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Positive inotropic effects induced by carbachol in rat atria treated with islet-activating protein (IAP) — association with phosphatidylinositol breakdown

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1 To elucidate the functional consequences of phosphatidyl inositol (PI) breakdown produced by activation of the muscarinic receptor of the atrial muscle, and to clarify the subtypes of the muscarinic receptor involved, the effects of muscarinic agonists and antagonists on mechanical function were studied in atria isolated from rats given intravenous islet-activating protein (IAP; 50 $\mu g\,kg^{-1}$ body weight) 48–72 h before the experiments.

2 The negative chronotropic and inotropic actions of carbachol (CCh) were attenuated and positive inotropic effects (62.5 ± 5.8% above basal level) were observed with 10^{-5}–10^{-3} M CCh. Oxotremorine did not produce positive inotropic effects even in doses as high as 3×10^{-4} M. High doses of carbachol produced positive chronotropic effects, although the effects were weak.

3 Propranolol (10^{-7} M) did not modify the positive inotropic effect of carbachol observed in IAP-treated atria, nor was there any change in the tissue cyclic AMP levels after carbachol.

4 High doses (10^{-5}–10^{-3} M) of carbachol produced PI breakdown in the absence and presence of IAP. Oxotremorine (3×10^{-4} M) did not produce PI breakdown. In the presence of oxotremorine, the positive inotropic effects and PI breakdown by carbachol were abolished.

5 The positive inotropic effect of carbachol was readily antagonized by atropine but pirenzepine and gallamine exhibited only weak antagonist effects.

6 These results suggest that a muscarinic agonist such as carbachol can produce a positive inotropic effect in IAP-treated atria, in association with PI breakdown, through activation of a muscarinic receptor which shows some similarity to that previously identified in smooth muscles.

Introduction

In recent years much attention has been directed towards the breakdown of phosphatidylinositol (PI) as a transduction mechanism connecting the activation of receptors with cellular responses (Downes & Michell, 1982; Berridge & Irvine, 1984). It has been observed, in atria, ventricles and cardiomyocytes (Brown & Brown, 1983; 1984; Brown *et al.*, 1985; Masters *et al.*, 1985; Ransnäs *et al.*, 1986; Woodcock *et al.*, 1987), that the activation of the muscarinic receptor or α_1-adrenoceptor by respective agonists could cause phosphatidylinositol (PI) breakdown. However, which cellular responses are connected to the PI breakdown in the heart remains to be clarified. The aim of the present study was to elucidate the functional consequences of the PI breakdown produced by muscarinic agonists in the heart. Isolated atrial preparations of the rat were used, and the negative inotropic and chronotropic effects of muscarinic agonists were eliminated by pretreating the preparations with islet-activating protein (IAP), an exotoxin produced by *Bordetella pertussis* (Ui, 1984). IAP is the substance that was shown to lead to a progressive ADP-ribosylation of a cellular guanine nucleotide-binding (G) protein, Gi (mediates inhibition of adenylate cyclase) or Go (unknown function), and a resultant elimination of the regulatory fumction of the protein (Katada & Ui, 1982a, b). Diminution by IAP-treatment of the negative inotropic and chronotropic effects of carbachol was demonstrated by Endoh *et al.* (1985). As positive inotropic effects were observed in association with PI breakdown, we studied the subtype of muscarinic receptor coupled to this positive inotropic action by use of selective antagonists.

Methods

Preparation of the rat atrial muscles

Male rats of the Wistar strain (250–350 g) were given IAP (50 $\mu g\,kg^{-1}$ body weight, unless otherwise stated), dissolved in 0.2 ml of 0.9% w/v NaCl solution, intravenously (IAP-treatment). The same amount of 0.9% w/v NaCl solution alone was given intravenously to control rats. Between 48 and 72 h after the injection of IAP or saline, hearts were excised, and atria were divided carefully into the right and left halves. The right atrium, which retained spontaneous rhythm, was used to assess the chronotopic action of the drugs. The initial resting tension was set at 0.2–0.3 g, and the rate of the spontaneous contraction of this preparation was recorded on an ink-writing oscillograph by means of a linearly-recording tachograph. The left atrium was further dissected into two parts, the central half of which was cut off and the remaining outer half was used to evaluate inotropic effects. This preparation was stimulated electrically by a square-wave pulse stimulator at a frequency of 1 Hz with voltages approximately 30% above the threshold (duration = 1 ms). The tension of the preparation was kept at 0.2–0.3 g throughout the course of the experiment. The preparations were mounted in a 10 ml organ bath and their contractile tension was recorded on an ink-writing oscillograph with the aid of a strain-gauge transducer and a carrier-amplifier. The bathing solution used was a Krebs-Henseleit solution of the following composition ($mmol\,l^{-1}$): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $NaHCO_3$ 24.9, $MgSO_4$ 1.6, KH_2PO_4 1.2 and glucose 12. The temperature of the solution was maintained at 32°C. The solution was aerated with a mixture of 95% O_2 and 5% CO_2. After dissection all preparations were allowed to equilibrate for one hour before the addition of any drugs.

