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Evidence from studies employing radioactively labelled fatty acids that the stimulation of flux through the diacylglycerol pool is an early action of vasopressin on hepatocytes

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1. In isolated hepatocytes prelabelled with [^{14}C]-arachidonic, -stearic, -linoleic, -oleic or -palmitic acids, vasopressin increased the amount of radioactivity present in diacylglycerols. The largest increase was observed in cells labelled with arachidonic or stearic acids. 2. In cells prelabelled with [^{14}C]- or [^{3}H]-arachidonic acid, the onset of the increase in radioactivity in diacylglycerols induced by vasopressin was slow, the increase was partly dependent on the presence of extracellular Ca^{2+}, and was associated with an increase in radioactivity present in phosphatidic acid which was more rapid in onset. Vasopressin decreased the amount of [^{3}H]arachidonyl-phosphatidylinositol 4,5-bisphosphate, but the magnitude of this decrease was less than 10% of the observed increase in radioactivity in [^{3}H]arachidonyl-diacylglycerol. 3. The concentration of vasopressin which gave half-maximal increase in [^{14}C]arachidonyl-diacylglycerol at low extracellular Ca^{2+} was 10-fold higher than that which gave half-maximal stimulation of $^{45}Ca^{2+}$ efflux. Phenylephrine, but not glucagon, also increased the amount of [^{14}C]arachidonyl-diacylglycerol. 4. It is concluded that an early action of vasopressin on the liver cell is to increase the flux of carbon from phospholipids, including the phosphoinositides, to diacylglycerols.

INTRODUCTION

An early event in the actions of vasopressin, angiotensin II and α_1-adrenergic agonists on liver cells is an increase in the hydrolysis of phosphoinositides (Billah & Michell, 1979; Kirk & Michell, 1981; Kirk *et al.*, 1981; Michell *et al.*, 1981; Creba *et al.*, 1983; Litosch *et al.*, 1983; Thomas *et al.*, 1983; Rhodes *et al.*, 1983; Seyfred & Wells, 1984). It has been shown that the actions of these agonists are accompanied by increases in the concentrations of inositol polyphosphates (Burgess *et al.*, 1984; Thomas *et al.*, 1984; Charest *et al.*, 1985), total diacylglycerols (Hughes *et al.*, 1984; Bocckino *et al.*, 1985; Bouscarel & Exton, 1986; Preiss *et al.*, 1986), and, in hepatocytes labelled with [^{14}C]arachidonic acid, ^{14}C-labelled diacylglycerols (Takenawa *et al.*, 1982; Thomas *et al.*, 1983; Hughes *et al.*, 1984). Although evidence has recently accrued to support the proposal that diacylglycerol acts as an intracellular messenger through activation of protein kinase C in a number of cell types (reviewed by Nishizuka, 1984), in hepatocytes the effects of agonists on diacylglycerol metabolism have not been well characterized.

The aim of the present experiments was to investigate the effects of vasopressin on the formation of diacylglycerols in hepatocytes, using cells in which the lipids are labelled with [^{14}C]- or [^{3}H]-arachidonic acid. This fatty acid is one of the two major fatty acid constituents of the phosphoinositides (Holub *et al.*, 1970; Holub & Kuksis, 1971; Allan & Cockcroft, 1983). The effects of vasopressin on [^{14}C]arachidonyl-diacylglycerol have been compared with those of the hormone on ^{14}C-labelled diacylglycerols in cells labelled with other fatty acids. Since vasopressin stimulates the activity of phosphoinositidase C, a phospholipase C specific for phosphoinositides (Creba *et al.*, 1983; Downes & Michell, 1985; Uhing *et al.*, 1986), and it has been shown that diacylglycerol formed by the action of exogenous phospholipase C mimics the action of vasopressin in decreasing the activity of glycogen synthetase (Blackmore *et al.*, 1986), the effects of vasopressin on [^{14}C]arachidonyl-diacylglycerol were also compared with those of an exogenous phospholipase C. The results indicate that an increase in flux from phosphoinositides and other phospholipids to a pool of diacylglycerols is an early action of vasopressin on the hepatocyte. A preliminary account of part of the present work has been published in abstract form (Pickford & Barritt, 1984, 1985).

