Further identification of multiple responses mediated by dopamine in the CNS of *Planorbis corneus*

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Intracellular recordings from neurones, receiving monosynaptic contacts from a dopamine-containing (DA-containing) neurone in the central ganglia of the gastropod mollusc *Planorbis corneus*, revealed that there are at least three DA-mediated responses. These are 'fast' excitatory postsynaptic potentials (E_fPSPs) (200 ms), 'slow' excitatory postsynaptic potentials (E_sPSPs) (900 ms), and inhibitory postsynaptic potentials (IPSPs) (200–900 ms). Various combinations of these synaptic potentials were recorded from postsynaptic neurones: E_sPSPs, E_fPEPs, E_sE_fPSPs, or E_fIPSPs. Neurones receiving such connections also responded appropriately to iontophoresized DA with a 'fast' depolarization (E_fPSPs), a 'slow' depolarization (E_sPSPs), or a hyperpolarization (IPSPs). These responses could be distinguished on the basis of function (excitation or inhibition), duration, rate of desensitization, and sensitivity to apomorphine, D-LSD, and tubocurarine. The neuroleptic drugs (DA antagonists) haloperidol, fluphenazine, and metoclopramide reduced both excitatory and inhibitory DA transmission. This investigation strongly supports the hypothesis that DA is the transmitter mediating multiple synaptic responses in *Planorbis*.

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Des enregistrements intracellulaires de neurones recevant des contacts monosynaptiques d'un neurone contenant de la DA dans le ganglion central du mollusque gastropode *Planorbis corneus*, ont démontré qu'il existe au moins trois types de réponses dépendantes de la DA. Celles-ci sont 'rapides' E_fPSPs (200 ms), 'lentes' E_sPSPs (900 ms) et IPSPs (200–900 ms). Des combinaisons variées de ces potentiels synaptiques ont été enregistrées sur un neurone postsynaptique: E_sPSPs, E_fPSPs, E_sE_fPSPs, ou E_fIPSPs. Les neurones recevant de telles connexions ont aussi répondu de façon appropriée à la DA ionophorésée avec une dépolarisation 'rapide' (E_fPSPs), une dépolarisation 'lente' (E_sPSPs) ou une hyperpolarisation (IPSPs). Ces réponses se distinguent selon la fonction (excitation ou inhibition), la durée, le taux de désensibilisation et la sensitivité à l'apomorphine, au D-LSD et à la tubocurarine. Les drogues neuroleptiques (antagonistes DA) halopéridol, fluphénazine, et métoclopramide réduisent à la fois la transmission excitatrice et inhibitrice de la DA. Ces recherches appuient fortement l'hypothèse voulant que la DA soit le transmetteur médiateur de multiples réponses synaptiques chez le *Planorbis*.

[Traduit par le journal]

Introduction

In the central ganglia of molluscs a number of direct chemical synapses between identified neurones have been studied to the extent that the transmitter substances, for example ACh (Gerschenfeld 1973) and 5HT (Cottrell and Macon 1974; Gerschenfeld and Paupardin-Tritsch 1974*b*), have been identified. Unlike vertebrate preparations these molluscan ganglia provide the advantage that microelectrodes may be inserted into both pre- and postsynaptic neurones. Furthermore, known concentrations of drugs can be added to the preparation while recording from the neurones *in situ* within the central ganglion.

Berry and Cottrell (1975) identified a series of monosynaptic connections in the CNS of the mollusc *Planorbis corneus* and these synapses probably use DA as their transmitter. This system of neuronal

Abbreviations: DA, dopamine; Ach, acetylcholine; 5HT, serotonin; CNS, central nervous system; EPSP, excitatory postsynaptic potential; EIPSP, biphasic excitatory–inhibitory postsynaptic potential; IPSP, inhibitory postsynaptic potential; E_sPSP, 'slow' excitatory postsynaptic potential; E_fPSP, 'fast' excitatory postsynaptic potential; D-LSD, lysergic acid diethylamide.

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connections consists of an identified DA-containing neurone which makes excitatory and inhibitory connections with a number of postsynaptic (follower) neurones. These workers have found that DA, among a number of possible transmitter candidates, best mimicked the evoked synaptic potentials. In addition, several drugs (for example, tubocurarine and ergometrine) that are known to block responses of *Aplysia* neurones to iontophoretically applied DA (Ascher 1972) also reduced or abolished the appropriate synaptic potentials. Since the original identification of this dopaminergic system, it has been demonstrated that neuroleptic drugs, such as haloperidol and fluphenazine, are also capable of blocking responses of *Aplysia* neurones to the iontophoretic application of DA (Heiss *et al.* 1976). The actions of these drugs in mammals are usually attributed to a blockade of DA receptors (Iversen 1975). Evidence such as this has been used to suggest that molluscan and mammalian DA receptors may be pharmacologically similar (Cools and Van Rossum 1976).

Multiple synaptic potentials are evoked in follower neurones by activation of the presynaptic (DA-containing) neurone. Either EPSPs, IPSPs, or biphasic (EIPSPs) are observed in follower neurones. In the present investigation we report the responses of follower neurones to iontophoretically applied DA and also the effects of various DA receptor agonists and antagonists upon DA-evoked or synaptic responses in the *Planorbis* dopaminergic system. An abstract reporting some of these findings has already been published (MacDonald 1976).

Methods

Specimens of *Planorbis corneus* were obtained from Gerrard and Co., East Preston, Sussex, U.K. and kept in aquaria at room temperature (20–22°C). Experiments were performed on the isolated subesophageal ganglionic ring which consists of a single visceral ganglion as well as one pair each of parietal, pleural, and pedal ganglia (left and right). Each ganglionic ring was pinned to the plastic base of a 1.5-ml perspex chamber and immersed in a continuous flow (about 10 ml/min) of *Planorbis* saline (pH 8; temperature 20–22°C; Na, 42.6; K, 1.3; Ca, 4.5; Mg, 1.5; Cl, 46.6; HCO_3, 6.9 (m*M*). This saline matches the ionic composition of *Planorbis* haemolymph (Berry 1972).

