On the influence of Ca-DTPA on δ-aminolevulinic acid dehydratase in rats

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Summary. In rats red cell δ-aminolevulinic acid dehydratase is inhibited by Ca-DTPA only after treatment with toxic doses or with fractionated therapeutic doses. Zn-DTPA does not influence the activity of the enzyme even after administration of high doses.

δ-Aminolevulinic acid dehydratase (ALAD), one of the important enzymes in heme synthesis, is present in various mammalian organs, especially in the erythrocytes. In vitro the enzyme is inactivated easily by chelating agents, like EDTA or DTPA. In vivo, however, contradictory results have been reported. Long-term treatment with 100 μmoles/kg and day Ca-DTPA did not affect the urinary excretion of δ-aminolevulinic acid (ALA) in rats[2]. This holds also for rabbits injected with 600 μmoles/kg and day Ca-EDTA[3]. These findings were taken as evidence that the chelates do not impair the ALAD-activity in vivo, and are in keeping with the fact that Ca-EDTA (600 μmoles/kg infused for 3 h) evoked in rats only a marginal inhibition of ALAD in erythrocytes and liver[4]. In baboons, however, Ca-DPTA (30 μmoles/kg and day) caused a significant decrease of ALAD activity in red cells[5,6]. As ALAD is considered to be a Zn-requiring enzyme[7,8] an inhibition by Ca-chelates of the type mentioned seems possible. In order to clarify these questions, we conducted a systematic study on the effect of Ca-DTPA on ALAD as dependent on dose and time. Furthermore we investigated for the first time the activity of ALAD under the influence of Zn-DTPA which of course should not be able to exert an inhibition of the enzyme by Zn removal.

Materials and methods. Young male rats of the Heiligenberg strain with a body weight of 130 ± 0.5 g were injected i.p. with Ca- and Zn-DTPA, respectively. At different times afterwards the animals were sacrificed, the blood removed and stored at 0 °C until assayed. With fractionated treatment the animals were injected every 2 h for 5 consecutive days, the cumulative dose is indicated in the table. After the end of the treatment ALAD was determined according to Burch and Siegel[9] and the enzyme activity calculated as nmoles porphobilinogen per h. and ml packed cell volume.

Results and discussion. The administration of Ca-DTPA leads to an inhibition of red cell ALAD only after toxic doses or with fractionated treatment (table). Zn-DTPA does not have any effect on the enzyme in either case. These results confirm those of Hofmann and Segewitz[2] and of Tanabe[3], but they differ from the findings of Cohen[5,6]. An explanation for this discrepancy could be sought in an interspecies difference, as negative results with therapeutic doses of Ca-DTPA have been observed in rodents only. The inactivation of ALAD by the chelate with fractionated treatment in the therapeutic dose range (table) confirms the findings of Hammond[4]. This result again emphasizes the enhanced toxicity of Ca-DTPA when applied in a protracted treatment schedule, as already reported by us in an earlier paper[10]. This enhanced toxicity is ascribed to the increased metal depletion in various organs which in turn is due to the maintainance of a high plasma level of the chelate[11].

The absence of such an effect of Zn-DTPA also points to an action of the Ca-chelate by metal, probably Zn, depletion. There remains, however, some doubt whether the chelate exerts a direct action on the enzyme: 1. The distribution spaces of chelate and enzyme are different, the chelate being distributed only in the extracellular space[12] whereas ALAD is an intracellular enzyme. 2. There is a delay of 24 h before the enzyme inhibition becomes noticeable. At this time, however, the chelate has already left the body for some time[12]. These considerations lead to the conclusion that Ca-DTPA does not act on ALAD itself but possibly on some step of the enzyme-synthesis. This view is corroborated by the results of Finelli et al.[8] in his investigation on the Zn-dependence of ALAD in rats.

Chelate (dose, mmoles/kg)	Days after treatment 1	2	3	4	5	6
Control	601 ±21	602 ±31	563 ±30	592 ±24	569 ±45	612 ±51
Ca-DTPA						
0.5	600 ±40	644 ±33	607 ±47	555 ±65	618 ±40	732 ±53
1	603 ±21	446* ±12	393* ±26	468* ±15	620 ±21	
2	598 ±15	391* ±45	416* ±38	339* ±32	516 ±60	581 ±34
4	538 ±28	311* ±27	265* ±21	258* ±15	500 ±29	629 ±43
8	500** ±19	262* ±16	120* ±20	33* ±9	40* ±5	44* ±6
0.5 fractionated (0.02 per injection)	456* ±39	390* ±34	449* ±34	639 ±39		
Zn-DTPA						
8	561 ±35	560 ±31	561 ±30	578 ±18	613 ±31	
2.5 fractionated (0.1 per injection)	601 ±15	605 ±38	556 ±12	568 ±40		

ALAD in erythrocytes (nmoles PBG per h and ml packed cell volume) after chelate treatment. Mean values ± SE. Control group 30, other groups 6 animals.
*, **Significantly different from corresponding control values.
*$p < 0.01$, **$p < 0.05$.

1 Acknowledgment. The expert technical assistance of Miss H. Olinger is gratefully acknowledged.

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