The acylation of proteins by xenobiotic amphipathic carboxylic acids in cultured rat hepatocytes

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Three xenobiotic amphipathic carboxylates, namely MEDICA 16, nafenopin and bezafibrate, which differ remarkably in their hydrophobic backbones, were found to acylate membrane and cytosolic liver proteins in cultured rat hepatocytes. The acylation patterns observed were time- and dose-dependent, and the acylated residue consisted of the original xenobiotic. The acylation patterns generated by the three xenobiotic carboxylates included common proteins which were acylated by the three xenobiotics (e.g. proteins of 32, 52, 56 and 72 kDa) as well as unique proteins which were specifically acylated by the respective xenobiotics. The acylation of liver proteins by either MEDICA 16 or nafenopin remained unaffected under conditions where protein synthesis was completely inhibited by cycloheximide. Protein acylation thus offers a common mode of action of xenobiotic amphipathic carboxylates, which may, however, result in diverse xenobiotyl–protein adducts. The xenobiotyl-acylated proteins might be involved in triggering some of the biological effects exerted by xenobiotic amphipathic carboxylates employed as hypolipidaemic effectors, peroxisomal proliferators or preadipocyte convertors.

INTRODUCTION

The covalent acylation of proteins by long-chain fatty acids has been reported to occur in many pro- and eukaryotic-cell types and to involve a wide variety of proteins, such as the vesicular-stomatitis-virus G protein (Schmidt *et al.*, 1979), the Rous-sarcoma-virus pp60src protein (Sefton *et al.*, 1982), the catalytic subunit of cyclic AMP-dependent protein kinase (Carr *et al.*, 1982), human apolipoprotein A-I (Hoeg *et al.*, 1986), the insulin receptor (Hedo *et al.*, 1987) and others (Omary & Trowbridge, 1981; Olson *et al.*, 1984). Under conditions where the acylating fatty acids were formed *de novo* from acetate, all of the fatty acyl residues linked to proteins could essentially be accounted for by myristate, palmitate and stearate (Towler & Glaser, 1986).

Protein acylation by long-chain fatty acids has been mostly studied by using either palmitate or myristate as the radioactive labels, and the respective acylation patterns observed were found to differ with respect to the proteins undergoing acylation, the association between acylation and protein synthesis and the types of the covalent bonds formed. Thus, on the basis of selectivity towards hydroxylamine, palmitate was reported to be primarily acylated via an ester (thioester) bond, whereas myristate was claimed to be essentially acylated in an amide bond (Magee & Courtneidge, 1985). Also, palmitoylated proteins were mainly associated with membranes, whereas myristoylated proteins were either soluble or membrane-bound (Magee & Courtneidge, 1985; Olson & Spizg, 1986). Moreover, on the basis of sensitivity to cycloheximide, protein myristoylation was co-translational, whereas palmitoylation was a post-translational event (Magee & Courtneidge, 1985; Olson & Spizg, 1986). The acylation patterns of fatty acids

other than myristate and palmitate (Marinetti & Cattieu, 1982) still remain to be investigated.

Protein acylation by long-chain fatty acids requires prior thioesterification to their respective long-chain fatty acyl-CoA products, which may then serve as the immediate acylating nucleophiles (Riendeau & Guertin, 1986; Bizzozero *et al.*, 1987). The acylated proteins may be deacylated by a respective microsomal acylesterase (Berger & Schmidt, 1986), and the combined action of the acylating and deacylating activities may thus form a substrate cycle between the two possible functional forms of acylated proteins. The precise function of acylated proteins is still obscure. Thus the acylated fatty acids could affect the intracellular transport or the final destination of respective acylated proteins or, alternatively, acylation might determine the catalytic activity of acylated enzymes or the modulatory capacity of regulatory proteins. Indeed, acylation was reported to be obligatory for maturation of the stomatitis virus (Schmidt & Schlesinger, 1980) or the transforming activity of the myristoylated pp60src protein (Kamps *et al.*, 1985).

