OCTOPAMINERGIC MODULATION OF THE FOREWING STRETCH RECEPTOR IN THE LOCUST

LOCUSTA MIGRATORIA

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Summary

Modulatory actions of various biogenic amines and peptides on the locust forewings stretch receptor (SR) were examined. The response of the SR to sinusoidal wing movements was unaffected by physiological concentrations (5×10^-8 mol l^-1) of the peptides AKHI, AKHII, proctolin and FMRFamide. The biogenic amine octopamine, however, enhanced the SR response in a dose-dependent manner when injected into the haemolymph of an almost intact animal or perfused over an isolated thorax preparation in which head, abdomen, gut and the entire central nervous system were removed (threshold at 5×10^-8 mol l^-1, maximal effect at 5×10^-4 mol l^-1 DL-octopamine). The SR was as sensitive to d-octopamine, the naturally occurring isomer of octopamine, as it was to DL-octopamine. Serotonin was equal to octopamine in effectiveness, followed in order of potency by synephrine, metanephrine and tyramine. Dopamine was ineffective. Phentolamine, but not DL-propranolol, antagonized the action of octopamine. The threshold of the modulatory effect of octopamine on the SR suggests that the increased haemolymph octopamine level which occurs during flight is sufficient to increase the SR activity. Two observations suggest that dorsal unpaired median (DUM) cells are involved in the octopaminergic modulation of the SR during flight: (1) selective stimulation of these cells modulated the SR response and this effect was blocked by phentolamine; and (2) a number of DUM cells were activated during flight. These results suggest that the SR activity is enhanced by octopamine following the onset of flight. Since the SR is involved in the control of wing beat frequency, the modulation of the SR might influence the generation of the motor pattern in flying locusts.

Introduction

Over the past decade it has been well established that circulating hormones, in particular various biogenic amines and peptides, influence many aspects of motor

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behaviour; e.g. the development of behaviour (Levine and Truman, 1983), the
initiation of behaviour (Trimble and Barker, 1984; Wieland and Gelperin, 1983)
and the generation of rhythmic behaviour (Harris-Warrick and Cohen, 1985;
Hooper and Marder, 1987; Flamm and Harris-Warrick, 1986a,b; Tuersley and
McCrohan, 1988). The flight behaviour of insects has been extensively studied as a
model for motor behaviour, and recent attention has been directed towards the
possible modulation of flight behaviour by octopamine. Octopamine has been
shown to be involved in the regulation of levels of haemolymph lipid, an important
energy source for flight muscles (Wheeler and Goldsworthy, 1985). It acts as an
adipokinetic neurohormone (Orchard et al. 1981, 1982), stimulating an early
increase in haemolymph lipid concentration during flight (Orchard and Lange,
1985a; Orchard, 1987), and it acts as a neurotransmitter controlling the release of
two peptidergic adipokinetic hormones, AKH I and II (see Orchard, 1987), which
are important for lipid mobilization during long-duration flights (Goldsworthy and
Wheeler, 1984; Orchard, 1987). Octopamine is also involved in the neural control
of flight performance. It modulates neuromuscular transmission and muscle
contraction of various flight muscles and enhances their power output (Klaassen
and Kammer, 1985; Fitch and Kammer, 1986; Klaassen et al. 1986; Candy, 1978;
Whim and Evans, 1988; Malamud et al. 1988). The flight muscles may be
responding to the elevation of haemolymph octopamine levels which occurs in the
first few minutes of flight (Goosey and Candy, 1980; Bailey et al. 1983) and/or to
octopamine locally released from certain dorsal unpaired median (DUM) cells.
These cells are octopaminergic (Evans and O'Shea, 1977, 1978; Morton and
Evans, 1984; Orchard and Lange, 1985b, 1987), and project to flight muscles
(Kutsch and Schneider, 1987; Watson, 1984) where they modulate neuromuscular
transmission and muscle contraction (Whim and Evans, 1988; Malamud et al.
1988). Besides these peripheral effects of octopamine, it has also been shown to
have effects on the central nervous system. Injection of octopamine into specific
regions of the thoracic ganglia can evoke and maintain flight rhythmic activity in
locusts (Sombati and Hoyle, 1984a; Stevenson and Kutsch, 1987, 1988) and moths
(Kinnamon et al. 1984; Claassen and Kammer, 1986). The flight motor pattern can
be initiated by octopamine in completely isolated locust ganglia (Stevenson and
Kutsch, 1987). Finally, octopamine may affect transmission in sensory pathways
coming from wing proprioceptors into the thoracic ganglia, since the responsive-
ness to wing stimulation is enhanced after injection of octopamine into the thoracic
ganglia of the moth (Kinnamon et al. 1984).

