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PRELIMINARY COMMUNICATIONS

EFFECT OF MORPHINE ON HEPATIC LIPID METABOLISM

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The subcutaneous implantation of morphine pellets has been shown as an effective method in inducing tolerance to and physical dependence on morphine in the mouse (1). Although the primary action of this narcotic drug is its analgesic effect in the central nervous system (2), possible alteration of liver functions may occur during the course of initial drug metabolism. In a preliminary study, we noticed that mice implanted with morphine pellets developed pale-colored livers within 24 hr after drug administration (3). However, most liver returned to normal after 3-9 days, even though morphine was continuously released into the system. Subsequent morphologic examination indicated a large increase in lipid droplets in hepatocytes corresponding to the gross changes observed (4). Lipid droplets' accumulation was thought to be due mainly to an increase in the triacylglycerol level in the hepatocytes.

In the present study, gross morphologic and ultrastructural changes are verified biochemically through analysis of the lipids in morphine-treated liver and comparison with that treated with placebo. In addition, activity of oleoylCoA:1-acyl-glycerophosphocholine (GPC)* acyltransferase (EC 2.3.1.23) in liver microsomes was also assayed. Morphine may alter hepatic functions by interacting with the membrane components, thus changing the normal transport functions. The assay of acyltransferase is thought to be important because, when coupled with phospholipase A_2, these enzymes may play a role in regulating the turnover of membrane phosphoglycerides (5).

MATERIALS AND METHODS

Male ICR mice, weighing 25-30 g, were obtained from Charles River, Wilmington, MA. Animals were maintained in an environment supplied with food and water *ad lib*. In the first experiment, groups of 12 were implanted with specially formulated morphine (75 mg base) pellets for 12 and 24 hr (1). The control mice were implanted with placebo pellets for 24 hr In the second experiment, one group of 6 mice was each implanted with a morphine pellet for 3 days. The other group of 6 mice was each implanted with 3 morphine pellets for 9 days by

*Abbreviations used: GPC, glycerophosphocholines; and TG, triacylglycerols.

giving a new pellet every third day. Controls received 3 placebo pellets similarly for 9 days

After decapitation, liver was dissected and the wet weight obtained. Liver was homogenized in 20 ml of 0.25 M sucrose containing 1 mM $MgCl_2$, 1 mM EDTA and 50 mM Tris-HCl (pH 7.4) A 5-ml portion of the liver homogenates was taken from each sample for lipid analysis. The remaining portion was subjected to differential speed centrifugation for isolation of microsomes. Briefly, liver homogenates were centrifuged at 1000 *g* for 10 min to sediment the cellular debris. The supernatant solution was centrifuged at 13,500 *g* for 20 min and the supernatant fraction was further centrifuged at 100,000 *g* for 60 min to obtain a microsomal pellet.

Assay of oleoylCoA:1-acyl-GPC acyltransferase activity

The microsomal pellet was resuspended in 5 vol. (by wt) of 0.25 M sucrose solution to obtain a protein concentration of about 1 mg/ml. Protein was determined by the method of Lowry *et al.* (6). Assay of oleoylCoA:1-acyl-GPC acyltransferase activity was similar to that described for brain microsomes (7). Approximately 100 µg of the microsomal protein was added to each incubation system containing: [^{14}C]oleoylCoA (0.05 µCi in 5 nmoles, New England Nuclear, Boston, MA), 1-acyl-GPC (50 mM) (Sigma Chemical Co., St. Louis, MO) and 0.25 M sucrose with 50 mM Tris (pH 7.4), to a total volume of 0.5 ml. 1-Acyl-GPC was originally dissolved in chloroform and was evaporated to dryness under nitrogen prior to the addition of other components. Incubation for each sample in duplicate was carried out for 10 min at 37°. Reaction was terminated by adding to the incubation mixture 2 ml of chloroform-methanol (2:1, v/v) with immediate mixing. The organic phase was taken to dryness and products of the reaction were separated by T.L.C. (7). Zero time incubation was used as background activity which was subtracted from each sample.

