Acetylcoenzyme A and the Synthesis of Acetylcholine in Neurones: Review of Recent Progress

S. Tuček

Institute of Physiology, Czechoslovak Academy of Sciences, Videňská 1083, 142 20 Prague, Czechoslovakia

Abstract. A review of recent progress in the investigation of the following two problems is given: (a) the origin of acetyl groups in the acetylcoenzyme A which is used for the synthesis of acetylcholine in mammalian nerve cells; (b) the role of acetylcoenzyme A in the control of acetylcholine synthesis. The data reviewed are mainly those that have been published after the reviews by Quastel (1978), Tuček (1978) and Jope (1979).

Key words: Acetylcholine — Acetylcoenzyme A — Cholinergic neurones — Citrate — Acetylcarnitine

The substrates for the synthesis of acetylcholine (ACh) in cholinergic neurones are acetylcoenzyme A (acetyl-CoA) and choline. The questions about the origin and supply of these substrates for the synthesis of ACh and their role in its control are of both theoretical and practical interest: it is well known that in the case of dopamine, another neurotransmitter, the discovery of the way how to support its synthesis by increasing the supply of its precursor, has radically improved the life of patients with parkinsonism. Problems concerning the origin of the acetyl-CoA which is used to synthesize ACh in neurones were outstandingly clearly analysed by Quastel (1978) on the cholinergic symposium in La Jolla in 1977; more recent summaries were published by Tuček (1978) and Jope (1979). The purpose of the present contribution is to evaluate recent progress in the field, without trying to give a comprehensive review of earlier literature.

It appears firmly established that glucose and pyruvate are the main sources of acetyl groups in the acetyl-CoA used to synthesize ACh in mammalian brain (Browning and Schulman 1968; Nakamura et al. 1970; Tuček and Cheng 1970, 1974; Lefresne et al. 1973). Acetate, on the contrary, seems to be the source of acetyl groups for the synthesis of ACh in some other mammalian organs or tissues (cornea — Fitzgerald and Cooper 1967; skeletal muscle — Dreyfus 1975) and in the nerves of lower vertebrates and invertebrates (electric organ of Torpedo — Israël and Tuček 1974; lobster nerve — Cheng and Nakamura 1970; neurones of *Aplysia* — Baux et al. 1979). Some progress has been achieved recently in

experiments with mammalian brain tissue regarding the question of how the intramitochondrial, pyruvate-generated acetyl-CoA passes the inner mitochondrial membrane on its way to the nerve-ending cytosol, where the synthesis of ACh takes place.

The intramitochondrial acetyl-CoA is probably first transformed to some other compound — citrate, glutamate, acetyl-carnitine, acetate etc. — which is then transported across the very selectively permeable inner mitochondrial membrane, and transformed again to acetyl-CoA in the outer mitochondrial space or extramitochondrially. Among the substances considered for the role of acetyl carriers for the synthesis of ACh, acetylcarnitine now appears a much stronger candidate than it did several years ago. Lefresne et al. (1977) were unable to dilute the radioactivity in labelled ACh, produced in synaptosomes from labelled glucose, by adding excess unlabelled acetylcarnitine to the incubation medium. This was interpreted as evidence against the role of acetylcarnitine in the supply of acetyl groups for the synthesis of ACh. However, in experiments with rat striatal slices and (^{14}C-acetyl) carnitine, Doležal and Tuček (1981) revealed that this compound was very efficiently used for the synthesis of ACh (with 2/3 the efficiency of the utilization of glucose, and considerably better than acetate or citrate). This finding neatly corresponded to the observation that selective degeneration of cholinergic nerve fibres to the nc. interpeduncularis leads to a selective decrease in the activities of choline acetyltransferase (ChAT), pyruvate dehydrogenase and carnitine acetyltransferase (Sterri and Fonnum 1980). It must be said, however, that in similar experiments with cholinergic denervation of the hippocampus, Szutowicz et al. (1982b) observed a selective decline in the activities of ChAT and ATP citrate lyase (the enzyme producing acetyl-CoA from citrate), and not of carnitine acetyltransferase. The reason for the difference between the results obtained by Sterri and Fonnum (1980) and Szutowicz et al. (1982b) is not apparent.

