CELLULAR ASPECTS OF INVERTEBRATE NEUROPHARMACOLOGY[1]

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INTRODUCTION

The subject of this article lies within the traditional range of questions which might be designated as pharmacological aspects of invertebrate neurobiology. We will not attempt to cover the entire area of chemical transmission in invertebrate nervous systems. This review is to call attention to the plurality of the chemical transmission mechanisms within a simple nervous system and to search for information on the origin of the pharmacological heterogeneity of synapses which appears to be a fundamental feature of nervous systems including that of man. Consideration will be given to results which can be interpreted in terms of cellular pharmacology. Emphasis will be on the species best investigated at the unit level, comparative data being drawn in to clarify how far the elected species are representative.

It should be noted that a comparative study should not necessarily adopt its assumptions and systems of classification from vertebrate pharmacology and neurology. Cholinergic receptors (ChR) are usually described in terms of vertebrate muscle pharmacology ("Muscarinic" and "nicotinic" receptors)—a classification which does not apply to invertebrates (1). The use of inadequate definitions delays the accumulation of data and working out of appropriate classifications of ChR's although some work has been done in this important area (2). Another invalid though widely accepted generalization is the assumption that ACh inactivating enzymes fall into two categories, viz., acetylcholinesterase (EC 3.1.1.7) of neurons, and cholinesterase (EC 3.1.1.8) of glial cells. Comparative data demonstrate that enzymes often do not fit this classification. Thus the single enzyme of the squid brain combines the properties of both mammalian cholinesterases; moreover, brain cholinesterases of decapod and octopod cephalopods are chemically and pharmacologically different enzymes (3–5). Since the presence of an ACh inactivating enzyme at junctional areas is considered to be a relatively late

[1] Abbreviations: ACh (acetylcholine); CA (catecholamine); ChR (cholinergic receptor); CNS (central nervous system); DA (dopamine); EPSP (excitatory postsynaptic potential); GABA (gamma-amino butyric acid); Gl (glutamate); IPSP (inhibitory postsynaptic potential); 5-HT (5-hydroxytryptamine).

acquisition of cholinergic transmission (6), this addition to the transmission mechanism may well have developed independently in different zoological groups using similar or different enzymes.

Additional difficulties arise from the assumption that neurons and nervous systems in invertebrates have the same functional organization as those in vertebrates. The discovery of "independent axons" in molluscan nerve cells (7), indicates that in some cases processes of invertebrate central neurons identified by classic neurohistology as dendrites are actually axons. Further, Nicholls & Baylor showed that the CNS of invertebrates may contain cell bodies of primary sensory neurons (8). This indicates that not all neurons which send their axons to the periphery are efferent nerve cells.

These and other possible peculiarities of invertebrates should be taken into account when considering the results of chemical and physiological investigations.

Worms

Hirudo medicinalis.—The medicinal leech is cheap and available and its massive dorsal muscle is one of the best preparations for assay of ACh. The muscle is also used for the bioassay of 5-HT which, unlike ACh, relaxes it. Ganglia of the ventral nerve cord are uniformly arranged and contain some nerve cells large enough to be visually identified. Our knowledge of general physiology of the glia is mainly derived from work on giant glial cells of the leech. This fortunate combination of merits has resulted in much progress in pharmacological investigations at the unit level.

Single cells of the longitudinal dorsal muscle have been studied with intracellular microelectrodes. The iontophoretic application of ACh either caused general depolarization of the muscle cell membrane or increased the frequency of small rhythmic depolarizations, both effects resulting in the production of action potentials. Application of 5-HT produced a decrease of spontaneous EPSP's and of small potentials resembling miniature end plate potentials (9). No heterogeneity of the muscle cell population has been detected in microelectrode experiments (9). The results of the investigation of ACh-ChR interaction *in vitro* provide a basis for the conclusion that two ChR's occur in the dorsal muscle (10). The same conclusion has been drawn from study of contractile responses of the whole muscle preparation. Succinylcholine and decamethonium caused contraction equal to that caused by potassium which is known to eliminate the electrochemical gradient. On the other hand, the maximal responses to these drugs were only 70 per cent of those obtained with ACh, carbachol, and nicotine (11). An estimate of the kinetics suggests that the two groups of cholinergic agonists act on different receptors (12).

