Tight connection between choline transport and phosphatidylcholine synthesis in MDCK cells

Philippe ZLATKINE, Christine LEROY*, Gert MOLL and Christian LE GRIMELLEC

Institut National de la Santé et de la Recherche Médicale, Unité 426 'Physiologie du tube rénal', L. M. E., Faculté de Médecine Xavier Bichat, Université Paris VII, 16 rue Henri Huchard, BP 416, 75870 Paris Cedex 18, France

In MDCK cells, choline uptake, the first step in the CDP-choline pathway for the biosynthesis of choline-containing phospholipids and osmolytes, occurs via both a transport system highly specific for choline and a non-specific pathway. The specific choline carrier is present at the apical domain of cells grown on dishes and is sodium-independent. Growing the cells on a permeant support results in the preferential localization of the specific choline carrier at the basolateral domain. To characterize the relationships between the choline uptake sites and the synthesis of phosphatidylcholine, MDCK cells were incubated with [*Me*-³H]choline and/or [*Me*-¹⁴C]choline for various times (up to 36 h) and the incorporation of label into phospholipids and water-soluble molecules was determined. For cells grown on dishes, addition of [*Me*-³H]choline at the apical side was followed by rapid incorporation of the label into the successive intermediates

of the CDP-choline pathway. A comparable situation was found when growing the cells on a permeant support and adding the labelled choline at the basolateral side of the culture. On the other hand, radioactive choline added to the apical bath entered the CDP pathway to only a very low extent. Efflux experiments on cells loaded with choline from either the apical or the basolateral side demonstrate the existence of intracellular pools of choline. Addition of hemicholinium-3, an inhibitor of the specific choline carrier, markedly reduced the metabolism of choline taken up by the cells on the basolateral side but had no effect on that transported at the apical side. These results strongly suggest the existence of a tight connection between the entry of choline through the specific choline carrier and phosphatidylcholine synthesis in MDCK cells.

INTRODUCTION

As in other mammalian tissues, phosphatidylcholine (PC) is the most abundant phospholipid class in the kidney, where it can account for about half of the total cellular phospholipid content [1]. Increased biosynthesis of PC is one of the earliest responses to growth signals in renal cells. During potassium depletion, acute renal failure or compensatory renal growth, the increase in PC biosynthesis precedes cellular division [2]. The major route of PC synthesis is the CDP-choline pathway [3], which starts with phosphorylation of choline by a cytosolic choline kinase. Synthesis of CDP-choline is then catalysed by CTP:phosphocholine cytidyltransferase, the regulatory enzyme of the CDP pathway, which is either cytosolic or bound to the cytosolic face of the endoplasmic reticulum [4,5]. Finally, the phosphocholine moiety is transferred to diacylglycerol by the membrane-bound enzyme CDP-choline:1,2-diacylglycerol choline phosphotransferase. Evidence suggests that, in glioma cells, there is a channelling of intermediates of PC biosynthesis, where choline kinase may be linked in a metabolon with phosphocholine cytidyltransferase and choline phosphotransferase [6]. To what extent this compartmental organization for PC biosynthesis exists in other cell systems is a major issue to address for the understanding of the regulation of PC synthesis [7].

Choline uptake by renal cells is the first step in the major pathway of biosynthesis of choline-containing phospholipids and osmolytes [8–10]. Choline uptake occurs in MDCK cells, as in liver and intestinal cells [11,12], both via a highly specific carrier system and by a non-specific pathway. The choline carrier present at the apical domain of MDCK cells grown on dishes becomes preferentially localized at the basolateral domain when cells are grown on a permeant support [13]. This makes MDCK

cells a very suitable model for studying the possible channelling of PC synthesis in cells. The present study was thus undertaken to characterize the relationships between the choline uptake sites and the synthesis of PC via the CDP pathway. Our results indicate that, under physiological conditions of culture, biosynthesis of choline lipids in MDCK cells occurs preferentially from choline taken up from the basolateral side of the cells. They suggest the existence of channelling between specific choline uptake and the synthesis of PC, and support the view that the CDP-choline pathway is organized as a metabolon.

