Synapses of Adrenergic Fibres

A large number of adrenergic fibres are known to reach the gut and to branch inside the ganglia of intramural plexuses[1,2] and make direct contact with glandular and muscular effectors[3,4]. The presence of adrenergic nerve terminals (proved to contain noradrenaline) is well known, though these are not true terminals in the anatomical sense, but varicosities, giving the axons a beaded appearance. The varicosities are thought to be the active points, where the release and the uptake of transmitter occur[5,6]. Since the morphological specializations used to recognize a synapse have not been clearly distinguished either at these adrenergic nerve terminals or on the adjacent effectors, the existence of synapses at this level has been questioned[7], and a diffuse release and effect of the transmitter has been proposed[7]. The question arises therefore whether, inside the myenteric plexus, adrenergic fibres establish with the intramural neurones morphologically differentiated synaptic contacts or whether the relationship is not an intimate one. This problem could not be settled with the fluorescence light microscopy method for detecting adrenergic fibres and electron microscopy was used to detect any fine structural relationships.

Small pieces of ileum of guinea-pigs were fixed in 5% glutaraldehyde in 0.1 cacodylate buffer at pH 7.3, post-fixed in 1.25% osmium tetroxide and embedded in Araldite. Sections were stained with uranyl acetate and lead citrate and observed in a Siemens 1A microscope.

The adrenergic fibres are identified by their high content of the so-called small granulated vesicles (SGV), 40–70 nm in diameter, with an extremely dense granule[8], and it has been shown[9] that the fibres containing the SGV can be labelled by ^{3}H-noradrenaline.

Numerous adrenergic fibres are observed; at the varicosities their diameter is 2–4 μm. Besides SGV, which constitute the major vesicular population, they contain at least two other kind of vesicles: small agranular vesicles, 40–60 nm in diameter, and a few scattered large vesicles, 80–180 nm in diameter, containing moderately dense granules. Mitochondria and small clusters of glycogen-like granules are frequently observed.

The majority of adrenergic fibres make direct contact with the perikarya of intramural neurons. The apposed membranes of the adrenergic varicosity and the intramural nerve cell appear thickened and the general aspect is that of a typical interneuronal synapse[10]. The majority of these adrenergic contacts are axosomatic

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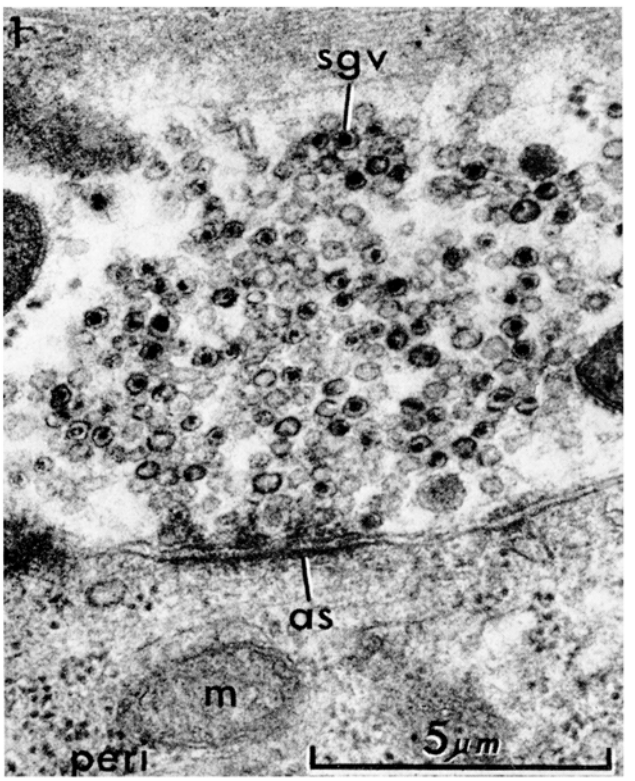

