

## OCTOPAMINERGIC MODULATION OF THE MEMBRANE POTENTIAL OF THE SCHWANN CELL OF THE SQUID GIANT NERVE FIBRE

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### SUMMARY

1. The actions of octopamine on the Schwann cells of the giant nerve fibre of the tropical squid are described.

2. The pharmacology of the receptors mediating the actions of octopamine has been investigated in terms of stereospecificity, amine specificity and interactions with a range of agonists and antagonists. The receptors are maximally activated by D(–)-octopamine and share many of the characteristics of OCTOPAMINE<sub>2</sub> class receptors in other preparations.

3. The octopamine receptors appear to mediate their actions by increasing the intracellular levels of cyclic AMP in the Schwann cells.

4. Low concentrations of octopamine potentiate the actions of the nicotinic cholinergic activation system of the Schwann cells.

5. The results are discussed in terms of the possible physiological role of octopamine in the modulation of Schwann cell activity during stressful conditions when the giant axon system is likely to be used at a high frequency to facilitate the escape response of the squid.

### INTRODUCTION

Biogenic amines are now known to be capable of binding to specific receptors on glial cells and of altering their physiological properties (see Van Calker & Hamprecht, 1980), in addition to their well known roles as neurotransmitters effecting chemical communication between neurones. At present very little information is available on the functional significance of the actions of biogenic amines on glial cells and on their complex dynamic reciprocal interactions with neurones. The bulk of the work in this field has been performed in culture using clonal glial cell lines or primary cultures of glial cells from perinatal brain tissue (see Van Calker & Hamprecht, 1980). Relatively pure cultures can now be obtained, and it has recently

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been shown that the  $\beta$ -adrenergic responses of such purified astrocytes from rat brains can be modulated by neuropeptides (Rougon, Noble & Mudge, 1983).

To study the effects of biogenic amines on the functional interactions between glial cells and neurones, it is essential to study an intact system where the anatomical relationships are preserved, as in recent studies on the hyperpolarizing actions of 5-hydroxytryptamine on the glial cells in the neuropile of leech segmental ganglia (Walz & Schlue, 1982) and on the action of octopamine to reduce the potassium permeability of the glia forming the insect blood brain barrier (Schofield & Treherne, 1985).

In the present study we have examined the effects of the biogenic amine octopamine on the neuronal-glial interactions in the giant nerve fibre from the stellate nerve of the tropical squid, *Sepioteuthis sepioidea* (see Villegas, 1981, 1984). The Schwann cells in this preparation have the capacity to synthesize (Heumann, Villegas & Herzfeld, 1981) and to store (Villegas & Jenden, 1979) large quantities of acetylcholine. The latter is released from the Schwann cell in response to an unknown chemical signal (which may be glutamate, see Villegas, 1984) released from the axon during neural activity. A long-lasting hyperpolarization of the Schwann cell membrane potential due to an increase in potassium conductance is induced by the released acetylcholine feeding back onto nicotinic cholinergic receptors on the Schwann cell (Villegas, 1974, 1975). The latter appear to mediate their actions by increasing the intracellular levels of cyclic AMP in the Schwann cells (Evans, Reale & Villegas, 1985).

We have chosen octopamine because it was first identified in the posterior salivary glands of the octopus (Erspamer & Boretti, 1951) and has since been shown to be a normal constituent of all nervous systems examined (see Harmar, 1980; Talamo, 1980; Evans, 1980, 1985; Robertson, 1981) including the stellate nerves of another species of squid, *Loligo vulgaris* (Juorio & Molinoff, 1974). In addition, octopamine has been demonstrated to function as a neuromodulator, as well as a true neurotransmitter and neurohormone, in a number of invertebrates including insects (Orchard, 1982; Evans, 1980, 1985), crustaceans (Kravitz *et al.* 1976, 1984) and molluscs (Walker, Ramage & Woodruff, 1972; Carpenter & Gaubatz, 1974; Kobayashi & Hasimoto, 1982). Further, as mentioned above, octopamine has been shown to be capable of modulating the properties of glial cells in the insect nervous system (Schofield & Treherne, 1985).

The present paper will describe the effects of octopamine on the Schwann cells of the squid giant axon and compare the pharmacological properties of the octopamine receptors mediating these effects with those of octopamine receptors in other preparations. Studies on the mode of action of the octopamine receptors on the squid Schwann cell will be described, together with studies on the octopaminergic modulation of the cholinergic response system of the squid Schwann cells.

#### MATERIALS AND METHODS

Giant nerve fibres with a diameter of 300–400  $\mu\text{m}$  were dissected in sea water from the hindmost stellar nerve of the squid *Sepioteuthis sepioidea*. Giant axons with their

surrounding Schwann cell sheaths were then isolated and cleaned of adhering bundles of small nerve fibres by dissection in artificial sea water (see below). All experiments were carried out at room temperature (20–22°C). Electrophysiological techniques were as described previously and involved the successive measurement of electrical potentials of a series of Schwann cells by brief impalements from inside the axon (Villegas, 1972, 1973, 1975).

Drugs were superfused over the preparation dissolved in artificial sea water containing (in mmol l<sup>-1</sup>): NaCl, 442; KCl, 10; CaCl<sub>2</sub>, 11; MgCl<sub>2</sub>, 45; and Tris-HCl buffer, 10 (pH 8.00). All the superfused solutions were continuously bubbled with a mixture of 95 % O<sub>2</sub> and 5 % CO<sub>2</sub>. Stock solutions of forskolin were made by dissolving 1 mg in 100 µl ethanol. Control experiments show that solutions containing up to 1 % (v/v) ethanol have no effect on the Schwann cell membrane potential (Villegas, Sevcik, Barnola & Villegas, 1976).

Axon-free nerve fibre sheaths were used for measurements of cyclic AMP levels. They were obtained as described previously (Villegas & Jenden, 1979). Briefly a pair of fine scissors was inserted into the isolated and cleaned giant nerve fibre (see above) and the axon was cut lengthwise. Isolation, cleaning and slitting of the fibre took 15–25 min. Immediately after slitting, the axon sheath, carefully handled with fine stainless steel forceps, was transferred to a large volume of artificial sea water (5 ml) and shaken gently to remove the axoplasm. It was then transferred to the incubation solution for 5 min during which time the solution was agitated by being continuously bubbled with a mixture of 95 % O<sub>2</sub> and 5 % CO<sub>2</sub>. After soaking, the sheath was blotted on filter paper and homogenized in an ice-cold concentrated hydrochloric acid: absolute ethanol mixture (1:60 v/v) (Horn & McAfee, 1977). In most experiments two samples were pooled to achieve greater accuracy. The cyclic AMP levels were assayed in the sheath extracts by radioimmunoassay using a commercial cyclic AMP assay kit (New England Nuclear). Protein determinations were carried out according to Lowry, Rosebrough, Farr & Randall (1951) using bovine serum albumin as standard.

We would like to acknowledge the gift of samples of the following drugs from pharmaceutical companies: clonidine HCl (Boehringer Ingelheim), naphazoline HCl, phentolamine mesylate (Ciba), isoprenaline sulphate (Riker), metoclopramide HCl (Beecham). We would also like to thank Dr M. D. Armstrong for his kind gift of samples of D(-)- and L(+)-octopamine and Dr Michael Raftery for kindly supplying the purified  $\alpha$ -bungarotoxin. Forskolin was obtained from Calbiochem and all other drugs were obtained from the Sigma Chemical Co.

