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

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Diadenosine 5′,5‴-P^1,P^3-triphosphate (Ap_3A) and diadenosine 5′,5‴-P^1,P^4-tetraphosphate (Ap_4A) induce distinctive patterns of $[Ca^{2+}]_i$ oscillations in single rat hepatocytes. We show here that $[Ca^{2+}]_i$ oscillations induced by Ap_3A and ADP are indistinguishable and that $[Ca^{2+}]_i$ oscillations induced by Ap_4A closely resemble those induced by ATP. These similarities embrace the following: (1) ADP and Ap_3A invariably induce $[Ca^{2+}]_i$ transients of short duration (approx. 9 s). Ap_4A, 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_4A is similar to that induced by ATP in the same hepatocyte. (2) Elevated intracellular cyclic AMP concentration modulates Ap_3A-induced transients, like ADP-induced transients, through an increase in both the peak $[Ca^{2+}]_i$ and the frequency of the transients. In contrast, Ap_4A-induced transients, like ATP-induced transients, develop an increased duration or a sustained rise in $[Ca^{2+}]_i$, with no rise in peak $[Ca^{2+}]_i$. (3) Ap_3A-induced transients, like ADP-induced transients, are abolished by low concentrations of the phorbol ester 4β-phorbol 12,13-dibutyrate (PDB; 5–10 nM), whereas long Ap_4A-induced transients, like long ATP-induced transients, are refractory to high concentrations of PDB (100 nM). We propose that the $[Ca^{2+}]_i$ oscillations induced in rat hepatocytes by Ap_3A are mediated by the same purinoceptor that mediates the effects of ADP, whereas the oscillations induced by Ap_4A are mediated by the same purinoceptor(s) that mediate the effects of ATP.

INTRODUCTION

Diadenosine 5′,5‴-P^1,P^3-triphosphate (Ap_3A) and diadenosine 5′,5‴-P^1,P^4-tetraphosphate (Ap_4A) 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_3A, Ap_4A, Ap_5A and Ap_6A are stored in the dense bodies of platelets and, upon platelet aggregation, are released into the extracellular milieu [2–4]. In chromaffin cells, Ap_4A, Ap_5A [5] and Ap_6A [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_2-purinoceptors (as defined by the classification of Burnstock and Kennedy [15]) for ADP and ATP. Thus, ATP and Ap_4A have been reported to activate the same receptor to induce cation currents in rat sensory neurons [16]. Ap_6A acts on P_{2x}-purinoceptors to stimulate contraction of the urinary detrussor muscle in human bladder [17]. In chromaffin cells Ap_4A and Ap_5A act via a putative P_{2y}-purinoceptor [8] to evoke an increase in cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) [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_4A and Ap_5A 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_3A and Ap_4A 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_4A whose agonist affinity series differed from any previously described P_2-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_4A 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_3A and Ap_4A stimulate glucose output and a transient net release of Ca^{2+} [23]. In isolated

Abbreviations used: Ap_3A, diadenosine 5′,5‴-P^1,P^3-triphosphate; Ap_4A, diadenosine 5′,5‴-P^1,P^4-tetraphosphate; Ap_5A, diadenosine 5′,5‴-P^1,P^5-pentaphosphate; Ap_6A, diadenosine 5′,5‴-P^1,P^6-hexaphosphate; $[Ca^{2+}]_i$, cytosolic concentration of free Ca^{2+}; L858051, 7β-desacetyl-7β-[γ-(*N*-methylpiperazino)butyryl]forskolin; PDB, 4β-phorbol 12,13-dibutyrate.

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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 $[Ca^{2+}]_i$ oscillations in rat hepatocytes are mediated via distinct dipurinoceptors, or whether Ap_3A and Ap_4A are able to exert their effects on $[Ca^{2+}]_i$ via binding to rat hepatocyte purinoceptors for ADP and ATP.

Many cell types respond to agonists acting via the phosphoinositide 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 α_1-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 V_1 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 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_{2y}-purinoceptor 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 hepatocytes; 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 intracellular 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 $[Ca^{2+}]_i$ oscillations 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 phorbol ester 4β-phorbol 12,13-dibutyrate (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, 'ATP_S', and (iii) a receptor which mediates ATP-induced transients of long duration, 'ATP_L' [34].

