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Original Investigations

Psychotropic Drug Influences on Brain Acetylcholine Utilization*

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Abstract. The cholinergic antisynthesis agent HC-3 was given intraventricularly to young male rats 20—30 days old to deplete brain acetylcholine (ACh). The rate of HC-3 induced depletion of ACh was used as an index of ACh utilization. Total brain ACh was determined following various doses of chlordiazepoxide, pentobarbital, chlorpromazine, methotrimeprazine, imipramine, morphine, *d*-amphetamine, scopolamine, LSD-25, and phencyclidine given i.p. alone and after intraventricular administration of HC-3. It was found that psychotropic drugs have marked differential effects on the rate of HC-3 induced ACh depletion.

Key words: Acetylcholine — *d*-Amphetamine — Chlordiazepoxide — Chlorpromazine — Imipramine — LSD-25 — Methotrimeprazine — Morphine — Phencyclidine — Psychotropic Drugs — Scopolamine.

Introduction

It is well known that steady state levels of substances in living tissues may not reflect their functional turnover. In autonomic ganglia the preganglionic nerve endings can synthesize acetylcholine (ACh) on demand so that total levels remain the same even during high frequency stimulation (MacIntosh, 1963). However, in the central nervous system synthesis of ACh cannot keep up with excessive demand. For example, the lowest levels of brain ACh exist during convulsions and the highest during anesthesia (Richter and Crossland, 1949; Crossland and Merrick, 1952). Both MacIntosh (1963) and Sattin (1966) demonstrated that neuronal ACh exists in several pools in bound and free forms. The bound forms have been called stabile and labile. Crossland and Slater (1968) studied the effects of various psychotropic drugs on "bound" and "free" brain ACh. They have shown some remarkable changes in the bound to free ACh ratio with different drugs, especially morphine.

Another important measure is brain ACh turnover. The turnover of any compound can be determined only if certain criteria are fulfilled (see

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Neff *et al.*, 1969). One technique is to use a drug to stop synthesis of the biogenic compound of interest. This effect must be immediate to obtain meaningful quantitative data. The best known cholinergic antisynthesis agent is hemicholinium-3 (HC-3). Inasmuch as the mechanism of action of HC-3 is still debated, and it certainly does not act instantaneously, it cannot be used in measuring true brain ACh turnover. However, it can be used as a tool to measure relative rates of ACh depletion. This manuscript describes some data obtained on brain ACh depletion following intraventricular (i. vent.) HC-3 given to rats treated with various psychotropic drugs.

Methods

Young albino male Holtzman rats from 20—30 days of age were used. The animals were on a 12:00 P.M. to 7:00 A.M. dark and 7:00 A.M. to 12:00 P.M. light cycle. HC-3 bromide was given i. vent. using diethyl ether-air anesthesia. Psychotropic drugs were given i. p. immediately after the injection of HC-3 i. vent. and termination of anesthesia. Animals were given various doses of HC-3 and equimolar NaBr and sacrificed by guillotine. The brain minus the cerebellum was removed and bioassayed for ACh using a modification (Dren and Domino, 1968) of the method of Stone (1955). All drug dosage was calculated as base.

Results

A dose of 20 μg total of HC-3 produced about a 55% depletion of brain ACh one-half hour after i. vent. injection with a relatively low mortality. Five minutes after this dose of HC-3 brain ACh dropped rapidly from a control post-ether mean ± S.E. of 18.9 ± 0.5 to 13.8 ± 0.3 nmol/g for a depletion rate of 5.1/5 min or 1.2 nmol/g/min. A second slower rate of ACh depletion occurred during the subsequent 25 min, as the ACh level dropped from 13.8 ± 0.3 to 10.1 ± 0.1 for a depletion rate of 3.7/25 or 0.15 nmol/g/min. The time course of brain ACh depletion following 20 μg i. vent. HC-3 is illustrated in Fig. 1. These data are in agreement with those of Hebb *et al.* (1964) for intracaudate and Slater (1968) for i. vent. injections of this drug.