## RESULTS

### *The effect of octopamine*

The monophenolic biogenic amine octopamine can induce a long lasting hyperpolarization of the membrane potential of the Schwann cell of the squid giant axon. The effects of octopamine are dose-dependent (Fig. 1A,B), increasing concentrations giving larger and longer-lasting effects. The threshold concentration for an

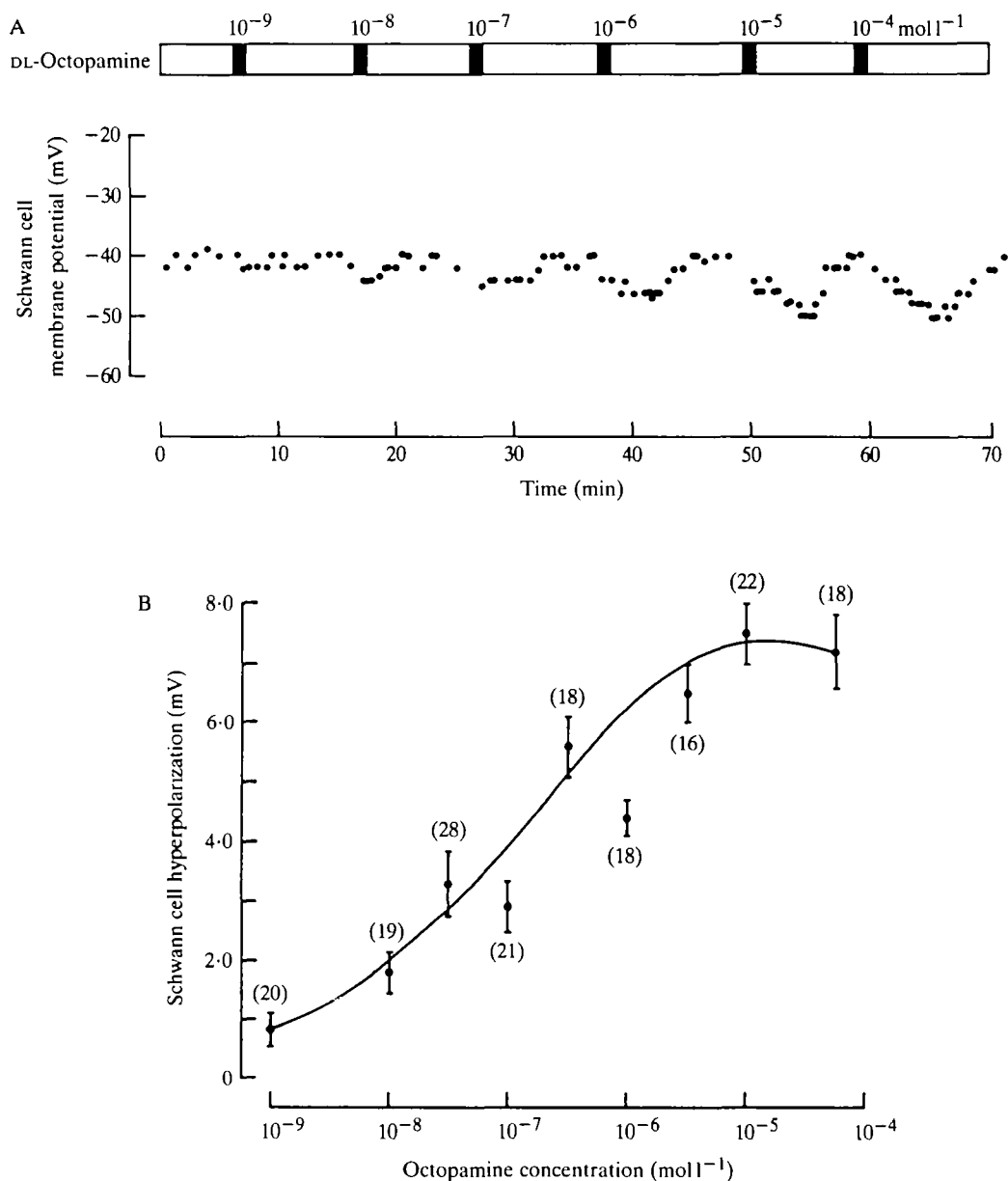


Fig. 1. The effect of DL-octopamine on the Schwann cell membrane potential. (A) A typical example of the effect of 1-min pulses of various concentrations (filled bars). Each point represents the potential difference recorded in a different Schwann cell. (B) Dose-response curve for the hyperpolarizing effect of DL-octopamine. The values represent the difference  $\pm$  standard error between the membrane potential before octopamine application and the maximal response to the 1-min pulse. The numbers in brackets represent the total number of cells penetrated at each concentration and each solution was tested on at least four nerve fibres.

observable effect of DL-octopamine lies between  $10^{-8}$  and  $10^{-9}$  mol l $^{-1}$ , whilst maximal effects occur in the range of  $10^{-5}$  mol l $^{-1}$ .

The membrane potential of the Schwann cell of the squid giant axon is also known to hyperpolarize in response to acetylcholine released from the Schwann cell in response to an unidentified chemical signal released by the giant axon (Villegas, 1981, 1984). This effect can be mimicked in a dose-dependent way by carbachol, the non-metabolizable analogue of acetylcholine (Villegas, 1974; Evans *et al.* 1985). To rule out the possibility that octopamine is mediating its effects by an action on the same receptor as carbachol we examined the effect of octopamine in the presence of  $\alpha$ -bungarotoxin, a specific irreversible blocker of the cholinergic receptors on the squid Schwann cell (Villegas, 1975). After exposure of a preparation to  $10^{-8}$  mol l $^{-1}$   $\alpha$ -bungarotoxin for 10 min, a treatment that blocks the actions of a 1-min pulse of  $10^{-6}$  mol l $^{-1}$  carbachol, the effect of a 1-min pulse of DL-octopamine persists (Fig. 2). In general the effects of a 1-min pulse of octopamine take a longer time to reach a maximal effect than the corresponding effect of a 1-min pulse of carbachol.

#### Stereospecificity of responses

Octopamine exists in two stereoisomeric forms, D(-) and L(+), with the former being the naturally occurring isomer in the octopus (Erspamer, 1952) and in other invertebrates (Goosey & Candy, 1980; Starratt & Bodnaryk, 1981). The stereospecificity of the octopamine-mediated hyperpolarization of the membrane potential

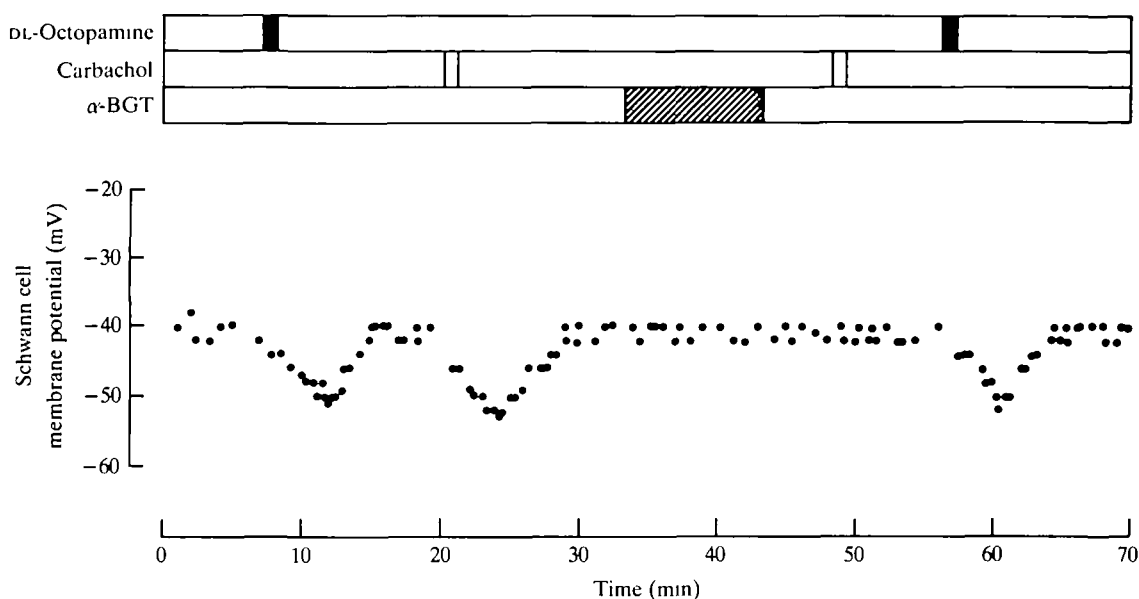


Fig. 2. The effect of a 10-min pulse of  $10^{-8}$  mol l $^{-1}$   $\alpha$ -bungarotoxin ( $\alpha$ -BGT) (hatched bar) on the responses of the Schwann cell membrane potential to 1-min pulses of  $10^{-5}$  mol l $^{-1}$  DL-octopamine (filled bar) and  $10^{-6}$  mol l $^{-1}$  carbachol (open bar). The  $\alpha$ -bungarotoxin pulse blocks the effect of carbachol but not the response to octopamine. Each point represents the potential difference recorded in a different Schwann cell.

