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SHORT COMMUNICATIONS

Changes in plasma free fatty acids and hepatic enzymes following traumatic injury in the rat

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Rauckman *et al.* [1] have recently described the use of infrarenal aortic ligation to produce a standardized traumatic injury in rats. The effects of this model trauma on hepatic enzymes were assessed by *in vitro* determinations of two drug-metabolizing enzymes, hepatic cytochrome P-450 and FAD-monooxygenase*. Activities of both enzymes decreased within 12 hr after trauma in comparison with control and sham-operated animals, suggesting that this particular animal model had potential value in studying trauma-induced changes in drug metabolism.

fatty acids and discusses the relevance of the rat injury model to the study of drug metabolism in injured human patients.

Lewis (LEW) and Fischer 344 (F-344) rats were purchased from the Charles River Breeding Laboratories (Wilmington, MA). Wistar–Furth (WF) rats were obtained from Harlan–Sprague–Dawley (Madison, WI). Each group of inbred rats consisted only of males and at the time of the study were approximately 6 weeks old, weighing an average of 110 g.

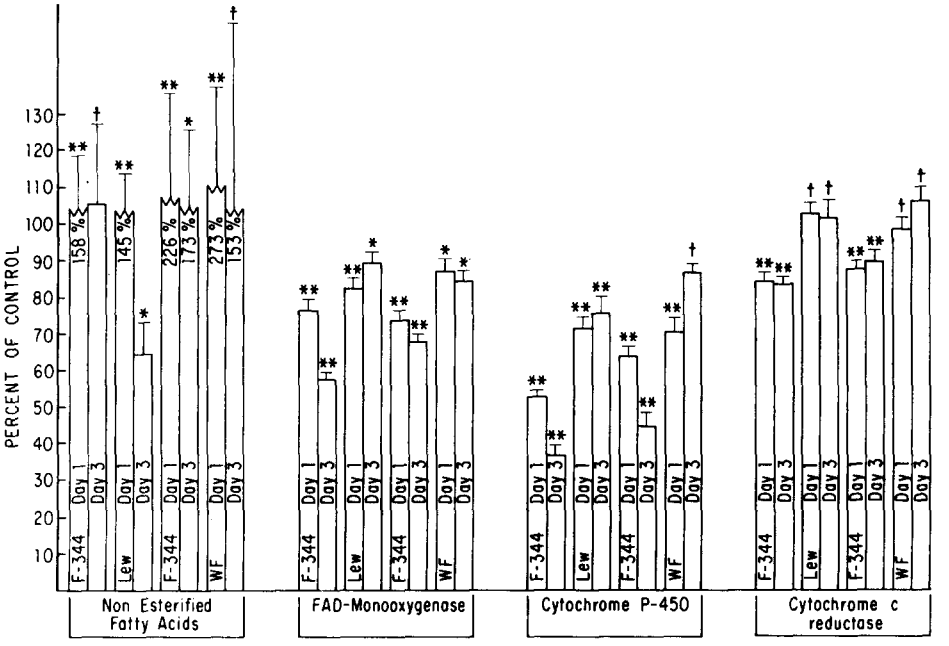

Fig. 1. Changes in plasma non-esterified fatty acids and hepatic enzymes, relative to controls, days 1 and 3 post-infrarenal aortic ligation in inbred rats. Strains are Fischer 344 (F-344), Lewis (Lew), and Wistar–Furth (WF). Error bars represent the standard error. Levels of significance are: (**) $P < 0.01$, (*) $P < 0.05$, (†) not significant. Data were analyzed by one-way analysis of variance, followed by Dunnett's test [7].

The Sprague–Dawley outbred rats used in the previous studies showed a high degree of individual variation in control and post-traumatic activities of cytochrome P-450 and FAD-monooxygenase and levels of plasma free fatty acid. The use of inbred rat strains apparently eliminates much of the individual variability so that there is less overlap between measured values of enzyme activities in control and traumatized animals. This communication describes changes in four hepatic enzymes and plasma free

Animals were maintained on ground corn-cob bedding with Purina rodent chow (No. 5001) and tap water *ad lib.* for at least 7 days before trauma. Aorta ligations and hepatic microsomal preparations were performed as described by Rauckman *et al.* [1]. Cytochrome P-450, FAD-monooxygenase, microsomal protein, and cytochrome *c* reductase determinations were performed using established methods [2–5]. Plasma free fatty acid methyl esters were quantitated by gas chromatography [6].

Table 1 shows the results of plasma free fatty acid and hepatic enzyme determinations in Lewis, Fischer 344, and Wistar–Furth inbred rats. Two separate experiments with Fischer 344 rats are shown to illustrate the consistency of the results with groups of animals used at different times. Clearly there is a marked elevation of plasma free fatty acids on day 1 post-trauma in all the groups with an apparent reversion to control levels generally occurring by day 3 (Fig. 1). All groups exhibited significant decreases in cyto-

* This flavoprotein in older literature was referred to as the microsomal mixed function amine oxidase or as the microsomal *N*-oxidase. The substrates for this monooxygenase are, however, not restricted to amines and a trivial name based on the nature of the prosthetic group avoids problems of broad substrate specificity.

