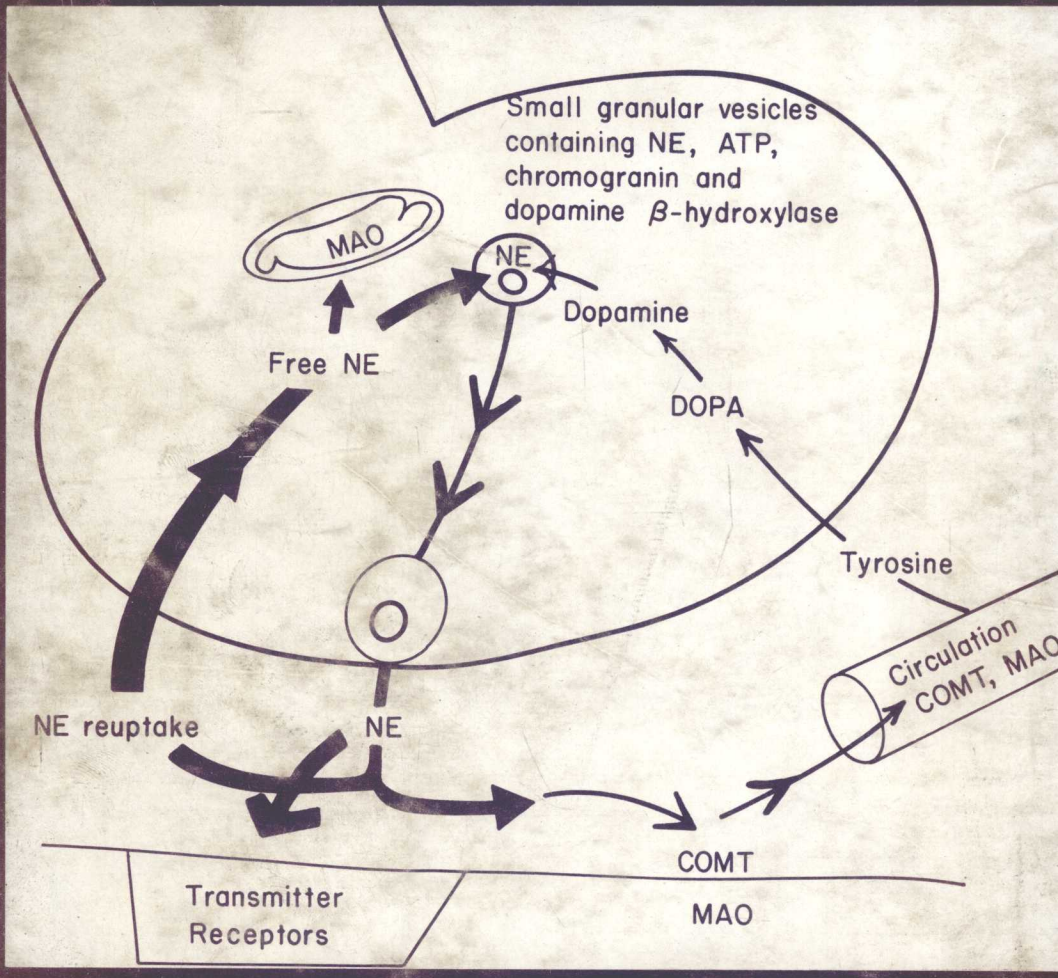
Chemical Pharmacology of the Synapse



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Chemical Pharmacology of the Synapse

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Preface

The analysis of synaptic function proceeds at an ever increasing rate. The basic contributions of workers from diverse fields, including biochemistry, chemistry, pharmacology and physiology, have made possible the attempts currently being made to provide a molecular description of the chemical transmission process. However, this multidisciplinary approach and the extremely rapid rate of development of this area raise a number of serious communication problems. It is increasingly difficult for workers to keep abreast of the developments in neurobiology and, more particularly, it is difficult for the new worker in the field to obtain an adequate perspective against which to align current developments.

