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STRESS-INDUCED ALTERATIONS IN MICROSOMAL DRUG METABOLISM IN THE ADRENALECTOMIZED RAT

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Abstract—Cold stress, phenobarbital and hydrocortisone treatment have been examined for their effects on microsomal drug metabolizing enzymes in the adrenalectomized rat. Adrenalectomy alone markedly reduces the rate of metabolism of ethylmorphine, aniline and hexobarbital. The administration of hydrocortisone or the exposure of adrenalectomized animals to cold stress results in an increase in ethylmorphine and aniline metabolism, but only a further decrease in the rate of hexobarbital metabolism. Phenobarbital stimulates the metabolism of all three compounds in adrenalectomized rats. These findings indicate that both stress and phenobarbital can bring about changes in drug metabolism independent of the presence of an intact adrenal. It is further suggested that phenobarbital and stress produce their changes in enzyme activity through different mechanisms since phenobarbital treatment results only in stimulatory activity while stress causes either an increase or a decrease depending upon the drug pathway examined. The possible role of extra-adrenal steroids mediating the stress-produced enzyme induction is discussed.

INSCOE and Axelrod[1] were among the first to demonstrate that stress could affect microsomal drug-metabolizing enzymes. They found that by subjecting rats to a cold environment for several days the hydroxylation of antipyrine was increased while the *N*-demethylation of meperidine was decreased. Rupe *et al.*[2] and Driever and Bousquet[3] reported that short term (2·5 hr) stress, such as hind limb ligation, causes a decrease in hexobarbital sleeping time. A subsequent report indicated that blood levels of several drugs were decreased more in stressed animals than in controls.[4]

Bousquet *et al.*[5] have concluded that it is not possible to influence the hexobarbital sleeping time in adrenalectomized or hypophysectomized animals. On this basis they suggested that the effects of stress on drug metabolism are probably mediated over the pituitary-adrenal axis. On the other hand, drug-produced enzyme induction appears to be independent of the adrenal-pituitary system.[6] Juchau *et al.*[7] supported this thesis by showing induction of benzpyrene hydroxylase in a perfused liver where no adrenal or pituitary influence was possible. It was of interest, therefore, to compare the effects of stress and drug treatment on hepatic enzymes in animals lacking an intact pituitary adrenal axis.

METHODS

Animals

Immediately upon arrival male Wistar rats weighing between 200 and 300 g were

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placed in uncrowded cages with food and tap water provided *ad libitum*. The lights in the animal room were placed on a 12 hr on–off cycle and the animals were kept in this quiet and relatively stress-free environment for at least 5 days.

Treatments

Stressed animals were housed separately in stainless steel cages for 4 days in a ventilated cold room maintained at 4°. Drug treatments were as follows: phenobarbital sodium, 40 mg/kg, i.p. for 5 days; hydrocortisone, 50 mg/kg, s.c. for 3 days. Twenty-four hr before sacrifice the animals were returned to stress-free conditions and all treatments halted. The animals were killed by decapitation.

Adrenalectomy

Adrenal glands were removed under ether anesthesia through a mid-dorsal incision in the skin and bilateral openings in the body wall. The adrenal artery was crushed to minimize bleeding after which the entire gland was removed using curved forceps. The body wall openings were closed with silk sutures; the skin was juxtaposed with clamps. Adrenalectomized rats were given 0·9% saline plus a standard laboratory chow *ad libitum*.

Adrenalectomized animals which were to receive drug treatment or be subjected to cold stress were maintained in stress free conditions for 2–3 days post-operatively before receiving additional treatment. This procedure allowed a certain amount of metabolic equilibrium or stabilization to occur and permitted a greater number of adrenalectomized animals to survive the rigors of cold exposure.

Tissue preparation

Livers were excised immediately after decapitation, weighed and homogenized in approximately 2 vol. 1·15% KCl for 1 min in a Waring–Blendor. The homogenate was centrifuged at 9000 *g* for 20 min at 4° to sediment membrane fragments, nuclear material and mitochondria. The resulting supernatant was adjusted in volume with 1·15% KCl so that each ml of solution contained the microsomes derived from 250 mg of fresh liver. The above procedures were carried out at 4° and generally required not more than 1 hr. After preparation the homogenate was placed in a clean glass container and packed in ice until used.

