SHORT COMMUNICATION

Effects of Vitamins K_1 and K_3 on Adenosine Nucleotide Content of Rat Tissues

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SUMMARY

Vitamin K_3 treatment considerably reduces the ATP content and ATP/ADP ratio in liver and heart in rats. Vitamin K_1 decreases the ATP level only in the liver. Correlations may exist between the uncoupling activity measured *in vivo* and the anti-inflammatory action of vitamins K.

Vitamins K_1 and K_3 have been found to inhibit significantly various experimental inflammations and anaphylactic shock in guinea pigs (1). Antiinflammatory action rests chiefly on the inhibition of proliferative phases of inflammation, the synthesis of new connective tissue (2). In connection with the study of the mechanism responsible for antiinflammatory action, the importance of the uncoupling effect on oxidative phosphorylation was studied. According to Whitehouse (3), in the case of nonsteroid antiinflammatory agents very close correlations exist between *in vitro* uncoupling effect and clinical efficacy.

Martius and Nitz-Litzow (4) reported that in the liver of animals with vitamin K deficiency oxidative phosphorylation is uncoupled. In their view the lowered protein (prothrombin) synthesis in the liver, which leads to the hemorrhagic tendency, is due to an uncoupling effect of vitamin K deficiency. Other investigators were unable to confirm the uncoupling effect of vitamin K deficiency (5, 6), but the important role of naturally occurring quinones (vitamin K_1, ubiquinone) in the synthesis of high energy phosphate bonds was supported. Chen and Dallam (7) suggested that natural vitamin K (K_1) participates as an intermediate in mitochondrial oxidative phosphorylation. Vitamin K_1 has no uncoupling effect and does not act on mitochondrial ATPase. On the contrary, naphthoquinones with shorter side chains than vitamin K_1 have shown strong uncoupling and ATPase-stimulating effects.

In our experiments both vitamins K_1 and K_3 have displayed antiinflammatory effects (1). Assuming that *in vivo* the measured adenine nucleotide content is the best indicator of oxidative phosphorylation, the effects of vitamins K_1 and K_3 on the ATP and ADP content of rat tissues was estimated. Male Wistar rats weighing from 150 to 180 g were used in groups of seven of equal body weight. The rats were treated subcutaneously once daily for 5 days with vitamin K_1 (Konakion® Roche) or a propylene glycol solution of vitamin K_3. Two hours after the last injection the animals were killed by decapitation. The blood was collected in ice cold perchloric acid in a Potter homogenizer, and the exact quantity was determined after homogenization by reweighing. Immediately after the blood samples were obtained, the liver and heart were removed rapidly and frozen in liquid nitrogen. After pulverization, the samples were extracted with perchloric acid as de-

TABLE 1
Effect of vitamins K on adenosine nucleotide content of rat tissues

Treatment and daily doses, s.c.	Tissue	Adenine nucleotide	Adenine nucleotide content (μmoles/g ± SEM)		ATP:ADP ratio	
			Controls	Treated	Controls	Treated
Vitamin K_1 30 mg/kg	Blood	ATP	0.348 ± 0.14	0.380 ± 0.02	2.78	3.07
		ADP	0.125 ± 0.02	0.124 ± 0.03		
	Liver	ATP	3.878 ± 0.24	3.130 ± 0.16	7.04	4.25[a]
		ADP	0.551 ± 0.04	0.737 ± 0.08		
	Heart	ATP	3.784 ± 0.36	4.050 ± 0.34	7.32	8.02
		ADP	0.517 ± 0.04	0.505 ± 0.04		
Vitamin K_3 10 mg/kg	Blood	ATP	0.284 ± 0.02	0.326 ± 0.02	2.27	2.49
		ADP	0.125 ± 0.02	0.131 ± 0.06		
	Liver	ATP	3.448 ± 0.24	1.692 ± 0.14	6.64	2.40[a]
		ADP	0.519 ± 0.06	0.703 ± 0.05		
	Heart	ATP	3.916 ± 0.22	1.712 ± 0.18	5.92	2.72[a]
		ADP	0.661 ± 0.04	0.628 ± 0.05		

[a] Significant difference from the controls ($p < 0.01$).

scribed by Maitra and Estabrook (8). Fluorimetric analyses of ATP and ADP were performed by the enzymic method of Greengard (9).

As shown in Table 1, ATP content and the ATP:ADP ratio were considerably reduced by vitamin K_3 in liver and heart. ADP was increased only in liver; in heart it remained unchanged. It was only in liver that three times larger doses of vitamin K_1 brought about differences in adenine nucleotide content. Under the influence of vitamin K_1, hepatic ATP and the ATP:ADP ratio were significantly reduced, while the ADP content increased. Adenine nucleotide blood levels were not changed by vitamin K treatment.

The strong *in vitro* uncoupling effect of vitamin K_3 has been confirmed *in vivo* by our results. The finding that the hepatic ATP level is influenced only by more massive doses of vitamin K_1 than of vitamin K_3 indicates that it is not the original molecule that is effective. Most probably vitamin K_1 is metabolized in the liver to a metabolite with shorter side chain, having an uncoupling effect like that of vitamin K_3. This metabolite is insufficient to bring about demonstrable uncoupling outside the liver in other organs with ample ATP synthesis. Presumably this is the reason why vitamin K_1, unlike vitamin K_3, is nontoxic. In the connective tissue, however, where the cellular density and metabolic activity are very low, the uncoupling metabolite of vitamin K_1 may nevertheless produce an uncoupling action of such measure as to exert a chronic antiinflammatory effect.

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