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

ROBERT E. STITZEL and RAYMOND L. FURNER

Department of Pharmacology, College of Medicine, West Virginia University, Morgantown, W.Va., U.S.A.

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Abstract—Stress alone and in combination with phenobarbital has been investigated for its stimulatory effects on hepatic microsomal drug metabolism in the rat. Stress (cold) caused increases in the rate of *p*-hydroxylation of aniline. *N*-dealkylation of ethylmorphine, and increases in adrenal ascorbic acid content. The stimulatory actions of stress on microsomal metabolism and ascorbic acid levels are additive with those produced by phenobarbital. It is tentatively concluded that stress and phenobarbital appear to act through different mechanisms in inducing increases in enzyme activity, although each treatment may have a common final step, namely an increased net synthesis of enzyme protein.

THE RESPONSE to stress differs from most other biologic reactions in that, although it is nonspecifically produced, the alterations it effects in body function are quite specific. Activation of the pituitary–adrenal axis, thymic involution, adrenal hypertrophy, and gastric ulcer formation are all well documented physiological responses to stressor agents.[1–4] However, metabolic consequences of exposure to stress, although recently studied by Rupe *et al.*[5] and Bousquet *et al.*,[6] have not been as extensively investigated. The present study was undertaken to evaluate the effects of stress (cold exposure) on hepatic drug-metabolizing enzymes.

METHODS

Preparation of animals

Male Wistar rats weighing between 200 and 300 g were used. Immediately upon arrival the animals were placed in uncrowded cages and food and tap water were provided *ad libitum*. The lights in the animal room were placed on a 12-hr on–off cycle and only authorized personnel were allowed admittance. The rats were maintained in this relatively stress-free condition for at least 5 days.

The stressed animals were housed separately in stainless steel cages placed in a ventilated cold room maintained at 4° ± 0·5°. After removal from the cold room the rats were again placed in the stress-free environment and sacrificed by decapitation 24 hr later. A 24-hour post-stress sacrifice time was chosen so that any cold-induced thyroid activation would be minimized. All enzyme assays were performed immediately after decapitation.

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Two other groups of animals, one of which was placed in the cold for 4 days, received daily i.p. injections of phenobarbital sodium in a dose of 40 mg/kg for 5 days. Both groups were sacrificed 24 hr after the last injection.

Control rates of drug metabolism were determined either along with each experimental group or controls were examined at various intervals after the initial maintenance period.

Preparation of tissue samples

The livers were removed, rinsed in cold 1·15% KCl, and homogenized in approximately 2 vol. KCl. Homogenization at 4° was carried out in a Waring-blender. A 9000-*g* fraction that contained microsomal and soluble enzymes was prepared from the homogenate by using a high-speed angle centrifuge (20 min at 4°). Each fraction was adjusted in volume with 1·15% KCl so that 1 ml contained the equivalent of 250 mg fresh liver.

Assays in vitro

Drug-metabolizing enzyme activity was determined by incubating 1 ml of the 9000-*g* fraction with appropriate contractors. A Dubnoff shaking incubator was used at 37° with air as the gaseous phase. All observations were made during a time interval when reaction rates were linear (12 min). Final concentrations of the cofactors added were: triphosphopyridine nucleotide (4×10^{-4} M), glucose 6-phosphate (5×10^{-3} M), nicotinamide (2×10^{-2}M), and $MgCl_2$ (5×10^{-3} M). All concentrations were at or above optimal levels, and the final volumes of all incubation mixtures were brought to 5 ml with 0·1 M phosphate buffer, pH 7·4.

The pathways studied and substrate concentrations in micromoles per 5 ml incubate were: the *N*-dealkylation of ethylmorphine[7] (5 μmole) and the hydroxylation of aniline[8] (10 μmole). Adrenal ascorbic acid content was measured by the dinitrophenylhydrazine procedure of Lowry *et al.*[9]

RESULTS

Effects of cold stress on microsomal enzyme activity

The data in Fig. 1 and 2 demonstrate the effects of cold exposure on the metabolism of aniline and ethylmorphine. Six or 12 hr of exposure to cold caused an initial depression of ethylmorphine metabolism, whereas more prolonged exposure leads to a

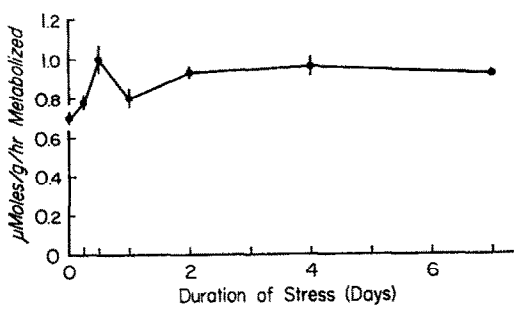

FIG. 1. Effect of stress on the *p*-hydroxylation of aniline by liver microsomal enzymes. Values in the figure indicate metabolism by 9000-*g* fraction expressed as average μmole of drug metabolized/g/hr ± S.E. The means are based on at least 10 animals at each point.

significant increase in the rate of ethylmorphine metabolism, until a peak value is reached at about 4 days ($P < 0{\cdot}001$). The rate at this point is about 32 per cent above the control rate.

