Volume 264, number 1, 130–134 FEBS 08412 May 1990

Extracellular ATP activates receptor-operated cation channels in mouse lacrimal acinar cells to promote calcium influx in the absence of phosphoinositide metabolism

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Received 23 March 1990

In exocrine acinar cells a variety of neurotransmitters (e.g. acetylcholine) stimulate phosphatidylinositol 4,5-bisphosphate hydrolysis elevating intracellular calcium to activate calcium-dependent membrane currents (outward K^+ and inward Cl^-). This study shows that in lacrimal acinar cells extracellular application of ATP is also associated with outward and inward current responses; these, however, are not the result of phosphoinositide metabolism. ATP directly activates receptor-operated cation channels which permit influx of Na^+ and Ca^+ (the inward current). The elevation in $[Ca^{2+}]_i$ which results is sufficient to activate the outward K^+ current. ATP thus promotes Ca^+ influx in the absence of phosphoinositide metabolism.

Extracellular ATP; Purinergic receptor; Receptor-operated channel; Calcium influx; Lacrimal acinar cell

1. INTRODUCTION

It is now recognised for exocrine acinar cells, as for a wide range of electrically unexcitable tissues, that the calcium-mobilizing effects of a number of different receptors, such as the cholinergic muscarinic or α-adrenergic, are due to the activation of a common transduction mechanism which regulates the hydrolysis of phosphatidylinositol 4,5-bisphosphate to yield the two second messengers, inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG) [1,2]. The inositol polyphosphate (IP_3) and the product of its phosphorylation inositol 1,3,4,5-tetrakisphosphate (IP_4) have been implicated as intracellular second messengers regulating for both the release of calcium from internal stores and calcium influx from the extracellular to intracellular fluid in the rodent lacrimal acinar cells [3–6]. A number of years ago the first evidence was presented suggesting that extracellular adenosine triphosphate (ATP) might also act as a calcium-mobilizing agonist in exocrine acinar cells [7]. ATP was shown to promote a range of responses which were dependent on the presence of calcium in the extracellular medium. At that time the role of the inositol polyphosphates in control of calcium release from internal stores and influx from extracellular fluid was not established but the conclusion was that ATP acting on P_2 type purinergic receptors was most probably activating an identical mechanism to that operated by the cholinergic muscarinic and α-adrenergic receptors. There are reports of P_2 purinergic receptor regulation of PIP_2 hydrolysis [8,9]. More recently a report has appeared [10] which confirms, in rat parotid acinar cells, the changes in membrane conductance that are promoted by extracellular ATP and draws attention to the similarity between the ATP effects and the calcium-dependent current responses evoked by the phosphoinositide-coupled cholinergic muscarinic receptors. The study also directly demonstrated the elevation in cytosolic free calcium concentration ($[Ca^{2+}]_i$) that is associated with ATP stimulation. Surprisingly, however, the study reported that ATP, unlike other calcium-mobilizing agents, had little if any effect on the turnover of phosphatidylinositol. Those authors concluded that ATP increased $[Ca^{2+}]_i$ by a mechanism that was distinct and different from that shared by the classical phospholipase C-coupled receptor agonists. In murine thymocytes ATP gives rise to an elevation in $[Ca^{2+}]_i$ that has been attributed to Ca^{2+} influx in the absence of phosphoinositide metabolism [11]. Benham and Tsien have also reported [12] that ATP can directly activate a receptor-operated Ca^{2+}-permeable channel in vascular smooth muscle. In the present study we use the patch-clamp technique of whole-cell and single channel current recording to investigate in more detail the current response of enzymatically isolated mouse lacrimal acinar cells to extracellular application of ATP. The evidence confirms that ATP does activate a mechanism

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Published by Elsevier Science Publishers B.V. (Biomedical Division)

distinct from that of the other calcium-mobilizing agonists.

