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Oxidative drug metabolism in liver microsomes from uremic rats

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Oxidative drug metabolism in liver microsomes from uremic rats. Uremia was achieved by subtotal nephrectomy in 50 to 70 day old male rats weighing 252.0±20.1 g. Nephrectomized rats and sham-operated controls were sacrificed six days later. After liver microsomes had been isolated, microsomal cytochrome P-450 and cytochrome b_5 content as well as microsomal specific activity for *N*-demethylation of aminopyrine, *O*-demethylation of *p*-nitroanisole and *p*-hydroxylation of acetanilide were measured. Serum urea concentration rose to 370.0± 80.0 mg/100 ml. The serum creatinine rose to 4.8±1.2 mg/100 ml. In sham-operated controls serum urea and creatinine concentrations were approximately 50 and 0.6 mg/100 ml, respectively. Uremic rats, in contrast to sham-operated controls, lost about 25% of their initial body wt. In uremic rats and in sham-operated controls with caloric deficiency there was a significant decrease of total microsomal protein to 51% of the value measured in sham-operated controls fed ad libitum. Absolute and relative liver wet wt decreased more in caloric deficient controls than in uremic rats, whereas microsomal protein content per g liver was lower in uremic rats. In comparison with sham-operated controls, there was a pronounced decrease in the specific activity of liver microsomes from uremic rats for the demethylation of aminopyrine and *p*-nitroanisole and for the *p*-hydroxylation of acetanilide. The microsomal cytochrome P-450 content was also significantly diminished, whereas the microsomal cytochrome b_5 content was not influenced, but there was stimulation of the *O*-demethylation of *p*-nitroanisole to 125.1% when compared to controls fed ad libitum. Four intraperitoneal injections of 6 mg δ-aminolevulinic acid/kg body given to uremic rats normalized the cytochrome P-450 content and significantly stimulated the specific activity for demethylation of *p*-nitroanisole, whereas demethylation of aminopyrine, *p*-hydroxylation of acetanilide and cytochrome b_5 content were not altered. In sham-operated controls none of the measured parameters were influenced by δ-aminolevulinic acid pretreatment. It is assumed that the reduction of cytochrome P-450 content in liver microsomes from uremic rats is caused by a deficiency of δ-aminolevulinic acid which leads to disturbances in cytochrome synthesis. The observed decrease in the ability of liver microsomes from uremic rats to metabolize aminopyrine, *p*-nitroanisole and acetanilide is not merely due to reduced cytochrome P-450 content.

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Métabolisme oxidatif des médicaments dans les microsomes hépatiques de rats urémiques. Chez des rats mâles, agés de 50 à 70 jours poids 249,6±19,6 g, l'urémie a été obtenue par néphrectomie subtotale. Six jours plus tard les rats néphrectomisés et les contrôles ont été sacrifiés. La fraction microsomiale a été isolée par centrifugation de l'homogénat hépatique. Après quoi les auteurs ont mesuré le contenu microsomial en cytochrome P-450 et b_5 ainsi que l'activité spécifique de *N*-déméthylation de l'aminopyrine, de *O*-déméthylation du *p*-nitroanisole et de la *p*-hydroxylation de l'acetalinide. Pendant la période post opératoire l'urée sérique a atteint 370,0±80 mg/100 ml et la créatinine sérique 4,8±1,2 mg/100 ml chez les animaux néphrectomisés alors que les valeurs respectives étaient de 50 et 0,6 mg/100 ml chez les animaux contrôles sham opérés. A la différence des contrôles les rats urémiques avaient perdu environ 25,0% de leur poids corporel initial. Chez les rats urémiques comme chez les controles soumis à un déficit calorique il a été observé une diminution significative des protéines totales microsomiales à 51% de la valeur obtenue chez les contrôles nourris ad libitum. Le poids du foie, absolu et relativ a plus diminué chez les contrôles dénutris que chez les rats urémiques alors que les protéines microsomiales par g de foie étaient inférieures chez les rats urémiques. Par comparaison avec les contrôles il a été observé une diminution importante de l'activité spécifique des microsomes hépatiques des rats urémiques pour la déméthylation de l'aminopyrine, la déméthylation du *p*-nitroanisole et pour la *p*-hydroxylation de l'acétalinide. Le contenu microsomial en cytochrome P-450 avait aussi diminué significativement alors que le contenu microsomal en cytochrome b_5 n'était pas modifié. Chez les contrôles dénutris la déméthylation de l'aminopyrine, la *p*-hydroxylation de l'acétalinide et le cytochrome P-450 microsomial n'étaient pas modifiés mais il y avait une stimulation de la *O*-déméthylation du *p*-nitroanisole à 195,1% par comparaison aux contrôles nourris ad libitum. Quatre injections intrapéritonéales de 6 mg d'acide δ-aminolévulinique par kg de poids chez les rats urémiques ont normalisé le contenu en cytochrome P-450 et stimulé significativement l'activité spécifique de la déméthylation du *p*-nitroanisole alors que la déméthylation de l'aminopyrine, la *p*-hydroxylation de l'acétanilide et la contenu en cytochrome b_5 n'étaient pas modifiés. Chez les contrôles aucune de ces activités n'a été influencée par l'acide δ-aminolévulinique. Il est supposé que la réduction du contenu en cytochrome P-450 des microsomes hépatiques des rats urémiques est déterminée par un déficit en δ-ALA qui conduit à une perturbation de la synthèse du cytochrome. La diminution de la cpacité des microsomes hépatiques à métaboliser l'aminopyrine, le

