hepatocytes was characterized as belonging to the P_{2Y}-subclass of purinoceptor (Keppens & De Wulf, 1986) as originally defined by Burnstock & Kennedy (1985). Activation of a single receptor species would then be predicted to yield $[Ca^{2+}]_i$ transients of consistent duration in response to either nucleotide. However, ADP and ATP induce $[Ca^{2+}]_i$ transients with very different durations in the majority of hepatocytes (Dixon *et al.*, 1990). Thus, ADP consistently produces transients of short duration (approx. 9–10 s). In contrast, ATP can elicit three different types of response in hepatocytes. One group of hepatocytes respond to ATP with transients of short duration, indistinguishable from those induced by ADP. A second group produce transients of much longer duration (up to 2 min), whereas in the final group, ATP induces transients of variable duration within a single response (Dixon *et al.*, 1990). We have previously reported additional agonist-specific differences between ADP and ATP, in the effects of high agonist concentrations (Cobbold *et al.*, 1988), and in the modulatory effects of elevated adenosine 3′5′-cyclic monophosphate (cyclic AMP) levels (Green *et al.*, 1994) and co-addition of α,β-MeATP (Dixon *et al.*, 1993). We have argued that these differences in the oscillatory responses of single hepatocytes to ADP and ATP are inconsistent with their effects being mediated by a single receptor (Dixon *et al.*, 1990; 1993; Green *et al.*, 1994). Indeed, we have proposed that the receptor mediating the response to ADP is distinct from the receptor(s) mediating the effects of ATP (Green *et al.*, 1994).

Here we have examined the effects of 2-meSATP on $[Ca^{2+}]_i$ in single aequorin-injected rat hepatocytes. By exploiting the

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differences we have previously reported in the $[Ca^{2+}]_i$ responses of hepatocytes to ADP and ATP, we demonstrate that, rather than acting as an analogue of ATP, 2-meSATP in fact mimics precisely the actions of ADP.

Methods

Hepatocyte preparation

Single hepatocytes were isolated from fed, male Wistar-strain rats (150–250 g) by collagenase perfusion as described previously (Woods *et al.*, 1987). Briefly, the rat was killed by cervical disolocation and the hepatic portal vein was cannulated During an initial Ca^{2+}-free perfusion, the liver was dissected out of the animal. The liver was subsequently perfused on a re-circulating basis for 20 min with collagenase (0.04% w/v; Boehringer) and Ca^{2+} (3.8 mM). The perfusion rate was 30 ml min^{-1} throughout. The cells were harvested and incubated at 37°C at low density (approx. 10^3 cells ml^{-1}) in 2% type IX agarose (Sigma) in William's medium E (WME; Gibco). Single hepatocytes were transferred to 0.1 mm path length microslides containing 1% type VII agarose (Sigma) which was subsequently gelled at 4°C for 2 min. The cells were then held at 37°C under a layer of liquid paraffin.

Aequorin preparation

An exhaustive description of the aequorin technique is provided in Cobbold & Lee (1991). A stock solution of aequorin was prepared by dissolving 1 mg aequorin in 7 μl of Ca^{2+}-free buffer (10 mM EDTA, 10 mM PIPES, pH 7.0). Small aliquots (approx. 200 nl) were dialysed on a micro-scale against injection buffer (150 mM KCl, 1 mM PIPES, 100 μM EDTA, 25 μM EGTA), to reduce the concentrations of KCl and EDTA. The dialysed aequorin was held as a droplet under liquid paraffin.

Microinjection procedure and data acquisition

Freshly-pulled pipettes were filled with aequorin solution by simply dipping the tip for a few seconds in the droplet of aequorin solution. Individual hepatocytes were injected with aequorin solution to approx. 0.5% of the cell volume, and transferred in the microslide to a perfusable cup held at 37°C, positioned under a cooled, low-noise photomultiplier, and superfused with WME, to which agonists were added. Photon counts were sampled every 50 ms by computer. At the end of an experiment, the total aequorin content of each cell was determined by discharging the aequorin by lysing the cell. The data were normalized retrospectively by computer, by calculating the photon counts per second divided by the total counts remaining. *In vitro* calibration data of the aequorin signal in terms of $[Ca^{2+}]_i$ were calculated by determining the rate of consumption of aequorin in Ca^{2+}-EGTA buffers mimicking the intracellular milieu, with $[Ca^{2+}]$ ranging from 10^{-8} M to 10^{-5} M, and 1 mM free Mg^{2+} (Cobbold & Rink, 1987). The computed fractional rate of aequorin consumption could then be plotted as $[Ca^{2+}]_i$ using exponential smoothing with time constants: for resting $[Ca^{2+}]_i$, 12 s; for transients, 1 s.

