PHARMACOLOGY OF CENTRAL SYNAPSES[1,2]

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At synapses, morphology and histochemistry blend with pharmacology and electrophysiology to provide a description of the transmission process. Because of this disciplinary convergence, it appeared to us that a review article dealing exclusively with the pharmacological aspects of central-synaptic transmission might be inadequate and potentially misleading. Hence, we have reviewed the available evidence with an interdisciplinary perspective, although ultimately relying in large measure upon morphological and biochemical sources. In the field of pharmacology proper, we have restricted our survey to investigations aimed at the characterization of central transmitters. These efforts, begun with the well-known contributions of Amin, Crawford & Gaddum (1), Feldberg (132), Vogt (305), Brodie & Shore (36), von Euler (124), Bogdanski et al. (30), and Carlsson et al. (53), have been lately directed toward the recognition of transmitters operating specific central synapses, through the use of a technique developed by Curtis & Eccles (75) that makes it possible to record the bioelectrical activity of a given neuron while administering a drug in its immediate extracellular environment. This approach simplifies recognition of the site of drug action, and, for this reason, we have confined our report to this type of pharmacological evidence. Additionally, we drew from studies on synapses in the peripheral nervous system, whenever similar information on central synapses was unavailable or inadequate. In these choices, we were guided principally by our own current interests. This bias and the editorial restrictions on space must have caused us to omit much that other reviewers would have stressed or, at least, included. We find comfort in the fact that the subject of central-synaptic transmission has been treated extensively in two recent books (116, 223) and in several review articles (16, 71, 113–115, 134, 145, 160, 212, 267, 268) which should be consulted for additional factual information and viewpoints.

It is currently assumed that central synapses, in the main, operate chemically through the release of transmitter substances from presynaptic nerve endings. Morphologically, most central synapses join axon terminals to dendrites (97, 150, 151, 154, 165, 180, 238–240, 245), but axosomatic

[1] The survey of literature pertaining to this review was completed in June, 1964.

[2] The following abbreviations are used: ACh (acetylcholine); ATP (adenosine triphosphate); CNS (central nervous system); DOPAmine (dihydroxyphenylethylamine); GABA (γ-aminobutyric acid); 5-HT (serotonin); NE (norepinephrine); and substance P (allegopregnam-3β,17α,20α-triol).

synapses are not uncommon (29, 97, 98, 172, 207, 224), and dendrodendritic (303, 304) and axo-axonic synapses also have been observed (28, 152, 289). The morphological and functional complexity of central synapses is hardly amenable to broad generalizations. However, strictly for the sake of expediency, we have organized our presentation on the basis of the morphological components observable at all central synapses.

Axon Terminals

Constituents.—Electron microscopy has shown axon terminals to contain mitochondria, small amounts of axoplasm, and a large number of spherical outlines some 400 Å in diameter—the synaptic vesicles (102, 150, 151, 238, 239). Since the latter retain circular outlines regardless of the plane of sectioning (240) and stereoscopic specimen tilting (257) and since they can be visualized as spheres after their extrusion from peripheral nerves (125), there is little doubt that they exist as such and do not represent cross-sectional outlines of endoplasmic tubules. Moreover, the existence of vesicles as true morphological and functional entities is supported by *in vitro* studies of monoamines uptake (219, 287). Finally, although ultraminced hydrophobic lipoprotein membranes do tend to form spherical micelles when placed in aqueous phase, such synthetic vesicles are more heterogeneous in appearance than true synaptic vesicles (315). Neurofilaments and other complex endocellular structures also may be present in nerve endings, depending upon the animal species, the area of the central nervous system (CNS) examined, and the method of tissue fixation utilized (32). Partial biochemical characterization of some of these nerve-ending components has become possible through the introduction of subcellular fractionation procedures (170, 310–312).

Fractionation studies.—Initially, the endogenous biogenic amines acetylcholine (ACh), norepinephrine (NE), serotonin (5-HT), and dihydroxyphenylethylamine (DOPAmine) appeared to be present in brain fractions containing mitochondria (54, 99, 103, 104, 227, 242, 248, 307, 308, 311, 315). Improvements in the fractionation procedure and especially the development of the density gradient technique (310) led to the separation of a fraction with the highest concentration of nondiffusible amines, which electron microscopy showed to consist mainly of pinched-off nerve endings (155), recently named synaptosomes (315). These particles are separable from myelin and mitochondria by virtue of their intermediate density (105, 106, 315). The nerve endings in brain tissue apparently possess sufficient mechanical strength (312) to withstand tissue homogenization. Hence, although torn away from their axons and postsynaptic elements during this procedure, they remain sufficiently intact to enclose the terminal axoplasm, the vesicles, and one or more mitochondria; and they often carry with them a portion of the subsynaptic membrane (97, 105, 106, 312, 314).