Table 1. Free fatty acid and hepatic enzyme drug-metabolizing activities in rats following infrarenal aortic ligation

Rat strain	N*	FAD-monooxygenase (nmoles·mg^{-1}·min^{-1})	Cytochrome P-450 (nmoles/mg)	Cytochrome *c* reductase (nmoles·mg·$^{-1}$·min^{-1})	Non-esterified fatty acids (μmoles/l)
Wistar-Furth					
Control	8	2.58 ± 0.38	0.407 ± 0.068	51.25 ± 2.40	62 ± 25
Day 1	8	2.25 ± 0.21†	0.283 ± 0.043‡	50.45 ± 4.34§	169 ± 46‡
Day 3	8	2.18 ± 0.19†	0.349 ± 0.026§	54.14 ± 5.15§	96 ± 91§
Fischer 344					
Control	8	2.28 ± 0.14	0.521 ± 0.053	54.08 ± 2.99	129 ± 33
Day 1	7	1.71 ± 0.17‡	0.269 ± 0.018‡	45.08 ± 3.62‡	203 ± 52‡
Day 3	6	1.27 ± 0.12‡	0.187 ± 0.034‡	44.68 ± 2.44‡	136 ± 69§
Lewis					
Control	8	1.91 ± 0.21	0.410 ± 0.048	41.46 ± 3.55	123 ± 44
Day 1	8	1.55 ± 0.14‡	0.291 ± 0.037‡	42.45 ± 2.61§	175 ± 35‡
Day 3	8	1.68 ± 0.13†	0.307 ± 0.053‡	42.02 ± 4.70§	79 ± 29†
Fischer 344					
Control	8	1.89 ± 0.12	0.426 ± 0.036	48.21 ± 3.65	84 ± 26
Day 1	8	1.37 ± 0.13‡	0.270 ± 0.032‡	41.92 ± 2.89‡	189 ± 55‡
Day 3	8	1.17 ± 0.11‡	0.187 ± 0.044‡	42.76 ± 3.93‡	145 ± 48‡

* N equals the number of individual rats. Data are means ± S.D.
† $P < 0.05$, compared to control.
‡ $P < 0.01$, compared to control.
§ Not different.

chrome P-450 content and FAD-monooxygenase activity 1 day post-trauma, with recoveries toward control levels occurring by day 3 in the Lewis and Wistar–Furth animals. The Lewis rats had greater decreases in FAD-monooxygenase activity and P-450 content on day 3 than on day 1. Significant changes in cytochrome *c* reductase activity 1 and 3 days post-trauma were observed only in the Fischer 344 rats.

We were able to acquire statistically significant data demonstrating post-traumatic elevation of non-esterified fatty acids by using inbred rats rather than the outbred (Sprague–Dawley) animals used in our original experiments [1]. The genetic homogenity of these inbred animals apparently results in diminished inter-individual variations in both non-esterified fatty acids and in the activity of several hepatic drug-metabolizing enzymes. Because of this, significant data may be obtained using relatively small groups of animals. The increased susceptibility of the hepatic drug-metabolizing enzymes to trauma in the F-344 animals is noteworthy. Unfortunately, the lack of data concerning differences in normal biochemical variables between various strains of inbred rats does not allow us to speculate why the F-344 animals are more sensitive.

The acute elevation in post-trauma plasma free fatty acid levels in animal models correlates with decreased activity in several hepatic drug-metabolizing enzymes. Elevated levels of plasma free fatty acids have been observed previously in human trauma victims [8] and, to an extent, correlate directly with the severity of the injury [9]. The rise in plasma free fatty acids in our animal injury model suggests its use in the study of traumatic injury in human patients; furthermore, the alterations in hepatic drug-metabolizing enzymes indicate that the animal model may be a relevant technique for concomitant study of the effects of drug-dosing regimens in traumatized patients. In certain patients, particularly those with multi-system injury, a respiratory distress syndrome is observed that appears to be related to alterations in fat metabolism [10]. Possibly there are also marked decreases in certain drug-metabolizing enzymes. Since these enzymes participate in the biotransformation of drugs, such changes in activity profiles could lead to serious drug related complications. In humans, traumatic injury might lead to a decreased biotransformation and elimination rate, higher blood levels, and possibly toxic effects from a supposedly therapeutic drug dose.

How fatty acids are implicated in this metabolic dysfunction is not known, but our animal studies have shown a correlation between the enhanced levels and post-traumatic hepatic metabolic dysfunction. Finally, the study of elevated fatty acid levels and decreased activity of drug-metabolizing enzymes in the injured patient is important in the assessment of those individuals who are at risk for possible respiratory and drug related complications.

