Ann. Rev. Pharmacol. Toxicol. 1982. 22:435-63

CHOLINERGIC SYSTEM IN BEHAVIOR: THE SEARCH FOR MECHANISMS OF ACTION

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INTRODUCTION

". . . any theory of behavior is at present, and must be for some time to come, a molar theory. This is because neuroanatomy and physiology have not yet developed to a point such that they yield principles which may be employed as postulates in a system of behavior theory . . ." (1). Some 40 years ago this statement by an internationally respected behavioral scientist recognized the importance of events intervening between changes in the environment, stimuli (S), and an organism's (O) responses (R) to them. Relations between S → R are dependent upon intervening events and are influenced by them. R cannot be predicted solely on information about S; understanding the characteristics of O is essential, even when the simplest of Rs are involved. Although advances in concepts and technologies have enabled very significant strides in knowledge about the biochemical, electrophysiological, and morphological properties of living organisms, there remain challenging questions about relations between the "molecular" events of these properties and the "molar" characteristics which constitute a fourth property of organisms, the behavioral property (2).

The present review is concerned with examining speculative ideas and hypotheses about mechanisms of action by which the cholinergic neurotransmitter system may be involved in the behavior of living organisms as they cope with changes in their external and internal environments. Because most of the cholinergic receptors within the mammalian central nervous system (CNS) appear to be muscarinic (3), emphasis in this review will be

0362-1642/82/0415-0435$02.00

upon muscarinic mechanisms. As a result of empirical tests of hypotheses generated by ideas about such mechanisms some hypotheses appear to have been falsified, others remain for further study, and still others have come to light which have only just begun to be tested. There is little doubt that even newer ideas will result from further knowledge of the characteristics of the cholinergic system and of effects of its manipulation on both normal and abnormal behaviors.

The "Integrated" Organism

For the biomedical sciences the organism in its biosphere constitutes a total "system." The multivariate and highly integrated organization of living organisms has led to conceptualization in terms of multilevels, of hierarchies of "subsystems." Within limits these subsystems are self-regulatory and self-correcting. Changes in one may lead to subsequent changes in others. Relations between all of them are reversible in the sense that alterations in one direction may lead to feedback changes in the opposite direction. Outcomes for the total system may range from death when the capacities of self-regulating processes are exceeded to changes in the overall behavior of an organism, acute or chronic, which affect its capabilities to make normal adjustments to its environment. Underlying essential plasticities are not only the "homeostatic" mechanisms of Claude Bernard, Sherrington, and Cannon, but also mechanisms which provide substrates for such behavioral processes as habituation, tolerance, and learning and memory, processes which have been referred to as "behavioral homeostasis" (4). For example, water deprivation produces blood borne and sensory changes in the body which increase the general activity of the organism. The activity is not random, but directed toward the goal of seeking water using behavioral responses ranging from relatively simple drinking to more complex responses acquired through past learning. Other incentives that motivate behavior may be learned. Regardless of the simplicity or complexity of an $S \rightarrow O \rightarrow R$ relation it involves many dynamic biochemical and electrophysiological events taking place at many morphological sites within the organism.

Although many events intervene between the stimulus and subsequent behavioral effects, some may be more critical to the end results than others. The discovery of the role of acetylcholine (ACh) at neuromuscular junctions left no doubt about the fact that the cholinergic system is involved in behavior. Muscular movements characterize most behaviors, certainly all those perceived by an independent observer and, some would argue, even in processes of thinking which involve subvocal activity in the larynx. Later discoveries indicated that the cholinergic system has broader involvements in behavior than solely in an organism's motor outputs. Interests of re-

searchers and clinicians have come to center particularly on roles the system may play in the complex activities of the CNS and especially the brain, which may be reflected in a wide variety of behaviors.

The modern era in the scientific study of behavior began in the early nineteenth century with questions about reactions of the total organism to stimuli in the environment, questions about "psychophysical" relations as they became known. Behavioral responses to changes in the physical or psychosocial environments were seen as initiated by quantitative relationships between the changes and events activated in appropriate sensory modalities of the responding organism. The relationship was perceived in a manner analogous to the concept of the pharmacological receptor which arose from experimentation during the late nineteenth and early twentieth centuries. In general terms, a drug in combination with a receptor produces a drug-receptor complex, a relationship which often is reversible. Such interaction with functional macromolecular components of the organism initiates the series of biochemical and electrophysiological changes which characterize the overall response to the agent: the drug-receptor complex provides a stimulus that sets into motion subsequent events which are drug-independent (5). "Whatever effects a drug produces in a biological system must be regarded as ultimate consequences of physicochemical interactions between that drug and functionally important molecules in the living organism (6)." Changes in the behavior of the total organism are the end points of special interest in the present review. Physicochemical interactions at macromolecular levels, e.g. the cholinergic neurotransmitter system, may affect behavior at molar levels. Early in the modern development of neuropsychopharmacology Kety (7) pointed to "... a very important principle in pharmacology.... We cannot expect drugs to introduce anything new into the mind or into behavior, but merely to accentuate or to suppress functions in behavior which are already present." The objective of the paragraphs to follow is to examine evidence which may help to identify primary relations between the cholinergic system and behavior, mechanisms of action that appear to be essential to such "functions in behavior."

General Methodological Approaches

Figure 1 is a schematic representation of the cholinergic system. It is possible that variation in any component of the system could be involved in variations of behavior: availability of precursors, their transport to sites where synthesis of ACh occurs, activity of the synthesizing enzyme choline acetyltransferase (CAT), storage and release of ACh, activity of the inactivating enzyme cholinesterase (ChE), sensitivity of postsynaptic receptors, high affinity choline (Ch) uptake, and modulating roles of presynaptic

receptors. Evidence that any one of these is involved requires the demonstration that it varies concomitantly with variations in behavior.

The major approach in the search for such relations has involved manipulation of some aspect of the system, as the independent variable, and observation of concomitant changes in some behavior pattern(s), as the dependent variable. Pharmacological "tools" have provided the principle means for manipulation, although changes in cholinergic functions following morphological lesions have also been employed. Examples of these various manipulations and their behavioral effects will be discussed below.

In other approaches behavior has served as the independent variable. Because behavior is characterized by its plasticity, particular behavioral patterns may be manipulated to seek concomitant changes in the neurotransmitter system.

Useful information has also resulted from the examination of innately determined individual differences in behavior. "Logically all genetic effects on behavior involve biochemistry (8)." In some instances genetic faults affecting neurotransmitter systems have been studied. In other investigations trends in the ontogeny of such systems have been related to development of behavior patterns.