2. MATERIALS AND METHODS

Exorbital lacrimal glands were excised from adult male mice and acinar cells enzymatically isolated [4–6]. After dispersion and washing, the cells were resuspended in a standard extracellular saline solution of the following composition (mM): NaCl 140, KCl 4.7, Hepes 10, glucose 10, $MgCl_2$ 1.13, $CaCl_2$ 1.2, at pH 7.2. Whole-cell or cell-attached single channel currents were recorded from single isolated lacrimal acinar cells at room temperature, 22–24°C. A list LM-EPC 7 (List Electronics, Darmstadt) patch-clamp amplifier was employed and the signal displayed on a storage oscilloscope and simultaneously recorded for subsequent analysis on magnetic tape (Racal 4DS, Southampton). The open probability of the channels was determined by digitization of the recordings at 8 kHz (CED 1401, Cambridge, UK) for input to a Tandon microcomputer and analysis of open probabilities (software by CED, Cambridge, UK). The standard intracellular solution contained the following (mM): KCl 140, $MgCl_2$ 1.13, Hepes 10, glucose 10, EGTA 0.5, Na_2ATP 0.5. Any modifications to these standard intracellular and extracellular solutions are indicated in the figure legends.

3. RESULTS AND DISCUSSION

The whole-cell current recording mode was used initially to investigate the membrane current responses of the single lacrimal acinar cells upon stimulation by either ATP at 1 mM or ACh at 1 μM. The threshold concentration for ATP-induced current responses was 200 μM (100 μM was ineffective, $n = 4$). 1 mM ATP was employed throughout this study as a sub-maximal stimulus but one which avoided the desensitization which was pronounced at higher concentrations. Adenosine ($n = 3$) and ADP ($n = 4$) were both ineffective at 1 mM. Quinidine sulphate (1 mM; $n = 6$) abolished [7,13] the outward and inward currents induced by ATP. Fig. 1B shows the experimental protocol employed whereby the current responses of the cells can be monitored at 3 different voltages. ATP and ACh are seen to evoke increases in both the outward (ACh = 790 ± 50 pA; ATP = 305 ± 66 pA) and inward (ACh = 162 ± 20 pA; ATP = 280 ± 50 pA) current components associated with depolarizing and hyperpolarizing pulses, respectively. The responses appear qualitatively very similar as was reported for the rat parotid acinar cells stimulated by the same agonists [10]. When the nature and time course of the responses are examined in more detail, however (Fig. 1C), important differences are to be seen between the current responses to ATP and ACh, respectively. At 0 mV both ATP and ACh evoke outward current responses. At −40 mV ACh still evokes an outward current but ATP now gives rise to an inward current. At −80 mV both agonists give rise to inward currents but that induced by ATP is more rapid in onset than that in response to the ACh. The time for half-maximal activation of the inward currents was significantly different for ACh and ATP; 2.87 ± 0.38 and 0.46 ± 0.06 s, respectively. The current responses to the two agonists clearly indicate that identical mechanisms are not being activated in each case.

Fig. 2 shows that the differences in the current responses of the lacrimal acinar cells to stimulation by ATP and ACh are due to a difference in the permeabilities activated by the two agonists. Both these agonists give rise to an outward current component that is susceptible to blockade by extracellular application of tetraethylammonium (TEA; 10 mM, $n = 8$). The TEA sensitivity of the ACh response is now well characterised in exocrine acinar cells and the outward current is known to be due to activation of the large conductance K^+ channels (Maxi K^+ channels) in the basolateral membranes [14,15]. These channels are activated by the elevation in $[Ca^{2+}]_i$ that results from stimulation of phosphoinositide-coupled receptors. The susceptibility of the ATP-induced outward current to TEA is shown here for the first time and indicates that it is most probably due to activation of the same, Maxi K^+, channels as for ACh. Fig. 2B shows that the ionic mechanisms underlying the inward current responses are not identical for the ATP and ACh responses. The inward current response evoked by ACh is abolished in the absence of Cl^-. It has been shown for lacrimal, as for other exocrine acinar cells [16,17], that the inward current component in response to ACh is due to activation of calcium-dependent Cl^- channels. These Cl^- channels are only activated at higher $[Ca^{2+}]_i$ than the K^+ channels and the Cl^- current response generally lags that of the K^+ current [18] as is seen in the present study. Fig. 2 shows that the inward current component induced by ATP is independent of Cl^-. The inward current in response to ATP persists in the absence of Cl^- and in experiments in which the ratio of internal and external Cl^- were varied there was no correlation between the Cl^- equilibrium potential and the reversal potential of the ATP-induced current response (Cl^- substituted either by sulphate, $n = 9$, or gluconate, $n = 6$). The ATP-induced current response is not then mediated by Cl^-. In 3 experiments the Na^+ in the extracellular bathing solution was replaced by 110 mM Ca^{2+} and ATP (1 mM) still evoked a large inward current of between 0.9 and 1.3 nA. There is a clear difference in the permeability pathways activated by the two different agonists suggesting that they do not utilise identical transduction mechanisms to achieve their effects. This is reflected in Fig. 2C in which the effects of removal of external calcium are investigated. ACh is known to elevate calcium in lacrimal acinar cells by releasing calcium from internal stores and by stimulating calcium influx across the surface membrane. These effects are mediated by the inositol polyphosphate products of PIP_2 hydrolysis. In the absence of extracellular calcium, ACh can evoke a transient inward and outward current response (Fig. 2C) which is due to the release of calcium from internal

