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Characterization of the effects of 2-methylthio-ATP and 2-chloro-ATP on brain capillary endothelial cells: similarities to ADP and differences from ATP

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1 Brain capillary endothelial cells responded to 2-methylthio-ATP (2MeSATP) by large increases in $[Ca^{2+}]_i$ (EC_{50} = 27 nM) that were partially dependent on the presence of extracellular Ca^{2+} and that were not associated with a measurable production of inositol phosphates.

2 2-chloro-ATP (2ClATP) raised $[Ca^{2+}]_i$ in a biphasic manner. At low concentrations, intracellular Ca^{2+} mobilization was not associated with a measurable production of inositol phosphates. At concentrations > 30 μM, 2ClATP activated phospholipase C.

3 The actions of 2ClATP, 2MeSATP and ADP on $[Ca^{2+}]_i$ were additive to those of ATP and UTP. Non-additive actions of 2MeSATP and of low concentrations of ADP or of 2ClATP were observed.

4 Cross desensitizations of the actions of ADP, 2MeSATP and 2ClATP were observed. None of them desensitized cells to the action of ATP.

5 It is concluded that 2MeSATP and low concentrations of 2ClATP and ADP induce intracellular Ca^{2+} mobilization by acting via an atypical P_{2y} purinoceptor that is not coupled to phospholipase C. At high concentrations, 2ClATP also activates phospholipase C and further increases $[Ca^{2+}]_i$ probably by acting on P_{2u} purinoceptors.

Keywords: Blood brain barrier; purinoceptor; 2-chloro-ATP; 2MeSATP; 2-methylthio-ATP

Introduction

Phosphorylated adenine nucleotides play an important role in modulating a variety of cellular functions in the cardiovascular system (Pirotton *et al.*, 1993; Gordon, 1986). Their action is mediated via at least five distinct P_2 purinoceptors (Burnstock & Kennedy, 1985; Gordon, 1986). P_{2x} receptors are receptor operated channels that are mainly found in vascular smooth muscle cells. P_{2y} receptors are phospholipase C-coupled receptors that are more specific for 2-methylthio-ATP (2MeSATP) than for adenosine 5′ triphosphate (ATP) and that are found in aortic endothelial cells. P_{2t} receptors are adenosine 5′ diphosphate (ADP)-specific receptors of platelets. P_{2z} receptors specifically recognize the tetrabasic form of ATP and are responsible for the permeabilizing effects of ATP in various transformed cell lines. Finally the recently identified P_{2u} receptors recognize ATP and uridine 5′ triphosphate (UTP) and couple to phospholipase C (O'Connor *et al.*, 1991). The structures of P_{2y} and P_{2u} receptors have recently been elucidated. Both receptors belong to the family of G protein coupled, seven transmembrane domain receptors (Lustig *et al.*, 1993; Webb *et al.*, 1993).

In a previous study we analyzed the responses of rat brain capillary endothelial cells (BCEC) to nucleotides and provided evidence for the presence of two types of receptors for nucleotides: (i) a P_{2u}-like receptor coupled to phospholipase C and (ii) an ADP-specific receptor the occupancy of which induced a mobilization of intracellular Ca^{2+} stores without activation of phospholipase C (Frelin *et al.*, 1993). In this paper we analyze the actions of 2MeSATP and 2-chloro-ATP (2ClATP) on BCEC. 2MeSATP is a well known agonist of P_{2y} receptors in aortic endothelial cells (Motte *et al.*, 1993; Wilkinson *et al.*, 1993). 2ClATP and 2MeSATP are antagonists of ADP responses mediated by P_{2t} receptors in platelets (Cusack & Hourani, 1982; Hall & Hourani, 1993). 2ClATP is also a potent P_{2y} agonist (Needham *et al.*, 1987).

