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THE CHARACTERIZATION OF PRESYNAPTIC OCTOPAMINE RECEPTORS MODULATING OCTOPAMINE RELEASE FROM AN IDENTIFIED NEURONE IN THE LOCUST

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Summary

Octopamine release has been demonstrated from the dorsal unpaired median neurone to the locust extensor-tibiae muscle (DUMETi) in response to high-[K$^+$] saline. Here, we provide evidence for the existence of presynaptic inhibitory autoreceptors for octopamine on the DUMETi terminals and report on their pharmacological profile. Octopamine release was initiated by exposure to high-[K$^+$] saline (0.1 mol l^{-1}) and measured using a radioenzyme assay for octopamine. Octopamine receptor antagonists (10^{-4} mol l^{-1}) potentiated the high-[K$^+$]-mediated release of octopamine with the following rank order of potency: phentolamine = metoclopramide > mianserin = chlorpromazine > cyproheptadine > yohimbine. Octopamine receptor agonists (10^{-4} mol l^{-1}) inhibited the high-[K$^+$]-mediated release of octopamine with the following rank order of potency: naphazoline > tolazoline > clonidine. Thus, the octopamine autoreceptors on the DUMETi terminals are much closer pharmacologically to the pre-and postsynaptic OCTOPAMINE$_2$ receptors in the locust extensor-tibiae muscle preparation than to the OCTOPAMINE$_3$ receptors from the locust central nervous system. The results suggest that there is likely to be more than one type of insect neuronal octopamine receptor. It is also likely that presynaptic modulation of octopamine release may be confined to octopamine receptors since a wide range of other putative modulatory substances did not produce this effect.

Key words: biogenic amine, octopamine, insect, dorsal unpaired median neurone, transmitter release, autoreceptor, locust, *Schistocerca gregaria.*

Introduction

Octopamine is an important neuromodulatory transmitter in both the central and peripheral nervous systems of insects and also functions as a circulating neurohormone in insects (Evans, 1985). Many of the functions for octopamine in insects suggest that it carries out roles equivalent to those of the catecholamines (norepinephrine and epinephrine) released by the sympathetic nervous system of vertebrates. The actions of octopamine in insects are mediated *via* multiple pharmacologically distinct subclasses of octopamine receptor which are coupled to different second messenger systems (Evans, 1981, 1993; Evans and Robb, 1993; Roeder, 1994).

Initial work on the pharmacological characterization of insect octopamine receptor subtypes was carried out on the extensor-tibiae neuromuscular preparation from the hindleg of the locust (see Evans, 1980, 1985). In this preparation, an identified octopaminergic dorsal unpaired median neurone (DUMETi) (Evans and O'Shea, 1977, 1978; Stevenson *et al.* 1992) modulates neuromuscular transmission from the slow motoneurone (SETi) to this muscle (Evans and O'Shea, 1977; O'Shea and Evans, 1979). DUMETi is one of a group of peripherally projecting octopaminergic dorsal unpaired median (DUM) cells (Siegler *et al.* 1991; Stevenson *et al.* 1992) that, together with a group of non-octopaminergic DUM interneurones (Thompson and Siegler, 1991; Stevenson *et al.* 1992), represent the progeny of a single median neuroblast lineage (Goodman and Spitzer, 1979; Campbell *et al.* 1995).

The octopamine receptors in the locust extensor-tibiae preparation were divided into the OCTOPAMINE$_1$ receptors, which modulate a myogenic rhythm of contraction *via* changes in intracellular [Ca^{2+}], and OCTOPAMINE$_{2A}$ and OCTOPAMINE$_{2B}$ receptors, which, respectively, modulate transmitter release from the presynaptic terminals of SETi and modulate the relaxation rate of twitch tension (Evans, 1981). Both types of OCTOPAMINE$_2$ receptor mediate their effects *via* the activation of adenylyl cyclase activity (Evans, 1984*a*,*b*). More recently, it has been suggested that the octopamine receptors that mediate changes in cyclic AMP levels in the locust central nervous system may correspond to a pharmacologically distinct OCTOPAMINE$_3$ subtype (Roeder and Gewecke, 1990; Roeder, 1992, 1995; Roeder and Nathanson, 1993).

Octopamine release has been demonstrated from DUMETi in response to high-[K^+] saline and to neuronal stimulation (Morton and Evans, 1984). Both forms of octopamine release were shown to be sensitive to the concentration of Ca^{2+} in the medium bathing the extensor muscle. In addition, the release of octopamine mediated by DUMETi stimulation was shown to be frequency-dependent. Preliminary evidence has been provided for the existence of presynaptic autoreceptors for octopamine on the terminals of DUMETi (Morton and Evans, 1984). Evidence has also been provided for the presence of octopaminergic autoreceptors on the cell bodies of cockroach DUM cells from the fifth abdominal ganglion, where they modulate ionic currents and spiking frequency (Achenbach *et al.* 1997). Here, we report on the pharmacological characterization of the presynaptic inhibitory octopamine autoreceptors on the DUMETi terminals and on the specificity of the presynaptic modulation of octopamine release from this preparation. A brief account of some of this work has already been published in abstract form (Evans and Howell, 1997).

Materials and methods

Animals

Adult female locusts (*Schistocerca gregaria*), 7–14 days after their final moult, were used in all experiments since the extensor-tibiae muscles of female locusts have been shown to contain higher levels of octopamine than those of males (Morton and Evans, 1983). Animals were obtained from crowded laboratory cultures fed on wheat seedlings. Small batches of animals were left undisturbed for 1–2 days after removal from the main culture before use to avoid any depletion of the octopamine levels in their extensor-tibiae muscles (Davenport and Evans, 1984*a*,*b*; Evans, 1981).

