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MULTIPLE RECEPTOR TYPES FOR OCTOPAMINE IN THE LOCUST

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SUMMARY

1. Three different pharmacological classes of octopamine receptor mediate the actions of octopamine on the locust extensor–tibiae neuromuscular preparation. A receptor classification scheme is proposed based on the results of detailed studies with agonists and antagonists.

2. Octopamine$_1$ class receptors mediate the slowing of a myogenic rhythm found in a specialized proximal bundle of muscle fibres. Octopamine$_{2A}$ class receptors mediate the increase in amplitude of slow motoneurone twitch tension and octopamine$_{2B}$ class receptors mediate the increase in relaxation rate of twitch tension induced by firing either the fast or the slow motoneurones.

3. Octopamine$_1$ receptors can be distinguished from the 2A and 2B classes since chlorpromazine (and yohimbine) are much better blocking agents than metoclopramide at the former receptors, whereas the converse is true for the latter class. Also clonidine is a more effective agonist than naphazoline for the former receptors and the converse is true for the latter class.

4. Octopamine$_{2A}$ can be distinguished from octopamine$_{2B}$ receptors since metoclopramide, mianserin and cyproheptadine show a strong preference for blocking the former class. Also naphazoline is a much better agonist than tolazoline at the former receptors and tolazoline is a much better agonist than clonidine at the latter.

5. The results are discussed in terms of the location of the various classes of octopamine receptors, their possible relationship to vertebrate α-adrenoreceptors, and the significance of the results for studies on octopamine receptors in the vertebrate central nervous system.

INTRODUCTION

Octopamine is now recognized as an important biogenic amine in arthropods where it fulfils many of the functions of adrenaline and noradrenaline in vertebrates. It has been shown to act as a circulatory neurohormone in crustaceans (Evans, Kravitz, Talamo & Wallace, 1976; Evans, Kravitz & Talamo, 1976; Kravitz, Battelle, Evans, Talamo & Wallace, 1976) and in insects (Candy, 1978; Goosey & Candy, 1980). In insects it also acts as a neuromodulator or local neurohormone, modulating the effectiveness of neuromuscular transmission (Evans & O'Shea, 1977, 1978; O'Shea & Evans, 1979; Buchan & Evans, 1980). A true neurotransmitter role for octopamine is yet to be proven, but suggestive evidence has been provided from the firefly light

organ (Carlson, 1968; Robertson & Carlson, 1976; Nathanson, 1979). Furthermore the role of octopamine in the central nervous tissues of insects has yet to be established (see Evans, 1980*a*).

To date evidence for the role of octopamine in vertebrates is scarce (Hicks, 1977; Hicks & McLennan, 1978*a*, *b*; Dao & Walker, 1980). This seems due largely to the difficulties involved in distinguishing between the effects of noradrenaline and octopamine. Undoubtedly the development of specific antagonists and agonists for octopamine receptors, that would distinguish them from other aminergic receptors, would greatly facilitate such studies.

In the present paper the pharmacological profiles of the receptors mediating three specific responses to octopamine in the extensor–tibiae neuromuscular preparation from the hind leg of the locust are described. This preparation has the advantage of possessing an identified octopaminergic neurone (Evans & O'Shea, 1977, 1978) designated DUMETi (Dorsal Unpaired Median cell to Extensor–Tibiae muscle) with a cell body located in one of the central ganglia of the insect nervous system (Hoyle, Dagan, Moberly & Colquhoun, 1974). The axon of this neurone bifurcates in the ganglion to give symmetrical processes which project peripherally, one to the extensor–tibiae muscle of the left hind leg and the other to the corresponding muscle in the right hind leg. The branches of this neurone in the muscle form 'blindly ending neurosecretory terminals' rather than specific neuromuscular junctions (Hoyle *et al.* 1974). This contrasts with the situation for the processes of the three identified central motoneurones to each extensor muscle (Pearson & Bergman, 1969; Hoyle & Burrows, 1973) which form typical neuromuscular junctions with the muscle fibres they innervate. Thus the effects of stimulating the identified octopaminergic neurone can be compared with the effects of exogenous application of octopamine and other potential agonists and antagonists. The three octopamine responses that have been studied are (1) the inhibition of a myogenic rhythm of contraction and relaxation located exclusively in a proximal bundle of muscle fibres in the extensor–tibiae muscle (Hoyle, 1975; Evans & O'Shea, 1978), (2) the potentiation in the amplitude and (3) the increase in the rate of relaxation of twitch tension induced in the muscle by firing the slow motoneurone to the extensor–tibiae (SETi) (Evans & O'Shea, 1977; O'Shea & Evans, 1979). Responses (2) and (3) represent the summed responses of all the muscle fibres of the extensor–tibiae that are innervated by the SETi motoneurone (see Hoyle, 1978 for description of SETi innervation pattern). The results indicate the presence of multiple types of octopamine receptors, as has been described for other aminergic receptors (see Berridge, 1980; and also Snyder & Goodman, 1980). The different classes of octopamine receptor have many pharmacological properties in common but none the less can be distinguished on the basis of differences in their responses to specific agonists and antagonists. A brief account of some of this work has already been published (Evans, 1980*b*).

METHODS

Experiments were performed at room temperature (21 °C) on adult *Schistocerca americana gregaria* (formerly *S. gregaria*) of either sex. The locusts were obtained from crowded laboratory cultures fed on wheat seedlings. Small batches of animals were left for 1–2 h before use after removal from the main culture.

Tension in the extensor–tibiae muscle of a hind leg was measured almost isometrically by means of a tension transducer attached to the distal apodeme. Octopamine responses of the myogenic rhythm and of the twitch tension induced by firing the slow motoneurone were measured as described previously (Evans & O'Shea, 1978; O'Shea & Evans, 1979). An operational amplifier signal differentiator was used to measure continuously the rates of contraction and relaxation of neurally evoked tension (Buchan & Evans, 1980).

Miniature end-plate potentials (m.e.p.p.s) were recorded intracellularly from extensor–tibiae muscle fibres using glass micro-electrodes filled with either 2M-K acetate or 3M-KCl which had DC resistances in saline of 15–30 MΩ. The majority of recordings were made from the muscle fibres of the distally located accessory extensor bundle which are innervated only by the slow motoneurone (SETi) and the common inhibitor (CI) (Hoyle, 1978; Evans & O'Shea, 1978). Recordings were also made from slow fibres of the 'fan' region, likewise innervated only by SETi and CI, and from fibres exclusively innervated by the fast motoneurone from 'region *b*' of the muscle (nomenclature of Hoyle, 1978).

DUMETi was stimulated antidromically by a pair of hook electrodes placed on the contralateral extensor–tibiae nerve ($5b_1$, nomenclature of Pringle, 1939). This technique allows the initiation of a higher frequency of spikes in the DUMETi axons than can be induced by passing depolarizing current into the DUMETi soma through a recording electrode (O'Shea & Evans, 1979). The motoneurones of the extensor–tibiae muscle were excited by stimulating the peripheral roots containing their axons with pairs of hook electrodes (nerve 3*b* for SETi and nerve 5 for the fast motoneurone, FETi) (O'Shea & Evans, 1979).

All drugs were dissolved in physiological isotonic saline (pH 6·8) containing 140 mM-NaCl, 10 mM-KCl, 4 mM-$CaCl_2$, 4 mM-$NaHCO_3$, 6 mM-NaH_2PO_4 (Usherwood & Grundfest, 1965) plus 90 mM-sucrose.

Drugs were superfused directly onto the surface of the extensor–tibiae muscle, except in the experiments where antagonists were used to block the action of DUMETi. In the latter case continuous superfusion appeared to prevent the demonstration of consistent effects to repeated bursts of DUMETi stimulation. Thus in this experiment 50 μl saline was applied to the surface of the extensor muscle and replaced by appropriate solutions during the course of the experiment (see Results for details).

Locusts extensor–tibiae neuromuscular preparations invariably respond to the introduction of a pulse of octopamine into the superfusate with increase in the amplitude and relaxation rate of SETi-induced twitch tension (O'Shea & Evans, 1979). During the course of the present study it was noticed that occasionally preparations either exhibited no change in these parameters at all, or gave responses of much reduced magnitude than usual to a standard pulse of DL-octopamine. In such cases it was always observed that the rate of relaxation and amplitude of twitch tension were initially very high, in the range normally only observed during the application of octopamine. It was found that these 'initially potentiated' preparations could be converted to 'normal' preparations by superfusing the muscle with 10^{-6} M-phenotolamine for 10 min. Once the phentolamine was washed off, the preparation showed octopamine sensitivity. If other animals from the same batch of 'initially potentiated' locusts were allowed to remain undisturbed for a period of 1–2 h before testing, they again exhibited 'normal' responses to octopamine. The 'initially potentiated' locusts were most often observed when taken from cages in which the animals had consumed all the food provided and had become hyperactive. Under such conditions it seems that an endogenous activator of the octopamine receptors, probably octopamine itself, is released, which results in a potentiation of the amplitude and relaxation rate of SETi-induced twitch tension. It is thus important in the study of octopamine responses to ensure that the animals used have not been subjected to any 'stressful' conditions which might interfere with measurements of the response to a particular stimulus regime. In the rest of this paper only results from animals that were initially not potentiated are reported.