EXPERIMENTAL

Methods

The isolation of hepatocytes from fed male rats, incubation of hepatocytes (Hughes *et al.*, 1984) and measurement of $^{45}Ca^{2+}$ release at 0.1 mM extracellular Ca^{2+} (Whiting & Barritt, 1982) were performed as described previously. Hepatocytes labelled with [^{14}C]- or [^{3}H]-arachidonic acid or other ^{14}C-labelled fatty acids were prepared as described by Hughes *et al.* (1984). The amounts of labelled fatty acids employed were: [^{14}C]arachidonic acid, 0.5 μCi (4.5 nmol)/ml; [^{3}H]arachidonic acid, 5.5 μCi (0.3 nmol)/ml; [^{14}C]stearic acid, 1.0 μCi (20 nmol)/ml; [^{14}C]linoleic acid, 0.33 μCi

Abbreviations used: PtdIns(4,5)P_2, PtdIns4P and PtdIns, phosphatidylinositol 4,5-bisphosphate, phosphatidylinositol 4-monophosphate and phosphatidylinositol.

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Table 1. Distribution of radioactivity in neutral lipids and in phospholipids isolated from hepatocytes labelled separately with [^{14}C]-arachidonic, -stearic, -linoleic, -palmitic or -oleic acid

Labelling of hepatocytes with [^{14}C]fatty acids, extraction of lipids and measurement of the amount of radioactivity in a given lipid species were performed as described in the Experimental section. The amount of radioactivity in a given lipid is expressed as a percentage of the total amount of radioactivity in the lipid extract. Values for the total amount of radioactivity in the lipid extracts, expressed as d.p.m./mg wet wt. of cells, were: 6600±200 (8) (arachidonic acid), 32000±3000 (7) (stearic acid), 10400±100 (3) (linoleic acid), 9900±2400 (3) (palmitic acid) and 27000±1000 (3) (oleic acid). Lipids extracted from approx. 11 mg wet wt. of cells were chromatographed.

		Radioactivity in lipid (% of radioactivity in total lipid extract)				
Lipid	Labelled fatty acid...	Arachidonic acid	Stearic acid	Linoleic acid	Palmitic acid	Oleic acid
Diacylglycerol		1±0.1 (8)	2.7±0.2 (7)	2.8±0.6 (3)	5.5±0.8 (3)	4.7±0.3 (3)
Triacylglycerol		21±2 (8)	36±2 (7)	45±0.2 (3)	58±5 (3)	65±2 (3)
Phosphatidylcholine		40±2 (6)	22±2 (5)	27±0.4 (3)	16±3 (3)	8.2±0.3 (3)
Phosphatidylinositol		6.2±0.3 (6)	7.3±0.3 (5)	1.3±0.2 (3)	1.5±0.2 (3)	1.5±0.4 (3)
Phosphatidylserine		1.7±0.1 (6)	1.3±0.1 (5)	0.14±0.05 (3)	0.19±0.02 (3)	0.24±0.1 (3)
Phosphatidyl-ethanolamine		16±1 (6)	11±1 (4)	11±2 (3)	9±2 (3)	6 (1)

(6 nmol)/ml; [^{14}C]oleic acid, 1 μCi (18 nmol)/ml; [^{14}C]palmitic acid, 0.33 μCi (6 nmol)/ml. Each fatty acid was dissolved in dimethyl sulphoxide. The labelling period was 30 min. After the addition of bovine serum albumin (1 mg/ml), the cells were washed and suspended in fresh incubation buffer in order to remove labelled fatty acids present in the extracellular medium (Hughes *et al.*, 1984). Cells (30 mg wet wt./ml) labelled with a given fatty acid were incubated for 5 min at 37 °C before the addition of vasopressin or vehicle (control incubations). Samples were removed at the times indicated for the measurement of the amount of radioactivity present in the phospholipids.

Hepatocytes were labelled with ^{32}P by incubation with [^{32}P]P_i (10 μCi/ml) in the presence of 1.3 mM-Ca^{2+}. After 60 min, hormone or vehicle was added and incubation continued. Samples for the extraction of lipids were removed at 0 (before addition of hormone), 1 and 15 min.

