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INVESTIGATIONS INTO THE ACETYLCHOLINE OUTPUT FROM THE CEREBRAL CORTEX OF THE CAT IN THE PRESENCE OF HYOSCINE

BY

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Many centrally acting cholinoceptive receptor blocking drugs reduce the level of cerebral acetylcholine (Ach) in the rat (Giarman & Pepeu, 1962). Of the drugs tested hyoscine hydrobromide was the most effective and the reduction in Ach content was restricted to the cerebral hemispheres (Giarman & Pepeu, 1964). The decrease of brain Ach caused by hyoscine was also shown to occur in the guinea-pig (Beani, Bianchi & Megazzini, 1964) and in the cat (Deffenu, Mantegazzini & Pepeu, 1966).

At the same time as this group of investigations were in progress, Mitchell (1963) and Szerb (1964) found an increased release of Ach from the cerebral cortex in sheep, cats, and rabbits when atropine was administered, either systemically or into the fluid within a Perspex cup applied to the cortex. Polak (1965) showed that hyoscine and atropine given intravenously, and also intraventricular hyoscine, produce a similar increase in the output of Ach into the effluent from perfused cerebral ventricles.

The purpose of the work reported in the present paper was to investigate the suggestion that hyoscine increases the output by preventing the binding of Ach to receptor sites during activity in cholinergic nerve endings impinging upon cortical neurones.

METHODS

Thirty-six adult cats of both sexes were used. A tracheal cannula was inserted under ether anaesthesia, the head was clamped in a stereotaxic apparatus and transections were made by means of a stereotactically orientated spatula, either at mid-pontine pre-trigeminal level or at collicular level. In some experiments, immediately after a transection at mid-pontine pre-trigeminal level, the cats were hemisected at collicular level following the procedure of Cordeau & Mancia (1958). Shortly after the transection the skull was opened bilaterally over a large part of the frontal and parietal areas. The cats were allowed to recover from the anaesthetic for 3 hr and during this time they resumed spontaneous respiration. The temperature was carefully maintained at 37±0.2° C. Those cats in which either haemorrhages or oedema occurred were discarded.

The collecting cups were made according to the method of Mitchell (1963). After carefully opening the dura, one or two Perspex cylinders covering 1 cm^2 of cortex were lowered on to the somatosensory or the parietal areas of both hemispheres by means of an adjustable electrode carrier. The cylinders were filled with 1 ml. Ringer solution (NaCl 9.0, KCl 0.42, $CaCl_2$ 0.24, $NaHCO_3$ 0.5, glucose 1.0 g/l.) containing 100 μg/ml. physostigmine sulphate.

Every 15 min the solutions were removed from the collecting cups and immediately bioassayed on the dorsal muscle of the leech in a 3 ml. bath according to the method of Murnaghan (1958). The upper end of the muscle was connected to a small mirror which reflected a beam of light on to a graduated scale which allowed an amplification of 200 times. The samples collected from the cups were diluted with distilled water (1 vol up to 1.4 vol) in order to make them isotonic with leech Ringer solution. They were compared with standard solutions of Ach chloride containing the same amount of physostigmine. Each substance added to the collecting cups during the experiments was tested on the leech muscle in order to detect any influence it might have on the response of the muscle to Ach.

That the active substance released into the cups was Ach or some very similar choline ester was supported by the following facts: (*a*) when physostigmine was omitted from the solution in the cups no active substance or, at most, small irregular amounts, could be detected; (*b*) the cup samples were inactivated when they were treated with four drops of 0.1 N NaOH, kept at room temperature for 30 min and neutralized with 0.1 N HCl; (*c*) the samples were not active when d-tubocurarine chloride (3×10^{-6} g/ml.) was present in the fluid bathing the leech muscle.

The electrical activity of the cortex was recorded by means of screw electrodes inserted into the skull over the occipital lobes and with silver ball electrodes from inside the collecting cups. An indifferent electrode was inserted on the mid-line of the frontal bone.

In two experiments, the amount of hyoscine in the collecting cup at the end of 15 min of contact with the cortex was estimated on isolated guinea-pig's ileum, in oxygenated Tyrode solution at 37° C containing mecamylamine hydrochloride 1×10^{-6} g/ml., morphine chloride 1×10^{-5} g/ml. and diphenydramine 1×10^{-7} g/ml. The inhibition caused by the cup solution of hyoscine on the contraction induced by 200 ng Ach chloride was compared with that caused by a standard solution of hyoscine.

