RESEARCH COMMUNICATION

Nuclear diacylglycerol is increased during cell proliferation *in vivo*

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Highly purified nuclei were prepared from livers and kidneys of rats undergoing compensatory hepatic or renal growth, the former being predominantly by cellular proliferation, and the latter mostly by cellular enlargement. In liver, an increase in nuclear diacylglycerol (DAG) concentration occurred between 16 and 30 h, peaking at around 20 h. At the peak of nuclear DAG production a specific translocation of protein kinase C to the nucleus could be detected; no such changes occurred in kidney. There was no detectable change in whole-cell DAG levels in liver, and the increase in DAG was only measurable in nuclei freed of their nuclear membrane. Overall, these results suggest that there is a stimulation of intranuclear DAG production, possibly through the activation of an inositide cycle [Divecha, Banfić and Irvine (1991) EMBO J. **10**, 3207–3214] during cell proliferation *in vivo*.

INTRODUCTION

We have demonstrated recently in a cultured cell line (Swiss 3T3 fibroblasts) the existence of a nuclear polyphosphoinositide cycle distinct from, and under a separate control from, the 'classic' plasma membrane cycle [1]. Other work on cell lines has suggested that this nuclear inositide cycle may be widespread [2–4], and several groups have reported the presence of phosphoinositidase C in nuclei [5–7]. However, our knowledge of the significance of this cycle in normal cell proliferation is presently limited by the fact that the studies have been carried out in cultured cells stimulated with purified growth factors and, as is revealed below, these may be misleading in some aspects. Here, we have employed a system where cell proliferation is stimulated *in vivo* (regenerating rat liver [8]), and have compared this with another compensatory *in vivo* trophic response in which there is mostly only cell enlargement and little cell proliferation (compensatory renal growth [9]). We show that in the former response, but not the latter, there is a marked and precisely timed production of nuclear diacylglycerol (DAG) accompanied by a translocation of protein kinase C (PKC) to the nucleus.

MATERIALS AND METHODS

Liver fractions and enzyme markers

Isolation of liver and kidney nuclei was based on a modification of an original procedure by Thomas et al. [10], as described in our previous paper [1]. Leucine arylamidase activity was determined as described in a commercial kit (Merckotest no. 3359), Na^+,K^+-ATPase was measured as described by Berner and Kinne [11]. The activities of succinate:cytochrome *c* oxidoreductase and KCN-resistant NADH oxidoreductase were determined according to Fleischer and Fleischer [12] and Sottocasa et al. [13] respectively. The activity of 5′-nucleotidase was measured as described by Burnside and Schneider [14]. DNA and proteins were determined by the methods of Burton [15] and Bradford [16] respectively.

Operational techniques

Male Wistar rats (150–250 g body wt.) were used in all experiments. Partial hepatectomy was performed under light ether narcosis and two-thirds of the liver was surgically removed [17]. Left-side unilateral nephrectomy was performed by a dorsolateral approach also performed under ether narcosis [18]. All control animals were sham operated. Lipid extraction and mass assays of DAG, PtdInsP_2, PtdIns$P$ and PtdIns were exactly as described in [1].

RESULTS AND DISCUSSION

The purity of nuclei isolated from liver and kidney was estimated by the determination of marker enzymes (see the Materials and methods section) and by electron microscopy (results not shown). Most marker enzymes were at levels so low that quantification was difficult, and electron microscopy showed no other obvious components present. The exception in this respect was that for endoplasmic reticulum KCN-resistant NADH oxidoreductase; the specific activity for this enzyme in nuclear preparations (original homogenate activity, 0.42 ± 0.02 μmol/mg of protein per ml and 0.29 ± 0.03 μmol/mg of protein per ml for liver and kidney respectively, defined as 1) was 0.17 ± 0.01 and 0.21 ± 0.02 for liver and kidney respectively, which decreased to 0.10 ± 0.01 and 0.14 ± 0.01 respectively when the nuclei were washed with 1% (v/v) Triton X-100 plus 1% deoxycholate (DOC) (the μg of DNA/mg of protein ratios for liver and kidney nuclei were 12.3 ± 0.6 and 9.3 ± 0.5 respectively in the absence of detergent wash, and 18.9 ± 0.4 and 14.2 ± 0.6 if washed). The presence of KCN-resistant NADH oxidoreductase may be taken as evidence for residual endoplasmic reticulum contamination that is finally removed by detergents, but it is equally likely that it is present in the nuclear membrane, which is also removed by Triton plus DOC.

