ing equivalents for the enzymatic reaction in cultured bovine adrenal medullary cells [17, 18]. In view of these facts, it seems possible that the inhibitory effect of hydralazine on the formation of NE may be due to inhibition of either the transport of DA into granules or the supplementation of reducing equivalents for the hydroxylation cycle. However, the 50% inhibitory concentration (IC_{50}) of hydralazine calculated from the results in Figs. 2 and 3 was found to be approximately 300 μM for the hydroxylation of tyrosine (Fig. 2A) and 30 μM for the conversion of DA to NE (Figs. 2B and 3B); these values were shown to be almost similar to the IC_{50} for TH and DBH (approximately 100 and 20 μM respectively) obtained from our previous experiments using the isolated enzymes [12]. This fact, therefore, seems to indicate that the inhibitory effect of hydralazine on the formation of both radioactive DA and NE from [^{14}C]tyrosine may be due to inhibition of TH and DBH, the rate-limiting enzymes in CA biosynthesis, by this drug within the intact cell.

In summary, the present studies show that hydralazine caused an inhibitory effect on basal and stimulated CA biosynthesis in cultured bovine adrenal medullary cells and also suggest that this drug may be able to suppress the hydroxylation of tyrosine to DOPA and the conversion of DA to NE as a result of the direct inhibition of both TH and DBH *in vivo*.

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Transducing signals involved in the activation of resting tonsillar B cells*

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Purified G0 human B cells provide a good system for investigating the biochemistry of signal transduction pathways involved in their activation and proliferation. Resting B cells can be stimulated to proliferate by a number of different agents which include complete mitogens, such as SAC (*Staphyloccus auerus* Cowan strain 1), immobilised anti-immunoglobulin and EBV, and incomplete mitogens such as $F(ab)_2$ fragments of anti-immunoglobulin which require the presence of T cell derived products such as B-cell growth factor (BCGF) to complete the cell cycle. Polyclonal mitogens have been shown to stimulate the turnover of phosphatidylinositol lipids in lymphocytes (reviewed in [1]). Recently, several workers have shown that hydrolysis of phosphatidylinositol 4,5 bis-phosphate (PI 4,5-P2) occurs in T cell derived lines [2, 3] or in purified T cells and B cells when surface receptors are crosslinked by polyclonal mitogens [4–6]. A general role for inositol phospholipid metabolism in the control of cell growth is supported by the observation that binding of the mitogen platelet derived growth factor (PDGF) to 3T3 cells results in the breakdown of PI 4,5-P2 [7]. Whether this hydrolysis is an invariant corollary of B cell mitogenesis and the analysis of the signalling processes achieved by the component arms of this hydrolytic pathway are the focus of this investigation. Analysis of the various stages of B cell activation was undertaken using monoclonal antibodies to cell surface antigens which identify various putative receptors.

Materials and methods

SAC was obtained from Calbiochem-Behring (Cambridge, U.K.), ionomycin from Calbiochem (La Jolla, CA), and TPA, 1,2-diolein, propidium iodide and acridine orange were obtained from Sigma (Poole, Dorset). DiC8 was prepared by the method of Davis *et al.* [8] from dioctanoylphosphatidylcholine which was obtained from Avanti Polar Lipids, Inc. [^{3}H]thymidine, [^{3}H]uridine and [^{3}H]inositol were obtained from Amersham Int. (Amersham, U.K.). The monoclonal antibodies used were as follows: BK19.9 identifies an antigen expressed by rapidly dividing cells which is structurally similar to but serologically distinct from the transferrin receptor. MHM6 identifies the B-lineage restricted CD23p45 antigen also known as Blast-2. A2 recognises the transferrin receptor (Tf-R) and 11EF7 an antigen expressed on activated B lymphocytes. Analysis of surface antigen expression was

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performed using an indirect rosette assay and as described previously [9]. The purification of resting B cells and their growth analysis were as described previously [10]. The incubation and labelling of B cells with [^{3}H]inositol and the subsequent analysis of the water soluble inositol phosphates were as described previously [6]. Cell cycle analysis using a FACS IV cell sorter was performed by staining with propidium iodide [11] or acridine orange and dual channel analysis [12].