RESULTS

Ach output in mid-pontine pre-trigeminal cats and in "cerveau isolé" cats

Table 1 illustrates the marked differences of Ach output, in the third collection period from the beginning of the experiment, between 15 cats transected at mid-pontine pre-trigeminal level and 7 cats transected at collicular level. The first group of animals showed activated electroencephalogram pattern and ocular reactions typical of the waking

TABLE 1

OUTPUT OF ACh EXPRESSED AS ng/15 min/cm² ± S.E. FROM THE CEREBRAL CORTEX OF CAT, THIRD COLLECTION PERIOD FROM START OF EXPERIMENT

	Midpontine Pretrigeminal Transection		Cerveau Isolé	
EEG	Activated		Synchronized	
Hemisphere	Left	Right	Left	Right
Experiments (No.)	15	7	7	3
ACh	19·8±1·4*	18·5±1·2†	7·5±0·6*	6·4±0·5†

* The differences between these values are significant for $P<0{\cdot}001$.

† The differences between these values are significant for $P<0{\cdot}01$.

state and a high Ach output. The cats transected at collicular level showed a synchronized electroencephalogram, miosis, and a low Ach output. Table 1 also demonstrates that insignificant differences were found between symmetrically opposite areas of the two hemispheres in cats with complete transections. The cups were applied either on the somato-sensory or on the parietal areas, and both regions showed similar output in agreement with the observation of Mitchell (1963).

Figure 1 confirms the differences of Ach output between the two types of preparation and demonstrates that the output is practically constant over a period of at least 2 hr and does not show spontaneous variations.

In cats with an activated electroencephalogram the local application of physostigmine caused the appearance of high voltage spiking from the lead in the cups, and this tended to disappear in about 30 min.

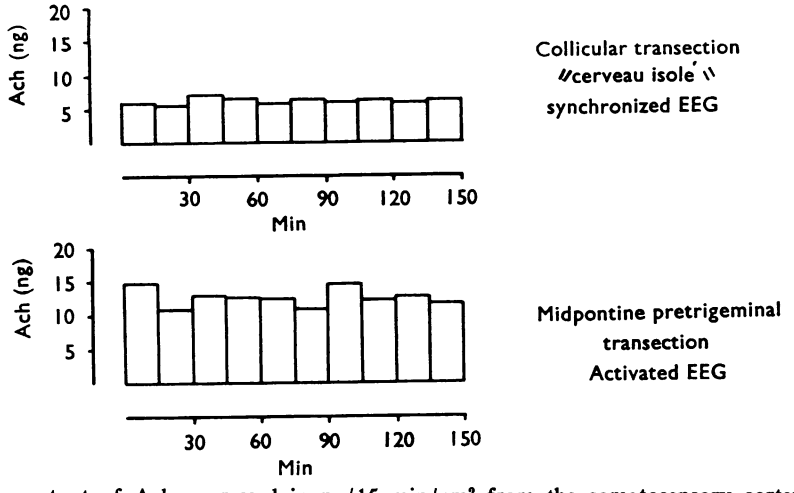

Fig. 1. The output of Ach expressed in ng/15 min/cm² from the somatosensory cortex of a cat transected at collicular level and of a cat transected at mid-pontine pre-trigeminal level; 15 min collection periods.

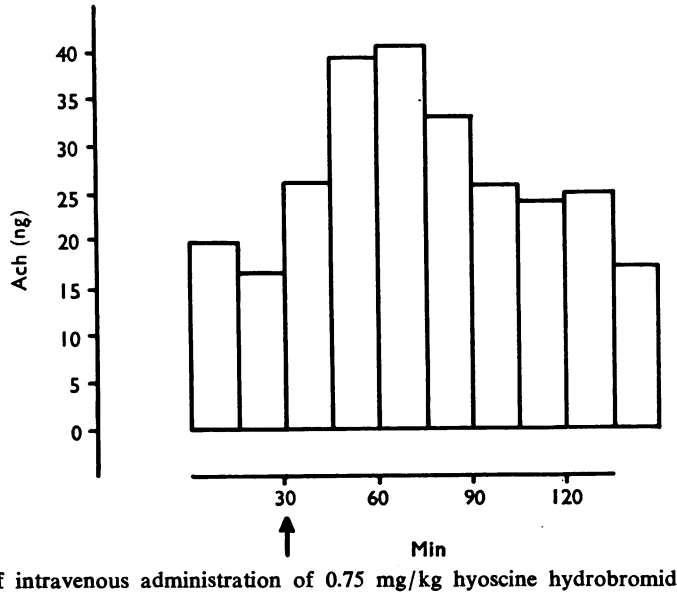

Fig. 2. Effect of intravenous administration of 0.75 mg/kg hyoscine hydrobromide (at the arrow) on the output of Ach, expressed as ng/15 min/cm², from the parietal cortex of a cat transected at mid-pontine pre-trigeminal level.

