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Morphine inhibition of drug metabolism in the rat

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CHRONIC morphine administration to rats is known to produce inhibition of morphine metabolism by the pathways of N-demethylation and glucuronide formation, coincident with the development of morphine tolerance.[1, 2] Axelrod[1] has suggested that morphine tolerance may be a biochemical correlate of the decreased ability of tolerant animals to N-demethylate morphine. This has been disputed most recently by Elison *et al.*[3] upon the basis of enzyme kinetic studies relative to rates of demethylation of several narcotic drugs. Recent studies in our laboratories,[4] in which we have used a four-day pretreatment of rats with morphine to block pituitary ACTH release caused by 'stress' situations, have suggested that morphine may be a nonspecific inhibitor of drug metabolism. The morphine inhibition can, in the intact animal, be overcome with ACTH and is not noted with compounds (barbital) that do not undergo extensive metabolism. Results of studies *in vitro* and *in vivo* bearing on this question are presented.

Male Holtzman rats weighing between 190 and 220 g were used in all experiments and were maintained on commercial laboratory chow with free acess to water. Animals were treated daily for four days with morphine sulfate (20 mg/kg) or saline (1 ml/kg) by intraperitoneal injection. This pretreatment regimen with morphine has been shown by Munson and Briggs[5] to block pituitary activation (ACTH release) by 'stress' situations, whereas the adrenal remains responsive to exogenous ACTH. Groups of control and morphine-pretreated animals were taken 24 hr after the last dose of morphine and either sacrificed for determination *in vitro* of hexobarbital and morphine metabolism, or intact animals from these groups were given hexobarbital (100 mg/kg), meprobamate (300 mg/kg), or barbital (250 mg/kg) by intraperitoneal injection for determination of duration of drug response. In another experiment morphine-pretreated animals received ACTH (100 mU/animal) by intravenous injection 2·5 hr before receiving hexobarbital; controls received an equal volume of saline by injection before hexobarbital. Hexobarbital and morphine metabolism was studied in liver slice preparations from control and morphine-pretreated rats.

Morphine-pretreated animals sleep for significantly longer intervals than do control animals after hexobarbital or meprobamate, whereas barbital sleeping time is not affected. Further, it is possible to overcome the effect of morphine pretreatment by the administration of ACTH prior to hexobarbital.

This is consistent with the known action of morphine in blocking pituitary–adrenal activity by preventing ACTH release. We have previously shown that stimulation of pituitary–adrenal activity by stress situations results in highly significant *decreases* in the duration of action of drugs which are metabolized (hexobarbital, meprobamate, pentobarbital, zoxazolamine), whereas the duration of action of drugs such as phenobarbital and barbital is not altered, suggesting that the effect is related to increased drug metabolism. The results of studies *in vivo* are presented in Table 1. Table 2 shows

TABLE 1. EFFECT OF MORPHINE PRETREATMENT ON DURATION OF DRUG ACTION IN THE RAT*

Duration of drug action (minutes ± S.E.)			
Hexobarbital		Meprobamate	Barbital
Control	Morphine-pretreated	Control	Control
25·7 ± 1·4	47·8 ± 1·4	96·8 ± 3·2	211 ± 2·6
(7)†	(7)	(6)	(6)
Morphine-pretreated	Morphine-pretreated + ACTH	Morphine-pretreated	Morphine-pretreated
47·8 ± 1·4	31·6 ± 1·8	128 ± 6·0	228 ± 10·1
(7)	(7)	(4)	(6)
$P < 0.0005$	$P < 0.0005$	$P < 0.005$	$P > 0.1$

* Drugs administered 24 hr after last dose or morphine or saline by intraperitoneal injection. Duration of response measured as period of loss of righting reflex. ACTH administered by intravenous injection 2·5 hrs prior to hexobarbital, controls receiving saline at same time period. All animals pretreated for 4 days either with morphine sulfate (20 mg/kg, i.p.) or saline (1 ml/kg, i.p.).