Materials

Aequorin was provided by Prof. O. Shimomura (Marine Biological Laboratory, Woods Hole, MA 02543, U.S.A.). ADP, ATP and adenosine 5'-[α,β-methylene]-triphosphate were obtained from Sigma, and 2-methylthioATP from ICN Biomedicals. Intracellular cyclic AMP levels were raised by direct activation of adenylate cyclase with the chemically modified forskolin, 7β-desacetyl-7β-[-γ-(N-methylpiperazino)butyryl] forskolin (L858051; Calbiochem).

Results

Single aequorin-injected hepatocytes responded to extracellular ADP and ATP in the concentration range 0.6–5 μM, with series of repetitive transients in $[Ca^{2+}]_i$, as previously observed (Dixon *et al.*, 1990).

Characterization of $[Ca^{2+}]_i$ transients induced by 2-meSATP

We have studied the effects of 2-meSATP on single aequorin-injected hepatocytes: 32/32 cells responded to 2-meSATP with transients of short duration indistinguishable from those induced by ADP. The duration of 2-meSATP-induced transients was consistent both within the same hepatocyte, and also between hepatocytes; the cell-to-cell variability in the pattern of transients observed for ATP was not seen. Figure 1 depicts the $[Ca^{2+}]_i$ transients induced by 2-meSATP and allows a comparison with ADP- and ATP-induced transients within the same hepatocyte. In this cell the short duration transients generated in response to 2-meSATP were in marked contrast to the long duration transients induced by ATP. The differences observed were not due to the changing response of the cell as the subsequent addition of ATP to the cell, again resulted in the generation of transients of long duration (not shown). Hepatocytes were more sensitive to 2-meSATP than ADP as illustrated in Figure 1; transients were recorded at comparable frequencies in response to 2.5 μM ADP and 1 μM 2-meSATP. The threshold for transient generation by 2-meSATP was between 0.1–0.5 μM, about 0.5 μM lower than for ADP in the same cell.

Extracellular metabolism of 2-meSATP is unlikely to account for the observed differences between the effects of 2-meSATP and ATP. In the present studies, the single hepatocyte is held in isolation from all other cells and is constantly superfused with medium, thereby providing a continuous supply of fresh agonist and simultaneously removing any breakdown products. In addition, we have previously argued that hydrolysis in the superfusate is not significant as inclusion of an ATP-regenerating system in the superfusate did not affect the hepatocyte response to ATP (Dixon *et al.*, 1990).

Effects of high concentrations of 2-meSATP

We have previously shown that hepatocytes respond to high ATP concentrations (above a threshold of approx. 5–10 μM) with an initial rapid rise in $[Ca^{2+}]_i$ peaking at over 1 μM. This is followed by a slowly-decaying fall, returning to the resting

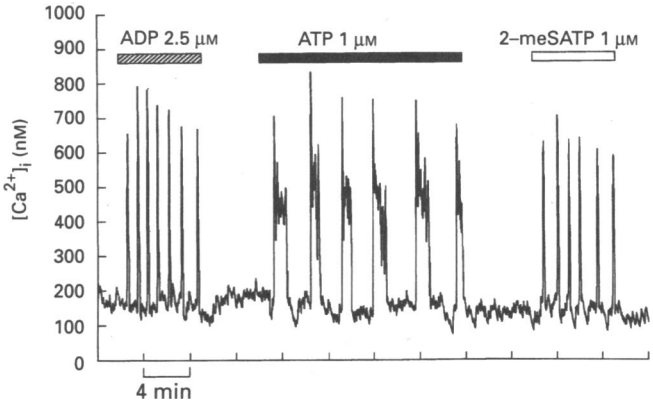

Figure 1 $[Ca^{2+}]_i$ transients induced by 2-methylthioATP (2-meSATP) resemble those induced by ADP, and not those induced by ATP. A single aequorin-injected hepatocyte responsed to 2.5 μM ADP with $[Ca^{2+}]_i$ transients of short duration. Subsequently, 1 μM ATP induced transients of long duration. Stimulating the hepatocyte with 1 μM 2-meSATP evoked transients of short duration, similar to those induced by ADP.

