Short-term starvation increases calcidiol-24-hydroxylase activity and mRNA level in rat kidney

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The renal mitochondrial calcidiol-24-hydroxylase activity and the corresponding cytochrome P-450 mRNA level were measured in rats subjected to short-term starvation alone or in combination with calcitriol treatment. Short-term starvation of 24 and 48 h increased the mRNA level by five- and sixfold, respectively. The 24-hydroxylase activity increased by five- and threefold, respectively. Treatment with calcitriol markedly increased the enzyme activity about 20-fold and the mRNA level about sixfold. In rats subjected to calcitriol treatment combined with 24 h of starvation, a significant further increase in enzyme activity was observed. The mRNA levels increased but the difference was not significant statistically. The results indicate that the mechanism by which starvation stimulates the enzymes is different, at least in part, from that behind the stimulatory effect of calcitriol.

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The quantitatively most important serum calcitriol metabolite, calcidiol, is hydroxylated in the kidney to form the biologically active form calcitriol, (24R)-hydroxycalcidiol or (23R)-hydroxycalcidiol (1). The possible biological roles of the two latter compounds are not clear and the possibility has been discussed that they are physiologically unimportant products of a degradative pathway (1). The calcidiol-24- and 1α-hydroxylases are regulated in a reciprocal fashion in the rat (2) and a similar situation seems to exist between the 23- and the 1α-hydroxylases in the guinea pig (3). The 1α- and 24-hydroxylations are regulated strictly by several factors, the most important being parathyroid hormone (PTH), serum phosphate and calcitriol itself (1). The mitochondrial 24-hydroxylase is a mixed-function oxidase containing a specific species of cytochrome P-450, ferredoxin and ferredoxin reductase. The cDNA of the cytochrome P-450 component was cloned recently (4) and the structure of the gene clarified (5). It was shown to belong to a new cytochrome P-450 family with only 30% homology with previously reported cytochrome P-450s. We have observed previously that the guinea pig renal 23- and 24-hydroxylase activities can be stimulated markedly by starvation (3) but the regulatory mechanism for this is not known. In the present study it is shown that starvation stimulates the renal 24-hydroxylase also in the rat and that the increase in activity is paralleled with a similar increase in mRNA level. For reasons of comparison, we studied also the effect of calcitriol treatment on the cytochrome P-450_{24} mRNA level and enzyme activity. The effect of starvation on a possible regulatory factor, serum levels of calcitriol, also was determined.

Materials and methods

Animals

The studies were performed on adult male rats of the Sprague-Dawley strain weighing about 200 g at the beginning of the experiments. The animals were fed a commercial standard pellet diet containing 1.0% calcium, 0.7% phosphate and 1500 IU calcitriol/kg.

In the short-term starvation experiments, animals were left for 24, 48 or 72 h without food but with free access to water. Treatment with calcitriol was given as ip injections (1 µg/kg +body wt.day⁻¹) on three consecutive days (3). All animals appeared healthy during the experiments.

Methods

The 24-hydroxylase activity was measured in kidney mitochondria prepared as described in Ref. 6. The mitochondrial pellet was resuspended in an incubation medium consisting of 50 mmol/l TRIS/acetate buffer (pH 7.4), 10 mmol/l MgCl₂, 12 mmol/l isocitrate and 2.5 µg of diphenyl-p-phenylenediamine. Incubations were performed at 37°C for 30 min with 10 µg of
calcidiol. The reaction was terminated by the addition of 1 ml of acetonitrile and 4000 cpm of $[^{3}H](24R)$-hydroxycalcidiol (3, 6). Quantitation of the product formed was performed by straight-phase HPLC (8% propan-2-ol in hexane) after isolation by Sep-Pak C$_{18}$ and reversed-phase HPLC (10% water in methanol) (3). Recovery was generally of the order of 40%. Protein was determined by the method of Lowry et al. (7).