Reaction mixtures

Complete reaction mixtures of 5 ml total volume were incubated in 25 ml beakers in a Dubnoff Metabolic Shaker at 37° with air as the gaseous phase. According to preliminary experiments reaction rates were linear during the first 12 min and sufficient product formed to accurately measure metabolic rates, therefore this interval was used. Each vessel contained the following constituents: NADP, 2·0 μmole; glucose-6-phosphate, 25 μmole; nicotinamide, 100 μmole; magnesium chloride, 25 μmole. Forty-nine μmole of semicarbazide hydrochloride were added to the reaction mixture when the *N*-demethylation of ethyl-morphine was measured. All cofactors were added in amounts which were at or above optimal levels. The pH of the incubation mixture was adjusted to 7·4 with 0·1M phosphate buffer. One of the following substrates was also added to the appropriate incubation mixture: ethylmorphine (5 μmole), aniline (10 μmole) or hexobarbital (3 μmole). When the substrate was ethylmorphine or aniline, 1 ml of the enzyme-containing supernatant was added to start the reaction; when hexobarbital was the substrate, twice that amount was used.

Determination of enzyme activity

The reaction was always started by adding the 9000 *g* fraction to the reaction mixture and was stopped 12 min later either by the addition of 20% zinc sulfate(*N*-demethylation of ethylmorphine[9]), transferring 3 ml of the incubation mixture to a flask containing 30 ml of heptane and 4 ml of NaCl saturated citrate buffer[10]) or by transferring 4 ml of the mixture to 30 ml of diethyl ether[11]). The assays were then carried out as described in these procedures.

RESULTS

Effects of cold stress on hexobarbital metabolism in unoperated animals

Fig. 1 demonstrates that exposure of intact rats to an environmental temperature of 4° results in a depression in the rate at which hexobarbital is metabolized. The

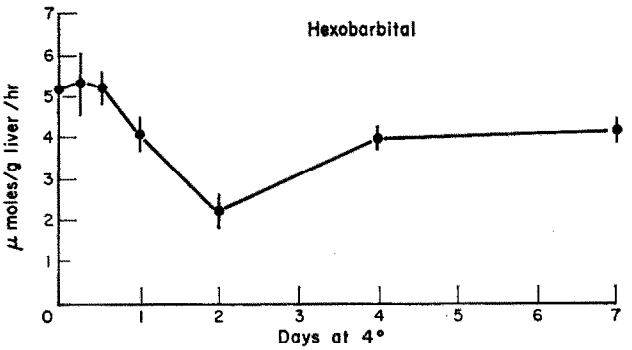

FIG. 1. The effect of cold stress on the *in vitro* metabolism of hexobarbital by intact rats. Vertical bars are S.E. and each point represents the mean of at least 5 animals. Time intervals of 2, 4 and 7 days are significantly different from control values ($P < 0.05$).

first signs of a depression in rate were seen after 1 day while the maximal depression occurred on the second day. Exposure to cold for periods longer than 2 days resulted in a gradual return of the rate towards control values, however, even after 7 days the rate was still significantly ($P < 0.05$) depressed (Fig. 1).

During the first 24 hr after adrenalectomy there was a pronounced fall in liver weight which gradually returned towards normal and then stabilized at almost control values (Fig. 2). Adrenalectomy also produced a gradual increase in thymus weight (Fig. 2).

Effect of adrenalectomy in untreated animals

The effects of adrenalectomy on drug-metabolizing enzymes were followed by making observation 1, 2, 3 and 8 days post-operatively. The most profound changes occurred with the first 72 hr. The *in vitro* rates of hexobarbital, ethylmorphine and aniline metabolism were all significantly ($P < 0.01$) depressed within the first 24 hr, although maximum depression did not occur until 48 hr (Fig. 3). The rates of metabolism then remained relatively stable for the next 6 days.