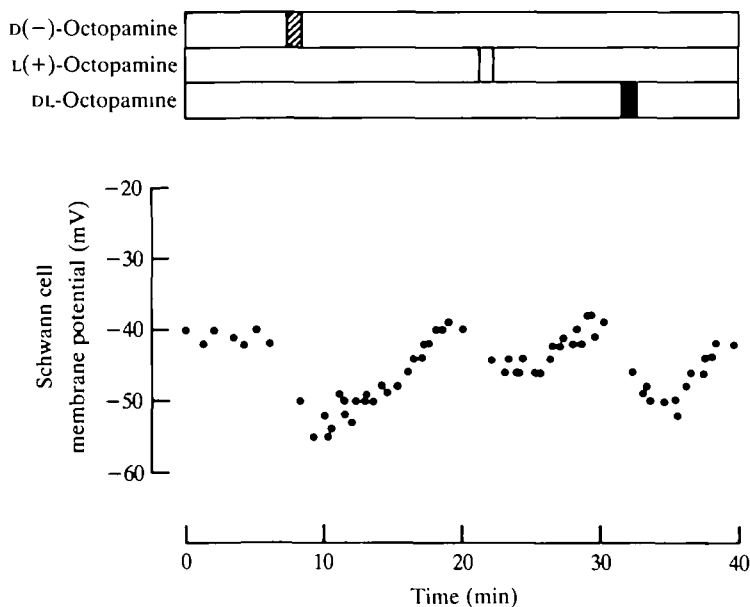


Fig. 3. The stereospecificity of the octopamine responses of the Schwann cell membrane potential to 1-min pulses at a concentration of  $10^{-5} \text{ mol l}^{-1}$  of D(-)-octopamine (hatched bar), L(+)-octopamine (open bar) and DL-octopamine (filled bar). Each point represents the potential difference recorded in a different Schwann cell.

of the Schwann cell of the squid was investigated in experiments in which a preparation was successively exposed to pulses (1 min,  $10^{-5} \text{ mol l}^{-1}$ ) of the D(-) and the L(+) isomers of octopamine followed by a control pulse of DL-octopamine (Fig. 3; Table 1). The D(-) isomer is more potent than both the L(+) isomer and the DL-isomeric mixture giving responses of larger magnitude and longer duration.

#### *Amine specificity of responses*

The nervous system of the squid contains, in addition to octopamine (Juorio & Molinoff, 1974), large amounts of other biogenic amines such as noradrenaline, dopamine and 5-hydroxytryptamine (Robertson & Juorio, 1976). Thus the specificity of the aminergic receptor on the squid Schwann cell was examined in a series of experiments where the effectiveness of 1-min pulses of various amines, structurally related to octopamine, was compared to the effects of 1-min pulses of DL-octopamine at a concentration of  $10^{-5} \text{ mol l}^{-1}$  (Table 1). It can be seen that of the compounds tested, only tyramine produces a response equal to that of DL-octopamine, but that even this compound is much less effective than the D(-) isomer of octopamine. Weaker, but significant, hyperpolarizing effects are also given by L-adrenaline, L-noradrenaline and phenylethanolamine. Small responses are also obtained with 5-hydroxytryptamine (5-HT) but it should be noted that at high concentrations 5-HT has been suggested to be capable of acting as a partial agonist of a variety of other octopamine receptors (e.g. in locust muscle, Evans, 1981). However synephrine, the *N*-methylated analogue of octopamine, only gives very small responses in some preparations (three out of eight) whilst in others it produces no observable effect.

In additivity experiments concentrations of DL-octopamine and tyramine, which alone give maximal responses, are not additive when given together either in terms of the maximal hyperpolarization obtained or of its duration (Fig. 4). These findings together with the finding that the dose-response curve for tyramine (not shown) is superimposable upon that for DL-octopamine (Fig. 1B), with similar maximal responses, are consistent with the idea that both amines are likely to be acting at the same receptor site. Of the compounds tested in this survey the maximal hyperpolarizing activity is observed with D(-)-octopamine.

#### *Action of agonists*

To characterize the receptors mediating the actions of octopamine on the membrane potential of the Schwann cell of the squid giant axon, the ability of a range of synthetic agonists to mimic this action was examined. It can be seen from (Fig. 5A,B) that drugs such as naphazoline and clonidine, which are effective  $\alpha$ -adrenoreceptor agonists in vertebrates and effective agonists of locust octopamine receptors (Evans, 1981), also mimic the actions of octopamine on the receptors of the squid Schwann cell. Naphazoline is more potent than clonidine at hyperpolarizing the Schwann cell. In contrast isoprenaline, a  $\beta$ -adrenoreceptor agonist, is ineffective at producing a hyperpolarization of the Schwann cell at concentrations up to  $10^{-4} \text{ mol l}^{-1}$  (Fig. 5C).

#### *Action of antagonists*

The response profiles of the receptors mediating the actions of octopamine have been further characterized in experiments where a range of antagonists have been used to block the octopamine responses. Metoclopramide is a very potent blocker of the actions of octopamine (Fig. 6A). A 1-min pulse of  $10^{-5} \text{ mol l}^{-1}$  DL-octopamine is

Table 1. *Actions of amines on Schwann cell membrane potential*

Amine	Hyperpolarizing response $\pm$ S.E. (mV)	(N)
D(-)-Octopamine	$12.3 \pm 0.3$	3
DL-Octopamine	$8.8 \pm 0.3$	17
Tyramine	$8.6 \pm 0.3$	8
L(+)-Octopamine	$4.0 \pm 0.1$	3
L-Adrenaline	$3.2 \pm 0.6$	5
L-Noradrenaline	$2.8 \pm 0.5$	4
Phenylethanolamine	2.0, 3.0	2
5-Hydroxytryptamine	$2.3 \pm 0.3$	3
DL-Synephrine	$0.8 \pm 0.4$	8
Dopamine	0	2
L-Tyrosine	0	2

The results are expressed as the mean  $\pm$  standard error of the maximum hyperpolarization produced by the introduction of a 1-min pulse of the amine into the superfusate at a concentration of  $10^{-5} \text{ mol l}^{-1}$ . *N* = the number of observations for each amine on different nerve fibres.

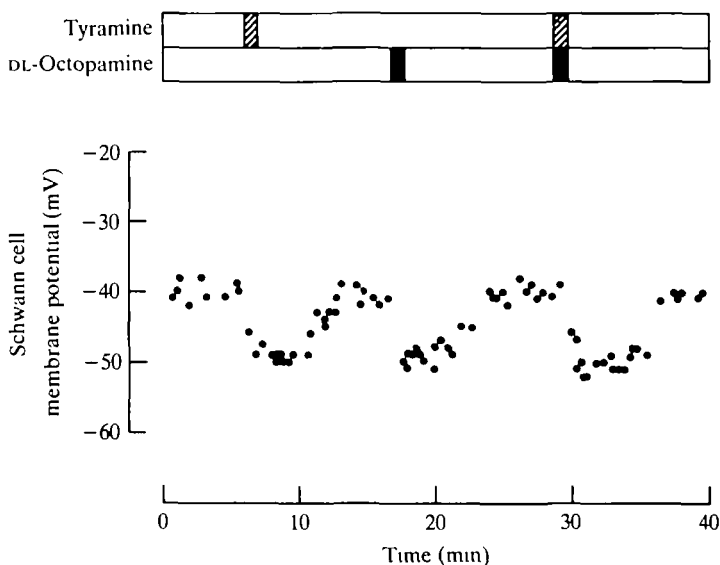


Fig. 4. Additivity experiment to show the effects on the Schwann cell membrane potential of 1-min pulses at a concentration of  $10^{-5} \text{ mol l}^{-1}$  of DL-octopamine (filled bar) and tyramine (hatched bar). Each point represents the potential difference recorded in a different Schwann cell.

almost completely blocked in the presence of  $10^{-7} \text{ mol l}^{-1}$  metoclopramide. Phentolamine, the  $\alpha$ -adrenergic blocking agent, is slightly less potent (Fig. 6B) than metoclopramide whilst DL-propranolol (Fig. 6C) only shows a slight blocking action at very high concentrations ( $10^{-4} \text{ mol l}^{-1}$ ). The actions of 1-min pulses of  $10^{-5} \text{ mol l}^{-1}$  tyramine are also blocked completely in the presence of  $10^{-6} \text{ mol l}^{-1}$  phentolamine (not shown).

The identification of blocking agents for the action of octopamine on the Schwann cell of the squid giant axon suggested that we could provide further evidence for separate sites of action of acetylcholine and octopamine in this system. However, in initial experiments with phentolamine we were surprised to find that at a concentration of  $10^{-6} \text{ mol l}^{-1}$  it blocked the actions of both carbachol (Fig. 7A) and 100-Hz stimulation of the giant axon (Fig. 7B) on the membrane potential of the Schwann cell. Further experiments (not shown) reveal that the effect of a 1-min pulse of  $10^{-6} \text{ mol l}^{-1}$  carbachol can be completely blocked in the presence of  $10^{-7} \text{ mol l}^{-1}$  phentolamine and that this blocking action of phentolamine has a threshold of between  $10^{-8}$  and  $10^{-9} \text{ mol l}^{-1}$ . This finding raised the possibility that some form of endogenous octopaminergic modulation might be required before the cholinergic system could express its effects. Alternatively, it might mean that the cholinergic receptors on the Schwann cell are unusual in that they are blocked by phentolamine. To distinguish between these two possibilities we examined the relative blocking actions of metoclopramide on 1-min pulses of  $10^{-6} \text{ mol l}^{-1}$  carbachol and  $10^{-6} \text{ mol l}^{-1}$  DL-octopamine (Fig. 7C). It can be seen that metoclopramide almost completely blocks the action of DL-octopamine whilst that of carbachol is not affected.

