Volume 280, number 2, 202–206 FEBS 09491 March 1991

ADONIS 001457939100201B

Effect of 7β-hydroxycholesterol on astrocyte primary cultures and derived spontaneously transformed cell lines

Cytotoxicity and cholesterogenesis

P. Behr, A. Kupferberg, C. Leray, P.F. Urban and M. Mersel

Centre de Neurochimie du CNRS et Unité 44 de l'INSERM, 5 rue Blaise Pascal, 67084 Strasbourg, France

Received 14 January 1991

The correlation between the lethal effect of 7β-hydroxycholesterol (7β-OH-CH) on spontaneously transformed cell lines derived from rat astrocyte primary cultures (normal cells) and de novo cholesterogenesis was investigated. Both 7β-OH-CH and 7-keto-CH were not cytotoxic on normal cells but 7β-OH-CH affected markedly the viability of the transformed cells. The use of [^{14}C]acetate or [^{14}C]mevalonate indicated that 7-keto-CH inhibits de novo cholesterogenesis upstream of 3-hydroxy-3-methylglutaryl CoA reductase (HMGR) in both cell types whereas 7β-OH-CH also inhibits downstream of HMGR. The accumulation of two radiolabelled products X_1 and X_2 between mevalonate and CH was found in unsaponifiable neutral lipids extracted from 7β-OH-CH treated transformed cells. HPLC and GC-MS revealed that X_1 and X_2 are not lanosterol and 24,25-epoxylanosterol, respectively. Incubation of the transformed cells with X_1 and X_2 did not affect their viability. Our data demonstrate that, under our experimental conditions, 7β-OH-CH cytotoxicity is not linked to the inhibition of de novo cholesterogenesis in cultured glial transformed cells.

Astrocyte culture; 7β-Hydroxycholesterol; Cytotoxicity; Cholesterogenesis; 3-Hydroxy-3-methylglutaryl CoA reductase

1. INTRODUCTION

The 7β-hydroxycholesterol (7β-OH-CH) is a metabolite of cholesterol (CH), present in minute amounts in human plasma and biliary fluid [1,2] and secreted by rat liver epithelial cell lines [3]. In vitro studies have shown that 7β-OH-CH exert a cytotoxic activity on proliferating and tumoral cells (for review see [4]) such YAC-1 and RDM-4 lymphomas, HTC cells, ZHC cells, heart fibroblasts and liver epithelial cells [5–8]. The lethal effect consisted either of rapid lysis or loss of adhesion and 'necrosis' of the cells.

With regard to the action mechanism of this compounds, two hypotheses prevail: (i) the incorporation of 7β-OH-CH in the plasma membrane of transformed and tumoral cells may affect its fluidity, function and stability [9]; (ii) 7β-OH-CH, as other oxysterols, inhibits 3-hydroxy-3-methylglutaryl-CoA reductase activity [10] and thus affects de novo cholesterogenesis required for cell proliferation at G1 and/or M phases [11,12].

Recently, we have demonstrated that 7β-OH-CH was cytotoxic to spontaneously transformed cell lines derived from neonatal rat astrocyte, but not to the mother cells [13]. Further investigations indicated that the decline in plasma membrane CH/phospholipid (PL) is not the unique cause of 7β-OH-CH cytotoxicity. We have also demonstrated that the transformed cells esterified 7β-OH-CH on C-3-OH by naturally occurring fatty acids and that the extent of esterification correlates with the degree of 7β-OH-CH cytotoxicity [13–15]. However, the effect of 7β-OH-CH on de novo CH biosynthesis in our biological model remained unclear and thus, the relationship between 7β-OH-CH cytotoxicity and CH biosynthesis pathway has been addressed in this report.