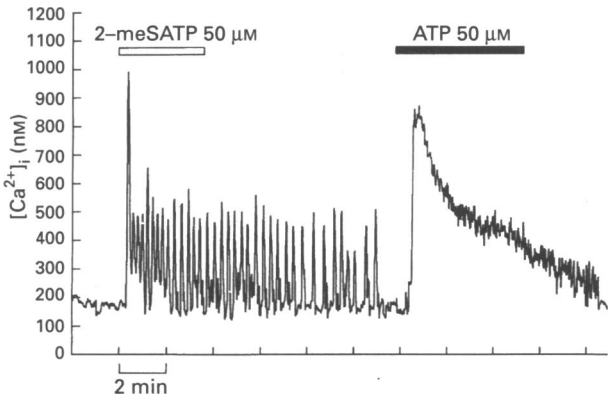

Figure 2 Differences in the $[Ca^{2+}]_i$ response of a single rat hepatocyte to high concentrations of ATP and 2-meSATP. A single aequorin-injected hepatocyte exposed to 50 μM 2-meSATP generated transients at high frequency, which were initially superimposed upon an elevated inter-transient $[Ca^{2+}]_i$. When the same cell was stimulated with 50 μM ATP, it responded with a rise in $[Ca^{2+}]_i$ followed by a slow fall in $[Ca^{2+}]_i$ back to the resting level.

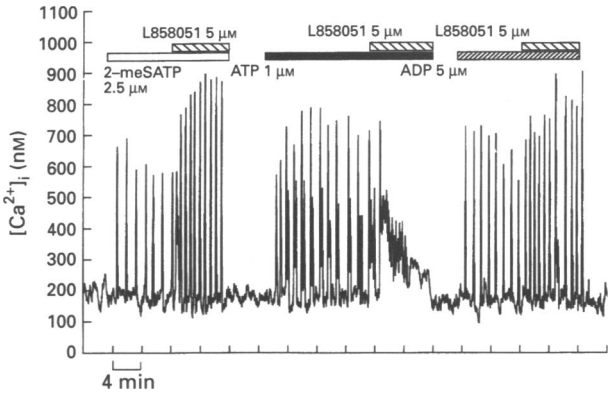

Figure 3 Effects of elevated intracellular cyclic AMP concentration on transients induced by 2-meSATP, ADP and ATP. A single aequorin-injected hepatocyte responded to 2.5 μM 2-meSATP with short duration transients. Elevation of intracellular cyclic AMP levels by application of L858051 resulted in an increase in the peak height and frequency of transients. ADP-induced transients in the same cell were similarly affected by this treatment. In contrast, application of L858051 in combination with ATP resulted in a sustained rise in $[Ca^{2+}]_i$.

$[Ca^{2+}]_i$ in 15–20 min. In contrast, similar high concentrations of ADP induce transients at high frequency, although these are initially superimposed on an elevated inter-transient $[Ca^{2+}]_i$ (Cobbold *et al.*, 1988). We show here that in 5/5 hepatocytes the response to a high concentration of 2-meSATP (50 μM) was similar to that induced by ADP, with the continued production of transients, and in contrast to the sustained rise in $[Ca^{2+}]_i$ induced by this concentration of ATP. This difference is illustrated clearly in Figure 2, which depicts the effect of 50 μM ATP and 50 μM 2-meSATP in the same hepatocyte.

Modulatory effects of elevated cyclic AMP concentration on $[Ca^{2+}]_i$ transients induced by 2-meSATP

Elevating intracellular cyclic AMP levels has different modulatory effects on ATP- and ADP-induced $[Ca^{2+}]_i$ transients (Green *et al.*, 1994). Thus, the peak height and frequency of transients induced by ADP are enhanced by experimental elevation of intracellular cyclic AMP. In contrast, ATP-induced transients undergo either an increase in duration of individual transients or conversion into a sustained rise in $[Ca^{2+}]_i$ (Green *et al.*, 1994). In the present study, intracellular cyclic AMP levels were elevated by direct activation of adenylate cyclase by co-application of 5 μM L858051, a chemically-modified forskolin derivative. The modulatory effects of L858051 on 2-meSATP-induced transients were studied in 4 hepatocytes. In 3/4 cells, elevated intracellular cyclic AMP concentrations stimulated an increase in the frequency and peak height of 2-meSATP-induced transients, with no effect on the duration of individual transients. (The final cell was unaffected by the co-addition of L858051.) The similarity of the modulatory effects of elevated cyclic AMP concentration of 2-meSATP- and ADP-induced transients within the same cell, is illustrated in Figure 3, which additionally describes the contrasting modulation of ATP-induced transients.