Earlier experimental work has led to controversy with regard to the role of citrate in the supply of acetyl groups in cholinergic neurones. Labelled atoms from citrate were not incorporated into ACh both in experiments *in vitro* (Nakamura et al. 1970; Lefresne et al. 1977; Cheng and Brunner 1978; Benjamin and Quastel 1981) and *in vivo* (Tuček and Cheng 1970, 1974), and the addition of unlabelled citrate did not alter the synthesis of labelled ACh from labelled glucose (Szutowicz et al. 1981). On the other hand, results obtained in experiments with the synthesis of ACh from glucose labelled by ^{14}C and ^{3}H in position 6 (a loss of 1/3 of ^{3}H compared to ^{14}C on the way from glucose to ACh) were most conveniently explained on the assumption that two-carbon units from glucose, forming finally the acetyl group in ACh, have to pass obligatorily through the stage of citrate (Sollenberg and Sörbo 1970). Although the results obtained with double-labelled glucose were confirmed in a more recent work by Sterling and O' Neill (1978), a considerable body of evidence has now accumulated supporting the view that

citrate is the source of a certain proportion (1/4—1/3), but not of all acetyl groups in brain ACh.

The evidence in support of this comes from experiments with (—)-hydroxycitrate, a selective inhibitor of ATP citrate lyase, the extramitochondrial enzyme which would necessarily be involved in the supply of acetyl-CoA for the synthesis of ACh if citrate were the source of acetyl groups. Results of experiments with (—)-hydroxycitrate obtained in various laboratories are summarized in Table 1. The inhibition of ATP citrate lyase has never been found to diminish the synthesis of ACh by more than 40%. At the same time, there is good ground to assume that ATP citrate lyase was completely inhibited by the concentrations of (—)-hydroxycitrate used; this is particularly evident from the studies of Tuček et al. (1981) and Szutowicz et al. (1982a). It is interesting to note that the inhibition of ATP citrate lyase was associated with a loss of acetyl-CoA which seems to be quite considerable. In experiments described by Říčný and Tuček (1982), the total amount of acetyl-CoA in striatal slices was diminished by 25—38% after incubation with 2.5 mmol/l (—)-hydroxycitrate. Assuming that the extramitochondrial acetyl-CoA was only affected, one can guess that the concentration of acetyl-CoA in the cytoplasm diminished to less than 1/2 of its usual level — provided that ATP citrate lyase is uniformly distributed and uniformly inhibited in various cellular and subcellular compartments in the slices. (It is not known how much acetyl-CoA is intra- and how much is extramitochondrial in the brain; in the adipocytes, the extramitochondrial acetyl-CoA was estimated to represent 36—72% of the total pool (Paetzke—Brunner et al. 1978)).

The reason why radioactive label from citrate, added to the incubation media or injected *in vivo* is so poorly incorporated into ACh, remains unclear. There is no doubt that citrate is very actively taken up by the cells or synaptosomes and is metabolized (Tuček and Cheng 1974; Gibson and Shimada 1980; Szutowicz et al. 1981, 1982a). The reason may be that citrate is taken up into some other — noncholinergic — compartment, but particularly in experiments with synaptosomes it is difficult to say what this compartment might be.

Table 1. Inhibitory effect of (—)-hydroxycitrate on the synthesis of ACh from glucose

Reference	Tissue	Concentration of (—)-hydroxycitrate (mmol/l)	Inhibition %
Sterling and O'Neill (1978)	Rat cortical slices	7.5	33
Endemann and Brunengraber (1980)	Perfused rat diaphragm	5	10—40
Gibson and Shimada (1980)	Rat cortical slices	2.5	22
	Rat brain synaptosomes	2.5	25
Tuček et al. (1981)	Rat striatal slices	2.5	33
Sterling et al. (1981)	Rat cortical slices	5	35
Szutowicz et al. (1981)	Rat brain synaptosomes	1	20—39
Říčný and Tuček (1982)	Rat striatal slices	5	29

It has repeatedly been proposed that ATP citrate lyase is preferentially associated with ChAT and the cholinergic nerve endings, but the evidence for this is controversial. In experiments with central cholinergic denervation, Szutowicz et al. (1982b) obtained results supporting a specific association of the two enzymes, while Sterri and Fonnum (1980) reported observations contradicting it. Similarly comparison of regional and subcellular distribution of ChAT and ATP citrate lyase indicated a correlation in the localization of the two enzymes in experiments of Szutowicz et al. (1982c) but not in those reported by Harvey et al. (1982). During postnatal development of the rat brain, the activity of ChAT was found to increase 14 fold, and that of ATP citrate lyase to diminish by 26% (Szutowicz et al. 1982a). The view that ATP citrate lyase is more active in the cholinergic than in other neurones is supported by measurements made on single neurones by Hayashi and Kato (1978). Although the activity of ATP citrate lyase in cerebellar synaptosomes (low in ChAT) is lower than in other parts of the brain (Szutowicz and Łysiak 1980), the parameters of citrate metabolism (particularly, the flux through ATP citrate lyase) are the same in cerebral and cerebellar synaptosomes (Szutowicz et al. 1982a). It was found possible to manipulate the activity of ATP citrate lyase and to change its contribution to the synthesis of ACh in the synaptosomes, and yet this did not affect the total synthesis of ACh which remained constant (Szutowicz et al. 1981).