Effect of intravenous and intracarotid administration of hyoscine on Ach output

Figure 2 shows the output of Ach from the parietal cortex of a cat transected at mid-pontine level following the injection of 0.75 mg/kg hyoscine hydrobromide. The drug also caused mydriasis and electroencephalogram changes including slow waves and spindle bursts disappearing in about 90 min. Similar effects on Ach output and on the electroencephalogram and pupils were seen after the injection of 16 μg hyoscine at the rate of 1 μg/min through the lingual artery into the carotid artery, according to the procedure described by Deffenu *et al.* (1966) but they were localized to the side of the injection.

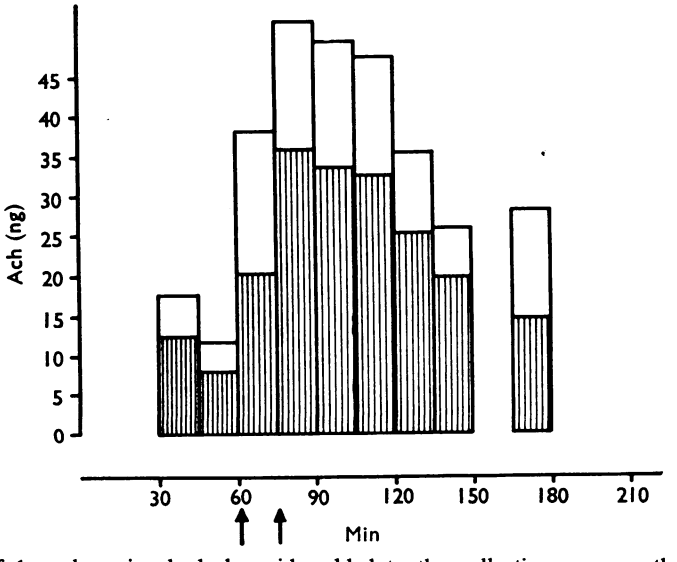

Fig. 3. Effect of 1 μg hyoscine hydrobromide added to the collecting cups on the output of Ach from the somatosensory cortex of the right (white) and left (shaded) hemispheres of a cat transected at mid-pontine pre-trigeminal level and with a left hemisection at collicular level. Acetylcholine expressed as ng/15 min/cm^2. Hyoscine added between the two arrows.

Effect of local administration of hyoscine on Ach output

Table 2 shows the maximum output of Ach observed after the addition of 1 μg hyoscine to the collecting cups applied to the cortex of pre-trigeminal mid-pontine cats and of "cerveau isolé" cats. The ratio between the values of the spontaneous output

TABLE 2

MAXIMUM EFFECT OF HYOSCINE (1 μg) APPLIED LOCALLY ON ACh OUTPUT EXPRESSED AS ng/15 min/cm^2 $\pm$ S.E.

EEG Pattern	Experiments (No.)	ACh Release		Ratio	*P*
Synchronized	4	Spontaneous After hyoscine	8·0$\pm$ 1·9 30·0$\pm$ 7·0	3·75	<0·05
Activated	7	Spontaneous After hyoscine	17·1$\pm$ 1·5 68·7$\pm$15·5	4·0	<0·01

before hyoscine and those of the maximum output after the drug is very similar in both groups of cats.

In Fig. 3 hyoscine was applied to cups on both hemispheres of a cat in which the electroencephalogram pattern and Ach output were made asymmetrical by means of a unilateral midbrain hemisection following the complete mid-pontine transection. It may be seen that the increase of Ach output after hyoscine is proportional to the initial spontaneous output and that the effect of the drug lasts more than 90 min. In this, as in most experiments, the increase of Ach output was associated with small changes in the electroencephalogram recorded from the cup lead, such as the appearance of small slow waves of medium voltage and, in some instances, of spindle bursts.