Determination of myo-inositol 1-phosphate (myo-Ins 1P)

Breakdown of phosphatidylinositol was assessed according to the procedure of Brown & Brown (1983) and Ransnäs *et al.* (1986). Atria were transferred to vessels containing the continuously oxygenated buffer solution, and were equilibrated for about 1 h at 32°C in a shaking water bath. Then the incubation medium was replaced by fresh medium containing 4 $\mu Ci\,ml^{-1}$ myo[2-^{3}H]-inositol, 17.1 Ci $mmol^{-1}$. After 120 min of incubation with myo[2-^{3}H]-inositol, drugs or control solutions were added. In experiments where myo-Ins 1P accumulation was measured, LiCl (10 mM) was added just before the addition of the drugs or the control solution. The preparation was incubated for 30 min in the medium bubbled with 95% O_2 and 5% CO_2 at 32°C. Incubation was terminated by rinsing the preparation in ice-cold saline and tissues were quickly blotted. Pairs of atria (one left and one right) were frozen in liquid nitrogen and extracted later (Brown & Brown, 1983).

Samples were homogenized in 3 ml of $CHCl_3/CH_3OH/H_2O$ (5:10:4, v/v). A two-phase system was obtained with the addition of $CHCl_3$ and H_2O to make the solution of $CHCl_3/CH_3OH/H_2O$ (10:10:5, v/v). Samples were centrifuged at 1600 *g* for 10 min and 2.0 ml of the upper (aqueous) phase was transferred to a column containing approximately 175 mg of an anion exchange resin (Bio-Rad AG 1-X8, 100–200 mesh, formate form). Columns were washed with a total of 10 ml of H_2O. Labelled myo-Ins 1P was eluted with 8 ml of 200 mM ammonium formate/100 mM formic acid. The radioactivity was counted in Aquasol (NEN) using a scintillation spectrometer.

Cyclic AMP assay

After incubation for 120 s at 32°C in the Krebs-Henseleit solution, muscles were removed from the organ bath and frozen immediately in liquid nitrogen. The muscles were homogenized in 1.5 ml of 0.1 N HCl. The homogenates were heated at 100°C for 3 min and centrifuged at 4°C. Adenosine 3′:5′-cyclic monophosphate (cyclic AMP) content of the supernatants was determined by the radioimmunoassay method described by Honma *et al.* (1977). After dissolving the tissue in 0.5 N NaOH, protein was determined using the Lowry method.

Data analysis

From the dose-response curves obtained, pA_2 values were calculated for each compound using the following equation:

$$pA_2 = -\log \frac{B}{\frac{A'}{A} - 1}$$

where A is the molar concentration of carbachol needed to produce a half maximum response without antagonists and A′, the concentration needed to produce the same response in the presence of the antagonist, and B is the molar concentration of the antagonist.

Materials

Drugs used were carbachol chloride, atropine sulphate (Wako Pure Chem., Japan), gallamine triethiodide (Sigma), oxotremorine (Aldrich Chem.

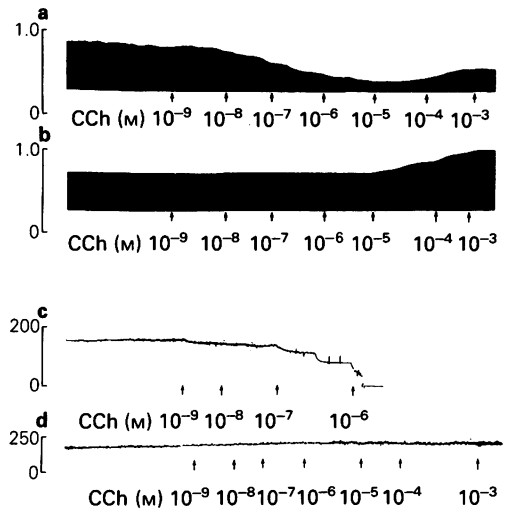

Figure 1 A typical record showing the effects of carbachol (CCh) on the rat atria. (a and b) Changes in tension (g) recorded in the left atria. (c and d) Frequency of contraction (beats min^{-1}) recorded in the right atria. (a and c) Control atria, (b and d) IAP-treated atria.