Because of the variety of synaptic sites present in any sample of brain tissue, the synaptosome fractions from homogenates of the whole brain or

even from particular regions of the brain can hardly be expected to be morphologically or functionally homogeneous (219, 312). These fractions, moreover, may be contaminated by other brain constituents. Hence, biochemical studies on synaptosome fractions require checks on the purity of the sample to remove doubts on the potential contribution of mitochondria, microsomes, and other contaminants. Electron microscopy can be used for this purpose (100, 245, 315); enzymatic activities known to be associated with either microsomal or mitochondrial structures (104, 105, 181, 315) also have been used as markers for checks on purity (215, 248–250, 262). Preferably, both methods should be employed concomitantly, since each is subject to interpretative pitfalls. Positive staining of synaptosomes to increase tissue contrast for electron microscopy can lead to extensive disruption of synaptosomes and to the release of their vesicular content (315), making some fractions appear to be falsely homogeneous. Negative staining of synaptosomes, however, would not seem to have this disadvantage (312). Chemical markers can be used confidently, only if the selected enzymes can be shown to be highly specific for particular intracellular components.

Harvested synaptosomes are very labile and can be delivered of their contents by freezing and thawing (262), hypotonic media (225, 227, 228, 246, 248, 249, 314), or sound (96, 220). Although attention is presently focused mainly on the relationship between possible transmitters and the synaptic vesicles, other studies are equally relevant. Hence, synaptic membranes and mitochondria can be separated, and their biochemical properties can be individually investigated (312). Conversely, intact synaptosomes can be used to study the kinetics of uptake of endogenous amines (219, 310). The latter approach, however, suffers from the limitations imposed by the heterogeneity of the central nerve endings contained in synaptosome fractions. In the periphery, where greater homogeneity of nerve endings could be assumed, synaptosomes are, unfortunately, not a component of subcellular fractionation (226).

Vesicles and Transmitters

ACh (100, 103, 202, 229, 260, 310, 315), NE (96, 100, 220, 229, 249), 5-HT (96, 100, 219, 220, 228, 229, 260, 262, 263), histamine (47, 225), DOPAmine (202), and substance P (263, 311, 312) have been shown to be associated with (or bound to) vesicles liberated from synaptosomes obtained from homogenized brains of mice (309), rats (246, 250, 260), guinea pig (225, 227, 229, 262, 263, 310), rabbit (310), dog (202, 225), sheep (262, 263), pig, and cow (96, 220). Glutamate and γ-aminobutyric acid (GABA) are present in all fractions and do not appear to be more concentrated in synaptosomes. Hence, they are thought to be present in the cytoplasm in a free form (263, 312, 321). Absence of localization of a substance in synaptosomes would *a priori* exclude its transmitter potentiality. Nevertheless, the possibility that glutamate and GABA also may be bound in intact nerve endings and simply more easily releasable during separation procedures has not yet been

ruled out. The bound amines cling to the vesicles until set free by the physical treatment, some being more easily releasable than others (202, 263, 315): DOPAmine > Norepinephrine > Histamine > 5-HT > ACh.

Acetylcholine appears to be associated with larger more translucent vesicles (157), while the smaller more dense vesicles seen or isolated from pineal gland (246, 316, 321), hypothalamus (96), cuneate nucleus (274), and spinal cord (29) may be related to catecholamines (220, 254, 255). However, synaptic terminals of both central (224, 274) and peripheral junctions (254, 255) are often found to contain more than one type of vesicle. This observation seems to imply that more than one transmitter may be stored in a single peripheral or central nerve ending, in contrast with what one would expect on the basis of Dale's law (87, 112, 115). From pharmacological and histochemical evidence, Burn & Rand (39–42) and Koelle (190–192) have independently proposed a two-step transmission process for peripheral autonomic nerve endings: ACh released from a nerve ending would act on the same nerve ending to release more ACh (190, 306) or norepinephrine (39–42) which would subsequently affect the postsynaptic site. Although the pharmacological evidence for this hypothesis has not gone unquestioned (244), a multiple-step transmission sequence at certain central synapses is an intriguing possibility.

