Brit. J. Pharmacol. (1962), **19**, 226–234.

DRUG-INDUCED CHANGES IN BRAIN ACETYLCHOLINE

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(*Received March* 27, 1962)

In rats, drug-induced depression of the central nervous system has been shown generally to be associated with an elevation in level of total acetylcholine in the brain. This generalization held true for a wide variety of depressant drugs with one notable exception: the subacute administration of reserpine, with which there was an increase in cerebral acetylcholine after the first dose, but a return to normal levels after subsequent doses, despite continued depression of the animals. Reduction in the level of total acetylcholine in the brain followed the administration of certain convulsants (pentylenetetrazole and 3,5-dimethylbutylethylbarbiturate); but no change was seen after the administration of several mildly exciting agents. The notable exceptions to this generalization were atropine and scopolamine, which significantly lowered brain acetylcholine in doses producing mild excitation in only some of the animals and no gross manifestations in the rest.

The level of total acetylcholine in the brain has been shown to vary with the functional activity of the brain. Such variations have been related to physiological changes in nervous activity such as sleep or wakefulness (Richter & Crossland, 1949), to the action of neuropharmacological agents (Tobias, Lipton & Lepinat, 1946; Richter & Crossland, 1949; Elliott, Swank & Henderson, 1950; Wajda, 1951), and to certain physical stresses, such as electrical stimulation (Richter & Crossland, 1949), cerebral trauma (Kovach, Fonyo & Halmagyi, 1957), and changes in temperature (Anand, 1952).

The purpose of these experiments has been to gather further information on the correlation between drug-induced behavioural changes in the rat and the content of total acetylcholine in the brain. In some experiments, correlations with total 5-hydroxytryptamine in the brain were also sought.

METHODS

Adult male rats (albino Wistar), 150 to 200 g, were killed by decapitation at the time stated in each experiment. The entire brain (excluding cerebellum, olfactory lobes and pituitary) was removed quickly and total acetylcholine was extracted by the method of Smallman & Fisher (1958). The acetylcholine content of the extract was estimated within 48 hr by means of a preparation of the isolated rectus abdominis muscle of the frog; the muscle

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was bathed in frog-Ringer solution containing physostigmine (1 mg/100 ml.). The values of acetylcholine are expressed in terms of acetylcholine chloride. The effect of the various drugs used was carefully examined on the rectus abdominis preparation in order to compensate for any influence on the response of the muscle to acetylcholine. In some experiments, brains were halved along the central sulcus and one-half was used to determine total acetylcholine, while the other half was used to estimate the 5-hydroxytryptamine level. Extraction of 5-hydroxytryptamine was accomplished by a modification of the method of Amin, Crawford & Gaddum (1954) and estimates of the content of 5-hydroxytryptamine were made by bioassay on the heart of *Mercenaria (Venus) mercenaria.* Dry extracts were kept at −16° C and assays were carried out within 72 hr. All drugs were administered intraperitoneally (in 0.1 to 0.4 ml.).

RESULTS

Drugs producing an increase in brain acetylcholine

Table 1 shows increases in total acetylcholine of the brain after the administration of various drugs to rats. The increases are expressed as % changes from the controls; absolute values are reported in subsequent tables.

The greatest increase was observed after the administration of a barely toxic dose of tetraethylpyrophosphate, a strong anticholinesterase agent, the effect of which on levels of acetylcholine has been reported previously (Stone, 1957). These animals exhibited signs which often terminated in convulsions and may be attributed to accumulation of acetylcholine in the brain.

TABLE 1

PERCENTAGE INCREASE IN ACETYLCHOLINE CONTENT OF RAT BRAIN PRODUCED BY SEVERAL DRUGS

Drug	Dose mg/kg intra-peritoneally	Time of sacrifice after administration (hr)	% increase in acetylcholine	Remarks
TEPP (Tetraethyl-pyrophosphate)	1	0·5	117 ($P<0·001$)	Toxic manifestations; convulsions
Methylpentynol (Methylparafynol)	500	0·2	90 ($P<0·001$)	Anaesthesia
Hydroxydione ("Viadril")	60	0·75	54 ($P<0·001$)	Anaesthesia
Morphine	50	0·5	47 ($P<0·001$)	Severe depression
Pentobarbitone	30	1·0	21 ($P<0·02$)	Sleep
Reserpine	2·5	24	20 ($P<0·001$)	Sedation
Phenylcyclohexyl-piperidine ("Sernyl")	20	0·33	15 ($P<0·05$)	Excitation; tremor

The increase of total acetylcholine caused by the group of central nervous system depressants in the table (methylpentynol, hydroxydione, morphine, pentobarbitone and reserpine) was roughly proportional to the degree of depression of the central nervous system. In all rats there was pronounced reduction in motor activity. The pentobarbitone-induced rise in brain acetylcholine agrees with the observations of other investigators (Elliott *et al.*, 1950; Richter & Crossland, 1949; Crossland & Merrick, 1954).

