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Invited Paper

Alpha-1-adrenergic stimulation of phosphoinositide breakdown in cultured neonatal rat ventricular myocytes

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Summary

The regulation of and the intracellular events following α_1-adrenergic receptor stimulation in the myocardium still remain to be disclosed. The effect of α_1-adrenergic stimulation on phosphoinositide breakdown was studied in cultured neonatal rat ventricular myocytes. Phenylephrine (30 μM) stimulated inositolphosphates formation, but only in the presence of 10 mM LiCl this could be measured. The increase was antagonized by prazosin (1 μM) but not by propranolol (1 μM). The variability in proportional distribution of the three inositolphosphates is discussed.

Abbreviations: PIP_2 Phosphatidylinositol 4,5-bisphosphate, PI Phosphatidylinositol, IP_3 Inositol 1,4,5,-trisphosphate, IP_2 Inositol 1,4-bisphosphate, IP_1 Inositol 1-monophosphate, DG Diacylglycerol, PKC Calcium/phospholipid-dependent protein kinase, PhE Phenylephrine.

Introduction

In ventricular myocardium both α_1- and β-adrenergic agonists provoke an increase of slow inward calcium current and enhance the contractile force [1, 2]. However qualitative and quantitative differences between the responses of the heart to α_1-agonists and β-agonists are prominent. The well-known mechanism underlying β-adrenoceptor stimulation – activation of adenylate cyclase followed by cyclic AMP-dependent protein phosphorylation – does not seem to apply to α_1-adrenoceptors.

In isolated rat ventricles [3], papillary muscle [4] and adult cardiomyocytes [5] stimulation of α_1-adrenoceptors was reported to enhance phosphoinositide breakdown. Although in these preparations the PI cycle of other cell types could have contributed, myocardial α_1-adrenergic receptors may be coupled to a phospholipase C which upon activation hydrolyses PIP_2 into IP_3 and DG. IP_3 is thought to mobilize Ca^{2+} from intracellular stores [6, 7] and DG is an activator of protein kinase C (PKC).

In our study of α_1-adrenergic stimulation we used primary cultures of neonatal rat ventricular myocytes. Our preparation contained a near to homogenous population of spontaneously beating cardiac muscle cells. The activity of PI-phospholipase C was measured as the accumulation of IP_3 and its breakdown products IP_2 and IP_1. Dephosphorylation of IP_1 was blocked by LiCl.

Experimental procedures

Primary heart cell culture

Cardiac cells were isolated from ventricles of 2–4 day old Wistar rats by stepwise trypsinization according to Yagev *et al.* (1984) [9]. A near to homogenous suspension of cardiomyocytes was obtained by two successive periods of 30 and 90 min preplating. Finally 1.8×10^6 myoblasts were seeded per Petridish (Greiner, TC 35/10), which after 24–26 h incubation (37° C, 5% CO_2, 95% hum.) resulted in a confluent monolayer of beating cardiomyocytes. Every 48 h the growth medium (Ham F-10 (Gibco), supplemented with 10% FCS, 10% HS, 2×10^5 U/l penicillin and 0.2 g/l streptomycin (Boehringer); and 135 mg/l $CaCl_2 \cdot 2H_2O$) was changed. Experiments were done on the third or fifth day after plating.

Cell incubation

After decanting the growth medium cardiomyocytes were rinsed twice with 1 ml incubation buffer (130 mM NaCl, 4.7 mM KCl, 1.3 mM $CaCl_2$, 20 mM $NaHCO_3$, 0.44 mM NaH_2PO_4, 1.1 mM $MgCl_2$, 0.2% D-glucose, 37° C, gassed, pH 7.4) followed by preincubation in 0.5 ml 6.7 Ci [2-^{3}H-]*myo*-inositol/ml buffer for 2 h. After this period the cells were rinsed twice with 0.5 ml buffer and incubated in buffer, 10 mM LiCl and other additions as indicated, at a final volume of 1 ml.

Incubations were terminated by rinsing with 0.5 ml ice-cold buffer immediately followed by addition of 0.5 ml methanol/13 M HCl (100:1 v/v).

