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INFLUENCE OF ANTIMUSCARINIC SUBSTANCES ON *IN VITRO* SYNTHESIS OF ACETYLCHOLINE BY RAT CEREBRAL CORTEX

BY

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When a solution of a cholinesterase inhibitor is brought into contact with the exposed cerebral cortex or the ventricular surface of the caudate nucleus of a living animal, acetylcholine (ACh) is liberated into this fluid. There is an increase in the amount of ACh released when atropine is added to the solution or injected intravenously (Mitchell, 1963 ; Szerb, 1964 ; Polak, 1965).

In the following experiments the influence of atropine and some atropine-like drugs was studied on the release and synthesis of ACh by slices of cortex from rat brain, treated with a cholinesterase (ChE) inhibitor. In certain conditions atropine was found to enhance the release of ACh from the tissue into the incubation medium and this increased release was associated with an increased synthesis of ACh. Some other antimuscarinic compounds also stimulated ACh output.

Preliminary reports of some of the results of this investigation have been published elsewhere (Polak and Meeuws, 1966 ; Polak, 1967).

METHODS

Female albino rats (160–190 g) were lightly anaesthetized with ether and decapitated. The brains were immediately removed from the skulls and placed in ice-cold oxygenated medium. In the cold room one slice in the dorsal plane was prepared from both hemispheres by means of a Stadie-Riggs microtome and weighed on a torsion balance. The thickness of the slices was about 0.5 mm. Portions of 71–208 mg of tissue were pre-incubated for 60 or 90 min at 37° C in 25 ml. vessels containing 2.5 ml. of medium to which soman (5×10^{-6}M) had been added in order to inactivate the ChE. Six or seven vessels with slices were incubated simultaneously. In the experiments in which pre-incubation lasted 90 min, the medium was replaced by freshly prepared medium containing soman at the end of each half hour and the tissue was rinsed once before the experiment started. In the latter type of experiments the drug under investigation was present in the medium during the last 30 min of pre-incubation.

At the start of the experiment the pre-incubation medium was replaced by 2.5 or 5 ml. of medium to which extra KCl and drugs had or had not been added. At the end of the incubation period, which lasted either 60 or 30 min, the media were collected in graduated centrifuge tubes. The slices were rinsed once and then extracted with HCl for the determination of their ACh (" total extractable ACh ") content according to the method of Elliott, Swank & Henderson (1950). The

washings were added to the incubation media. In many experiments one portion of tissue was extracted at the beginning of the experiment, that is immediately after pre-incubation, in order to determine the ACh content at zero-time. The extracts and media were frozen after adjusting the pH to 4 and stored until assayed for ACh.

The composition of the medium was as follows (mM): NaCl, 118.5; $NaHCO_3$, 24.9; KCl, 4.7; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $MgSO_4$, 1.2; glucose, 10. Except when stated otherwise, soman (5×10^{-6}M) was present in the medium during both pre-incubation and incubation. In some experiments physostigmine sulphate was used instead of, or in addition to, soman. The medium was kept in equilibrium with an atmosphere of 95% oxygen and 5% carbon dioxide and the vessels were shaken continuously during pre-incubation and incubation. When extra KCl was added no osmotic compensations were made.

The ACh-like activity of the extracts and of the incubation media was estimated by bioassay on the dorsal leech muscle, treated with physostigmine, against an ACh chloride standard solution and expressed in terms of μg of this salt per gram of tissue (wet weight). No exhaustive attempts to confirm the identity of ACh were performed, but suitable dilutions of either alkali treated (pH near 14 during 120 min) and subsequently neutralized samples (Feldberg, 1945) or of the experimental medium (containing drugs which might be destroyed by alkali treatment) were added to the standard solution in all assays in order to correct for substances other than ACh, which might influence the sensitivity of the assay preparation. In several experiments known amounts of ACh were added to an extract after its ACh content had been determined, and a determination of the ACh content of the mixture was performed in order to detect possible sensitizing or desensitizing substances which might have appeared or disappeared as a result of the treatment with alkali. There was no indication of the presence of such substances. In a few experiments *d*-tubocurarine was found to antagonize the effects of an extract to the same extent as those of equipotent doses of the standard solution.

Presentation of the results and statistical evaluation

All results are presented as means of at least four observations ± S.E.M.; the numbers of observations are given in the figures. Either Student's *t* test or Wilcoxon's rank sum test at the 5% level of significance was used to decide whether differences were significant.

The activities of the optical stereo-isomers of hyoscyamine, 3-quinuclidinyl benzylate and (−)-hyoscine in stimulating ACh release from slices of cerebral cortex were compared by plotting the log concentrations against the differences between the amounts of ACh released from samples of slices incubated in the presence and in the absence of the drug under investigation. Four experiments were made for each drug concentration, and in each experiment three test samples containing the drug and three control samples were set up together. The ACh content of the samples was assayed separately, and each test sample was paired with a different control sample, chosen at random. Potency ratios with 99% fiducial limits were determined according to Finney (1952).

