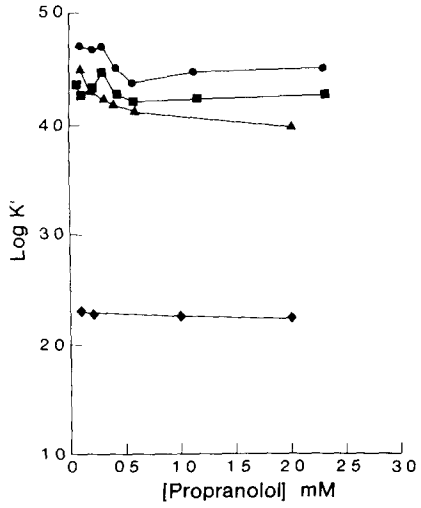

Fig 2 Log molar apparent partition coefficients (K') of propranolol as a function of propranolol concentration in model and biological membranes· (●) erythrocytes, (■) erythrocyte ghosts, (▲) DMPC liposomes, and (◆) *n*-octanol–aqueous buffer solution Temperature 37°, pH 7 4

partially protein-depleted, underwent greater membrane perturbation and possibly increased exposure of acidic phospholipids to propranolol and electrostatic binding which could account for the slight rise in log K' in the low propranolol concentration region The approximate constance of log K' at higher concentrations implies that the environment for partitioning was similar in all three cell membrane systems with respect to bilayer structure and characteristics

In summary, studies of the uptake and partition coefficient of propranolol in erythrocytes, ghosts, and liposomes demonstrate subtle changes occurring in the lipid matrix up to about 0 6 mM propranolol These changes were mediated by protein and acidic phospholipids In erythrocytes, interaction between propranolol and protein appeared to improve the stability of the cell membrane before sufficient propranolol had partitioned into the lipid matrix to cause extensive disruption In ghost cells, perturbation of the membrane, likely through interaction of propranolol with acidic phospholipids, caused fluctuation in log K' but otherwise log K' exhibited behavior similar to that in erythrocytes, although uptake and partitioning of propranolol was less The uptake of propranolol was an order of magnitude less in liposomes compared to erythrocytes, but the uniform concentration dependence of log K' and the osmotic fragility of liposomes in the presence of propranolol [8] indicate that the hydrophobic interaction plays a major role in creating membrane disturbances [14] The *n*-octanol-buffer system does not serve as a suitable model in this instance to describe the non-specific membrane-stabilizing activity of propranolol

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Effects of alloxan on *S*-adenosylmethionine metabolism in the rat liver

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In the rat liver up to 40% of the phosphatidylcholine is synthesised by the transmethylation pathway being *S*-adenosyl-l-methionine (AdoMet)* the methyl donor [1] This pathway is modulated by a variety of hormones and by the levels of AdoMet and *S*-Adenosyl-l-homocysteine (AdoHcy) (reviewed in [2]) AdoHcy is a competitive inhibitor of the transmethylation reaction Therefore, the ratio AdoMet/AdoHcy must be carefully controlled to maintain the cellular necessities of phosphatidylcholine In the liver of alloxan-diabetic rats the content of phosphatidylcholine is lower than in control animals [3, 4] The activity of the enzyme phospholipid methyltransferase is also reduced in liver microsomes of alloxan-diabetic rats [5] We have measured the levels of AdoMet and AdoHcy and the synthesis of phosphatidylcholine by the transmethylation pathway in hepatocytes isolated from alloxan-

* Abbreviations used AdoMet, *S*-adenosyl-L-methionine AdoHcy, *S*-adenosyl-L-homocysteine, AdoMet-synthetase, *S*-adenosyl-L-methionine synthetase

diabetic rats Furthermore, we have determined the activities of the enzymes *S*-adenosylmethionine synthetase (AdoMet-synthetase) and phospholipid methyltransferase in the rat liver from alloxan-diabetic and normal rats The present results show drastic changes in the metabolism of AdoMet in alloxan-diabetic rats being these changes counteracted by insulin treatment

Materials and methods

Male normally fed Wistar rats (250–400 g) were used in all experiments Rats were made diabetic by injection through the peneal vein of 60 mg/kg body weight of alloxan [6] Two days after alloxan injection, a group of rats received subcutaneously 2 u/day insulin NPH (Nordish Gentofte) After 6 days animals were used for experimentation

