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The effect of adrenalectomy on hepatic mixed function oxidase activity in female rats

JEFFREY R. FRY*, CLIVE G. WILSON, *Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Clifton Boulevard, Nottingham NG7 2UH*

It has been demonstrated that the livers of adrenalectomized male rats have an impaired ability to metabolize Type I drug substrates of the cytochrome P450-dependent mixed function oxidase (MFO) system whereas the ability to metabolize Type II substrates is unaffected (Kato & Gillette 1965; Marshall 1971; Stripp et al 1971). On the other hand, it has been reported that the livers of adrenalectomized female rats show no such defect in the ability to metabolize Type I substrates (Kato & Gillette 1965; Stripp et al 1971), and it has been suggested that this effect of adrenalectomy in male rats is mediated through impairment of the androgen-stimulating effects known to occur in male rats (Kato 1977). As the sex difference in drug metabolism is believed to be confined to the rat and, to a much lesser extent, the mouse, it has been suggested from the results with the female rats that humans with adrenal insufficiency are unlikely to have an impaired hepatic ability to metabolize drugs (Kato 1977). The male and female rats used in these early investigations were studied 4 days post adrenalectomy but as it is known that there are sex differences in the turnover of hepatic cytochrome P450 in the rat (female rats not showing the rapid first phase of cytochrome P450 degeneration observed in male rats, Levin et al 1975), it was considered possible that this period was too short to demonstrate conclusively the effect of adrenalectomy on the MFO system of female rats. We have therefore studied in-vivo and in-vitro certain aspects of the hepatic MFO system in female rats 7–8 days post adrenalectomy, using typical Type I (7-ethoxycoumarin (7EC), hexobarbitone (HB)) and Type II (*p*-chloro-*N*-methylaniline (PCMA)) substrates. We have also studied the longer-term effects of adrenalectomy, and for this reason some animals were maintained for 47–50 days post adrenalectomy before measurement of hepatic MFO activity.

Methods

Female [illegible] rats (initially ca 100 g) were obtained from the University of Nottingham Joint Animal Breeding Unit, Sutton Bonington, Leics, U.K. Bilateral adrenalectomy was performed under halothane anaesthesia and control rats were sham-operated under identical conditions. The animals were left for 7–8 or 47–50 days and throughout this time they were allowed free access to standard laboratory diet and 1% NaCl in the drinking water (to compensate for loss of sodium ions due to loss of adrenals and, hence, mineralocorticoid control). Liver 10 000 *g* supernatants and microsomal fractions were prepared as described previously (Litterst et al 1975; Fry 1981) and assayed for protein and cytochrome P450 content, and 7-EC *O*-de-ethylase and PCMA *N*-demethylase activities by published methods (Lowry et al 1951; Joly et al 1975; Greenlee & Poland 1978; Aitio 1978; Kupfer & Bruggeman 1966).

In the sleeping time studies the animals were injected i.p. with HB at 100 mg kg^{-1} in water adjusted to pH 10·2 with NaOH, and the sleeping time was recorded from the loss to the recovery of the righting reflex. Throughout the sleeping time the animals were maintained at 37 °C by being kept on a heated table.

Success of the adrenalectomy was checked by visual examination immediately after death. Statistical comparison of the results was performed by means of an unpaired Student's *t*-test (Campbell 1967).

* Correspondence.

Table 1. Body and liver weights and hepatic MFO activity of female Wistar rats 7–8 days after sham-operation ('Control rats') or adrenalectomy.

	Control rats	Adrenalectomized rats
Body weight (g)	126·5 ± 1·7 (17)	126·6 ± 1·2 (19)
Liver weight (g)	5·97 ± 0·17(12)	4·93 ± 0·14(12)***
Cytochrome P450 content		
(nmol g^{-1} liver)	4·50 ± 0·42(6)	3·00 ± 0·27(6)**
7-EC *O*-de-ethylase activity		
(nmol product g^{-1} liver min^{-1})	15·07 ± 2·43(6)	8·67 ± 1·47(6)*
(nmol product $nmol^{-1}$ P450 min^{-1})	3·48 ± 0·56	2·89 ± 0·46
PCMA *N*-demethylase activity		
(nmol product g^{-1} liver min^{-1})	92·6 ± 6·5(6)	61·2 ± 6·9(6)***
(nmol product $nmol^{-1}$ P450 min^{-1})	21·0 ± 1·3	20·3 ± 1·5
HB sleeping time (min)	43·2 ± 3·1(5)	72·4 ± 5·3(7)***

Values are mean ± s.e. with the numbers of rats given in brackets.
Test values significantly different from control values at $^{*}P < 0·05$; $^{**}P < 0·02$. $^{***}P < 0·01$.

Table 2. Body and liver weights and hepatic MFO activity of female Wistar rats 47–50 days after sham-operation ('Control rats') or adrenalectomy.

	Control rats	Adrenalectomized rats
Body weight (g)	186·7 ± 1·8	189·5 ± 8·0
Liver weight (g)	6·68 ± 0·25	6·10 ± 0·47
Cytochrome P450 content (nmol g^{-1} liver)	5·38 ± 0·77	6·08 ± 0·46
7-EC *O*-de-ethylase activity (nmol product g^{-1} liver min^{-1})	22·3 ± 2·2	25·4 ± 1·8
PCMA *N*-demethylase activity (nmol product g^{-1} liver min^{-1})	77·8 ± 3·8	71·9 ± 4·2

Values are mean ± s.e. of 6 rats.

Results and discussion

At 7–8 days post-operation, the adrenalectomized rats showed no change in body weight compared with the control rats, but there was a significant decrease in liver weight (Table 1), probably due to the loss of glycogen and associated water that occurs following adrenalectomy (Baxter & Forsham 1972). Adrenalectomy produced no change in the protein content of the microsomal fraction (data not shown) but the cytochrome P450 content was significantly decreased. Both enzyme activities were decreased following adrenalectomy when measured on a g liver basis and the magnitude of these decreases paralleled that for cytochrome P450 (P450, 33% decrease, 7-EC *O*-de-ethylase, 42% decrease, PCMA *N*-demethylase, 34% decrease). When the apparent turnover numbers (i.e. nmol product $nmol^{-1}$ P450 min^{-1}) of the enzymes were calculated it was observed that there were no significant differences between control and adrenalectomized rats, thus indicating that the loss in enzyme activity was due solely to the loss in cytochrome P450 content. The results also indicate that the loss in enzyme activity is not confined to either Type I or Type II substrates. Cytochrome P450-dependent MFO activity in-vivo was also decreased as judged by a prolongation of the HB sleeping time.

By 47–50 days post-adrenalectomy, the decreases in liver weight and hepatic MFO activity noted at 7–8 days post-adrenalectomy were completely reversed (Table 2).

In conclusion, these studies have clearly demonstrated that female rats, at 7–8 days post-adrenalectomy, have an impaired ability to metabolize drugs via the MFO system by virtue of a loss of hepatic cytochrome P450. It is probable that this loss of cytochrome is due, in turn, to an enhanced induction of haem oxygenase as has been recently reported for male rats (Sardana et al 1980). These decreases in hepatic cytochrome P450 content and related MFO activity are transient, the changes being reversed within 47–50 days after adrenalectomy. Whether this reversion of hepatic MFO activity to normal levels reflects adjustment of the animals to a lack of glucocorticoids or to the synthesis of glucocorticoids by extra-adrenal sites, remains to be determined.

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