Incubation of muscles

Extensor-tibiae muscles from locust hindlegs were removed from the femur and rinsed in saline containing (in $mmol\,l^{-1}$): 150 NaCl, 6 $CaCl_2$, 4 $KHCO_3$, 4 KH_2PO_4 and 2 $MgCl_2$ (Usherwood and Grundfest, 1965; modified by Evans, 1978). Groups of six muscles were placed in 1 ml of saline for 3 min, and the saline was then removed (normal saline sample). The saline was replaced with 1 ml of high-[K^+] saline (high-[K^+] saline sample) containing various concentrations of K^+ (0.05, 0.1 or 0.15 $mol\,l^{-1}$ K^+). Ca^{2+}-free saline was obtained by substitution of Ca^{2+} with 0.006 $mol\,l^{-1}$ Mg^{2+}, and 0.01 $mol\,l^{-1}$ Co^{2+} saline was obtained by the addition of 0.01 $mol\,l^{-1}$ Co^{2+} to normal saline. In experiments to investigate the effectiveness of drugs on octopamine release, drugs were included in both the normal saline sample and the high-[K^+] saline sample at a concentration of 10^{-4} $mol\,l^{-1}$. In experiments to investigate the time course of octopamine release, 1 min sample periods were used and three exposures of 1 min each to normal saline were followed by three exposures of 1 min each to high-[K^+] saline.

Octopamine extraction

Octopamine was extracted from the saline samples by passing them through an ion-exchange column (Dowex 50W, 100–200 mesh size, 2.56 cm×0.5 cm internal diameter, H^+ form). The column was washed using 5 ml of water before octopamine was eluted with 5 ml of ammonium hydroxide and lyophilized under vacuum (Savant). The residue was redissolved in 25 μl of 0.01 $mol\,l^{-1}$ formic acid. The samples were stored frozen until assayed.

Radioenzymatic assay for released octopamine

The radioenzymatic assay for octopamine used in this study was based on that described by Molinoff *et al.* (1969) as modified by Evans (1978). It resulted in a sensitivity of twice background levels for the assay of 10–15 pg (0.1 pmol) based on a DL-octopamine standard. The assay is based on the amount of radiolabelled *N*-methyl-octopamine (synephrine) produced from endogenous octopamine and [methyl-^{3}H]*S*-adenosyl methionine by the enzyme phenylethanolamine-*N*-methyltransferase. Using an [^{3}H]octopamine standard, the recovery from the Dowex columns was greater than 80 % for all the types of saline used. The authenticity of the radioenzyme assay products was confirmed by thin-layer chromatography as described by Evans (1978), and the presence of drugs in the saline did not interfere with the assay.

Chemicals

[Methyl-^{3}H]*S*-adenosyl methionine was obtained from Amersham. Phenylethanolamine-*N*-methyltransferase, unlabelled *S*-adenosyl methionine, DL-octopamine, octopamine agonists and antagonists were obtained from Sigma. Where compounds were not water-soluble, the compound was first dissolved in dimethyl sulphoxide (DMSO) and appropriate controls carried out. The final concentration of DMSO was never greater than 0.1 %

Results

K^+-stimulated release

The K^+-stimulated release of endogenous octopamine from the terminals of the DUMETi neurone on the locust extensor-tibiae muscle was characterized (Fig. 1). The amount of octopamine released was dependent upon the concentration of [K^+] in the medium. The release plateaued at a K^+ concentration of 0.1 $mol\,l^{-1}$ (Fig. 1A), and this concentration was used in all subsequent experiments. Fig. 1B shows the time course of the release of endogenous octopamine by 0.1 $mol\,l^{-1}$ K^+. A low background level of release was found in the first three 1 min sample periods in control saline. In the presence of a high [K^+], the maximum amount of octopamine was released in the first minute of stimulation and the amount released then subsequently declined in the following two sample periods. Thus, in the rest of the present study, the amount of octopamine released during a 3 min period of exposure to 0.1 $mol\,l^{-1}$ K^+ was used to characterize the pharmacology of the response. The K^+-stimulated release of octopamine was Ca^{2+}-sensitive (Fig. 1C). It was blocked by the removal of external Ca^{2+} ($0Ca^{2+}$) and by the addition of