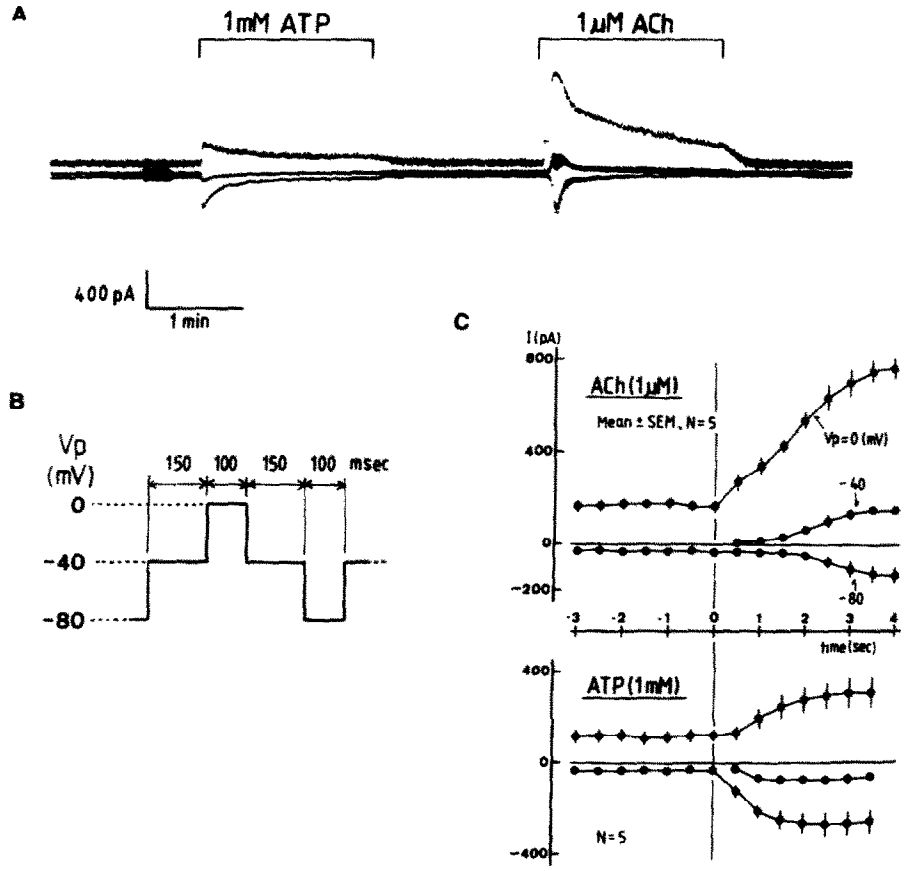

Fig. 1. Whole-cell current recording from single lacrimal acinar cell. The cell is bathed in the standard extracellular solution and the pipette is filled with the standard internal solution. (A) Shows the current responses evoked in the same cell by the extracellular application of ATP and ACh. Outward currents shown as upward, and inward current as downward, deflections. (B) Illustrates the hyperpolarizing and depolarizing pulse protocols imposed from the holding potential of −40 mV. (C) Shows on an expanded time-base the mean time-course, amplitude and direction of the currents evoked by ACh and ATP at the different voltages.

stores. It is also shown in Fig. 2 that in the absence of extracellular calcium, ATP still evokes an inward current but the outward current component is almost if not totally abolished ($n = 12$). This suggests that ATP is incapable of releasing intracellular calcium and that the transduction mechanisms is not that of the phosphoinositide-coupled receptors.