Double-barrelled microelectrodes filled with 2 *M* potassium citrate (5–10 mΩ) were inserted into the soma of the DA neurone (left pedal ganglion) and into the soma of one of its followers (visceral or left parietal ganglia). One barrel was connected to a variable direct current source to permit alteration of membrane potential when required. For example, to evaluate membrane input resistance, square (hyperpolarizing) pulses could be passed through this barrel, or in the case of the DA neurone, depolarizing pulses could be delivered to evoke a train of action potentials of constant frequency and duration (this resulted in the elicitation of EPSPs and IPSPs (summated potentials) of constant amplitude and duration). A 500-MΩ resistor was placed in series with the electrode to maintain constant current pulses. The other barrel of the electrode was used to record membrane potential on a Gould–Brush pen recorder and a Tektronix 502 oscilloscope. Electrodes demonstrating a large degree (>200 kΩ) of coupling between barrels were discarded.

DA was applied iontophoretically to the vicinity of follower neurones using single-barrelled micropipettes (DA HCl, 0.5–2 *M*; pH 5–6). Positive (cationic) current pulses (0.1–2.0 s, 50–600 nA) were applied to these micropipettes from a Grass stimulator by placing a 100-MΩ resistor in series with the phoretic pipette. The current passed between the pipette and earth was monitored to make certain that the current of each pulse remained constant. A retaining current was also applied to minimize drug leakage. Due to the rapid oxidation of DA solutions, pipettes were filled just before use and they were discarded after each experiment. The resistances of these DA pipettes ranged from 5 to 30 MΩ and tip diameters were about 0.5–1.5 μm. In some cases ACh (1 *M*, pH 5) was also applied iontophoretically in a manner similar to that of DA to test the specificity of certain DA antagonists.

Agonists and antagonists studied were dissolved in *Planorbis* saline and perfused continuously through the bathing chamber. A multiple stopcock allowed selection of the appropriate drug solution and a suction-controlled outflow of the chamber permitted a rapid change from saline to drug-containing saline. The following drugs were applied in this manner: apomorphine HCl (Sandoz Pharmaceuticals), haloperidol (Jassen Pharmaceuticals), fluphenazine HCl (E.R. Squibb and Sons), metoclopramide (courtesy of Dr. P. Jenner), tubocurarine and D-LSD (25) (Burroughs Wellcome). The pH of each solution was appropriately adjusted and each drug tested was applied in steps of increasing concentration through the ranges indicated in the results.

Results

Berry and Cottrell (1975) have applied DA to follower neurones by adding it directly to the bath or by simply allowing it to diffuse from the tip of a broken pipette; however, no attempt was made to apply it iontophoretically. We found that to evoke responses to iontophoresized DA the position of the pipette tip was critical. If the tip was positioned at various regions around the soma no response to DA phoresis was observed until the tip was juxtaposed to the axon hillock. (This region is pigmented in these neurones and it is easily located even in small neurones.) This observation indicated that the somata of follower neurones were insensitive to DA. *Aplysia* neurones are similar in this respect and DA receptors may be located as far as 1 mm away from the parent soma (Ascher 1972). Swann and Carpenter (1975) also have reported that the DA receptors are remote from the soma but they were able to overcome this problem by penetrating the neuropile of the ganglion. This was not possible in

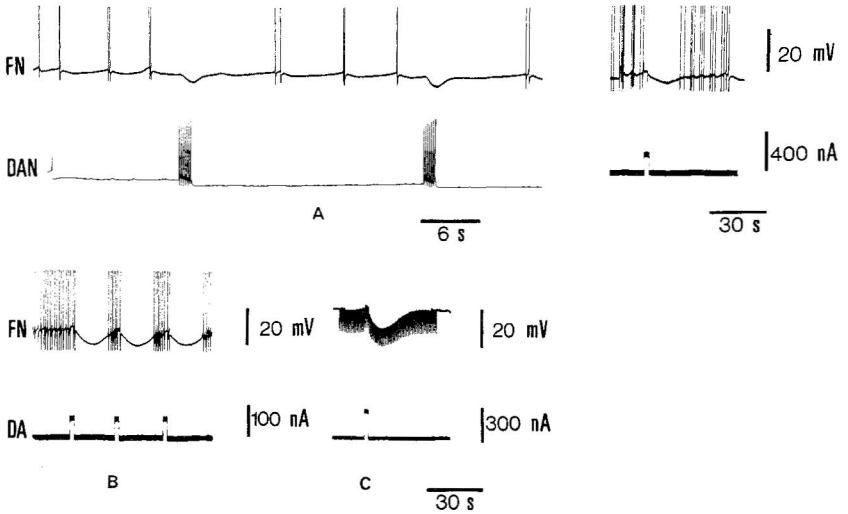

FIG. 1. Inhibitory responses of *visceral* follower neurones (held at resting level) to stimulation of the DA neurone or to iontophoretically applied DA. Summated IPSPs and the response of the same neurone to a DA pulse are shown (A), repeated pulses of DA demonstrated no desensitization of this response (B). An increase in conductance underlies this response because a fall in input resistance can be observed. This is demonstrated by a reduction in the amplitude of constant current (hyperpolarizing) pulses passed during DA phoresis (C). ABBREVIATIONS: responses of follower neurone (FN), of the DA neurone (DAN), and of the monitored iontophoretic current (DA). These abbreviations apply to all subsequent figures.

Planorbis due to the structure of the connective capsula covering the visceral and parietal ganglia. Removal or penetration of this capsula resulted in the destruction of the underlying neurones. None the less, because the DA receptors must be somewhat close to the hillock region, it is possible to record responses to phoresized DA without removing the capsula and without physically contacting the preparation with the phoretic pipette. A check was made to eliminate direct current effects on the neurones simply by reversing the iontophoretic current. If the membrane failed to follow a negative current pulse it was unlikely that a positive pulse would produce an artifact. Furthermore, since only follower neurones responded to pulses of DA, and then only when applied to a restricted region of the follower neurone, it is not likely that pH changes were responsible for any of the responses. The saline itself would also have had some capacity to buffer the ejected DA.

