Tyrosine Aminotransferase: Activation or Repression by a Stress* (33579)

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It has been amply demonstrated that administration of adrenocortical hormones to the rat will result in increased activity of a group of liver enzymes, among which are tyrosine aminotransferase (L-tyrosine:2-oxoglutarate amino transferase, IUB 2.6.1.5) and tryptophan pyrrolase (tryptophan oxygenase, L-tryptophan:oxygen oxidoreductase, IUB 1.13.1.12) (1). That activation of the pituitary–adrenal system by stress produces comparable results is less clear. The literature pertaining to stress-activation or induction of these enzymes is sparse and generally limited to stresses such as fasting in which many ancillary metabolic alterations are also to be expected (2). We have previously reported (3) that these enzymes are not elevated following adrenocortical activation by 30 min on a reciprocating shaker—a stress involving noise, rapid physical agitation, and presumably an emotional response akin to fear. Furthermore, imposition of the same stress to adrenalectomized rats was followed by a clear reduction in enzymic activities, a phenomenon that could be prevented by concurrent hypophysectomy (4). We suggested that in response to that particular stress a mechanism was activated inhibiting the transaminase-inducing effects of adrenocortical hormones, that the pituitary was involved, and that such hormonal repression may play as important a role in physiological adaptation as that attributed to enzyme induction. In the present study we demonstrated that a stress may call forth either induction or repression depending upon the experimental procedures, and that tyrosine amino transferase and tryptophan pyrrolase respond differently.

Methods. Male Sprague-Dawley rats, derived from the Berkeley-Pacific strain and bred in our laboratory, were used. Animals were anesthetized with 5 mg/100g i.p. sodium pentobarbital. One group was not treated further. A second group was stressed by midline laparotomy and exposure of the viscera. A third group was adrenalectomized through the same midline incision. The wounds were closed with clips and the animals were sacrificed 4 hr later.

In separate experiments rats, adrenalectomized or adrenalectomized and hypophysectomized under pentobarbital anesthesia, were again anesthetized 7 days later, and half the group were stressed by laparotomy and treated as above.

Animals were sacrificed by decapitation and trunk blood was collected. The livers were rapidly removed, rinsed with saline and blotted, weighed, and homogenized in 5 vol of cold 0.15 *M* KCl, neutralized with KOH. The homogenates were centrifuged for 30 min in a Spinco L-2 at 0.5° at 105,000*g*, and the clear supernatant solution was used for determination of enzymic activity. Tyrosine aminotransferase, tryptophan pyrrolase, adrenal corticosterone, serum corticosterone, and protein concentration were measured by methods previously described (3). Tryptophan was determined by the method of Denckla and Dewey (5).

Results and Discussion. Both tyrosine aminotransferase and tryptophan pyrrolase in the liver of animals stressed by midline laparotomy were significantly increased after 4 hr (Table I). However, the enzymes in animals

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TABLE I. Effects of Laparotomy or Adrenalectomy on Hepatic Enzymes of the Rat.

	N	Mean ± SE		
		Control	Laparotomy	Adrenalectomy
TA[a]	3	3.88 ± 1.44	7.76 ± 0.82 (<.01)[d]	1.65 ± 0.33
TA + PP[a]	6	16.29 ± 1.67	49.47 ± 3.07 (<.001)	12.19 ± 3.00
TP[a]	6	5.95 ± 1.06	12.32 ± 2.43 (<.025)	6.11 ± 1.40
TP + He[a]	6	20.70 ± 3.52	32.41 ± 7.13	13.21 ± 2.18
Adrenal corticoids[b]	6	18.2 ± 7.5	30.6 ± 4.4	6.6 ± 2.2
Serum corticoids[c]	6	17.5 ± 6.8	43.9 ± 1.7 (<.05)	6.0 ± 0.7

[a] TA—tyrosine aminotransferase activity expressed as μmoles/min/g of protein; PP—pyridoxal phosphate, 0.13 m*M*; TP—tryptophan pyrrolase activity expressed as μmoles/hr/g of protein; He—hematin, 0.15 μ*M*. All enzyme activities were measured 4 hr after surgical treatment.

[b] Adrenal corticoids are expressed as μg/g of adrenal and were measured 0.5 hr after laparotomy in a different group of rats than that used for enzyme activity determinations, or in the excised glands of the adrenalectomized group.

[c] Serum corticoids are expressed as μg/100 ml serum and were measured 0.5 hr after laparotomy or adrenalectomy.

[d] Significant differences from the control group are indicated in parentheses.

subjected to similar surgical stress during the course of adrenalectomy did not respond, but were found to be at lower levels than the anesthetized controls or at about the same level as in unanesthetized, untreated animals. Thus, contrary to what was observed with shaker stress (3), infection (6), and fasting (2), laparotomy was effective in increasing both corticoids and these two liver enzymes, and the enzyme increases were probably mediated by adrenal corticoids. We have suggested elsewhere that enzymic response to stress will be the resultant of the activating effects of corticoids and/or substrate and cofactors, and the inhibitory effects of other hormones such as growth hormone. What happens in a particular stress will, therefore, depend upon the effectiveness of that stress in mobilizing these divergent systems. The acute effects of laparotomy in the intact rat suggest a predominance of corticoid activation.

When, however, the animals were subjected to laparotomy stress 1 week after adrenalectomy, a different picture emerged. As shown in Table II tyrosine aminotransferase activity was repressed, measurements of activity in the presence or absence of added pyridoxal phosphate showed an equal repression. These results suggest a lesser amount of total enzyme protein following laparotomy but no change in the pyridoxal phosphate saturation of the enzyme, although we cannot exclude the possibility of the production of an enzyme inhibitor. Of interest, also, is that a comparison of the activity of aminotransferase in the intact control animals shown in Table I and the adrenalectomized controls of Table II clearly shows the effect of pentobarbital anesthesia on the enzyme activity and indicates the dependence of this effect on the presence of the adrenal gland.