Lipids were extracted as described by Billah *et al.* (1980) (diacylglycerols, triacylglycerols and phospholipids) or Creba *et al.* (1983) [PtdIns(4,5)P_2, PtdIns4P, PtdIns and phosphatidic acid]. Diacylglycerols and triacylglycerols (Billah *et al.*, 1980); phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine and PtdIns (Halenda & Rubin, 1982); PtdIns(4,5)P_2 and PtdIns4P (Rebecchi & Gershengorn, 1983); and PtdIns and phosphatidic acid (Guy & Murray, 1983) were separated by t.l.c. The area of the t.l.c. plate corresponding to a given lipid was scraped off and mixed with 4 ml of ACS II scintillation fluid. The amount of radioactivity present in the total lipid extract and in each lipid extracted from the t.l.c. plate was determined using a Mark III liquid-scintillation counter fitted with a d.p.m. accessory (Searle Analytic Inc.) for ^{14}C-labelled samples, and a Beckman LS3801 liquid scintillation counter (Beckman Instruments Inc., Irvine, California) for ^{3}H-labelled samples. In each case, values of c.p.m. were converted to d.p.m. by using an appropriate quench curve.

Since the amount of radioactivity incorporated into total cellular lipids varied from one cell preparation to another, the radioactivity present in each lipid spot extracted from the t.l.c. plate was expressed as a fraction of that present in the total lipid extract. In the case of diacylglycerol, the sum of radioactivity present in the 1,2- and 1,3-isomers was determined.

Except where indicated otherwise, the results are expressed as the mean±S.E.M. for the number of experiments indicated. Degrees of significance were determined by Student's t test. Comparisons which yielded values of $P > 0.05$ were considered to be not significant.

Materials

^{14}C- and ^{3}H-labelled fatty acids, $^{45}Ca^{2+}$ and ACS II scintillation fluid were obtained from Amersham Australia, and [arginine]vasopressin, phospholipase C (from *Clostridium perfringens*) and glycerolipid standards from Sigma Chemical Co. [^{32}P]P_i was provided by Biotechnology Research Enterprises, Adelaide, South Australia. All other chemicals were of the highest grade available and were obtained from the sources described previously (Hughes *et al.*, 1984).

RESULTS

The distribution of radioactivity in lipids extracted from hepatocytes labelled with [^{14}C]arachidonic acid and with other [^{14}C]fatty acids is shown in Table 1. A relatively higher proportion of radioactivity is present in phosphatidylinositol and phosphatidylserine in cells labelled with arachidonic or stearic acids compared with the proportion present in these phospholipids in cells labelled with the other fatty acids. For cells labelled with oleic or palmitic acid, a larger proportion of the radioactivity is present in triacylglycerols and in diacylglycerols compared with the proportion present in these lipids in hepatocytes labelled with arachidonic or stearic acids. Hepatocytes treated with [^{12}C]arachidonic acid instead of the [^{14}C]fatty acid exchanged $^{45}Ca^{2+}$ at 0.1 mM extracellular Ca^{2+} and responded to vasopressin

Table 2. Effects of vasopressin on the amount of radioactivity present in phospholipids extracted from hepatocytes labelled with [^{3}H]arachidonic acid or [^{32}P]P_i

Hepatocytes were labelled with [^{3}H]arachidonic acid or [^{32}P]P_i and incubated in the presence of 1.3 mM-$CaCl_2$ as described in the Experimental section. Lipids extracted from approx. 6.7 mg wet wt. of cells were chromatographed. For each incubation, the amount of radioactivity present at 1 or 15 min after addition of vasopressin or vehicle is expressed as a percentage of that present at 0 min (before addition of vasopressin or vehicle). ND indicates that the value was not determined. For cells labelled with [^{3}H]arachidonic acid, the amount of radioactivity present in diacylglycerol, phosphatidic acid, PtdIns(4,5)P_2, PtdIns4P and PtdIns at 0 min was 1620±160 (4), 360±30 (4), 47±8 (11), 125±27 (11) and 360±30 (8) d.p.m./mg wet wt. of cells, respectively. For cells labelled with [^{32}P]P_i, the amounts of radioactivity present in PtdIns(4,5)P_2 and PtdIns4P were 1440±410 (9) and 1080±410 (9) d.p.m./mg wet wt. of cells, respectively. The values of P, determined using Student's t-test for unpaired samples, for a comparison of the value obtained in the presence of vasopressin with the corresponding value obtained for control cells are: *$P < 0.05$, **$P < 0.01$, ***$P < 0.002$.