Inhibitory Responses

Follower neurones in the *visceral ganglion* characteristically responded with IPSPs to intracellular stimulation of the DA neurone (Fig. 1A). Although the location of follower neurones on the ventral surface can be judged approximately, the only definite means of identification is their response to the DA neurone. Visceral neurones that failed to respond to activation of the DA neurone (not followers) were insensitive to the iontophoretic application of DA regardless of the position of the pipette tip (>50 neurones tested). Follower neurones receiving IPSPs from the DA neurone were hyperpolarized by DA (47 neurones tested) and this change in potential was associated with a decrease in membrane input resistance (Fig. 1C). Phoresis of DA successfully evoked responses only when pulses of greater than 1-s duration (>100 nA) were applied. A 2-s pulse of DA evoked a hyperpolarization and cessation of spontaneous action potentials ranging in duration from 30 to 90 s (Fig. 1). Large currents were required to evoke such responses (compared with depolarizations, see below), probably as a consequence of the electrical coupling between these follower neurones. The electrotonic synapses have a loading effect which reduces the amplitude of potentials evoked in any one neurone (Berry and Pentreath 1977). These inhibitory responses could not be reversed with hyperpolarization of the membrane and this may also be a property of the electrical coupling of visceral follower neurones.

Repetitive pulses of DA produced responses of consistent duration and amplitude (Fig. 1B) indicating a resistance to desensitization by this response. Bath-applied DA does not demonstrate much if any desensitization of this response in *Planorbis* (Berry

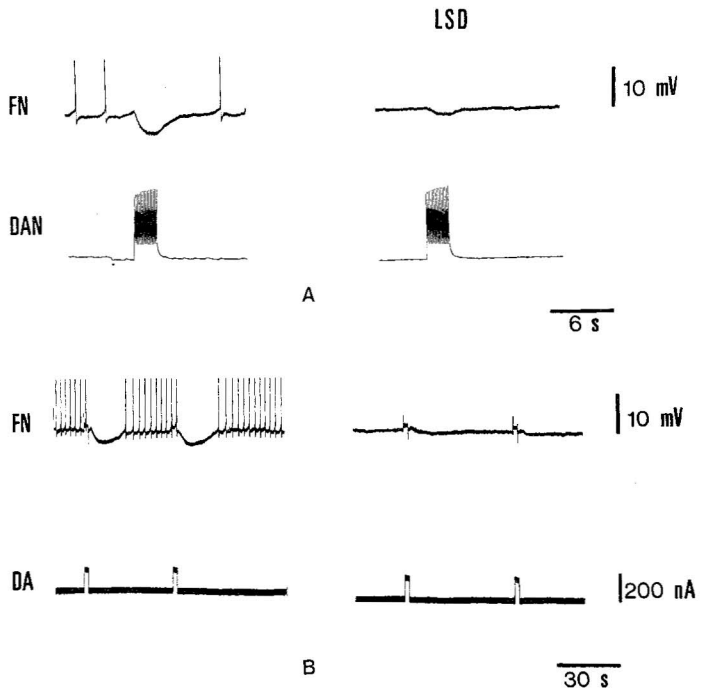

FIG. 2. The effects of D-LSD upon IPSPs (A) and responses to phoretically applied DA (B) on the same follower neurone. Responses before the perfusion of D-LSD are shown on the left and in the presence of this drug on the right (5 μM, 25 min). D-LSD almost completely blocked these responses but a hyperpolarization of the membrane gradually developed (10 mV) which resulted in the suppression of spontaneous action potentials. If the membrane was depolarized to its control level, in the presence of D-LSD, these responses were still blocked or greatly reduced.

and Cottrell 1975) nor of comparable responses of *Aplysia* neurones (Asher 1972).

Iontophoresis of the DA agonist apomorphine is reported to suppress the activity of mammalian CNS neurones that are similarly affected by DA (Zarzecki *et al.* 1976). However, we found that perfusion of this drug at concentrations ranging from 10 to 100 μM (11 neurones tested) failed to suppress spontaneous activity (1 mM apomorphine actually depolarized and excited neurones) and did not influence membrane polarization, input resistance, dopaminergic IPSPs, or hyperpolarizing responses to phoresized DA. Also, other authors have reported that apomorphine fails to mimic the hyperpolarizing actions of DA (Struyker-Boudier *et al.* 1968) on molluscan neurones.

Sato and Sawada (1975) have demonstrated that D-LSD in low concentrations (1–10 μM) was able to selectively antagonize hyperpolarizing DA responses of *Aplysia* neurones without affecting depolarizing responses. In the *Planorbis* system we found that D-LSD (5 μM, three neurones tested) abolished IPSPs (Fig. 2A) elicited by stimulation of the DA neurone and hyperpolarizing responses to iontophoretically applied DA (Fig. 2B). However, this drug also hyperpolarized (5–10 mV) the follower neurones and decreased their input resistance. These actions were characterized by a slow onset (15–30 min) and by long periods to recovery (>3 h). Similar 'agonist'-like effects have been reported for related ergots such as ergometrine (Ascher 1972; Berry and Cottrell 1975) and as a consequence it is unreasonable to suggest that these drugs are specific antagonists of hyperpolarizing DA responses. At the concentration used by us, D-LSD failed to affect excitatory responses of follower neurones (slow EPSPs, see below) or depolarizing responses to DA phoresis (slow depolarization, see below, three neurones tested).