EXPERIMENTAL

Materials

[*methyl*-³H]Choline chloride (81.8 Ci/mmol) and [*methyl*-¹⁴C]choline chloride (55 mCi/mmol) were obtained from Amersham. All other biochemicals used were of the highest purity available and were obtained from regular commercial sources.

Cell culture

MDCK cells (passages 74–76) were grown to confluence on plastic dishes and on 0.4 mm-pore-size Transwell supports (Costar), in a 1:1 (v/v) mixture of Dulbecco's modified Eagle's medium (DMEM) (choline concentration 29 μ M) and Ham's F12 medium (choline concentration 101 μ M) supplemented with 10% fetal calf serum, at 37 °C, as previously described [13].

Radiolabelling of choline-containing lipids and water-soluble precursors

Determination of choline incorporation into lipids and water-soluble precursors was performed by incubating cells with

Abbreviations used: PC, phosphatidylcholine; P-Chol, phosphocholine; GPC, glycerophosphocholine; HC-3, hemicholinium-3; DMEM, Dulbecco's modified Eagle's medium.

* To whom correspondence should be addressed.

radiolabelled choline added to the serum-free 1:1 DMEM/Ham mixture, which contains initially 65 μM choline (TechGen International).

Cells grown on plastic dishes

Monolayers of MDCK cells grown on plastic dishes were washed four times with serum-free medium at 37 °C prior to incubation at the same temperature in 2 ml of serum-free DMEM/Ham mixture containing 2 $\mu\text{Ci/ml}$ [^3H]choline. Incorporation was stopped by adding 2 ml of ice-cold substrate-free medium. Monolayers were rapidly washed four times with this solution. The cells were scraped off and the lipids extracted.

Cells grown on filters

Choline incorporation by MDCK cells grown on a permeant support was measured 3–4 days after confluence was reached, in a medium identical to that used for experiments on cells attached to plastic dishes. Cells were washed four times with serum-free DMEM/Ham mixture before incubation at 37 °C with radiolabelled choline (2 $\mu\text{Ci/ml}$) either at the apical (800 μl) or at the basolateral (2 ml) side of the filters. A preliminary experiment in which either 1.5 ml or 800 μl was added to the apical chamber showed no difference in the transport and incorporation of choline into the cells. For some experiments, double-labelling was performed by adding simultaneously [^{14}C]choline at the apical side and [^3H]choline at the basolateral side of MDCK cells. After the incubation period, filters were washed four times with ice-cold substrate-free medium and removed; the cells were scraped off with a razor blade and the lipids were extracted. The effect of hemicholinium-3 (HC-3) on choline incorporation was determined by adding 500 μM of this choline structural analogue to the incubation medium either at the apical or at the basolateral side of the culture.

Pulse–chase experiments

MDCK cells grown on filters were pulsed for 30 min with 2 $\mu\text{Ci/ml}$ [^3H]choline in DMEM/Ham mixture, added at either the apical or the basolateral side of the cell monolayer, as described above. After the pulse period, cells were rapidly washed with DMEM/Ham and further incubated in the same medium, at 37 °C, for the chase period. After the chase, the media from the upper and lower chambers of the wells were collected, the filters were washed and removed, and the radioactivity was determined by liquid scintillation counting.

Determination of choline uptake

Choline uptake by MDCK cells was determined at 37 °C from 5 min [^3H]choline uptakes (5 $\mu\text{Ci/ml}$; 65 μM) as previously described [13].

Extraction and analysis of lipids and water-soluble compounds

Lipids were extracted using the method of Bligh and Dyer [14], slightly modified as previously described [15]. The phase containing the lipid extracts was evaporated to dryness under nitrogen and solubilized in chloroform/methanol (2:1, v/v). TLC was performed on a pre-coated silica gel thin layer plate (Whatman LK5) impregnated with boric acid using chloroform/ethanol/water/triethylamine (30:35:6:35, by vol.) [16]. Individual lipid components were detected under UV light after spraying with primulin and identified by comparison with authentic standards. The spots were scraped off and the radioactivity was determined.

Radioactivity associated with choline-containing water-soluble compounds was determined as described by Sanghera and Vance [17].

Data analysis

Results are expressed as means $\pm$ S.E.M. of three to five independent experiments, in each of which cell sampling was done in triplicate. Comparisons were performed by using Student's *t* test; *P* < 0.05 was considered significant.

