Cytosolic free Ca\(^{2+}\) oscillations induced by diadenosine 5',5''-P\(^1\),P\(^3\)-triphosphate and diadenosine 5',5''-P\(^1\),P\(^4\)-tetraphosphate in single rat hepatocytes are indistinguishable from those induced by ADP and ATP respectively

Anne K. GREEN,* Peter H. COBBOLD and C. Jane DIXON
Department of Human Anatomy and Cell Biology, University of Liverpool, Liverpool. L69 3BX, U.K.

Diadenosine 5',5''-P\(^1\),P\(^3\)-triphosphate (Ap\(^5\)A) and diadenosine 5',5''-P\(^1\),P\(^4\)-tetraphosphate (Ap\(^4\)A) induce distinctive patterns of [Ca\(^{2+}\)]\(_\text{cyt}\) oscillations in single rat hepatocytes. We show here that [Ca\(^{2+}\)]\(_\text{cyt}\), oscillations induced by Ap\(^5\)A and ADP are indistinguishable and that [Ca\(^{2+}\)]\(_\text{cyt}\), oscillations induced by Ap\(^4\)A closely resemble those induced by ATP. These similarities embrace the following: (1) ADP and Ap\(^5\)A invariably induce [Ca\(^{2+}\)]\(_\text{cyt}\) transients of short duration (approx. 9 s). Ap\(^5\)A, like ATP, can induce, depending upon the individual cell, either transients of short duration (approx. 9 s), transients of much longer duration or a mixture of short and long transients within a single response. We show here that the pattern of oscillations induced by Ap\(^5\)A is similar to that induced by ATP in the same hepatocyte. (2) Elevated intracellular cyclic AMP concentration modulates Ap\(^5\)A-induced transients, like ADP-induced transients, through an increase in both the peak [Ca\(^{2+}\)]\(_\text{cyt}\) and the frequency of the transients. In contrast, Ap\(^5\)A-induced transients, like ATP-induced transients, develop an increased duration or a sustained rise in [Ca\(^{2+}\)]\(_\text{cyt}\), with no rise in peak [Ca\(^{2+}\)]\(_\text{cyt}\). (3) Ap\(^5\)A-induced transients, like ADP-induced transients, are abolished by low concentrations of the phorbol ester 4\(\beta\)-phorbol 12,13-dibutyrate (PDB; 5–10 nM), whereas long Ap\(^5\)A-induced transients, like long ATP-induced transients, are refractory to high concentrations of PDB (100 nM). We propose that the [Ca\(^{2+}\)]\(_\text{cyt}\), oscillations induced in rat hepatocytes by Ap\(^5\)A are mediated by the same purinoceptor that mediates the effects of ADP, whereas the oscillations induced by Ap\(^5\)A are mediated by the same purinoceptor(s) that mediate the effects of ATP.

INTRODUCTION

Diadenosine 5',5''-P\(^1\),P\(^3\)-triphosphate (Ap\(^5\)A) and diadenosine 5',5''-P\(^1\),P\(^4\)-tetraphosphate (Ap\(^4\)A) are the most abundant of the diadenosine polyphosphates, a family of naturally occurring molecules consisting of two adenosine groups linked by a variable number of phosphate groups. Diadenosine polyphosphates have been detected in a wide variety of cells [1] and are stored at high concentration in platelets and chromaffin cells. Ap\(^5\)A, Ap\(^4\)A, Ap\(^6\)A and Ap\(^7\)A are stored in the dense bodies of platelets and, upon platelet aggregation, are released into the extracellular milieu [2–4]. In chromaffin cells, Ap\(^5\)A, Ap\(^7\)A [5] and Ap\(^6\)A [6] are co-stored with AMP, ADP, ATP and catecholamines. It has been estimated that, following release from platelets and chromaffin cells, diadenosine polyphosphates could be present at micromolar concentrations in the extracellular fluid [7,8]. Since diadenosine polyphosphates could reach physiologically significant concentrations and, compared with ATP, have relatively long half-lives in blood [9], it is apparent that they are well-suited to their emerging role as extracellular effectors. Indeed, diadenosine polyphosphates have been shown to modulate a number of biological processes including platelet aggregation [10–12], catecholamine release from chromaffin cells [13] and vasoregulation [4,7,14].

