Effects of Morphine on Plasma Lipids in the Rabbit

Reports of morphine effects on lipid metabolism are sparse. They usually deal with the immediate effect of the narcotic in rats, no effects having been described in rabbits. In this paper we report the effects of morphine on various lipid fractions in rabbit plasma. Because of the development of tolerance and dependance phenomena the metabolic effects of morphine are multiple and complex. We have therefore been careful to note the effects of the initial injection in a non-tolerant animal as well as those which appear in chronically intoxicated and abstinent animals.

Materials and methods. Male rabbits (Fauve de Bourgogne) weighing 2.5 kg were divided into 3 experimental groups. The blood of 115 animals was analyzed. Morphine chlorhydrate (Chaix et Du Marais) was injected i.m. Plasma free fatty acids (FFA) were measured by a colorimetric method[1].

In one experimental group of 13 animals wey have used a modified thin layer silicagel chromatography technique to determine plasma phospholipids, triglycerides, and free and esterified cholesterol. After spotting, 2 successive migrations are carried out. The first uses chloroform-methanol-water (65:25:4, v/v/v) and is stopped approximately 3 cm from the starting line. This first run carries the phospholipids approximately 1.5 cm from the start and thus facilitates their determination with densitometry. After the plate is dried, the second migration is carried out in the same direction for the whole length of the plate with a mixture of petroleum ether-ethyl ether-acetic acid (85:15:0.8, v/v/v). This procedure allows an excellent separation of the different lipid fractions, and gives subsequent densitometry results with better than 90% accuracy.

Results and discussion. 1. Effects of morphine on plasma FFA concentration in rabbits. We have determined plasma FFA as well as other plasma constituents (sugars and corticosterone)[2] in 102 animals after the initial injection of morphine, during a chronic intoxication (5–10 mg/kg lasting 21 days) and after withdrawal from morphine. Results are given in Figure 1.

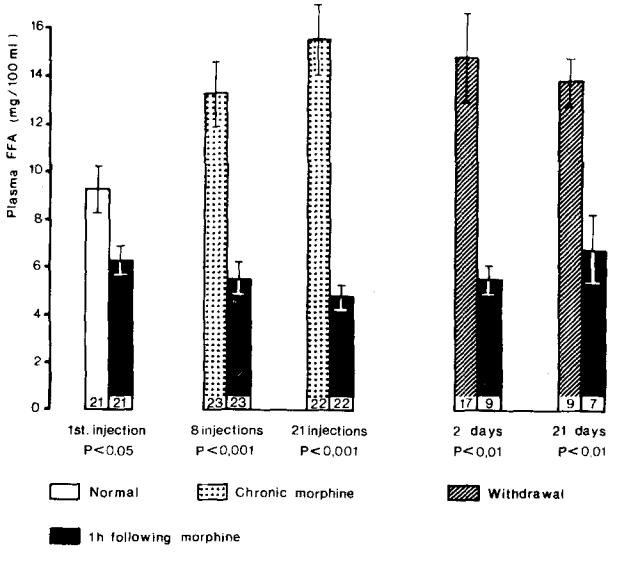

Fig. 1. Changes in plasma FFA and effects of morphine during chronic intoxication and withdrawal in rabbits. Mean value ± SE, p is given by Student's t-test, the number in the bars indicates the number of animals.

During the 3 weeks of daily morphine administration, each new injection causes a more than 50% drop in plasma FFA within 60 min. This effect lasts approximately 6 h. It should be noted that, even 24 h after a given injection, the plasma FFA levels in intoxicated animals are almost double the control values. The maximum value recorded was 40 mg/100 ml. In control rabbits deprived of food for 15 h , plasma FFA average 9.2 ± 1.1/100 ml. This basal value rises to 18.8 ± 2.9 by the 21st day of repeated injections but morphine retains its depressive effect because a new injection always lowers FFA levels (6.3 ± 1.1 mg/100 ml after 1 h). After withdrawal from morphine, the elevated plasma FFA concentration as well as the depressive effect of a new injection of the drug persist as long as 3 weeks after cessation of regular injections.