RESULTS

PC synthesis in MDCK cells grown on a solid support

Growing MDCK cells on plastic dishes in the presence of [^3H]choline resulted in a time-dependent incorporation of choline into lipids and water-soluble compounds (Figure 1). After 3 h of incubation, 1944 pmol of choline/mg of protein had been incorporated into the lipophilic fraction. PC accounted for 85 % of the labelled lipid species, whereas sphingomyelin and lyso-PC accounted for 11.5 % and 3.5 % of the total respectively. The corresponding amount of choline and its metabolites in water-soluble compounds was 933 pmol/mg of protein, with phosphocholine (P-Chol), the first intermediate in the CDP pathway, representing 75 % of total water-soluble compounds. CDP-choline, glycerophosphocholine (GPC), a product of PC metabolism, and choline accounted for $5 \pm 2\%$, $10 \pm 2\%$ and $8 \pm 2\%$ respectively of the water-soluble radioactivity. In accordance with the literature [18], most of the label ($75 \pm 0.1\%$) was recovered in the lipophilic phase after 36 h of incubation with radiolabelled choline. These results confirm that, as in other renal cells [2], PC biosynthesis in MDCK cells occurs via the CDP pathway with choline as a precursor

PC synthesis in MDCK cells grown on a permeant support

Although present at the apical domain of MDCK cells grown on dishes, the specific choline carrier becomes preferentially localized at the basolateral domain of cells grown on a permeant support

Figure 1 Choline incorporation into phospholipids and water-soluble compounds by MDCK cells grown on dishes

[^3H]Choline (2 $\mu\text{Ci/ml}$) incorporation from the apical side of MDCK cells grown on plastic dishes was measured as a function of time in total lipid (●) and water-soluble (■) extracts. The data are means $\pm$ S.E.M. of three independent experiments.

Table 1 Choline incorporation into total hydrophobic and water-soluble extracts from filter cultures after 36 h of incubation

MDCK cells grown on filters were incubated for 36 h with ^3H - and ^{14}C -radiolabelled choline ($1\ \mu\text{Ci/ml}$) added simultaneously at the apical and basolateral side of the filters respectively. The incorporation of choline was determined in the hydrophobic and water-soluble phases after extraction. The data were corrected for counting efficiency (21.5% for ^3H and 40% for ^{14}C), spillover of ^{14}C into ^3H (9%), and the difference in total choline concentration between the apical ($101\ \mu\text{M}$) and the basolateral ($65\ \mu\text{M}$) chambers of the culture. The data are means $\pm$ S.E.M. of three independent experiments.

Labelling site	Incorporation (nmol/36 h per mg of protein)		
	Hydrophobic	Water-soluble	Ratio
Apical	14.2 ± 2.03	9.4 ± 0.4	2.2 ± 0.2
Basolateral	121 ± 5	54.3 ± 3.2	1.48 ± 0.12

Figure 2 Choline incorporation into phospholipids and water-soluble compounds by MDCK cells grown on filters

Incorporation of radiolabelled choline ($2\ \mu\text{Ci/ml}$) from the apical (^{14}C ; $\square$, $\circ$) and from the basolateral (^3H ; $\blacksquare$, $\bullet$) side of the filters was measured as a function of time in total lipid ($\circ$, $\bullet$) and water-soluble ($\square$, $\blacksquare$) extracts. Data were corrected as in Table 1, and are means $\pm$ S.E.M. of three independent experiments.

[13]. MDCK cells were grown as a monolayer on filters and were incubated in the presence of radiolabelled choline in order to study the relationships between the uptake sites and the incorporation of choline. For long-duration incubations (36 h) with cells grown on a permeant support, comparison of parallel experiments where [^3H]choline ($65\ \mu\text{M}$ choline final concentration) was added at either the apical or the basolateral side of the culture revealed that apical label incorporation represented less than 15% of the corresponding basolateral incorporation. Similar results were obtained using [^{14}C]choline ($101\ \mu\text{M}$ choline final concentration) as substrate in the medium (results not shown). Experiments using simultaneous labelling with [^3H]choline in the lower chamber ($65\ \mu\text{M}$) and [^{14}C]choline in the upper chamber ($101\ \mu\text{M}$) showed that, even with a favourable apical to basolateral gradient, choline incorporation from the apical side was much lower than that from the basolateral side of the cells (Table 1). Moreover the ratio of hydrophobic to water-soluble compounds was higher for the label added at the basolateral side than for that added at the apical side.

