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SYNAPSES OF A GIANT SEROTONIN NEURONE AND A GIANT DOPAMINE NEURONE: STUDIES USING ANTAGONISTS

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Summary—Many important discoveries in neuropharmacology, as in other fields of science, have followed the introduction of new techniques. It is also true to say, however, that advances can be made using existing techniques to yield more information by choosing simple, more easily studied, neuronal systems extant in the animal kingdom.

The purpose of this paper is to describe two simple systems for studying the neuronal roles of serotonin and dopamine and to illustrate their usefulness for pharmacological studies by presenting some data obtained with blocking drugs.

Synapses of two symmetrically placed giant serotonin neurones

There is a giant serotonin-containing neurone in each cerebral ganglion of the snail *Helix pomatia*. The serotonin within these neurones has been detected histochemically, by bioassay (COTTRELL and OSBORNE, 1970) and by microchromatography (OSBORNE and COTTRELL, 1971). The cells can convert 5-hydroxytryptophan (5-HTP) to serotonin *in vitro* (COTTRELL and POWELL, 1972) and take up 5-HTP (PENTREATH and COTTRELL, 1972, 1973) and manufacture serotonin *in vivo* (OSBORNE, 1973).

Experiments made with intracellular electrodes on isolated central nervous system preparations show that each giant serotonin cell makes excitatory synaptic connexions with three identified giant neurones in each buccal ganglion (Fig. 1A). Stimulation of either giant serotonin cell to fire spikes results in excitatory postsynaptic potentials (epsps) in the middle and posterior buccal cells (Fig. 1B). The connexion of each giant serotonin cell onto each middle cell is probably monosynaptic because the injection of the giant serotonin cells with tetraethylammonium, which prolongs the action potential, increases the amplitude of the epsps (presumably by an increase in transmitter release, KATZ and MILEDI, 1967) and high Ca medium (physiological solution containing 60 mM $CaCl_2$), which greatly increases spike threshold and therefore blocks polysynaptic connexions, does not interfere with transmission from the giant serotonin cells to the middle buccal cells (COTTRELL, 1970; COTTRELL and MACON, 1974). Unlike the situation in the middle buccal cells, individual epsps are not seen in the anterior cells in response to each action potential fired by the giant serotonin cells. The response to giant serotonin cell firing is delayed in the anterior cells. A fourth cell receiving inhibitory input from each giant serotonin cell has also been located in each ganglion (Fig. 1C).

Pharmacological studies have been made on the giant serotonin cell, the middle cells and on the giant serotonin cell–middle cell connexions. The giant serotonin cells are depo-

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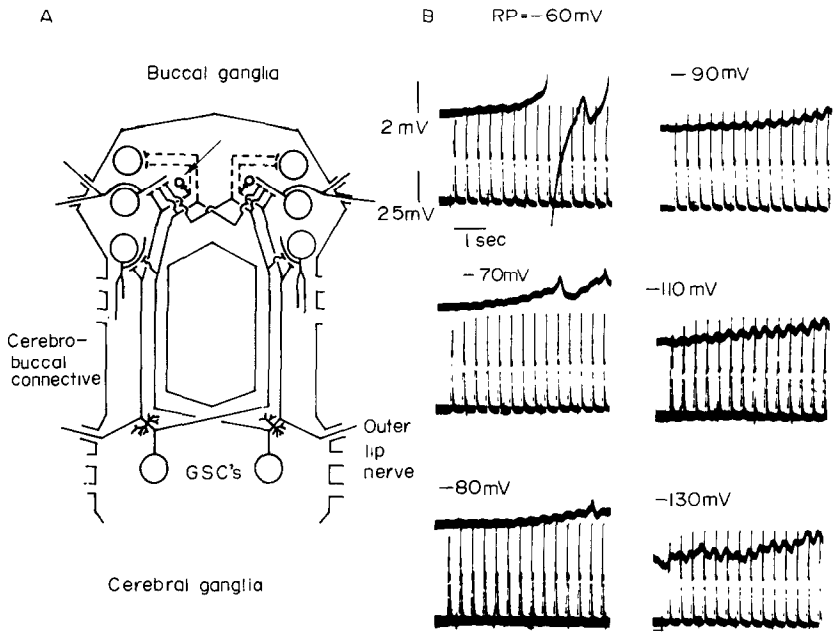