Phospholipid	Vasopressin (nM)	Time (min)	Radioactivity in phospholipid (% of amount present at 0 min) [^{3}H]Arachidonic acid	[^{32}P]P_i
Diacylglycerol	0	1	105±2 (4)	–
		15	102±2 (4)	–
	10	1	96±3 (4)	–
		15	119±7 (4)*	–
Phosphatidic acid	0	1	106±3 (8)	ND
		15	109±3 (7)	ND
	10	1	127±7 (8)**	ND
		15	129±5 (8)***	ND
PtdIns(4,5)P_2	0	1	104±4 (13)	113±7 (9)
		15	101±8 (11)	96±5 (5)
	10	1	93±3 (14)*	74±5 (13)***
		15	88±3 (11)	80±6 (5)*
PtdIns4P	0	1	104±4 (13)	111±10 (9)
		15	105±5 (11)	98±6 (4)
	10	1	97±3 (14)	82±5 (13)***
		15	104±4 (12)	90±8 (6)
PtdIns	0	1	104±1 (8)	ND
		15	105±2 (8)	ND
	10	1	105±2 (7)	ND
		15	105±4 (7)	ND

by releasing $^{45}Ca^{2+}$ (cf. Whiting & Barritt, 1982) in a manner similar to untreated cells (results not shown). This result indicates that the conditions employed to label cells with fatty acid did not significantly impair the response of the cells to vasopressin.

In hepatocytes labelled with [^{3}H]arachidonic acid and subsequently washed to remove extracellular [^{3}H]arachidonic acid, vasopressin increased the amount of radioactivity in both diacylglycerol and phosphatidic acid (Table 2). While the increase in [^{3}H]phosphatidic acid was evident at 1 min, no increase in [^{3}H]diacylglcyerol was observed at this time (Table 2). Treatment with vasopressin for 1 min decreased the amount of radioactivity present in PtdIns(4,5)P_2 (Table 2). No decrease was observed in the amounts of [^{3}H]PtdIns4P or [^{3}H]PtdIns (Table 2). As reported by others (Creba *et al.*, 1983; Thomas *et al.*, 1983; Rhodes *et al.*, 1983; Litosch *et al.*, 1983; Seyfred & Wells, 1984), vasopressin decreased the amount of radioactivity in both PtdIns(4,5)P_2 and PtdIns4P in cells labelled with [^{32}P]P_i (Table 2). In four experiments, the amount of radioactivity present in [^{3}H]diacylglycerol increased from 1740±70 d.p.m./mg wet wt. of cells (mean±S.E.M.) at 0 min to 2070±160 ($P < 0.01$) at 15 min after addition of vasopressin, while that in [^{3}H]phosphatidic acid increased from 395±60 at 0 min to 520±30 ($P < 0.002$) at 15 min after addition of vasopressin. By comparison, in the absence of vasopressin the total amount of radioactivity present in PtdIns(4,5)P_2 and PtdIns4P was 47±8 and 125±27 d.p.m./mg wet wt. of cells.

The effect of vasopressin on ^{14}C-labelled diacylglycerols in hepatocytes labelled with [^{14}C]arachidonic acid was compared with that in cells labelled with other fatty acids. The results (Table 3) show that the treatment of cells with vasopressin for 15 min caused a significant increase in the amount of radioactivity present in diacylglycerols for each of the four additional fatty acids tested. The magnitude of the effect with cells labelled with stearic acid is comparable with that for cells labelled with arachidonic acid. The smallest increase was observed with cells labelled with palmitic acid (Table 3). Treatment with vasopressin for 15 min caused no increase in the amount of radioactivity in triacylglycerols in cells labelled with [^{14}C]arachidonic acid, or with any of the other labelled fatty acids tested.