p-nitroanisole et l'acétalinide n'est pas seulement liée à la diminution du contenu en cytochrome P-450.

Previously published investigations in uremic male rats have reported a decrease in the specific activity of liver microsomes for demethylation of aminopyrine and a diminished microsomal cytochrome P-450 content in the absence of other functional alterations of the endoplasmic reticulum of the liver. There was no interference with glucuronidation of 4-methylumbelliferone, glucose-6-phosphatase activity or microsomal cytochrome b_5 content [1, 2]. From these results it can be concluded that uremia causes a specific disturbance of microsomal mixed-function oxidation, which plays a basic role in oxidative drug metabolism. Results of Kato and Gilette [3] Castro et al [4] and Leber, Degkwitz and Staudinger [5] have demonstrated that in many abnormal physiological states such as starvation, adrenalectomy and scurvy, the activity of the mixed-function oxidation system is influenced.

Despite the well known species and sex differences in oxidative drug metabolism, the results depend on the substrates and pathways studied. According to the findings of Kato and Gilette [3], starvation of male rats for three days leads to a decrease in the specific activity of liver microsomes for demethylation of aminopyrine and hydroxylation of hexobarbital, but stimulates *O*-demethylation of *p*-nitroanisole and *p*-hydroxylation of aniline. Adrenalectomy of male rats diminished the demethylation of aminopyrine and the hydroxylation of hexobarbital in liver microsomes, whereas the metabolism of aniline and zoxazolamine is not altered. Furthermore, liver microsomes from male rats exposed to an atmosphere containing 5% O_2 for three hours had decreased activities for the metabolism of aminopyrine and hexobarbital, but *p*-hydroxylation of aniline was not influenced. Similar alterations were found in liver microsomes from male rats after castration. Lastly, differences in the decreased metabolism of coumarin [6], acetanilide [7] and aminopyrine [5] have been observed in scorbutic male guinea pigs.

Considering these results it seemed necessary to determine whether uremia altered the demethylation of aminopyrine solely or whether it also altered the metabolism of other substrates undergoing mixed-function oxidation. Therefore, we measured the *O*-demethylation of *p*-nitroanisole and *p*-hydroxylation of acetanilide in addition to the demethylation of aminopyrine and the microsomal P-450 content in liver microsomes from nephrectomized male rats. In an earlier publication [8] we reported that in male nephrectomized rats pretreated for five days with phenobarbital, there was stimulation of the *N*-demethylation of aminopyrine to a level that was similar to that in normal controls.

The stimulatory influence of phenobarbital on mixed-function oxidation takes place at different sites of the reaction chain. Phenobarbital stimulates the synthesis of cytochrome P-450 by increasing heme synthesis. In rats treated with phenobarbital there is an increase in the activity of microsomal flavoprotein TPNH-cytochrome c reductase which is at least partly responsible for reduction of the substrate-cytochrome P-450 complex. In addition the substrate-binding capacity of cytochrome P-450 is augmented and the initial rate of substrate-induced P-450 reduction by NADPH is enhanced.

