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EFFECTS OF MORPHINE ON THE GLUCONEOGENIC ENZYMES FROM RAT LIVER *IN VIVO*

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Abstract—The acute and chronic effects of morphine on the four obligatory gluconeogenic enzymes in rat liver have been studied *in vivo*. Glucose-6-phosphatase and phosphoenolpyruvate carboxykinase activities are increased in rats which have received a single intraperitoneal injection of morphine 15 hr prior to sacrifice. No change is observed in any of the four hepatic enzymes when the animals are killed 5 hr after the injection. The activities of glucose-6-phosphatase, pyruvate carboxylase and phosphoenolpyruvate carboxykinase from chronically morphinized rats do not differ from those of normal control, but that of fructose-1,6-diphosphatase is increased.

PREVIOUS work by Walsh *et al.* [1–3] has shown that morphine acts as a pseudohormone, participating in the control of metabolism. Since the chemical structure of morphine bears similarities to both adrenaline and steroids,[4,5] it is likely that morphine may exert similar effects on cellular metabolism as the above hormones. It is well established that adrenaline enhances the overall pathway of gluconeogenesis in adult rat liver,[6] and that the steroid, hydrocortisone, elevates the activities of the four obligatory gluconeogenic enzymes.[7–9] Thus it is of interest to examine the effect of morphine on the gluconeogenic enzymes in both acute and chronically treated animals.

MATERIALS AND METHODS

Chemicals. G6P was purchased from Boehringer Mannheim Corp. F-1, 6-diP, PGI, G6PD, β-mercaptoethanol, NADP, NADH, PEP, IDP, ATP, Acetyl CoA, and MDH were purchased from Sigma Chemical Co. (St. Louis, Mo.). $MgSO_4$, EDTA, sodium pyruvate and Tris (hydroxymethyl)-aminomethane were purchased from E. Merck Ag. (Darmstadt). $NaH^{14}CO_3$(CFA-3) was obtained from Amersham (England).

Animals. Virgin female albino rats of 140–180 g at the time of decapitation were used. Chronically morphinized animals had received daily intraperitoneal (i.p.) injection of morphine (30 mg/kg body wt) for 6 weeks while normal control animals received only saline injection instead of morphine. Animals receiving a single dosage of morphine (30 mg/kg body wt) intraperitoneally were killed 5 or 15 hr after the injection according to the condition stated in the experiment. All animals had unrestricted access to food and water.

Preparation of liver extract. For assays of glucose-6-phosphatase, fructose-1,6-diphosphatase, phosphoenolpyruvate carboxykinase, livers were homogenized in 4 vol. of ice-cold 0·25 M sucrose solution and the supernatant fluid was obtained by centrifuging the homogenate for 1 hr at 54,000 *g* at 4° in a Spinco ultracentrifuge (rotar

No. 40.2). The whole homogenate was used for glucose-6-phosphatase assay while the supernatant fluid was used for fructose-1,6-diphosphatase and phosphoenolpyruvate carboxykinase assays.

For assay of pyruvate carboxylase, liver homogenate was prepared at room temperature in 3 vol. of 400 mM Tris buffer containing 0·1 mM EDTA and 0·5 mM ATP adjusted to pH 7·8. Homogenate was freeze-dried and the solid was resuspended in the same volume of water. Suspension was then centrifuged at 25,000 *g* for 2 hr at 10° and the supernatant was diluted in a 1:10 ratio for enzyme assay.

Enzyme assays. Glucose-6-phosphatase was assayed by the method described by Yeung *et al.*[10] Fructose-1,6-diphosphatase was assayed by the spectrophotometric method of Underwood and Newsholme.[11] Pyruvate carboxylase was assayed by the method of Utter and Keech[12] with modification as described by Yeung *et al.*[10] Phosphoenolpyruvate carboxykinase was assayed by the method of Chang and Lane.[13]

Protein determination. Protein concentration was determined by the method of Lowry *et al.*[14]

RESULTS

Acute and chronic effects of morphine administration on glucose-6-phosphatase. Acute treatment of rats with morphine 5 hr prior to sacrifice has no effect on glucose-6-phosphatase activity. However as the period of exposure to the drug is prolonged to 15 hr, there is a significant increase in activity (Table 1), indicating that this enzyme is induced gradually. The activity from chronically morphinized rats shows no significant difference from those of normal control.