Amphipathic xenobiotics consisting of a carboxylate function carried on a variable hydrophobic backbone have been shown to have diverse effects on cellular functions. Thus many of these compounds act *in vivo* as potent hypolipidaemic effectors by modulating key steps in the synthesis and degradation of lipoproteins (Odonkor & Rogers, 1983; Bar-Tana *et al.*, 1985). In addition, xenobiotic amphipathic carboxylates were repeatedly reported to induce liver, kidney and adipose peroxisomal proliferation in rodents and other species (Moody & Reddy, 1978; Reddy *et al.*, 1982), followed by liver enlargement as a result of hypertrophy and hyperplasia of liver cells (Reddy & Lalwani, 1985). The

Abbreviations used: DMSO, dimethyl sulphoxide; PAGE, polyacrylamide-gel electrophoresis; PMSF, phenylmethanesulphonyl fluoride.

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induction of peroxisomal proliferation by xenobiotic amphipathic carboxylates could also be observed in cultured rat hepatocytes incubated in the presence of the added respective proliferators (Gray *et al.*, 1983; Hertz *et al.*, 1985). Furthermore, carboxylate xenobiotics were recently reported to act as potent convertors of epididymal preadipocytes into mature fat-loaded adipocytes (Brandes *et al.*, 1986, 1987). The diversity of these biological effects could not be accounted for by a single mode of action shared by the variety of the xenobiotic amphipathic carboxylates involved. The acylation of proteins by xenobiotic amphipathic carboxylates might, however, offer a unified signalling mechanism that could still allow for diversity based on the individual protein-xenobiotic adducts formed.

The proposed acylation of proteins by xenobiotic carboxylates was verified in the present study by analysing the acylation of liver proteins by nafenopin {2-methyl-2-[*p*-(1,2,3,4-tetrahydro-1-naphthyl)phenoxy]propionic acid} and bezafibrate (2-{4-[2-(4-chlorobenzamido)ethyl]phenoxy}-2-methylpropionic acid), serving as fibrate model compounds, as well as by MEDICA 16 (β,β' -tetramethyl-substituted hexadecane- α,ω -dioic acid; Bar-Tana *et al.*, 1985; Rose-Kahn & Bar-Tana, 1985). The three xenobiotics induce peroxisomal proliferation with a concomitant liver growth (Moody & Reddy, 1978; Hertz *et al.*, 1988), preadipocyte conversion (Verrando *et al.*, 1981; Brandes *et al.*, 1986) and hypolipidaemia (Best & Duncan, 1970; Bar-Tana *et al.*, 1985, 1987a). The possible causal relationship protein acylation and the biological effects exerted by xenobiotic amphipathic carboxylates is discussed.

EXPERIMENTAL

Cell cultures and labelling conditions

Cultured rat hepatocytes were prepared as described by Berry & Friend (1969) with modifications as previously described (Hertz *et al.*, 1985). Cells (1.5×10^6) were plated on collagen-pre-coated 35 mm-diameter Falcon tissue-culture dishes in 1 ml of RPMI 1640 medium containing 15% (v/v) foetal-calf serum, 1 m-unit of insulin, 10 μ g of cortisol, 50 μ g of streptomycin sulphate and 50 μ g of penicillin G. At 90 min after plating, the culture medium was replaced by fresh medium and the cells further incubated for additional 2 h. After this time the medium was replaced by 1 ml of RPMI 1640 medium containing 5% foetal-calf serum, insulin, cortisol, streptomycin sulphate and penicillin G as described above and the respective radioactive xenobiotic carboxylate or the respective native long-chain fatty acid as specified. MEDICA 16 was added as a sodium salt. Nafenopin was added in DMSO at a final DMSO concentration of 0.5% (v/v). After incubation for the specified time periods the medium was removed, the cultured cells were rinsed five times with saline (0.9% NaCl) and scraped off the plate with 0.3 ml of saline into 7.5 ml of chloroform/methanol (2:1, v/v). The suspension was thoroughly mixed, centrifuged, and the protein precipitate was washed three times with chloroform/methanol (2:1, v/v). The washed protein pellet was dissolved in electrophoresis buffer [0.0625 M-Tris/HCl buffer (pH 6.8)/10% (w/v) glycerol/2.3% (w/v) SDS]. The samples were boiled for 2 min and stored at -20°C until being subjected to SDS/PAGE. SDS/PAGE was carried out in 10%-(w/v)-polyacrylamide gels as described by Laemmli