Thus recent findings are consistent with the earlier hypothesis that ACh is a chemical mediator at excitatory motor junctions in the longitudinal dorsal muscle; 5-HT is a possible candidate as an inhibitory mediator though it

should be noted that no IPSP's were registered from muscle cells (9, 13). Histochemically, very thin varicose fibers containing 5-HT were found intermingled with the muscle cells in longitudinal, circular, and dorso-ventral muscles as well as in some nonsomatic muscles. These terminals are believed to be branches of the 5-HT containing axon of the "colossal" cell of Retzius (14). The presence of 5-HT in the cell body of the Retzius neuron was demonstrated in several laboratories by means of Falck-Hillarp fluorescent technique (14–16). However, the finding of branches of the Retzius axon in antagonistic locomotor muscles makes the interpretation of the functional role of 5-HT in the Retzius cell somewhat difficult.

Besides "colossal" cells, four smaller neurons in each segmental ganglion also contain 5-HT. Their axons branch in the neuropile thus forming presumably serotoninergic central synapses. The six neurons containing 5-HT correspond to what were previously called "chromaffin cells." Cells of this chemical type are more numerous in the subesophageal and anal complexes of ganglia (14, 16). Histochemically, no catecholamine (CA) has been found by Kerkut and collaborators in the ventral nerve cord (15), but other authors gave descriptions, coinciding in some details, of preterminal varicose fibers containing a primary CA. None of the cell bodies in the cord reveal characteristic green fluorescence, whereas fluorescing pericarya occur in the cerebral and subesophageal ganglia. A single large strongly fluorescing cell is constantly situated within a cluster of nonmonoaminergic neurons in each paired segmental nerve (14, 16).

Electrophysiological data demonstrate that the neuron population is heterogenous. The cells of Retzius differ in their membrane properties from some identifiable large neurons of a segmental ganglion which characteristically have fast and large action potentials (17, 18). These latter cells are placed symmetrically, seven on each side of the ganglion. A thorough investigation has shown that they are primary sensory neurons providing the CNS with tactile information from the skin. Unlike the Retzius cells which display only EPSP's, these cells show IPSP's as well, and possess an electrogenic sodium pump which serves to extrude sodium ions after a period of activity. The pump is inhibited by strophanthidin (8, 19). The list of identified cells has been extended by the discovery of motoneurons capable of producing contraction of longitudinal or circular muscles of the body wall (20).

Retzius cells have been the only ones systematically investigated with respect to their pharmacological receptors. Kerkut & Walker showed that ACh stimulated the spontaneous activity of the Retzius cell whereas benzoquinonium (mytolon) and decamethonium were the most potent antagonists of this action. The depolarizing effect of ACh was also reduced by atropine or tubocurarine, the latter being the weakest antagonist (21). It should be remembered that tubocurarine is the strongest antagonist in the dorsal muscle. Gerasimov confirmed and extended these observations. He showed that orthodromic responses of the cell as well as responses to applied ACh could

be blocked by atropine but not by *d*-tubocurarine. Giant glial cells are also sensitive to ACh which in a high concentration produces depolarization (22).

Both 5-HT and dopamine (DA) hyperpolarize the membrane of the Retzius cell and can depress the spontaneous or ACh-induced activity. Cells are insensitive to other compounds tested such as norepinephrine, epinephrine, isoprenaline, GABA, Gl, ergothioneine, imidazolic acetic acid, and histamine (21). Desensitization to repeatedly applied ACh has been observed (21, 22). Since serotoninergic presynaptic axons occur in the neuropile, 5-HT might be the natural transmitter substance of neurons inhibiting the Retzius cell. Of theoretical interest in this connection is the suggestion of Kerkut & Walker that a substance can synaptically act on a cell which releases the same substance from its own synaptic endings (21). Though normally no IPSP's can be detected from the Retzius cell (17, 21), their appearance in a Ca-free solution (17) indicates the existence of inhibitory input.

To summarize, ACh, 5-HT, and a primary CA may be transmitter substances in leech synapses. No epinephrine which had previously been regarded as responsible for chromaffin reaction of some nerve cells was found in the nervous system. The ChR of the Retzius cell differs from those of dorsal muscle cells and the list of pharmacologically distinct ChR's in the leech might be extended—at least, classic data of Gaskell on lateral vessels should be remembered in this connection (23).