In experiments with shorter incubation times (Figure 2),

Table 2 Determination of choline uptake: measurement of specific and non-specific transport

Specific and non-specific transport are expressed as a percentage of the total apical or basolateral uptake. Uptake experiments for determination of choline transport were performed as described in the Experimental section. The data represent means $\pm$ S.E.M. of three independent experiments.

Labelling site	Transport (% of total)	
	Specific	Non-specific
Apical	21.6 ± 9.0	78.3 ± 8.9
Basolateral	89.0 ± 0.9	11.0 ± 1.0

addition of labelled choline to the basolateral side of the cells resulted in a high incorporation of the label into both phospholipids ($3134\ \text{pmol/3 h per mg of protein}$) and water-soluble compounds ($5721\ \text{pmol/3 h per mg of protein}$). In accordance with the data obtained for cells grown on dishes, PC accounted for 85% of the labelled lipid species, and most of the labelling in water-soluble compounds was recovered in P-Chol ($3571\ \text{pmol/mg of protein}$). The relative proportions of labelled choline ($9 \pm 2\%$), P-Chol ($75 \pm 3\%$), CDP-choline ($5 \pm 2\%$) and GPC ($8 \pm 1\%$) were similar to those determined in cells grown on Petri dishes. On the other hand, adding [^{14}C]choline at the apical side of cells grown on a permeant support resulted in very limited incorporation into both phospholipids ($94\ \text{pmol/mg of protein}$) and water-soluble compounds ($166\ \text{pmol/mg of protein}$) after 3 h. These results indicate that under 'physiological' conditions of culture, i.e. culture on filters, biosynthesis of PC occurs preferentially from the basolateral side of MDCK cells.

Relationship between choline uptake and PC biosynthesis

Choline enters MDCK cells using at least two different mechanisms: a transport system specific for choline (apparent $K_m = 43\ \text{mM}$) which is sodium-independent, and a non-specific system [13]. In accordance with our previous data concerning cells grown on filters, total [^3H]choline uptake from the apical side was about 10 times lower than that from the basolateral side (1098 ± 98 versus $11507 \pm 198\ \text{c.p.m./5 min}$). Non-specific transport accounted for 78% of the choline that entered the cells through the apical membrane, compared with only 11% when radiolabelled choline was added at the basolateral side of the filters (Table 2). Thus choline enters MDCK cells essentially via the non-specific system at the apical side and via its specific carrier-mediated process at the basolateral side.

The relationship between choline uptake and PC biosynthesis was examined in double-labelling experiments. [^3H]Choline taken up from the basolateral side of the cells was rapidly phosphorylated to P-Chol (Table 3). The P-Chol/choline ratio was close to unity after a 15 min incubation and rose to a value of 9, comparable with that obtained in cells grown on plastic dishes, after 3 h. The formation of labelled P-Chol was much slower and markedly decreased for [^{14}C]choline added to the apical side of filters (Table 3). Addition to the basolateral medium of HC-3, an inhibitor of the specific choline transport system in MDCK cells, inhibited up to 66% of both uptake [13] and incorporation of [^3H]choline into lipids ($57 \pm 2.6\%$ and $61 \pm 6.5\%$ for 1 and 3 h of incubation respectively) and water-soluble compounds ($66 \pm 2.9\%$ and $51 \pm 4.6\%$ for 1 and 3 h of incubation respectively). Double-labelling experiments indicated that only the basolateral

Table 3 Time-dependence of labelled phosphocholine/choline ratio

MDCK cells grown either on filters or on plastic dishes were incubated with radiolabelled choline ($2 \mu\text{Ci/ml}$) added to either the apical or the basolateral side of the culture. At the end of the incubation cells were washed and water-soluble compounds were extracted (see the Experimental section). After TLC the spots were counted for radioactivity. The data represent means $\pm$ S.E.M. of three to five independent experiments.

