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Effect of adrenergic agonists on phosphoinositide breakdown in rat skeletal muscle preparations

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Adrenergic regulation of phosphoinositide breakdown in rat skeletal muscle was investigated in 30-min incubations with 10 mM LiCl. In rat hemidiaphragms, prelabelled with D-*myo*-[2-^{3}H]inositol, addition of α-agonists (epinephrine, norepinephrine, phenylephrine) induced a 5–8-fold increase of [^{3}H]inositol monophosphate accumulation. This could be prevented by inclusion of α-antagonists (phentolamine, prazosin). β-Agonists and/or β-antagonists had no effect. Similar experiments with isolated flexor digitorum brevis muscle fibers yielded confirmatory results. Functional integrity of β-receptor mediated processes was suggested by the β-agonist-induced increase of glucose 6-phosphate in hemidiaphragms and cAMP in fiber preparations. The results indicate that phosphoinositide breakdown in differentiated rat skeletal muscle is, at least in part, under α-adrenergic control.

Phosphatidylinositol; α-Agonist; (Rat diaphragm, Muscle fiber)

1. INTRODUCTION

Receptor-mediated activation of membrane bound phospholipase C, which catalyses the hydrolysis of phosphatidylinositol 4,5-bisphosphate, generates two second messengers. Diacylglycerol thus formed can activate protein kinase C, whereas inositol 1,4,5-trisphosphate is involved in the regulation of intracellular Ca^{2+} fluxes. The latter compound can be rapidly inactivated and catabolized to inositol by specific monoesterases (review [1]).

Using LiCl, a specific inhibitor of inositol phosphate hydrolysis at the inositol monophosphate step, α-adrenergic mechanisms have been shown to stimulate phosphoinositide breakdown in fat cells [2], hepatocytes [3], BC3H-1 cells [4], smooth muscle [5] and heart [6]. Whether this also applies to differentiated skeletal muscle is unknown.

This communication reports on a stimulatory effect of α-adrenergic agonists on inositol phosphate formation in hemidiaphragms and isolated flexor digitorum brevis muscle fibers of rat.

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2. MATERIALS AND METHODS

Unless otherwise noted, all chemicals were purchased from Merck (Darmstadt), Serva (Heidelberg) or Sigma (München). Phentolamine was from Ciba (Wehr) and prazosin was from Pfizer (Karlsruhe). Enzymes and coenzymes were from Boehringer (Mannheim) and D-*myo*-[2-^{3}H]inositol (577–620 kBq/nmol), L-3-phosphatidyl-[2-^{3}H]-inositol (603 kBq/nmol) and ^3H_2O standard from Amersham (Braunschweig).

Fed male rats were used (100–150 g body wt). Hemidiaphragms were prelabelled with [^{3}H]inositol (1 μM, 300 kBq/nmol) by incubation in an about 10-fold volume (w/v) of carbogen saturated

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(95% O_2/5% CO_2) Krebs-hydrogen carbonate buffer (KRB), pH 7.4, as modified in [2], for 150 min (37°C, shaking water bath). After washing with KRB, muscles were incubated in fresh inositol-free buffer, containing 10 mM LiCl and antagonist, if necessary. After 10 min, the reaction was started by addition of agonist solution and continued for 30 min. Thereafter, muscles were quickly withdrawn, washed twice in ice-cold KRB, blotted with filter paper, and freeze-stopped in liquid N_2. Tissue was weighed and pulverized in a percussion mortar together with a 3-fold volume of water. 200 mg powder were extracted with 750 μl $CHCl_3/CH_3OH$ (1:2, v/v) followed by 250 μl water and 250 μl $CHCl_3$. After centrifugation (8000 × g, 2 min), the radioactivity incorporated into lipid and water-soluble metabolites was determined (recovery 95 ± 2%, n = 8) as described in [2].

