ACETYLCOENZYME A AND THE CONTROL OF THE SYNTHESIS OF ACETYLCHOLINE IN THE BRAIN

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Abstract. Experimental work concerning the relation between the availability of acetylcoenzyme A and the synthesis of acetylcholine in the brain is reviewed and discussed from the viewpoint of the "mass action" ("reaction equilibrium") hypothesis of the control of acetylcholine synthesis.

INTRODUCTION

Acetylcholine (ACh) acts as a transmitter of impulses on many synapses both in the central and the peripheral nervous system and is continually synthesized in the presynaptic endings and terminal varicosities of cholinergic axons (reviews 5, 32, 48). It has been noted very early in the investigations of cholinergic mechanisms that the synthesis of ACh is finely controlled and adjusted to actual needs. A classical example of this control has been described by Richter and Crossland (42); they stimulated the brain cells in rats with large electrodes placed on the skull and measured the content of ACh in the brain at different intervals during and after stimulation and during and after subsequent convulsions. It was a salient feature of their observations that there was not only a rapid decline in the level of ACh in the brain during
stimulation, but also a rapid return to the "resting" level of ACh when the stimulation stopped; a new fall of brain ACh occurred during subsequent convulsions, but it was again followed by a rapid restoration of "resting" concentration. These pioneer experiments very clearly demonstrated that the cells of the brain are capable to replenish rapidly their ACh reserves once they had been depleted, without, however, producing more ACh than required for the maintenance of a steady "resting" level.

During the 32 years separating us from the publication of Richter's and Crossland's work, a vast evidence accumulated concerning various physiological, biochemical, ultrastructural, biophysical and pharmacological aspects of the synthesis of ACh in the nerve terminals and its control; nevertheless the mechanisms responsible for the control of ACh synthesis and for the maintenance of stable ACh levels have not been fully identified and defined. Problems in their understanding have been analysed in many reviews (5, 19, 24, 25, 31, 32, 48). A large body of experimental evidence appears to be best explained on assumption that, at the ultimate level, the synthesis of ACh in the cholinergic neurons is controlled by the law of mass action and that, at rest, the concentration of ACh in the compartment of its synthesis corresponds to the equilibrium between the concentrations of substrates and products in the reaction catalysed by choline acetyltransferase (ChAT; EC 2.3.1.6) (17, 39, 48).

In accord with this view, the concentration of ACh in the compartment of its synthesis is expected to depend on the concentrations of choline, coenzyme A (CoA) and acetyl-CoA. It has been indeed observed that the concentration of ACh in the brain and other organs is increased after the administration of large doses of choline (4, 20, 28, 40, but see references 9, 11, and 36 for contrary evidence) and that it is diminished after the supply of choline into the nerve cells had been inhibited (6, 21).

In our contribution we wish to summarize experimental evidence related to the question of how the synthesis and content of ACh in the brain are affected by changes in the availability of acetyl-CoA for the synthesis. Full account of the experiments performed in our laboratory has been or will be published elsewhere (7a, 43, 44 and Říčný and Tuček, in preparation).

**ACETYL-CoA AND ACETYLCHOLINE IN THE BRAIN IN EXPERIMENTAL AVITAMINOSIS B₁**

Since thiamine pyrophosphate is a coenzyme of pyruvate dehydrogenase (EC 1.2.4.1), experimental deficiency of thiamine (avitaminosis
B1) would be expected to interfere with the production of acetyl-CoA from pyruvate, the most important source of acetyl-CoA in the brain, and consequently with the production of ACh. The synthesis of ACh has indeed been found to be impaired in brain slices from thiamine-deficient pigeons incubated in vitro (33). In experiments with thiamine-deficient rats (37, 45) and with isolated sympathetic ganglia exposed to the antagonists of thiamine (38), the transmission in sympathetic ganglia was "fatigued" more easily than in control experiments, in good accord with the later discovered (46) impairment of the synthesis of the transmitter. However, attempts to directly correlate data on the availability of acetyl-CoA and on the content of ACh in the brain in experimental avitaminosis B1 yielded controversial results. Heinrich et al. (22) found that the concentration of acetyl-CoA was diminished by 42% and that of ACh by 35% in the brain of thiamine deficient rats. On the other hand, Reynolds and Blass (41) observed no effect of thiamine deficiency on the content of either acetyl-CoA or ACh in the brain. It appears that the degree of thiamine deficiency and its actual effect on the activity of the pyruvate dehydrogenase complex will have to be examined in more detail in future attempts to analyse the relations between acetyl-CoA and ACh using the model of the avitaminosis B1.