Correspondence address: P. Behr, Centre de Neurochimie du CNRS et Unité 44 de l'INSERM, 5 rue Blaise Pascal, 67084 Strasbourg, France

2. MATERIALS AND METHODS

2.1. *Primary cultures of astrocytes (normal cells)*

Astrocyte primary cultures derived from neonatal rat brain hemispheres were obtained according to Sensenbrenner et al. [16]. The cells were seeded at a density of 2×10^5 on 35 mm diameter culture dishes (Falcon 3001, USA). Culture medium (2 ml) (Dulbecco's Modified Eagles, DMEM; Gibco, Grand Island, NY) was supplemented with 10% fetal calf serum (FCS; Gibco). Cells were grown in a 95% air/5% CO_2 humid atmosphere at 37°C and medium was changed at 5 days intervals.

2.2. *Spontaneously transformed cell lines (transformed cells)*

Spontaneously transformed cell lines were derived from the primary cultures by repetitive subculturing as reported previously [17]. To each subculture a passage number (P) was attributed and P101, P102, P103, P105, P106 and P117 were obtained by trypsinization of the confluent previous passages with 0.05% trypsin and 0.04% Tyrode-KCl. The harvested cells were seeded at a density of 10×10^4 cells per 35 mm culture dishes in DMEM containing 10% FCS. Cells were grown as described for the primary cultures.

Published by Elsevier Science Publishers B. V.

2.3. *Morphological studies and cell viability*

Cells treated with 7-keto-cholesterol (7-keto-CH) or with 7β-OH-CH were observed with a Nikon phase-contrast microscope and photographed with a Nikon camera. The cell viability was tested by the trypan blue method and the cell number was determined as previously described [18].

2.4. *Effect of 7-keto-CH and 7β-OH-CH on 3-hydroxy-3-methylglutaryl CoA reductase (HMGR) activity*

Growing or confluent cell cultures (13 or 19 days in culture for normal cells, respectively, and 2 or 5 days in culture for transformed cells, respectively) were treated with 30 μM 7-keto-CH (Sigma, USA) or 30 μM 7β-OH-CH (Sigma, USA) dissolved in 8 μl ethanol. After 24 h, cells were incubated either with 20 μCi of sodium [2-^{14}C]acetate (53 mCi/mmol; Amersham, UK) in 100 μl H_2O or with 5 μCi of DL-dibenzylethylendiamine [2-^{14}C]mevalonate (44 mCi/mmol; Amersham, UK) in 100 μl NaCl 9% for 2 h. Cells were washed with 9% NaCl, detached from the culture substratum by means of a rubber policeman and submitted for radioactive steroids analysis. When experiments were undertaken for detecting radioactive accumulation products between mevalonate and CH, transformed cell cultures were incubated with 1 μCi [2-^{14}C]mevalonate for 16 h.

2.5. *Lipid extraction and radioactive steroid analysis*

Lipids were extracted according to Folch et al. [19] and submitted to bidimensional thin-layer chromatography (TLC) on precoated silica 60 plates (Merck, Germany) and to autoradiography as reported previously [13,20]. Silica gel areas corresponding to the radioactive spots were scraped in 500 μl of H_2O, dissolved in 10 ml scintillator (Biofluor, NEN, USA) and counted in a scintillation counter (United Technologies Packard Tri-Carb 4000 series, USA). For the detection of radioactive metabolites accumulating downstream of HMGR, the lipids were extracted in the presence of 0.08% butylated hydroxytoluene and submitted to saponification in 0.2 ml of a 0.5 M NaOH ethanolic solution for 30 min at 60°C; 0.2 ml of H_2O was added and the unsaponified lipids extracted twice with 1 ml of hexane. The organic phase was washed with 0.8 ml of 48% ethanol containing 0.25 M NaOH and evaporated under a nitrogen stream. The dry residue was submitted to monodimensional TLC; the solvent system was petroleum ether (b.p. 40-60°C)/acetone(8:2 v/v).

