J. exp. Biol. **155**, 203–217 (1991)
Printed in Great Britain © *The Company of Biologists Limited 1991*

CHARACTERIZATION OF RECEPTORS MEDIATING THE ACTIONS OF DOPAMINE ON AN IDENTIFIED INHIBITORY MOTONEURONE OF THE COCKROACH

BY JULIAN P. L. DAVIS* AND ROBERT M. PITMAN†

The Gatty Marine Laboratory, University of St Andrews, St Andrews, Fife KY16 8LB, Scotland

Accepted 13 July 1990

Summary

1. The effects of a number of dopaminergic agonists and antagonists upon the soma of a prothoracic inhibitory motoneurone of the cockroach (*Periplaneta americana*) have been recorded under voltage-clamp conditions.

2. Dopamine generates inward currents that are extremely voltage-dependent: currents increase rapidly at membrane potentials more negative than about −120 to −150 mV and also show a peak at membrane potentials of approximately −20 mV. As a result of this voltage-dependence, dopamine induces a region of negative resistance in the current–voltage relationship of the neurone.

3. The dopaminergic agonists apomorphine, bromocryptine, ergometrine and A-6,7-DTN mimic the action of dopamine on this neurone, all having a similar voltage-dependence to that of dopamine. The selective D-1 receptor agonist SK&F 82526 and the D-2 agonist LY 171555, however, were both inactive on the preparation.

4. Responses to dopamine were suppressed by a number of D-1 and D-2 receptor antagonists, indicating that the pharmacological profile of the dopamine-sensitive receptor in this insect preparation is different from that of vertebrate dopamine receptors.

Introduction

Until recently, insect central neuropharmacology has focused mainly upon receptors for acetylcholine (ACh) and gamma-aminobutyric acid (GABA), which are both major neurotransmitter candidates in the insect central nervous system (CNS) (see Pitman, 1985). Considerably less is known about other compounds which may act as important neurotransmitters or modulators in this system, such as the catecholamines noradrenaline and dopamine. These have both been

* Present address: Department of Anatomy and Physiology, University of Dundee, Dundee DD1 4HN, Scotland.

† To whom reprint requests should be addressed.

Key words: dopamine receptors, dopaminergic agonists and antagonists, voltage-clamp, insect, cockroach, *Periplaneta americana*.

detected in the CNS of a number of different species. In the cockroach CNS, for example, the concentration of dopamine in the thoracic ganglia is about 2.7 pmol mg^{-1} tissue wet mass, which is approximately an order of magnitude greater than that of noradrenaline (Dymond and Evans, 1979). Adrenaline, in contrast, is below the level of detection. Catecholamines can also be localized histochemically in the cockroach brain (Frontali, 1968; Klemm, 1976, 1982) and ventral nerve cord (Fleming and Pitman, 1983; Baker and Pitman, 1989). Although there appear to be relatively few catecholamine-containing neurones in the ventral nerve cord, catecholamine-containing processes arborize extensively in the neuropiles of all the ventral ganglia (Fleming and Pitman, 1983; Baker and Pitman, 1989).

At least some of the actions of dopamine in the cockroach CNS may be mediated through elevation of the intracellular cyclic AMP concentration, since dopamine-sensitive adenylate cyclase activity has been detected in the nerve cord (Nathanson and Greengard, 1973, 1974; Harmar and Horn, 1977; Downer *et al.* 1985). Although noradrenaline can also stimulate such activity, it appears to do so by binding cyclase-linked receptors for dopamine, octopamine and serotonin. The insect CNS, therefore, appears to lack a specific noradrenaline-sensitive adenylate cyclase (Nathanson and Greengard, 1973; Harmar and Horn, 1977).

In the majority of electrophysiological studies, catecholamines have been found to excite insect central neurones (Gahery and Boistel, 1965; Kerkut *et al.* 1969; Walker *et al.* 1980). One exception to this is the report by Steiner and Pieri (1969) that ionophoretically applied dopamine inhibited neuronal activity in the ant brain. Recently, dopamine has been shown to depolarize and excite the soma of a prothoracic common inhibitory motoneurone (Pitman and Fleming, 1985; Pitman and Baker, 1989; Pitman and Davis, 1988). Currents evoked by dopamine from this neurone are unusual, however, in that they are extremely voltage-dependent, going through a minimum near the normal resting potential of the neurone, but increasing when the membrane potential is more positive or more negative than this (Pitman and Davis, 1988). Calcium apparently plays an important role in the generation of these dopamine-stimulated currents, since they are abolished in Ca^{2+}-free solution or in the presence of Cd^{2+} or verapamil. However, other ions may also contribute, since alterations in the external concentrations of Na^+, K^+ or Cl^-, in some instances, can affect responses.

The action of dopamine upon the prothoracic common inhibitory motoneurone does not appear to be mediated via a rise in intracellular cyclic AMP concentration, since dopamine responses were unaltered under conditions which interfere with this second messenger system (Pitman and Fleming, 1985; Pitman and Baker, 1989). In this motoneurone, dopamine appears to act *via* receptors distinct from those for noradrenaline or octopamine, since responses to each of these compounds are affected differentially by a number of pharmacological antagonists (Pitman and Davis, 1988). In mammals, central dopamine receptors can be divided into two types, D-1 and D-2 (Kebabian and Calne, 1979). Activation of D-1 receptors leads to stimulation of an adenylate cyclase and a rise in intracellular

cyclic AMP, while D-2 receptor activation may produce a fall in the cyclic AMP concentration or leave it unaltered. These two mammalian dopamine receptor types can also be distinguished according to their differing pharmacological profiles. If receptors sensitive to dopamine in the insect CNS have similar properties to mammalian dopamine receptors, the effects of dopamine on the cockroach common inhibitory motoneurone might be expected to involve activation of D-2 receptors, a process which does not produce a rise in intracellular cyclic AMP level. If this were the case, the pharmacological profile of the insect receptor should also conform to that of a mammalian D-2 receptor.