I would like to acknowledge the gift of samples of the following drugs from pharmaceutical companies: clonidine HCl, tramazoline HCl, trimizolin HCl, L-Adrianol, WB101 (Boehringer Ingelheim); tolazoline HCl, naphazoline HCl, phentolamine mesylate (CIBA); promethazine HCl, chlorpromazine HCl (May & Baker): *cis*(*z*)-flupenthixol 2HCl, *trans*(*E*)-flupenthixol 2HCl (Lundbeck); methoxamine HCl (Burroughs Wellcome); ergometrine hydrogenmaleinate, dihydroergocryptine mesilate, clozapine (Sandoz); isoprenaline sulphate (Riker); cyproheptadine HCl (Merck, Sharp & Dohme); azapetine sulphate, chlordiazepoxide HCl (Roche); phenoxybenzamine HCl

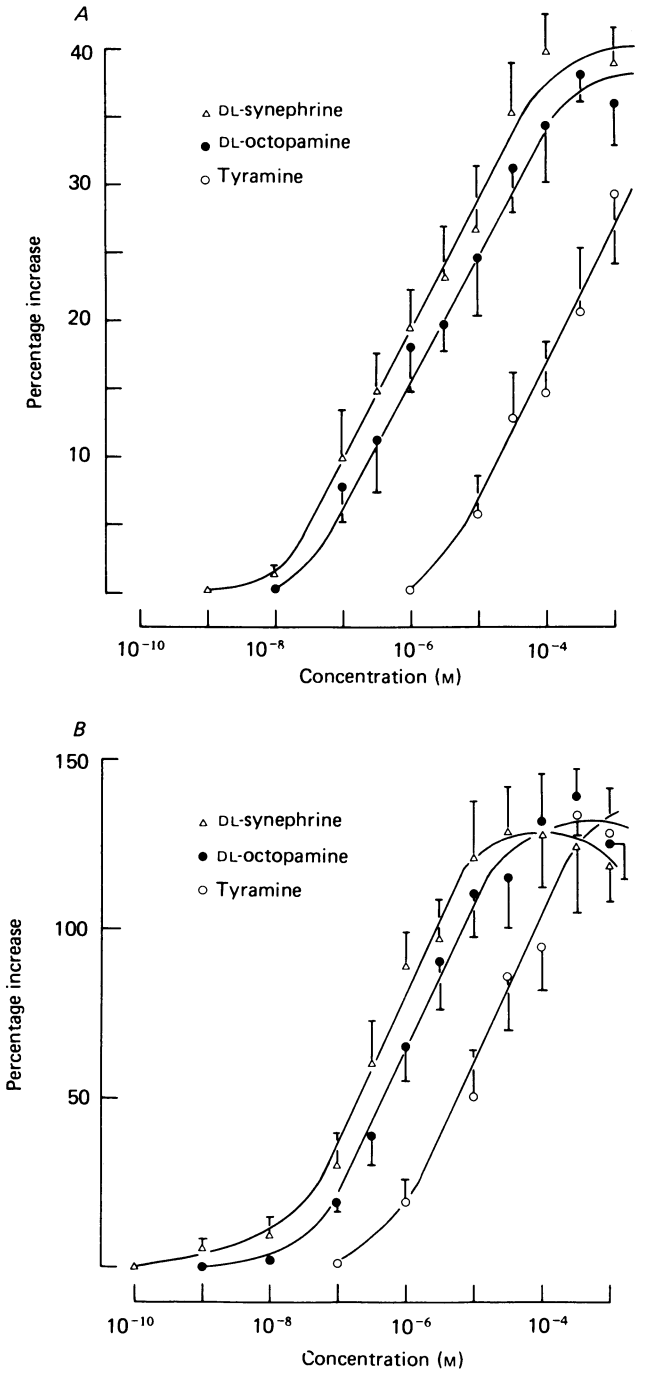


Fig. 1. Dose–response curves for the actions of the phenolamines on slow motoneurone (SETi) neuromuscular transmission in the extensor-tibiae muscle of the locust hindleg. *A*, maximal effects on twitch amplitude, *B*, maximal effects on the relaxation rate of twitch tension for different amine concentrations. SETi was fired at a frequency of 1 Hz and each of the amines was introduced into the superfusate for a period of 30 s. Each point represents the mean of at least four determinations and the bars represent standard errors.

(Smith, Kline & French); prazosin HCl (Pfizer); metoclopramide HCl (Beecham); fluphenazine HCl (Squibb); mianserin HCl (Organon). I would also like to thank Dr M. D. Armstrong for his kind gift of samples of D(−)- and L(+)-octopamine. All other drugs were obtained from Sigma Chemical Co.

RESULTS

(*1*) *Amine specificity of responses*

The receptors mediating the slowing of the myogenic rhythm exhibit a specificity for monophenolic amines such as octopamine and synephrine, the *N*-methylated analogue of octopamine (Evans & O'Shea, 1978). At a concentration of 10^{-6} M the latter two biogenic amines are the only ones reported, from a wide range of related compounds, to be capable of potentiating the amplitude and relaxation rate of SETi-induced twitch tension (O'Shea & Evans, 1979). A more detailed study of the dose dependency of the latter two effects is shown in Fig. 1 for synephrine and octopamine, and also for tyramine, the non-β-hydroxylated precursor of octopamine. The increases in amplitude and rate of relaxation of twitch tension are dose-dependent for each of the three amines, the sensitivity decreasing in the order synephrine, octopamine, tyramine, In additivity experiments (not shown) concentrations of octopamine and tyramine which alone gave maximal responses were not additive when given together. Similar results were obtained when the additivity of synephrine and octopamine responses were examined. These findings, together with the parallel dose–response curves and similar maximal responses are consistent with the idea that the three amines are likely to be acting at the same receptor sites.

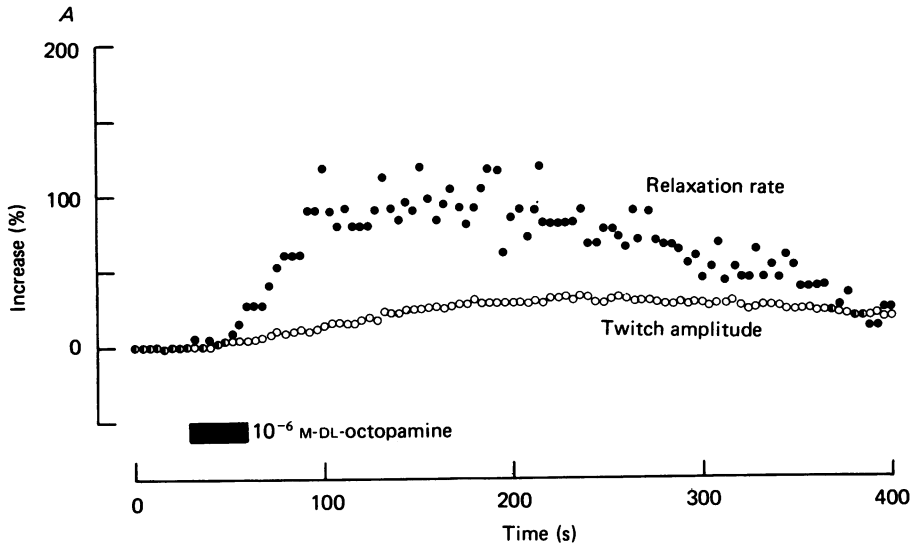
A previous report (O'Shea & Evans, 1979) suggested that the indolalkylamine, 5-hydroxytryptamine (5-HT) had no effects on SETi-induced twitch tension at a concentration of 10^{-6} M. However in the present study, higher concentrations of 5-HT (in the range 10^{-5}–10^{-4} M) were applied to the preparation and an increased rate of relaxation of twitch tension was observed, with an 80% maximal increase. Under these circumstances the response to 5-HT (10^{-5} M) could not be blocked by gramine (10^{-5} M), a selective 5-HT blocker, but was antagonized by phentolamine (10^{-5} M), an α-adrenoreceptor blocking agent that also blocks octopamine receptors (see below). This suggests that at high concentrations 5-HT may be acting as a partial agonist of the octopamine receptors mediating the increased rate of relaxation of twitch tension. The converse effect has also been shown to occur in the extensor–tibiae muscle, since high concentrations of octopamine will activate the 5-HT receptors responsible for increasing the frequency of the myogenic rhythm found in the fibres of the specialized proximal muscle bundle (Evans & O'Shea, 1978).