Other possible sites of action for octopamine, which have not yet been
examined, are wing proprioceptors. This modulation would have important
functional implications, since the generation of the flight rhythm is influenced by
the activity of three wing proprioceptors, the tegula (Pearson and Wolf, 1988), the
campaniform sensilla (Horsmann and Wendler, 1985) and the stretch receptor
(Möhl, 1985a,b). In the present study we have examined the effect of octopamine
on one of these wing proprioceptors, the stretch receptor (SR). The exact location
of this single-celled SR at the base of each wing is known (Pfau, 1982), as is its
central projection into the thoracic ganglia (Altman and Tyrer, 1977). In the intact flying locust the SR is rhythmically active at the beginning of each wing depression (Möhl, 1985a; Wendler, 1982), and stimulation of the SR can reset and entrain the flight rhythm (Pearson et al. 1983; Möhl, 1985b; Reye and Pearson, 1987). The SR is known to excite monosynaptically depressor motoneurones (Burrows, 1975) as well as several interneurones involved in the generation of the flight rhythm (Reye and Pearson, 1987). We report here that this primary afference is itself modulated by octopamine in concentrations similar to those occurring in the haemolymph at the onset of flight. One source of this octopamine may be spill-over from octopaminergic DUM cells, since extracellular stimulation of DUM cells was found to modulate the activity in the SR. Furthermore, intracellular recordings in deafferented and intact flying locusts revealed that DUM cells are activated during flight. A preliminary report of some of these data has been published (Ramirez et al. 1989).

Materials and methods

Animals

Adult male and female Locusta migratoria from colonies at the University of Toronto and at the University of Alberta were used. The locusts were maintained at 30°C and fed on fresh wheat seedlings and bran. All experiments were performed at room temperature (22–24°C).

Stretch receptor recordings

To examine the effect of various drugs on the forewing SR in almost intact animals, the locusts were first fixed with wax, ventral side up, on a steel holder (Fig. 1A). The legs, but not the wings, were removed. A small window was cut into the sternal cuticle above the mesothoracic ganglion to expose the prothoracic nerve 6 and mesothoracic nerve 1D2, both of which contain afferents from the forewing SR (Altman and Tyrer, 1977). A suction electrode was placed on one of these nerves (electrode 1, Fig. 1). Both nerves were cut proximal to the ganglia to ensure that only afferent activity was recorded. Following this procedure, the wound was covered with Parafilm, which was then waxed onto the sternal cuticle. A 10 μl micropipette, connected to a peristaltic pump, was inserted into a small hole (1–2 mm in diameter) which was cut into the sternal cuticle posterior to the metathoracic antecosta (Fig. 1). 10 μl of a known concentration of drug was injected at a rate of 1 ml min⁻¹ through the micropipette into the haemolymph. Assuming that these adult locusts contain roughly 200 μl of haemolymph (Loughton and Tobe, 1969; I. Orchard, unpublished results), the drug would be diluted by a factor of 20. This factor was included in all concentrations mentioned in this study. Because the response of the SR saturates at high octopamine concentrations (see Results) we took precautions to minimize the endogenous haemolymph octopamine level. To diminish the stress-induced high level of octopamine (see, for example, Orchard et al. 1981; Davenport and Evans, 1984), the
preparation was left untouched for 1–2 h after dissection. An accumulation of octopamine and other drugs was avoided by waiting 1 h between drug injections. The experiment was stopped after injection of high octopamine concentrations ($5 \times 10^{-5} \text{mol L}^{-1}$ or higher). Since all tracheae were left intact, this preparation was easily maintained for 12 h without any change in the SR recording quality.

A similar preparation was used to examine the spill-over effect of octopamine released by DUM cells. Since DUM cells are the only efferent insect neurones which have bilateral projections into both left and right peripheral nerves, they can be selectively stimulated by placing suction electrodes (electrode 2, Fig. 1) on a nerve contralateral to the SR recording site (electrode 1, Fig. 1). To avoid muscle contractions on the stimulated side, the stimulated nerves were cut distal to the suction electrode. This stimulation arrangement should cause a release of octopamine ipsilateral to the SR recording site without causing contractions of ipsi- and contralateral muscles. This was necessary to avoid any unspecific activation of the SR by reflexes or muscle twitches. The spill-over effect of different DUM cell populations was examined by stimulating three different nerves (N1, N3 and N5) with pulses of 2 ms duration for 15 s at 10 Hz. The transection of the ipsilateral N1 proximal to the ganglion, as described above for the SR recording procedure, was omitted if the contralateral N1 was stimulated.

