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THE INFLUENCE OF CENTRALLY ACTING CHOLINOLYTIC DRUGS ON BRAIN ACETYLCHOLINE LEVELS

BY

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A number of centrally acting cholinolytic drugs reduced levels of cerebral acetylcholine in the rat. Among its naturally occurring analogues, hyoscine had the greatest potency, producing a decrease of 31% at a dose of 0.63 mg/kg. Atropine methyl nitrate, which acts as a cholinolytic drug in the periphery, had no effect on brain acetylcholine levels. The fall in acetylcholine produced by hyoscine was greatest after 60 min and disappeared at about 120 min. The animals tended to show a partial tolerance to this effect of hyoscine when the drug was administered repeatedly. The reduction in acetylcholine after hyoscine was restricted to the cerebral hemispheres, and did not appear in subcortical regions of the brain. Hyoscine had no influence on the net synthesis of acetylcholine by acetone-extracted powder of rat brain. In a series of four synthetic cholinolytic drugs, only the two with conspicuous psychotomimetic actions in man produced a decrease in brain acetylcholine comparable to that seen with hyoscine and related alkaloids.

Previous work in this (Giarman & Pepeu, 1962) and in another (Berry & Starz, 1956) laboratory has established that large doses of atropine and hyoscine in the rat produce significant reductions in total level of acetylcholine in the whole brain, while causing either no gross change in behaviour, or occasionally a pattern of alternating excitation and depression. This was of interest to us, because decreases in brain acetylcholine had been reported previously to occur only during convulsions. This paper describes an investigation in some detail of the effect of hyoscine and related compounds on cerebral acetylcholine levels.

METHODS

Adult male rats (150 to 200 g) were decapitated at appropriate times after intraperitoneal injection of the compounds being investigated. The brains (excluding cerebella, olfactory lobes and pituitary glands) were quickly removed and the acetylcholine was extracted by trichloracetic acid, after the method of Smallman & Fisher (1958). The acetylcholine content of the extracts was estimated within 48 hr by bioassay on the frog isolated rectus abdominis muscle preparation, which is highly resistant to the cholinolytic effect of atropine and related compounds when maintained in the presence of physostigmine (1 mg/100 ml. of frog-Ringer solution).

Previous investigations in this laboratory (Pepeu, Schmidt & Giarman, 1963) and in those of Crossland & Redfern (1963) and of Szerb (1963) have demonstrated that such extraction

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and bioassay procedures give an estimate of acetylcholine activity of which 90% or more is due to acetylcholine or very closely related derivatives.

In some experiments the brain (removed as described above) was divided into three parts by careful dissection at 4° C: the cerebral hemispheres were dissected away and considered one part, while the rest of the brain was subdivided into two parts by a section rostral to the corpora quadrigemina.

The drugs were dissolved in from 0.1 to 0.5 ml. of 0.9% saline and injected intraperitoneally. Control animals, examined with each experiment, were given equivalent volumes of 0.9% saline by the same route. The doses of all drugs have been expressed as salts.

The determination of choline acetylase activity was done by a modification of the method of Smallman (1958). The powdered residue of an acetone extract was prepared from the pooled brains of ten rats and used as a source of the enzyme. Final concentrations of components of the incubation mixture were as follows (mM): L-cysteine hydrochloride 20, $MgCl_2\,6H_2O$ 5, KCl 27, potassium acetate 10, choline chloride 10, adenosine triphosphate $4H_2O$ (dipotassium) 3, Coenzyme A (Calbiochem.) 0.2, phosphate buffer (*p*H 6.8) 7, and physostigmine sulphate 0.25. Hyoscine hydrobromide in a concentration of 10 μg/ml. was added to some samples.

The mixtures, with a final volume of 2.0 ml., were incubated in air at 37° C in a Dunoff metabolic shaker for 30 min. The reaction was stopped by adding 0.5 ml. of 0.33 N-hydrochloric acid and the samples were diluted to 10 ml. with frog-Ringer solution and bioassayed on the frog rectus abdominis muscle preparation. A standard solution of acetylcholine chloride was made in a corresponding dilution of incubate in which acetylcholine had been destroyed by adding a few drops of 0.33 N-sodium hydroxide solution, boiling for 5 min and neutralizing with hydrochloric acid.

RESULTS

Effects of hyoscine and related alkaloids

Table 1 shows the decrease of brain acetylcholine caused by different single doses of hyoscine, atropine, atropine methyl nitrate and homatropine, 30 min after administration of these drugs.

