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Effect of maternal morphine administration on fetal and neonatal rat liver tyrosine aminotransferase

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While numerous studies exist concerning the biochemical pharmacological effects following acute and chronic administration of narcotics, there is still a lack of information regarding offspring of opiate-dependent animals.

Among the variety of effects elicited by morphine, those involving amino acid and protein metabolism [1] are of particular intrest since they could influence fetal and newborn development. In a previous paper [2] we showed that acute administration of morphine in the rat results in a rise of liver tyrosine aminotransferase (L-tyrosine: 2-oxoglutarate aminotransferase, EC 2.6.1.5.), an enzyme which intervenes in the first step of tyrosine metabolism. With chronic administration of the narcotic, a tolerance develops to this enzymatic effect. On the other hand, it is well known that tyrosine aminotransferase (TAT) has, in fetal-neonatal liver, a developmental pattern which is sensitive to pharmacological manipulation [3]. We have therefore extended our investigation on TAT in order to ascertain whether changes are seen in enzyme levels of fetal and newborn rats after acute and chronic maternal administration of morphine.

Pregnant Sprague-Dawley (Nossan) rats were obtained 6 days after fertilization, caged separately and given food and water *ad lib*. The environmental conditions were standardized (22 ± 2°; 12 hr artificial lighting per day). Eight days after fertilization the rats were randomized into three groups. The first group received, subcutaneously, 20 mg/kg morphine (HCl, C. Erba), at 8.00 hr daily for 12 days. The animals of the second group were given saline subcutaneously for 11 days and then, at 8.00 hr of the 12th day, a single dose of morphine, 20 mg/kg/s.c. The rats of the third group (controls) received saline only. As a comparison, the same schedule of treatment was adopted for three groups of non-pregnant adult female rats of the same strain. Six hr after last administration all the pregnant and non-pregnant rats were killed by decapitation. The abdominal cavity was opened and the livers were removed from all the rats for TAT analysis. The fetuses were removed from pregnant rats and their livers were collected, for the same purpose, into three pools according to the maternal treatment. A separate group of pregnant rats, receiving either morphine (8 animals) or saline (8 animals) during the entire period of gestation, were allowed to deliver naturally. Within 24 hr of birth, the livers from their offspring were analogously pooled and tested for TAT. Tyrosine aminotransferase (TAT) activity was determined in whole liver homogenate, in the presence of pyridoxal-5-phosphate, by the method described by Kenney [4] and expressed as μmoles of *p*- hydroxyphenyl-piruvate/100 mg/hr. P values were calculated by the Student's *t*-test.

As shown in Table 1, morphine acutely administered to non-pregnant rats increases liver TAT levels. With chronic administration of the narcotic a tolerance develops to the enzymatic effect. These data confirm, therefore, our previous results [2]. We know, at present that there are at least two independent stimuli for TAT increased synthesis: the corticosteroid and the substrate inductions [5]. Morphine does influence corticosteroid secretion: single doses of narcotic generally stimulate adrenal responses whereas prolonged administration produces depression of the basal levels of corticosteroid secretion [6]. The dual effect of morphine we have seen after acute and chronic administration on liver TAT levels may be the consequence of the dual effect of the narcotic on corticosteroids. The mechanism by which morphine influences corticosteroid secretion is not clearly defined but evidence exists suggesting that it could act at a central site, probably through the stimulation and inhibition of the synthesis or output of releasing factor(s) responsible for the control of the pituitary adrenal system [7]. The enzyme induction is not statistically evident in pregnant rats; in all these animals of the three groups of treatment, enzyme levels were already initially high. The reason for high levels of TAT in livers of pregnant rats may be ascribed to their elevated concentration of corticosteroids [8, 9]. Morphine administration is practically ineffective on TAT induction either because these high corticosteroid concentrations impede, by feed back mechanism, further secretion of ACTH by morphine or because liver TAT cannot be induced over a certain level. Studies on enzyme induction carried out by Wicks [10] with hydrocortisone in pregnant rats support this latter hypothesis.

Table 1. Effects of acute and chronic administration of morphine on liver tyrosine aminotransferase (TAT) of pregnant and non-pregnant rats

	No of rats	Treatment	Dose (mg/kg/s.c. per day)	Treatment period (days)	TAT (μmoles of *p*-hydroxyphenylpyruvate 100mg per 1 hr)*
Non-pregnant rats	8	Saline	—	12	12.38 ± 1.37
	8	Morphine	20	1	25.71 ± 1.21†
	8	Morphine	20	12	14.99 ± 0.87
Pregnant rats	8	Saline	—	12	22.15 ± 2.75‡
	8	Morphine	20	1	26.28 ± 2.44‡
	8	Morphine	20	12	28.21 ± 3.21‡

* 6 hr after last administration. Results are expressed as mean ± S.E.M.

† $P < 0.05$ compared with respective controls.

‡ $P < 0.05$ compared with non-pregnant controls.

Table 2. Liver tyrosine aminotransferase activity (TAT) in fetal and newborn rats after acute and chronic maternal treatment with morphine

	No of observations*	Maternal treatment	Dose (mg/kg/s.c. per day)	Treatment period of mothers (days)	TAT (μmoles of p-hydroxyphenylpyruvate 100 mg per 1 hr)
Fetal rats	10	Saline		12	0.60 ± 0.04
	8	Morphine	20	1	1.05 ± 0.22
	8	Morphine	20	12	0.75 ± 0.06
Newborn rats (within 24 hr of birth).	8	Saline		13–14	15.63 ± 1.67
	10	Morphine	20	13–14	30.02 ± 3.21†

* This refers to the number of separate pools of livers rather than individual livers.
† $P < 0.05$ compared with respective controls.

As shown in Table 2, enzyme activity in the fetal livers from control rats is much lower than that of adult animals; after maternal administration of morphine a modest TAT increase is seen which, however, is not statistically significant. The placenta may be a source of repressors of TAT during gestation since extracts of rat placenta are capable of blocking TAT induction by hydrocortisone in explants of fetal liver [10]. Moreover, as shown in Table 2, the TAT activity is fully evident in newborn rats from saline-treated animals allowed to deliver naturally, thus confirming the placenta as a possible source of TAT repressors. TAT activity in newborn rats from morphine-tolerant animals allowed to deliver naturally is at high mean levels (Table 2). Since in adult rats morphine withdrawal is followed, within 24–48 hr, by a marked increase in circulating corticosteroids [11], it may be suggested that a similar effect of increased adrenal activity occurs in newborn rats, due to sudden lack of maternal supplied morphine, with a consequent stimulus of TAT synthesis.

In conclusion the present results provide evidence of altered tyrosine aminotransferase (TAT) patterns in the livers of pups of morphine-treated animals.

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