2.6. *HPLC*

HPLC (Waters Associates) was performed with a 5 μm silica lichrosorb reversed-phase column (3.9 mm × 15 cm) using methanol (Merk, Germany) as eluent with a flow rate of 1 ml/min and a pressure of 1000 p.s.i. The labeled compounds were detected at 220 nm by a Spectrachrom 100 spectrophotometer (Spectra, USA) and by a Flo-One counter (Radiomatic Instruments, USA) coupled to the column output. Retention times of 25-OH-CH (Sigma, USA), 7-keto-CH, 24,25-epoxylanosterol (a generous gift from Dr F. Schuber), desmosterol (Sigma, USA), lanosterol (Sigma, USA) and CH (Sigma, USA) were 2.10, 3.10, 3.10 and 6.50, 7.55 and 9.05 min, respectively.

2.7. *GC-MS spectrometry*

Mass spectra were obtained on a GC-MS (Finnigan-Mat, Model 4021, USA) operated in the electron impact mode with an electron energy of 70 eV. Injector, separator, transfer line and ion source temperatures were 300, 270, 270 and 250°C respectively. Gas chromatography was performed with a 25 m (0.32 mm internal diameter) fused silica capillary column coated with SE54 liquid phase (Film 0.2 μm, methylsilicone). Helium N55 grade (Air Liquide, France) was used at a pressure of 1 bar as carrier gas. The column temperature was 220°C for 0.1 min, programmed from 220 to 290°C (3°C/min) and maintained at 290°C for 1 min. The underivated samples were injected using a ROS moving needle injector. The injection solvent was chloroform and samples were eluted free from contaminants at 245°C.

Protein determination was performed according to Lowry et al. [21].

3. RESULTS

3.1. *Cytotoxic effect of 7β-OH-CH on cultured transformed cells*

Neither the viability, nor the overall morphology of 13 and 19 days cultured normal cells were affected by 30 μM 7β-OH-CH. In contrast, this treatment affected 2 days in culture transformed cells: after 24 h cell bodies retracted progressively and, after 48 h, started to detach from the culture substratum (cell number and protein content were reduced by 60%). The lethal effect was delayed by 24 h when 5 days in culture transformed cells were treated with 30 μM 7β-OH-CH. Replacement of 7β-OH-CH by 30 μM 7-keto-CH was not lethal on both normal and transformed cell types. This model which demonstrates that neither 7β-OH-CH, nor 7-keto-CH are cytotoxic on normal cells and that 7β-OH-CH exclusively affected the transformed cells allowed us to investigate the relationship between 7β-OH-CH cytotoxicity and cholesterogenesis blockage.

3.2. *Effect of 7β-OH-CH on HMGR activity*

Endogenous HMGR activity was assayed by evaluating the incorporation of either radiolabelled [^{14}C]acetate (upstream of HMGR) or [^{14}C]mevalonate (downstream of HMGR) in de novo synthesized CH; the difference in percent inhibition of radioactivity incorporated in CH between cells incubated either with the former or with the latter radioactive precursor measures the extent of HMGR activity inhibition. Control experiments indicated that the extent of [^{14}C]acetate and [^{14}C]mevalonate uptake in the total lipid extract, which reached a plateau and 2 h incubation, was not modified by the addition of 7β-OH-CH and 7-keto-CH (7% and 0.2% of the added radioactivity for acetate and mevalonate, respectively). It is noteworthy that incorporation of [^{14}C]acetate and [^{14}C]mevalonate in CH by dividing transformed cell cultures was about 3-4-fold higher than when the cultures reached confluency (legend to Table I).

Table I shows that treatment of normal, dividing and quiescent cells, and of dividing transformed cells with 30 μM 7-keto-CH reduces essentially the incorporation of [^{14}C]acetate in CH. In contrast to 7-keto-CH, 7β-OH-CH affects the incorporation of both [^{14}C]acetate and [^{14}C]mevalonate in CH; however, the extent of inhibition of label incorporation in CH is higher when the cells were incubated with [^{14}C]acetate than with [^{14}C]mevalonate (Table I). It is noteworthy that for confluent transformed cell cultures both 7-keto-CH and 7β-OH-CH act in a similar way: the percent inhibition of [^{14}C]acetate incorporation in CH was equal to that of [^{14}C]mevalonate.