In writing this book we have been particularly conscious of the latter point and have attempted to provide a general and comparative view of synaptic function. To this end we have organized the material into five broad chapters. Chapter I attempts to give a basic perspective of synaptic function and includes comparative discussions of synapse morphology, transmitter pathways and localization, receptor classification, synaptic control mechanisms and the trophic function of the synapse. Subsequent chapters deal with quantitative aspects of neurotransmitter-receptor interactions, structure-activity relationships, the ionic and metabolic consequences of neurotransmitter-receptor interaction and the isolation of receptors.

It is our hope that the book will be of use not only to neurobiologists but also to workers from other fields who may wish to obtain a general view of synaptic function. Of necessity, in a book of this size we have not been able to deal comprehensively with the topics listed above and we are acutely aware of our own deficiencies in many of the areas discussed. Nonetheless, we believe that we have provided adequate documentation so that the interested reader may pursue topics in greater detail.

We wish to thank our wives for their understanding during the writing of this book and one of us (D.J.T.) wishes to express his gratitude to Professor N. B. Chapman and his colleagues in the Department of Chemistry, University of Hull for the hospitality extended during a sabbatical visit when Chapters II and III were written. Our thanks are due also to numerous colleagues for many helpful discussions and criticisms. All errors and omissions remain of course, the responsibilities of the authors.

D. J. Triggle
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November 1, 1975

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Chapter 1

Structure and function of the synapse

C. R. TRIGGLE and D. J. TRIGGLE

Prior to the proposal and acceptance of the neurone theory – pre-eminently associated with Ramón y Cajal (1852–1934) – which established that the nervous system is made up of isolated independent neurones, there could have been little incentive to investigate the possibility of any specific chemical basis of neurone activity. The establishment of the protoplasmic discontinuity – termed the synapse by Sherrington – did however indicate the necessity of defining the molecular basis of neuronal communication and information flow and processing in the nervous system. Since then the continued interplay of anatomy, physiology, pharmacology and biochemistry has provided the intellectual cutting edge for the dissection of the molecular components of the nervous system.

The concept of chemical transmission

The basic experimental observation that synaptic communication might utilize specific chemical agents was made by Elliot (1904) who, much struck by the similarities between the effects of epinephrine, known to occur in the adrenal medulla, and those of sympathetic stimulation, suggested that, "adrenaline (epinephrine) might be the chemical stimulant liberated on each occasion when the impulse arrives at the periphery." This splendid suggestion was not received with any great enthusiasm and the possible reasons for this have been discussed by Dale (1960). Although the concept of chemical transmission was courageously extended to parasympathetic nerves by Dixon (1906, 1907; see Dale, 1935), who endeavoured to isolate a transmitter, by Hunt and Taveau (1906), who noted the powerful cardiodepressor activity of acetylcholine, and by Dale (1914), who noted the similarities between acetylcholine and parasympathetic activity, it was not until the classic experiments of Loewi (1921, 1922) that it was clearly established that chemical agents liberated on stimulation of the vagus nerve to one frog heart could, when administered to a second frog heart, mimic

the effects of parasympathetic stimulation. Even then it was not until Dale and Dudley (1929) actually isolated acetylcholine from spleen extracts that the inhibitory "vagusstoff" and acetylcholine were recognized to be the same agent.

This brief historical introduction, treated more adequately elsewhere (Dale, 1953; Eccles, 1964; McLennan, 1970), will suffice to suggest the first great difficulty in the study of synaptic transmission, the identification of the neurotransmitter. The half-century subsequent to Loewi's work has led to the realization that comparatively few molecules appear to serve as neurotransmitters throughout the animal kingdom. These agents include the phenylethylamines (norepinephrine, epinephrine and dopamine), the indole, 5-hydroxytryptamine, the imidazole, histamine, acetylcholine, a number of amino acids, including glycine, glutamic, aspartic and γ-aminobutyric acids, and possibly also such agents as the polypeptide substance P, prostaglandin E and adenosine triphosphate (Fig. 1.1; Table I.1). This list is quite possibly incomplete since the transmitters for the majority of synapses have simply not been identified. Since the transmitter function of many of these molecules is spread throughout the animal phyla, it must be presumed that it evolved fairly early (p. 107). However, as will become apparent in subsequent sections, the sites at which a given transmitter is utilized and the mechanisms by which it produces its effects may differ considerably between species, and predictions by analogy of the identity or activity of a particular transmitter may be totally erroneous. Hence, a precise identification of a synaptic transmission process must, as with any other system of chemical recognition, establish the identity of both the transmitter and the specific cellular molecule – the receptor – with which it interacts to initiate the observed response.