Results and discussion

Table 1 and Fig. 1 summarise the activation status of purified resting B cells as revealed by a number of different parameters. One of these parameters, the accumulation of inositol trisphosphate (IP_3) after 5 min, results from the stimulated hydrolysis of PI4,5-P2. It can be seen that hydrolysis of this inositol phospholipid was achieved by SAC and anti-immunoglobulin which both cross-link cell surface immunoglobulin. Significantly, EBV virus, which induced an early RNA and DNA synthesis, did not hydrolyse PI 4,5-P2 at any time during the first 2 hr (data not shown) following incubation of B cells with virus which is internalised by endocytosis within minutes. In order to look for a latent perturbation of inositol lipid metabolism, the rate of [^{3}H]inositol incorporation into phospholipid was assessed at various timepoints between 0.5 hr and 48 hr. An increase in inositol phospholipid turnover was not observed at any time-point after stimulating B cells with EBV. These data suggest that the cellular signalling events subsequent to EBV stimulation act post-PI 4,5-P2 hydrolysis. It is important to note that the increased turnover in PI induced by anti-immunoglobulin observed at 5 min ceased by 24 hr and was severely diminished at 6 hr. Capping and internalisation of surface immunoglobulin may explain the cessation of inositol phospholipid perturbation. Furthermore, the lack of a sustained hydrolysis of inositol phospholipids may explain the failure of anti-immunoglobulin to promote complete mitogenesis. Evidence for this concept comes from the recent findings of Rothstein *et al.* who showed that cytochalasin D, which effectively disrupts microfilaments, together with anti-immunoglobulin provide a complete mitogenic signal [13].

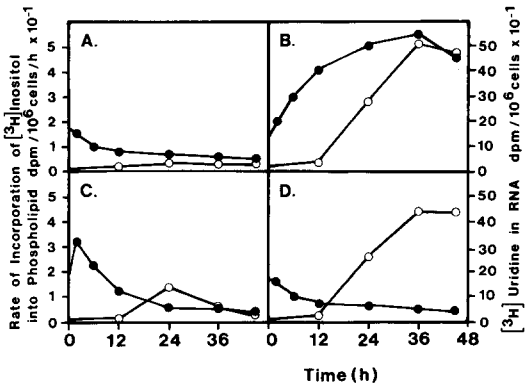

Fig. 1. The rate of incorporation of [^{3}H]inositol into phospholipids (●) and the uptake of [^{3}H]uridine into RNA (○) is shown at various timepoints after stimulation of purified tonsillar B cells by a number of potential mitogens: control (A), *Staphylococcus aureus* Cowan strain 1 at 1:20,000 dilution (B), Soluble fragments of F(ab)2 of anti-immunoglobulin at 15 μg/ml (C) and Epstein–Barr virus, B95.8 strain (D). The methods of analysis and cell culture are as described in Materials and Methods.

The surface marker analysis showed that stimulation of B cells by anti-immunoglobulin, SAC and EBV resulted in the appearance of various antigens at the cell surface (see also Fig. 2). These exocytotic processes do not necessarily involve PI 4,5-P2 hydrolysis as is seen in other systems [14, 15]. Both the BK19.9 and MHM6 antigens appeared at the cell surface when all three agonists were used to stimulate B-cells though only SAC and anti-Ig caused hydrolysis of PI 4,5-P2. The hydrolysis of PI 4,5-P2 leads to the generation of diacylglycerol and IP_3, which liberates calcium from intracellular stores. Changes in calcium and/or diacylglycerol analogue levels have been shown to activate the enzyme protein kinase C and/or cause transposition of this enzyme from the cytoplasm to the plasma membrane. Of particular interest, in view of the above considerations, is to what extent protein kinase is activated when B cells are stimulated to divide by EBV.

A summary of the data presented in Fig. 1 and Table 1 and reported previously by us [6, 16] is shown in Fig. 2. The figure shows the sequence of cellular events throughout the G0 to G2 and M phases of the cell cycle and at what point and to what extent various agonists promote cellular changes. The sustained hydrolysis of inositol phospholipids appears to be necessary for complete mitogenesis other than when induced by EBV. Production of diacylglycerol and calcium mobilisation are key signals as shown by the ability of diacylglycerol analogues (TPA, OAG and DiC8) and the calcium ionophores ionomycin and A23187 to stimulate B-cell growth when used in combination but not when added alone (see Fig. 2). Other studies suggest that protein kinase C activation is a key event in mitogenesis [17, 18] and synergy between diacylglycerol analogues and

Table 1. Extent of PI 4,5-P2 hydrolysis and DNA synthesis and cell surface antigen expression induced by potential B cell mitogens