The precision of any statement that behavior measure y is some function of cholinergic event x will vary with the accuracy with which x and y can be specified. Among the reasons why the cholinergic system has witnessed a revival of interest are the technological advances which enable its various components to be specified and measured. Included among these are: techniques for the microwave fixation of brain tissue in situ; for chemical assays of ACh and Ch, e.g. by combined gas chromatography mass spectrometry (GCMS); for radioligand labeling of postsynaptic receptors; and, for study-

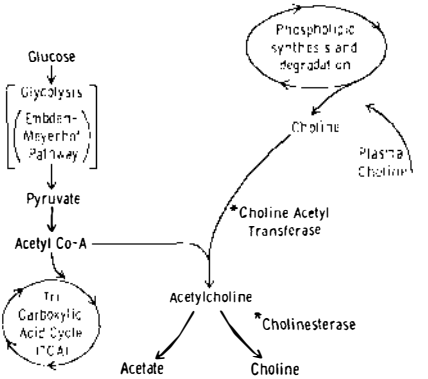

Figure 1 Schematic representation of the various components of the cholinergic neurotransmitter system.

ing the uptake of Ch and the synthesis and release of ACh in vivo and in vitro, the latter involving synaptosomal preparations.

The wealth of different behavior patterns from which to choose those to be measured in any specific study presents problems of selection. The research literature shows that two approaches to the problems have received most attention. One is based upon an a priori classification of behavior, sometimes accepting results of factor analyses of a wide variety of behavior as the starting point. Behavior patterns "representative" of one or more of the classes are included in the behavioral test battery. A second approach begins with some systematic model of behavior. A long time favorite, derived from models in communication theory, analyzes behavior in terms of such processes as sensory input, central fixation and storage, central retrieval or readout, and motor output. Measures of behavior which differentiate among these processes are then selected. Whatever the basis for selection each measure must have validity, i.e. it must measure the behavior it is supposed to measure, and it must be capable of being applied consistently, i.e. it must have reliability without which variability of measurement may mask existing relations.

Evidence for Involvement of the Cholinergic System in Behavior

In the Epilogue to his 1974 critical review of information about "Drug and Chemical Stimulation of the Brain" Myers (9) stated: "Most impressive is the singular fact that ACh is the only substance that can influence every physiological or behavioral response thus far examined." Several other recent reviews have presented evidence for involvement of the cholinergic system, at least in particular aspects of behavior. As commented earlier, activity of ACh at neuromuscular junctions must be involved in behaviors characterized by motor activity, and from the standpoint of an independent observer this includes all behavior. Cholinergic involvement in other aspects of behavior has now been documented in a wealth of research reports. Because this is an assertion from which the present discussion of mechanisms of action proceeds, no attempt will be made to summarize the evidence yet again. A number of review articles have already subjected the evidence to detailed scrutiny (10–16).

PHARMACOKINETICS

Although the primary concern of the present review is with mechanisms of action, the use of chemical agents as tools in research makes it desirable to comment briefly on matters relating to the absorption, biotransformation, distribution, localization, and elimination of such agents. The pharmacoki-

netics of agents affecting cholinergic systems are such as to require carefully differentiated analyses in order to specify particular mechanisms of action. For example, the anticholinesterases act at all cholinergic synapses: central and peripheral, muscarinic and nicotinic. Products from the biotransformation of these agents (e.g. eseroline from physostigmine; fluoride anion from the organophosphate, DFP) may produce their own effects. Antimuscarinic agents competitively block presynaptic as well as postsynaptic muscarinic receptors, actions which, because they oppose each other, may partly account for differences among such agents in their effects on behavior. Both precursors in the synthesis of ACh, Ch, and acetylcoenzyme A (AcCoA) also act in other capacities.

Innate characteristics of body fluids may affect the distribution of an agent and, hence, its effects on behavior. This is shown clearly in a series of experiments on sex differences following pharmacological manipulation of the cholinergic system (17). The results suggest that female animals are less sensitive to DFP because of their higher levels of serum ChE to which DFP administered peripherally binds, thus reducing the amount available for penetration into the brain.

Another example of the involvement of pharmacokinetic factors, referred to as ". . . the first fully documented observation of a heritable modification of a pharmacologic response . . ." (18), is the presence of atropinesterase in some, but not all, lines of rabbits. Recognition of the genetically based variation came from observations that some lines can thrive on belladonna leaves without physical or behavioral abnormalities. The search for a mechanism of action underlying the variation led to observations that atropine, an alkaloid of the belladonna plant, is inactivated in vitro by rabbit serum and that inactivation is due to an enzymatic destruction of the agent. Later study showed that the serum and liver of some, but not all, stocks of rabbits contain atropinesterase that hydrolyzes atropine. Thus, the biotransformation of atropine when the enzyme is present prevents or reduces the agent's major action, i.e. competitive antagonism of the actions of ACh and other muscarinic agonists.

Observations of these kinds indicate the importance of considering pharmacokinetic processes as possible confounding variables in the search for cholinergic mechanisms of action underlying behavior.

GENETIC DETERMINANTS

Observable and measurable behavioral traits constitute an organism's behavioral phenotype. The systems concept discussed earlier emphasizes that behavioral phenotypes interact with morphological and biochemical phenotypes in the integrated organism. In studying the origin and development

of any phenotype, genetic and environmental determinants must both be considered. Within a population the phenotypic variance, P, among individuals is closely associated with differences in genetic determinants, G, and differences in nongenetic (environmental or acquired) determinants, E, and may be influenced by interactions between the two: $P = G + E + f(G, E)$. Approaches to discovering the contributions of each arise from this relationship. The genotypic approach takes a known difference in heredity and studies its influences on behavior. The phenotypic approach starts with known individual differences in behavior and seeks to discover the genetic factor, if any, which underlies the variations. Because all genetic effects on behavior involve biochemistry, a thorough search with either approach leads eventually to consideration of biochemical mechanisms of action.

Illustrative of the experimental study of genetic determinants as a method for investigating relations between specific components of the cholinergic system and behavior is an experimental program, still underway, reported by Overstreet and colleagues (19). The program involves the use of assortative mating techniques. Several studies have reported success in breeding lines of animals which differ significantly in a target behavior, e.g. emotionality (20), general activity (21), maze (22), and conditioned avoidance (23) learning. Selection for high and low levels of ChE activity in the rat cerebral cortex has been reported and large phenotypic differences between inbred strains of mice in ChE activity has also been noted (24). The Overstreet program is unique in seeking to develop distinct lines of rats which differ in *interactions* between the behavioral and neurochemical phenotypes. Male and female rats of the original parental generation were continuously distributed in terms of measures of several physiological and behavioral variables when administered a standard dose of the anticholinesterase agent, DFP. DFP is ". . . particularly valuable as an investigative tool . . . [because of] . . . the virtually irreversible inactivation it produces by alkylphosphorylation of AChE and certain other esterases; its high lipid solubility, resulting in penetration into the CNS; and its relative specificity" (25). DFP has been shown to have differential effects on a wide variety of behaviors, affecting some and not others (26). By intermating males and females least reactive to DFP and also mating those most reactive it was possible to develop two new lines of animals, a resistant R-line and a sensitive S-line, which began to differ from each other by the second filial generation. The differences have now been maintained through 14 generations. The two lines also differ in the rates at which they develop tolerance to chronic administration of DFP, with R-line animals reaching asymptotic recovery levels more rapidly than S-line subjects. Assays of several areas from the brains of animals from the two lines, following injection into the tail vein of a deuterium variant of Ch and sacrifice by microwave fixation (27), have shown no significant

differences in ACh synthesis or in ACh or Ch levels. Other experiments using radioligand binding techniques (28) are looking for possible differences between lines in the numbers or affinities of muscarinic ACh receptors (mAChRs). Plans also include in vitro studies of Ch uptake, ACh synthesis, and release in synaptosomal preparations. This rather detailed description of a research program which is still underway is intended to illustrate the uses to which animal models can be put in applying experimental genetic analysis to the search for cholinergic mechanisms of action underlying behavior.