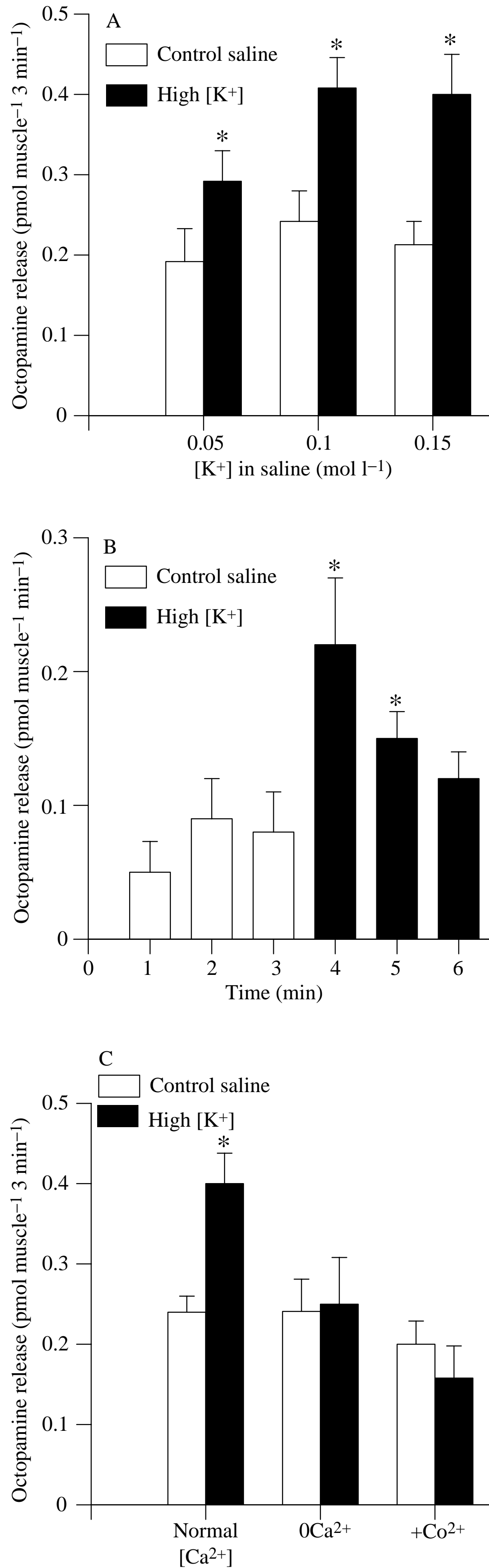

Fig. 1. The K^+-stimulated release of endogenous octopamine from the terminals of the DUMETi neurone on the locust extensor-tibiae muscle. (A) The amount of octopamine released is dependent upon the $[K^+]$ of the medium. The release plateaued at a K^+ concentration of $0.1\,mol\,l^{-1}$, and this concentration was used in all subsequent experiments. (B) The time course of octopamine release from the extensor-tibiae muscle in response to a high $[K^+]$. A low background level of release occurs in control saline, and in the presence of high-$[K^+]$ saline the maximum amount of octopamine is released in the first minute of stimulation. (C) The K^+-stimulated release of octopamine is Ca^{2+}-sensitive. It is blocked by removal of external Ca^{2+} ($0Ca^{2+}$) and by the addition of $10\,mmol\,l^{-1}$ Co^{2+} ($+Co^{2+}$), which competes with Ca^{2+} for Ca^{2+} entry channels. Values are means + S.E.M., N=3. An asterisk indicates a value that is significantly different from the value in control saline in A and C and from the value at 3 min in control saline in B (t-test; P<0.05).

$0.01\,mol\,l^{-1}$ Co^{2+} ($+Co^{2+}$), which competes with Ca^{2+} for Ca^{2+} entry channels.

The effects of antagonists

A range of known antagonists of insect octopamine receptors (Evans, 1981, 1993; Evans and Robb, 1993) potentiated both the basal and the K^+-stimulated release of endogenous octopamine from the terminals of the DUMETi neurone on the extensor-tibiae muscle. This suggests that the DUMETi terminals express inhibitory octopaminergic autoreceptors.

A comparison of the effectiveness of a range of antagonists ($10^{-4}\,mol\,l^{-1}$) at stimulating octopamine release indicated that phentolamine, metoclopramide, mianserin and chlorpromazine were effective inhibitors of the inhibitory octopaminergic autoreceptors (Fig. 2A). The antagonist pharmacological profile of these receptors is similar to that of the $OCTOPAMINE_{2A}$ receptors present on the presynaptic terminals of the locust slow extensor-tibiae motoneurone to this muscle. The ability of phentolamine to potentiate both basal and K^+-stimulated release of endogenous octopamine is dose-dependent with an effective threshold of approximately $10^{-5}\,mol\,l^{-1}$ for the potentiation of K^+-stimulated release (Fig. 2B).

The effect of agonists

A range of known agonists of insect octopamine receptors (Evans, 1981, 1993; Evans and Robb, 1993) inhibited both the basal and the K^+-stimulated release of endogenous octopamine from the locust extensor-tibiae muscle, further confirming the presence of inhibitory octopaminergic autoreceptors on the terminals of the DUMETi neurone (Fig. 3A).

A comparison of the effectiveness of a range of agonists ($10^{-4}\,mol\,l^{-1}$) at inhibiting octopamine release indicated that naphazoline inhibited both basal and K^+-stimulated release, whilst tolazoline and clonidine inhibited only basal octopamine release. This pharmacological profile is again consistent with the presence of $OCTOPAMINE_2$-like autoreceptors on the DUMETi terminals.

The octopamine receptor agonists and antagonists act in a competitive manner on the presynaptic octopamine receptors on the DUMETi terminals. Both phentolamine (10^{-4} mol l^{-1}) and metoclopramide (10^{-4} mol l^{-1}) reduced the inhibition of K^+-stimulated release of endogenous octopamine mediated by naphazoline (10^{-4} mol l^{-1}) (Fig. 3B).