A decrease in the extracellular Ca^{2+} concentration from 1.3 to 0.1 mM decreased the effect of vasopressin on [^{14}C]arachidonyl-diacylglycerol by about 50% (Table 3). Investigation of the time course and dose–response for the effects of vasopressin on ^{14}C-labelled diacylglycerol was conducted at 0.1 mM extracellular Ca^{2+}, the concentration employed in previous studies of hormone-

Table 3. Effect of vasopressin on the amount of radioactivity present in diacylglycerols isolated from hepatocytes labelled separately with different fatty acids

Hepatocytes labelled with a given fatty acid were prepared as described in the Experimental section. Incubation of labelled hepatocytes in the presence of 10 nM-vasopressin or vehicle for 15 min, extraction of diacylglycerol, and measurement of the amount of radioactivity present in diacylglycerol were performed as described in the Experimental section. Lipids extracted from 12 mg wet wt. of cells were chromatographed. For cells labelled with [^{14}C]arachidonic acid, the values are the means ± S.E.M. of the results obtained from four separate incubations conducted using one cell preparation. The results shown represent those obtained with one of two cell preparations which both gave similar results. For cells labelled with each of the other fatty acids, the values are the means ± S.E.M. of those obtained from four separate incubations conducted using one cell preparation. The values of P, determined using Student's t-test for unpaired samples, are: $*P < 0.01$, $**P < 0.001$.

^{14}C-labelled fatty acid	Concentration of extracellular Ca^{2+} (mM)	Radioactivity in diacylglycerols (d.p.m./12 mg wet wt. of cells)		Radioactivity in diacylglycerols in presence of vasopressin (% of control)
		Control	Vasopressin	
Arachidonic	1.3	3650 ± 140	4800 ± 70**	132
Arachidonic	0.1	3480 ± 70	4120 ± 110*	118
Stearic	1.3	4160 ± 90	5620 ± 80**	135
Linoleic	1.3	11470 ± 190	13730 ± 180**	120
Oleic	1.3	9660 ± 160	11620 ± 220**	120
Palmitic	1.3	5290 ± 90	5850 ± 140*	111

induced Ca^{2+} release from intracellular stores (Whiting & Barritt, 1982).

The time course for the effect of vasopressin on the amount of radioactivity in [^{14}C]arachidonyl-diacylglycerol is shown in Fig. 1. The maximum increase of 20% was reached after about 15 min exposure to the hormone. The earliest time at which a significant difference was observed was 2 min. A second addition of vasopressin at 15 min after the first did not further increase the amount of radioactivity in the diacylglycerols (results not shown). This indicates that the plateau in the time course is unlikely to be due to degradation of vasopressin, although it may be due to desensitization of the response to vasopressin (Bréant *et al.*, 1981). The increase in the amount of radioactivity in diacylglycerols was not associated with detectable changes in the amount of radioactivity in phosphatidylcholine, phosphatidylethanolamine or phosphatidylserine (results not shown).

The concentration of vasopressin which gave half-maximal increase in the amount of radioactivity in [^{14}C]arachidonyl-diacylglycerols was approx. 1.0 nM, compared with a value of 0.1 nM that gave half-maximal stimulation of $^{45}Ca^{2+}$ efflux (Fig. 2). In hepatocytes labelled with [^{14}C]arachidonic acid the amount of radioactivity present in diacylglycerols in cells treated for 15 min with phenylephrine or glucagon was 112 ± 1% ($n = 5$) ($P < 0.05$) and 98 ± 2% ($n = 4$) of the radioactivity present at 0 min, respectively, compared with a value of 98 ± 5% ($n = 5$) in the absence of hormone.

Exposure of hepatocytes labelled with [^{14}C]arachidonic acid to phospholipase C from *Clostridium perfringens* (1 unit/ml) for 5 or 10 min increased the amount of radioactivity in diacylglycerols to 410 ± 200% and 370 ± 150% ($n = 3$) of the value at 0 min, respectively, compared with values of 100 ± 4% and 110 ± 8%, respectively, for control cells. Similar results were obtained with hepatocytes labelled with [^{14}C]stearic acid (results not shown). No change in the amount of radioactivity in triacylglycerols was detected (results not shown).