These results provide additional evidence that the hyperpolarizing actions of carbachol and octopamine are produced by separate receptor systems and that further, phentolamine, normally an aminergic blocking agent, can block the nicotinic cholinergic receptors on the Schwann cell of the squid.

#### *Mode of action of octopamine*

The membrane potential of the Schwann cell of the squid giant axon can be modulated by changes in the cyclic AMP levels within the Schwann cell and evidence has been presented that the nicotinic receptor of the squid Schwann cell is likely to mediate its effects by a mechanism that activates adenylate cyclase (Evans *et al.* 1985). Since octopamine also induces a long lasting hyperpolarization of the Schwann cell membrane potential, as does activation of the nicotinic receptor, we investigated the possibility that octopamine may also mediate its effects in this preparation by increasing cyclic AMP levels.

If octopamine acts on a receptor on the Schwann cell which activates adenylate cyclase then its actions should be potentiated by inhibitors of the enzyme, phosphodiesterase, that breaks down cyclic AMP. Fig. 8A shows that theophylline can potentiate the hyperpolarizing effect of octopamine on the Schwann cell membrane potential. In the presence of a sub-threshold dose of theophylline ( $10^{-8} \text{ mol l}^{-1}$ ) the effect of a submaximal dose of DL-octopamine ( $10^{-6} \text{ mol l}^{-1}$ ) is potentiated in both amplitude and time course. On return to artificial sea water (ASW) the response to the octopamine pulse gradually returns to its initial control value.

Theophylline, however, is a methylxanthine which has been reported to bring about a release of calcium from internal stores in muscle and mammalian neurones (see Neering & McBurney, 1984) in addition to its actions on phosphodiesterase. Thus we also investigated the effects of papaverine, a non-methylxanthine phosphodiesterase inhibitor, on the membrane potential of the Schwann cell of the squid. Fig. 8B shows that 1-min pulses of papaverine induce a long-term hyperpolarization of the Schwann cell membrane potential as does theophylline (Evans *et al.* 1985). The effect of papaverine is dose-dependent with a threshold of between  $10^{-7}$  and  $10^{-8} \text{ mol l}^{-1}$  for a 1-min pulse introduced into the superfusate. A subthreshold dose of papaverine ( $10^{-8} \text{ mol l}^{-1}$ ) also potentiates both the amplitude and time course of the effect of a submaximal dose of DL-octopamine ( $10^{-6} \text{ mol l}^{-1}$ ) (Fig. 8C). The size of the octopamine response gradually declines back to control levels when the preparation is returned to ASW. Fig. 8C is a continuation of the experiment in Fig. 8B.

The experiments with theophylline and papaverine suggest that octopamine mediates its actions on the Schwann cell membrane potential by an increase in cyclic nucleotide levels but does not indicate whether cyclic AMP or cyclic GMP levels are being changed. We therefore examined the effects of the diterpene compound forskolin, which can specifically increase the sensitivity of adenylate cyclase to activation by various agonists in a number of preparations (Seamon & Daly, 1981). Fig. 8D shows that the effect of a 1-min pulse of  $10^{-6} \text{ mol l}^{-1}$  DL-octopamine is also potentiated in both amplitude and time course when applied to the preparation in the

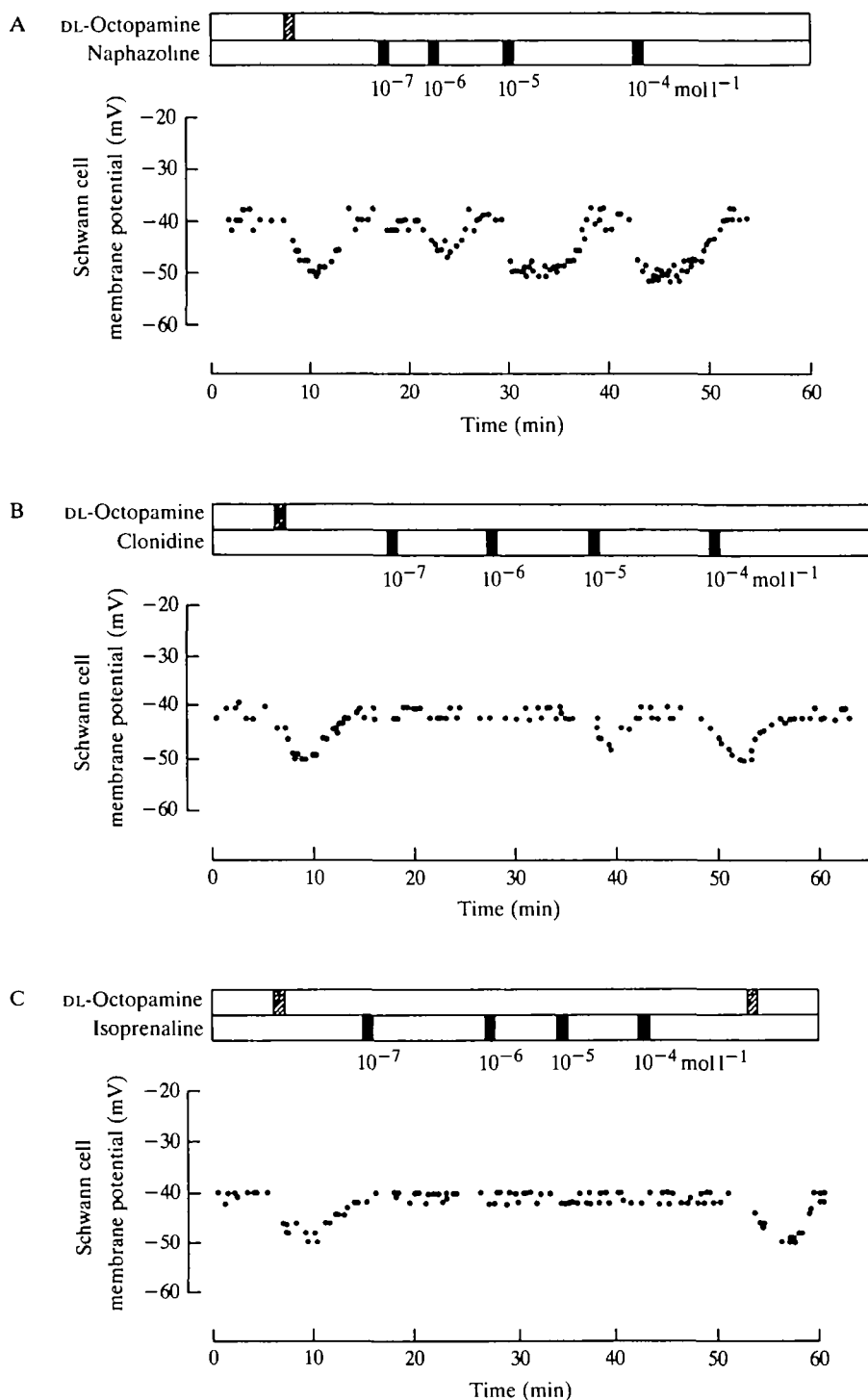


Fig. 5. The effect of various concentrations of agonists on the Schwann cell membrane potential compared to the effect of a 1-min control pulse of  $10^{-5} \text{ mol l}^{-1}$  DL-octopamine (hatched bars). Each point represents the potential difference recorded in a different Schwann cell. (A) The effects of 1-min pulses of naphazoline (filled bars). (B) The effects of 1-min pulses of clonidine (filled bars). (C) The effects of 1-min pulses of isoprenaline (filled bars).