It seems likely from this and other evidence that citrate is the source of about one third of the acetyl groups used for the synthesis of ACh and that there must be other (parallel) sources of acetyl groups. The total amount of ATP citrate lyase present in the brain certainly could produce much more acetyl-CoA than needed for the synthesis of ACh (Szutowicz and Łysiak 1980); however, it still is difficult to be quite sure that a sufficient amount of the enzyme is where it must be if it has to participate in the synthesis of ACh, i.e. in the cholinergic nerve endings. If the enzyme is there, then it is the output of citrate from the mitochondria that limits the utilization of citrate for the synthesis of ACh (Szutowicz et al. 1982c).

The possibility that acetate (formed in the cytoplasm, entering the nerve terminals from the extracellular fluid, or arriving as an "acetyl carrier" from the mitochondria) might be of physiological importance for the synthesis of ACh, received additional consideration in experiments on striatal slices (Doležal and Tuček 1981) and sympathetic ganglion (Kwok and Collier 1982). Its utilization in the brain slices was low. In the ganglion at rest the utilization of acetate was quite high (higher than that of glucose). However, during preganglionic stimulation the synthesis of ACh from glucose increased several fold, whereas that from acetate diminished. These observations are in line with earlier evidence indicating that acetate is unlikely to make a substantial contribution to the synthesis of ACh.

The utilization of 3-hydroxybutyrate for the synthesis of ACh in cortical slices was evaluated as low by Sterling et al. (1981), but this may be just a matter of

concentrations: the incorporation of labelled atoms from (6-^{3}H)glucose was about 40 times higher than that from 3-hydroxy(3-^{14}C)butyrate, the concentration of which in the incubation medium was about 40 times lower than that of glucose. Thus, the cholinergic neurones seem quite capable of utilizing 3-hydroxybutyrate for ACh production, but the physiological role of this substrate is likely to be low in view of its concentrations in the body fluids and in the cells.

An interesting way how to overcome the problem of acetyl groups transfer from the mitochondria has been proposed by Lefresne et al. (1978a, b, c). These authors suggested that, in addition to the mitochondrial pyruvate dehydrogenase (located in the mitochondrial matrix), there is another pool of the enzyme located extramitochondrially, either in the cytosol or in the membranes of cholinergic nerve terminals; in the membrane the enzyme might be in a complex with the carrier for choline and with ChAT. This suggestion is based on very indirect evidence — several types of experiments on synaptosomes in which it proved possible to dissociate the utilization of pyruvate for the synthesis of CO_2 from its utilization for the synthesis of ACh. The explanation is put forward that the experimental measures applied (e.g., exposure to α-cyanocinnamate followed by exposure to bromopyruvate) inhibited a pool of pyruvate dehydrogenase responsible for the supply of acetyl-CoA for the synthesis of ACh while the pool of pyruvate dehydrogenase associated with the Krebs cycle continued to function. It is not easy to propose alternative and reliable explanations for the findings described, but it would be even more difficult to accept the hypothesis of extramitochondrial pyruvate dehydrogenase without further and more direct experimental evidence. So far, no evidence has been reported indicating the presence of extramitochondrial pyruvate dehydrogenase in the neurones, and based on subcellular fractionations. No pyruvate dehydrogenase was found in cytosolic fractions by Polak et al. (1978) and by Szutowicz and Łysiak (1980). The fact that α-cyanocinnamate itself produces parallel decreases of ACh and CO_2 synthesis from pyruvate (Lefresne et al. 1978c; Jope and Jenden 1980) speaks against the hypothesis; since α-cynocinnamate inhibits the transport of pyruvate into the mitochondria, the production of CO_2 occurring intermitochondrially in the Krebs cycle should be more affected than the hypothetically extramitochondrial production of acetyl-CoA for the synthesis of ACh.

In 1967, Tuček reported that the output (leakage? transport?) of acetyl-CoA from brain mitochondria incubated *in vitro* greatly increases in the presence of Ca^{2+} ions and diminishes in the presence of ATP or ADP; he tentatively associated these changes with mitochondrial swelling and contraction. These observations raised the question whether similar changes in the leakage of acetyl-CoA from the mitochondria might occur in intact cells, either during spontaneous cycles of mitochondrial swelling and contraction, or under the influence of Ca^{2+} influx into the cells (e.g., into the presynaptic nerve terminals during their activity). Polak et