The biological effects of diadenosine polyphosphates on a number of cells and tissues have been attributed to interactions with known P\(_\text{2x}\)-purinoceptors (as defined by the classification of Burnstock and Kennedy [15]) for ADP and ATP. Thus, ATP and Ap\(^5\)A have been reported to activate the same receptor to induce cation currents in rat sensory neurons [16]. Ap\(^5\)A acts on P\(_\text{2x}\)-purinoceptors to stimulate contraction of the urinary detrusor muscle in human bladder [17]. In chromaffin cells Ap\(^5\)A and Ap\(^4\)A act via a putative P\(_\text{2x}\)-purinoceptor [8] to evoke an increase in cytosolic free Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_\text{cyt}\)) [18] and an activation of protein kinase C [19]. In contrast, however, it has been proposed that, in some cells and tissues, diadenosine polyphosphates may act via unique receptors highly specific for diadenosine polyphosphates. For example, it has been suggested that Ap\(^5\)A and Ap\(^4\)A may act via a separate sub-type of receptors, distinct from that for ATP, to induce contraction of rat vas deferens [20]. In intact rabbit hearts, Ap\(^5\)A and Ap\(^4\)A exert specific effects that are not seen in response to adenosine, AMP, ADP or ATP; it was thus suggested that specific receptors for the diadenosine polyphosphates may exist on endothelial and/or smooth-muscle cells of the vascular wall of coronary arteries [7]. On the basis of displacement binding studies, which revealed the presence of binding sites for Ap\(^5\)A whose agonist affinity series differed from any previously described P\(_{\text{2x}}\)-purinoceptor, the existence on rat brain synaptosomes of a unique purinoceptor with high affinity for diadenosine polyphosphates has been suggested [21]. Indeed, a unique membrane receptor for Ap\(^5\)A has been identified in mouse brain. This dipurinoceptor was also detected in several other mouse tissues, including heart, muscle and liver [22].

In perfused isolated rat liver, Ap\(^5\)A and Ap\(^7\)A stimulate glucose output and a transient net release of Ca\(^{2+}\) [23]. In isolated

---

Abbreviations used: Ap\(^5\)A, diadenosine 5',5''-P\(^1\),P\(^3\)-triphosphate; Ap\(^4\)A, diadenosine 5',5''-P\(^1\),P\(^4\)-tetraphosphate; Ap\(^6\)A, diadenosine 5',5''-P\(^1\),P\(^6\)-hexaphosphate; Ap\(^7\)A, diadenosine 5',5''-P\(^1\),P\(^7\)-heptaphosphate; [Ca\(^{2+}\)]\(_\text{cyt}\), cytosolic concentration of free Ca\(^{2+}\); L899051, 7\(\beta\)-desacetyl-7\(\beta\)-[\(\gamma\)-(N-methylpiperazino)butyryl]forskolin; PDB, 4\(\beta\)-phorbol 12,13-dibutyrate.

* To whom correspondence and reprint requests should be addressed.
hepatocytes, a series of diadenosine polyphosphates stimulate a
dose-dependent activation of glycogen phosphorylase similar to
that observed with ATP [24]. Extracellular ADP and ATP act on
rat hepatocytes to stimulate the hydrolysis of PtdIns(4,5)P_2 and
subsequent mobilization of Ca^{2+} [25]. We have previously shown
that Ap_3A and Ap_4A, acting as the uncleaved diadenosine
polyphosphates, induce oscillations in cytosolic free Ca^{2+}
concentration ([Ca^{2+}]_i) in single rat hepatocytes [26] which resemble
those induced by ADP and ATP [27]. However, the identity of
the receptor(s) mediating the effects of Ap_3A and Ap_4A in rat
hepatocytes is unknown. The aim of the present study was to
determine whether the Ap_3A- and Ap_4A-induced [Ca^{2+}]_i oscilla-
tions in rat hepatocytes are mediated via distinct di purino-
ceptors, or whether Ap_3A and Ap_4A are able to exert their
effects on [Ca^{2+}]_i, via binding to rat hepatocyte purinoreceptors
for ADP and ATP.