Cellular Site of Origin of Transmitter

The cellular site of origin of enzymes for transmitter synthesis is unclear, but it has been shown that tyrosinase (234), decarboxylase (100), and DOPAmine-β-oxidase (188) are bound to vesicles. It has been proposed that vesicles could originate from the Golgi apparatus (302) or from mitochondria (149) and may be subsequently transported down neurotubules (101, 271, 302) through the axoplasm. Recent histochemical studies on hypothalamus (50, 127), spinal cord (49), and sympathetic ganglia (162, 164) utilize the formation of fluorescent material occurring when catecholamines and 5-HT-containing tissue are exposed to formaldehyde. Catecholamines are transformed into 3-4-dihydroisoquinolines (127) yielding a yellow-green fluorescence, while triptamine is transformed into 3-4-dihydronorharman (126, 127) which causes a yellow fluorescence. In monaminergic nerves, there is a diffuse fluorescence of cell bodies and axons but a more intense fluorescence at various sites which are thought to correspond to synaptic contacts, while no fluorescence is usually observed in the preterminal part of the axon (127). If the transmitter is the source of the fluorescence, these findings suggest that the transmitter is not manufactured exclusively in the nerve ending. In the periphery, the enzymes for norepinephrine biosynthesis are contained within the vesicles that store catecholamines (234), suggesting a close functional relationship between storage and biosynthesis (58). The same would not seem applicable to ACh, since, in cholinergic nerves, cholinacetylase is not found associated with the storage vesicles (312).

The presence of vesicles in a given presynaptic terminal is usually

regarded as strong suggestive evidence for chemical transmission at that synapse. However, vesicles morphologically similar to those seen in presynaptic terminals have also been found postsynaptically (258), as well as in presynaptic terminals (17, 167, 258) at certain invertebrate junctions where there is evidence for electrical rather than chemical transmission (16, 17, 139–143, 158, 217, 218, 257–259). It has also been reported that subcellular fractions of rat brain (presumably vesicles) take up and bind added ACh (45) or 5-HT (215) with high affinity. Considering that vesicular sequestration might be a general cytological phenomenon, it is clear that morphological similarities among vesicles cannot be automatically equated with identity of their biochemical constitution or function. On the other hand, binding, in the jargon of biological sciences, covers such an array of biochemical mechanisms (from nonspecific adsorption to highly specialized, stereospecific, transport mechanisms) that the demonstration of *in vitro* uptake does not necessarily reflect a functionally significant biological phenomenon. At any rate, the possibility that the vesicles could also function in the re-uptake of the transmitter after its release should be considered, particularly in view of recent evidence (12, 31, 37, 174) indicating that reuptake and storage are an important process in the inactivation of norepinephrine released from peripheral nerve endings.

Presynaptic Membrane

Ultimately, the transmitter must cross the presynaptic membrane and the synaptic cleft, en route to its postsynaptic site of action. Information on the fine morphology of the presynaptic membrane is now available (98, 105, 151, 165, 166, 313), but practically nothing is known of its biochemical properties. While there is evidence (108, 177, 183) that Ca^{++} is an important cofactor in the passage of transmitter across the presynaptic membrane, the modality of its participation is, as yet, undetermined. Conceivably, the synaptosomes may provide suitable material for studies of this type.

Synaptic Cleft

If the movement of the transmitter within the synaptic cleft takes place by passive diffusion (112, 116), it is clear that the kinetics of the diffusion should be influenced by the geometry of the cleft and, at least for ACh, by the spatial distribution of catabolic enzymes. Despite variations introduced by the type of fixative used and the technique of its administration (151, 154, 165, 256, 273), a cleft width of 100 to 300 Å is commonly observed, although tighter junctions have been described (16, 17, 257–259; cf. also 241) in structures for which there is physiological evidence of electrical transmission. As for the shape of synaptic clefts, various relationships of presynaptic and postsynaptic elements have been reported (97), and intrasynaptic networks or channels have been described (103). Histochemical data on the synaptic distribution of cholinesterase at central synapses are still meager (95, 147, 189, 277, 298), but studies on its distribution at periph-

eral synapses show that this enzyme may be located on either presynaptic or postsynaptic membranes or on both presynaptic and postsynaptic elements, depending upon the structure studied (192). Were this finding applicable to central synapses, it is conceivable that variations in the spatial distribution of catabolic enzymes and/or in the shape of synaptic clefts could contribute to variations in the duration of synaptic potentials, for example between those of spinal motor (112) and of pyramidal hippocampal neurons (116, 182). Other factors, such as active re-uptake of the transmitter in nerve endings (12, 37, 174) and the association constant between transmitter and receptor sites may similarly contribute to these differences. Moreover, the suggestion (112) that the action potential itself may considerably aid in the removal of transmitters of appropriate charge from the receptor sites also may be considered.