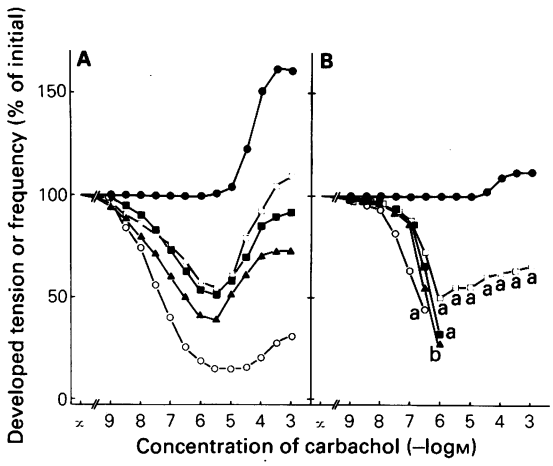

Figure 2 Dose-effect relationship for the effects of islet-activating protein (IAP) treatment on the chronotropic and inotropic effects of carbachol in rat atria. The concentrations of IAP were 0 (○), 0.5(▲), 5(■), 15(□) and 50(●) $\mu g\,kg^{-1}$. Intropic and chronotropic effects were assessed in left (A) and right (B) atria, respectively. The data are means from six experiments. (a) Two out of six atria ceased to beat. (b) One out of six atria ceased to beat.

Co.), (−)-isoprenaline hydrochloride (Nikken Kagaku, Japan), (±)-propranolol hydrochloride (Sumitomo Chem. Co., Japan), myo[2-^{3}H]-inositol (17.1 Ci $mmol^{-1}$) (New England Nuclear), IAP (Kaken Pharmaceutical Co., Japan) and pirenzepine (Boehringer Mannheim).

Results

Figure 1 is a representative record showing the effects of carbachol on the rat isolated atria. In the control atria low concentrations of carbachol (10^{-8}–10^{-5} M) produced negative inotropic and chronotropic effects. In IAP-treated atria the negative inotropic and chronotropic effects were abolished, and the positive inotropic action was observed at higher concentrations (10^{-5}–10^{-3} M). However, only minimal positive chronotropic action was observed.

Figure 2 shows the concentration-response curves for the inotropic and chronotropic action of carbachol and the effects of IAP treatment. IAP was injected intravenously in doses of 0.5–50 $\mu g\,kg^{-1}$ body weight to each animal 48–72 h before isolation of the heart. In the control left atria, carbachol produced negative inotropic effects in concentrations up to 10^{-6} M. The depression of contraction amounted to 32 ± 5% ($n = 6$) of the initial tension. At concentrations higher than 10^{-6} M, a dose-dependent increase in tension was observed. The frequency of contraction of the spontaneously beating right atria was likewise decreased and the atria ceased to beat in the presence of 10^{-6} M carbachol. In atria treated with IAP, a dose-dependent attenuation of the negative inotropic and chronotropic effects of carbachol was observed, and the positive inotropic effects became manifest in a dose-related manner in preparations from animals treated with IAP (0.5–5 $\mu g\,kg^{-1}$). With 50 $\mu g\,kg^{-1}$ IAP, only the positive inotropic effect was observed. The contractile tension attained was 160.9 ± 4.9% ($n = 6$) of the control tension. There was no change in the time to peak tension, nor was there any change in the time course of relaxation. The decrease in frequency of contraction was likewise attenuated, and a slight positive chronotropic effect was observed after 50 $\mu g\,kg^{-1}$ IAP. However, the increase in the frequency amounted to only 13 ± 6% ($n = 6$) over the control even with 10^{-3} M carbachol. In the presence of physostigmine the positive inotropic effect was also produced by acetylcholine in IAP-treated atria (data not shown).

As shown in Figure 3 the positive inotropic action of carbachol (10^{-5}–10^{-3} M) was not influenced by 10^{-7} M propranolol. Nor was there any increase in the level of cyclic AMP after administration of carbachol (10^{-6} M, 10^{-3} M) in either the control or the IAP-treated left (Figure 4) and right atria (data not shown), whereas isoprenaline (10^{-7} M) induced an