† Animals per group.

TABLE 2. EFFECT OF MORPHINE PRETREATMENT ON DRUG METABOLISM IN THE RAT*

Treatment	Substrate metabolized ± S.E.	
	Hexobarbital (μmoles/g liver/60 min)	Morphine (μmoles/g liver/90 min)
Controls (saline, 1 ml/kg, i.p. for 4 days)	0·95 ± 0·12	0·65 ± 0·04
Morphine sulfate (20 mg/kg, i.p. for 4 days)	0·71 ± 0·08 $P < 0.1$	0·51 ± 0·06 $P < 0.1$

* Six animals per group. Liver slices incubated in 5·0 ml of 0·1 M phosphate buffer, pH 7·4, containing either 1·7 μM of hexobarbital or 1·0 μM of morphine at 37° for 60 or 90 min, respectively, using oxygen as the gas phase. Analysis for unchanged hexobarbital in the incubation medium by the method of Cooper and Brodie[9] and for unchanged morphine by a modification of the method of Shideman and Kelly.[10]

that the morphine pretreatment regimen inhibits drug metabolism when hexobarbital (side-chain oxidation) and morphine (glucuronide formation) are used as substrates.

The data suggest that morphine may be a nonspecific inhibitor of drug metabolism in the rat and that the mechanism of the inhibition is related to morphine block of endocrine function. It is known that adrenalectomized rats show depressed activity of drug metabolism which can be reversed by treatment with corticosteroids.[6] Under the conditions of morphine pretreatment used in these experiments the circulating level of corticosteroids would be low and could not be increased, owing to block of ACTH release. The mechanism(s) by which corticosteroids regulate drug metabolism in the intact animal has not been elucidated, but it is not unreasonable to assume that induction of enzyme synthesis may be involved, considering the well-established fact that levels of several liver enzymes (tryptophan pyrrolase, glutamic–alanine transaminase, tyrosine transaminase) are markedly elevated by corticosteroids.[7, 8]

Our data lead us to question further the significance of a relationship between morphine tolerance and morphine metabolism in the rat. It appears, rather, that morphine may be a nonspecific inhibitor of drug metabolism by virtue of its endocrine effects.

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Effect of stimulation and catecholamines on glucose-6-phosphate content of intact skeletal muscle*

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RECENT observations of phosphorylase have led to awakened interest in the possible involvement of hexosephosphates in the regulation of contractility. Phosphorylase is the enzyme that catalyzes the reaction: glycogen + inorganic phosphate $\rightleftarrows$ glucose-1-phosphate.[1] As a resultant of altered phosphorylase activity, one may expect to find changes in hexosephosphate levels in tissue, and it has been proposed that such changes may be related to muscle contraction.[2]

Our laboratory[3] has provided evidence that several cardiotonic agents known to increase phosphorylase activity in cardiac muscle do indeed produce increases in glucose-6-phosphate levels in the heart concomitant with their action. Related experiments on skeletal muscle contraction in particular are definitive but fragmentary.

It has been established that electrical stimulation and epinephrine increase both phosphorylase activity and hexosephosphate content of skeletal muscle.[4] Other studies indicate that epinephrine elevates the glucose-6-phosphate content of isolated diaphragm and that exogenously added hexosephosphate improves the contraction of the potassium-depressed diaphragm.[2]

To help clarify the possible role of hexosephosphates in muscle contraction, this report supplements the paucity of data in this area, particularly in studies *in vivo*, and provides some further quantitation for the previously cited basic observations relative to the effects of stimulation and epinephrine on the hexosephosphate content of skeletal muscle.

Male Wistar strain rats (generously donated by Dr. Edward Muntwyler, Department of Biochemistry), weighing approximately 300 g, were anesthetized with 90 mg pentobarbital sodium/kg administered intraperitoneally. The anterior tibial muscles were exposed and placed under a tension of 10 g. A maximal stimulus of 500 μsec duration was applied directly to the muscle for the times and

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