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Methods

BCEC were prepared as previously described (Vigne *et al.*, 1989). Cells were grown in Dulbecco's modified Eagle's medium (GIBCO) supplemented with 10% foetal calf serum (Dutscher, Strasbourg, France), 100 units ml^{-1} penicillin and 100 μg ml^{-1} streptomycin. For intracellular Ca^{2+} measurements, cells were loaded with 5 μM indo-1/AM for 2 h in complete culture medium at 37°C. After dissociation from the culture dishes, cells were centrifuged at low speed and suspended into an Earle's salt solution (composition, mM: NaCl 140, KCl 5, $CaCl_2$ 1.8, $MgSO_4$ 0.8, glucose 5, buffered at pH 7.4 with N-2-hydroxyethylpiperazine-N′-2-ethane sulphonic acid-NaOH 25). Flow cytometric analysis of the indo-1 fluorescence was performed as described previously (Frelin *et al.*, 1993) using a FacStar Plus (Becton-Dickinson). Indo-1 fluorescence ratio were measured in single cells and collected in real time at a rate of 500 cells s^{-1}. Means of 1000–3000 individual measurements were computed and collected at different times following the addition of agonists. The values of the fluorescence ratio given by the computer are arbitrary values. The technique, because it reduces the incidence of the cell to cell variability in the responses, is well suited for a pharmacological analysis of the action of Ca^{2+} mobilizing agents (Frelin *et al.*, 1993). To define dose-response curves for agonists, cells were treated with the desired concentration of agonists and analyzed. The indo-1 fluorescence ratio of 1000 cells, sampled during 1.5 s, 20 s after the addition of agonists were collected and the mean ratio computed. Each experiment was repeated at least three times. Low $[Ca^{2+}]$ (50 nM) solutions were prepared using 0.5 mM $CaCl_2$ and 1.2 mM ethyleneglycol *bis*-(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid. All experiments were performed at 20°C.

To measure the production of inositol phosphates, BCEC were grown to confluency in six well plates and labelled to equilibrium with 2 μCi ml^{-1} of *myo*-[2-^{3}H]-inositol in complete culture medium. After washing with Earle's salt solution, cells were incubated for 10 min at 37°C in a 110 mM NaCl, 40 mM LiCl modified Earle's salt solution. After 5 min

of exposure to agonists, the radioactivity incorporated into total inositol phosphates (InsP) was determined as previously described (Frelin *et al.*, 1993).

Proteins were determined according to Bradford (1976).

Means ± s.e.mean are shown. When no error bar is presented in the Figures, it was smaller than the size of the points. Dose-response curves were fitted to a four parameter logistic function using the SigmaPlot software.

Materials

2ClATP and 2MeSATP were purchased from Research Biochemicals Inc. Other nucleotides were from the Sigma Chemical Co. Indo-1/AM was from Calbiochem. Endothelin-1 was from Neosystems (Strasbourg, France). *myo*-[2-^{3}H] inositol (19 Ci mmol^{-1}) was from Amersham.

Results

Figure 1a shows that the addition of 1 μM 2MeSATP to BCEC increased $[Ca^{2+}]_i$ in a manner partially dependent on the presence of external Ca^{2+}. The dose-response curve for the action of 2MeSATP on $[Ca^{2+}]_i$ is presented in Figure 1b. It was monophasic with an EC_{50} value of 27 ± 4 nM. Figure 2a shows that like 2MeSATP, 2ClATP raised $[Ca^{2+}]_i$ in a manner partially dependent on the presence of external Ca^{2+}. The dose-response curve for 2ClATP action on $[Ca^{2+}]_i$ is shown in Figure 2b. It extended over at least five orders of magnitude and showed a marked shoulder at 3 to 10 μM.

We next measured the production of total inositol phosphates. Figure 3 shows that 2MeSATP had no effect on InsP production up to the highest concentration tested (1 mM).

