ACTIVITY OF THIAMINE PYROPHOSPHATE-CONTAINING RATS AFTER HYDROXYTHIAMINE ADMINISTRATION

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NADPH-generating enzymes are generally accepted as having an important role in supplying steroid hydroxylation reactions with reducing equivalents [9, 12]. At the same time it has been shown that a reduction in the activity of thiamine pyrophosphate-containing (TPP-containing) enzymes as a result of a deficiency of the enzyme leads to disturbance of steroid hormone biosynthesis in the adrenal cortex [2, 11]. However, the relative importance of each of the TPP-containing enzynes of the adrenals in the provision of the necessary components and energy for steroid production has not been explained. It is a remarkable fact that in the principal reactions of NADP reduction catalyzed by malate dehydrogenase (MDH), isocitrate dehydrogenase (IDH), and glucose-6-phosphate dehydrogenase (G6PDH), together with 6-phosphogluconate dehydrogenase (6PGDH), products are formed which serve as substrates for the pyruvate dehydrogenase complex (PDC), the oxoglutarate dehydrogenase complex (OGDC), and transketolase (TK), respectively. All these circumstances necessitate a comprehensive study of the TPP-dependent enzyme systems of the adrenals [6, 13] and their interaction with NADPH-generating enzymes.

The aim of this investigation was to study the order and degree of inhibition of TPP-containing enzymes in the adrenals after parenteral administration of the known antivitamin, hydroxythiamine (HT) [3], and also to discover how this is reflected in activity of coupled NADP-dependent dehydrogenases and on the plasma 11-hydroxycorticosteroid (11-HCS) level, which is an integral parameter of adrenocortical function.

EXPERIMENTAL METHOD

The control and all experimental groups consisted of 16 male albino rats weighing 110-130 g. HT (from Ferak, West Berlin) was dissolved in 0.85% NaCl and injected intraperitoneally in a dose of 1 mmole/kg. The rats were decapitated 2, 4, 8, and 12 h after injection of the antivitamin. The adrenals were homogenized with 0.15 M KCl solution (1:10) by means of a Teflon pestle. To prepare each homogenate four adrenal glands were taken from two animals of a homogeneous group. Cytosol and mitochondrial fractions were obtained by differential centrifugation [5]. The thrice washed mitochondria were destroyed by the addition of Triton X-100 to 0.2% concentration [15]. Activity of PDC [6], OGDC [13], MDH [12], and IDH [15] in the mitochondrial extracts was determined. As well as the last two of these enzymes, activity of TK [4], G6PDH, and 6PGDH [10] also was determined in the cytosol. The velocity of the enzyme reactions was recorded on a Specord UV VIS spectrophotometer with thermostatically controlled cuvette holder (30°C). The total 11-HCS concentration in the blood plasma was determined fluorometrically [14].

EXPERIMENTAL RESULTS

The study of activity of all three main TPP-dependent enzymes at short intervals after injection showed that activity of OGDC was the first to fall (after 2 h), and that inhibition of OGDC was very intensive and prolonged (Table 1). A significant fall in PDC activity occurred later, 4 h after injection of HT, and its activity returned to normal after 12 h. As regards TK, located in the hyaloplasm of the adrenocorticocytes, its inhibition was not observed

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TABLE 1. Enzyme Activity (in mmoles substrate/min/mg protein) in Rat Adrenals at Different Time Intervals after Injection of HT in a Dose of 1 mmole/kg (M ± m, n = 8)

Enzymes	Control	After injection of HT			
		2 h	4 h	8 h	12 h
Mitochondrial:					
PDC	6,41±1,01	5,14±0,18	3,08±0,33**	3,63±0,20*	5,71±0,19
PGDC	17.8±1,0	11,4±0,7***	3,79±0,50***	3,85±0,31***	5,78±0,63***
MDH	5,23±0,48	5,01±0,36	5,46±0,81	—	5,07±0,80
IDH	48,7±1,3	42,9±1,6*	31,3±1,3***	30,2±2,1***	43,9±1,9
Cytosol:					
TK	23,9±1,4	21,7±0,9	22,1±0,7	18,0±1,6*	19,7±0,7*
G6PD	56,1±1,8	69,9±4,3	67,0±3,2*	83,5±7,0**	77,7±6,0**
6-PGD	63,8±6,4	48,9±4,7	49,8±2,3	50,1±9,1	42,0±6,4*
MDH	48,4±2,3	44,4±2,1	52,7±2,3	49,6±1,9	39,2±0,7**
IDH	131,7±11,0	124,5±5,1	137,7±7,1	130,4±3,2	119,6±3,7

Legend. *P < 0.05, **P < 0.01, ***P < 0.001.