Since a diminished cytochrome P-450 content was found in liver microsomes of uremic rats, studies were intiated to determine whether the decreased activity of the mixed-function oxidation system was due to a disturbance in the synthesis of cytochrome P-450 which is regulated by the extent of heme synthesis [17]. A regular synthesis of the heme percursor δ-aminolevulinic acid from glycine and succinyl-CoA is necessary for normal heme synthesis. Phenobarbital stimulates synthesis of δ-aminolevulinic acid and heme by inducing the rate-limiting enzyme, δ-aminolevulinic acid synthetase.

The present investigation was designed to determine whether the decreased microsomal cytochrome P-450 content and mixed-function oxidation activity in uremic rats that can be stimulated by pretreatment with phenobarbital is due to disturbances in heme synthesis caused by a deficiency of δ-aminolevulinic acid. To clarify this question nephrectomized rats and controls were pretreated with δ-aminolevulinic acid.

Methods

Male SIV rats (Ivanovas, Kislegg, Allgau) 50 to 70 days old weighing approximately 250 g were housed four to six per cage and fed a commercial diet (Altromin standard) ad libitum. Uremia was achieved by subtotal nephrectomy. Anesthesia was initiated by intraperitoneal injection of hexobarbital-Na, 10 mg per 100 g body wt and continued with diethylether. After opening the abdomen the right kidney was completely extirpated. Enough of the left kidney was removed to leave 12 to 15% of the original weight of both kidneys together. During nephrectomy both adrenals were left intact. In the control animals that were sham-operated, both kidneys were revolved one after the other and replaced in the abdomen. The incisions were sutured as in the nephrectomized animals. The remaining remnant of the left kidney was treated with Histoacryl (Fa. Braun, Melsungen) to prevent bleeding. In the sham-operated controls, Histoacryl was layed on identical parts of the decapsulated left kidney. Use of an additional group of control animals was necessary because the nephrectomized rats, in contrast to sham-operated controls, exhibited a decrease in body wt during the postoperative phase. A second group of sham-operated controls were given a restricted amount of food so that they lost an equal amount

of body wt during the same time interval as nephrectomized rats. Thus, the non-specific influence of a loss in body wt could be distinguished from the more specific influence of uremia.

To determine the effect of δ-aminolevulinic acid on oxidative drug metabolism, uremic rats and sham-operated controls were treated with four intraperitoneal injections of δ-aminolevulinic acid (6 mg/kg body wt) 48, 36, 24, and 12 hr before sacrifice.

Six days after surgery the uremic rats and the control rats were sacrificed by exsanguination under ether anesthesia. Blood was collected for estimation of serum urea and creatinine.

Isolation of liver microsomes. Livers were removed and homogenized in a Potter homogenizer with five volumes of 0.1 M Tris-HCl buffer (pH 7.4) containing 0.25 M saccharose [5]. Cell debris, nuclei and mitochondria were removed by a 20 min centrifugation at 12000 × G using an SS-34 rotor in a Servall refrigerated centrifuge. The supernatant was centrifuged for 60 min at 105000 × G in a Spinco L 50 centrifuge. The microsomal pellet was suspended in 0.15 M KCl and recentrifuged to remove adventitious hemoglobin. The washed microsomal fraction was suspended in 0.1 M Tris-HCl buffer at pH 7.4 (0.5 ml/g liver).

Estimation of microsomal protein. The protein content of the liver microsomal suspension was estimated by using the biuret reagent method of Beisenherz et al [9]. The accompanying non-specific turbidity was eliminated by adding KCN [10].