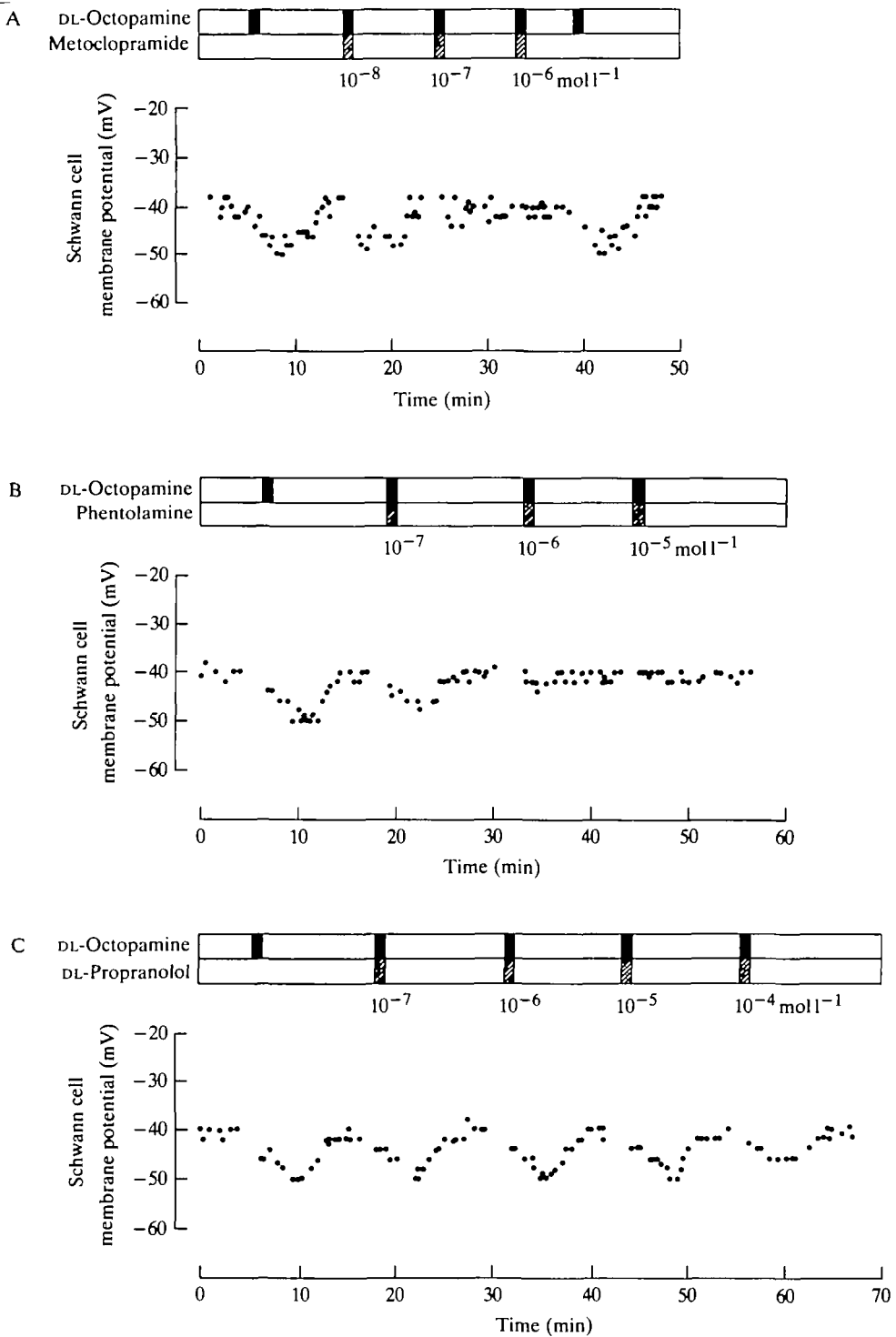


Fig. 6. The effect of various concentrations of antagonists on the Schwann cell membrane potential response to a 1-min pulse of  $10^{-5}$  mol l<sup>-1</sup> DL-octopamine (filled bars). Each point represents the potential difference recorded in a different Schwann cell. (A) The effect of 1-min pulses of metoclopramide (hatched bars). (B) The effect of 1-min pulses of phentolamine (hatched bars). (C) The effect of 1-min pulses of DL-propranolol (hatched bars).

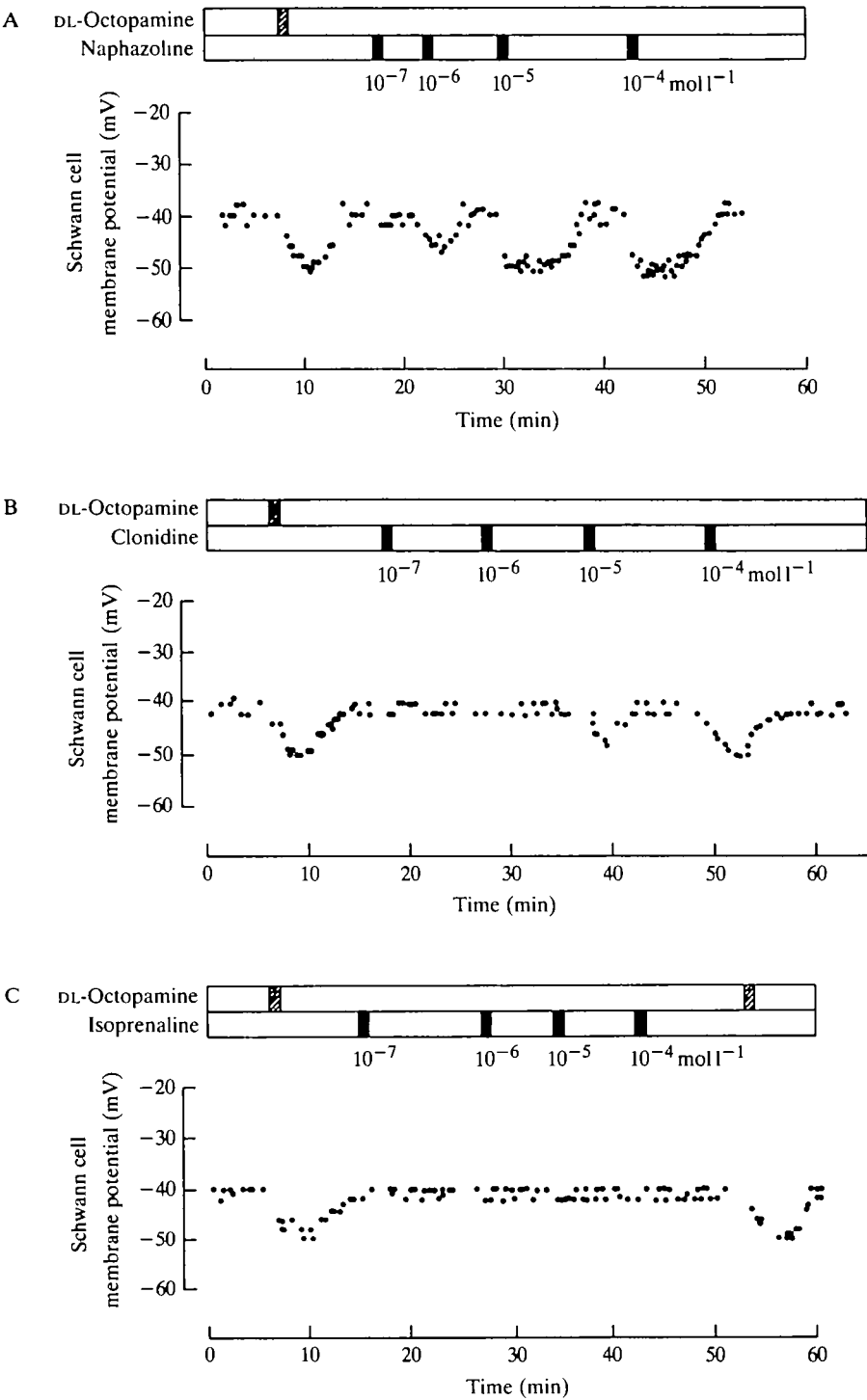


Fig. 5. The effect of various concentrations of agonists on the Schwann cell membrane potential compared to the effect of a 1-min control pulse of  $10^{-5} \text{ mol l}^{-1}$  DL-octopamine (hatched bars). Each point represents the potential difference recorded in a different Schwann cell. (A) The effects of 1-min pulses of naphazoline (filled bars). (B) The effects of 1-min pulses of clonidine (filled bars). (C) The effects of 1-min pulses of isoprenaline (filled bars).

presence of  $2.5 \times 10^{-8} \text{ mol l}^{-1}$  forskolin. The latter concentration of forskolin does not alter the Schwann cell membrane potential when given alone (Evans *et al.* 1985). The size of the octopamine response gradually returns to control levels when the preparation is returned to ASW.

The fact that octopamine produces its hyperpolarizing effects on the Schwann cell of the squid giant axon in the presence of  $\alpha$ -bungarotoxin suggests that the actions of octopamine are directly on the Schwann cell rather than being mediated *via* an effect on the giant axon or an indirect effect *via* the activation of the cholinergic pathway. This is supported by the observation that octopamine and tyramine, at a concentration of  $5 \times 10^{-4} \text{ mol l}^{-1}$ , have no significant influence on the cyclic AMP content of the axoplasm of squid giant axons (Baker & Carruthers, 1984). It is further supported by our preliminary results on the direct measurement of cyclic AMP levels in the isolated sheath of the giant axon (Table 2). It can be seen that, after a 5-min incubation in the presence of DL-octopamine, the cyclic AMP levels in the isolated sheaths increase by around 100%.

