α_1-Adrenergic activation of brown adipocytes leads to an increased formation of inositol polyphosphates

Eewa Nånberg and James Putney, jr*

Department of Metabolic Research, The Wenner-Gren Insitute, University of Stockholm, Biologihus F3, S-106 91 Stockholm, Sweden and **Department of Phamacology, Medical College of Virginia, Richmond, VA 23298-0001, USA*

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α_1-Adrenergic activation of isolated brown adipocytes causes a rapid mobilization of intracellular Ca^{2+}. The cells also respond with an increased turnover of inositol lipids. The present work demonstrates that α_1-adrenergic stimulation of brown adipocytes results in phospholipase C-mediated breakdown of phosphatidylinositol bisphosphate to form inositol trisphosphate. The rate of appearance of inositol trisphosphate is sufficiently rapid for it to mediate or contribute to Ca^{2+} mobilization in these cells.

(Brown adipocyte) *α_1-Adrenoceptor* *Inositol polyphosphate* *Li^+*

1. INTRODUCTION

Previous studies have shown that α_1-adrenoceptor activation causes mobilization of intracellular Ca^{2+} in brown adipocytes [1]. This α_1-adrenergic effect is dependent on the presence of extracellular Na^+ [1] and may be associated with an increased Na^+ influx. Since the abundant mitochondria in brown adipocytes possess an active Na^+/Ca^{2+} exchange mechanism [2], it has been suggested that the α_1-adrenoceptor-induced Ca^{2+} mobilization could arise secondarily to Na^+ entry [1,3,4].

α_1-Adrenergic stimulation of brown adipocytes also induces an increased turnover of PtdIns [5,6]. It is generally believed that agonist-activated turnover of PtdIns reflects phospholipase C-mediated breakdown of phosphatidylinositol 4,5-bisphosphate to diacylglycerol and inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3]. Ins(1,4,5)P_3 has been shown to cause Ca^{2+} release in several different types of permeabilized cells [7–9] and from microsomal fractions from hepatocytes [10] and insulinoma cells [11]. Ins(1,4,5)P_3 also caused EGTA-inhibited activation of pyruvate dehydrogenase in isolated white fat cells [12], which may be indicative of intracellular Ca^{2+} release.

The aim of this work was to determine whether α_1-adrenergic activation of brown adipocytes results in an increase in cellular Ins(1,4,5)P_3, in the light of the possibility that this putative messenger could contribute to Ca^{2+} mobilization in these cells.

Abbreviations: $InsP_3$, inositol trisphosphate; PtdIns, phosphatidylinositol

2. MATERIALS AND METHODS

2.1. *Isolation of brown adipocytes*

Brown adipocytes were isolated by collagenase digestion of tissue obtained from adult golden hamsters, as described [1]. Cell viability was routinely about 90%, estimated by staining with Alcian blue.

2.2. *Incubation of brown adipocytes and labelling of inositol lipids*

All incubations were performed in Krebs-Ringer bicarbonate buffer containing 2% bovine serum albumin, 10 mM glucose and 10 mM fructose at

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pH 7.4 (37°C) and under a gas phase of 5% CO_2 in O_2 (pH was continuously monitored with phenol red). Inositol lipids were labelled by incubating the adipocytes (1.0×10^6 cells/ml) for 90 min in medium containing 10 μCi/ml *myo*-[^{3}H]inositol. Extracellular radioactivity was removed by centrifugation and the cells were incubated for an additional 20 min at 0.5×10^6 cells/ml. 1 μM propranolol was added and the suspension divided into aliquots for experimental incubations. To some of the aliquots drugs were added. 1 ml samples were taken from these incubations, and the reactions stopped by precipitation with 2 ml of 4.5% perchloric acid. Perchloric acid was added to controls (t = 0) prior to the addition of drugs. The samples were left on ice for 10 min and then centrifuged. The supernatants were neutralized with 3 ml of 0.5 M KOH/9 mM $Na_2B_4O_7$ (pH 8). Representative aliquots were extracted with chloroform-methanol to determine total radioactivity in cellular phospholipids [13].

2.3. *Separation of inositol phosphates*

The water-soluble inositol phosphates were separated by anion-exchange chromatography on Dowex-AG1 as described by Berridge et al. [14]. Radioactivity in fractions corresponding to inositol phosphate (InsP), inositol bisphosphate ($InsP_2$) and $InsP_3$ were determined and expressed as a percentage of the radioactivity in total lipids, or as a percentage of control (t = 0) radioactivity.

2.4. *Materials*

myo-[^{3}H]Inositol was obtained from American Radiochemicals, Dowex-AG1 from Bio-Rad, norepinephrine bitartrate, propranolol-HCl and yohimbine-HCl from Sigma. Prazosin-HCl was a gift from Pfizer.