TABLE 1. THE ACUTE AND CHRONIC EFFECTS OF MORPHINE ON HEPATIC GLUCOSE-6-PHOSPHATASE

State of rats	No. of rats	G6Pase activity (mean ± S.E.M.)	Difference
N	4	1·55 ± 0·08	0·02 ± 0·12
M (5 hr)	4	1·53 ± 0·09	(N.S.)
N	7	1·57 ± 0·06	0·24 ± 0·08
M (15 hr)	7	1·81 ± 0·06	($P \approx 0.01$)
N	6	1·40 ± 0·08	0·09 ± 0·11
Cm	8	1·31 ± 0·08	(N.S.)

Enzyme activities are expressed in μmoles Pi formed/mg protein per hr.
N = Normal control; M = morphine-treated (acute effect); Cm = chronically morphinized.

Acute and chronic effects of morphine administration on fructose-1,6-diphosphatase. Table 2 demonstrates that treatment of rats with morphine 5 and 15 hr prior to sacrifice produces no change in fructose-1,6-diphosphatase activity. However, after chronic morphinization, there is an elevation in activity. The mean difference (+1·02) between the normal control and that from chronically morphinized rats being significant.

Acute and chronic effects of morphine administration on pyruvate carboxylase. As shown in Table 3, there is no significant change in pyruvate carboxylase activity following either acute or chronic morphinization.

Acute and chronic effects of morphine administration on phosphoenolpyruvate carboxykinase. Table 4 shows that the activity of phosphoenolpyruvate carboxykinase

TABLE 2. THE ACUTE AND CHRONIC EFFECTS OF MORPHINE ON HEPATIC FRUCTOSE-1,6-DIPHOSPHATASE

State of rats	No. of rats	FDPase activity (mean ± S.E.M.)	Difference
N	4	3·69 ± 0·13	0·25 ± 0·42
M (5 hr)	4	3·94 ± 0·09	(N.S.)
N	4	3·97 ± 0·20	0·17 ± 0·22
M (15 hr)	6	3·80 ± 0·13	(N.S.)
N	6	3·57 ± 0·12	1·02 ± 0·28
Cm	10	4·59 ± 0·21	($P < 0·01$)

Enzyme activities are expressed in μmoles substrate utilized/mg protein per hr.
N = Normal control; M = morphine-treated (acute effect); Cm = chronically morphinized.

is dramatically elevated in rats which have been given a single injection of morphine 15 hr prior to sacrifice. However in an experiment not reported here, no significant difference in enzyme activity was demonstrated in rats which had received a single injection of morphine 5 hr prior to sacrifice.

In chronically treated animals, the activity of phosphoenolpyruvate carboxykinase is not significantly different from that of normal rats.

The activities of the enzyme from the two groups of normal animals are different as they are from rats of different ages.

DISCUSSION

Of the four gluconeogenic enzymes examined, only glucose-6-phosphatase and phosphoenolpyruvate carboxykinase show significant increase 15 hr after a single dose of morphine (Tables 1 and 4) whereas no change is detected on the activities of fructose-1,6-diphosphatase and pyruvate carboxylase (Tables 2 and 3). It is unlikely that the changes observed with glucose-6-phosphatase and phosphoenolpyruvate carboxykinase are due to activation of the two enzymes as no significant change in activity is observed on any of the four enzymes in liver from rats which were sacrificed 5 hr after morphinization. Thus the effect observed is more likely to be a result of enzyme induction.

It is well established that phosphoenolpyruvate carboxykinase is the rate-limiting enzyme of gluconeogenesis. Of the four gluconeogenic enzymes, the formation during development of glucose-6-phosphatase and fructose-1,6-diphosphatase are about the slowest. Ballard and Hanson[15] have reported that in developing rat liver, there is

TABLE 3. THE ACUTE AND CHRONIC EFFECTS OF MORPHINE ON HEPATIC PYRUVATE CARBOXYLASE

State of rats	No. of rats	Pyruvate carboxylase activity (mean ± S.E.M.)	Difference
N	6	6·93 ± 0·31	0·35 ± 0·40
M (15 hr)	4	6·58 ± 0·20	(N.S.)
N	4	6·05 ± 0·06	0·15 ± 0·08
Cm	4	6·20 ± 0·06	(N.S.)

Enzyme activities are expressed in μmoles malate formed/mg protein per hr.
N = Normal control; M = morphine-treated (acute effect); Cm = chronically morphinized.