In Table 1 it is shown that the increase in nuclear DAG mass that occurs during compensatory hepatic growth (see Figure 1) can only be measured when the nuclei were prepared in the presence of detergents so that their DAG content was lower. We interpret this as meaning that in nuclei prepared without detergents, DAG in the membranous material that detergents remove does not increase, and so the changes are obscured. This is a similar observation to that described in [1], and it implies an

Abbreviations used: DAG, diacylglycerol; PKC, protein kinase C; DOC, deoxycholate.

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Table 1 Concentrations of DAG, PtdInsP_2, PtdIns$P$, expressed in pmol/mg of protein, and PtdIns, expressed in nmol/mg of protein, in cells and nuclei prepared either without detergent (O) or in the presence of Triton and DOC (T + D)

Abbreviations: Sham, sham operated; Comp, compensatory organ hypertrophy is taking place. All results were obtained 24 h after operation. Results are given ± S.E.M., and are from four independent experiments, each conducted on duplicate animals.

Preparation	Liver				Kidney			
	DAG (pmol/mg of protein)	PtdInsP_2 (pmol/mg of protein)	PtdIns$P$ (pmol/mg of protein)	PtdIns (nmol/mg of protein)	DAG (pmol/mg of protein)	PtdInsP_2 (pmol/mg of protein)	PtdIns$P$ (pmol/mg of protein)	PtdIns (nmol/mg of protein)
Cells								
Sham	200 ± 28	356 ± 37	703 ± 11	10.56 ± 0.4	217 ± 21	712 ± 43	1200 ± 99	15.7 ± 0.7
Comp	192 ± 17	328 ± 21	697 ± 41	11.2 ± 0.3	221 ± 14	716 ± 28	1158 ± 79	16.3 ± 1.1
Nuclei O								
Sham	157 ± 11	305 ± 28	578 ± 23	10.5 ± 0.7	163 ± 21	503 ± 21	807 ± 12	15.7 ± 0.7
Comp	189 ± 30	291 ± 32	569 ± 26	10.2 ± 0.9	191 ± 31	553 ± 31	798 ± 41	15.4 ± 0.2
Nuclei T + D								
Sham	89 ± 11	204 ± 18	420 ± 21	10.9 ± 0.6	102 ± 12	413 ± 21	622 ± 31	16.1 ± 0.4
Comp	211 ± 30*	178 ± 14	413 ± 30	11.2 ± 0.3	113 ± 16	397 ± 36	610 ± 21	15.7 ± 0.6