Rat liver microsomes were isolated and the activity phospholipid methyltransferase determined as previously described [7] AdoMet-synthetase activity was determined in the liver cytosol as described in [8] Hepatocytes were isolated by perfusion with collagenase as previously described [9] Phospholipid methylation was determined by measuring the incorporation of [^{3}H]-methyl groups from [^{3}H-methyl]-methionine into phospholipids as mentioned previously [10] To determine the levels of AdoMet and AdoHcy hepatocytes were labeled with 2 μCi/ml [^{35}S]-methionine At various times cells were homogenized with 10% sulfosalicilic acid and then centrifuged at 10000 *g* for 10 min in a Beckman microfuge Supernatants were pipetted into clean tubes and stored under liquid nitrogen until used for the analysis of AdoMet and AdoHcy The levels of AdoMet and AdoHcy were measured by HPLC as described [11, 12] Blood glucose was determined by the glucose-oxidase method [13]

Results

As reported by Hoffman *et al* [5], phospholipid methyltransferase activity in microsomes from alloxan-diabetic rats with blood glucose levels of 530 ± 37 mg/100 ml was lower than in normal rats (Table 1) The reduction was about 70% and was not observed in alloxan-diabetic rats treated with insulin In contrast with these results, AdoMet-synthetase activity was about 2-fold higher in alloxan-diabetic rats than in normal animals Again, these changes were not observed in alloxan-diabetic rats treated with insulin (Table 1) The effect of alloxan-treatment on phospholipid methyltransferase and AdoMet-synthetase was on the V_{max} of the enzyme without showing any effect on the apparent K_m for respectively AdoMet and methionine (not shown)

The incorporation of methyl groups from [^{3}H-methyl]-methionine into phospholipids was however, the same in hepatocytes isolated from alloxan-diabetic rats than in normal animals (Fig 1) No differences were observed in the incorporation of radioactivity into phosphatidylcholine, phosphatidyl-*N*-monomethylethanolamine or phosphatidyl-*N*,*N*-dimethylethanolamine between alloxan-diabetic and normal rats (not shown) To explain this apparent contradiction, lower microsomal phospholipid methyltransferase activity and similar phospholipid methylation in intact hepatocytes, we have measured the levels of AdoMet and AdoHcy in alloxan-diabetic and normal rats

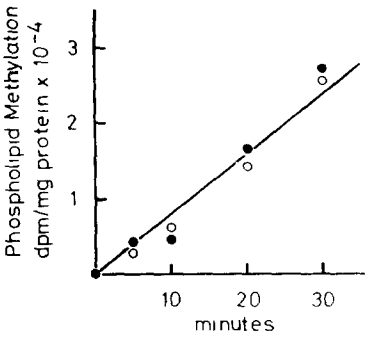

Fig 1 Phospholipid methylation by intact hepatocytes isolated from normal (○) and alloxan-treated diabetic (●) rats At time zero 1 μCi/ml [^{3}H-methyl]-methionine was added and at the indicated times the incorporation of [^{3}H]-methyl groups into phospholipids determined as described under Methods Results are the average of three independent experiments in duplicate

In rat hepatocytes isolated from both alloxan-diabetic and normal animals, the incorporation of radioactivity from [^{35}S]-methionine into AdoMet + AdoHcy reached a steady state level about 10 min after the addition of the labeled precursor (Fig 2) The incorporation of radioactivity into AdoMet + AdoHcy was about 5-fold greater in alloxan-diabetic than in normal rats (Fig 2, Table 2) which agrees with the data showing a higher AdoMet-synthetase activity in diabetic animals The increased incorporation of radioactivity into AdoMet + AdoHcy was however, not observed in alloxan-diabetic rats treated with insulin (Table 2) The ratio AdoMet/AdoHcy was the same in alloxan-diabetic rats than in normal animals and was not modified by the injection of insulin to diabetic rats (Table 2)

Hepatocytes isolated form normal rats respond to the addition of physiological doses of glucagon with a rapid stimulation of phospholipid methyltransferase activity [9, 14] This effect was, however, never observed with hepatocytes isolated from alloxan-diabetic rats (Fig 3) Similarly, the addition of high doses of cAMP which in normal rats also produce a fast activation of phospholipid methyltransferase [9], failed to produce this effect in alloxan-diabetic rats (not shown) Alloxan-diabetic rats treated with insulin were unable to increase its methyltransferase activity in response to glucagon (Fig 3)

Table 1 Phospholipid methyltransferase and *S*-adenosylmethionine-synthetase activities in the liver from normal, alloxan-diabetic and alloxan-diabetic insulin treated rats

