Biochem. J. (1980) **188**, 683–688
Printed in Great Britain

Relation between the Content of Acetyl-Coenzyme A and Acetylcholine in Brain Slices

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(*Received 8 November 1979*)

Slices of rat caudate nuclei were incubated *in vitro* in media containing, among other constituents, three different concentrations of glucose (0.5, 2 and 10mM), 0.2mM-choline, paraoxon as an inhibitor of cholinesterase, and 5mM- or 30mM-K^+. After 30 and 60min of incubation, the concentrations of acetyl-CoA, acetylcholine and choline in the tissue and of acetylcholine in the incubation medium were measured. The content of acetyl-CoA in the slices varied in direct relation to the concentration of glucose in the incubation medium. The content of acetylcholine in the slices and, in experiments with high K^+, also the amount of acetylcholine released into the incubation medium varied in direct relation to the concentration of glucose in the incubation medium and to the concentration of acetyl-CoA in the slices; the relation between the concentrations of acetyl-CoA and of acetylcholine in the slices was linear. It was concluded that the availability of acetyl-CoA had a decisive influence on both the rate of synthesis of acetylcholine and its steady-state concentration. The observations accord with the view that, at the ultimate level, the synthesis of acetylcholine is controlled by the Law of Mass Action.

Several mechanisms have been proposed to explain the control of the synthesis of acetylcholine and the maintenance of its stable concentration in cholinergic neurons (for review, see MacIntosh & Collier, 1976; Tuček, 1978). Three views have received most attention: (*a*) that the synthesis of acetylcholine is primarily controlled by the high-affinity uptake of choline; (*b*) that the control depends on the inhibition of choline acetyltransferase by the products of the reaction that it catalyses, i.e. by acetylcholine and CoA; (*c*) that at the sites of acetylcholine synthesis the concentrations of the substrates and products of the reaction catalysed by choline acetyltransferase approximate to equilibrium and the synthesis of acetylcholine is governed by the Law of Mass Action (Potter *et al.*, 1968; Glover & Potter, 1971). In an authoritative review, Collier (1977) stated that the last hypothesis 'is attractive but remains to be tested'.

The Mass-Action hypothesis for the control of acetylcholine synthesis is supported by observations indicating that an increase in the concentration of choline in the tissues leads to an increase in acetylcholine content (Kuntscherová, 1972; Haubrich *et al.*, 1974; Cohen & Wurtman, 1975; but for a contrary view see also Eckernäs *et al.*, 1977; Pedata *et al.*, 1977; Flentge & Van den Berg, 1979), and that influences interfering with the transport of choline into the nerve terminals decrease the concentration of acetylcholine in them (Hebb *et al.*, 1964; Collier *et al.*, 1972). The relation between the concentration of acetyl-CoA and acetylcholine in the brain and the tissues with cholinergic innervation is of evident interest, but it has not been investigated except in studies concerning the effects of thiamin deficiency (Heinrich *et al.*, 1973; Reynolds & Blass, 1975), yielding contradictory results.

In the present study, we examined the relation between the concentrations of acetyl-CoA and acetylcholine in slices of rat caudate nuclei incubated in media with different concentrations of glucose. Preliminary communication of the results has been made previously (Říčný & Tuček, 1980*a*; Tuček *et al.*, 1980).

Methods

Wistar rats weighing about 200g were used. Their caudate nuclei were cut into slices with a McIlwain tissue chopper (McIlwain & Rodnight, 1962) set at 0.4mm. Pooled tissue from two animals, weighing 110–140mg, was incubated in 0.8ml of a Krebs–Ringer solution containing low (5mM) or high