ACETYL-COA AND ACETYLCHOLINE IN BRAIN SLICES INCUBATED WITH VARIOUS CONCENTRATIONS OF GLUCOSE

To induce changes in the availability of acetyl-CoA in the slices of rat caudate nuclei, we incubated them for 30 or 60 min in a physiological saline solution containing various concentrations of glucose (0.5, 2 and 10 mM); the incubation medium contained a high concentration of choline (0.2 mM), an inhibitor of cholinesterase (0.058 mM diethyl-p-nitrophenyl phosphate), and either 5 mM or 30 mM K+. At the end of the incubation, the concentrations of acetyl-CoA and of ACh in the slices, of ACh in the incubation medium, and of choline in the slices were measured (43).

The content of acetyl-CoA in the slices was found to be directly proportional to the content of glucose in the incubation medium. After 60 min incubation, with 5 mM K+ it was 1.30, 1.85 and 4.10 nmol/g in 0.5, 2 and 10 mM glucose, respectively. Values observed after the incubation with 30 mM K+ showed a similar dependence on the concentration of glucose. The content of ACh in the slices incubated with 5 mM K+ was 83, 154, and 267 nmol/g in the presence of 0.5, 2, and 10 mM glucose; similar values were found in experiments with 30 mM K+. The release of ACh into the medium was low in experiments with 5 mM K+ and marked dependence on the concentration of glucose was
not evident; in experiments with 30 mM K⁺, however, the amount of ACh was highly dependent on the concentration of glucose in the medium, being 127, 407 and 863 nmol/g in 0.5, 2 and 10 mM glucose, respectively.

Plotting individual values of ACh concentrations in the slices against the concentrations of acetyl-CoA yielded straight lines, corresponding to the equations:

1. \[ [ACh] = 47.3 [\text{acetyl-CoA}] + 56.3 \] (in the presence of \(5\) mM K⁺),
2. \[ [ACh] = 58.5 [\text{acetyl-CoA}] + 31.6 \] (in the presence of \(30\) mM K⁺).

In experiments with \(5\) mM K⁺, it might have been suspected that the concentration of ACh in the tissue was affected by depolarization of the neurons possibly occurring during incubations with low concentrations of glucose. The relations between acetyl-CoA and ACh were very similar, however, in the presence of the physiological \(5\) mM and of the depolarizing \(30\) mM concentration of K⁺. The analysis of the results indicated that, under the conditions used, the availability of acetyl-CoA exerted a decisive influence both on the rate of synthesis and on the steady-state concentration of ACh in the slices.

**ACETYL-COA AND ACETYLCHOLINE IN BRAIN SLICES INCUBATED WITH METABOLIC INHIBITORS**

Another attempt to investigate the relations between the content of acetyl-CoA and the synthesis of ACh in the brain has been made in experiments on slices of rat nuclei caudati incubated in the presence of inhibitors (44 and Říčný and Tuček, in preparation). The inhibitors used were sodium bromopyruvate, 3,4-dinitrophenol, sodium cyanide, and sodium (−)-hydroxycitrate.

The slices were incubated 60 min in a physiological saline solution containing 10 mM glucose, 0.05 mM choline, 0.058 mM diethyl-p-nitrophenyl phosphate, 6.2 or 31.2 mM K⁺, and various concentrations of the inhibitors. After incubations with 0.25–1 mM bromopyruvate, an inhibitor of the pyruvate dehydrogenase complex, the content of acetyl-CoA in the slices was diminished by 31–86% in the presence of low K⁺ and by 51–89% in the presence of high K⁺; at the same time, the content of ACh in the slices was lowered by 14–86% in low K⁺ and by 27–83% in high K⁺, and the amount of ACh released into the incubation medium was by 45–72% decreased.

The concentrations of acetyl-CoA and of ACh in the slices changed in parallel, although not to the same degree, also after incubations with sodium cyanide. In experiments with 0.05 mM sodium cyanide and 6.2 mM K⁺, the concentration of acetyl-CoA in the slices was increased
by 35% and that of ACh by 25%; the reason of the increase is unknown, but the parallelism in the change of acetyl-CoA and ACh concentrations is conspicuous. In a 0.5 mM concentration and with 6.2 mM K⁺, sodium cyanide reduced the concentration of acetyl-CoA by 28%, but that of ACh by 90% — a finding which apparently confirms earlier electron microscopic data (23) that the neurons are more susceptible to damage by cyanide than the glial cells. In experiments with 31.2 mM K⁺ and 0.5 mM sodium cyanide, the amounts of acetyl-CoA and ACh present in the slices and of ACh released into the medium were diminished by 63, 83, and 82%, respectively.