The Nerve Ending as a Functional Unit

We have, thus far, spoken of the nerve ending as an aggregate of parts. Since each of these component parts contributes to the properties of the nerve endings, it is fairly obvious that each should be considered as a potential site of drug interaction. Nevertheless, the nerve ending operates as one functional unit for the secretion of a transmitter at chemically mediated synapses. The nature and sequence of the biochemical steps in transmitter release at central synapses are unknown. Current views on this subject must perforce be extrapolated from the available studies [recently extensively reviewed (35, 55, 57, 58, 306, 320)] on peripheral cholinergic and adrenergic synapses, since only limited information is available (38, 300) on norepinephrine turnover in the central nervous system. These studies are briefly considered here as a potentially useful paradigm but it is with the understanding that their applicability to central transmission is mostly conjectural at the present time. It should be noted, moreover, that occasional difficulties arise in the interpretation of kinetic studies on peripheral synapses with tracer doses of radioisotopically labeled norepinephrine because of uncertainties regarding the meaning given by different workers to the term tracer dose (55). In order for studies of this type to yield physiologically relevant data, it would seem that the tracer dose used should be below that required for threshold pharmacological effects. Additionally, the stereospecificity of uptake mechanisms should be borne in mind (214, 235) and the results interpreted considering the contribution made to the early part of the time curves of labeled noripinephrine tissue levels by the *d* form present in the racemic mixtures of the injected radioisotope (214). Lastly, without denying the usefulness of investigations carried out in non-steady-state conditions, it would seem that turnover rates of transmitters may best be studied after equilibration has been achieved (58, 232, 300). Nevertheless, studies with labeled norepinephrine, on the whole, indicate that the neurohormone is far from being just stored in nerve endings in a static way but is, instead, in a constant state of rapid flux between intracellular compartments (58).

The turnover rate, binding, and metabolism of norepinephrine in different intracellular compartments can be studied following parenteral administration of labeled norepinephrine of high specific activity (38, 58, 65, 193, 230, 282) or of a labeled precursor (300). The diphasic decline of the specific activity in adrenergically innervated organs has been interpreted (58, 232) as evidence that endogenous norepinephrine is stored in an open two-compartment system, similar to that described by Zilversmit & Shore (322). Comparable experiments on ACh are hampered by the unavailability of highly labeled ACh and of sufficiently sensitive methods for its chemical determination (56), and, possibly, by a more limited participation of an uptake mechanism than found at the adrenergic nerve endings. However, calculations based on the rate of depletion and restoration of ACh in a sympathetic ganglion after treatment with hemicholinium (19, 212) seem to indicate that, in peripheral nerve endings, ACh also is stored in more than one intracellular compartment.

The available data can be interpreted to indicate that newly synthesized molecules of norepinephrine and ACh diffuse from the site of synthesis into a storage pool where the neurohormone is protected from metabolizing enzymes. This storage site presumably corresponds to synaptic vesicles where norepinephrine may be, in part, complexed with adenosine triphosphate (ATP), or a protein, or both (52, 249, 279, 287). Most of the norepinephrine stored in the nerve ending is not readily available for utilization (bound), although some must be readily available (free), held within the boundary of the nerve ending through a stereospecific mechanism (214) which freely acts on the amine (58). This mobile pool of transmitter would be in chemical equilibrium with the nonmobile portion. When utilization exceeds synthesis, the shift in equilibrium would cause mobilization of the transmitter from the storage compartment which, in turn, would increase the rate of biosynthesis until the steady-state level is restored. Since synthesis is continuous, when the utilization rate is reduced, the neurohormone would leak into the proximity of metabolizing enzymes.

Many variants of this scheme have been suggested (12, 35, 48, 59, 161, 174, 232, 276, 282, 300). Hopefully, these efforts will eventually lead to a better understanding of the mode of action of drugs and the mechanisms concerned with the synthesis, storage, and metabolism of transmitters at peripheral and central synapses. Of special usefulness would be drugs that block the synthesis of the transmitter, since they would facilitate the study of the kinetics of its uptake and utilization. It has been reported (19) that hemicholinium may block ACh synthesis by interfering with choline uptake, but it is also possible (211) that hemicholinium may act as a false transmitter. Tyrosine hydroxylase may be the limiting step of norepinephrine synthesis (234). If this were so, blockade of tryosine hydroxylase should then limit norepinephrine synthesis and indirectly produce exhaustion of the substrate if synaptic function is maintained. There is recent evidence (281) that this is a promising lead.

The Origin of Miniature Postsynaptic Potentials

It is currently believed (cf. 116) that miniature postsynaptic potentials recorded from striated (89–91, 109–111, 131, 203) and smooth (43, 44) muscle fibers, sympathetic ganglia (22, 23), and, apparently, from spinal and cortical motoneurons (184, 201, 204) represent the spontaneous discharge of synaptic vesicles (91, 183). Collision of a vesicle with the presynaptic membrane would result in the emptying of its content into the synaptic cleft, each vesicle thus contributing a quantum of transmitter (91, 183).

