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Evidence for the convergence of β-adrenergic and muscarinic signalling systems at a post-receptor site

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The β-adrenergic agonist isoproterenol stimulates inositol trisphosphate (IP_3) formation and cytosolic Ca^{2+} ($[Ca^{2+}]_i$) mobilization in rat parotid acini via a cAMP-dependent process. Atropine, a muscarinic antagonist, inhibited these isoproterenol responses without affecting isoproterenol-induced amylase secretion or peak $[Ca^{2+}]_i$ and IP_3 responses elicited by α_1-adrenergic stimulation with epinephrine. Atropine had no effect on isoproterenol-induced $[Ca^{2+}]_i$ responses in a cell line which lacked muscarinic receptors and did not alter β-adrenoreceptor ligand binding. These results suggest that the inhibition by atropine results from a post-receptor effect on cAMP-mediated stimulation of phosphatidylinositol 4,5 bisphosphate (PIP_2) hydrolysis.

Ca^{2+} mobilization; Cyclic AMP; Parotid; Receptor crosstalk; Atropine

1. INTRODUCTION

Siginal transduction is an intricate process whereby an extracellular stimulus is translated into a cellular response [1-6]. This includes binding of an agonist to its receptor, binding and activation of a specific G protein to this hormone-receptor complex (i.e. release of GαGTP), stimulation of an effector enzyme by the activated G protein, and finally generation of an appropriate second messenger. Several steps in this process can be independently regulated. For example, the binding of an agonist to its receptor can be dissociated from receptor-G protein binding [7-13]. Recent reports have shown evidence for interactions between distinct components of cellular second messenger systems (e.g. cAMP, IP_3, protein kinase C, $[Ca^{2+}]_i$) [4,14-17]. For example, in different cell types cAMP can inhibit [18] or activate [19-21] the IP_3 generating system. We have provided examples of the latter effect in both rat parotid acinar cells [20] and B82 mouse fibroblasts [22]. In this report, we show that atropine, acting at muscarinic receptors, can specifically inhibit $[Ca^{2+}]_i$ mobilization induced by isoproterenol, acting at β-adrenoreceptors, and by 8 BrcAMP by a mechanism likely involving regulation of PIP_2 hydrolysis at a common post-receptor site.

2. MATERIALS AND METHODS

Parotid acinar cells were prepared from male rats by enzymatic digestion using procedures and sources for chemicals described previously [20]. For $[Ca^{2+}]_i$ measurements, cells were incubated with 20 μM quin 2-AM (Calbiochem) and fluorescence changes determined with an SLM 8000 spectrofluorimeter as reported [20]. At this concentration of quin 2, there is no significant buffering of $[Ca^{2+}]_i$ (i.e. pattern and magnitude are similar to results with fura 2) and no alteration in cell fluid secretory capacity (Ca^{2+}-activated Cl^- efflux) [23]. PIP_2 hydrolysis was followed as IP_3 formation and was determined 30 s after agonist stimulation of cells as described [20]. Amylase secretion was followed for 30 min using a modified Bernfeld assay [20,23,24]. The binding of [^{3}H]quinuclidinyl benzilate ([^{3}H]QNB, 33 Ci/mmol; New England Nuclear) and [^{125}I]iodocyanopindolol ([^{125}I]CYP, 22,000 Ci/mmol) to cell membranes was performed as previously described [10,25]. B82 cells were maintained in culture as previously described [10]. Data were analyzed for statistical significance using a Student's *t*-test assuming a normal distribution of values.

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3. RESULTS AND DISCUSSION

Isoproterenol (200 μM) and 8 BrcAMP (2 mM) increase $[Ca^{2+}]_i$ in rat parotid acinar cells (fig.1A,C). Atropine (0.1 μM) totally inhibits the $[Ca^{2+}]_i$ elevation induced by 10 μM carbachol but is without effect on the isoproterenol-mediated response. However, when the atropine concentration is increased to 10 μM, both the carbachol and isoproterenol-mediated changes in $[Ca^{2+}]_i$ are abolished (fig.1B). In addition, 10 μM atropine blocks the elevation of $[Ca^{2+}]_i$ elicited by 8 BrcAMP (fig.1D), suggesting that the inhibitory effect of atropine is manifested after cAMP formation. Furthermore, 10 μM atropine does not inhibit either basal or isoproterenol-stimulated amylase secretion from acinar cells (table 1), demonstrating that the muscarinic antagonist does not non-specifically block β-adrenoreceptor mediated events.