Many cell types respond to agonists acting via the phos-
phoinositide signalling pathway by the generation of [Ca^{2+}]_i
oscillations [28]. Single rat hepatocytes, microinjected with the
Ca^{2+}-sensitive photoprotein aequorin, exhibit series of repetitive
[Ca^{2+}]_i transients whose frequency is modulated by agonist
concentration [29]. The duration of the individual transients is
dependent upon the receptor species activated and is consistent
between hepatocytes. For example, activation of the \( \alpha \)-adrenoceptor with either phenylephrine or adrenaline induces
transients of identical duration in rat hepatocytes (approx. 5–7 s)
[30]. Similarly, vasopressin and oxytocin, acting at the same \( \alpha \)-
receptor on rat hepatocytes, induce transients of the same
duration (approx. 10–12 s), despite oxytocin having a 500-fold
lower affinity for the receptor [31]. The variability in the transient
duration results from differences in the rate of fall of [Ca^{2+}]_i, from
its peak [32]. We have previously shown that ADP and ATP,
thought to act through the same P_2X-receptor in liver [33],
induce [Ca^{2+}]_i transients of different duration in the majority of
hepatocytes. ADP invariably induces transients of short duration
(approx. 9 s). In response to ATP, however, three different
oscillatory [Ca^{2+}]_i responses are distinguishable between hepato-
cytes; ATP induces either short transients indistinguishable
in terms of duration from those induced by ADP, transients of
a much longer duration or a mixture of short and long transients
within a single response [27]. Furthermore, elevated intracellular
cyclic AMP concentration exerts different modulatory effects on
[Ca^{2+}]_i oscillations induced by ADP and ATP. Elevated intra-
cellular cyclic AMP levels enhance the frequency and peak
[Ca^{2+}]_i, of ADP-induced transients. In contrast, the elevation of
intracellular cyclic AMP levels in hepatocytes producing [Ca^{2+}]_i,
occulations in response to ATP stimulates either an increase in
the duration of transients or a sustained rise in [Ca^{2+}]_i, [34].
In addition, we have recently demonstrated that ADP- and
ATP-induced transients are differentially sensitive to the phor-}

\[ \text{PDB} \] [35]. These data are not consistent with ADP and ATP acting via a single receptor
species. It is particularly noteworthy that short ATP-induced transients, although indistinguishable in terms of duration from
ADP-induced transients, are modulated differently by both
treatments described above. We have thus proposed the existence
of three functionally distinct receptors on the hepatocyte cell
surface: (i) an ADP receptor, (ii) a receptor which mediates
ATP-induced transients of short duration, \( \text{ATP}^+ \), and (iii) a
receptor which mediates ATP-induced transients of long dura-
tion, ‘ATP^−’ [34].

Here we report several similarities between the oscillatory
[Ca^{2+}]_i, responses to Ap_3A and ATP, and to Ap_4A and ADP. On
the basis of these similarities we suggest that the [Ca^{2+}]_i oscilla-
tions induced by Ap_3A and Ap_4A in single rat hepatocytes are
mediated by the same receptors that mediate the oscillatory
[Ca^{2+}]_i responses to ADP and ATP respectively.

RESULTS AND DISCUSSION

Comparison of [Ca^{2+}]_i transients induced by ADP and Ap_3A

Single aequorin-injected hepatocytes responded to ADP and
Ap_3A, in the range 0.6–10 \( \mu \)M by the generation of series of repetitive [Ca^{2+}]_i,
thanents whose frequency was dependent on agonist concentration. In agreement with previous observations
[26,27] the transients were consistent in duration and the duration
of each transient was short (approx. 9 s). Figure 1 shows typical
ADP- and Ap_3A-induced [Ca^{2+}]_i, transients recorded in the same
single hepatocyte.