Materials used

Commercially obtained ACh chloride, atropine sulphate, lysergic acid diethylamide (LSD), cocaine hydrochloride, physostigmine sulphate, phenobarbital sodium, tropine and tropic acid were used. Because the chemical purity of tropine was considered doubtful, thin-layer chromatography on silica gel was performed and the presence of about 0.1% hyoscine was detected. Soman (3,3-dimethyl-*n*-butyl 2-methylphosphonofluoridate) and Ro 2-3308 (3-quinuclidinyl benzylate) were synthesized in the Chemical Laboratory RVO-TNO, where the sulphates of (−)- and (+)-hyoscyamine were also prepared. The $[\alpha]_{589}^{20}$ of the (−)-hyoscyamine was −27.5°, that of the (+)-hyoscyamine +25.7°. If it is assumed that the optical purity of the (−)-isomer was 100% (compare Werner & Miltenberger, 1959, and Rosenblum & Taylor, 1954) it can be calculated that the (+)-hyoscamine was contaminated with 3% (−)-hyoscyamine.

For the sake of comparison of the activities of different drugs the concentrations of the sulphates of atropine, (−)- and (+)-hyoscyamine and physostigmine are expressed in the text as molar concentrations of the alkaloid moieties, that is M should be read as g-equiv/l.

RESULTS

In the experiments in which the slices were pre-incubated for 60 min (Figs. 1, 5 and 6 and Table 1) ACh production per gram of tissue during incubation in a 25 mM KCl medium was consistently greater than that in the experiments, in which the pre-incubation period was 90 min (Figs. 2, 3 and 4). In these, however, the medium was replaced at the end of each 30 min during the pre-incubation. This may have induced a loss of tissue fragments or constituents which would explain the difference in the results.

Medium with 4.7 mM KCl

When, after pre-incubation for 1 hr with soman, the slices of rat cerebral cortex were incubated in a medium containing 4.7 mM KCl, ACh was released from the tissue into the bath fluid. Because the amounts of ACh extracted from the slices at the end of the incubation were about the same as or slightly larger than immediately after pre-incubation, synthesis of ACh must have occurred. This is evident from the experiments of Fig. 1 in which the ACh extractable from the slices rose from 6.7 ± 0.3 μg/g to 7.3 ± 0.5 μg/g, and in which 0.66 ± 0.07 μg of ACh per gram of tissue was in addition released into the bath fluid.

Increased KCl concentrations

In confirmation of the results obtained by Mann, Tennenbaum & Quastel (1939), release and synthesis of ACh were found to be enhanced on raising the KCl concentration in the medium (Fig. 1). After incubation for 1 hr in a medium containing 25 mM KCl the ACh of the tissue amounted to 7.6 ± 0.6 μg/g and 4.2 ± 0.2 μg/g was released into the medium. This is about six times as much as during incubation in a 4.7 mM KCl medium. When the KCl concentration was raised to 50 mM, release and synthesis of ACh were stimulated even more ; the ACh concentration of the tissue reached 13.9 ± 1.4 μg/g ; in addition ACh 19.4 ± 1.4 μg/g was released into the medium.

Atropine

As shown in Fig. 1, atropine in a concentration of 3×10^{-6}M had no effect on the synthesis of ACh when the concentration of KCl in the medium was 4.7 mM, but enhanced synthesis when the concentration was 25 or 50 mM. With 25 mM KCl in the medium, the amounts of ACh released increased from 4.2 ± 0.2 to 13.5 ± 1.5 μg/g during 1 hr incubation, and at the end of this period the ACh content of the tissue slices had risen from 7.6 ± 0.6 to 13.2 ± 0.9 μg/g. With 50 mM KCl in the medium the release increased from 19.4 ± 1.4 to 24.7 ± 1.3 μg/g, but the ACh content of the tissue slices had risen insignificantly only, from 13.9 ± 1.4 to 15.6 ± 1.1 μg/g.

Figure 2 illustrates the effects of different concentrations of atropine on ACh synthesis in slices of cerebral cortex incubated in a medium containing 25 mM KCl. Atropine was effective in a concentration as low as 6×10^{-9}M.