al. (1978) reported that Ca^{2+} ions increased the release from mitochondria of a precursor of acetyl groups, which was later also identified as acetyl-CoA (Polak, personal communication). Ca^{2+} ions apparently act upon the mitochondrial membranes: their effect on the output of acetyl-CoA becomes abolished following treatment of the membranes with ether (Tuček 1967) or Triton X-100 (Benjamin and Quastel 1981). To be sure that Ca^{2+} acts by altering mitochondiral permeability and not by changing the activity of pyruvate dehydrogenase (which it might about by actinating the pyruvate dehydrogenase phosphate phosphatase — Denton et al. 1975), Říčný and Tuček (1983) compared the effects of Ca^{2+} on the output of acetyl-CoA from brain mitochondria with its effects on the formation of $^{14}CO_2$ from (1-^{14}C)pyruvate in the mitochondria. In mitochondria with undamaged membranes, 1 mmol/l of Ca^{2+} brought about a 4-to-6-fold increase in the release of acetyl-CoA accompanied by only 30% increase in the activity of pyruvate dehydrogenase.

In view of this discrepancy and of the effect of detergents (see above) it is evident that Ca^{2+} acts on mitochondrial membranes, but the mechanism of its action is not clear. Does it open some specific gates, or activate a specific carrier? Does it increase mitochondrial permeability by activating phospholipase A (as suggested by Benjamin and Quastel 1981)? Or does it bring about mechanical damage of the membranes during excessive swelling? An answer should be found to these questions, and also to the question whether the concentrations of Ca^{2+} required to alter the permeability of mitochondrial membranes inside the cells (when the mitochondria are in their natural environment) are the same or lower than those required in experiments with isolated mitochondria. Only in case that lower concentrations of Ca^{2+} are sufficient could the phenomenon be of physiological significance.

Under certain conditions, citrate (McLennan and Elliott 1950; Gibson and Shimada 1980; Doležal and Tuček 1981; Benjamin and Quastel 1981; Říčný and Tuček 1982) and EGTA (Benjamin and Quastel 1981; Říčný and Tuček 1982) were found to diminish the synthesis of ACh in brain slices or synaptosomes. This effect cannot be explained by chelation of extracellular Ca^{2+} and blockade of ACh release since it occurs also in Ca^{2+}-free media (Doležal and Tuček 1981; Benjamin and Quastel 1981; Říčný and Tuček 1982). It has been proposed (Říčný and Tuček 1982) that citrate and EGTA act by chelating Ca^{2+} ions inside the cells, and that a lowering of the (already low) Ca^{2+} concentration in the cytosol has an opposite effect than its increase and diminishes the output of acetyl-CoA from the mitochondria. The output of acetyl-CoA from purified brain mitochondria into the incubation medium was indeed found to be lower at an estimated Ca^{2+} concentration of 1×10^{-9} mol/l than at an estimated Ca^{2+} concentration of 1×10^{-7} mol/l (which is approximately the concentration expected in the cytosol) (Říčný and Tuček 1983).

To summarize, the information available at present gives more support to the view that there are several parallel ways in which acetyl groups from the intramitochondrial (pyruvate-generated) acetyl-CoA are transported for the extramitochondrial synthesis of ACh than to the view that there is just one specific pathway. Citrate seems to be the source of about 1/3 of the extramitochondrial acetyl-CoA utilized for this purpose. Acetylcarnitine is another very likely source of extramitochondrial acetyl groups, whereas the other candidates for the role of acetyl carriers, such as glutamate, acetylaspartate or acetate, seem rather speculative (with at least as much evidence negating as supporting their role). The possibility that, under conditions of an increased cytoplasmic Ca^{2+} concentration, acetyl-CoA itself is transferred or leaks through the inner mitochondrial membrane, deserves more investigation; if this mechanism worked, it could provide an efficient means of autoregulation of ACh synthesis.

The possible participation of acetyl-CoA supply in the control of the synthesis of ACh in cholinergic neurones has been an important aspect of several investigations reported recently. In thiamine-deficient rats, the synthesis of ACh occurring in a sympathetic ganglion during preganglionic stimulation was found to be diminished by more than 60% in comparison with controls (Sacchi et al. 1978); the activity of ChAT in the ganglion was not changed, and it seems justified to conclude that the impairment of ACh synthesis was due to a diminished supply of acetyl-CoA by pyruvate dehydrogenase, (of which thiamine pyrophosphate is a coenzyme). Behavioral changes occurring during thiamine deficiency together with changes in the synthesis of ACh in the brain have been described by Barclay et al. (1981).

