Ann. Rev. Pharmacol. Toxicol. 1977. 17:369–86

PHARMACOLOGICAL IMPLICATIONS OF BRAIN ACETYLCHOLINE TURNOVER MEASUREMENTS IN RAT BRAIN NUCLEI

❖6686

D. L. Cheney and E. Costa
Laboratory of Preclinical Pharmacology, National Institute of Mental Health, Saint Elizabeths Hospital, Washington DC 20032

INTRODUCTION

The action of drugs on cholinergic mechanisms can be evaluated electrophysiologically (1–3) or biochemically (4–7). In brain, there are a few cholinergic pathways amenable to an electrophysiological investigation. Among them are the septal-hippocampal (8) and the habenulo-interpeduncular pathways (9). However, most of the important cholinergic mechanisms of brain elude this method of study. For instance, it is not possible to study electrophysiologically the intrinsic cholinergic systems of striatum and *N. accumbens.* Striatum and *N. accumbens* are the subject of greater interest in psychopharmacology because it is currently believed that by understanding the way drugs act on these intrinsic cholinergic systems we may learn how to minimize the extrapyramidal side effects of antipsychotics (10). Thus, with the present technical development it appears possible that progress in this area will stem from biochemical studies rather than from electrophysiological research.

The action of drugs on cholinergic systems in the brain can be inferred by measuring the acetylcholine (ACh) turnover rate in vivo (4, 5), or the V_{max} of choline (Ch) uptake (11) or the rate of ACh release using a push-pull cannula (6). The use of ACh turnover rate measurements to estimate the cholinergic neuronal activity stems from the belief that the turnover rate of ACh increases or decreases when the activity of cholinergic neurons is enhanced or depressed, respectively. Historically, this belief developed as a result of the demonstration that in cat

superior cervical ganglion the turnover rate of ACh at rest never exceeded 20% of the maximum rate that could be attained during electrical stimulation of preganglionic nerves (12). Since the ACh stored in cat sympathetic ganglia is located in terminal axons, the ganglion was a good model to correlate the neuronal activity with the ACh turnover rate. Unfortunately the whole brain cannot be used as a model for this correlation because of the intricate network of cholinergic neurons.

It is highly unlikely that drugs would simultaneously change the activity of every cholinergic neuron in brain in the same direction unless they act directly on Ch acetyltransferase (CAT) or acetylcholinesterase. Hence, the nonuniformity and intricacy of the brain cholinergic network make measurements of ACh turnover in whole brain devoid of any significance in the study of the action of analgesics, neuroleptics, hypnotics, etc. In contrast, measurements of ACh turnover rates in specific brain nuclei containing small, intrinsic cholinergic neurons or important cholinergic projections are of value in understanding how drugs change the cholinergic function at the level of specific synapses. Furthermore, when such studies are carried out in several nuclei of the brain simultaneously one might be able to elucidate whether a drug given chronically or acutely acts primarily on cholinergic mechanisms or whether the involvement of cholinergic neurons is secondary to a drug-induced modification of the synaptic input mediated by other transmitters, i.e. serotonin, dopamine, γ-aminobutyric acid (GABA), substance P, etc. These results allow drug actions to be described in terms of their effects on the integration of different synaptic mechanisms. Thus a given drug effect may be expressed in terms of the changes in the biochemical correlate of various transmitter systems in selected brain nuclei. Only recently have suitable methods become available to measure ACh content (13) and its turnover rate in brain parts or nuclei (14, 15) in which the ACh and Ch content have been stabilized by killing the rats with microwave radiation focused on the skull (16). This procedure kills the animals instantaneously and rapidly inactivates the enzymes responsible for the synthesis and degradation of ACh and its precursor, Ch (16). Moreover, the use of microwave radiation has imposed a reevaluation of older reports on drug effects on ACh and Ch content of brain nuclei. This review attempts to bring into focus the new concepts that are emerging from currently ongoing experiments to study the molecular dynamics of cholinergic transmission.

METHODS TO MEASURE ACh TURNOVER RATE

Over the past several years the methods proposed to study the regulation of cholinergic transmission in vivo have required the labeling of the ACh and Ch stored in neurons by injecting labeled precursors of ACh and by measuring simultaneously the changes with time in the specific activities of Ch and ACh (4, 5, 14, 15, 17–22). In addition to these isotopic methods, various nonisotopic approaches have been suggested to estimate the fractional rate constant of ACh efflux (6, 23–29). Some of the nonisotopic methods used drugs to instantaneously inhibit synthesis, metabolism, or transport of ACh, its precursor, or metabolites (30). A flaw common to all the methods involving the use of inhibitors derives from the widely spread assump-

tion that drugs are so specific that even in high doses they selectively inhibit a given metabolic step. Obviously such specificity is exceptional; more often drugs that inhibit ACh metabolism exert collateral actions on other processes which directly or indirectly regulate a number of other metabolic steps. The isotopic methods which use tracer doses of a precursor of ACh are devoid of such an assumption but may be plagued by other conceptual flaws. Therefore, they are not devoid of problems; in fact they include a number of other tacit assumptions concerning the dynamics and compartmentation of ACh and its precursors which are open to critique. The assumption implicit in all of these techniques, whether isotopic or nonisotopic, is that at steady state the newly taken up pool of Ch is in rapid equilibrium with the pool of Ch that is the precursor of ACh. Moreover, it is assumed that the rates of synthesis and degradation of ACh are in equilibrium, and therefore principles of steady state kinetics can be used to calculate ACh turnover rate.

Nonisotopic Methods

RELEASE RATES OF ACh MacIntosh (12) reported that in the absence of changes in tissue concentrations of ACh and after inhibition of ACh metabolism, the efflux of ACh from a perfused superior cervical ganglion of cats would describe the rate of ACh renovation in the ganglion. He further suggested that this same approach might be valid in brain (see 31). A rate constant for ACh release from brain slices was determined by measuring the release of ACh elicited by electrical stimulation in the presence or absence of eserine and/or atropine (23). However, in view of the rapid postmortem increase in tissue Ch concentrations (16, 32) and considering that an increase in Ch content (33, 34) increases ACh synthesis because CAT is not saturated, the heuristic value and physiological significance of methods which measure in vitro the ACh turnover rate by measuring release rates of ACh are open to question. A more reliable estimation of the dynamic state of ACh stores can be obtained by in vivo perfusion of limited areas of exposed cerebral cortex (24–26), cerebellum (27), striatum (28), and hippocampus (29) of rabbits, cats, and rats using specifically constructed cups perfused with Ringer's solution. However, with this perfusion method the size of the tissue being perfused is unknown. Hence, it is impossible to calculate turnover rate because the number of neurons contributing to the ACh efflux may change during the experiment and because it is impossible to prove that steady state is maintained during perfusion when drugs are administered. The use of the push-pull cannulae (6), which limit perfusion to small areas, has been preferred, but even in this case it is practically impossible to estimate the turnover rate of the ACh stores being studied. However, with the push-pull cannula, neurotransmitter efflux from various brain regions of the awake, gallamine-immobilized, or unrestrained cat can be measured [see references in (6)]. The effluxes can be compared in the presence or absence of various drugs, which, if needed, can even be added to the perfusion fluid. However, intervening edema or other dynamic problems may change the size of the brain tissue from which the efflux is being collected.

Since the amount of tissue perfused cannot be quantitated with any degree of accuracy, the ACh dynamics can be expressed neither in terms of fractional rate constant nor as a turnover rate. Moreover, it is almost impossible to assess whether during a push-pull cannula operation the ACh store in the tissue perfused is maintained at steady state.