Fig. 1. A: Diagram of the cerebral and buccal ganglia of *Helix pomatia* showing the giant serotonin cells and follower neurones. The approximate position of the neurone receiving inhibitory input is shown with an arrow. The processes of the different cells were traced either by dye injection or by electrophysiological methods. The exact points of contact between the cells are not known, but the synapses are probably axo-axonic (cf. TAUC, 1967). B: Responses of the left middle neurone to stimulation of the left giant serotonin cell. The records at the top left show that at the resting membrane potential, repetitive firing of the giant serotonin cell (resulting from applied depolarizing pulses and shown in the lower record of each pair) brought about small depolarizing potentials in the middle cell, which summed and led to the firing of an action potential. When the middle cell was artificially hyperpolarized and firing in the middle cell blocked, the size of the small depolarizing potentials (epsps) was increased.

larized and stimulated by acetylcholine (ACh) and there is some evidence that these cells (previously called giant metacerebral cells) may receive excitatory cholinergic inputs (KANDEL and TAUC, 1966). Stimulation of the external peritentacular nerve can result in a biphasic, depolarizing–hyperpolarizing, response which is mimicked by glutamate (SZCZEPANIAK and COTTRELL, 1973) (see Fig. 2A).

Studies designed to elucidate the possible transmitter role of serotonin within the giant serotonin cells have been made by determining the pharmacological properties of the middle cells and investigating the giant serotonin cell—middle cell connexions. Serotonin at a concentration of 5×10^{-7}M depolarizes and stimulates the middle cells. Serotonin applied iontophoretically similarly depolarizes the middle cells; this response rapidly diminishes with repetitive applications of serotonin, presumably due to desensitization of the receptors (COTTRELL and MACON, 1974) (see Fig. 2B). The serotonin potential is blocked by a number of agents including: lysergic acid diethylamide (LSD-25, 10^{-4}M), bromolysergic acid diethylamide ($2{\cdot}5 \times 10^{-4}$M), methysergide ($2{\cdot}5 \times 10^{-4}$M), morphine (2×10^{-5}M), tubocurarine ($1{\cdot}5 \times 10^{-4}$M) and low levels of serotonin itself (5×10^{-8}M). Some of these agents have been tested on the epsps elicited by the giant serotonin cells.

The concentration of LSD-25 (10^{-4}M) required to abolish the epsps resulted in a considerable depolarization and activation of the middle cells; consequently it was not poss-