These findings indicate that (i) 7-keto-CH and 7β-OH-CH inhibit de novo CH synthesis in normal and transformed cultured glial cells, (ii) 7-keto-CH acts mainly at HMGR activity step whereas 7β-OH-CH af-

Table I

Inhibition of de novo cholesterol biosynthesis in rat astrocyte primary cultures and derived spontaneously transformed cell lines treated either with 7β-OH-CH or 7-keto-CH

		7β-OH-CH (30 μM)		7-keto-CH (30 μM)	
		[^{14}C]acetate	[^{14}C]mevalonate	[^{14}C]acetate	[^{14}C]mevalonate
Normal cells (a)	DC	95%	60%	90%	20%
	CC	80%	36%	90%	15%
Transformed cells (b)	DC	95%	70%	90%	25%
	CC	63%	75%	80%	80%

The cells were treated as described in Materials and Methods and the data is expressed as percent inhibition of radioactivity found in cholesterol. The radioactivity (cpm/mg protein) found in CH extracted from controls was 7000, 9300, 39462 and 7100 for normal, dividing and confluent, cultures and transformed, dividing and confluent, cultures respectively when treated with [^{14}C]acetate; in the case of [^{14}C]mevalonate the radioactivity found in CH was 700, 1050, 3200 and 620, respectively. (a) Normal cells treated at 13 and 19 days in culture. (b) Transformed cells treated at 2 or 5 days in culture (P102, P103 and P105). DC = dividing cultures; CC = confluent cultures; 7β-OH-CH = 7β-hydroxycholesterol; 7-keto-CH = 7-keto-cholesterol. These values are means of two independent determinations for normal cells and three for transformed cells carried out in duplicate, for which standard errors ≤ 10%.

fects also cholesterogenesis downstream of HMGR.

Thus we may infer that 7β-OH-CH cytotoxicity does not correlate with cholesterogenesis inhibition since (i) 7-keto-CH and 7β-OH-CH do not alter the development of the cultured normal cells but inhibit CH biosynthesis, (ii) 7-keto-CH which is not lethal to transformed cells inhibits CH biosynthesis.

3.3. *Effect of 7β-OH-CH downstream of HMGR activity in the transformed cells*

Since 7β-OH-CH inhibits also CH biosynthesis downstream of HMGR in transformed cells (Table I), we have concentrated on the accumulation of radioactive metabolites between mevalonate and CH. Autoradiography of TLC plates revealed the presence of two radioactive compounds (X_1 and X_2) when dividing transformed cells were treated with 7β-OH-CH and incubated with [^{14}C]mevalonate. Treatment of the transformed cells with 7-keto-CH did not show a significant accumulation of X_1 and X_2 (Fig. 1). The R_f of X_1 corresponds to lanosterol whereas that of X_2 is slightly lower than that of 24,25-epoxylanosterol (Fig.

