Br. J. Pharmacol. (1990), **101**, 829–834 © Macmillan Press Ltd, 1990

Effects of carbachol and (−)-N^6-phenylisopropyladenosine on myocardial inositol phosphate content and force of contraction

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1 The effects of carbachol and the A_1-adenosine receptor agonist (−)-N^6-phenylisopropyladenosine (PIA) on force of contraction and inositol lipid metabolism were studied in electrically driven left auricles and papillary muscles isolated from guinea-pig hearts. Both carbachol and PIA (0.01–10 μM) had concentration-dependent negative inotropic effects in auricles. In papillary muscles PIA had no inotropic effect. Carbachol also had no inotropic effect at low concentrations (0.01–1 μM) but at 10–100 μM it exerted a slight positive inotropic effect.

2 In auricles and papillary muscles both carbachol and PIA concentration-dependently increased inositol trisphosphate (IP_3; significant at 1 μM). Accordingly phosphatidylinositol bisphosphate (PIP_2), the precursor of IP_3, was reduced. All effects of carbachol and PIA were antagonized by atropine (10 μM) and 1,3-dipropyl-8-cyclopentylxanthine (DPCPX; 20 μM) respectively, indicating receptor-mediated effects.

3 In auricles the negative inotropic effects of carbachol and PIA preceded the increase in IP_3.

4 In papillary muscles the increase in IP_3 preceded the slight positive inotropic effect of carbachol, indicating that the M-cholinoceptor-mediated increase in IP_3 and force of contraction may be related. However, PIA showed a comparable increase in IP_3 but no inotropic effect, indicating a dissociation between those parameters.

5 In conclusion, in previous studies a close relation between increases in IP_3 and force of contraction has been shown after α_1-adrenoceptor stimulation. The present study with carbachol supports this view. However, the present data for PIA could not show such a close relationship, questioning the role of IP_3 as an endogenous regulator of force of contraction.

Introduction

The M-cholinoceptor agonist carbachol and the A_1-adenosine receptor agonist (−)-N^6-phenylisopropyladenosine (PIA) have different effects on myocardial contractility in different parts of the heart. In auricles both agents exert pronounced negative inotropic effects. In papillary muscles carbachol and PIA have slight positive and no inotropic effects at high concentrations respectively. But in ventricular tissue both PIA and carbachol reduce force of contraction in the presence of agents that increase adenosine 3′:5′-cyclic monophosphate (cyclic AMP) levels such as isoprenaline (Böhm *et al.*, 1984; 1985; Löffelholz & Pappano, 1985; Brückner *et al.*, 1985; Linden *et al.*, 1985; Endoh, 1987). Furthermore, cholinoceptor agonists reportedly cause an enhanced incorporation of [^{32}P]-phosphate into phosphatidylinositol in the heart (Quist, 1982; Brown & Brown, 1983). By use of [^{3}H]-inositol, an increased inositol phosphate formation after stimulation with carbachol has been demonstrated in preparations of chick, rat and guinea-pig hearts (Brown *et al.*, 1985; Brown & Jones, 1986; Scholz, 1989). The initial step in the inositol lipid metabolism is a phospholipase C-mediated hydrolysis of phosphatidylinositol bisphosphate (PIP_2) resulting in the generation of the two presumed second messengers diacylglycerol (DG) and inositol trisphosphate (IP_3; Berridge & Irvine, 1984; 1989). DG activates a protein kinase C (Nishizuka, 1986) while IP_3 releases calcium from intracellular stores in many tissues (for review see Berridge & Irvine, 1984; 1989; Scholz, 1989). It is still a matter of debate whether or not IP_3 releases calcium from cardiac sarcoplasmic reticulum (Hirata *et al.*, 1984; Movsesian *et al.*, 1985), although there is evidence that IP_3 is indeed an intracellular calcium mobilizing agent in cardiac muscle (Nosek *et al.*, 1986; Fabiato, 1986; Kentish *et al.*, 1990). The existence of inositol lipid metabolism has also been shown in the human heart (Kohl *et al.*, 1989).

