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Reduced carnitine and ketogenesis in the pivampicillin treated rat*

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Abstract—Pivampicillin (630 mg/kg body wt) given daily by stomach tube induced carnitine deficiency in the rat. The carnitine concentrations after 24 days were significantly reduced to (mean ± SD) 34 ± 2, 27 ± 7, 70 ± 18, 75 ± 16 and 49 ± 4% of controls in plasma, liver, muscle, heart and kidney, respectively, without any further reduction after 36 days. Pivampicillin treatment reduced the carnitine concentrations in the liver of the 48 hr fasted rat to about 1/2 of the controls after 6 days. The concentration of β-hydroxybutyrate was significantly reduced up to 14 days of treatment, and again increased. There was no significant difference in the free fatty acid concentrations between treated and control rats. Thus, pivampicillin treatment induced carnitine deficiency in the rat, but not as pronounced as seen in humans. This is possibly caused by adjustment of bacterial flora in the gut or altered renal mechanisms. The pivampicillin-treated rat, therefore, is not a useful model for pronounced carnitine deficiency in humans.

Pivaloyloximethyl-esterified antibiotics pivampicillin (Pondocillin®) and pivmecillinam (Selexid®) induce carnitine deficiency in humans due to loss of urine carnitine as the pivaloylcarnitine ester [1–3]. Carnitine has a pivotal role in fatty acid metabolism [4]. There has, so far, existed no good animal model for human carnitine deficiency. The purpose of this study, therefore, was to investigate pivampicillin-induced carnitine deficiency in the rat as a possible model for carnitine deficiency and its possible effects on fatty acid metabolism and ketogenesis.

Materials and Methods

Animals. Male, Wistar rats (weighing 220–420 g) were supplied from Møllegaard Breeding Center (L.I. Skensved, Denmark). They were fed on a standard laboratory animal chow (Ewos-Alab Brood Stock Feed-R3), housed three in each cage and adapted for 5 days before the experiments. The animals were randomized into treatment and control groups. The control rats had free access to the stock diet and water. Pivampicillin (630 mg/kg body wt) was given by stomach tube (gavage) every day at 8 a.m. to animals in the treatment group. Starved animals were fasted for 48 hr before killing. Water was allowed *ad lib.* during the period of fasting. All animals were killed between 8 and 10 a.m. The animals were anaesthetized by intramuscular injection of midazolam (Dormicum "Roche") combined with fentanyl/fluanison (Hypnorm "Janssen"), 0.15 mL of 100% dilution/100 g body wt. The animals were killed after 20 min of anaesthesia in the experiments where free fatty acids (FFA†) were measured. Blood was collected in heparin or EDTA glass. The heart ventricles, samples of liver, muscle and kidney were removed and frozen immediately with a Wollenberger clamp cooled in dry ice. Skeletal muscle was obtained from the hind leg. The experiments were done according to the regulation of experimental research on animals in Norway.

Assay procedures. Plasma and tissue carnitine concentrations were measured by a radiochemical assay [5, 6] modified as described [7]. FFA in plasma were quantitated by enzymic assay [8] using commercially available assay kits (WAKO NEFAC-test) and a Cobas Bio centrifugal analyser and plasma β-hydroxybutyrate was determined by a kinetic method [9, 10].

Chemicals. Pivampicillin base was supplied by Dr W. O. Godtfredsen from Leo Pharmaceutical Product, Ballerup, Denmark. Pivampicillin suspension (126 mg/mL) was prepared by pivampicillin base (12.6 g) and methylcellulose (0.25%) to 100 mL. [1-^{14}C]Acetyl-CoA (sp. radioact. 10 μCi/μmol) was obtained from Amersham (Amersham, U.K.). Enzymes, coenzymes and other substrates were purchased from the Sigma Chemical Co. (St Louis, MO, U.S.A.) and Boehringer (Mannheim, Germany). Commercial kit, WAKO NEFAC-test was supplied by Wako Chemicals GmbH (Germany).

Statistical analysis. An incomplete block analysis of variance (ANOVA) was applied for statistical testing of all data except the data from experiment 2 (Table 1) which had been tested for group difference with a two-sample Student's *t*-test.