The time course of aniline metabolism after exposure of rats to 4° is somewhat different from that seen for ethylmorphine. The hydroxylation of aniline shows an

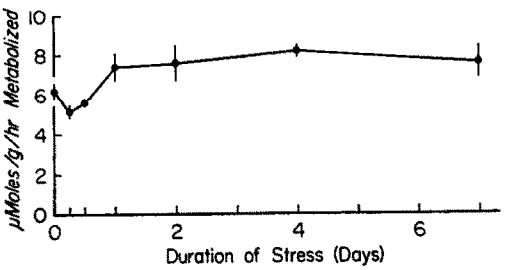

FIG. 2. Effect of stress on the *N*-dealkylation of ethylmorphine by liver microsomal enzymes. Values in the figure indicate metabolism by the 9000-*g* fraction per hr ± S.E. The means are based on at least 10 animals at each point.

immediate increase in rate with no initial depression. A relatively stable rate of metabolism is established after 4 days of cold exposure. This figure is approximately 39 per cent above the control rate.

Effects of cold stress on adrenal ascorbic acid content

We tested the effects of stress on adrenal ascorbic acid content, since it has been shown that agents that cause enzyme induction can increase ascorbic acid content.[10]

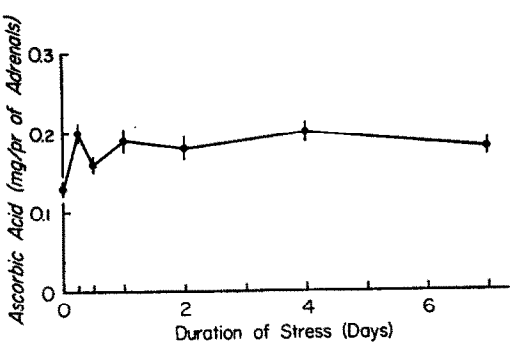

FIG. 3. Effect of stress on the content of ascorbic acid in the rat adrenal gland. Values in the figure indicate ascorbic acid content per pair of adrenal glands ± S.E.M. These determinations are based on at least 10 rats at each point.

Animals subjected to ambient temperature of 4° showed a marked elevation in adrenal ascorbic content (Fig. 3). Exposure for as little as 6 hr produced a 54 per cent increase in ascorbic acid levels. Levels continued to remain significantly ($P < 0{\cdot}001$) elevated during 7 days' exposure to decreased temperature.

Effects of cold stress and phenobarbital used in combination as compared to the effects of either agent alone

Optimal doses of phenobarbital (40 mg/kg, i.p., for 5 days) and optimal stress conditions (4 days at 4°) were used. Table 1 demonstrates that the combination of stress and phenobarbital administration produced a greater increase in aniline hydroxylation, ethylmorphine dealkylation, and adrenal ascorbic acid content than did either

TABLE 1. STIMULATORY EFFECTS OF STRESS AND PHENOBARBITAL ON HEPATIC DRUG-METABOLIZING ENZYME ACTIVITY AND LIVER WEIGHT WHEN GIVEN ALONE OR IN COMBINATION

Treatment	Ethylmorphine*	Aniline*	Ascorbic acid†	Liver wt.††
Control (14)	6.24 ± 0·43	0·70 ± 0·03	0·13 ± 0·01	4·14 ± 0·09
Stress‡ (10)	8·18 ± 0·41§¶	0·97 ± 0·04§	0·20 ± 0·01	4·12 ± 0·15§
Phenobarbital** (5)	9·08 ± 0·11§¶	1·83 ± 0·11	0·17 ± 0·01§¶	4·77 ± 0·04§¶
Stress + phenobarbital (5)	9·68 ± 0·33§	2·05 ± 0·12	0·23 ± 0·01§	5·50 ± 0·13§

* Values in the table indicate metabolism by supernatant fraction expressed as average μmole of drug metabolized per g per hr.
† Values in the table indicate mg per pair of adrenal glands.
‡ Rats were kept at 4° for 4 days.
§ $P < 0·01$ as compared with control.
¶ $P < 0·05$ as compare with stress + phenobarbital-treated animals.
** Animals received 40 mg phenobarbital sodium/kg, i.p., daily for 5 days.
†† g/100 g of body wt.

procedure alone. Table 1 also shows that the combination of phenobarbital and stress or phenobarbital alone caused an increase in liver weight. However, stress alone produced no such increase in hepatic weight at any of the time periods studied.

A comparison of the combined treatment of stress and phenobarbital (Table 1) reveals a significantly ($P < 0·05$) elevated rate of ethylmorphine metabolism, ascorbic acid content, and liver weight over that produced by either treatment alone. However, the rate of aniline hydroxylation, although higher than control, was about equally elevated by phenobarbital or stress + phenobarbital.