Stereo-isomers of atropine

The effect of the stereo-isomers of atropine on ACh release was investigated on incubation in a medium containing 25 mM KCl. As shown in Fig. 3, the log dose-response

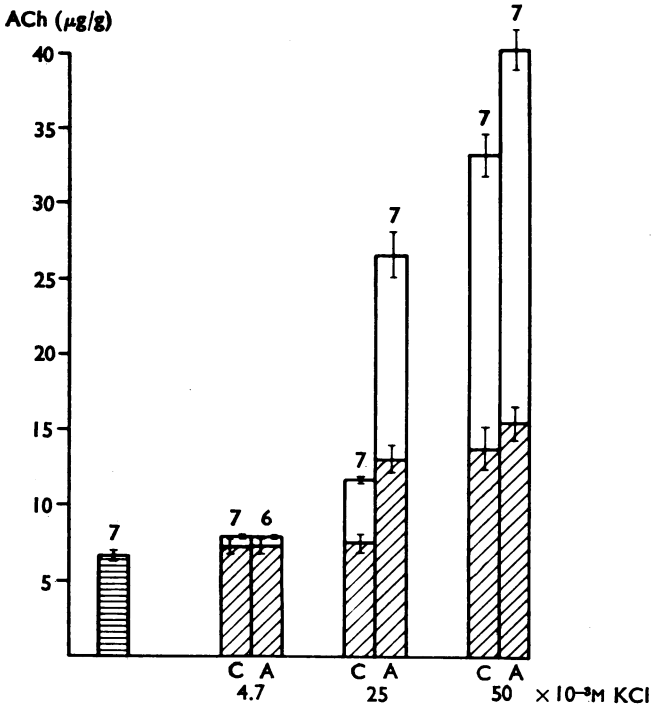

Fig. 1. Influence of atropine on synthesis and release of ACh by slices of cerebral cortex. Portions of 89–154 mg of tissue. Pre-incubation (60 min in 2.5 ml. of medium with 4.7 mM KCl) and incubation (60 min in 2.5 ml. of medium with different KCl concentrations) in the presence of 0.005 mM soman. Incubation with (A) and without (C) 3×10^{-6}M atropine. Mean amounts of ACh extracted ±S.E.M. and released ±S.E.M. (μg/g). In this and the following figures the numbers of observations are indicated above or beside each column. , ACh content at 0 min ; , ACh content at 60 min ; □, ACh released during 60 min. In all figures vertical lines indicate 2×S.E.M.

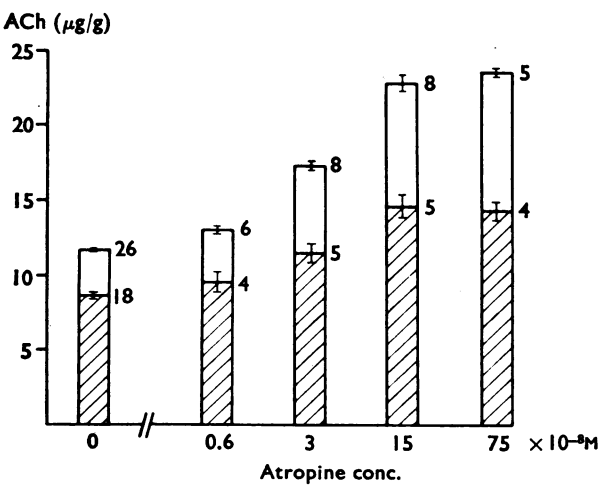

Fig. 2. Influence of atropine on synthesis and release of ACh by slices of cerebral cortex. Portions of 124–172 mg of tissue. Pre-incubation (90 min in 2.5 ml. of medium with 4.7 mM KCl) and incubation (60 min in 2.5 ml. of medium with 25 mM KCl) in the presence of soman 0.005 mM. Mean quantities of ACh extracted ±S.E.M. and released ±S.E.M. (μg/g). □, ACh released during 60 min ; , ACh content slices after 60 min.

curves of the two stereo-isomers were linear and parallel, but (−)-hyoscyamine was 16 (99% fiducial limits 11–25) times as potent as (+)-hyoscyamine. On the assumption that the (+)-stereo-isomer was contaminated with about 3% of its optical antipode (see METHODS), the actual potency ratio between the two isomers would be nearer to 30.

Ro 2-3308 and (−)-hyoscine

The antimuscarinic compound Ro 2-3308, which has strong hallucinogenic properties (Bell & Gershon, 1964) as well as (−)-hyoscine stimulated ACh release in a 25 mM KCl medium. The regression lines obtained with these substances were linear and parallel to those of hyoscyamine. Ro 2-3308, the effect of which is shown in Fig. 3, was 4.6 (99% fiducial limits 3.0–7.4) times as effective as (−)-hyoscyamine and stimulated ACh release in a concentration as low as 1.2×10^{-9}M. The potency of (−)-hyoscine was 0.56 (99% fiducial limits 0.30–1.00) times that of (−)-hyoscyamine. Its regression line is not presented in Fig. 3 because of its vicinity to that of (−)-hyoscyamine, but its effect is shown in the block diagram of Fig. 4.