Malhotra & Pundlik (1959) found that reserpine increased the acetylcholine content of the dog brain. In our experiments with rats, reserpine caused a small but

statistically significant increase in cerebral acetylcholine. This rise was accompanied by the characteristic depression resulting from an overdose of this drug ; the animals, however, maintained some spontaneous motility. With repeated daily administration of reserpine for periods up to 8 days, the cerebral level of acetylcholine rose on the second day, and then returned to normal, despite the deepening degree of depression which was observed in the animals (Table 2). The changes in cerebral

TABLE 2

EFFECT OF CHRONIC ADMINISTRATION OF RESERPINE (0·5 MG/KG, INTRAPERITONEALLY PER DAY) ON CEREBRAL ACETYLCHOLINE AND 5-HYDROXYTRYPTAMINE OF THE RAT*

* These animals were sacrificed 20 hr after the last injection of reserpine. † The difference between these two values is 15% and is statistically significant ($P<0·001$)

	No. of animals	No. of days	Acetylcholine (μg/g ±s.d.)	5-Hydroxytryptamine (μg/g)
Controls	10	—	†2·80±0·14	0·355
Reserpine	7	2	†3·21±0·22	0·175
Reserpine	4	4	2·72±0·19	0·060
Reserpine	4	6	2·76±0·18	0·053
Reserpine	4	8	2·85±0·19	0·085

acetylcholine induced by chronically administered reserpine did not parallel in time the decrease in cerebral 5-hydroxytryptamine (cf. Table 2).

Garattini & Valzelli (1958) and Sulser & Brodie (1960) have demonstrated that, when rats are exposed to an environmental temperature of 4° C for 2 to 4 hr before the administration of reserpine and maintained at this temperature after the injection, the effect of the drug is greatly reduced. Such animals do not show the general depression, ptosis, miosis, and diarrhoea which are typically elicited by reserpine in animals at room temperature. In addition, the reserpine-induced decrease in the levels of 5-hydroxytryptamine in the tissues is almost entirely prevented in animals kept in the cold, while the decrease in cerebral catecholamines can still be observed (Sulser & Brodie, 1960). Table 3 shows the effect of a single intraperitoneal injection

TABLE 3

EFFECT OF RESERPINE (2·5 MG/KG INTRAPERITONEALLY) AT DIFFERENT TEMPERATURES ON THE LEVEL OF ACETYLCHOLINE AND 5-HYDROXYTRYPTAMINE IN THE BRAIN

* The difference between these two values, 19%, is statistically significant ($P<0·001$)

	No. of animals	Temperature (°C)	Acetylcholine (μg/g ±s.d.)	5-Hydroxytryptamine (μg/g±s.d.)
Control	9	22	2·71±0·29*	0·294±0·018
Reserpine	11	22	3·25±0·16*	0·099±0·044
Reserpine	6	4	2·96±0·11	0·210±0·032
Control	5	4	2·80±0·14	0·342±0·056

of reserpine on the levels of 5-hydroxytryptamine and acetylcholine in the brains of rats kept at 4° C and at 22° C. The animals were sacrificed 20 hr after the administration of reserpine.

Under these conditions, the increase in brain acetylcholine appears to coincide with depression of the animals and decrease in 5-hydroxytryptamine content of the brain.

Hydroxydione, a steroid with anaesthetic properties, has been used in short surgical procedures (Laubach, P'An & Rudel, 1955). Following its intraperitoneal administration, there was a rise in cerebral acetylcholine, which was found to correlate with the anaesthetic state. It was maximal at the greatest depth of anaesthesia and disappeared within 1 min after arousal (Table 4). A similar rapid decrease of brain acetylcholine toward the normal level following anaesthesia by pentobarbitone has been reported by Elliott *et al.* (1950).