We report here the effects of a range of dopaminergic agonists and antagonists upon dopamine-sensitive receptors in the soma membrane of the prothoracic common inhibitory motoneurone of the cockroach.

Materials and methods

Recordings were made from a common inhibitory motoneurone (designated D_3 by Iles, 1976) in the prothoracic ganglion of adult male cockroaches (*Periplaneta americana* L.). The preparation was set up for intracellular recordings under current-clamp or voltage-clamp in a manner similar to that described previously for the metathoracic ganglion (Pitman, 1975). Desheathed prothoracic ganglia were placed in an experimental chamber containing circulating oxygenated physiological saline. Normal saline had the following composition: NaCl 214 mmol l^{-1}; KCl 3.1 mmol l^{-1}; $CaCl_2$ 9.0 mmol l^{-1}; Tes buffer (pH 7.2) 10 mmol l^{-1}. Pharmacological agonists and antagonists were dissolved in normal physiological saline without osmotic compensation. Because dopamine is relatively unstable in solution, in the majority of experiments it was dissolved in normal physiological saline containing 1 g/100 ml sodium metabisulphite.

Neurones were impaled under visual control with two microelectrodes filled with 2 mol l^{-1} potassium acetate. One microelectrode was used to monitor membrane potential and the other to pass current through the neurone. Recording microelectrodes had a resistance of 20–30 MΩ, while current-passing microelectrodes were normally bevelled to a resistance of 8–15 MΩ.

During impalement of neurones with intracellular microelectrodes, current-clamp recordings were made with a standard unity-gain high-input impedance amplifier and bridge system. To voltage-clamp the neurone, the output from the voltage-recording amplifier was fed into the input of a laboratory-built high-voltage (±100 V) inverting amplifier with an adjustable gain and frequency response. The output of this amplifier was connected to the current-passing intracellular microelectrode. To generate command steps, pulses from a Tektronix 162 waveform generator and Tektronix 161 pulse generator were applied to the input of this inverting feedback amplifier. Current was monitored by a current-to-voltage converter connected to the preparation bath. Drug responses were displayed on a Tektronix 5103N oscilloscope, and permanent records recorded on a Gould 220 pen recorder.

In most instances agonists were applied locally to the motoneurone cell body from a micropipette by pressure pulses (7–170 kPa; 50–500 ms) using a Picospritzer II system (General Valve Corp.). The tip of the drug-filled micropipette was positioned over the nerve cell body under visual control. Bath application of agonists and antagonists was achieved by adding a 0.3 ml sample to the bath in such a way that the drug was diluted by mixing before it reached the preparation. Concentrations of these agents are expressed as the final concentration after dilution in the volume of the experimental bath (3 ml). To wash out bath-applied drugs, saline was perfused through the experimental chamber at a rate of $10\,ml\,min^{-1}$.

The following drugs were used: dopamine hydrochloride (Sigma and Aldrich), apomorphine hydrochloride (Sigma), bromocryptine mesylate (2-bromo-alpha-ergocryptine methane sulphonate) (Sigma), ergometrine maleate (Sigma), ADTN hydrobromide [(±)-2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene hydrobromide] (Research Biochemicals), fenoldopam (SK&F 82526 methane sulphonate) (Smith Kline & French), LY 171555 (quinpirole hydrochloride) (Lilly Research), fluphenazine hydrochloride (Squibb), (+)-butaclamol hydrochloride (Research Biochemicals), cis-(Z)-flupenthixol dihydrochloride (Lundbeck), SCH 23390(7-chloro-8-hydroxy-3-methyl-1-phenyl-1,2,3,4,5-pentahydro-3-benzazepine maleate) (Schering-Plough Corp. Inc.), metoclopramide monohydrochloride (Sigma), spiroperidol (spiperone) (Janssen), haloperidol (Janssen), YM 09151-2 [*N*-(1-benzyl-2-methyl pyrrolidin-3-yl)-5-chloro-2-methoxy-4-methyl-aminobenzamide] (Yamanouchi Pharmaceutical), sulpiride (Sigma) and chlorpromazine hydrochloride (Sigma).

Results

Under current-clamp conditions, dopamine depolarizes the soma of the prothoracic common inhibitory motoneurone D_3 (Pitman and Davis, 1988; Pitman and Fleming, 1989). In voltage-clamp recordings dopamine responses are revealed as inward currents that are relatively small when dopamine is applied at membrane potentials close to the normal resulting potential (−55 to −60 mV) of the neurone (Fig. 1). The magnitude of inward currents generated by dopamine, however, increased dramatically at membrane potentials in the region of −120 to −150 mV. There was also an increase in dopamine currents at more positive potentials. It was not possible to observe dopamine responses directly at potentials more positive than those shown in Fig. 1, since prolonged command steps of this magnitude caused membrane currents to become unstable. To overcome this problem, currents generated by brief command pulses in the absence of dopamine were subtracted from currents generated by identical voltage steps applied at the peak of responses to dopamine. Currents obtained by such subtractions represent the current produced by dopamine at any given command potential. Fig. 2 illustrates graphically this method of determining the current–voltage relationship for dopamine obtained by the above method, showing the voltage-dependence of

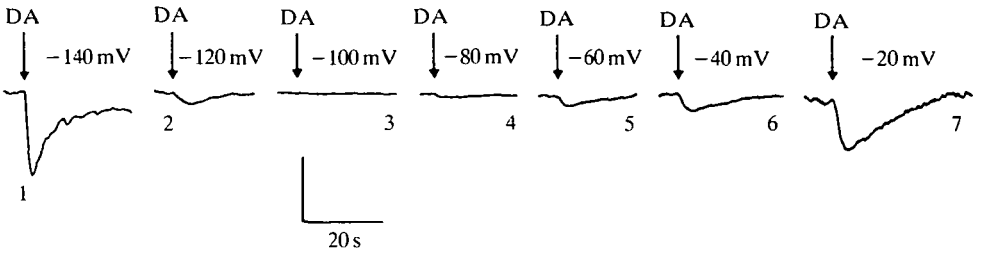

Fig. 1. Effect of command potential upon currents evoked by local application of dopamine (DA) to the soma of the prothoracic common inhibitory motoneurone D_3 under voltage-clamp conditions. Inward currents are shown as downward deflections. Command potentials at which responses were obtained are given above each trace. Brief pressure pulses (150 ms, 70 kPa) were applied to a micropipette with its tip positioned close to the soma membrane. Current calibrations: trace 1, 50 nA; trace 2, 2 nA; traces 3,4,5, 1 nA; trace 6, 5 nA; trace 7, 10 nA. Holding potential, −60 mV.