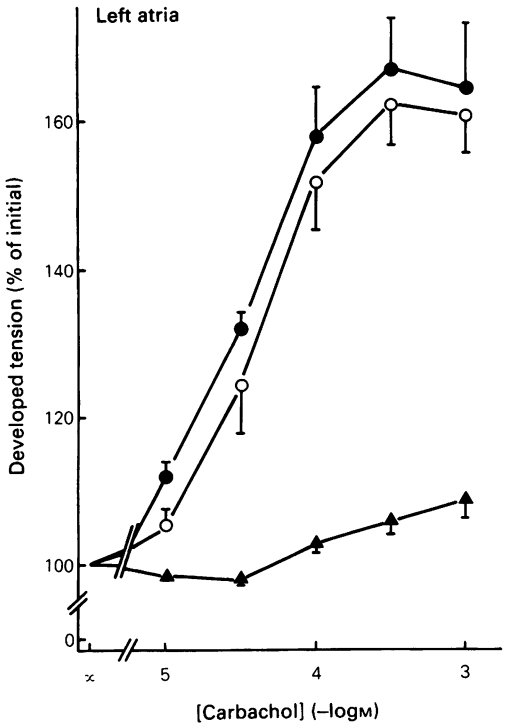

Figure 3 Effects of propranolol and atropine on the positive inotropic action of carbachol in islet-activating protein (IAP)-treated left atria. IAP-treated left atria were incubated with 10^{-7} M propranolol (●), 10^{-7} M atropine (▲) or vehicle (○) for 30 min before administration of carbachol. Incubation with 10^{-7} M propranolol or 10^{-7} M atropine caused no changes in the basal level of tension. The data are mean from six experiments; vertical lines indicate s.e.

increase in cyclic AMP. Thus, the release of noradrenaline or histamine was excluded as a mechanism of the positive inotropic effects of carbachol. However, atropine (10^{-7} M) blocked the positive inotropic action of carbachol (Figure 3).

To examine the breakdown of PI, the atria were pre-labelled with myo[^{3}H]-inositol and the incorporation of ^{3}H to myo-Ins 1P was measured in the presence of 10 mM LiCl. Addition of the higher concentrations of carbachol (10^{-5}–10^{-3} M) to the incubation media resulted in an accumulation of myo-Ins 1P (Figure 5) both in the right and the left atria irrespective of whether the preparation was treated with IAP or not. Carbachol 10^{-3} M produced an increase of about 150% over the basal value. The concentrations of carbachol causing myo-Ins 1P accumulation corresponded to those causing a positive inotropic action in IAP-treated atria (Figure 2). The negative inotropic and chronotropic effects of another muscarinic agent, oxotremorine (10^{-9}–10^{-5} M) were also abolished by IAP-treatment. However, a significant positive inotropic effect was not observed with oxotremorine; even with 3×10^{-4} M oxotremorine the tension developed was only 106.1 ± 1.7% of the initial tension (Figure 6). Consistent with this observation, we found no accumulation of myo-Ins 1P (Table 1). Furthermore, the positive inotropic effect of carbachol (10^{-3} M) was depressed in the presence of oxotremorine (3×10^{-4} M), in association with the reduction of the accumulation of myo-Ins 1P.

To characterize the muscarinic receptor involved in the positive inotropic effect of carbachol, three muscarinic receptor antagonists, atropine, pirenzepine and gallamine, were tested for their effectiveness in antagonizing the positive inotropic and the negative inotropic action of carbachol. These compounds produced parallel rightward shifts in the carbachol dose-response curve. The affinities of the antagonists towards the receptor subserving the negative and the positive inotropic effects, expressed as pA_2 values, in IAP-treated atria are shown in Table 2. The antagonistic effects of pirenzepine and gallamine on the muscarinic receptors coupled to the positive inotropic effect of IAP-treated atria were weaker than the

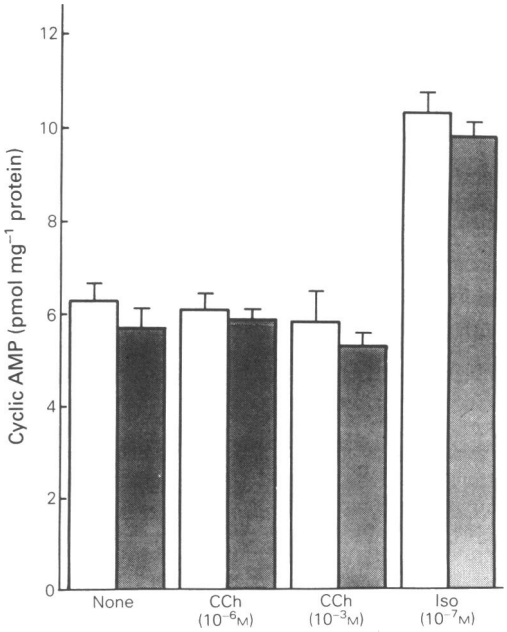

Figure 4 Effect of carbachol (CCh) and isoprenaline (Iso) on the cyclic AMP level in the rat left atria. Open columns: control; stippled columns: islet-activating protein (IAP)-treated atria. For details see Methods section. The data are mean from five experiments; vertical lines indicate s.e.