Incubation time (min)	Phosphocholine/choline		
	Cells grown on filters		Cells grown on dishes
	Apical labelling	Basolateral labelling	
15	0.26 ± 0.06	0.99 ± 0.29	2.07 ± 0.59
30	0.22 ± 0.03	1.21 ± 0.44	2.00 ± 0.52
60	0.60 ± 0.21	2.41 ± 1.04	3.21 ± 0.96
120	0.84 ± 0.18	4.36 ± 1.80	6.00 ± 1.73
180	1.60 ± 0.80	9.37 ± 3.20	9.80 ± 2.70

Table 4 Effect of HC-3 on choline incorporation into lipids and water-soluble compounds by MDCK cells grown on filters

Cells were incubated for 5 h with [^{14}C]choline (apical side) and [^3H]choline (basolateral side) ($1 \mu\text{Ci/ml}$), either in the absence or in the presence of HC-3 ($500 \mu\text{M}$) at both sides of the culture. The arbitrary units correspond to the data expressed in c.p.m. corrected for counting efficiency (21.5% for ^3H and 40% for ^{14}C), spill-over of ^{14}C into ^3H (9%), and the difference in the total choline concentration between the apical ($101 \mu\text{M}$) and the basolateral ($65 \mu\text{M}$) chambers of the culture.

Labelling site	Incorporation of choline (arbitrary units)			
	Lipids		Water-soluble compounds	
	Control	+ HC-3	Control	+ HC-3
Apical	1332	1251	3607	2905
Basolateral	15713	7680	25824	11902

Table 5 Choline release from MDCK cells grown on filters

Cells were pulsed for 30 min with radiolabelled choline ($2 \mu\text{Ci/ml}$) added at either the apical or the basolateral side of the filter. Total cell labelling from apical and basolateral incorporation after the pulse period was 3217 ± 72 c.p.m. and 29028 ± 725 c.p.m. respectively. Then, cells were chased for 30 min with fresh medium as described in the Experimental section. Choline release is expressed as a percentage of total radiolabelled choline incorporated from the apical or the basolateral side of the culture. The data represent means $\pm$ S.E.M. of three independent experiments.

Labelling site	Choline release (% of total incorporated)		
	Total from cells	Through membrane	
		Apical	Basolateral
Apical	88.5 ± 2.4	56.7 ± 7.3	31.8 ± 4.2
Basolateral	65.8 ± 4.1	6.5 ± 3.3	59.3 ± 1.5

choline incorporation into lipids and water-soluble compounds was affected by HC-3 addition (Table 4).

Choline release from MDCK cells grown on filters

To further determine the fate of intracellular water-soluble compounds, cells were pulsed with [^3H]choline ($2 \mu\text{Ci/ml}$) for

30 min, then chased with fresh growth medium. In this series of experiments total cell labelling from apical and basolateral incorporation after the pulse period was 3217 ± 72 c.p.m. and 29028 ± 725 c.p.m. respectively (Table 5). The release of labelled compounds was completed within 30 min. No radioactivity other than choline was detected in the medium from a 30 min chase. The total release of choline was significantly higher from apical-side-pulsed cells than from basolateral-side-pulsed cells (Table 5). Furthermore, choline efflux from apical-side-pulsed cells occurred at both the apical and the basolateral side of the monolayer. This contrasted with the situation found for basolateral-side-pulsed cells, where the relative amount of choline released into the apical medium was very low (Table 5).

DISCUSSION

The present experiments demonstrate that PC biosynthesis occurs preferentially at the basolateral side of MDCK cells grown on filters, and that specific choline transport and choline metabolism through the CDP pathway are tightly connected. They further suggest that the sodium-independent choline carrier present in MDCK cells may be the first component of a metabolon linking choline uptake to PC biosynthesis via the CDP pathway.

