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# REVIEW

# NEUROTRANSMITTERS IN THE CEPHALOPOD BRAIN

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**Abstract**—1. The presence of possible neurotransmitter substances in the central nervous systems of cephalopod molluscs is reviewed.

2. The evidence suggests that acetylcholine; the catecholamines dopamine and noradrenaline; and 5-hydroxytryptamine are possible neurotransmitters.

3. The evidence is less good for other amines or amino acids having a similar role.

## INTRODUCTION

Cephalopods are amongst the most active of invertebrates; they are predators, hunting chiefly by sight and they have a highly developed nervous system. They are able to analyse visual and tactile information; to make rapid muscle responses; to make complex colour and luminescent changes and they also have remarkable powers of learning.

Many scientists, particularly over the past two decades have examined the neurological basis of some of these behaviour patterns, and in *Octopus* especially, but also in *Sepia* and *Loligo*, a considerable amount of information has been amassed relating brain structure to the animal's behaviour. One may note the anatomical studies of Young on *Octopus* (1971) and on *Loligo* (1974, 1976, 1977); the electrical stimulation studies on *Sepia* performed by Boycott (1961); the visual and tactile discrimination experiments of Sutherland (e.g. 1969) and of Wells (e.g. 1974); and a wide variety of learning experiments (e.g. Boycott & Young, 1950; Wells, 1966), which are reviewed by Sanders (1975). Other workers have examined the brain chemistry of cephalopods and there is an extensive literature on this subject. The present article attempts to review the evidence concerning the presence of possible neurotransmitters in the cephalopod central nervous system (CNS).

In cephalopods, there is ultrastructural evidence that most, if not all, synapses in the CNS are chemical rather than electrical (Gray & Young, 1964). However, it must be emphasised that *the chemical identity of the transmitter is not known for any synapse in the cephalopod brain.* To prove that a chemical functions as a neurotransmitter at a particular junction, a number of criteria have been proposed. These criteria, which are principally based on work on the vertebrate peripheral nervous system (Curtis, 1961; McLennan, 1963; Eccles, 1964) are reviewed in detail by Werman (1966). The ideal way to identify a possible neurotransmitter is to demonstrate that when applied to an excitable cell it mimics the endogenous transmitters liberated at that cell's synapses. Experiments of this nature have not been performed in the cephalopod CNS. In the peripheral nervous system there has been some elegant work on the transmitter at the stellate ganglion (see for example Miledi, 1972, and reviews by Gerschenfeld, 1973, and by Kehoe & Marder, 1976); and extensive work on the control of chromatophores has postulated innervation with cholinergic excitatory fibres (Florey, 1966; Florey & Kriebel, 1969). In the CNS, evidence as to whether certain chemicals act as neurotransmitters relies principally on whether they are present themselves and whether their synthesising and degradative enzymes are present in nervous tissue. It is evidence of this sort that will be considered in the present review, for three separate classes of chemicals:

(1) Acetylcholine and associated enzymes;
(2) Biogenic amines and associated enzymes;
(3) Amino acids.

There has been no suggestion of purines or peptides having a neurotransmitter function in cephalopods, although an endecapeptide called eloidisin, which is chemically very similar to substance P, has been extracted from the posterior salivary glands (Erspamer & Anastasi, 1962; Erspamer & Falconieri Erspamer 1962).

Although nowadays there is a standard terminology for the different parts of the brain (Young, 1971), based on that of Dietl (1878), the terminology employed by the original authors will have to be used throughout this review. A diagram of the divisions of the cephalopod brain is shown in Fig. 1. The relationships of the different genera of cephalopods mentioned in the text are shown in Fig. 2.

## ACETYLCHOLINE AND ASSOCIATED ENZYMES

In vertebrates, acetylcholine meets almost all the criteria as a neurotransmitter at the neuromuscular junction, in autonomic ganglia and at post-ganglionic parasympathetic nerve endings (see review edited by Fields, 1977).