As an example of the problems involved in the push-pull cannula experiments, we recall that atropine increases the rates of ACh efflux from the cerebral cortex when given either systemically (12, 26, 35–39) or topically (35, 37–41). It has, therefore, been concluded that atropine increases ACh turnover rate. However a number of laboratories have reported that atropine also lowers ACh content in brain structures (42–45). Thus the increased rate of ACh release following atropine reflects the decreased steady state content while synthesis is unchanged. Although push-pull cannula experiments give useful indications, one must be prepared to deal with the problem that drugs may increase efflux rates as they are compensating for decrease in steady state; thereby the synthesis rate of ACh is constant (45).

INHIBITION OF ACh SYNTHESIS In brain the fractional rate constant of a transmitter efflux can be measured from the slope of the decline of the transmitter content elicited by complete and instantaneous blockade of its synthesis (30). By definition, this rate constant multiplied by the ACh content at steady state yields the turnover rate. Hemicholinium C-3 (HC-3), which is believed to block ACh synthesis (46), has been injected intraventricularly to measure the turnover rate of brain ACh (47). However, it appears that the main action of HC-3 is not a blockade of synthesis but a competitive inhibition of Ch uptake (46, 49). In addition, HC-3 may inhibit ACh synthesis by competing with Ch for acetylation (49), by blocking Ch kinase (50), or by diverting Ch to phospholipid formation (51). Since HC-3 inhibits Ch uptake and does not inhibit CAT completely and instantaneously, the essential requirements of the postulate for the use of HC-3 in the measurement of ACh turnover are not fulfilled. In line with this conclusion is the observation that the decline of ACh after HC-3 injection is not exponential but reaches a plateau after partial depletion at 30 min postinjection.

Calculations of ACh turnover rate from the ACh decline elicited by HC-3 thus far have been made from a single time point taken at a fixed interval after HC-3 injection (47) without assessing whether or not the ACh declines at maximal rates and follows first order kinetics. Thus what has been published on the ACh turnover using HC-3 is not adequate and the method does not have great future potential.

POSTMORTEM CHANGES IN ACh CONTENT Some investigators have proposed that in rats killed by decapitation drugs may change brain ACh content when they have changed the ACh turnover rate before death (52, 53). Thus, an increase in striatal ACh content in rats which were decapitated at a certain time after the injection of dopamine receptor agonists (1, 54–58) has been interpreted to indicate a decrease in the turnover rate of striatal ACh. On the other hand, it has been reported that a decrease of striatal ACh content following the injection of dopamine receptor antagonists (1, 54, 56, 58) or after cerebral hemisection (1) indicates an

increase in ACh turnover rate. When the rats are killed by microwave irradiation which stabilizes Ch content, the injection of dopamine receptor agonists or antagonists fails to change striatal ACh content (10, 59).

When the enzymes involved in ACh metabolism are not inactivated instantaneously and irreversibly, the ACh content of brain structures may change considerably because of the rapid postmortem metabolism of ACh and the increase of brain Ch content (16). Focused microwave irradiation (16) prevents the postmortem accumulation of free Ch in brain and blocks ACh destruction. Since the stability of ACh levels depends on the method used to kill the rats, predictions made concerning the turnover rate of ACh based on ACh content may or may not be correct. A decrease of striatal ACh content occurs after injection of ACh receptor blockers although they do not decrease ACh turnover rate (10, 59).

CHANGES IN V_{MAX} OF SODIUM-DEPENDENT HIGH AFFINITY Ch UPTAKE Various lines of evidence suggest that sodium-dependent high affinity Ch uptake may be a rate-limiting step in the synthesis of ACh (60–62). This uptake (60, 61, 63, 64) appears to play an important role in the functioning of cholinergic terminals where it is located (65, 66), shows a specificity for a free hydroxyl group and a quaternary nitrogen (67), and does not transport ACh (68–70). Since it is functionally coupled to CAT (62) and to a releasable pool of ACh (71) the Ch uptake is an integral part of the regulation of ACh turnover (72). Hence, changes in the V_{max} of the high affinity Ch uptake measured in vitro after drug treatment may indicate changes in the ACh synthesis which have occurred in vivo following drug treatment (73, 74).

Unfortunately this method does not give a quantitative measure of synthesis rate. It only indicates the direction of the change. At least theoretically it appears possible to decrease or increase the availability of Ch without changing the V_{max} of Ch uptake by changing the affinity of the uptake for Ch. This method is rapid and simple and gives some important information on neuronal activity. Since in these studies fresh tissue must be used, the rapid postmortem increase in tissue Ch content might introduce artifacts and cause problems in interpretation. It must be emphasized that data available on drug effects on Ch uptake (74) parallel the data on the action of drugs on ACh synthesis obtained by other methods (25, 26, 31, 45, 75–80).

Isotopic Methods

GENERAL CONSIDERATIONS The rate of ACh efflux from endogenous stores, including the efflux from neuronal stores, can be measured by labeling the ACh at steady state (17). The rate-limiting step of ACh synthesis is the uptake of Ch (60–62). This Ch in part is transported from plasma and in part is generated from the hydrolysis of ACh. Since this recycling of Ch could cause a reutilization of the label which would interfere with the estimation of ACh turnover based on principles of steady state kinetics, one must minimize the interference created by the recycling of labeled Ch (4, 5).

Calculations of the efflux rates of ACh are made by applying principles of steady state kinetics. Since the labeling procedures do not alter the steady state of the

precursor, we are monitoring the rate of synthesis and degradation of ACh by measuring the change with time of the abundance of label in Ch and ACh. It is imperative in this method that the total amounts of Ch and ACh do not change while the relative abundance of a given isotope in the chemical composition of Ch and ACh changes. Furthermore, this approach requires that the enzymes responsible for the synthesis and degradation of ACh and Ch are instantaneously inactivated at the time of sacrifice, preferably with microwave irradiation. Even the 20 seconds that are required to freeze the brain following the immersion of rats in liquid nitrogen could cause a variability in the measurement of Ch concentrations which would not be tolerable for the measurement of ACh turnover with isotopic methods.

Although these methods eliminate some of the drawbacks associated with the nonisotopic methods, the isotopic methods are based on the following assumptions which are not yet entirely documented by experimental evidence (4, 5, 19, 22):

1. Plasma Ch is assumed to be preferentially transferred to a pool of free brain Ch which in turn equilibrates with the various metabolic pools of brain Ch at a rate proportional to their intrinsic metabolic rates. The pool size of Ch that functions as a precursor for ACh is very small. Since the rate of ACh synthesis is fast, the specific activity of free brain Ch would mirror with a certain accuracy the specific activity of Ch serving as ACh precursor.
2. A simple kinetic model based on a single open metabolic compartment of ACh store is assumed. Some equations are derived from this assumption and used to calculate turnover.
3. With the constant rate of label infusion and the time limits proposed in this model, the feedback due to recycling of labeled Ch formed from the hydrolysis of ACh is insignificant and fails to exceed the noise of the methods available to measure isotopic abundance.
4. The rate of conversion of free brain Ch into brain ACh is assumed to be much more rapid than the turnover rates which describe the other Ch metabolic pathways.

PULSE INJECTION OF ISOTOPIC LABEL This approach utilizes the measurement of the specific radioactivity of ACh and its precursor Ch or the percentage of incorporation of deuterated ACh and Ch at various times after the pulse injection of labeled Ch or a labeled precursor of Ch (17–22). Both radioactive and stable isotopes of Ch have been used to pulse label brain ACh and measure the turnover rate of ACh in whole mouse brain (21, 75), whole rat brain (17), or in large brain regions (78).