#### *Octopaminergic modulation of cholinergic responses*

Octopamine functions as a neuromodulator in a number of invertebrate preparations (Kravitz *et al.* 1976; Evans & O'Shea, 1977; O'Shea & Evans, 1979; Kobayashi & Hasimoto, 1982). We therefore examined its effects on the hyperpolarization of the squid Schwann cell membrane potential produced by activation of the nicotinic cholinergic receptors. The submaximal hyperpolarizing effects of 1-min pulses of  $10^{-7} \text{ mol l}^{-1}$  carbachol are potentiated in both amplitude and time course when given in the presence of subthreshold doses ( $10^{-8} \text{ mol l}^{-1}$ ) of DL-octopamine (Fig. 9A). The cholinergic system of the squid Schwann cell can also be activated by acetylcholine released from the Schwann cell in response to an unidentified chemical signal released by the giant axon during neural activity (see Villegas, 1981, 1984). The prolonged hyperpolarization induced in the Schwann cell upon stimulation of the giant axon is also potentiated in the presence of a subthreshold dose ( $10^{-8} \text{ mol l}^{-1}$ ) of DL-octopamine (Fig. 9B).

### DISCUSSION

#### *Pharmacology of the receptor mediating octopamine responses*

The octopamine-induced long lasting hyperpolarization of the Schwann cell of the squid giant axon is mediated *via* a receptor system that is independent of the nicotinic cholinergic receptor system described previously in this cell (Villegas, 1981,

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Fig. 7. The effects of antagonists on the cholinergic receptors of the Schwann cell. Each point represents the potential difference recorded in a different Schwann cell. (A) The blocking action of  $10^{-6} \text{ mol l}^{-1}$  phentolamine (hatched bar) on the effect of 1-min pulses of  $10^{-6} \text{ mol l}^{-1}$  carbachol (filled bars). (B) The blocking action of  $10^{-6} \text{ mol l}^{-1}$  phentolamine (hatched bar) on the hyperpolarization of the Schwann cell caused by stimulation of the giant axon for 1-min periods at 100 Hz. (C) The blocking action of  $10^{-6} \text{ mol l}^{-1}$  metoclopramide (hatched bar), on the effects of 1-min pulses of  $10^{-6} \text{ mol l}^{-1}$  DL-octopamine (filled bars) and  $10^{-6} \text{ mol l}^{-1}$  carbachol (open bars).

1984). The octopamine receptors on the Schwann cell have many similarities, and some differences, with the octopamine receptors described previously in insects (Harmar & Horn, 1977; Nathanson, 1979; Evans, 1981), in crustaceans (Battelle & Kravitz, 1978) and in other molluscs (Dougan & Wade, 1978*a,b*; Batta, Walker & Woodruff, 1979).

The octopamine receptors on the squid Schwann cell are relatively stereospecific for the D(-) isomer of octopamine, as are all other octopamine receptors studied. In