Other drugs

Relatively high concentrations of LSD and phenobarbital did not influence ACh release from the isolated cerebral cortex incubated in a 25 mM KCl medium. Cocaine and tropic acid, two drugs chemically related to atropine but without its antimuscarinic activity, were also ineffective. They were tested in a concentration of about 10^{-6}M. The result is shown in the block diagram of Fig. 4, which also illustrates the effect of tropine. This breakdown product of atropine did stimulate ACh output when applied in high concentrations, but chromatographic analysis showed it to be contaminated with about 0.1% hyoscine. This may explain its stimulating effect.

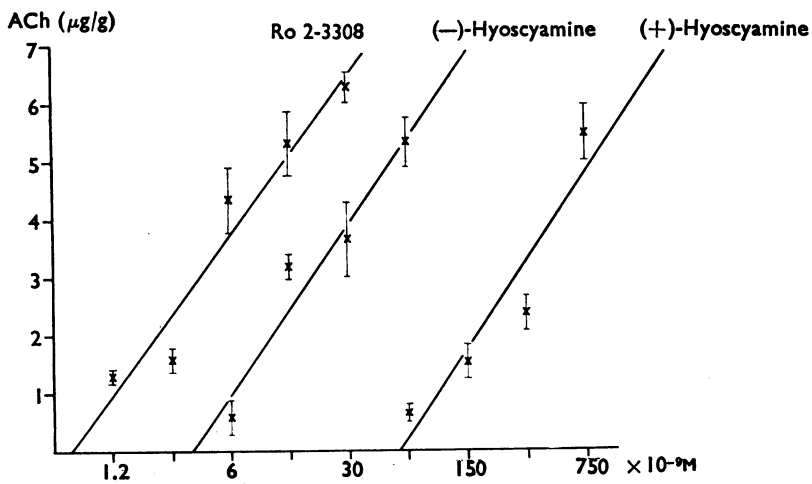

Fig. 3. Influence of (−)- and (+)-hyoscyamine and of Ro 2-3308 on release of ACh from slices of cerebral cortex. Portions of 103–163 mg of tissue. Pre-incubation and incubation as in Fig. 2. Abscissa: drug concentration. Ordinate: ACh release in the presence of the drug minus that in a randomized paired control. Presented are means of four observations ±S.E.M. and the calculated least square regression line of each drug.

Acetylcholine depleting effect of atropine in strong concentrations

In the presence of 25 and 50 mM KCl, atropine in concentrations of 6×10^{-9} to 3×10^{-6}M was shown to increase not only the release of ACh but also the ACh content

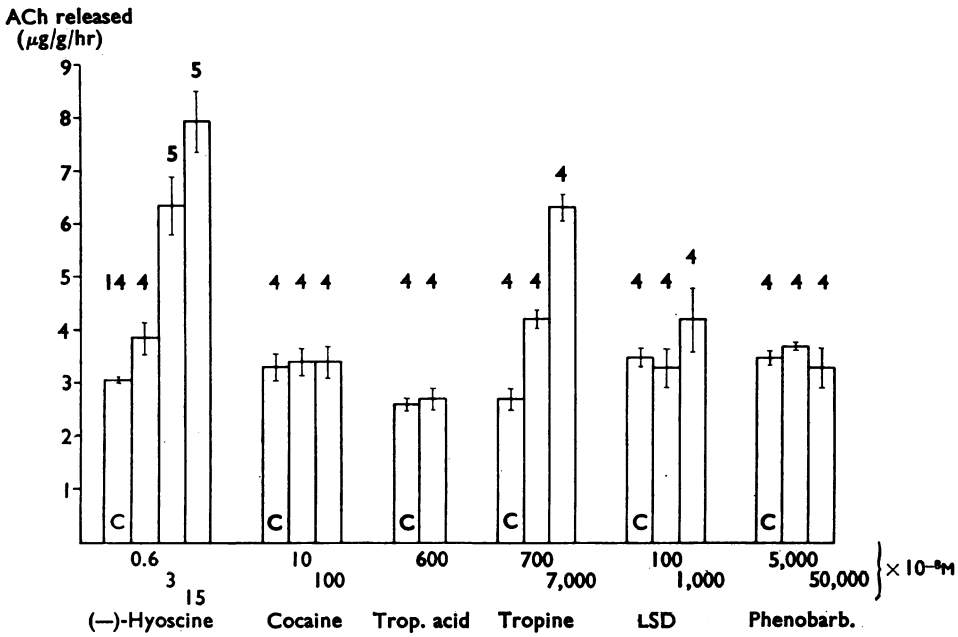

Fig. 4. Influence of several drugs on release of ACh from slices of cerebral cortex. Portions of tissue of 96–150 mg. Pre-incubation and incubation as in Fig. 2. Mean amounts of ACh released ±S.E.M. (μg/g). C, Control; □, in presence of drug.