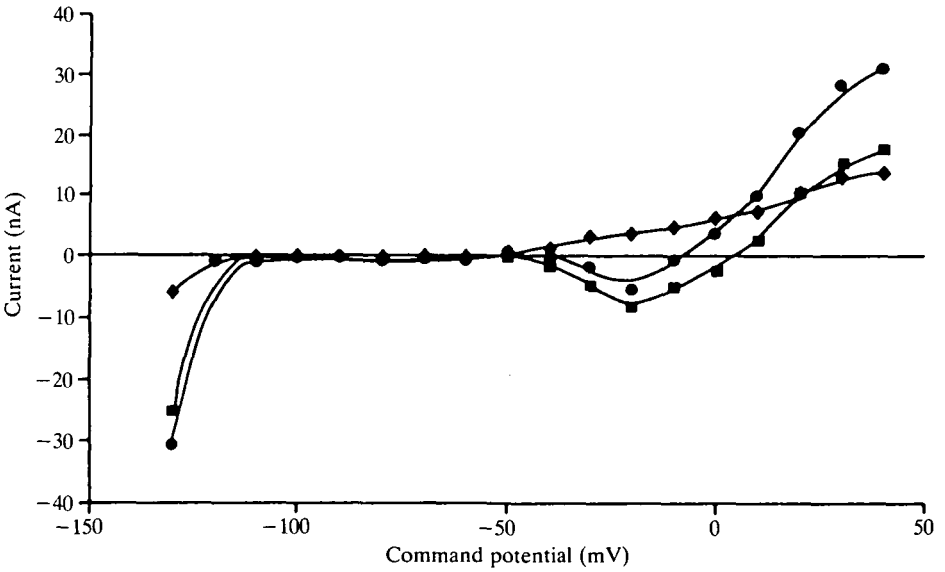

Fig. 2. Voltage-dependence of dopamine currents recorded from the neurone under voltage-clamp conditions (■). Dopamine-evoked currents obtained by subtraction of the current–voltage relationships obtained before (◆) and during (●) application of dopamine.

dopamine currents. Dopamine currents normally reached a minimum at membrane potentials between −60 mV and −100 mV, but increased dramatically as the membrane potential approached −120 to −150 mV. At progressively more positive command potentials, inward dopamine currents increased to a maximum in the region of −10 to −20 mV before declining then reversing at approximately 0 mV.

Dopaminergic agonists

To determine the characteristics of the receptor that mediated the responses to dopamine in this preparation, the effects of a number of different mammalian dopaminergic agonists were tested. The morphines apomorphine and bromocryptine, the dopaminergic ergot ergometrine and the naphthalene A-6,7-DTN (ADTN) all depolarized the neurone in current-clamp recordings. Voltage-clamp recordings revealed that responses to all four compounds had a similar voltage-dependence to that of the currents evoked by dopamine; responses were minimal at potentials close to the resting potential of the neurone, but increased as the membrane potential was shifted to more positive or more negative values. The current–voltage relationships of the above agonists, determined in a manner similar to that described for dopamine, showed a steep rise at membrane potentials of −140 to −160 mV and a peak in inward current at about −10 to −20 mV (Fig. 3). The current finally reversed and became outwardly directed between 0 and +15 mV.

Since it is extremely difficult to quantify the concentration of drugs reaching the neuronal membrane when applying agents by brief pressure application, the relative potencies of different dopaminergic agonists was determined by bath application of these compounds in the manner described in Materials and methods. Threshold concentrations were established by determining the minimum concentration of agonist necessary to produce a discernible response. Using this

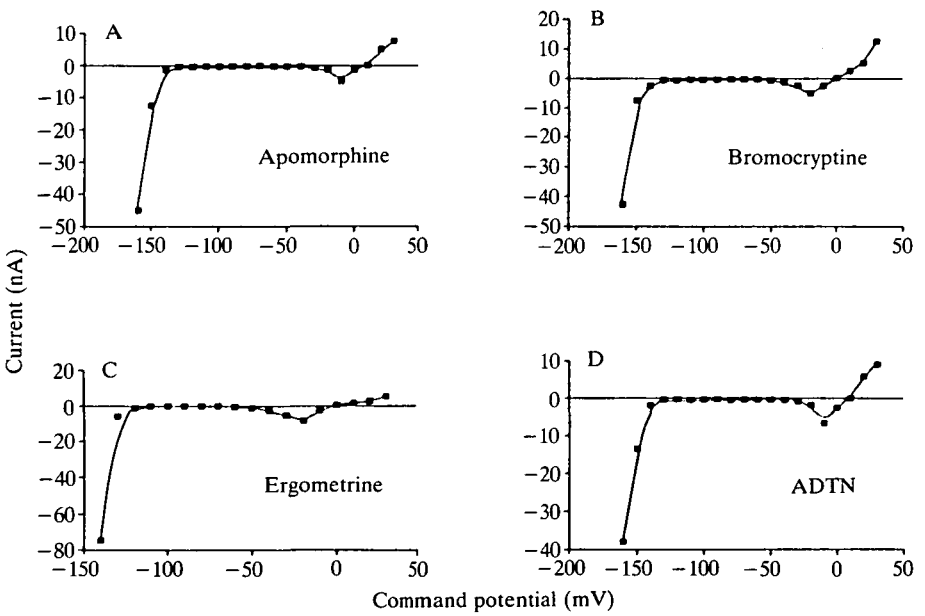

Fig. 3. Current–voltage relationships of responses to (A) apomorphine, (B) bromocryptine, (C) ergometrine and (D) ADTN recorded under voltage-clamp conditions. Agonist-evoked currents were determined as described for Fig. 2.