(1970), radioactive molecular-mass markers (Amersham International) being used. After electrophoresis, the gels were fixed in 7% (v/v) acetic acid in water, were subsequently impregnated in Amplify (Amersham International), dried, and exposed to preflashed X-ray film (Hyperfilm-MP; Amersham International) at -70°C .

Subcellular fractionation

The subcellular distribution of the acylated proteins was determined by subjecting the cultured labelled cells to homogenization and differential centrifugation before SDS/PAGE. Thus, subsequent to labelling, the cells were washed with saline and scraped off the plate into a homogenization buffer containing 0.25 M-sucrose, 10 mM-Tris/HCl buffer, pH 7.4, 5 mM-MgCl₂, 1 mM-PMSF and soybean trypsin inhibitor (10 μ g/ml). The cells suspension was homogenized by 40 strokes of a tight pestle in a Dounce glass homogenizer and the homogenate was centrifuged for 10 min at 4°C and 700 *g*. The debris/nuclear fraction was discarded and the postnuclear supernatant was further centrifuged in a Beckman 50 Ti rotor for 60 min at 105 000 *g* to yield the particulate and soluble fractions. The respective fractions were delipidated and subsequently subjected to SDS/PAGE as described above.

Identification of the acylated xenobiotic residue

After SDS/PAGE the gel section containing the protein bands within the molecular-mass range 10–80 kDa was subjected to overnight electroelution in a dialysis tube containing the electrophoresis running buffer. The eluted proteins were then dialysed extensively against water and freeze-dried. The respective non-labelled xenobiotic was added as carrier to the freeze-dried powder, and the mixture was subjected to acid hydrolysis in 6 M-HCl *in vacuo* for 20 h at 110°C . The acid hydrolysate was extracted three times with diethyl ether, and half of the ether extract was subjected to reverse-phase t.l.c. on RP 18 plates (Merck). MEDICA 16- and nafenopin-derived residues were eluted with dioxan/water/formic acid (16:4:1, by vol.) and acetic acid/acetonitrile/water (11:6:3, by vol.) respectively. The other half of the ether extract was subjected to exhaustive basic hydrolysis for 48 h in 2 M-KOH in 90% ethylene glycol under reflux (Gilman & Avakian, 1946). The hydrolysate was acidified, extracted three times with ether, and the ether extract was subjected to reverse-phase t.l.c. as described above. After chromatography, the respective scraped bands were counted for radioactivity in 25% Lumax to toluene.

Materials

MEDICA 16 was synthesized as previously described (Bar-Tana *et al.*, 1985). Details of the synthesis of β,β' -tetramethyl-substituted 8,9-hexadecene- α,ω -dioic acid will be provided by J. B.-T. on request. Nafenopin was from CIBA-Geigy. Bezafibrate was from Boehringer-Mannheim. RP-18 plates for reverse-phase t.l.c. were from Merck. [9,10- ^3H]Palmitic acid (40 Ci/mmol) and [9,10- ^3H]myristic acid (40 Ci/mmol) were from American Radiolabeled. [8,9- ^3H]MEDICA 16 and [U- ^3H]nafenopin were obtained by tritiating β,β' -tetramethyl-substituted 8,9-hexadecene- α,ω -dioic acid and nafenopin respectively (Nuclear Research Center, Negev, Israel). [U- ^{14}C]Bezafibrate was from Boehringer-Mannheim,