In summary, Lewis, Wistar–Furth, and Fischer 344 inbred rats have been evaluated as trauma injury models following infrarenal aorta ligation. These animals are superior to the commonly used outbred Sprague–Dawley rats in that highly significant statistical changes in several hepatic enzymes, as well as in plasma free fatty acids, were shown to occur following trauma. The changes in the Fischer 344 animals were reproducible with respect to cytochrome P-450 content, FAD-monooxygenase activity, and cytochrome *c* reductase activity. Plasma free fatty acid levels reverted to average control levels by day 3 post-injury. The inbred rat injury model appears to be useful for the study of drug metabolism and plasma free fatty acid changes after injury, and may be relevant to studies in injured human patients.

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Activation of soluble striatal tyrosine hydroxylase in the rat brain after CDPcholine administration

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Since its introduction as an antiparkinsonian drug, cytidine 5'diphosphorylcholine (CDPcholine) has been the subject of a number of studies. The pharmacological effect of exogenous CDPcholine may be derived from a biochemical improvement of the impaired lipid metabolism in the brain. Moreover, CDPcholine increases dopamine level and decreases serotonin level in the whole mouse brain, leaving norpinephrine content unchanged [1]. We found that CDPcholine increases the level and synthesis of dopamine in the striatum and decreases the Ceveland synthesis serotonin in the brainstem of the rat brain [2]. The striatal dopaminergic activation induced by CDPcholine is correlated with the specific inhibitory uptake of dopamine in synaptosomal homogenate of rat striatum, observed *in vitro* and *in vivo* [3]. The present paper examines the *in vitro* and *in vivo* effects of CDPcholine on the activity of tyrosine hydroxylase, the rate-limiting enzyme in the synthesis of catecholamines.

Methods

Preparation of tissues. CDPcholine (SIGMA) was dissolved in saline and injected i.v. up to a total volume of 2 ml per kilogram of body weight; rats were administered different doses, and then killed by cervical dislocation at different times after administration; their brains were removed and striatum dissected according to the method of Glowinski and Iversen [4]. Brain tissues were homogenized in 10 vol. (v/w) ice cold 50 mM Tris–HCl buffer pH 6.2 containing 0.2% Triton × 100 using a Glass–Teflon homogenizer (clearance 0.025 cm). The homogenates were centrifuged at 40,000 *g* for 20 min at 4° and the supernatant fluid was gently stirred to obtain a uniform suspension, the source of soluble tyrosine-hydroxylase.

Assay in vitro. Tyrosine-hydroxylase activity was assayed *in vitro* by measuring the formation of tritiated water from 3,5 ditritiotyrosine, as described by Nagatsu *et al.* [5] and modified by Levitt *et al.* [6]; standard incubation mixture contained 0.5 to 1.0 mg protein, 15 μM ^{3}H-tyrosine, 1.1 mM 2-amino-4 hydroxy-6-7-dimethyltetrahydropteridine ($DMPH_4$), 50 mM 2-mercaptoethanol, 0.435 mM $FeSO_4$ in a Tris–acetate 0.11 mM pH 6.1 buffer, and CDPcholine at different concentrations up to a final volume of 300 μl. Incubation was started by shaking at 37° and lasted for a period of 20 min. The reaction was stopped by addition of 50 μl acetic acid; the mixture was centrifuged at 5000 *g* for 5 min and 300 μl of the supernatant were placed on to a Dowex 50 H^+ form column (4 ml of a melanger with water 1/1 v/v). 1 ml of H_2O was run through the column prior to use) and washed with 3 × 600 μl water. The effluent and washings were collected into a scintillation vial and 10 ml Unisolve® solution added. Radioactivity was determined by scintillation counting. Protein in the soluble suspension was estimated by the method of Lowry *et al.* [7] and results of tyrosine hydroxylation determination were expressed as nmoles H_2O formed/hour/mg protein.

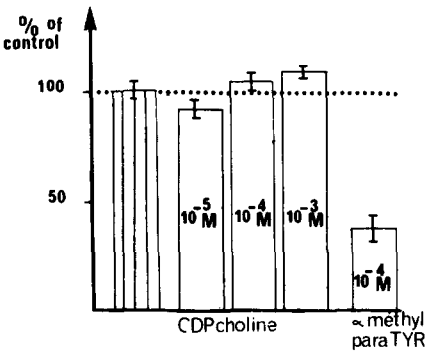

Fig. 1. The *in vitro* effect of CDPcholine on striatal tyrosine hydroxylase measured *in vitro*. CDPcholine (10^{-5} to 10^{-3} M) was added to standard incubation mixture containing 0.5 to 1.0 mg protein of the rat striatum homogenate, 15 μM [^{3}H]tyrosine, 1.1 mM $DMPH_4$, 50 mM 2 mercaptoethanol, 0.435 mM $FeSO_4$, in a Tris–acetate 0.11 mM pH 6.1 buffer. After 20 min incubation at 37° the reaction was stopped by addition of 50 μl acetic acid. The tritiated water formed from 3,5-ditritiotyrosine was separated through a Dowex 50 H^+ form column. α-Methyl paratyrosine (10^{-4} M) was used as the reference inhibitor. Results are expressed as percentage of control values.