Enzyme assays: N-demethylation of aminopyrine. The reaction mixture contained in a final volume of 1.5 ml included 0.15 M phosphate buffer (pH 7.4), 0.5 mM NADP, 5 mM isocitrate, 0.03 ml isocitratedehydrogenase (20 mg protein/2 ml), 4 mM $MgCl_2$ and 5 mM aminopyrine. The final microsomal protein content was 2 mg/ml. Blanks contained equivalent volumes of buffer in place of the aminopyrine. After a five minute incubation at 37° C, the reaction was terminated by the addition of 0.5 ml of 10% trichloroacetic acid. The precipitated protein was removed through centrifugation. Formaldehyde in the supernatant derived from aminopyrine during the incubation was estimated using the method of Nash [13].

p-Hydroxylation of acetanilide. The reaction mixture contained in a final volume of 1 ml included 0.1 M Tris-HCl buffer (pH 7.4), 2 mg microsomal protein, 8 mM acetanilide and a NADPH generating system consisting of 2 mM NADP, 20 mM isocitrate, 4 mM $MgCl_2$ and 0.12 ml isocitratedehydrogenase (20 mg/2 ml). After a 15 min incubation at 37° C, the reaction was stopped by adding 2.0 ml of distilled water at 80° C. The protein was precipitated by incubation at 80° C for three min. After centrifugation the supernatant was acidified with sulfuric acid and saturated with NaCl. The phenols formed during the incubation period were extracted into peroxide-free diethylether. The ether phase was evaporated and the dry residue dissolved in 0.2 ml diethylether. The formation of *N*-acetyl-*p*-aminophenole was estimated after separation of the reaction products by thin layer chromatography (Kieselgel HF_{254}, Merck, Darmstadt) as described by Degkwitz and Staudinger [7]. Blanks contained equivalent volumes of buffer in place of acetanilide. To inhibit esterase activity of liver microsomes, paraoxon in a final concentration of 10^{-5} M was added to the reaction mixture.

O-demethylation of p-nitroanisole. The reaction mixture consisted of 10^{-3} M *p*-nitroanisole dissolved in 0.15 M phosphate buffer (pH 7.85), 2 mg microsomal protein, an NADPH generating system (0.5 mM NADP, 5 mM isocitrate, 0.03 ml isocitratedehydrogenase (20 mg/2 ml), 4 mM $MgCl_2$) and enough buffer to give a final volume of 2 ml. The initial velocity of *p*-nitrophenole formation was measured according to the method of Netter and Seidel [11] following the increase in absorbancy at 405 mμ at 37° C. Nonenzymatic *p*-nitrophenole formation was negligible.

Estimation of microsomal cytochromes. The cytochrome P-450 content was determined measuring the dithionite reduced plus CO minus dithionite reduced difference spectrum of liver microsomes from 450 to 490 mμ as described by Omura and Sato [14]. The microsomal cytochrome b_5 content was determined from the NADH reduced minus oxidized difference spectrum from 425 to 409 mμ according to Omura and Sato [14]. The final protein concentration in the sample and reference cuvette was 0.5 to 1.0 mg/ml. Each measurement was done in duplicate, the second time with one-half the protein concentration.

Statistical evaluation. The X-test of Van der Waerden and Nievergelt was used [12]. Differences were assumed to be significant when P was less than 0.01.

Specific enzyme activities. Specific enzyme activities were expressed as nmole product × mg microsomal protein^{-1} × min^{-1}. Cytochrome P-450 content was expressed as $OD_{450-490} \times$ mg protein$^{-1} \times$ cm^{-1}, cytochrome b_5 content as $OD_{425-409} \times$ mg protein$^{-1} \times$ cm^{-1}.

Urea and creatinine concentration. Measurements were carried out in an autoanalyzer utilizing standard methods.

Results

Table 1 shows the effect of nephrectomy on body weight, absolute and relative wet weight of liver, absolute and relative yield of microsomal protein and serum creatinine and urea concentration in male rats. As mentioned previously the rise in concentration of serum urea and creatinine was accompanied by a considerable loss of body weight (up to 75%), absolute wet weight of liver and relative wet weight of liver per 100 g body weight. The latter finding was also observed in caloric deficient controls. In

Table 1. Characteristic observations in control and uremic rats[a]