2. Effects of morphine on other plasma lipids. This experiment involved 13 rabbits, each serving as its own control. 3 h after the initial dose, morphine exhibits a substantial depressive effect on several lipid constituents of the plasma. Figure 2 demonstrates that phospholipid concentration clearly drops ($p < 0.01$), this drop being more than 40% in some cases. In addition, a significant ($p < 0.05$) drop in plasma triglycerides was found. Free and esterified cholesterol concentrations did not change significantly.

After repeated injections, the effects of morphine become less pronounced, and tolerance develops towards its depressive effects, the drug has practically no effects on plasma lipids.

3. Effects of food withdrawal. Morphine effects in rabbits during a prolonged fast. Appetite is spontaneously reduced in morphine-treated animals. In order to eliminate the food factor, we have studied fasting rabbits divided into 2 groups: 8 were simply deprived of food and served as controls; the other 12 were also fasted but received daily injections (5 mg/kg, i.m.) of morphine. After 4 days of fasting, morphine elicited a drop in plasma phospholipids and triglycerides ($p < 0.01$). After 8 days of fasting, tolerance develops towards the depressive effect of morphine on phospholipids and triglycerides. In addition, basal levels of free and esterified cholesterol were significantly higher in morphine-treated than in control animals ($p < 0.05$), this is responsible for the hyperlipemia ($p < 0.05$), in the morphinized group of fasting rabbits.

With regard to plasma lipids, the phenomena of tolerance and reversal of action analogous to those already described for carbohydrates[3], with corresponding changes in the activity of various endocrine glands, have been found[4]. Our results demonstrate a postmorphinic hypolipemia due to reduced plasma levels of phospholipids and triglycerides, thus confirming our preliminary experiments[5,6]. If the chronic intoxication is continued, tolerance develops towards the depressive effect on lipemia but not towards the immediate effect of morphine on FFA. Moreover, in tolerant rabbits the FFA concentra-

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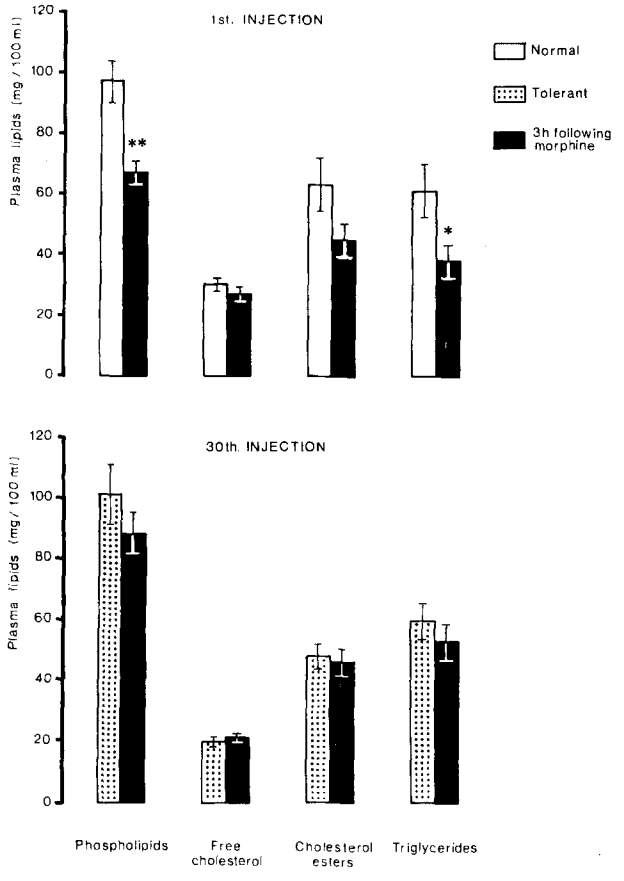

Fig. 2. Changes in morphine effects in various lipid fractions of rabbits plasma during chronic intoxication. Mean values ± SE, p is given by Student's t-test. * $p < 0.01$. ** $p < 0.05$.

tions are clearly higher than in controls. Finally, in animals withdrawn from morphine for several days or even weeks, the FFA concentration remains elevated, as does the immediate hypolipemic effect of the drug.