The present resurgence of interest in the search has also been stimulated by developments at the human clinical level, some of which involve genetic analyses of behavioral disorders in relation to abnormalities in the cholinergic system (13). Huntington's disease (29) is an example which illustrates directions in which research, greatly aided by new analytical tools, has taken. Genetically Huntington's disease is an autosomal dominant disorder, manifested in involuntary chorieform movements and dementia. It occurs in two forms. The more frequent of the two develops progressively with symptoms appearing in the fourth or fifth decade of life. Onset of the second form is earlier and has a rapid course, often characterized by rigid hypokinetic movements, seizures, and mental retardation. Brains of patients who have died with the major form show morphological changes consisting of a loss of small neurons in the striatum and degenerative changes in the cerebral cortex, globus pallidus, and subthalamic nuclei (30). Accompanying changes in neurotransmitter systems have also been observed: gamma-aminobutyric acid (GABA) and its synthetic enzyme were consistently reduced (31) and significant decreases in CAT activity found (32). Radioligand studies using the reversible anticholinergic quinuclidinyl benzilate ([^{3}H]-QNB) have shown alterations in synaptic neurotransmitter receptor binding sites to be due to a decrease in the number of sites and not to changes in receptor affinity (33). Results of such research clearly link specific components of the cholinergic system, i.e. CAT activity and postsynaptic receptor sensitivity, with a genetically determined behavioral abnormality. They also lend strong support to the importance of interactions among intervening events (in the present example between ACh and GABA neurotransmitter systems) for full understanding of mechanisms of action underlying behavior.

DEVELOPMENTAL DETERMINANTS

In general, most neuronal function in the neonatal organism is incompletely developed. There is evidence that synthesis, storage, release, and the capacity to inactivate neurotransmitters are less well developed compared to the adult. It also appears that in the immature, developing brain

the blood brain barrier is generally not so effective, allowing penetration of chemicals that might only be manifested in the adult by peripheral effects.

Ontogenetic Changes in the Cholinergic System

During recent years the ontogeny of the cholinergic neurotransmitter system has been receiving special attention. As yet unpublished results of research conducted at the University of California, Los Angeles (S. G. Butcher and D. J. Jenden, personal communication) using animal subjects has shown that levels of ACh increase only slightly in the rat striatum during the first postnatal week. Rapid increase follows during the second and third weeks. The synthesis of labeled ACh from deuterium-labeled Ch is poorly developed in the neostriatum and the cortex during the first 2 weeks. Adult levels of ACh are reached between the third and fourth weeks. These states are paralleled by changes in the activity of the synthetic enzyme, CAT, in the striatum (34). During the first 3 weeks of life levels of endogenous Ch are slightly higher than adult Ch levels in the rat striatum and frontal cortex (S. G. Butcher and D. J. Jenden, personal communication). Because this is a period of rapid synaptogenesis, some of the Ch may be incorporated into phospholipids involved in the formation of synapses (35). Using radioligand binding techniques to study the ontogeny of muscarinic receptors in rat brain Kuhar and co-workers (36) have shown that different agonist binding sites develop at different rates. There was a 5–6 day lag in the appearance of high affinity sites, a lag which continued to be observed up to 20 days with rates of increase being about the same throughout this period.

Studies of changes in components of the cholinergic system in human brain at various states of development have been carried out on postmortem material. The research is complicated by such factors as genetic heterogeneity, postmortem changes, presence of disease processes, and the treatment received during the premortem period. However, certain components of the cholinergic system have been shown to be relatively stable after death, e.g. CAT, AChE, and mAChR. Research (37) focusing on these components has shown an order of cholinergic development with receptor preceding neurotransmitter enzyme and with marked regional differences. In the cerebral cortex CAT activity and QNB binding sites increased with age, reaching adult values relatively late in development. In the cerebellum these components attained peak values during gestation and then showed a marked fall to adult levels during the early postnatal period. AChE activity was lower in the cerebral cortex than in cerebellum. Developmental increases occurred in both areas, with activity in the former increasing initially to a level higher than the final adult value.

Concomitant Changes of Behavior

That the ontogeny of the cholinergic system should be related to trends in the development of behavior is a reasonable hypothesis, given the established interactions of the two described earlier. For many years clinical experience has provided dramatic evidence that infants may respond abnormally to doses of drugs which are found to be appropriate for older children and adults. Comparative studies have shown ". . . a distinct parallel between human and rat (and other mammalian) development. Both are altricial, with a large part of physical and neurological development occurring in the postnatal period, and both pass through periods of intense hyperactivity in the normal course of development" (38).

Some examples of concomitant variations between trends in development of the cholinergic system and behavior may contribute to an understanding of mechanisms of action involved. Earlier it was noted that, in the rat, the synthesis of ACh from Ch is poorly developed in the neostriatum and the cortex during the first two weeks postpartum, with adult levels being reached between the third and fourth weeks. In isolation the rat shows hyperactivity at about 10 days postpartum which peaks at 15 days and declines precipitously to adult level by 20 to 25 days (38). An analogous concordance of events has been reported for behavior of the human child, the hyperactivity being observable at 18–20 months neonatally and decreasing sharply during the 3 to 5 year period (39). Such relationships suggest the hypothesis that activity of the cholinergic system is involved in suppression of behavior, probably acting in opposition to an excitatory system. Changes in the acquisition and retention of a new behavioral response also vary with age. In a typical experiment (40) rate of acquisition of a passive avoidance response increased significantly as a function of age; adults were also markedly superior in retention.