[8,9-³H]MEDICA 16 was radioactively pure as determined by silicic acid t.l.c. in light petroleum (b.p. 40–60 °C)/diethyl ether/acetic acid (70:10:1, by vol.) and RP-18 t.l.c.) in dioxan/water/formic acid (16:4:1, by vol.) [U-³H]Nafenopin was radioactively pure as determined by silicic acid t.l.c. in xylene/butan-2-one/acetic acid (50:50:3, by vol.) and RP-18 t.l.c. in acetic acid/acetonitrile/water (11:6:3, by vol.) [U-¹⁴C]Bezafibrate was radioactively pure as determined by silicic acid t.l.c. in xylene/butan-2-one/acetic acid (50:50:3, by vol.).

RESULTS

The acylation of liver proteins by either MEDICA 16 or nafenopin was studied in cultured rat hepatocytes incubated in the presence of the respective radioactive xenobiotics. The acylated proteins were separated by SDS/PAGE and could thus be identified by their respective molecular masses. As shown in Fig. 1, the acylation of liver proteins by MEDICA 16 was time- (Fig. 1a) and dose- (Fig. 1b) dependent and resulted in

Fig. 1. Acylation of liver proteins by MEDICA 16

Cultured rat hepatocytes were incubated in the presence of [8,9-³H]MEDICA 16, and the delipidated proteins were subsequently separated by SDS/PAGE as described in the Experimental section. Each lane contained 100 μg of protein. (a) Time-dependency: the cells were incubated for 10, 30, 60, 180, 360 min and 20 h (lanes 1–6 respectively) in the presence of 50 μM-[8,9-³H]MEDICA 16 (12 Ci/mmol). (b) Concentration-dependency: the cells were incubated for 6 h in the presence of 5, 10, 20, 50 and 100 μM-[8,9-³H]MEDICA 16 (16 Ci/mmol) (lanes 1–5 respectively).

defined acylated bands which varied in molecular mass over the range of 10–100 kDa. The time course of protein acylation by MEDICA 16 (Fig. 1a) was a function of the specific protein band concerned, and the resulting acylation patterns observed were therefore dependent on the respective incubation periods in the presence of radioactive MEDICA 16. Thus the 13, 23, 42 and 66 kDa proteins appeared to be already acylated within 10 min of incubation, whereas acylation of the 32, 36, 52, 56 and 72 kDa proteins was negligible during the first 60 min of incubation, but became significantly pronounced later on. Thus the acylation pattern observed during the first 60 min of incubation consisted essentially of the 13, 23, 42 and 66 kDa bands, whereas that observed after 6–20 h of incubation was dominated by the 13, 32, 52 and 56 kDa bands and was sustained upon further incubating the culture for 48 h in the presence of the radioactive label (results not shown). The acylation capacity of MEDICA 16 (Fig. 1b) was evaluated by incubating the cultured cells in the presence of increasing concentrations of the xenobiotic for a time period of 6 h,

Fig. 2. Acylation of liver proteins by nafenopin

Cultured rat hepatocytes were incubated in the presence of (U-³H) nafenopin and the delipidated proteins were subsequently separated by SDS/PAGE as described in the Experimental section. Each lane contained 100 μg of protein. (a) Time-dependency: the cells were incubated for 1, 3, 6 and 20 h (lanes 1–4 respectively) in the presence of 25 μM-[U-³H]nafenopin (20 μCi/mmol). (b) Concentration-dependency: The cells were incubated for 6 h in the presence of 5, 10, 25 and 50 μM-[U-³H]nafenopin (20 Ci/mmol) (lanes 1–4 respectively).

as the acylation pattern generated during this time period consisted of early as well as lately acylated protein bands. As shown in Fig. 1(b), protein acylation by MEDICA 16 was characterized by a threshold below which the acylation capacity was almost negligible, followed by an increase in the acylation capacity within the concentration range 20–100 μM . This pattern was observed irrespectively of the specific protein bands concerned and could perhaps reflect the non-covalent binding of the amphipathic drug to binding proteins present in the culture medium (e.g. foetal-calf-serum albumin), with a concomitant decrease in the free dioic acid.