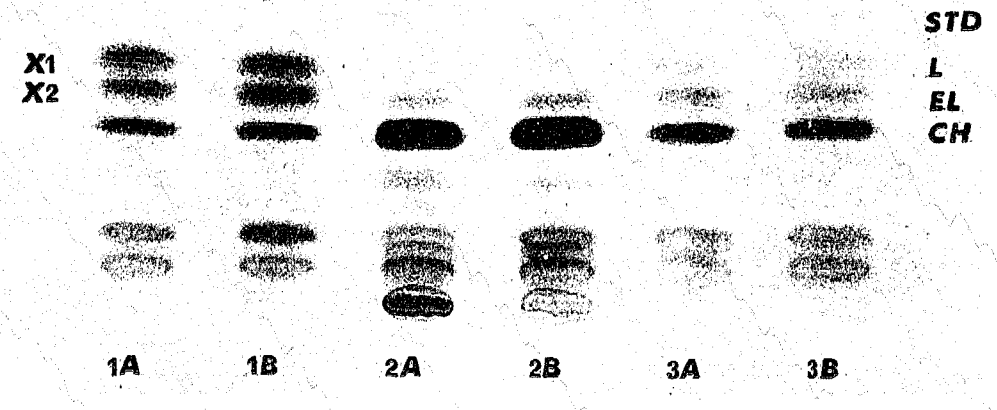

Fig. 1. Autoradiography of ^{14}C-radiolabelled unsaponifiable neutral lipids extracted from dividing transformed cells (A: P106; B: P117) treated either with 7β-OH-CH (lane 1) or with 7-keto-CH (lane 3) and with [^{14}C]mevalonate as described in Materials and Methods. The chromatogram was exposed to X-ray film for 4 days. (Lane 1A) 12 000 cpm; (lane 1B) 15 000 cpm; (lanes 3A) 11 000 cpm; (lane 3B) 11 000 cpm. Lanes 2A (30 000 cpm) and 2B (32 000 cmp) represent the autoradiography of the extracted, and chromatographed, unsaponifiable neutral lipids from cells treated with [^{14}C]mevalonate only. STD = standards; L = lanosterol; EL = 24,25-epoxylanosterol; CH = cholesterol.

1). In the case of confluent transformed cell cultures, the extent of X_1 and X_2 in control, 7β-OH-CH, and 7-keto-CH treated cells was similar and low. HPLC and GC-MS analysis indicate that X_1 and X_2 are not eluted as lanosterol and 24,25-epoxylanosterol. The retention times under HPLC analysis are 4.10 min for X_1 and 4.45 min for X_2 whereas it is 3.10 min and 7.55 min for lanosterol and 24,25-epoxylanosterol, respectively. Under GC-MS analysis, X_1 is eluted at 8.38 min and shows a major peak (*m/e*: 375) whereas lanosterol is eluted at 20.22 min with a characteristic molecular peak (*m/e*: 426). Thus X_1 and X_2 seem less polar than lanosterol and 24,25-epoxylanosterol under our experimental HPLC and GC-MS conditions.

These observations raised the question whether X_1 or/and X_2 are implicated in 7β-OH-CH cytotoxicity. Extraction of X_1 and X_2 from the silica with chloroform/methanol/water (5:5:1 v/v) and incubation of the cultured cells with amounts of X_1 and X_2 (dissolved in 8 μl ethanol) which were equivalent to those synthesized by a same quantity of cells did not alter their viability.

4. DISCUSSION

Astrocyte cultures provide a valuable system for testing the cytotoxicity of 7β-OH-CH and elucidating its action mechanism since this cell type possesses a proliferative potential which is involved in certain pathological processes such as reactive gliosis and the production of glioblastomas. We took advantage of the fact that neither 7β-OH-CH nor 7-keto-CH are cytotoxic on the normal cells, on the one hand, and that, on the other hand, 7β-OH-CH exclusively affects the transformed cells for examining the correlation between 7β-OH-CH cytotoxicity and inhibition of de novo cholesterogenesis. We focus particularly on HMGR, the rate-limiting enzyme of the sterol synthetic pathway, which catalyzes the conversion of HMG-CoA to mevalonate.

Our results indicating a high level of cholesterogenesis in cultured dividing cells and the inhibition of de novo cholesterogenesis by both 7β-OH-CH and 7-keto-CH agrees with the findings of other authors [22,23]. We have also demonstrated a differential action of 7β-OH-CH and 7-keto-CH on CH biosynthesis: 7-keto-CH acts on HMGR activity whereas 7β-OH-CH affects also the pathway downstream of HMGR. Interestingly enough, that 7β-OH-CH and 7-keto-CH affected CH biosynthesis downstream of HMGR activity in confluent transformed cell cultures may be attributed to the dynamic and physical state of the cultures growing as layers overlapping one another. Nevertheless, our findings demonstrate clearly that correlation between oxysterol cytotoxicity and de novo CH biosynthesis could not be established. Maltese et al. and Langan et al. have shown the relationship between sterol biosynthetic pathway and DNA synthesis and cellular proliferation in developing normal and tumoral astrocyte cultures [11,24]; however, differences in the biological model as well as in the culture conditions should be kept in mind. This may also explain the fact that 25-OH-CH-cytotoxicity on C6 cells corresponds to the decline of membrane CH/PL [24] whereas the cytotoxicity of 7β-OH-CH on our transformed cells does not always correlate with CH/PL decrease [14]. Our model also seems useful for investigating the effect of oxysterols on HMGR activity; recently, Shaw et al. [25] have reported that the use of human hepatoblastoma cell line (Hep-G2) may not be the appropriate cell system for studying the inhibition of HMGR activity by CI-981, BMY-21950, lovastatin, provastatin and fluvastatin.