In one series of experiments the effect of internal application of agents which are reported to interfere with PIP_2 hydrolysis were tested. Neomycin (0.1 mM) [19] and compound 48/80 (10 μg/ml) [20] were both included in the solution filling the pipette and dialysing the cell interior. As seen in Fig. 3, both these agents results in the eventual blockade of the current's response to the two phosphoinositide-coupled agonists, ACh and Nad. In the same cells at a time when the ACh and Nad responses are totally blocked the ATP responses persist (neomycin $n = 4$; 48/80 $n = 5$). This indicates that the ATP effects are independent of PIP_2 breakdown.

Single channel currents were recorded from cell-attached patches on lacrimal acinar cells (Fig. 4). Two species of ion channels were recorded. In 16 of 78 patches activity was recorded in a large conductance (127 ± 6 pS at 0 mV) channel which was voltage sensitive and showed under physiological ionic gradients a rectification in the current-voltage relationship and an extrapolated reversal potential of about −60 mV. These findings are entirely consistent with the properties of the now well-characterised Maxi K^+ channel as reported for lacrimal acinar cells [14,15,18]. In 3 out of 18 patches another smaller conductance channel was observed. This channel had a very low open probability at all voltages, had a conductance of about 30 pS and a linear current-voltage plot with a reversal potential of close to 0 mV (Fig. 4). The characteristics of this channel are similar to those of the non-discriminating (equally permeable to both Na^+ and K^+) cation channel which has already been described in this preparation [18]. The open probability of this channel when observed was only 0.019 ± 0.012 ($n = 3$) at a potential of +90 mV. The occurrence and open probability of this channel was, however, very much greater when ATP was included in the solution filling the recording pipette, i.e. bathing the outside surface of the mem-

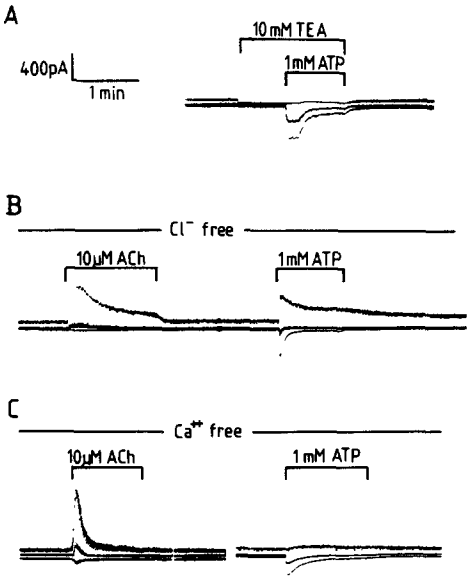

Fig. 2. (A) Whole-cell current response to ATP with 10 mM TEA in the standard external solution. The outward current component is abolished in the presence of TEA. (B) Responses to ACh and ATP in the absence of Cl^- from both the external and internal solutions, i.e. Cl^- free. (C) In the absence of extracellular Ca^{2+} (no calcium but 1 mM EGTA added) ACh evokes a normal but transient current response. In a different cell it is seen that ATP evokes only inward current.

brane patch. In the presence of 0.5 mM ATP the open probability was increased to 0.098 ± 0.02 (n = 5) and in the presence of 1 mM ATP the open probability was 0.45 ± 0.04 (n = 5) and channel activity was now recorded in almost 50% of the patches tested (28 out of 62 patches). The open probability of the channel was not increased by the application of either ACh or ATP to the extra-patch membrane, i.e. applied to the solution bathing the cells. The data indicate that ATP had to be in contact with the external surface of the patch