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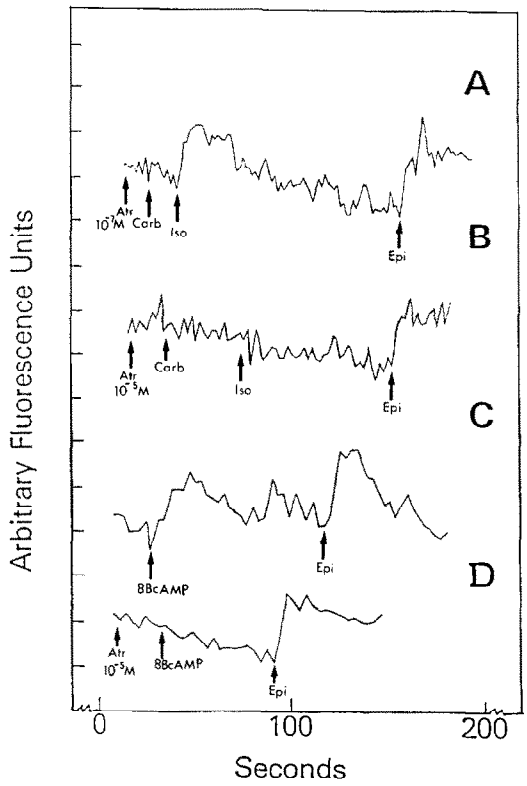

Fig.1. Effect of atropine on stimulated $[Ca^{2+}]_i$ mobilization in rat parotid acinar cells. Data shown are representative quin 2 fluorescence traces of 3-5 experiments performed. Experiments shown in traces C and D were performed in the presence of 5 mM EGTA. The following agents were added at the arrows: Atr, 10^{-7} M (0.1 μM atropine), Atr, 10^{-5} M (10 μM atropine), Carb (10 μM carbachol), Iso (200 μM isoproterenol), Epi (10 μM epinephrine), 8 BrcAMP (2 mM 8 BrcAMP).

Changes in $[Ca^{2+}]_i$ are somewhat distal to receptor activation and may be affected by several Ca^{2+} flux processes. Therefore, we examined the effect of atropine on PIP_2 hydrolysis (IP_3 formation) induced by isoproterenol. The data (table 1) clearly demonstrate that 10 μM atropine inhibits the isoproterenol-induced elevation of IP_3. This suggests that the atropine inhibition of isoproterenol-stimulated $[Ca^{2+}]_i$ changes likely results secondarily from the inhibition of PIP_2 hydrolysis. The effects of atropine on β-adrenergic responses do not extend to α_1-adrenergic events. The peak $[Ca^{2+}]_i$ change (fig.1B,D) obtained after epinephrine (10 μM) stimulation (806 ± 149 nM, $n = 8$), is unaffected by atropine (774 ± 144 nM, $n = 9$). Similarly, epinephrine-mediated IP_3 generation (table 1; ~2 fold basal) remains unchanged in the presence of atropine. Although epinephrine is a mixed agonist, acting on both α- and β-adrenergic receptors [26] the $[Ca^{2+}]_i$ mobilization responses induced by 10 μM epinephrine are primarily α_1-adrenergic receptor-mediated [27]. The results discussed above support the specific nature of the inhibitory effects of atropine on isoproterenol and cAMP induced $[Ca^{2+}]_i$ mobilization and IP_3 generation.

We next examined the possible effect of atropine on isoproterenol-induced $[Ca^{2+}]_i$ changes in B82 cells, a mouse fibroblast cell line which lacks muscarinic receptors. B82 cells can mobilize $[Ca^{2+}]_i$ and increase IP_3 levels after β-adrenergic stimuli [22]. As shown in fig.2, atropine has no effect on isoproterenol-induced $[Ca^{2+}]_i$ changes in B82 cells. This finding strongly suggests that the inhibitory effect of atropine is dependent upon the presence of muscarinic receptors. Although we observed that 10 μM atropine was without effect on isoproterenol-induced amylase secretion, and thus unlikely to interfere with ligand binding to the β-adrenoreceptor, this possibility was directly examined. Atropine (0.1, 1 and 10 μM) had no significant effect on the specific binding of [^{125}I]CYP to B82 cell membranes (96 ± 8%, 92 ± 9%, and 90 ± 8%, respectively, of control, 100%, values). Specific binding of [^{125}I]CYP was ~65% of total binding in these studies. Similarly, it is conceivable that our findings in parotid cells were a result of isoproterenol (or 8 BrcAMP) actions on the muscarinic receptor. Accordingly, we measured the binding of [^{3}H]QNB to parotid membranes in the absence or presence of either 200 μM isoproterenol (± ATP) or 2 mM 8 BrcAMP. The specific binding of [^{3}H]QNB was unaffected by these agents (97.4-99.0% of control, 100%, values), whereas carbachol, at a 20-fold lower concentration than isoproterenol, displaced ~35% of the specifically bound [^{3}H]QNB. Specific binding of [^{3}H]QNB was 50–60% of total binding in these studies. In addition, since it has been reported that rat parotid acinar cell preparations may contain contaminating nerve terminals [28], we assessed the possibility that 200 μM isoproterenol might induce the release of acetycholine from these contaminants. This situation would mean that the isoproterenol-induced, and