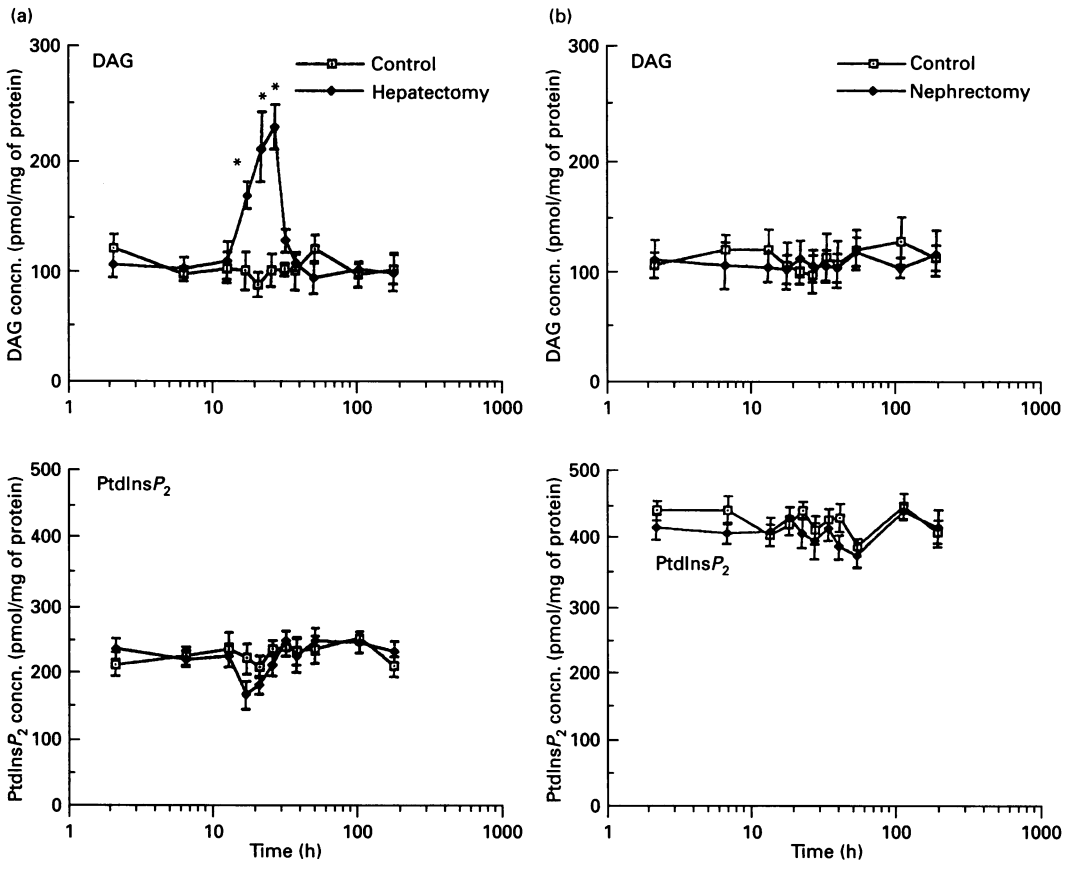

Figure 1 Time course of changes of DAG and PtdInsP_2 concentration in nuclei following partial hepatectomy (a) or unilateral nephrectomy (b)

Each result represents the mean value ± S.E.M. from dupliate determinations from four different experiments. Asterisks indicate significant difference from control.

intranuclear location for these biochemical changes (see also [2]). Note also that the nuclear lipids are a very small percentage (< 5%, results not shown) of the total cellular content, so the changes observed in the nuclear lipids would not be detectable in whole cells (as is shown in Table 1).

A full time course of nuclear lipid changes after partial hepatectomy is shown in Figure 1. An increase in the nuclear DAG mass could be observed between 16 and 24 h after partial hepatectomy, whereas no significant changes in the mass levels of PtdIns, PtdIns$P$ and PtdInsP_2 could be observed, although a

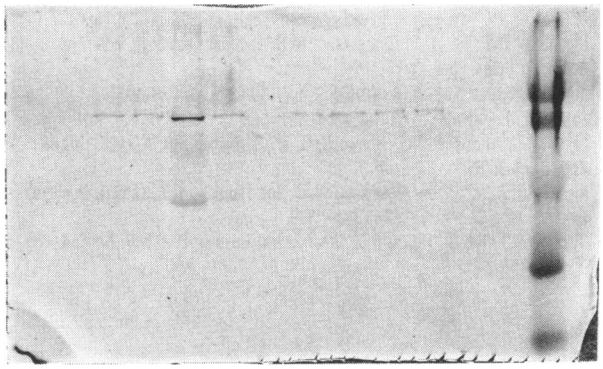

Figure 2 Translocation of PKC to nuclear and non-nuclear fractions

At 20 h after sham operation, partial hepatectomy or unilateral nephrectomy, cells were lysed using a non-detergent hypotonic lysis buffer, and nuclear and post-nuclear fractions were isolated as described in [1]. Protein (50 mg) was subjected to SDS/PAGE, transferred to nitrocellulose and probed with anti-PKC antibody: lane 1, liver nuclear preparation from sham-operated animal; lane 2, liver post-nuclear membranes from sham-operated animal; lane 3, liver nuclear preparation from partially hepatectomized animal; lane 4, liver post-nuclear membranes from partially hepatectomized animal; lane 5, kidney nuclear preparation from sham-operated animal; lane 6, kidney post-nuclear membranes from sham-operated animal; lane 7, kidney nuclear preparation from unilaterally nephrectomized animal; and lane 8, kidney post-nuclear membranes from unilaterally nephrectomized animal. The antibody used was raised to a consensus sequence to α_1, β, γ and δ PKCs (see [1], where the same antibody was used).

