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MORPHINE INHIBITION OF THE INSULIN-INDUCIBLE FORM OF HEPATIC TYROSINE AMINOTRANSFERASE

SUMANTRA DAS, TAPAN K. CHATTERJEE and JAGAT J. GHOSH*
Department of Biochemistry, University College of Science, Calcutta 700 019, India

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Abstract—Morphine treatment in normal intact rats caused a dose-dependent increase in hepatic tyrosine aminotransferase (TAT) activity, as demonstrable up to 2 hr of exposure to the opioid alkaloid. However, such increase in TAT activity was invariably preceded by a prior decline in the enzyme level, as observed after 15 min of morphine treatment. Such an initial decline in activity was not demonstrable in diabetic animals. Further studies indicate that morphine inhibited the insulin-induced increase in TAT activity, a phenomenon which could be reversed by the opioid antagonist naloxone. The results suggest an opioid control mechanism in the regulation of the insulin-inducible form of TAT and indicate the possibilities of a trophic role of endogenous opiates in gluconeogenesis.

The mechanism of action of morphine in the regulation of hepatic tyrosine aminotransferase (EC 2.6.1.5) activity is not well documented. Studies on the effect of morphine on isoenzymes of tyrosine aminotransferase (TAT), from 6 hr after initial morphine exposure up to 72 hr, indicate a hydrocortisone-like property of the opioid alkaloid in elevating TAT activity, in addition to its ability to increase cAMP and cortisone, two factors also responsible for the elevation of the enzyme activity [1]. Observations in the present study indicate that such morphine-induced elevation of TAT activity in intact animals is preceded by an initial sharp fall in the enzyme level when exposed for a shorter period. Further studies suggest that such a fall in TAT activity may be due to inhibition of the insulin-inducible form of the enzyme. However, morphine is without effect on glucocorticoid-induced TAT activity. Control of the insulin-inducible form of TAT is not well understood [2]. Present observations demonstrate a possible involvement of opioids in such a control. Furthermore, since TAT is an important gluconeogenic enzyme, observations suggest a new role for opiates in gluconeogenesis.

MATERIALS AND METHODS

Preparation of animals. Male albino rats [125 ± 10 (S.D.) g, Charles Foster strain] were maintained on a 12-hr light–dark cycle. Since TAT activity exhibits marked dietary fluctuations [3], the animals were fed with laboratory stock diet containing 20% protein between 8:00 a.m. and 2:00 p.m., and experiments were conducted 4 hr later. Adrenalectomy was performed using the dorsal approach under light ether anesthesia. Subsequently, the animals were maintained on 0.9% saline and used 1 week after surgery. Rats were made diabetic by treatment with streptozotocine (Upjohn) as reported previously [4]. A 4% solution of streptozotocine was prepared in 0.1 M citrate buffer, pH 4.0, and injected intraperitoneally at a dose of 80 mg/kg within 2 min of preparation. After 7 days, animals were tested for the development of hyperglycemia by estimating the blood glucose level in tail vein blood by the glucose oxidase–peroxidase method of Bergmeyer and Bernt [5]. Animals were used subsequently.

Drug preparation. Morphine sulfate (Dey's Medical), naloxone (Endo), dexamethasone (Wyeth) and insulin-regular (Boots) were used during the study. Drugs were diluted to appropriate concentrations in 0.1 ml of normal saline. Morphine was injected subcutaneously while all other drugs were administered via the intraperitoneal route.