Effect of phenobarbital treatment in adrenalectomized animals

When adrenalectomized animals were injected with phenobarbital, there was a marked increase in the metabolism of ethylmorphine, aniline and hexobarbital

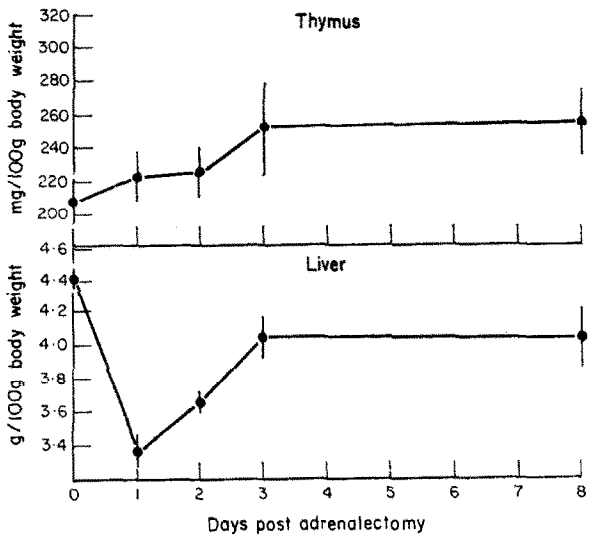

FIG. 2. The effect of adrenalectomy on liver and thymus weight.
Vertical bars are S.E. and each point represents the mean of at least five animals.

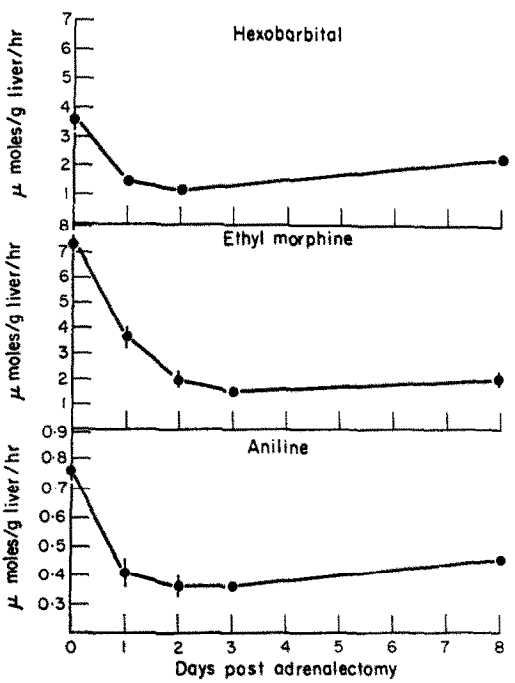

FIG. 3. The effect of adrenalectomy on the *in vitro* metabolism of hexobarbital, aniline and ethylmorphine.

Vertical bars are S.E. and each point represents the mean of at least five experiments. Values indicated for days 1, 2, 3 and 8 are significantly different from controls ($P < 0{\cdot}05$).

compared to adrenalectomy alone (Table 1). Ethylmorphine and aniline metabolism were increased to a mean rate above that found in the unoperated, untreated control animals, while hexobarbital metabolism was returned to approximately control levels by phenobarbital treatment.

TABLE 1. EFFECT OF ADRENALECTOMY, DRUG-TREATMENT AND COLD STRESS ON HEPATIC MICROSOMAL ENZYMES

Treatment	Hexobarbital	Ethylmorphine	Aniline*
Control (9)	3·59 ± 0·40†	7·26 ± 0·28§	0·73 ± 0·04†
Adrex (5)	2·36 ± 0·09	2·02 ± 0·21	0·45 ± 0·01
Adrex + Phenobarbital (8)	3·17 ± 0·23*	9·11 ± 0·19§	1·26 ± 0·03§
Adrex + Four days cold (6)	0·77 ± 0·11§	3·28 ± 0·14§	0·63 ± 0·02§
Adrex + Hydrocortisone (10)	1·90 ± 0·12†	3·14 ± 0·15‡	0·57 ± 0·01‡

Phenobarbital was injected (40 mg/kg) for 5 days with the last injection given 24 hr before the animals were sacrificed. Hydrocortisone was injected (50 mg/kg, s.c.) as a suspension in corn oil for three days with the last injection being given 24 hr before sacrifice. The injection schedules were such that all treatments were stopped on the eighth day after adrenalectomy (Adrex). The numbers in parenthesis represent the number of animals used for each determination. Control and experimental animals were used randomly.

* Values in the table indicate metabolism expressed as average micromoles of drug metabolized/g/hr ± S.E.

The following symbols indicate that the values were significantly different from the 8 day post-adrenalectomized animals (Adrex): †$P < 0.05$; ‡$P < 0.01$; §$P < 0.001$.

Effect of cold stress on adrenalectomized animals

Exposure of adrenalectomized rats to a temperature of 4° resulted in an increase in aniline and ethylmorphine metabolism of roughly 50 per cent compared to adrenalectomy alone, but served only to further depress the metabolism of hexobarbital (Table 1). The increase in aniline and ethylmorphine metabolism, although returning towards normal, did not reach control levels.