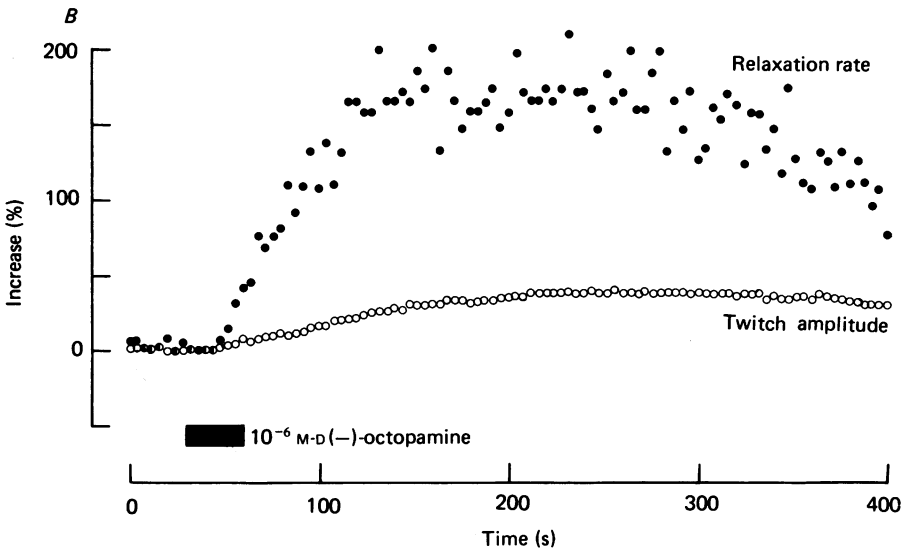
(*2*) *Stereospecificity of responses*

Octopamine exists in two stereoisometric forms, L(+) and D(−), the latter being the naturally occurring isomer in the locust (Goosey & Candy, 1980). The stereospecificity of the octopamine-mediated potentiation of the amplitude and the relaxation rate of SETi-induced twitch tension was investigated in an experiment in which a preparation was successively exposed to pulses (30s at 10^{-6} M) of the DL (Fig. 2*A*), the D(−) (Fig. 2*B*) and the L(+) (Fig. 2*C*) isomers of octopamine. The D(−) isomer is more potent than the L(+) at potentiating both parameters, and the responses to the D(−) isomer are also slightly greater than the corresponding ones to the control

pulse of the DL-isomeric mixture. Further experiments (not shown) indicate that the exact magnitude of the responses obtained in such an experiment depend on the order of presentation of the different isomers. The application of either a pulse of D(−)-or DL-octopamine immediately after exposure of the preparation to a pulse of L(+)-octopamine on many occasions leads to an increase in the sizes of responses to the former isomers. This latter effect may be due to a non-stereospecific interaction of the octopamine isomers with an inactivation mechanism.

The receptors mediating the slowing of the myogenic rhythm in the extensor muscle are also stereospecific for the naturally occurring D(−) isomer of octopamine. D(−)-octopamine (10^{-7}M) caused a $65{\cdot}8 \pm 3{\cdot}6\,\%$ ($n = 4$) reduction in the frequency of the myogenic rhythm during a 5 min exposure whilst an equivalent pulse of





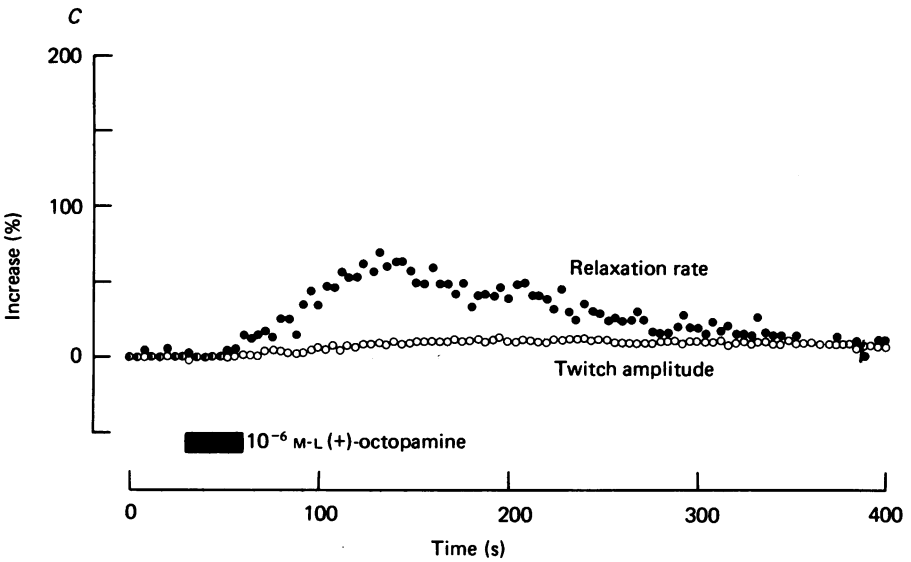


Fig. 2. Stereospecificity of effects of octopamine on the amplitude of twitch tension and rate of relaxation of twitches generated by the stimulation of the SETi motoneurone at 1 Hz. *A*, a 30 s pulse of 10^{-6}M-DL-octopamine. *B*, a 30 s pulse of 10^{-6}M-D(−)-octopamine *C*, a 30 s pulse of 10^{-6}M-L(+)-octopamine. All data points are normalized to the initial value prior to the pulse of the octopamine isomer for comparison and represent results obtained from a single preparation with a 15 min wash period between the application of the different isomers.

TABLE 1. Action of agonists on myogenic rhythm

Drug	Effect at 10^{-7} M (%) ± S.E.	EC_{50}
Clonidine	100	$6{\cdot}8 \times 10^{-10}$
Tolazoline	100	$1{\cdot}5 \times 10^{-9}$
Tramazolin	100	$1{\cdot}7 \times 10^{-9}$
L-Adrianol	79·2 ± 8·6	$1{\cdot}0 \times 10^{-8}$
Naphazoline	83·0 ± 6·8	$1{\cdot}2 \times 10^{-8}$
DL-octopamine	75·7 ± 6·2	$1{\cdot}5 \times 10^{-8}$
Methoxamine	80·3 ± 0·6	$1{\cdot}7 \times 10^{-8}$
L-phenylephrine	57·2 ± 3·0	$5{\cdot}0 \times 10^{-8}$
Dihydroergocryptine	60·3 ± 3·6	$5{\cdot}5 \times 10^{-8}$
Dihydroergotamine	56·6 ± 9·5	$6{\cdot}0 \times 10^{-8}$
Ergotamine	46·7 ± 6·6	$1{\cdot}2 \times 10^{-7}$
Ergometrine	41·4 ± 7·5	$2{\cdot}4 \times 10^{-7}$
Ergocryptine	41·7 ± 0·7	$3{\cdot}1 \times 10^{-7}$
Isoprenaline	0	—

Results are expressed as the mean reduction in frequency ± S.E. (n = at least 3) caused by the application of a 5 min pulse of each drug at a concentration of 10^{-7} M in the presence of 10^{-6}M-gramine. EC_{50} is the concentration required to reduce the frequency of the rhythm by 50 % and was obtained from log dose–response curves.

L(+)-octopamine caused only a reduction of 28·2 ± 5·6 % ($n = 4$). In the same batch of locusts DL-octopamine (10^{-7} M) caused a 65·6 ± 5·2 % ($n = 4$) reduction in frequency. In these experiments gramine (10^{-6} M) was added to each preparation superfusate to prevent any interference from the stimulation of the accelerating receptors on the myogenic bundle (see Evans & O'Shea, 1978).

(3) Action of agonists at octopamine receptors

To characterize the receptors mediating the actions of octopamine on the myogenic rhythm and on twitch tension a wide range of potential agonists have been applied to the extensor–tibiae muscle preparations.

(a) Effects on receptors slowing myogenic rhythm

The effectiveness of a range of agonists was determined from dose–response curves. The compounds are listed in Table 1 in the order of their concentration required to produce a 50 % reduction in the frequency of the myogenic rhythm. Drugs such as clonidine, naphazoline, and methoxamine which are effective α-adrenoreceptor agonists in vertebrates, are also effective agonists of the locust octopamine receptors. An unusual feature of the latter receptor is that tolazoline is a very effective agonist, whilst it is usually considered to be an antagonist of most α-receptors in vertebrates. However, a recent study shows that tolazoline also has agonist activity on the post-synaptic α-adrenoreceptors of the smooth muscle of the cat nictitating membrane (Arbilla & Langer, 1978). The responses of the ergot alkaloids were also different from their effects on α-adrenoreceptors in vertebrates since they all acted as weak agonists of the octopamine receptors, despite the fact that dihydroergocryptine is generally regarded as a potent α-adrenergic antagonist. However, Williams, Mullikin & Lefkowitz (1976) have shown that the latter drug has both agonist and antagonist properties at α-adrenergic receptors in uterine smooth muscle. By contrast isoprenaline, a β-adrenergic agonist, was ineffective at slowing the myogenic rhythm at concentrations up to 10^{-5} M.