Enzyme assay. Rats were killed by decapitation. Liver was perfused *in situ* via hepatic artery with 10 ml of cold isotonic saline. The blanched liver was then carefully dissected out and kept under cold conditions. TAT activity was determined by the method of Chan and Cohen [6] with slight modifications as reported previously from this laboratory [7]. Liver was homogenized in 3 vol. of 0.1 M KH_2PO_4 buffer, pH 7.6, containing 0.4 mM pyridoxal-5′-phosphate and 1 mM dithiothreitol, using ten strokes of a Potter–Elvehjem homogenizer. Homogenate was centrifuged at 105,000 *g* for 90 min at 4°, and the supernatant fraction was used directly for enzyme assay. The reaction mixture contained 12 μM tyrosine, 60 μM α-ketoglutarate, 1.2 μM pyridoxal phosphate, 3 μM diethyl dithiocarbamate and 300 μM potassium phosphate in a total volume of 3.0 ml at pH 7.6. *p*-Hydroxy phenyl pyruvate formed was estimated colorimetrically by using Brigg's reaction. Protein was measured by the biuret method [8].

RESULTS

The effect of morphine on hepatic tyrosine aminotransferase activity of intact animals was determined

* Send all correspondence to: Professor J. J. Ghosh, Department of Biochemistry, University College of Science, 35, Ballygunge Circular Road, Calcutta 700 019, India.

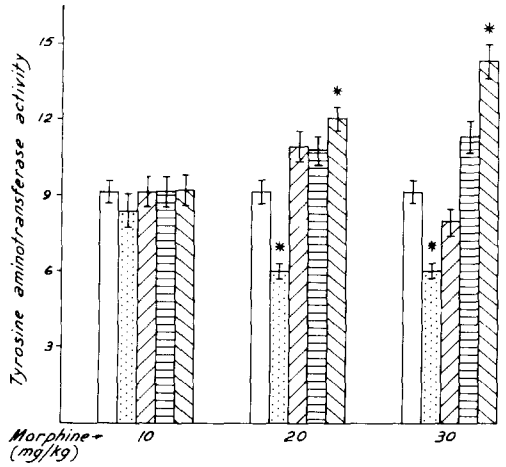

Fig. 1. Effect of morphine on hepatic aminotransferase (TAT) activity of intact rats. Morphine (s.c.) was injected at a dose of 10, 20 or 30 mg/kg body weight. For each dose, TAT activity was determined at 15, 30, 60 and 120 min of morphine treatment. Details of the assay procedure are provided in Materials and Methods. TAT activity is expressed as μmoles of *p*-hydroxy phenyl pyruvate formed per mg of protein per hr of incubation. Columns and vertical bars are means and S.E. for four separate determinations. Key: zero min/control (□), 15 min (⬚), 30 min (▨), 60 min (▤) and 120 min (▩); (*) significantly different from control ($P < 0.005$).

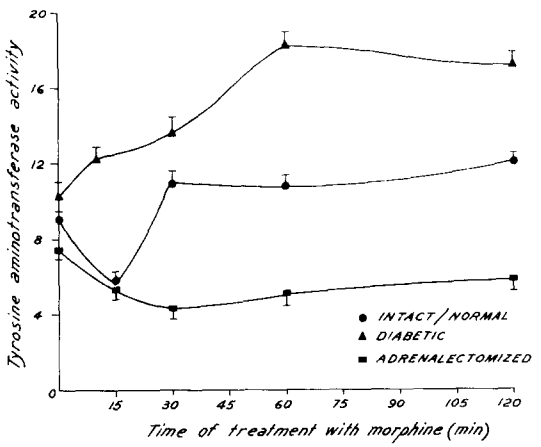

Fig. 2. Effect of morphine on hepatic aminotransferase activity in normal, diabetic, and adrenalectomized rats. Details of the assay procedure are provided in Materials and Methods. Activity is expressed as μmoles of *p*-hydroxy phenyl pyruvate formed per mg of protein per hr of incubation. Ordinate shows activity of the enzyme during different times of treatment with morphine (20 mg/kg, s.c.) as compared to zero time control value. Each point is the mean of four different experiments. Bars show standard error.

at 15 min, 30 min, 1 hr and 2 hr exposure to the opioid using three different doses (Fig. 1). Morphine treatment caused marked elevation of TAT activity as demonstrable at the longer treatment schedules. Moreover, such an activation exhibited dose-dependent characteristics, a higher dose of morphine having a greater effect on TAT level. However, although TAT activity showed a progressive increase with increase in the time of morphine treatment, such increase was invariably preceded by an initial decline in the enzyme activity as observed during 15 min of treatment.