As can be seen from Fig. 1.1 there is a considerable diversity in the structures of molecules serving a putative neurotransmitter function. Most of these molecules are of rather simple structure but the possible neurotransmitter role of the peptide substance P is of great interest since it suggests a relationship between the neurotransmitter function and the endocrine function of peptides (Iversen, 1974a, b). Thus the hormone vasopressin, released, along with other nonapeptide hormones, from hypothalamic neurones terminating in the pituitary gland, produces profound effects on neuronal excitability (Nicoll and Barker, 1971) in addition to its antidiuretic action (Frieden and Lipner, 1971). Similarly, the releasing factor peptides carried from hypothalamic nuclei through the blood to the noninnervated adenohypophyseal cells and responsible for hormone release (growth hormone, prolactin, etc.) may also have significant effects on neuronal excitability. Thus, thyrotropin releasing hormone (TRH) and melanocyte stimulating hormone-release-inhibiting hormone (MIH) have a variety of behavioral effects (Iversen, 1974a, b), and very large concentrations of TRH are found outside of the hypothalamus. Substance P found in a variety of tissues such as the intestine, dorsal roots of spinal nerves and the brain including the

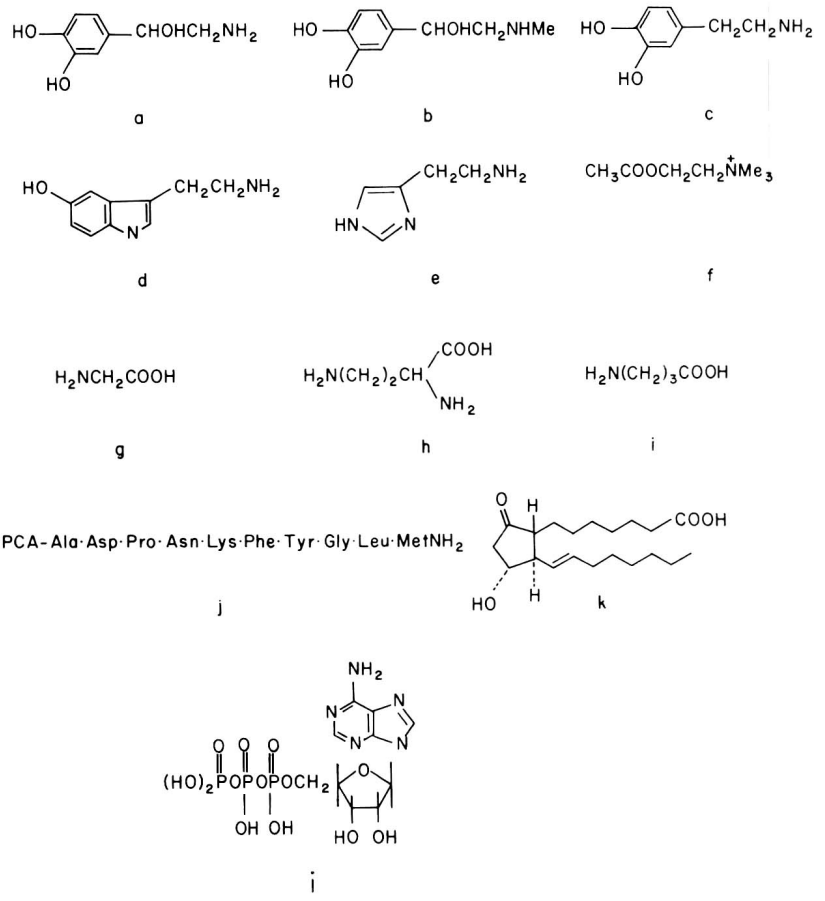

FIG. 1.1. Structural formulae of neurotransmitters. (a) Norepinephrine; (b) epinephrine; (c) dopamine; (d) 5-hydroxytryptamine; (e) histamine; (f) acetylcholine; (g) glycine; (h) glutamic acid; (i) λ-aminobutyric acid; (j) substance P; (k) prostaglandin E; (l) adenosine triphosphate.