of the tissue slices. On raising the atropine concentration to 3×10^{-5}M, however, this increase of ACh in the tissue slices became smaller, and with a concentration of 3×10^{-4}M the ACh content actually decreased. In Fig. 5 the effects of atropine 3×10^{-4} and 3×10^{-6}M are compared at different KCl concentrations of the medium. The initial ACh content of the tissue slices was 6.6 ± 0.2 μg/g. It fell to 5.5 ± 0.3 and 5.3 ± 0.2 μg/g on incubation with atropine 3×10^{-4}M at a KCl concentration of either 25 or 50 mM, whereas at these KCl concentrations it rose to 13.7 ± 0.7 and 17.0 ± 0.7 μg/g on incubation with atropine 3×10^{-6}M. In the presence of 4.7 mM KCl, atropine in either concentration had scarcely any effect on the ACh content of the tissue slices.

In the experiments with 25 mM KCl the sum of the amounts of ACh released into the medium and extracted from the tissue after incubation with 3×10^{-4}M atropine was about the same as after incubation with 3×10^{-6}M and much larger than in the absence of atropine (compare Fig. 1). Thus in this condition both atropine concentrations stimulated the synthesis of ACh to the same extent. On the other hand, in a medium containing 50 mM KCl the combined amount of ACh found in medium and tissue slices was smaller on incubation with 3×10^{-4}M atropine than with 3×10^{-6}M and equal to that found without atropine (compare Fig. 1).

Acetylcholine depleting effect of physostigmine

Physostigmine reduced the ACh content of the tissue slices incubated in a 25 or 50 mM KCl medium without stimulating synthesis of ACh. The effect was obtained whether

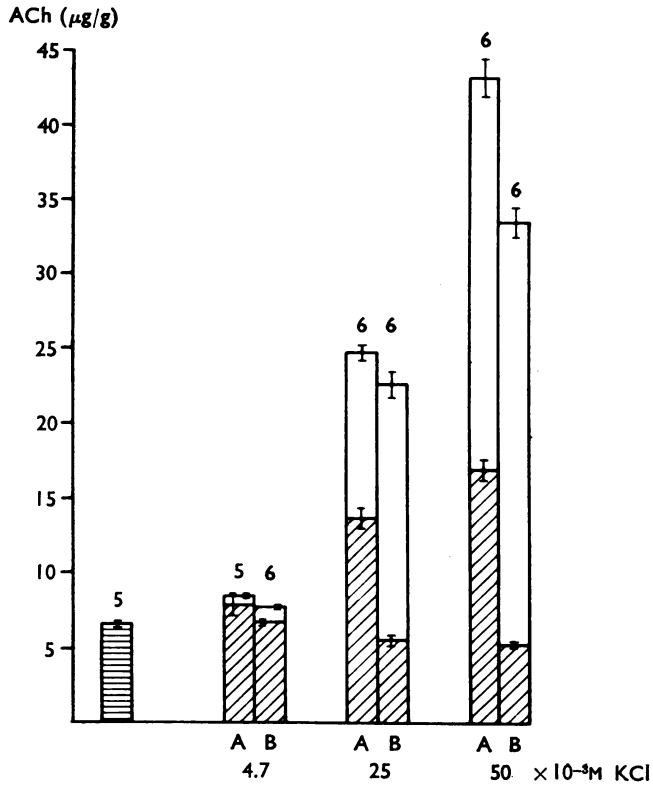

Fig. 5. Influence of high concentrations of atropine on ACh extractable and released from slices of cerebral cortex. Portions of 108–167 mg of tissue. Pre-incubation and incubation as in Fig. 1, except that incubation was with atropine 3×10^{-6}M (A) or 3×10^{-4}M (B). ▤, ACh content slices at 0 min; ▨, ACh content slices at 60 min; □, ACh released during 60 min.

soman was present in the medium or not. As in the experiments with atropine, depletion did not occur in a 4.7 mM KCl medium.

Table 1 illustrates the depletion produced with different concentrations of physostigmine in the presence of soman and with a 25 mM KCl concentration in the medium. Physostigmine was effective in concentrations lower than or similar to those used conventionally to inactivate the cholinesterase of brain tissue in *in vitro* experiments. The reduction in the ACh content of the tissue slices was associated with a corresponding increase in the amount of ACh released; there was thus no evidence for increased synthesis.