Two reduced preparations were used to examine whether octopamine had a direct influence on the activity of the SR. An isolated thorax preparation (Fig. 1B) was used to demonstrate that the SR is modulated directly by octopamine and not

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**Fig. 1.** Preparations for examining the effects of various drugs on the response of the forewing stretch receptor (SR) to sinusoidal wing movements. (A) Preparation used to study the effect of various drugs in an almost intact locust by injecting small volumes (10 µL) into the circulating haemolymph. (B) Reduced thorax preparation, in which head, abdomen, gut and the entire central nervous system were removed. The preparation is constantly perfused with saline. Both preparations are described in detail in the text.
indirectly by any other released hormone. The legs, the hindwings, the head, the abdomen, the gut and the entire central nervous system were removed. The thorax was mounted ventral side up and was continuously perfused with saline (1 ml min⁻¹) (Fig. 1B). In this preparation, the attachment of the SR to the body wall was not altered so SR activity could always be recorded and the effect of the examined drug was highly reproducible. A completely isolated SR was examined in 10 animals, to exclude the possibility that the SR was indirectly modulated by contracting muscles. In these preparations the left half of the thorax was mounted dorsal side up in a Petri dish. The legs, the hindwing and all muscles and ganglia were removed, but the thoracic body wall, the forewing, the SR and nerve 1D2 were left intact. In this type of preparation the SR was only loosely attached to the body wall and in many cases it was therefore very difficult or impossible to activate the SR. However, in a number of preparations SR activity could still be evoked by wing movements. These preparations were completely covered and constantly perfused with saline (1 ml min⁻¹). The effect of drugs was then examined by exchanging the incoming saline for one containing the appropriate drug. This procedure increased the drug concentration in the perfusion bath without causing a mechanical disturbance of the SR.

Stretch receptor stimulation and data analysis

For all preparations, the SR was stimulated in the same manner. The forewing was waxed on a metal lever connected to a pen motor and driven by a function generator (Fig. 1). The wing was moved with sinusoidal movements up and down, and the movements were monitored by the output signal of the function generator. Before each set of experiments, the frequency, amplitude and the angle of the wing to the body were adjusted to achieve a constant number of spikes phase-locked to the imposed wing movements (between 1 and 4 spikes per wing movement). In most cases the SR was stimulated at low frequencies between 1 and 10 Hz, mainly to keep the amount of information stored on the computer as small as possible. However, in some cases we stimulated the SR at higher frequencies (between 15 and 20 Hz). The extracellularly recorded SR spikes were fed on-line via a window discriminator into a computer (Neurograph model STA-1, Medical Instruments Corp. or DEC LSI 11/23). All data were stored on diskette and plotted in a sequential spike histogram. The bin width for the histograms was either 1 or 2 s. The number of events occurring in the intervals 1 min before and 4–5 min after drug injection was averaged by the computer and the difference between these values was expressed as a percentage increase or decrease.

Preparations for DUM cell recording and staining

A preparation as described by Robertson and Pearson (1982) was used to record intracellularly from DUM cells during flight-like rhythmic activity. The animals were mounted dorsal side up on a cork board, legs and wings were removed, and the thorax was opened by a dorsal incision. The mesothoracic ganglion was exposed by removing the gut and small muscles overlying the ganglion. The
ganglion was ventrally supported by a stainless-steel platform. Deafferented flight rhythmic activity was evoked either by blowing frontal wind towards the animal or by sudden changes in the light conditions.

A preparation as described by Wolf and Pearson (1988) was used to record intracellularly from DUM cells in intact, tethered, flying animals. The locusts were mounted ventral side up on a steel holder. The legs, but not the wings, were removed and a small window was cut into the sternal cuticle to expose the mesothoracic ganglion. The ganglion was supported and flight was evoked as described above.

In both preparations wing elevator and depressor muscles are activated in an alternating manner (Robertson and Pearson, 1982; Wolf and Pearson, 1988). Rhythmic activity in the flight system was monitored by electromyograph (EMG) recordings of hindwing depressor muscles 129 or 127. Owing to cross-talk from adjacent elevator muscles, alternating activity could usually be seen in the depressor EMG alone. In a few cases rhythmic activity was monitored by placing a bipolar hook electrode on nerve N1D1 to record from the dorso-longitudinal muscle 112, a wing depressor. Intracellular recordings from DUM neurones were obtained either from their neuropile processes or from their somata. D.c. records were stored on an FM tape recorder and examined later. Glass electrodes were filled with a 5 % solution of Lucifer Yellow in distilled water: electrode resistances varied between 60 and 200 MΩ. Dye was injected by passing negative current (5–8 nA) for up to 30 min. The ganglia were processed as described by Robertson and Pearson (1982) and the neurones were identified and named as described by Watson (1984).