Other annelides.—Microelectrode recordings from nerve cells of the leech, *Aulastoma gulo,* demonstrate the same pattern of physiological heterogeneity as that in the medicinal leech, peculiar features of the Retzius cells coincide in the two species (24). In an oligochaete, *Lumbricus terrestris,* the exact homologue of the Retzius cell of leeches cannot be recognized; each segmental ganglion of the earthworm contains at least 50 cells which exhibit the specific fluorescence of 5-HT and occupy constant positions (15, 25–28). There are striking anatomical and chemical similarities between a single pair of cells which contain a primary CA and are situated in each segmental ganglion of the earthworm at the origin of the second nerve (25, 28), and the above mentioned single CA-containing cell in paired segmental nerves of the leech. It seems very likely that these cells are of common origin. Using an improved extraction procedure Rude found dopamine to be the predominant CA in the ventral nerve cord of the earthworm (29). A primary CA is also present in numerous epidermal neurons which send their axons to segmental nerves and further to the neuropile of the ventral nerve cord. Fibers of this chemical type can be found in certain muscles including that of the pharyngeal wall (25, 28). A biogenic monoamine was reported to be present in motor nerve endings in the longitudinal muscle of the body wall in two species of earthworms (26, 27). Indirect indications that terminals originate from 5-HT containing central neurons are discussed by Myhrberg (28). Indeed, 5-HT contracts this muscle and relaxes the gizzard muscle. These muscle preparations also exhibit differences in properties of their ChR's (30).

In a polychaete, *Nephthys,* the general pattern of distribution of monoaminergic neurons is fundamentally the same as that observed in leech and earthworm. Namely, cells containing 5-HT predominate in the ventral nerve cord whereas neurons containing a primary CA predominate in the supraesophageal ganglion (brain) and form an extensive epidermal sensory system (31).

Other worms.—In a planarian, *Dendrocoelum lacteum,* a primary CA can be visualized in subepithelial bipolar nerve cells and in neurons forming a plexus in the wall of the pharynx. As in annelides, monoaminergic neurons of this worm constitute a minority of the population of nerve cells in ganglia (32). The head ganglion of another planarian, *Phagocata oregonensis,* is reported to contain two to four pairs of cells displaying the yellow fluorescence of 5-HT (33). A remarkable feature of a nemertine, *Lineus sanguineus,* is that it has no peripherally situated monoaminergic neurons; all cell bodies containing a primary CA lie within the CNS, some of them sending their axons to the periphery (34). The nematode *Ascaris suum* is comparable to *L. sanguineus* in this respect; no monoaminergic axons have been found in longitudinal nerves bearing motor fibers which innervate somatic muscles (35).

Thus, the constancy of both positions and connections of monoaminergic neurons in worms so far investigated indicates that the detailed chemical structure of the nervous system is determined within a species. Moreover, groups of neurons and sometimes individual cells showing identical or close chemical and anatomical characteristics can be found in different species. This allows the fate of homologous neurons to be traced from one species to another within taxonomic units of relatively high rank.

The somatic neuromuscular junction of ascarids seems to be the only nonannelide worm preparation studied pharmacologically at the cellular level. Data on physiology, pharmacology, and the peculiar morphology of this junction reviewed by DeBell (36) indicate its general pharmacological similarity to cholinergic motor junctions in the dorsal muscle of the leech. The suggestion that excitatory neuromuscular transmission in nematodes is cholinergic is in accordance with histochemical demonstration of the high activity of an ACh hydrolysing enzyme at the junction (37). Since *d*-tubocurarine produces an increase of transmembrane potential in the muscle cell, spontaneous contractions of the body wall muscle seem to be caused by the constant release of ACh from motor nerve endings (38). The same interpretation has been given to the origin of spontaneous contractions of somatic muscles in the earthworm (39).

The nature of the inhibitory action of the nervous system on ascaride somatic muscle remains obscure. Using the electronmicroscope, it was found that in the junctional area there were two morphologically distinct types of synaptic vesicles in the same presynaptic axon, rather than two types of axons (40). Both excitation and inhibition could be mediated by the same axon, the effect depending on the rate of stimulation. Peculiar EPSP's which become IPSP's at higher frequencies of stimulation have been discovered in

molluscs (41). Actually, the inhibition of ascaride somatic muscle is known to be produced by a high frequency stimulation applied at the level of the circumesophageal ring (42).

Numerous descriptions of the effects of drugs upon whole helminths or strip preparations are controversial; the possibility that nervous elements interfere with pharmacological reactions of such muscle preparations is discussed by Shishov (42).

The esophagus of ascarids is another convenient system for pharmacological experiments at the cellular level (43). An electrogenic sodium pump of this preparation was shown to be activated by epinephrine (44).

ARTHROPODS

Crustacea Decapoda.—Large decapods such as crayfish, lobster, and crab, are the arthropods which have contributed most to cellular pharmacology. The relatively large size of many central and peripheral nerve cells, distinct muscles composed of thick fibers and supplied by a small number of excitatory and inhibitory axons, are the main features useful in explaining crustacean neural and muscular mechanisms in unit terms. The neuropharmacological characteristics of some preparations, such as the lobster giant axon and the crayfish abdominal stretch receptor neuron, are so well known that they will not be discussed because of space limitations.