Excitatory Responses (Slow)

Examination of the responses of neurones in the *left parietal* ganglion demonstrated that only follower neurones responded to iontophoretically applied DA (25 follower neurones tested, >150 nonfollowers tested) and only if the pipette tip was placed close to the axon hillock. The majority of these follower neurones (see below for exceptions) responded with individual E_sPSPs (slow) of about 900 ms in duration (Fig. 3F) to a

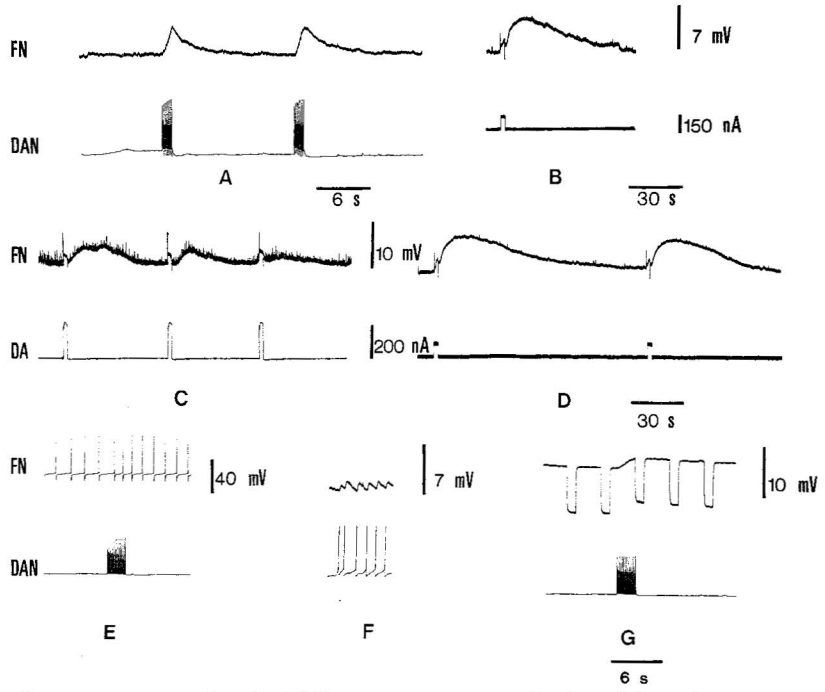

FIG. 3. Slow excitatory responses of parietal follower neurones to activation of the DA neurone or to phoretic pulses of DA. Summated E_sPSPs and a slow depolarization of the same neurone are shown (A,B). This neurone was hyperpolarized by 60 mV beyond its resting membrane potential. Little excitation of these neurones was apparent at resting level (E). Single spikes in the DA neurone evoked individual E_sPSPs with a duration of about 900 ms (F). Desensitization was observed with some neurones (C) but others were relatively resistant to the application of several pulses of DA (D). A small decrease of input resistance was usually observed (G). Calibrations for B apply for D as well.

single spike in the DA neurone. As was the case with visceral follower neurones, identification was possible only by recording a response to activation of the DA neurone. These E_sPSPs (summated) were consistently smaller in magnitude than comparable inhibitory responses. At resting membrane potential responses usually caused only a small increase in the spike activity recorded from the soma (Fig. 3E). To evoke detectable depolarizing responses in parietal follower neurones (Fig. 3A), it was necessary to hyperpolarize the membrane by 50–100 mV beyond the resting levels. It was difficult to maintain stable membrane potentials for the long periods required to test drugs under these artificial conditions. Furthermore, in many preparations tested (particularly during the winter months) no EPSPs were found whereas IPSPs were always found in every preparation. E_sPSPs produced only small changes in membrane potential and input resistance (Fig. 3G). This evidence suggests that conductance of the soma membrane was only marginally affected by events occurring at the synaptic and DA-receptive regions that are remote from the recording site.

The iontophoretic application of a 2-s DA pulse depolarized follower neurones for a period of 30–90 s (slow depolarization, Fig. 3B) and the amplitude of the phoretic potential approximated that of the maximal summated EPSPs (see Figs. 3 A and B and 5 C and D for examples) at various levels of membrane potential. Reversal potentials for the EPSPs and the phoretic responses could not be determined due to membrane rectification (see Ginsborg 1973).

In a manner similar to previous observations for *Aplysia* neurones (Ascher 1972), repeated pulses of DA were often associated with a gradual fading of subsequent responses (desensitization) (Fig. 3C). Not all neurones demonstrated such a desensitization, however, and some maintained a stable response to the application of repeated DA pulses (Fig. 3D). This difference in tendency to desensitization may be a consequence of the occurrence of two excitatory DA receptors on the same neurone (see below).

Contrary to previous reports that the DA agonist apomorphine is able to mimic the depolarizing actions of DA upon molluscan neurones, we found that most parietal follower neurones (E_sPSPs) were