Table 1. *Summary showing the relative potencies of bath-applied pharmacological agonists on the membrane properties of the soma of the cockroach prothoracic common inhibitory motoneurone*

Compound	Threshold concentration ($mol\,l^{-1}$)	Mammalian target receptor	Effect on insect neurone
Dopamine	10^{-4}	D-1/D-2	+ (17)
ADTN	10^{-4}	D-2	+ (6)
Ergometrine	10^{-3}	D-2	+ (6)
Apomorphine	10^{-3}	D-2	+ (7)
Bromocryptine	10^{-3}	D-2	+ (7)
SK&F 82526	$<10^{-4}$	D-1	− (5)
LY 171555	$<10^{-4}$	D-2	− (7)

+=agonistic; −=no effect.
* Final concentration following bath application.
Number of experiments performed with each compound is shown within brackets.

method, it was found that the relative potencies of the active dopaminergic agonists were apparently ADTN>ergometrine>apomorphine>bromocryptine. The potency of ADTN was similar to that of dopamine.

Although the preparation responded to dopamine, apomorphine, bromocryptine and ergometrine, which are considerably more effective on mammalian D-2 receptors, than upon D-1 receptors (Kebabian and Calne, 1979; Offermeier and van Rooyen, 1982), the partial ergoline LY 171555, a selective D-2 receptor agonist, was inactive when locally applied to the neurone in concentrations up to $10^{-4}\,mol\,l^{-1}$. The preparation was also insensitive to the benzazepine D-1 receptor agonist SK&F 82526 at concentrations up to $10^{-4}\,mol\,l^{-1}$. Thus, although the insect receptor investigated here responded well to relatively non-selective dopaminergic agonists, these two selective agonists were completely inactive at the concentrations tested. Unfortunately, the limited solubility of LY 171555 and SK&F 82526 precluded use of these compounds at higher concentrations.

The relative potencies of bath-applied dopaminergic agonists are given in Table 1.

Dopaminergic antagonists

The action of dopamine on the prothoracic common inhibitory motoneurone could be suppressed by a range of mammalian dopaminergic antagonists. To determine the potencies of different antagonists in blocking the action of dopamine, a standard response was obtained by pressure application. Antagonist was then applied to the bath in samples of ascending concentration until a point was reached at which agonist responses were virtually eliminated. Responses to dopamine were reversibly suppressed by the non-selective antagonist (+)-

butaclamol (10^{-7} mol l^{-1}). Although the phenothiazine antagonist fluphenazine (10^{-6} mol l^{-1}) also reversibly blocked the action of dopamine in the preparation, the effectiveness of the related phenothiazine chlorpromazine as an antagonist could not be established, because this compound had major irreversible effects upon the resting properties of the neurone which varied from one preparation to the next. Following application of chlorpromazine at concentrations ranging between 10^{-9} and 10^{-4} mol l^{-1}, the membrane potential could undergo marked hyperpolarization or depolarization which was frequently accompanied by a drastic increase in membrane conductance.

Among the antagonists tested, the mixed D-1/D-2 receptor antagonist flupenthixol was the most potent, suppressing dopamine responses at concentrations in the region of 10^{-9} mol l^{-1}. Responses could also be reversibly suppressed by the selective D-1 antagonist SCH23390 (10^{-6} mol l^{-1}). The receptor mediating dopamine responses in this preparation, however, did not appear to distinguish between D-1 and D-2 antagonists, since dopamine-stimulated currents could be blocked by the butyrophenone D-2 receptor antagonists haloperidol and spiroperidol or by the benzamide YM 09151-2 (Fig. 4). While the antagonism of dopamine responses produced by haloperidol or YM 09151-2 could be at least partially reversed, the action of spiroperidol was completely irreversible at all concentrations that reduced the magnitude of responses to dopamine. Unlike YM 09151-2, the benzamide antagonist metoclopramide, at concentrations up to 10^{-4} mol l^{-1}, failed to suppress dopamine responses. The effectiveness of the related antagonist sulpiride could not be evaluated because, like chloropromazine, it produced large non-specific effects upon the resting properties of the neurone in the absence of applied dopamine. These changes included changes in membrane potential and a large rise in membrane conductance.

The relative potencies upon the preparation of the dopaminergic antagonists tested are given in Table 2.

Discussion

It has been shown previously that the actions of dopamine upon the cockroach common inhibitory motoneurone are mediated by receptors with pharmacological characteristics that differ from those of receptors that mediate responses to octopamine, noradrenaline or ACh (Pitman and Davis, 1988). In view of those observations, it is highly probable that specific dopamine receptors are present in this preparation. The results presented here further strengthen this case, since the action of dopamine on this preparation may be mimicked by a number of classical dopaminergic agonists and blocked by specific antagonists.