Atwood has critically discussed morphological and physiological aspects of neuromuscular mechanisms and has concluded that the striking diversity of contractile and electrical properties of somatic muscle fibers ("slow" and "fast" fibers) is not based on differences in the chemical nature of transmitter substances in their corresponding motor nerve endings. Instead, the functional features of different motor endings seem to correlate with their morphological organization, fast ones being arranged so as to produce a large output of transmitter over a brief period of time (45). Recent data indicate that the functional differences in question at least are not based on a different duration of the synaptic action of the transmitter substance (46). Evidence that glutamate (Gl) may be the excitatory transmitter in somatic muscles is regarded by Atwood as suggestive (45). There remains however some degree of scepticism and uncertainty in this respect. Florey & Woodcock reported that in a crab nerve muscle preparation Gl stimulates motor nerve terminals, thus causing increased quantal release of natural transmitter. Threshold levels of Gl for its stimulating effect appear to be no greater than concentrations which have been found in hemolymph. While these results do not rule out some contributions by a postsynaptic effect of Gl, they reinforce doubts regarding its role as a transmitter substance (47).

Effects of inhibitory axons on somatic muscles are generally believed to be mediated by GABA. However the late Voskresenskaya regarded the inhibitory nerve as a regulating system exerting both stimulating and inhibiting influences on muscle contractions; she suggested that these nerve fibers of arthropods might be adrenergic (39).

Atwood has reviewed the existing knowledge of peripheral inhibition in crustacean somatic muscles; pharmacological aspects of the subject can be summarized as follows. Electron lucent synaptic vesicles of inhibitory and excitatory axons are different, the former being significantly smaller and more elongated. Endings of both types occur close together, and, correspondingly, GABA and Gl receptors occupy the same restricted area on the muscle cell. The two types of receptors are pharmacologically and kinetically different: contrary to the Gl receptor, the GABA receptor does not display desensitization to the transmitter and can be effectively blocked by picrotoxin. GABA produces both post- and presynaptic inhibition by means of an increase in membrane conductance, mainly for chloride. The effect of postsynaptic inhibition is to drive the muscle cell membrane potential displaced by EPSP towards the chloride equilibrium potential which is close to the resting level. The effect of presynaptic conductance increase is to reduce the amplitude of the nerve terminal potential which is thought to control the release of the excitatory transmitter (48).

Some recent investigations further elucidate the role of GABA in inhibitory control of somatic muscle. Iversen & Kravitz demonstrated that an uptake of GABA from the extracellular space could be responsible for terminating the synaptic action of GABA in the lobster nerve muscle preparation. Indeed, no enzymatic inactivation of GABA has been found as yet in this or other type of junction, neither do GABA receptors undergo desensitization. The uptake system is highly selective for GABA and capable of transporting it from the extracellular space against a concentration gradient. The cellular location of the GABA uptake remains unknown. A separate uptake mechanism for Gl has been found in the same preparation (49).

Microassay of single nerve cell pericarya of lobster abdominal ganglia showed that cell bodies of inhibitory neurons had a high GABA content whereas those of excitatory motor cells had a low one, Gl being about equally concentrated in pericarya of both types. The grouping of neurons with common transmitter chemistry, and the characteristic patterns of location and peripheral connections of individual identifiable cells indicate that the chemical, anatomical, and physiological structure of the CNS is determined (50). This suggestion is also supported by the results of histochemical localization of monoaminergic neurons in the CNS of the crayfish *Astacus astacus;* a large number of neurons containing a primary CA have been found in fore-part regions of the CNS thus far investigated, whereas the number of cells containing 5-HT was very small, cells and fibers of both types occupying constant positions (26, 51).

Nerve fibers containing a primary CA, probably norepinephrine, have been reported to be present in plexa innervating circular and longitudinal muscle fibers in the hind gut of the crayfish (52). The decapod hind gut is known to contract on application of norepinephrine and epinephrine (53). Contractions are inhibited by GABA while the hind gut muscle as well as that of the heart is not responsive to Gl; ACh seems to have an indirect

action on these muscle preparations (see 54). In contrast to somatic muscles, nonsomatic ones probably constitute a pharmacologically heterogeneous group.

Other arthropods.—Multiple evidence indicates that the crustacean pharmacological pattern is fundamentally representative for the whole phylum.

Intracellular recordings from somatic muscle fibers of the horseshoe crab *Limulus polyphemus* (Chelicerata Merostomata) have shown that Gl produces excitation; GABA mimics the action of inhibitory axons, and picrotoxin blocks its effects (55).

