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Effects of chronically administered morphine on rat liver tyrosine aminotransferase

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Morphine, as well as other opiates, has a dual action on corticosteroid secretion. Single doses of narcotic generally stimulate adrenal cortical responses whereas prolonged administration produces depression of the basal levels of corticosteroid secretion. These effects have been clearly ascertained in the rat by measuring the classical parameters of adrenal activity, namely, the plasma corticosterone and the adrenal ascorbic acid levels [1, 2]. Paroli and Melchiorri [3] have also found an initial increase in corticosteroid urinary excretion in rats treated with morphine, followed by a decreased steroid excretion in a successive stage of the narcotic administration. Taken together, the data indicate a tendency toward the development of a tolerance to the hormonal effect of morphine, like that which is obtained for some of its other pharmacological effects, above all for analgesia.

In previous papers we showed that an acute administration of morphine, as well as of many other drugs acting on the CNS [4, 5], results in a rise of liver tyrosine aminotransferase (L-tyrosine: 2-oxoglutarate aminotransferase, EC 2.6.1.5). Since the levels of this enzyme are regulated either by its substrate or by circulating corticosteroids [6], we tried to ascertain whether continual morphine administration normalizes the liver enzyme levels, in the rat.

Female albino rats of the Sprague Dawley strain with an initial weight of 150 ± 20 g were used. They were fed a standard diet and water *ad lib*. The environmental conditions were standardized (22 ± 2 : 12 hr artificial lighting per day). The rats were randomized into five groups of 10 animals each. The first group of rats received 20 mg/kg morphine (HCl, C. Erba), subcutaneously, at 8.00 hrs; 6 hr after the administration, the rats were killed. The second, third, fourth and fifth groups of rats were treated with morphine, 20 mg/kg/s.c., at 8.00 hrs daily for 7, 11, 14 and 18 days, respectively; the rats were killed 6 hr after the last administration. Controls animals received saline only.

Tyrosine aminotransferase (TAT) activity was determined in whole liver homogenate, in the presence of pyridoxal-5-phosphate, by the method described by Kenney [7] and expressed as μmoles of *p*-hydroxyphenyl-pyruvate/100 mg/hr. P values were calculated by the Student's *t*-test.

Morphine, acutely administered, increased the levels of tyrosine aminotransferase (TAT) in liver (Table 1), confirming our previous results [4]. Rats treated for 7 days with morphine still had high TAT levels, but from the 11th day the enzymatic stimulation was reduced and completely disappeared by the 14th day, when the enzyme levels were similar to those of the control groups. Normalization of tyrosine aminotransferase levels supports, therefore, the hypothesis that the continual administration of morphine might resut in a development of a tolerance to the enzymatic effect of the narcotic. Our findings agree with those of others who have described a depressed adrenal response

Table 1. Liver tyrosine aminotransferase (TAT) after acute or chronic treatment with morphine

No. of rats	Treatment	Dose (mg/kg per day)	Treatment period (days)	TAT (μmoles *p*-hydroxyphenylpyruvate/100 mg per 1 hr)*	% increase
10	Saline	—	1	13·68 ± 0·65	
10	Morphine	20	1	26·08 ± 0·57†	+ 90·6
10	Saline		7	14·17 ± 1·16	
10	Morphine	20	7	26·39 ± 1·30†	+ 86·2
10	Saline		11	16·80 ± 0·93	
10	Morphine	20	11	26·06 ± 1·87†	+ 55·1
10	Saline	—	14	13·01 ± 0·93	
10	Morphine	20	14	13·48 ± 1·87†	− 3·6
10	Saline		18	14·57 ± 1·81	
10	Morphine	20	18	14·26 ± 1·40	− 2·1

* 6 hr after last morphine administration. Results are expressed as mean ± S.E.M.
† $P < 0.05$ compared with respective controls.

in rats undergoing a 6-day treatment with the same dose of morphine [8], in contrast to the acute effect of the analgesic [2].