The close resemblance of the cardiac effects of M-cholinoceptor and A_1-adenosine receptor agonists (for review see Endoh, 1987) led us to compare the concentration-dependent and time-dependent effects of carbachol and PIA on different products of inositol lipid metabolism and on force of contraction in auricles and papillary muscles from guinea-pigs. The aim of the study was twofold. Firstly, since in previous studies a close relation between increase in IP_3 and force of contraction has been shown after α_1-adrenoceptor stimulation (Poggioli *et al.*, 1986; Schmitz *et al.*, 1987a) the present study investigates this phenomenon by a comparative study of the effects of carbachol and PIA. Secondly, since it has been shown in auricles that pertussis toxin treatment converted the negative inotropic effect of carbachol into a positive inotropic effect (Tajima *et al.*, 1987) the effects of carbachol and PIA on inositol phosphates were also studied in auricles.

Some of these results were presented at the 29th Spring Meeting of the German Society of Pharmacology and Toxicology (Scholz *et al.*, 1988a).

Methods

Force of contraction

The experiments were performed on electrically driven (frequency 1 Hz, duration 5 ms, intensity 20% greater than threshold) left auricles and papillary muscles isolated from guinea-pigs (body weight 200–250 g). The animals were killed by a blow on the neck and bled from the carotid arteries. The preparations were attached to a bipolar platinum stimulating electrode and suspended individually in 10 ml glass tissue chambers for recording contractions as described previously (Scholz *et al.*, 1988b). All animals were pretreated with reserpine (5 mg kg^{-1} i.p. 18 h before they were killed) to

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prevent interference from endogenous catecholamines. The bathing solution was a modified Tyrode solution containing (mM) NaCl 119.8, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1.05, NaH_2PO_4 0.42, $NaHCO_3$ 22.6, Na_2EDTA 0.05, ascorbic acid 0.28, glucose 5.0. It was gassed continuously with 95% O_2 plus 5% CO_2 and maintained at 35°C with a pH of 7.4. The force of contraction was measured with an inductive force transducer (W. Fleck, Mainz, FRG). Each muscle was stretched to the length at which force of contraction was maximal. The resting force (approximately 10 mN in the auricles and 5 mN in the papillary muscles) was kept constant throughout the experiment. After mechanical stabilization the substances were added.

Determination of inositol lipid products

Electrically driven left auricles and papillary muscles were labelled for 6 h with 20 μCi ml^{-1} of [^{3}H]-inositol in 10 ml bathing solution (composition see above), gassed with 95% O_2 plus 5% CO_2. Then they were washed for 10 min in [^{3}H]-inositol-free bathing solution and preincubated for 30 min with adenosine deaminase (1 μg ml^{-1}; only in the experiments with PIA to exclude interference from endogenous adenosine; Böhm *et al.*, 1985) and for 10 min with lithium chloride which was present throughout the remainder of the experiments (10 mM; to facilitate the measurement of phosphoinositide products; Scholz *et al.*, 1988b). Thereafter, the muscles were incubated in bathing solution containing carbachol or PIA (plus adenosine deaminase). At the end of each experiment the muscles were frozen in liquid nitrogen, homogenized with a microdismembrator (Braun, Melsungen, FRG), followed by the addition of 1 ml of chloroform/methanol/ hydrochloric acid (100:200:2) and extracted with water (310 μl) and chloroform (310 μl). The inositol phosphates (inositol phosphate, IP_1; inositol bisphosphate, IP_2; inositol trisphosphate, IP_3) were eluted from Dowex 1X8 anion exchange columns (formate form) according to the method of Berridge *et al.* (1983) as described previously (Schmitz *et al.*, 1987b; Scholz *et al.*, 1988b). The phospholipids (phosphatidylinositol, PI; phosphatidylinositol phosphate, PIP; phosphatidylinositol bisphosphate, PIP_2) were washed and dried under a stream of nitrogen. Thereafter they were separated on h.p.t.l.c. silica-gel plates (impregnated with potassium oxalate) running in one dimension with chloroform/methanol/acetone/acetic acid/ water (40:13:15:12:7). Thereafter the silica-gel plates were placed into a vessel with iodine vapour and identified by co-chromatographed standards. The radioactively labelled products were counted in a liquid scintillation counter.