(b) Effects on receptors that modulate SETi twitch tension

The effects of a range of agonists in potentiating the amplitude and the relaxation rate of SETi-induced twitch tension are shown in Table 2 for a drug concentration of 10^{-5} M. The EC_{50} values given represent the concentration of each drug required to produce 50 % of the maximal response to octopamine and were obtained from dose–response curves prepared for each drug.

In general the same drugs are effective in producing these responses as are effective agonists of the receptors slowing the myogenic rhythm. There are, however, a few differences in the rank order of potency of the drugs, with clonidine being less effective than naphazoline at the receptors potentiating twitch tension and the converse being true for the receptors on the myogenic bundle. Methoxamine, dihydroergocryptine and isoprenaline did not potentiate either parameter of twitch tension up to a concentration of 10^{-4} M, which contrasts with the agonistic actions of the two former compounds on the myogenic bundle octopamine receptors.

A comparison of the results in Table 2 with those in Table 1 indicates that the receptors mediating the increase in relaxation rate and the increase in amplitude of

twitch tension show more similarities to each other in their pharmacological profiles than either of them do to that of the receptors mediating the slowing of the myogenic rhythm. The two former receptor types can however be distinguished by a comparison of their EC_{50} ratios for each drug. This ratio indicates that the effective agonists fall into three classes. Tolazoline and tramazolin appear to be relativey better agonists of the increases in relaxation rate than of the increases in the amplitude of twitch tension. Naphazoline on the other hand is an example of a drug where the inverse is true. The ratios for clonidine and L-phenylephrine are much closer to the unity value of octopamine itself, indicating that they are equally effective agonists at both sites.

TABLE 2. Action of agonists on SETi twitch tension

	Amplitude		Relaxation rate		Ratio $\frac{EC_{50}\text{ amplitude}}{EC_{50}\text{ relaxation}}$
Drug	% increase at 10^{-5}M	EC_{50}	% increase at 10^{-5}M	EC_{50}	
Naphazoline	58·0 ± 2·7	$1{\cdot}3 \times 10^{-8}$	125·0 ± 11·0	$2{\cdot}2 \times 10^{-7}$	0·06
Tolazoline	25·0 ± 1·2	$3{\cdot}2 \times 10^{-6}$	111·8 ± 4·6	$6{\cdot}0 \times 10^{-7}$	5·33
DL-octopamine	24·5 ± 4·2	$3{\cdot}3 \times 10^{-6}$	110·2 ± 11·0	$2{\cdot}0 \times 10^{-6}$	1·65
Tramazolin	22·0 ± 5·7	$6{\cdot}0 \times 10^{-6}$	105·4 ± 10·2	$9{\cdot}0 \times 10^{-7}$	6·67
Clonidine	22·0 ± 3·9	$6{\cdot}4 \times 10^{-6}$	67·6 ± 10·4	$2{\cdot}0 \times 10^{-5}$	0·32
L-phenylephrine	19·0 ± 3·0	$1{\cdot}3 \times 10^{-5}$	55·8 ± 4·5	$6{\cdot}0 \times 10^{-5}$	0·22
Methoxamine	0	—	0	—	—
Isoprenaline	0	—	0	—	—
Dihydroergocryptine	0	—	0	—	—

Results are expressed as the mean percentage increase ± S.E. (n = at least 5) in the amplitude and relaxation rate of SETi twitch tension when 30s pulses of each of the drugs are introduced into the superfusate of the extensor tibiae muscle. EC_{50} is the concentration of the drug required to produce 50% of the maximal response obtained to octopamine. SETi was fired at a frequency of 1 Hz.

(4) *Action of antagonists at octopamine receptors*

The response profiles of the octopamine receptors have been further analysed in experiments where a range of antagonists have been used to block the octopamine responses.

(a) *Effects on receptors slowing myogenic rhythm*

The relative effectiveness of a series of drugs in blocking the actions of a 5 min pulse of 10^{-7} M-DL-octopamine on the myogenic rhythm is shown in Table 3. The experiments were performed in the presence of 10^{-6} M-gramine to prevent any interference from the activation of the 5-HT receptors that increase the frequency of the rhythm. A 2 min pre-wash in the drug was included in the superfusate before the test pulse of octopamine. The results are expressed in the order of their EC_{50} concentration, which represents the concentration of a drug required to reduce the response to 10^{-7} M-DL-octopamine by 50%. The EC_{50} concentration was obtained from dose–response curves.

The α- adrenergic blocking agents such as WB4101 and phentolamine were the most potent antagonists tested and had very similar EC_{50} values. In addition the phenothiazine derivatives, promethazine and chlorpromazine, were effective blocking

agents, as were cyproheptadine and the dibenzodiazepine derivative, clozapine. The benzodiazepine derivative, chlordiazepoxide, however, was without effect at concentrations up to 10^{-5} M. Other α-adrenergic antagonists such as yohimbine and phenoxybenzamine were effective blockers, but prazosin and mianserin were much weaker blockers. The β-adrenergic blocking agents, such as propranolol and dichloroisoproterenol had weak and insignificant blocking activities respectively. Similarly the antipsychotic drugs, flupenthixol and fluphenazine, that block dopamine receptors, were relatively poor antagonists of the octopamine receptors. Metoclopramide, another drug that blocks some dopamine receptors (Jenner, Marsden & Peringer, 1975; Kehr & Debus, 1979; Dougan & Wade, 1978*a*) and has also been reported to block some molluscan octopamine receptors (Dougan & Wade, 1978*a*), was ineffective at blocking the octopamine responses of the myogenic rhythm. Further, at concentrations above 10^{-5} M it appears to be a partial agonist of the octopamine receptors mediating these responses.

TABLE 3. Action of antagonists on octopamine responses of myogenic rhythm

Drug	% decrease in response at 10^{-6}M	EC_{50}
WB4101	100	$9{\cdot}0 \times 10^{-10}$
Phentolamine	100	$1{\cdot}9 \times 10^{-9}$
Promethazine	100	$5{\cdot}0 \times 10^{-9}$
Chlorpromazine	100	$2{\cdot}6 \times 10^{-8}$
Cyproheptadine	87·9 ± 9·2	$3{\cdot}7 \times 10^{-8}$
Azapetine	57·2 ± 3·3	$1{\cdot}1 \times 10^{-7}$
Clozapine	79·8 ± 5·2	$2{\cdot}4 \times 10^{-7}$
Yohimbine	77·5 ± 6·2	$2{\cdot}8 \times 10^{-7}$
DL-propranolol	63·5 ± 12·7	$5{\cdot}4 \times 10^{-7}$
Phenoxybenzamine	84·1 ± 2·2	$5{\cdot}8 \times 10^{-7}$
Trimazolin	52·5 ± 11·1	$8{\cdot}5 \times 10^{-7}$
Cis(z)-flupenthixol	37·8 ± 3·6	$2{\cdot}7 \times 10^{-6}$
Prazosin	43·7 ± 2·9	$4{\cdot}4 \times 10^{-6}$
Mianserin	0	$4{\cdot}5 \times 10^{-6}$
Fluphenazine	10·2 ± 3·2	$7{\cdot}1 \times 10^{-6}$
Trans (E)-flupenthixol	3·9 ± 1·7	—
Dichloroisoproterenol	0	—
Metoclopramide	0	—

The results are expressed as the percentage reduction in response ± S.E. ($n = 3$) to a 5 min pulse of 10^{-7} M-DL-octopamine induced by the presence of the drug at a concentration of 10^{-6} M. Gramine was present in the superfusate at a concentration of 10^{-6} M throughout the experiment. The drugs were introduced into the superfusate for a 2 min pre-wash period before the addition of the octopamine, in addition to their presence during the octopamine pulse itself. EC_{50} is the concentration of the drug required to reduce the response to 10^{-7} M-DL-octopamine by 50%.