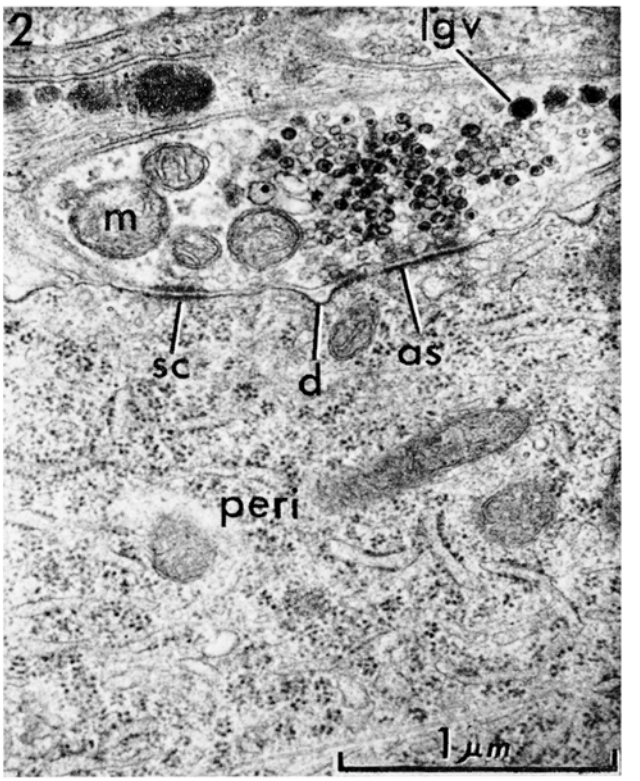

Fig. 1. A nerve terminal filled with small granulated vesicles makes a synapse with an intramural nerve cell body of the myenteric plexus. sgv, small granulated vesicles; m, mitochondrion; as, asymmetrical contact; peri, perikaryon.

Fig. 2. A similar nerve terminal at lower magnification to show asymmetrical (as) and symmetrical contacts (sc), large granulated vesicles (lgv), and also the dips (d) in the postsynaptic membrane. peri, perikaryon; m, mitochondrion.

synapses (Figure 1); a single nerve fibre may contact a cell body at several points forming typical 'en passage' synapses. The thickenings of pre- and post-synaptic membranes is asymmetric, the post-synaptic thickening being more prominent. On the cytoplasmic side of the pre-synaptic membrane clusters of vesicles lie close to the membrane; the majority of these are electron lucent round or slightly flattened vesicles, and only a minority contain a dense granule, whereas the proportion of granulated vesicles is greater inside the varicosity. The synaptic cleft is about 20–25 nm wide and is always open along its full extent and never fuses to form a 'tight' junction. Symmetrical thickenings of apposed membranes, without associated aggregations of vesicles, frequently occur between the adrenergic terminal and the same nerve cell body with which it establishes asymmetrical contacts (Figure 2). Similar symmetrical contacts are established between the nerve fibre and surrounding glial cell processes. The synaptic membranes may appear in cross section as 2 parallel lines, but more often the post-synaptic region of the cell body protrudes for a distance up to 1 μm into the pre-synaptic knob. The post-synaptic membrane frequently shows an invagination with dense material on the cytoplasmic side. Whether these are permanent invaginations similar to those found in the motor end plates or whether they are invaginations which originate from the opening of coated vesicles, remain to be decided.

These observations show that in the myenteric plexus adrenergic fibres establish mainly axosomatic contacts, with the morphological features of typical synaptic junctions. A direct action of adrenergic fibres on intramural nerve cells, already suggested on the basis of fluorescence microscopy observations[1–4], is thus substantiated on morphological grounds, each fibre making a number of discrete synaptic contacts. The large size of the intramural nerve cells and the presence of numerous adrenergic terminals coupled with easy anatomical accessibility of these structures makes the myenteric plexus a valuable model for the study of adrenergic transmission.