The classic papers of Bacq (Bacq, 1935a,b; Bacq & Mazza, 1935a,b) were the first to catalogue the acetylcholine content of many organs of *Octopus vulgaris.* Indeed, this was the first occasion that pure acetylcholine was isolated from an invertebrate, and the quantity that was found in the brain (77 μg/g wet wt) led Bacq (1935c) to suggest that acetylcholine was possibly a neurotransmitter in *Octopus.*

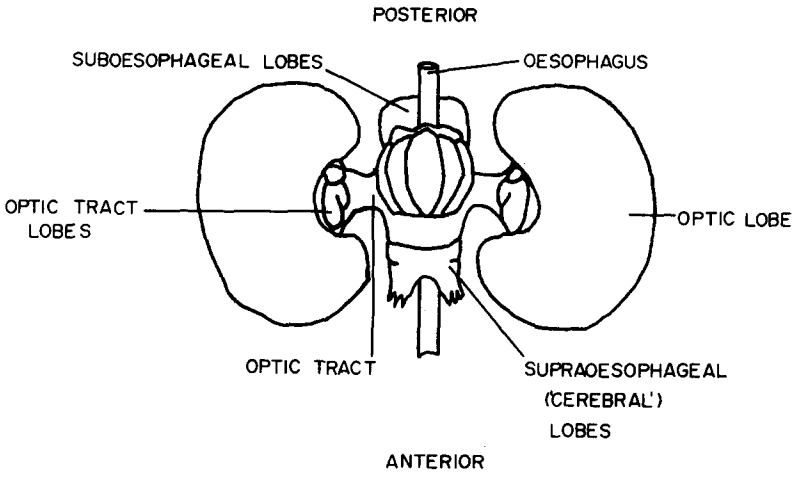



Fig. 1. Diagram of an entire octopus brain from the dorsal aspect (from Young, 1964).

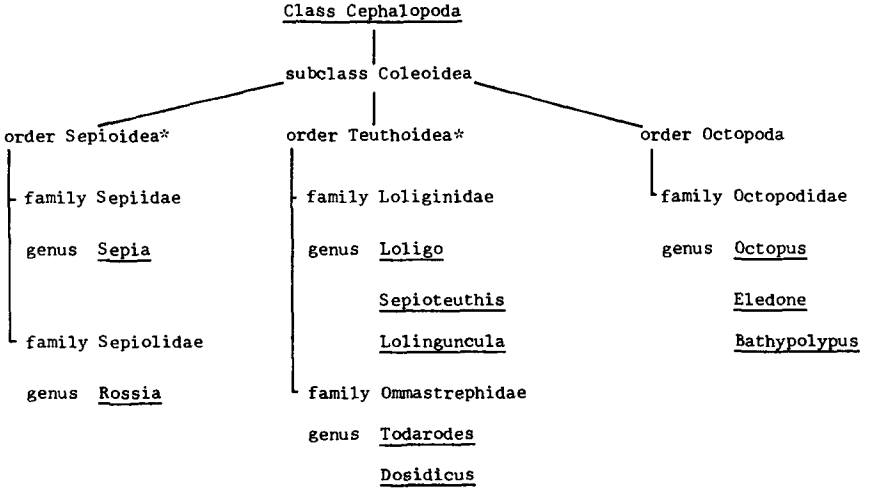
Subsequently, high concentrations of acetylcholine, its synthesising enzyme: cholineacetyltransferase (choline acetylase) and its hydrolysing enzyme: acetylcholinesterase, have been discovered in the brains of many cephalopods. Corteggiani (1938), for example, examined free and bound acetylcholine in the cerebral ganglia of *Octopus vulgaris* and *Sepia officinalis* and claimed that the bound amine constituted from 65% to 70% of the total acetylcholine.

The brain of the squid *Loligo pealeii* has been shown to be a very rich source of cholineacetyltransferase (Nachmansohn & Weiss, 1948; Berman *et al.*, 1953) and has been used as the source material for enzyme specificity tests (Korey *et al.*, 1951). Acetylcholinesterase has also been found in high concentrations in the same tissues (Nachmansohn & Meyerhof, 1941).