Another important issue is whether these receptors resemble mammalian dopamine receptors, since any unique pharmacological features of the insect receptor may make it an important potential target for the design of novel insecticides. Dopamine receptors in the mammalian CNS have been divided into two classes (Kebabian and Calne, 1979), which have been distinguished both in

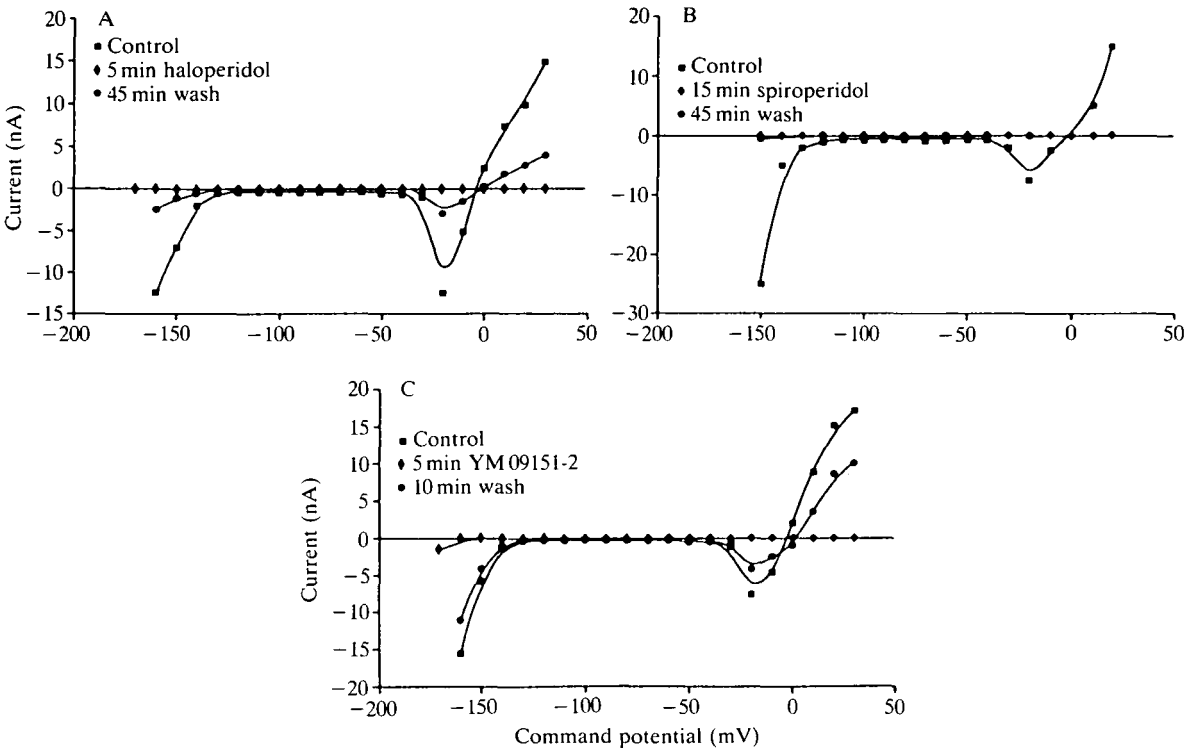

Fig. 4. Block of dopamine-evoked currents by the D-2 receptor antagonists haloperidol, spiroperidol and YM 09151-2. (A) ■ control; ◆ 5 min haloperidol (10^{-9} mol l^{-1}); ● 45 min wash produced partial reversal of blockade by haloperidol. (B) ■ control; ◆ 15 min spiroperidol (10^{-6} mol l^{-1}); ● 45 min wash resulted in no restoration of the response to dopamine. (C) ■ control; ◆ 5 min YM 09151-2 (10^{-6} mol l^{-1}); ● 10 min wash resulted in partial return of dopamine-evoked currents.

terms of their pharmacological profiles and the second messenger system they employ; D-1 receptor activation is frequently associated with a rise in intracellular cyclic AMP concentration, while activation of D-2 receptors is not.

The pharmacological profile of receptors in the insect inhibitory motoneurone preparation does not conform well to that of either mammalian D-1 or D-2 classes, since dopamine responses could be completely blocked by the D-1 receptor antagonists flupenthixol and SCH 23390 or by the D-2 receptor antagonists haloperidol, spiroperidol and YM 09151-2. Conversely, some compounds such as SK&F 82526, LY 171555 and metoclopramide, which are potent dopaminergic agents in mammals, are inactive on this insect preparation. The receptor, however, does resemble the mammalian D-2 receptor in that apomorphine, bromocryptine and ergometrine all act as agonists, which is more characteristic of their action at such sites than upon D-1 receptors, where they normally act as antagonists or partial agonists (Markstein *et al.* 1978; Trabucchi *et al.* 1976; Kebabian and Calne, 1979).

Table 2. *Summary of the actions of dopaminergic antagonists upon dopamine currents recorded from the cell body of the cockroach prothoracic common inhibitory motoneurone*

Compound	Blocking concentration (mol l^{-1})	Mammalian target receptor	Effect on insect neurone
(+)-Butaclamol	10^{-7}	D-1/D-2	+ (7)
Fluphenazine	10^{-6}	D-1/D-2	+ (9)
Flupenthixol	10^{-9}	D-1/D-2	+ (6)
Chlorpromazine	$>10^{-9}$	D-2	? (11)
SCH 23390	10^{-6}	D-1	+ (8)
Haloperidol	10^{-9}	D-2	+ (6)
Spiroperidol	10^{-5}	D-2	++ (7)
YM 09151-2	10^{-6}	D-2	+ (8)
Metoclopramide	$<10^{-4}$	D-2	− (9)
Sulpiride	$>10^{-6}$	D-2	? (7)

+=reversible block; ++= irreversible block; −=no effect; ?=non-specific effects. Number of experiments performed with each compound is shown within brackets.

Like mammalian D-2 receptors, the receptors on the insect neurone studied here do not appear to produce their effects *via* an increase in intracellular cyclic AMP concentration; neither the resting properties of the neurone nor dopamine responses were significantly affected by application of dibutyryl cyclic AMP, the phosphodiesterase inhibitor isobutyl methylxanthine (IBMX) or the adenylate cyclase activator forskolin (Pitman and Fleming, 1985; Pitman and Baker, 1989). Therefore, in this respect, the receptor studied here shows similarities to the mammalian D-2 receptor, even though it differs significantly in its pharmacological profile.