Flexor digitorum brevis muscle fibers were prepared essentially as described in [7]. After incubation of muscles in KRB for 200 min with [^{3}H]inositol (1 μM, 600 kBq/nmol), collagenase (0.5 U/ml), and ampicillin (0.1 mg/ml), a crude single cell suspension was obtained by trituration with a Pasteur pipette. The suspension was washed four times with KRB by centrifugation (15 × g, 90 s). Thus, an about 1000-fold enrichment of fibers in relation to the mononuclear cells was obtained. As determined by trypan blue exclusion, the viability of fibers was largely maintained (60%, cf. [7]). 0.2 ml of enriched fiber suspension (about 5×10^4 fibers/ml) was mixed with 10 μl of LiCl solution (0.2 M) containing antagonist, if necessary, and further incubated, as described above. The reaction was terminated and extraction performed exactly as described for muscle powder.

Tissue water content (81.6 ± 1.0%, n = 16) was determined in separate experiments and remained unaffected by epinephrine treatment. Glucose 6-phosphate content of incubated hemidiaphragms was enzymatically determined in perchloric acid extracts [8]. Production of cAMP in membrane preparations of enriched fiber suspensions was estimated as given in [9].

3. RESULTS

Incorporation of radioactivity from 1 μM [^{3}H]inositol into lipid and water-soluble muscle metabolites increased at an essentially constant rate up to 4 h of incubation. Similar results were obtained in 3-h incubations with [^{3}H]inositol from 1 to 80 μM (not shown). Thus, standard prelabel-

Table 1

Effect of various adrenergic agonists on [^{3}H]inositol monophosphate accumulation in incubations of prelabelled rat hemidiaphragms with LiCl

Condition	[^{3}H]Inositol monophosphate accumulation (dpm/mg dry wt)			
	–	Phentolamine (10 μM)	Prazosin (1 μM)	Propranolol (10 μM)
Control	101 ± 15	118 ± 35	125 ± 35	110 ± 13
Epinephrine (10 μM)	809 ± 194	156 ± 26	140 ± 9	688 ± 108
Norepinephrine (10 μM)	694 ± 96	157 ± 31	n.d.	n.d.
Phenylephrine (10 μM)	444 ± 130	100 ± 14	108 ± 14	515 ± 46
Isoprenaline (1 μM)	94 ± 8	124 ± 35	121 ± 38	110 ± 12

Hemidiaphragms were prelabelled by incubation with [^{3}H]inositol. 10 mM LiCl and antagonist in fresh medium were applied 10 min prior to a 30-min treatment with agonist. The reaction was terminated by freeze-stopping of tissue in liquid N_2. Water-soluble metabolites were extracted and radioactivity incorporation determined after anion chromatographic separation (see section 2 for details). The concentrations of drugs were as indicated. Results are means ± SD from 3–14 separate incubations; n.d., not determined

ling conditions were deliberately chosen to obtain sufficient incorporation of radioactivity for reliable measurements. In incubations of prelabelled hemidiaphragms in the absence of LiCl, radioactivity was recovered in phosphatidylinositol, glycerophosphoinositol, inositol tris-, bis- and monophosphate (Ins-P) fractions and amounted to 2561 ± 564, 49 ± 11, 28 ± 11, 44 ± 17 and 36 ± 3 dpm/mg dry wt (n = 8), respectively. With LiCl, given exclusively or in combination with other drugs, only the increase of radioactivity in Ins-P proved to be statistically significant ($p <$ 0.001 by Student's t-test).

The effects of various agonists and antagonists are shown in table 1. Ins-P accumulation in hemidiaphragms was considerably stimulated by α-agonists. As tested with epinephrine, this was due to a linear increase over the 30-min incubation period (not shown). Stimulation was totally inhibited in the presence of α-antagonists. In contrast, neither β-agonists nor β-antagonists had any effect.

In comparative experiments with intact flexor digitorum brevis muscles, prelabelled for 150 min, phenylephrine induced a 4.2 ± 0.8-fold increase in Ins-P (n = 4). This corresponded to the data for hemidiaphragms (cf. table 1).

Whether α-agonist effects were actually due to hormonal regulation of striated muscle metabolism, was examined with prelabelled fiber preparations which were virtually free from mononuclear cells. In fact, epinephrine induced a 2–3-fold increase in Ins-P formation (2.0 ± 0.3-fold in crude preparations, n = 3) which could be completely blocked by the α-antagonist phentolamine (fig.1).