Augustinnson (1949) examined the substrate specificity of the cholinesterase from *Loligo* central ganglia and found that acetylcholine was hydrolysed at a higher rate than butyryl- or propionyl-choline, and that the activity-substrate concentration relationships were similar to those obtained with enzyme preparations from the electric organs of eels.

Feldberg *et al.* (1951) found that large amounts of acetylcholine were synthesised in the brain of *Sepia officinalis*, although none was synthesised in the retina or optic nerves. Florey (1963) noted that in *Octopus dofleini* there were enormous differences in the acetylcholine content of various parts of the nervous system and that there were almost parallel variations in the acetylcholinesterase content. A major review of the distribution of acetylcholine and acetylcholinesterase in *O. dofleini* showed that both were present in considerably higher quantities in the CNS than in peripheral organs, the concentrations being particularly high in the optic and supraoesophageal lobes; and also that acetylcholinesterase hydrolysed acetylcholine much more readily than butyryl- or benzoylcholine (Loe & Florey, 1966).

High concentrations of cholinesterase have also been found in the brain of *Todarodes pacificus*




* Sometimes combined as order Decapoda.

Fig. 2. Summary of the classification of Cephalopoda (adapted from Voss, 1977) showing all genera mentioned in this review.

(= *Ommastrephes sloanei pacificus*; Voss—personal communication). The optic lobes have been shown to hydrolyse 10–16 mmols ACh/g tissue/hr, which agrees closely with the value obtained from the electric organ of *Electrophorus electricus*, a commerical source of the enzyme. In contrast, the corresponding value for the cerebral ganglia is only 3 mmol ACh/g tissue/hr (Turpaev *et al.*, 1968). These workers also showed differences in the substrate specificity between decapod and octopod cholinesterases, which was also confirmed by work on the heart muscle and haemolymph of *Octopus*, *Todarodes* and *Rossia pacifica* (Grigor'eva *et al.*, 1968). Only the octopod enzyme can be considered as a true acetylcholinesterase whilst the decapod enzyme is a broad-substrate, non-specific cholinesterase (see review by Sakharov, 1970). This difference in enzyme specificity has also been noted from histochemical studies by Barlow (1977) and by Tansey (1978).

Soviet researchers working on the Pacific coast have used *Todarodes* brain extensively for enzyme research. This work has included studies on enzyme inhibition by anti-cholinesterase agents (Rozengart, 1968) and by ammonium compounds (Kupriyanov *et al.*, 1973); studies on the structural surface of the enzyme (Kulieva *et al.*, 1971); and experiments on substrate specificity (Bogolyubova *et al.*, 1972).

Similarly, American workers on the Atlantic coast have used cholineacetyltransferase from the head ganglia of *Loligo* for studies on enzyme activation (Prince, 1967); for studies on isoenzyme reactivity (Prempeh *et al.*, 1972); and as test material in an enzyme purification study (Husain & Mautner, 1973).

An enzyme capable of hydrolysing DFP (diisopropylfluorophosphate), a potent inhibitor of cholinesterase (Koelle, 1975), has also been found in the brain, liver and giant axon of *Loligo pealeii* (Hoskin, 1969; Hoskin & Long, 1972; Garden *et al.*, 1975). The physiological effect of this DFP-ase remains obscure, and, although it has been suggested as acting as a barrier to the penetration of cholinesterase inhibitors to the conducting membranes of nerves (Nachmansohn & Neumann, 1975), its predominant localisation in the axoplasm does not support this view (Hoskin, 1976). Experiments on the optic nerves of squid have shown that organophosphorus compounds such as DFP can interact specifically with components of the nerve membrane other than cholinesterases, and it is therefore possible that the DFP-ase may play a similar non-cholinergic role (Woodin, 1974).

A study of the cardioactive agents extracted from the organs of *Octopus bimaculatus*, which, incidentally failed to show 5-HT, showed considerable quantities of acetylcholine in the optic and cerebral ganglia (Agarwal *et al.*, 1972). Injections of acetylcholine itself, as well as drugs known to affect the cholinergic system, into the cephalic aorta of *Eledone cirrosa* (Chichery & Chanelet, 1971) and *Sepia officinalis* (Chichery & Chanelet, 1972, 1973) showed a variety of peripheral effects, which may indicate the presence of cholinergic neurons in the central nervous system.