There is evidence that behavioral effects of pharmacological manipulation of specific components of the cholinergic system are dependent on the state of development. Administration of the ACh antagonist, scopolamine, to animals at neonatal ages from 10 to 15 days had no motor effect, but increased activity in those at 20 to 25 days postpartum (41, 42). Differential effects on spontaneous alternation behavior of manipulating the cholinergic system with scopolamine and the reversible anticholinesterase, physostigmine, have led to the conclusion that certain age-dose level relations ". . . were consistent with the hypothesis that cholinergic inhibitory mechanisms in the brain develop gradually . . ." (43): rates of responding were not disrupted by scopolamine nor facilitated by physostigmine at ages below about 24 days. Experiments involving administration of anticholinesterase agents have established that the young are generally much more susceptible

than adults (44–47). These agents, acting as indirect agonists, suppress behavior in the adult in a dose-dependent manner. Changes in median lethal dose (LD_{50}) values with age appear to be due to incomplete initial development of enzymes which catalyze metabolism of the agent. Effects of the direct ACh agonist, pilocarpine, in producing behavioral abnormalities, i.e. catalepsy, have also been shown to be related to developmental state (48): pilocarpine did not produce cataleptic responses in 10 day old rats, but did in animals 15 to 20 days old when cholinergic substrates of catalepsy appear to have reached functional maturity.

Trends in the development of the cholinergic system and of behavior show high concordances which suggest that the maturation of at least certain behaviors parallels maturation of the transmitter system. The findings also suggest that a role of cholinergic functioning within the CNS is to suppress or inhibit behavior.

CHOLINERGIC MECHANISMS IN THE ADULT

The vast majority of research into cholinergic mechanisms of action underlying behavior has chosen to study adult subjects, thereby eliminating confounding developmental processes of the kinds discussed above. As knowledge of the cholinergic system has been refined it has become possible to vary different components of the system with increasing selectivity, while measuring concomitant effects on behavior. In examining progress made using this general approach it is not intended to review the burgeoning literature in full. Examples chosen to illustrate various major points could, in most instances, be supplemented with others of similar value.

Precursor Availability

Levels of ACh in the mammalia CNS remain remarkably stable even under experimental manipulations of the cholinergic system, suggesting an effective mechanism for regulatory control (49). ACh synthesis in nerve terminals depends upon the availability of its precursors, Ch and AcCoA, at the proper site. This involves both sources of the precursors and their transport to the site. Progress in the development of knowledge about these neurochemical features of the cholinergic system has been examined in several recent reviews (50–52).

CHOLINE TRANSPORT Ch availability at the nerve terminal for synthesis of ACh is regulated by a high affinity transport system (HAChTS). The system is sodium- and temperature-dependent and appears to exist primarily in cholinergic nerve terminals, the sites of ACh synthesis. There also is

some evidence that a low affinity transport system may be involved in taking Ch to the sites, but this does not appear to be a major process in maintaining ACh levels in nerve endings (52). The generally accepted position at present is that the uptake of Ch by HAChTS is a rate limiting step in the synthesis of ACh.

Discovery of pharmacological means for manipulating the HAChTS has opened the way for experimental studies of its involvement in behavior. Hemicholinium-3 (HC-3) has served as the pharmacological tool in a recent series of experiments (53, 54). Injection by the cerebroventricular (i.vt.) route overcame the difficulty that HC-3 does not readily penetrate the blood-brain barrier when administered peripherally. By the i.vt. route HC-3 reaches the walls of the ventricles and accumulates in the caudate nucleus and the hippocampus, where it becomes associated with nerve endings and inhibits HAChT (55). Effects of doses given covered a range of ACh levels in whole brain from normal to 20% of normal, when the rate of decrease approaches an asymptote (56). Included in the behaviors studied were: multiple stimulus rating scales, which provided a standardized technique for measuring reactions to five different stimulus situations; reactivity in a situation involving inescapable electric shock; performance of a simple operant response under conditions of 23.5 hours water deprivation; and acquisition and retention of a discrete trial conditioned avoidance response (CAR). The possibility that any behavioral effects observed could be attributed to directly affected peripheral sensory or motor changes was unlikely because of the i.vt. route of administration. Analyses of the several behaviors showed that neither changes in specific motivational states nor generalized changes in motivation were affected by the HC-3 treatments. There were no significant differences among dosage groups in locomotor capabilities. Two hours after injection during peak effects of the drug, subjects at high dose levels showed very marked hyper-reactivity when exposed to the multiple stimulus assay conditions. In the CAR situation HC-3-treated animals who did not learn on day 1 showed very considerable savings in learning on day 2. In this instance inhibiting HAChT in brain affected the central processing of information: incoming information arrived centrally and was stored; deficiencies occurred in its readout, i.e. in the use to which it was put. Results of these kinds add further support to the conclusion that effects on behavior of manipulating the cholinergic system are selective rather than nonspecific.

There also is evidence that changes in behavior may affect high affinity Ch uptake (57), i.e. behavior affecting the uptake system as well as the system affecting behavior. The behavior observed consisted of a simple operant response for food reward. Experimental animals were given 9 days of practice; control subjects were not. Assays of hippocampi and striata,

involving incubation of homogenates with [^{3}H]-Ch, showed that Ch transport into striatal homogenates were not affected, but that transport into hippocampal tissue was very significantly greater for the experimental than the control subjects. "The results support the notion that the high affinity uptake of choline may vary depending on the behavioral situation" (57).

CHOLINE De novo synthesis of Ch in the mammalian brain takes place slowly, if at all. Two basic sources of Ch are available: Ch and phosphatidylcholine (lecithin) in the diet and the methylation of phosphatidylethanolamine in the liver to form lecithin (58, 59). Both lipid-bound and unbound forms of Ch are transported to brain via the blood. Phospholipids may be catabolized to free Ch. This general series of events is shown schematically in Figure 1. Present evidence supports the conclusion that the normal mammalian brain is amply supplied with Ch from these sources. Effects on behavior of manipulating the Ch supply either by augmenting ("loading") or by decreasing its normal sources is now attracting considerable attention.

Evidence has been accumulating for many years that cholinergic functions are involved in the behavioral processes of learning and memory (15, 60). Memory impairment occurring during the aging process is a particularly important aspect of these processes. Decreases in brain CAT activity and in muscarinic receptor binding indicates that both pre- and postsynaptic cholinergic degeneration may occur during the normal aging process, the changes being exaggerated in patients with Alzheimer's disease. In a recent review of precursor loading Jenden (51) has stated that, despite the abundance of Ch in normal brain, it is ". . . quite possible that pathological or functional states exist in which a temporary or extended deficiency may develop in the supply of Ch for ACh synthesis." The processes of aging may involve such a functional state. A recent report of studies designed to evaluate effects of dietary Ch on age-related changes in memory has provided information of particular interest in this regard. Central to the findings was the hypothesis that ". . . it might be possible to modulate the *rate* at which memory impairments occur with age by varying the availability of dietary choline" (61). Mice served as subjects, being placed on purified diets either deficient or enriched in free Ch. Four and a half months later they were trained and tested for retention in a single-trial, passive avoidance task. Their performance was compared with animals of various ages maintained on control diet. Results showed a dramatic deficit in retention in senescent animals and marked differences between the memories of the Ch-deficient and Ch-enriched groups, the latter (then 13 months old) performing as well as 3 month old mice and the former as poorly as the senescent animals. These findings suggest that the efficacy of precursor loading may be, at least in part, a function of the time frame over which

it occurs, and this, in turn, a function of the state of the mechanism(s) affecting its availability at the site of ACh synthesis.