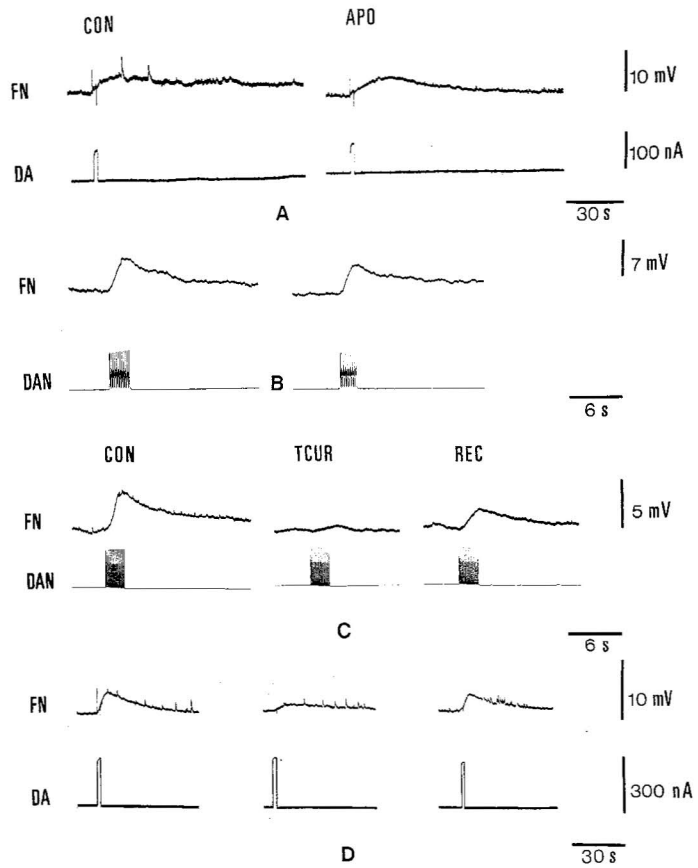

FIG. 4. Action of apomorphine and tubocurarine upon E_sPSPs and slow depolarizing responses to DA. Apomorphine (50 μM, 20 min) had no effect on E_sPSPs (B) or slow DA depolarizations (A). Membrane potential and input resistance were also unchanged by apomorphine. Tubocurarine (100 μM, 8 min) reduced E_sPSPs (C) and slow depolarizing DA responses (D, 100 μM, 10 min). ABBREVIATIONS: CON, control responses before drug application; APO, in the presence of apomorphine; TCUR, in the presence of tubocurarine; REC, recovery of responses after 20 min of washing.

not influenced by this drug. Membrane potential, input resistance, E_sPSPs (summated) (Fig. 4B), and DA phoretic responses (slow depolarization) (Fig. 4A) were not affected by this drug in concentrations ranging from 1 to 100 μM (10 neurones tested). The dopaminergic responses of these neurones (five tested) were rapidly (5–10 min) reduced or abolished by tubocurarine (100 μM) (Fig. 4 C and D). This concentration of tubocurarine has previously been shown to have no affect upon visceral follower IPSPs (Berry and Cottrell 1975). However, Carpenter *et al.* (1977) have suggested that tubocurarine (100 μM) blocks the Na^+ and Cl^- iontophores of *Aplysia* neurones. In our experiments we cannot be certain, therefore, that the blockade of these EPSPs was a result of an action of tubocurarine on DA receptors or on the receptor-linked iontophores.

Biphasic Responses (Fast Excitatory Responses)

Visceral follower neurones occasionally responded to DA neurone activation with a biphasic response consisting of a small depolarization (about 200 ms in duration) followed by a much longer lasting hyperpolarization identical with the previously described summated IPSPs (Fig. 5A). Such a response with a 'fast' E_fPSP of an amplitude of up to 1 mV at resting membrane potential is very rare, though Berry and Cottrell (1975) have shown that many visceral followers possess such E_fPSPs but the amplitude is so small as to be barely detectable. Unlike the results that have been obtained by Ascher (1972), we have not seen discrete biphasic responses (depolarization–hyperpolarization) to phoretically applied DA. However, when some visceral follower neurones were hyperpolarized to about −100 mV, which resulted in a virtual disap-

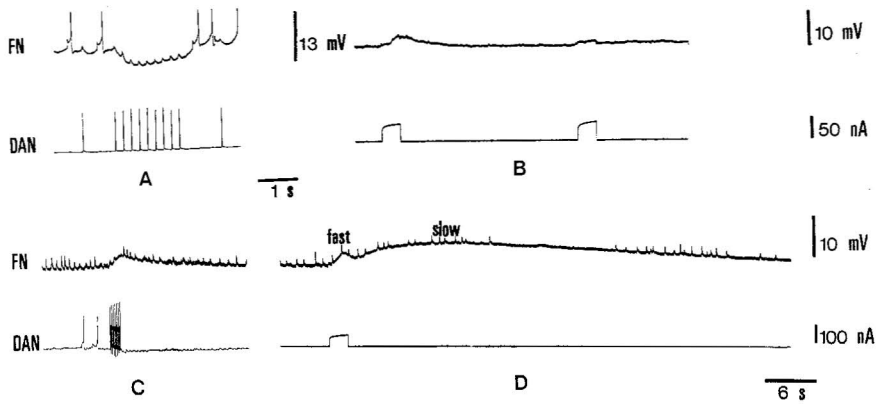

FIG. 5. Biphasic responses of *visceral* follower neurones (A,B) and a *parietal* neurone (C,D). E_fPSPs of about 200 ms duration are superimposed upon summated IPSPs (A). This neurone was recorded at resting level but application of a DA pulse did not reveal a depolarizing component. If such neurones were hyperpolarized (B, held at -100 mV) the hyperpolarization was replaced with a fast depolarizing response to DA. A second pulse of DA illustrates the rapid desensitization of this response (5 min of washing required for full recovery), indicating that this is not a residual inhibitory response. The *parietal* neurone whose synaptic responses are shown (C) demonstrated both a fast and a slow depolarization to DA phoresis (D). The initial fast component was desensitized by subsequent pulses while the slow component remained constant in size (not shown).

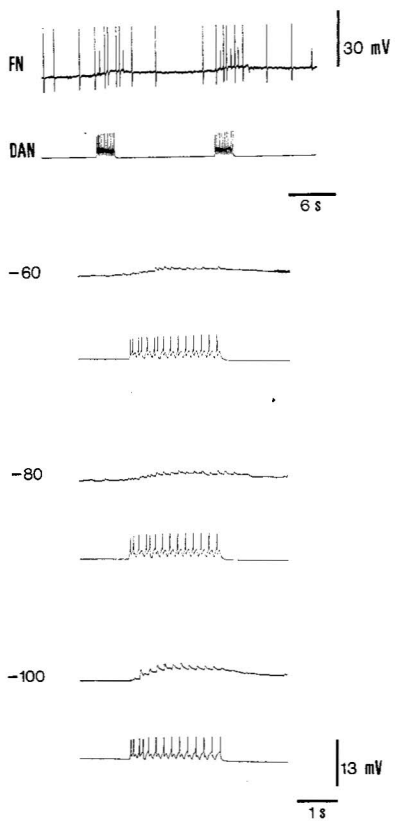

FIG. 6. Biphasic (E_fE_sPSPs) responses of a *parietal* follower neurone recorded at various levels of membrane potential. At the observed resting potential (-55 mV) these E_fE_sPSPs increased spike activity unlike the case of E_fIPSPs which never evoke spikes in the soma (Berry and Cottrell 1975). At -100 mV distinct 200-ms E_fPSPs are superimposed upon a summated slow potential (E_sPSPs).