![Figure 1 ADP and Ap_3A induce [Ca^{2+}]_i oscillations of short duration](image-url)
Table 1  A comparison of the oscillatory [Ca\(^{2+}\)] responses induced by ATP and Ap4A within the same single rat hepatocyte

<table>
<thead>
<tr>
<th>Numbers of individual hepatocytes</th>
<th>ATP-induced transients of short duration</th>
<th>ATP-induced transients of variable duration</th>
<th>ATP-induced transients of long duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ap4A-induced</td>
<td>13</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>oscillations of short duration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ap4A-induced</td>
<td>1</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>oscillations of variable duration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ap4A-induced</td>
<td>0</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td>oscillations of long duration</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Comparison of [Ca\(^{2+}\)] transients induced by ATP and Ap4A within the same individual hepatocyte

Single aequorin-injected hepatocytes responded to extracellular ATP, within the concentration range 1–10 \(\mu\)M, by the generation of oscillations in [Ca\(^{2+}\)], similar to those described previously [27]. Thus three classes of hepatocytes were distinguishable: (i) hepatocytes in which extracellular ATP induced [Ca\(^{2+}\)], oscillations of short duration (approx. 9 s), (ii) hepatocytes which responded to ATP with [Ca\(^{2+}\)], oscillations that were consistently of longer duration, and (iii) hepatocytes in which ATP induced a mixture of short and long transients within a single response. ATP was previously considered to be unique in its ability to induce a variable oscillatory [Ca\(^{2+}\)], response between hepatocytes; other agonists always elicit [Ca\(^{2+}\)], transients of consistent duration, characteristic of the stimulating agonist [32]. However, we have recently described a similar variability in the pattern of oscillations induced in hepatocytes by Ap4A [26]. Here we compare the oscillatory [Ca\(^{2+}\)], responses to ATP and Ap4A by aequorin measurements of [Ca\(^{2+}\)], oscillations induced by ATP and Ap4A in the same individual hepatocyte. As a control, a characteristic response to phenylephrine, i.e. repetitive [Ca\(^{2+}\)], transients of approx. 5–7 s duration, was recorded in all hepatocytes before addition of ATP or Ap4A. Single hepatocytes responded to Ap4A, within the concentration range 1–10 \(\mu\)M, by the generation of oscillations in [Ca\(^{2+}\)]. Table 1 relates the pattern of oscillations induced by Ap4A to the pattern of oscillations induced by ATP within the same individual hepatocyte. In the majority of hepatocytes, the oscillatory [Ca\(^{2+}\)], response to Ap4A closely resembled that induced by ATP in the same single hepatocyte. Thus, as illustrated in Figure 2(a), the majority of hepatocytes which generated short [Ca\(^{2+}\)], transients in response to ATP responded to Ap4A with similar short transients (13/14 cells). In the majority of hepatocytes in which ATP induced long transients, Ap4A also induced similar long transients (22/24 cells), as shown in Figures 2(b) and 2(c). As previously described, in some hepatocytes the falling phase of long ATP-induced transients is composed of discrete secondary oscillations [27,34]. The ability to induce secondary oscillations within [Ca\(^{2+}\)], transients in rat hepatocytes was previously considered to be exclusive to ATP [34]. However, as illustrated in Figure 2(c), in all hepatocytes in which ATP induced [Ca\(^{2+}\)], oscillations of this type, Ap4A also induced [Ca\(^{2+}\)], oscillations in which the falling phase was similarly composed of secondary oscillations (n = 11). In the group of hepatocytes which responded to ATP by the generation of a mixture of short and long transients within a single ‘variable’ response, Ap4A induced a similar variable response (Figure 2d; 11/11 cells).

We have thus shown that, in the majority of hepatocytes, the response to Ap4A, in terms of the duration and profile of [Ca\(^{2+}\)], transients induced, very closely resembles that recorded in the same cell in response to ATP. Therefore, not only does Ap4A share the ability of ATP to induce a variable response between hepatocytes, but within a single hepatocyte Ap4A induces the same class of response as that induced by ATP. These findings thus suggest that Ap4A-induced [Ca\(^{2+}\)], oscillations may be mediated via the same receptor(s) that mediate the [Ca\(^{2+}\)], response to ATP in rat hepatocytes.