Table 1 Effects of oxotremorine on the carbachol-induced positive inotropic action and accumulation of myo[^{3}H]-inositol 1-phosphate ([^{3}H]-Ins 1P) in islet-activating protein-treated left atria

	Contraction (% of control)	[^{3}H]-*Ins 1P* (c.p.m. mg^{-1} wet wt)
Control	100	3.51 ± 0.21
Carbachol (10^{-3} M)	160.9 ± 4.9	8.60 ± 0.84
Oxotremorine (3×10^{-4} M)	106.1 ± 1.7	3.80 ± 0.47
Carbachol (10^{-3} M) + oxotremorine (3×10^{-4} M)	106.2 ± 5.8**	4.10 ± 0.29**

The data are mean ± s.e. from 5–6 experiments.
** Significantly different from the responses induced by carbachol 10^{-3} M ($P < 0.01$).

antagonism of the muscarinic receptors coupled to the negative inotropic effect.

Discussion

In IAP-treated rat left atria carbachol produced a dose-dependent positive inotropic effect and a dose-dependent breakdown of phosphatidylinositol, as evidenced by an accumulation of myo-inositol 1-phosphate. The concentrations of carbachol producing these two effects were similar (Figure 2, Figure 5). Furthermore, another muscarinic agonist oxotremorine, which did not produce an accumulation of myo-Ins 1P, did not produce a significant positive inotropic action in IAP-treated atria. Also the positive inotropic effects of carbachol were suppressed in the presence of oxotremorine in association with the depression of the accumulation of myo-Ins 1P (Table 1). The muscarinic receptor antagonists used, gallamine, pirenzepine (Table 2) and atropine (Table 2 and Figure 3), antagonized the positive inotropic effects, while propranolol (10^{-7} M) was without effect. Thus, it is clear that the receptor involved is a muscarinic receptor. This is the first clear-cut demonstration that the positive inotropic effects are

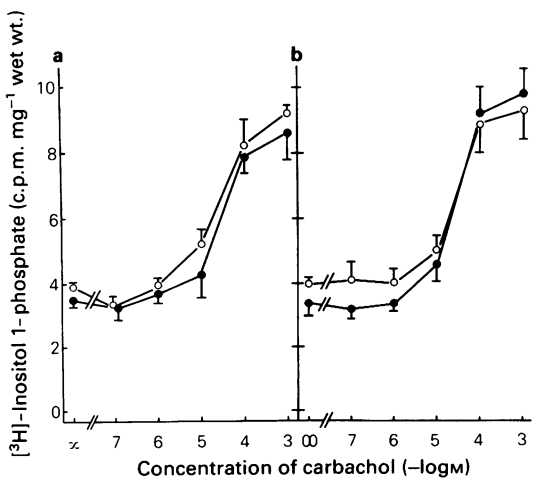

Figure 5 Accumulation of myo[^{3}H]-inositol 1-phosphate by carbachol in the rat atria. Control (○) or islet-activating protein-treated (●) rat left (a) and right (b) atria were incubated with each concentration of carbachol for 30 min in the medium with 10 mM LiCl at 32°C as described in Methods. The data are mean from five experiments; vertical lines indicate s.e.

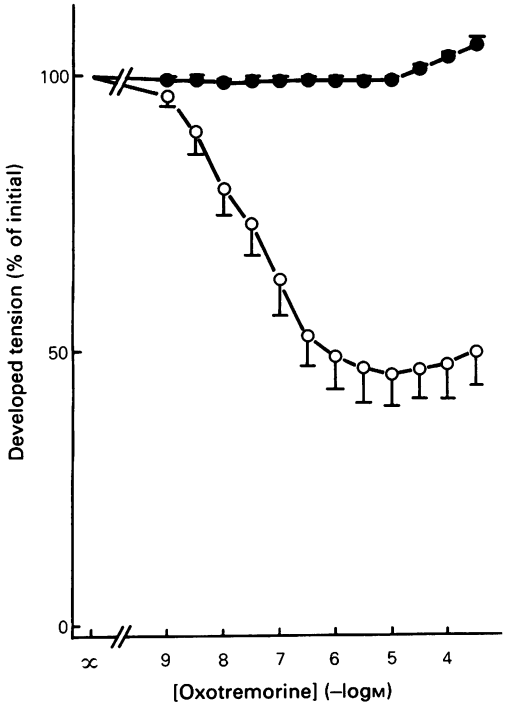

Figure 6 Inotropic effect of oxotremorine in the rat isolated left atria. Control (○) or islet-activating protein-treated (●) left atria were incubated with oxotremorine as described in Methods. The data are mean from five experiments; vertical lines indicate s.e.