Effect of hydrocortisone treatment on adrenalectomized animals

Hydrocortisone treatment of adrenalectomized animals brought about an elevation in the rate of aniline and ethylmorphine metabolism, but further depressed the metabolism of hexobarbital (Table 1). Under our experimental conditions hydrocortisone was not able to fully restore the rates of aniline and ethylmorphine, however.

DISCUSSION

It has been previously demonstrated in this laboratory that cold stress could elevate the rate of metabolism of ethylmorphine and aniline.[8] The metabolism of these compounds was increased to a maximum of approximately 35 per cent during a 4 day exposure to cold. The present studies indicate that exposure to cold does not always result in an increased rate of metabolism of foreign compounds since the rate of disappearance of hexobarbital was diminished. This agrees well with the findings of Inscoe and Axelrod[1] who reported both an increase and a decrease in rate after cold stress depending upon which enzyme system was studied. However, studies using a different form of stress[5] showed only increased clearance of compounds from the blood. The above findings suggest that the type of stress employed may be an important

factor in determining the metabolic response elicited. This problem is being investigated and will be the subject of a future communication.

The *in vitro* rates of metabolism of hexobarbital, ethylmorphine and aniline were all reduced following adrenalectomy. Increasing the circulating steroid levels by administering hydrocortisone was able to elevate the rates of aniline and ethylmorphine metabolism, although hexobarbital metabolism was further depressed. Remmer[12, 13] has reported that adrenalectomy lowers the activity of liver microsomal enzymes and that administration of prednisolone was able to increase the activity of these enzymes. The initial precipitous fall in the rate of metabolism after adrenalectomy might be partially explained by the marked decrease in liver weight which occurred during the first 24 hr post-operatively. Loss of liver enzyme protein, possibly due to an initial diminished food intake, cannot be the entire explanation, however, since liver weights subsequently returned to near normal levels while drug metabolizing enzyme activity was still depressed.

When adrenalectomized animals were subjected to cold stress, the metabolism of ethylmorphine and aniline were increased compared to unstressed adrenalectomized animals. Hexobarbital metabolism, however, was further depressed by exposure to cold. Apparently cold stress can produced alterations in the activity of drug-metabolizing enzymes whether or not the adrenal glands are present. This suggests that cold stress-mediated enzyme induction is not totally dependent upon the pituitary-adrenal axis. This is in contrast to the findings of Bousquet *et al.*[5] who found that an intact adrenal-pituitary system was necessary for stress-caused induction. It is important to note, however, that their findings were in connection with a short term (2·5 hr) stress while that considered in this report was of a considerably longer duration. It is also possible that changes in hexobarbital sleeping time do not always reflect alterations in microsomal activity.

We have previously suggested that stress and drug-produced induction of microsomal enzymes appear to be brought about through different mechanism.[8] Additional support for such a hypothesis has been provided by the studies in which adrenalectomized animals were given phenobarbital or subjected to cold stress. While ethylmorphine and aniline metabolism were elevated by both pocedures, hexobarbital metabolism was increased by the former and further decreased by the latter. It may be that phenobarbital can stimulate a wide variety of drug pathways, whereas stress can stimulate the metabolism of some compounds, but not others. Such a selectivity has been shown for chemical induction between phenobarbital and chlordane[14] on the one hand and the poly-cyclic hydrocarbons[15] on the other.

The exact mechanism by which stress brings about this increase in aniline and ethylmorphine and a decrease in hexobarbital metabolism in adrenalectomized animals cannot be stated at this time. The release of extra-adrenal steroids by stress may be a contributing factor. Such a hypothesis is supported by our observation that exogenous steroids given to adrenalectomized rats mimics the metabolic effects of stress described above. The participation of steroids other than glucocorticoids cannot be ruled out, however. It should be remembered that after adrenalectomy all sources of steroids are not eliminated since the sex steroids are still present. Quinn *et al.*[16] has shown that male and female rats show different rates of drug metabolism and that these differences could be minimized by treating the animals with the appropriate hormone of the opposite sex. This suggests that the sex steroids may be linked to

drug metabolizing enzymes, at least in the rat, in an intimate manner. An examination of the effects of stress in adrenalectomized and castrated animals might provide additional information on this point.

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