The results presented here indicate either that this insect preparation possesses a single class of dopamine-sensitive receptor with pharmacological properties distinct from either mammalian D-1 or D-2 receptors or that two separate receptor populations coexist on the neurone. The latter suggestion is the less likely, for two major reasons. First, if the neurone possessed two types of receptor pharmacologically similar to those of mammals, any one antagonist which selectively blocked a single class of those receptors should only partially suppress dopamine responses. Second, responses could be completely abolished both by the D-1 antagonists SCH 23390 and flupenthixol and by the D-2 antagonists haloperidol, spiroperidol and YM 09151-2.

Although this is the first report on the pharmacological profile of receptors that mediate the electrophysiological effects of dopamine on an identified insect central neurone, dopamine receptors of cockroach salivary glands have already been characterized in considerable detail. Dopamine mimics the effects of nerve stimulation in this preparation, where it appears to act as the neuroglandular transmitter substance. The glandular response to dopamine includes two indepen-

dent events which have been studied in detail. First, it stimulates secretion of saliva from the gland and, second, it causes hyperpolarization of acinar cells (for reviews, see House and Ginsborg, 1979, 1982). Dopamine-evoked salivary secretion apparently utilizes cyclic AMP as a second messenger (Gray *et al.* 1984) while acinar cell hyperpolarization results from an increase in potassium conductance which appears to involve mobilization of calcium from an intracellular store rather than a rise in cyclic AMP level (Ginsborg *et al.* 1980). A number of selective D-1 or D-2 agonists can produce acinar cell hyperpolarization. However, the actions of all the agonists were blocked by the D-1 receptor antagonist SCH 23390 but not by the D-2 antagonist sulpiride (A. M. Evans and K. L. Green, in preparation). A range of antagonists exhibit similar absolute and rank order potencies in blocking either secretory or electrophysiological response, D-1 receptor antagonists being more effective than D-2 antagonists. The order of potency in blocking acinar cell hyperpolarization was as follows: chlorpromazine>haloperidol>SCH 23390>metoclopramide. Domperidone and sulpiride were inactive. These observations indicate that both responses result from activation of a single class of receptor which resembles the mammalian D-1 subtype (Evans and Green, 1990*a*,*b*). It appears, therefore, that in this preparation, activation of a dopamine D-1 receptor can generate two distinct responses *via* different second messenger pathways.

A direct comparison between the dopamine receptor of the cockroach salivary gland and that on the prothoracic common inhibitory motoneurone described here is hard to make, since the range of antagonists tested on the two preparations differed. However, it does seem that, while the receptor in salivary glands resembles the mammalian D-1 subtype, this does not appear to be true of the neuronal receptor since the D-2 receptor antagonist haloperidol was approximately 1000-fold more potent than the D-1 antagonist SCH 23390 in blocking dopamine responses in this preparation, and SCH 23390 was essentially equipotent with the D-2 antagonist YM 09151-2. The antagonistic potencies of chlorpromazine and sulpiride on the two preparations could not be compared because both these compounds produced marked non-specific effects on the properties of the common inhibitory motoneurone in the absence of applied dopamine.

The finding that dopamine-sensitive receptors in the insect CNS have pharmacological properties distinct from those of mammalian dopamine receptors is similar to some observations that have been made on dopamine responses in molluscan neurones; Bokisch and Walker (1988) studying hyperpolarizing responses to dopamine in neurones of the snail *Helix aspersa*, and Audesirk (1989) studying hyperpolarizing and biphasic responses in *Lymnaea stagnalis* neurones have each concluded that the receptors on these neurones cannot be placed into the mammalian receptor classification scheme on the basis of their pharmacological properties. Failure to observe conformity with the mammalian classification scheme, however, does not appear to reflect any gradual evolution in the pharmacology of invertebrate receptors, since considerable variation occurs even

among molluscan dopamine receptors. Thus, Bokisch and Walker (1988) found that SCH 23390 could block inhibitory responses to dopamine in *Helix* while Audersirk (1989) detected no antagonistic effects of this compound in *Lymnaea*. The insect receptors characterized in this report appear to be distinct from either of these molluscan receptors, since ergometrine, apomorphine and bromocryptine act as agonists on this insect preparation but serve as antagonists on *Helix* neurones, while apomorphine has no effect at all on the receptors of *Lymnaea* RPeD1 and B-2 cells. Conversely, SCH 23390 blocks the action of dopamine on insect neurones but is without effect on RPeD1 and B-2 neurones. The insect receptor and the *Lymnaea* receptors studied by Audesirk (1989), however, do have a number of characteristics in common. First, neither appears to involve cyclic AMP as a second messenger, and, second, both the insect and *Lymnaea* receptors are insensitive to related specific D-1 agonists (SK&F 82526 and SK&F 38393) and the D-2 agonist LY 171555.

Further diversity of invertebrate dopamine receptors is revealed by work on growth hormone producing cells of *Lymnaea*, which indicates that they possess two types of receptor that do have similar characteristics to those of mammals (Stoof *et al.* 1985). Depolarization and an increase in membrane excitability evoked by dopamine is mediated by D-1-like receptors, while activation of D-2-like receptors produces membrane hyperpolarization. Cyclic AMP mimicked the effects of D-1 receptor activation and antagonized events mediated by D-2 receptors in this preparation (Stoof *et al.* 1985; de Vlieger *et al.* 1986). Despite the general similarity of these receptors to those of mammals, the D-2 receptors of these two groups do show some differences in their pharmacological profiles (Werkman *et al.* 1987). In view of these observations, it appears that a number of different types of dopamine receptor exist on molluscan neurones, some of which fit into the mammalian dopamine receptor classification scheme, while others do not. It is interesting that, over the past few years, evidence has begun to emerge indicating that, even in mammals, both D-1 and D-2 receptors can be divided into a number of subtypes which may exhibit characteristic differences in their pharmacology or in the second messenger pathway through which they operate (for a review see Andersen *et al.* 1990). It is possible, therefore, that further research will reveal a similar diversity of dopamine receptors in insects.