Cholinesterases have been demonstrated histochemically in the optic lobes of a number of genera (Drukker & Schadé, 1967); in the nerve trunk and nerve fibres emerging from the macula of *Octopus* (Vinnikov *et al.*, 1968; Vinnikov, 1969); in the CNS of *Sepia officinalis* (Chichery & Chichery, 1974; Tansey, 1978) and in parts of the CNS of *Octopus vulgaris* (Barlow, 1971, 1977; Tansey, 1978). There has been no ultrastructural localisation in the CNS; the only work at this level has been peripherally, in the giant nerve fibres of *Sepioteuthis sepioidea* (Villegas & Villegas, 1974).

The quantities of acetylcholine and associated enzymes in the cephalopod CNS are so large that several authors have speculated that it may play a general role in nerve metabolism rather than having a specific function in synaptic transmission. This idea has been dispelled to some extent by work on the subcellular distribution of these chemicals, using the synaptosome isolation technique of Jones (1967). Synaptosome preparations made by Florey (1967) and by Florey & Winesdorfer (1968) from the optic lobes of *Octopus dofleini* revealed that a large proportion of bound acetylcholine was associated with fractions containing nerve endings. Indeed, the acetylcholine content of these endings was nearly one hundred times greater than that of corresponding fractions from mammalian brains.

Similarly, in the CNS of *Loligo pealeii*, part of the acetylcholine extracted was located in a fraction containing vesicles similar to those found in nerve endings (Heilbronn *et al.*, 1971), whilst subcellular studies of the same tissue revealed the presence of considerable quantities of acetylcholine, cholinesterase and cholineacetyltransferase (Welsh & Dettbarn, 1972). It has also been shown that synaptosome preparations from the optic lobes of *L. pealeii* and *L. forbesii* have a very high affinity uptake system for choline, which may be specific for cholinergic terminals and is particularly sensitive to metabolic inhibitors (Barker *et al.*, 1974; Whittaker *et al.*, 1972; Dowdall & Simon, 1973). Similar work with *Sepia officinalis* has shown that butyrylcholine-pyrene derivatives are extremely potent inhibitors of this choline uptake system (Dowdall *et al.*, 1976).

There is, therefore, much evidence that considerable quantities of acetylcholine (see Table 1) and its associated enzymes are present in cephalopod nervous tissue. Of particular importance is the ultrastructural work that has located these chemicals within synaptosomes and nerve terminals. Despite the lack of physiological evidence of function, we can conclude that there is a strong probability that acetylcholine acts as a neurotransmitter in the cephalopod CNS.

## BIOGENIC AMINES AND ASSOCIATED ENZYMES

There is also good evidence for the presence of other possible neurotransmitters in the cephalopod CNS. In this section we shall consider the catecholamines noradrenaline and dopamine, and also 5-hydroxytryptamine (5-HT), histamine, tyramine and octopamine.

In mammalian nervous systems, noradrenaline is accepted by most investigators as the neurotransmitter of the post-ganglionic sympathetic neuron, and there is also evidence that it functions as a transmitter in the CNS (e.g. see review by Molinoff & Axelrod, 1971). Dopamine, a precursor of noradrenaline, has also been postulated as a central transmitter in verte-

brates (e.g. Siggins, 1978; Bunney & Aghajanian, 1978) and in gastropods, lamellibranchs and insects (Walker & Kerkut, 1978). 5-HT principally produces inhibitory effects in the mammalian nervous system (Aghajanian *et al.*, 1975) and has been proposed as the neurotransmitter in the lateral eyes of *Limulus* (Adolph & Ehinger, 1975) and in the bovine retina (Thomas & Redburg, 1979). In the mammalian CNS there is evidence that histamine qualifies as a central neurotransmitter (Schwartz, 1975; Green *et al.*, 1978), whilst para-tyramine has been proposed as a "synaptic activator" (Boulton, 1978). Octopamine has been considered to be a "false" transmitter in the mammalian nervous system because it is taken up, stored in and released from catecholaminergic terminals, following the inhibition of monoamine oxidase (Hicks, 1977), but it has been postulated as a transmitter in some invertebrates (Axelrod & Saavedra, 1977) especially in the lobster, where it is found in considerable quantities (Wallace *et al.*, 1974).