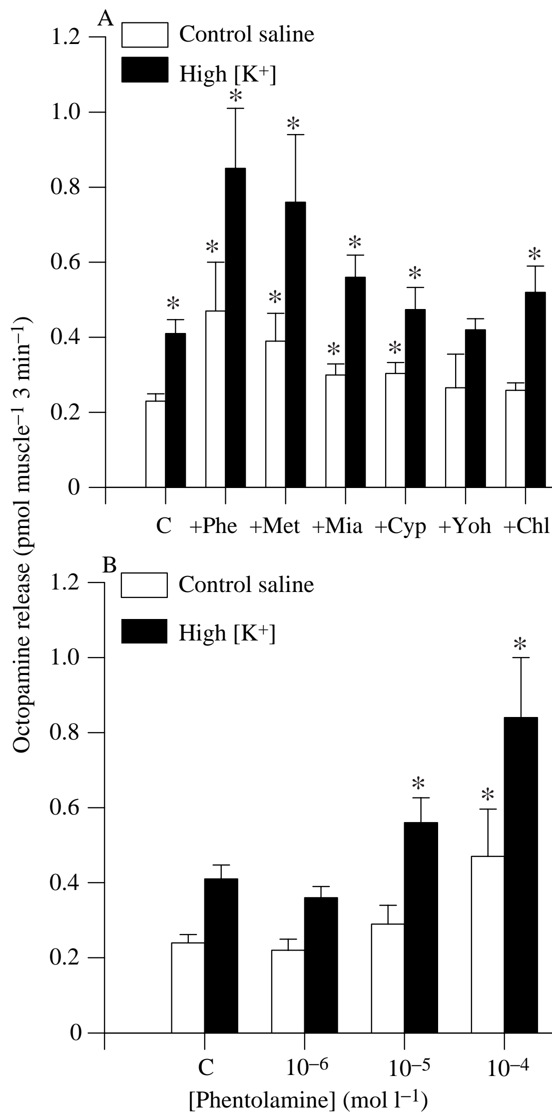

Fig. 2. Known antagonists of insect octopamine receptors potentiate both the basal and the K^+-stimulated release of endogenous octopamine from the terminals of the DUMETi neurone on the extensor-tibiae muscle. This suggests that the DUMETi terminals express inhibitory octopaminergic autoreceptors. (A) A comparison of the effectiveness of a range of antagonists (10^{-4} mol l^{-1}) at stimulating octopamine release indicates that phentolamine (Phe), metoclopramide (Met), mianserin (Mia) and chlorpromazine (Chl) are effective inhibitors of the inhibitory octopaminergic autoreceptors. The other antagonists used were cyproheptadine (Cyp) and yohimbine (Yoh). The antagonist pharmacological profile of these receptors is similar to that of the $OCTOPAMINE_{2A}$ receptors present on the presynaptic terminals of the locust slow extensor-tibiae motoneurone to this muscle. (B) The ability of phentolamine (Phe) to potentiate the release of basal and K^+-stimulated release of endogenous octopamine is dose-dependent, with an effective threshold of approximately 10^{-5} mol l^{-1}. Values are means + S.E.M., N=3. An asterisk indicates a value that is significantly different from the control value (t-test; P<0.05). C, control value.

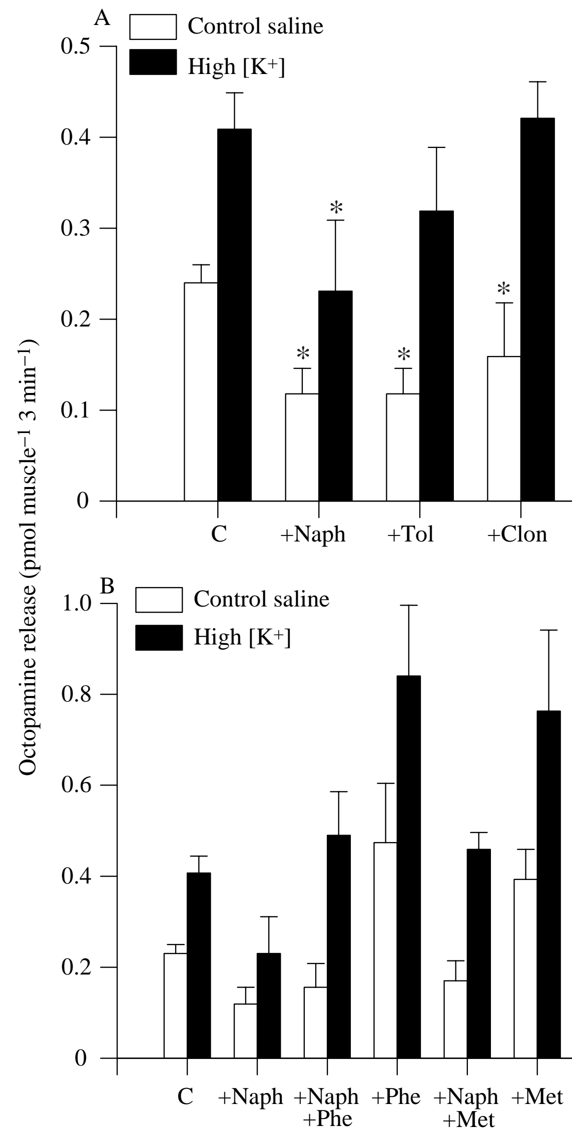

Fig. 3. Known agonists of insect octopamine receptors inhibit both the basal and the K^+-stimulated release of endogenous octopamine from the locust extensor-tibiae muscle, further confirming the presence of inhibitory octopaminergic autoreceptors on the DUMETi terminals. (A) A comparison of the effectiveness of a range of agonists (10^{-4} mol l^{-1}) at inhibiting octopamine release indicates that naphazoline (Naph) inhibits both basal and K^+-stimulated release, whilst tolazoline (Tol) and clonidine (Clon) inhibit only basal octopamine release. This pharmacological profile is again consistent with the presence of $OCTOPAMINE_2$-like inhibitory autoreceptors on the DUMETi terminals. An asterisk indicates a value that is significantly different from the control value (t-test; P<0.05). (B) The octopamine receptor agonists and antagonists act in a competitive manner on the presynaptic octopamine receptors on the DUMETi terminals. Both phentolamine (Phe) (10^{-4} mol l^{-1}) and metoclopramide (Met) (10^{-4} mol l^{-1}) reduce the inhibition of K^+-stimulated release of endogenous octopamine mediated by naphazoline (Naph) (10^{-4} mol l^{-1}). Values are means + S.E.M., N=3. C, control value.

