Psychopharmacologia (Berl.) 25, 291–298 (1972). LSD 2321

Original Investigations

Psychotropic Drug Influences on Brain Acetylcholine Utilization*

Edward F. Domino and Ann E. Wilson

Michigan Neuropsychopharmacology Research Program,
Department of Pharmacology
University of Michigan, Ann Arbor, and Lafayette Clinic, Detroit, U.S.A.

Received October 6, 1971; Final Version February 14, 1972

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

* Supported in part by grant MH-11846, USPHS.

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

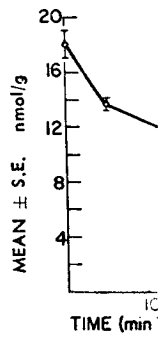


Fig. 1. Time course of brain a
cholinium-3 in young rats. A
rats and brain ACh measure

LSD-25, 0.2 mg/kg. The
and are listed in Table
± S.E. brain level of A
slanted vertical bars th
Thus, the open vertical
control bar for the effect
slanted vertical bars sh
the drug effect on HC
student "*t*" tests were d
probabilities. A series of
post diethyl ether, i.p. sa
The data are given in T
were observed. Because
impossible to run daily
cular day, separate exp
randomly run to insure.

It can be noted that
ACh but caused only a s
at 50 mg/kg, i.p. ($P <$
caused a slight ($P < 0.0$
induced depletion; whe
increase in brain ACh
chlorpromazine and m
doses of 10 mg/kg prod
morphine produced a d
depletion ($P < 0.001$).
reduced steady state

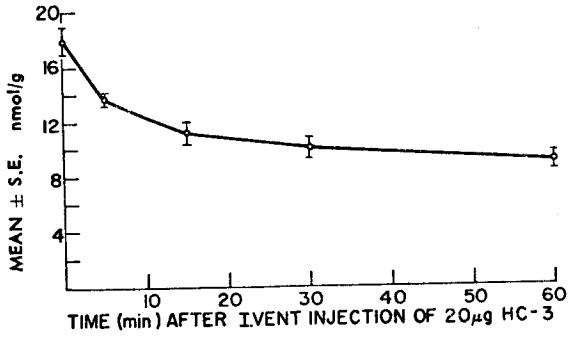


Fig. 1. Time course of brain acetylcholine depletion following intraventricular hemicholinium-3 in young rats. A dose of 20 μg i.vent. HC-3 was given to 20—30 day old rats and brain ACh measured at different times. At least 8 animals are in each group

