Regulation of the Na,K-ATPase [4, 6] and AChE levels by ACh in the same membranes was not shown to be completely identical in form. Previously arguments had been adduced [3] in support of the possibility that ACh may be supplied to the nerve cell and synthesized in the cell during depolarization, after which it may participate in the regulation of transcription. If this possibility *in situ* is denied, of all the probable metabolic intermediaries of ACh not one activator of AChE synthesis is left.

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ACTIVATION OF LIVER MITOCHONDRIAL CITRATE SYNTHETASE BY NORADRENALIN AND CYCLIC AMP

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UDC 577.15.024.612.452.018

KEY WORDS: noradrenalin; cyclic AMP; citrate synthetase.

Citrate synthetase (CS) catalyzes a strongly exergic and, *in vivo*, probably irreversible reaction [11]. CS is regulated by many intracellular metabolites [9, 11, 12, 14] and, for that reason, it is regarded as a primary control point of the Krebs' cycle [5, 8, 9]. Starting out from the principles of obligatory regulation of all biologically important processes by hormones and cyclic nucleotides [1], it could be postulated that CS is regulated by catecholamines (CA) and cyclic AMP. However, the action of CA has not been studied. So far as cyclic AMP is concerned, there has been only one report [6] that it has no effect on purified CS of *Rhodopseudomonas*. This is natural because the effects of cyclic AMP are usually indirect in character.

In the investigation described below the action of CA and cyclic AMP were studied on liver CS.

EXPERIMENTAL METHOD

Experiments were carried out on 64 male Wistar rats weighing 150-200 g and 15 (CBA × C57BL)F_1 mice. In the experiments *in vivo*, to reduce liberation of endogenous CA, the ganglioolytic pirilen (pempidine) was injected 1.5 h beforehand in a dose of 10 mg/kg, followed by noradrenalin (NA) in a dose of 11 μmoles/kg 15 min before the investigation. In the experiments *in vitro*, the regulators were incubated with the homogenate for mitochondria for 6 min at 27°C in the presence of 10^{-3} M theophylline, but in the experiments with NA, with the addition of ascorbate. Mitochondria were isolated in medium containing 0.25 M sucrose, 1 mM EDTA, and 10 mM Tris-HCl, pH 7.5, at 9000g. Mitochondria were disintegrated by osmotic shock in distilled water (1 min), after which CS was determined [3]. Measurements were made at 25°C on an SF-26 spectrophotometer. There were 6-13 experiments in each series.

EXPERIMENTAL RESULTS

Injection of NA activated CS (Table 1). NA gave a similar effect when incubated with rat liver homogenates. This shows that the effects of the neurohormone is not mediated through

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(Presented by Academician of the Academy of Medical Sciences of the USSR S. S. Debov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 93, No. 1, pp. 41-43, January, 1981. Original article submitted April 5, 1981.

0007-4888/82/9301-0048$07.50

TABLE 1. Effect of NA on Rat Liver CS Activity (in nanomoles citrate/mg mitochondrial protein) (M ± m)

Experimental conditions	Control	Experiments	Activation, %	P
Injected in vivo (11 μmoles/kg)	59,0±2,8	73,0±5,1	24,0±9,8	<0,05
Incubation with homogenate (10^{-6} M)	51,0±4,7	57,0±4,8	13,0±2,3	<0,001

TABLE 2. Activation of Rat Liver CS by NA and Cyclic AMP on Incubation of Regulators with Various Cell Fractions (M ± m)

Activator	Whole homogenate	Mitochondria
NA	13,0±2,3*	3,3±2,0
Cyclic AMP	18,0±3,9*	11,0±0,6*

Legend. Activation expressed in % of control level (62-74 nmoles citrate/min/mg mitochondrial protein); *P < 0.05.

other physiological systems but is aimed directly at the liver tissue.

After further simplification of the system — incubation of NA with mitochondria — no effect now was observed (Table 2), evidence of the involvement of an extramitochondrial factor. It was natural to suppose that this factor is cyclic AMP. On incubation both with homogenates and with isolated mitochondria, cyclic AMP did in fact activate CS (Table 2). Under the combined influence of NA and cyclic AMP activation of CS (15.0 ± 1.9%) was observed, and this effect did not differ from that of cyclic AMP alone (P > 0.6). This is evidence in support of the view that the effect of CA is realized through cyclic AMP.

The activating effect of cyclic AMP on CS on incubation with rat liver mitochondria was confirmed by "blind" experiments (P < 0.05) and the same effect was observed on mouse liver mitochondria (21.0 ± 8.1%; P < 0.05).

Activation of CS may be the reason for accumulation of citrate in the blood after administration of CA *in vivo* [10].

Activation of CS by physiological concentrations of CA and cyclic AMP, observed in this investigation, is most interesting because CS is not merely a key enzyme of the Krebs' cycle, but it is also a "trigger" enzyme responsible for introducing most of the metabolites of all types of metabolism into the cycle. Together with data showing activation of mitochondrial NAD-isocitrate dehydrogenase by CA and cyclic AMP [3, 4], these results show that control of the Krebs' cycle by these regulators is branched in character. This is evidently an important factor in the stimulation of the overall oxygen consumption of the body [7] and mitochondrial restoration [2, 3] by CA and cyclic AMP, and ultimately in the provision of energy for the "biochemical mobilization" which is characteristic of CA and cyclic AMP. This is probably an essential factor in the raising of resistance to different types of stress.

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