Modulatory effects of elevated cyclic AMP concentration on [Ca\(^{2+}\)], transients induced by Ap4A and Ap4A

The hepatocyte oscillatory [Ca\(^{2+}\)], responses to Ap4A and Ap4A were further characterized by exploitation of the phenomenon of receptor specificity in the modulatory effect of experimentally elevated cyclic AMP concentration on [Ca\(^{2+}\)], oscillations [34,38,39].

Elevation of intracellular cyclic AMP levels by direct activation of adenylate cyclase by co-application of the forskolin derivative L858051 (5 \(\mu\)M) stimulated an increase in the frequency and peak [Ca\(^{2+}\)], of Ap4A-induced transients (7/7 cells), as shown in Figure 3. As previously reported [34], L858051 (2–10 \(\mu\)M) stimulated an increase in the frequency and peak [Ca\(^{2+}\)], of ADP-induced transients. In agreement with previous observations [34], L858051 alone had no effect on [Ca\(^{2+}\)], (results not shown).

Ap4A-induced oscillations were also potentiated by the co-application of L858051; however, the modulatory effects of elevated intracellular cyclic AMP concentration on individual [Ca\(^{2+}\)], transients differed markedly from the effect on Ap4A-induced transients. As shown in Figure 4(a), the co-application of L858051 (5 \(\mu\)M) to hepatocytes responding to Ap4A alone by the generation of short transients stimulated an increase in the duration of the transients (7/7 cells). In hepatocytes responding to Ap4A alone with transients of long duration, the co-application of L858051 (5 \(\mu\)M) stimulated either an increase in the duration of each transient (7/8 cells; Figure 4b) or a sustained rise in [Ca\(^{2+}\)], (1/8 cells; Figure 4c). Elevation of intracellular cyclic AMP concentration (by co-application of 5 \(\mu\)M L858051) in hepatocytes responding to Ap4A alone by the generation of transients of variable duration stimulated an increase in the duration of transients (6/6 cells). A typical result is shown in Figure 4(d). As described previously [34], the co-application of L858051 exerted similar modulatory effects on ATP-induced transients. Thus, in hepatocytes responding to ATP by the generation of short [Ca\(^{2+}\)], transients or transients of variable duration, L858051 (2–10 \(\mu\)M) stimulated an increase in the duration of each transient. In hepatocytes generating long transients in response to ATP, L858051 stimulated either an increase in the duration of each transient or a sustained rise in [Ca\(^{2+}\)]. In some hepatocytes, the prolongation of Ap4A-induced transients was accompanied by an increase in the number of secondary oscillations within the falling phase of each transient (see Figures 4a, 4b and 4d). A similar effect on ATP-induced transients was previously reported [34].

These data thus demonstrate a further difference between the oscillatory [Ca\(^{2+}\)], responses to Ap4A and Ap4A. Instead, the
A. K. Green, P. H. Cobbold and C. J. Dixon

Figure 2 The oscillatory \([\text{Ca}^{2+}]\) response of a single hepatocyte to \(\text{Ap}_4\text{A}\) resembles that to ATP

Single aequorin-injected hepatocytes were superfused with ATP and \(\text{Ap}_4\text{A}\) for the periods indicated. Single hepatocytes responded to both ATP and \(\text{Ap}_4\text{A}\) by the generation of (a) \([\text{Ca}^{2+}]\) transients of short duration, (b) transients of long duration, (c) transients of long duration in which the falling phase is composed of prominent secondary oscillations, and (d) transients of variable duration. Time constants: for resting \([\text{Ca}^{2+}]\), 10 s; for transients, 1 s.