Table 1. *The effect of potential modulators on the release of endogenous octopamine from the terminals of the DUMETi neurone in the locust extensor-tibiae muscle in the presence of 10^{-5} mol l^{-1} phentolamine*

	Octopamine release (pmol muscle^{-1} 3 min^{-1})		
Compound (10^{-4} mol l^{-1})	Background	K^+-stimulated	*N*
None (control)	0.29±0.05	0.56±0.07	3
Classical transmitters			
5-HT	0.24±0.02	0.49±0.02	3
ACh	0.26±0.02	0.48±0.09	3
Carbachol	0.31±0.03	0.50±0.06	3
Neuropeptides			
Proctolin	0.34±0.07	0.50±0.08	3
AKH II	0.25±0.02	0.56±0.04	3
SCP_B	0.28±0.04	0.62±0.06	3
F1	0.28±0.02	0.53±0.03	3
FMRF-NH_2	0.25±0.03	0.63±0.07	3
FLRF-NH_2	0.27±0.03	0.60±0.05	3
YGGFMRF	0.28±0.01	0.57±0.06	3
Opioid-like peptides			
δ Ligand Leu-enk	0.31±0.02	0.61±0.04	3
δ Ligand Met-enk	0.26±0.02	0.56±0.06	3
δ Agonist DADLE	0.33±0.02	0.64±0.03	3
δ Agonist DPDPE	0.28±0.08	0.66±0.05	3
μ/δ Ligand β-end	0.33±0.04	0.53±0.07	3
κ Ligand Dyn 1-13	0.25±0.05	0.60±0.04	3
PDH	0.25±0.03	0.65±0.08	3
Amino acid			
Taurine	0.28±0.04	0.49±0.01	3
Purine			
Adenosine	0.31±0.04	0.65±0.08	3

5-HT, 5-hydroxytryptamine; ACh, acetylcholine; AKH, adipokinetic hormone; SCP_B, small cardioactive peptide B; F1, lobster FMRFamide-like peptide; Leu-enk, leucine enkephalin; Met-enk, methionine-enkephalin; β-end, β-endorphin; Dyn 1-13, Dynorphin 1-13; PDH, pigment dispersing hormone.

Values are means ± S.E.M.

Specificity of presynaptic modulation of octopamine release

The ability of a range of putative modulatory agents (10^{-4} mol l^{-1}), which have been suggested to be co-localised with octopamine in DUM cells in various insect species or to have presynaptic modulatory effects in other systems, were compared for their ability to modulate octopamine release from the terminals of the DUMETi neurone. These experiments (Table 1) were all performed in the presence of 10^{-5} mol l^{-1} phentolamine to block any effects on the inhibitory octopaminergic autoreceptors and to potentiate the amount of endogenous octopamine released, but similar results (not shown) were obtained in experiments conducted in the absence of added phentolamine. None of the range of classical insect neurotransmitters or neuropeptides tested produced any significant change in the basal or K^+-stimulated release of endogenous octopamine from the locust extensor-tibiae muscle. These results suggest that the inhibitory presynaptic modulation of octopamine release from the terminals of the DUMETi neurone may be specific for presynaptic octopaminergic autoreceptors.

Discussion

Presynaptic inhibitory octopaminergic autoreceptors are present on the terminals of the octopamine-containing modulatory neurone DUMETi, which innervates the extensor-tibiae muscle of the locust hindleg. The antagonist pharmacological profile of these receptors (Table 2) is much more similar to that of the $OCTOPAMINE_{2A}$ presynaptic octopamine receptors present on the terminals of the slow excitatory motoneurone (SETi) to the same muscle (Evans, 1981) than to that of the $OCTOPAMINE_3$ receptors suggested to be present in the locust central nervous system (Roeder and Gewecke, 1990; Roeder, 1992, 1995; Roeder and Nathanson, 1993). It is also different from that of the $OCTOPAMINE_1$ receptor subtype, which inhibits the myogenic rhythm of contraction and relaxation and is found in a bundle of slow muscle fibres at the proximal end of the locust extensor-tibiae muscle (Evans, 1981). The results of the present study suggest that it is inappropriate to designate all locust neuronal octopamine receptors as belonging to the $OCTOPAMINE_3$ subtype (cf. Roeder and Nathanson, 1993). It is clear that octopamine receptors of the $OCTOPAMINE_{2A}$ subclass appear to be present on the presynaptic terminals of both DUMETi (present study) and SETi (Evans, 1981). In addition, it should be noted that any biochemical characterization of octopamine receptors from the insect central nervous system is likely to include a component from the receptors present on

Table 2. *A comparison of the antagonist pharmacological profiles of octopamine receptor subtypes*

Receptor class	Pharmacological profile	Location	Reference
Type 1	Phe>Chl>Cyp>Yoh>Mia>Met	Extensor muscle	Evans (1981)
Type 2A	Met=Mia=Phe>Cyp≫Chl≫Yoh	SETi terminals	Evans (1981)
Type 2B	Phe>Met≫Mia>Cyp>Chl>Yoh	Extensor muscle	Evans (1981)
Type 3	Mia>Phe>Chl>Met>Yoh	Central nervous system	Roeder (1994)
DUMETi (type 2A?)	Phe=Met>Mia=Chl>Cyp>Yoh	DUMETi terminals	Present study

Phe, phentolamine; Chl, chlorpromazine; Cyp, cyproheptadine; Yoh, yohimbine; Mia, mianserin; Met, metoclopramide.

non-neuronal cell types, such as the perineurial glial cells that form the insect central nervous system blood–brain barrier (Schofield and Treherne, 1985, 1986).

The presence of presynaptic autoreceptors regulating the release of neurotransmitter from synapses is well established for a range of vertebrate aminergic systems (see Verhage *et al.* 1994). For instance, inhibitory presynaptic α-adrenergic receptors are present on the terminals of sympathetic noradrenergic neurones (Starke *et al.* 1989; Jackish *et al.* 1992), and presynaptic inhibitory D_2-like dopamine receptors are present on the terminals of dopaminergic neurones in the striatum (Iannazzo *et al.* 1997) and in the substantia nigra and ventral tegmental area (Mercuri *et al.* 1997). Inhibitory presynaptic autoreceptors for 5-hydroxytryptamine have also been reported on the A_1 serotonin-containing neurones in the central nervous system of the lobster *Homarus vulgaris* (Ma and Weiger, 1993). However, the phenomenon is not confined to biogenic amine receptors since inhibitory presynaptic muscarinic receptors are thought to be able to reduce the release of acetylcholine from central cholinergic synapses in synaptosomal preparations from the insect central nervous system (see Breer, 1990). In addition, the neuropeptide bucculin, which is released from the B15 and B16 motoneurones to the accessory radula closer muscle of *Aplysia californica*, can feed back onto the nerve terminals and inhibit the release of acetylcholine (see Weiss *et al.* 1993). Further, the α, β and γ peptides produced from the egg-laying hormone precursor of *Aplysia* can feed back onto the bag cells from which they are released to produce an autoexcitatory action on the cells (Brown and Mayeri, 1989; Kauer *et al.* 1987; Rock *et al.* 1986).