The scheme described in the previous section suggests an alternative explanation for the apparent quantal release of transmitter from nerve endings. The alternative would utilize the evidence that Ca^{++} has an ubiquitous role (cf. 107, 176) participating in the maintenance of transmembrane potential (236, 291, 292), transmitter release (183), and transmitter action at postsynaptic sites (122, 183). Ca^{++} could be pictured as occluding potential transmembrane channels through which the transmitter could leak out. In this case, any factor that would momentarily displace some of these ions from their membrane sites (e.g., a spontaneous process associated with the resting metabolism of the nerve cell or an action potential) could result in diffusion of transmitter ions from the mobile pool in the nerve ending along their concentration gradient. The kinetics of this escape would principally reflect the quantity of divalent ion displaced, the duration of the displacement, and the concentration gradient for the transmitter. In the resting state, these factors may be presumed to be relatively constant for any one nerve ending, thus giving the appearance of quantal transmitter release. One could, therefore, envision the vesicles merely as storage sites for restocking that portion of transmitter in the mobile pool utilized by the nerve activity.

The Postsynaptic Membrane

Resuming our analysis of the structural elements of the synapse, we proceed to the neuronal membrane. Two of its parts are readily recognizable morphologically: subsynaptic and nonsynaptic portions. In theory, the subsynaptic regions should be amenable to further classification on the basis of the kind of neurohormone by which they are activated, the direction of the response elicited by the neurohormone, and many related pharmacological criteria. In practice, the neuronal membrane continues to defy all attempts at its classification. Conceptually, separation of the postsynaptic membrane into at least two distinct parts (subsynaptic regions and the rest) seems unavoidable once some functional specialization of the subsynatpic region is postulated. However, such a specialization may actually be achieved, at least at the neuromuscular junction, through the suppression of transmitter receptiveness of the membrane adjacent to the subsynaptic portion (resulting somehow from the activity of the nerve ending) rather than through the possession by the subsynaptic membrane of specialized receptors (61). In this context, it may be noted that sensitivity of denervated muscle fibers to ACh has been

found to extend the length of the fiber once its motor supply has degenerated (185, 186). In recent years, Grundfest has collected a large body of evidence favoring a difference in the property of electrical responsiveness of subsynaptic and nonsynaptic regions (158–160). However, it is possible (16, 116) that differences in membrane responsiveness to drugs or to electrical stimuli may reside in the molecular makeup of individual pores of the membrane, which may be well dispersed among pores of different characteristics rather than conglomerated in certain areas or patches of the membrane as conventionally assumed. Should this be the case, it would seem that exploration of the physicochemical matrix for the response may well be beyond our present technology.

The apparent lack of ultrastructural differentiation between contiguous portions of the cell membrane confronts us with the dilemma that, although we cannot identify its receptors, we have impressive indirect evidence for their existence as functional entities (138). Yet, for the present, we can only describe them in operational terms, that is: (*a*) the receptor is assumed to be a specific molecular structure of the effector cell; and (*b*) the drug must react at this site to elicit a specific cellular response. The latter would reflect either the number of receptors occupied (9, 10, 11, 138) or the rate of association between drug and receptor (243). Needless to say, these concepts do not uncover the essence of the phenomenon but merely provide a formal description of the relevant kinetic data. From the electrophysiological standpoint, the occurrence of alterations in transmembrane potential, resulting from a redistribution of ions due to changes in membrane conductance brought about by the transmitter, provides convenient means for the detection of the phenomenon and a vocabulary for its description. Yet, the cause and effect relationship between these ionic fluxes and the biochemical event at the synapse remains conjectural (113, 129). Bearing in mind the possible functional significance of the subsynaptic cytoplasmic specializations in the form of plates (97), cones (153), vacuoles (29, 165, 319), and lamellated bodies (173) that have been described, one wonders if the membrane-conductance changes brought about by the transmitter may not be just a fortunate epiphenomenon of its action. If, on the other hand, bioelectric ion redistribution is an essential phase of synaptic function, as the available evidence overwhelmingly suggests (112–116), it still may not be an end in itself. Considering the large changes in the output of transmitter from nerve endings resulting from experimentally (90, 205) or synaptically induced (117–120, 135) changes in their resting potential, one wonders if ionic shifts across the cell membrane may not also assist in the regulation of the metabolism of the cell through enzyme activation, free-water redistribution, or other parameters of cell function.

Glia

Lastly, let us at least be aware of the glia. By various estimates they are the most numerous cells in the brain (46, 148, 165), and they undoubtedly

contribute to subcellular brain fractions, although the extent of their contamination is unknown and generally not considered (309). An intimate glial-neuronal interdependence has been repeatedly suggested (146, 148, 178, 179). Such interdependence is indicated by several lines of evidence. For example, glia cells enclose all nonjunctional surfaces of presynaptic and postsynaptic elements (168, 208–210); they respond *in vitro* to electrical stimuli (175); they can contain structures similar to presynaptic vesicles at junctional sites of denervated muscle (18); and they can undergo ultrastructural changes after administration of a convulsant drug (179). On the other hand, destruction of glia was not found (200) to alter the electrophysiological behavior of leech nerve cells *in vitro*, an observation which seems directly contrary to the postulated close glial-neuronal interdependence. Possible differences in the properties of glia, *in vitro* and *in vivo*, and in the manifestation of its contribution to the function of nerve cells in the two experimental situations may make the study of glial-neuronal interdependence technically difficult. Nevertheless, this relationship must be clarified. Until it is, glia cannot simply be ignored as a potential central site of drug action.