TABLE 2. 11-HCS Concentration (in μmoles/liter) in Blood Plasma of Rats at Various Time Intervals after Injection of HT (M ± m, n = 16)

Experimental conditions	11-HCS
Control	0,566±0,051
After injection of HT	
2 h	0,624±0,053
4 h	0,376±0,039*
8 h	0,411±0,025*
12 h	0,351±0,023**

Legend. *P < 0.01, **P < 0.001.

until 8 h after the injection and it was weaker than inhibition of activity of mitochondrial TPP-dependent polyenzyme systems. The mechanism of inhibition of these enzymes after entry of HT into the body is linked with pyrophosphorylation of that compound in the tissues by thiamine kinase [3], after which it can replace TPP in the chloride form [4]. The high competitive ability of hydroxythiamine pyrophosphate (HTPP) compared with TPP was demonstrated by the writers using highly purified PDC from bovine adrenal glands [7]. Homogeneous OGDC from the same source retains endogenous TPP more strongly [13]. Despite differences in the strength of binding of PDC and OGDC with TPP, the injected pseudocoenzyme precursor HT leads to more marked inhibition of activity of the second complex. The explanation of this may be the higher rate of turnover of the coenzyme *in vivo* in the case of OGDC than with PDC and, consequently, greater incorporation of HTPP into the active center. For some other tissues, a decrease in oxoglutarate dehydrogenase activity has not been discovered in response to entry of HT into the body [3]. The strong effect of the antivitamin on OGDC from the adrenals is evidence of the existence of organic differences at the level of the same enzyme system.

After injection of HT activity of mitochondrial IDH fell simultaneously with OGDC in the adrenals (Table 1), but by a lesser degree. It can be postulated that OGDC was inhibited first, and this was reflected in the function of the adjacent IDH, and led to a change in its activity. This effect of injection of HT was undoubtedly indirect, for no inhibition of NADP-dependent IDH by the antivitamin HT or by its mono- and diphosphate derivatives in concentrations of 10^{-5}-10^{-7} M, which are realistic for tissues, was observed *in vitro*. Cytosol IDH, which has no direct connection with mitochondrial OGDC, was indifferent to injection of HT. Meanwhile other NADP-dependent dehydrogenases modified their activity at different times after HT injection. If inhibition of 6-PGD activity can be tentatively explained by its functional connection with TK, which was inhibited a little starting with 8 h after injection, the earlier activation of G6PD is not quite clear.

Changes in enzyme activity in the adrenals are accompanied by lowering of the 11-HCS concentration in the peripheral blood plasma (Table 2), reflecting adrenocortical function [9]; this effect began, moreover, 4 h after beginning of the experiment, before activity of TK and

6-PGD was inhibited (Table 1). The decrease in steroid production, postulated on the basis of the blood 11-HCS level, in response to injection of HT is thus in all probability connected with disturbance of the functioning of the 2-oxo-acid dehydrogenase complexes in the adrenals. The importance of the role of PDC and OGDC in metabolism of the adrenal cortex is not confined, however, to their close coupling with NADP-dependent dehydrogenases. These complex polyenzyme systems, lying at the crossroads of the main metabolic pathways, reduce NAD which is later utilized in the transhydrogenase reaction, with NADPH generation, and in the tissue respiration chain with the formation of ATP [1]. Besides, acetylcoenzyme A, a structural component for biosynthesis of steroid molecules, is formed in the pyruvate dehydrogenase reaction, and succinyl-coenzyme A, utilized for the construction of heme, a component of such an important link in the steroid hydroxylating system as cytochrome P-450, is formed in the oxoglutarate dehydrogenase reaction [1].

The results obtained with the use of HT confirm that 2-oxo-acid dehydrogenase complexes with their complex mechanisms of regulation [6, 8] can be sensitive objects for exerting action on metabolism and, consequently, on adrenocortical function..

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