(*b*) *Effects on receptors that modulate SETi twitch tension*

In general the same drugs inhibit the actions of octopamine on SETi twitch tension (see Table 4) and its actions on the myogenic rhythm. There are, however, three striking differences between the sets of results. First, metoclopramide is a very potent antagonist of the octopamine receptors modulating both the amplitude and the relaxation rate of SETi twitch tension. This contrasts with its lack of effect on

TABLE 4. Action of antagonists on octopamine potentiation of SETi twitch tension

	Amplitude		Relaxation rate		EC_{50} amplitude
Drug	Blocking effect at 10^{-5} M (%)	EC_{50}	Blocking effect at 10^{-5} M (%)	EC_{50}	EC_{50} relaxation
Metoclopramide	79·3 ± 4·3	$1{\cdot}0 \times 10^{-6}$	51·1 ± 7·6	$9{\cdot}5 \times 10^{-6}$	0·11
Mianserin	100	$1{\cdot}2 \times 10^{-6}$	43·0 ± 8·6	$2{\cdot}0 \times 10^{-5}$	0·06
Phentolamine	62·6 ± 6·3	$1{\cdot}4 \times 10^{-6}$	63·5 ± 4·2	$3{\cdot}9 \times 10^{-6}$	0·36
Cyproheptadine	61·6 ± 6·6	$2{\cdot}2 \times 10^{-6}$	13·1 ± 3·9	$5{\cdot}1 \times 10^{-5}$	0·04
Clozapine	65·8 ± 1·1	$5{\cdot}0 \times 10^{-6}$	28·2 ± 5·4	$3{\cdot}8 \times 10^{-5}$	0·13
Phenoxybenzamine	22·0 ± 3·2	$5{\cdot}9 \times 10^{-5}$	20·0 ± 2·1	$3{\cdot}4 \times 10^{-4}$	0·17
WB4101	29·7 ± 4·9	$1{\cdot}3 \times 10^{-4}$	33·0 ± 3·0	$8{\cdot}9 \times 10^{-5}$	1·46
Chlorpromazine	28·6 ± 4·3	$1{\cdot}6 \times 10^{-4}$	27·0 ± 2·9	$7{\cdot}0 \times 10^{-5}$	2·29
DL-propranolol	25·6 ± 5·2	$2{\cdot}9 \times 10^{-4}$	11·0 ± 3·0	$4{\cdot}0 \times 10^{-4}$	0·73
Prazosin	0	—	11·8 ± 2·1	$> 5{\cdot}0 \times 10^{-4}$	—
Yohimbine	0	—	0	—	—

Results are expressed as the percentage reduction in response ± S.E. to a standard 30 s pulse of 10^{-6} M-DL-octopamine caused by the presence of the blocking agents at a concentration of 10^{-5} M. EC_{50} is the concentration of each drug required to reduce the response by 50% and was obtained from log dose–response curves. Each blocking drug was applied for a 1 min pre-wash period as well as during the octopamine pulse itself. SETi was fired at a frequency of 1 Hz. $n = 4$ except for prazosin and yohimbine where $n = 3$.

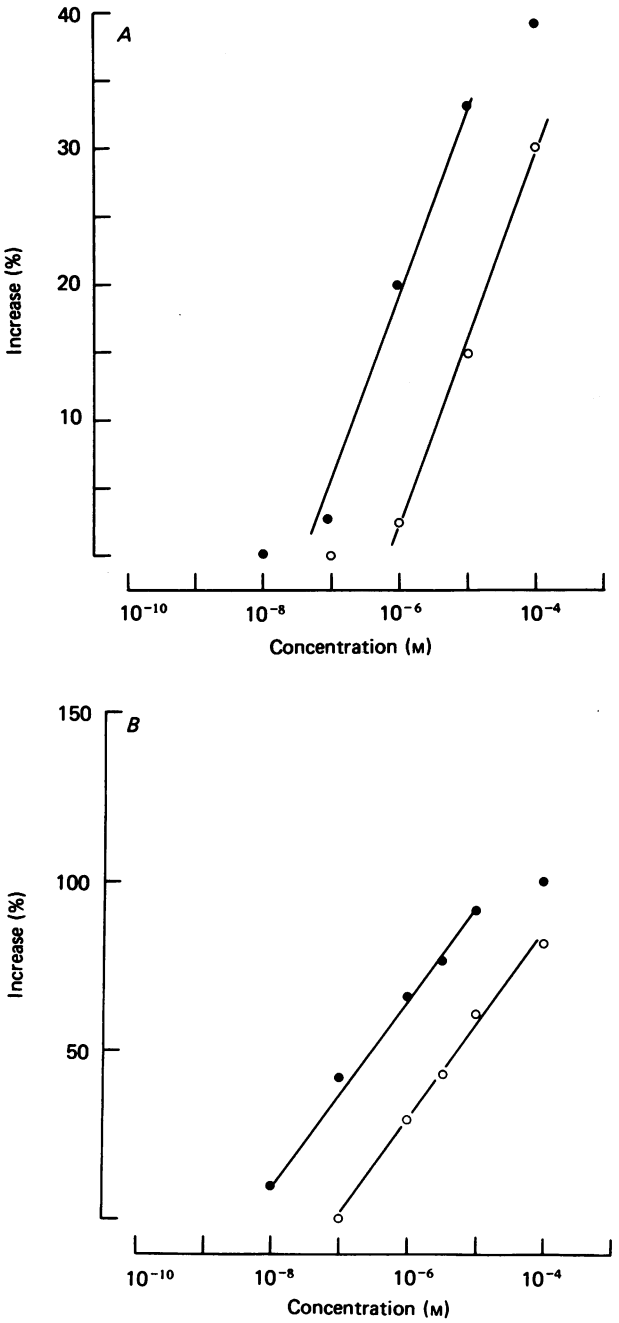


Fig. 3. Dose–response curves for the actions of DL-octopamine on the amplitude (*A*) and relaxation rate of twitch tension (*B*) induced by firing SETi at a frequency of 1 Hz, in the presence (○) and absence (●) of 10^{-5}M-phentolamine. The plots show the result obtained on a typical preparation where 30s pulses of increasing concentrations of octopamine were applied with a 10 min wash period between pulses.

the myogenic rhythm responses. Secondly, mianserin, a poor blocking agent of the myogenic rhythm responses, is a very effective blocker of the actions of octopamine on SETi twitch tension. Thirdly, yohimbine, an effective blocker of the myogenic rhythm responses, has no blocking effect on the actions of octopamine on SETi twitch tension. In general α-adrenergic blocking agents are again more potent than β-adrenergic blocking agents at antagonizing the actions of octopamine on SETi twitch tension. Phentolamine, however, is a more potent blocker than WB4101 whereas both appear to be almost equally potent blockers of the effects of octopamine on the myogenic rhythm.

The blocking actions of the antagonists are competitive with octopamine since, as shown for phentolamine in Fig. 3, the presence of the drug shifts the dose–response curve for octopamine to the right without significantly altering its slope. The affinity constant, K, of this inhibitor for the receptors can be obtained by matching responses before and during its application (Bowser-Riley, House & Smith, 1978). Under such conditions the equipotent dose ratio X, is given by $X-1 = K[I]$ where I is the concentration of the antagonist (Schild, 1949). For the example shown in Fig. 3 where $X = 14$ and the phentolamine concentration used was 10^{-5} M, $K = 1{\cdot}4\ \mu M^{-1}$ for both the antagonism of the receptors causing increase in relaxation rate and those causing the increase in twitch amplitude. Estimates of the maximal rates of increase in relaxation rate and of increase in twitch amplitude were obtained from plots of V against V/S for the data shown in Fig. 3. The maximal rates calculated were identical in the presence and absence of 10^{-5} M-phentolamine for both the increase in twitch amplitude ($V_{max} = 38{\cdot}2\%$) and the increae in relaxation rate ($V_{max} = 105{\cdot}2\%$).

An estimate of the relative effectiveness of the different compounds on the increase in twitch amplitude and increase in relaxation rate responses can be obtained by a comparison of the EC_{50} ratios for each of the drugs. The antagonists fall into three classes: first, those with ratios much less than unity, such as cyproheptadine and mianserin, that are more effective blockers of the action of octopamine on the increase in amplitude than on the increase in relaxation rate of twitch tension; secondly those such as WB4101 and chlorpromazine where the converse is true; thirdly, the remaining drugs tested which fall into a class of intermediate selectivity.

Thus the studies with antagonists again indicate that the receptors mediating the responses to octopamine in the modulation of the amplitude and relaxation rate of SETi twitch tension are more similar to each other than to the receptors mediating the slowing effects of octopamine on the myogenic rhythm. However, the receptors mediating the increased amplitude of twitch tension and increased rate of relaxation exhibit a differential sensitivity to a range of blocking agents.

(*5*) *Action of agonists and antagonists of octopamine on spontaneous m.e.p.p.s*

Octopamine can also increase the frequency but not the amplitude of m.e.p.p.s recorded in muscle fibres innervated by SETi and this response can be blocked by the α-adrenergic blocking agent, phentolamine, but not by the β-adrenergic blocking agent, propranolol (O'Shea & Evans, 1979). These findings suggest the presence of presynaptic octopamine receptors on the terminals of the SETi motoneurone which may account for the octopamine-mediated increase in the amplitude of SETi-induced twitch tension. The pharmacological profile of these presynaptic receptors has been further examined.