The first of these amines to be associated with the cephalopod CNS was histamine. Sereni (1929) noted that intravascular injection of histamine caused chromatophore expansion and "general excitement" in *Octopus vulgaris* and *Eledone moschata*; he considered that the effect was mediated by the CNS. Ungar *et al.* (1937) showed that the central ganglia of *Octopus vulgaris* contained considerable amounts of histamine but made no deductions as to its physiological role. A crude attempt to do so, by Jullien *et al.* (1957) revealed nothing that could be unequivocally related to the CNS. It is interesting to note, however, that Scuka (1971) found that histamine had a suppressive effect on the resting and action potentials of the squid giant axon.

Histamine has also been found in the cerebral ganglia of *Octopus conispadicus* (Boldyrev & Lebedev, 1972), in the optic lobes of *Eledone moschata* (Bertaccini, 1964) and, associated with *N*-acetyl-histamine, in the CNS of the giant Pacific squid *Dosidicus gigas*

Table 1. Possible neurotransmitters in the cephalopod CNS

| | ACh | Ala | Asp | DA | GABA | Glu | Gly | His | 5HT | NA | Oct | Tau | Tyr |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Dosidicus gigas* | | | | | | | | D<br>E | D<br>E | | | | |
| *Eledone cirrosa* | | G<br>H | G | A<br>E | F<br>G(−)<br>H(−) | F<br>G<br>H | F | | A<br>E | A<br>E | | E<br>F | |
| *moschata* | | | | | | | | D | D<br>E | E | E | | |
| *Loligo forbesii* | B<br>C | | | | | | | | | | | | |
| *pealeii* | B<br>C<br>D<br>E | | C | A | | C | C | | A<br>B<br>C<br>E | A<br>B<br>C | | | |
| *vulgaris* | | | | | | | | | E | E | E | | |
| *Octopus bimaculatus* | E | | E | | | | | E | E(−) | | | | |
| *briareus* | | | | | | | | | E | | | | |
| *conispadicus* | | E | | | E(−) | | | | | | | | |
| *dofleini* | B<br>D<br>E | | | E | | | | | E | | E | | E |
| *macropus* | | | | E | | | | | | E | E | | |
| *vulgaris* | B<br>D<br>E | | | A<br>B<br>E | | | | D<br>E | A<br>D<br>E | A<br>B<br>E | B<br>E | | |
| *Sepia esculenta* | | | E | | E(−) | E | | | | | | | |
| *officinalis* | C<br>D<br>E | | | A<br>E | | | | | E | A<br>E | E | | |

Chemical abbreviations

ACh—acetylcholine
Ala—alanine
Asp—aspartate
DA—dopamine
GABA—γ-aminobutyric acid
Glu—glutamate
Gly—glycine
His—histamine
5-HT—5-hydroxytryptamine
NA—noradrenaline
Oct—octopamine
Tau—taurine
Tyr—tyramine

Techniques

A = histochemistry
B = synaptosome localisation
C = synaptosome uptake mechanism
D = extraction—bioassay
E = extraction—chemical assay
F = extraction—microchromatography
G = brain slices—*in vitro* isotope labels
H = *in vivo* isotope labels
(−) = chemical not detected or below sensitivity level of technique

(Roseghini & Ramorino, 1970). A histaminase has also been extracted from the optic lobes, renal appendages and pancreas of *E. cirrosa* (Boadle, 1969), but the substrate specficity of the enzyme differed so widely from the histaminase found in vertebrates that it has been suggested that the cephalopod enzyme is a broad substrate monoamine oxidase (*ibid.*).