Table 1

Effect of atropine on agonist-stimulated IP_3 formation and amylase secretion in rat parotid acinar cells

Treatment	IP_3 (% inositol pool)	Amylase (% release)
Control (no addition)	0.111 ± 0.007 (16)	15
Isoproterenol (200 μM)	0.157 ± 0.012 (12)*	100
Epinephrine (10 μM)	0.208 ± 0.010 (3)*	n.d.
Atropine (10 μM)	0.084 ± 0.005 (4)	16
Atropine (10 μM) + isoproterenol (200 μM)	0.093 ± 0.017 (5)	134
Atropine (10 μM) + epinephrine (10 μM)	0.219 ± 0.020 (5)*	n.d.

IP_3 and amylase were determined as described in the text. Data for IP_3 formation are the mean ± SE for the number of experiments shown in parentheses. IP_3 levels which are significantly different ($P < 0.01$), by Student's t-test, from control values are indicated by an asterisk. Amylase data are the average of two separate experiments and are expressed relative to the amount released detected with isoproterenol-stimulated samples (i.e., 100 %). In these experiments isoproterenol-stimulated amylase release was 45% in 30 min; n.d. = not determined

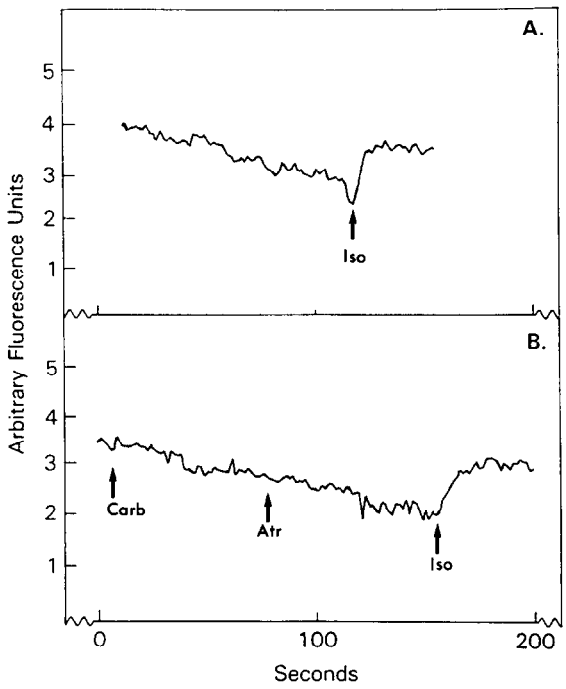

Fig.2. Effect of atropine on β-adrenoreceptor-induced elevations of $[Ca^{2+}]_i$ in B82 cells. Data shown are representative quin 2 fluorescence traces of 3 experiments performed. Iso (200 μM isoproterenol), Atr (10 μM atropine) and Carb (10 μM carbachol) were added at the arrows.

atropine-inhibited, changes in $[Ca^{2+}]_i$ and IP_3 were only indirectly elicited. However, when parotid acinar cells were incubated in the presence of acetylcholine esterase (0.1–5 U/ml), there was no effect on isoproterenol-stimulated $[Ca^{2+}]_i$ mobilization events (not shown).

In aggregate, our data demonstrate that the muscarinic antagonist atropine, by a mechanism which requires the presence of muscarinic receptors, can inhibit the stimulation of PIP_2 hydrolysis induced by the β-adrenergic agonist isoproterenol (via cAMP). We have previously suggested that cAMP likely acts at a site distal to the muscarinic receptor. An important implication of our present findings is that atropine, in addition to its well-established pharmacological ability to displace cholinergic agents from muscarinic receptors, can induce post-receptor effects. This is further supported by our data which show that atropine does not inhibit cAMP mediated IP_3 generation, by a mechanism which is independent of the muscarinic receptor. It is tempting to speculate that the inhibitory effect of atropine occurs at the level of receptor–G protein interactions. This conclusion is consistent with observations made on [^{3}H]QNB binding to cardiac membranes in the absence or presence of GTP by Burgisser et al. [29]. Their results suggested that the QNB–muscarinic receptor binding process involved a G protein interaction (i.e. a post-muscarinic receptor change brought about by an antagonist). They proposed a 'reciprocal binding' model to describe the effect of GTP on [^{3}H]QNB receptor binding, which is also supported by data obtained with other systems [30–35]. Accordingly, we suggest, based on our findings, that there is a post-receptor convergence of the mechanisms involved in the regulation of PIP_2 hydrolysis via the muscarinic receptor and another intracellular signalling system (cAMP-linked).

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