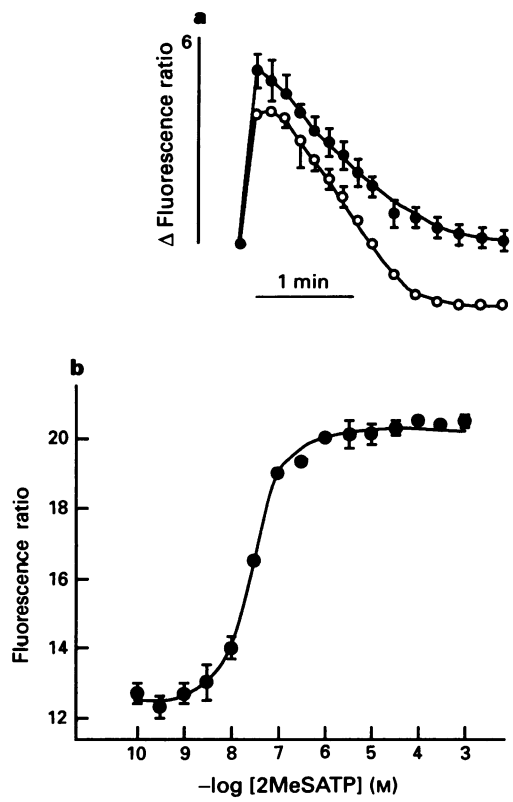

Figure 1 The action of 2-methylthio-ATP (2MeSATP) on $[Ca^{2+}]_i$: (a) Changes in indo-1 fluorescence ratio observed after the addition of 1 μM 2MeSATP. Experiments were performed in the presence of 1.8 mM Ca^{2+} (●) or of 50 nM Ca^{2+} (○). (b) Dose-response curve for the action of 2MeSATP on $[Ca^{2+}]_i$. Mean indo-1 fluorescence ratio was measured 20 s after the addition of the nucleotide to the cells. Means ± s.e. ($n = 3$) are shown.

Similarly, 2ClATP had no action up to concentrations of 10 μM. At higher concentrations, 2ClATP increased InsP production up to 1.8 fold. Under the same conditions ATP (30 μM) and endothelin-1 (100 nM) increased InsP production 2 and 2.6 fold (Figure 3). An obvious possibility for these results is that 2ClATP had two distinct actions on BCEC. At low concentrations it increased $[Ca^{2+}]_i$ in the absence of

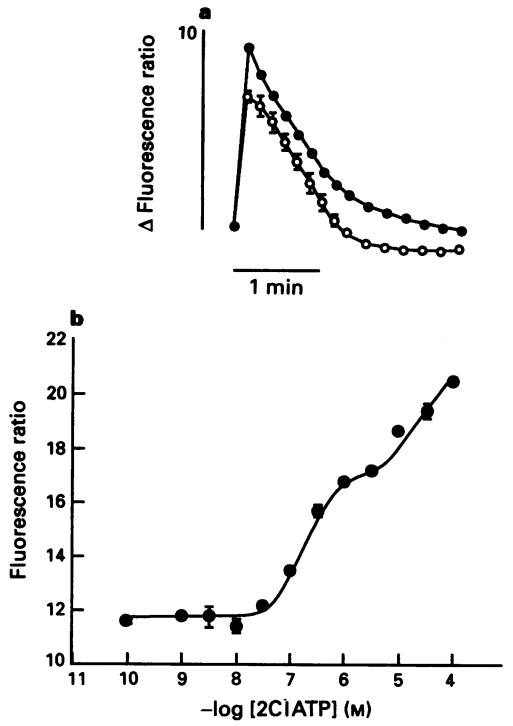

Figure 2 The action of 2-chloro-ATP (2ClATP) on $[Ca^{2+}]_i$: (a) Changes in indo-1 fluorescence ratio observed after the addition of 1 μM 2ClATP. Experiments were performed in the presence of 1.8 mM Ca^{2+} (●) or of 50 nM Ca^{2+} (○). (b) Dose-response curve for the action of 2ClATP on $[Ca^{2+}]_i$. Mean indo-1 fluorescence ratio were measured 20 s after the addition of the nucleotide to the cells. Means ± s.e. ($n = 3$) are shown.

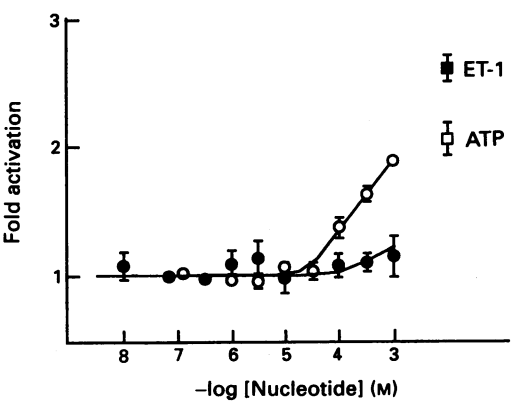

Figure 3 The action of 2-methylthio-ATP (2MeSATP) and 2-chloro-ATP (2ClATP) on inositol phosphates (InsP) production. Cells were exposed for 5 min to the indicated concentrations of 2MeSATP (●) and 2ClATP (○) and the production of total InsP was measured as described in Methods. The figure also shows for comparison the effects of 100 nM endothelin-1 (ET-1) and of 30 μM ATP in the same cells. Means ± s.e. ($n = 8$) are shown. It was checked that the clones used in these experiments responded to low concentrations of 2MeSATP and 2ClATP by large changes in $[Ca^{2+}]_i$.