The first work on catecholamines and indolealkylamines and their metabolism was performed by Blaschko and his co-workers, who, having found *Octopus* and *Sepia* livers to be a very rich source of amine oxidase (Blaschko & Hawkins, 1952a,b), examined *Octopus* brain only to find quite low levels of the enzyme (Blaschko, 1952). Further work on amine oxidase, particularly from *Sepia* liver but also from the brains of *Sepia officinalis* and *Loligo forbesii*, has revealed the wide substrate specificity of the enzyme. The enzyme from decapod nervous system oxidised not only tyramine and related compounds such as octopamine and β-phenyl-alanine, but also aliphatic monoamines, long chain diamines, tryptamine and particularly 5-HT (Blaschko & Philpot, 1953; Blaschko & Himms, 1954).

Florey & Florey (1953) noted that aqeuous extracts of the optic, cerebral and stellate ganglia of *Sepia* increased cardiac frequency in cephalopods. They concluded that the active agent was identical to enteramine or 5-HT. This substance had already been extracted from the posterior salivary glands of *Octopus vulgaris* and *O. macropus*, and chemically characterised, using no fewer than 30,000 pairs of glands from *O. vulgaris* (Erspamer, 1948; Erspamer & Asero, 1953).

The 5-HT content of the optic lobes of *Eledone moschata* and *Octopus vulgaris* were examined in more detail by Piccinelli (1958), who found that administering reserpine caused a conspicuous decrease of 5-HT in the optic lobes although there was no release of the endogenous 5-HT from the posterior salivary glands. Similar work by Bertaccini (1964), also on the optic lobes of *E. moschata*, confirmed the depletion of 5-HT by reserpine and also noted that the amount of the amine present varied seasonally. A quantitative evaluation of 5-HT in the nervous system of a number of invertebrates showed that although the cephalopods examined did contain 5-HT in their brains, the range of values was lower than for other molluscs (Welsh & Moorhead, 1960; Mirolli & Welsh, 1964). 5-HT has also been extracted from the optic lobes and central brain of *Dosidicus gigas* (Roseghini & Ramorino, 1970). A piece of indirect evidence for 5-HT in the brain of *O. vulgaris* comes from the effects of phenothiazines, substances well known as tranquillisers that are known to block the actions of 5-HT (Byck, 1975). When injected into *Octopus* these drugs caused a slowing of movement and apparently "sleep" (Katona & Wolleman, 1964).

The catecholamines noradrenaline and dopamine were isolated from the brain of *Eledone cirrosa* by Cottrell (1967). A more detailed quantitative analysis of noradrenaline, dopamine and 5-HT in the different lobes of the cephalopod brain was undertaken by Juorio (1971). This work on *Octopus vulgaris* and *E. cirrosa* reported that the amines were stored in reserpine-sensitive granules and were present in sufficient quantities to act as neurotransmitters. Incidentally, the concentrations of adrenaline in the brain were below the limits of sensitivity of the technique employed (*ibid.*).

Further work on amine distribution has revealed the presence of octopamine in reserpine-sensitive granules and in the same density gradients as noradrenaline and dopamine (Juorio & Molinoff, 1971, 1974). These authors postulated that these three chemicals may be confined within the same nerve endings, or comprise separate octopaminergic, dopaminergic and noradrenergic nerve endings (Juorio & Molinoff, 1974). Octopamine has also been reported in the brain of *Bathypolypus arcticus* (Hicks & O'Dor, 1977) and, as its name suggests, it was first isolated from *Octopus vulgaris*, from the posterior salivary glands (Erspamer & Boretti, 1951). Para-tyramine has also been extracted from the brain of *O. vulgaris* (Juorio & Philips, 1975) and, like the catecholamines, 5-HT and octopamine, it was found to be stored in reserpine-sensitive granules and to be inhibited by monoamine oxidase.

Histochemical localisation of noradrenaline, dopamine and 5-HT in the brain of *Octopus vulgaris* has been shown by Matus (1973), and in the brains of a number of cephalopod genera by Tansey (1978, 1979), and in the cephalopod "cerebellum" (Messenger & Tansey, 1979). Characteristic green–yellow aminergic fluorescence has also been reported along the optic gland nerves of *O. bimaculatus* and *O. apollyon* (Nishioka *et al.*, 1970) although this has not been confirmed in other *Octopus* species or in other cephalopod genera (Tansey, 1978, 1979).

