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Effects of morphine and pentobarbitone on acetylcholine synthesis by rat cerebral cortex

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Summary

1. The synthesis of carbon-14-labelled acetylcholine (^{14}C-ACh) from carbon-14 uniformly labelled glucose (U-^{14}C-D-glucose) under different conditions was studied.

2. The ability of cerebral cortex slices and minces from morphine-treated and pentobarbitone-treated rats incubated in 4 mM K^+ medium to form ^{14}C-ACh was markedly reduced as compared with those from control animals.

3. The ability of slices from drug-treated and control animals incubated in 31 mM K^+ medium to form ^{14}C-ACh was similar.

4. Cerebral cortex homogenates from both groups of animals in either 4 or 31 mM K^+ medium formed similar amounts of ^{14}C-ACh.

5. These findings add further support to the hypothesis that the concentration of ACh at the site of synthesis governs the rate of formation of the neurotransmitter.

Introduction

Changes in the concentration of acetylcholine (ACh) in the brain have been observed when the functional activity of the organ is altered by certain drugs. For example, the administration of central nervous system depressants such as morphine and pentobarbitone to rats has been associated with an increase in the ACh content of their brains, while administration of the stimulant pentylenetetrazole has been associated with a decrease in ACh content (Giarman & Pepeu, 1962 ; Crossland & Slater, 1968). However, the concentration of ACh in the brain is generally well maintained within relatively narrow limits. This has been assumed to be related to a depression of the process of ACh formation by ACh itself. That the concentration of ACh in the vicinity of the site of formation may regulate the rate of ACh synthesis has been reported (Sharkawi & Schulman, 1969a). We have shown that the formation of ^{14}C-ACh by rat cerebral cortex slices was depressed when their ACh content was increased by prior administration of morphine.

The question whether this observation would be seen with other drugs that increase the brain content of ACh such as pentobarbitone was examined in the present study. These drugs are believed to diminish the release of ACh from nervous tissue (Crossland, 1953). The diminished liberation of ACh would result in the accumulation of the neurotransmitter and consequently could depress the process of ACh formation. If this is the case, the presence of an agent that causes

the release of ACh, and thus decreases its concentration in the vicinity of the site of formation, should reverse the inhibition of ACh synthesis. This hypothesis was examined by studying ^{14}C-ACh formation by cerebral cortex slices from drug-treated and control animals in the presence and absence of a releasing agent (K^+) in the incubation medium. Another means of decreasing the ACh concentration in the vicinity of the site of synthesis would be to disrupt the integrity of the cells. For this purpose, ^{14}C-ACh formation by cerebral cortex homogenates from drug-treated rats was compared with those from untreated animals.

Methods

Male Holtzman rats weighing between 200 and 480 g were decapitated. Morphine-treated rats were decapitated 90 min after subcutaneous injection of morphine sulphate (50 mg/kg calculated as free base). Pentobarbitone-treated animals were decapitated 60 min after intraperitoneal injection of 50 mg/kg pentobarbitone sodium. The brains were immediately removed and placed in ice-cold saline. Cerebral cortex slices were prepared as described by McIlwain & Rodnight (1962). Cortex minces were prepared by the method of Gardiner (1961). Homogenates of cerebral cortex were made in three volumes of ice-cold saline containing physostigmine sulphate (2×10^{-4}M) as a cholinesterase inhibitor.

Portions of 200 ± 10 mg (wet weight) of slices, minces, or aliquots of homogenates containing 100 mg (wet weight) of cerebral cortex were placed in 50 ml vessels containing 3 ml of the incubation medium (pH 7·4). Two forms of incubation media were used. The composition of the first medium was as follows (mM): NaCl, 130 ; KCl, 4 ; $CaCl_2$, 2 ; $NaHCO_3$, 25 ; U-^{14}C-D-glucose, 5. This medium will be referred to as 4 mM K^+ medium. The composition of the second medium was as follows (mM): NaCl, 103 ; KCl, 31 ; $CaCl_2$, 2 ; $NaHCO_3$, 25 ; U-^{14}C-D-glucose, 5. This medium will be referred to as 31 mM K^+ medium. Both media contained physostigmine sulphate (2×10^{-4}M). The medium was equilibrated with 95% oxygen and 5% carbon dioxide and the vessels were shaken at 37° C in a Dubnoff shaker for 30 minutes.