The possibility that Ch loading may be effective in altering behavior when the cholinergic system is malfunctioning is illustrated in another series of experiments (62, 63) designed to study effects of deanol (2-dimethylaminoethanol). Under normal conditions deanol is a remote precursor of Ch, which, in large doses, increases Ch levels in plasma and brain but does not normally increase ACh levels (51). In the above experiments deanol administered by itself in otherwise untreated animals produced no change in any of the behaviors measured. However, when injected cerebroventricularly in combination with hemicholinium, the most potent synthetic inhibitor of HAChT, deanol suppressed behavioral effects of HC-3, i.e. suppressed hyper-reactivity. When ACh levels were depressed by interference with the HAChT, deanol compensated for abnormal behavioral consequences, although it had no behavioral effects under normal conditions.

The hypothesis that the cholinergic system may be precursor-dependent in its more general functioning is still under test. The results of one extensive series of experiments have provided evidence suggesting that Ch, administered either as Ch chloride or as phosphatidylcholine, ". . . by raising blood and brain Ch levels, can be a major determinant of the rate of ACh synthesis and, probably, of the amount of the neurotransmitter released when cholinergic neurons are depolarized" (64). That Ch and ACh levels are significantly elevated in the hippocampal area of the rat has been shown under conditions where subjects had ad lib access to a Ch-deficient diet for 8 days before being injected intraperitoneally with 60 mg/kg of Ch chloride in aqueous solution (65). Compared to comparable controls experimentally treated animals showed a significant elevation of both Ch and ACh levels in the hippocampus and caudate, the former reaching peak values about 20 min after injection. Other investigators have not been able to confirm the conclusion that Ch administration significantly alters brain ACh levels, although it may have other central cholinergic effects. In one such study (66) comparable groups of animal subjects were maintained for 2 weeks on one of the following diets: Ch-deficient, standard Ch, or Ch-supplemented. General activity was assessed during a 30 min trial in a symmetrical Y-maze. Analyses of various brain areas showed a general lack of effect of the increased Ch availability on ACh levels. Animals on Ch-deficient diet showed a decrease of ACh level to 85% of control values in the striatum only, a state restored by acute administration of Ch. No significant behavioral changes were noted in these subjects. By contrast, those with Ch-supplemented diets showed a significant hyperactivity (186% of controls), although their ACh levels were comparable to that of control subjects. "Results suggest that dietary choline is intimately involved with central

cholinergic mechanisms, but effects may not be mediated through alterations in neurotransmitter levels" (66). In these experiments measurement of changes in behavior provided clues about pharmacological effects which would not otherwise have been noted, and directed attention to the possibility that mechanisms of action other than those conventionally considered may be involved.

The possibility that "precursor loading" could be helpful in the treatment of behavior disorders which appear to be related to a cholinergic deficit has given rise to a burst of investigations using both patients (e.g. suffering from tardive dyskinesia and Huntington's disease) and normal human subjects. Evaluations of the present success of this therapeutic strategy (13) are equivocal, with suggestions that more needs to be known about the dynamics of the system in humans which provides the brain with Ch for ACh synthesis. Information from clinical sources does not yet provide the kinds of information about relations between Ch deficiency or loading and behavior required for precise statements to be made.

ACETYL-CoA As illustrated in Figure 1, glucose is normally the ultimate source of AcCoA required for the synthesis of ACh in mammalian brain and pyruvate is the immediate precursor. Radioactive glucose or pyruvate lead to formation of ACh labeled in the acetyl moiety under both in vitro and in vivo conditions. Maintenance of constant levels of ACh depends upon unimpaired production of AcCoA (67, 68), ACh synthesis being sensitive to reductions in glucose or pyruvate even when the concentrations of other products, e.g. ATP, remain unchanged.

There are behavioral concomitants of abnormalities in these events, as evidenced, for example, by the results of clinical investigations of such disorders as Wernicke-Korsakoff syndrome, Friedreich's ataxia, pyruvate decarboxylase deficiency, and hereditary intermittent cerebellar ataxia. In cases of the latter, symptoms appear under conditions of stress and are paralleled by increased pyruvate in blood, urine, and cerebrospinal fluid (69). Blass & Gibson (70) have recently summarized findings which indicate that even minimal impairment of glucose oxidation by the brain leads to impairment in ACh synthesis and may be reflected in impairment of behavior. Restrictions either on the supply of glucose or of oxygen affect the normal source of AcCoA which is dependent upon the oxidation of pyruvate derived from glucose by glycolysis. Early studies of its behavioral effects demonstrated that mild hypoxia impairs human performance of both psychomotor and cognitive tasks (71) and produces a general euphoria. More recent evidence has added deficiencies in judgment and memory to the list. There is evidence to suggest that impaired carbohydrate oxidation and impaired cholinergic function may occur in the brains of patients with

senile dementia. The wide range of behaviors affected indicates involvement of the glucose-pyruvate pathway generally and one of its products, AcCoA, in particular in the impairment of cerebral function. "It is tempting to speculate that damage to cholinergic systems due to chronic, low-grade hypoxia is a common mechanism in many common disorders including senile dementia" (70).

Efforts to study behavioral consequences of varying particular steps in the glucose to AcCoA pathway have been limited by the lack of precise pharmacological "tools." Studies of effects of thiamine (vitamin B_1) deficiency, the best known partial defect in the pyruvate dehydrogenase enzyme complex, on a variety of behavior patterns illustrate one attempt to be more specific (72). Three littermate-matched groups of rats were involved: one maintained on a thiamine-deficient diet; another caloric-control group on an adequate diet, but paired with animals in the first group for total daily food intake; and a general control group on ad-lib diet adequate in thiamine. Results showed differential effects on behavior. There were no significant effects of thiamine deficiency prior to the onset of polyneuritis on measures of general activity, maze learning, discrimination learning, or avoidance conditioning. The deficiency was, however, associated with exaggerated reactions during exposure to stress, when thiamine-deficient animals showed significantly greater response rigidity and displacement activity.

CAT Activity

As indicated in Figure 1, CAT catalyzes the final step in the synthesis of ACh. The point was made earlier that CAT appears unlikely to be a rate-limiting step in ACh synthesis because of its presence in large excess of maximum turnover rates of ACh and because its inhibition has little effect on ACh levels (73, 74). Despite this rationale several experiments have been designed to study behavioral effects of inhibiting CAT activity.