measurable InsP production. At high concentrations (>30 μM) it further increased $[Ca^{2+}]_i$ by activating phospholipase C. Very similar results have been previously reported for ADP (Frelin *et al.*, 1993).

A useful way to assess receptor heterogeneity is to look for additive responses. Additive actions of maximally effective concentrations of agonists are expected if they bind to different receptor sites. Non additive actions are expected if they bind to the same receptor. They are also expected if they bind to different sites but maximally increase $[Ca^{2+}]_i$ by depleting intracellular Ca^{2+} stores. However, one difficulty with this approach is that ADP and 2ClATP which have a complex action of $[Ca^{2+}]_i$ (e.g. Figure 2) may recognize more than one type of receptor. For this reason, dose-response curves for each nucleotide were established in the presence of fixed concentrations of 2MeSATP, 2ClATP or ADP. In a first series of experiments, cells were treated at the same time with 1 μM 2MeSATP and different concentrations of ATP, UTP, ADP or 2ClATP. At a concentration of 1 μM, 2MeSATP induced maximum InsP-independent intracellular Ca^{2+} changes (Figure 1b). Figure 4 shows that ATP and UTP still increased $[Ca^{2+}]_i$ in the presence of 2MeSATP. EC_{50} values were 7.9 ± 1.4 μM and 4.3 ± 1.9 μM for ATP and UTP respectively. These values were close to the EC_{50} values measured in the absence of 2MeSATP (ATP: 10 μM, UTP: 15 μM; Frelin *et al.*, 1993). Thus the presence of 2MeSATP hardly altered the responses to ATP and UTP. Figure 4 further shows that in the presence of 1 μM 2MeSATP, ADP and 2ClATP increased $[Ca^{2+}]_i$ but only at concentrations >10 μM. EC_{50} values were 0.15 ± 0.02 mM and 0.19 ± 0.06 mM for ADP and 2ClATP respectively. A conspicious effect of 2MeSATP was to transform the biphasic action of 2ClATP (Figure 2b) into a monophasic action (Figure 4). It suppressed the actions of low concentrations of 2ClATP that are independent of the formation of InsP and left unaltered the action of large concentrations of 2ClATP that are associated with an activation of phospholipase C. 2MeSATP also transformed the biphasic action of ADP into a monophasic, InsP-associated action.

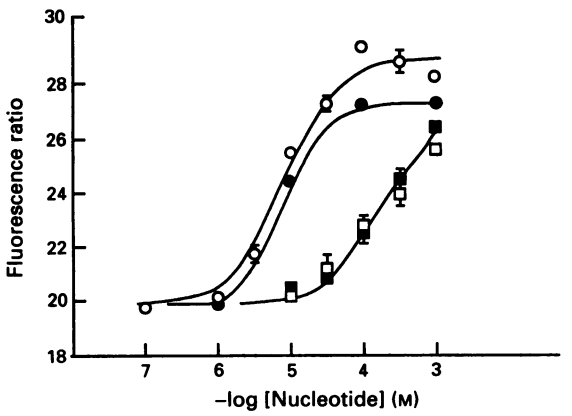

Figure 4 The action of nucleotides on $[Ca^{2+}]_i$ in the presence of 2-methylthio-ATP (2MeSATP). Cells were exposed at the same time to 1 μM 2MeSATP and different concentrations of ATP (○), UTP (●), ADP (■) and 2-chloro-ATP (2ClATP) (□). The mean indo-1 fluorescence ratio was measured 20 s later and plotted as a function of the concentration of nucleotide used. The mean indo-1 fluorescence ratio in the absence of agonists was 10. Means ± s.e. ($n = 3$) are shown. All experiments shown were performed on the same batch of cells and are directly comparable.