Here we report several similarities between the oscillatory $[Ca^{2+}]_i$ responses to Ap_4A and ATP, and to Ap_3A and ADP. On the basis of these similarities we suggest that the $[Ca^{2+}]_i$ oscillations 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.

MATERIALS AND METHODS

Single hepatocytes were isolated from fed 150–250 g male Wistar rats by collagenase digestion as described previously [32]. Collagenase was from Boehringer. Microdialysis and microinjection of aequorin, data acquisition and analysis were described previously [36]. The experimental medium, Williams medium E (Gibco) gassed with CO_2/air (1:19), was superfused over single hepatocytes at 37 °C. ADP, ATP, Ap_3A, Ap_4A and 7β-desacetyl-7β-[γ-(*N*-methylpiperazino)butyryl]forskolin (L858051) were added to this medium. 4β-Phorbol 12,13-dibutyrate (PDB) and the inactive phorbol ester 4α-phorbol 12,13-dibutyrate (4α-PDB) were dissolved in dimethyl sulphoxide to give 10 mM stocks. Aliquots were added to the experimental medium to give required concentrations. ADP, ATP, Ap_3A and Ap_4A were from Sigma. PDB and 4α-PDB were from LC Laboratories. L858051 (Calbiochem) is a chemically modified forskolin with an enhanced stability and water solubility, whose adenylate cyclase-stimulating activity and binding capacity are approximately equal to those of forskolin [37].

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 μM by the generation of series of repetitive $[Ca^{2+}]_i$ transients 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.

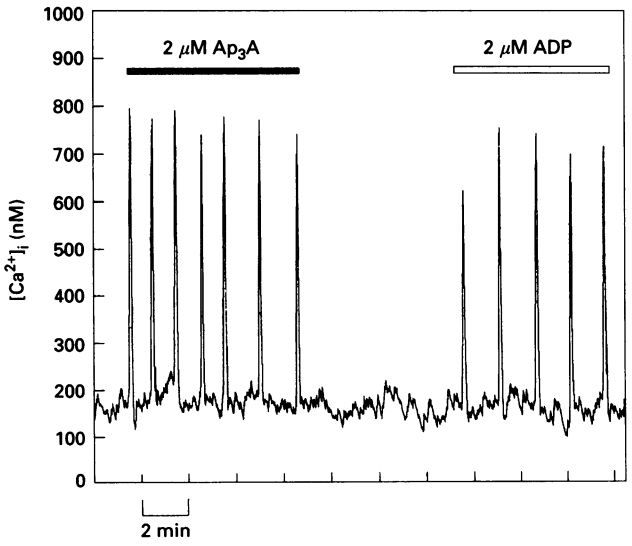

Figure 1 ADP and Ap_3A induce $[Ca^{2+}]_i$ oscillations of short duration

A single aequorin-injected hepatocyte was superfused with ADP and Ap_3A for the periods indicated. As shown in this example, hepatocytes invariably respond to ADP and Ap_3A by the generation of $[Ca^{2+}]_i$ transients of short duration (approx. 9 s). Time constants: for resting $[Ca^{2+}]_i$, 10 s; for transients, 1 s.

Table 1 A comparison of the oscillatory $[Ca^{2+}]_i$ responses induced by ATP and Ap_4A within the same single rat hepatocyte

Oscillations in $[Ca^{2+}]_i$ were recorded from single aequorin-injected hepatocytes superfused with ATP and Ap_4A as illustrated in Figure 2. The duration of $[Ca^{2+}]_i$ transients induced by Ap_4A is related to the duration of $[Ca^{2+}]_i$ transients induced by ATP within the same single hepatocyte.

	Numbers of individual hepatocytes		
	ATP-induced transients of short duration	ATP-induced transients of variable duration	ATP-induced transients of long duration
Ap_4A-induced transients of short duration	13	0	0
Ap_4A-induced transients of variable duration	1	11	2
Ap_4A-induced transients of long duration	0	0	22

Comparison of $[Ca^{2+}]_i$ transients induced by ATP and Ap_4A within the same individual hepatocyte