Pharmacological manipulations of CAT activity have used trans-4-(1-naphthylvinyl) pyridine (NVP) as a CAT inhibitor. Interpretation of results depends upon the specificity with which NVP acts, evidence for which is equivocal. In one study (75) reversible blocks of muricidal behavior in rats and fighting behavior in mice previously housed in isolation were observed. Treatment with NVP did not affect the hyper-irritability of rats with septal lesions. All these behaviors have often been termed "aggressive." The differences in effects of the treatment provide an opportunity to point out that generalizations about cholinergic involvement in some very broad category of behavior may be confusing and untenable. Other investigators (76) have reported that NVP administered i.vt. in mice impaired acquisition of passive avoidance responses. Observations that inhibition of CAT activity did not suppress hyper-reactivity but did impair learning are consistent with

behavioral effects of other manipulations which suppress activity of cholinergic systems and suggest that such systems in brain have inhibiting functions in controlling response output.

Reductions in both CAT and AChE activities occur with aging and have been reported in patients with Alzheimer's disease without losses in other enzymes involved in neurochemical processes. The changes were found to be located in the amygdala, hippocampus, and cerebral cortex. The possibility that they may be related to neuropathological effects on behavior and with intellectual impairment (15) suggests that the "... concept of Alzheimer's disease as a cholinergic system failure may have important consequences for research on this condition" (77).

ACh Release

Steps involved in the nerve-impulse release of ACh have been described in detail in several recent reviews (78, 79). The process may be inhibited by such biological "tools" as botulinum neurotoxin (Botx). In a series of experiments involving observations of several behavior patterns (R. W. Russell and C. B. Gundersen, submitted for publication) Botx was used for this purpose. Botx is a polypeptide neurotoxin which acts presynaptically to cause almost complete inhibition of stimulus-evoked transmitter release from cholinergic nerve endings (79). In the experiments Botx at doses ranging from 0.01 to 100.0 times the LD_{50} for intraperitoneal (i.p.) administration was injected directly into the ventricles of rat brain. Observations were made under two conditions, i.e. with and without the peripheral protection of antiserum. LD_{50}s estimated from linear log-probit plots showed a decrease in mortality of more than 30 times under the antiserum conditions. Low doses of the neurotoxin did not produce significant changes in normal behavior. As dose increased, Botx without antiserum was followed by a behavioral symptomatology dominated by flaccid paralysis and respiratory distress. At higher doses there were a few cases of bizarre behaviors, e.g. gnawing on cages, and some animals became hyper-reactive to stimuli in the various test situations. These signs were present only occasionally and then in very mild form in animals at high dose levels when the neurotoxin i.vt. was accompanied by antiserum i.p. Within the range of doses which did not induce some degree of incapacitation there were two observations worthy of special note. First, a finding that there were no significant dose-related differences in the number of trials to reach the criterion for acquisition of a conditioned avoidance response until the highest dose was reached suggests that there is a critical level for the inhibition of ACh release below which the cholinergic system is able to cope with behavioral demands, i.e. there is a margin of safety for the release process. The second point concerns the specificity of the behavioral effect when it

did occur: until they were incapacitated, animals persisted in making the innate escape response to shock at rates not significantly different from rates of control subjects, indicating that the effect of inhibiting ACh release was on the learned but not the innate response.

Inactivation of ACh

For ACh to be effective as a neurotransmitter it must be activated within the time limits required for the normal response characteristics of the biological units upon which it acts. As indicated in Figure 1, AChE is responsible for hydrolizing ACh which is released from the nerve terminal. Presumably inhibition of the inactivating enzyme leads to accumulation of endogenous ACh at its receptor sites and thus produces effects similar to excessive stimulation of receptors.

Anticholinesterase (anti-ChE) agents have received much attention from researchers and the general public because of their extensive applications as toxic agents, particularly in the form of agricultural insecticides. Reports (80, 81) of human occupational or adventitious exposures describe general symptoms which include: loss in discrimination performance, difficulty in concentration and expressing thoughts, confusion, disorientation, and signs of anxiety and depression. Early experiments (26) using animal models concluded that exposure to anti-ChEs produced differential effects on behavior, some behavior patterns being affected and others not. Behaviors affected involved the extinction of old responses which were no longer appropriate in coping with new environmental demands. Dose-effect data revealed a critical brain AChE activity level at about 45% of normal below which the behavior was significantly affected. These behaviors were considerably more sensitive to effects of anti-ChEs than were other, pathological signs. Such observations have been replicated in many more recent studies of a variety of anti-ChE agents using different routes of entry and more sophisticated behavioral analyses. Improved techniques for neurochemical analyses of effects of anticholinesterase agents on the cholinergic system have been applied in the investigation of relations between neurochemical and behavioral variables. The search for mechanisms of action involved in the development of behavioral tolerance to chronic decreases in AChE activity illustrates the direction in which research is now proceeding.

Observations involving both human subjects and animal models have shown that tolerance develops during chronic exposure to ChE inhibitors. Traditionally tolerance has been defined in terms of three major characteristics: an acute change in the criterion variable, a diminution in the effect with repeated administration of a fixed dose, and reinstatement of the original effect by an increase in dose. To these may be added the fact that tolerant subjects react quite differently than nontolerant subjects to challenges by

other chemical agents. Results of investigations of chronic exposures to anti-ChE compounds with different chemical structures have been sufficiently similar to warrant a general summary of their effects. Early parasympathomimetic signs of reduced ChE activity decrease with chronicity of treatment and disappear even as the enzyme activity continues to be depressed, as evidenced in serum, red blood cells, and brain. Measurements of a wide variety of innate and acquired behaviors have shown that the same chronic treatment leads to differential effects, i.e. some behaviors show tolerance and others do not. For those variables that do develop tolerance there are limits when exposures are sufficiently high to exceed the plasticity of the system and to produce gross pathological signs and incapacitation. Below these limits, magnitude of acute effects and duration of exposure (before tolerance is complete) are dose-dependent (82). Studies in which subjects who have recovered to pretreatment behavioral baselines have been "challenged" with other agents have made it quite clear that there remain long lasting biochemical effects in the absence of overt symptoms.