Groups of eight young rats were given i.p. a psychotropic drug alone or the same drug plus 20 μg of i.vent. HC-3, and sacrificed 30 min later each morning between 8:30 and 9:30 A.M. and brain ACh measured that day. Thirty minutes after i.vent. HC-3 was chosen for sacrifice because the fall in brain ACh is beginning to plateau (see Fig. 1). The drugs included chlordiazepoxide, 10 and 50 mg/kg; pentobarbital, 10 and 50 mg/kg; chlorpromazine, methotrimeprazine, imipramine, morphine and phencyclidine, 10 mg/kg; *d*-amphetamine and scopolamine, 2 mg/kg; and

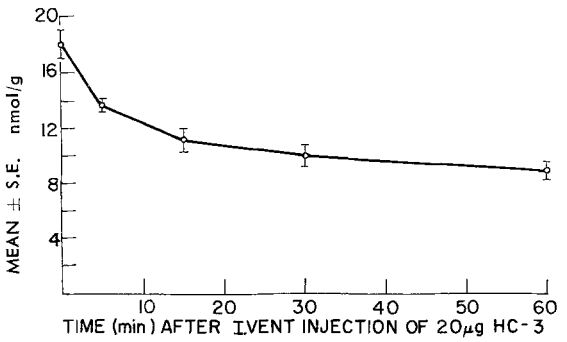

Fig. 1. Time course of brain acetylcholine depletion following intraventricular hemicholinium-3 in young rats. A dose of 20 μg i.vent. HC-3 was given to 20—30 day old rats and brain ACh measured at different times. At least 8 animals are in each group

LSD-25, 0.2 mg/kg. The data obtained are plotted as a bar graph in Fig. 2 and are listed in Table 1. The open vertical bars represent the mean ± S.E. brain level of ACh in control saline injected animals, and the slanted vertical bars that after HC-3 for the psychotropic drug listed. Thus, the open vertical bars should be compared to the upper horizontal control bar for the effects of the drug on steady state brain ACh, and the slanted vertical bars should be compared to the lower horizontal bar for the drug effect on HC-3 induced ACh depletion. Group comparison student "*t*" tests were determined. The asterisks indicate the significance probabilities. A series of control animals were run including no injection, post diethyl ether, i.p. saline and post diethyl ether, and equimolar NaBr. The data are given in Table 2. No significant differences in brain ACh were observed. Because of this, and the fact that it was technically impossible to run daily controls with each drug treatment on any particular day, separate experiments were performed. Control assays were randomly run to insure experimental validity.

It can be noted that large doses of chlordiazepoxide increased brain ACh but caused only a small antagonism of HC-3 induced ACh depletion at 50 mg/kg, i.p. ($P < 0.05$). Employed in sedative doses, pentobarbital caused a slight ($P < 0.05$) increase in brain ACh and no change in HC-3 induced depletion; when given in anesthetic doses, it produced a marked increase in brain ACh alone and after HC-3 ($P < 0.01$). In contrast, chlorpromazine and methotrimeprazine in large behaviorally effective doses of 10 mg/kg produced no significant effect, while the same dose of morphine produced a dramatic antagonistic effect against HC-3 induced depletion ($P < 0.001$). Imipramine, *d*-amphetamine and scopolamine reduced steady state brain ACh and further enhanced its depletion