The above observations led us to consider the possibility that other substances released by DUM cells might also feed back and alter the release of octopamine from DUMETi. Thus, FMRFamide-like peptides, which are co-localized with octopamine in some locust DUM cells (Stevenson and Pflüger, 1994), were tested on octopamine release from DUMETi, but no effects were observed. Similarly, 5-hydroxytryptamine, which has been localized immunochemically in a subpopulation of DUM cells in *Rhodnius prolixus* (Orchard *et al.* 1989) and has been shown to have an effect on the excitability of the somatic membrane of DUM neurones in the cockroach thoracic ganglia (Washio and Tanaka, 1992), also had no effect on octopamine release from DUMETi. Proctolin has also been detected immunocytochemically in DUM cells in the cricket *Gryllus bimaculatus* (Yasuyama *et al.* 1992) and the cockroach *Periplaneta americana* (Amat *et al.* 1997) and has been shown to have an effect on the excitability of the somatic membrane of DUM neurones in the cockroach (Walker *et al.* 1980; Washio and Tanaka, 1992). However, it again had no effect on octopamine release from DUMETi in the locust. It should be noted that it appears highly unlikely, on the basis of current evidence, that the locust DUMETi neurone contains any 5-hydroxytryptamine, proctolin or FMRFamide-like peptides. Further, taurine, which has been suggested on the basis of immunocytochemical evidence to co-exist with octopamine in insect DUM neurones (Nürnberger *et al.* 1993), to inhibit the stress-induced elevation of octopamine levels in the haemolymph of the cockroach *Periplaneta ameriocana* (Hayakawa *et al.* 1987*a*,*b*) and to be co-released with octopamine (Hayakawa *et al.* 1987*a*), again had no effect on octopamine release from DUMETi under the conditions used in the present study. In addition, adipokinetic hormone (AKH)-like peptides, which increase low-voltage activation of Ca^{2+} currents in DUM neurones in *Periplaneta americana* (Wicher and Penzlin, 1994), acetylcholine, which also modulates DUM neurone activity (Tribut *et al.* 1994), and adenosine, which has been suggested as a possible co-released transmitter with octopamine from DUM cells (Evans and Myers, 1986), had no effect on octopamine release from DUMETi.

A range of opioid-like peptides have been shown to modulate slow neuromuscular transmission in the locust hindleg extensor-tibiae muscle (Howell, 1995). They potentiate the rate of relaxation and amplitude of slow-motoneurone-induced twitch tension in this muscle, and the effects are blocked by phentolamine as well as naloxone. This suggests the possibility that these opioid-like peptides could be mediating their effects *via* a release of octopamine from the DUMETi terminals in this muscle. However, in the present investigation, no effect on the release of endogenous octopamine could be demonstrated with the range of ligands used that are specific for the different opioid receptor subtypes.

It would appear that the presynaptic modulation of the release of endogenous octopamine from the terminals of the DUMETi neurone may be highly specific for octopamine itself. However, before this suggestion can be confirmed, it will be necessary to rule out the effects of any potentially co-released modulators, such as neuropeptides, from the DUMETi terminals in this preparation.

In functional terms, DUMETi appears to be specifically activated during particular behaviours involving the parallel activation of motoneurones to the extensor-tibiae muscle (Burrows and Pflüger, 1995). It presumably modulates the performance of the muscle to increase the amount of tension generated by a burst of action potentials in the slow motoneurone. It also removes any maintained tension in the muscle so that the motor output more closely matches the neuronal input pattern and thus converts the muscle from a postural to a dynamic response mode (Evans and Siegler, 1982). This raises the question of the physiological significance of inhibitory presynaptic autoreceptors for octopamine on the terminals of the DUMETi neurone. It seems likely, by analogy with the well-described inhibitory actions of presynaptic α-adrenergic receptors on the terminals of noradrenergic sympathetic neurones in vertebrates (Starke *et al.* 1989; Jackish *et al.* 1992), that these presynaptic octopamine autoreceptors on the DUMETi terminals would function to limit the release of octopamine at times of intense activity in the neurone. Under such conditions, high levels of octopamine are likely to accumulate in the local environment of the nerve terminals. Alternatively, these receptors are also likely to be able to limit the amount of octopamine released

from the nerve terminals of DUMETi during periods of stress, when octopamine levels are raised in insect haemolymph (Davenport and Evans, 1984*a*,*b*). Future research in this area will need to concentrate on the determination of local octopamine concentrations in the immediate vicinity of the extensor-tibiae muscle during stressed and non-stressed states of the animal. In addition, the physiological effects of individual repeated bursts of DUMETi stimulation need to be assessed during different periods of prolonged activity in this muscle.

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References

ACHENBACH, H., WALTHER, C. AND WICHER, D. (1997). Octopamine modulates ionic currents and spiking in dorsal unpaired median (DUM) neurons. *Neuroreport* **8**, 3737–3741.

AMAT, C., SINAKEVITCH-PEAN, I. AND HUE, B. (1997). Proctolin-like immunoreactivity in dorsal paired median neurons generating plateau action potentials in cockroach *Periplaneta americana*. *Neurosci. Res.* **29**, 263–268.