Earlier data on the role of Gl and GABA in neuromuscular mechanisms in insects are summarized in several review articles (54, 56–58). Further details can be found in the most recent publications. There is no ultrastructural difference in the synaptic vesicles of motor axons producing fast and slow EPSP's in somatic muscle cells, but synapse bearing terminals of the two types are differently arranged; the vesicles are of the same kind as those of crustacean motor endings (59). Among a number of amino acids tested, L-glutamic acid was the most active substance on nerve muscle preparations of the locust, grasshopper, and cockroach. The presence of two acidic groups and one amino group was shown to be essential for excitatory activity, the position of the latter being of importance (60). If Gl receptors of insect and decapod muscle are compared, many common features in location, kinetics, etc., can be found (60–63). Contrary to earlier suggestions, the insect hemolymph seems to contain very little "free" Gl (60), this finding does not agree with the above cited supposition that Gl operates as a body fluid factor modulating synaptic transmission (47).

It appears that the pharmacology of nonsomatic nerve muscle preparations is quite different. Though precise details are rare, some observations suggest the presence of unknown transmitter substances at insect visceral nerve muscle junctions (64, 65). Special methods of cellular pharmacology now being applied to arthropod visceral organs will permit establishment of the site of action of drugs tested.

The cockroach heart is insensitive to Gl and GABA. Efferent nerve endings of three distinct types have been found on muscle cells, their transmitter substances remaining obscure. Neurons of the heart ganglion are believed to control miogenic automatism of the heart, they rather than muscle cells are the site of action of ACh and cholinomimetics. There are no cholinergic presynaptic terminals in the ganglion, and the high sensitivity of some neurons to ACh is supposed to be related to their mechanoceptive properties, just as in the abdominal stretch receptor neuron of crayfish (66–69).

On the contrary, GABA inhibits the hearts of the stomatopode, *Squilla,* and of *Limulus*. The automatism of these hearts is neurogenic, the postsynaptic membrane of pacemaker neurons receiving an inhibitory input from the CNS; this membrane is the site of action of GABA and its antagonist, picrotoxin (70, 71).

Generally, GABA and, correspondingly, picrotoxin are effective only on

cells which receive inhibitory innervation, whereas identical cells lacking such input are insensitive to these drugs. Besides somatic muscle cells and neurons of heart ganglion, abdominal stretch receptors of Cretacea and Insecta follow this rule (72). The sensitivity seems to be induced by inhibitory axons since it disappears after denervation (39). On the other hand, denervated muscle cells remain sensitive to G1 (61, 62). In general, denervated structures persist and augment their sensitivity to transmitter substances, the behaviour of the GABA receptor thus being highly specific. The GABA operating inhibitory system is probably a *de novo* acquisition of arthopods having no correlates in other invertebrate phyla. The GABA receptor seems to be additive to innate pharmacological receptors of cells which may have different natures and origins.

Physiological evidence indicates the presence of adrenergic peripheral synapses in insects. Adrenergic substances triggering luminescence of the isolated luminescent organ of the firefly show many properties typical of drug receptor interactions (73). In the insect CNS, numerous cells containing CA display a characteristically constant pattern of distribution which resembles that in the crayfish CNS (26, 74, 75). Klemm has found four neurons apparently containing 5-HT in a ganglion of the stomatogastric nervous system in trichopteran insects (76).

In addition to a CA (or CA's), ACh operates as a synaptic transmitter in insect CNS. Recent microelectrode data on the sensitivity of certain central neurons to ACh (77, 78) seem to remove the last obstacles to acceptance of this role for ACh, which also fits other fundamental criteria (58, 79). Certain restricted parts of insect neuropile are rich in presynaptic arborizations of fibers giving a selective reaction with pseudoisocyanine; it is clear that this "neurosecretory dye" reveals a peculiar type of synaptic material; the pattern of distribution of these specific synaptic fields remains constant from species to species (79).

Molluscs

The presence of an extensive system of efferent nerve cells outside the CNS makes for considerable difficulty in the interpretation of data obtained on molluscan muscle preparations. For example, the accelerating nerve influence upon the snail heart is reported to be a sequential event consisting of several distinct cellular and pharmacological links (80). Though the literature on the pharmacology of peripheral transmission in molluscs is voluminous, investigations at the unit level are just beginning (81, 82). On the contrary, pharmacological characteristics of central neurons and synapses are extremely complete and detailed, the investigations being facilitated by anatomical features of the CNS in opisthobranch and pulmonate gastropods, namely by the presence of large and giant nerve cells. Of a number of species used, the best investigated are an opisthobranch, sea hare (*Aplysia*), and pulmonate land snails of the genus *Helix,* in particular *H. pomatia* and *H. (Cryptomphallus) aspersa.*

Aplysia *and* Helix.—Abdominal and pleural ganglia of the sea hare, and

the dorsal aspect of the subesophageal ganglion of the land snail are mainly used in microelectrode experiments. These parts of the two CNS's have a common origin as derivatives of the visceral loop. The fact that the two populations of cells consist of neurons of identical pharmacological types suggests that an extensive homology of specific cell groups and possibly individual cells may be revealed.