The injections of radiolabeled Ch necessary to label the ACh content of small brain areas may change the steady state of Ch; hence phosphorylcholine has been used to bypass this difficulty (4, 5, 14, 19, 22, 76, 81). In fact in plasma and brain there is a large pool of phosphorylcholine (83, 83). Perhaps in brain tissue phosphorylcholine may be even a natural precursor of brain Ch (84). Radioactive phosphorylcholine efficiently labels brain Ch (19).

With pulse injections of labeled phosphorylcholine one can measure the ACh turnover rate in samples of brain tissue weighing about 100–300 mg (22). However, it is impossible to label ACh in brain nuclei with pulse injections of isotopically labeled phosphorylcholine because the amount of ^{14}C or deuterium that can be incorporated into ACh at steady state is too small to be detectable.

CONSTANT RATE INFUSION OF DEUTERIUM OR ^{14}C LABELED PHOSPHORYLCHOLINE Intravenous infusion of radiolabeled Ch or phosphorylcholine at constant rate for several minutes allows for a sufficient labeling of endogenous stores of Ch and ACh, and ACh turnover rate can be measured in various regions of rat brain (4, 5, 20). Although this approach is valid to measure ACh turnover rates in 100–200 mg brain samples, it cannot be used to measure the turnover rate of ACh in brain samples of 0.5–10 mg tissue (34; G. Racagni and D. L. Cheney, unpublished observations). To measure ACh turnover rate in such small tissue samples mass fragmentography and deuterium labeling must be used. A constant rate infusion with deuterated phosphorylcholine can label the Ch and ACh content in tissues weighing about 1 mg without changing the steady state content of either ACh or Ch (14, 15). Considering that a brain nucleus usually contains a few picomoles of ACh, the detection of a labeling of 10% of the molecules of Ch and ACh implies a measurement of the variant atomic species of molecules in the range of 200–300 femtomoles. This range is the lower limit of mass fragmentographic detection of ACh (85).

ACTIONS OF DRUGS ON ACh TURNOVER

Parasympathomimetics

Muscarinic agonists appear to increase ACh content in brain of rats or mice whether killed by microwave irradiation or by decapitation (10, 45, 76, 78, 86, 87). Pilocarpine (86), arecoline (86), and oxotremorine (18, 76) reduce the conversion of labeled precursor into ACh in mouse brain. Since this reduced conversion does not compensate for the increased brain ACh content, the turnover rate of ACh is decreased. It should be noted that the V_{max} of low K_m Ch uptake (74) and the release of ACh are also reduced by oxotremorine (87). The biosynthesis of ACh in cerebellum, medulla oblongata, midbrain, hippocampus, and cerebral cortex (88) is decreased by oxotremorine. Analgesics, dopamine receptor agonists, and GABA receptor agonists reduce the ACh turnover in certain structures, but not in others (see specific references later). In contrast, oxotremorine reduces ACh turnover in every structure tested and by the same extent (78). This finding suggests that Ch uptake is regulated by ACh hydrolysis occurring in the synaptic cleft, which in turn regulates the ACh turnover rate. It can be inferred that when ACh receptors are occupied by the agonist, little or no ACh is released and hydrolized. As a result of some unknown regulatory mechanism when the ACh synthesis is not utilized, the ACh content of the tissue increases and the ACh synthesis is reduced. By analogy with dopamine

neurons this finding suggests that there are presynaptic cholinergic receptors which regulate ACh synthesis. Since this regulatory mechanism is diffuse to every cholinergic synapse, one might infer that these regulatory receptors may be closely associated with Ch uptake located presynaptically.

Acetylcholinesterase inhibitors increase the brain concentration of ACh (10, 45, 76, 89–91) even when the animals are killed by microwave irradiation (10, 75). Physostigmine reduced the Ch uptake in whole mouse brain and in rat hippocampus (74) and decreases the ACh turnover rate in mouse brain (76). Perhaps these acetylcholinesterase inhibitors reduce Ch uptake thereby causing a decrease in ACh synthesis.

The ganglionic stimulant, nicotine (92), increases the ACh release from cerebral cortex while the ganglionic blocker, hexamethonium (93), reduces the release of ACh from neocortex.

Parasympatholytics

Muscarinic receptor blockers increase the ACh release from the cerebral cortex (36, 38, 94–97), caudate (96), and hippocampus (96). These drugs also increase the V_{max} of the high affinity Ch uptake (11), lower the ACh content of striatum (45, 98), and increase the rate of conversion of labeled Ch into labeled ACh (45, 98) in mouse brain. Since this increase in the rate of conversion of labeled Ch to ACh is counterbalanced by the reduction in the size of the ACh pool, no change in the turnover rate of ACh ensues. Other anticholinergics, benztropine, trihexyphenidyl, and clozapine, in high doses decrease ACh striatal concentrations and increase the fractional rate constant for ACh efflux without changing the turnover rate of ACh in rat striatum (10). Some reports have suggested that clozapine in high oral doses reduces ACh turnover rate in cerebral cortex (99).

Inhibitors of Acetylcholine Synthesis

Only a few drugs have been found that will acutely inhibit Ch acetyltransfease in vivo (100). The most specific of these is 4-(1-napthylvinyl) pyridine (100, 101). This drug decreases the ACh content of mice killed by quick freezing in liquid nitrogen (45) but fails to change the brain content of ACh in mice killed by microwave irradiation (102). However, in animals killed with microwave, napthylvinylpyridine depresses the conversion of intravenous labeled Ch into ACh (45, 102). That the brain ACh content is not lowered by an extensive inhibition of CAT suggests that this enzyme is not rate limiting (60, 62, 103) and must be inhibited almost totally before the ACh content is decreased.

Hemicholinium competitively blocks the Ch transport in cholinergic axon terminals, thereby limiting the synthesis of ACh available for release (93, 104). Intraventricular injections of HC-3 deplete brain ACh by 80% (77). This depletion can be prevented by prior administration of Ch (77). While CAT inhibition does not reduce brain ACh, HC-3 injection reduces the ACh content to 20% of the level of nontreated rats and the ACh turnover rate by 70% (24, 31). This finding suggests that Ch plays an important role in the regulation

uptake is reduced (16, 61) the release of ACh from neocortex is also diminished.

Barbiturates and Anesthetics

Pentobarbital inhibits the release of ACh from the cerebral cortex of rabbit (39) and sheep (36) and increases the ACh content of cortex (105), striatum (105), and whole brain of rats (43, 89). In anesthetic doses barbiturates reduce the Ch uptake (74, 105) and decrease ACh turnover rate in mouse brain (18, 105–107). In rats pentobarbital inhibits the rate of brain ACh decline following intraventricular HC-3 (47), reduces the Ch uptake in cerebral cortex (11, 74, 105), hippocampus (11, 94), and hypothalamus (11), but not in striatum (74, 105) or in brain stem (105), and reduces the rate of conversion of labeled Ch into ACh in cerebral cortex and in striatum (105). Thus, pentobarbital does not alter striatal ACh turnover rate while it depresses the cortical and, perhaps, hippocampal turnover rate of ACh. This is a clear indication that barbiturates change ACh turnover by an indirect, perhaps transsynaptic mechanism. Similar inferences can also be made for other general anesthetics, thus raising the possible theoretical suggestion that a mimicry of some endogenous inhibitory transmitters is a possible mechanism of action of some anesthetics.