Total cellular RNA was isolated from rat kidney slices by the LiCl–urea method (8). Electrophoresis of total RNA in agarose gels containing formaldehyde and blotting of the separated RNA onto nylon membranes (Hybond N, Amersham, UK) was carried out by standard procedures (9). For the hybridization, cDNA probes for rat kidney 24-hydroxylase (4) and human glyceraldehyde 3-phosphate dehydrogenase (10) were labelled with $^{32}$P using the Pharmacia Oligolabelling kit (Pharmacia, Uppsala, Sweden). Hybridization of the blots with labelled probes was done according to Gehring et al. (11), and the blots were exposed thereafter to Fuji New RX X-ray films at $-70^\circ$C. The mRNA was estimated by densitometry and the data expressed as the ratio between 24-hydroxylase mRNA and glyceraldehyde 3-phosphate dehydrogenase mRNA. The different treatments did not affect significantly the levels of glyceraldehyde 3-phosphate dehydrogenase mRNA.

Serum levels of calcitriol were determined using a commercial radioreceptor assay from INC STAR (Stillwater, MN) using a calf thymus receptor. Serum levels of vitamin D-binding protein (DBP) were determined by quantitative rocket immunoelectrophoresis (12). The electrophoresis was carried out on

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**Fig. 1.** The effect of short-term starvation on the renal 24-hydroxylase activity (c.) and the kidney cytochrome P-450$_{c24}$ mRNA level (■) level. Measurements of enzyme activity and cytochrome P-450 mRNA are described in the text. The means of 6–9 animals are given and expressed as a percentage of the control. Asterisks represent significant differences compared to control animals.

**Fig. 2.** Effects of starvation in combination with calcitriol treatment on the 24-hydroxylase activity (A) and the cytochrome P-450$_{c24}$ mRNA level (B) in rat kidney. contr: control rats, starv: starved rats. 24 h: 1,25; calcitriol-treated rats: 1,25 + starv: calcitriol-treated rats starved for 24 h. Treatment and assay of enzyme activity and mRNA are described in the text. Data are given as means of 5–9 animals and expressed as a percentage of the control. Asterisks represent significant differences compared to control animals.
Table 1. Serum levels of calcitriol and vitamin D-binding protein (DBP) in starved rats.

<table>
<thead>
<tr>
<th></th>
<th>Calcitriol (ng/l)</th>
<th>DBP (arb. units)</th>
<th>“Free calcitriol index”</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>65 ± 3</td>
<td>0.33 ± 0.02</td>
<td>0.18 ± 0.02</td>
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<tr>
<td>(N = 5)</td>
<td></td>
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<tr>
<td>Starved, 24 h</td>
<td>54 ± 3</td>
<td>0.28 ± 0.01*</td>
<td>0.19 ± 0.01</td>
</tr>
<tr>
<td>(N = 8)</td>
<td></td>
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<tr>
<td>Starved, 48 h</td>
<td>30 ± 2*</td>
<td>0.19 ± 0.01*</td>
<td>0.16 ± 0.01</td>
</tr>
<tr>
<td>(N = 4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Starved, 72 h</td>
<td>39 ± 3*</td>
<td>0.22 ± 0.01*</td>
<td>0.18 ± 0.06</td>
</tr>
<tr>
<td>(N = 6)</td>
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*p < 0.05 compared to control rats.

agarose gels containing 2% of an anti-rat DBP serum (13). A pooled control rat serum was used as standard. The results are expressed in arbitrary units. The non-protein-bound fraction of calcitriol was expressed as the ratio between calcitriol and DBP, in analogy with “the free calcitriol index” (14).

Data are given as means ± s.e.m unless stated otherwise. The Kruskal–Wallis test and the Mann–Whitney U test were used for evaluation of the data.