BREER, H. (1990). Molecular biology of cholinergic synapses in insects. In *Molecular Insect Science* (ed. H. H. Hagedorn, J. G. Hildebrand, M. G. Kidwell and J. H. Law), pp. 31–39. New York: Plenum Press.

BROWN, R. O. AND MAYERI, E. (1989). Positive feedback by autoexcitatory neuropeptides in neuroendocrine bag cells of *Aplysia*. *J. Neurosci*. **9**, 1443–1451.

BURROWS, M. AND PFLÜGER, H.-J. (1995). Action of locust neuromodulatory neurons is coupled to specific motor patterns. *J. Neurophysiol.* **74**, 347–357.

CAMPBELL, H. R., THOMPSON, K. J. AND SIEGLER, M. V. S. (1995). Neurons of the median neuroblast lineage of the grasshopper: A population study of the efferent DUM neurons. *J. comp. Neurol.* **358**, 541–551.

DAVENPORT, A. P. AND EVANS, P. D. (1984*a*). Stress induced changes in the octopamine levels of insect haemolymph. *Insect Biochem.* **14**, 135–143.

DAVENPORT, A. P. AND EVANS, P. D. (1984*b*). Changes in haemolymph octopamine levels associated with food deprivation in the locust, *Schistocerca americana gregaria*. *Physiol. Ent.* **9**, 269–274.

EVANS, P. D. (1978). Octopamine distribution in the insect nervous system. *J. Neurochem.* **30**, 1009–1013.

EVANS, P. D. (1980). Biogenic amines in the insect nervous system. *Adv. Insect Physiol.* **15**, 317–473.

EVANS, P. D. (1981). Multiple receptor types for octopamine in the locust *J. Physiol., Lond.* **318**, 99–122.

EVANS, P. D. (1984*a*). A modulatory octopaminergic neurone increases cyclic nucleotide levels in locust skeletal muscle. *J. Physiol., Lond.* **348**, 307–324.

EVANS, P. D. (1984*b*). The role of cyclic nucleotides and calcium in the mediation of the modulatory effects of octopamine on locust skeletal muscle. *J. Physiol., Lond.* **348**, 325–340.

EVANS, P. D. (1985). Octopamine. In *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, vol. 11 (ed. G. A. Kerkut and L. I. Gilbert), pp. 499–530. Oxford: Pergamon Press.

EVANS, P. D. (1993). Molecular studies on insect octopamine receptors. In *Comparative Molecular Neurobiology* (ed. Y. Pichon), pp. 286–296. Basel, Switzerland: Birkhäuser Verlag.

EVANS, P. D. AND HOWELL, K. M. R. (1997). Characterization of presynaptic octopamine receptors modulating octopamine release from an identified neurone in the locust. *Soc. Neurosci. Abstr.* **23**, 1235.

EVANS, P. D. AND MYERS, C. M. (1986). Peptidergic and aminergic modulation of insect skeletal muscle. *J. exp. Biol.* **124**, 143–176.

EVANS, P. D. AND O'SHEA, M. (1977). An octopaminergic neurone modulates neuromuscular transmission in the locust. *Nature* **270**, 257–259.

EVANS, P. D. AND O'SHEA, M. (1978). The identification of an octopaminergic neurone and the modulation of a myogenic rhythm in the locust. *J. exp. Biol.* **73**, 235–260.

EVANS, P. D. AND ROBB, S. (1993). Octopamine receptor subtypes and their modes of action. *Neurochem. Res*. **18**, 869–874.

EVANS, P. D. AND SIEGLER, M. V. S. (1982). Octopamine mediated relaxation of maintained and catch tension in locust skeletal muscle. *J. Physiol., Lond.* **324**, 93–112.

GOODMAN, C. S. AND SPITZER, N. C. (1979). Embryonic development of identified neurones: Differentiation from neuroblast to neurone. *Nature* **280**, 208–214.

HAYAKAWA, Y., BODNARYK, R. P. AND DOWNER, R. G. H. (1987*a*). Effect of taurine on excitation-induced changes in brain and hemolymph octopamine levels in *Periplaneta americana. Archs Insect Biochem. Physiol.* **5**, 129–137.

HAYAKAWA, Y., DOWNER, R. G. H. AND BODNARYK, R. P. (1987*b*). Taurine inhibits octopamine-stimulated cyclic AMP production in cockroach. *Biochim. biophys. Acta* **929**, 117–120.

HOWELL, K. M. R. (1995). The control of octopamine release from an identified modulatory neurone in the locust. PhD thesis, University of Cambridge.

IANNAZZO, L., SATHANANTHAN, S. AND MAJEWSKI, H. (1997). Modulation of dopamine release from rat striatum by protein kinase C: interaction with presynaptic D_2-dopamine autoreceptors. *Br. J. Pharmac.* **122**, 1561–1566.

JACKISH, R., HUANG, H. Y., RENSING, H., LAUTH, D., ALLGAIER, C. AND HERTTING, G. (1992). α_2-Adrenoceptor mediated inhibition of exocytotic noradrenaline release in the absence of extracellular Ca^{2+}. *Eur. J. Pharmac.* **226**, 245–252.

KAUER, J. A., FISHER, T. E. AND KACZMAREK, L. K. (1987). Alpha bag cell peptide directly modulates the excitability of the neurons that release it. *J. Neurosci.* **7**, 3623–3632.

MA, P. M. AND WEIGER, W. A. (1993). Serotonin-containing neurons in lobsters: the actions of γ-aminobutyric acid, octopamine, serotonin and proctolin on activity of a pair of identified neurons in the first abdominal ganglion. *J. Neurosci.* **69**, 2015–2029.

MERCURI, N. B., SAIARDI, A., BONCI, A., PICETTI, R., CALABRESI, P., BERNARDI, G. AND BORRELLI, E. (1997). Loss of autoreceptor function in dopaminergic neurons from dopamine D_2 receptor deficient mice. *Neuroscience* **79**, 323–327.