Alterations in adrenal and thymus weights after exposure to cold

That exposure to cold is a stress is borne out by the pattern of alterations found in adrenal and thymus weights (Fig. 4). Exposure to 4° resulted in thymic involution and adrenal hypertrophy. The maximal decrease in thymus weight occurred after 4 days' exposure; the maximum adrenal hypertrophy occurred after 1 to 2 days.

DISCUSSION

The demonstration by Rupe *et al.*[5] and Bousquet *et al.*[6] that rats subjected to unilateral hindlimb ligation showed decreased sleeping times after barbiturate administration suggested that stress (hindlimb ligation) could affect the metabolism of these depressant drugs. The results of their work provide indirect evidence, however, since only change in blood level were examined. It was felt that a more direct examination of enzyme activity under conditions of stress would be of interest. Also, their choice of a stress produced by hindlimb ligation left open the possibility that the release of substances from the ischemic leg was responsible for the results obtained. In addition,

another form of stress was used in order to determine whether stress-induced alteration in drug metabolism is a general phenomenon or whether it is limited to stress produced by physical trauma.

Animals were kept at 4° for various times ranging from 6 hr to 7 days and then sacrificed 24 hr after removal from the stress situation. The enzymes involved in the

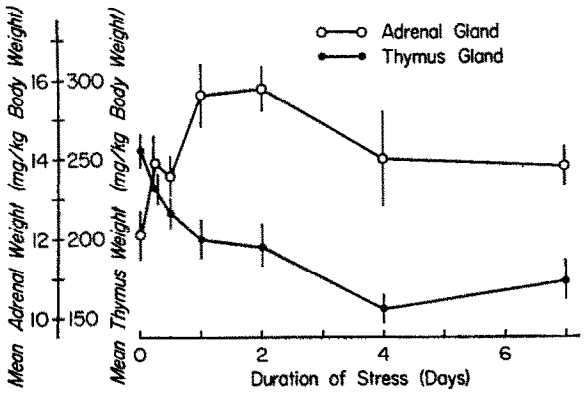

FIG. 4. The effect of various periods of cold stress upon thymus and adrenal gland weights. Values in the figure indicate average weight of organ per 100 g body weight ± S.E.; ○—○ adrenal weight; ●—● thymus weight.

N-dealkylation of ethylmorphine and the hydroxylation of aniline were shown to be stimulated after 1, 2, 4, and 7 days of exposure to cold. The rate of metabolism of these compounds was increased to a maximum of approximately 35 per cent during these time intervals. Mean liver weights showed no tendency to increase during these periods. Such a finding could be interpreted as an indication that an increased rate of drug metabolism is not due to an increased enzyme synthesis. However, small changes in enzyme activity, such as those reported in the present study, may not be reflected in increases in liver weight, since this is perhaps too crude a measure of increased net synthesis of enzyme protein.

The increase in aniline hydroxylase activity in stressed rats occurred sooner than that observed for the *N*-dealkylase enzyme. Whereas 12 hr of cold exposure caused no increase in the rate of ethylmorphine metabolism, it produced significant ($P < 0{\cdot}001$) stimulation of aniline hydroxylation. Apparently the time course of stress-produced induction is different for different microsomal enzymes. Differences in the rate of increase in the activities of hepatic enzymes have also been observed by Shimazu[11] and Kenney *et al.*[12]

The stress-induced metabolic changes just discussed are similar to those classically observed after phenobarbital treatment. The present authors, therefore, undertook to discover whether the mechanisms of action are also similar. The method used was suggested by the work of Hart and Fouts.[13] These investigators observed that a combination of phenobarbital and chlordane produced no greater increase in microsomal activity than did either agent administered separately and concluded that these two agents probably produced their stimulatory effects through the same mechanism.

We therefore undertook to compare the combined and separate effects of phenobarbital administration and stress.

When optimal doses of phenobarbital amd maximal stress conditions (4 days at 4°) were used in combination, the resultant enzyme activities for the metabolism of aniline and ethylmorphine were greater than those seen after either agent alone. Livers from stressed animals showed no increase in weight, but those taken from animals given phenobarbital alone or in combination with stress showed significant increases. Both these findings suggest that stress and phenobarbital produce their increases in enzyme activity through different mechanisms. Additional confirmatory evidence was provided by an examination of the ascorbic acid content of the adrenal gland. Although both cold stress and phenobarbital caused an increase in ascorbic content, a combination of the two procedures resulted in a greater increase than either treatment alone.

By demonstrating that metabolic changes in a stress situation are not peculiar to physical trauma and cannot be due to differences in drug absorption alone, the present study provides evidence that stress *per se* directly stimulates liver enzymes. These findings also provide suggestive evidence that the subcellular mechanism involved in stress-related enzyme induction may be different from that produced by phenobarbital, since the effect of these two different procedures appeared to be additive. However, since Driever and Bousquet[14] have shown that actinomycin D can prevent the induction produced by hindlimb ligation, increased protein synthesis may be at least one common step in both phenobarbital and stress-induced enzyme induction.

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