Ketamine decreases the turnover rate of ACh in caudate and hippocampus without changing that of the cerebral cortex or the thalamic-hypothalamic region (108). Chloralose (31) decreases the release of ACh from neocortex. The local anesthetic, cocaine (94), also decreases the release of ACh from cerebral cortex. The gaseous anesthetics, ether (36), cyclopropane (36), and halothane-N_2O (35) have been reported to inhibit the release of ACh from cerebral cortex in sheep and cat. Halothane also decreases the turnover rate of ACh measured by infusion of isotopic ACh precursors in cerebral cortex, caudate, hippocampus, and thalamic-hypothalamic region (108). In contrast, enflurane decreases the ACh turnover rate only in cerebral cortex (108).

In fact, other nongaseous anesthetics also depress ACh turnover rate in various regions of rat brain. Chlorohydrate and γ-butyrolactone cause a 50–80% reduction in Ch uptake in hippocampus (11), and chlorohydrate reduces the turnover rate of ACh also in the whole brain (109).

Narcotic Analgetics

Narcotic analgetics inhibit the release of ACh from cerebral cortex of cats (24, 31) and mice (110) and from striatum of cats (28). Morphine also inhibits the HC-3–induced decline of rat brain ACh content and the uptake of Ch in rat occipital cortex but fails to change the uptake of Ch in rat striatum (74). Furthermore, an ED_{50} for analgesia of morphine, meperidine, viminol R_2, and azidomorphine decreases the turnover rate of ACh measured by infusion of isotopic precursors in cerebral cortex and hippocampus of rats but not in rat striatum (111) or whole mouse brain (112). In more detailed studies performed in tissue punches from brains of morphine-treated rats, an ED_{80} dose of morphine for analgesia decreased the ACh turnover rate in *N. accumbens,* cerebral cortex, and hippocampus but failed to change that of striatum, septum, dorsal raphe, or locus coeruleus (15). The observation that morphine reduces the ACh turnover rate in the hippocampus but not in

the septum raises an interesting point. The septum contains the cell bodies of the cholinergic neurons that innervate the hippocampus (1). The transsynaptic mechanisms triggered by opiate receptor activation may control the synthesis and degradation of the ACh in terminals located in hippocampus but not that of ACh in cell bodies. If this were the case the metabolism of ACh in terminals would change in parallel with changes in neuronal activity, whereas no changes of ACh turnover would occur with activity changes in cell bodies. A similar conclusion was reached by measuring uptake of Ch: uptake changes with turnover rate of ACh in terminals (70) but not in cell bodies (69).

Psychotomimetics

Tetrahydrocannabinol decreases the release of ACh from cerebral cortex, reduces the Ch uptake in hippocampus but not in cerebral cortex (74), diminishes the HC-3–elicited depletion of ACh in whole brain (113), and reduces the conversion of labeled Ch into ACh in slices of rat striatum, hypothalamus, and cerebral cortex (114). Further studies are needed to assess the reliability of predictions concerning the activity of cholinergic neurons made from measurements of conversion rates of Ch into ACh in slices of brain. Judging from studies on tetrahydrocannabinol, conversion of Ch into ACh in brain slices and uptake studies of Ch yield discrepant results. Since uptake studies have a reasonable level of predictability (see Table 1) one must suspect that as a result of the changes in the sizes of the Ch pool the conversion in vitro fails to yield reliable information concerning drug actions on ACh from turnover.

Ethyl alcohol (115) decreases the release of ACh from neocortex.

Dopamine Receptor Agonists

As we mentioned in our discussion of the effects of anesthetics on ACh turnover, many drugs may alter the ACh turnover rate through an action on synaptic mechanisms which control the activity of the cholinergic neurons. In striatum, there is evidence for an interaction between DA and cholinergic neurons (116, 117). Thus, (+)-amphetamine (25, 118), a DA releaser, and L-DOPA (119), which is a potent dopamine receptor agonist, decrease the turnover rate of ACh in striatum but not in cortex. Similar results have been obtained with push-pull cannulae except by measuring the ACh release from cerebral cortex of guinea pig and cat it has been found that (+)-amphetamine and L-DOPA release ACh (118, 119). Stimulation of dopaminergic receptors directly with apomorphine fails to alter the turnover rate

Table 1 Comparison of drug effects on sodium-dependent high affinity Ch uptake (51) and ACh turnover rate (phosphorylcholine infusion) (4, 64, 89)

	Ch uptake			ACh turnover rate		
	Cortex	Caudate	Hippocampus	Cortex	Caudate	Hippocampus
Haloperidol		↑		→	↑	↓
Morphine	↓	→	↓	↓	→	↓
Pentobarbital	↓	→	↓	↓	→	

of ACh in rat cerebral cortex but reduces the ACh turnover rate in striatum (80). Also amantadine (118), which releases catecholamines from their intraneural storage site and possibly inhibits their reuptake, decreases the release of ACh from neocortex. Although the results obtained with Ch uptake, push-pull cannula, and isotopic measurement of ACh turnover show a good correlation, apomorphine fails to decrease the Ch uptake in striatum (74) whereas it decreases the ACh turnover rate in striatum (80).

Dopamine Receptor Blockers

When dopaminergic receptors are blocked by either chlorpromazine or haloperidol the turnover rate (79) and release (52) of ACh increases in striatum but not in cortex. Using mice and high doses of chlorpromazine, Jenden (120) demonstrated a decrease in the ACh turnover of whole brain. In more detailed studies using pharmacological doses of chlorpromazine it was found that this drug and haloperidol increase the ACh turnover rate of *N. caudatus* and *N. accumbens* but fail to change that of *N. interpeduncularis*, amygdala, raphe dorsalis, locus coeruleus, and septum (10). Clozapine, another potent dopaminergic receptor blocker endowed with antipsychotic activity, fails to change ACh turnover rate in caudate and *N. accumbens*; this apparent discrepancy was explained by the muscarinic receptor blockade elicited by clozapine (10). In fact the increase of ACh turnover elicited by haloperidol in striatum can be reduced by administration of either muscarinic receptor blockers or clozapine.

Haloperidol, clozapine, and chlorpromazine fail to change ACh turnover in substantia nigra but decrease the turnover rate of ACh in globus pallidus (121). Since drugs which share a defined pharmacological property on dopamine receptors affect differently the ACh turnover in specific brain nuclei, it is possible to retain some of the pharmacological effects associated with dopamine receptor blockade and to eliminate other effects by increasing certain pharmacological characteristics of the molecules. This appears to be the case in the association of the ACh and dopamine receptor–blocking activity of clozapine. Clozapine possesses anticholinergic and antidopaminergic actions and retains the antipsychotic activity that derives from the dopamine receptor–blocking action but loses the cataleptogenic action of pure dopamine receptor blockers. It is important to note that dopaminergic receptor blockers can increase the turnover of striatal ACh when they fail to block muscarinic receptors. In contrast, all the antipsychotics with dopamine receptor–blocking activity reduce the turnover rate of ACh in globus pallidus whether or not they block cholinergic receptors. These results suggest that the increase of ACh turnover in striatum is an index of the extrapyramidal liability of antipsychotics and that the decrease of ACh turnover in globus pallidus is a characteristic property related to the antipsychotic action.