An example of the effect of a 30s pulse of 10^{-6} M-DL-octopamine on the m.e.p.p. frequency recorded in a muscle fibre from the accessory extensor bundle, which is only innervated by SETi and the common inhibitor, is shown in Fig. 4. The octopamine pulse gives a maximum increase in the m.e.p.p. frequency of 35·0% (Fig. 4*A*) (average increase at this concentration is $26{\cdot}6 \pm 2{\cdot}99\%$, $n = 21$) but has no significant effect on the amplitude distribution of the m.e.p.p.s in this fibre (Fig. 4*B* and *C*). Similar recordings from muscle fibres of the fan region of the extensor–tibiae,

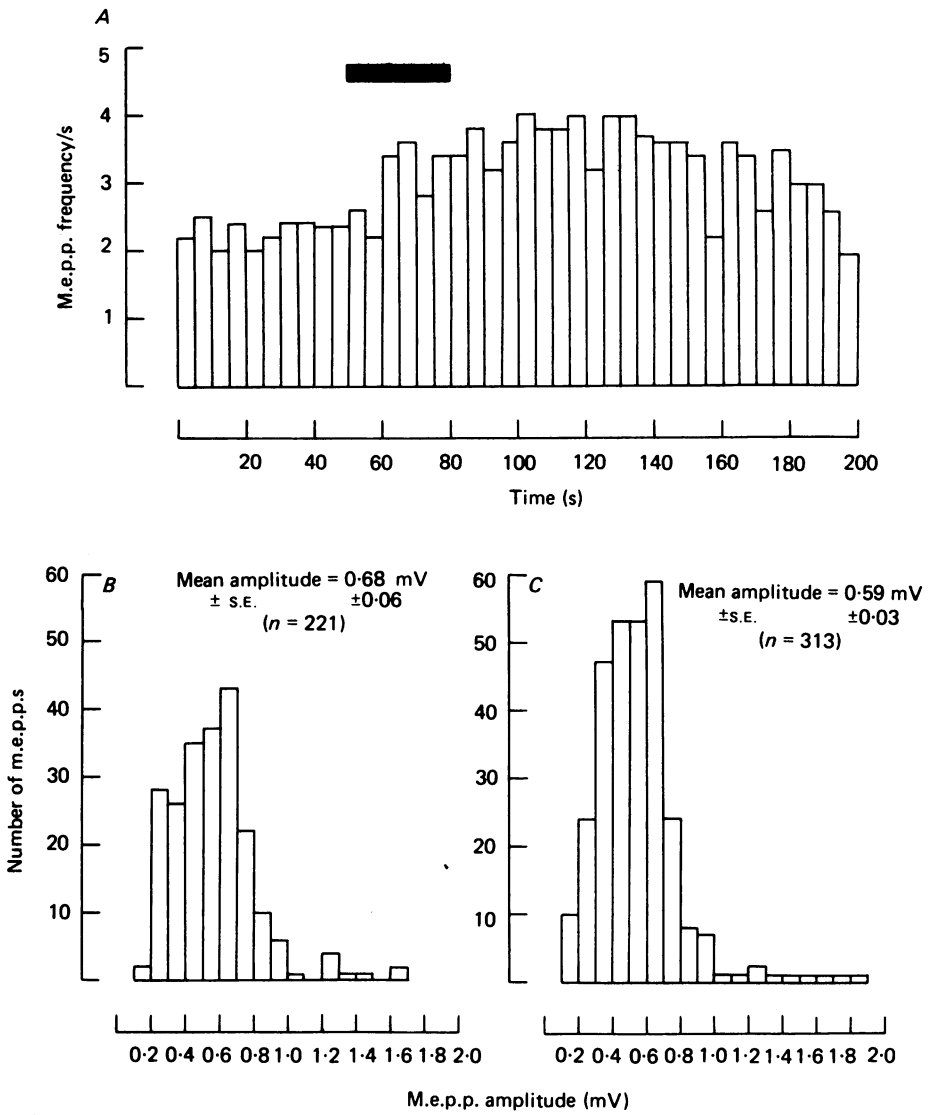


Fig. 4. Effect of 10^{-6}M-DL-octopamine (black bars) on the spontaneous release of neurotransmitter from the terminals of the SETi motoneurone to slow distal fibre of the extensor–tibiae muscle. *A*, a plot of the frequency of m.e.p.p.s (mean frequency per second of 5 consecutive seconds) against time. *B* and *C*, respectively, amplitude histograms of m.e.p.p.s for periods of 1·5 min before and after application of the octopamine pulse.

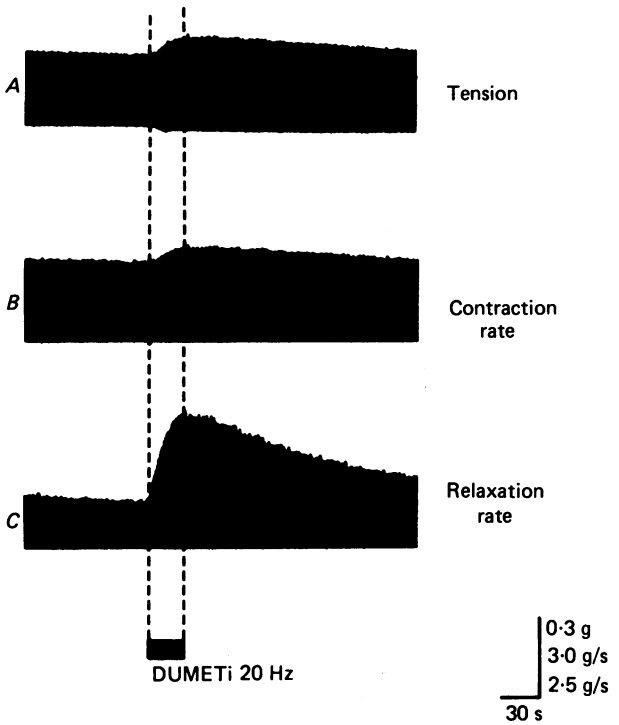


Fig. 5. Effect of firing DUMETi at 20 Hz for 30 s (black bar) on SETi neuromuscular transmission. SETi was fired at 1 Hz. Trace *A* shows effect on amplitude of twitch tension. Traces *B* and *C* show effects on rates of contraction and relaxation of twitch tension respectively. Note the similarity in the time course and magnitude of the response in traces *A* and *B*.

TABLE 5. Effect of drugs on octopamine-mediated increase in SETi m.e.p.p. frequency

(*a*) Agonists

Drug	Increase in frequency (%)
Naphazoline	11·5±0·25
Tolazoline	10·9±3·5
Clonidine	9·5±0·7

(*b*) Antagonists

Drug	Inhibition (%)
Metoclopramide	100
Phentolamine	83·0±7·9
Cyproheptadine	49·4±6·4
Chlorpromazine	0
WB4101	0

(*a*) The results are expressed as the percentage increase in m.e.p.p. frequency ± S.E. ($n = 3$) produced by the application of a 30 s pulse of the drug at a concentration of 10^{-5} M.

(*b*) Results expressed as the percentage inhibition ± S.E. ($n = 3$) of the response to a 30 s pulse of 10^{-6} M-DL-octopamine by the presence of the drugs at a concentration of 10^{-5} M in the superfusate. Each antagonist was applied to the preparation for a 1 min pre-wash prior to its joint application with the octopamine pulse. All results (*a* and *b*) were obtained by comparing the m.e.p.p. frequency in a 90 s period before octopamine, or other agonist application, with that of a similar period after the end of the agonist application.

which also receive only a slow excitatory innervation (Hoyle, 1978), also demonstrate a similar increase in m.e.p.p. frequency with no change in m.e.p.p. amplitude when superfused with octopamine. Fibres innervated solely by the fast excitatory axon (e.g. those in region 'b' of the muscle; Hoyle, 1978) did not show any significant increase in frequency or amplitude of m.e.p.p.s in the presence of octopamine at concentrations up to 10^{-5} M. Thus the octopamine-mediated increase in m.e.p.p. frequency appears to be a specific property of the slow motoneurone terminals.

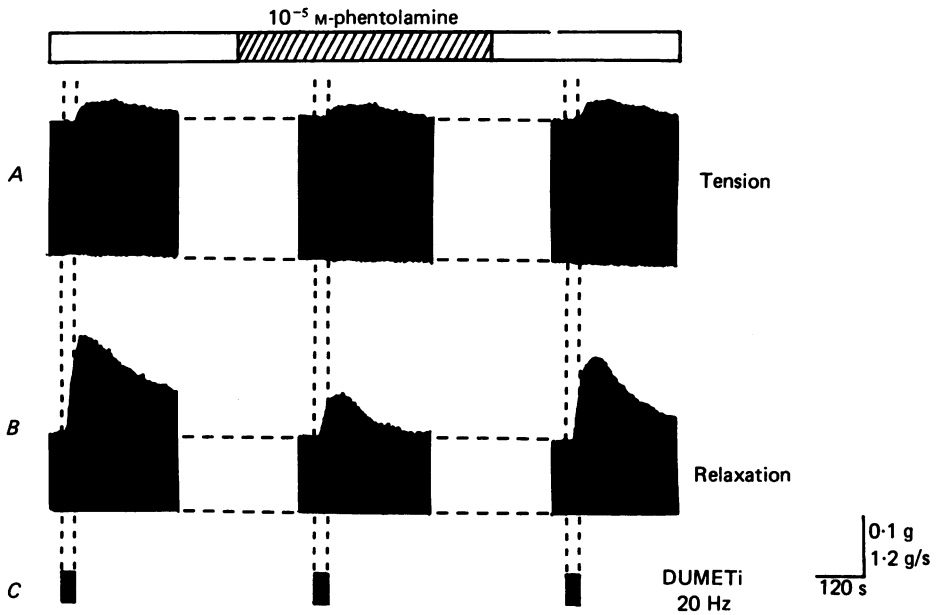


Fig. 6. Blocking action of phentolamine (10^{-5} M) (hatched bar) on the effects of firing DUMETi at 20 Hz for 30 s (black bar) on amplitude (trace *A*) and relaxation rate (trace *B*) of SETi-induced twitch tension. SETi fired at 1 Hz. The first effects of DUMETi stimulation were obtained during the addition of a 50 μl aliquot of isotonic saline to the exposed surface of the extensor–tibiae muscle. The saline was then replaced by an equal volume of saline containing 10^{-5}M-phentolamine and allowed to stand for 5 min before obtaining the second set of responses to DUMETi stimulation, which were reduced in magnitude. The phentolamine-containing saline was replaced by three changes of fresh saline during a 5 min wash period, after which time a third set of responses to DUMETi stimulation was obtained.

