REGULATION OF THE MICROSOMAL ACETYLCHOLINESTERASE LEVEL IN NERVE CELLS BY ACETYLCHOLINE AND CYCLIC NUCLEOTIDES

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Acetylcholinesterase (AChE) of nerve tissue is a protein with a short half-life [9]. The study of regulation of AChE biosynthesis is important from the standpoint of relations between functional load and enzyme activity, transformation of the nervous impulse into metabolic signals, and so on. Views on the adaptive synthesis of AChE in response to an increase in the quantity of substrate (acetylcholine — ACh) cannot yet satisfactorily explain such new facts as, for example, the increase in activity of the enzyme within the cells under the influence of cyclic AMP [1, 8], whereas it is known that cyclic GMP is a metabolic precursor of ACh. It has also been found that ACh is a regulator of the expression of other, noncholinesterase, genes of nerve cells, for example the Na,K-ATPase gene [2-6], and in this respect it is an intracellular hormone. However, it is not known whether ACh has any direct effects, independently of cyclic GMP, on expression of the AChE gene.

In the present investigation it was accordingly decided to study the effect of ACh, cyclic GMP, and cyclic AMP on the level of microsomal AChE activity in nerve cells in a model (cell-free) system developed previously [4, 6] to study regulation of expression of the Na,K-ATPase gene.

EXPERIMENTAL METHOD

Albino rats weighing 150-250 g were used. The whole brain, except the cerebellum and caudal part of the medulla, was homogenized in a 2.45 M sucrose solution in a volume of 7 ml per brain. The homogenate was centrifuged for 75 min at 27,000g. The residue containing nuclei was resuspended in optimal medium for synthesis of nuclear RNA [4, 6]. An equal volume of microsomal-cytoplasmic fractions was added to aliquots of the nuclear suspension (2.5 ml). This latter suspension was obtained by centrifugation of the homogenate (2.5 ml of 0.3M sucrose per brain) at 8000 g for 10 min. The experimental samples contained either ACh (10^{-6}-10^{-3} M), or cyclic AMP (10^{-6} M), or cyclic GMP (10^{-6} M), or puromycin (25 μg/ml). In cases when the action of ACh was studied against the background of puromycin or actinomycin D (50 μg/ml) all samples (control and experimental) contained the antibiotic.

The samples were incubated for 60 min with periodic stirring, an equal volume of 0.3 M sucrose was added, and the mixture was centrifuged for 7 min at 4000g. The supernatant was drawn off and centrifuged for 75 min at 27,000g. The residue of microsomes was resuspended in 1.5-3 ml of 0.1 M phosphate buffer, pH 7.8, containing 0.075 M NaCl and 0.04 M $MgCl_2$. The AChE activity was determined in the microsomal suspension [7]. Other details of the method were described previously [2-6].

The control level of microsomal AChE activity was 3.9-13.1 μmoles acetylcholine/mg protein/h; butyrylthiocholine was virtually not hydrolyzed by the microsomal preparations. Since the control level of enzyme activity varied from experiment to experiment, the final results in each experiment were expressed as percentages of the control.

EXPERIMENTAL RESULTS

As a result of preincubation of the cell-free system with ACh (10^{-6}-10^{-3} M) the microsomes isolated from it had a higher level of AChE activity (Table 1). The maximal increase

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TABLE 1. AChE Activity of Microsomes Preincubated in Cell-Free Systems with Addition of ACh, Cyclic AMP, and Cyclic GMP (M ± m)

Variant of cell-free system	AChE activity, % of control
Complete system ± ACh	
10^{-6} M	111±4 (9)*
10^{-5} M	124±5 (25)*
10^{-4} M	111±6 (15)
10^{-3} M	105±6 (14)
Complete system + puromycin (25 μg/ml)	91±3 (18)*
Complete system + actinomycin D (50 μg/ml) + ACh (10^{-5} M)	95±4 (10)
Complete system + puromycin (25 μg/ml) + ACh (10^{-5} M)	96±5 (17)
System without nuclei + ACh (10^{-5} M)	82±8 (9)*
Complete system + cyclic AMP (10^{-6} M)	81±4 (14)*
Complete system + cyclic GMP (10^{-6} M)	89±5 (17)**

Legend. Number of experiments in parentheses; *P < 0.01, **P < 0.05.

was observed when ACh was present in a concentration of 10^{-5} M (25% after incubation for 60 min), i.e., in a concentration corresponding to the intracellular level. With a further increase in the ACh concentration to 10^{-4}-10^{-3} M not only did the effect not increase, but it was substantially reduced. Similar dependence on ACh concentration also was found for RNA synthesis in isolated nerve cell nuclei [3].

If the system was preincubated with puromycin, the microsomes subsequently isolated from it had a lower level of AChE activity (Table 1). This fact suggests that spontaneous synthesis of AChE takes place in the cell-free system and that this synthesis is accelerated by the addition of ACh to the system. That this conclusion is correct is confirmed, first, by the fact that incorporation of ^{14}C-amino acids into microsomal membrane proteins, which is sensitive to actinomycin [2], was activated by ACh in this cell-free system and, second, by the absence of effect of ACh on the AChE level in the presence of actinomycin D and puromycin, inhibitors of transcription and translation respectively (Table 1).

If the apparatus for RNA synthesis, i.e., the nuclei, were removed from the system, its preincubation with ACh significantly lowered the level of activity of the enzyme in the microsomes (Table 1). Since ACh did not have this effect in the presence of puromycin, i.e., when translation was blocked, it is reasonable to suppose that ACh inhibits translation of messenger RNA coding the structure of AChE. The action of ACh on AChE activity in the microsomes is evidently dual in character and consists of activation of synthesis of messenger RNA or its processing in the nucleus and inhibition of translation of the corresponding RNA. These factors evidently are the components of a single intracellular system, equipped with abundant feedback, for regulating the enzyme level. Since ACh induces an increase in AChE activity in the microsomes in a complete system, its effect on synthesis of processing of messenger RNA can be regarded as the principal component, and its effect on translation as a correcting influence.

Preincubation of the cell-free system with cyclic nucleotides caused a decrease in the level of enzyme activity (Table 1). Because of these facts, cyclic nucleotides cannot be regarded as possible intermediaries in the inducing action of intranuclear ACh on the AChE level. They are also evidence that the increase in AChE activity resulting from addition of cyclic AMP to whole tissue [1] is mediated through separate mechanisms, perhaps through intercellular interaction.

The data described above suggest that acetylcholine regulation of the level of microsomal AChE activity in the nerve cell is a multicomponent system. The main component in this system is the direct action of ACh on synthesis of the AChE gene product or on its processing, and the other components — cyclic nucleotides and the effect of ACh itself on translation — are correcting elements.

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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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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