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(30 mM) concentrations of K^+ ions and different concentrations of glucose. The low-K^+ medium contained 123 mM-NaCl, 5 mM-KCl, 1.2 mM-$MgCl_2$, 2.5 mM-$CaCl_2$, 1.2 mM-sodium phosphate buffer, pH 7.4, 25 mM-$NaHCO_3$, 0.058 mM-diethyl *p*-nitrophenyl phosphate (Mintacol, Bayer, West Germany), 0.2 mM-choline chloride and glucose as indicated; it was equilibrated with a mixture of O_2/CO_2 (19:1). In the high-K^+ medium, the concentration of KCl was increased to 30 mM and that of NaCl was correspondingly diminished. The incubation was performed in an atmosphere of O_2/CO_2 (19:1) at 38°C. After 30 or 60 min, the slices were removed from the incubation medium and homogenized in 5% (w/v) trichloroacetic acid. Their acetyl-CoA, choline and acetylcholine content was then determined by using the radio-enzymic procedure with choline acetyltransferase as described previously (Říčný & Tuček, 1980*b*); method III of the acetyl-CoA assay was used as described by Říčný & Tuček (1980*b*). The principle of the procedure applied is the conversion of tissue acetyl-CoA into [^{14}C]acetylcholine, and of tissue choline and acetylcholine into [^{14}C]acetylcholine. The content of acetylcholine in the incubation medium was measured by bioassay on guinea-pig ileum.

Results

The results obtained after incubations lasting 30 min are summarized in Fig. 1. The content of acetyl-CoA in the slices varied depending on the concentration of glucose both in the low-K^+ and in the high-K^+ incubation medium; it was highest with 10 mM-glucose and lowest with 0.5 mM-glucose. A similar relation was found between the content of acetylcholine in the slices and the concentration of glucose in the medium. In low-K^+ incubations, no correlation was apparent between the amount of acetylcholine released into the medium and the concentration of glucose; in high-K^+ incubations, the release of acetylcholine into the medium was directly proportional to the concentration of glucose in the medium. The concentration of choline in the slices was little affected by variations in the concentration of glucose in the medium in experiments with low K^+; with high K^+, a higher choline content was found after incubations with the lowest (0.5 mM) concentration of glucose.

After incubations lasting 60 min (Fig. 2), the results were qualitatively similar. Again, the highest content of acetyl-CoA and acetylcholine in the tissue was found after incubations with the highest concentration of glucose, and the lowest values were observed after incubations with the lowest concentration of glucose. The same relation was discovered between the release of acetylcholine into

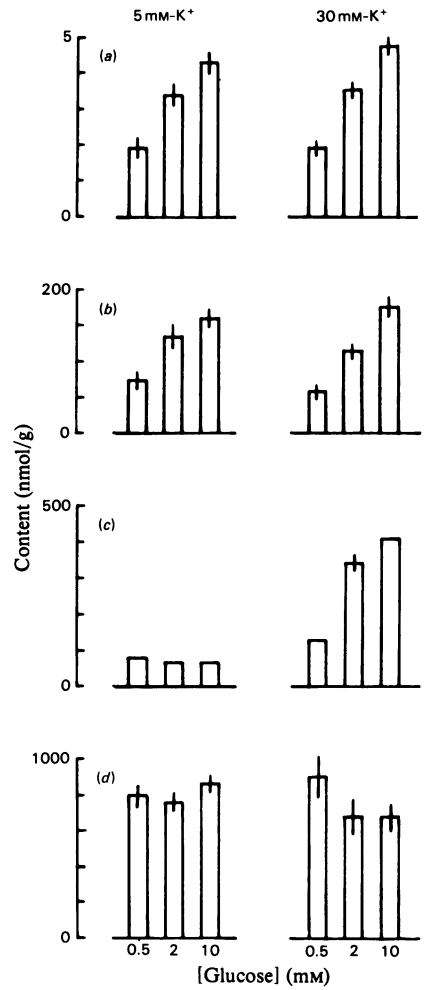

Fig. 1. *Content of acetyl-CoA (a), acetylcholine (b) and choline (d) in slices of caudate nuclei and of acetylcholine in the incubation medium (c) after 30 min incubation in the presence of 0.5 mM-, 2.0 mM- or 10.0 mM-glucose and of 5 mM- or 30 mM-K^+*

Results shows means ± S.E.M. for three observations. In several experimental groups the S.E.M. was too small to be shown on the scale used.

the medium and the concentration of glucose in the medium in experiments with 30 mM-K^+, although not with 5 mM-K^+. The content of choline in the slices was highest at 0.5 mM- and lowest at 10 mM-glucose.