Single aequorin-injected hepatocytes responded to extracellular ATP, within the concentration range 1–10 μM, by the generation of oscillations in $[Ca^{2+}]_i$ similar to those described previously [27]. Thus three classes of hepatocytes were distinguishable: (i) hepatocytes in which extracellular ATP induced $[Ca^{2+}]_i$ oscillations of short duration (approx. 9 s), (ii) hepatocytes which responded to ATP with $[Ca^{2+}]_i$ 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 $[Ca^{2+}]_i$ response between hepatocytes; other agonists always elicit $[Ca^{2+}]_i$ 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 Ap_4A [26]. Here we compare the oscillatory $[Ca^{2+}]_i$ responses to ATP and Ap_4A by aequorin measurements of $[Ca^{2+}]_i$ oscillations induced by ATP and Ap_4A in the same individual hepatocyte. As a control, a characteristic response to phenylephrine, i.e. repetitive $[Ca^{2+}]_i$ transients of approx. 5–7 s duration, was recorded in all hepatocytes before addition of ATP or Ap_4A. Single hepatocytes responded to Ap_4A, within the concentration range 1–10 μM, by the generation of oscillations in $[Ca^{2+}]_i$. Table 1 relates the pattern of oscillations induced by Ap_4A to the pattern of oscillations induced by ATP within the same individual hepatocyte. In the majority of hepatocytes, the oscillatory $[Ca^{2+}]_i$ response to Ap_4A 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 $[Ca^{2+}]_i$ transients in response to ATP responded to Ap_4A with similar short transients (13/14 cells). In the majority of hepatocytes in which ATP induced long transients, Ap_4A 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 $[Ca^{2+}]_i$ 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 $[Ca^{2+}]_i$ oscillations of this type, Ap_4A also induced $[Ca^{2+}]_i$ 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, Ap_4A induced a similar variable response (Figure 2d; 11/11 cells).

We have thus shown that, in the majority of hepatocytes, the response to Ap_4A, in terms of the duration and profile of $[Ca^{2+}]_i$ transients induced, very closely resembles that recorded in the same cell in response to ATP. Therefore, not only does Ap_4A share the ability of ATP to induce a variable response between hepatocytes, but within a single hepatocyte Ap_4A induces the same class of response as that induced by ATP. These findings thus suggest that Ap_4A-induced $[Ca^{2+}]_i$ oscillations may be mediated via the same receptor(s) that mediate the $[Ca^{2+}]_i$ response to ATP in rat hepatocytes.

Modulatory effects of elevated cyclic AMP concentration on $[Ca^{2+}]_i$ transients induced by Ap_3A and Ap_4A

The hepatocyte oscillatory $[Ca^{2+}]_i$ responses to Ap_3A and Ap_4A were further characterized by exploitation of the phenomenon of receptor specificity in the modulatory effect of experimentally elevated cyclic AMP concentration on $[Ca^{2+}]_i$ 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 μM) stimulated an increase in the frequency and peak $[Ca^{2+}]_i$ of Ap_3A-induced transients (7/7 cells), as shown in Figure 3. As previously reported [34], L858051 (2–10 μM) stimulated an increase in the frequency and peak $[Ca^{2+}]_i$ of ADP-induced transients. In agreement with previous observations [34], L858051 alone had no effect on $[Ca^{2+}]_i$ (results not shown).

Ap_4A-induced oscillations were also potentiated by the co-application of L858051; however, the modulatory effects of elevated intracellular cyclic AMP concentration on individual $[Ca^{2+}]_i$ transients differed markedly from the effect on Ap_3A-induced transients. As shown in Figure 4(a), the co-application of L858051 (5 μM) to hepatocytes responding to Ap_4A alone by the generation of short transients stimulated an increase in the duration of the transients (7/7 cells). In hepatocytes responding to Ap_4A alone with transients of long duration, the co-application of L858051 (5 μM) stimulated either an increase in the duration of each transient (7/8 cells; Figure 4b) or a sustained rise in $[Ca^{2+}]_i$ (1/8 cells; Figure 4c). Elevation of intracellular cyclic AMP concentration (by co-application of 5 μM L858051) in hepatocytes responding to Ap_4A 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 $[Ca^{2+}]_i$ transients or transients of variable duration, L858051 (2–10 μM) 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 $[Ca^{2+}]_i$. In some hepatocytes, the prolongation of Ap_4A-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 $[Ca^{2+}]_i$ responses to Ap_3A and Ap_4A. Instead, the