Several hypotheses have been proposed about the neurochemical mechanism(s) of action underlying tolerance development to anti-ChE agents. Among those eliminated after tests of their validity have been: nonspecific metabolic changes, end-product inhibition of ACh synthesis, and neurochemical redundance with activity shunted into another, noncholinergic pathway (83). The possibility that reduction in AChE activity could induce changes at presynaptic subcellular levels has been studied using in vitro and in vivo preparations. The former studies involved the use of techniques for subcellular fractionation and for integrated gas chromatography mass spectrometry to measure endogenous and tracer variants of Ch and ACh in synaptosomes prepared from the brains of rats at various stages of tolerance development (84). No statistically significant differences were found among treatment groups in the total concentration of ACh or Ch, the synthesis of ACh, or the high affinity transport of Ch. In the in vivo studies (85) rats at the same stages of tolerance were given pulse intravenous injections of $[^2H_4]$-Ch 1 min before death by microwave irradiation of the head. Homogenates from the whole brain or from regions were assayed by GCMS. Significant increases (15% above control values) in total brain ACh were seen 4–48 hr after an acute injection and after 1–22 days of chronic administration. Total brain Ch did not vary concomitantly with the development of tolerance: levels were significantly decreased for 4–24 hr and were significantly greater than control values 10 and 22 days after chronic administration. No changes were seen in ACh synthesis or Ch uptake, indicating that the behavioral tolerance could not be due to end-product inhibition. Psychopharmacological evidence suggested (26) that the more probable mechanisms(s) of tolerance to anti-ChE compounds might involve changes in

muscarinic receptors. Direct evidence has come from several laboratories that postsynaptic mAChRs are indeed involved: tolerance development is associated with a decrease in numbers of postsynaptic mAChRs (86). Whether the affinity of receptors for agonists may also change requires further study (87). The possible involvement of presynaptic receptors is unknown.

Examples of this kind indicate the complexities of a search for mechanisms of action which may be involved when manipulation of one component of the cholinergic system is reflected in changes of behavior. Yet it is through such manipulations of the system that the nature of its relation to behavior may be understood.

Cholinergic Receptors

Evidence has already been presented which indicates that some forms of behavioral plasticity are related to changes in cholinergic receptors, e.g. behavioral changes during early ontogenesis, tolerance development, and pathological conditions such as Huntington's disease. The existence of specialized macromolecular components, receptors, on postjunctional membranes is an essential element in neurohumoral transmission. Development of techniques for specific binding of radiolabeled ligands to particular receptors provides a means of quantifying receptors and thus of determining concomitant variations in them and in measures of behavior. Receptor adaptations to the kinds of centrally acting drugs which are used as tools in manipulations of behavior have recently been examined in detail (88).

NICOTINIC RECEPTOR Although the present review is concerned primarily with muscarinic receptors, no discussion of roles of the cholinergic system in behavior would be complete without some reference to advances in knowledge about nicotinic receptors (nAChR). nAChRs of skeletal muscle are involved in all overt behavioral acts. Advances in knowledge about nAChRs have resulted primarily from the availability of alpha-neurotoxins isolated from snake venom, which bind with high affinity to the ACh binding site of nAChRs of muscle. Radiolabeled alpha bungarotoxin, a nicotinic antagonist, is widely used for research purposes.

The most striking illustration of the roles of nAChRs in behavior has come from recent studies of myasthenia gravis, a disorder involving progressive muscular weakness which may gradually increase until fatal (89). Normal nerve transmission between motor nerves and muscle is blocked. Some 10 years ago it was observed that there occurred a decrease in nAChRs at neuromuscular junctions in myasthenia gravis (90). It was also observed that rabbits immunized with purified nAChR protein developed muscular weakness similar to that characteristic of the disorder (91). The

subsequent development of a valid experimental model has added further evidence to clinical studies that the pathophysiology of myasthenia gravis has an autoimmune basis. Anti-nAChR antibodies, found in the sera of patients suffering from the disorder, play a central role in impairing neuromuscular transmission. The effect is a deficit on the motor output side of behavior. Evidence has also been presented that anti-nAChR antibodies may have CNS effects (92).

MUSCARINIC RECEPTOR The fact that it has not been feasible to discuss effects on behavior of other features of the cholinergic system without referring to mAChRs testifies to the essential roles served by the receptors in cholinergic system-behavior interactions. Although increasing attention to these roles can be predicted for the future, few so far meet the requirement of presenting data on *both* alterations in receptors and tolerance development under identical drug conditions. Specifications for the proper research design of studies in this area have been proposed by Overstreet & Yamamura (93).

In addition to the examples of involvement of mAChRs in cholinergic system-behavior relations discussed above, two further matters raised in the recent literature deserve attention. The suggestion has been made that mAChRs may be involved in the tolerance and physical dependence that develop to such agents as ethanol and barbiturates. In a study (94) of mice exposed to chronic ethanol treatment that produced physical dependence, [^{3}H]-QNB binding in the hippocampal and cortical areas of withdrawn animals revealed a significant elevation in numbers of mAChRs. There was a return to normal in 24 hr, when behavioral signs of withdrawal, e.g. seizures and tremors, were no longer evident. Results of another study (95) show that 3 days of abstinence following chronic barbiturate treatment to rats was associated with significant increases in [^{3}H]-QNB binding sites in tissue from the striatum and from the midbrain-medulla-cerebellum. Saturation studies pointed to significantly more mAChR (B_{max} 56% above control values) in the latter. No differences were found in affinity. Earlier findings that tolerance to barbiturates is associated with supersensitivity to pilocarpine and that HC-3 prevents and reverses tolerance to them has indicated a role for ACh in the development of tolerance to this class of compounds. Cholinergic receptors may be involved in the process. Such involvement suggests the possibility of interactions at the receptor level between cholinergic and noncholinergic neurotransmitter systems in the overall mechanism of action of drugs like the barbiturates. For example, GABA-like effects associated with the barbiturates do not preclude selective interaction with the cholinergic system. This kind of interaction has recently been reported between cholinergic and dopaminergic system

in vitro; dopamine agonists modify the binding properties of striatal mAChR (96).

Another matter of importance concerns the reported involvement of cholinergic receptors in the aging process: "The determination of postsynaptic cholinergic receptor density is a quantitative chemical correlate of aging in the rodent brain" (97). Earlier in the present review evidence was discussed for increases in QNB binding sites during early ontogenesis, changes which parallel behavioral development. The decline of behavioral functions with normal or pathological aging suggests that these developmental trends in the CNS may be reversed during aging. Evidence from research with animal models indicates that the number of nuerons in the brain does not decline as a function of age (98). Experiments (97) designed to study the possibility that more subtle changes may be involved have shown decreases in numbers of [^{3}H]-QNB binding sites after mice had reached the age of 18 months. There were no changes in affinity. It is proposed that: "When receptor numbers are reduced to a critical level, functional impairment will begin to appear and parallel the further decrease of receptors" (97).

MORPHOLOGICAL DETERMINANTS

The preceding discussion has emphasized the dynamics of neurochemical events in the cholinergic system. Clearly manipulation of any one of these events may affect a wide range of behaviors, yet not all behaviors are affected simultaneously. More specific relations exist between the neurochemical event, the behavior elicited, and the demands of the situation with which the organism must cope. It has been implied above that such specificity is related to the site of action, as well as to the mechanism of action. The unique distributions of various transmitter pathways and the complexities of their interactions are apparent in histochemical studies of the brain (99). Mapping of mAChRs has become possible using autoradiographic localization of [^{3}H]-QNB binding sites (100), making morphological localization of the cholinergic system even more specific. The importance of interactions between behavioral, biochemical, and morphological properties is a keystone in the "systems" approach to the study of living organisms: "Neuroanatomical features are just as important as the biochemical in the neurochemistry of behavior" (16).