Direct measurements of the content of acetyl-CoA and ACh in striatal slices and of the release of ACh from them revealed a direct relationship between the availability of acetyl-CoA and the rate of synthesis or the steady-state concentration of ACh in the slices, independent of whether the changes in the availability of acetyl-CoA had been produced by limitations in the supply of glucose (Říčný and Tuček 1980), by inhibitors of mitochondrial metabolism (Říčný and Tuček 1981), or by inhibition of extramitochondrial ATP citrate lyase (Říčný and Tuček 1982). These observations are in good accord with (and provide an experimentally based explanation of) earlier observations of a close correlation between the rates of pyruvate decarboxylation and ACh synthesis in brain prisms incubated with metabolic inhibitors, low O_2 or low glucose (Gibson et al. 1975; Gibson and Blass 1976a), and of profound effects of hypoxia (Weiler et al. 1979; Ksiezak and Gibson 1981a,b) or metabolic inhibitors (Jope and Jenden 1977; Jope et al. 1978; Jope 1981) on the synthesis of ACh in brain slices or synaptosomes.

Results similar in appearance but very probably entirely different in their underlying mechanism, have been reported from experiments investigating the rate of cerebral ACh synthesis under *in vivo* conditions in animals exposed to different

types of hypoxia (Gibson and Blass 1976b; Gibson et al. 1978, 1981a; Gibson and Duffy 1981; Shimada 1981). The common finding was that the synthesis of ACh in the brain (measured by the incorporation of label from glucose or choline into ACh) was considerably diminished during hypoxia. This was apparently true even when the hypoxia-induced increases in brain choline and lactate (both capable of diluting the injected labelled substrates) were taken into account. However, the concentration of ACh in the brain did not change, except in the highest degrees of hypoxia. ACh concentration is the result of an equilibrium between ACh release and ACh synthesis and, as a rule, the synthesis of ACh is regulated so as to compensate for losses occurring by its release (for reviews see MacIntosh and Collier 1976; Tuček 1978). If the synthesis of ACh were diminished and the release unchanged, the content of ACh should be lowered. It is most likely that the primary change occurring during hypoxia is a decrease in ACh release; some of the mechanisms which might be responsible have been discussed by Gibson and Peterson (1982). Changes in ACh release (slowing down of ACh turnover) are also likely to be to a certain extent responsible for some of the observations indicating that the rate of ACh synthesis in the brain declines with age (Gibson et al. 1981b), in accordance with the observed impairment of K^+-stimulated, Ca^{2+}-dependent ACh release from brain slices in old rats (Gibson and Peterson 1981). In future work it seems substantial to distinguish in situations mentioned in this paragraph how much the diminished ACh release is due to alterations of the release apparatus itself, and how much it is due to decreased excitatory or increased inhibitory inputs into cholinergic neurones.

Close correlations between the synthesis of ACh and the rates of pyruvate decarboxylation (Gibson et al. 1975; Gibson and Blass 1976a) and the tissue content of acetyl-CoA (Říčný and Tuček 1980, 1981) support the view that the availability of acetyl-CoA plays a role in the control of the synthesis and steady-state levels of ACh in the cells. There is growing consensus that the reaction catalysed by ChAT, i.e.

$$\text{choline} + \text{acetyl-CoA} \leftrightharpoons \text{ACh} + \text{CoA}$$

is usually close to equilibrium in the nerve terminals, particularly thanks to the presence of comparatively high amounts of ChAT (Potter et al. 1968; Tuček 1978; Jenden 1979). Decrease in the availability of acetyl-CoA is then likely to influence the steady-state levels of ACh by altering the reaction equilibrium; at lowered steady-state levels, the rates of ACh release will also be lowered, and this is likely to be one of the causes of diminished rates of the synthesis. Another cause of diminished rates of the synthesis may be changes in the efficiency of the enzymatic catalysis itself since intracellular concentrations of acetyl-CoA are generally below the values of Km of ChAT for acetyl-CoA (for review see Tuček 1978).

Although the *in vitro* results referred to above leave little doubt about the

influence of acetyl-CoA availability on the synthesis and steady-state levels of ACh in experiments *in vitro*, it is still difficult to say to what extent changes in the supply of acetyl-CoA are likely to influence the synthesis of ACh under conditions *in vivo*. An *in vivo* experimental situation in which the supply of acetyl-CoA apparently was the rate-limiting factor in the synthesis of ACh has been described by Doležal and Tuček (1982). After the injection of a large dose of atropine, the release of ACh from central neurones is greatly increased because of the blockade of presynaptic muscarinic receptors taking part in the control of the release. This leads to a decrease in the content of ACh in the brain. By administering a large dose of glucose before atropine, it proved possible to diminish the atropine-induced depletion of ACh. It seems likely that glucose acted by improving the supply of acetyl-CoA. This observation suggests that, under conditions of an increased demand on the synthesis of ACh, the supply of acetyl-CoA may become rate-limiting in brain neurones, in the same way as it has been shown for the other precursor, i.e. choline (Wecker et al. 1978; Wecker and Schmidt 1980; Schmidt and Wecker 1981; Jope 1982; Trommer et al. 1982).