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

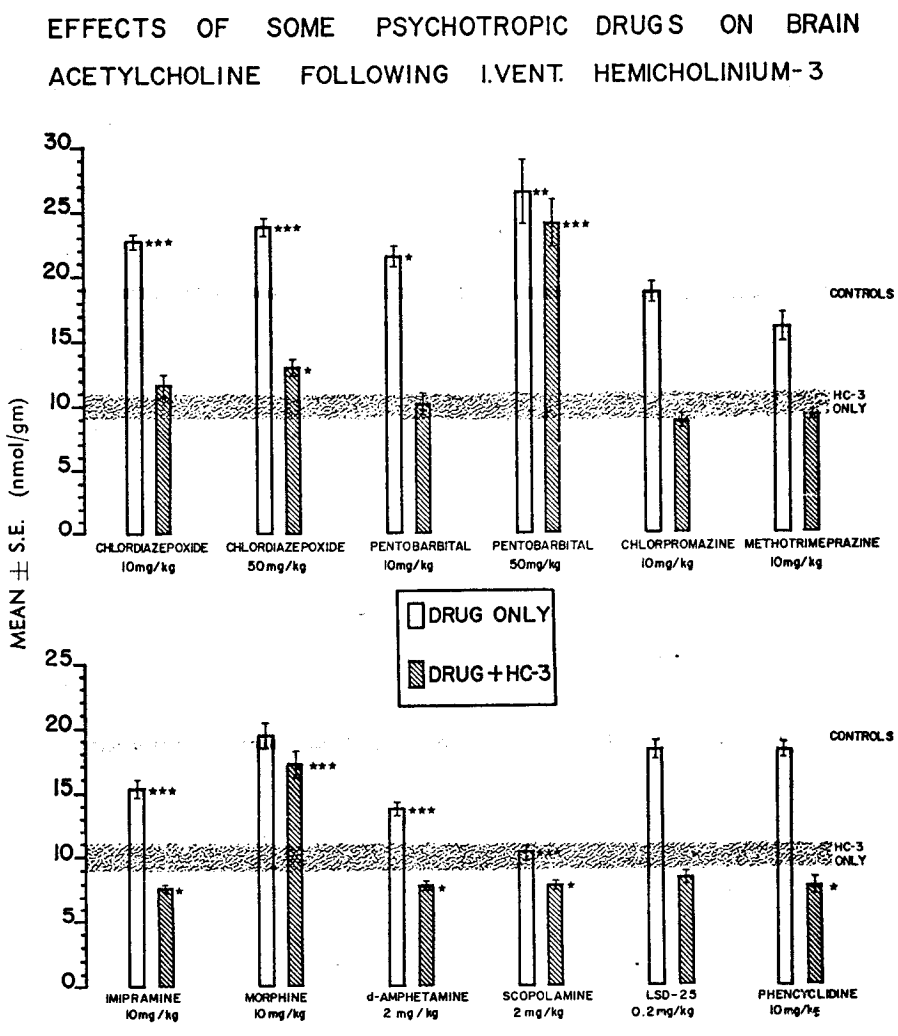


Fig. 2. Effects of some psychotropic drugs on brain acetylcholine following intraventricular hemicholinium-3 in young rats. The mean ± S.E. levels of brain ACh of normal rats given saline is illustrated by the upper horizontal bar and that after 20 μg i.vent. HC-3 by the lower horizontal bar. The open 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

Effects of some psychotropic d

Drug	Dose mg/kg
0.9% Saline	9
Chlordiazepoxide	10
	50
Pentobarbital	10
	50
Chlorpromazine	10
Methotrimeprazine	10
Imipramine	10
Morphine	10
d-Amphetamine	2
Scopolamine	2
LSD-25	0.2
Phencyclidine	10

N.S. — Not significant
drug alone and HC-3 alone
i.p. and HC-3 i.vent. and th

Table 2. *Lack of effect o*

Treatment
Control
No injections
Post diethyl ether
I.P. saline
Post diethyl ether
I. vent.
NaBr

N.S. — Not significan
injections.

following HC-3. LSD-2
ACh, while phencyclidi
depletion.

These results in you
brain barrier and liver
with data obtained in a
oxide in a dose of 10 mg
ing 50 mg/kg caused a

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*

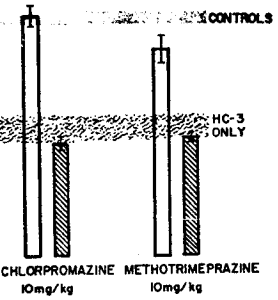
Treatment	Number	Mean ± S.E. nmol/g	Significance
Control No injections	8	18.1 ± 1.3	
Post diethyl ether I.P. saline	8	18.9 ± 0.5	N.S.
	9	18.7 ± 0.4	N.S.
Post diethyl ether I. vent. NaBr	8	17.4 ± 0.9	N.S.

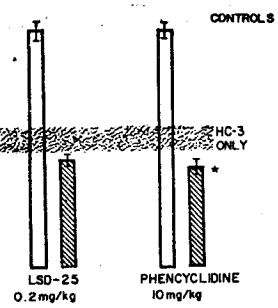
N.S. — Not significant student "*t*" test group comparison to control of no injections.

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

RUGS ON BRAIN
EMICHOLINIUM-3





ylcholine following intra-
S.E. levels of brain ACh of
zontal bar and that after
vertical bars represent the
p. and the slanted vertical
data are expressed as mean
, ** < 0.01, *** < 0.001
als are in each group

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 antirelea
brain (Beleslin and Polak

Our data also indicat
depletion of brain ACh fo
of ACh turnover. The va
are much lower than the
obtained by Richter and
deplete brain ACh in the
et al. (1969) using labelle
found that anesthesia
10 nmol/g/min. Although
cessation of synthesis to
over, it is a useful tool t
ACh utilization.