Figure 3 Elevation of intracellular cyclic AMP levels enhances the peak \([\text{Ca}^{2+}]\) and frequency of \([\text{Ca}^{2+}]\) oscillations induced by extracellular \(\text{Ap}_4\text{A}\)

A single aequorin-injected hepatocyte responding to \(\text{Ap}_4\text{A}\) by the generation of \([\text{Ca}^{2+}]\) transients was co-supplied with 5 \(\mu\text{M} \ L858051\) for the period indicated. Time constants: for resting \([\text{Ca}^{2+}]\), 10 s; for transients, 1 s.

response to \(\text{Ap}_4\text{A}\) resembles that to ADP, whereas the response to \(\text{Ap}_4\text{A}\) resembles that to ATP.

Effects of phorbol ester on \(\text{Ap}_4\text{A}-\) and \(\text{Ap}_4\text{A}\)-induced \([\text{Ca}^{2+}]\) transients

Consistent with the inhibition of ADP-induced \([\text{Ca}^{2+}]\), oscillations by low concentrations of PDB (5–10 nM) [35], the co-application of PDB (5 nM) abolished (6/12 hepatocytes) or caused a decrease in frequency (6/12 hepatocytes) of \(\text{Ap}_4\text{A}\)-induced \([\text{Ca}^{2+}]\), oscillations. Of the six hepatocytes in which 5 nM PDB caused a decrease in frequency of the transients, application of 10 nM PDB caused the abolition (4/6 hepatocytes) or a further decrease in frequency (2/6 hepatocytes) of \(\text{Ap}_4\text{A}\)-induced \([\text{Ca}^{2+}]\) transients. Figure 5 shows a typical result in which \(\text{Ap}_4\text{A}\)-induced \([\text{Ca}^{2+}]\), oscillations were promptly abolished by the co-application of 5 nM PDB. As a control, the inactive phorbol ester 4a-PDB (10 nM) had no effect on \(\text{Ap}_4\text{A}\)-induced \([\text{Ca}^{2+}]\), oscillations (3/3 hepatocytes).

As described previously, ATP-induced \([\text{Ca}^{2+}]\), oscillations of long duration were resistant to PDB, even, in the majority of hepatocytes, at greatly elevated concentrations (100 nM) [35]. We therefore decided to examine the impact of PDB on long
Ap4A-induced [Ca\(^{2+}\)] Oscillations. The co-application of 5 nM PDB to 15/16 hepatocytes generating [Ca\(^{2+}\)] transients of long duration in response to Ap4A had no effect on the duration of the inter-transient interval (Figure 6). However, consistent with the effect of 5 nM PDB on long ATP-induced [Ca\(^{2+}\)] oscillations [35], there was a small decrease in the duration of individual long Ap4A-induced oscillations in eight of these 15 hepatocytes. (In the remaining cell the duration of the inter-transient interval was increased.) Furthermore, higher concentrations of PDB failed to abolish long Ap4A-induced oscillations in the majority of hepatocytes; 11/13 hepatocytes exposed to 25 nM PDB continued to generate long Ap4A-induced [Ca\(^{2+}\)] oscillations, with no alteration in the inter-transient interval. (In one hepatocyte the inter-transient interval was increased by the application of 25 nM PDB; in the remaining cell the oscillations were abolished.) Of ten hepatocytes in which the long Ap4A-induced [Ca\(^{2+}\)] oscillations were resistant to 25 nM PDB, long Ap4A-induced [Ca\(^{2+}\)] oscillations in seven hepatocytes were similarly resistant to the co-application of 100 nM PDB. (In the remaining 3/10 hepatocytes the Ap4A-induced transients were abolished by the co-application of 100 nM PDB.) Figure 6 shows a typical result in which long Ap4A-induced [Ca\(^{2+}\)] oscillations were resistant to the co-application of PDB (5–100 nM).