Results

The effect of short-term starvation was studied both on 24-hydroxylase activity and cytochrome P-450_{cc24} mRNA levels. The study included four groups of rats: control rats (N = 6) and rats starved for 24 h (N = 9), 48 h (N = 6) and 72 h (N = 6) (Fig. 1). All the starved groups had significantly increased mRNA levels and 24-hydroxylase activities compared to the control group. The maximal 24-hydroxylase activity was observed at 24 h of starvation and it preceded the maximal mRNA level, which was at 48 h. By 72 h of starvation both parameters had decreased but were still elevated compared to the control level.

In rats treated with calcitriol (N = 6), the 24-hydroxylase activity increased about 20-fold and the mRNA level by about sixfold compared to the control (Fig. 2). When this treatment was combined with 24-h starvation (N = 5), enzyme activity increased significantly from 2292 ± 293% to 3949 ± 608% (p < 0.01). The mRNA levels increased from 664 ± 135% to 1015 ± 156%. However, this latter increase did not reach statistical significance (Fig. 2).

A possible regulatory factor, serum calcitriol, also was measured in starved rats (Table 1). Starvation significantly decreased the total circulating levels of calcitriol. However, the DBP levels decreased in parallel, resulting in an unchanged “free calcitriol index”.

Discussion

The present study demonstrates a stimulatory effect of starvation on both the cytochrome P-450_{cc24} mRNA level and the 24-hydroxylase activity in rat kidney. We have observed previously that the guinea pig renal 23-hydroxylase activity is stimulated markedly by starvation (3). In the guinea pig, 23-hydroxylation is more dominant than 24-hydroxylation and stimulation of 23-hydroxylation is accompanied by a decrease in 1α-hydroxylation (3). The cDNA corresponding to the cytochrome P-450_{cc24} has not been cloned yet but there are reports indicating that the 24- and the 23-hydroxylases are closely related (15). Increased 24-hydroxylase activity in starved rats has been observed (Dr M Warner, unpubl. obs.) but to our knowledge no studies have been published.

Calcitriol is a well-known inducer of 24-hydroxylase activity. Ohshima et al. showed initially that the cytochrome P-450_{cc24} mRNA increased after calcitriol administration (14). Armbrecht and Boltz reported recently that cytochrome P-450_{cc24} mRNA was increased 3 h after a single high-dose injection of calcitriol (16). The present study confirms the previous reports that the mRNA level increases after calcitriol treatment (16, 17). The treatment increased enzyme activity about 20-fold whereas the mRNA level only increased about sixfold. In the starvation experiments the increase in enzyme activity and mRNA level were in the same range. This may indicate that a mechanism other than increased protein synthesis is involved in the stimulatory effect of calcitriol. When 24-h starvation was added to the treatment with calcitriol, a significant further increase in enzyme activity was observed. The mRNA level also increased but the difference did not reach statistical significance. That the stimulatory mechanism of starvation is different from that of calcitriol was supported also by the finding that the serum “free calcitriol index” was unchanged by starvation.

In order to investigate further the stimulatory mechanism of starvation, we attempted also to study the effects of starvation in diabetic rats. It has been reported that vitamin D metabolism is altered in diabetes, with enhanced 24-hydroxylation and depressed 1α-hydroxylation (18). According to a recent report by Armbrrecht et al., there is an eightfold increase in the cytochrome P450_{cc24} mRNA level in diabetic rats (17). In preliminary experiments we have been able to confirm a two- to threefold increase in 24-hydroxylase activity and an eightfold increase in the mRNA level in diabetic rats. However, we were unable to get reproducible results from streptozotocin-induced diabetic rats who were additionally subjected to starvation. The data obtained had a large interindividual variation and no conclusion could be made on whether starvation could stimulate further the 24-hydroxylase in diabetic rats. Theoretically, an involvement of insulin and/or insulin-like growth factor I (19) in the regulation of vitamin D metabolism in both starvation and diabetes is an attractive possibility and needs further investigation. Insulin-like growth factor I
decreases during starvation (19) and it has been shown to mediate the stimulatory effect of hypophosphataemia on the 1α-hydroxylase (20). Whether or not the marked starvation-induced upregulation of the 24-hydroxylase enzyme system is of physiological importance remains to be established.

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