Synthesis of PC via the CDP pathway in MDCK cells

Labelled choline taken up by MDCK cells grown on dishes was rapidly incorporated into the intermediates of the CDP pathway, i.e. P-Chol and CDP-choline. Choline taken up from the basolateral side of MDCK cells grown on filters was also rapidly metabolized to P-Chol. A comparable time-dependent situation, with the amount of labelled P-Chol higher than that of choline and in large excess over CDP-choline, has been reported for various cells [19–21], including BHK cells [22]. P-Chol constitutes a relatively stable pool and the formation of PC occurs as a rather delayed process [23]. The rate-limiting conversion of P-Chol into CDP-choline and the rapid conversion of CDP-choline into PC [24–26] are in accordance with the high degree of P-Chol labelling and the low degree of CDP-choline labelling that we observed. Considering the time course for PC synthesis, P-Chol formation via either the action of a phospholipase C, active in the kidney medulla [27], or the degradation of GPC [28,29] was unlikely to account for the high level of labelled P-Chol measured during the first 2 h of incubation. GPC radiolabelling in MDCK cells was in accordance with evidence that kidney cells possess the ability to synthesize GPC from choline via PC and lyso-PC [30]. It has been reported that, after 1 h of incubation, inner medullary collecting duct (IMCD) cells in suspension incorporated 90% of labelled choline in PC and 7% in GPC [30]. This suggests that PC synthesis and metabolism are slower processes in MDCK cells than in IMCD cells.

Channelling of PC synthesis in MDCK cells

Whereas cells grown on dishes readily incorporated choline into the intermediates of the CDP pathway and into phospholipids, labelled choline provided at the apical side of cells grown on filters was poorly incorporated into cellular PC, even after 36 h of incubation. Thus, despite an apical total choline uptake that was significantly higher (750 versus 282 pmol/5 min per mg of protein), the amount of choline incorporated from the apical side into PC precursors was about 20 times lower in filter-grown cells than in cells grown on dishes. In contrast to cells grown on dishes, however, choline enters the apical side of cells grown on filters essentially via a non-specific mechanism that is not sensitive

to HC-3 [13]. On the other hand, for cells on filters the amount of choline incorporated into metabolites (in pmol/mg of protein) was 30-fold higher from the basolateral side than from the apical side. In cells grown on filters the specific choline carrier, which is HC-3 sensitive, is preferentially located on the basolateral membrane domain [13], and the basolateral choline uptake (about 10 times the apical uptake) essentially occurs through it. These data strongly suggest the existence of a tight connection between specific choline transport and PC synthesis in MDCK cells.

A decrease in the specific radioactivity of choline entering from the apical side caused by the large influx of choline from the basolateral side might also have decreased, and delayed, the incorporation of the apical radiolabel into the successive intermediates of the CDP pathway. This possible explanation for the low incorporation of apical choline in cells grown on filters was ruled out by the results of experiments with HC-3. HC-3 significantly decreased both the uptake of [³H]choline and its incorporation into PC from the basolateral side, but had no effect on ¹⁴C incorporation from the apical side of the cells. This absence of an effect on apical ¹⁴C incorporation further indicated that HC-3 was not acting through an inhibition of choline kinase. Moreover, the difference between the apical and basolateral incorporation of choline into PC was observed even in the presence of a favourable upper to lower chamber choline concentration gradient.

Using intact and electroporabilized glial cells, George et al. [6] have reported that the precursors of PC synthesis are not freely diffusible, with the individual steps of the CDP pathway being functionally linked. In these cells, specific choline transport is coupled to channelled phospholipid synthesis. The parallel between the localization of the specific choline carrier and the metabolism of choline, the inhibition by HC-3, and the observation that choline which enters the apical side of cells grown on filters, essentially via a non-specific mechanism, is poorly incorporated into the CDP pathway, suggest that channelling of choline from its specific carrier to PC synthesis may also occur in MDCK cells. In accordance with this hypothesis, the existence of intracellular pools of choline in MDCK cells was demonstrated by the fact that choline entering from the basolateral side was poorly mixed with choline entering from the apical side: choline efflux from cells loaded from the basolateral side occurred from the same compartment, whereas more than one-third of the choline loaded from the apical side was released into the basolateral bath. It is noteworthy that the existence of different pools of intracellular choline was previously reported in rat liver [31] and heart [32]. It has also been suggested that, in BHK cells, the phosphorylation of choline occurs when it is transported into the cells, with the cellular choline pool not being involved in PC synthesis [22].

In conclusion, as a result of their ability to change the localization/activity of their specific choline transport system from the apical to the basolateral membrane domain with changing culture conditions, MDCK cells could provide a convenient model for further studies on choline channelling to PC biosynthesis.