Riassunto. Nel plesso mienterico dell'ileo di cavia sono presenti numerose fibre adrenergiche. Queste fibre, riconosciute al microscopio elettronico per il contenuto in piccole vesicole a granulo denso, formano tipiche giunzioni sinaptiche, principalmente axo-somatiche, con i neuroni intramurali.

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[11] I am grateful to Professors J. Z. YOUNG and E. G. GRAY for their advice and support in this work. A grant from the Wellcome Trust is gratefully acknowledged.

Effect of Drugs on Excitatory and Inhibitory Potentials in *Helix aspersa*

There is good evidence for chemical transmission between nerve cells in molluscan ganglia[1]. Acetylcholine is both an inhibitory and an excitatory transmitter of synaptic activity[2,3]. 5-Hydroxytryptamine (5-HT) is an excitatory transmitter[4], while dopamine is an inhibitory transmitter[5]. The present study makes use of one dopamine inhibitory pathway, one acetylcholine excitatory pathway and one 5-HT excitatory pathway in the suboesophageal ganglionic mass of the snail, *Helix aspersa*. The two excitatory pathways produce excitatory postsynaptic potentials (EPP) on presynaptic stimulation, while the dopamine pathway produces an inhibitory postsynaptic potential (IPP). The effect of pretreatment with 6 compounds on these potentials is the subject of the present investigation.

Materials and methods. All experiments were carried out on the isolated brain of the snail, *Helix aspersa*. All compounds were injected into the snail haemocoel in a volume of 0.2 ml distilled water except for 6-hydroxydopamine which was dissolved in ascorbic acid solution to prevent oxidation. Stimuli were applied to the appropriate nerve at a frequency of 1.2 Hz. The voltage was selected to give a unitary monosynaptic response. Three parameters were measured: the size of the potential after a single stimulus which was called the initial height; following repetitive stimulation the EPP declined to a constant amplitude while the IPP increased to a constant amplitude, these values were called the final heights; the number of stimuli required to reach this final height was also recorded. 20 values for each parameter were determined for the control untreated snails. The experimental values were obtained from 7 preparations for each drug. The significance (p) for each result is shown beneath each pair of histograms. The control animals' histogram is unshaded while the experimental animals' histogram is shaded. The effect on the initial height is shown in the left pair of histograms (a) and the effect on the final height is shown on the right in (b), Figure 1.

Results and discussion. α-methyl-5-hydroxytryptophan (α-methyl-5-HTP), Figure 1A, 200 μg per snail injected 60 min before experiment, reduced both the initial and final heights of the 5-HT EPP by about 40%. Pretreatment with this compound had no effect on the number of stimuli required to give the final height of the EPP. This effect suggests that α-methyl-5-HTP may be converted to α-methyl-5-HT and released as a false transmitter following presynaptic stimulation, rather than only causing depletion of transmitter store by inhibition of 5-HT synthesis. In this latter case there should be a shortening in the time course to reach the final height. This compound did not significantly change the height of either the acetylcholine EPP or the dopamine IPP.

Pretreatment with p-chlorophenylalanine (Figure 1B), 5 mg per snail 24 h before experiment, reduced the initial and final heights of the 5-HT EPP by about 50% and also decreased the number of stimuli required to give the final height. This suggested that this compound was depleting the stores of 5-HT, probably by inhibiting the enzyme tryptophan hydroxylase[6]. This result confirmed spectrophotofluorimetric determinations where the same dose of p-chlorophenylalanine reduced the 5-HT level in the snail brain from 4.5 μg/g tissue to 2.5 μg/g tissue[7]. This compound did not significantly alter the other potentials.

Pretreatment with reserpine (Figure 1C), 3 single doses of 350 μg given at 24 h intervals and then experimented on 24 h after the final dose, reduced the initial and final heights of the 5-HT EPP by about 50%. This result agreed with the observation that reserpine reduced the size of the 5-HT EPP in the snail buccal ganglia[8]. Reserpine also significantly reduced the final height of