Effect of α,β-MeATP on 2-meSATP-induced $[Ca^{2+}]_i$ transients

We have previously reported that ATP-induced $[Ca^{2+}]_i$ transients were specifically potentiated by the co-addition of α,β-MeATP and that ADP-induced transients were unaffected by this treatment (Dixon *et al.*, 1993). We show here that the co-addition of 25 μM α,β-MeATP to 8/8 hepatocytes producing transients in response to 2-meSATP did not affect the cellular response. The effect of co-addition of 25 μM α,β-MeATP on ATP-induced transients was tested in 5 of these 8 hepatocytes.

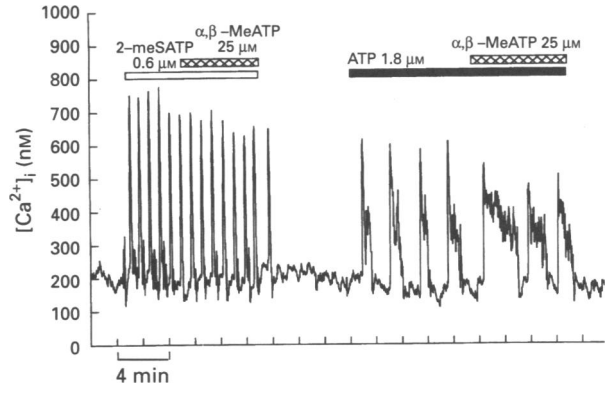

Figure 4 Effect of co-addition of α,β-MeATP on $[Ca^{2+}]_i$ transients induced by 2-meSATP and ATP. A single aequorin-injected hepatocyte responded to 0.6 μM 2-meSATP with $[Ca^{2+}]_i$ transients of short duration, which were unaffected by the addition of 25 μM α,β-MeATP. The same hepatocyte was subsequently stimulated with 1.8 μM ATP. Co-addition of 25 μM α,β-MeATP prolonged the duration of individual ATP-induced $[Ca^{2+}]_i$ transients.

In all 5 cells ATP-induced transients were potentiated by this treatment; discrete transients were converted into a sustained rise in $[Ca^{2+}]_i$ (2/5 hepatocytes) or the duration of individual transients was prolonged (3/5 hepatocytes; Figure 4).

Discussion

Single cell studies yield much information about the physiological, second-by-second consequences of activation of receptors, events that are masked in studies of cell populations. In addition, problems associated with agonist hydrolysis are avoided. Thus, single aequorin-injected hepatocytes reveal differences in the duration of $[Ca^{2+}]_i$ transients induced by ADP and ATP (Dixon *et al.*, 1990). We have previously shown additional agonist-specific differences between ADP and ATP, in the effects of high agonist concentrations (Cobbold *et al.*, 1988), and in the modulatory effects of elevated cyclic AMP levels (Green *et al.*, 1994) and co-addition of α,β-MeATP (Dixon *et al.*, 1993). These differences have been exploited here to characterize the hepatocyte response to the nucleotide 2-meSATP, which has been used extensively as an ATP analogue, and is widely regarded as the most potent agonist at the

P_{2Y}-purinoceptor (P_{2Y1}; Cusack, 1993; Fredholm *et al.*, 1994; Abbracchio & Burnstock, 1994). We demonstrate here that the effects of 2-meSATP closely resemble those of ADP, and are in contrast to the effects of ATP. Thus, as outlined in the Introduction, ATP induces transients which show variability in their duration between cells (Dixon *et al.*, 1990); no such cell-to-cell differences were observed in the response of hepatocytes to 2-meSATP which, like ADP, induced transients of consistent duration, both within the same cell, and also between hepatocytes. In addition, the transients induced by both 2-meSATP and ADP are modulated similarly by the addition of both forskolin, to increase intracellular cyclic AMP levels, and the methylated derivative of ATP, α,β-MeATP. These modulatory effects are in contrast to the effects on ATP-induced transients. The possible mechanism underlying the receptor-specific regulation of the hepatocyte Ca^{2+} oscillator by these two modulatory agonists have been discussed previously (Dixon *et al.*, 1993; Green *et al.*, 1994). Importantly for the present study, the effects of the modulatory agonist were the same for transients induced by both 2-meSATP and ADP.