In our earlier work (Giarman & Pepeu, 1962) atropine sulphate, in a dose of 50 mg/kg, was found to decrease brain acetylcholine by 33%. It is clear that a similar reduction can be obtained with a dose of 5 mg/kg, but not with 1 mg/kg. The quaternary form, atropine methyl nitrate, is ineffective in a dose of 2.63 mg/kg. It is clear from these results that, while homatropine is of the same order of potency as atropine, hyoscine is the most potent drug in this group. This last drug, in a dose of 0.63 mg/kg, produced a considerable reduction in cerebral acetylcholine levels.

Demonstration that the cholinolytic agents do not interfere with the bioassay

The following facts prove that the cholinolytic agent that may be extracted from the brain is not responsible for the reduction in acetylcholine level found in such brain extracts.

(1) The decrease in acetylcholine produced by 400 mg of atropine per kg was smaller than that seen after 25 mg/kg (Giarman & Pepeu, 1962).

(2) The addition of atropine to a standard solution of acetylcholine does not alter the known potency of the solution on the frog rectus abdominis muscle preparation treated with physostigmine.

(3) The decrease in brain acetylcholine after hyoscine (0.67 mg/kg) is not greater when the extracts are bioassayed on the small intestine of the guinea-pig, a preparation which is highly sensitive to cholinolytic drugs. Our results showed a 20% reduction in brain acetylcholine, which is similar to the 31% reduction found with the assay on the rectus preparation (Table 1).

TABLE 1

INFLUENCE OF HYOSCINE AND RELATED ALKALOIDS ON BRAIN ACETYLCHOLINE LEVELS IN THE RAT

Doses are expressed as acid salts, and were dissolved in 0·9% saline and injected intraperitoneally. Acetylcholine levels are means with standard deviations. N.S., not significant. *Equimolar doses, equivalent to 2·5 mg/kg of atropine sulphate

Drugs	No. of rats	Dose (mg/kg)	Acetylcholine (μg/g)	Change (%)	Significance of change (*P*)
None	31	—	2·87±0·26	—	—
Atropine sulphate	4	1·00	2·57±0·26	−13	N.S.
Atropine sulphate	10	5·00	1·94±0·44	−33	<0·01
Atropine sulphate	5	25·00	1·64±0·20	−42	<0·001
Atropine sulphate	6	50·00	2·02±0·41	−29	<0·01
Atropine methyl nitrate	6	2·63*	2·54±0·38	−12	N.S.
Hyoscine hydrobromide	9	0·63	1·99±0·37	−31	<0·01
Hyoscine hydrobromide	4	3·15*	1·81±0·28	−37	<0·01
Homatropine hydrobromide	5	2·50*	1·89±0·27	−34	<0·01
Homatropine hydrobromide	5	25·00	1·98±0·30	−31	<0·01

(4) Authentic acetylcholine added to extracts of brain from animals that had received hyoscine (1 mg/kg) was found to have a potency equivalent to the amount added, when assayed on the guinea-pig ileum.

Time-course of the effect of hyoscine on brain acetylcholine levels

Hyoscine hydrobromide (0.63 mg/kg) was given to a group of twenty rats. Four of these animals were killed at each of the times shown in Fig. 1, and the level of

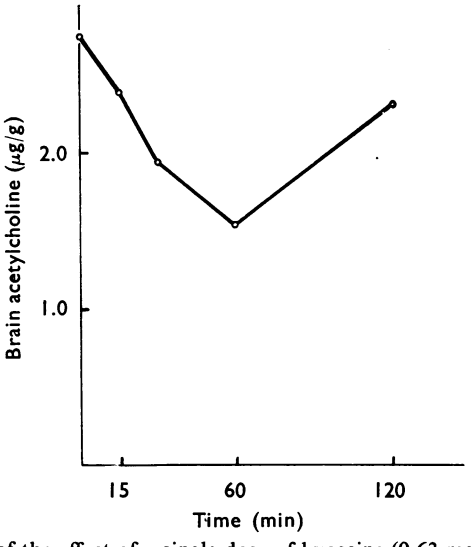

Fig. 1. The time course of the effect of a single dose of hyoscine (0·63 mg/kg, intraperitoneally) on brain acetylcholine levels in the rat. Each point is the mean value for four animals.

acetylcholine in the brain was determined. The reduction in brain acetylcholine was greatest at 60 min, after which time it tended to grow less and to disappear at around 120 min. In this context, White & Boyajy (1960) have reported that the behavioural effects of hyoscine in the rabbit last 1 to 3 hr, but that electroencephalographic changes persist for longer periods.