DISCUSSION

The results indicate that vasopressin increases the flux of carbon from one or more precursor species to diacylglycerol and phosphatidic acid. The dose–response curve for the effect of vasopressin on [^{14}C]diacylglycerol at low extracellular Ca^{2+} is similar to that for the effect of the hormone on the hydrolysis of PtdIns(4,5)P_2 (Creba *et al.*, 1983; Rhodes *et al.*, 1983; but see Thomas *et al.*, 1983). This comparison, together with the observation that 1 min exposure to vasopressin decreases [^{3}H]arachidonyl-PtdIns(4,5)P_2, indicates that the hydrolysis of PtdIns(4,5)P_2 by phospholipase C contributes to the increase in radioactivity in diacylglycerol. However, other explanations for the similarity in the dose–response curves cannot be completely eliminated because the comparison is between the maximum increase in the amount of radioactivity in diacylglycerol (measured at 15 min) and the initial rate of PtdIns(4,5)P_2 hydrolysis (Creba *et al.*, 1983).

The following observations indicate that it is unlikely that the increased flux of carbon to diacylglycerol is due solely to the hydrolysis of PtdIns(4,5)P_2 by phospholipase C. Firstly, the increase in radioactivity in diacylglycerols was greater than the total amount of radioactivity present in PtdIns(4,5)P_2 plus PtdIns4P. Secondly, substantial increases in [^{14}C]diacylglycerols were observed in cells labelled with linoleic, oleic or palmitic acids. These are only minor constituents of the phosphoinositides (Akesson, 1969; Holub *et al.*, 1970; Holub & Kuksis, 1978; Allan & Cockcroft, 1983). Thirdly, in cells labelled with [^{3}H]arachidonic acid, the effect of vasopressin was diminished by a decrease in the extracellular Ca^{2+} concentration. However, previous studies have shown that the hydrolysis of PtdIns(4,5)P_2 is independent of increases in the cytoplasmic Ca^{2+}

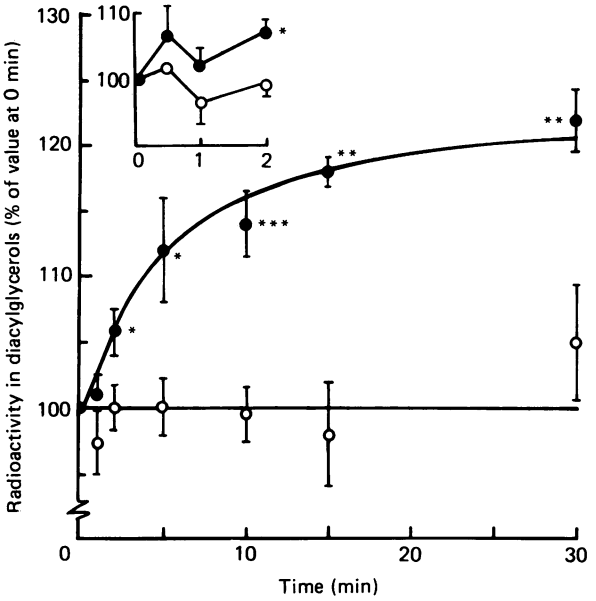

Fig. 1. Time-course for the effect of vasopressin on the amount of radioactivity in diacylglycerols in hepatocytes labelled with [^{14}C]arachidonic acid

Labelling of hepatocytes with [^{14}C]arachidonic acid, incubation in the presence of 0.1 mM extracellular Ca^{2+} and in the presence (●) or absence (○) of 10 nM-vasopressin, and measurement of the amount of radioactivity in diacylglycerols at different times after the addition of vasopressin, were performed as described in the Experimental section. In both control and vasopressin-treated cells, the amount of radioactivity present at any given time is expressed as a percentage of that present at 0 min (time of addition of vasopressin or vehicle). The amount of radioactivity present in diacylglycerols at 0 min (sum of the 1,2- and 1,3-isomers), was 123±9 (8) d.p.m./mg wet wt. of cells. The inset shows the values obtained during the first 2 min. The values are the means±S.E.M. of three to seven determinations made on separate cell preparations. The values of P, determined for paired comparisons, are: $*P < 0.05$, $**P < 0.01$, $***P < 0.005$.