At the end of the incubation period, the incubation vessels were cooled to 0° C. The ^{14}C-ACh content of the vessels was estimated by the method of Browning & Schulman (1968). This method involves extraction of ACh from the tissue by homogenization in fresh incubation medium (pH 4) and heating in a boiling water bath for 10 min after the addition of ACh carrier. The mixture of labelled and unlabelled esters is purified by ion exchange, after which ACh is isolated as the chloroaurate salt. ACh chloroaurate is plated on Pyrex planchets and its ^{14}C content is measured.

The pre-incubation ACh content of brain cortex from morphine-treated and pentobarbitone-treated rats was assayed using the frog rectus abdominus muscle sensitized with physostigmine sulphate (10^{-5}M). Extraction of ACh was performed as described by McIntosh & Perry (1950).

Results

Influence of morphine and pentobarbitone on ACh content and ^{14}C-ACh formation

Figure 1 shows that the ACh content of rat brain cortex from morphine-treated and pentobarbitone-treated rats was about 40% greater than that of control animals.

Figures 1 and 2 also show that the ability of cerebral cortex minces and slices from drug-treated animals to form ^{14}C-ACh is markedly reduced when incubated in 4 mM K^+ medium. However, when 31 mM K^+ medium was used for incubation, slices from drug-treated animals formed as much ^{14}C-ACh as slices from untreated rats (Fig. 2). Incubation in 31 mM K^+ medium markedly increased the amount of ^{14}C-ACh formed. This increase has been shown to be entirely due to a marked increase in the amount of ^{14}C-ACh formed by slices and then released into the incubation medium (Sharkawi & Schulman, 1969a).

Influence of morphine and pentobarbitone on the formation of ^{14}C-ACh by homogenates

Figure 3 shows that the inhibitory effect of morphine and pentobarbitone on the formation of ^{14}C-ACh is not seen when cerebral cortex homogenates were used instead of slices or minces. Homogenates from drug-treated animals formed as much ^{14}C-ACh as those from control animals whether incubated in 4 or 31 mM K^+ medium. These data also show that the stimulating effect of increased K^+ concentration on ACh formation is not seen with homogenates.

Discussion

The ACh content of the whole brain of the rat has been shown to increase after the administration of morphine and pentobarbitone (Giarman & Pepeu, 1962; Crossland & Slater, 1968). Results in Fig. 1 show that the ACh content of rat brain cortex is markedly increased after the administration of these drugs. However,