Observations	Control rats $N=25$	Rats with caloric deficiency $N=25$	Rats with uremia $N=25$
Initial body wt, *g*	250.5 ±16.8	249.6 ±19.6	252.0 ±20.1
Final body wt, *g*	252.2 ±22.7 ×	193.7 ±13.5°	188.5 ±15.6°
Liver wet wt, *g*	11.3 ± 1.5 ×	5.9 ± 0.4°	7.0 ± 0.7 +
g liver/100 g body wt	4.5 ± 0.5 ×	3.0 ± 0.4°	3.7 ± 0.3 +
Total microsomal protein, *mg*	141.9 ±25.3 ×	69.2 ±13.7°	72.3 ±16.2°
mg microsomal protein/g liver	13.2 ± 1.1 ×	11.7 ± 0.9°	10.3 ± 1.0 +
Serum urea, *mg/100 ml*	50.0 ± 3.2 ×	52.1 ± 4.8 ×	370.0 ±80.4°
Serum creatinine, *mg/100 ml*	0.6 ± 0.1 ×	0.5 ± 0.2 ×	4.8 ± 1.2°

[a] $P<0.01$ when × is compared with °, and ° is compared with +.

addition, in comparison to the normal rats, there was a 51% decrease of the total microsomal protein in uremic rats as well as in the caloric deficient controls. This was due to a loss in the wet weight of the liver as well as to a decrease in microsomal protein/g liver. In caloric deficient controls there was a more severe decrease in absolute and relative liver wet weight when compared to uremic rats. In uremic rats the microsomal protein content per g liver was reduced more than in the controls with caloric deficiency.

Table 2. Daily food consumption per animal before operation and up to the sixth day of the postoperative period[c]

Experimental groups	Before surgery	After surgery	% of control
Controls[a], *g* $N=25$	25.2±2.3 ×	24.9±2.0 ×	100
Uremic rats[a], *g* $N=25$	25.8±3.0 ×	4.4±1.0°	17.7
Caloric defiency[b], *g* $N=25$	25.0±2.4 ×	3.0±0.3 +	12.0

[a] Spontaneous food consumption.
[b] Rats received only the quantity of food indicated in the table.
[c] $P<0.01$ when × compared with °, and ° compared with +.

As demonstrated in Table 2, the loss of body weight was caused to a high degree by a reduction in spontaneous food consumption following nephrectomy, which was not observed in sham-operated controls. To cause a decrease in body weight in sham-operated controls that was similar to that of the nephrectomized animals, their daily food consumption must not exceed approximately 68% of the quantities measured in uremic animals. Whenever sham-operated animals received the same amount of food as nephrectomized rats, their body weight diminished less, but the reduction was still significant during the postoperative period (approximately 10 to 14%/six days).

Table 3 shows that the loss of body weight observed in caloric deficient controls alone did not influence the specific activities of *N*-demethylation of aminopyrine and *p*-hydroxylation of acetanilide, nor the microsomal cytochrome P-450 and b_5 content, whereas a 25% increase in the specific activity for *O*-demethylation of *p*-nitroanisole

Table 3. Microsomal cytochrome content and specific enzyme activities in liver microsomes from uremic and control rats: Influence of δ-ALA pretreatment[b]

Observations	Cytochrome P-450[c]	Cytochrome b_5[d]	Aminopyrine	*p*-Nitroanisole	Acetanilide
Controls fed ad libitum	0.062±0.005 × $N=25$	0.079±0.002 × $N=25$	8.0±0.8 × $N=10$	2.03±0.2 × $N=25$	0.88±0.08 × $N=15$
Controls with caloric deficiency	0.059±0.004 × $N=25$	0.082±0.005 × $N=25$	7.6±0.6 × $N=10$	2.54±0.3° $N=25$	0.92±0.07 × $N=15$
Uremic rats	0.042±0.005° $N=25$	0.076±0.006 × $N=25$	5.2±0.7° $N=10$	1.27±0.3°° $N=25$	0.61±0.09° $N=15$
Uremic rats + δ-ALA	0.058±0.006 × $N=20$	0.078±0.008 × $N=20$	5.9±0.6 + $N=10$	1.66±0.2 ++ $N=20$	0.70±0.08 + $N=10$
Controls fed ad libitum + δ-ALA	0.061±0.006 × $N=20$	0.083±0.007 × $N=20$	7.8±0.9 × $N=10$	2.14±0.3 × $N=20$	0.90±0.07 × $N=15$
Controls with caloric deficiency + δ-ALA	0.060±0.006 × $N=20$	0.080±0.010 × $N=20$	7.7±0.7 × $N=10$	2.50±0.2° $N=20$	0.91±0.06 × $N=10$