It is difficult to explain the effects on plasma lipids by changes in endocrine activity even though these effects occur simultaneously with changes in hormonal secretion. The initial injection of morphine elicits ACTH secretion as well as that of glucagon[4]. It is known that these 2 hormones mobilize fatty acids; however, we have observed the opposite effect on FFA. A single dose of morphine also causes a secretion of corticosteroids[2] whose immediate effects in rabbits are to lower plasma lipids[7]. It is possible that under these conditions the postmorphinic hypolipemia results from a hypersecretion of corticosterone. Nevertheless this explanation is not very satisfying when one considers the case of chronically treated animals in which morphine retains its depressive effect on plasma FFA and in which the drug actually depresses rather than increases the secretion of corticosterone[2]. In chronically morphinized rabbits, the FFA level in blood withdrawn 24 h after a given injection is abnormally high. At the present time we have no explanations for this.

To conclude, it is very difficult to supply a purely endocrinological explanation for the effects of morphine on blood lipids. Other experiments are necessary to elucidate the actions of morphine as well as the changes in action during chronic intoxication and withdrawal.

Résumé. Chez le Lapin, 1 h après une injection de morphine on note une baisse du taux des phospholipides, des triglycérides et des acides gras libres (AGL) du sang. Au cours du traitement chronique cet effet dépressif ne s'observe plus qu'au niveau des AGL dont le taux est anormalement élevé 24 h après la dernière injection.

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Effect of Long Term Lithium Treatment on Brain Fumarase Activity

The specificity of lithium therapy for manic-depressive psychosis is by now undisputed[1,2]; however the mechanism of this remarkable effect is still far from a satisfactory explanation[3,4]. Inspite of there being little or no effect on most enzymes studied[4], our previous communications reported the activation of succinate dehydrogenase[5] and the inhibition of aconitase[6] in the brains of mice treated with Li_2CO_3. These results led us to study the effect of Li^+ on brain fumarase (fumarate hydratase, E.C. 4.2.1.2)activity.

Material and methods. The experiments were carried out with male Swiss mice (mean initial body weight 20 g) maintained in a standard balanced diet ad libitum. To the control group of mice, distilled water was given. The other group of animals received as drinking water a solution containing 100 mg Li_2CO_3/l. After a period of 132 days of experimentation, the mice were killed by cervical dislocation and the brains removed quickly and stored at −20 °C until used. Brain homogenates (10%) were prepared in ice-cold 0.1 M phosphate buffer, pH 7.4, and the fumarase activity was determined by a modification of the spectrophotometric method of RACKER[7]. The final volume was 2.0 ml including 1.0 ml of 0.1 M sodium L-malate, pH 7.4, 0.95 ml of 0.1 M phosphate buffer, pH 7.4, and 0. 05 ml of brain homogenate to start the reaction. Incubation was carried out at 37 °C for 10 min and the reaction was stopped by the addition of 2.0 ml of 0.5 M $HClO_4$. A control was prepared for each sample by the addition of $HClO_4$ and homogenate to the buffered substrate at time zero. Spectrophotometric determinations in the supernatants were made at 240 nm in a Shimadzu QV-50 spectrophotometer equipped with cells of 10 mm light path. The enzymatic activity follows a zero order kinetics and it is proportional to concentrations of the homogenate up to 0.25 ml. One unit of enzymatic activity is equivalent to a change in optical density of 0.001 in 10 min at 37 °C. Total proteins in the homogenates

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