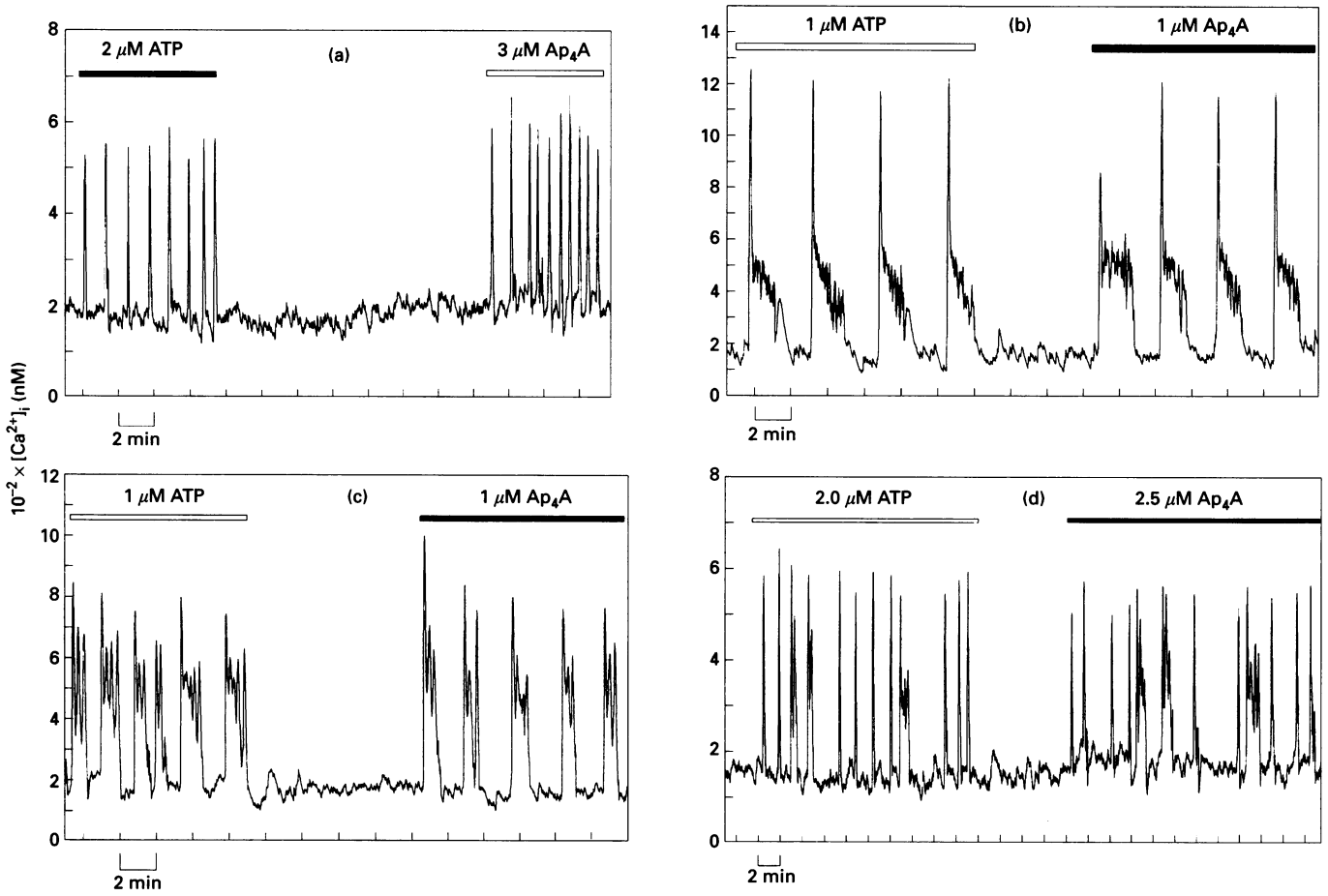

Figure 2 The oscillatory $[Ca^{2+}]_i$ response of a single hepatocyte to Ap_4A resembles that to ATP

Single aequorin-injected hepatocytes were superfused with ATP and Ap_4A for the periods indicated. Single hepatocytes responded to both ATP and Ap_4A by the generation of (**a**) $[Ca^{2+}]_i$ 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 $[Ca^{2+}]_i$, 10 s; for transients, 1 s.