The acylation pattern observed in the presence of added nafenopin (Fig. 2) was similarly characterized by defined proteins which appeared to be acylated as a function of incubation time (Fig. 2a) and nafenopin concentrations (Fig. 2b). Nafenopin appeared to acylate fewer liver proteins than those acylated by MEDICA 16 and no nafenopin-acylated proteins could be identified during incubation periods shorter than 3 h.

The detection of common acylated proteins shared by the acylation patterns of MEDICA 16 and nafenopin was further pursued by generating the respective acylation patterns under equivalent experimental conditions and using the same culture preparation. The observed acylation patterns were also compared with those generated by palmitate and myristate. As shown in Fig. 3, the 13, 32, 52, 56 and the 72 kDa bands appeared to be shared by the acylation patterns of both, nafenopin and MEDICA 16. The 32, 52 and 56 kDa bands were also observed in the acylation pattern generated by bezafibrate, in spite of its low specific radioactivity. Besides the apparently shared proteins, some other acylated proteins were specifically labelled by one xenobiotic, but not by the others. For example, the MEDICA 16-acylated bands of 23, 36, 42, 44 and 66 kDa were not shared by nafenopin and, similarly, the

61 kDa band of nafenopin and bezafibrate could not be identified within the acylation pattern generated by MEDICA 16. It is worth noting that the 32 kDa protein also appeared to be acylated in the presence of palmitate and myristate, whereas most other palmitate- and myristate-acylated bands appeared to lack their counterparts in the acylation patterns generated by MEDICA 16 or nafenopin. The extent of protein acylation by native or xenobiotic amphipathic carboxylates did not correlate with the fractional abundance of these proteins in the liver cell (Fig. 3, lane 6).

The identity of the acylated xenobiotic residues in MEDICA 16-acylated proteins was verified by subjecting the acylated protein bands in the molecular-mass range 10–80 kDa to acid hydrolysis *in vacuo*, followed by an exhaustive basic hydrolysis as described in the Experimental section. The combined hydrolysis resulted in recovering most of the acylated radioactivity within a spot which co-chromatographed with authentic MEDICA 16. Hence, most of the acylated xenobiotic residues in MEDICA 16-acylated bands appears to consist of MEDICA 16 *per se* rather than its degradation product. The identity of the acylated xenobiotic residues in nafenopin-acylated bands could not be verified by acid hydrolysis, since it resulted in degradation of authentic nafenopin.

The identity of the proteinaceous residues acylated by MEDICA 16 could be partially inferred from the partition and chromatographic characteristics of the products recovered in the acid hydrolysate of MEDICA 16-acylated proteins. Thus, after acid hydrolysis, 75% of the total acylated MEDICA 16 radioactivity could be extracted into diethyl ether, whereas the other 25% were recovered in the water phase. Of the ether extract, only 25% of the radioactivity co-chromatographed with authentic MEDICA 16 in reverse-phase t.l.c., whereas

Fig. 3. Acylation of liver proteins by MEDICA 16, nafenopin, bezafibrate, palmitate and myristate

Cultured rat hepatocytes were incubated for 6 h in the presence of either 0.45 mM-[U- ^{14}C]bezafibrate (28 Ci/mol), 50 μM -[8,9- ^3H]MEDICA 16 (12 Ci/mmol), 25 μM -nafenopin (20 Ci/mmol), 500 μCi of [9,10- ^3H]palmitate (40 Ci/mmol) or 500 μCi of [9,10- ^3H]myristate (40 Ci/mmol) (lanes 1–5 respectively). The delipidated proteins were separated by SDS/PAGE as described in the Experimental section. The exposure time for the bezafibrate lane was three times longer than for the other lanes. Lane 6, Coomassie Blue staining. Each lane contained 100 μg of protein.