3. RESULTS AND DISCUSSION

Norepinephrine (10 μM) caused a modest, but statistically significant increase in [^{3}H]$InsP_3$ 1 min after its addition ($P < 0.05$; n = 5); data are shown in fig.1. Also shown are the effects of norepinephrine when 20 mM LiCl [15] was present 20 min prior to, and during, stimulation. Li^+ did not affect the basal or initial (1 min) increase in $InsP_3$, but markedly potentiated the increase in $InsP_3$ level 5 and 10 min after the addition of

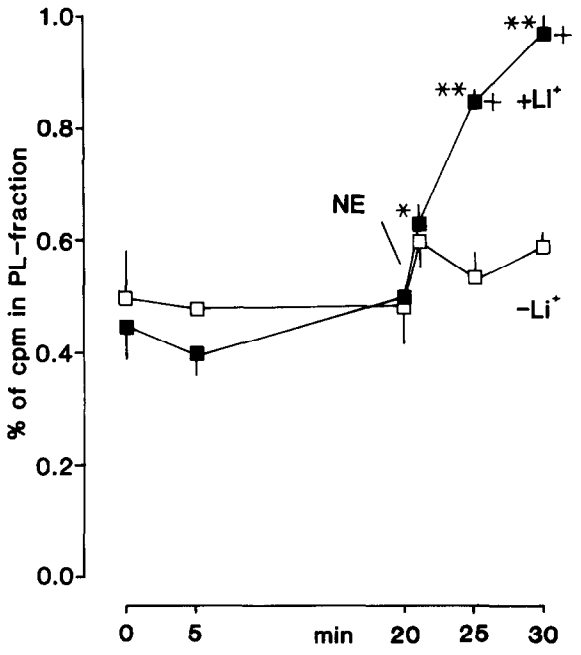

Fig.1. Effect of Li^+ on basal and agonist-stimulated levels of $InsP_3$ in brown adipocytes. [^{3}H]Inositol-loaded cells were resuspended in fresh buffer and incubated with or without 20 mM LiCl for 20 min (controls received 20 mM NaCl). At 20 min, 1 μM propranolol followed by 10 μM norepinephrine were added and the incubation continued for another 10 min. (□) – Li^+, (■) + Li^+. The radioactivity in the $InsP_3$ fraction is here calculated as % of the cpm in the total phospholipid fraction. Points shown are means from 3 parallel experiments (± SE). (*) and (**) show significant increases above the pre-norepinephrine level at 20 min ($P < 0.05$ and < 0.01, respectively); + shows significant effect of Li ($P < 0.05$); Student's paired t-test.

norepinephrine. A similar effect was noted for $InsP_2$ and InsP, but for InsP the relative changes due to hormone or Li^+ were much smaller (not shown; cf. fig.2). This concentration of Li^+ did not affect cell viability and only slightly (≤ 10%) suppressed the norepinephrine-induced respiratory response (this response is mainly a β-adrenergic effect but the slight suppression was sustained when the α_1-adrenergic component of respiration was inhibited by prazosin; thus it was not due to a selective inhibition of the α_1-component).

In the experiments, 1 μM propranolol was routinely added to diminish the oxygen consumption of the cells and thus ensure an adequate ox-

ygen supply. Garcia-Sainz et al. [5,16] have reported an increased incorporation of ^{32}P into PtdIns in the presence of a high concentration of propranolol. This is probably due to inhibition of phosphatidic acid phosphohydrolase; however, in preliminary experiments (not shown), 1 μM propranolol did not affect the incorporation of [^{3}H]-inositol into inositol phosphates in this system.

Fig.2 shows the time course of the effect of 10 μM norepinephrine, in the presence of 20 mM Li^+, on [^{3}H]$InsP_3$, [^{3}H]$InsP_2$ and [^{3}H]InsP levels in brown adipocytes. Changes in InsP levels were, relative to the control level, smaller than for $InsP_2$ and $InsP_3$ and thus statistically less reliable (note the difference in ordinate scales). Norepinephrine induced an increase in both $InsP_2$ and $InsP_3$ already at the earliest time point examined, 15 s. This indicates that polyphosphoinositide hydrolysis may initiate inositol lipid cycling in brown adipocytes and that earlier reports on increased PtdIns synthesis being stimulated by α-adrenergic activation [5,6] may be understood as a resynthesis after polyphosphoinositide breakdown, as shown for a number of other cell systems [17,18]. The increases in both $InsP_2$ and $InsP_3$ were unaffected by the α_2-adrenoceptor antagonist yohimbine (0.1 μM) but were completely blocked by the α_1-adrenoceptor antagonist, prazosin, at the same concentration. This result is consistent with the reported findings of Garcia-Sainz et al. [5] and of Mohell et al. [6] who showed that prazosin was a much more potent blocker than yohimbine of the phenylephrine-stimulated PtdIns turnover. The increase in $InsP_3$ is at least as rapid as the previously described α_1-adrenergic Ca^{2+} mobilization in these cells [1], suggesting that release of Ca^{2+} by $InsP_3$ could well contribute to this response.