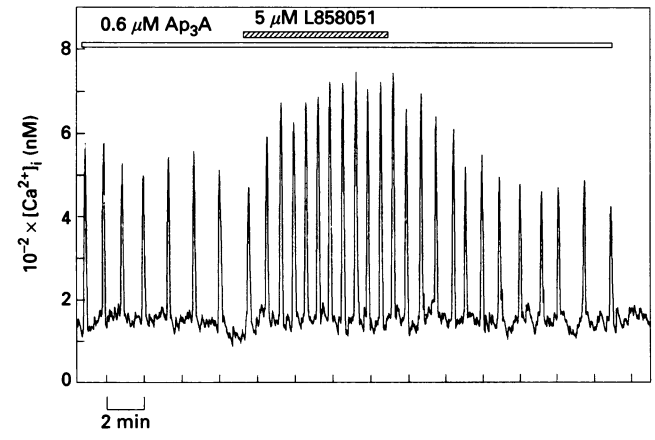

Figure 3 Elevation of intracellular cyclic AMP levels enhances the peak $[Ca^{2+}]_i$ and frequency of $[Ca^{2+}]_i$ oscillations induced by extracellular Ap_3A

A single aequorin-injected hepatocyte responding to Ap_3A by the generation of $[Ca^{2+}]_i$ transients was co-supplied with 5 μM L858051 for the period indicated. Time constants: for resting $[Ca^{2+}]_i$, 10 s; for transients, 1 s.

response to Ap_3A resembles that to ADP, whereas the response to Ap_4A resembles that to ATP.

Effects of phorbol ester on Ap_3A- and Ap_4A-induced $[Ca^{2+}]_i$ transients

Consistent with the inhibition of ADP-induced $[Ca^{2+}]_i$ 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 Ap_3A-induced $[Ca^{2+}]_i$ 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 Ap_3A-induced $[Ca^{2+}]_i$ transients. Figure 5 shows a typical result in which Ap_3A-induced $[Ca^{2+}]_i$ oscillations were promptly abolished by the co-application of 5 nM PDB. As a control, the inactive phorbol ester 4α-PDB (10 nM) had no effect on Ap_3A-induced $[Ca^{2+}]_i$ oscillations (3/3 hepatocytes).

As described previously, ATP-induced $[Ca^{2+}]_i$ 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