The effects of a series of selected agonists and antagonists on the octopamine-mediated increase in SETi m.e.p.p. frequency are shown in Table 5. At a concentration of 10^{-5} M the rank order of effectiveness of the agonists is naphazoline > tolazoline > clonidine, which is the same as for their effects on the increase in amplitude of SETi twitch tension. The rank order of the antagonists is metoclopramide > phentolamine > cyproheptadine, which is again the same as for their effects on the increase in amplitude of SETi twitch tension. The receptors mediating the actions of octopamine on the increase in SETi m.e.p.p. frequency appear to be very similar pharmacologically to those mediating the octopamine-induced increase in SETi twitch amplitude.

(6) Effects of antagonists on the actions of DUMETi

One of the criteria that needs to be established before a particular compound can be accepted as the transmitter of an identified neurone is that the same antagonists should block the actions of the exogenously applied transmitter as block the effects of stimulating the identified neurone. The effects of stimulating the DUMETi neurone at 20 Hz for 30 s on the amplitude of SETi-induced twitch tension and its rates of contraction and relaxation are shown in Fig. 5. In a similar fashion to the effects of octopamine application (O'Shea & Evans, 1979; Buchan & Evans, 1980) DUMETi increases twitch amplitude and the contraction rate by similar magnitudes and over parallel time courses, whilst the effect on relaxation rate is much more pronounced and has a shorter latency and time to peak. Phentolamine, a potent blocking agent for the effects of exogenously applied octopamine, can also effectively reduce the effects induced by DUMETi stimulation in a reversible manner (Fig. 6). The effects of phentolamine are dose-dependent. Similar results can be obtained by using WB4101, cyproheptadine or metoclopramide instead of phentolamine. Thus the same drugs that block the effects of exogenously applied octopamine also block the actions of stimulating the identified octopamine-containing neurone, a finding which is consistent with the conclusion that octopamine is the neuromodulatory compound released from the terminals of the DUMETi neurone.

DISCUSSION

General properties of octopamine receptors

The octopamine receptors in the locust extensor–tibiae neuromuscular preparation have many similarities with octopamine receptors from other insects (Harmer & Horn, 1977; Nathanson, 1979), from crustaceans (Battelle & Kravitz, 1978) and from molluscs (Dougan & Wade, 1978*a*, *b*; Batta, Walker & Woodruff, 1979).

The octopamine receptors in the locust preparation are stereospecific for the D(−) isomer, as are all other octopamine receptors studied (Kravitz *et al.* 1976; Harmer & Horn, 1977; Battelle & Kravitz, 1978; Dougan & Wade, 1978*a*, *b*). However, the relative potency ratio of the D(−) isomer to the L(+) isomer varies considerably from one experimental preparation to the next. It ranges from a value of between 2 and 10 in the present study on locust receptors, to exceed a value of 200 in cockroach brain adenylate cyclase assays (Harmer & Horn, 1977). DL-octopamine is not significantly less potent than the D(−) isomer (Harmer & Horn, 1977; Battelle & Kravitz, 1978; present study) and the latter has been shown to be the naturally occurring form of octopamine in the octopus (Erspamer, 1952) and the locust (Goosey & Candy, 1980).

Octopamine receptors are maximally stimulated by phenolamine derivatives rather than phenylamines or catecholamines. Synephrine, the *N*-methylated analogue of octopamine, is a very potent agonist of all octopamine receptors examined. In the locust, dose–response curves reveal that it is consistently more potent than octopamine itself, confirming earlier observations at selected concentrations (Evans & O'Shea, 1978; O'Shea & Evans, 1979). Similarly, synephrine is more potent than octopamine at inducing light production in firefly light organs (Carlson, 1968), in stimulating

adenylate cyclase activity and in decreasing the clotting time of lobster haemocytes (Battelle & Kravitz, 1978). In contrast, the potency of these two phenolamines is reversed in the stimulation of octopamine-sensitive adenylate cyclase activity in homogenates of cockroach brain (Harmer & Horn, 1977) and of firefly lanterns (Nathanson, 1979). Thus, the increased potency of synephrine with respect to octopamine appears to be a property restricted to responses obtained by the application of the amines to intact cellular preparations. This raises the problem faced in all pharmacological studies on intact preparations, namely that different drugs may have a differential access to the receptor sites or may be inactivated at different rates by enzymes or specific uptake systems. The latter possibility was suggested (Evans, 1980*b*) as a possible explanation for the reversed potency of octopamine and synephrine in certain insect preparations, since *N*-methylated biogenic amines are taken up less readily than their non-methylated precursors by a high-affinity uptake system for biogenic amines in cockroach nerve cord (Evans, 1978). However, preliminary experiments with desimipramine (10^{-6} M), a potent inhibitor of the high-affinity uptake of octopamine (Evans, 1978), show that it does not increase the responses of the locust extensor–tibiae neuromuscular preparation to octopamine or change its potency ratio to synephrine. Further, at higher concentrations (10^{-5}M and above) desimipramine acts as a competitive inhibitor of the octopamine receptors and reduces the responses to both octopamine and synephrine (P. D. Evans, unpublished). These results suggest that inactivation by a high-affinity uptake mechanism is unlikely to be interfering with the pharmacological profiles of the octopamine receptors in the present study. The possibility of a differential enzymatic breakdown of octopamine and synephrine has not yet been examined.

Octopamine receptors are selectively blocked by the same drugs that block α-adrenoreceptors in vertebrates. In the present study, the previously described blocking actions of phentolamine, an α-adrenergic blocking agent, on octopamine responses in the locust extensor–tibiae muscle (Evans & O'Shea, 1978; O'Shea & Evans, 1979) have been shown to be competitive in nature, with an affinity constant of 1·4 μM^{-1}. As pointed out by Bowser-Riley *et al.* (1978) values in this range are some 100–1000 times less than is usually found for α-receptors (Furchgott, 1972) but it is in the same range as they observed for the action of phentolamine on the catecholamine receptors in the cockroach salivary gland. Octopamine receptors have also been shown to be blocked by phentolamine in preference to propranolol, a β-adrenergic blocking agent, in a variety of other invertebrate preparations (Harmer & Horn, 1977; Battelle & Kravitz, 1978; Konishi & Kravitz, 1978; Nathanson, 1979; Batta *et al.* 1979). Contrary to these observations Hoyle (1975) states that β-blocking agents block the actions of biogenic amines in slowing the myogenic rhythm in the locust extensor-tibiae muscle, but he does not give the identity of the agents used or their concentrations. The octopamine receptors described in the present study are all blocked by clozapine, a potent α_2-adrenergic antagonist (Berthelsen & Pettinger, 1977) which also blocks octopamine receptors in the heart of the mollusc *Tapes watlingi* (Dougan & Wade, 1978*b*). The results of the present study are in close agreement with the detailed pharmacological profile provided for the antagonism of the octopamine-sensitive adenylate cyclase activity in homogenates of cockroach brain (Harmer & Horn, 1977). It thus appears that octopamine receptors have at least some similarities in certain aspects of their binding sites with vertebrate α-adrenergic receptors.

TABLE 6. A classification of octopamine receptors

Receptor class	Octopamine$_1$	Octopamine$_{2A}$		Octopamine$_{2B}$	
Antagonists	Chlorpromazine (> yohimbine) ≫ metoclopramide	Metoclopramide ≫ chlorpromazine (> yohimbine)			
			EC_{50}		EC_{50}
		Cyproheptadine	2 μM	Cyproheptadine	50 μM
		Mianserin	1 μM	Mianserin	20 μM
		Metoclopramide	1 μM	Metoclopramide	10 μM
Agonists	Clonidine ≫ naphazoline	Naphazoline ≫ clonidine			
		Naphazoline ≫ tolazoline		Tolazoline ≫ clonidine	
Modulatory function in locust	Myogenic rhythm	Amplitude of SETi twitch tension		Rate of relaxation of SETi and FETi twitch tension	
Location	Post-synaptic on muscle fibres	Presynaptic on SETi nerve terminals (?)		Post-synaptic on muscle fibres	

(> represents a difference of a factor of 10, and ≫ a difference of a factor of 50 or more)

Evidence for multiple classes of octopamine receptor

The presence of three distinct classes of octopamine receptor is suggested by the present pharmacological findings in the locust. The three classes of receptor appear to be indistinguishable in terms of their stereospecificity or structure-specificity to biogenic amines, but they can be distinguished by the use of detailed studies on the actions of agonists and antagonists.