hypothalamus (von Euler and Gaddum, 1931; Chang and Leeman, 1970; Otsuka *et al.*, 1972; Takahashi *et al.*, 1974), has a very powerful excitant action on spinal cord motoneurones (Konishi and Otsuka, 1974) and cerebral cortex and cuneate nucleus neurones (Phillis and Limacher, 1974; Krnjević and Morris, 1974); it may well be an excitatory transmitter involved in sensory neurone function.

Three hypothalamic–adenohypophyseal pepetides, TRH, luteinizing hormone–releasing hormone (LH–RH) and growth hormone-release inhibiting hormone (SRIF), have been shown to have powerful neurone depressant effects at several brain levels including the cerebral and cerebellar cortex and brain stem

TABLE I.1. General distribution of putative neurotransmitter function[a]

Neurotransmitter	Site(s)
Acetylcholine	Mammalian peripheral nervous system; autonomic preganglionic fibers; parasympathetic postganglionic fibers; motor fibers to skeletal muscle. Mammalian central nervous system: widespread distribution including Renshaw cells, cerebellum , cerebral cortex, thalamus, lateral and medial geniculate nuclei, caudate nucleus. Probably wide-spread in invertebrate central and peripheral nervous systems.
Dopamine	Mammalian central nervous system: (see Fig. 1.12) Insect and mollusc central nervous system.
Norepinephrine	Mammalian peripheral nervous system: sympathetic postganglionic fibers; ganglionic interneurones; adrenal medulla. Mammalian central nervous system: (see Fig. 1.12). Invertebrate central nervous system.
Epinephrine	Adrenal medulla; Generally widespread but usually in much lower concentrations than NE.
Histamine	Vertebrate CNS: hypothalamus, midbrain.
5-Hydroxytryptamine	Vertebrate central nervous system: (see Fig. 1.11). Vertebrate peripheral nervous system. Invertebrate nervous system.
γ-Aminobutyric acid	Vertebrate central nervous system: cerebellum, cortex, hippocampus, retina (?) spinal cord. Invertebrate peripheral nervous system: annelid and insect crustacean neuro-muscular junctions.
Glutamic acid	Vertebrate central nervous system: spinal cord Invertebrate peripheral nervous system: insect and crustacean neuromuscular junction.
Glycine	Vertebrate central nervous system: spinal cord.
Aspartic acid	Vertebrate central nervous system: spinal cord.
Substance P	Widespread distribution through vertebrate central nervous system and peripheral nervous system; including substantia nigra and dorsal root ganglion (spinal cord).
Adenosine triphosphate	Vertebrate peripheral nervous system

[a]This table represents no more than a brief summary of what are believed to be the most important locations of transmitter function. It is to be realized that not all of the agents listed have *established* neurotransmitter function at all of the sites listed. Transmitter action and localization presents many difficulties in the vertebrate central nervous system and much of the work reported for invertebrates is quite cursory. Summaries of available data are to be found in Bradley (1968), Phillis (1970) and Albers *et al.*, (1972). Comprehensive comparative analyses of neurotransmitter status have been given by Gerschenfeld (1973) and Michelson (1972) and the role of ATP as a transmitter is discussed by Burnstock (1972).

(Renaud *et al.*, 1975) adding further weight to the evidence that these agents may act both as hormones and neurotransmitters.