Drugs

Substances used were carbamoylcholine chloride (Sigma, St. Louis, U.S.A.), (−)-N^6-phenylisopropyladenosine (Boehringer, Mannheim, FRG), atropine sulphate (Merck, Darmstadt, FRG), 1,3-dipropyl-8-cyclopentylxanthine (DPCPX, a gift from Dr M.J. Lohse, Heidelberg, F.R.G.), LiCl (Merck, Darmstadt, FRG), phosphoinositides (Sigma, St. Louis, U.S.A.), myo-[2-^{3}H]-inositol (20 Ci mmol^{-1}, Amersham, Braunschweig, FRG), AG 1X8 anion exchange resin (formate form; Bio-Rad Laboratories, München, FRG), h.p.t.l.c.-silica-gel plates 60 (Merck, Darmstadt, FRG), Ready-Value scintillation cocktail (Beckmann, München, FRG). All other chemicals were of analytical or best grade commercially available. All substances were freshly dissolved in prewarmed and pregassed bathing solution. Deionized and twice distilled water was used throughout.

Statistics

The values presented are means ± s.e.mean. Statistical significance was estimated with Student's *t* test for unpaired observations. A *P* value of less than 0.05 was considered significant.

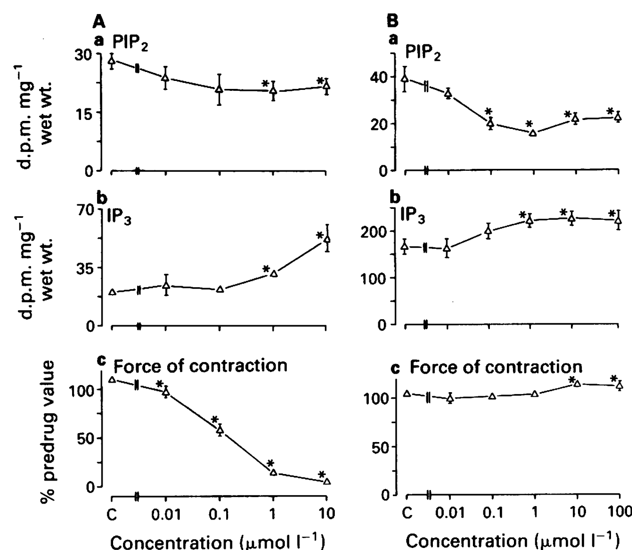

Figure 1 Effects of carbachol in guinea-pig auricles (A) and papillary muscles (B). Shown are concentration-response curves for the effects of carbachol on inositol lipid products (a,b) or force of contraction (c) of guinea-pig isolated electrically driven heart muscle preparations in the presence of lithium chloride (10 mM). Ordinates: phosphatidylinositol bisphosphate (PIP_2; a), inositol trisphosphate (IP_3; b) in d.p.m. mg^{-1} wet weight and force of contraction as a percentage of predrug value (c). Abscissae: Concentration of carbachol in μM. The pre-carbachol value of force of contraction was 2.9 ± 0.3 mN (n = 25; A(c)) and 1.3 ± 0.1 mN (n = 32; B(c)). The incubation time was 5 min for each drug concentration. C = control. n = 4–6 for (A) and 5–7 for (B). * $P < 0.05$ vs control.

Results

Concentration-dependent effects of carbachol

Auricles Figure 1A(a,b) shows concentration-response curves for the carbachol-induced effects on inositol lipid metabolism in guinea-pig left auricles. Accumulation of IP_3 or degradation of PIP_2 began at 1 μM carbachol. At 10 μM carbachol, the highest concentration investigated, the effects apparently did not reach a maximum. The concentration-dependence of the effect of carbachol on force of contraction is shown in Figure 1A(c). The negative inotropic effect was significant at 0.01 μM of carbachol. At 10 μM carbachol the myocardial force decreased to about 4.9% of the predrug value. All effects of carbachol (10 μM) on inositol lipid metabolism and force of contraction were blocked by the M-cholinoceptor-antagonist atropine (10 μM; Table 1A). PI remained unchanged under all conditions.