drop in PtdInsP_2 is suggested. The large rise in DAG concentrations, with only a small decrease in PtdInsP_2, might suggest a source of DAG other than polyphosphoinositides (e.g. phosphatidylcholine as suggested by Leach et al. [4]), and at present we cannot rule that out; however, it is a common observation for the 'classic' (plasma membrane) inositide cycle that compensatory phosphorylation of PtdIns obscures a drop in PtdInsP_2 levels, especially over a time course of the length observed here (see e.g. [19]). The increase in nuclear DAG levels coincides remarkably closely with the increase in nuclear phosphoinositidase C activity observed in regenerating rat liver by Kuriki et al. [5]. Finally, in Swiss 3T3 cells, we were able to detect a (larger) decrease in nuclear PtdInsP_2 [1], and overall it seems most likely, until we have evidence to the contrary, that a similar mechanism is involved here.

At the same time as the increase in nuclear DAG occurred, there was no detectable change in whole-cell DAG (Table 1), supporting the conclusion drawn above that this response is unlikely to be due to contamination of the nuclei with plasma membrane (see [1] for further discussion). Following unilateral nephrectomy, no changes in DAG and inositol lipid mass levels in the nuclei isolated from the remnant kidney could be observed throughout the whole experimental period (2 h –7 days) (Table 1 and Figure 1), and these results suggest that it is cell proliferation, rather than the change in tissue size that accompanies compensatory organ hypertrophy, which is associated with stimulated nuclear DAG production.

In Swiss 3T3 cells [1] and IIC9 cells [4], the increase in nuclear DAG is accompanied by a translocation of PKC to the nucleus. We have looked for a specific nuclear translocation of PKC at the peak of the nuclear DAG generation (20 h), and the result (Figure 2) shows that such an event can be observed while there is no detectable translocation to non-nuclear membranes. This is consistent with no evidence for stimulated plasma membrane DAG production at this time (Table 1), and it argues against any non-specific effect causing the nuclear PKC increase, such as an overall increase in cellular PKC. We should note that because detection of DAG increases requires DOC/Triton-washed nuclei (above, Table 1), but Western blotting necessitates the absence of DOC (see [1]), we are not able to show both effects on the same nuclear preparation; thus, as with Swiss 3T3 cells [1], we can only assert that PKC has migrated to the nucleus, rather than to within the nucleus. Finally, the isoenzyme of PKC involved here is not delineated by this antibody, and the identification of this, and of the precise timing of the translocation, will be interesting areas for subsequent extensive exploration.

In conclusion, compensatory hepatic growth is a very informative model for natural cell proliferation [8], and comparison with cell cultures may help us to understand the relevance of the nuclear DAG production described in those latter systems [1–4]. In Swiss 3T3 cells, the nuclear DAG production and PKC translocation appear to be transient, and to be over by 1 h [1] (although we cannot be sure that a low level of stimulation does not continue [20]). This early response has not been easy to reconcile with the most likely potential targets for nuclear PKC, which include lamins, DNA polymerase and topoisomerase II (see [20] for review). However, in regenerating rat liver (Figure 1) the increase in nuclear DAG levels starts at around 16 h, is maximal at 20–25 h and is probably over by 40 h. This shows a remarkably close parallel with DNA synthesis and also with the beginning of mitosis [21], and it is later than the increase in nuclear protein kinase activity (suggested to be predominantly protein kinase A) which was documented by Laks et al. [22]. There is a report of a small increase in nuclear PKC activity in regenerating liver [23] at 4 h, and we cannot presently exclude an earlier, less easily detectable, stimulation of nuclear DAG formation that might be coincident with this. Finally, it is relevant to re-iterate that the DAG formation in Figure 1 coincides remarkably well with the transient appearance of an increased phosphoinositidase C activity in nuclei of regenerating rat liver which was observed by Kuriki et al. [5], an observation that we have confirmed (N. Divecha, H. Banfić, and R. F. Irvine, unpublished work). These authors also showed that activation of α DNA polymerase started at this time [5], and we should note that, in addition to a possible control of this enzyme by PKC [20], Ins(1,4)P_2 [24] has been implicated in DNA polymerase activation.

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