In a separate group of experiments, the content of acetyl-CoA, acetylcholine and choline was determined in slices of caudate nuclei immediately after they had been prepared, without any incubation. The values obtained (mean ± S.E.M.) were 5.00 ± 0.12 nmol/g for acetyl-CoA, 39.9 ± 2.3 nmol/

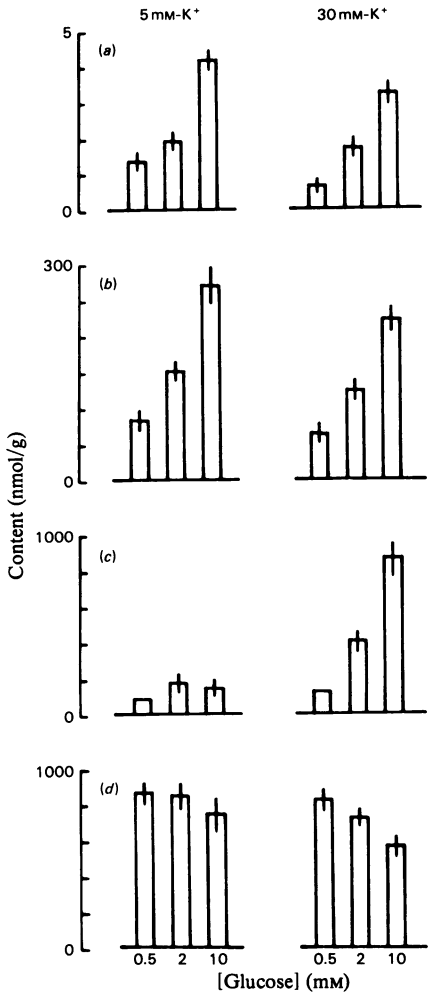

Fig. 2. *Content of acetyl-CoA (a), acetylcholine (b) and choline (d) in slices of caudate nuclei and of acetylcholine in the incubation medium (c) after 60 min incubation in the presence of 0.5 mM-, 2.0 mM or 10.0 mM-glucose and of 5 mM- or 30 mM-K^+*

Results are means ± S.E.M. for six to nine observations. In two groups the S.E.M. was too small to be shown on the scale used.

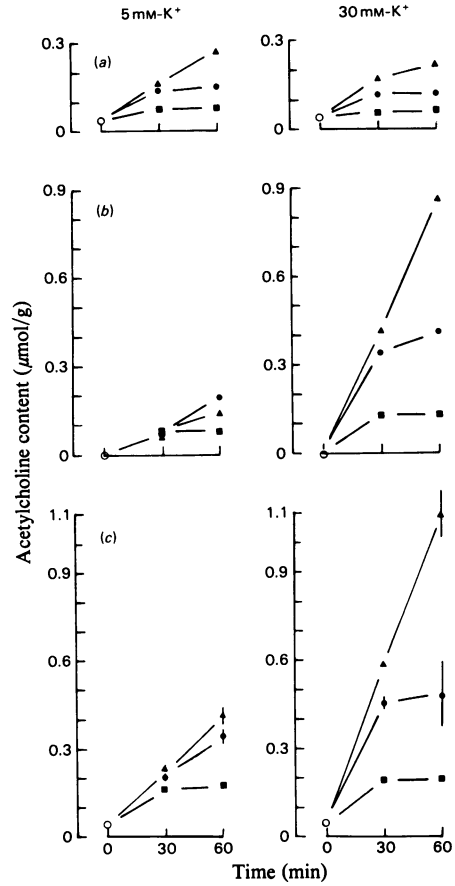

Fig. 3. *Changes in the content of acetylcholine in slices of caudate nuclei (a), in the incubation medium (b) and in the sum of the two values (c) after 30 and 60 min incubation in the presence of different concentrations of glucose and of 5 mM- or 30 mM-K^+*

The graph is based on data given in Figs. 1 and 2. Slices of caudate nuclei were incubated in the presence of 0.5 mM- (■), 2.0 mM- (●) or 10.0 mM-glucose (▲); individual symbols are means for four (zero time), three (30 min) or six to nine observations (60 min). Vertical bars at the symbols in the lowest section of the graph indicate S.E.M. values; in some cases its value was too small to be shown on the scale used. Values of S.E.M. for the content of acetylcholine in the tissue (*a*) and in the medium (*b*) are given in Figs. 1 and 2. The mean content of acetylcholine in the slices at time zero was 39.9 ± 2.3 nmol/g.