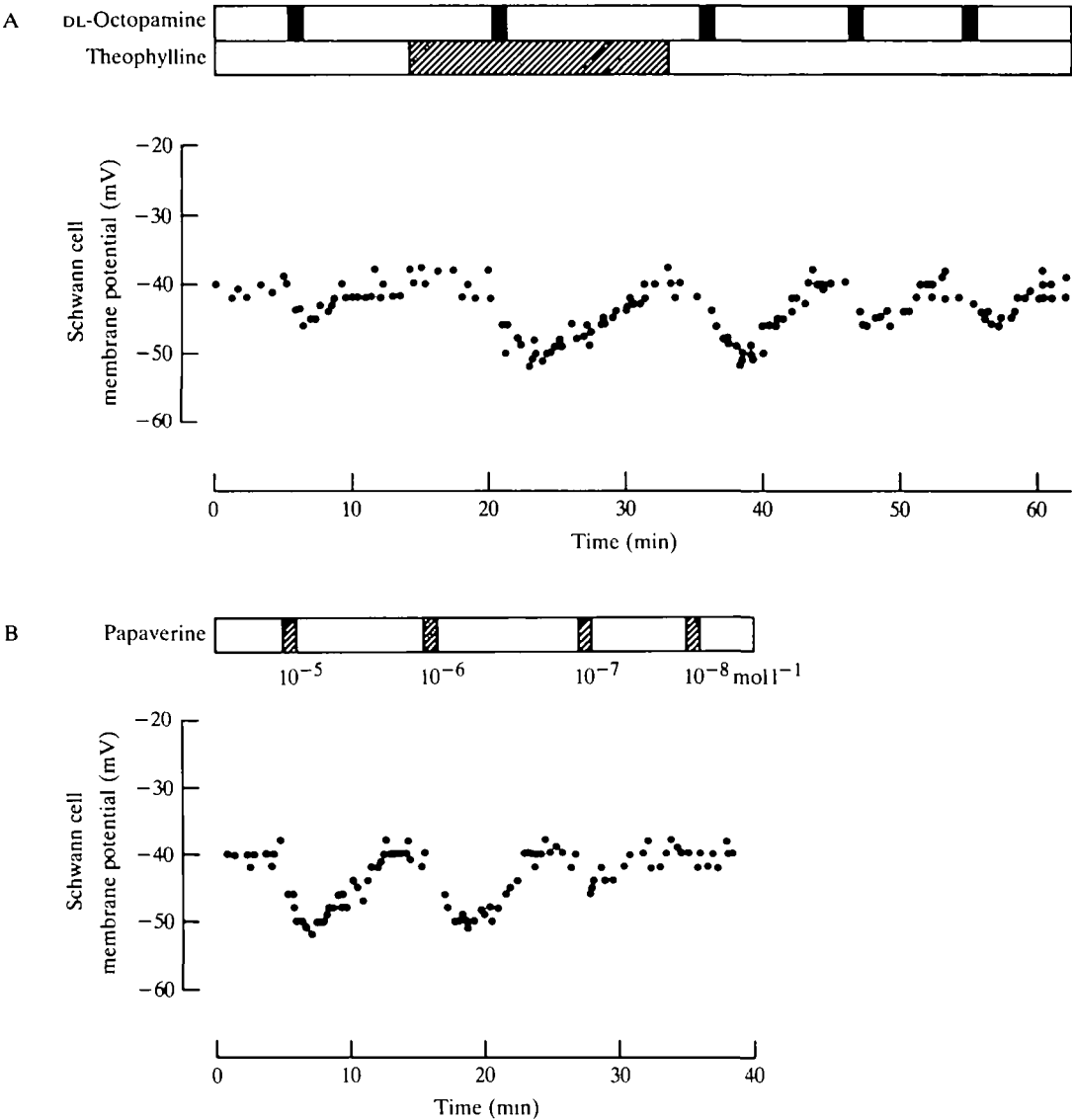


Fig. 8. For legend see facing page

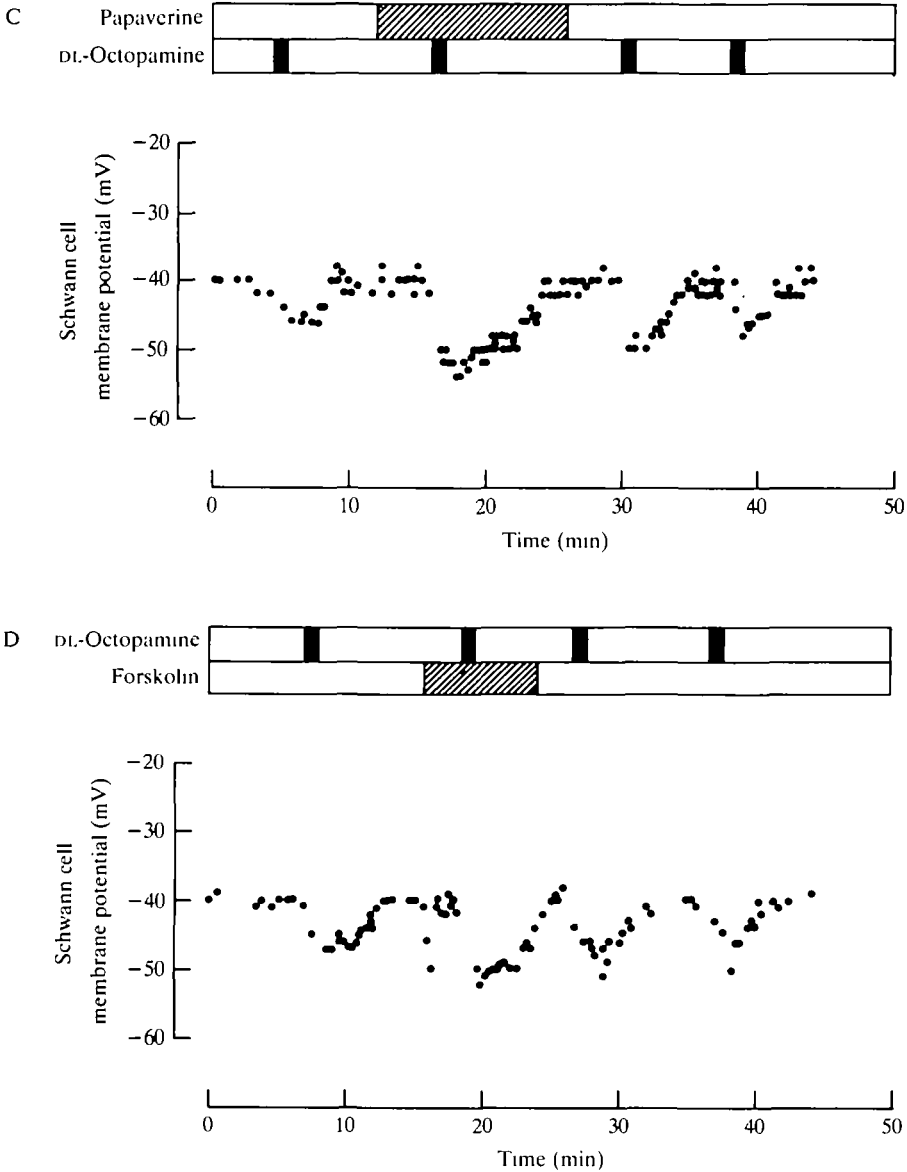


Fig. 8. Studies on the mode of action of octopamine on the membrane potential of the Schwann cell. Each point represents the potential difference recorded in a different Schwann cell. (A) The potentiating effect of  $10^{-8} \text{ mol l}^{-1}$  theophylline (hatched bar) on the effects of 1-min pulses of  $10^{-6} \text{ mol l}^{-1}$  DL-octopamine (filled bars). (B) The effect of 1-min pulses of papaverine at various concentrations (hatched bar). (C) Continuation of experiment shown in B, to show the potentiating effect of  $10^{-8} \text{ mol l}^{-1}$  papaverine (hatched bar) on the effects of 1-min pulses of  $10^{-6} \text{ mol l}^{-1}$  DL-octopamine (filled bars). (D) The potentiating effect of  $2.5 \times 10^{-8} \text{ mol l}^{-1}$  forskolin (hatched bar) on the effects of 1-min pulses of  $10^{-6} \text{ mol l}^{-1}$  DL-octopamine.

fact D(−) octopamine, which is the naturally occurring isomer of octopamine in another cephalopod mollusc, the octopus (Erspamer, 1952), was the most potent compound we tested on the squid receptors. It was almost three times more effective than the L(+) isomer of octopamine and about 50% more effective than the DL isomeric mixture. In other preparations the relative potency ratio of the D(−) isomer to the L(+) isomer varies considerably, ranging from a value of 2 to exceed a value of 200 (Harmar & Horn, 1977; Evans, 1981, 1985).

Amine specificity studies indicate that the squid Schwann cell octopamine receptors are maximally sensitive to phenolamines rather than phenylamines or catecholamines. Unusually, however, compared with the majority of other octopamine receptors studied, synephrine, the *N*-methylated analogue of octopamine, was not an effective agonist whilst tyramine was equally as effective as DL-octopamine. In most intact cell systems synephrine is either more potent or equipotent to octopamine (see Evans, 1985), whilst in most tissue homogenates used for studies on phenolamine-activated adenylate cyclase octopamine is more potent than synephrine (Harmar & Horn, 1977; Nathanson, 1979). Another receptor system in which tyramine is equally effective with octopamine and both are much more effective than synephrine is the high affinity uptake system for octopamine in the cockroach nerve cord (Evans, 1978). However, it is very difficult to see how such an uptake system itself could explain the results obtained in the present study on the squid. In the only other study of the effects of octopamine on glial cell function (Schofield & Treherne, 1985) octopamine was about twice as effective as synephrine in changing the permeability of the insect perineurial blood brain barrier. At present only one other octopamine system has been described where synephrine has no apparent effect. This is the excitation-induced hypertrehalosemic response of the cockroach where injections of octopamine increase haemolymph levels of trehalose whereas injections of synephrine are not effective (Downer, 1979). It should be remembered, however, that the rank order of potency of the three phenolamines in any intact tissue will reflect differences in their ease of access and also in their rate of inactivation. Nonetheless,

Table 2. *Cyclic AMP content of isolated sheaths from squid giant nerve fibre*

Cyclic AMP content (pmol mg <sup>-1</sup> protein)		
Controls	(1)	3.7
	(2)	2.9
	(3)	1.2
	(4)	2.8
	(5)	2.2
	(6)	3.5
Mean ± S.E. = 2.7 ± 0.4		
10 <sup>-5</sup> mol l <sup>-1</sup> DL-Octopamine	(1)	5.2
	(2)	4.6
	(3)	6.1
Mean ± S.E. = 5.3 ± 0.4		

The controls were incubated for 5 min in artificial sea water and the experimental nerves were incubated for 5 min in artificial sea water containing 10<sup>-5</sup> mol l<sup>-1</sup> DL-octopamine. All solutions were aerated during the incubation with a mixture of 95% O<sub>2</sub> and 5% CO<sub>2</sub>.

the pharmacological similarities of the squid receptors with other octopamine receptors (see below), the fact that they are stereospecific for the D(-) isomer of octopamine and the presence of octopamine in squid stellate nerves (Juorio &