TABLE 4

RELATION OF ANAESTHESIA INDUCED BY HYDROXYDIONE (60 MG/KG, INTRAPERITONEALLY) TO THE LEVEL OF ACETYLCHOLINE IN THE BRAIN OF THE RAT

Functional state of animals	No.	Brain acetylcholine (μg/g$\pm$s.d.)
Normal (controls)	12	2·76$\pm$0·30
During anaesthesia (45 min after drug)	6	4·23$\pm$0·26
One minute after regaining righting reflex (ataxic)	3	2·84 (2·70–2·98)

The influence of steroid hormones on cerebral acetylcholine has been studied by Torda & Wolff (1952), who demonstrated an increased ability of brain of cortisone-treated rats to synthesize acetylcholine. In view of their finding and the effect produced by hydroxydione, the level of acetylcholine in the brain was examined in rats treated with glucocorticoids and adrenocorticotrophic hormone in order to detect possible variations in the neurohumour not related to the anaesthetic state. Table 5 summarizes these data.

TABLE 5

EFFECT OF SOME STEROIDS AND OF ADRENOCORTICOTROPHIC HORMONE ON THE LEVEL OF ACETYLCHOLINE AND 5-HYDROXYTRYPTAMINE IN THE BRAIN OF THE RAT

* These animals were sacrificed 45 min after the administration of hydroxydione, while the animals were in surgical anaesthesia. † This value is significantly different from the mean control value (0·55) at $P<0·01$, derived from " Student's " t table

Drug	No. of animals	Dose (mg/kg/day)	Duration of treatment	Acetylcholine level (μg/g $\pm$s.d.)	5-Hydroxytryptamine level (μg/g)	Remarks
None	12	—	—	2·76$\pm$0·30	0·55	—
Cortisone	4	50	9 days	2·67$\pm$0·16	—	Decreased adrenal weight
Cortisone	6	50	20 days	2·82$\pm$0·29	0·81†	Decreased adrenal weight
Triamcinolone	6	25	6 days	3·05$\pm$0·22	0·57	Weight loss
Hydroxydione (" Viadril ")	6	60	Single dose*	4·23$\pm$0·26	0·48	Anaesthesia
Adrenocorticotrophic hormone	6	2 u./kg	14 days	2·93$\pm$0·33	0·64	Increased adrenal weight

The repeated intraperitoneal administration of cortisone, triamcinolone or adrenocorticotrophic hormone did not influence the level of acetylcholine in the brain, but cortisone significantly elevated the level of cerebral 5-hydroxytryptamine while hydroxydione did not influence the cerebral level of this amine.

Interest in phenylcyclohexylpiperidine (" Sernyl ") has arisen from the complex action of this psychotomimetic agent on the central nervous system. Chen (1958)

reported that in monkeys this drug produced primarily sedation and depression, while in rats and mice excitement was the predominant effect observed. Griefenstein, Devault, Yoshitake & Gajewski (1958) have reported that "Sernyl" is an analgesic and psychotomimetic agent in human beings. In our experiments on rats the intraperitoneal injection of 20 mg/kg caused excitation, tremors and ataxia. Sacrificed 20 min after the injection, these animals barely showed a statistically significant increase in the levels of cerebral acetylcholine.

Drugs producing a decrease in brain acetylcholine

A number of investigators found that the occurrence of convulsions, induced either pharmacologically or electrically, is associated with a lowering of cerebral acetylcholine (cf. Feldberg, 1957). Accordingly, several drugs or pairs of compounds which elicit in the rat various degrees of excitation of the central nervous system have been investigated with respect to their effect upon the levels of acetylcholine in the brain. Results are reported in Table 6.

TABLE 6

EFFECT OF A VARIETY OF CENTRAL NERVOUS SYSTEM STIMULANTS ON CEREBRAL ACETYLCHOLINE

* DL-5-Hydroxytryptophan. † DL-Dihydroxyphenylalanine. ‡ This value differs significantly from the control at a level of $P<0.01$. § This value differs significantly from the control at a level of $P<0.02$

Drugs	No. animals	Dose (mg/kg, intraperitoneally	Time	Acetylcholine μg/g ±s.d.)	% decrease	Remarks
None	10	—	—	2·86±0·16	—	
Pentylenetetrazole (Metrazole)	6	75	12 min	2·21±0·24‡	22·7	Convulsions
3,5-Dimethylbutyl-ethyl barbiturate	8	20	10 min	2·26±0·32§	21·0	Convulsions
Lysergic acid diethylamide	6	0·2	2 hr	2·91±0·22	—	Mild excitation
Iproniazid	5	100	9 hr	3·03±0·31	—	No symptoms
Iproniazid+5-hydroxy-tryptophan*	4	100 100	16 hr 1 hr	2·85±0·26	—	Tremors, some motor incoordination
Iproniazid+DOPA†	4	100 250	16 hr 1 hr	2·81±0·32	—	Dyspnoea, marked excitation