For example, in the CNS of *Aplysia,* there is a single giant nerve cell possessing a peculiar pattern of impulse activity ("parabolic bursts") and characteristic pharmacological features; this is the Br-cell of Arvanitaki & Chalazonitis (83), cell 3 of Strumwasser (84) and R15 of the Kandel group (85). The cell RPa1 of *H. pomatia* appears to be the only subesophageal neuron possessing a similar set of properties (86). With a little care the exact coincidence of the relative positions of the two cells in their corresponding ganglia can be discerned since each one lies within a particular cell group of supraintestinal origin (87, 88) at its caudodorsal aspect close to the root of the nerve which supplies osphradium in both opisthobranches and water pulmonates. It might be added that the only "parabolic burster" of the nudibranch *Tritonia,* cell 22 (89), occupies a position of similar origin (87). No doubt the three cells are not only similar but homologous, though the animals are very different.

The heterogeneity of nerve cells can be revealed by means of different methods and criteria. The most elaborate classification based on differences in synaptic input of neurons and in their responses to ACh has been summarized by Tauc (90); it includes the following pharmacological types of cells: D, H, DINHI, CILDA (DILDA plus HILDA), and an apparently nonuniform group of neurons insensitive to ACh.

As far as responses to amino acids are concerned, four types of cells can be distinguished in *Helix* (91).

Histochemical methods also reveal distinctive types of cells and of their terminal axons. Using a new microassay for the ACh synthesizing enzyme, choline acetyltransferase (E.C.2.3.1.6), Giller & Schwartz have found a good correlation between the functional architecture of the *Aplysia* abdominal ganglion and the regional distribution of the enzyme; a substantial concentration of the enzyme was found in two identified giant neurons of which one is known as cholinergic (92). Cells and fibers containing a primary CA, apparently dopamine, and others containing 5-HT were visualized in *Helix* ganglia (93, 94). An anatomically distinct group of neurons containing both dopamine and 5-HT is believed to occur in *Helix,* the suggestion being based on the fact that both DOPA (3,4-dihydroxyphenyl alanine) and 5-hydroxytryptophan produce an increase of specific monoamine fluorescence of these cells when injected in snails (94). High activity of glucose 6-phosphate dehydrogenase is characteristically revealed in an identifiable group of axons in the *Helix* neuropile (95); one more type of presynaptic endings in gastropods definitely demonstrated for the first time in *Tritonia* (96) is that displaying fluorescence induced by pseudoisocyanine.

Neurons also differ in their physiological characteristics such as the rectifying properties of the cell membrane (100), its oscillatory properties (7, 83), etc. Contrary to the bulk of the cell population, some neurons can produce action potentials in sodium free solutions and, correspondingly, differ in their sensitivity to tetrodotoxin (97–99); such neurons constitute a distinct group of supraintestinal origin which might be easily identified in *Helix* and other pulmonates. Physiological criteria for identification of specific neurons are discussed in several articles (7, 86, 101).

To bridge gaps between different systems of classification, maps of identifiable cells are of paramount importance. At present, the most elaborate map is that of the abdominal ganglion of *Aplysia* (85, 102, 103); mapped and characterized both pharmacologically and physiologically are some subesophageal neurons of *H. aspersa* (104, 105) and *H. pomatia* (86). As yet, little is known of the way in which different characteristics are linked. Sensitivity to 5-HT was reported to be linked with "anomalous rectification" of cell membrane, all neurons in question being of the CILDA type (106). There is no action of Gl on H-cells, while among D-cells some are depolarized and others hyperpolarized by the drug (107). It is to be hoped that future research may elucidate the ultimate number of chemically distinct nerve cell types.

There now remains the need to review some of the recent developments related to cholinergic and monoaminergic transmission.

The phenomenon of "two-component inhibition" of some identifiable neurons of *Aplysia* convincingly demonstrates that the apparent differences between cholinergic synapses may, at least in part, result from the variations in properties of ChR's. The IPSP in question consists of an earlier fast component and late slow one. Electrical response of the cell to ACh or carbachol has the same two-component form, whereas other cholinomimetics imitate only the first component of the synaptic response. The first fast component can be selectively blocked by *d*-tubocurarine, the slow one by tetraethylammonium, the ionic mechanisms of the two components being likewise different (108–110). Another identifiable cholinergic junction is also believed to possess two ChR's, one producing depolarization and another which hyperpolarizes the postjunctional membrane. The sign of synaptic effect is determined by frequency of presynaptic impulses; it has been suggested that this is due to lower threshold sensitivity of one of the receptors and to its capability of being desensitized by accumulating ACh (41). Desensitization of gastropod neurons to ACh was assumed to be the consequence of release of a ChR blocking substance induced by ACh; this interpretation is based on studies of the mechanism of desensitization of the molluscan heart to ACh (6, 111).