In many cells in which 2-meSATP acts as a potent agonist, the maximum response to this nucleotide is lower than that to ATP. Thus, the maximum $[Ca^{2+}]_i$ achieved by stimulation with 2-meSATP is lower than that with ATP in populations of human umbilical vein cells (Carter *et al.*, 1988), submandibular salivary cell line, ST_{885} cells (Gibb *et al.*, 1994) and Chinese hamster cultured ovary cells (Iredale & Hill, 1993). Similarly, Ca^{2+}-dependent processes including prostacyclin production in pig aortic endothelial cells (Needham *et al.*, 1987), relaxation of pre-contracted rat aorta (Dainty *et al.*, 1990; O'Connor *et al.*, 1991) and bovine aorta (Wilkinson *et al.*, 1994), and contraction of rat colon (Bailey & Hourani, 1990) demonstrate lower maximum responses to 2-meSATP, compared with ATP. When exposed to high concentrations of ATP, hepatocytes respond with a sustained rise in $[Ca^{2+}]_i$, in contrast to the high frequency transients induced by high concentrations of 2-meSATP. When measured in a population of cells the high frequency transients would translate into a lower maximum response, compared with a sustained rise in $[Ca^{2+}]_i$. This difference in the effects of 2-meSATP and ATP at high concentrations in single rat hepatocytes is therefore in keeping with results from population studies.

Keppens & De Wulf (1991) reported that 2-meSATP is the most potent glycogenolytic ATP analogue in rat hepatocytes despite, even at high concentrations, inducing only very small increases in Ins(1,4,5)P_3 formation, compared with ATP. This may underlie the continued production of transients in response to high concentrations of 2-meSATP demonstrated here; the Ins(1,4,5)P_3 levels required to achieve a sustained rise in $[Ca^{2+}]_i$ are never reached. Significantly, Keppens *et al.* (1993) demonstrated that the ADP analogue, ADPβS, similarly induced only small increases in Ins(1,4,5)P_3 levels; high concentrations of ADP, like 2-meSATP, give rise to transients at high frequency, not a sustained rise in $[Ca^{2+}]_i$ (Cobbold *et al.*, 1988).

Qualitative differences in the effects of 2-meSATP and ATP have prompted many investigators to conclude that 2-meSATP and ATP are acting at distinct receptors in their systems (Dainty *et al.*, 1990; Keppens & De Wulf, 1991; O'Connor *et al.*, 1991; Iredale & Hill, 1993; Gibb *et al.*, 1994; Murgo *et al.*, 1994; Vigne *et al.*, 1994). This conclusion has been supported in some studies by additivity and desensitization experiments. Thus, additive actions of maximally effective concentrations of 2-meSATP and ATP have been observed in rat brain capillary endothelial cells (BCEC, Vigne *et al.*, 1994) and human megakaryocytic Dami cells (Murgo *et al.*, 1994). In addition, BCEC desensitized by 2-meSATP remain responsive to ATP (Vigne *et al.*, 1994). In keeping with these findings, the results from the present study indicate that 2-meSATP acts at a receptor on rat hepatocytes which is distinct from the ATP receptor(s). However, we extend this to propose that the receptor through which 2-meSATP mediates its effects is in fact an ADP receptor. This proposal is consistent with results from additivity experiments which reveal that the effects of 2-meSATP and ADP are non-additive in brain capillary endothelial cells (Vigne *et al.*, 1994) and Dami cells (Murgo *et al.*, 1994). ADP-desensitized or 2-meSATP-desensitized BCEC no longer respond to either 2-meSATP or ADP, respectively (Vigne *et al.*, 1994). In many of the systems in which a lower maximum response has been recorded for 2-meSATP compared with ATP, a similar lower maximum response has been recorded in response to ADP, or its analogue ADPβS (Needham *et al.*, 1987; Carter *et al.*, 1988; Dainty *et al.*, 1990; O'Connor *et al.*, 1991; Wilkinson *et al.*, 1994), indicating that the same receptor could be involved in mediating their effects. In addition, in many systems where 2-meSATP is either a poor agonist or inactive, ADP, or ADPβS, have a similar profile (Brown *et al.*, 1991; Iredale *et al.*, 1992; Murrin & Boarder, 1992; Raha *et al.*, 1993; Merten *et al.*, 1993).

