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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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.

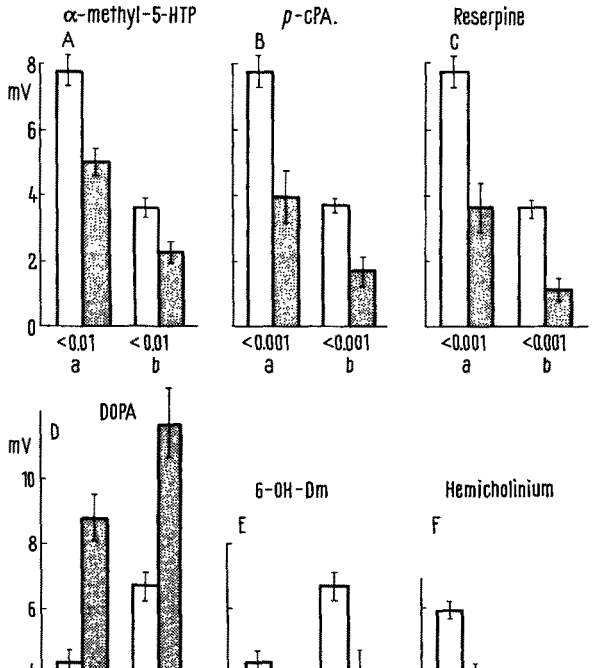
Results and discussion. α-methyl-5-hydroxytryptophan (α-methyl-5-HTP), Figure 1 A, 200 μg per snail injected 60 min before experiment, reduced both the initial and final heights of the 5-HT EPP by about 40%. Pretreatment with this compound had no effect on the number of stimuli required to give the final 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 1 B), 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 1 C), 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

the dopamine EPP, p value of < 0.05, and the number of stimuli required for the final height of the EPP, p value of < 0.01. These results are in agreement with spectrophotofluorimetric determinations following reserpine pretreatment where the same dose of reserpine reduced the 5-HT and dopamine levels in the snail brain from 4.5 µg/g tissue to 1.7 µg/g tissue respectively[7]. Reserpine acts on the vesicle bound 5-HT[9] and it can be assumed it is acting in this way in the snail. Reserpine reduced the number of stimuli required for the constant height of the 5-HT EPP but this was not significant. Reserpine had no other significant effects.

Pretreatment with L-3-4-dihydroxyphenylalanine (L-DOPA), Figure 1D, 200 µg per snail 60 min before experiment, increased both the initial and final heights of the dopamine IPP by about 100%. It had no other significant effect. An increase in the availability of the precursor would be expected to give rise to an increase in the amount of transmitter, and possibly the number of vesicles produced, and hence released following stimulation. The present finding confirmed previous work where following DOPA pretreatment the level of dopamine in the snail brain rose from 6.6 µg/g tissue to 9.5 µg/g tissue[10].



Histograms to show the effect of pretreatment with 6 compounds on synaptic potentials. In each case (a) refers to the initial height and (b) to the final height. The experimental histograms are hatched. The significance (p) is shown under each pair of histograms. The standard error is indicated by a bar. The control histograms are compiled from 20 observations and each drug histogram is compiled from 7 observations. The ordinate is expressed in mV. A) α-methyl-5-hydroxytryptophan (α-methyl-5-HTP) on 5-HT EPP; B) p-chlorophenylalanine (p-cPA) on 5-HT EPP; C) reserpine on 5-HT EPP; D) L-3-4-dihydroxyphenylalanine (L-DOPA) on dopamine IPP; E) 6-hydroxydopamine (6-OH-Dm) on dopamine IPP; F) hemicholinium on acetylcholine EPP.

Pretreatment with 6-hydroxydopamine (Figure 1E), 3 single doses of 350 µg given at 24 h intervals 14 days before experiment, reduced the initial and final heights of the dopamine EPP by about 50%. It had no effect on the number of stimuli required for the final IPP height. This compound at low doses depleted dopamine probably by replacing it presynaptically thus acting as a false transmitter[11]. In higher doses it caused degeneration of catecholamine stores which was irreversible in the rat brain[12]. In the present study 6-hydroxydopamine failed to abolish the IPP as would be expected if it was causing degeneration. Further studies are required with this compound in the snail.

Pretreatment with hemicholinium (Figure 1F) 5 mg per snail 16 h before experiment, reduced the initial and final heights of the cholinergic EPP. The time course for the EPP final height was also reduced, p value of < 0.01. Hemicholinium is known to inhibit the uptake of choline presynaptically into cholinergic terminals[13], and thus inhibit the synthesis of acetylcholine[14]. It has been found that hemicholinium reduced cholinergic EPP produced in Renshaw cells following afferent stimulation[15]. The results from the present study suggest that in the snail hemicholinium depletes acetylcholine presynaptically. Hemicholinium had no significant effect on either the IPP or the 5-HT EPP.

The present study shows that pretreatment of the animal with specified drugs brings about clear and repeatable changes in the size of the postsynaptic potentials. These results provide further evidence that acetylcholine, 5-HT and dopamine are synaptic transmitters in the snail brain.

Résumé. Des produits chimiques furent injectés à l'escargot *Helix aspersa*. Leur effet fut enregistré en potentiels postsynaptiques. L'hemicholinium réduit l'amplitude d'un potentiel postsynaptique d'excitation cholinergique. L'α-Methyl-5-HTP et le p-chlorophenylalanine ou résperpine font décroître l'amplitude d'un potentiel postsynaptique d'exication 5-HT, le 6-Hydroxydopamine diminue celle d'un potentiel postsynaptique d'inhibition dopamine tandis que le DOPA l'augmente.

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