The use of [^{14}C]mevalonate concomitantly with 7β-OH-CH or 7-keto-CH revealed the accumulation of two radiolabelled compounds downstream of HMGR activity in transformed cells treated with 7β-OH-CH only. In spite of R_f values similar to lanosterol and 24,25-epoxylanosterol according to TLC and autoradiography, HPLC and GC-MS indicated that X_1 and X_2 are not lanosterol and 24,25-epoxylanosterol, respectively. Gupta et al. [26] have demonstrated that the incubation of IEC-6 cultures with ketoconazole inhibits HMGR activity with the accumulation of lanosterol and 24,25-epoxylanosterol. Treatment of dividing transformed cells with X_1 and X_2 did not affect their viability. Moreover, treatment of confluent cultures with 7β-OH-CH affected the cellular viability whereas X_1 and X_2 accumulate faintly.

These data which demonstrate the inhibition of de novo cholesterogenesis downstream of HMGR activity, exclude the involvement of this phenomenon in the cytotoxic action mechanism of 7β-OH-CH. These findings together with our previous report [15] strengthen the hypothesis that the esterification of 7β-OH-CH on C-3-OH by Acyl-CoA: cholesterol actyltransferase activity (ACAT) in the transformed cells, is directly linked to the cytotoxic effect of 7β-OH-CH. The fact that the esterification of 7β-OH-CH occurs in the endoplasmic reticulum (ER) since ACAT is localised in this organelle, prompted us to study the effect of 7β-OH-CH and/or of 7β-OH-cholesteryl-3-esters on ER key functions, such as protein glycosylation. Our preliminary results (data not shown) indicate that treatment of transformed cells with 7β-OH-CH reduced markedly the labelling of membrane proteins recognized by cerebellar soluble lectin (CSL), a lectin specific to mannose-enriched glycan residues [27]. In contrast, when cells were treated with 7-keto-CH or with 7β-OH-CH and an inhibitor of ACAT, the decrease in membrane proteins labelled with CSL was not observed. Since the presence of dolichylphosphate (DP) in ER is a prerequisite for primary protein glycosylation, on the one hand, and DP, synthesized downstream of HMGR,

possesses its own regulation in the brain [28,29] on the other, we may hypothesize that the mechanism of action of 7β-OH-CH is linked to its esterification on C-3 which in turn alters the glycosylation process of some native peptides on ER. Experiments are now underway to examine this possibility.

Acknowledgements: This work was partially funded by Grant 6282 from l'Association pour la Recherche contre le Cancer. We are pleased to thank Dr J.P. Zanetta for testing CSL and for helpful discussion, Mrs L. Schellenbaum and Mr. R. Bersuder for expert technical assistance and Mrs V. Ulrich for typing this manuscript.