Pharmacological Methodologies

Turning to the consideration of the synapse as a target for drug molecules, it is clear that our most pressing immediate objective in neuropharmacology is the identification of transmitters at as many central synapses as possible. Only after this goal has been achieved can we place in their proper perspective the multitude of reports on the central effects of drugs related to peripheral transmitters. Without intention of casting aspersion on any particular study, we confess to reticence when confronted with evidence allegedly implying the existence of a central synapse operated by transmitter A, because of electroencephalographic or behavioral changes produced by parenteral, intrathecal, or interstitial injection of substance B which is an antagonist of A in autonomic effector organ C, although not an antagonist of A in organ D or species E. At first sight, it would also seem paradoxical to resort to sophisticated techniques for extracellular or even intracellular unit recording to study, allegedly on the recorded unit, the effects of a substance administered by one or another route a few or a few hundred synapses away. In reality, there are a number of practical considerations that justify the wide usage (e.g., 4, 5, 8, 88, 121, 137, 203, 206, 222, 280, 288) of this approach, but the interpretation of results thus obtained, in terms of their relevancy to specific effects at a given synapse, is clearly difficult and in most cases impossible. When the complexity of central synaptic arrangements, the presence of diffusional and enzymatic barriers, and all possible sources of indirect drug effects are taken into account, it becomes clear that the method of choice should ideally be one permitting comparison of the conductance change induced by restricted drug application to an individual synapse with the change induced on the same neuron by the transmitter released from the nerve ending. This type of very discrete analysis is possible in the *in vitro*

study of certain junctions [e.g., muscle end plate (89–94, 130, 131, 183–187, 237); frog sympathetic ganglia (20–23); Aplysia ganglion cells (136, 293)] for which all required electrodes for intracellular recording and extracellular drug administration can be independently brought into suitable relation with themselves and with the synaptic site under visual control. However, comparable investigation of independently tested, individual, mammalian central synapses *in vivo* is technically unfeasible at present.

The best that can be currently achieved is the study of the conductance changes induced by the electrophoretic administration of a drug in the close proximity of the nerve cell, by means of concentric electrodes that permit us to record intracellularly from a neuron while a drug is administered extracellularly in its proximity (68, 79, 80, 83, 85). In such experimental conditions, the drug is not applied to a single synapse, but rather to a relatively large portion of the cell membrane as well as to whatever nerve terminals, glial cells, or neighboring units happen to be interposed between the tip of the extracellular, drug-containing, electrode and the recorded unit. However, because of the difficulty of recording intracellularly from neurons of the central nervous system, this method is not eminently practicable. In most cases, one is forced to record extracellularly rather than intracellularly from the tested unit, a difference entailing much loss of highly relevant information on the intimate cause of the cell's response to stimulation of synaptic pathways and to administration of drugs in its immediate external environment.

Needless to say, the data obtained with this technique [microelectrophoresis (72)] must be interpreted judiciously (66, 67, 267, 268), since the administered substance may act (*a*) on the postsynaptic membrane, mimicking, potentiating, or blocking the action of the transmitter; (*b*) on presynaptic terminals, causing or blocking the release of the transmitter; (*c*) on nonsynaptic membrane, bringing about changes independently from synaptic processes; or (*d*) on a neighboring unit, functionally related with the one under observation, indirectly causing changes in its behavior. Nevertheless, when used for the study of neuronal organizations in which physiological understanding of local synaptic relationships is sufficiently advanced, this technique has provided partial pharmacological characterization of certain synapses in spinal cord (75, 76), lateral geniculate (70, 73, 74), and olfactory bulb (24, 26, 269). Relying, as it does at present, upon electrophoresis for the controlled ejection of the appropriate ion from the tip of the microelectrode, the microelectrophoretic technique is limited in the choice of drugs that can be tested to those that ionize well in aqueous solution at a pH compatible with physiological conditions. For this reason and to overcome other inherent limitations of the method, it is often advantageous to supplement microelectrophoretic data with other types of pharmacological evidence at the unit level. One useful combination (26, 269) is the use of the microelectrophoretic technique to study the responsiveness of neurons to suspected and naturally released transmitter, before and after parenteral administration of a drug that selectively and completely depletes the storage site of a transmitter.