Effect of repeated injections of hyoscine

Hyoscine hydrobromide was given intraperitoneally to twelve experimental animals in a dose of 1.0 mg/kg, at 7 a.m., 2 p.m. and 11 p.m. every day for 6 days. Each day (except the 3rd) two animals were killed 1 hr after the dose given at 7 a.m., and the brain acetylcholine content determined. Acetylcholine in brains of unmedicated animals was determined in parallel with each of the experimental animals. Results of this experiment are summarized in Table 2.

TABLE 2

EFFECT OF REPEATED ADMINISTRATION OF HYOSCINE ON BRAIN LEVELS OF ACETYLCHOLINE IN THE RAT

Hyoscine hydrobromide, 1 mg/kg, was injected intraperitoneally, three times daily for 6 days. Times relate to the first injection of hyoscine

No. of injections	Time (hr)	Reduction in acetylcholine (%)
1	1	34
4	25	18
10	73	15
13	97	16
16	121	16
19	145	24

These results indicate that, after the initial response of cerebral acetylcholine to a single effective dose of hyoscine, subsequent doses of the drug, given at approximately 8-hourly intervals, lead to an attenuated response, seen after four drug administrations and maintained at about the same level for nineteen injections.

Regional localization of the effect of hyoscine on brain acetylcholine levels

Loeb, Magni & Rossi (1959) have implicated a suprathalamic site of action of atropine, while Longo (1956) had demonstrated that atropine and hyoscine block primarily corticopetal effects of electrical stimulation of brain structures. It was, therefore, of interest to us to attempt to localize the decrease in acetylcholine in the rat brain that follows the administration of hyoscine. For this study the brain was divided into three large parts, as described above. Results are summarized in Table 3. Clearly, the effect of hyoscine on brain acetylcholine levels is restricted to the cerebral hemispheres, and does not occur in subcortical regions. The fact that the decrease in total acetylcholine in the cerebrums of the treated animals is less than that observed in the whole brain (Table I) is probably due to the small number of animals in this study.

Effect of hyoscine on choline acetylase activity in rat brain

One possible explanation for the decrease of brain acetylcholine caused by these drugs is that they may inhibit the formation of acetylcholine by choline acetylase.

TABLE 3

INFLUENCE OF HYOSCINE ON ACETYLCHOLINE LEVELS IN DIFFERENT REGIONS OF THE RAT BRAIN

Hyoscine hydrobromide, 0·63 mg/kg, was always given. *Cut at the level of the corpora quadrigemina, so that the latter remained with the caudal mesencephalon and metencephalon. Acetylcholine levels are means with standard deviations. N.S., not significant

Part of Brain	Drug	No. of estimates	Acetylcholine (μg/g)	Change (%)	Significance of change (*P*)
Cerebrum	None	4	2·28±0·24		
	Hyoscine	4	1·50±0·33	−35	<0·01
Rostral mesencephalon*	None	5	2·89±0·49		
	Hyoscine	4	2·49±0·61	−14	N.S.
Caudal mesencephalon* and metencephalon	None	5	2·90±0·29		
	Hyoscine	4	3·17±0·58	+9	N.S.

We have, therefore, studied the influence of hyoscine on the net synthesis of acetylcholine by an enriched preparation of acetone-extracted powder of rat brain, under the conditions described above. The amount of acetylcholine formed (mean and standard deviation, five experiments) was 1.70 ± 0.05 mg/hr/g, which compares favourably with that obtained by Smallman (1958) with acetone-extracted residue from rabbit brain. The addition of hyoscine hydrobromide (10 μg/ml.) did not alter this production of acetylcholine.

Effect of synthetic cholinolytic compounds on brain acetylcholine levels

In view of the recognized psychotomimetic potency of hyoscine and atropine, it seemed of interest to investigate effects on brain acetylcholine levels of a small series of synthetic cholinolytic compounds shown by Abood, Ostfeld & Biel (1959) to have marked psychotomimetic and peripheral cholinolytic properties. Of the four drugs tested only the two that had demonstrated psychotomimetic potency in man affected brain levels or acetylcholine (Table 4). The reductions in acetylcholine by these compounds were similar in magnitude to those elicited by effective doses of atropine and hyoscine (Table 1). Of the two compounds that did not affect brain levels of acetylcholine, one has a quaternary nitrogen and is active as a cholinolytic

TABLE 4

EFFECTS OF CERTAIN SYNTHETIC CHOLINOLYTIC COMPOUNDS ON BRAIN ACETYLCHOLINE LEVELS IN THE RAT

Each drug was given intraperitoneally in 0·9% saline in a dose of 10 mg/kg, and the animals were killed 30 min later. Acetylcholine levels are means with standard deviations. N.S., not significant