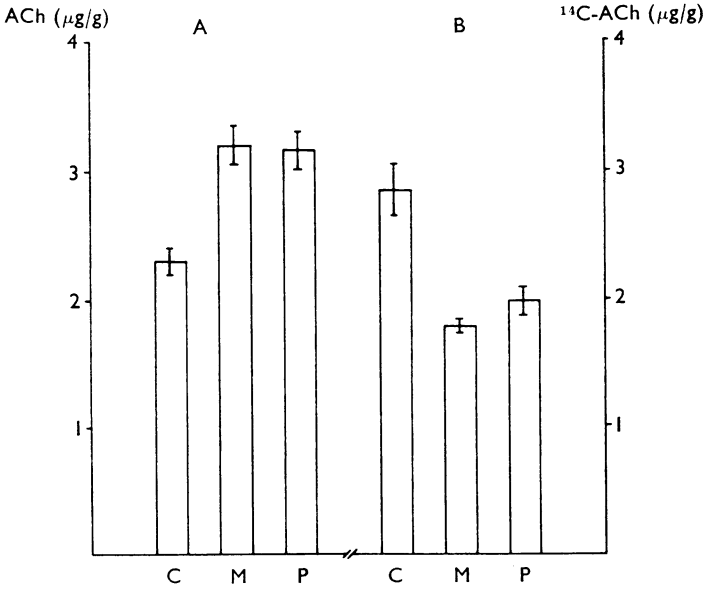

FIG. 1. Influence of morphine and pentobarbitone administration on the ACh content of rat cerebral cortex (A), and on the formation of ^{14}C-ACh by rat cerebral cortex minces (B). ACh content of aliquots of cortex from control and drug-treated rats before incubation was determined by bioassay. Portions of 200±10 mg (wet weight) of minces were incubated in 3 ml of 4 mM K^+ medium for 30 min at 37° C. C, Control; M, morphine-treated; P, pentobarbitone-treated.

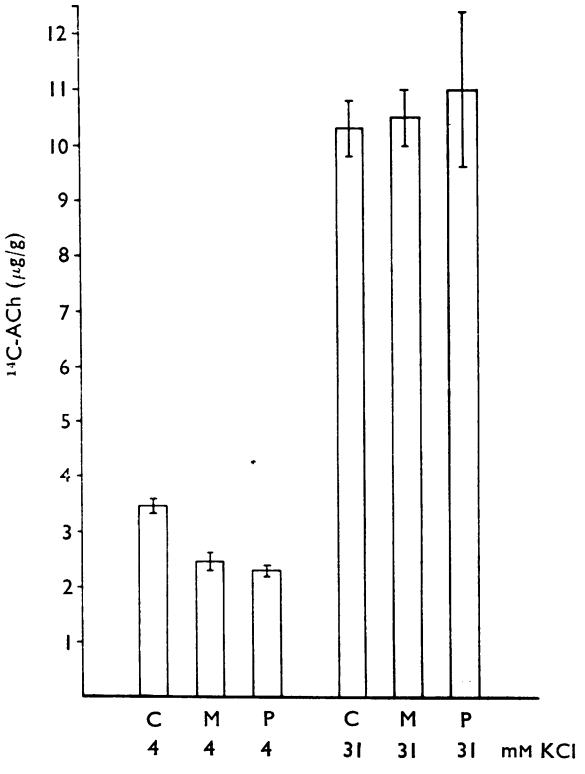

FIG. 2. Formation of ^{14}C-ACh by cerebral cortex slices from control and drug-treated rats. Portions of 200+10 mg (wet weight) of slices were incubated in 3 ml of 4 or 31 mM K^+ medium for 30 min at 37° C. C, Control; M, morphine-treated; P, pentobarbitone-treated.

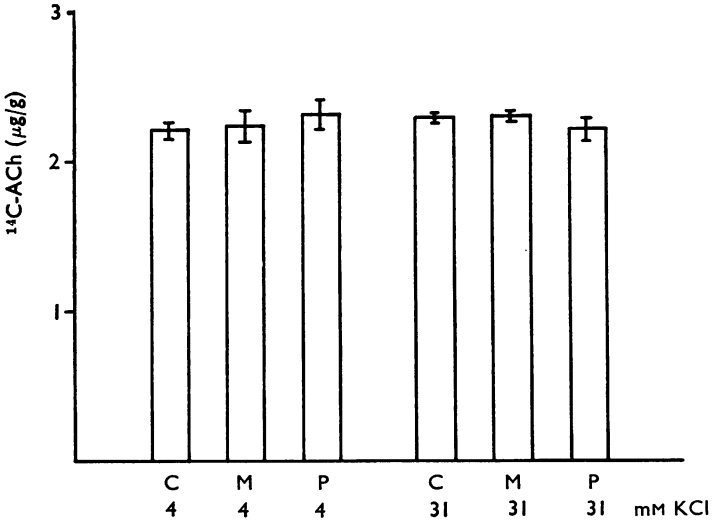

FIG. 3. Formation of ^{14}C-ACh by cerebral cortex homogenates from control and drug-treated animals. Aliquots of homogenates containing 100 mg (wet weight) were incubated in 3 ml of 4 mM or 31 mM K^+ medium for 30 min at 37° C. C, Control; M, morphine-treated; P, pentobarbitone-treated.