Table 2 Potencies of atropine, pirenzepine and gallamine as antagonists at receptors coupled to the negative inotropic action in control rat atria and the positive inotropic action in islet-activating protein (IAP)-treated rat atria

	pA_2 values	
	Negative inotropic effect in control atria	*Positive inotropic effect in IAP-treated atria*
Atropine	9.52 ± 0.07	7.84 ± 0.10**
Pirenzepine	6.86 ± 0.09	5.92 ± 0.11**
Gallamine	6.80 ± 0.12	5.71 ± 0.17**

Left atria were incubated with pirenzepine (3×10^{-6} M) or gallamine (10^{-5} M) for 30 min before administration of carbachol. Incubation with atropine, pirenzepine or gallamine alone caused no changes in the basal level of tension. Difference in the affinities of antagonist were analysed as described in Methods. The data are means ± s.e. from five experiments.

** Significantly different from the pA_2 values of pirenzepine or gallamine for the negative inotropic action in the control atria ($P < 0.01$).

induced through activation of muscarinic cholinoceptors and that they are closely related to changes in PI breakdown. Although it has been shown that α_1-adrenoceptor and muscarinic cholinoceptor activation cause PI breakdown in atria, ventricle and cardiomyocytes (Brown & Brown, 1983; 1984; Brown *et al.*, 1985; Masters *et al.*, 1985; Ransnäs *et al.*, 1986; Woodcock *et al.*, 1987), the functional consequences of the PI breakdown have never been clarified. It is possible that inositol 1,4,5-trisphosphate induced, through the PI breakdown process, the release of calcium from intracellular storage sites which then produces the positive inotropic effects. Hirata *et al.* (1984) and Nosek *et al.* (1986) found that inositol 1,4,5-trisphosphate could mobilize Ca^{2+} from the cardiac sarcoplasmic reticulum. Through the breakdown of PI, diacylglycerol can also be produced which can indirectly modify Ca^{2+}-transport process through activation of protein kinase C (Nishizuka, 1984). Stimulation of Ca^{2+} sequestration by cardiac sarcoplasmic reticulum by way of phosphorylation of phospholamban by protein kinase C has been demonstrated (Movsesian *et al.*, 1984). PI breakdown was also produced by high concentrations of carbachol even in the control atria, but the positive inotropic effect was observed only in the IAP-treated atria due probably to the overwhelmingly stronger negative inotropic effect.

In this study, the negative inotropic action of carbachol was abolished by IAP-treatment, while PI breakdown remained unchanged (Figures 2 and 5). This is in agreement with the results obtained by Masters *et al.* (1985) in cultured chick heart cells. According to them, IAP-treatment inhibited the muscarinic receptor-mediated attenuation of cyclic AMP accumulation by isoprenaline, but did not inhibit the muscarinic receptor-mediated PI breakdown. Thus, G protein may or may not be involved in the PI breakdown induced by muscarinic receptor activation in the atria, but it is not sensitive to IAP. Although it has been shown, in guinea-pig neutrophils (Ohta *et al.*, 1985) and rat mast cells (Nakamura & Ui, 1985), that the receptor-linked activation of phospholipase C was mediated by IAP-sensitive G protein, Hepler & Harden (1986) provided direct evidence for the involvement of IAP-insensitive G protein in the carbachol-stimulated PI breakdown in 1321N1 human astrocytoma cells.

Available evidence suggests the existence of 3 types of muscarinic receptor (Birdsall & Hulme, 1983). The first receptor type is found in sympathetic ganglia and in the CNS (so-called neuronal receptors). It has a high affinity for pirenzepine and is strongly antagonized by pirenzepine (Brown *et al.*, 1980). The second type is found in the myocardium with low affinities for pirenzepine, and is selectively affected by gallamine (Clark & Mitchelson, 1976). This receptor is regulated by guanine nucleotides and connected to the opening of K^+ channels (Pfaffinger *et al.*, 1985) or the inhibition of adenylate cyclase (Hazeki & Ui, 1981; Masters *et al.*, 1985). Stimulation of this receptor subtype causes a negative inotropic effect in cardiac muscle. The third type of receptor is found in smooth muscle with low affinity for pirenzepine and is not strongly affected by gallamine. Stimulation of the third type of receptor results in increases in cytosolic Ca^{2+}.

Although the transduction mechanism of the third type of receptor is not yet clear, it has recently been suggested that PI breakdown may be part of a receptor transduction process controlling receptor-operated increases in cytosolic Ca^{2+} in smooth muscles (Baron *et al.*, 1984; Sekar & Roufogalis, 1984; Best *et al.*, 1985; Grandordy *et al.*, 1986). The receptor coupled to the positive inotropic effect found in the present study is different from the second type, as the antagonism by gallamine was sig-

nificantly less when compared with the receptor coupled to the negative effect in the heart (Table 2). As the antagonism by pirenzepine at this receptor was weak compared with that at the 'neuronal' receptor, it cannot be equated with the first type, either (Table 2). Furthermore, it is shown in this study that the effector system of the receptor subserving the positive inotropic effect is connected with PI breakdown. Thus, the receptor-effector system subserving the positive inotropic effect resembles the system found in smooth muscle, i.e. the system with the third type of receptor, although the affinity of the receptor, of the present experiment, for atropine was found to be rather low (Table 2).