This potentially useful approach suffers from the limited number of drugs of this type, the limited knowledge of their mode of action, and the need for lengthy recording from any given unit in order to distinguish between sought and unwanted effects of the drug.

It is clear, then, that none of the currently available techniques for the pharmacological study of central synapses is fully satisfactory. The one finally chosen in any carefully conceived investigation must ultimately reflect an essentially personal choice of what seems to be the lesser of many evils. Cognizant that these limitations also apply to the technique of microelectrophoresis (267, 268), we conclude that it represents, nevertheless, a major step in the right direction, towards a discrete pharmacological analysis of cellular events, assistance in the characterization of central synapses and in the identification of central transmitters. Obviously, much more than mere pharmacological evidence is required for identification of central transmitters.

Transmitter Identification

A number of criteria (67, 116, 133, 144, 242) have been suggested for the characterization of a given substance as a transmitter at a given synapse. Unfortunately, some of these criteria cannot be met at the present time for central synapses, and it may be unrealistic (133) to require that they should be. Nevertheless, three major criteria, each amenable to further subdivision, clearly stand out: (*a*) the substance and the appropriate enzyme systems for its biosynthesis and its metabolism must be present and in the right locale; (*b*) the suspected substance and the transmitter must have identical actions and similar pharmacological characteristics on the postsynaptic cell; and (*c*) the transmitter released from the nerve ending must be shown to be identical to the suspected substance. This concluding section will consider the applicability of the reviewed data to these criteria.

First criterion.—Subcellular fractionation studies are likely to turn out evidence relevant to the first criterion through the analysis of synaptosome constituents. Thus far, they have uncovered the usual ACh, norepinephrine, 5-HT, DOPAmine, histamine, glutamate, GABA, and substance P (47, 96, 103, 202, 220, 225, 227, 229, 249, 260, 262, 263, 310–312, 315, 321) in central nerve terminals. Recognition of other chemicals of possible transmitter significance presumably awaits the development of appropriate biological test systems. Currently, these chiefly consist of a number of autonomic effector organs from a variety of species. Conceivably, central synapses may prove more rewarding for this search. The recent use of Renshaw cells for biological detection of ACh extracted from the brain and administered by the microelectrophoretic technique (264) is a first step in this direction. However, because brain homogenization is required as a necessary step in subcellular fractionation procedures, this technique does not permit correlation of specific axon terminals with particular synapses. On the other hand, developments in light and fluorescence histochemistry (2, 3, 49–52, 86, 126,

128, 162–164, 169, 171, 189–192, 278, 294–297, 301) and in the newer art of electron-microscopic histochemistry (13, 60, 95, 233, 261, 265, 266, 298, 299, 317) hopefully may lead to intracellular localization of transmitters or enzymes for their biosynthesis and metabolism.

Second criterion.—The second criterion concerns the demonstration of identical action and similar pharmacology for the transmitter and its suspected chemical analog. The technique of microelectrophoresis can be of obvious assistance in this regard, providing that the relevant synaptic pathway can be selectively activated. Unfortunately, this is a rare instance indeed, even for the best-studied central synaptic sites.

Most studies (6, 7, 14, 25, 27, 33, 63, 64, 74, 77, 81, 195–198, 221, 270, 277, 283, 284) have merely served to demonstrate responsiveness of units in many regions of the brain to one or another endogenous amine and to investigate the effects on the response of synergists and antagonists of the suspected transmitter. In the main, these studies showed that, while some units do respond to the administered amine in the direction of facilitation or depression, other units do not. For a number of reasons (268), we feel that such evidence is only suggestive when positive, and meaningless when lack of drug effects is observed. Even so, these data provide a starting point for further characterization of the mechanisms responsible for the cell's responsiveness to the administered chemical substances. Since anesthesia modifies the responsiveness of at least some (14, 25, 221, 253, 277), if not all (34), neurons to the endogenous amines, it may be fruitful to repeat, in unanesthetized conditions, those studies which is anesthetized animals had shown absence in a given region of neurons responsive to endogenous amines. In this regard, the inability of spinal motorneurons and interneurons to respond to administration by microelectrophoresis of catecholamines (69, 80) is puzzling, in view of recent histochemical evidence (49) showing their presence in certain structures of the spinal cord (cf. also 4, 216).