In Fig. 6 the effect of 8×10^{-4}M physostigmine is compared with that of 5×10^{-6}M soman. The experiments with the two inhibitors of cholinesterase were not performed simultaneously so no significance can be attached to small differences observed. Pre-incubation was either with physostigmine or with soman, and with 4.7 mM KCl in both conditions. The mean ACh content of the tissue slices after the pre-incubation period was 6.3 ± 0.4 μg/g in the presence of physostigmine and 6.9 ± 0.4 μg/g in the presence of soman. When incubation was continued for 1 hr the ACh content of the tissue slices incubated with physostigmine remained practically unchanged (6.5 ± 0.2 μg/g) whereas it rose to 7.7 ± 0.3 μg/g in the slices incubated with soman. On increasing the KCl concentration to 25 mM, the ACh content of the tissue decreased during the incubation period

TABLE 1

EFFECT OF PHYSOSTIGMINE ON ACh EXTRACTED FROM SLICES OF CEREBRAL CORTEX AND RELEASED INTO THE MEDIUM

Portions of 71–208 mg of tissue. Pre-incubation (60 min in 2.5 ml. of medium containing 4·7 mM KCl) and incubation (30 min in 5 ml. of medium containing 25 mM KCl) in the presence of soman (0·005 mM). Each figure represents one observation.

Physostigmine concentration (M)	ACh (μg) per gram of tissue		
	In slices	Released into bath	Total
5×10^{-5}	5·1	3·9	9·0
	5·0	4·4	9·4
1×10^{-4}	3·8	5·6	9·4
2×10^{-4}	4·0	7·3	11·3
4×10^{-4}	3·9	7·3	11·2
	5·4	5·3	10·7
8×10^{-4}	4·5	5·4	9·9
1×10^{-3}	4·8	6·4	11·2
	4·7	5·9	10·6
2×10^{-3}	6·4	4·8	11·2
	5·6	4·4	10·0
	5·5	4·4	9·9
Mean	4·9	5·4	10·3
No physostigmine	7·4	3·5	10·9
	6·0	2·9	8·9
	6·5	2·8	9·3
	8·6	2·5	11·1
	9·2	2·6	11·8
	6·9	2·6	9·5
	8·4	3·1	11·5
	7·7	3·4	11·1
Mean	7·6	2·9	10·5

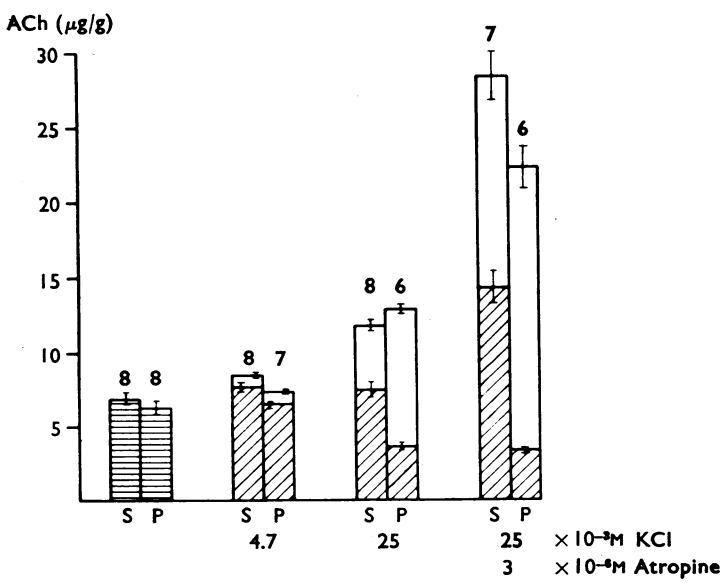

Fig. 6. Influence of potassium and atropine on ACh content of slices of cerebral cortex and on release of ACh into the medium during incubation with either soman or physostigmine. Portions of 136–171 mg of tissue. Pre-incubation and incubation as in Fig. 1, except that either 5×10^{-6}M soman (S) or 8×10^{-4}M physostigmine (P) was present in the medium. ▤, ACh content slices at 0 min; ▨, ACh content slices at 60 min; □, ACh released during 60 min.

to 3.7 ± 0.2 μg/g in the physostigmine experiments, but in the soman experiments it increased to about the same extent as during incubation with 4.7 mM KCl (7.5 ± 0.5 μg/g). Whether incubation was with physostigmine or soman, however, synthesis of ACh was about equal because the ACh depletion of the tissue slices in the presence of physostigmine was associated with a corresponding increase in the release of ACh into the medium.

The last two columns of the block diagram of Fig. 6 show the effect of atropine added to the incubation medium as well. Synthesis of ACh was stimulated, but whereas the ACh content rose to 14.3 ± 1.1 μg/g in the tissue slices incubated with soman it fell to 3.3 ± 0.2 μg/g in those incubated with physostigmine. Although this depletion was associated with a larger release of ACh into the medium (19.0 ± 1.4 μg/g) than in the corresponding soman experiments (14.1 ± 1.6 μg/g), the total amounts of ACh synthesized were smaller.