It is proposed to designate the octopamine receptors mediating the slowing of the myogenic rhythm octopamine$_1$ class receptors, to distinguish them from the octopamine$_2$ class receptors mediating the modulatory actions of octopamine on SETi-twitch tension. The effects of octopamine on the amplitude and on the relaxation rate of SETi twitch tension are mediated by two pharmacologically distinct classes of receptor which it is proposed to designate octopamine $_{2A}$ and octopamine$_{2B}$ class receptors respectively. Table 6 summarizes the most striking pharmacological differences between the three classes of octopamine receptor which are of use in determining the class of octopamine receptor present in other tissues.

Octopamine$_1$ receptors can be distinguished from the corresponding class 2A and 2B receptors since chlorpromazine (and yohimbine) are much better blocking agents than metoclopramide at the former receptors whereas the converse is true for the latter class of receptors. This distinction can be further reinforced by agonist studies since clonidine is more effective than naphazoline at stimulating octopamine$_1$ receptors and the converse is true for class 2A and 2B receptors.

In the present study the subdivision of the octopamine$_2$ class receptors was achieved by a comparison of the ratio of effective doses of various agonists and antagonists, since some drugs exhibited a preference for one subclass over the other. Thus metoclopramide, mianserin and cyproheptadine show a strong preference for blocking the receptors of the octopamine$_{2A}$ subclass over the receptors of the octopamine$_{2B}$ subclass. A converse but much weaker preference is shown by WB4101 and chlorpromazine for octopamine$_{2B}$ receptors. This distinction can again be reinforced by agonist studies, since naphazoline is a much better agonist than tolazoline at octopamine$_{2A}$ receptors, and tolazoline is a much better agonist than clonidine at octopamine$_{2B}$ receptors.

Thus octopamine receptors on the locust extensor–tibiae neuromuscular preparation can be subdivided into multiple classes which can be distinguished pharmacologically. This parallels the subdivisions of other biogenic amine receptor types (see Berridge, 1980; Snyder & Goodman, 1980). At present not enough detailed pharmacological information is available on the agonist and antagonist profiles of octopamine in other preparations to know how general is the above classification.

Location of different classes of octopamine receptors

In any neuromuscular preparation drug responses may be obtained due to the activation of either pre- or post-synaptic receptors. In the locust extensor–tibiae muscle preparation, the octopamine$_1$ class of receptors mediating the slowing of the myogenic rhythm are located post-synaptically on the muscle fibres of the proximally located myogenic bundle. In addition a post-synaptic location is also found for the octopamine$_{2B}$ class receptors mediating the increased rate of relaxation of twitch

tension. The latter receptors probably have a widespread distribution throughout the extensor muscle since they affect twitch tension generated by both FETi and SETi (O'Shea & Evans, 1979). Further, since the muscle fibres of the myogenic bundle are innervated by SETi (Hoyle, 1978) and contribute to the twitch tension generated by this motoneurone, it is possible that these fibres have a mixture of octopamine$_1$ and octopamine$_{2B}$ receptors spread over their surfaces. Additional studies on the isolated but innervated myogenic bundle (May, Brown & Clements, 1979) are required to resolve this point.

Not so strong an argument can be made for the location of the octopamine$_{2A}$ receptors mediating the increase in SETi twitch-tension amplitude. Twitch amplitude could be increased presynaptically by the release of increased amounts of transmitter or post-synaptically by effects on excitation–contraction coupling. Since the extensor– tibiae muscle possesses a large population of muscle fibres that are dually innervated by the FETi and SETi motoneurones, and since the octopamine induced increase in twitch amplitude (O'Shea & Evans, 1979), together with the increased rate of twitch contraction rate (P. D. Evans, unpublished) is restricted to the SETi motoneurone, this argues for a presynaptic location of the octopamine$_{2A}$ receptors. Equally, the fact that the octopamine-mediated increase in twitch amplitude, and the concomitant increase in contraction rates of twitch tension, are of similar amplitude and time course to each other, but not to those of the increase in relaxation rates of twitch tension, is also consistent with the idea that presynaptic octopamine receptors mediate the increase in SETi twitch amplitude (Buchan & Evans, 1980). Further, the presence of presynaptic octopamine receptors on the terminals of the SETi motoneurone has been demonstrated by the fact that octopamine increases SETi m.e.p.p. frequency but not amplitude (O'Shea & Evans, 1979). In the present paper a preliminary survey of the octopamine receptors mediating the increase in m.e.p.p. frequency shows that they are similar pharmacologically to the octopamine$_{2A}$ receptors. Thus the available evidence suggests that the octopamine-induced potentiation of SETi twitch amplitude is mediated by presynaptic octopamine$_{2A}$ receptors on the terminals of the SETi motoneurone.

Relationship between α-adrenoreceptors and octopamine receptors

Octopamine receptors have many more similarities with vertebrate α-adrenoreceptors than they do with vertebrate β-adrenoreceptors in terms of both antagonist and agonist activities. Octopamine$_1$ receptors are blocked more strongly by phentolamine, clozapine and yohimbine than by phenoxybenzamine and prazosin, indicating a similarity with the α_2-subclass of adrenoreceptors (Berthelson & Pettinger, 1977; Langer, 1980). Octopamine subclass 2A and 2B receptors are also blocked by clozapine but are not blocked by yohimbine. In terms of agonist activity all three classes of octopamine receptor are again similar to vertebrate α_2-adrenoreceptors, since clonidine and tramazolin are more potent than phenylephrine and methoxamine (Berthelsen & Pettinger, 1977).

The parallel between vertbrate α-adrenoreceptors and octopamine receptors does not appear, on the basis of present evidence, to extend to the mode of action of the systems they subsequently activate. In general α-receptors are thought to mediate their actions via changes in calcium permeability, activation of guanylate cyclase

activity and even inhibition of adenylate cyclase activity (see Berridge, 1980), whereas octopamine receptors are reported to increase adenylate cyclase activity (see Evans, 1980*a*, *b*, for references). It remains possible, however, that the different classes of octopamine receptor may mediate their actions through different mechanisms, as has been suggested for multiple forms of other aminergic receptors (Berridge, 1980).

In view of the increasing diversity of α-adrenergic receptor subclasses and the marked parallels between octopamine receptors and α-adrenoreceptors, it seems important to realise that many of the ligands used to study and to isolate vertebrate α-adrenoreceptors may have a poor specificity and also react with octopamine receptors. The two classes of receptor can, however, be distinguished by reference to their relative affinities for phenolamines and catecholamines, the octopamine receptors exhibiting a preference for amines with a single hydroxyl on the aromatic ring and the α-adrenoreceptors exhibiting a preference for amines with two hydroxyl groups on the aromatic ring. Thus in future studies on the isolation and pharmacology of presumed α-adrenoreceptors in vertebrates it will be essential to verify that a single population of receptors is being studied and not a mixture of octopamine receptors and α-adrenoreceptors. The above results suggest that any classification of aminergic receptors should reflect the close relationship between octopamine receptors and α-adrenoreceptors.

Significance for studies on octopamine receptors in vertebrates

The presence of specific octopamine receptors in the vertebrate central nervous system has been implied from experiments where octopamine and noradrenaline have antagonistic actions on neurones in the spinal cord and cortex (Hicks & McLennan, 1978*a*, *b*) and in the thalamus (Dao & Walker, 1980). However, information on the pharmacological characteristics of these vertebrate octopamine receptors is very limited. To date only the actions of a few antagonists have been reported in different preparations. Dao & Walker (1980) report that cyproheptadine antagonizes octopamine actions in the rat thalamus, which is similar to the actions of this drug on octopamine actions in insect preparations (Harmer & Horn, 1977; present study). In contrast Hicks & McLennan (1978*a*) were unable to demonstrate a blocking action of metoclopramide on the octopamine responses they obtained in the rat spinal cord and they conclude that 'drugs which have been reported as (octopamine) antagonists at certain invertebrate syapses are unlikely to be useful in the mammalian central nervous system'. This conclusion, based on a study of a limited number of drugs, in limited areas of the brain, neglects the possibility that multiple classes of octopamine receptor may exist in the vertebrate brain, with a variable distribution of the receptor types in different brain regions. It seems possible that the octopamine receptors described by Hicks & McLennan (1978*a*, *b*) could be similar to the octopamine$_1$ receptors in the locust since neither class of receptor are blocked by metoclopramide and, further, metoclopramide appears to have some agonist activity at each type of receptor.

The information provided in the present study on the pharmacological distinction of three specific classes of octopamine receptor in locusts should be of use in establishing whether a similar classification of octopamine receptors can be made in the vertebrate central nervous system.

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