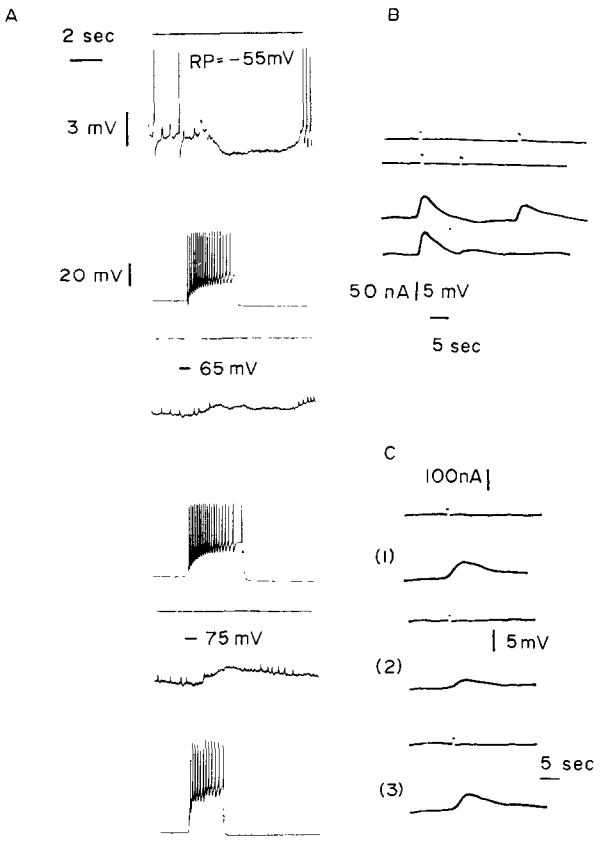

Fig. 2. A: Giant serotonin cell-elicited hyperpolarizing response observed in a medium size neurone in the left buccal ganglion. The response was inverted by artificially hyperpolarizing the cell. The buccal cell membrane potential is shown in the upper, and the giant serotonin cell membrane potential in the lower, recording of each pair. B: Response of the middle cell (lower two records) to repeated iontophoretic application of serotonin. The left middle cell was artificially hyperpolarized (to −65 mV) to avoid spike firing. The current pulses (upper records) were 30 nA for 500 msec. The effect of the second application of serotonin within 10 sec of the first was greatly reduced, but when the pulse interval was 25 sec (above), there was only a slight reduction in the second potential. C: Blocking effect of morphine sulphate (2×10^{-5} M) on the middle cell serotonin potential. The upper trace in each pair of records is the current pulse (30 nA for 500 msec) and the lower trace the cell membrane potential (−65 mV). Response in normal physiological solution (1), after exposure to morphine sulphate for 12 min (2) and after 10 min washing (3).

ible to establish whether the blockade was specific to the receptors or whether it was a non-specific membrane effect. Methysergide ($2{\cdot}5 \times 10^{-4}$ M) also abolished the giant serotonin cell-elicited epsps and had much less depolarizing action on the middle cell. However, further studies showed that methysergide could block conduction of giant serotonin cell action potentials along the cerebro-buccal connectives (cf. bromolysergic acid, see GERSCHENFELD and STEFANI, 1966). Morphine (2×10^{-5} M), however, blocked the epsps without depolarizing the middle cells or blocking the giant serotonin cell action potential conduction, thus providing some evidence that serotonin is the transmitter substance released (see Fig. 2C). Nevertheless, the giant serotonin cell-elicited epsps were not blocked

by low levels of tryptamine (10^{-6}M) nor serotonin itself (10^{-7}M) sufficient to antagonize the response to iontophoresed serotonin. This discrepancy may be due to the position of the synaptic receptors deep in the tissue and therefore less accessible than those responding to iontophoresed serotonin. Another factor may be the uptake of serotonin, and perhaps related compounds, by fine nerve terminals in the neuropile (PENTREATH and COTTRELL, 1972, 1973).

Reduction in the external Na concentration resulted in a diminution of the serotonin potential. Similarly the giant serotonin cell-elicited epsps were reduced in size and lost in low Na solution although action potentials could still be recorded. (Persistence of action potentials in reduced Na has been noted in other snail neurones, e.g. GERASIMOV, 1964; KERKUT and GARDNER, 1967; MEVES, 1968.) Both the serotonin potential and the giant serotonin cell-mediated epsps therefore appear to result in an increase in the permeability to Na.

In agreement with the view that serotonin is released from the giant serotonin cells to mediate the epsps in the middle cells, reserpine causes a progressive diminution of the epsps with repetitive stimulation (COTTRELL and MACON, 1974).

Thus available data suggest that serotonin is the transmitter substance released from the endings of the giant serotonin cells and that the connexions described offer many important advantages for analysing further the suspected transmitter role of serotonin and the drugs thought to influence its levels or actions in nervous tissue.

Synapses of a giant dopamine neurone

This system is made up of a giant dopamine-containing neurone and several different follower neurones in the central nervous system of the water snail *Planorbis corneus* (BERRY and COTTRELL, 1973a).

MARSDEN and KERKUT (1970) first described a giant green-fluorescing cell in the left pedal ganglion of *Planorbis corneus* using amine fluorescence histochemistry. The colour was indicative of a primary catecholamine and Marsden and Kerkut concluded that the neurone contained dopamine (DA) rather than noradrenaline because of the preponderance of DA in extracts of the ganglia. More recent microbiochemical studies have shown that the perikaryon of the giant green-fluorescing cell contains 5·4 ± 0·6 pmol of DA, but no detectable noradrenaline (POWELL and COTTRELL, 1974). Microspectrofluorometric studies similarly provide evidence for the presence of DA and lack of noradrenaline (B. FALCK, personal communication).

Processes of the giant dopamine cell have been traced by dye-injection, and electrophysiological, experiments (Fig. 3A). Axon branches pass through the left pleural ganglion into the left parietal and visceral ganglia. In these ganglia there are many neurones which receive synaptic input from the giant dopamine cell. Action potentials elicited in the giant dopamine cell result in the appearance of epsps in some follower neurones (Fig. 3B), inhibitory postsynaptic potentials (ipsps) in others (Fig. 3C) and biphasic, depolarizing-hyperpolarizing, potentials in other neurones (Fig. 3D). Experiments made using high Ca physiological solution suggest that each type of response recorded results from monosynaptic connexions.