Increased enzyme synthesis represents a phenomenon of 'enzymatic adaptation', that is, it indicates the capacity of the animals to adapt to changing conditions. Even if, at present, we know little about the purpose of this enzymatic adaptation (also exhibited by tryptophan oxidase), we know that there are at least two independent stimuli for the adaptive response: the corticosteroid and the substrate inductions [6]. A great variety of stimuli, chemical and environmental (the latter generally associated with fear, hunger, pain and cold exposure [4]), give the signal, via the hypothalamic-pituitary system, for corticosteroid secretion thus inducing the enzyme synthesis.

The mechanism by which morphine influences corticosteroid secretion (and consequently TAT synthesis) is not clearly defined, but evidence suggests that it exerts its effect at a central site in the region of the hypothalamus [9], probably through the stimulation and inhibition of the synthesis or output of releasing factors (RFs) responsible for the control of the pituitary-adrenal system[10]. The intervention of morphine on RFs might be direct or indirect through the biogenic amines, since these transmitters are implicated in the mechanism of RFs secretion [11, 12] and evidence exists that their turnover rate is modified by acute and chronic morphine administration [13].

Morphine may also influence TAT synthesis by acting at other sites. It has been shown, for instance, with experiments *in vitro*, that morphine interferes with the corticosteroid-binding capacity of rat liver slices [14].

In any case the present results give evidence of a supplementary pharmacological tool suitable for demonstrating both the adrenal response to narcotics and the onset of a tolerance to their chronic administration.

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Modification of protein–lipid interactions in the Gunn rat by treatment of microsomal UDP-glucuronyltransferase with diethylnitrosamine*

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The activities of UDP-glucuronyltransferases (EC 2.4.1.17) in liver microsomes from homozygous Gunn rats, in contrast to normal rats, are insensitive to treatments which modify the fluidity of the lipid phase of the membrane [1, 2]. The defect in the function of UDP-glucuronyltransferases in homozygous Gunn rats thus includes abnormal regulatory properties as well as abnormal rates of catalysis. Of great potential significance for understanding of the defect in these rats is the fact that the carcinogen diethylnitrosamine (DEN) activates microsomal UDP-glucuronyltransferase (EC 2.4.1.17) [3]. Addition of DEN to microsomes from Gunn rats corrects the deficiencies in the rates of glucuronidation of *o*-aminophenol [3], *o*-aminobenzoate [3] and *p*-nitrophenol.

Since it seems as if DEN corrects the defect in the catalytic function of UDP-glucuronyltransferase from Gunn rats, DEN also might correct the abnormalities of regulatory function, that is, produce a form of the enzyme that is activated on subsequent modification of the fluidity of the membrane lipids by treatment with phospholipases and detergents. This possibility has been investigated by determining whether DEN modifies the effect of lipid–protein interactions on the properties of UDP-glucuronyltransferase in liver microsomes from homozygous Gunn rats.

Homozygous female Gunn rats were obtained from the colony maintained by Dr. M. M. Thaler, University of California Medical Center, San Francisco. Techniques for the preparation of microsomes, and partially purified phospholipase A, and assay methods have been described previously [1]. Details of conditions of treatment of microsomes with detergents and phospholipase A, and conditions for enzyme assays are given in the legends and text. Proteins were measured with the biuret method [4].

Prior treatment of microsomes from Gunn rats with DEN produces a form of UDP-glucuronyltransferase which is activated on subsequent treatment with phospholipase A (Table 1). DEN activates UDP-glucuronyltransferase in microsomes from normal as well as Gunn rats, but does not modify the activating effect of phospholipase A on enzyme from normal rats. Addition of DEN to microsomes from Gunn rats also allowed for activation of UDP-glucuronyltransferase by Triton X-100 (Table 2). The effect of Triton on activity was biphasic (Fig. 1), which is typical of that obtained with normal microsomes. This is a signifi-

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