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SHORT COMMUNICATIONS

The influence of atropine on the release and uptake of acetylcholine by the isolated cerebral cortex of the rat

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WHEN a solution of a cholinesterase inhibitor is brought into contact with the cerebral cortex or with the ventricular surface of the caudate nucleus of cats, acetylcholine (ACh) is released into this fluid. Addition of atropine to this solution or its systemic administration produces a strong increase in this output of ACh.[1-3] It has been suggested that the effect of atropine might be explained by competition between atropine and ACh for certain sites of uptake in the CNS[2,-4]. In the present investigation the influence of atropine on the release and uptake of ACh by the rat brain cortex *in vitro* was studied. It was found that atropine enhanced the release of ACh from rat brain cortex slices into the medium in which they were incubated, provided that this medium contained 25 mM KCl. Two additional observations were made: (1) when ACh was added to the medium a considerable uptake of ACh into the slices took place; (2) this uptake could be inhibited by atropine, but the concentrations of atropine needed for this inhibitory action were more than hundred times larger than those in which atropine enhanced the release of endogenous ACh from the tissue. These observations do not seem to support the idea that atropine causes an increase in the output of ACh from the brain by impeding (re)absorption of released ACh.

METHOD

Portions of about 150 mg rat brain cortex slices, pretreated with the cholinesterase inhibitor soman (0·005 mM), were incubated during 1 hr at 37° in 2·5 ml medium saturated with 95% O_2 and 5% CO_2. The composition of this medium was as follows (mM): NaCl 118·5, $NaHCO_3$ 24·9, KCl 4·7 or 25, $CaCl_2$ 2·5, KH_2PO_4 1·2, $MgSO_4$ 1·2, glucose 10. Unless mentioned otherwise soman (0·005 mM) was present in the medium during the whole experiment. In some experiments atropine, ACh and/or eserine sulphate were added to the medium at the beginning of the incubation period. The brain cortex slices were about 0·4 mm thick. After 1 hr the tissue was rinsed three times, and extracted with HCl by the method of Elliott *et al.*[5] Other samples of tissue were extracted immediately after pretreatment with soman. The ACh activity of the extracts and of the incubating fluids was estimated by bio-assay on the eserinised dorsal leech muscle against an ACh chloride standard solution and expressed in terms of this salt. Adequate dilutions of alkali treated and subsequently neutralized[6] extracts or dilutions of (modified) Krebs solution with or without atropine or eserine were added to the standard solutions to correct for substances other than ACh which might influence the sensitivity of the assay preparation. In some experiments smaller portions of tissue (about 75 mg) were incubated during a shorter time (30 min) in larger volumes (5 ml) of the medium containing 25 mM KCl.

RESULTS AND DISCUSSION

During incubation of the cerebral cortex slices ACh was set free into the medium. In a medium containing 25 mM KCl about five times as much ACh was released as in a 4·7 mM KCl medium. This confirms the results of Mann *et al.*[7] The ACh content of the tissue did not change during incubation in either medium. Addition of 1 μg/ml atropine sulphate to the 25 mM KCl medium resulted in a further threefold enhancement of ACh release, as well as in a rise of the ACh content of the tissue (Table 1). In other experiments atropine sulphate in a concentration of 0·05 μg/ml also increased the ACh output and 10 μg/ml did not produce a larger effect than 1 μg/ml. No significant atropine effect was observed in a medium with 4·7 mM KCl.

Uptake of added ACh was studied in experiments in which soman treated cerebral cortex slices were incubated in medium containing 4·7 mM KCl or 25 mM KCl or 25 mM KCl + 1 μg/ml atropine. ACh was added to the bath (final concentration 4 μg/ml) at the beginning of the incubation period. The

ACh concentrations of the tissue and the medium after one hour were estimated and compared with controls in which rat cerebral cortex had been incubated in the same media but without the addition of ACh (Table 2, columns a, b and c). The results show that there was a considerable uptake of added ACh into the tissue against a concentration gradient and that atropine did not significantly inhibit this uptake in a concentration in which it maximally enhanced the output of endogenous ACh. As a

TABLE 1. THE MEAN CONCENTRATIONS ± S.E. OF ACh IN RAT BRAIN CORTEX SLICES IMMEDIATELY AFTER PRE-INCUBATION WITH SOMAN AND AFTER 1 HR OF INCUBATION IN MEDIA OF DIFFERENT COMPOSITION AND THE MEAN AMOUNTS ± S.E. OF ACh RELEASED

Composition incubation medium	ACh content slices (μg/g)		ACh released into bath (μg/g/60 min)
	immediately after pre-incubation with soman	after 1 hr incubation	
Krebs with KCl 4·7 mM	6·9 ± 0·38 (8)	7·7 ± 0·28 (8)	0·83 ± 0·083 (8)
Krebs with KCl 25 mM		7·5 ± 0·50 (8)	4·3 ± 0·35 (8)
Krebs with KCl 25 mM + atropine (1 μg/ml)		14·3 ± 1·08 (7)	14·1 ± 1·56 (7)

The numbers of experiments are given between brackets. In each experiment about 150 mg tissue was incubated in 2·5 ml medium.

consequence of the uptake the ACh concentration of the medium decreased. This decrease was particularly evident in the experiments in normal Krebs solution in which the endogenous ACh production was relatively small. It was less conspicuous in the medium with 25 mM KCl and least in the 25 mM KCl medium with atropine, in which there was a large endogenous ACh production.