These observations are particularly interesting in relation to ASCHER's (1972) iontophoresis studies in another gastropod species *Aplysia californica*. Ascher showed that different neurones could respond with depolarizing, hyperpolarizing or biphasic, depolarizing-hyperpolarizing potentials, i.e. similar responses to those seen in *Planorbis* when the giant

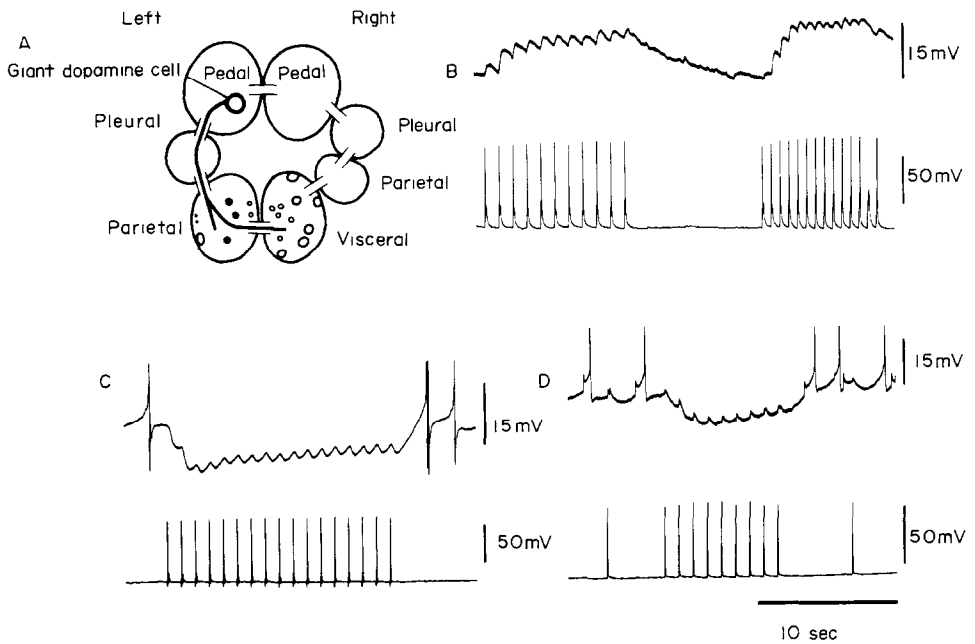

Fig. 3. A: Diagram of the dorsal surface of the suboesophageal ganglionic ring in *Planorbis corneus* showing the giant dopamine cell, which lies on the ventral surface, and the approximate positions of its follower neurones. The axon of the giant dopamine cell was traced as far as the parietal ganglion (about 1 mm) by injection of cobalt, and through to the visceral ganglion electrophysiologically. Open and closed circles represent follower cells receiving inhibitory and excitatory input respectively from the giant dopamine cell. Records (B)–(D) show the three different types of response (upper record of each pair) to stimulation of the giant dopamine cell. B: Excitatory responses of a parietal ganglion cell (hyperpolarized by 30 mV to −90 mV in order to increase the amplitude of the epsps; at the resting membrane potential the epsps summed and gave rise to action potentials in the parietal ganglion cell). C: Inhibitory responses of a visceral ganglion cell. D: Biphasic responses of a visceral ganglion cell.

dopamine cell is stimulated. In *Aplysia* the biphasic DA response could only be observed after iontophoretically applied DA; hyperpolarization alone was seen in these neurones when DA was generally applied to the preparation. General application of DA to *Planorbis* neurones results in depolarization of those neurones in which epsps are seen during giant dopamine cell stimulation and hyperpolarization of those which respond to giant dopamine cell activation by exhibiting ipsps or the biphasic response. Thus, although iontophoresis experiments have not been made in *Planorbis*, there is evidence for the presence of at least two types of synaptic DA receptors, one type resulting in depolarization and the other in hyperpolarization. Furthermore there appear to be more than one type of DA receptor on some follower neurones; these are probably responsible for the biphasic synaptic potentials recorded. Glutamate also hyperpolarized the cells which responded with ipsps or biphasic potentials to giant dopamine cell activation.