Very recently a peptide has been isolated from mammalian brain that may well function as the endogenous opiate ligand (Terenius and Wahlström, 1975a, b; Hughes, 1975; Kosterlitz and Hughes, 1975; Pasternak *et al.*, 1975). It has long been realized that the pharmacological actions of the narcotic agonists and antagonists together with the established structure-activity relationship of these agents indicates the existence of an opiate receptor in the central nervous system and in some peripheral locations (i.e., guinea-pig ileum) where an identical structure-activity relationship to that seen in the CNS can be deduced (Creese and Snyder, 1975). The existence of such a receptor, confirmed by its recent partial isolation (Snyder, 1975) suggests that there should exist an endogenous ligand active at these receptors: the recently isolated peptide may fulfill such a function. This peptide has a molecular weight of 1000–1200, it has a CNS distribution paralleling that of the opiate receptors, it mimics the action of morphine-like agonists in inhibiting contractions of guinea-pig ileum and its binding behaviour is similar to that of the opiate agonists in that it is decreased by Na^+ ions and stands in competitive relationhip to the narcotic antagonists.

The identification of neurotransmitters and neurotransmitter pathways

The early difficulties in defining neurotransmitters have already been noted yet the pioneering experiments of Loewi indicate two of the fundamental criteria that have to be satisfied to establish a neurotransmitter pathway, namely, release and collectability in adequate amounts from presynaptic nerve terminals and the establishment of identity of action in every respect of the putative transmitter molecule and synaptic activity. In their most rigorous sense these are extremely difficult criteria to fulfill* although they are most closely fulfilled for the intensively studied actions of acetylcholine at the skeletal neuromuscular junction.

Although these two criteria are without doubt the most important for establishing a transmitter pathway (Werman, 1966, 1972) there are other properties, mechanisms and pathways that are also characteristic of chemical transmission. The following list would appear to represent the general

*In retrospect, it may be argued that the small but important discrepancies noted between the actions of epinephrine and muscarine and sympathetic and parasympathetic stimulation respectively contributed significantly to the initial reluctance to accept chemical transmission (Dale, 1953).

characteristics of such transmission processes:

1. the presence of a presynaptic biosynthetic pathway;
2. the presence of a presynaptic transmitter;
3. the presence of a presynaptic transmitter storage mechanism;
4. the presence of a specific transmitter release mechanism;
5. the presence of specific postsynaptic receptors;
6. the presence of specific mechanisms to terminate transmitter action.

These general characteristics of synaptic transmission are schematically represented in Fig. 1.2. In view of the difficulties of applying the fundamental criteria of collectability and identity of action these general characteristics of synaptic transmission have also been employed as criteria of transmitter action. It is to be emphasized that the general ease of applicability of these criteria varies considerably between transmitter candidates and becomes acute with agents such as the amino acids and adenosine triphosphate that serve a general metabolic role in addition to their putative transmitter function. A discussion of these general properties of synaptic transmission together with brief reference to the morphology of synapses will serve as an introduction to the detailed analyses of transmitter action at the molecular level that are presented in subsequent chapters.

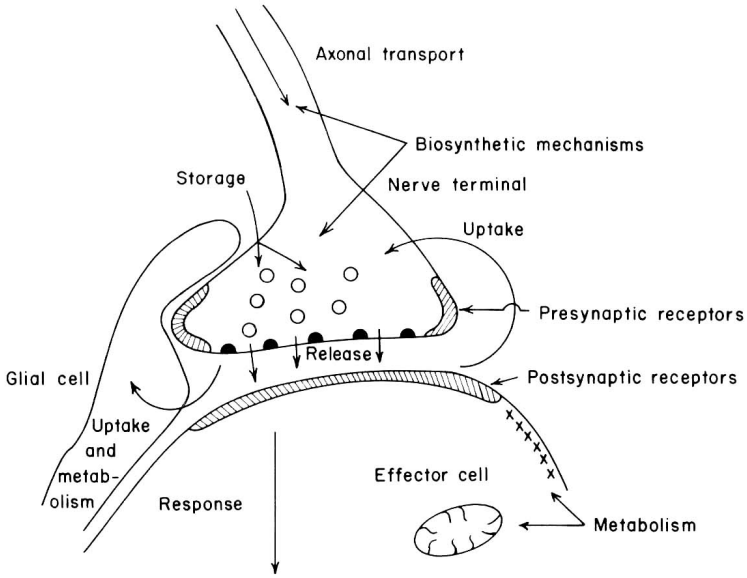

FIG. 1.2. Schematic representation of synaptic function.