The findings suggest that a decrease in the level of acetylcholine in the brain follows the administration of a central nervous system stimulant only when it produces convulsions (cf. pentylenetetrazole and 3,5-dimethylbutylethyl barbituric acid). Intense excitation as produced by dihydroxyphenylalanine (DOPA) after pre-treatment with iproniazid, characterized by dyspnoea, hypermotility and aggressiveness, but without convulsions, did not change the cerebral levels of acetylcholine. Similarly, 5-hydroxytryptophan, when given after iproniazid, produced only mild changes in behaviour and caused no alteration in the level of cerebral acetylcholine.

The action of atropine on the central nervous system is still rather obscure. Gaddum (1953) considers this drug a medullary stimulant which leads to depression; in fact, it has been demonstrated that atropine can prolong anaesthesia induced by barbiturates (Mensch & de Jongh, 1959). In man, the central actions of atropine

are equally complex, ranging from the production of a toxic psychosis to deep coma. Such properties have been made use of therapeutically in some patients with mental illness (Forrer & Miller, 1958). Table 7 shows the effect of atropine and related compounds on cerebral acetylcholine in the rat. With all dosages of atropine tested there was a decrease in the levels of cerebral acetylcholine, regardless of the gross manifestations of toxicity produced. Indeed, at the lowest dose tested, 50 mg/kg, which produced mydriasis and only a slight increase in motor activity, the effect on the level of acetylcholine in the brain was most pronounced. Similarly, scopolamine, in a dose of 50 mg/kg, caused no gross manifestations of toxicity in the rat, although the level of acetylcholine in the brain was reduced. These effects of atropine and scopolamine on brain acetylcholine were detectable between 10 and 30 min after the drugs were administered.

TABLE 7

THE INFLUENCE OF ATROPINE AND SOME OTHER COMPOUNDS ON BRAIN ACETYLCHOLINE

* All changes listed were significant when subjected to " Student's " t test

Drugs	No. of animals	Dose (mg/kg, intraperitoneally)	Time (min)	Acetylcholine μg/g $\pm$s.d.	% change*	Remarks
None	17	—	10	2·95±0·18	—	—
Atropine	7	50	10	1·99±0·12	−33	No gross manifestations or mild excitation
Atropine	6	100	10	2·29±0·23	−23	No manifestations
Atropine	8	400	10	2·18±0·21	−27	Excitation alternate with depression; tremors; convulsions
Scopolamine	4	50	10	1·98±0·21	−33	No gross manifestations or mild excitation
Atropine plus Pentobarbitone	5	50 30	10 30	4·24±0·78	+44	Deep sleep (anaesthesia ?)
Pentobarbitone	5	30	30	3·43±0·22	+16	Sleep
Benactyzine	5	25	20	2·73±0·34		Mild excitation

Although atropine and pentobarbitone produce opposite effects on cerebral acetylcholine, atropine does not antagonize the pentobarbitone anaesthesia. Pretreatment of the rats with atropine (50 mg/kg) 10 min prior to the administration of the barbiturate not only increased the duration of the anaesthetic state caused by pentobarbitone (30 mg/kg) but also increased the elevation in level of acetylcholine in the brain.

Benactyzine possesses from one-twentieth to one-tenth the activity of atropine, with respect to inhibition of peripheral cholinergic impulses (Ing, Dawes & Wajda, 1945 ; Larsen, 1955) ; on the central nervous system it exhibits effects which are reminiscent of the tranquillizers (Larsen, 1955). In a dose equimolar to that of atropine, benactyzine caused no significant change in the level of acetylcholine in the brain.

Schueler (1961) has suggested that hemicholinium inhibits the synthesis of acetylcholine, and Birks & Macintosh (1961) have shown that, in the cat, the inhibition of synthesis of acetylcholine by hemicholinium leads to a decreased output of acetylcholine in the perfusate from the perfused superior cervical ganglion *in situ*.

Hemicholinium in doses of from 90 to 200 mg/kg, administered intraperitoneally in rats, caused general depression, dyspnoea and muscular tremors; 30 min after the administration of the drug at the peak of these manifestations, there were no significant changes in cerebral acetylcholine (mean of 2.88 ± 0.30 μg/g in 10 animals, as compared to a mean control value of 2.95 ± 0.18 μg/g in 17 animals).