β-Receptor-mediated regulation was examined by treatment of incubated diaphragms with 10 μM epinephrine for 10 min. Glucose 6-phosphate content was 0.23 ± 0.02 μmol/g of muscle wet wt compared to 0.09 ± 0.01 and 0.07 ± 0.02 μmol/g in controls and in the presence of 10 μM propranolol, respectively (n = 3). As is shown in fig.2, in membrane preparations of isolated muscle fibers, β-agonists induced a several fold increase of cAMP formation.

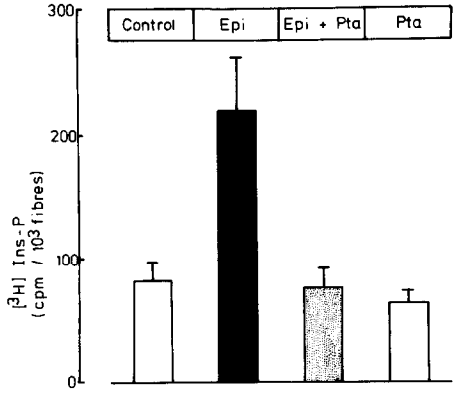

Fig.1. Effect of adrenergic stimulation on [^{3}H]inositol monophosphate accumulation (Ins-P) in incubations of prelabelled flexor digitorum brevis muscle fibers with LiCl. Muscles were incubated with [^{3}H]inositol and collagenase and enriched fiber suspensions prepared as detailed in section 2. 10 mM LiCl and 10 μM phentolamine (Pta) were applied 10 min prior to a 30-min treatment with epinephrine (Epi, 10 μM). Extraction and chromatographic separation of [^{3}H]Ins-P were performed as given in section 2. Results (means ± SD of triplicate samples) of one experiment, typical for three, are shown.

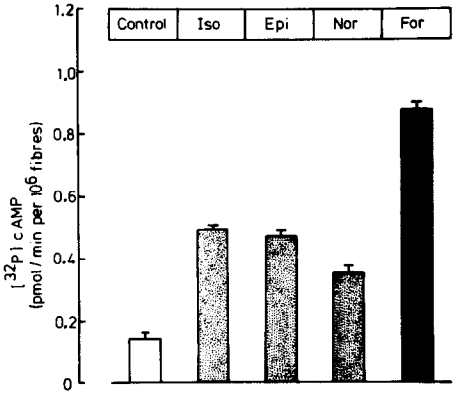

Fig.2. Effect of catecholamines on cAMP production in membrane preparations of isolated flexor digitorum brevis muscle fibers. Enriched fiber suspensions were prepared as given in the legend to fig.1, but the tritium label was omitted. Membranes were prepared and assayed for adenylate cyclase activity in 10-min incubations according to [9] in the absence (control) or presence of hormone (10 μM): isoprenaline, Iso; epinephrine, Epi; norepinephrine, Nor. The effect of forskolin (For, 33 μM) is included for comparison. [^{32}P]cAMP formation was calculated on the basis of the specific radioactivity of [α-^{32}P]ATP in the assays. Means ± SD of quadruplicate samples from one experiment, typical of two, are shown.

4. DISCUSSION

It was the aim of the present study to establish whether or not catecholamines might influence differentiated skeletal muscle phosphoinositide turnover. Taken together, the present results suggest that in the rat (i) catecholamines are involved and (ii) effects are mediated by α_1-receptors. This is compatible with the results of a recent investigation, in which the occurrence of α_1-receptors in skeletal muscle could be demonstrated, whereas α_2-receptors were apparently absent [10].

In prelabelled rat hemidiaphragms, epinephrine-induced accumulation of Ins-P was linear for 30 min, and slight increases of radioactivity in the Ins-P_3 fraction were found, even after short hormone treatment in the minute range. Whether Ins-P accumulation was actually a consequence of α_1-receptor-mediated enhancement of Ins-P_3 formation, as has been proposed for rat heart [6], remains to be established. The present experiments provided no conclusive, i.e. statistically significant, results, presumably due to some experimental limitations, e.g. impossibility of reaching a steady state in phospholipid labelling, variation of data and, possibly, small changes in intracellular Ins-P_3 steady-state concentrations.

Catecholamine-induced increase in Ins-P_3 seems, however, an attractive possibility, as in skeletal muscle, Ins-P_3 might be involved in excitation-contraction coupling [11] and has been suggested to increase Ca^{2+} sensitivity of the contractile apparatus [12].

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