g for acetylcholine and 383.8 ± 24.3 nmol/g for choline; these values obtained on sliced tissue differ from the values *in vivo* (see Tuček, 1979; Freeman & Jenden, 1976), but they provide information about the biochemical conditions in the tissue at the moment when the incubation is begun. From comparison with results obtained after 30 and 60 min of incubation (Figs. 1 and 2), it is evident that the content of acetyl-CoA in the slices was slightly diminished after incubations with 10 mM-glucose and 5 mM-K^+ (a decrease to 4.32 nmol/g after 30 min and 4.10 nmol/g after 60 min); with 10 mM-glucose and 30 mM-K^+, the value decreased to 4.75 nmol/g after 30 min and to 3.18 nmol/g after 60 min. The content of acetyl-CoA in the slices decreased much more

during incubations with low concentrations of glucose. The content of acetylcholine and choline in the tissue found at the end of the incubations was higher in all experimental groups than the initial content given above; the time course of changes in the content of acetylcholine in the tissue, in the medium and in the tissue plus medium taken together is shown in Fig. 3. With 0.5 mM- and 2 mM-glucose, most of the increase in the content of acetylcholine in the slices occurred during the first 30 min of incubation, whereas during the second 30 min the content of acetylcholine changed very little. With 10 mM-glucose, the content of acetylcholine continued to increase also during the second 30 min of incubation, rising in the presence of 5 mM-K^+ to 156.8 nmol/g after 30 min and to 267.2 nmol/g after 60 min incubation; in the presence of 30 mM-K^+, the content of acetylcholine in the slices increased to 175.0 nmol/g after 30 min and to 224.3 nmol/g after 60 min.

A plot (Fig. 4) of individual values of acetylcholine content in the slices against corresponding values of acetyl-CoA content, as recorded in the same experiments, suggests that a linear relationship prevailed between the two values under the conditions used in the present work. The following equations were calculated for the regression lines from results plotted in Fig. 4:

(1) in the presence of 5 mM-K^+

$$y = 47.3x + 56.2;$$

(2) in the presence of 30 mM-K^+

$$y = 58.5x + 31.6$$

where y represents the concentration of acetylcholine and x that of acetyl-CoA in the tissue.

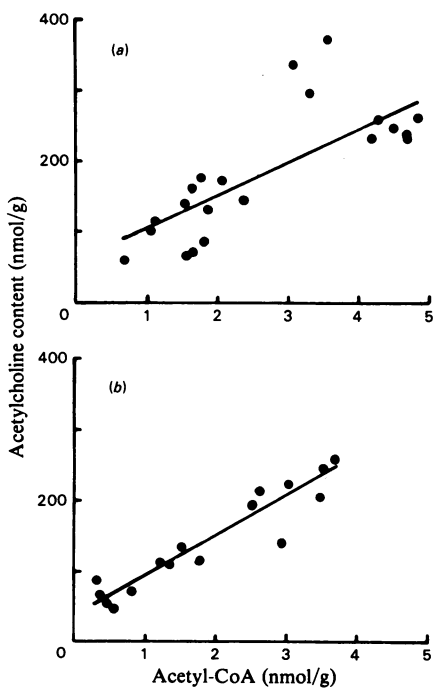

Fig. 4. *Correlation between the content of acetyl-CoA and acetylcholine in slices of caudate nuclei after 60 min incubation in the presence of 0.5 mM-, 2.0 mM- or 10 mM-glucose and of 5 mM- (a) or 30 mM-K^+ (b)*
Each point is the result of one experiment. The straight lines represent the calculated regression lines; their equations are given in the text.

Discussion

It is clear from the results that the content of acetyl-CoA was considerably diminished in slices of caudate nuclei incubated in the presence of low concentrations of glucose. The incubations were intentionally performed with a comparatively high concentration (0.2 mM) of choline in the medium which is about 20 times the concentration of choline present in the blood plasma (see Tuček, 1978, for review). In this way, it was possible to investigate changes in acetylcholine content in the slices in a situation when the availability of one of its precursors, i.e. acetyl-CoA, was subjected to large variations, while the availability of the other precursor, i.e. choline, was comparatively stable. The low values of acetylcholine content observed at low glucose concentrations are clearly not a consequence of a lack of choline; the content of choline in the slices reached the highest values after incubations with the lowest concentrations of glucose. Apparently, the low amounts of acetylcholine must be interpreted as a consequence of a lack of acetyl-CoA.