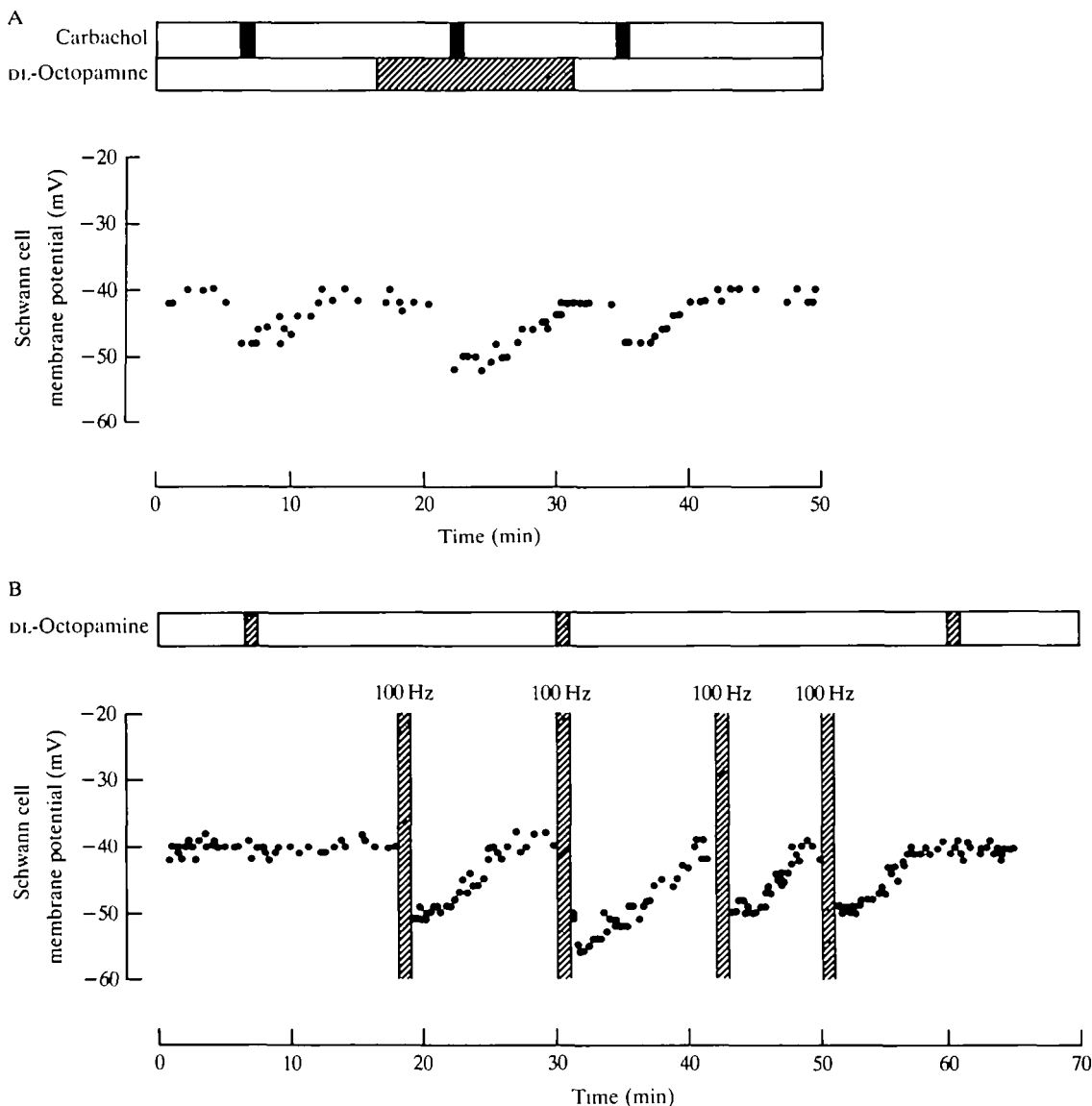


Fig. 9. Octopaminergic modulation of cholinergic responses of Schwann cell membrane potential. Each point represents the potential difference recorded in a different Schwann cell. (A) The effect of  $10^{-8} \text{ mol l}^{-1}$  DL-octopamine (hatched bar) on the effect of 1-min pulses of  $10^{-7} \text{ mol l}^{-1}$  carbachol (filled bars). (B) The effect of a 1-min pulse of  $10^{-8} \text{ mol l}^{-1}$  DL-octopamine (hatched bar) on the hyperpolarizing response of the Schwann cell membrane potential to stimulation of the giant axon at 100 Hz for 1-min periods.

Molinoff, 1974) combine to suggest that octopamine, rather than tyramine, is probably the natural activator of these receptors in the intact animal.

Multiple classes of octopamine receptor, which can be pharmacologically distinguished from one another, have been described in the locust extensor tibiae muscle (Evans, 1981). In the squid Schwann cell, studies with a range of agonists and antagonists indicate that the octopamine receptors have similarities with the OCTOPAMINE<sub>2</sub> subclass (Evans, 1981). Thus metoclopramide is a better blocking agent than phentolamine, and naphazoline is a better agonist than clonidine on these receptors.

The observation that the cholinergic receptors on the squid Schwann cell are also blocked by phentolamine is also unusual. These cholinergic receptors are classed as nicotinic since they are blocked by  $\alpha$ -bungarotoxin and curare, and since nicotine is a more effective agonist than muscarine (Villegas, 1975). However they are different from classical nicotinic cholinergic receptors in many respects. Their activation induces a hyperpolarization as a result of an increased potassium conductance (see Villegas, 1981, 1984) rather than a depolarization due to an increased sodium conductance. In addition, their activation also appears to stimulate a mechanism that increases cyclic AMP levels (Evans *et al.* 1985). Both these features are usually associated with muscarinic rather than nicotinic acetylcholine receptors in many other neuronal and muscle preparations. The blocking of the squid cholinergic receptors by the  $\alpha$ -adrenergic blocking agent phentolamine may indicate that these receptors also have some similarities with classical muscarinic receptors, since monoclonal antibodies have detected a possible structural homology between muscarinic receptors and  $\alpha_1$ -adrenergic receptors (Venter, Eddy, Hall & Fraser, 1984). In addition, phentolamine and other 2-substituted imidazolines are known to have some direct effects on a variety of vertebrate muscarinic receptors (see Weiner, 1980).

#### *Mode of action of octopamine receptors*

The octopamine receptors on the Schwann cell of the squid appear to mediate their actions by increasing the intracellular levels of cyclic AMP. The actions of octopamine are potentiated by the phosphodiesterase inhibitors theophylline and papaverine, presumably by slowing down the rate at which the receptor-activated increase in cyclic AMP levels is metabolized. In addition, the diterpene compound forskolin, which increases the sensitivity of the adenylate cyclase complex to receptor activation (Seamon & Daly, 1981; Daly, 1984; Seamon & Wetzel, 1984), also potentiates the actions of octopamine by increasing the amount of cyclic AMP generated by a given dose of octopamine. The above conclusion is supported by the fact that changes in the intracellular level of cyclic AMP by mechanisms that bypass receptor activation, and also the application of cyclic AMP analogues, can induce a hyperpolarization of the Schwann cell membrane potential (Evans *et al.* 1985). In addition, preliminary measurements reveal that cyclic AMP levels in isolated glial sheaths from squid axons can be increased after incubation in the presence of octopamine. The conclusion that octopamine directly increases intracellular levels of

cyclic AMP within the Schwann cells and not by an action on the giant axon is supported by the findings of Baker & Carruthers (1982, 1984) that octopamine, at concentrations up to  $5 \times 10^{-4} \text{ mol l}^{-1}$ , does not increase the cyclic AMP levels of extruded axoplasm from the giant axon of the squid, *Loligo forbesi*. Further, the actions of octopamine are not mediated indirectly *via* an activation of the cholinergic system, since they are not blocked by  $\alpha$ -bungarotoxin.

The role of cyclic AMP in mediating the long-term, receptor-induced hyperpolarization of the squid Schwann cells has many parallels with other systems. Thus, cyclic AMP changes can alter ion channel permeability in many preparations (see Siegelbaum & Tsien, 1983). In addition many biogenic amines can increase cyclic AMP levels in cultures of vertebrate glial cells (see Van Calcar & Hamprecht, 1980; Rougon *et al.* 1983). The only other known action of octopamine on a glial cell is its ability to reduce the potassium permeability of the glia forming the insect blood brain barrier, although its mode of action in that preparation has not yet been determined (Schofield & Treherne, 1985).

The actions of octopamine in a variety of other preparations are also mediated *via* an increase in cyclic AMP levels induced by receptors with similar pharmacological properties to those of the squid Schwann cell. Thus octopamine receptors increase cyclic AMP levels to produce a long-term modulatory effect on neuromuscular transmission and muscle contraction in insect skeletal muscle (Evans, 1984*a,b*), to produce light production in the light organ of the firefly (Nathanson & Hunnicutt, 1979; Nathanson, 1979) and to produce a variety of physiological effects in lobster haemolymph, heart and exoskeletal muscle (Battelle & Kravitz, 1978). In addition OCTOPAMINE<sub>2</sub> subclass receptors probably also increase adenylate cyclase activity in homogenates of locust central nervous system (Morton, 1984) and of *Drosophila* heads (Uzzan & Dudai, 1982).

#### *Octopaminergic modulation of cholinergic function*

Modulatory actions of octopamine have been described in a number of different invertebrate preparations (Evans & O'Shea, 1977; O'Shea & Evans, 1979; Kravitz *et al.* 1976; Kobayashi & Hasimoto, 1982; Barber, 1982; Orchard & Lange, 1985). In the squid a complex mechanism of interaction occurs between the giant axon and its surrounding sheath of Schwann cells (see Villegas, 1984). The axon releases an unknown chemical message (possibly glutamate, see Villegas, 1984) that stimulates the Schwann cell to release acetylcholine. The acetylcholine feeds back onto the nicotinic cholinergic receptors on the Schwann cell which cause an increase in the cyclic AMP levels in the Schwann cell (Evans *et al.* 1985). It is likely that the use of a cyclic AMP second messenger system in this response pathway amplifies the original chemical signal released by the giant axon so that the Schwann cell can produce a coordinated biochemical and physiological response. This cholinergic system is potentiated by low doses of octopamine which by themselves have no direct action on the membrane potential of the Schwann cell. The mechanism of this potentiation is not clear at the present time since both the cholinergic and octopaminergic systems appear to mediate their actions *via* increased levels of cyclic AMP. Usually two

receptor systems using the same second messenger system do not potentiate one another. An exception to this does, however, occur in cultured astrocytes from the rat cortex where both noradrenaline and vasoactive intestinal peptide increase cyclic AMP levels when applied alone but produce a synergistic effect when applied together (Rougon *et al.* 1983).

At present the source of the octopamine in the intact squid responsible for the modulation of the Schwann cell membrane potential is not known. The stellate nerve of the squid, which contains the giant axon used in the present studies, has been shown to contain low levels of octopamine in another species of squid, *Loligo vulgaris* (Juorio & Molinoff, 1974). Thus co-activation of the octopaminergic neurones in the stellate nerve and the giant axon could be responsible for this octopaminergic modulation. Alternatively octopamine has been shown to act as a circulatory neuro-hormone in both crustaceans (see Kravitz *et al.* 1976, 1980, 1984) and insects (see Evans, 1980; Orchard, 1982). In the locust, octopamine levels in the haemolymph have been shown to increase in response to various stressful circumstances (Orchard, Loughton & Webb, 1981; Davenport & Evans, 1984*a,b*). Since the giant axon system of the squid is only likely to be used at a high frequency during the escape response of the animal under stressful circumstances, a similar stress-induced increase of octopamine in squid haemolymph would be ideally timed to increase the responsiveness of the squid Schwann cell to its cholinergic activation system.

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