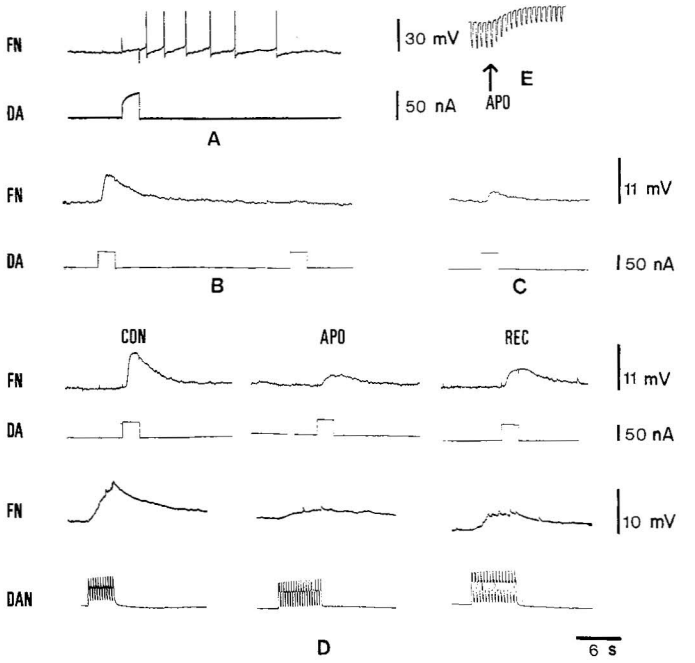

FIG. 7. Top. Characteristics of fast responses of *parietal* follower neurones to stimulation of the DA neurone and to DA pulses. A 2-s pulse of DA evoked a fast depolarization of several follower neurones that was capable of exciting the neurone to threshold when the membrane was hyperpolarized just below resting potential (A). This response demonstrated a rapid and long-lasting desensitization (B) which required 5 min of washing for complete recovery. A partial recovery is seen after 3 min of washing (C). Bottom. The effect of apomorphine on E_fPSPs and fast depolarizing responses to DA. Apomorphine greatly reduced E_fPSPs and fast depolarizations (D) but apomorphine itself depolarized the membrane and decreased membrane input resistance (E). Membrane potential was adjusted to control levels in the presence of apomorphine. Desensitization or an increase in membrane conductance probably produced these reductions in responses. ABBREVIATIONS: CON, responses before drug application; APO, E_fPSPs (apomorphine perfusion, 5 μM, 10 min), fast depolarization (apomorphine perfusion, 1 μM, 20 min); REC, partial recovery of responses after 10 min of washing.

pearance of the hyperpolarizing response (these responses do not reverse), a small depolarization (2–5 s in duration) was observed (Fig. 5B). This response demonstrated a very rapid rate of desensitization (Fig. 5B) and recovery took about 5 min even though the preparation was continually perfused with normal saline. Similar 'fast' E_fPSPs were also seen in parietal neurones without the presence of hyperpolarizations. Only four such neurones were found. Two of these demonstrated a biphasic response (fast depolarization – slow depolarization) to DA iontophoresis (Fig. 5D) and one clearly demonstrated both 'fast' E_fPSPs superimposed upon a 'slow' (summated) E_sPSPs (E_fE_sPSPs, Fig. 6). Both the 'fast' E_fPSP and the 'slow' E_sPSPs increased with progressive membrane hyperpolarization and unlike E_fIPSPs, these E_fE_sPSPs were functionally excitatory (Fig. 6). Two of the neurones demonstrated no apparent slow response and consisted only of the fast response (Fig. 7). These fast responses could be distinguished from the slow responses on the basis of duration of the response (Fig. 5 B and D, Fig. 6, and Fig. 7), the rate of desensitization (Fig. 5B, Fig. 7 B and C), and by susceptibility to apomorphine (Fig. 7D). In the case of three of these parietal neurones we were able to demonstrate that apomorphine (1–10 μM) depolarized the membrane, decreased membrane input resistance (Fig. 7E), and reduced subsequent fast E_fPSPs and fast depolarizations to DA phoresis (Fig. 7D). It was difficult at times to determine if the EPSPs (summated) represented either fast or slow EPSPs primarily because the fast E_fPSPs evoked by low frequent stimulation of the DA neurone may not be of sufficient amplitude. This is again probably due to the remote location of the DA receptors from the soma. Thus, in the case of the neurone in Fig. 5 C and D it could not be determined from the synaptic potential whether or not the EPSP was fast or slow. DA phoresis demonstrated that this neurone possessed both fast and slow DA responses. Neither of these excitatory responses could be reversed by depolarization due to membrane rectification (Ginsborg 1973).

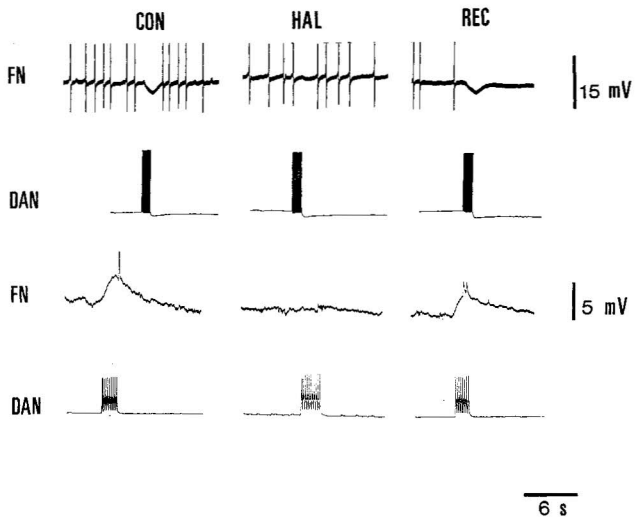

FIG. 8. Action of haloperidol upon IPSPs and E_sPSPs. This drug reduced both types of PSPs. ABBREVIATIONS: CON, responses before drug application; HAL, IPSPs (haloperidol perfusion, 50 μM, 25 min), EPSPs (haloperidol perfusion, 50 μM, 30 min); REC, recovery of responses with washing (IPSPs, 30 min; EPSPs, 25 min).