It might be argued that, in experiments with 5 mM-K^+ and low glucose, the content of acetylcholine in the slices diminished because of depolarization caused by the lack of energy and consequent inability of the cells to maintain the membrane potential; the depolarization would cause the release of acetylcholine into the incubation medium. Such interpretation is not supported, however, by the results of measurements of the content of acetylcholine in the incubation medium. Furthermore, experiments with 30 mM-K^+ indicate that the correlation between the content of acetylcholine in the slices and the concentration of glucose in the medium is preserved under conditions when the neurons are depolarized.

A closer analysis of the results suggests that a correlation exists between the availability of acetyl-CoA in the tissue and both the rate of acetylcholine synthesis and its content at a steady state. During

the first 30min of incubation, the synthesis of acetylcholine (i.e. the increase in its content in the slices plus the medium) occurred under all incubation conditions used (Fig. 3); it is clear from comparison with data in Fig. 1 that the rate of acetylcholine synthesis was directly related to the concentration of acetyl-CoA in the slices. During the second 30min of incubation, the sum of acetylcholine in the tissue and the medium continued to increase in all slices except those incubated with 5mM-K^+ and 0.5mM-glucose (Fig. 3); again, the rate of synthesis was directly related to the content of acetyl-CoA in the slices. A steady concentration of acetylcholine has been reached, however, in the slices themselves (without the medium) after 30min of incubation with 0.5mM- and 2mM-glucose, both with 5mM- and 30mM-K^+ (Fig. 3). A comparison of the content of acetyl-CoA and acetylcholine in these slices indicates that the direct relation between the content of acetyl-CoA and acetylcholine in the tissue, observed under conditions when the content of acetylcholine had been increasing, was also maintained under conditions when the concentration of acetylcholine reached a steady value.

Present results do not yet permit the analysis of the kinetics of acetylcholine synthesis in the sliced brain tissue in strictly quantitative terms. The concentrations of acetyl-CoA, acetylcholine and choline that have been measured apply to the tissue as a whole, including its extracellular water, glial cells and non-cholinergic neurons. Of the three substances measured, only acetylcholine would be restricted to cholinergic neurons under physiological conditions, but cannot be assumed to be confined to these neurons in the conditions of our experiments, mainly because of the presence of a cholinesterase inhibitor in the incubation medium. Furthermore, it is unlikely that acetylcholine is uniformly distributed throughout the cholinergic neurons, and the relation between the amount of acetylcholine present in the intracellular compartments of its synthesis and storage is not known (and may vary with time during incubations). Variables of this kind may explain why the ratios between the concentrations of acetylcholine, acetyl-CoA and choline are not the same after 30 and 60min of incubation and at various concentrations of glucose and K^+.

The main conclusion that can be drawn from the present results is that the rate of acetylcholine synthesis in slices of brain tissue incubated *in vitro* and the content of acetylcholine found in them under conditions approaching a steady state depend on their content of acetyl-CoA. This conclusion is in good accord with a number of recent observations indicating that the rate of acetylcholine synthesis or the content of acetylcholine in the brain are altered under conditions of hypoxia and hypoglycaemia (Gibson & Blass, 1976*a*; Gibson *et al.*, 1978), under the influence of metabolic inhibitors (Gibson *et al.*, 1975; Gibson & Blass, 1976*b*; Jope *et al.*, 1978; Lefresne *et al.*, 1978) and after starvation (Kuntscherová, 1972). It may help to explain the high sensitivity of synaptic transmission to the lack of glucose (Nicolescu *et al.*, 1966; Dolivo, 1974) and its changes in thiamin-deficient animals (Perri *et al.*, 1970*a*,*b*; Sacchi & Perri, 1973). The present findings are in agreement with the view that, at the ultimate level, the synthesis of acetylcholine is controlled by the Law of Mass Action and that changes in the availability of both precursors play a role in its regulation under physiological and pathological conditions.

We thank Dr. Ann Silver and The Wellcome Trust for assistance in providing some of the reagents.

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