Some pharmacological studies have been made on the synaptic potentials elicited by stimulation of the giant dopamine cell. ASCHER (1972) observed that the depolarizing responses to DA iontophoresis could be blocked with tubocurarine but not hexamethonium. The effect of tubocurarine on the giant dopamine cell-elicited biphasic synaptic responses is shown in Figure 4A. The depolarizing response is abolished without any noticeable effect

on the summed hyperpolarizing response. Tubocurarine also had some antagonistic effect on the lone epsps mediated by the giant dopamine cell, but it was less effective and its effect was less predictable than against the depolarizing phase of the biphasic response. Hexamethonium (10^{-3} M) did not block the depolarizing synaptic potentials.

WALKER, WOODRUFF, GLAIZNER, SEDDEN and KERKUT (1968) observed that ergometrine could have an antagonistic effect on generally applied DA in some neurones of *Helix aspersa*. ASCHER (1972) confirmed this finding but also showed that ergometrine can have a non-specific effect on membrane resistance, which necessitates careful control exper-

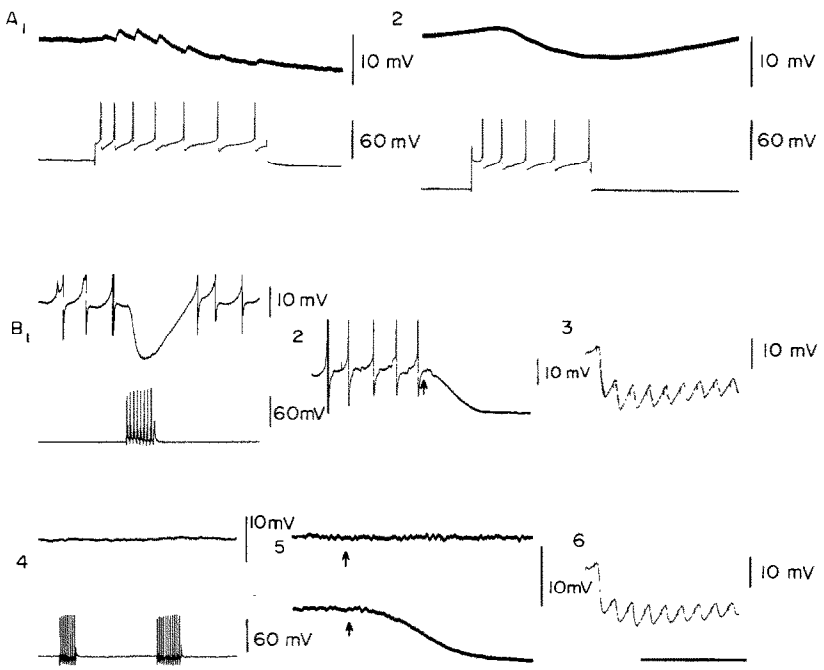

Fig. 4. A: Effect of tubocurarine on biphasic synaptic potentials (b.p.s.p.s) produced in a visceral ganglion cell (upper trace of each pair) by stimulation of the giant dopamine cell. (A1) Control; (A2) tubocurarine, 10^{-4} M. Tubocurarine blocks the depolarizing phase without affecting the hyperpolarizing phase of biphasic synaptic potentials at concentrations as high as 10^{-3} M. The visceral ganglion cell was hyperpolarized by 20 mV to −85 mV in order to increase the amplitude of the depolarizing phase (the amplitude of the hyperpolarizing phase is consequently reduced). Full recovery occurred after 30 min of washing. Hexamethonium (10^{-3}M) did not block the depolarizing phase. B: Effects of ergometrine on the inhibitory response of a visceral ganglion cell to stimulation of the giant dopamine cell, and to applied dopamine and glutamate. (B 1–3) Control responses; (B 4–6) effects of ergometrine, 2×10^{-6}M, on the responses. (B1) ipsps produced in a visceral ganglion cell (upper) by stimulation of the giant dopamine cell. (B2) Hyperpolarizing response of the same visceral ganglion cell to dopamine (arrow). (B3) Compound ipsps in the visceral ganglion cell evoked by repetitive stimulation (note stimulus artefacts) of a parietal nerve. (B4) Ergometrine abolishes ipsps from the giant dopamine cell. (B5) Ergometrine also abolishes the response of the visceral ganglion cell to dopamine (arrow, upper record) but the hyperpolarizing response to glutamate (arrow, lower) remains. (B6) The amplitude of ipsps produced by stimulation of the parietal nerve is little affected by the ergometrine. Note that ergometrine abolishes spontaneous firing (by hyperpolarizing the visceral ganglion cell). The controls show that the blockade of transmission from the giant dopamine cell is not the result of a non-specific action on membrane resistance. Similar specific blockade of transmission was obtained with $2{\cdot}5 \times 10^{-4}$M 6-hydroxydopamine. This antagonist had the advantage that complete recovery occurred usually within about 20 min of washing whereas recovery from ergometrine occurred only after at least 1 hr of washing and was often incomplete. Time calibration: 2 sec for B3, B6; 10 sec for other records.