Papillary muscles Figure 1B shows the results obtained in papillary muscles. Carbachol had no inotropic effect at low concentrations (0.01–1 μM; Figure 1B(c)) but at 10 to 100 μM it exerted a slight positive inotropic effect, up to 115% of control. Carbachol concentration-dependently increased IP_3 (significant at 1 μM). Accordingly PIP_2 was reduced (Figure 1B(a,b)). These effects were also blocked by atropine (10 μM; Table 1B).

Time-dependent effects of carbachol

Auricles The time course of the inositol lipid metabolism in the absence and presence of carbachol (10 μM) is shown in Figure 2A(a,b). Carbachol increased IP_3 to about 226% of control at 5 min. PIP_2 decreased within 5 min to about 73% of control. Figure 2A(c) shows the time course of force of contraction. Carbachol exerted a strong negative inotropic effect which could already be detected at 10 s, reached maximum at 1 min and remained nearly constant thereafter. Thus, in auricles the negative inotropic effect of carbachol preceded the increase in IP_3.

Table 1 Effects of carbachol (CCh, 10 μM) and carbachol in the presence of atropine (Atr, 10 μM) on inositol lipid products (d.p.m. mg^{-1} wet weight) or force of contraction (in % of predrug value) of electrically driven left auricles (A) or papillary muscles (B) in the presence of lithium chloride (10 mM)

	Control	*CCh*	*CCh + Atr*
(A) *Guinea-pig left auricles* (*n* = 6)			
IP_1	101 ± 11	152 ± 9*	102 ± 11
IP_2	30 ± 3	97 ± 9*	28 ± 1
IP_3	20 ± 2	47 ± 8*	19 ± 2
PI	1125 ± 142	1110 ± 104	1171 ± 192
PIP	46 ± 4	29 ± 3*	44 ± 4
PIP_2	31 ± 3	22 ± 2*	31 ± 3
Force	109 ± 1.6	4.9 ± 2.7*	104 ± 6.1
(B) *Guinea-pig papillary muscles* (*n* = 6)			
IP_1	262 ± 38	376 ± 37*	284 ± 34
IP_2	195 ± 26	288 ± 25*	181 ± 28
IP_3	139 ± 13	199 ± 19*	150 ± 15
PI	4998 ± 680	4332 ± 392	4175 ± 230
PIP	494 ± 74	280 ± 51*	492 ± 46
PIP_2	434 ± 47	221 ± 60*	393 ± 62
Force	105 ± 1.5	115 ± 1.4*	102 ± 0.2

All 6 inositol lipid products were measured in each muscle. The products determined were inositol phosphate (IP_1), inositol bisphosphate (IP_2), inositol trisphosphate (IP_3), phosphatidylinositol (PI), phosphatidyl inositol phosphate (PIP) and phosphatidylinositol bisphosphate (PIP_2). The pre-carbachol value of force of contraction was 2.8 ± 0.5 mN in auricles and 1.5 ± 0.3 mN in papillary muscles respectively. * Denotes significant differences versus control ($P < 0.05$). The incubation time was 5 min. *n* = number of preparations.

Papillary muscles The slight positive inotropic effect of carbachol (10 μM; Figure 2B(c)) was significant at 3 min amounting to about 115% of control. The increase in IP_3 was significant at 2 min, reached a maximum at 10 min and was accompanied by a decrease of PIP_2 (Figure 2B(a,b)). Thus, in papillary muscles the increase in IP_3 preceded the increase in force of contraction induced by carbachol.

Concentration-dependent effects of (−)-N^6-phenylisopropyladenosine

Auricles Figure 3A(a,b) shows concentration-response curves for the PIA-induced effects on inositol lipid metabolism in guinea-pig left auricles. Accumulation of IP_3 or degradation of PIP_2 began at 0.1–1 μM PIA. The concentration-dependence of the effect of PIA on force of contraction is shown in Figure 3A(c). The negative inotropic effect was significant at 0.01 μM PIA. At 10 μM PIA the force of contraction decreased to about 5% of the predrug value. All effects of PIA (10 μM) on inositol lipid metabolism and force of contraction were blocked by the A_1-adenosine receptor-antagonist DPCPX (20 μM; Table 2A). DPCPX was used, because it is a potent and selective A_1-adenosine receptor antagonist (700 fold A_1-selectivity; Lohse *et al.*, 1987; Leyen *et al.*, 1989). PI remained unchanged under all conditions.