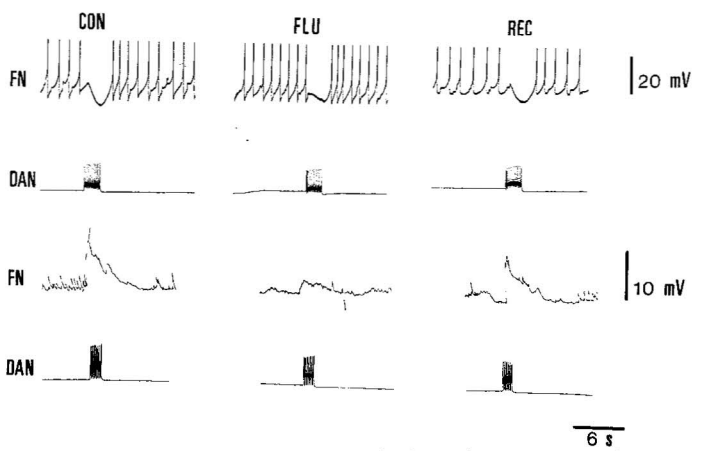

FIG. 9. Action of fluphenazine upon IPSPs and E_sPSPs. Fluphenazine reduced both types of PSPs. ABBREVIATIONS: CON, responses before drug application; FLU, 100 μM, 30 min exposure to fluphenazine; REC, recovery of responses with washing (IPSPs, 30 min; EPSPs, 15 min).

Effects of Neuroleptics on IPSPs and Slow EPSPs

Heiss *et al.* (1976), studying responses of *Aplysia* neurones, have demonstrated a reduction or blockade of excitatory and inhibitory responses to iontophoretically applied DA in the presence of haloperidol or fluphenazine (10–100 μM) without any effect on responses to 5HT or ACh. We found that haloperidol (IPSPs, six neurones; E_sPSPs, three neurones), fluphenazine (IPSPs, six neurones; E_sPSPs, three neurones), and another neuroleptic with DA antagonist activity, metoclopramide (IPSPs, six neurones; E_sPSPs, three neurones) at concentrations ranging from 50 to 100 μM reduced IPSPs and (or) abolished E_sPSPs (see Figs. 8, 9, and 10). Excitatory responses were usually reduced to a greater extent than inhibitory responses, possibly because excitatory responses were weaker; however, a similar selectivity has also been reported by Heiss *et al.* (1976). We also found that the onset of reduction was relatively long (compared with tubocurarine, for example) and it required 20–40 min of drug perfusion. Recovery was usually delayed for about 1 h even though the ganglia were continuously washed. This is a similar time course of action as compared with the previous reported results with DA phoresis (Heiss *et al.* 1976).

Perfusion with these drugs at concentrations ranging from 125 to 150 μM was accompanied by

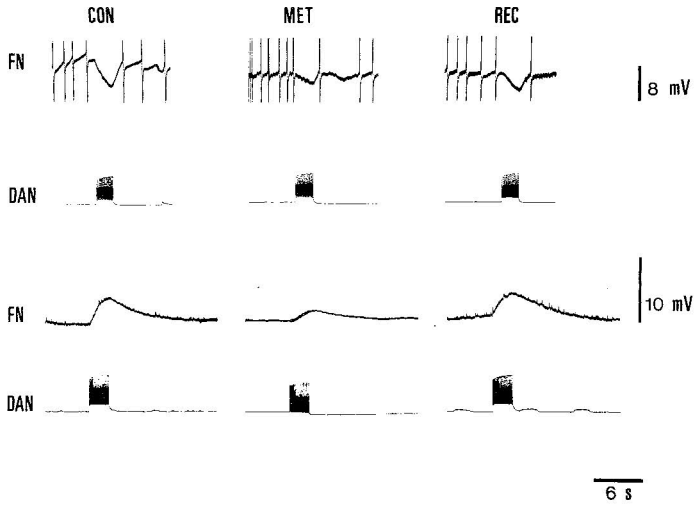

FIG. 10. Action of metoclopramide upon IPSPs and E_sPSPs. This drug reduced both types of PSPs. ABBREVIATIONS: CON, responses before drug application; MET, IPSPs (50 μM, 25 min exposure to metoclopramide), EPSPs (100 μM, 20 min exposure to metoclopramide); REC, recovery of responses after 35 min of washing.

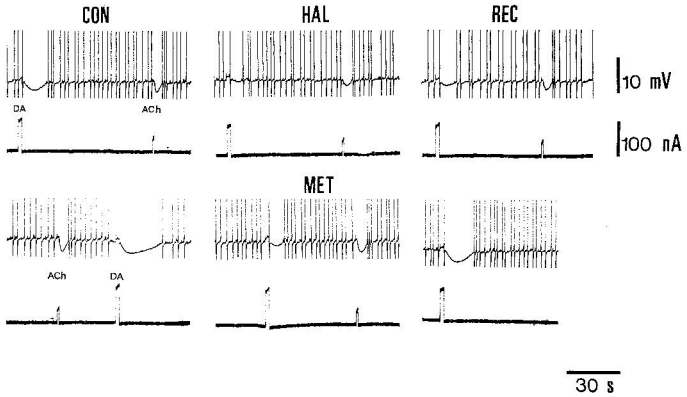

FIG. 11. Specific effect of haloperidol and metoclopramide on DA hyperpolarizations. Haloperidol reduced responses to both putative transmitters (ACh and DA) but the ACh responses reappeared soon after washing whereas DA responses were depressed for much longer periods (see text). Metoclopramide had a similar effect but responses to ACh were usually not reduced to the same extent. ABBREVIATIONS: CON, responses before drug perfusion; HAL, perfusion of haloperidol, 75 μM, 30 min; REC, recovery after 20 min of washing; MET, perfusion of metoclopramide, 75 μM, 25 min; REC, recovery after 30 min of washing.