iments for meaningful data on DA receptors. Ergometrine at a concentration of 2×10^{-6}M antagonized the giant dopamine cell-elicited ipsps and the hyperpolarizing effect of DA without blocking the hyperpolarizing response to glutamate or the ipsps resulting from stimulating another inhibitory input, from a parietal nerve (Fig. 4B). The results of these and similar experiments can therefore be interpreted in terms of a blockade of the hyperpolarizing DA receptors and a release of DA from the endings of the giant dopamine cell.

6-Hydroxydopamine (6-OHDA) is known to deplete catecholamines from neurones (see MALMFORS and THOENEN, 1971). During the course of a series of experiments designed to study possible presynaptic effects of 6-OHDA, it was observed that it could also block the ipsps elicited by the giant dopamine cell by a postsynaptic action. Available data indicate that 6-OHDA specifically blocks the DA hyperpolarizing receptors (BERRY and COTTRELL, 1973b). The depolarizing DA response and the giant dopamine cell-mediated epsps are also antagonized, but these are less sensitive to 6-OHDA than the hyperpolarizing responses.

The results of the electrophysiological studies on the giant dopamine cell provide clear evidence for a role of DA as a transmitter and for the presence of at least two types of synaptic DA receptors on follower cells. Furthermore both types of receptors appear to be present on some follower cells. The depolarizing phase of the biphasic potentials can be selectively blocked with tubocurarine; lone giant dopamine cell-mediated epsps are less sensitive to tubocurarine blockade. The ipsps and the DA hyperpolarizing response can be blocked with ergometrine and 6-OHDA.

DISCUSSION

One way to circumvent many of the serious technical problems encountered in attempting to analyse the possible transmitter role of active substances in neurones is to select a suitable simple neuronal system. Two such systems have been described. In each it is possible to locate a specified giant neurone, with known biogenic amine content, for electrophysiological experiments and to record from follower neurones in selected regions of the central nervous system. Consequently it has been possible to repeat experiments on the same neuronal connexions. Because the presynaptic neurones (and some of the postsynaptic neurones) are large, microbiochemical experiments can be made on them and they can be readily located for electron microscope and autoradiographic studies.

The results obtained so far provide evidence for a transmitter role of serotonin and DA in these systems. However, to establish this view beyond doubt it will be necessary to show release of the amines during activation of the presynaptic neurones and to compare the precise modes of action of the amines with the transmitter substances released. It is important in the case of the presumed dopaminergic synapses to compare the ion permeability changes produced by DA and those occurring during synaptic activity.

Finally, it is interesting to consider the presence of different synaptic receptors to DA, and perhaps also to serotonin (cf. PAUPARDIN-TRITSCH and GERSCHENFELD, 1973) on postsynaptic neurones and the probable existence of two different synaptic receptors to DA on certain follower neurones in *Planorbis* (cf. ASCHER, 1972). There are now several cases of multiphasic responses and multiphasic synaptic potentials occurring in single neurones of gastropod molluscs, for example to ACh and presumed cholinergic inputs (TAUC, 1967; KEHOE, 1972; WACHTEL and KANDEL, 1971), and to glutamate application and the pre-

sumed presynaptic release of this agent (SZCZEPANIAK and COTTRELL, 1973). The interesting question arises: is this phenomenom restricted to certain central neurones of molluscan, and perhaps other invertebrate species (as perhaps a mechanism for economizing on interneurones?), or does it occur in central neurones of other phyla, perhaps even in mammalian species? This question raises the problem of the general significance of experimental results obtained with gastropod neurones and those of other invertebrate species. There are obviously some major differences between neurones found in the different phyla, and care must be taken in comparing results obtained in the invertebrates with those obtained in the vertebrates. However, available information does suggest that many of the same transmitter substances are used in arthropod, molluscan and vertebrate species, and it should be remembered that fresh data obtained with invertebrate neurone preparations in the past have often yielded new insights and prompted new approaches to the study of the mammalian nervous system.

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