It may be mentioned here that all antipsychotics increase GABA turnover in globus pallidus (122), suggesting that the GABA output from striatum to pallidus is facilitated by antipsychotics and that perhaps these GABA neurons may impinge on cholinergic neurons causing their inhibition (122). By measuring turnover rate of various transmitters in different nuclei and by obtaining the profiles of drug action on various synaptic mechanisms, it is possible to foster our understanding and make

predictions from animal studies on the therapeutic action and the intensity of possible side effects of various antipsychotics.

GABAergic Receptor Stimulants

Diazepam and muscimol reduce the ACh turnover rate measured by infusion of an isotopic precursor of ACh in cortex and midbrain but fail to change that of ACh in striatum or hippocampus (123). Since muscimol and diazepam (124, 125) activate GABA receptors, these results invite speculation on the possibility that GABA regulates ACh transsynaptically in cortex and midbrain but not in striatum and hippocampus.

Central Nervous System Stimulants

Strychnine, picrotoxin, and pentylenetetrazol are thought to exert their primary action through noncholinergic transmitter systems. Nevertheless, they all have similar actions on cholinergic mechanisms. Strychnine (25), which may act by blocking glycine receptors (126), increases ACh release from the cerebral cortex (25). Pentylenetetrazol (96, 123, 128) and hexamethylenetetrazol (25, 29, 36) increase ACh release from cerebral cortex, caudate, and hippocampus and increase the low K_m Ch uptake of brain homogenates (11, 74).

CONCLUSION

Various techniques have been employed to study how drugs affect the dynamic regulation of cholinergic function. The various studies carried out with these techniques have contributed new ways to study the action of drugs on the CNS. By measuring ACh turnover rate in various brain nuclei the profiles of the synaptic actions of drugs can be traced. Thus side effects and therapeutic activity of drugs can be categorized in terms of their action at various synapses in different nuclei of rat brain. Of the techniques discussed in this review the measurement of the V_{max} of the low K_m uptake of Ch and the measurement of deuterium incorporation into Ch and ACh of various nuclei of rat brain, following the infusion at constant rate with deuterated phosphorylcholine, appear to be the methods of choice for these studies. Table 1 illustrates the analogies between the results obtained with the Ch uptake and deuterium incorporation measurements. Although there are some discrepancies, the number of analogies is encouraging. A drawback of the uptake method is the limited sensitivity (10 mg tissue or more) and the impossibility of relating quantitatively this response to the amount of drug injected. Although more complicated, the mass fragmentographic method gives reliable dose-response relationships and allows measurements in 0.5 mg tissue samples. By understanding the profile of drug actions on various synaptic mechanisms of brain nuclei it might be possible to design new screening procedures whereby unwanted side effects of therapeutically useful drugs can be predicted and therefore eliminated in careful structure-activity studies. With the present understanding of neuropharmacology this appears to be an important practical contribution of the in vitro turnover rate measurements of ACh.

Literature Cited

1. Rommelspacher, H., Kuhar, M. 1975. Effects of dopaminergic drugs and acute medial forebrain bundle lesions on striatal acetylcholine levels. *Life Sci.* 16: 65–70
2. Gage, F. H., Olton, D. S. 1975. Hippocampal influence on hyperreactivity induced by septal lesions. *Brain Res.* 98:311–25
3. Kuhar, M. J., DeHaven, R. N., Yamamura, H. I., Rommelspacher, H., Simon, J. R. 1975. Further evidence for cholinergic habenulo-interpeduncular neurons: Pharmacologic and functional characteristics. *Brain Res.* 97:265–75
4. Racagni, G., Cheney, D. L., Trabucchi, M., Wang, C., Costa, E. 1974. Measurement of acetylcholine turnover rate in discrete areas of the rat brain. *Life Sci.* 15:1961–75
5. Cheney, D. L., Trabucchi, M., Racagni, G., Costa, E. 1974. Effects of acute and chronic morphine on regional rat brain acetylcholine turnover rate. *Life Sci.* 15:1977–90
6. Bartholini, G., Stadler, H., Gadea-Ciria, M., Lloyd, K. G. 1976. The use of the push-pull cannula to estimate the dynamics of acetylcholine and catecholamines within various brain areas. *Neuropharmacology* 15:515–19
7. Dross, K., Kewitz, H. 1972. Concentration and origin of choline in the rat brain. *Naunyn Schmiedebergs Arch. Pharmacol.* 274:91–106
8. Lewis, P. R., Shute, C. C. D. 1967. The cholinergic limbic system: Projections to hippocampal formation, medial cortex, nuclei or the ascending cholinergic reticular system, and the subfornical organ and supra-optic crest. *Brain Res.* 90:521–42
9. Kataoka, K., Nakamura, Y., Hassler, R. 1973. Habenulointerpeduncular tract: A possible cholinergic neuron in rat brain. *Brain Res.* 62:264–67
10. Racagni, G., Cheney, D. L., Trabucchi, M., Costa, E. 1975. *In vivo* actions of clozapine and haloperidol on the turnover rate of acetylcholine in rat striatum. *J. Pharmacol. Exp. Ther.* 196: 323–32
11. Simon, J. R., Atweh, S., Kuhar, M. J. 1976. Sodium-dependent high affinity choline uptake: A regulatory step in the synthesis of acetylcholine. *J. Neurochem.* 26:909–22
12. MacIntosh, F. C. 1963. Synthesis and storage of acetylcholine in nervous tissue. *Can. J. Biochem. Physiol.* 41: 2555–71
13. Cheney, D. L., LeFevre, H. F., Racagni, G. 1975. Choline acetyltransferase activity and mass fragmentography measurement of acetylcholine in specific nuclei and tracts of rat brain. *Neuropharmacology* 14:801–9
14. Costa, E., Cheney, D. L., Racagni, G., Zsilla, G. 1975. An analysis at synaptic level of the morphine action in striatum and n. accumbens: Dopamine and acetylcholine interactions. *Life Sci.* 17:1–8
15. Zsilla, G., Racagni, G., Cheney, D. L., Costa, E. 1977. Constant rate infusion of deuterated phosphorylcholine to measure the effects of morphine on acetylcholine turnover rate in specific nuclei of rat brain. *Neuropharmacology.* In press
16. Guidotti, A., Cheney, D. L., Trabucchi, M., Doteuchi, M., Wang, C., Hawkins, R. A. 1974. Focussed microwave radiation: A technique to minimize post mortem changes in cyclic nucleotides, DOPA and choline and to preserve brain morphology. *Neuropharmacology* 13:1115–22
17. Dross, K., Kewitz, H. 1966. Der einbau von i.v. zugefuhrtem cholin in das acetylcholin des gehirns. *Naunyn-Schmiedebergs Arch. Pharmakol. Exp. Pathol.* 255:10–11
18. Schuberth, J., Sparf, B., Sundwall, A. 1969. A technique for the study of acetylcholine turnover in mouse brain *in vivo*. *J. Neurochem.* 16:695–700
19. Hanin, I., Cheney, D. L., Trabucchi, M., Massarelli, R., Wang, C. T., Costa, E. 1973. Applications of principles of steady state kinetics to measure acetylcholine turnover rate in rat salivary glands: Effects of diafferentation and duct ligation. *J. Pharmacol. Exp. Ther.* 187:68–77
20. Saelens, J. K., Simke, J. P., Allen, M. P., Conroy, C. A. 1973. Some of the dynamics of choline and acetylcholine metabolism in rat brain. *Arch. Int. Pharmacodyn.* 203:305–12
21. Jenden, D. J., Choi, L., Silverman, R. W., Steinborn, J. A., Roch, M., Booth, R. A. 1974. Acetylcholine turnover estimation in brain by gas chromatography-mass spectrometry. *Life Sci.* 14: 55–63
22. Cheney, D. L., Costa, E., Hanin, I., Trabucchi, M., Wang, C. T. 1975. Application of principles of steady state ki-