DISCUSSION

The evidence obtained in earlier studies appeared to justify the generalization that during sedation, sleep and anaesthesia the level of acetylcholine in the brain increases, while during convulsions it decreases (Feldberg, 1957). The data presented here bear out the findings that increased cerebral levels of acetylcholine are associated with drug-induced depression of the central nervous system, and would indicate further that this increase is roughly proportional to the degree of depression of the central nervous system. On the other hand, results obtained with atropine, with DOPA after iproniazid and with "Sernyl" cast some doubt on the notion that a decrease in the levels of acetylcholine in the brain is certain to accompany drug-induced excitation and convulsions.

With the anticholinesterase, tetraethylpyrophosphate, the striking increase in the level of acetylcholine in the brain almost certainly is attributable to reduced destruction of acetylcholine. On the other hand, the mechanism of the increase produced by the various depressants of the central nervous system is more obscure. Experiments on the effect of two of these depressants (reserpine, methylpentynol) on the acetylcholinesterase of brain (by the method of Michel, 1949) have ruled out inhibition of this enzyme as an explanation of the elevation in brain acetylcholine. It is reasonable to assume that utilization of the neurohormone is reduced in the depressed brain, although there is no rigorous proof of this. Another possibility is that some or all of these depressants can reduce or prevent the release of acetylcholine from its "bound" form. This is an attractive hypothesis, especially with respect to the mechanism of the methylpentynol-induced elevation in cerebral acetylcholine, in view of the demonstration by Marley & Paton (1959) that this sedative-hypnotic agent inhibits the release of acetylcholine from the perfused superior cervical ganglion of the cat.

Whatever mechanism is operative in drug-induced increases in the levels of acetylcholine in the brain, the most striking aspect of this phenomenon is the relative efficiency of acetylcholine metabolism in maintaining the level of the neurohormone within relatively narrow limits. For example, with such a potent anticholinesterase as tetraethylpyrophosphate, the brain level of acetylcholine rarely increases more than 100%, while it is not uncommon to find increases of 200% or more in the levels of 5-hydroxytryptamine or catecholamines in the brain when monoamine oxidase is inhibited, as, for example, with iproniazid. This imposition of a ceiling on the level of acetylcholine achievable in the brain may be related to a depression of the synthetic process by acetylcholine itself. Moreover, our experiments with the glucocorticoids and adrenocorticotrophic hormone clearly indicated the stability of the metabolism of acetylcholine in brain. Treatment of the rats with massive doses of cortisone for 20 days produced a significant increase in the level of

5-hydroxytryptamine, but no change in the amount of acetylcholine in the same brain.

With the various depressants investigated, elevation of the level of acetylcholine in the brain was closely correlated with the appearance of depression and, indeed, with the extent of this depression. With hydroxydione, in which the rise in acetylcholine level was observed during the anaesthetic state, the acetylcholine level fell again within 1 min as the animal regained consciousness. Another example was observed in the cold-stressed animals which received reserpine. They were not depressed by reserpine, and showed no depletion of brain 5-hydroxytryptamine and no change in the amount of acetylcholine in the brain. According to Malhotra & Pundlik (1959) there is in fact a remarkable agreement between the electroencephalographic changes caused by reserpine, in the dog, and the level of acetylcholine in different areas of the brain.

After atropine, Wikler (1952) found a behavioural alerting in the presence of a sleep-like electroencephalograph pattern. Our finding of a fall in brain acetylcholine after atropine, therefore, would correlate with the behavioural effect (alerting with eventual convulsions) rather than with the electroencephalograph pattern. The sleep-like electroencephalograph, on the other hand, would accord with the potentiation by atropine of the sedation and of the elevation of brain acetylcholine produced by pentobarbitone.

The failure to find any changes in the level of acetylcholine in the brain after hemicholinium may be related to the inability of this compound to alter acetylcholine synthesis in the brain appreciably in the time allowed in the present experiments. Very recently, Metz (1962) has shown that hemicholinium produces only a gradual decline in the total acetylcholine content in the pons and medulla of the dog, which becomes most marked at 140 min. It is possible that the manifestations of toxicity seen in our experiments with this substance were due largely to effects upon acetylcholine synthesis at peripheral sites.

This work was aided by grant B-940 from the National Institute of Neurological Diseases and Blindness, U.S. Public Health Service.

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