	Blood glucose (mg/100 ml)	PMTase (pmoles/min/mg)	AdoMet-synthetase (nmoles/min mg)
Normal (N = 6)	115 ± 2	158 ± 24	0 62 ± 0 02
Alloxan-diabetic (N = 6)	530 ± 37	48 ± 6	1 02 ± 0 07
Alloxan-diabetic insulin treated (N = 6)	192 ± 28	151 ± 19	0 50 ± 0 03

Results are the mean ± S E M of six independent determinations in triplicate

Table 2 Levels of AdoMet and AdoHcy in hepatocytes isolated from normal, alloxan-diabetic and alloxan-diabetic insulin-treated rats

	AdoMet + AdoHcy (dpm/min/mg)	AdoMet/AdoHcy
Normal (N = 8)	11460 ± 1853	1 84 ± 0 12
Alloxan-diabetic (N = 8)	52743 ± 9654	1 73 ± 0 51
Alloxan-diabetic insulin treated (N = 8)	6382 ± 283	1 98 ± 0 35

Results are the mean ± S E M of eight independent determinations in duplicate The levels of AdoMet and AdoHcy were determined 20 min after the addition of [^{35}S]-methionine to the cell suspension

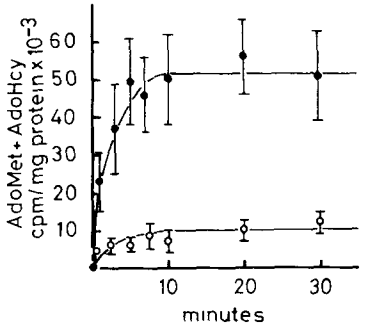

Fig 2 Levels of AdoMet and AdoHcy in hepatocytes isolated from normal (○) and alloxan-treated diabetic (●) rats At time zero, cells received 2 μCi/ml [^{35}S]-methionine and at the indicated times samples were taken and the levels of AdoMet and AdoHcy determined as described under Methods Results are the mean ± S E M of four independent experiments

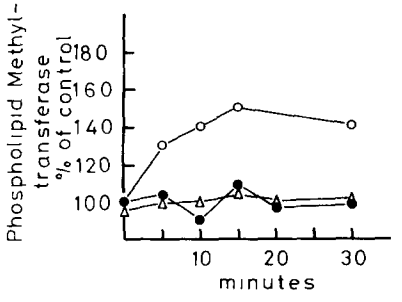

Fig 3 Effect of glucagon addition on phospholipid methyltransferase in hepatocytes isolated from normal (○), diabetic (●) and insulin-treated diabetic (△) rats In each condition, phospholipid methyltransferase activity of glucagon-treated hepatocytes was compared with the corresponding activity of the non-stimulated cells, which received the arbitrary value of 100% One hundred per cent corresponds to 34 6, 15 3 or 36 2 pmoles/min/mg protein in respectively normal, diabetic and insulin-treated diabetic rats At time zero, hepatocytes were stimulated with 1 μM glucagon and the activity phospholipid methyltransferase determined as mentioned under Methods Results are the average of three independent experiments in duplicate

Discussion

The present results confirm the data of Hoffman *et al* [5] showing a decreased phospholipid methyltransferase activity in microsomes from alloxan diabetic rats Surprisingly, phospholipid methylation in hepatocytes isolated from both normal and diabetic rats was, however, the same under both conditions This apparent controversy can be explained considering that the activity AdoMet-synthetase and the levels of AdoMet, but not the ratio AdoMet/AdoHcy, are higher in alloxan-diabetic rats than in normal animals If in normal hepatocytes phospholipid methyltransferase is not working at saturating levels of AdoMet, an increase in the intracellular concentration of AdoMet, like that shown here for alloxan-diabetic rats, would cause a concomitant increase in the rate of phospholipid methylation The concentration of AdoMet in rat liver has been reported to be about 50 μM [15] and the apparent K_m for AdoMet for the conversion of phosphatidylethanolamine to phosphatidyl-*N*-monomethylethanolamine is 58 μM, phosphatidyl-*N*-monomethylethanolamine to phosphatidyl-*N*,*N*-dimethylethanolamine is 65 μM and phosphatidyl-*N*,*N*-dimethylethanolamine to phosphatidylcholine is 96 μM [16] These results indicate that in rat liver phospholipid methyltransferase is not working at saturating levels of AdoMet Therefore, an increase in the concentration of AdoMet maintaining constant the AdoMet/AdoHcy ratio must stimulate phospholipid methylation The ratio AdoMet/AdoHcy under our conditions is similar to that reported by others for rat liver [17] It is interesting to note that, despite the large changes in AdoMet-synthetase activity and AdoMet levels in hepatocytes from alloxan-diabetic rats, these cells maintain a constant AdoMet/AdoHcy ratio This is important since pharmacological manipulation of this ratio has been shown to alter many cell functions (reviewed in [2])