The unusual voltage-dependence of dopamine-evoked currents suggests that dopamine may be exerting a novel role in neuronal function. Dopamine currents are small and inwardly directed at membrane potentials in the region of the normal resting potential of the neurone, but become much larger at more depolarized potentials. It may be expected, therefore, that activation of dopaminergic synapses would only produce significant physiological actions when the neurone has been depolarized by some other means. One possible function of such currents could be to contribute to burst generation in a manner similar to that in some neurones of the crustacean stomatogastric ganglion, in which dopamine can change the membrane properties of some neurones in such a way that their burst-generating ability is enhanced (Marder and Eisen, 1984; Flamm and Harris-

Warrick, 1986). The effects of dopamine upon ionic currents responsible for burster properties have also been studied in the *Aplysia* neurone R_{15}. This molluscan neurone exhibits a slow inward calcium current which generates a region of negative slope resistance in the current–voltage relationship of the cell and confers burster properties (Wilson and Wachtel, 1978; Lotshaw and Levitan, 1988). Dopamine inhibits bursting in this neurone by suppressing the slow calcium current, hence eliminating the negative slope region from the current–voltage relationship.

Dopamine has the converse effect upon the cockroach common inhibitory motoneurone studied here, since it causes the appearance of a region of negative resistance in the current–voltage relationship. Such a change, in principle, could enable the neurone to acquire burster properties or the ability to generate plateau potentials in response to brief synaptic inputs. However, application of dopamine under current-clamp conditions did not induce such properties in the neurone. At most, prolonged exposure to dopamine occasionally caused the neurone to produce action potentials in bursts (Pitman and Baker, 1989). These observations indicate either that the role of dopamine is different from that proposed above or that expression of such properties is conditional upon some other, as yet unknown, factors. Recent evidence does indicate that some excitatory motoneurones in the cockroach can generate plateau potentials. These, however, do not appear to be modulated by dopamine (J. C. Hancox and R. M. Pitman, personal observation).

This work was supported by a grant from the Science and Engineering Research Council. We wish to thank the following companies for gifts of chemicals: H. Lundbeck (flupenthixol), Schering-Plough Corp. Inc. (SCH 23390), Smith Kline & French (SK&F 82526), Lilly Research (LY 171555), Squibb (fluphenazine), Janssen (spiroperidol and haloperidol) and Yamanouchi Pharmaceutical (YM 09151-2).

References

ANDERSEN, P. H., GINRICH, J. A., BATES, M. D., DEARRY, A., FALARDEAU, P., SENOGLES, S. E. AND CARON, M. G. (1990). Dopamine receptor subtypes: beyond the D_1/D_2 classification. *Trends pharmac. Sci.* **11**, 231–236.

AUDESIRK, T. E. (1989). Characterization of pre- and postsynaptic dopamine receptors in *Lymnaea*. *Comp. Biochem. Physiol.* **93**C, 115–119.

BAKER, J. R. AND PITMAN, R. M. (1989). Localization of biogenic amine-containing neurones in the ventral nerve cord of the cockroach (*Periplaneta americana*). *Comp. Biochem. Physiol.* **92**C, 237–243.

BOKISCH, A. J. AND WALKER, R. J. (1988) Quantitative antagonist studies on dopamine inhibitory responses of *Helix aspersa* neurones. *Comp. Biochem. Physiol.* **89**C, 121–126.

DE VLIEGER, T. A., LODDER, J. C., STOOF, J. C. AND WERKMAN, T. R. (1986). Dopamine receptor stimulation induces a potassium dependent hyperpolarizing response in growth hormone producing neuroendocrine cells of the gastropod mollusc *Lymnaea stagnalis*. *Comp. Biochem. Physiol.* **83**C, 429–433.

DOWNER, R. G. H., GOLE, J. W. D. AND ORR, G. L. (1985). Interaction of formamidines with octopamine-, dopamine- and 5-hydroxytryptamine-sensitive adenylate cyclase in the nerve cord of *Periplaneta americana*. *Pestic. Sci.* **16**, 472–478.

DYMOND, G. R. AND EVANS, P. D. (1979). Biogenic amines in the nervous system of the

cockroach, *Periplaneta americana:* association of octopamine with mushroom bodies and dorsal unpaired median (DUM) neurones. *Insect Biochem.* **9**, 535–545.

EVANS, A. M. AND GREEN, K. L. (1990*a*). Pharmacological characterization of the dopamine receptor mediating the secretory response of the cockroach salivary gland *in vitro*. *Comp. Biochem. Physiol.* (in press).

EVANS, A. M. AND GREEN, K. L. (1990*b*). Characterization of the dopamine receptor mediating the hyperpolarization of cockroach salivary gland acinar cells *in vitro*. *Brit. J. Pharmac.* (in press).

FLAMM, R. E. AND HARRIS-WARRICK, R. M. (1986). Aminergic modulation in lobster stomatogastric ganglion. II. Target neurons of dopamine, octopamine and serotonin within the pyloric circuit. *J. Neurophysiol.* **55**, 866–881.

FLEMING, J. R. AND PITMAN, R. M. (1983). Histochemical localization of biogenic amines in cockroach neurones. *J. Physiol., Lond.* **334**, 98P–99P.

FRONTALI, N. (1968). Histochemical localization of catecholamines in the brains of normal and drug-treated cockroaches. *J. Insect Physiol.* **14**, 881–886.

GAHERY, Y. AND BOISTEL, J. (1965). Study of some pharmacological substances which modify the electrical activity of the sixth abdominal ganglion of the cockroach, *Periplaneta americana*. In *The Physiology of the Insect Central Nervous System* (ed. J. E. Treherne and J. W. L. Beament), pp. 73–78., London: Academic Press.

GINSBORG, B. L., HOUSE, C. R. AND MITCHELL, M. R. (1980). On the role of calcium in the electrical responses of cockroach salivary gland cells to dopamine. *J. Physiol., Lond.* **303**, 325–335.