Drugs	No. of rats	Acetylcholine (μg/g)	Significance of change (*P*)	Change (%)	Remarks
None	31	2·87±0·26	—	—	—
1-Methylpiperid-3-yl benzilate (J.B. 336)	6	1·75±0·05	0·2>*P*>0·01	−40	Psychotomimetic
1,1-Dimethylpiperid-3-yl benzilate (J.B. 330)	5	2·85±0·35	N.S.	—	Peripheral cholinolytic only
1-Ethylpyrrolidin-2-ylmethyl α-cyclopentyl-α-phenylglycollate (J.B. 8099)	6	1·82±0·25	<0·001	−36	Psychotomimetic
3,4-Dimethylpiperazin-1-yl *N*-propylbenzilate (J.B. 8035)	6	2·63±0·38	N.S.	−9	Centrally active cholinolytic with no psychotomimetic action

agent only in the periphery, and the other has been described as a centrally active cholinolytic drug without psychotomimetic potency (Abood, personal communication).

DISCUSSION

This work demonstrates that centrally acting cholinolytic drugs, possessing recognized psychotomimetic and amnesic effects in man, cause a fall in total brain levels of acetylcholine in the rat. This kind of alteration of cerebral acetylcholine level has been associated with certain drug- and electrically-induced convulsions (Richter & Crossland, 1949 ; Feldberg, 1957) ; but, with these cholinolytic drugs, the neurochemical change was not correlated with any specific gross behavioural change.

The potency of these drugs in causing an alteration in brain acetylcholine level seemed to correlate with their potency in inducing " amnesia " in the rat (Domer & Scheuler, 1960) and electrophysiological changes in the rabbit (Longo, 1956). In such studies hyoscine is generally ten- to fifteen-times as active as atropine, and in our investigation hyoscine was more potent than atropine in reducing brain acetylcholine.

Among the synthetic cholinolytic agents in this study, there was a close correlation between ability to reduce brain acetylcholine and psychotomimetic activity in man (Abood, Biel & Cannon, 1961). It is not our contention that all psychotomimetic drugs cause a change in cerebral acetylcholine level ; indeed, neither lysergic acid diethylamide nor mescaline alters acetylcholine levels (Freedman, Giarman & Pepeu, unpublished). Among certain cholinolytic psychotomimetic drugs, however, it is likely that psychotomimetic effects are linked with alterations in brain acetylcholine level. While this effect on brain acetylcholine seems from this work to be restricted to a fairly specific group of drugs, it is of interest that the anti-Parkinsonian drug, caramiphen, has been shown recently to produce a similar reduction in brain acetylcholine (Pepeu, 1963). In view of this and the known anti-Parkinsonian action of atropine and hyoscine, other anti-Parkinsonian drugs might be expected to demonstrate the same effect on brain acetylcholine levels.

It has been observed in monkeys with chronically implanted electrodes that small doses of hyoscine produce a relatively greater stimulating effect than larger doses (Domino & Hudson, 1959). Moreover, in a comparison of central effects produced by different doses of atropine and hyoscine, White & Boyajy (1961) reported that maximal electroencephalographic and behavioural changes were obtained with relatively low doses, suggesting that these alkaloids saturate central receptors in a manner analogous to their known peripheral actions. These findings agree with our results on brain acetylcholine. In fact, 5 mg/kg of atropine produced a reduction in brain acetylcholine almost as great as that produced by 25 mg/kg ; and in a previous work it was observed that 50 mg/kg of atropine had a more pronounced effect than 100 or 400 mg/kg (Giarman & Pepeu, 1962).

With respect to the mechanism by which hyoscine and related compounds can cause a fall in brain acetylcholine, our results have ruled out inhibition of choline acetylase activity as a possibility. There are three other possibilities which remain to be considered.

(1) These drugs might enhance activity of acetylcholinesterase. While it must be conceded that this is an unlikely possibility and difficult to prove, a preliminary study indicated that there is no change in acetylcholinesterase activity in the presence of hyoscine. Furthermore, it has already been established that atropine actually inhibits acetylcholinesterase (Lüllmann, Förster & Westermann, 1952).

(2) These drugs might produce a blockade of central acetylcholine receptors as they do to peripheral acetylcholine receptors. This would lead to a movement of physiologically liberated acetylcholine to acetylcholinesterase and destruction, as well as to points distant from the sites of storage and release. It seems likely that this mechanism is similar to that which may be responsible for some of the observations of Mitchell (1963), who reported an increased spontaneous output of acetylcholine from the parietal cortex of the sheep after atropine (5 mg/kg, intravenously).

(3) These drugs might alter storage sites of acetylcholine in a manner leading to reduced uptake of newly synthesized acetylcholine or to increased release of bound acetylcholine, or both.

The net result in any case would be a reduction in total acetylcholine. The latter two possibilities are being investigated.

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