References

Barclay L. L., Gibson G. E., Blass J. P. (1981): Impairment of behavior and acetylcholine metabolism in thiamine deficiency. J. Pharmacol. Exp. Ther. **217,** 537—543

Baux G., Simonneau M., Tauc L. (1979): Transmitter release: ruthenium red used to demonstrate a possible role of sialic acid containing substrates. J. Physiol. (London) **291,** 161—178

Benjamin A. M., Quastel J. H. (1981): Acetylcholine synthesis in synaptosomes: Mode of transfer of mitochondrial acetyl coenzyme A. Science **213,** 1495—1497

Browning E. R., Schulman M. O. (1968): (^{14}C)Acetylcholine synthesis in cortex slices of rat brain. J. Neurochem. **15,** 1391—1405

Cheng S.—C., Brunner E. A. (1978): Alteration of tricarboxylic acid cycle metabolism in rat brain slices by halothane. J. Neurochem. **30,** 1421—1430

Cheng S.—C., Nakamura R. (1970): A study on the tricarboxylic acid cycle and the synthesis of acetylcholine in the lobster nerve. Biochem. J. **118,** 451—455

Denton R. M., Randle P. J., Bridges B. J., Cooper R. H., Kerbey A. L., Pask H. T., Severson D. L., Stanbie D., Whitehouse S. (1975): Regulation of mammalian pyruvate dehydrogenase. Mol. Cell. Biochem. **9,** 27—53

Doležal V., Tuček S. (1981): Utilization of citrate, acetylcarnitine, acetate, pyruvate and glucose for the synthesis of acetylcholine in rat brain slices. J. Neurochem. **36,** 1323—1330

Doležal V., Tuček S. (1982): Effects of choline and glucose on atropine-induced alterations of acetylcholine synthesis and content in the caudate nuclei of rats. Brain Res. **240,** 285—293

Dreyfus P. (1975): Identification de l'acétate comme précurseur du radical acétyle de l'acétylcholine des jonctions neuro-musculaires du rat. C. R. Acad. Sci. Paris, D, **280,** 1893—1894

Endemann G., Brunengraber H. (1980): The source of acetyl coenzyme A for acetylcholine synthesis in the perfused rat phrenic nerve-hemidiaphragm. J. Biol. Chem. **255,** 11091—11093

Fitzgerald G. G., Cooper J. R. (1967): Studies on acetylcholine in the corneal epithelium. Fed. Proc. Am. Soc. Exp. Biol. **26,** 651

Gibson G. E., Blass J. P. (1976a): A relation between (NAD^+)/(NADH) potentials and glucose utilization in rat brain slices. J. Biol. Chem. **251,** 4127—4130

Gibson G. E., Blass J. P. (1976b): Impaired synthesis of acetylcholine in brain accompanying mild hypoxia and hypoglycemia. J. Neurochem. **27,** 37—42

Gibson G. E., Duffy T. E. (1981): Impaired synthesis of acetylcholine by mild hypoxic hypoxia or nitrous oxide. J. Neurochem. **36,** 28—33

Gibson G. E., Peterson C. (1981): Aging decreases oxidative metabolism and the release and synthesis of acetylcholine. J. Neurochem. **37,** 978—984

Gibson G. E., Peterson C. (1982): Decreases in the release of acetylcholine *in vitro* with low oxygen. Biochem. Pharmacol. **31,** 111—115

Gibson G. E., Shimada M. (1980): Studies on the metabolic pathway of the acetyl group for acetylcholine synthesis. Biochem. Pharmacol. **29,** 167—174

Gibson G. E., Jope R., Blass J. P. (1975): Decreased synthesis of acetylcholine accompanying impaired oxidation of pyruvic acid in rat brain minces. Biochem. J. **148,** 17—23

Gibson G. E., Shimada M., Blass J. P. (1978): Alterations in acetylcholine synthesis and cyclic nucleotides in mild cerebral hypoxia. J. Neurochem. **31,** 757—760

Gibson G. E., Peterson C., Sansone J. (1981a): Decreases in amino acid and acetylcholine metabolism during hypoxia. J. Neurochem. **37,** 192—201

Gibson G. E., Peterson C., Jenden D. J. (1981b): Brain acetylcholine synthesis declines with senescence. Science **213,** 674—676

Harvey S. A. K., Leong S. F., Clark J. B. (1982): Noncorrelation of choline kinase or ATP citrate lyase with cholinergic activity in rat brain. J. Neurochem. **39,** 1481—1484