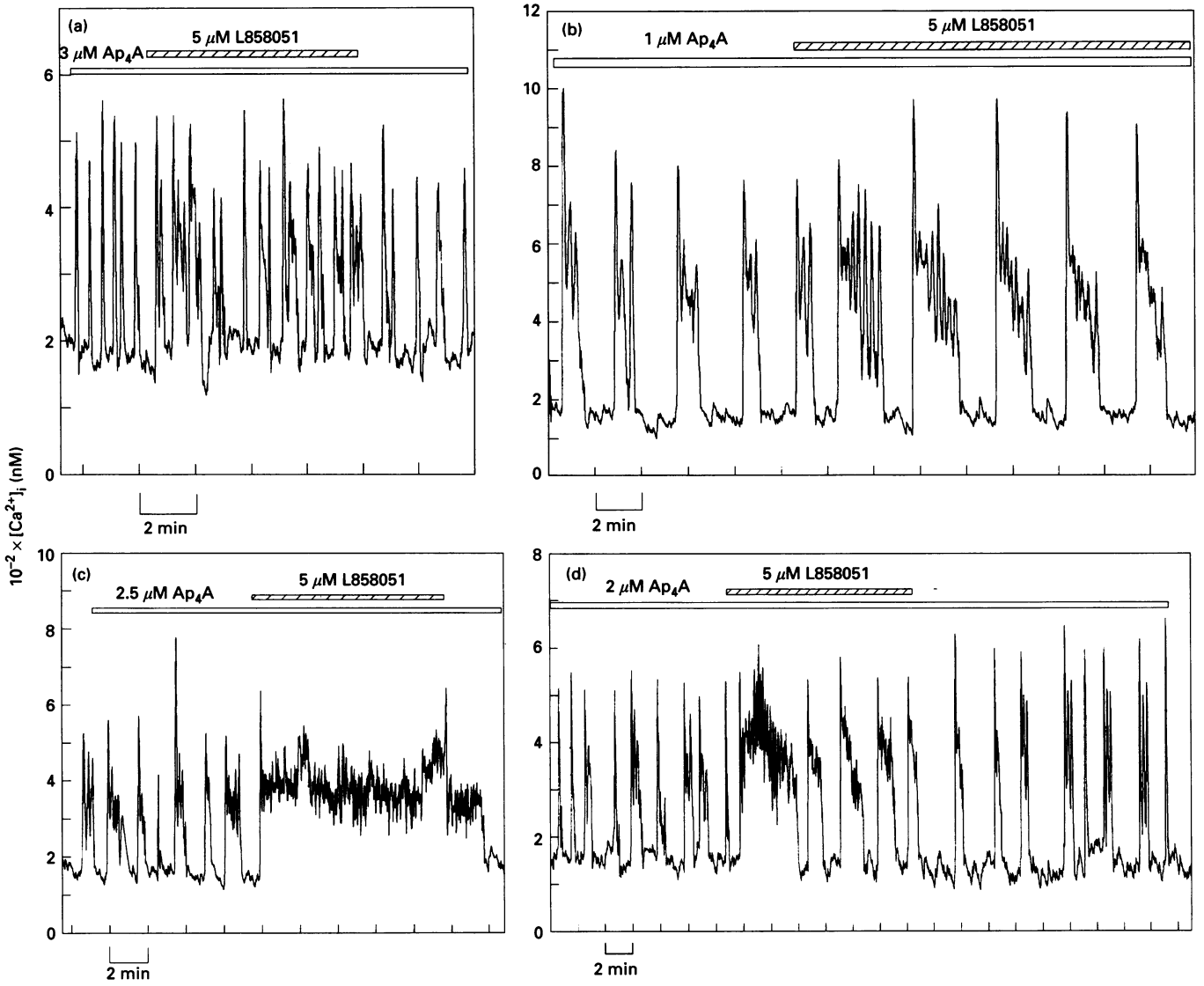

Figure 4 Elevation of intracellular cyclic AMP levels modulates Ap_4A-induced $[Ca^{2+}]_i$ oscillations via either an increase in the duration of individual transients or a sustained rise in $[Ca^{2+}]_i$

Single aequorin-injected hepatocytes responded to Ap_4A by the generation of (**a**) $[Ca^{2+}]_i$ transients of short duration, (**b**) and (**c**) transients of long duration, and (**d**) transients of variable duration. The intracellular cyclic AMP concentration was elevated by the co-application of 5 μM L858051 for the periods indicated. Time constants: for resting $[Ca^{2+}]_i$, 10 s; for transients, 1 s.

Ap_4A-induced $[Ca^{2+}]_i$ oscillations. The co-application of 5 nM PDB to 15/16 hepatocytes generating $[Ca^{2+}]_i$ transients of long duration in response to Ap_4A 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 $[Ca^{2+}]_i$ oscillations [35], there was a small decrease in the duration of individual long Ap_4A-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 Ap_4A-induced oscillations in the majority of hepatocytes; 11/13 hepatocytes exposed to 25 nM PDB continued to generate long Ap_4A-induced $[Ca^{2+}]_i$ 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 Ap_4A-induced $[Ca^{2+}]_i$ oscillations were resistant to 25 nM PDB, long Ap_4A-induced $[Ca^{2+}]_i$ oscillations in seven hepatocytes were similarly resistant to the co-application of 100 nM PDB. (In the remaining 3/10 hepatocytes the Ap_4A-induced transients were abolished by the co-application of 100 nM PDB.) Figure 6 shows a typical result in which long Ap_4A-induced $[Ca^{2+}]_i$ oscillations were resistant to the co-application of PDB (5–100 nM).

The resistance of long ATP-induced $[Ca^{2+}]_i$ oscillations and long Ap_4A-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 Ap_3A-induced oscillations. Mechanisms by which receptor-specific regulation of the hepatocyte $[Ca^{2+}]_i$ 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 $[Ca^{2+}]_i$ responses to ATP and Ap_4A

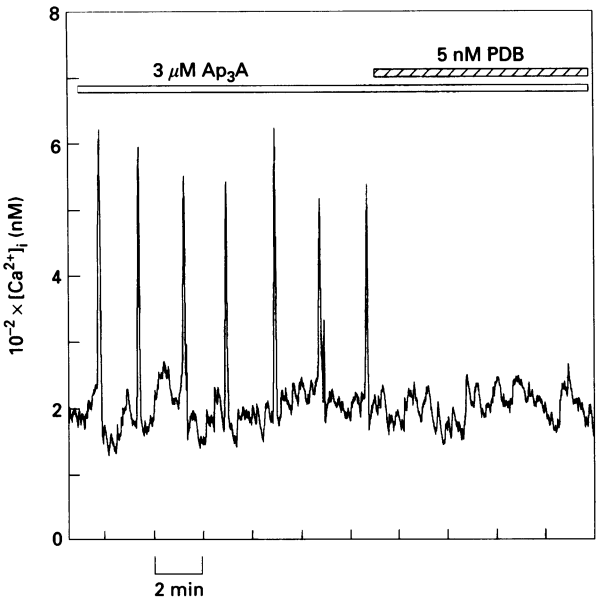

Figure 5 Ap_3A-induced $[Ca^{2+}]_i$ oscillations are inhibited by low concentrations of PDB