effects such as membrane depolarization, an increase in the frequency of spontaneous action potentials, and also some reduction in spike height. These effects are probably associated with nonspecific or 'local anaesthetic' actions of high concentrations of these drugs (see Heiss *et al.* 1976). However, the lower concentrations used to reduce synaptic potentials did not produce such nonspecific actions and input resistance was unaltered by the neuroleptics.

Responses to iontophoresized ACh (Fig. 11; tested on at least three neurones for each neuroleptic) were occasionally reduced although to an extent small in comparison with DA responses. The time course of reduction of ACh responses also differed from those of DA in that ACh responses were reduced within 2–3 min of drug application and reversed after 5–10 min of washing. This indicated, at least to a certain extent, a more selective action of these agents on DA responses in agreement with Heiss *et al.* (1976). Metoclopramide did not affect responses to ACh to the same extent as haloperidol (Fig. 11) or fluphenazine (not shown). Because of the technical difficulties involved, depolarizing iontophoretic responses to DA and ACh were not studied.

Discussion

In the vertebrate peripheral nervous system it has

TABLE 1. Characterization of dopamine responses

DA response	Hyperpolarization (IPSP)	Fast depolarization (E_fPSP)	Slow depolarization (E_sPSP)
Duration of response to a 2-s DA pulse	30–90 s	2–5 s	30–90 s
Duration (PSP)	0.2–0.9 s	0.2 s	0.9 s
Desensitization	None	Rapid	Little or moderate
Tubocurarine, 100 μM	No effect	Reduced*	Reduced
Apomorphine, 1–10 μM	No effect	Mimicked and reduced	No effect
D-LSD, 5 μM	Mimicked and reduced	?	No effect
Haloperidol, 50–100 μM	Reduced	?	Reduced
Fluphenazine, 50–100 μM	Reduced	?	Reduced
Metoclopramide, 50–100 μM	Reduced	?	Reduced

*Berry and Cottrell (1975).

long been recognized that ACh produces opposite functional effects (inhibition or excitation) at different synapses (Loewi 1933; Dale 1937) and this dual action of ACh has also been clearly demonstrated within the CNS of molluscs (see Gerschenfeld 1973). Furthermore, in molluscs, ACh inhibitions and excitations are mediated by pharmacologically distinct receptors which may even be present on the same neurone. Other transmitter substances such as 5HT can activate as many as six distinct receptors of which at least four are associated with synaptic potentials mediated by 5HT (Gerschenfeld and Paupardin-Tritsch 1974*a*, 1974*b*). Therefore, it is not surprising that at least three distinct responses to phoresised DA were recorded from *Planorbis* follower neurones. The characteristics of these phoretic responses and their related synaptic potentials are summarized in Table 1.

The transmitter mediating the synaptic potentials of follower neurones is most probably DA. This hypothesis is supported by the following evidence. (*1*) Each DA neurone contains significant amounts of DA (5.5 ng) but no detectable noradrenaline (Powell and Cottrell 1974). (*2*) This neurone can synthesize 3,4-dihydroxyphenylalanine and DA from tyrosine but not noradrenaline (Osborne *et al.* 1975). (*3*) The terminal processes of the DA neurone can be autographically labelled after injection of tritiated DA into the soma (Pentreath and Berry 1975) and these processes can be found in the vicinity of follower neurones. (*4*) The connections between the DA neurone and its followers are monosynaptic and only bath-applied DA (not 5HT, noradrenaline, glutamate, and ACh) mimics these synaptic potentials in a consistent manner (Berry and Cottrell 1975). (*5*) The properties of responses to DA phoresis are identical with those of the predicted synaptic potentials, and DA agonists and antagonists have the expected actions on these synaptic potentials (this investigation).

The release of DA as a consequence of activation of the DA neurone has yet to be demonstrated. Also a mechanism for inactivation or uptake of DA would have to be found before a complete acceptance of this hypothesis. Nonetheless, a considerable degree of evidence now supports the role of DA as neurotransmitter in this system.

The *Planorbis* dopaminergic system presents a number of advantages for the pharmacological study of DA receptors (see Introduction). Unfortunately, quantitative analysis of DA responses is greatly restricted by membrane rectification and electrical coupling between neurones. Perhaps the most serious difficulty to overcome is the problem of recording from the perikarya conductance changes occurring remotely on axons deep within the neuropile. In the case of the fast E_fPSP, these problems are compounded by the low frequency of their occurrence.

Keeping these limitations in mind we examined the effects of several centrally acting substances on DA as well as synaptic responses. Our results are more qualitative than quantitative but they do reveal some interesting similarities between the molluscan and the mammalian dopaminergic systems. For example, the neuroleptic drugs haloperidol, fluphenazine, and metoclopramide reduce transmission in both *Planorbis* and mammalian dopaminergic systems. On the other hand, we have found a complex variety of dopaminergic responses in *Planorbis* wherein apomorphine is a possible agonist of one of two excitatory responses and D-LSD and ergometrine (at concentrations studied) are perhaps mixed 'agonist–antagonist' of the inhibitory response alone. It is difficult to make such comparisons, however, as most studies of vertebrate DA receptors are based upon behavioral or biochemical criteria rather than upon direct electro-

physiological examination. The possibility remains that multiple DA-mediated synaptic potentials are characteristic of both the mammalian and molluscan CNS (Cools and Van Rossum 1976).

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