In the columns (d), (e) and (f) of Table 2 the same results as in the columns (a), (b) and (c) respectively are expressed as total amounts of ACh found in the brain tissue and in the medium together. Since in the experiments to which column (f) refers, 10 μg ACh had been added to each incubation mixture, the figures found here should be ten higher than the corresponding figures in column (e). Actually the results show that there was a reasonable recovery of the added ACh. It would appear that the added ACh was distributed in about the same way between the tissue and the medium in all three media, and that the differences in the results were brought about by superposition of this distribution on the changes in the concentration of endogenous ACh in the tissues and the media, produced by the addition of KCl and atropine to the medium.

The effect of atropine sulphate on the uptake of added ACh into slices of cerebral cortex was also studied in a series of experiments in which the concentration of the added ACh was kept practically constant during the time of incubation. This was achieved by incubating smaller amounts of tissue (about 75 mg) in larger volumes (5 ml) during a shorter time (30 min). This precaution facilitated the evaluation of the atropine effects. The experiments were performed in a medium containing 25 mM KCl. In a concentration of 10 μg/ml atropine sulphate inhibited the uptake of ACh into the slices by about 25 per cent and in a concentration as high as 100 μg/ml it caused an inhibition of about 70 per cent. This agrees with the findings of Creese and Taylor[8] who demonstrated uptake of ^{3}H-carbachol into slices of rat brain cortex and inhibition of this uptake by atropine. The fact that in the experiments of these investigators 1 μg/ml of the atropine base, corresponding to 1·2 μg/ml of the sulphate, was about as effective as 10 μg/ml of the sulphate in our experiments might be related to the fact that the concentration of the ^{3}H-carbachol in their experiments was much smaller (0·15 μg/ml) than that of the ACh in our experiments (4 μg/ml).

Other experiments were performed with rat cerebral cortex in a medium containing eserine sulphate (0·4 mM) as the cholinesterase inhibitor, since this substance has been used by most other investigators.[1, 2, 5, 7, 9] The results of these experiments were similar to those obtained with soman, as far as the influence of KCl and atropine on the output of ACh is concerned, but the uptake of added ACh

TABLE 2. ACh CONCENTRATIONS AND TOTAL AMOUNTS OF ACh IN SLICES IMMEDIATELY AFTER PRE-INCUBATION WITH SOMAN AND IN SLICES AND MEDIUM AFTER 1 HR INCUBATION OF ABOUT 150 mg RAT BRAIN CORTEX IN 2·5 ml KREBS OF DIFFERENT COMPOSITIONS

	Concentrations of ACh (μg/g and μg/ml)					Total amounts of ACh (μg)			Recovery added ACh
	Before incubation	After 1 hr incubation				Before incubation	After 1 hr incubation		
	(a)	No ACh added (b)		ACh (4 μg/ml added) (c)		(d)	No ACh added (e)	10 μg ACh added (f)	(f) − (e)
	slices	slices	medium	slices	medium	slices	slices + medium	slices + medium	
Normal Krebs	6·1	8·5	0·065	31	2·3	0·9	1·3	10·0	8·7
	8·7	8·8	0·047	30	2·3	1·3	1·5	10·2	8·7
	7·0	8·3	0·034	28	2·0	1·0	1·4	9·5	8·1
	7·2	7·9	0·039	34	2·5	1·0	1·3	11·2	9·9
Mean	7·3	8·4	0·046	31	2·3	1·1	1·4	10·2	8·9
25 mm KCl Krebs		8·5	0·21	35	2·9		1·7	12·1	10·4
		9·2	0·22	29	2·5		1·9	10·9	9·0
		7·9	0·19	32	3·4		1·5	12·9	11·4
		7·8	0·20	29	2·8		1·5	11·2	9·7
Mean		8·4	0·21	31	2·9		1·7	11·8	10·1
25 mM KCl Krebs + Atropine 1 μg/ml		15	1·2	40	3·3		5·2	13·9	8·7
		16	0·60	31	3·6		3·7	13·6	9·9
		14	0·62	40	4·1		3·7	15·7	12·0
		18	0·80	35	3·6		4·4	13·8	9·4
Mean		16	0·81	37	3·7		4·3	14·3	10·0

* Every figure is the result of one experiment.

was extremely small and during incubation of cortex tissue in an eserine sulphate medium containing 25 mM KCl with or without atropine the ACh concentration of the tissue fell to about 4 μg/ml. In subsequent experiments on soman treated brain cortex slices it appeared that eserine sulphate (0·4 mM) strongly inhibits the uptake of ACh. This explains why Elliott and Henderson[9] who 15 years ago demonstrated uptake of ACh into brain slices in an eserine sulphate medium, found only a comparatively small effect.

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Carbamylaspartate, a new agent against acute ammonia intoxication*

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INTRODUCTION

In recent years studies have been carried out in our Laboratory on the mechanism of acute ammonia intoxication[1] in which some amino acids showed a distinct protective effect.[2,3] Ornithine-aspartate mixture was able to suppress in the rat the toxic effects of a LD_{50} of ammonium acetate when injected intraperitoneally. L-aspartate alone was almost inactive; it became very active when associated with L-ornithine which by itself showed already much activity. The mechanism of this protection was consistent with an enhancement of the Krebs-Henseleit urea cycle.[4] L-arginine protected even better rats intoxicated with ammonia;[3] however, its mechanism of protection cannot be entirely explained on the basis of an enhancement of Krebs-Henseleit urea cycle.[5]

The present paper deals with the effect of carbamylaspartate (CA)† on acute ammonia intoxication. CA is an intermediate compound in the pathway leading to pyrimidine nucleotides.[6] Its role in urea synthesis has been recently discussed.[7, 8]

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† The following abbreviations are used in this paper: CA—carbamylaspartate; AA—ammonium acetate.