	IP_3 (5 min)	(^{3}H) thymidine 60–72 hr	BK19.9		MHM6		Tf-R		11EF-7	
			6 hr	24 hr	6 hr	24 hr	24 hr	48 hr	24 hr	48 hr
Control	25	190	4	1	2	2	1	3	0	0
αIg (15 μg/ml)	298	210	33	58	40	60	4	4	1	0
SAC (1:20,000)	365	135, 392	42	63	46	57	7	45	0	18
EBV (B95.8)	27	98, 738	52	62	54	68	8	40	1	35
TPA (0.1 ng/ml)	25	258	30	49	42	56	4	2	0	2
Ionomycin (0.8 μg/ml)	26	85	40	37	42	40	3	5	0	0
TPA + Ionomycin	28	216, 195	71	79	66	65	11	59	3	37
OAG 60 nM	24	195	—	—	—	—	—	—	—	—
DiC_8 60 nM	25	183	—	—	—	—	—	—	—	—
OAG + Ionomycin	20	63, 422	—	—	—	—	—	—	—	—
DiC_8 + Ionomycin	18	185, 340	—	—	—	—	—	—	—	—

Levels of IP3 are given as dpm/10^6 cells collected from Li+ treated cells after 5 min. [^{3}H]thymidine incorporation is given as dpm/microtitre well. BK19.9, MHM6, Tf-R and 11EF7 data are the % positive cells. Cell preparation, labelling and extraction procedures are as described in Materials and Methods. (−) signifies experiment not done.

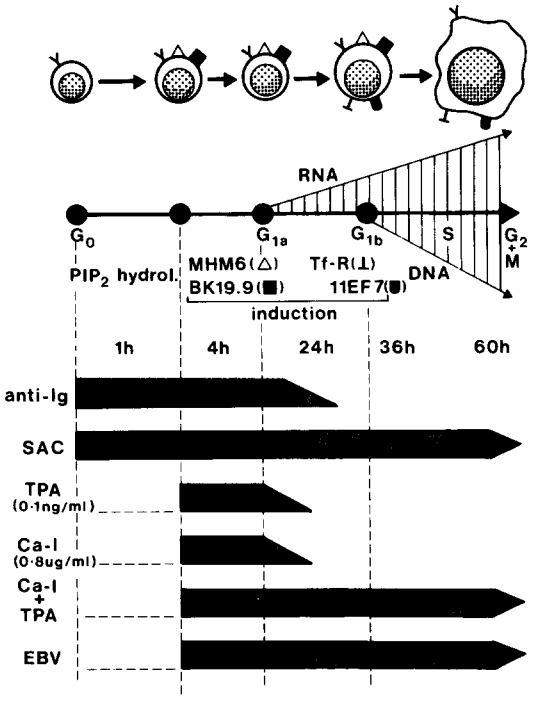

Fig. 2. Shows diagrammatically the temporal progression and stage of activation reached by potential B cell proliferative agents. The cell surface antigens depicted are; surface immunoglobulin (Y), MHM6 antibody (Δ), BK19.9 antibody (■), transferrin receptor, Tf-R, (⊥) and 11EF7 antibody (●). Blast cells show a reduced expression of both MHM6 and BK19.9 antigens. The diagram summarises data from this paper (see Table 1). The preparation of cells, labelling procedures and dose of agents used are as described in Materials and Methods and in Fig. 1 and Table 1.

calcium ionophores has been reported in studies of the redistribution of protein kinase C from a soluble to a particulate phase in a cell free systems [17, 19]. Furthermore, TPA activation of protein kinase C promotes events seen in growth factor induced mitogenesis such as actin depolymerisation and phosphorylation of vinculin, the transferrin, EGF and insulin receptors [20–24]. As well as the identity and location of substrate proteins for protein kinase C, the form of the enzyme *per se* has to be taken into consideration. The early work of Nishizuka's group showed that protein kinase C, while attached to the membrane, is cleaved by a calcium dependent protease (reviewed in [25]). The kinase active fragment is then independent of diacylglycerol and calcium control but shows the same substrate specificity as the pro-enzyme [25, 26]. In conclusion, several recent papers [19, 26–29] suggest that cellular processes which transpose protein kinase C to the membrane and activate or moderate proteolytic cleavage of the enzyme provide multiple controls on protein kinase C activity. Our own studies suggest that during mitogenesis the former and/or both processes require sustained production of protein kinase C agonists.

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