The response of tryptophan pyrrolase to laparotomy in the week adrenalectomized rat was somewhat different from the aminotransferase response. Total enzyme (holoenzyme and apoenzyme) appeared unchanged, for no difference in activity was seen when measured with saturating amounts of hematin. The degree of endogenous saturation with cofactor differed considerably, however, increasing from about 35% in the controls to near 70% in the stressed animals. Such increased cofactor saturation is known to follow induction by substrate but in that case increased levels of total enzyme are seen (7). Direct measurements of tryptophan concentration in liver showed no differences between control and stressed rats. Increased cofactor availability could also explain the increased saturation, but Greengard (8) proposed that increased cofactor concentrations would sti-

TABLE II. Effect of Laparotomy on Hepatic Enzymes in Adrenalectomized Rats (1 week).

	Mean ± SE		
	Control	Laparotomy	Probability
TA[a]	0.95 ± 0.20 (17)[b]	0.53 ± 0.09 (21)	<0.05
TA + PP[a]	8.44 ± 0.89 (17)	3.96 ± 0.44 (21)	<0.005
TP[a]	5.66 ± 0.83 (17)	13.88 ± 0.90 (20)	<0.005
TP + He[a]	18.20 ± 0.76 (4)	20.14 ± 0.63 (4)	—

[a] TA — tyrosine aminotransferase activity expressed as μmoles/min/g of protein; PP—pyridoxal phosphate, 0.13 m*M*; TP—tryptophan pyrrolase activity expressed as μmoles/hr/g of protein; He—hematin, 0.15 μ*M*. All enzyme activities were measured 4 hr after laparotomy.

[b] Number of animals indicated within parentheses.

mulate production of more apoenzyme from preexisting templates. We suggest the possibility that whatever mechanism is activated by laparotomy to repress aminotransferase activity in these animals, also prevents the increase of apotryptophan pyrrolase that would normally accompany the conversion of apoenzyme to holoenzyme by cofactor. Since it has been shown that the cofactor-induced apoenzyme increase is not dependent upon synthesis of new RNA (8), such repression would necessarily take place somewhere in the translation portion of the protein synthetic pathway, a phenomenon previously suggested by Tomkins, *et al.* (9). Seidman, *et al.* (10) studied the induction of pyrrolase by hydrocortisone in the regenerating liver of rats adrenalectomized 5–7 days before treatment. They observed a depression of response to administered hydrocortisone in sham-operated animals (corresponding to our laparotomized animals) and attributed the depression to the operative procedure. Their findings support our suggestion that laparotomy activates systems that interact with adrenal corticoids.

It is not yet clear what the agent for the repression may be. We have previously reported that the pituitary is involved in a somewhat analogous repression (4). In the present study it was again possible to implicate the pituitary gland. Table III demonstrates that animals hypophysectomized and adrenalectomized for 1 week did not show any change in tryptophan pyrrolase activity following laparotomy and that, although still responding to some extent, the degree of repression of aminotransferase activity is considerably lessened. Under these conditions the saturation of the aminotransferase with its cofactor, pyridoxal phosphate, is increased. If the increased cofactor saturation is the result of increased availability of pyridoxal phosphate, then the lessened repression observed may be the result of interaction between the still active inhibitory mechanism and the increased pyridoxal phosphate. The pituitary may, then, exert part of its effect indirectly through control of the pyridoxal phosphate availability. The lessened repression following hypophysectomy may also, however, result from the loss of pituitary hormones, of which one—growth hormone—inhibits activation of aminotransferase when administered together with adrenal corticoids (11, 12). Of paramount importance in this regard is the altered response of chronically adrenalectomized rats

TABLE III. Effect of Laparotomy on Hepatic Enzymes in Adrenalectomized–Hypophysectomized Rats (1 week).

	Mean ± SE		
	Control[b]	Laparotomy[b]	Probability
TA[a]	2.52 ± 0.38	2.45 ± 0.19	—
TA + PP[a]	12.03 ± 0.69	9.50 ± 0.40	<0.005
TP[a]	8.11 ± 1.25	8.55 ± 1.48	—
TP + He[a]	15.09 ± 1.53	16.28 ± 1.49	—

[a] TA — tyrosine aminotransferase activity expressed as μmoles/min/g of protein; PP—pyridoxal phosphate, 0.13 m*M*; TP—tryptophan pyrrolase activity expressed as μmoles/hr/g of protein; He—hematin, 0.15 μ*M*. All enzyme activities were measured 4 hr after laparotomy.

[b] $N = 8$ for both groups.

to stress demonstrated here. Because of this, interpretation and comparison of published results is extremely difficult. For example, Kenney (12) in evaluating the effects of growth hormone on tyrosine aminotransferase in liver used 1 or 2 day adrenalectomized rats; Csányi and Greengard (13), in a similar study, used 5–10 day adrenalectomized animals; and we have used both intact (11) and variously adrenalectomized (4) rats for the study of the inhibitory nature of growth hormone and other pituitary factors. If all our studies are directed toward elucidating the physiological responses of normal animals, it would seem essential to know the time course of the changes in response of systems under investigation after previous treatment.

Summary. Four hr following laparotomy stress to the intact rat the activity of liver tyrosine aminotransferase is increased two to threefold. Simultaneous removal of the adrenal prevents the increase. A marked repression of aminotransferase activity follows imposition of the same stress to rats adrenalectomized 1 week earlier. Hyperphysectomy eliminates some, but not all, of this laparotomy-induced repression in 1 week adrenalectomized rats.

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