The hyperpolarizing action of ACh on some neurons includes the stimulation of the electrogenic sodium pump (112). Otherwise, the effect of ACh on H-cells is generally accepted to be mainly the result of an increase of chloride permeability (113, 114) which can be blocked by copper ions

(115). As to ionic mechanisms of ACh action on D-cells, there is no equivocal opinion (116, 117), and cells themselves referred to as D-cells seem to be different.

More than 50 per cent of the neurons investigated by Glaizner were inhibited by ACh, whereas only one of the 44 cells tested was depolarized by dopamine (104), the data contradicting an earlier suggestion that DA mediates synaptic excitation of H-cells (90). The excitatory action of DA appears to be a relatively rare event. Receptors of cells hyperpolarized by DA had been first qualified as similar to alpha-adrenergic receptors of vertebrates (117), but later they were recognized as true DA receptors (118). Some of these cells are also inhibited by norepinephrine; these receptors are pharmacologically distinguishable from DA receptors (119). In DILDA-cells of *Aplysia,* both synaptic long lasting inhibition and DA-induced hyperpolarization are the result of an increase in potassium permeability; in addition, in the cell R15, DA appears to stimulate an electrogenic pump (120). There are similar findings for a neuron of *H. aspersa* (121) which possesses an oxygen sensitive electrogenic sodium pump (122); the size, position, peculiar pattern of activity, and pharmacological properties of this big cell (123) indicate that it is the homologue of "parabolic burster" (cell RPa1) of *H. pomatia* and, therefore, of cell R15 of *Aplysia.* Stimulation of an electrogenic pump by DA or synaptic input is referred to by some other authors (112, 124). Since the pump is a rather sluggish mechanism, it can be responsible for the long duration of the peculiar synaptic inhibition of CILDA-cells. An alternative explanation is to suppose that there is a defect in the mechanism of DA reuptake from the synaptic cleft.

Some recent findings give additional support for the role of 5-HT as a mediator at synapses producing excitation of CILDA-cells. These cells display EPSP's of two types, fast and slow. The fast EPSP's, as well as the excitatory action of ACh, are antagonized by hexamethonium, the slow ones are blocked by drugs which antagonize the effect of 5-HT (lysergic acid diethylamide, tryptamine, excess of 5-HT) (106). Nerve and other tissues can take up 5-HT from the incubation medium and release it when electrically stimulated (125). The relation of these findings to synaptic transmissions has been doubted since it was shown that 5-HT taken up from the medium located in the glial sheath of neurons rather than in the synaptic neuropile (126). However the possibility of dislocation of 5-HT in the process of fixation and further treatment of tissue samples was not ruled out in the latter work.

Other gastropods.—Willows elaborated a detailed cellular map of the CNS of the nudibranch *Tritonia* (89, 127), however, very large neurons of this otherwise well studied mollusc were not investigated pharmacologically. On the contrary, neuropharmacological investigations of the fresh water snails, *Lymnaea stagnalis* and *Planorbarius corneus,* which are sometimes interesting from a chemical point of view, were carried out mainly though not always (128) on random nonidentified cells. Some contradiction in qual-

ification of ChR's of the pond snail (129, 130) and garden snail (131–133) occurs but this is difficult to discuss since it is not known if the neurons investigated are comparable.

In principle, it seems obvious that the extensive homology at the unit level can be established by means of both electrophysiological and histochemical methods. A striking example of histochemical identification is found in the paired ventral metacerebral cells of *Helix* which contain 5-HT; the presence of this amine allowed us to find these cells in brains of different families of stylommatophoran pulmonates. In the nudibranch, *Dendronotus,* there is also a single pair of 5-HT containing giant cells in a corresponding area (134) (they correspond to cells 8 and 19 of the above mentioned map of *Tritonia*). Finally, a pair of giant cells containing 5-HT was found in cerebral ganglia of the basommatophoran snail, *Lymnaea* (135). Cells containing DA can be likewise followed histochemically from species to species, from pulmonates to opistobranches and prosobranches (95, 135).