[a] $P<0.01$ when × compared with °, × with +, × with °°, × with ++, °° with ++, ° with °°, and ° against ++; $P<0.05$ when ° compared with +.
[b] Specific enzyme activities: nmoles product × mg microsomal protein^{-1} × min^{-1}.
[c] Cytochrome P-450: $OD_{450-490}$ × mg microsomal protein^{-1} × cm^{-1}.
[d] Cytochrome b_5: $OD_{425-409}$ × mg microsomal protein^{-1} × cm^{-1}.

was achieved. In contrast there was a significant decrease in microsomal cytochrome P-450 content and specific activities for the *N*-demethylation of aminopyrine, the *p*-hydroxylation of acetanilide and the *O*-demethylation of *p*-nitroanisole in liver microsomes from nephrectomized rats. The cytochrome b_5 content remained unchanged as noted previously [1].

The results of pretreatment with δ-aminolevulinic acid in uremic rats are also shown in Table 3. The diminished cytochrome P-450 content returned to normal levels. The *O*-demethylation of *p*-nitroanisole was significantly stimulated but was not returned to normal levels. The *N*-demethylation of aminopyrine and the *p*-hydroxylation of acetanilide were not significantly altered and cytochrome b_5 content was not influenced. Pretreatment of control animals did not alter the measured enzyme activities and cytochromes. Wet weight of liver, microsomal protein content and other parameters shown in Table 1 were not influenced in any group.

Discussion

As stated previously (1, 2) the loss of body weight following nephrectomy was a specific effect of uremia and did not appear to be due to the anesthesia or the operation itself. The decrease of body weight would not appear to be solely the result of a reduced food consumption. In uremic rats additional factors such as diminished food utilization and/or an increased rate of metabolic turnover probably occured. The deficiency in daily caloric consumption had different effects in sham-operated controls when compared to nephrectomized animals. Although both groups of animals had lost the same amount of body weight, caloric deficiency alone caused a more severe loss of absolute and relative wet weight of liver, whereas the amount of microsomal protein per g liver was higher than in uremic rats. Obviously, in uremic animals the wet cot of the liver decreased more by a decrease in the microsomal protein than in controls fed restricted food quantities.

Further studies are necessary to clarify why the protein content of liver microsomes from uremic rats is decreased. Preliminary results from our laboratory suggest that both protein synthesis and protein degradation are altered in liver microsomes from uremic rats (unpublished observations). The results mentioned above lead to the conclusion that the metabolic changes in the liver of uremic rats are not the same as those in livers of sham-operated controls fed reduced quantities of food.

There existed pronounced differences in the specific enzyme activities and microsomal P-450 content per mg microsomal protein between uremic rats and animals kept on a caloric deficient diet. Caloric deficiency itself did not alter the microsomal cytochrome P-450 and b_5 content and the specific activities for demethylation of aminopyrine and *p*-hydroxylation of acetanilide, but the specific activity of the *O*-demethylation of *p*-nitroanisole was significantly increased by approximately 25%. In contrast there was a decrease of the microsomal cytochrome P-450 content and of all measured specific enzyme activities in uremic rats. Thus, the capacity of the liver to metabolize substrates by the mixed-function oxidation reaction system is limited by the specific enzyme activities (per mg microsomal protein) and the total amount of microsomal protein in the liver. From the decrease of liver microsomal protein to 51% and the reduction of specific enzyme activity to an average of 65% of the value measured in control rats fed ad libitum, it can be concluded that the *in vivo* metabolism of substrates per minute through the mixed-function oxidation pathway should theoretically decrease to 32% of the value obtained in normal rats. No data concerning this fact have been available until now. Additional experiments are now in progress to determine whether this value is correct or if additional *in vivo* factors must be considered.