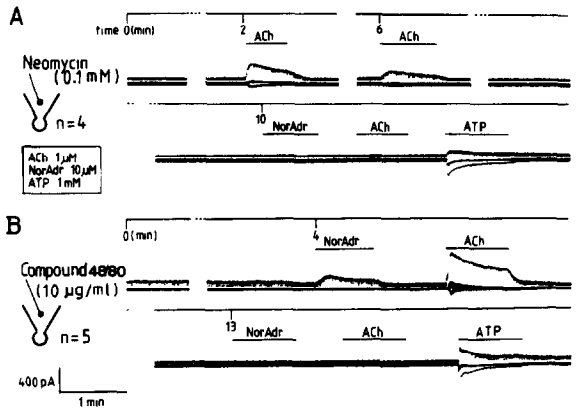

Fig. 3. Neomycin (A) and compound 48/80 (B) are applied internally via the patch pipette and within 10 min of establishing the whole-cell recording configuration the responses to ACh and Nad are abolished. In the same cells the responses to ATP persist even after blockade of the ACh and Nad effects.

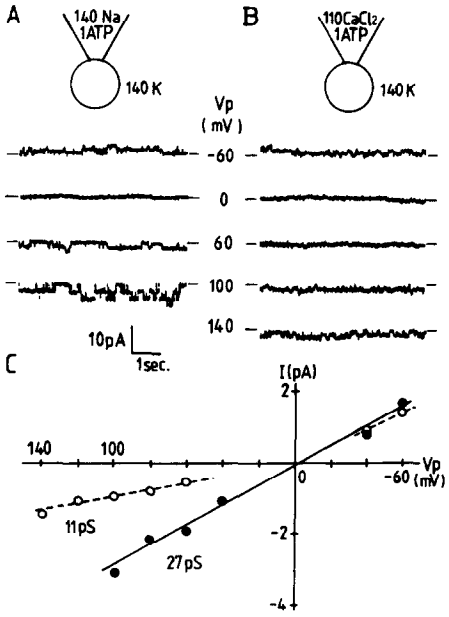

Fig. 4. Single channel currents recorded from cell-attached patches of membrane. The cells are bathed in a high (140 mM) K^+ solution, i.e. depolarized to 0 mV. The electrically isolated patches of membrane are repolarized by voltage-clamp of the patch pipette (transmembrane potential = $-V_p$). (A) Shows the single channel current amplitude at different voltages when the pipette is filled with the normal extracellular solution (Na^+ = 140 mM). The inward currents are thus due to Na^+, the outward currents due to the exit of intracellular K^+. (B) When the Na^+ in the recording pipette is replaced with Ca^{2+} (110 mM) the inward currents (now carried by Ca^{2+}) persist but with a reduced amplitude. (C) The current/voltage plot for the recordings of (A) and (B) showing the reduced single channel conductance when the inward current is carried by Ca^{2+} instead of Na^+.

of membrane to directly activate these cation channels, i.e. receptor-operated channels. When the Na^+ in the recording pipette was replaced by 110 mM Ca^{2+}, the inward currents seen at hyperpolarizing patch potentials were still observed although with the single channel conductance reduced to around 10 pS (Fig. 4). At depolarising potentials in the same patches, outward currents were observed with a conductance that was restored to the 30 pS seen in the control experiments. These results indicate that the cation channels activated by ATP have a significant conductance to Ca^{2+} which could allow for calcium influx in the absence of phosphoinositide metabolism.

ATP activates outward and inward currents in the isolated mouse lacrimal acinar cells. The outward current is due to activation of the voltage- and calcium-activated K^+ channel. The inward current is due to activation, not of Cl^- channels as is the case for the phosphoinositide-coupled receptors, but due to activation of a cation-selective channel which allows for entry of Na^+ and importantly calcium ions. It is the resulting elevation in $[Ca^{2+}]_i$ which in turn activates the K^+ channels. The presence of the non-discriminating cation

channels has previously been reported in exocrine acinar cells [15] though in lacrimal glands their role in stimulus-secretion coupling has been unclear. They have not been shown to be activated during normal physiological stimuli (the inward current response to ACh is totally abolished in the absence of Cl^-) and they require much higher $[Ca^{2+}]_i$ than either the K^+ or Cl^- channels for activation. The present study, demonstrating that they can function as receptor-operated channels with a significant permeability for Ca^{2+} under conditions where physiological ionic gradients exist, suggests a unique role for these channels in the regulation of exocrine secretion.

Acknowledgements: This work was supported by grants from the Wellcome Trust (UK) and Cystic Fibrosis Foundation (USA) to D.V.G.

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