One problem in relating levels of $InsP_3$ as determined by anion exchange to biological responses is the recent finding that the tritiated material formed in some cells is a mixture of isomers, specifically Ins(1,4,5)P_3 and Ins(1,3,4)P_3 [19, 20,22]. In different systems, Ins(1,4,5)P_3 has been shown to release intracellular Ca^{2+} [17]. While Ins(1,3,4)P_3 has not been tested directly, there is indirect evidence that it does not mobilize Ca^{2+} [20]. In the present study, [^{3}H]$InsP_3$ was not resolved into the 2 isomers. There are interesting parallels, however, between these data and those of Burgess et al. [20] and Rubin [21] with liver and

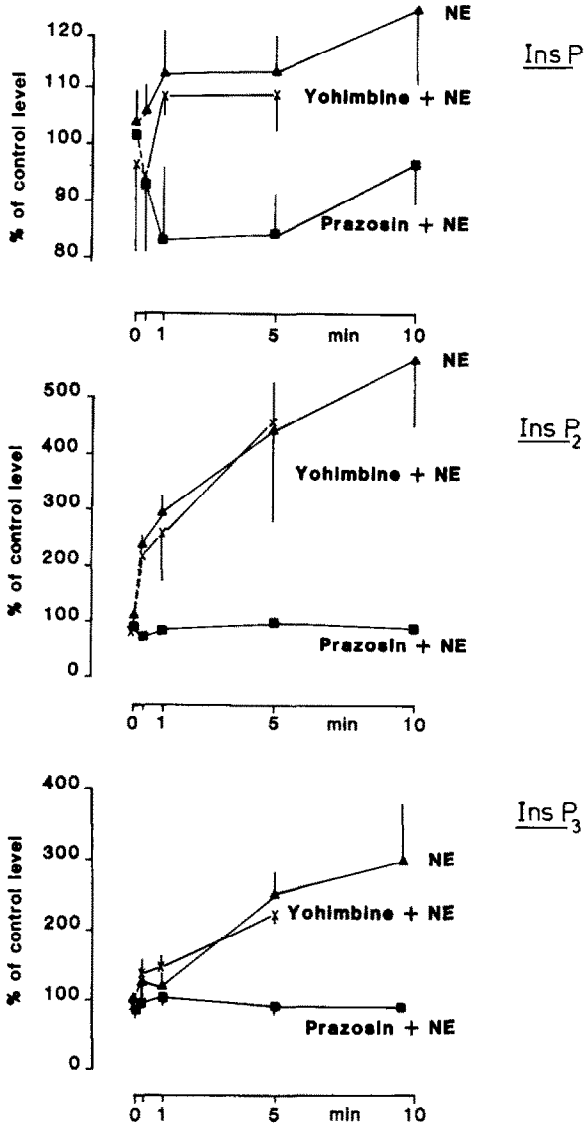

Fig.2. Effect of norepinephrine on the formation of inositol phosphates in brown adipocytes. [^{3}H]Inositol-loaded cells were preincubated for 20 min in the presence of 20 mM LiCl. At 20 min, 1 μM propranolol was added and after another minute, 0.1 μM prazosin, 0.1 μM yohimbine or buffer. Immediately thereafter 10 μM norepinephrine was added. The amount of [^{3}H]inositol phosphates was measured and is presented as % of control values (100% thus corresponds to the level of the inositol phosphates in the batch, before the additions). The data represent mean values from 3 experiments; bars indicate ± SE. Observe that the scale is adjusted so that the absolute amount of cpm is identical for all 3 panels (mean % of total phospholipid label was 28.7, 0.72, and 0.28 for InsP, $InsP_2$ and $InsP_3$, respectively).

exocrine pancreas which permit some speculation on the isomers formed. In the pancreas, as for the brown fat cells, Li^+ potentiates [^{3}H]InsP_3 accumulation. In pancreas, this potentiation occurs after 2 min of stimulation [21] and is totally accounted for by the Ins(1,3,4)P_3 isomer, Ins (1,4,5)P_3 not being affected by Li^+ at all [20]. This finding is consistent with reports that during the initial minute after receptor activation, only the Ins(1,4,5)P_3 isomer is increased [20,22]. Thus, the pattern of [^{3}H]InsP_3 production in the brown adipocyte would suggest that the initial increase, in the first minute or so, is largely Ins(1,4,5)P_3, while the Li^+-sensitive material which accumulates with more prolonged stimulation may be primarily Ins(1,3,4)P_3. Studies are currently under way to examine more directly these isomers in the brown adipocyte system.

In summary, these results demonstrate that inositol lipid turnover in the brown adipocyte results from an α_1-adrenoceptor-mediated phosphodiesteratic breakdown of polyphosphoinositides, liberating soluble inositol phosphates. The formation of InsP_3 is rapid enough to enable it to contribute to the previously described intracellular Ca^{2+} mobilization in these cells.

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