Synapse Structure

The basic features of the chemically transmitting synaptic junction are the presence of the synaptic cleft, some 100–600 Å in width, and specialized presynaptic and postsynaptic membranes. Although a detailed analysis of synapse structure will not be attempted here (for such analyses, Peters *et al.*, 1970; Gray 1971; Pappas and Purpura, 1972 and Pfenninger, 1973 may be consulted), brief consideration may be helpful in establishing, at an elementary level, some morphological correlates of the chemical transmission mechanism since although the gross morphology of synapses may differ quite markedly, at the fine structural level there is much in common.

In most neurones, three distinct regions may be distinguished: a cell body which contains the nucleus, a number of twisting and ramifying dendrites, and a single relatively straight process, sometimes covered in a myelin sheath, termed

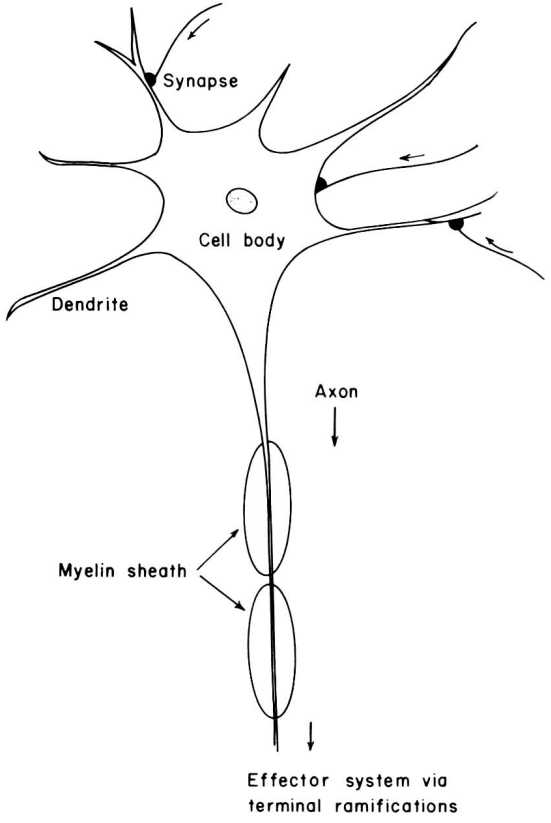

FIG. 1.3. Schematic representation of neurone.

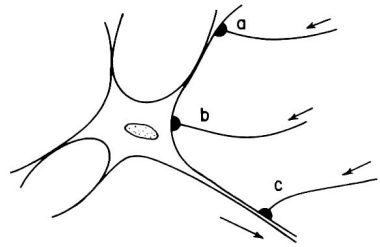

FIG. 1.4 Neurone showing (a) axo-dendritic synapses, (b) axo-somatic synapses and (c) axo-axonal synapses.

the axon which transmits, through its terminal ramifications, the cell response to the effector system (Fig. 1.3).