Figure 5 shows the results of similar experiments performed in the presence of 1 μM 2ClATP. At this concentration, 2ClATP increased $[Ca^{2+}]_i$ to a large extent (Figure 2b) but had no action on the formation of InsP (Figure 3). In the presence of 1 μM 2ClATP, ATP and UTP still increased $[Ca^{2+}]_i$. EC_{50} values were 21 ± 2 μM and 13 ± 3 μM for ATP and UTP respectively, close to the values previously observed in the absence of 2ClATP. Thus low concentrations of 2ClATP hardly modified the responses to ATP and UTP. Figure 5 further shows that 2ClATP almost completely suppressed the action of 2MeSATP. It also prevented low concentrations (<10 μM) of ADP from raising $[Ca^{2+}]_i$ and transformed its biphasic action into a monophasic action. The EC_{50} value for the action of ADP on $[Ca^{2+}]_i$ in the presence of 2ClATP was 0.24 ± 0.03 mM. At these concentrations, ADP induced a measurable production of InsP (Frelin *et al.*, 1993).

Figure 6 shows the results of similar experiments performed in the presence of 10 μM ADP. This concentration induced maximum InsP-independent Ca^{2+} mobilization (Frelin *et al.*, 1993). As previously reported (Frelin *et al.*, 1993), ADP did not prevent ATP from increasing $[Ca^{2+}]_i$, thus indicating additive actions. The EC_{50} value for the action of ATP in the presence of ADP was 11 ± 2 μM. Figure 6 further shows that ADP completely suppressed the actions of

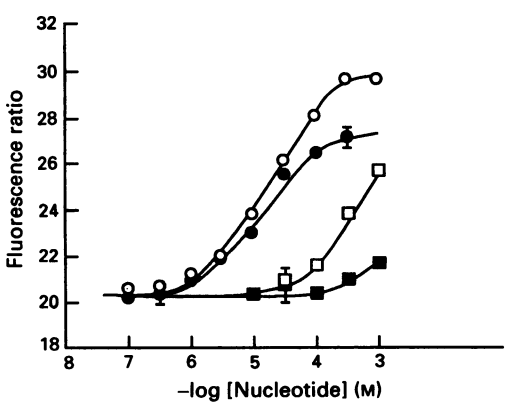

Figure 5 The action of nucleotides on $[Ca^{2+}]_i$ in the presence of 2-chloro-ATP (2ClATP). Cells were exposed at the same time to 1 μM 2ClATP and different concentrations of ATP (○), UTP (●), ADP (□) and 2-methylthio-ATP (2MeSATP) (■). The mean indo-1 fluorescence ratio was measured 20 s later and plotted as a function of the concentration of nucleotide used. The mean indo-1 fluorescence ratio in the absence of agonists was 10. Means ± s.e. ($n = 3$) are shown. All experiments shown were performed on the same batch of cells and are directly comparable.

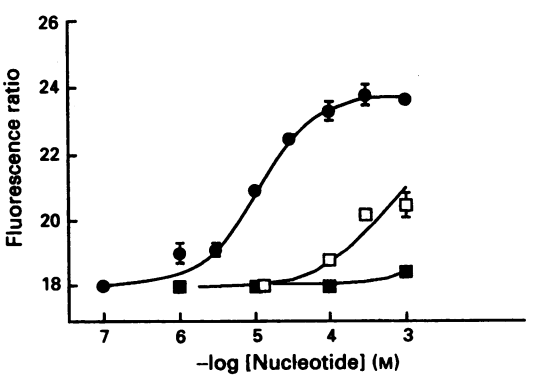

Figure 6 The action of nucleotides on $[Ca^{2+}]_i$ in the presence of ADP. Cells were exposed at the same time to 10 μM ADP and different concentrations of ATP (●), 2-methylthio-ATP (2MeSATP) (■) and 2-chloro-ATP (2ClATP) (□). The mean indo-1 fluorescence ratio was measured 20 s later and plotted as a function of the concentration of nucleotide used. The mean indo-1 fluorescence ratio in the absence of agonists was 10. Means ± s.e. ($n = 3$) are shown. All experiments shown were performed on the same batch of cells and are directly comparable.