The effects of morphine (20 mg/kg) on TAT activity of intact, adrenalectomized, and diabetic rats are shown in Fig. 2. In contrast to intact animals, morphine treatment in adrenalectomized rats caused a decrease in TAT activity which did not increase significantly with increase in treatment time. In diabetic animals, however, morphine caused marked elevation of TAT activity from control value which

Table 1. Effect of morphine on insulin- and glucocorticoid-induced elevation of hepatic tyrosine aminotransferase (TAT) activity: action of naloxone

Treatment*	TAT activity†
Control (saline)	9.13 ± 0.62
Insulin (2 units)	30.17 ± 2.15‡
Insulin (2 units) + morphine (20 mg)	11.15 ± 1.10§
Insulin (2 units) + morphine (10 mg)	19.69 ± 2.13
Insulin (2 units) + morphine (20 mg) + naloxone (5 mg)	12.32 ± 2.07§
Insulin (2 units) + morphine (20 mg) + naloxone (20 mg)	33.56 ± 2.91
Insulin (2 units) + naloxone (20 mg)	28.66 ± 1.87
Dexamethasone (1 mg)	27.37 ± 2.12‡
Dexamethasone (1 mg) + morphine (20 mg)	29.41 ± 1.97
Dexamethasone (1 mg) + morphine (10 mg)	26.22 ± 2.35
Naloxone (20 mg)	11.56 ± 0.97

* All drug treatments were for 1 hr. Figures within parentheses indicate the dose per kg body weight used. Morphine was injected subcutaneously while all other drugs were administered via the intraperitoneal route.

† TAT activity is expressed as μmoles of *p*-hydroxy phenyl pyruvate formed per mg of protein per hr of incubation. Estimation of enzyme activity is described in Materials and Methods. Results are mean ± S.E.M. for four separate determinations.

‡ Significantly different ($P < 0.001$) from control (saline).

§ Significantly different ($P < 0.001$) from animals treated with insulin.

increased with increase in the time of exposure to the opioid. Moreover, the degree of activation of TAT in diabetic animals was much greater than in normal animals. However, unlike normal animals, such activation of TAT activity in diabetic rats was not preceded by an initial decrease in the enzyme level at the shorter treatment schedules.

The effects of morphine on insulin and glucocorticoid induction of TAT activity are presented in Table 1. Both insulin and dexamethasone caused about a 3-fold increase in the enzyme activity from control levels. However, morphine at a dose of 20 mg/kg completely inhibited the insulin-stimulated elevation of TAT activity while a 10 mg/kg dose could only partially inhibit the said elevation. Neither of the doses of morphine had any significant effect on the dexamethasone-induced increase in the activity of TAT. The complete inhibition of the insulin-inducible form of the enzyme by morphine could be blocked by the simultaneous administration of 20 mg/kg naloxone, while a 5 mg/kg dose of the antagonist had no effect. Naloxone, by itself, had no effect on the TAT activity of both control as well as insulin-treated animals.

DISCUSSION

Wong *et al.* [1] reported that a single morphine injection increases forms II, III and IV of tyrosine aminotransferase in rats at 6 hr elevations which last up to 24 hr following exposure to the opioid. The authors concluded that such elevations may be due to the combined action of increased cAMP and corticosterone levels. In addition, these workers also observed a delayed stimulatory effect of morphine on TAT activity at 48 hr which reached a maximum at 72 hr. A probable implication of the pseudohormone-like property of morphine was suggested for this late effect.