In two experiments in which the amount of hyoscine present in the collecting cups at the end of 15 min of contact with the cortex was estimated, 84% and 86% of the added amount was recovered. Of 1 μg of drug about 150 ng penetrate into the vascular and extracellular fluids and into the brain tissues.

Figure 4 shows the dose-effect relationship obtained by plotting the dose of hyoscine added to the collecting cups against the maximum % increase of Ach output. Each point is the average of at least three observations. It appears that the effect of hyoscine increases over a narrow range of doses and that the curve tends to flatten rapidly.

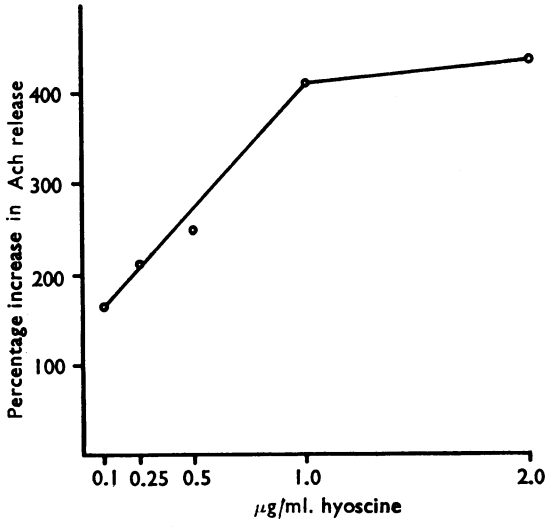

Fig. 4. Dose-effect relationship between the doses of hyoscine hydrobromide (abscissa) added to the collecting cups and the maximum percentage increase of Ach output (ordinate) from the cortex after the drug.

Effect of mecamylamine on Ach output

Mecamylamine blocks nicotinic receptors in the central nervous system (Knapp & Domino, 1962) and doses of 1.2 to 5.0 mg/kg affect the behaviour in mice (Oliverio, 1966). The possible effect of this drug on Ach release was investigated by local applica-

tion of a dose which exerts anti-nicotinic properties on the isolated guinea-pig's ileum. Ten micrograms of mecamylamine hydrochloride added to the collecting cups in 5 experiments did not affect Ach release or change the electrocorticogram recorded from the cups. Higher concentrations of mecamylamine could not be used since they affected the response of the leech muscle to Ach.

Effect of local anaesthetics on Ach output

Two local anaesthetics, cocaine hydrochloride 1% v/v in eserinized Ringer solution and butylamino benzoyl diethylaminoethanol hydrochloride (Farmocaina) 0.1% v/v in eserinized Ringer solution without bicarbonate, were investigated for their effect on the spontaneous output of Ach and on the output after hyoscine. Acetylcholine was determined in the samples collected immediately before and after the application of the anaesthetics. The samples containing the anaesthetics were discarded since they caused a strong contraction of the leech muscle. Table 3 shows the effect of cocaine and Farmocaina added to the cup of one hemisphere. Both spontaneous output and the output

TABLE 3

EFFECT OF LOCAL ANAESTHETICS ON ACh OUTPUT FROM THE CEREBRAL CORTEX OF CAT EXPRESSED AS ng/cm²/15 min

Experiment No.	1		2		3	
Hemisphere	Left	Right	Left	Right	Left	Right
	—	—	22·8	20·9	21·6	22·9
	—	—	Hyoscine→52·3	→49·5	Hyoscine→51·3	→53·6
			1 μg		1 μg	
	19·7	17·3	93·8	71·0	72·7	78·5
	Cocaine		Cocaine		Farmocaina	
	1%	16·3	1%	57·8	0·1%	48·5
	7·0	15·5	21·2	77·2	21·5	45·2
	7·3	15·8	21·7	44·2	15·9	60·2

after hyoscine are depressed, as demonstrated by the occurrence of a marked difference between the releases from the two hemispheres after their application. In 2 experiments 0.5 μg hyoscine and cocaine 1% were simultaneously applied on the cortex and the increase of Ach output caused by hyoscine was markedly delayed. Furthermore, in 2 experiments in which Farmocaina 0.1% and hyoscine 0.5 μg were simultaneously applied, the effect of the latter drug was completely suppressed.

