Blood and Brain Lactic Acid Levels in Morphinized Rats

The narcotic effect of morphine is accompanied by many metabolic disturbances, some of which have received little attention. In the present study we report changes in lactic acid levels in blood and in cerebral cortex of rats following a single dose of morphine, in chronic morphinized and in abstinent animals.

Materials and methods. Male Wistar rats weighing from 200 to 250 g were used in these experiments. Morphine chlorhydrate (Chaix et Du Marais) was injected i.m. in doses ranging from 10 to 50 mg/kg. Blood samples were obtained by section of the jugular vein. Lactic acid levels of blood and of cerebral cortex tissue were determined by the method of BARKER and SUMMERSON[1].

Since morphine treatment leads to either the development of tolerance or even to a reversal of action[2], we thought it necessary to examine not only the effects of a single dose, but also the changes in these effects during a period of chronic treatment followed by withdrawal. To avoid variations due to circadian rhythms, all animals were injected and bled at 09.00 h following a 9-hour fast.

Results. Administration of a single dose of morphine (20 mg/kg) in rats induced a 40% increase in blood lactic acid concentration. The Figure a) represents the results obtained in a typical experiment. Moreover, in a group of 10 rats, provided with intra-aortic cannulas so that each animal served as its own control and blood was sampled directly at the level of the heart, we have injected the same dose of morphine and obtained comparable results. In cannulated rats the normal value was 14.7 $\pm$ 1.4 mg/100 ml of blood, which rose to 25.6 $\pm$ 3.2 mg/100 ml 1 h following the administration of the drug (20 mg/kg). Since no significant changes in blood pyruvic acid concentration were found, the L/P ratio was enhanced in morphine-treated rats. The effect of the first dose was temporary; 6 h after the injection, the blood concentration of lactic acid returned to normal.

In cerebral cortex tissue (Figure b) the data show an increase in the concentration of lactic acid following the injection of morphine. The normal level remained stable at 59.5 $\pm$ 1.5 mg/100 g fresh weight; it rose slightly but significantly ($p < 0.001$) to 77.5 $\pm$ 1.5 mg/100 g fresh weight 1 h following the injection of a single dose of morphine (20 mg/kg) and to 94.8 $\pm$ 2.1 mg/100 g fresh weight after a dose of 50 mg/kg.

In chronically morphinized rats, doses were increased from 20 to 50 mg/kg over 3 weeks. Tolerance occured in about 1 week. It is interesting to note that after tolerance to morphine developed, blood and brain lactic acid levels were above normal when samples were taken 24 h after the last injection.

Finally, after withdrawal, the abnormally high concentrations found in the blood and brain, as well as the tolerance towards the immediate effect of the drug, persisted for at least 1 to 2 weeks.

Discussion. Results show that, under our experimental conditions, administration of morphine induced a significant increase in the concentration of lactic acid in blood and in cerebral cortex of the rat without increasing that of pyruvic acid. The increase in brain lactate after morphine has been previously described in the rat[3]. Tolerance developed quickly towards these effects and chronically treated rats had abnormally high blood and brain levels of lactic acid for a relatively long time after withdrawal.

The accumulation of lactate might result from excessive cellular production, from a deficiency of hepatic metabolism or from a decrease in tissue utilization. During the period of the first few injections, lactate accumulation would be expected if some of the properties of the drug

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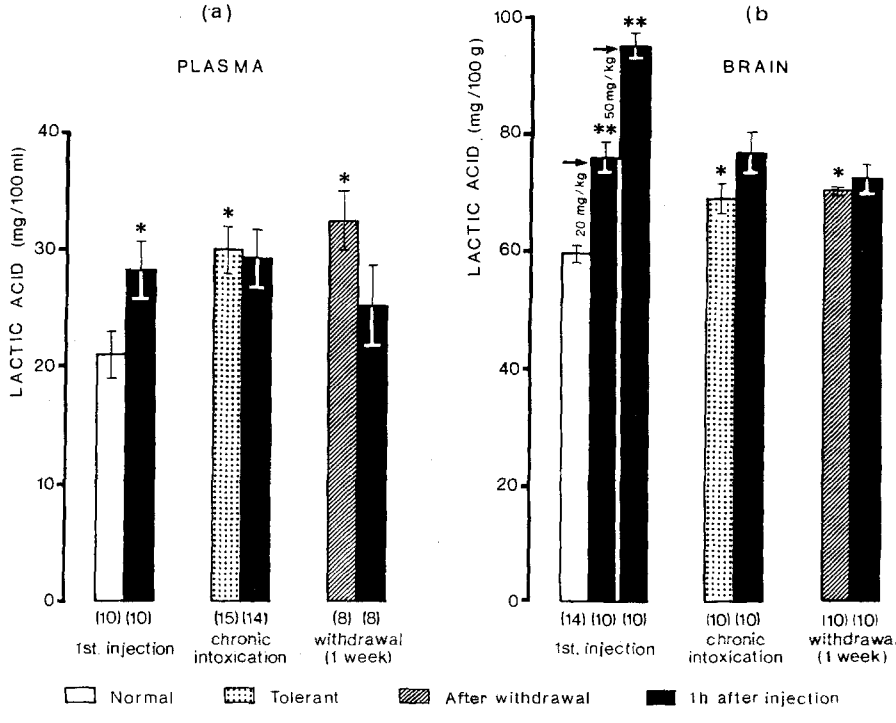

Changes in plasma (a) and in brain (b) lactic acid levels induced by acute and chronic morphine treatment and by withdrawal from morphine in rats. In (a) the dose of morphine is 20 mg/kg. The number of animals is in parentheses. Values are plotted as mean $\pm$ S.E. p is given by Student's t-test. $*p < 0.01$ and $**p < 0.001$ vs normal.

are taken into consideration: morphine is known to have striking depressive effects on the blood circulation and on respiration, resulting in a certain degree of anoxia.