Fig. 4. Subcellular distribution of MEDICA 16- and nafenopin-acylated proteins in cultured rat hepatocytes

Cultured rat hepatocytes were labelled for 6 h with either 25 μM -[U- ^3H]nafenopin (NAF; 20 Ci/mmol) or 50 μM -[8,9- ^3H]MEDICA 16 (MED; 12 Ci/mmol) and were subsequently homogenized and subjected to differential centrifugation as described in the Experimental section. The delipidated proteins of the particulate (P) and soluble (S) fractions were separated by SDS/PAGE. Each lane contained 80 μg of protein.

Fig. 5. Effect of cycloheximide on acylation of the liver proteins

Cultured rat hepatocytes were labelled for 6 h by 50 μM -[8,9- ^3H]MEDICA 16 (12 Ci/mmol) (lanes 1 and 2), 25 μM -[U- ^3H]nafenopin (20 Ci/mmol) (lanes 3 and 4), 500 μCi of [9,10- ^3H]palmitate (40 Ci/mmol) (lanes 5 and 6) and 500 μCi of [9,10- ^3H]myristate (40 Ci/mmol) (lanes 7 and 8) in the absence (–) or presence (+) of 50 μg of cycloheximide/ml (CYC). The delipidated proteins were separated by SDS/PAGE as described in the Experimental section. Each lane contained 80 μg of protein.

most of the radioactivity (75%) was accounted for by a compound(s), less polar than MEDICA 16, having an R_F value of 0.05 as compared with that for MEDICA 16 of 0.59. By subjecting the ether extract to exhaustive basic hydrolysis under conditions suitable for hydrolysing an interfered amide or ester bond (Gilman & Avakian, 1946), all of the ether-extract radioactivity co-chromatographed with authentic MEDICA 16. The resistance of the acylated MEDICA residue to acid hydrolysis could result from the combined interference of the proteinaceous acylated residue and the β,β' -substitutions of MEDICA 16. It should be pointed out that the bond type formed in MEDICA 16-acylated proteins could not be verified by hydroxylamine treatment, since methyl esters of MEDICA 16 proved to be resistant to hydroxylamine treatment as a result of the interference exerted by the β,β' -substitution.

The subcellular localization of the major MEDICA 16- and nafenopin-acylated liver proteins was determined by subjecting the labelled cultured cells to homogenization, followed by differential centrifugation before SDS/PAGE. As shown in Fig. 4, the 13, 32 and the 52 kDa bands consisted of soluble acylated proteins, whereas the 56 kDa band consisted of a particulate protein.

The association between protein acylation by xenobiotics and protein synthesis was evaluated by studying the acylation patterns observed in the presence of added cycloheximide. As shown in Fig. 5, the acylation of liver proteins by either MEDICA 16 or nafenopin

remained unaffected in the presence of cycloheximide under conditions where methionine incorporation was completely inhibited (results not shown). Thus protein acylation by xenobiotic amphipathic carboxylates reflects a post-translational event. The sustained acylation of liver proteins in the presence of cycloheximide was also observed in the case of palmitate, but not myristate, thus corroborating similar results previously observed in other cell types (Magee & Courtneidge, 1985; Towler & Glaser, 1986).

DISCUSSION

Liver proteins were found in the present study to undergo acylation by three xenobiotic amphipathic carboxylates which differ remarkably in their hydrophobic backbones. The specific structural constraints contributed by the hydrophobic backbone appear to determine the final acylation pattern observed in each case. Protein acylation may thus offer a common mode of action of xenobiotic amphipathic carboxylates which allows, however, for diversity based on the individual xenobiotyl-protein adducts formed. Furthermore, it is tempting to propose that *non*-xenobiotic endogenous amphipathic carboxylates having an hydrophobic bone which differs remarkably from that of aliphatic palmitate or myristate (e.g., retinoic acid, prostaglandins or leukotrienes) might also participate in protein acylation similar in nature to that reported here for xenobiotic carboxylates.