In IAP-treated right atria, carbachol did not produce changes in the spontaneous frequency of contraction (Figure 2) in spite of the myo-Ins 1P accumulation (Figure 5). In this respect the positive inotropic effect of muscarinic cholinoceptor stimulation resembles the positive inotropic effect of α_1-adrenoceptor stimulation. Both are not associated with an increase in the frequency of contraction and are linked with PI breakdown (Brown & Brown, 1983; Brown *et al.*, 1985). α_1-Adrenoceptor stimulation is responsible for part of the positive inotropic action caused by noradrenaline (Wenzel & Su, 1966; Govier, 1967) However, the physiological roles of the positive inotropic effect induced by high concentrations of carbachol in IAP-treated rat atria in association with PI breakdown is not clear. It may represent a feedback system that competes with the excessive negative action induced by stimulation of the muscarinic receptor.

In conclusion, the studies presented here demonstrate that the muscarinic agonist, carbachol, could produce positive inotropic effects in atria through activation of the muscarinic receptor linked to PI breakdown and that the cholinoceptor involved, resembles that previously identified in smooth muscle, although atropine seems to possess a relatively low affinity.

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References

BARON, C.B., CUNNINGHAM, M., STRAUSS III, J.F. & COBURN, R.F. (1984). Pharmacomechanical coupling in smooth muscle may involve phosphatidylinositol metabolism. *Proc. Natl. Acad. Sci. U.S.A.*, **81**, 6899–6903.

BERRIDGE, M.J. & IRVINE, R.F. (1984). Inositol trisphosphate, a novel second messenger in cellular signal transduction. *Nature*, **312**, 315–321.

BEST, L., BROOKS, K.J. & BOLTON, T.B. (1985). Relationship between stimulated inositol lipid hydrolysis and contractility in guinea-pig visceral longitudinal smooth muscle. *Biochem. Pharmacol.*, **34**, 2297–2301.

BIRDSALL, N.J.M. & HULME, E.C. (1983). Muscarinic receptor subclasses. *Trends. Pharmacol. Sci.*, **4**, 459–463.

BROWN, D.A., FORWARD, A. & MARSH, S. (1980). Antagonist discrimination between ganglionic and ileal muscarinic receptors. *Br. J. Pharmacol.*, **71**, 362–364.

BROWN, S.L. & BROWN, J.H. (1983). Muscarinic stimulation of phosphatidylinositol metabolism in atria. *Mol. Pharmacol.*, **24**, 351–356.

BROWN, J.H. & BROWN, S.L. (1984). Agonists differentiate muscarinic receptors that inhibit cyclic AMP formation from those that stimulate phosphoinositide metabolism. *J. Biol. Chem.*, **259**, 3777–3781.

BROWN, J.H., BUXTON, I.L. & BRUNTON, L.L. (1985). α_1-Adrenergic and muscarinic cholinergic stimulation of phosphoinositide hydrolysis in adult rat cardiomyocytes. *Circ. Res.*, **57**, 532–537.

CLARK, A.L. & MITCHELSON, F. (1976). The inhibitory effect of gallamine on muscarinic receptors. *Br. J. Pharmacol.*, **58**, 323–331.

DOWNES, P. & MICHELL, R.H. (1982). Phosphatidylinositol 4-phosphate and phosphatidylinositol 4,5-bisphosphate: lipids in search of a function. *Cell Calcium*, **3**, 467–502.

ENDOH, M., MARUYAMA, M. & IIJIMA, T. (1985). Attenuation of muscarinic cholinergic inhibition by islet-activating protein in the heart. *Am. J. Physiol.*, **249**, (*Heart Circ. Physiol.* **18**), H309–H320.

GOVIER, W.C. (1967). A positive inotropic effect of phenylephrine mediated through alpha adrenergic receptors. *Life Sci.*, **6**, 1361–1365.

GRANDORDY, B.M., CUSS, F.M., SAMPSON, A.S., PALMER, J.B. & BARNES, P.J. (1986). Phosphatidylinositol response to cholinergic agonists in airway smooth muscle: relationship to contraction and muscarinic receptor occupancy. *J. Pharmacol. Exp. Ther.*, **238**, 273–279.