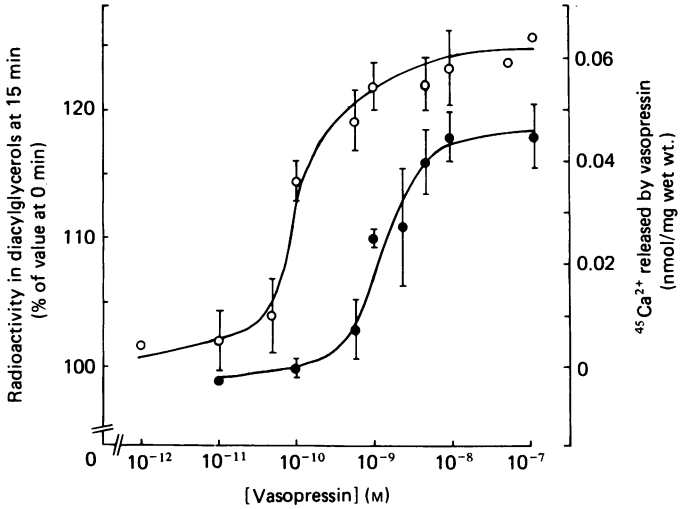

Fig. 2. Comparison of dose–response curves for the stimulation by vasopressin of the amount of radioactivity in diacylglycerols and of Ca^{2+} efflux

Diacylglycerols (○) were extracted from hepatocytes labelled with [^{14}C]arachidonic acid and incubated in the presence of 0.1 mM-Ca^{2+} and vasopressin for 15 min, as described in the legend of Fig. 1. The amount of $^{45}Ca^{2+}$ (○) released after 5 min exposure to vasopressin in cells pretreated with [^{12}C]arachidonic acid in place of [^{14}C]arachidonic acid and incubated in the presence of 0.1 mM-$^{45}Ca^{2+}$ was measured as described in the Experimental section. The values for the amount of [^{14}C]diacylglycerols are the means±S.E.M. of three to five or ten (10 nM-vasopressin) separate experiments. The values for the amounts of $^{45}Ca^{2+}$ released are the means±S.E.M. for three to five separate experiments.

concentration (Creba *et al.*, 1983). Other precursors of diacylglycerols may include PtdIns, phosphatidylcholine and glycolipids (Grove & Schimmel, 1982; Guy & Murray, 1982; Hughes *et al.*, 1984; Daniel *et al.*, 1986; Besterman *et al.*, 1986; Griendling *et al.*, 1986; Saltiel & Cuatrecasas, 1986).

The relatively long time taken for the vasopressin-induced increase in radioactivity in diacylglycerols to reach a maximum most likely reflects a slow increase in total diacylglycerols within the cell (Bocckino *et al.*, 1985; Blackmore *et al.*, 1986). The slow transfer of diacylglycerol from the plasma membrane to other sites within the cytoplasm [e.g. the endoplasmic reticulum and mitochondrial lipid storage sites (Saggerson & Bates, 1981; Vance & Vance, 1986) and other intracellular membranes] may also contribute to the observed slow increase in labelled diacylglycerol. However, the results obtained with exogeneous phospholipase C, which increased the amount of radioactivity in di-, but not tri-, acylglycerols, indicate that there is little transfer of diacylglycerols from the plasma membrane to intracellular sites of triacylglycerol synthesis. The rapid increase in labelled phosphatidic acid induced by vasopressin may reflect the small quantities of this phospholipid in hepatocytes (White, 1973) and a more specific localization of phosphatidic acid within the cell.

The effects of vasopressin on radioactively-labelled diacylglycerol in hepatocytes labelled with [^{14}C]- or [^{3}H]-arachidonic acid are consistent with a previous report that vasopressin causes a relatively slow increase in the proportion of arachidonic acid in total diacylglycerols (Hughes *et al.*, 1984). Differences in the observed time courses and dose–response curves for the effects of vasopressin on [^{14}C]diacylglycerol (the present results; Takenawa *et al.*, 1982; Thomas *et al.*, 1983) and total diacylglycerols (Hughes *et al.*, 1984; Bocckino *et al.*, 1985; Bouscarel & Exton, 1986; Preiss *et al.*, 1986) may be due to differences in the degree to which primary (Ca^{2+}-independent) and secondary (Ca^{2+}-dependent) effects of vasopressin on diacylglycerol formation are expressed.

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