Saline and drugs

All drugs were freshly dissolved before each set of experiments in physiologically isotonic saline (pH 7.0) containing (in mmol l⁻¹): NaCl, 150; KCl, 10; CaCl₂, 4; MgCl₂, 2; NaHCO₃, 4; sucrose, 90; trehalose, 5; Heps, 5. DL-octopamine, DL-propranolol, dopamine, metanephrine, serumphine, serotonin and tyramine were obtained from Sigma Chemical Co. and the peptides AKH1, AKHII, proctolin and FMRFamide were obtained from Peninsula Laboratories. We acknowledge the following gifts: phentolamine from CIBA-GEIGY Ltd, methysergide hydrogenmaleinate from Sandoz Ltd, and D-octopamine from Dr J. S. Kennedy, Department of Health and Human Services, Rockville, MD, USA.

Statistics

Significance was assessed with a Student's t-test, and data were considered significant throughout this study at \( P<0.05 \).

Results

Effects of octopamine on the stretch receptor

DL-Octopamine, injected into the haemolymph of almost intact locusts, cause
an increase in the response of the SR to imposed wing movements. In the example shown in Fig. 2, the SR responded to 4 Hz sinusoidal wing movements for a period of more than 1 h with 1 spike per wing depression, which did not change after injection of 10 µl of saline into the haemolymph. To demonstrate the constancy of this response, five oscilloscope sweeps were superimposed (Fig. 2A). A similar result was obtained in more than 20 such control experiments. Following the injection of DL-octopamine (final concentration, 5×10⁻⁷ mol l⁻¹), the SR response was modulated and the SR occasionally discharged at 2 spikes per wing depression.

Fig. 2. Effect of DL-octopamine on the SR response to sinusoidal wing movements in an intact locust. Upper traces: extracellular recording from the forewing SR. Lower traces: sinusoidal signal of the function generator used to drive the pen motor. Upward deflections, wing elevation; downward deflections, wing depression. (A) SR response after injection of 10 µl of isotonic saline. Five traces were superimposed. There is no variation in the number of SR spikes or in the timing of the SR spikes. (B) SR response after injection of 10 µl of 10⁻⁵ mol l⁻¹ DL-octopamine into the animal [estimated final concentration, (EFC) 5×10⁻⁷ mol l⁻¹]. Only one oscilloscope sweep is shown. The SR sometimes responded with two spikes to the same stimulus as in A. (C) SR response after injection of 10 µl of 10⁻³ mol l⁻¹ DL-octopamine into the animal (EFC 5×10⁻⁵ mol l⁻¹). One oscilloscope sweep is shown. The SR always responded with two spikes. The interval between these two spikes is decreased compared with the interval in B.
(Fig. 2B). If the octopamine level was further increased to a concentration of $5 \times 10^{-5} \text{mol l}^{-1}$, the same wing movements evoked, over several minutes, 2 spikes per wing depression (Fig. 2C). Besides an increase in the number of spikes per wing depression, we also observed a shortening of the spike interval within each burst. The interval between two spikes decreased from $50 \pm 1.2 \text{ms}$ at a concentration of $5 \times 10^{-7} \text{mol l}^{-1}$ (Fig. 2B) to $33 \pm 1.3 \text{ms}$ at a concentration of $5 \times 10^{-5} \text{mol l}^{-1}$ octopamine (Fig. 2C). It must be emphasized that the concentrations are estimated values, assuming that an adult locust contains 200 $\mu\text{l}$ of haemolymph and that the $10 \mu\text{l}$ of injected drug was equally diluted in this volume. This is almost certainly a conservative estimate for older animals.

To demonstrate and later quantify the changes in SR activity over longer periods, the number of spikes was plotted in sequential spike histograms (Fig. 3).