In rats with intra-aortic cannulas, we have noted that morphine leads to a 30% decrease in blood O_2 concentration and to a simultaneous increase of 20% in the CO_2 concentration. Moreover, some tissues incubated in vitro show an enhanced uptake of glucose without increased lactate production when morphine is added to the medium[4,5], very probably because there is no deficiency of oxygen in this medium. However, it is unlikely that the in vivo lactate excess results exclusively from cellular hypoxia. When the depressive effects of the drug on respiration are inhibited by nalorphine, lactate accumulation persists, but is reduced. A study on rats[6] shows nalorphine alone has effects as marked as those of morphine; but in the rats receiving both drugs simultaneously, the antagonism of nalorphine towards the respiratory depressive effect of morphine is evident. Under our experimental conditions, the lactate accumulation was only partly reduced. It is necessary to search for other causes of the high plasma lactate. It is possible that an increased secretion of adrenaline due to morphine administration is involved. Many studies on morphine-induced hyperglycemia have implicated this hypersecretion. We have conducted some additional experiments at the histochemical level[7] and found that morphine causes a brief but marked depletion of the granules of the adrenal medulla. Moreover preliminary experiments on rabbits show that the simultaneous administration of β-blockers (butoxamine, 25 mg/kg) completely inhibits the rise in plasma lactic acid level normally induced by morphine. In such a case, the increase of plasma lactate can reasonably be attributed to the stimulation of the sympathetic nervous system.

Although the effect of the first morphine doses can easily be explained, the situation in chronically treated rats is less clear. In these animals, development of a tolerance towards the immediate effect of the drug occured which corresponded to a gradual decrease in the activity in the adrenal medulla. Successive injections of morphine did not cause the depletion of pheochromic granules found with the first injections. In chronically intoxicated and in abstinent animals, plasma lactic acid concentration remained abnormally high, even 1 week after withdrawal. The adrenal medulla remained in an inhibited state but the blood sugar and the oxygen consumption were normal. Thus, a relationship between the high concentration of blood lactic acid and the secretory activity of the adrenal medulla was not seen, perhaps because the regulation of cellular metabolism is profoundly modified. For example, in vivo glycolysis of various organs of chronic morphine-treated animals was found to be significantly increased[8].

The increase of lactate production seems to be a symptom of a deep and lasting disturbance in cellular metabolism induced by morphine intoxication. Whatever the origin, the lactate excess is interesting to consider in cerebral tissue in which the biochemical composition is generally very stable. As a result of the lactate excess, disorders occur in the central nervous system, and these disorders might be related to the typical phenomena of tolerance and physical dependence.

Résumé. Chez le rat, une première dose de morphine (20 mg/kg) entraîne une hausse du taux de l'acide lactique du sang et du cortex cérébral. Si les injections sont répétées la tolérance se développe vis-à-vis de cet effet immédiat et de plus, les animaux chroniquement traités possèdent un taux de base d'acide lactique anormalement élevé. Cette anomalie persiste au moins une semaine après sevrage.

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Glycogen Metabolism and the Effect of Nitroglycerin on the Glycogen Metabolism in the Normal and Ischemic Canine Myocardium

Ischemia or anoxia of the heart causes acceleration of glycogenolysis and accumulation of the intermediates of glycolysis in the myocardium[1-6]. These studies were conducted on the assumption that the heart muscle must be homogeneous from the view point of metabolism. Jedeikin[7], however, reported that the level of glycogen and activity of glycogen phosphorylase in the endocardial layers were higher than those in the epicardial layers. This suggests that there are some differences in metabolism between the endo- and epicardial layers, especially when the heart is ischemic. The present study was conducted in an attempt to examine this possibility, and to study the effect of nitroglycerin on the myocardial metabolism.

Mongrel dogs anesthetized with pentobarbital were used. Under artificial respiration, one of the small branches of the coronary artery was ligated 5 min after i.v. injection of saline or nitroglycerin. Just before or after ligation of the small branch (1.5, 3, 7 and 30 min after the ligation), the heart was removed, and immediately frozen with freezing clamps. The endo- and epicardial portions of the left ventricle, which had been nourished by the small branch, were taken for determination of glycogen, glucose-6-phosphate (G6P), lactate, adenosinetriphosphate (ATP) and phosphocreatine (PCr), and activities of phosphorylase *a* and *b*. Results are shown in the Figure and the Table.

Results. 1. *Control (saline-injected) dogs.* In non-ischemic hearts, the levels of glycogen, G6P and lactate, and

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