DISCUSSION

The ability of atropine to increase the release of ACh from brain tissue has so far been demonstrated only in *in vivo* experiments. The present experiments show that atropine exerts this action also in incubated slices of rat cerebral cortex, but only on incubation in a high potassium medium. This accords with previous findings which indicate that isolated cerebral tissue more nearly approximates that in the living animal if the potassium concentration of the medium is raised (see Quastel, 1965).

The effect of atropine was obtained with a concentration as low as 6×10^{-9}M, suggesting that it is linked to the antimuscarinic property. This view is supported by the finding that (−)-hyoscyamine was about 30 times as potent as (+)-hyoscyamine, that other antimuscarinic drugs shared with atropine this ability to stimulate ACh release, and that a number of substances without antimuscarinic activity, such as cocaine, phenobarbital, tropic acid, tropine and LSD, had no appreciable effect on the release of ACh. The results obtained with phenobarbital differ, for reasons unknown, from those obtained by McLennan & Elliott (1951). They incubated rat brain slices in a 27 mM KCl medium and found that synthesis of ACh was stimulated by low and inhibited by high concentrations of phenobarbital.

The effect of atropine is dependent on the KCl concentration of the medium, but KCl itself increases the release of ACh from incubated slices of brain tissue (Mann *et al.*, 1939; Welsh & Hyde, 1944; McLennan & Elliott, 1950). According to the previous findings the effect is maximal at a concentration of 25 mM KCl; an increase to 50 mM KCl actually resulted in a diminution of ACh release (Mann *et al.*, 1938). In the present experiments the effect of KCl in increasing acetylcholine release was confirmed, but more ACh was produced when the medium contained 50 instead of 25 mM KCl. Differences in technique seem to be responsible for the discrepancy in these results. One factor may be the NaCl concentration of the medium which was reduced in the previous experiments in order to compensate for the increased KCl concentration. This was not done in the present experiments. Bhatnagar & MacIntosh (1967) pointed out that the inhibition of ACh synthesis obtained by Mann *et al.* with excessively high KCl concentrations may have been the result of the removal of the Na from the medium, and they produced evidence in favour of this view.

The increased release of ACh brought about by raised potassium concentrations is probably a direct consequence of the depolarization of nerve endings produced by this cation, analogous to the effect of depolarization of motor nerve endings in the isolated diaphragm by potassium or by an electrical current which results in an immediate increase in the resting output of ACh (Liley, 1956). Depolarization of cell membranes in slices of cerebral cortex either by potassium or by electrical pulses increases oxygen consumption, aerobic glycolysis and lactate formation (see McIlwain, 1966). It also leads to an accelerated conversion of pyruvate to acetyl-coenzyme A (Kini & Quastel, 1959), which may be directly linked to the stimulation of ACh synthesis by high potassium concentrations. The potassium induced increase in the metabolism is probably associated with energy requiring recovery processes involved in re-establishing the disturbed ionic gradients across the cell membrane.

It is known that the increase in metabolism induced by electrical pulses or potassium is much more sensitive to drugs and to changes in substrate than the " resting " metabolism (Quastel, 1965 ; McIlwain, 1966). Atropine and other antimuscarinic substances which were studied on this increased metabolism inhibited the oxygen consumption and glycolysis of slices of cerebral cortex stimulated by electrical impulses (McIlwain, 1951 ; O'Neill, Simon & Cummins, 1963). On the other hand McIlwain did not obtain inhibition of the oxygen consumption and glycolysis stimulated by increased potassium concentration, but O'Neill *et al.* observed the inhibiting effect of antimuscarinic substances also in this condition. The concentration of the antimuscarinic substances required to inhibit respiration augmented either by electrical impulses or by increased potassium concentration was of the order of 10^{-4}M. This is at least 1,000 times stronger than the concentration which, in the present experiments, produced maximal increase in the release and synthesis of ACh. The two phenomena therefore seem to be unrelated.

It has been postulated that atropine increases the release of ACh from the brain in the living animal by blocking its reabsorption or by displacing it from its stores, either of which would lead to a decreased ACh content of the tissue (Giarman & Pepeu, 1964 ; Szerb, 1964 ; Polak, 1965). In the present experiments, however, not only the release but also the synthesis of ACh was stimulated by low concentrations of atropine. This action of atropine probably is dependent on intact cellular structures, because in cell-free biochemical systems prepared from rat brain atropine or hyoscine were found not to influence the synthesis of ACh and acetyl-coenzyme A (Giarman & Pepeu, 1964 ; Schuberth, 1965).