MOLINOFF, P. B., LANDSBERG, L. AND AXELROD, J. (1969). An enzymatic assay for octopamine and other β-hydroxylated phenylethylamines. *J. Pharmac. exp. Ther*. **170**, 253–261.

MORTON, D. B. AND EVANS, P. D. (1983). Octopamine distribution in solitarious and gregarious forms of the locust, *Schistocerca americana gregaria*. *Insect Biochem*. **13**, 177–183.

MORTON, D. B. AND EVANS, P. D. (1984). Octopamine release from an identified neurone in the locust. *J. exp. Biol*. **113**, 269–287.

NÜRNBERGER, A., RAPUS, J., ECKERT, M. AND PENZLIN, H. (1993). Taurine-like immunoreactivity in octopaminergic neurones of the cockroach, *Periplaneta americana. Histochemistry* **100**, 285–292.

ORCHARD, I., LANGE, A. B., COOK, H. AND RAMIREZ, J.-M. (1989). A subpopulation of dorsal unpaired median neurons in the blood-feeding insect *Rhodnius prolixus* displays serotonin-like immunoreactivity. *J. comp. Neurol.* **289**, 118–128.

O'SHEA, M. AND EVANS, P. D. (1979). Potentiation of neuromuscular transmission by an octopaminergic neurone in the locust. *J. exp. Biol.* **79**, 169–190.

ROCK, M. K., SHOPE, S. B. AND BLANKENSHIP, J. E. (1986). Effects of synthetic bag cell and atrial gland peptides on identified nerve cell in *Aplysia. J. Neurosci.* **17**, 273–290.

ROEDER, T. (1992). A new octopamine receptor class in locust nervous tissue, the octopamine 3 (OA_3) receptor. *Life Sci.* **50**, 21–28.

ROEDER, T. (1994). Biogenic amines and their receptors in insects. *Comp. Biochem. Physiol*. **107**C, 1–12.

ROEDER, T. (1995). Pharmacology of the octopamine receptor from locust central nervous tissue (OAR3). *Br. J. Pharmac*. **114**, 210–216.

ROEDER, T. AND GEWECKE, M. (1990). Octopamine receptors in locust nervous tissue. *Biochem. Pharmac.* **39**, 1793–1797.

ROEDER, T. AND NATHANSON, J. A. (1993). Characterization of insect neuronal octopamine receptors (OA_3 receptors). *Neurochem. Res.* **18**, 921–925.

SCHOFIELD, P. K. AND TREHERNE, J. E. (1985). Octopamine reduces potassium permeability of the glia that form the insect blood–brain barrier. *Brain Res.* **360**, 344–348.

SCHOFIELD, P. K. AND TREHERNE, J. E. (1986). Octopamine sensitivity of the blood–brain barrier of an insect. *J. exp. Biol.* **123**, 423–439.

SIEGLER, M. V. S., MANLEY, P. E. AND THOMPSON, K. J. (1991). Sulphide silver staining for endogenous heavy metals reveals subsets of dorsal unpaired median (DUM) neurones in insects. *J. exp. Biol.* **157**, 565–571.

STARKE, K., GÖTHERT, M. AND KILBINGER, H. (1989). Modulation of neurotransmitter release by presynaptic autoreceptors. *Physiol. Rev*. **69**, 864–989.

STEVENSON, P. A. AND PFLÜGER, H.-J. (1994). Colocalization of octopamine and FMRFamide related peptide in identified heart projecting (DUM) neurones in the locust revealed by immunocytochemistry. *Brain Res.* **638**, 117–125.

STEVENSON, P. A., PFLÜGER, H.-J., ECKERT, M. AND RAPUS, J. (1992). Octopamine immunoreactive cell populations in the locust thoracic-abdominal nervous system. *J. comp. Neurol.* **315**, 382–397.

THOMPSON, K. J. AND SIEGLER, M. V. S. (1991). Anatomy and physiology of spiking local and intersegmental interneurons in the median neuroblast lineage of the grasshopper CNS. *J. comp. Neurol.* **305**, 659–675.

TRIBUT, F., DUVAL, A. AND LAPIED, B. (1994). Two distinct receptors are activated by arecoline on cockroach sixth abdominal ganglion DUM neurones. *J. exp. Biol.* **186**, 325–331.

USHERWOOD, P. N. R. AND GRUNDFEST, H. (1965). Peripheral inhibition in skeletal muscle of insects. *J. Neurophysiol.* **28**, 497–518.

VERHAGE, M., GHIJSEN, W. E. J. M. AND LOPES DA SILVA, F. H. (1994). Presynaptic plasticity: the regulation of Ca^{2+}-dependent transmitter release. *Prog. Neurobiol*. **42**, 539–574.

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

WASHIO, H. AND TANAKA, Y. (1992). Some effects of octopamine, proctolin and serotonin on dorsal unpaired median neurones of cockroach (*Periplaneta americana*) thoracic ganglia. *J. Insect Physiol.* **38**, 511–517.

WEISS, K. R., BREZINA, V., CROPPER, E. C., HEIERHORST, J., HOOPER, S. L., PROBST, W. C., ROSEN, S. C., VILIM, F. S. AND KUPFERMANN, I. (1993). Physiology and biochemistry of peptidergic cotransmission in *Aplysia. J. Physiol., Paris* **87**, 141–151.

WICHER, D. AND PENZLIN, H. (1994). Ca^{2+} currents in cockroach neurones: properties and modulation by neurohormone D. *Neuroreport* **5**, 1023–1026.

YASUYAMA, K., KIMURA, T. AND YAMAGUCHI, T. (1992). Proctolin-like immunoreactivity in the dorsal unpaired median neurons innervating the accessory gland of the male cricket, *Gryllus bimaculatus*. *Zool. Sci.* **9**, 53–64.