Results and Discussion

The total carnitine concentrations were significantly reduced ($P < 0.001$) after 24 days of treatment to (mean ± SD) 34 ± 2, 27 ± 7, 70 ± 18, 75 ± 16 and 49 ± 4% of controls in plasma, liver, muscle, heart and kidney, respectively (Table 1, experiment 1), without any further reduction after 36 days (Table 1, experiment 2). The plasma carnitine level was reduced and reached a steady state after 3 days of pivampicillin treatment, while the reduction in carnitine concentration in muscle, which is the major pool for carnitine in the body, came only slowly, similar to what has been observed in humans [2]. There was a rapid, continuous decrease in liver carnitine concentration during treatment. The liver carnitine pool equilibrates apparently with plasma carnitine and it is less protected than the carnitine in the heart and the muscle. The heart carnitine, however, differs from the muscle carnitine changes. This is most similar to what occurs in the humans, where muscle carnitine was reduced to 10% of normal levels without any symptoms from the heart [2]. A pronounced reduction in heart carnitine could not occur without deleterious consequences, as seen in diphtheria or Adriamycin-induced carnitine deficiency of the heart [11–13].

Pivampicillin treatment clearly induces a state of carnitine deficiency in the male rat, as seen from the reduction of liver and muscle carnitine up to 24 days. Simultaneously, the growing male rat (Table 1) increases carnitine in plasma and the organs as previously described [14]. Thus, two processes at least take place at the same time, where the carnitine loss induced by pivampicillin during the first 24 days is strong enough to reduce carnitine concentrations. We expected, therefore, that prolonged treatment for 36

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† Abbreviations: FFA, free fatty acids; β-OH, β-hydroxybutyrate.

Table 1. Carnitine concentrations (mean ± SD) in different organs of rats (N = 6) during pivampicillin treatment (630 mg/kg/day)

Days of treatment	Plasma (nmol/L)	Liver (nmol/g)	Muscle (nmol/g)	Heart (nmol/g)	Kidney (nmol/g)
Experiment 1					
0 day	67 ± 9	95 ± 13	371 ± 34	1079 ± 67	266 ± 37
3 days	38 ± 7	78 ± 11	372 ± 25	1169 ± 223	304 ± 48
6 days	36 ± 4	67 ± 8	323 ± 28	1026 ± 142	271 ± 27
14 days	32 ± 5	52 ± 6	290 ± 24	883 ± 65	255 ± 15
24 days	32 ± 2	29 ± 8	291 ± 35	1093 ± 269	216 ± 10
Control (24 days)	93 ± 9	108 ± 18	414 ± 82	1460 ± 197	445 ± 29
Significance*	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Experiment 2					
36 days	30 ± 6	34 ± 12	382 ± 33	834 ± 116	221 ± 51
Control (36 days)	82 ± 9	75 ± 13	293 ± 99	1157 ± 99	302 ± 37
Significance†	P < 0.05	P < 0.05	NS	P < 0.05	NS

* By ANOVA.
† By Student's *t*-test of two samples.
NS, not significant.

days would have induced a more pronounced deficiency, but this was not confirmed (Table 1). A possible explanation for this is that prolonged treatment changes the gut flora; adaptive process might occur with increased carnitine synthesis by growth of *Pseudomonas* AK 1 species [15–17].

The effect of pivampicillin treatment on ketogenesis was studied in rats fasted for 48 hr (Table 2).

Pivampicillin treatment reduced the total carnitine content in liver of the 48-hr fasted rat to about 1/2 of controls after 6 days. There was no further reduction of liver carnitine with time beyond 14 days as expected from the studies in the fed rat (Tables 1 and 2). Due to the weight reduction in the livers during the 48-hr fast (from 12 to 6–7 g), there was an increase in carnitine concentration given as nmol/g, but not when calculated as nmol/liver. This does not support mobilization of muscle carnitine toward the liver in the fasted rat [18]. This increase in liver carnitine concentration, however, diminished the induced depletion and also reduced the possible effects of carnitine deficiency in this model.