TABLE 4. THE ACUTE AND CHRONIC EFFECTS OF MORPHINE ON HEPATIC PHOSPHOENOL-PYRUVATE CARBOXYKINASE

State of rats	No. of rats	PEP carboxykinase activity (mean ± S.E.M.)	Difference
N	6	2·14 ± 0·14	1·43 ± 0·15
M (15 hr)	7	3·57 ± 0·24	(P < 0·001)
N	5	1·37 ± 0·18	0·01 ± 0·19
Cm	7	1·36 ± 0·11	(N.S.)

Enzyme activities are expressed in μmoles malate formed/mg protein per hr.
N = Normal control; M = morphine-treated (acute effect); Cm = chronically morphinized.

25-fold increase in the activity of phosphoenolpyruvate carboxykinase comparing with only 2–3·5-fold increase in the activities of glucose-6-phosphatase and fructose-1,6-diphosphatase over the same period after delivery. The present results also indicate that acute effect is mainly on phosphoenolpyruvate carboxykinase, being a 70 per cent increase while that of glucose-6-phosphatase is only about 15 per cent above the control.

Exton and Park[6] have studied the concentration of various metabolic intermediates in perfused rat liver in the presence of adrenaline. They have found that the level of malate remains unchanged while that of phosphoenolpyruvate is considerably elevated. They therefore concluded that phosphenolpyruvate carboxykinase is greatly enhanced whereas the activity of pyruvate carboxylase is unchanged. They have further shown that a similar pattern of changes can be observed after administration of cyclic 3′, 5′-AMP. Yeung and Oliver[16] have demonstrated that in foetal rats, both adrenaline and cyclic 3′,5′-AMP cause significant induction of phosphoenolpyruvate carboxykinase. Sutherland and Robinson[17] have demonstrated that the level of cyclic 3′,5′-AMP in rat liver is greatly enhanced following administration of adrenaline. Since morphine bears structural similarity to adrenaline, it may also act similarly in increasing the level of cyclic 3′,5′-AMP. A single dose of morphine has in fact been shown to bring about an increase in the level of ATP in rat brain,[18] and this may indirectly increase the level of cyclic 3′,5′-AMP which will subsequently bring about enzyme induction. However, it should be pointed out that there may be another mechanism whereby morphine exerts its inductive effect. This is related to the structural resemblance between morphine and glucocorticoids. As it has been demonstrated in various laboratories that glucocorticoids stimulate the activities of the gluconeogenic enzymes in adult rat liver,[7-9] it is thus possible that morphine may have a hydrocortisone-like effect.

With development of tolerance to morphine after chronic treatment, the animal has set up a new hormonal balance for regulation of metabolism. Hence those changes observed by acute administration are either absent or less marked in rats chronically treated with morphine. Miller *et al.*[19] have found that the pattern of changes in metabolite concentration observed in rat brains after morphine injection disappears in chronically morphinized rats. Furthermore, Dodge and Takemori[18] have found that the acute increase in ATP concentration after morphine injection vanishes in chronically morphinized rats. In the present study, we have also observed the disappearance of the inductive effects of glucose-6-phosphatase and phosphoenolpyruvate carboxykinase after chronic treatment. However, the activity of

fructose-1,6-diphosphatase from chronically morphinized animals is increased. One possible explanation appears to be that the concentration of morphine required to bring about induction of this enzyme is exceptionally high. In the case of acute administration, the level of morphine accumulating after a single injection is insufficient to affect the synthetic machinery of the enzyme. On chronic morphinization, the morphine gradually accumulates in the liver and when the level reaches a threshold, induction of the enzyme occurs. Another possible explanation may be that the accumulated morphine protects fructose-1,6-diphosphatase from degradation and hence the activity of fructose-1,6-diphosphatase after chronic treatment is increased.

It can be seen from Table 3 that there is no change in the activity of pyruvate carboxylase in either acute or chronically treated animals. Although it has been reported that the activity of the enzyme is elevated following glucocorticoid treatment,[7] Exton and Park [6] have failed to demonstrate an inductive effect of adrenaline on the pyruvate carboxylase system from metabolite studies. No attempt has been made to assess the relative potency of adrenaline and hydrocortisone in the induction of enzyme systems. However, it is true that the dosage of adrenaline required to bring about enzyme induction is much lower than that of hydrocortisone. Hence, it may be expected that morphine is ineffective in the pyruvate carboxylase system.

In conclusion, morphine has a definite effect on the induction of the gluconeogenic pathway and this lends further support to the previous proposal that morphine acts as a pseudohormone, participating in the control of metabolism.

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