A single aequorin-injected hepatocyte responding to Ap_3A by the generation of $[Ca^{2+}]_i$ oscillations was co-supplied with 5 nM PDB for the period indicated. Time constants: for resting $[Ca^{2+}]_i$, 10 s; for transients, 1 s.

exhibiting a marked difference from oscillations induced by other Ca^{2+}-mobilizing agonists. This observation provides further evidence in support of our proposal that ATP and Ap_4A act to mobilize Ca^{2+} in an identical manner, conceivably to the extent of sharing the same receptor(s).

We hypothesize that the $[Ca^{2+}]_i$ oscillations induced by Ap_3A and Ap_4A 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 $[Ca^{2+}]_i$ oscillations observed here in response to Ap_3A and Ap_4A, 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 Ap_3A and Ap_4A. We consider this unlikely, since, in contrast with ATP, which is completely degraded to adenosine in 30 s, Ap_4A 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 Ap_3A or Ap_4A 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‴-P^1,P^3-(P^1-thio)triphosphate (mixed isomers) and (S_pS_p)diadenosine 5′,5‴-P^1,P^4-dithiotetraphosphate, phosphorothioate analogues of Ap_3A and Ap_4A respectively, induce $[Ca^{2+}]_i$ oscillations in single rat hepatocytes [26]. Compared with Ap_3A and Ap_4A, these analogues show a much slower rate of cleavage by specific Ap_3A and Ap_4A hydrolases and non-specific phosphodiesterases [41]. It is thus apparent that the intact diadenosine polyphosphates are able to induce $[Ca^{2+}]_i$ oscillations in single rat hepatocytes.

The ability to respond to Ap_3A and Ap_4A may represent an important physiological role for the ADP and ATP receptors on rat 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 Ap_3A and Ap_4A. Ap_3A and Ap_4A 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, Ap_3A and Ap_4A 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*, Ap_3A and Ap_4A may have a more important role than ADP and ATP as extracellular effectors, particularly at targets remote from the release site. Indeed, Ap_3A and Ap_4A may represent the true physiological agonists of the previously postulated ADP, ATP_S and ATP_L receptors [27,34,35].

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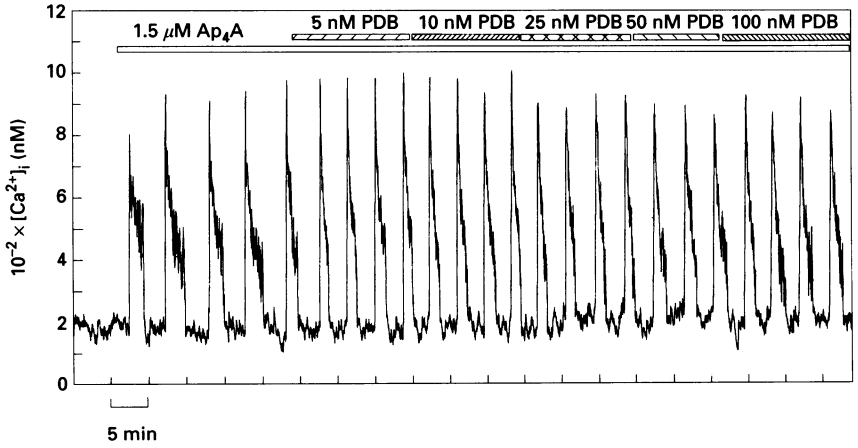

Figure 6 Ap_4A-induced $[Ca^{2+}]_i$ oscillations of long duration are not inhibited by PDB

A single aequorin-injected hepatocyte responding to Ap_4A by the generation of $[Ca^{2+}]_i$ oscillations of long duration was co-supplied with PDB (5–100 nM) for the periods indicated. Time constants: for resting $[Ca^{2+}]_i$, 10 s; for transients, 1 s.

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