Hayashi H., Kato T. (1978): Acetyl-CoA synthesizing enzyme activities in single nerve cell bodies of rabbit. J. Neurochem. **31,** 861—869

Israël M., Tuček S. (1974): Utilization of acetate and pyruvate for the synthesis of "total", "bound" and "free" acetylcholine in the electric organ of Torpedo. J. Neurochem. **22,** 487—491

Jenden D. J. (1979): An overview of choline and acetylcholine metabolism in relation to the therepeutic uses of choline. In: Nutrition and the Brain, vol. 5 (Eds. A. Barbeau, J. H. Crowdon, R. J. Wurtman), pp. 13—24, Raven Press, New York

Jope R. S. (1979): High affinity choline transport and acetyl-CoA production in brain and their roles in the regulation of acetylcholine synthesis. Brain Res. Rev. **1,** 313—344

Jope R. S. (1981): Acetylcholine turnover and compartmentation in rat brain synaptosomes. J. Neurochem. **36,** 1712—1721

Jope R. S. (1982): Effects of phosphatidycholine administration to rats on choline in blood and choline and acetylcholine in brain. J. Pharmacol. Exp. Ther. **220,** 322—328

Jope R. S., Jenden D. J. (1977): Synaptosomal transport and acetylation of choline. Life Sci. **20,** 1389—1392

Jope R. S., Weiler M. H., Jenden D. J. (1978): Regulation of acetylcholine synthesis: control of choline transport and acetylation in synaptosomes. J. Neurochem. **30,** 949—954

Jope R. S., Jenden D. J. (1980): The utilization of choline and acetylconzyme A for the synthesis of acetylcholine. J. Neurochem. **35,** 318—325

Ksiezak H. J., Gibson G. E. (1981a): Acetylcholine synthesis and CO_2 production from variously labeled glucose in rat brain slices and synaptosomes. J. Neurochem. **37,** 88—94

Ksiezak H. J., Gibson G. E. (1981b): Oxygen dependence of glucose and acetylcholine metabolism in slices and synaptosomes from rat brain. J. Neurochem. **37,** 305—314

Kwok Y. N., Collier B. (1982): Synthesis of acetylcholine from acetate in a sympathetic ganglion. J. Neurochem. **39,** 16—26

Lefresne P., Guyenet P., Glowinski J. (1973): Acetylcholine synthesis from (2-^{14}C)pyruvate in rat striatal slices. J. Neurochem. **20,** 1083—1097

Lefresne P., Hamon M., Beaujouan J. C., Glowinski J. (1977): Origin of the acetyl moiety of acetylcholine synthesized in rat striatal synaptosomes. Biochimie **59,** 197—215

Lefresne P., Beaujouan J.—C., Glowinski J. (1978a): Arguments en faveur de l'origine extramitochondriale du radical acétyle de l'acétylcholine synthétisée dans des synaptosomes du striatum du rat. C. R. Acad. Sci. Paris, D, **286,** 1165—1167

Lefresne P., Beaujouan J. C., Glowinski J. (1978b): Evidence for extramitochondrial pyruvate dehydrogenase involved in acetylcholine synthesis in nerve endings. Nature **274,** 497—500

Lefresne P., Beaujouan J. C., Glowinski J. (1978c): Origin of the acetyl moiety of acetylcholine in rat striatal synaptosomes: a specific pyruvate dehydrogenase involved in ACh synthesis. Biochimie **60,** 479—487

MacIntosh F. C., Collier B. (1976): Neurochemistry of cholinergic terminals. In: Neuromuscular Junction (Ed. E. Zaimis), Handbook of Experimental Pharmacology, New Series, vol. 42, pp. 99—228, Springer Verlag, Berlin

McLennan H., Elliott K. A. C. (1950): Factors affecting the synthesis of acetylcholine by brain slices. Am. J. Physiol. **163,** 605—613

Nakamura R., Cheng S.—C., Naruse H. (1970): A study on the precursors of the acetyl moiety of acetylcholine in brain slices. Biochem. J. **118,** 443—450

Paetzke-Brunner I., Schön H., Wieland O. H. (1978): Insulin activates pyruvate dehydrogenase by lowering the mitochondrial acetyl-CoA/CoA ratio as evidenced by digitonin fractionation of isolated fat cells. FEBS Lett. **93,** 307—311

Polak R. L., Molenaar P. C., Braggaar-Schaap P. (1978): Regulation of acetylcholine synthesis in rat brain. In: Cholinergic Mechanisms and Psychopharmacology (Ed. D. J. Jenden), pp. 511—524, Plenum Press, New York

Potter L. T., Glover V. A. S., Saelens J. K. (1968): Choline acetyltransferase from rat brain. J. Biol. Chem. **243,** 3864—3870