In most instances the presynaptic component is an axon or axon terminal which forms synaptic junctions on a muscle or gland cell or on another neurone. In the case of neurones, the axon terminal may be on a dendrite, cell body or axon forming axo-dendritic, axo-somatic or axo-axonal synapses (Fig. 1.4). However, it is not necessary that axons constitute the presynaptic element and dendro-dendritic, somato-dendritic and somato-somatic synapses are also known (Rall, 1967; Bodian, 1972; Pappas and Waxman, 1972; Purpura, 1972; Reese and Shepherd, 1972). This complex diversity of neuronal interaction does not represent mere caprice of organization and must be intimately concerned with aspects of neuronal integration. It has been suggested that of the commonest axo-dendritic and axo-somatic synapses the former are concerned with excitatory and the latter with inhibitory actions. This particular morphological classification appears to be an oversimplification (Pappas and Waxman, 1972): nonetheless, some morphological correlation with physiological function does appear possible on the basis of a more detailed examination of synapse structure. In addition to the two component synapses just discussed there also exist three component or serial synapses with axo-axo-dendritic or axo-axo-somatic contacts in the vertebrate central nervous system (Gray and Guillery, 1966; Gray, 1969a, b) and axo-axo-muscular junctions in crustacean muscles (Dudel and Duffler, 1961a, b) and it is likely that these are concerned with presynaptic inhibition whereby an inhibitory synapse modifies an excitatory synapse to reduce the release of excitatory transmitter (p. 68).

Presynaptic specializations

The most characteristic structural specialization of the presynaptic terminal is the presence of synaptic vesicles (De Robertis and Bennett, 1954; Palay, 1954) which represent the basic site of storage and release of the neurotransmitter

TABLE I.2. Sizes and staining properties of synaptic vesicles (Pfenninger, 1973)

Vesicles	Shape and size[a] Aldehyde $-OsO_4$	Aldehyde $-FE$	ZIO[b]	Staining of Contents UL, BIUL[c] E-PTA	$KMnO_4/OsO_4$ $K_2Cr_2O_7$
Cholinergic	*S*, ~500 Å	*S*, ~500 Å	+	−	−
Aminergic	*S*, ~500 Å	*S*, ~500 Å	+	−	+
Glycine	*F*	*S*, ~400 Å	+	−	−
Unidentified	*S*, *F*	*S*, ~300–600 Å	+	−	

[a]By EM using aldehyde-OsO_4 fixing or aldehyde and freeze etching (FE). [b]Zinciodide-osmium tetroxide (ZIO). [c]Uranyl acetate-lead hydroxide (UL); bismuth iodide-uranyl-lead (BIUL); ethanol-phosphotungstic acid (EPTA). For a discussion of the specificity of these histochemical reagents see Pfenninger (1973).

(p. 61). It is now clear that although the vesicles within a single class of terminal are more or less uniform in size, different classes of terminals may be characterized by different vesicle sizes, staining properties and shapes. Typically, vesicles have been described as spherical with diameters of 300–600 Å and distinguished by electron dense or electron-lucent centers that appear characteristic of adrenergic and cholinergic nerve terminals respectively (Table I.2). Subsequently it was realized, largely from work on CNS synapses, that the agranular vesicles were of two types that appeared after fixation as spherical (*S*) or flattened (*F*) respectively (Uchizono, 1965; Bodian, 1966, 1972; Akert *et al.*, 1972; Pfenninger, 1973). There appears to exist some correlation between vesicle size and shape and the transmitter type and Uchizono (1965, 1968) in fact suggested that *F* and *S* type vesicles may be characteristic of inhibitory and excitatory synapses respectively. This suggestion appears to have been confirmed for a number of synapses from central vertebrate and peripheral nervous systems (Gray, 1969a, b, 1971) and crustacean stretch receptors and neuromuscular junctions (Uchizono, 1967; Atwood, 1968) where the physiological evidence for excitation or inhibition exists. A further type of vesicle has been associated with the smooth muscle neurones proposed to use adenosine triphosphate as a transmitter (Burnstock, 1972). These are granular vesicles but are larger than the granular adrenergic vesicles, being some 800–2000 Å in diameter and have been termed large opaque vesicles. These vesicles are not depleted by various catecholamine depleting agents and hence can be distinguished from the adrenergic vesicles, and their distribution generally correlates with the presence of non-adrenergic non-cholinergic inhibitory transmission.

Synapses may be distinguished not only by the type of synaptic vesicles but also by a number of other characteristics. Gray (1959) reported that two types of synapse could be distinguished according to the width of the synaptic cleft and the extent of thickening on the pre- and postsynaptic membranes (Table I.3 and Fig. 1.5). These synapse types, termed Type 1 and Type 2 by Gray, appear