netics to the *in vivo* estimation of acetylcholine turnover rate in mouse brain. *J. Pharmacol. Exp. Ther.* 192:288–96
23. Szerb, J. C. 1975. The release of acetylcholine from central cortical slices in the presence or absence of an anticholinesterase. In *Cholinergic Mechanisms*, ed. P. G. Waser, 213–16. New York:Raven
24. Pepeu, G., Garau, L., Mulas, M. L., Marconcini-Pepeu, I. 1975. Stimulation by morphine of acetylcholine output from the cerebral cortex of septal rats. *Brain Res.* 100:677–80
25. Hemsworth, B. A., Neal, M. J. 1968. The effect of central stimulant drugs on acetylcholine release from rat cerebral cortex. *Br. J. Pharmacol.* 34:543–50
26. Celesia, G. G., Jasper, H. H. 1966. Acetylcholine released from cerebral cortex in relation to state of activation. *Neurology* 16:1053–63
27. Mitchell, J. F. 1960. Release of acetylcholine from the cerebral cortex and the cerebellum. *Proc. Physiol. Soc.* 155:22P
28. Yaksh, T. L., Yamamura, H. I. 1975. Blockade by morphine of acetylcholine release from the caudate nucleus in the mid-pontine pretrigeminal cat. *Brain Res.* 83:520–24
29. Smith, C. M. 1972. The relase of acetylcholine from the rabbit hippocampus. *Br. J. Pharmacol.* 45:172P
30. Costa, E., Neff, N. H. 1966. Isotopic and nonisotopic measurement of the rate of catecholamine biosynthesis. In *Biochemistry and Pharmacology of the Basal Ganglion*, ed. E. Costa, L. Cote, M. D. Yaks, 141–56. New York:Raven
31. Beleslin, D., Polak, R. L. 1965. Depression by morphine and chloralose of acetylcholine release from the cats brain. *J. Physiol. London* 177:411–19
32. Butcher, S. G., Butcher, L. L. 1974. Acetylcholine and choline levels in rat corpus striatum after microwave irradiation. *Proc. West. Pharmacol. Soc.* 17:37–39
33. Cohen, E. L., Wurtman, R. J. 1976. Brain acetylcholine: Control by dietary choline. *Science* 191:561–62
34. Racagni, G., Trabucchi, M., Cheney, D. L. 1975. Steady-state concentrations of choline and acetylcholine in rat brain parts during a constant rate infusion of deuterated choline. *Naunyn-Schmiedebergs Arch. Pharmacol.* 290: 99–105
35. Dudar, J. D., Szerb, J. C. 1969. The effect of topically applied atropine on resting and evoked cortical acetylcholine release. *J. Physiol.* 203:741–62
36. Mitchell, J. F. 1963. The spontaneous and evoked release of acetylcholine from the cerebral cortex. *J. Physiol.* 165:98–118
37. Phillis, J. W., Chong, G. C. 1965. Acetylcholine release from the cerebral and cerebellar cortices: Its role in cortical arousal. *Nature* 207:1253–55
38. Szerb, J. C. 1964. The effect of tertiary and quaternary atropine on cortical acetylcholine output and on the electroencephalogram in cats. *Can. J. Physiol. Pharmacol.* 42:303–14
39. Beani, L., Bianchi, C., Santinoceto, L., Marchetti, P. 1968. The cerebral acetylcholine release in conscious rabbits with semi-permanently implanted epidural cups. *Int. J. Neuropharmacol.* 7:469–81
40. Collier, B., Mitchell, J. F. 1966. The central release of acetylcholine during stimulation of the visual pathway. *J. Physiol. London* 184:239–54
41. Phillis, J. W. 1968. Acetylcholine release from the cerebral cortex: Its role in cortical arousal. *Brain Res.* 7:378–89
42. Holmstedt, B., Lundgren, G., Sundwall, A. 1963. Tremorine and atropine effects on brain acetylcholine. *Life Sci.* 10:731–39
43. Consolo, S., Ladinsky, H., Peri, G., Garattini, S. 1972. Effect of central stimulants and depressants on mouse brain acetylcholine and choline levels. *Eur. J. Pharmacol.* 18:251–55
44. Giarman, N. J., Pepeu, G. 1964. The influence of centrally acting cholinolytic drugs on brain acetylcholine levels. *Br. J. Pharmacol.* 23:123–26
45. Saelens, J. K., Simke, J. P. Schuman, J., Allen, M. P. 1974. Studies with agents which influence acetylcholine metabolism in mouse brain. *Arch. Int. Pharmacodyn. Ther.* 209:250–58
46. Gardiner, J. E. 1961. The inhibition of acetylcholine synthesis in brain by a hemicholinium. *Biochem. J.* 81:297–303
47. Domino, E. F., Wilson, A. E. 1972. Psychotropic drug influences on brain acetylcholine utilization. *Psychopharmacologia* 25:291–98
48. Buterbaugh, G. G., Spratt, J. L. 1968. Effect of hemicholinium on choline uptake in the isolated perfused rabbit heart. *J. Pharmacol. Exp. Ther.* 159:255–60
49. Rodriquez deLores Arnaiz, G., Zieher, L. M., DeRobertis, E. 1970. Neurochemical and structural studies on the

mechanism of action of hemicholinium-3 in central cholinergic synapses. *J. Neurochem.* 17:221–29