Papillary muscles In Figure 3B the same experiments are shown for the papillary muscle. PIA had no inotropic effect (0.01–100 μM; Figure 3B(c)) but PIA concentration-dependently increased IP_3 (significant at 1 μM) and PIP_2 was reduced (Figure 3B(a,b)). Again these effects were all blocked by DPCPX (20 μM; Table 2B).

Time-dependent effects of (−)-N^6-phenylisopropyladenosine

Auricles The time course of the inositol lipid metabolism in the absence and presence of PIA (10 μM) is shown in Figure 4A(a,b). PIA increased IP_3 to about 171% of control, significant at 5 min. PIP_2 was decreased within 5 min to about 67% of control. Figure 4A(c) shows the time course of force of contraction. PIA exerted strong negative inotropic effects which could already be detected at 10 s, and remained nearly constant from 1–15 min. Thus, in auricles the negative inotropic effect of PIA preceded the increase in IP_3.

Papillary muscles PIA (10 μM; Figure 4B) had no inotropic effect. In contrast, the increase in IP_3 was significant at 2 min, reached a maximum thereafter and was accompanied by a decrease of PIP_2. Thus, in papillary muscles the increase in

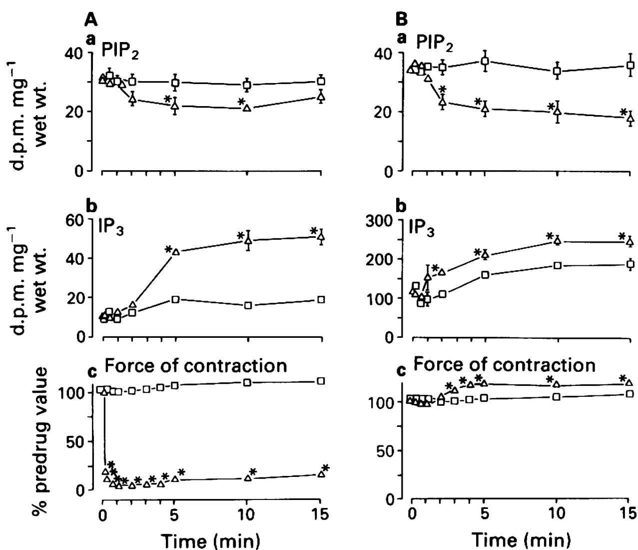

Figure 2 Effects of carbachol in guinea-pig auricles (A) and papillary muscles (B). Shown are time courses of inositol lipid products (a,b) or force of contraction (c) of guinea-pig isolated electrically driven heart muscle preparations in the absence (□) and presence (△) of carbachol (10 μM). All experiments were performed in the presence of lithium chloride (10 mM). Ordinates: phosphatidylinositol bisphosphate (PIP_2; a) inositol trisphosphate (IP_3; b) in d.p.m. mg^{-1} wet weight and force of contraction as percentage of predrug value (c). Abscissae: time of incubation with carbachol in min. The value of force of contraction at zero time was 3.9 ± 1.2 mN (*n* = 6; A(c)) and 1.6 ± 0.6 mN (*n* = 5; B(c)). *n* = 4–6 for (A) carbachol and *n* = 6 for (A) control; *n* = 5–7 for (B) carbachol and *n* = 6 for (B) control. * $P < 0.05$ vs control.