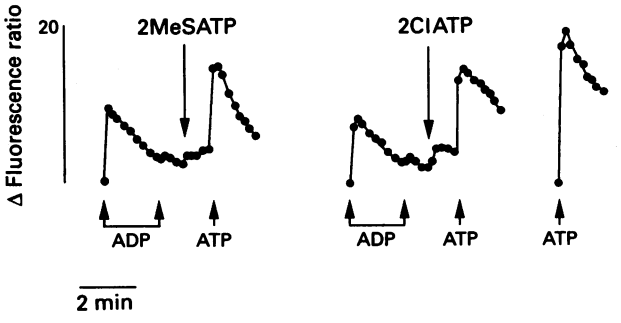

Figure 7 ADP desensitized cells to the actions of 2-methylthio-ATP (2MeSATP) and 2-chloro-ATP (2ClATP) but not to that of ATP. Cells were exposed twice to 3 μM ADP and then to 1 μM 2MeSATP, 1 μM 2ClATP and 0.3 mM ATP as indicated and changes in the indo-1 fluorescence ratio were monitored. The right panel shows for comparison, a Ca^{2+} transient obtained in the same batch of cells using 0.3 nM ATP. Each point was the mean of 1000 individual cell measurements.

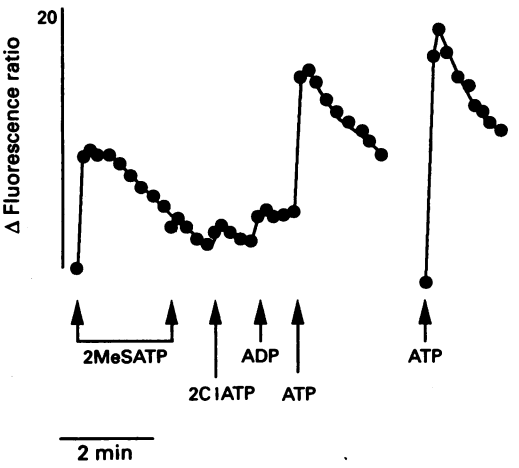

Figure 8 2-Methylthio-ATP (2MeSATP) desensitized cells to the actions of ADP and 2-chloro-ATP (2ClATP) but not to that of ATP. Cells were exposed twice to 1 μM 2MeATP and then to 1 μM 2ClATP, 3 μM ADP and 0.3 mM ATP as indicated and changes in the indo-1 fluorescence ratio were monitored. The right panel shows for comparison, a Ca^{2+} transient obtained in the same batch of cells using 0.3 mM ATP. Each point was the mean of 1000 individual cell measurements.

2MeSATP and of low concentrations of 2ClATP. ADP did not prevent large (>30 μM) concentrations of 2ClATP from raising $[Ca^{2+}]_i$. At these concentrations, 2ClATP induced a measurable production of InsP (Figure 3).

Taken together, the results presented in Figures 4 to 6 indicated that 2MeSATP, 2ClATP and ADP acted in very similar ways. They hardly modified the cellular responses to ATP and UTP. They prevented their high affinity, InsP independent responses but had no action on the low affinity responses, associated with a measurable production of InsP.

Another way to assess receptor heterogeneity is to perform desensitization experiments. We previously reported that ADP desensitized cells still responded to ATP (Frelin *et al.*, 1993). Figure 7 shows that a first treatment of BCEC with 3 μM ADP desensitized cells to further applications of ADP and also to the applications of 2MeSATP and 2ClATP. Yet desensitized cells still responded to 0.3 mM ATP by a large $[Ca^{2+}]_i$ transient. Figure 8 further shows that cells that had been treated with 1 μM 2MeSATP no longer responded to 1 μM 2ClATP or to 3 μM ADP, yet they retained large responses to 0.3 mM ATP. Finally, Figure 9 shows that 1 μM 2ClATP-treated cells were desensitized to the actions of