Our studies (Fig. 1), using a much shorter duration of morphine treatment, indicate that such activation of TAT was invariably preceded by an initial decrease in the enzyme level. Further observations (Fig. 2) demonstrated that, in adrenalectomized animals, morphine consistently depressed hepatic TAT level from control values. If glucocorticoids were the major contributors in morphine-induced elevation of TAT activity, it would be expected that, in adrenalectomized rats, morphine would not only fail to increase TAT activity but would produce no significant alteration in the enzyme activity from the control level. On the contrary, morphine treatment in adrenalectomized animals caused a decrease in TAT activity, thereby suggesting that other factors might be involved in the regulation of hepatic TAT activity by opioids. Two main possibilities emerge from these observations. One is that morphine, in addition to corticosterone release, may also stimulate the secretion of another TAT modifier which has an opposing, retarding influence on the enzyme activity. Alternatively, the opioid treatment may cause inhibition of a particular isozymic form of TAT which is inducible by a factor other than glucocorticoid. Either of these possibilities may explain the reason for the initial decline in the level of TAT (Fig. 1) during a shorter exposure of morphine. However, the second proposition seems more likely as there is no TAT modifier known to exert an inhibitory influence on the activity of the enzyme.

Reports suggest that the isozymic forms of TAT are regulated mainly by insulin in addition to corticosteroids [9]. Observations on the effect of morphine treatment in streptozotocine-treated diabetic rats (Fig. 2) showed marked elevations of TAT activity, the magnitudes of which were considerably higher than those observed during corresponding treatments in normal animals. Moreover, unlike in normal rats, such elevation of TAT in diabetic rats was not preceded by a fall in the enzyme level. Further work (Table 1) carried out demonstrated that morphine (20 mg/kg) could inhibit insulin-induced elevation of TAT activity but had no effect on the activity of the glucocorticoid-inducible form of the enzyme. Although a lower dose of morphine (10 mg/kg) could not completely block the elevation of TAT activity by insulin like the higher dose, it did demonstrate partial inhibition of the same. Such inhibition of the insulin-inducible form of TAT by the opiate agonist morphine appears to be an opiate-receptor mediated action since the opiate antagonist naloxone, at a dose of 20 mg/kg, completely reversed the observed inhibition. A smaller dose of naloxone (5 mg/kg), effective to attenuate analgesic response of morphine, was, however, without effect in antagonizing the said inhibition.

It appears that, perhaps at the earlier treatment period, morphine inhibited the basal insulin-induced TAT level which accounted for the initial decline in the enzyme activity during treatment in intact animals. Possibly at the later stages this was masked by the generation of corticosterone-induced TAT activity. A greater increase of the enzyme activity in diabetic rats as compared to non-diabetic animals may thus be due to an unopposed action of corticosterone on TAT level.

The regulation of the insulin-inducible form of TAT is not well understood. Recent reports indicate that lectins, a group of plant proteins, have a possible influence on the activity of this form of the enzyme [2]. Results of the present study demonstrate an opioid-dependent mechanism in the control of the insulin-inducible form of TAT. Recent evidences [10, 11] that the endogenous opiate β-endorphin is released from the pituitary into the blood in response to acute stress suggests a trophic role of the circulating β-endorphin in some target tissue or tissues since other anterior pituitary polypeptides are trophic hormones for single or multiple tissues of the body. Moreover, the presence of β-endorphin and related opioid peptides in various tissues outside the CNS, namely pancreas, adrenal, placenta, stomach, kidney, gut, eye, and pineal gland [12–14], has been reported. Furthermore, alteration of the secretory pattern of insulin, glucagon and somatostatin in the pancreas in the presence of glucose [15] and induction of ornithine decarboxylase in the kidney [16] are a few trophic actions of β-endorphin thus far reported. Our observations with the exogenous opioid, morphine, suggest the possibility of another such trophic behavior of opiates and give indications of a new role for endogenous opiates in gluconeogenesis.

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