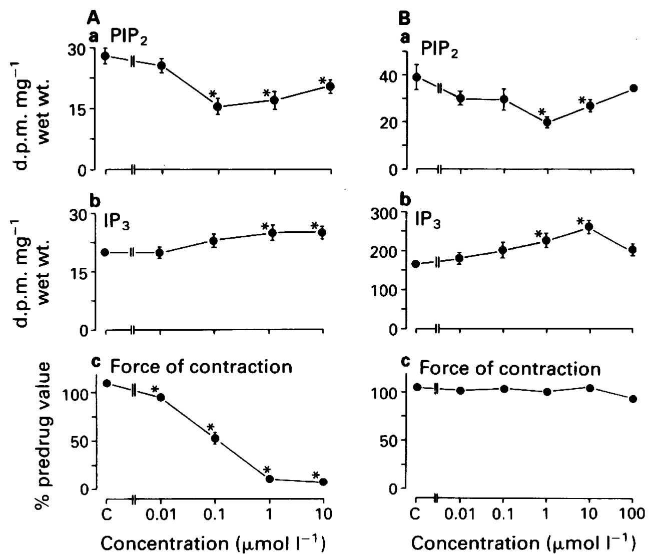

Figure 3 Effects of (−)-N^6-phenylisopropyladenosine (PIA) in guinea-pig auricles (A) and papillary muscles (B). Shown are concentration-response curves for the effects of PIA on inositol lipid products (a,b) or force of contraction (c) of guinea-pig isolated electrically driven heart muscle preparations in the presence of lithium chloride (10 mM). Ordinates: phosphatidylinositol bisphosphate (PIP_2; a) inositol trisphosphate (IP_3; b) in d.p.m. mg^{-1} wet weight and force of contraction as percentage of predrug value (c). Abscissae: Concentration of PIA in μM. The pre-PIA value of force of contraction was 3.4 ± 0.3 mN (*n* = 25; A(c)) and 1.4 ± 0.1 mN (*n* = 30; B(c)). The incubation time was 5 min for each drug concentration. C = control. *n* = 5–7 for (A) and (B). * $P < 0.05$ vs control.

Table 2 Effects of (−)-N^6-phenylisopropyladenosine (PIA, 10 μM) and PIA in the presence of 1,3-dipropyl-8-cyclopentylxanthine (DPCPX, 20 μM) on inositol lipid products (d.p.m. mg^{-1} wet weight) or force of contraction (in % of predrug value) of electrically driven left auricles (A) or papillary muscles (B) in the presence of lithium chloride (10 mM)

	Control	*PIA*	*PIA + DPCPX*
(A) *Guinea-pig left auricles* (n = 5)			
IP_1	94 ± 8	139 ± 9*	102 ± 9
IP_2	30 ± 2	51 ± 3*	28 ± 2
IP_3	20 ± 2	46 ± 2*	20 ± 2
PI	1044 ± 64	980 ± 107	1010 ± 60
PIP	51 ± 2	28 ± 1*	51 ± 3
PIP_2	29 ± 2	20 ± 2*	27 ± 1
Force	109 ± 1.6	8.5 ± 1.3*	99 ± 3.2
(B) *Guinea-pig papillary muscles* (n = 6)			
IP_1	311 ± 24	392 ± 26*	314 ± 15
IP_2	196 ± 17	278 ± 14*	209 ± 18
IP_3	175 ± 14	251 ± 21*	166 ± 14
PI	3936 ± 438	3907 ± 602	3792 ± 627
PIP	472 ± 45	336 ± 30*	461 ± 55
PIP_2	398 ± 25	253 ± 20*	377 ± 28
Force	105 ± 1.5	104 ± 1.9	104 ± 1.3

All 6 inositol lipid products were measured in each muscle. The products determined were inositol phosphate (IP_1), inositol bisphosphate (IP_2), inositol trisphosphate (IP_3), phosphatidylinositol (PI), phosphatidylinositol phosphate (PIP) and phosphatidylinositol bisphosphate (PIP_2). The pre-PIA value of force of contraction was 2.9 ± 0.5 mN in auricles and 1.4 ± 0.2 mN in papillary muscles respectively. * Denotes significant differences versus control ($P < 0.05$). The incubation time was 5 min. n = number of preparations.