Assay of [^{3}H-]inositolphosphates

Cells were scraped and kept overnight at −20° C in methanol/chloroform/13 M HCl (200:100:1 v/v). Phase separation was obtained in chloroform/methanol/2.5 M HCl (2:2:1 v/v). After rinsing the organic phase once with 1 ml synthetic upper phase (chloroform/methanol/0.6 M HCl, 3:48:47 v/v) the combined upper phases were diluted with water and applied to 1 ml Dowex 1X8 anion exchanger (formate form). Inositolphosphates were separated according to Berridge *et al.* (1982) [10]. One volume of eluate was mixed with two volumes of Instagel (Packard) and counted for radioactivity by liquid scintillation (Tricarb 2660 from Packard).

Statistical analysis

Data were evaluated for statistical significance by ANOVA and the Bonferroni t-test for multiple comparisons. $P < 0.05$ was considered significant.

Results

As is shown in Table 1 the addition of 30 μM PhE to ^{3}H-inositol labelled cardiomyocytes caused after 30 minutes an increase in ^{3}H-inositolphosphates accumulation only in the presence of Li^+. This increase was completely blocked by the specific α_1-adrenergic antagonist prazosin. The β-adrenergic antagonist propranolol had no effect on the PhE-stimulated inositolphosphates formation.

In two experiments we examined the proportional distribution of the three inositolphosphates. This was found to vary very much between the experiments: IP_1 43.0%–59.8%; IP_2 33.2%–30.3%; IP_3 23.8%–9.9%. In two other experiments we examined the same parameter after shorter incubation periods. Again a large variation: 1 min. IP_1 61.2%–49.6%; IP_2 25.4%–34.0%; IP_3 13.4%–16.4%; 6 min. IP_1 63.1%–61.0%; IP_2 24.3%–26.9%; IP_3 12.6%–12.1%. Although at each time point most of the label was present in IP_1, in every experiment the IP_1 was seen rising while the radioactivity in IP_3 and IP_2 was constant.

Discussion

Our findings show that myocardial α_1-adrenergic receptors are coupled to the phosphoinositol cycle. This is in agreement with other reports [3–5].

The fact that we were not able to show any rise in the level of IP_3 shortly (1 min) after stimulation

could be interpreted as an indication that not PIP_2 but PI is the substrate for agonist stimulated phospholipase C in myocardial cells. On the other hand the activity of the IP_3-and IP_2-phosphatases may be that high that the IP_3- and IP_2-peaks are too transient to be measured directly. The IP_1-phosphatase certainly must be very active as can be derived from the impossibility to measure inositolphosphates formation in the absence of Li^+ (Table 1). This monovalent cation acts as an inhibitor of this phosphatase [10]. The continuous formation of IP_1 should in this case be seen in consequence of IP_3-formation and -dephosphorylation.

How this could agree with the generally supposed second messenger action of IP_3 is not easily answered. During agonist-receptor binding there could exist a constant flow of IP_3 from its formation site to the calcium storage site. The IP_3-phosphatase may even be involved in the action of IP_3 on the calcium stores. But this is mere speculation and will stay this until some intracellular IP_3-binding site has been identified.

Of more importance as a second messenger (especially in heart muscle) may be the other PIP_2-breakdown product, which was not measured in our experiments: the diglyceride. It is supposed that DG activates PKC which has several protein substrate sites in the myocardium e.g. phospholamban [11]. Whether this phosphorylation actually occurs under α_1-adrenergic stimulation is at present not known. However it would indicate a common target of α_1- and β-adrenergic stimulation.

Table 1. Receptor specificity of phenylephrine-stimulated [^{3}H-]inositol phosphates formation in neonatal rat cardiomyocytes

Addition(s)	[^{3}H-]inositolphosphates (% of control ± S.E.M.)
None	93 ± 20
Li^+	100
Phenylephrine	116 ± 14
Li^+, phenylephrine	433 ± 83*
Li^+, phenylephrine, prazosin	117 ± 11
Li^+, phenylephrine, propranolol	356 ± 53*

Data are means ± S.E.M. of at least 6 samples. Control value (100%) is 322 ± 35 d.p.m./dish (n = 9). The concentrations used were 10 mM LiCl, 30 μM phenylephrine, 1 μM prazosin and 1 μM propranolol. Incubation time was 30 minutes. * $P<0.05$.

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