Beleslin, D., Polak, R. L.: D
release from the cat's bra
Crossland, J., Merrick, A. J.:
of brain. J. Physiol. (Lon
— Slater, P.: The effect of
content of rat brain. Brit
Dren, A. T., Domino, E. F.:
and brain acetylcholine
(1968).
Hebb, C. O., Ling, G. M., M
locally applied hemichol
nucleus. Nature (Lond.)
Mac Intosh, F. C.: Synthesis
J. Biochem. 41, 2555—2
Neff, N. H., Lin, R. C., Ngai,
serotonin in unanesthetiz
cology, pp. 92—109. E.
1969.
Paton, W. D. M.: The action
on acetylcholine output
macol. 11, 119—127 (19
Richter, D., Crossland, J.:
physiological states. Am
Sattin, A.: The synthesis an
13, 515—524 (1966).
Schuberth, J., Sparf, B., Su
turnover in mouse brain
Slater, P.: The effects of tr
content of rat brain. Int
— Hypothermia following
Brit. J. Pharmacol. 36,

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.

References

Beleslin, D., Polak, R. L.: Depression by morphine and chloralose of acetylcholine release from the cat's brain. J. Physiol. (Lond.) **177**, 411—419 (1965).

Crossland, J., Merrick, A. J.: The effect of anesthesia on the acetylcholine content of brain. J. Physiol. (Lond.) **125**, 56—66 (1954).

— Slater, P.: The effect of some drugs on the "free", and "bound" acetylcholine content of rat brain. Brit. J. Pharmacol. **33**, 42—47 (1968).

Dren, A. T., Domino, E. F.: Effects of hemicholinium (HC-3) on EEG activation and brain acetylcholine in the dog. J. Pharmacol. exp. Ther. **161**, 141—154 (1968).

Hebb, C. O., Ling, G. M., McGreer, E. G., McGeer, P. L., Perkins, D.: Effect of locally applied hemicholinium on the acetylcholine content of the caudate nucleus. Nature (Lond.) **204**, 1309—1311 (1964).

Mac Intosh, F. C.: Synthesis and storage of acetylcholine in nervous tissue. Canad. J. Biochem. **41**, 2555—2571 (1963).

Neff, N. H., Lin, R. C., Ngai, S. H., Costa, E.: Turnover rate measurements of brain serotonin in unanesthetized rats. In: Advances in Biochemical Psychopharmacology, pp. 92—109. E. Costa and P. Grungard, eds. New York: Raven Press 1969.

Paton, W. D. M.: The action of morphine and related substances on contraction and on acetylcholine output of coaxially stimulated guinea-pig ileum. Brit. J. Pharmacol. **11**, 119—127 (1957).

Richter, D., Crossland, J.: Variation in acetylcholine content of the brain with physiological states. Amer. J. Physiol. **159**, 247—255 (1949).

Sattin, A.: The synthesis and storage of acetylcholine in the striatum. J. Neurochem. **13**, 515—524 (1966).

Schuberth, J., Sparf, B., Sundwall, A.: A technique for the study of acetylcholine turnover in mouse brain *in vivo*. J. Neurochem. **16**, 695—700 (1969).

Slater, P.: The effects of triethylcholine and hemicholinium on the acetylcholine content of rat brain. Int. J. Neuropharmacol. **7**, 421—427 (1968).

— Hypothermia following intraventricular injection of hemicholinium-3 in rats. Brit. J. Pharmacol. **36**, 46—52 (1969).

Stone, W. E.: Acetylcholine in the brain. I. "Free," "bound," and total acetylcholine. Arch. Biochem. **59**, 181—192 (1955).

Wilson, A. E., Domino, E. F.: Inhibitory effects of morphine on brain acetylcholine depletion following intraventricular hemicholinium. Pharmacologist **12**, 294 (1970).

Dr. Edward F. Domino
Department of Pharmacology
University of Michigan
School of Medicine
Ann Arbor, Michigan 48104, U.S.A.

Psychopharmacologia (Ber

Alterations by Cen
of Self-Sti
by Tetrabenazi

Division of P
Pa

Received Novem

Abstract. The effects of
scopolamine (0.5 mg/kg), a
pression of self-stimulation
The antagonism of each co
of tetrabenazine (2 mg/kg).
and pentobarbital sodium
Against the suppressio
partial protection through
transient delaying action.
tion was given by *d*-amphe
Against physostigmine.
protection, and chlordiazep
Against the effect of pe
protection by *d*-amphetam
deficits, and a stimulant act
were still depressed. Chlord
These results are interp
pressed rates of respondin
produced by the compound
mitter-like substances.

Key words: Self-Stimula
mine — Pentobarbital — *d*

Compounds which l
stimulation behavior (
Cooper, 1970), and oth
increase SS (Stein, 1964
catecholamines, in part

* Supported by grant M