Because the same extremely low concentrations of atropine increased both synthesis and release of ACh the two effects appear to be coupled. A regulating negative feed-back mechanism between the ACh concentration in the tissue and the rate of ACh formation has been postulated by Mann *et al.* (1939) on the basis of the stimulating effect of potassium, but a second negative feed-back mechanism may exist between the ACh concentration in the environment of a store of ACh and the rate of its release. In favour of such a mechanism are the recent findings that the release of ACh from minced brain (Bhatnagar & MacIntosh, 1967) and from the synaptosome fraction of a brain homogenate (Hosein, Levy & Ko, 1967) is inhibited by the addition of ACh to the medium. If ACh receptors were involved in this negative feed-back system, their sensitivity might be reduced by atropine. Atropine would then stimulate the release and synthesis of ACh by blocking the inhibiting effect produced by the released ACh.

If reduction of the ACh content of the stores in the tissue slices were to act as the appropriate stimulus for synthesis of ACh one would expect increased synthesis of ACh to be always associated with a decreased ACh content of the tissue. Often, however, the reverse was found—for instance, when incubation was in a soman containing medium with a high KCl concentration or when antimuscarinic substances were added to such a medium. To reconcile these results with the theory that reduction of the ACh stores acts as the stimulus for synthesis, it is necessary to postulate that the ACh is stored in two compartments and that by determination of the ACh content of the slices a reduction in one compartment may not be revealed when accumulation in the other has occurred. The results obtained with physostigmine suggest that two such compartments exist and that reduction takes place in one.

When physostigmine was added to a medium containing a high KCl concentration and soman the ACh content of the tissue slices was found to decrease, but to a certain extent only. No further decrease occurred even when the physostigmine concentration used was increased manyfold. This result is in favour of the hypothesis of two ACh holding compartments, one being physostigmine-sensitive, the other physostigmine-resistant. Only the physostigmine-sensitive compartment would be depleted by physostigmine. Further, the finding that physostigmine produced no or only a slight decrease in the ACh content of the tissue slices incubated in a low potassium medium may indicate that in this condition nearly all ACh was present in the physostigmine-resistant compartment. On the other hand the rise which occurred in the ACh content of the tissue slices incubated with soman instead of physostigmine in conditions in which synthesis and release of ACh were stimulated—that is, in a high potassium medium, with or without atropine—must have taken place in the physostigmine-sensitive compartment, because it was not observed when in otherwise similar conditions soman was replaced by physostigmine.

Relatively high concentrations of atropine, in addition to their stimulating effect on ACh synthesis, appear to empty the physostigmine-sensitive compartment in the same way as physostigmine. This would explain why the rise in the ACh content of the slices incubated with 25 mM KCl and 3×10^{-6}M atropine was changed into a fall when the atropine concentration was raised to 3×10^{-4}M.

SUMMARY

1. A study was made of the influence of some antimuscarinic drugs on the release and synthesis of acetylcholine (ACh) by slices of cerebral cortex from rats after pre-incubation with the irreversible cholinesterase inhibitor soman.

2. During aerobic incubation at 37° C in a medium containing glucose and soman with 4.7 mM KCl, ACh was released into the bath and synthesized by the tissue. The release and synthesis increased when the potassium concentration of the medium was raised to 25 or 50 mM.

3. Atropine induced an additional increase in the release and synthesis of ACh on incubation in a high-potassium medium. This effect was obtained with atropine in a concentration as low as 6×10^{-9}M. On incubation in a low-potassium medium (4.7 mM) atropine did not have this effect.

4. The stimulation of release and synthesis of ACh by atropine seems to be linked to its anti-muscarinic properties. (−)-Hyoscyamine, which is known to have a much stronger antimuscarinic activity than (+)-hyoscyamine, was 30 times as potent in stimulating ACh release as its (+)-stereo-isomer. Hyoscine and Ro 2-3308, a hallucinogenic antimuscarinic drug, also stimulated the release of ACh. Ro 2-3308 was effective in a concentration as low as 2×10^{-9}M.

5. Drugs without antimuscarinic activity, such as cocaine, tropic acid, lysergic acid diethylamide and phenobarbital did not increase release and synthesis of ACh.

6. High concentrations of tropine stimulated ACh release but this effect was probably caused by contamination with hyoscine.

7. The increased release and synthesis of ACh which occurred with and without atropine in the presence of soman and a high potassium concentration in the medium were associated with an increase in the ACh content of the tissue slices. When physostigmine was added as well to the medium or replaced the soman the ACh content of the tissue slices decreased although the stimulating effect of potassium and atropine on release and synthesis of ACh still occurred.

8. Atropine in a high concentration (10^{-4}M) also decreased the ACh content of the tissue slices, yet release and synthesis of ACh were stimulated in the same way as with atropine in weak concentrations.

9. The results are discussed on the assumption that the ACh is stored in the tissue in two compartments, one being physostigmine-sensitive and the other physostigmine-resistant. Only the physostigmine-sensitive compartment is depleted by physostigmine or high concentrations of atropine.

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