Analysis of liver triacylglycerol (TG) content

Lipids from the 5-ml sample of liver homogenates were extracted by adding 5 vol. of chloroform-methanol (2:1, v/v). A portion of the lipid extract was applied to a T.L.C. plate which was developed with a solvent system containing hexane-ether-15 N NH_4OH (80:20:0.1, v/v). The TG band appeared at R_f 0.6 and was scraped into a test tube for converting the acyl groups to their methyl esters by alkaline-methanolysis (8). A known amount of heptadecanoyl methyl ester (C17:0) was added to each sample as standard for quantitative analysis. Fatty acid methyl esters were analyzed by G.L.C. (9) using a Hewlett Packard Research Gas Chromatograph (5830A) equipped with automatic digital integrator for peak area computation.

RESULTS AND DISCUSSION

Liver TG levels obtained at various time periods after morphine are shown in Table 1. A 6-fold increase in hepatic TG (based on liver weight) was observed at 12 hr after pellet implantation. This high level then started to decline to a 3-fold increase at 24 hr after morphine pellet implantation. The TG level continued to decrease to below controls at 3 days

but then returned to control level by 9 days. Except for the 9-day data, all changes observed were shown to be statistically significant by analysis of variance.

In liver microsomes, oleoylCoA:1-acyl-GPC activity was inhibited almost completely at 12 hr after morphine and the degree of inhibition correlated well with the TG increase (Table 1). Acyltransferase activity started to rise after this period so that by 3 days the enzymic activity was 2-fold higher than controls. Enzymic activity returned to control level at 9 days. Thus, the inhibition and stimulation of acyltransferase activity were related directly to accumulation and depletion of liver TG.

Table 1. Liver triacylglycerol levels and oleoylCoA:1-acyl-GPC acyltransferase activity in mice after morphine pellet implantation

Morphine treatment	N	Liver wt (g)	TG/g wt*	Acyltransferase† (nmoles/min/mg protein)
Experiment 1				
Placebo	12	1.94 ± 0.19	1.0 ± 0.40	1.90 ± 0.51
12 hr	11	1.34 ± 0.13‡	6.5 ± 2.30‡	0.14 ± 0.10‡
24 hr	12	1.53 ± 0.23‡	2.8 ± 0.98‡	0.46 ± 0.52‡
Experiment 2				
Placebo	6	1.97 ± 0.10	1.0 ± 0.43	1.94 ± 0.31
3 day	5	1.20 ± 0.15‡	0.6 ± 0.08§	4.35 ± 1.48‡
9 day	7	1.69 ± 0.32	1.0 ± 0.27	2.43 ± 0.75

* TG values are relative to placebo which is designated as 1.0.

† Assay of oleoylCoA:1-acyl-GPC acyltransferase activity of liver microsomes is described in text.

‡ Results are significantly different compared with controls, as shown by analysis of variance ($p < 0.001$).

§ Results are significantly different compared with controls, as shown by analysis of variance ($p < 0.05$).

From the study, we have obtained biochemical data to support the morphologic findings concerning deposition of lipid droplets in hepatocytes after morphine treatment (3,4). No morphologic or ultrastructural changes were found in the liver at all time periods after control mice were implanted with placebo. The 6-fold or higher increase in liver TG level at 12 hr after morphine implantation corresponded also to an inhibition of acyltransferase activity. The mechanism leading to this transient TG increase and subsequent depletion while morphine is continuously released into the body is not understood yet. However, we believe that this may be dependent on the ability of hepatocytes to mobilize TG. Since the

1-acyl-phosphoglyceride acyltransferases are membrane-bound enzymes known for their role in regulating membrane phospholipid turnover (5), an inhibition of the enzymic activity by morphine would result in alteration of other membrane transport activities. A number of amphiphilic compounds, such as local anesthetics, neuroleptic drugs and mitogens, have been shown recently to be potent inhibitors of the acyltransferase (10-12). Morphine may exert a similar inhibitory action on the enzyme. Such a potent effect of drug-membrane interaction in the *in vivo* system has not been demonstrated before. This model of study is profoundly interesting not only for understanding lipid metabolism with regard to drug-membrane interaction but also because it allows one to investigate the mechanism underlying the high degree of tolerance to the drug.

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