The acid metabolites of dopamine and 5-HT have been found in five species of cephalopods (Juorio, 1972; Juorio & Killick, 1972). Juorio & Barlow (1973) suggested, on the basis of radioactivity labelled conversions, that the brain of *Octopus vulgaris* contained enzymes capable of synthesising dopamine and noradrenaline; and in the following year, demonstrated species-specific variations in the ratio of catecholamines in the vertical lobe (Juorio & Barlow, 1974).

The optic lobe of *Loligo pealeii* produces an extremely useful synaptosome preparation that has been used extensively for neurotransmitters (Dowdall & Whittaker, 1973). This preparation has been shown to have specific high-affinity uptake systems for L-noradrenaline (Pollard *et al.* 1973) and for 5-HT (Feldman & Dowdall, 1973; Dowdall, 1974). Pollard *et al.* (1975) reported that the uptake of L-noradrenaline and 5-HT was blocked by the use of chloropromazine, an inhibitor of monoamine uptake and a common anti-psychotic drug, and suggested that the *Loligo* preparation could be very useful in basic pharmacological research on aminergic synapses.

In conclusion, there is a great deal of empirical evidence of the presence of possible neurotransmitter amines in the brain of cephalopods (see Table 1). The evidence that dopamine, noradrenaline and 5-HT are found in reserpine-sensitive granules, that enzymes capable of synthesising dopamine and noradrenaline have been located and that synaptosome uptake studies have shown uptake mechanisms for noradrenaline and 5-HT, suggests that the catecholamines and 5-HT may act as neurotransmitters. At the moment, the evidence for octopamine, histamine or tyramine having a similar role is less good.

## AMINO ACIDS

Several amino acids have been suggested as having a neurotransmitter function in the mammalian CNS (see review by Davidson, 1976). Before reviewing the situation in cephalopods, we shall briefly consider which amino acids have been suggested as neurotransmitters elsewhere. From neurophysiological evidence two classes of amino acids have been proposed; those with an excitatory effect, glutamic acid, aspartic acid, cysteic acid and homocysteic acid; and those with an inhibitory effect, γ-amino-butyric acid (GABA), glycine, taurine and β-alanine. Of these, all but cysteic and homocysteic acid have been reported in the cephalopod brain.

GABA has been postulated as an inhibitory transmitter at certain synapses in the mammalian CNS (Krnjević & Schwartz, 1967; Obata & Takeda, 1969; Roberts *et al.*, 1976) and at the lobster neuromuscular junction (Otsuka *et al.*, 1966). Glycine is principally thought to act as an inhibitory transmitter in the spinal cord (Aprison & Werman, 1965; Aprison *et al.*, 1970), whilst glutamate has been proposed as an excitatory transmitter in the spinal cord (Krnjević, 1970; Johnson, 1972) and as the transmitter in the parallel fibres of the cerebellum (Sandoval & Cotman, 1978). Miledi (1972) has reported evidence for specific glutamate receptors in the squid stellate ganglion. Taurine has been suggested as a neurotransmitter in the vertebrate retina (Mandel *et al.* 1976; Barbeau *et al.*, 1975); both D- and L-aspartate have been suggested as excitatory neurotransmitters from iontophoresis on cortical neurons (Krnjević & Phillis, 1963); and β-alanine may be an inhibitory transmitter in the mammalian CNS (Defeudis & Martin Del Rio, 1977).