50. Ansell, G. B., Spanner, S. 1975. The origin and metabolism of brain choline. See Ref. 23, pp. 117–29
51. Gomez, M., Domino, E. F., Sellinger, O. Z. 1970. Effect of hemicholinium on choline distribution *in vivo* in the canine caudate nucleus. *Biochem. Pharmacol.* 19:1753–60
52. Stadler, H., Lloyd, K. G., Gadea-Ciria, M. 1973. Enhanced striatal acetylcholine release by chlorpromazine and its reversal by apomorphine. *Brain Res.* 55:476–80
53. Guyenet, P. G., Agid, Y., Javoy, F., Beaujouan, J. C., Bossier, J., Glowinski, J. 1975. Effects of dopaminergic receptor agonists and antagonists on the activity of the neostriatal cholinergic system. *Brain Res.* 84:227–44
54. Sethy, V. H., VanWoert, M. H. 1974. Modification of striatal acetylcholine concentration by dopamine receptor agonist and antagonists. *Res. Commun. Chem. Pathol. Pharmacol.* 8:13–28
55. Ladinsky, H., Consolo, S., Garattini, S. 1974. Increase in striatal acetylcholine levels in vivo by Piribedil, a new dopamine receptor stimulant. *Life Sci.* 14:1251–60
56. McGeer, P. L., Grewaal, D. S., McGeer, E. G. 1974. Influence of noncholinergic drugs on rat striatal acetylcholine levels. *Brain Res.* 80:211–17
57. Consolo, S., Ladinsky, H., Garattini, S. 1974. Effect of several dopaminergic drugs and trihexyphenidyl on cholinergic parameters in rat striatum. *J. Pharm. Pharmacol.* 26:275–77
58. Guyenet, P., Agid, Y., Javoy, F., Beaujouan, J. C., Glowinski, J. 1974. Action selective des neuroleptiques sur les neurones cholinergiques du neostriatum chez le rat: Antagonisme vis-à-vis de l'apomorphine. *C. R. Acad. Sci. Paris* 278:2679–82
59. Cheney, D. L., Racagni, G., Zsilla, G., Costa, E. 1976. Differences in the action of various drugs on striatal acetylcholine and choline content in rats killed by decapitation or microwave radiation. *J. Pharm. Pharmacol.* 28:75–77
60. Yamamura, H. I., Snyder, S. H. 1973. High affinity transport of choline into synaptosomes of rat brain. *J. Neurochem.* 21:1355–74
61. Guyenet, P., Lefresne, P., Rossier, J., Beaujouan, J. C., Glowinski, J. 1973. Inhibition by hemicholinium-3 of C^{14} acetylcholine synthesis and H^3 choline high affinity uptake in rat striatal synaptosomes. *J. Mol. Pharmacol.* 9:630–39
62. Barker, L. A., Mittag, T. W. 1975. Comparative studies of substrates and inhibitors of choline transport and choline acetyltransferase. *J. Pharmacol. Exp. Ther.* 192:86–94
63. Haga, T., Noda, H. 1973. Choline uptake systems of rat brain synaptosomes. *Biochem. Biophys. Acta* 291:564–75
64. Carroll, P. T., Buterbaugh, G. G. 1975. Regional differences in high affinity choline transport velocity in guinea-pig brain. *J. Neurochem.* 24:229–32
65. Kuhar, M. J., Sethy, V. H., Roth, R. H., Aghajanian, G. K. 1973. Choline: Selective accumulation by central cholinergic neurons. *J. Neurochem.* 20:581–93
66. Pert, C. B., Snyder, S. H. 1974. High affinity transport of choline into the myenteric plexus of guinea pig intestine. *J. Pharmacol. Exp. Ther.* 191:102–8
67. Simon, J. R., Mittag, T. W., Kuhar, M. J. 1975. Inhibition of synaptosomal uptake of choline by various choline analogs. *Biochem. Pharmacol.* 24:1139–42
68. Kuhar, M. J., Simon, J. R. 1974. Acetylcholine uptake: Lack of association with cholinergic neurons. *J. Neurochem.* 22:1135–37
69. Suszkiw, J. B., Beach, R. L., Pilar, G. R. 1976. Choline uptake by cholinergic neuron cell somas. *J. Neurochem.* 26:1123–31
70. Suszkiw, J. B., Pilar, G. 1976. Selective localization of a high affinity choline uptake system and its role in ACh formation in cholinergic nerve terminal. *J. Neurochem.* 26:1133–38
71. Mulder, A. H., Yamamura, H. I., Kuhar, M. J., Snyder, S. H. 1974. Release of acetylcholine from hippocampal slices by potassium depolarization: Dependence on high affinity choline uptake. *Brain Res.* 70:372–76
72. Guyenet, P. G., Lefresne, P., Beaujouan, J. C., Glowinski, J. 1975. The role of newly taken up choline in the synthesis of acetylcholine in rat striatal synaptosomes. See Ref. 23, pp. 137–44
73. Simon, J., Kuhar, M. J. 1975. Impulse-flow regulation of high affinity choline uptake in brain cholinergic nerve terminals. *Nature* 255:162–63
74. Atweh, S., Simon, J. R., Kuhar, M. J. 1975. Utilization of sodium-dependent high affinity choline uptake *in vitro* as a measure of the activity of cholinergic neurons *in vivo*. *Life Sci.* 17:1535–44

75. Sparf, B. 1973. On the turnover of acetylcholine in the brain. *Acta Physiol. Scand. Suppl.* 397:1–47
76. Trabucchi, M., Cheney, D. L., Hanin, I., Costa, E. 1975. Application of principles of steady-state kinetics to the estimation of brain acetylcholine turnover rate: Effects of oxotremorine and physostigmine. *J. Pharmacol. Exp. Ther.* 194:57–64
77. Freeman, J. J., Choi, R. L., Jenden, D. J. 1975. The effects of homicholinium on behavior and on brain acetylcholine and choline in the rat. *Psychopharmol. Commun.* 1:15–27
78. Choi, R. L., Roch, M., Jenden, D. J. 1973. A regional study of acetylcholine turnover in rat brain and the effect of oxotremorine. *Proc. West. Pharmacol. Soc.* 16:188–90
79. Trabucchi, M., Cheney, D. L., Racagni, G., Costa, E. 1974. Involvement of brain cholinergic mechanisms in the action of chlorpromazine. *Nature* 249:664–66
80. Trabucchi, M., Cheney, D. L., Racagni, G., Costa, E. 1975. In vivo inhibition of striatal acetylcholine turnover by L-Dopa, apomorphine and amphetamine. *Brain Res.* 86:130–34
81. Cheney, D. L., Trabucchi, M., Hanin, I., Costa, E. 1975. Morphine dependence and *in vivo* turnover rate in whole mouse brain. *J. Pharmacol. Exp. Ther.* 195:288–95
82. Dawson, R. M. C. 1955. Phosphorylcholine in rat tissues. *Biochem. J.* 60:325–28
83. Ansell, G. B., Spanner, S. 1975. The origin and metabolism of brain choline. See Ref. 23, pp. 117–29
84. Ansell, G. B., Spanner, S. 1971. Studies on the origin of choline in the brain of the rat. *Biochem. J.* 122:741–50
85. Koslow, S., Racagni, G., Costa, E. 1974. Mass fragmentographic measurement of norepinephrine, dopamine, serotonin and acetylcholine in seven discrete nuclei of the rat tel-diencephalon. *Neuropharmacology* 13:1123–30
86. Haubrich, D. R., Reid, W. D., Gillette, J. R. 1972. Acetylcholine formation in mouse brain and effect of cholinergic drugs. *Nature New Biol.* 238:88–89
87. Szerb, J. C., Somogyi, G. T. 1973. Depression of acetylcholine release from cerebral cortical slices by cholinesterase inhibition and by oxotremorine. *Nature New Biol.* 241:121–22
88. Nordberg, A., Sundwall, A. 1976. Effects of oxotremorine on endogenous acetylcholine and on uptake and biotransformation of radioactive choline in discrete regions of mouse brain *in vivo*. *Biochem. Pharmacol.* 25:135–40
89. Giarman, N. J., Pepeu, G. 1962. Drug-induced changes in brain acetylcholine. *Br. J. Pharmacol.* 19:226–34
90. Pazzagli, A., Pepeu, G. 1964. Amnesic properties of scopolamine and brain acetylcholine in the rat. *Int. J. Neuropharmacol.* 4:291–99
91. Milosevic, M. P. 1969. Acetylcholine content in the brain of rats treated with paraoxon and pyridinium-2-aldoxime methylchloride. *J. Pharm. Pharmacol.* 21:469–70
92. Armitage, A. K., Hall, G. H., Sellers, C. M. 1969. Effects of nicotine on electrical activity and acetylcholine release from the cat cerebral cortex. *Br. J. Pharmacol. Chemother.* 35:152–60
93. Rao, K. S., Bhatt, H. V., Gopalakrishna, G., Haranth, P. S. R. K. 1970. Influences of intracarotid infusions of hexamethonium on acetylcholine release from perfused cerebral ventricles in anesthetized dogs. *Indian J. Med. Res.* 58:1279–84
94. Bartolini, A., Pepeu, G. 1967. Investigating into acetylcholine output from the cerebral cortex of the cat in the presence of hyoscine. *Br. J. Pharmacol. Chemother.* 31:66–73
95. Polak, R. L. 1965. Effect of hyposcine on the output of acetylcholine into perfused cerebral ventricles of cats. *J. Physiol. London* 181:317–23
96. Yaksh, T. L., Yamamura, H. I. 1975. The release *in vivo* of [^{3}H] acetylcholine from cat caudate nucleus and cerebral cortex by atropine pentylenetetrazol, K^+- depolarization and electrical stimulation. *J. Neurochem.* 25:123–30
97. Domino, E. F., Bartholini, A. 1972. Effects of psychotromimetic agents on the EEG and acetylcholine release from the cerebral cortex of brainstem transected cats. *Neuropharmacology* 11: 703–13
98. Lundholm, B., Sparf, B. 1975. The effect of atropine on the turnover of acetylcholine in the mouse brain. *Eur. J. Pharmacol.* 32:287–92
99. Haubrich, D. R., Wang, P. F. L., Herman, R. L., Clody, D. E. 1975. Acetylcholine synthesis in rat brain: Dissimilar effects of clozapine and chlorpromazine. *Life Sci.* 17:739–48
100. Smith, J. C., Cavallito, C. J., Foldes, F. F. 1967. Choline acetyltransferase inhibitors: A group of styrylpyridine ana-