When the appropriate synaptic pathway can be selectively stimulated, some of the requirements called for by the second criterion can be met. Currently, these studies provide the major source of evidence on the identity of transmitters at certain central synapses. However, for none of these synapses is the evidence complete in regard to the full implementation of the criteria for transmitter identification. The evidence for ACh mediation of Renshaw cell excitation by motorneuron axon collaterals (75, 76, 116) is too well known to require repeating here. Nevertheless, it is worth remembering that (*a*) the motorneuron and axon collaterals, thought to be responsible for the response (112, 114, 116), cannot be recognized anatomically (272); (*b*) we have only indirect proof that they actually contain ACh; (*c*) no information on the location of the enzymes for its biosynthesis and its metabolism is available; and (*d*) there is no direct evidence that ACh is actually released. A case could be made, also, for the probable transmitter function of ACh at certain cortical (197, 198, 283, 284), thalamic (6, 7, 74), and hippocampal (286) sites, and, possibly, in the caudate nucleus (25), although essentially

on circumstantial evidence, since the relevant cholinergic pathways are, as yet, undetected. Thus, in view of the variety of ACh responses observed in the many regions of the brain thus far explored, either in the direction of facilitation (6, 7, 27, 33, 63, 73, 77, 197, 221, 270) or depression (7, 14, 25, 27, 33, 253, 270) and comprising both nicotinic (7, 70, 76, 82, 270, 283) and muscarinic (7, 82, 198) types (i.e., blocked by curarizing or atropine-like drugs, respectively), it would seem somewhat premature to establish Renshaw cells as the pharmacological paragon for all central cholinergic synapses.

Neurons responding to microelectrophoretic administration of NE with an increase or a decrease in spontaneous discharge rate have also been found in the majority of central structures thus far explored, namely in the brainstem (33), hypothalamus (27), lateral geniculate (73), caudate nucleus (25), hippocampus (285), and cortex (195) of the cat, and in the olfactory bulb of the rabbit (14). For the latter structure, we have recently put forward pharmacological evidence (24, 26, 269) tending to incriminate norepinephrine in the recurrent inhibitory pathway from the lateral olfactory tract to mitral cells (15, 156, 247, 275, 318). Norepinephrine, as well as ACh and 5-HT, depressed olfactory neurons, but only drugs which block norepinephrine or deplete norepinephrine stores disrupted the inhibitory reflex arc.

Little can be said at present about the potential central functions of 5-HT, apart from noting that units responsive to its microelectrophoretic administration have been found at many central sites (7, 14, 25, 27, 195, 285) and that there is some indication that it may have a synaptic site of action on lateral geniculate units (71, 74). In the context of studies on 5-HT at central sites, our recent observation (26, 269) that lysergic acid diethylamide and 2-bromo lysergic acid diethylamide were more effective blockers of norepinephrine than of 5-HT responses of mitral cells in rabbit olfactory bulb may be worth bearing in mind. Only limited information is available on the effects of direct administration to individual neurons of DOPAmine (7, 25, 69, 73, 195) and of histamine (73, 80, 195) and none, as yet, on substance P. On the other hand, the literature on the central effects of amino acids, particularly glutamate and GABA, is abundant. However, the interpretation of these results with regard to their physiological significance is still highly controversial. For this reason, we are not prepared to discuss it here, but refer the reader to the original sources (6, 62, 66, 71, 73, 77, 78, 79, 83, 84, 85, 123, 196, 199, 251, 252, 286) and to the recent evidence that GABA is an inhibitory transmitter at neuromuscular junctions in crustacea (194, 290).

Third criterion.—While there is reason to expect that we may eventually fulfill the requirements of the first two criteria with presently available techniques, prospects are rather dim with regard to the demonstration that a particular substance is, in effect, released from a given nerve terminal. In the absence of this proof, all other types of evidence on transmitter characterization can only be construed as supportive. Thus far, only *en masse* techniques are available for the detection of the release, relying upon collection of fluid from the surface of the brain (213, 231) or from complex cannulae inserted

interstitially (144). Obviously, a far more discrete approach is necessary, but its obtainment is as yet unachieved.

Conclusions

It is customary to end a review with an appraisal of the latest progress in the field reviewed; this we find impossible to do. As the preceding sections have made clear, the pieces that compose the central synaptic puzzle are many. Some of these may already be in their proper place, but many would seem to be only partially fitted in or still only dimly perceived.

A major step forward would be the clarification of the biochemical factors that make it possible for a nerve ending to weave its way through brain structures until it finally makes a functionally meaningful contact with the right cell at the appropriate cellular locale. Similarly, in view of the evidence for both chemical and electrical forms of synaptic transmission in certain species, a re-evaluation of the evidence for our current belief that only chemical transmission occurs in the mammalian brain is paramount. Lastly, the role of excitant and depressant amino acids in synaptic transmission must be clarified. They are present in nerve endings, and they are exceedingly powerful substances; could they be indispensable links, either intermediate or final, in the release or action of the substances currently regarded as being transmitters? These and the many other problems raised, or alluded to, in the course of this article, must be resolved before we can attempt to put the synapse (and, hopefully, one day, even the brain) back together again, piece by piece.

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