REFERENCES

- White, D. A. (1973) in *Form and Function of Phospholipids* (Ansell, G. B., Dawson, R. M. C. and Hawthorne, J. N., eds.), vol. 3, pp. 441–482, Elsevier, Amsterdam
- Toback, F. G. (1984) *Am. J. Physiol.* **246**, F249–F259
- Kennedy, E. P. and Weiss, S. B. (1956) *J. Biol. Chem.* **222**, 193–214
- Weinhold, P. A., Rousifer, M. E., Williams, S. E., Brubaker, P. G. and Feldman, D. A. (1984) *J. Biol. Chem.* **259**, 10315–10321
- Pelech, S. L. and Vance, D. E. (1989) *Biochim. Biophys. Acta* **1003**, 284–292
- George, T. P., Morash, S. C., Cook, H. W., Byers, D. M., Palmer, F. B. St. C. and Spence, M. W. (1989) *Biochim. Biophys. Acta* **1004**, 283–291
- Vance, D. E. (1990) *Biochem. Cell. Biol.* **68**, 1151–1165
- Banfill, H., Pokrajac, N. and Irvine, R. F. (1985) *Biochim. Biophys. Acta* **833**, 223–228
- Nakanishi, T., Balaban, R. S. and Burgh, B. M. (1988) *Am. J. Physiol.* **255**, C181–C191
- Garcia-Perez, A. and Burgh, B. M. (1991) *Physiol. Rev.* **71**, 1081–1115
- Kessler, M., Acuto, O., Storelli, C., Murer, H., Muller, M. and Semenza, G. (1978) *Biochim. Biophys. Acta* **506**, 163–154
- Zeisel, S. H., Story, D. L., Wurtman, R. J. and Brunengraber, H. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 4417–4419
- Zlatkine, P., Moll, G., Blais, A., Loiseau, A. and Le Grimellec, C. (1993) *Biochim. Biophys. Acta* **1153**, 237–242
- Bligh, E. G. and Dyer, W. J. (1965) *Can. J. Biochem. Physiol.* **37**, 911–917
- Venien, C. and Le Grimellec, C. (1988) *Biochim. Biophys. Acta* **942**, 159–168
- Leray, C., Pelletier, X., Hemmendinger, S. and Cazenave, J. P. (1987) *J. Chromatogr.* **420**, 411–416
- Sanghera, J. S. and Vance, D. E. (1989) *Biochim. Biophys. Acta* **1003**, 284–292
- Slivka, S. R., Meier, K. E. and Insel, P. A. (1988) *J. Biol. Chem.* **263**, 12242–12246
- Yavin, E. (1976) *J. Biol. Chem.* **251**, 1392–1397
- Sunga, P. S., Pritchard, P. H. and Rabkin, S. W. (1988) *Biochem. Cell. Biol.* **66**, 231–237
- George, T. P., Cook, H. W., Byers, D. M., Palmer, F. B. St. C. and Spence, M. W. (1991) *J. Biol. Chem.* **266**, 12419–12423
- Whitehead, F. N., Trip, E. and Vance, D. E. (1981) *Can. J. Biochem.* **59**, 38–47
- Klein, J., Koppen, A., Löffelholz, K. and Schmitthener, J. (1992) *J. Neurochem.* **58**, 870–876
- Infante, J. P. (1977) *Biochem. J.* **167**, 847–849
- Pritchard, P. H. and Vance, D. E. (1981) *Biochem. J.* **196**, 261–267
- Pelech, S. L. and Vance, D. E. (1984) *Biochim. Biophys. Acta* **779**, 217–251
- Craven, P. A. and Derubertis, F. R. (1983) *J. Biol. Chem.* **258**, 4814–4823
- Abra, R. M. and Quinn, P. J. (1975) *Biochim. Biophys. Acta* **380**, 436–441
- Spanner, S. and Ansell, G. B. (1987) *Neurochem. Res.* **12**, 203–206
- Bauernschmitt, H. G. and Kinne, R. K. H. (1993) *Biochim. Biophys. Acta* **1148**, 331–341
- Sundler, R., Arvison, G. and Akesson, B. (1972) *Biochim. Biophys. Acta* **280**, 559–568
- Kiss, Z. (1977) *Biochem. J.* **168**, 387–391