logs. *Biochem. Pharmacol.* 16:2438–41
101. Salama, A. I., Goldberg, M. E. 1975. Elevation of amphetamine levels in rat brain after administration of the choline acetyltransferase inhibitor 4-(1-naphthylvinyl) pyridine (NVP). *Arch. Int. Pharmacodyn. Ther.* 215:197–201
102. Haubrich, D. R., Wang, P. F. L. 1976. Inhibition of acetylcholine synthesis by juglone and 4-(1-napthylvinyl) pyridine. *Biochem. Pharmacol.* 25:669–72
103. Hebb, C. 1972. Biosynthesis of acetylcholine in nerve tissue. *Physiol. Rev.* 52:918–57
104. Birks, R., MacIntosh, F. C. 1961. Acetylcholine metabolism of a sympathetic ganglion. *Can. J. Biochem.* 39:787–827
105. Trabucchi, M., Cheney, D. L., Racagni, G., Costa, E. 1975. Pentobarbital and *in vivo* turnover rate of acetylcholine in mouse brain and in regions of rat brain. *Pharmacol. Res. Commun.* 7:81–94
106. Trabucchi, M., Cheney, D. L., Hanin, I., Costa, E. 1973. Effect of various sedative drugs on levels of choline and acetylcholine and on turnover rate of acetylcholine in mouse brain. *Proc. APHA Acad. Pharm. Sci.*, San Diego, Abstr.
107. Nordberg, A., Sundwall, A. 1975. Effect of pentobarbital on endogenous acetylcholine and biotransformation or radioactive choline in different brain regions. See Ref. 23, p. 229
108. Ngai, S. H., Cheney, D. L., Finck, A. D. 1976. Effects of halothane, enflurane, and ketamine on acetylcholine concentrations and turnover in rat brain structures. *Proc. Ann. Meet. Am. Soc. Anesthesiol.*, Abstr., p. 427
109. Atweh, S., Kuhar, M. J. 1975. Effects of impulse flow alterations of the *in vivo* synthesis of ^{3}H-acetylcholine in the rat hippocampus. *Pharmacologist* 17:255
110. Jhamandas, K., Dickinson, G. 1973. Modification of precipitated morphine and methadone abstinence in mice by acetylcholine antagonists. *Nature New Biol.* 245:219–21
111. Zsilla, G., Cheney, D. L., Racagni, G., Costa, E. 1976. Correlation between analgesia and the decrease of acetylcholine turnover rate in cortex and hippocampus elicited by morphine, meperidine, viminol R_2 and azidomorphine. *J. Pharmacol. Exp. Ther.* 199: 662–68
112. Cheney, D. L., Costa, E., Hanin, I., Racagni, G., Trabucchi, M. 1975. See Ref. 23, pp. 217–28
113. Domino, E. F. 1971. Neuropsychopharmacologic studies of marihuana and natural THC derivatives in animals and man. *Ann. NY Acad. Sci.* 191:166–91
114. Friedman, E., Hanin, I., Gershon, S. 1976. Effect of tetrahydrocannabinols on ^{3}H-acetylcholine biosynthesis in various rat brain slices. *J. Pharmacol. Exp. Ther.* 196:339–45
115. Graham, D. T., Erickson, C. K. 1971. The effect of ethanol on the output of cerebral acetylcholine *in vivo*. *Fed. Proc.* 30:622 (Abstr.)
116. Anden, N. E., Bedard, P. 1971. Influences of cholinergic mechanisms on the function and turnover rate of brain dopamine. *J. Pharm. Pharmacol.* 23: 460–62
117. Stadler, H., Lloyd, K. G., Gadea-Ciria, M., Bartholini, G. 1973. Enhanced striatal acetylcholine release by chlorpromazine and its reversal by apomorphine. *Brain Res.* 55:476–80
118. Beani, L., Bianchi, C. 1973. Effect of amantadine on cerebral acetylcholine release and content in the guinea pig. *Neuropharmacology* 12:283–89
119. Pepeu, G., Bartholini, A. 1968. Effect of psychoactive drugs on the output of acetylcholine from the cerebral cortex of the cat. *Eur. J. Pharmacol.* 4: 254–63
120. Jenden, D. J. 1974. Effects of chlorpromazine on acetylcholine turnover in vivo. In *The Phenothiazines and Structurally Related Drugs*. ed. I. S. Forrest, C. J. Carr, E. Usdin. New York:Raven
121. Cheney, D. L., Zsilla, G., Costa, E. 1977. Dopamine and acetylcholine interactions in *N. accumbens, N. caudatus, Globus pallidus* and *Substantia nigra:* Pharmacological implications. In *Non-striatal Dopamine*, ed. E. Costa, G. L. Gessa, 179–86. New York:Raven
122. Mao, C. C., Marco, G., Revuelta, A., Bertilsson, L., Costa, E. 1977. The turnover rate of γ-aminobutyric acid in the nuclei of telencephalon: Implications in the pharmacology of antipsychotics and of a minor tranquilizer. *Biol. Psychiatry.* In press
123. Zsilla, G., Cheney, D. L., Costa, E. 1976. Regional changes in the rate of turnover of acetylcholine in rat brain receiving diazepam or muscimol. *Naunyn Schmiedebergs Arch. Pharmacol.* 294:251–55
124. Johnston, G. A. R., Curtis, D. R., DeGroat, W. C., Duggan, A. W. 1968. Central actions of ibotenic acid and

muscimol. *Biochem. Pharmacol.* 17: 2488–89
125. Costa, E., Guidotti, A., Mao, C. C., Suria, A. 1975. New concepts on the mechanism of action of benzodiazepines. *Life Sci.* 17:167–86
126. Curtis, D. R., Hosli, L., Johnston, G. A. R., Johnston, I. H. 1968. The hyperpolarization of spinal motoneurons by glycine and related amino acids. *Exp. Brain Res.* 5:238–62
127. Obata, K., Takeda, K., Shinozaki, H. 1970. Further study on pharmacological properties of the cerebellar-induced inhibition of Deiter's neurons. *Exp. Brain Res.* 11:327–42
128. Nicoll, R. A., Padjen, A. 1976. Pentylenetetrazol: An antagonist of GABA at primary afferents of the isolated frog spinal cord. *Neuropharmacology* 15: 69–72