The resistance of long ATP-induced [Ca\(^{2+}\)] oscillations and long Ap4A-induced oscillations to high concentrations of PDB is in marked contrast with the inhibition by low concentrations of PDB of phenylephrine-, vasopressin- [40], ADP-, short ATP-[35] and Ap4A-induced oscillations. Mechanisms by which receptor-specific regulation of the hepatocyte [Ca\(^{2+}\)] oscillator by protein kinase C may occur have been discussed previously [35]. It is clear that this phenomenon represents a further example of the rat hepatocyte oscillatory [Ca\(^{2+}\)] responses to ATP and Ap4A...
Figure 5  Ap₄A-induced [Ca²⁺], oscillations are inhibited by low concentrations of PDB

A single aequorin-injected hepatocyte responding to Ap₄A by the generation of [Ca²⁺], oscillations was co-supplied with 5 nM PDB for the period indicated. Time constants: for resting [Ca²⁺], 10 s; for transients, 1 s.

Figure 6  Ap₄A-induced [Ca²⁺], oscillations of long duration are not inhibited by PDB

A single aequorin-injected hepatocyte responding to Ap₄A by the generation of [Ca²⁺] oscillations of long duration was co-supplied with PDB (5–100 nM) for the periods indicated. Time constants: for resting [Ca²⁺], 10 s; for transients, 1 s.

...exhibiting a marked difference from oscillations induced by other Ca²⁺-mobilizing agonists. This observation provides further evidence in support of our proposal that ATP and Ap₄A act to mobilize Ca²⁺ in an identical manner, conceivably to the extent of sharing the same receptor(s).

We hypothesize that the [Ca²⁺], oscillations induced by Ap₃A and Ap₄A in rat hepatocytes are mediated by the same purinoceptors that mediate the effects of ADP and ATP respectively. It may, however, be argued that the [Ca²⁺], oscillations observed here in response to Ap₃A and Ap₄A, which, we have proposed, are mediated by the hepatocyte ADP and ATP receptors, are the result of action of ADP and ATP themselves, liberated by the extracellular metabolism of Ap₃A and Ap₄A. We consider this unlikely, since, in contrast with ATP, which is completely degraded to adenosine in 30 s, Ap₃A is only marginally degraded after 2 min in a suspension of hepatocytes [24]. Furthermore, in the present studies, the single hepatocyte is held in isolation from all other cells and is constantly superfused with medium, which thereby simultaneously provides a continuous supply of fresh Ap₃A or Ap₄A and removes any breakdown products. The accumulation of ADP and ATP at the cell surface is thus prevented. In addition, we have previously demonstrated that diadenosine 5',5''-P₁,P₂-(P¹-thio)triphosphate (mixed isomers) and (S₂,S₂)-diadenosine 5',5''-P₁,P₂-dithiotetraphosphate, phosphorothioate analogues of Ap₃A and Ap₄A respectively, induce [Ca²⁺], oscillations in single rat hepatocytes [26]. Compared with Ap₃A and Ap₄A, these analogues show a much slower rate of cleavage by specific Ap₃A and Ap₄A hydrolases and non-specific phosphodiesterases [41]. It is thus apparent that the intact diadenosine polyphosphates are able to induce [Ca²⁺], oscillations in single rat hepatocytes.

The ability to respond to Ap₃A and Ap₄A may represent an important physiological role for the ADP and ATP receptors on hepatocytes. If the distribution of these receptors is not restricted to rat hepatocytes, it must be considered that any cell type expressing these receptors is a potential target for the actions of Ap₃A and Ap₄A. Ap₃A and Ap₄A are longer-lasting signalling molecules than ADP and ATP and may be able to act upon target tissues relatively distant from their site of liberation [9]. Furthermore, compared with ADP and ATP, Ap₃A and Ap₄A are degraded more slowly by ecto-nucleotidases on the surface of various potential target cells [42]. It is therefore tempting to speculate that, in vivo, Ap₃A and Ap₄A may have a more important role than ADP and ATP as extracellular effectors, particularly at targets remote from the release site. Indeed, Ap₃A and Ap₄A may represent the true physiological agonists of the previously postulated ADP, ATP₈ and ATP₇ receptors [27,34,35].

We are grateful for funding from The Wellcome Trust.
REFERENCES


Received 16 February 1995/2 May 1995; accepted 5 May 1995