The concentration of β-hydroxybutyrate (β-OH) decreased with duration of treatment up to 14 days and increased again towards 36 days, with significant difference between treated and control rats. During prolonged treatment the β-OH concentration was higher in treated than in controls. The plasma FFA concentration was reduced with time, both in treated and control rats, with no significant difference between the groups (P = 0.19). The ratio β-OH/FFA was reduced significantly towards 14 days and increased again after 14 days of treatment, but did not differ between treated and control rats (Table 2).

Pivampicillin treatment has been reported to reduce the FFA concentration in humans [1], by the inhibitory effect of pivalic acid/pivaloylcarnitine on the adipose tissue and its release of FFA, which could not be confirmed in the rat experiments. In the fasting state, the plasma FFA increase by mobilizing from adipose tissue to liver for β-oxidation where the ketone bodies are produced. β-OH is quantitatively the predominant ketone body present in the blood in ketosis [19, 20]. In addition, an inhibition of

Table 2. Body weight, liver carnitine and plasma levels of β-OH and FFA in the fasted rats before and during pivampicillin treatment

	Body wt (g)	Liver carnitine (nmol)	FFA (mmol/L)	β-OH (mmol/L)	β-OH/FFA ratio
C 0 day	214 ± 15	1164 ± 234	0.90 ± 0.27	2.96 ± 0.73	3.53 ± 1.40
C 3 days	228 ± 3	904 ± 178	0.84 ± 0.28	2.09 ± 0.74	2.71 ± 0.60
T 3 days	225 ± 3	856 ± 116	0.87 ± 0.26	1.47 ± 0.47	1.71 ± 0.39
C 6 days	240 ± 7	950 ± 103	0.69 ± 0.22	1.45 ± 0.89	2.01 ± 0.63
T 6 days	240 ± 7	495 ± 74	0.95 ± 0.18	1.37 ± 0.53	1.52 ± 0.82
C 14 days	289 ± 11	932 ± 296	0.52 ± 0.25	0.67 ± 0.26	1.38 ± 0.26
T 14 days	266 ± 19	466 ± 104	0.69 ± 0.09	1.15 ± 0.37	1.66 ± 0.47
C 24 days	285 ± 18	1021 ± 157	0.46 ± 0.09	0.84 ± 0.16	1.83 ± 0.30
T 24 days	289 ± 11	760 ± 78	0.66 ± 0.22	1.55 ± 0.32	2.45 ± 0.48
C 36 days	361 ± 23	1190 ± 205	0.64 ± 0.28	1.23 ± 0.28	2.13 ± 0.76
T 36 days	362 ± 14	547 ± 91	0.63 ± 0.12	1.71 ± 0.41	2.73 ± 0.40
Significance	NS	P < 0.05	NS	P < 0.05	NS

Values represent mean ± SD, N = 6.
P < 0.05 by ANOVA.
NS, not significant.
C, control group; T, pivampicillin-treated group (630 mg/kg/day p.o.).

ketone body production through reduced liver carnitine could occur [1]. In addition to the absolute determination of β-OH concentration, the ratio of β-OH/FFA was calculated as an expression of capacity for ketogenesis during condition with possible variation in FFA influx to the liver.

There was a rapid reduction in β-OH after 3–6 days of treatment, which was most likely caused by reduction in liver carnitine. The increased β-OH concentration seen in the rat after prolonged treatment is difficult to explain from the reduction in the liver carnitine. It is uncertain, however, whether or not an increased concentration of pivaloylcarnitine and pivaloyl-CoA present in the mitochondria will inhibit ketone body formation as occurs with propionyl-CoA [4, 21, 22].

In conclusion, this study shows that pivampicillin treatment in the rat induces carnitine deficiency in the different tissues in a similar way to humans in short-term experiments. In humans, a carnitine deficiency develops after pivampicillin treatment with pronounced alteration in liver metabolism as hypoglycemia [2], which does not develop in the rat, possibly due to adjustment of the bacterial flora in the gut or an altered renal excretion mechanism. The pivampicillin-treated rat, therefore, is not a useful model for pronounced carnitine deficiency in humans.

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