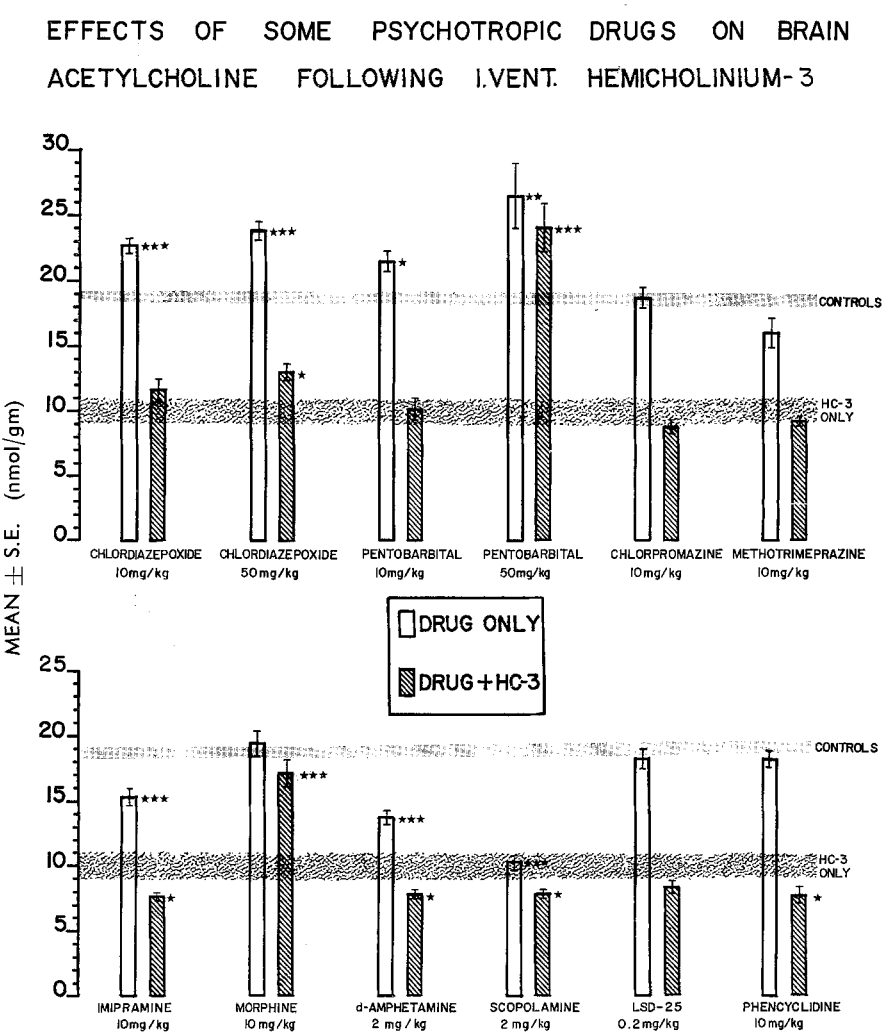

Fig. 2. Effects of some psychotropic drugs on brain acetylcholine following intraventricular hemicholinium-3 in young rats. The mean ± S.E. levels of brain ACh of normal rats given saline is illustrated by the upper horizontal bar and that after 20 μg i.vent. HC-3 by the lower horizontal bar. The open vertical bars represent the mean ± S.E. brain ACh $^1/_2$ h after the drug alone given i.p. and the slanted vertical bars represent that after the drug and HC-3 as noted. All data are expressed as mean ± S.E. brain ACh in nmol/g. P values are: * < 0.05, ** < 0.01, *** < 0.001 student "t" test group comparison. At least 8 animals are in each group

Table 1
Effects of some psychotropic drugs on steady state and HC-3 depleted brain acetylcholine

Drug	Dose mg/kg	Drug alone			Drug plus 20 μg HC-3		
		N	Brain ACh m ± S.E.	*P* value	*N*	Brain ACh m ± S.E.	*P* value
0.9% Saline	9	9	18.7 ± 0.4	—	9	10.0 ± 1.0	—
Chlordiazepoxide	10	16	22.3 ± 0.6	< 0.001	8	11.6 ± 0.8	N.S.
	50	8	23.8 ± 0.8	< 0.001	8	13.1 ± 0.7	< 0.05
Pentobarbital	10	8	21.4 ± 0.9	< 0.05	7	10.8 ± 0.9	N.S.
	50	9	26.6 ± 2.5	< 0.01	8	24.1 ± 1.8	< 0.001
Chlorpromazine	10	8	18.6 ± 0.8	N.S.	8	8.9 ± 0.5	N.S.
Methotrimeprazine	10	9	16.0 ± 1.1	N.S.	6	9.3 ± 0.8	N.S.
Imipramine	10	10	15.3 ± 0.6	< 0.001	8	7.6 ± 0.1	< 0.05
Morphine	10	8	19.4 ± 0.9	N.S.	12	17.2 ± 1.0	< 0.001
d-Amphetamine	2	8	13.8 ± 0.5	< 0.001	11	7.9 ± 0.3	< 0.05
Scopolamine	2	8	10.4 ± 0.4	< 0.001	11	7.8 ± 0.2	< 0.05
LSD-25	0.2	8	18.2 ± 0.8	N.S.	9	8.4 ± 0.4	N.S.
Phencyclidine	10	8	18.1 ± 0.5	N.S.	8	7.7 ± 0.4	< 0.05

N.S. — Not significant student "*t*" test group comparison to control saline vs drug alone and HC-3 alone vs drug plus HC-3. The psychotropic drugs were given i.p. and HC-3 i.vent. and the animals sacrificed $^1/_2$ h later.