Simple extractions from cephalopod brain have revealed the presence of a number of these amino acids. High levels of aspartic acid have been found in the brain and eyes of *Octopus bimaculatus* (Kittredge *et al.*, 1962), whilst D-aspartate has been found in the brain of *O. vulgaris* (D'Aniello & Giuditta, 1977). The brain of *Sepia esculenta* contains glutamic acid but practically no GABA or *N*-acetyl aspartic acid (Tsukada *et al.*, 1964). The enzyme that catalyses the glutamine–glutamate conversion, glutamate synthetase, has been shown to have high activity in the optic lobes of *O. vulgaris* and *Rossia pacifica* (Kleinschuster & Morris, 1972), and in *Eledone cirrosa* (Cory & Rose, 1969). Work on *E. cirrosa* showed that the optic lobes contained very little GABA, some glutamic acid and glycine and a great deal of taurine (Osborne, 1971, 1972). A similar distribution of amino acids has been shown in single axons of *S. officinalis* (Lewis, 1952). Osborne (1971) believed that the taurine principally played a role in osmoregulation, a belief that was shared by Simpson *et al.* (1959) from work on entire specimens of *Lolinguncula brevis*, a brackish-water cephalopod. Gould & Cottrell (1974) confirmed high levels of taurine in the brain of *Eledone* and also found proline.

An *in vitro* examination of the uptake of $^{14}C$-labelled glucose in slices of vertical and optic lobes of *Eledone cirrosa* showed that the major metabolites formed were lactate, carbon dioxide, glutamine and alanine, traces of aspartate and glutamate but only very little GABA (Cory & Rose, 1969; Chain, 1970). This work agrees with previous evidence that tricarboxylic acid cycle and transaminase enzymes are present in cephalopod brain (Drukker & Schadé, 1963, 1964; Pascoe & Schadé, 1967a,b).

A subsequent *in vivo* experiment in *Eledone cirrosa* confirmed the high production of alanine and low levels of GABA (Rose & Cory, 1970). The cerebral ganglia of *Octopus conispadicus* were also found to contain a large quantity of alanine, although GABA was not detected at all (Boldyrev & Lebedev, 1972). It is interesting to note, incidentally, that the livers of *O. vulgaris*, *E. moschata* and *Sepia officinalis* were incapable of deaminating GABA (Baret *et al.*, 1965). The very low levels of GABA in *Octopus* brain have been correlated with the low levels of its synthesising enzyme, glutamic acid decarboxylase (Roberts, 1964). In comparison with rat cerebral cortex (Beloff-Chain *et al.*, 1955a,b), the generation, uptake and utilisation of GABA in octopus brain is negligible (Cory, 1969; Cory & Rose, 1969). The same experiment in the gastropod mollusc, *Helix pomatia*, also revealed low GBA and high alanine production from glucose (Bradford *et al.*, 1969). There is evidence from vertebrate work that there are a number of different metabolic compartments in the brain, particularly with reference to glutamate and GABA metabolism (see Symposium Review by Balazs & Cremer, 1973). It has also been suggested that one of these cellular compartments is represented by the neuroglia (Rose, 1970; Iversen & Kelly, 1975) and as it is known that the CNS of the octopus contains relatively fewer glial cells than that of the rat (Young, 1964), this morphological difference may account for the differences in metabolism.

Squid synaptosomes from *Loligo pealeii* have been shown to have uptake mechanisms for aspartic and glutamic acids, glutamate and glycine (Pollard *et al.*, 1975) but no specific role has been defined for these acids.

Thus, high levels of some free amino acids are present in the cephalopod brain (see Table 1). Similar high levels are also found in other cephalopod tissues, for example muscle (Florkin, 1966) and may well be related to osmoregulation in a marine environment (Duchâteau *et al.*, 1952). There could be diverse metabolic reasons for their presence in cephalopod CNS, and there is little evidence that any of these amino acids act as neurotransmitters.

## CONCLUSION

In conclusion, it may be said that although there is a considerable amount of data about the presence of different possible neurotransmitter substances in the cephalopod CNS, much of it is inconclusive. Ultrastructural evidence from the supraoesophageal lobes of *Eledone* (Jones, 1970) has shown different populations of vesicles that may be associated with different neurotransmitter substances, and the evidence reviewed here suggests that there are at least four of these substances in the cephalopod brain. The ultrastructural localisation and the mechanisms of synaptosome uptake shown for acetylcholine, the catecholamines and 5-HT however make these chemicals strong candidates for such a role. It must be remembered, however, that at the moment, there is no physiological evidence of the mode of action of any of these substances in the cephalopod CNS.

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