HAZEKI, O. & UI, M. (1981). Modification by islet-activating protein of receptor-mediated regulation of cyclic AMP accumulation in isolated rat heart cells. *J. Biol. Chem.*, **256**, 2856–2862.

HEPLER, J.R. & HARDEN, T.K. (1986). Guanine nucleotide-dependent pertussis-toxin-insensitive stimulation of inositol phophate formation by carbachol in a membrane preparation from human astrocytoma cells. *Biochem. J.*, **239**, 141–146.

HIRATA, M., SUEMATSU, E., HASHIMOTO, T., HAMACHI T., & KOGA, T. (1984). Release of Ca^{2+} from a non-mitochondrial store site in peritoneal marcrophages treated with saponin by inositol 1,4,5-trisphosphate. *Biochem. J.*, **223**, 229–236.

HONMA, M., SAITOH, T., TAKEZAWA, J. & UI, M. (1977). An ultrasensitive method for simultaneous determination of cyclic AMP and cyclic GMP in small-volume samples from blood and tissue. *Biochem. Med.*, **18**, 257–273.

KATADA, T. & UI, M. (1982a). Direct modification of the membrane adenylate cyclase system by islet-activating protein due to ADP-ribosylation of a membrane protein. *Proc. Natl. Acad. Sci. U.S.A.*, **79**, 3129–3133.

KATADA, T. & UI, M. (1982b). ADP ribosylation of the spe-

cific membrane protein of C6 cells by islet-activating protein associated with modification of adenylate cyclase activity. *J. Biol. Chem.*, **257**, 7210–7216.

MASTERS, S.B., MARTIN, M.W., HARDEN, T.K. & BROWN, J.H. (1985). Pertussis toxin does not inhibit muscarinic-receptor-mediated phosphoinotide hydrolysis or calcium mobilization. *Biochem. J.*, **227**, 933–937.

MOVSESIAN, M.A., NISHIKAWA, M. & ADELSTEIN, R.S. (1984). Phosphorylation of phospholamban by calcium-activated, phospholipid-dependent protein kinase. *J. Biol. Chem.*, **259**, 8029–8032.

NAKAMURA, T. & UI, M. (1985). Simultaneous inhibitions of inositol phospholipid breakdown, arachidonic acid release, and histamine secretion in mast cells by islet-activating protein, pertussis toxin. *J. Biol. Chem.*, **260**, 3584–3593.

NISHIZUKA, Y. (1984). The role of protein kinase C in cell surface signal transduction and tumor promotion. *Nature*, **308**, 693–698.

NOSEK, T.M., WILLIAMS, M.F., ZEIGLER, S.T. & GODT, R.E. (1986). Inositol trisphosphate enhances calcium release in skinned cardiac and skeletal muscle. *Am. J. Physiol.*, **250** (*Cell Physiol.*, **19**), C807–C811.

OHTA, H., OKAJIMA, F. & UI, M. (1985). Inhibition by islet-activating protein of a chemotactic peptide-induced early breakdown of inositol phospholipids and Ca^{2+} mobilization in guinea pig neutrophils. *J. Biol. Chem.*, **260**, 15771–15780.

PFAFFINGER, P.J., MARTIN, J.M., HUNTER, D.D., NATHANSON, N.M. & HILLE, B. (1985). GTP-binding proteins couple cardiac muscarinic receptors to a K channel. *Nature*, **317**, 536–538.

RANSNÄS, L., GJÖRSTRUP, P., HJALMARSO, A., SJÖGREN, C. & JACOBSSON, B. (1986). Muscarinic receptors in mammalian myocardium: effects of atrial and ventricular receptors on phosphatidylinositol metabolism and adenylate cyclase. *J. Mol. Cell. Cardiol.*, **18**, 807–814.

SEKAR, M.C. & ROUFOGALIS, B.D. (1984). Muscarinic-receptor stimulation enhances polyphosphoinositide breakdown in guinea-pig ileum smooth muscle. *Biochem. J.*, **223**, 527–531.

UI, M. (1984). Islet-activating protein, pertussis toxin: a prove for functions of the inhibitory guanine regulatory component of adenylate cyclase. *Trends Pharmacol. Sci.*, **5**, 277–279.

WENZEL, D.G. & SU, J.L. (1966). Interactions between sympathomimetic amines and blocking agents on the rat ventricle strip. *Arch. Int. Pharmacodyn. Ther.*, **160**, 379–389.

WOODCOCK, E.A., LEUNG, E. & MCLEOD, J.K. (1987). A comparison of muscarinic acetylcholine receptors coupled to phosphatidylinositol turnover and to adenylate cyclase in guinea-pig atria and ventricles. *Eur. J. Pharmacol.*, **133**, 283–289.

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