GRAY, D. C., GINSBORG, B. L. AND HOUSE, C. R. (1984). Cyclic AMP as a possible mediator of dopamine stimulation of cockroach gland cells. *Q. Jl exp. Physiol.* **69**, 171–186.

HARMAR, A. J. AND HORN, A. S. (1977). Octopamine-sensitive adenylate cyclase in cockroach brain: effects of agonists antagonists and guanylyl nucleotides. *Molec. Pharmac.* **13**, 512–520.

HOUSE, C. R. AND GINSBORG, B. L. (1979). Pharmacology of cockroach salivary gland secretion. *Comp. Biochem. Physiol.* **63**C, 1–6.

HOUSE, C. R. AND GINSBORG, B. L. (1982). Properties of dopamine receptors at a neuroglandular synapse. In *Ciba Foundation Symposium*, vol. 88 (ed. D. Evered, M. O'Connor and J. Whelan), pp. 32–47. London: Pitman.

ILES, J. F. (1976). Organization of motoneurones in the prothoracic ganglion of the cockroach, *Periplaneta americana* (L.). *Phil. Trans. R. Soc. Ser.* B **276**, 205–219.

KEBABIAN, J. W. AND CALNE, D. B. (1979). Multiple receptors for dopamine. *Nature* **277**, 93–96.

KERKUT, G. A., PITMAN, R. M. AND WALKER, R. J. (1969). Iontophoretic application of acetylcholine and GABA onto insect central neurones. *Comp. Biochem. Physiol.* **31**, 611–633.

KLEMM, N. (1976). Histochemistry of putative neurotransmitter substances in the insect brain. *Prog. Neurobiol.* **7**, 99–169.

KLEMM, N. (1982). A modified glyoxylic acid–formaldehyde technique for histofluorescence of catecholamine-containing neurones in cryostat sections of the insect brain. *J. Histochem. Cytochem.* **30**, 398–400.

LOTSHAW, D. P. AND LEVITAN, I. B. (1988). Reciprocal modulation of calcium current by serotonin and dopamine in identified *Aplysia* neuron R15. *Brain Res.* **439**, 64–76.

MARDER, E. AND EISEN, J. S. (1984). Electrically coupled pacemaker neurons respond differently to same physiological inputs and neurotransmitters. *J. Neurophysiol.* **51**, 1362–1374.

MARKSTEIN, R., HERRLING, P. L., BUERKI, H. R., ASPER, H. AND RUCH, W. (1978). The effect of bromocriptine on rat striatal adenylate cyclase and rat brain monoamine metabolism. *J. Neurochem.* **31**, 1163–1172.

NATHANSON, J. A. AND GREENGARD, P. (1973). Octopamine sensitive adenylate cyclase: evidence for a biological role of octopamine in nervous tissue. *Science* **180**, 308–310.

NATHANSON, J. A. AND GREENGARD, P. (1974). Serotonin-sensitive adenylate cyclase in neural tissue and its similarity to the serotonin receptor: a possible site of action of lysergic acid diethylamide. *Proc. natn. Acad. Sci. U.S.A.* **71**, 797–801.

OFFERMEIER, J. AND VAN ROOYEN, J. M. (1982). Is it possible to integrate dopamine receptor

terminology? In *More About Receptors* (ed. J. W. Lamble), pp. 93–97. Amsterdam: Elsevier Biomedical.

PITMAN, R. M. (1975). The ionic dependence of action potentials induced by colchicine in an insect motoneurone cell body. *J. Physiol., Lond.* **247**, 511–520.

PITMAN, R. M. (1985). Pharmacology of the insect central nervous system. In *Comprehensive Insect Physiology, Biochemistry and Pharmacology* (ed. G. A. Kerkut and L. I. Gilbert), pp. 5–54. Oxford: Pergamon.

PITMAN, R. M. AND BAKER, J. R. (1989). Dopamine responses recorded from a common inhibitory motoneurone of the cockroach (*Periplaneta americana*). *Comp. Biochem. Physiol.* **92**C, 245–251.

PITMAN, R. M. AND DAVIS, J. P. L. (1988). Pharmacological differentiation of responses to dopamine, octopamine and noradrenaline recorded from a cockroach motoneurone. *Pestic. Sci.* **24**, 311–323.

PITMAN, R. M. AND FLEMING, J. R. (1985). The action of dopamine on an identified insect neurone. *Pestic. Sci.* **16**, 447–448.

STEINER, F. A. AND PIERI, L. (1969). Comparative electrophoretic studies of invertebrate and vertebrate neurones. In *Mechanisms of Synaptic Transmission* (ed. K. Akert and P. G. Waser), pp. 191–199. Amsterdam: Elsevier.

STOOF, J. C., DE VLIEGER, T. A. AND LODDER, J. C. (1985). Opposing roles for D-1 and D-2 dopamine receptors in regulating the excitability of growth hormone-producing cells in the snail *Lymnaea stagnalis*. *Eur. J. Pharmac.* **106**, 431–435.

TRABUCCHI, M., SPANO, P. F., TONON, G. C. AND FRATTOLA, L. (1976). Effect of bromocriptine on central dopaminergic receptors. *Life Sci.* **19**, 225–231.

WALKER, R. J., JAMES, V. A., ROBERTS, C. J. AND KERKUT, G. A. (1980). Neurotransmitter receptors in invertebrates. In *Receptors for Neurotransmitters, Hormones and Pheromones* (ed. D. B. Sattelle, L. M. Hall and J. G. Hildebrand), pp. 41–57. Amsterdam: Elsevier: Amsterdam.

WERKMAN, T. R., LODDER, J. C., DE VLIEGER, T. A. AND STOOF, J. C. (1987). Further pharmacological characterization of a D-2-like dopamine receptor on growth hormone producing cells in *Lymnaea stagnalis*. *Eur. J. Pharmac.* **139**, 155–161.

WILSON, W. A. AND WACHTEL, H. (1978). Prolonged inhibition in burst firing neurons: synaptic inactivation of the slow regenerative inward current. *Science* **202**, 772–775.