2,4-Dinitrophenol was found to reduce the concentration of acetyl-CoA and of ACh in the tissue. When used in a 0.2 mM concentration and with 6.2 mM K⁺, it lowered the concentrations of acetyl-CoA and of ACh by 41 and 57%, respectively; with 31.2 mM K⁺, the concentrations of acetyl-CoA and ACh were reduced by 57 and 41%, respectively. The release of ACh into the medium was increased, however, by 0.1 mM 2,4-dinitrophenol, although the concentrations of acetyl-CoA and ACh in the slices were lowered; presumably, the increase in the release of ACh was associated with the increased release of Ca²⁺ from the mitochondria to the cytosol occurring in the presence of 2,4-dinitrophenol (10).

Changes in the concentrations of acetyl-CoA and ACh in the slices were also observed after incubations with 31.2 mM K⁺ and (-)-hydroxycitrate, a compound known to act as a specific inhibitor of ATP-citrate lyase (EC 4.1.3.8; 3, 47, 51), the enzyme responsible for the formation of acetyl-CoA from citrate; when used in a 10 mM concentration, the inhibitor reduced the concentration of acetyl-CoA in the slices by 30% and that of ACh by 34% (Řičný and Tuček, in preparation).

THE EFFECT OF GLUCOSE ON ATROPINE-INDUCED DEPLETION OF BRAIN ACETYLCHOLINE

The results described in previous sections indicate that, under conditions of in vitro experiments, the rate of ACh synthesis depends on the availability of acetyl-CoA and suggests that the supply of acetyl-CoA might become rate-limiting for the synthesis of ACh also under conditions in vivo. Recently, Wecker and Schmidt (52) described experiments in vivo in which they were able to influence a pharmacologically induced depletion of brain ACh by a preliminary administration of choline, the other substrate for the synthesis of ACh. In their experiments, the content of ACh in the brain was diminished by injecting rats with atropine, known to increase the release of ACh from the nerve endings by acting upon presynaptic muscarinic receptors and thus interrupting
a negative feedback control of ACh release (review 34). When the animals were pretreated with a large dose of choline, the fall of brain ACh produced by atropine was either prevented or diminished. It appears from these experiments that the supply of choline becomes rate-limiting for the synthesis of ACh when the demand on the synthesis is augmented by an increase in transmitter release.

In our experiments (7a) we investigated the possibility to influence the atropine-induced depletion of cerebral ACh synthesis. As there is no doubt that the acetyl-CoA used for the synthesis of ACh in the brain originates from glucose (2, 7, 29, 49, 50), we assumed that it might be possible to improve the availability of acetyl-CoA by injecting the animals with a large dose of glucose. Rats were starved 24 h and then injected either with physiological saline or with glucose (20.2 mmol/kg body weight). After one hour, they were injected either with physiological saline or with atropine sulphate (25 mg/kg body weight). They were killed 20 min later and the concentration of ACh in their caudate nuclei was measured.

The injection of atropine brought about a 48% decrease in the content of ACh in caudate nuclei, whereas the injection of glucose alone did not have any effect upon it. If, however, the injection of glucose preceded that of atropine, the content of ACh in the caudate nuclei was diminished by 25% instead of 48%. Although the content of acetyl-CoA in the caudate nuclei has not been measured in these experiments, it seems very likely that glucose did act by improving the supply of acetyl-CoA for the synthesis of ACh.

CONCLUSIONS

Relations between the availability of acetyl-CoA in the brain and the synthesis of ACh are of both theoretical and practical interest. On a theoretical level, their investigation assists in the understanding of the mechanisms responsible for the control of the synthesis of ACh in cholinergic neurons. On a more practical level, there is the attractive possibility that it might become possible to promote or inhibit the synthesis of ACh and thus to influence the function of cholinergic synapses by suitably manipulating the supply of acetyl-CoA.

Experimental data reviewed in this article fit the concept of reaction equilibrium control of the synthesis of ACh. Together with the findings concerning the supply of choline and mentioned in the Introduction, they indicate that the synthesis of ACh can be influenced by changes in the supply of either of the two ACh precursors, i.e., of choline and acetyl-CoA. The close relation between the supply of acetyl-CoA and the syn-
thesis of ACh, as revealed in experiments with brain slices incubated with different concentrations of glucose or with metabolic inhibitors, provides explanation for earlier observations indicating that the synthesis of ACh in the brain is impaired by agents or conditions affecting the oxidative metabolism of the brain (12–16, 26, 27, 30) and that the transmission on cholinergic synapses is very sensitive to the lack of glucose (17, 18, 35). It appears certain that attempts to support the functioning of cholinergic synapses by improved supply of precursors for the synthesis of ACh should not be restricted to attempts to provide more choline (review 1), but should also exploit every possibility to promote the availability of acetyl-CoA.

REFERENCES