Quastel J. H. (1978): Source of acetyl group in acetylcholine. In: Cholinergic Mechanisms and Psychopharmacology (Ed. D. J. Jenden), pp. 411—430, Plenum Press, New York

Říčný J., Tuček S. (1980): Relation between the content of acetylcoenzyme A and acetylcholine in brain slices. Biochem J. **188,** 683—688

Říčný J., Tuček S. (1982): Acetylcoenzyme A and acetylcholine in slices of rat caudate nuclei incubated with (—)-hydroxycitrate, citrate, and EGTA. J. Neurochem. **39,** 668—673

Říčný J., Tuček S. (1983): Ca^{2+} ions and the output of acetylcoenzyme A from brain mitochondria. Gen. Physiol. Biophys. **2,** 27—37

Sacchi O., Ladinsky H., Prigioni I., Consolo S., Peri G., Perri V., (1978): Acetylcholine turnover in the thiamine-depleted superior cervical ganglion of the rat. Brain Res. **151,** 609—614

Schmidt D. E., Wecker L. (1981): CNS effects of choline administration: evidence for temporal dependence. Neuropharmacology **20,** 535—539

Shimada M. (1981): Alteration of acetylcholine synthesis in mouse brain cortex in mild hypoxic hypoxia. J. Neural Transmiss. **50,** 233—246

Sollenberg J., Sörbo B. (1970): On the origin of the acetyl moiety of acetylcholine in brain studied with a differential labelling technique using ^{3}H-^{14}C-mixed labelled glucose and acetate. J. Neurochem. **17,** 201—207

Sterling G. H., O'Neill J. J. (1978): Citrate as the precursor of the acetyl moiety of acetylcholine. J. Neurochem. **31,** 525—530

Sterling G. H., McCafferty M. R., O'Neill J. J. (1981): β-Hydroxybutyrate as a precursor to the acetyl moiety of acetylcholine. J. Neurochem. **37,** 1250—1259

Sterri S. H., Fonnum F. (1980): Acetyl-CoA synthesizing enzymes in cholinergic nerve terminals. J. Neurochem. **35,** 249—254

Szutowicz A., Łysiak W. (1980): Regional and subcellular distribution of ATP-citrate lyase and other

enzymes of acetyl-CoA metabolism in rat brain. J. Neurochem. **35,** 775—785
Szutowicz A., Bielarzyk H., Łysiak W. (1981): The role of citrate derived from glucose in the acetylcholine synthesis in rat brain synaptosomes. J. Biochem. **13,** 887—892
Szutowicz A., Kabata J., Bielarczyk H. (1982a): The contribution of citrate to the synthesis of acetyl units in synaptosomes of developing rat brain. J. Neurochem. **38,** 1196—1204
Szutowicz A., Srere P. A., Allen C. N., Crawford I. L. (1982b): Effects of septal lesions on enzymes of acetyl-CoA metabolism in the cholinergic system of the rat hippocampus. J. Neurochem. **39,** 458—463
Szutowicz A., Stepień M., Bielarczyk H., Kabata J., Łysiak W. (1982c): ATP citrate lyase in cholinergic nerve endings. Neurochem. Res. **7,** 799—810
Trommer B. A., Schmidt D. E., Wecker L. (1982): Exogenous choline enhances the synthesis of acetyloholine only under conditions of increased cholinergic neuronal activity. J. Neurochem. **39,** 1704—1709
Tuček S. (1967): The use of choline acetyltransferase for measuring the synthesis of acetyl-coenzyme A and its release from brain mitochondria. Biochem. J. **104,** 749—756
Tuček S. (1978): Acetylcholine Synthesis in Neurons. Chapman and Hall, London
Tuček S., Cheng S.—C. (1970): Precursors of acetyl groups in acetylcholine in the brain in vivo. Biochim. Biophys. Acta **208,** 538—540
Tuček S., Cheng S.—C. (1974): Provenance of the acetyl group of acetylcholine and compartmentation of acetyl-CoA and Krebs cycle intermediates in the brain *in vivo*. J. Neurochem. **22,** 893—914
Tuček S., Doležal V., Sullivan A. C. (1981): Inhibition of the synthesis of acetylcholine in rat brain slices by (—)-hydroxycitrate and citrate. J. Neurochem. **36,** 1331—1337
Wecker L., Dettbarn W.—D., Schmidt D. E. (1978): Choline administration: modification of the central actions of atropine. Science **199,** 86—87
Wecker L., Schmidt D. E. (1980): Neuropharmacological consequences of choline administration. Brain Res. **184,** 234—238
Weiler M. H., Misgeld U., Bak I. J., Jenden D. J. (1979): Acetylcholine synthesis in rat neostriatal slices. Brain Res. **176,** 401—406

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