Table 2. *Lack of effect of various control procedures on rat brain acetylcholine*

Treatment	Number	Mean ± S.E. nmol/g	Significance
Control No injections	8	18.1 ± 1.3	
Post diethyl ether	8	18.9 ± 0.5	N.S.
I.P. saline Post diethyl ether	9	18.7 ± 0.4	N.S.
I. vent. NaBr	8	17.4 ± 0.9	N.S.

N.S. — Not significant student "*t*" test group comparison to control of no injections.

following HC-3. LSD-25 in a dose of 0.2 mg/kg had no effect on brain ACh, while phencyclidine in a dose of 10 mg/kg increased HC-3 induced depletion.

These results in young rats (known to have a relatively poor blood-brain barrier and liver drug metabolizing enzymes) are to be contrasted with data obtained in adult rats (90 days and older) where chlordiazepoxide in a dose of 10 mg/kg produced no change in brain ACh, and following 50 mg/kg caused an increase to only 21.6 ± 0.9 nmol/g. Somewhat

similar data have been obtained with morphine. These effects of morphine are prevented by narcotic antagonists and are reduced in tolerant animals (Wilson and Domino, 1970).

Discussion

In any drug study involving intact animals at room temperature, one wonders what the influence of body temperature is on the phenomenon being investigated. It is well known that HC-3 causes a decrease in body temperature which can be influenced by psychoactive drugs including *d*-amphetamine, imipramine and desmethylimipramine (Slater, 1969). While we did not systematically measure rectal temperature under these drug conditions, it appears unlikely that a change in body temperature can account for the present findings. The fall in brain ACh induced by i.vent. HC-3 is not prevented by maintaining the body temperature of rats in a heated chamber (unpublished observations, Domino, 1972). Drugs such as *d*-amphetamine, scopolamine and small doses of morphine and LSD-25 are known to increase body temperature, yet they either had no effect or decreased brain ACh. Sedatives and anesthetics generally decrease body temperature, but increased brain ACh. Furthermore, Slater (1969) showed that at 30 min after HC-3 and *d*-amphetamine there was no significant elevation of body temperature. The increase occured about 1—2 h later. In addition, scopolamine in a rather large dose did not alter the body temperature decrease induced by HC-3. Although alterations in body temperature probably do not account for the changes observed, further research along these lines is clearly indicated. Because the psychotropic drugs were administered simultaneously with HC-3, an effect on the transport and disposition of HC-3 cannot be excluded. Further studies of this must also be undertaken.

With the information available to date, the most likely interpretation of the finding that various psychotropic drugs affect the rate of brain ACh depletion induced by HC-3 is that they alter ACh turnover as a result of their primary pharmacologic actions. The result that anesthetizing doses of pentobarbital completely prevented the HC-3 depleting effect is consistent with the findings of Schuberth *et al.* (1969). Morphine is especially effective in preventing brain ACh depletion in contrast to chlorpromazine. On the other hand, imipramine, *d*-amphetamine, scopolamine, and phencyclidine appear to enhance brain ACh depletion and therefore probably enhance its turnover. Of course, other explanations for these interactions with HC-3 are possible. Especially important is the fact that only morphine had a marked antihemicholinium action in doses that were only mildly sedative in contrast to the other central nervous system depressants studied. The most reasonable explanation is that this narcotic agonist reduced brain ACh turnover, a concept consistent with

its known ACh antirelease effects in guinea pig ileum (Paton, 1957) and brain (Beleslin and Polak, 1965).

Our data also indicate that, as expected, one cannot use the rate of depletion of brain ACh following i.vent. HC-3 as a quantitative measure of ACh turnover. The values we calculated of 1.2 and 0.15 nmol/g/min are much lower than the values for brain ACh turnover of 38 nmol/g/min obtained by Richter and Crossland (1949) using electroshock to rapidly deplete brain ACh in the rat, and of 50 nmol/g/min obtained by Schuberth *et al.* (1969) using labelled choline in the mouse. The latter investigators found that anesthesia dramatically reduced brain ACh turnover to 10 nmol/g/min. Although HC-3 fails to meet the criterion of immediate cessation of synthesis to be of value in quantitative studies of ACh turnover, it is a useful tool to study the effects of centrally acting drugs on ACh utilization.

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