In rat hepatocytes, 2-meSATP and ADPβS have similar effects; both stimulate only small increases in Ins(1,4,5)P_3 levels, and their glycogenolytic effects are readily abolished by phorbol esters (Keppens & De Wulf, 1991; Keppens *et al.*, 1993). However, based on the observation that 2-meSATP, unlike ADPβS, failed to counteract the rise in cyclic AMP achieved by glucagon, Keppens (1993) determined that these nucleotides were not acting at the same receptor. Many recent reports have demonstrated that single G-protein-linked receptor species can be linked to multiple effector systems (Milligan, 1993). Furthermore, one recent study demonstrated that occupation of a single cloned *Drosophila* receptor by either octopamine or tyramine differentially coupled it to inhibition of forskolin-stimulated increases in cyclic AMP levels and elevation of $[Ca^{2+}]_i$ (Robb *et al.*, 1994). It is conceivable therefore, that 2-meSATP and ADPβS share the same receptor on rat hepatocytes which is coupled to increases in $[Ca^{2+}]_i$ when occupied by either agonist, but additionally to the inhibition of glucagon-stimulated increases in cyclic AMP levels only when ADPβS is the agonist.

Whilst the biological actions of extracellular ATP have received much attention, ADP, by contrast, remains neglected, and has received little consideration independently of ATP as an extracellular agonist. This is curious when the sources of ADP, which give rise to sufficient concentrations to warrant its recognition as a physiological agonist, are well documented (Gordon, 1986; Dubyak & El-Moatassim, 1993). Apart from the platelet P_{2T} purinoceptor (P_{2Y3}), at which ADP is the sole agonist (Gordon, 1986; Dubyak & El-Moatassim, 1993), the possibility of ADP-specific receptors has not been widely entertained. Recently, however, examples of ADP-specific receptors have been reported. The presence of P_{2T} purinoceptors, whose distribution was previously believed to be restricted to platelets, has been demonstrated on K562 leukaemia cells (Murgo & Sistare, 1992) and human megakaryocytic Dami cells (Murgo *et al.*, 1994). Importantly, 2-meSATP was equipotent with ADP in both cell types (Murgo *et al.*, 1994). An ADP-specific receptor has been reported in rat osteoblastic cells (Reimer & Dixon, 1992). Palea *et al.* (1994) demonstrated the presence of a purinoceptor on human isolated urinary bladder detrusor muscle, which was preferentially activated by ADPβS; 2-meSATP was also an agonist for this receptor. A P_{2Y} purinoceptor (P_{2Y3}) that shows a preference for ADP over ATP has recently been cloned from chick brain (Barnard *et al.*, 1994a,b). The mammalian homologue has been isolated from rat brain, and the mRNA encoding this brain-derived recombinant receptor was reported to be present in some peripheral tissues, although whether these included the liver was not specified (Barnard *et al.*, 1994a,b); the effect of 2-meSATP on this cloned receptor was not reported.

2-meSATP has played an important, and central role in the development of purinoceptor classification. The present work, however, indicates that 2-meSATP and ADP activate a single receptor on rat hepatocytes, which is distinct from the receptor(s) mediating the effects of ATP. Murgo *et al.* (1994) concluded that Dami cells express two purinoceptors, one mediating the effects of UTP and ATP (a P_{2U} purinoceptor),

and the other mediating the effects of ADP and 2-meSATP (a P_{2T} purinoceptor). Since Keppens *et al.* (1992) determined that the effects of UTP and ATP were mediated by a single receptor on rat hepatocytes, it could be argued that hepatocytes, like Dami cells, express a combination of P_{2U} (P_{2Y2}) and P_{2T} (P_{2Y3}) purinoceptors. It is probable that many cells express heterogenous populations of receptors for ADP and ATP, and this could contribute, at least in part, to the great diversity of rank order of potency series observed. In conclusion, we believe that 2-meSATP has been used inappropriately as an ATP analogue, and as a consequence ADP-specific receptors remain unrecognized.

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