drug-induced changes in ACh concentration of the brain have been found to vary only within relatively narrow limits (Giarman & Pepeu, 1962). For example, the effect of different anaesthetics on the brain ACh concentration for any particular species is almost constant. Also it was not possible, by any treatment, to increase the amount of ACh extractable from brain tissue beyond that found in deep anaesthesia (Crossland & Merrick, 1954). This has led to the speculation that a regulating negative feedback mechanism, governed by the concentration of ACh at the site of synthesis, may be responsible for the maintenance of the concentration of the neurotransmitter in the brain within such relatively narrow limits. Our finding that increased pre-incubation concentration of ACh in cortex of drug-treated animals was associated with a decrease in the rate of formation of ^{14}C-ACh is in accord with this concept. Unpublished observations from this laboratory suggest that this may be a general phenomenon with drugs that increase brain ACh, other than cholinesterase inhibitors. Kaita & Goldberg (1969) found that inhibition of choline acetyltransferase by ACh occurred at a concentration of 10 mM and progressively increased with a concentration of 100 mM. This finding substantiates the concept of negative feedback regulation of the process of ACh formation.

The results also exclude the possibility that the drug-induced increase in ACh content of the rat brain is due to an increased rate of synthesis of the neurotransmitter. Under none of the present conditions did brain tissue from drug-treated animals form more ACh than those from untreated animals. On the contrary, a marked decrease in the amount of acetylcholine formed was seen in slices and minces incubated in 4 mM K^+ medium. ^{14}C-ACh formation by slices from control and drug-treated animals was similar when incubation was carried out in 31 mM K^+ medium. Also, cerebral cortex homogenates from either group of animals incubated in either medium formed similar amounts of the neurotransmitter.

It is generally accepted that the increase in ACh concentration in the brain, following the administration of the present drugs, is the result of reduced release of the neurotransmitter due to decreased neuronal activity (Crossland, 1953). Indeed, there is considerable evidence that morphine and anaesthesia reduce the release of ACh from nervous tissue. For example, morphine has been shown to reduce the release of ACh from the lateral ventricle and subarachnoid space of the cat (Beleslin & Polak, 1965 ; Beleslin, Polak & Sproull, 1965) and from rat cerebral cortex slices (Sharkawi & Schulman, 1969b). Reduced release of ACh from rat brain has been observed with barbiturate anaesthesia (Mitchell, 1963 ; Celesia & Jasper, 1966).

Reduced release of ACh results in increased concentration of the neurotransmitter in the neurone containing it. This increased concentration, by virtue of a negative feedback regulating mechanism, inhibits the process of formation of the neurotransmitter as seen in Fig. 1. However, drug-induced increase in ACh concentration in slices is reduced if incubation is carried out in a medium containing an agent which enhances the release of ACh. This will consequently lead to the recovery of the process of ACh formation. Hence, slices from drug-treated animals, despite their increased pre-incubation ACh content, form similar amounts of ACh as those from control animals, when incubated in 31 mM K^+ medium. That this is the case is clearly seen in Fig. 2. The fact that increased K^+ concentration enhances the release of ACh has been demonstrated in different systems. For example, increasing K^+ concentration of the medium enhances the release of ACh from isolated rat diaphragm (Mitchell & Silver, 1963) and from rat cerebral cortex slices (Sharkawi &

Schulman, 1969a). Also increasing K^+ concentration in perfusion fluid increases the liberation of ACh from the superior cervical ganglion of the cat (Brown & Feldberg, 1936).

The observation that drug treatment did not affect the ability of homogenates, incubated in either 4 or 31 mM K^+ medium, to synthesize ACh adds further support to the concept of negative feedback regulation of the process of ACh formation. Disruption of cellular integrity would make the concentration of ACh in the vicinity of the site of formation similar in incubation vessels containing homogenates from drug-treated and control animals. This observation also suggests that the cell membrane may be the site where inhibition of the release of ACh occurs.

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