REFERENCES

[1] Koopman, B.J., Van Der Molen, J.C., Wolthers, B.G. and Vandrepas, J.B. (1987) J. Chromatogr. 416, 1-13.
[2] Kandutsch, A. and Chen, H. (1973) J. Biol. Chem. 248, 8408-8417.
[3] Padieu, P., Maume, G., Hussein, N., Chessebeuf, M. and Tasconas, C. (1985) Biochim. Biophys. Acta 833, 245-261.
[4] Smith, L.L. and Johnson, B.H. (1989) Free Radical Biol. Med. 7, 285-332.
[5] Schroepfer, G.J., Shevill, B.C., Wang, K.S., Wilson, W.K., Kisic, A. and Clarkson, T.B. (1984) Proc. Natl. Acad. Sci. USA 81, 6861-6865.
[6] Cheng, K.P., Nagano, H., Luu, B., Ourisson, G. and Beck, J.P. (1977) J. Chem. Res. 217, 2501-2521.
[7] Mersel, M., Luu, B. and Ourisson, G. (1984) CR Acad. Sci. Paris 299, 221-225.
[8] Nordman, P., Chessebeuf-Padieu, M., Luu, B., Diez-Ibanez, M., Mack, G. and Mersel, M. (1989) Cell Biol. Toxicol. 5, 261-270.
[9] Hoffmann, P.C., Richman, C.M., Hsu, R.S., Chung, J., Scanu, A.M. and Yachnin, S. (1981) Blood 57, 164-169.
[10] Kandutsch, A.A., Chen, H.W. and Heiniger, H.J. (1978) Science 201, 498-501.
[11] Langan, T.J. and Volpe, J.J. (1981) J. Neurochem. 46, 1283-1296.
[12] Astruc, M., Roussillon, S., Defay, R., Descomps, B. and Crastes de Paulet, A. (1983) Biochim. Biophys. Acta 763, 11-18.
[13] Kupferberg, A., Teller, C., Behr, P., Leray, C., Urban, P.F., Vincendon, G. and Mersel, M. (1989) Biochim. Biophys. Acta 1013, 231-238.
[14] Kupferberg, A., Cremel, G., Behr, P., Van Dorsselaer, A., Luu, B. and Mersel, M. (1990) Molecular and Cellular Biochem. (in press).
[15] Kupferberg, A., Behr, P. and Mersel, M. (1990) Biochim. Biophys. Acta 1046, 106-109.
[16] Sensenbrenner, M., Devilliers, G., Bock, E. and Porte, A. (1980) Differentiation 17, 51-61.
[17] Mersel, M., Theley, G., Delaunoy, J.P., Rebel, G., Flory, E. and Mandel, P. (1985) CR Acad. Sci. Paris 301, 811-815.
[18] Mersel, M., Benenson, A., Delaunoy, J.P., Devilliers, G. and Mandel, P. (1983) Neurochem. Res. 8, 449-463.
[19] Folch, J., Lees, M. and Sloane-Stanley, G.H. (1957) J. Biol. Chem. 226, 497-509.
[20] Pelletier, X., Mersel, M., Freysz, L. and Leray, C. (1987) Biochim. Biophys. Acta 902, 223-228.
[21] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265-275.
[22] Richert, L., Castagna, M., Beck, J.P., Rong, S., Luu, B. and Ourisson, G. (1984) Biochem. Biophys. Res. Commun. 120, 192-198.
[23] Brown, M. and Goldstein, J.L. (1974) J. Biol. Chem. 249, 7306-7314.
[24] Maltese, W.A., Reitz, B.A. and Volpe, J.J. (1981) Cancer Res. 41, 3448-3452.
[25] Shaw, M.K., Newton, R.S., Sliskovic, R.D., Roth, B.D., Ferguson, E. and Krause, B. (1990) Biochim. Biophys. Res. Commun. 170, 725-734.
[26] Gupta, A., Sexton, R.C. and Rudney, H. (1986) J. Biol. Chem. 261, 8348-8356.
[27] Marschal, P., Reeber, A., Neeser, J.R., Vincendon, G. and Zanetta, J.P. (1989) Biochimie 71, 53-61.
[28] James, M.J. and Kandutsch, A. (1980) Biochim. Biophys. Acta 619, 432-435.
[29] Andersson, M., Elmberger, P.G., Edlund, C., Kristensson, K. and Dallner, G. (1990) FEBS Lett. 269, 15-18.