DISCUSSION

In agreement with the findings of Celesia & Jasper (1966) on intact curarized cats we have demonstrated that also in animals transected either at midpontine pre-trigeminal level or at collicular level, activated electroencephalogram pattern is associated with a high release of Ach from the cortex, synchronized electroencephalogram pattern with a lower Ach release. These results are in keeping with the observations of Pepeu & Mantegazzini (1964), who showed that Ach content was twice as high in cortical samples taken from cats showing a synchronized electroencephalogram before death than in samples from cats with activated electroencephalogram.

The intravenous, intracarotid, or local administration of hyoscine hydrobromide causes an increase of Ach output from the cortex in both groups of cats with activated or

synchronized electroencephalogram. It appears from our experiments that the same amount of hyoscine brings about the same relative increase of the output regardless of the amount of Ach spontaneously released. The effect of local application of hyoscine and the small amount of drug which penetrates the brain are evidence that an action on subcortical structures is not necessary in order to increase Ach output.

Local anaesthetics can decrease both the spontaneous output and the output enhanced by hyoscine and in some instances prevented completely the effect of this drug. It has been claimed that local anaesthetics decrease Ach release from isolated intestine by acting on the cholinergic nerve endings (Johnson, 1963). Mitchell (1963) showed that Ach diffusing from the brain into the cups is of cortical origin and it may be therefore assumed that local anaesthetics depress the release of brain Ach by acting on cortical cholinergic nerve endings.

A tentative explanation of the increase of Ach output following the administration of hyoscine should take into account several facts.

(*a*) At the concentrations used in our experiments hyoscine has no effect on choline-acetylase (Giarman & Pepeu, 1964). Cholinesterases are inhibited in our experiments by the presence of large amounts of physostigmine, and therefore an effect of hyoscine on Ach metabolism should be ruled out.

(*b*) The possibility that anticholinergic drugs increase Ach output by acting on the cerebral blood flow was ruled out by Szerb (1964) on the basis of the lack of effect of locally applied vasodilator and vasoconstrictor drugs on Ach output and on the absence of Ach in the sagittal sinus blood either before or after local application of atropine.

(*c*) Hyoscine may penetrate into the cholinergic nerve endings displacing stored Ach. If this were the case, a larger effect of hyoscine might be expected in " cerveau isolé " cats in which the spontaneous output is small and the cortical content of Ach is high. However, we have shown that hyoscine causes the same relative increase regardless of the amount of the spontaneous output.

(*d*) Hyoscine may prevent the binding of Ach released from the nerve endings to specific or unspecific receptors. However, Polak & Meeuws (1966) have shown that the uptake by rat brain slices of Ach added to the incubation medium is reduced by a concentration of atropine much larger than those in which atropine enhances the release of endogenous Ach from the tissue. Mecamylamine, which is an active anti-nicotinic agent, has no effect on Ach output from the cortex, suggesting that the increase of output is associated with the blockade of muscarinic receptors. Krnjević & Phillis (1963) demonstrated the presence of muscarinic receptors on neurones of the superficial layers of cat's cortex. However, it has been shown that Ach has a strong affinity for a number of chemical constituents of the brain (Green, 1962). Furthermore, the marked decrease in the brain level of Ach following the administration of hyoscine to rats (Giarman & Pepeu, 1964) and to cats (Deffenu *et al.*, 1966) suggests the involvement of a large number of receptor sites.

SUMMARY

1. The spontaneous output of acetylcholine from the cerebral cortex, estimated by bioassay, was higher in cats transected at midpontine pretrigeminal level, showing an activated electroencephalogram and ocular reactions typical of wakefulness, than in cats

transected at collicular level showing a synchronized electroencephalogram and a fissurate miosis. In both groups of cats the output was practically constant over a period of at least 2 hr.

2. Hyoscine hydrobromide administered either intravenously, intracarotid, or added to the collecting cups caused a long-lasting increase of the spontaneous output. The same dose of drug added to the collecting cups caused a similar percentage increase of the output, regardless of the amount of Ach spontaneously released.

3. A dose-effect relationship was determined by plotting the doses of hyoscine added to the collecting cups against the maximum percentage increase of Ach output.

4. Local anaesthetic compounds decreased the spontaneous output; when applied after hyoscine decreased the output enhanced by the latter drug, when together with hyoscine they delayed or prevented its effect.

5. The results are discussed in relation to previous findings that hyoscine also decreases Ach content of the brain and it is concluded that they support the hypothesis that hyoscine increases the output through the occupation of specific Ach receptors.

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