By analogy to the acylation of proteins by native long-chain fatty acids, the acylation of proteins by xenobiotic carboxylates is presumably mediated by the respective xenobiotic acyl-CoA derivative rather than the free carboxylic acid. Indeed, nafenopin as well as similar fibrates drugs were recently reported by Bronfman *et al.* (1986) to undergo an ATP-dependent CoA-thioesterification catalysed by preparations of rat liver microsomal long-chain fatty acyl-CoA synthase. Rat liver microsomal fractions were similarly found to catalyse an ATP-dependent CoA-thioesterification of MEDICA 16, with the formation of MEDICA 16 mono-CoA at either carboxylic and (R. Hertz & J. Bar-Tana, unpublished work). The apparent K_m and V_{max} for MEDICA 16 thioesterification amounted to 8.5 μM and 6 nmol min per mg of microsomal protein as compared to 8.0 μM and 30 nmol/min per mg of microsomal protein for palmitate. The substrate specificity of the microsomal long-chain fatty acyl-CoA synthase thus appears to be broad enough to account for the formation of diverse xenobiotic acyl-CoA derivatives. Furthermore, as a result of the α - or β,β' -substitutions, many of the concerned xenobiotic acyl-CoA derivatives are resistant to β -oxidation and may thus accumulate even under conditions which do not favour their formation.

The search for common acylated proteins shared by the acylation patterns of structurally different xenobiotic carboxylates is of special interest, since those proteins might be involved via their acylated forms in triggering hypolipidaemia, peroxisomal proliferation or preadipocyte conversion. Indeed, the 32, 52, 56 and 72 kDa acylated proteins might be considered tentatively as potential candidates which should be subjected now to further dissection of their structural identity by employing high-resolution techniques. This search may also be complemented by looking for palmitoylated or

myristoylated proteins which have their counterparts in the acylation patterns of xenobiotic carboxylates, since high concentrations of long-chain fatty acids were previously reported to induce moderate peroxisomal proliferation in cultured rat hepatocytes (Hertz *et al.*, 1985). Furthermore, the proteins acylated by a particular xenobiotic and which are not shared by other acylation patterns may be considered as potential mediators of the specific biological effects induced by that xenobiotic. For example, the unique effect of MEDICA 16 as an anti-obesity agent (Bar-Tana *et al.*, 1987), as well as its specific effect with respect to apolipoprotein C-III reduction (Bar-Tana *et al.*, 1988) are not shared by fibrates and might perhaps be triggered by singular MEDICA 16-acylated proteins. Similarly, some of the biological effects exerted by *non*-xenobiotic endogenous amphipathic carboxylates could be mediated by the acylation of selected proteins.