Insulin-treated alloxan-diabetic rats show normal phospholipid methyltransferase and AdoMet-synthetase activity These results strongly indicate that the effects of alloxan treatment on AdoMet metabolism are insulin-dependent and not due to other possible toxic effects of the drug Insulin-treated alloxan-diabetic rats still have an impaired phospholipid methyltransferase response to glucagon and cAMP It is important to note that insulin-treated rats are still diabetic and that insulin injection only partially compensates for their hormonal imbalance What is regulating phospholipid methyltransferase and AdoMet-synthetase activity in diabetes is not known The present results show that alloxan-treated rats have a low liver phospholipid methyltransferase and hepatocytes from these animals treated with glucagon do not have an increased methyltransferase activity The effect of alloxan-treatment can be a decreased insulin/glucagon ratio resulting in a high

serum free fatty acid concentration which, as reported [18], blocks phospholipid methyltransferase Whether the high serum levels of free fatty acids are responsible for the impaired phospholipid methyltransferase response to glucagon in diabetes remains to be determined

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Interaction between ellipticine derivatives and circular supercoiled DNA as revealed by gel electrophoresis. Possible relationship with the mechanisms of cytotoxicity

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Certain ellipticine derivatives are DNA intercalating cytotoxic agents with antitumoral activity against experimental tumors [1] and in clinical cases [2] Their mechanisms of action have been related to their DNA affinity as measured by competition with ethidium bromide [1], to their ability to be oxidized and to generate electrophilic intermediates [3] and lastly to their capacity to inhibit topoisomerase II activity [4] However, no relationship between cytotoxic activity and the above mentioned biochemical properties has been demonstrated for various substituted compounds Two possibilities should, therefore, be taken into consideration (1) modifications in different regions of the ellipticine molecule induce different series of compounds with different mechanisms of action, and (2) some biochemical property of the ellipticine structure, related to its cytotoxic effect, remains unknown In this paper, we report the ability of different substituted derivatives of ellipticine to modify the electrophoretic migration of circular supercoiled DNA This effect is due to unknown properties of the DNA–ellipticine binding, which may be related to the mechanism of cytotoxicity

Materials

The ellipticine derivatives were synthesized by Dr Dat-Xuong (Gif/Yvette), Dr Viel (Châtenay-Malabry) and Dr Lesco (Villejuif) PM2 supercoiled DNA was either prepared according to the method of Espejo [5], or purchased from Boehringer (Grenoble, France)

Methods

(1) *Electrophoresis* Sample preparation a PM2 stock solution was diluted in 1 mM EDTA, 10 mM sodium phosphate (pH 7) for (i) drug interaction, (ii) optical density measurements DNA (3.7×10^{-3} M in bases) was mixed at 4°, with ellipticine derivatives ($5–25 \times 10^{-6}$ M) Before loading the samples into the gel slots, the solution was mixed with a six-fold concentrated solution of 0.25 mg/ml bromphenol blue and 40% sucrose, as previously described [6]

Electrophoresis was carried out for 18 hr in an 0.8% agarose horizontal gel (Model H1, BRL, Rockville, MD) in Gary's buffer (36 mM Tris, 30 mM Na_2HPO_4, 1 mM EDTA, pH 8) at 60 V A photograph of the ethidium bromide stained gel was taken under u v excitation A densitometric scanning of the DNA bands on the negative of this photography was then carried out with the Joyce Loebl apparatus

The width of the DNA bands was evaluated by measuring the bases of the densitometric peaks The DNA migration was obtained by measuring the distance between the slot and the peak of the densitometric DNA band

(2) *Cytotoxic effects on L1210 cells in culture* The experimental protocol has been previously reported [7] The cells were exposed to increasing concentrations of drug during 48 hr and incubated in a 5% CO_2 atmosphere at 37° The drugs were dissolved in dimethylsulfoxide (1% final) The cell concentration was determined with a ZBI Coulter Counter The cytotoxicity was evaluated by measuring the drug concentration which decreases by 50% the L1210 cell growth rate after 48 hr All experiments were performed within a period of 8 mo

Results and discussion

The electrophoretic migration of PM2 circular DNA was studied when DNA and ellipticine derivative were both present in the gel slot ($0.13 <$ drug/base < 0.65) The