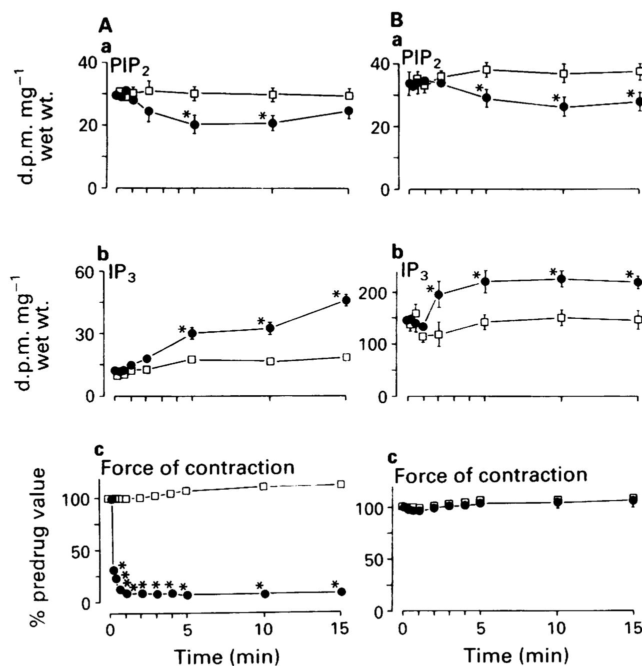

Figure 4 Effects of (−)-N^6-phenylisopropyladenosine (PIA) in guinea-pig auricles (A) and papillary muscles (B). Shown are time courses of inositol lipid products (a,b) or force of contraction (c) of guinea-pig isolated electrically driven heart muscle preparations in the absence (□) and presence (●) of PIA (10 μM). All experiments were performed in the presence of lithium chloride (10 mM). Ordinates: phosphatidylinositol bisphosphate (PIP_2; a) inositol trisphosphate (IP_3; b) in d.p.m. mg^{-1} wet weight and force of contraction as percentage of predrug value (c). Abscissae: time of incubation with PIA in min. The value of force of contraction at zero time was 3.7 ± 0.3 mN (n = 5; A(c)) and 1.7 ± 0.2 mN (n = 6; B(c)). n = 7–9 for (A) PIA and n = 6–9 for (A) control; n = 6–8 for (B) PIA and n = 8 for (B) control. * $P < 0.05$ vs control.

IP_3 resembles that induced by carbachol, while there is no inotropic effect.

Discussion

The present study shows that carbachol and PIA had similar effects on inositol lipid metabolism and on force of contraction in the mammalian heart. However, a close relation between increase in IP_3 and force of contraction could not be demonstrated. Carbachol or PIA decreased PIP_2 and PIP and increased the content of IP_3 and its congeners IP_2 and IP_1. All effects were blocked by the M-cholinoceptor antagonist atropine or the A_1-adenosine receptor antagonist DPCPX, indicating receptor-mediated effects. For clarity the effects of carbachol and PIA will be discussed separately.

Carbachol

In auricles (Figure 1A) there is an apparent dissociation between increase in IP_3, which supposedly is a second messenger for positive inotropic effects (Renard & Poggioli, 1987; Scholz *et al.*, 1988b), and force of contraction because carbachol had a negative inotropic effect. In addition, the negative inotropic effect was significant at lower concentrations than the increase in IP_3 and preceded the increase in IP_3. Thus, the increase in IP_3 after stimulation with carbachol is unlikely to be responsible for the negative inotropic effect. This is not surprising because the negative inotropic effect in auricles is due to an activation of atrial potassium channels through a guanine nucleotide binding protein (G-protein; Pfaffinger *et al.*, 1985; Böhm *et al.*, 1986). Carbachol increases potassium conductance, hyperpolarizes the membrane, decreases action potential duration and thereby reduces influx of calcium, leading to the negative inotropic effect.