These experiments have demonstrated that in liver microsomes from uremic rats not only the specific activity for *N*-demethylation of aminopyrine was decreased but that the specific activities for *O*-demethylation of *p*-nitroanisole and the hydroxylation of acetanilide were decreased. Aminopyrine and *p*-nitroanisole belong to the type I substrates which means that after addition of these substrates to liver microsomes in the difference spectrum there is a peak at 380 to 390 mμ and a trough at 420 to 430 mμ. In contrast, when acetanilide is added to rat liver microsomes, there is a modified type II difference spectrum, which means that a peak arises at 425 to 430 mμ and a trough at 380 to 390 mμ [15, 16].

Aminopyrine metabolism in rats is inducible by pretreatment with phenobarbital while *p*-hydroxylation of acetanilide is inducible by pretreatment with 3-methylcholanthrene and similar substances. The *O*-demethylation of *p*-nitroanisole can be stimulated by both groups of inducers. Our experiments have shown that the decreasing effect of uremia on oxidative drug metabolism in rat liver microsomes extended to all types of substrates undergoing mixed-function oxidation. This observation contrasts with the above mentioned results derived from less physiological states such as adrenalectomy, castration and starvation where the metabolism of either type I or type II substrates is usually altered while that of the other type is not influenced.

The microsomal cytochrome P-450 content and the specific activities for *p*-hydroxylation of acetanilide, *O*-demethylation of *p*-nitroanisole and *N*-demethylation of aminopyrine in microsomes from uremic rats were decreased to approximately 62 to 69% of the value measured in control animals fed ad libitum. This indicates that in liver microsomes from uremic rats oxidative drug metabolism was influenced relatively uniformly despite the difference in the types of substrates measured.

Earlier results have shown that microsomal mixed-function oxidation and cytochrome P-450 content in uremic rats can be stimulated as in normal rats by pretreating the animals with phenobarbital [8]. It is assumed [17] that phenobarbital augments cytochrome P-450 content by inducing the rate limiting enzyme in heme synthesis, δ-aminolevulinic acid synthetase, leading to a stimulation of δ-aminolevulinic acid synthesis and thus to an increase in heme synthesis. From Table 3 it can be seen that the decreased cytochrome P-450 content in liver microsomes from uremic rats could be normalized by pretreatment with δ-aminolevulinic acid during the last 48 hours before the animals were sacrificed. It is therefore concluded that the diminished cytochrome P-450 content is due to a disturbance in heme synthesis caused by a deficiency of δ-aminolevulinic acid in liver microsomes from uremic rats. In preliminary experiments with rats in which uremia was achieved by the ligation of both ureters, the specific activity of liver microsomes to demethylate aminopyrine and the microsomal cytochrome P-450 content was decreased as in liver microsomes from subtotally nephrectomized animals (unpublished observations). Thus, the disturbance in mixed-function oxidation is apparently due to the uremic condition *per se* and not to the reduction in kidney mass. Whether a deficiency of δ-aminolevulinic acid is also important with regard to uremia-induced anemia is not yet known.

Although microsomal cytochrome P-450 content remained normal when the uremic animals were pretreated with δ-aminolevulinic acid, such was not the case with oxidative drug metabolism. There was a slight but significant stimulation of specific activity for *O*-demethylation of *p*-nitroanisole, whereas *N*-demethylation of aminopyrine and *p*-hydroxylation of acetanilide were not influenced. These findings suggest that the decreased activity of the mixed-function oxidation system in liver microsomes from uremic rats is not merely due to the simultaneously observed reduction of cytochrome P-450 content.

According to traditional opinion, the mixed-function oxidation system consists of a reaction chain with several more or less independent links such as oxidation of cytochrome P-450, binding of substrates to oxidized cytochrome P-450 and reduction of the cytochrome P-450-substrate complex either directly or through the participation of microsomal flavoprotein NADPH-cytochrome c-reductase. The fact that phenobarbital pretreatment of uremic rats not only stimulates microsomal cytochrome P-450 content but also stimulates the substrate metabolism above the level obtained with untreated normal rats in contrast to pretreatment with δ-aminolevulinic acid, demonstrates that phenobarbital stimulates other links of the reaction chain mixed-function oxidation responsible for the uremia-induced decrease of oxidative drug metabolism. Further experiments are necessary to establish the site where uremia exerts its influence on the mixed-function oxidation system.

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