REFERENCES

- Bar-Tana, J., Rose-Kahn, G. & Srebnik, M. (1985) *J. Biol. Chem.* **260**, 8404–8410
- Bar-Tana, J., Tzur, R., Frenkel, B., Rose-Kahn, G., Mayorek, N. & Hertz, R. (1987) in *Recent Advances in Obesity Research*, vol. 5 (Berry, E. M., Blondheim, S. H., Eliahou, H. E. & Shafir, E., eds.), pp. 319–326, John Libbey and Co., London
- Bar-Tana, J., Rose-Kahn, G., Frenkel, B., Shafer, Z. & Fainaru, M. (1988) *J. Lipid Res.* **29**, 431–441
- Berger, M. & Schmidt, M. F. G. (1986) *J. Biol. Chem.* **261**, 14912–14918
- Berry, M. N. & Friend, D. S. (1969) *J. Cell. Biol.* **43**, 506–520
- Best, M. M. & Duncan, C. M. (1970) *Atherosclerosis* **12**, 185–192
- Bizzozero, O. A., McGarry, J. F. & Lees, M. B. (1987) *J. Biol. Chem.* **262**, 2138–2145
- Brandes, R., Arad, R. Bar-Tana, J. (1986) *Biochim. Biophys. Acta* **877**, 314–321
- Brandes, R., Hertz, R., Naishtat, S., Weil, S. & Bar-Tana, J. (1987) *Life Sci.* **40**, 935–941
- Bronfman, M., Amigo, L. & Morales, M. N. (1986) *Biochem. J.* **239**, 781–784
- Carr, S. A., Biemann, K., Shozo, S., Parmelle, D. C. & Titani, K. (1982) *Proc. Natl. Acad. Sci. U.S.A.* **179**, 6128–6131
- Gilman, H. & Avakian, F. (1946) *J. Am. Chem. Soc.* **68**, 2104–2105
- Gray, J. J. B., Lake, B. G., Beamand, J. A., Foster, J. R. & Gangolli, S. D. (1983) *Toxicol. Appl. Pharmacol.* **67**, 15–25
- Hedo, J. A., Collier, E. & Watkinson, A. (1987) *J. Biol. Chem.* **262**, 954–957
- Hertz, R., Arnon, J. & Bar-Tana, J. (1985) *Biochim. Biophys. Acta* **836**, 192–200
- Hertz, R., Bar-Tana, J., Sujatta, M., Pill, J., Schmidt, F. H. & Fahimi, H. D. (1988) *Biochem. Pharmacol.*, in the press
- Hoeg, J. M., Meng, M. S., Ronan, R., Fairwell, T. & Brewer, H. B., Jr. (1986) *J. Biol. Chem.* **261**, 3911–3914
- Kamps, M. P., Buss, J. E. & Sefton, B. M. (1985) *Proc. Natl. Acad. Sci. U.S.A.* **82**, 4625–4628
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
- Magee, A. I. & Courtneidge, S. A. (1985) *EMBO J.* **4**, 1137–1144
- Marinetti, G. V. & Cattieu, K. (1982) *Biochim. Biophys. Acta* **685**, 109–116
- Moody, D. E. & Reddy, J. K. (1978) *Am. J. Pathol.* **90**, 435–446
- Odonkor, J. M. & Rogers, M. P. (1983) *Biochem. Pharmacol.* **33**, 1337–1341
- Olson, E. N. & Spizg, G. (1986) *J. Biol. Chem.* **261**, 2458–2466
- Olson, E. N., Glaser, L. & Merlie, J. P. (1984) *J. Biol. Chem.* **259**, 5364–5367
- Omary, M. B. & Trowbridge, I. S. (1981) *J. Biol. Chem.* **256**, 4715–4718
- Reddy, J. K., Warren, J. R., Reddy, M. K. & Lalwani, N. D. (1982) *Am. N.Y. Acad. Sci.* **386**, 81–110
- Reddy, J. K. & Lalwani, N. D. (1985) *CRC Crit. Rev. Toxicol.* **12**, 1–58
- Riendeau, D. & Guerton, D. (1986) *J. Biol. Chem.* **261**, 976–981
- Rose-Kahn, G. & Bar-Tana, J. (1985) *J. Biol. Chem.* **260**, 8411–8415
- Schmidt, M. F. G. & Schlesinger, M. J. (1980) *J. Biol. Chem.* **255**, 3334–3339
- Schmidt, M. F. G., Bracha, M. & Schlesinger, M. J. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 1687–1691
- Sefton, B. M., Trowbridge, I. S., Cooper, J. A. & Scolnick, E. M. (1982) *Cell (Cambridge, Mass.)* **31**, 465–474
- Towler, D. & Glaser, L. (1986) *Biochemistry* **25**, 878–884
- Verrando, P., Negrel, R., Grimaldi, P., Murphy, M. & Ailhaud, G. (1981) *Biochim. Biophys. Acta* **663**, 255–265

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