In auricles the increase in potassium conductance conceivably overrides a possible IP_3-induced positive inotropic effect. Recently, it could be demonstrated (Tajima *et al.*, 1987; Kohl *et al.*, 1990) that pertussis toxin treatment converted the negative inotropic effect of carbachol (starting at 10 μM) in auricles into a positive inotropic effect which was still accompanied by an increase in inositol phosphates, indicating that 2 different G-proteins are involved: a pertussis toxin-sensitive G-protein which regulates potassium conductance at low concentrations of agonists and a different pertussis toxin-insensitive as yet unidentified G-protein which couples the M-cholinoceptor to the inositol-lipid-metabolism in the heart at high concentrations of agonists. Alternatively two different subtypes of M-cholinoceptors may be involved. The pertussis toxin-sensitive effect of carbachol on potassium conductance prevails over the pertussis toxin-insensitive effects on inositol lipid metabolism. Hence a negative inotropic effect of carbachol is normally observed despite an increase in IP_3. A positive inotropic effect of carbachol possibly due to the IP_3 increase can only be observed after elimination of the effect on potassium conductance with pertussis toxin. A pertussis toxin-insensitive G-protein has also been shown for the α_1-adrenoceptor-mediated effects on inositol lipid metabolism in the heart (Schmitz *et al.*, 1987b) and in other tissues (Cockroft, 1987; Rosenthal & Schultz, 1988). All effects of carbachol were blocked by atropine, indicating that the effects on force of contraction and inositol lipid metabolism are mediated via M-cholinoceptors.

In papillary muscles carbachol alone induced a slight positive inotropic effect and an increase in IP_3 content (Figure 1B). The increase in force developed slowly being first significant at 3 min whereas the increase in IP_3 was significant at 2 min (Figure 2B). It is evident that the increase in IP_3 preceded the increase in force, fulfilling a prerequisite for a second messenger role of IP_3. The concentration- and time-dependent effects of carbachol on force of contraction and IP_3 are compatible with an IP_3-mediated positive inotropic effect.

(−)-N^6-*phenylisopropyladenosine*

In auricles the effects of PIA on force of contraction and inositol lipid metabolism were similar to the effects of carbachol. The negative inotropic effect after stimulation with PIA was also faster (Figure 4A) and occurred at lower concentrations (Figure 3A) than did the increase in IP_3 content. This leads to similar conclusions. In brief, a possible IP_3-induced positive inotropic effect could be overriden by an increase in potassium conductance, leading to the negative inotropic effect. Moreover, DPCPX antagonized all effects of PIA, indicating an A_1-adenosine receptor-mediated effect (Leyen *et al.*, 1989).

In papillary muscles PIA had no inotropic effect but increased inositol phosphates and decreased phospholipids (Figure 3B). Thus, concentration- and time-dependent effects (Figure 4B) of PIA on inositol lipid products are comparable with the results observed with carbachol. However, the reason for the lack of effect of PIA on force of contraction is unclear. Firstly, it could be due to unspecific effects of the adenosine analogue, because the parent compound adenosine, like carbachol, has a slight positive inotropic effect at high concentrations (100 μM; Brückner *et al.*, 1985; Legsseyer *et al.*, 1988). Secondly, it could indicate a dissociation between increase in IP_3 and increase in force of contraction. Thirdly, compartmentation of IP_3, as has been shown for cyclic AMP in cardiomyocytes (Buxton & Brunton, 1983) cannot be excluded. In contrast to the present study, no stimulation of the inositol lipid metabolism in atrial and ventricular myocytes was found after stimulation with PIA (Leung *et al.*, 1986). However, a similar increase in IP_3 was found with adenosine in rat papillary muscles (Legssyer *et al.*, 1988).

In summary, atrial and ventricular M-cholinoceptors and adenosine A_1-receptors are both coupled to inositol lipid metabolism. In atrial tissues there is an apparent dissociation between increase in IP_3 and force of contraction. However, a possible IP_3 induced positive inotropic effect of carbachol can only be observed after elimination of the effect on potassium conductance. In ventricular tissues the positive inotropic and IP_3 increasing effects of carbachol revealed similar time- and concentration-dependencies and hence might be closely related. In contrast, PIA failed to cause an increase in force of contraction. Thus, the present data could not show a close relationship between increase in IP_3 and an increase in force of contraction as has been shown for α_1-adrenoceptor-stimulation.

This study was supported by the Deutsche Forschungsgemeinschaft and the Claussen Stiftung.

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(Received March 6, 1990
Revised July 11, 1990
Accepted August 4, 1990)