The experimental approach of stimulating CNS sites with choline agonists and antagonists has produced evidence of relations between neurochemical events in the sites and specific behavior patterns. Results of this approach are illustrated in experiments designed to study innate behaviors, studies which gave rise to a concept of "neurochemical coding of behavior"

(2, 9, 101). Early in the research it was discovered that differential chemical stimulation in the same circumscribed area of the hypothalamus elicited quite distinct and specific changes in certain innate behaviors. In satiated animal subjects cholinergic stimulation evoked drinking behavior and adrenergic stimulation evoked eating behavior (102). Evidence was later presented to show that direct cholinergic stimulation could be equivalent to deprivation in eliciting motivated behavior in support of the acquisition, retention, and extinction of new responses when water served as the reinforcement (103). By varying the intensity of stimulation (dose level of ACh or ACh agonist), dose-effect relationships for drinking behavior were found to be ogival in shape, fitted neatly by straight line log-probit plots. Such relationships would be expected to occur if there were a population of cholinergically-coded units involved in determining the total behavioral response output and if their thresholds were normally or independently distributed (2). As might be expected, the story has become more complex as new research has uncovered modulating processes and interactions between circuits with different codings (9). However, the point still remains that certain precise forms of behaviors may be elicited by stimulation of particular cholinoceptive neural circuits.

Another approach to the morphological localization of such interactions is to lesion particular CNS sites and to measure consequent effects on neurochemical events and on behavior. For example, it has been shown that in the rat, septal lesions are followed by significant neurochemical changes in the hippocampus, which receives cholinergic input from the septum. Destruction of the input reduces endogenous hippocampal ACh levels by more than 80%, Ch levels by 20–32%, and CAT activity by 85–90% (104). Maximum effects occurred by 4 days post-lesion and persisted when assayed 2 months later. These neurochemical effects are paralleled by significant changes in behavior. In the rat septal lesions produce hyperdypsia, daily water intake increases by about 50%, with trend analyses indicating a highly significant Pearson product-moment correlation coefficient of -0.73 (105). Septal lesions are also associated with other behavioral effects, one of the most prominent being hyper-reactivity to environmental stimulation, often interpreted as "aggressive behavior." The picture becomes complicated because hyper-reactivity disappears before other features of the septal syndrome, a phenomenon which could be accounted for in terms of compensatory changes in the cholinergic system or its interactions with other systems.

Studies of human clinical material buttress still further the importance of interactions between behavior, neurochemical events, and morphological sites of action. Patients with Huntington's disease, characterized behaviorally by involuntary choreiform movements and dementia, have been found

to have severe degeneration of neurons within the basal ganglia, whose neurotransmitters are ACh and gamma-aminobutyric acid. There occur marked reductions of [^{3}H]-QNB binding in the caudate nucleus and putamen of choreic brains, with no significant alterations reported in other brain areas (32). Patients suffering from Alzheimer's disease have significant losses in CAT activity in the caudate, putamen, frontal cortex, and hippocampus, with significant decreases in specific QNB binding in the hippocampus but not in other areas (106). "Behavioral correlates of cortical and hippocampal lesions make the cholinergic losses in these areas particularly relevant to the cognitive abnormalities of patients with Alzheimer's disease" (107).

There are many other examples from both preclinical and clinical research which support the view that cholinergic events occurring in particular areas of the nervous system are involved in providing substrates for specific behaviors (26).

CONCLUSIONS

Early in this review attention was called to the ubiquitous nature of the cholinergic system: "Most impressive is the singular fact that ACh is the only substance that can influence every physiological or behavioral response thus far examined" (9). From central to peripheral neurohumoral functions and from simple to complex behavioral acts the cholinergic system plays an essential role in the capability of living organisms to cope with the demands of constantly varying internal and external enviornments. Coping requires a finely tuned plasticity of cholinergic system-behavior interactions, which function well within their normal limits. Behavior is very sensitive to conditions in which the limits are exceeded, giving rise to abnormalities of clinical concern, and adverse effects of exposures to environmental chemicals which affect the cholinergic system are leading to the development of a discipline of behavioral toxicology (108).

It is clear from both experimental and clinical research that behavior may be affected by variations at any point in the cholinergic system. Variations at the same point may, under some conditions, provide the mechanism underlying normal adjustments to environmental circumstances (e.g. decrease in mAChRs during the adaptive process of tolerance development), yet under other conditions be involved in behavioral maladjustment (e.g. decrease in mAChRs in Huntington's and Alzheimer's disease). Although all features of the system are involved, some have greater margins of safety than others in the system's normal functioning as a substrate of behavior, e.g. behavioral effects of decreases in AChE activity do not appear until a

reduction of approximately 55% occurs. Central to its interactions with behavior are its sites of action. Different behaviors involve different sites. The cholinergic system is more selective than to exert its influence on some imaginary conglomerate described as "Behavior."

There is a danger in presenting a case of the present kind that one subsystem within the organism will appear to be operating on its own. The cholinergic system is *essential* to normal behavior, but it is *not sufficient* to support any behavior on its own. It has been possible in so brief a review to consider the wealth of interactions which may involve the cholinergic system with many other processes in even the simplest of behaviors.

Behavioral effects of variations in the cholinergic system are evidenced and have been measured as a multiplicity of different specific acts. General statements about mechanisms of action require that the many specific behaviors discussed in the present paper and the many more to be found in the literature be categorized in some meaningful manner. Behavioral biology has followed the course of other sciences by introducing theoretical constructs which serve to organize empirically similar behaviors into composite classes. Application of this approach in the present discussion suggests certain general statements. The cholinergic system is involved at the sensory input side, mediating selection from the mass of impinging stimuli those to which the organism will respond, a process known as "attention." The system is involved centrally with the processing, storage, and use of information in "learning" and "memory." It also contributes centrally to persistent behavioral traits constituting the "affective" characteristics of the individual organism, e.g. "mood" and "emotion." On the output side the system is not only responsible for functioning at neuromuscular junctions but is involved in the suppression or facilitation of behaviors which are already available in an organism's response repertoire.

ACKNOWLEDGMENTS

I wish to express my appreciation to the UCLA School of Medicine and especially to Dr. Donald J. Jenden, Professor and Chairman of the Department of Pharmacology, for the opportunities provided me to carry out my investigations of relations between activity of the cholinergic neurotransmitter system and behavior. Results of this research and of other recent studies conducted at the Flinders University of South Australia with support from the Australian Research Grants Committee are reported in the present review. Dr. Grant D. Schiller was a constructive critic as the manuscript developed. I am also indebted to Mrs. Flo Comes for her invaluable editorial work during final preparation of the manuscript.

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