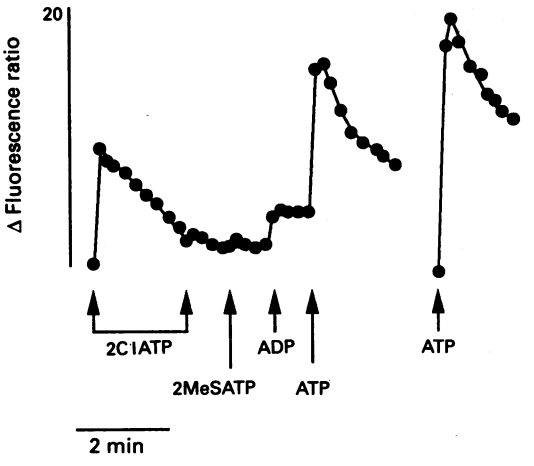

Figure 9 2-Chloro-ATP (2ClATP) desensitized cells to the actions of 2-methylthio-ATP (2MeSATP) and ADP but not to that of ATP. Cells were exposed twice to 1 μM 2ClATP and then to 1 μM 2MeATP, 3 μM ADP and 0.3 mM ATP as indicated and changes in the indo-1 fluorescence ratio were monitored. The right panel shows for comparison, a Ca^{2+} transient obtained in the same batch of cells using 0.3 mM ATP. Each point was the mean of 1000 individual cell measurements. Experiments shown in Figures 7–9 were performed on the same batch of cells and are therefore directly comparable. They are representative of three independent experiments.

2MeSATP and ADP but not to that of ATP. Thus cross desensitizations of the actions of ADP, 2MeSATP and 2ClATP were observed. None of these substances desensitized cells to the action of ATP.

Discussion

2MeSATP is a potent agonist of P_{2y} receptors in aortic endothelial cells (Motte *et al.*, 1993; Wilkinson *et al.*, 1993) and an antagonist of P_{2t} receptors of platelets (Hall & Hourani, 1993). Conversely, 2ClATP is an agonist of P_{2y} receptors (Needham *et al.*, 1987), a weak agonist of P_{2u} receptors (Lustig *et al.*, 1993) and an antagonist of P_{2t} receptors (Cusack & Hourani, 1982; Hall & Hourani, 1993). We previously described the presence in rat BCEC of two distinct receptor sites for nucleotides: (i) a P_{2u} purinoceptor for ATP and UTP and (ii) an ADP receptor that induced the mobilization of thapsigargin-sensitive intracellular Ca^{2+} stores in the absence of formation of inositol phosphates (Frelin *et al.*, 1993). This paper defines the actions of 2MeSATP and 2ClATP in BCEC and shows that the two nucleotides act in a manner similar to that of ADP and distinct from that of ATP.

Evidence that 2MeSATP and low concentrations of ADP have similar actions is as follows: (i) 2MeSATP (Figure 3) and low concentrations of ADP (Frelin *et al.*, 1993) induce intracellular Ca^{2+} mobilization in the absence of measurable InsP production. (ii) 2MeSATP hardly alters the cell responses to ATP and UTP but it prevents the action of low concentrations of ADP (Figure 4). Conversely ADP prevents the action of 2MeSATP but not that of ATP (Figure 6). (iii) 2MeSATP and ADP desensitize each other's action. 2MeSATP and ADP do not desensitize cells to the action of ATP (Figure 7–9).

Evidence that low concentrations of 2ClATP and ADP act similarly are as follows: (i) Low concentrations of 2ClATP (Figures 2 and 3) and ADP (Frelin *et al.*, 1993) induce intracellular Ca^{2+} mobilization in the absence of measurable InsP production. (ii) Low concentrations of 2ClATP do not prevent ATP or UTP from raising $[Ca^{2+}]_i$ with EC_{50} values

close to those observed in the absence of 2ClATP (Figure 5). Low concentrations of 2ClATP prevent the action of low concentrations of ADP (Figure 4). Conversely ADP prevents the action of low concentrations of 2ClATP but not that of ATP (Figure 6). (iii) 2ClATP and ADP desensitize each other's action. 2ClATP and ADP do not desensitize cells to the action of ATP (Figures 7–9).

The close similarities of the actions of 2MeSATP and of 2ClATP suggest that they may recognize the same receptor. The observations that 2MeSATP and low concentrations of 2ClATP have no additive actions (Figures 4 and 5) and desensitize each other's action (Figures 8 and 9) are fully consistent with this hypothesis.