Other molluscs.—Microelectrode recordings from central neurons of a bivalve, *Spisula,* indicate that the cell population is heterogenous (136). Since bivalve nerve cells are usually very small, further progress depends on selection of an appropriate preparation; the zebra mussel *Dreissena* that has relatively large neurons might be of interest in this connection (137).

The heterogeneity of nerve cell populations is also documented by histochemical localization of biogenic monoamines. They occur in a minor part of the cell population in *Anodonta* (93, 138). Sweeney systematically studied the distribution of these cells in *Sphaerium.* In part, they appear to be ectodermal primary sensory neurons, others lie in ganglia and send their axons into the neuropile. There is no evidence of monoaminergic motor innervation peripherally with the possible exception of the neurons of buccal and genital ganglia (139). On the other hand, the suggestion that 5-HT is a neurogenic relaxing factor in some bivalve muscles has been supported by a recent finding that the tonic contraction of the adductor muscle of *Anodonta,* taking place after the inhibition of 5-HT synthesis in the CNS, could be abolished by applying 5-HT to the ganglia (140).

In *Sphaerium,* amounts of DA and norepinephrine are rather equal (139), the results differing from earlier observations on *Anodonta* and *Spisula* that DA is the only or predominant CA. The two CA's or DA seem to be the most likely candidates for localization within the granular synaptic vesicles (141, 142).

Although the giant axon and giant synapse of the squid are widely used in general physiology and pharmacology, much of the chemistry of synaptic transmission in cephalopods remains obscure. The muscle apparatus of the chromatophore is a convenient system for studying nerve effector properties at unit level. The increase in miniature potential activity seen after application of ACh may indicate that at this junction ACh acts by releasing the natural transmitter substance; 5-HT augments the rate of both shortening and relaxation of muscle cells responding to nerve stimulation (143). In the

cephalopod CNS, cholinergic endings seem even more abundant than in the CNS of mammals. In the optic lobes of the octopus brain, the ACh content of the nerve ending fractions is nearly 100 times greater than that of corresponding fractions in the mammalian brain (144). Noncholinergic transmission probably occurs in the octopus brain as well. Although most of the synaptic vesicles seen in nerve ending particles obtained in fractionation experiments are of a granular type, about 4 per cent are granular, ranging in size from 300 to 1000 Å (145). It is noteworthy that granular vesicles of apparently monoaminergic endings in a bivalve mollusc are roughly of the same diameter (141).

Conclusion

Comparative data summarized in a number of reviews show that the various known and possible transmitter substances are widely distributed within the animal kingdom (33, 146, 147). There seems little doubt that every vertebrate and invertebrate nervous system is composed of neurons of different chemical types, both chemical output (releasing substance) and input (receptors) of a given neuron being specific.

The results reviewed in this article indicate that chemical heterogeneity of invertebrate nervous systems is highly ordered in regard to positions and connections of cells of a certain type. In different preparations of a given species, individual neurons or groups of neurons with identical characteristics can be recognized because of an invariant set of morphological, physiological, and pharmacological properties.

Further, it can be shown that specific nerve units of common origin maintain their fundamental pharmacological properties when traced from species to species and from genera to genera. The results obtained in microelectrode experiments are impressive though rather limited. Histochemical observations provide us with more abundant information that can be a subject for far-reaching comparison. Traced from coelenterates (26, 148) to higher worms, arthropods, and molluscs, cells containing primary CA(s) but not 5-HT behave as if to display the correctness of the classic A. and O. Hertwigs' scheme of the origin of the nervous system: bipolar sensory neurons of ectodermal epithelium are the initial point of the evolution, followed by different stages of their progressive ganglionization mainly at the cephalic end of the animal. Cells containing 5-HT are not related to ectodermal epithelium and lie mainly in parts of nervous systems other than cerebral ganglia. Of particular interest in this connection is the observation that in sea anemones, in addition to ectodermal neurons displaying green (CA) fluorescence, there occur entodermal cells with yellow-green (5-HT?) fluorescence (26).

Thus, the chemical heterogeneity of the nervous system is neither a peculiar property of higher animals, nor does it result from differentiation of the initially homogenous population of ganglionic cells at some stage of evolution of the invertebrate CNS.

To account for the occurrence of distinct chemical types of neurons in both diffuse and ganglionized nervous systems we may postulate that, at early stages of the evolution of Metazoa, different cells which had functioned as systems of chemical control developed independently cytological and physiological features of the neuron. These initially different cells then united into the nervous system, each keeping its basic specificity though acquiring new qualities in the course of further diversive evolution. In terms of this hypothesis, homologous cells and junctions from all species of animals are to have common background transmitter chemistry. An alternative point of view is that the chemical pattern of the nervous system, though being constant within a given phylum, may be radically different in other phyla (54).

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