Very similar properties have previously been reported in rat hepatocytes which respond to ATP, UTP, 2MeSATP and ADP by large increases in $[Ca^{2+}]_i$ and by increased glycogenolytic rates. First ATP and ADP induce different intracellular $[Ca^{2+}]_i$ oscillations (Dixon *et al.*, 1990). Second, while the actions of ATP and UTP are clearly associated to an activation of phospholipase C (Keppens *et al.*, 1992), those of ADβS and 2MeSATP are not (Keppens & DeWulf, 1991; Keppens *et al.*, 1993). Finally, it is worth noting that the EC_{50} value for the action of 2MeSATP on glycogen phosphorylase in hepatocytes (20 nM, Keppens & DeWulf, 1991) is close to the EC_{50} value for the action of 2MeSATP on $[Ca^{2+}]_i$ in BCEC (27 nM, Figure 1b). This could suggest that a similar receptor is expressed by rat BCEC and by rat hepatocytes. This receptor recognises low concentrations of 2MeSATP, 2ClATP and ADP and it induces intracellular Ca^{2+} mobilization in the absence of formation of inositol phosphates. Its pharmacological profile, defined from the InsP-independent actions of nucleotides on $[Ca^{2+}]_i$ is: 2MeSATP (EC_{50} = 27 nM, Figure 1b) > 2ClATP (EC_{50} ~0.3 μM, Figure 2b) > ADP (EC_{50} ~1 μM; Frelin *et al.*, 1993). This receptor although pharmacologically similar to P_{2y} receptors differs from known P_{2y} purinoceptors in that it is not coupled to phospholipase C. It differs from P_{2t} purinoceptors for 2ClATP and 2MeSATP act as agonists rather than as antagonists. It is unlikely a P_{2x} or P_{2z} purinoceptor for changes in $[Ca^{2+}]_i$ induced by 2MeSATP and 2ClATP are not suppressed when the external Ca^{2+} concentration is lowered (Figures 1 and 2) and mostly result from the mobilization of intracellular stores. This is also suggested by the observation that α, β methylene ATP, a potent agonist of P_{2x} receptors is inactive on BCEC (Frelin *et al.*, 1993). 2MeSATP is usually considered as a high affinity and specific agonist of P_{2y} receptors and is thought to act primarily by activating phospholipase C (Motte *et al.*, 1993; Wilkinson *et al.*, 1993; Webb *et al.*, 1993). It is worth noting that at low concentrations 2MeSATP may also act in a manner independent of the formation of inositol phosphates both in rat BCEC (this study) and in rat hepatocytes (Keppens & DeWulf, 1991).

At high concentrations, 2ClATP increases $[Ca^{2+}]_i$ in parallel to an activation of phospholipase C (Figures 2 and 3). The properties of the receptor involved can be defined from the experiments presented in Figures 5 to 7 in which the InsP-independent responses were masked by the use of low concentrations of ADP, 2MeSATP or 2ClATP. Under these conditions ATP (EC_{50} = 7–21 μM), UTP (EC_{50} = 4–13 μM), 2ClATP (EC_{50} = 0.19 mM) and ADP (EC_{50} = 0.15–0.24 mM) increase $[Ca^{2+}]_i$. Similar values have been found for their action on phospholipase C activity (ATP: 10 μM, UTP: 15 μM, 2ClATP: >0.1 mM, ADP: >0.1 mM) (Frelin *et al.*, 1993, Figure 2). Both profiles are typical of a P_{2u} receptor (Fine *et al.*, 1989; Pfeilschrifter, 1990; Brown *et al.*, 1991; O'Connor *et al.*, 1991; Lustig *et al.*, 1993; Raha *et al.*, 1993), suggesting that the InsP-dependent action of nucleotides was mediated by P_{2u} purinoceptors. Further evidence for the presence of a P_{2u} receptor on BCEC is provided by the observation that ATP and UTP have non-additive and cross-desensitizing actions (Frelin *et al.*, 1993). Finally, clear evidence that the P_{2u} receptor is distinct from the ADP receptor described above is that some of the cell clones used in this study responded to ATP and UTP but not to low concentrations of 2ClATP or of 2MeSATP (data not shown).

In conclusion, the results presented in this paper provide further support for the presence of at least two types of purinoceptors in rat BCEC: a P_{2u} purinoceptor and an atypical P_{2y} receptor that induces intracellular Ca^{2+} mobilization via a phospholipase C independent mechanism.

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