![Sequential spike histograms](image)

**Fig. 3.** Sequential spike histograms demonstrating the effect of dl-octopamine on the SR response to sinusoidal wing movements in an intact locust. (A) The SR response was increased after injection of $10 \mu\text{l}$ of $10^{-5} \text{mol l}^{-1}$ dl-octopamine (EFC $5 \times 10^{-7} \text{mol l}^{-1}$). SR activity reached a peak approximately 1.5 min after dl-octopamine injection and declined gradually over the next 14 min. (B) The SR response after injection of $10 \mu\text{l}$ of $10^{-3} \text{mol l}^{-1}$ dl-octopamine (EFC $5 \times 10^{-5} \text{mol l}^{-1}$). The increase in SR activity developed faster at this higher concentration and did not decline after reaching a peak. (C) Saturation of the SR response. No change in the SR activity occurred after injection of $10 \mu\text{l}$ of $10^{-3} \text{mol l}^{-1}$ dl-octopamine (EFC $5 \times 10^{-5} \text{mol l}^{-1}$). The SR responded very consistently to the sinusoidal wing movements. Bin width for A–C was 2 s.
SR activity usually increased 9–12 s after injection of 10 μl of octopamine into the haemolymph. However, the onset of this increase varied from animal to animal and occurred in a few animals as late as 2 min after injection. The increase in activity usually developed more rapidly at higher concentrations (Fig. 3B) than at lower concentrations (Fig. 3A). After reaching a peak the SR activity gradually declined. More than 15 min was necessary to reach the resting values if low concentrations were injected (Fig. 3A), and more than 30 min for higher concentrations. Such long-lasting responses are not surprising since these experiments were performed in almost intact animals in which the injected octopamine remained in the haemolymph until it was degraded by the animal’s uptake system (see, for example, Evans, 1981). The SR activity became saturated if octopamine was injected over short intervals, probably because of an accumulation of octopamine in the haemolymph. Typically the SR responded very consistently after reaching its activity peak, with a higher discharge rate than in the initial experiment (Fig. 3B). Further octopamine injections were, in such cases, ineffective (Fig. 3C). However, saturation of the SR was mostly avoided as described in Materials and methods.

Many experiments (e.g. Figs 2, 3) were performed in almost intact animals, thereby giving important insights into the question of whether the drugs are effective on the SR in the living animal. However, it was uncertain whether the effects observed were direct or indirect. For example, it is possible that octopamine, circulating in the haemolymph, causes the release of the adipokinetic hormone AKH from the corpora cardiaca (Orchard and Lange, 1985a). Thus, one possibility is that the modulation of the SR observed in the intact animal is not due to octopamine, but indirectly due to released AKH or another released hormone. To exclude such indirect effects we examined the effect of octopamine on the SR in a perfused thorax preparation in which the head, the abdomen, the gut and the entire central nervous system had been removed. Since the exact concentration of octopamine in this perfused preparation was known, it could also serve as a reference to examine whether the concentrations estimated in an intact locust were realistic. In this reduced preparation the SR was modulated by octopamine in exactly the same manner as in the intact animal, and the responses of the SR to sinusoidal wing movements were enhanced following perfusion with octopamine (Fig. 4). The examples in Fig. 4 also demonstrate that the SR responses were modulated when the SR was activated at a frequency of 1 Hz (Fig. 4A) or at a frequency of 18 Hz, as occurs during flight (Fig. 4B).

To examine whether the effects observed in an intact animal and a perfused thorax were quantitatively the same, we obtained dose–response curves for both preparations (Fig. 5). The EC$_{50}$ of $9 \times 10^{-7}$ mol l$^{-1}$ for the dose–response curve obtained in an intact animal was very similar to the EC$_{50}$ of $9.3 \times 10^{-7}$ mol l$^{-1}$ for the dose–response curve obtained in a perfused thorax preparation. This suggests that the dose-dependent modulation of the SR obtained in an intact animal is due directly to octopamine and not indirectly to any other released hormone. The first significant responses of the SR occurred at concentrations of $5 \times 10^{-8}$ mol l$^{-1}$
Fig. 4. Sequential spike histograms demonstrating the effect of DL-octopamine on the SR response to sinusoidal wing movements at two different frequencies. Data were obtained from a perfused thorax preparation. (A) The SR response to passive wing movements at a frequency of 1 Hz. SR activity was increased after perfusion with $10^{-4}$ mol$^{-1}$ DL-octopamine. (B) The SR response to passive wing movements at a frequency of 18 Hz (approximately the flight frequency). SR activity was increased after perfusion with $10^{-4}$ mol$^{-1}$ DL-octopamine. In A and B each point was an average of 10 consecutive stimulation cycles.

Fig. 5. Dose-response curves for the action of DL-octopamine on the SR response to sinusoidal wing movements, obtained in intact locusts ( ● ) and in perfused thorax preparations ( ○ ). Ordinate: percentage increase in the SR response after DL-octopamine injection. Each point represents the mean of the number of determinations indicated. Bars represent standard errors.
octopamine, with apparent maximal effects observed at concentrations of $5 \times 10^{-4} \text{ mol}l^{-1}$ (Fig. 5).

In about 20% of our experiments we found that SR activity was modulated by octopamine in a biphasic manner. The activity initially decreased and then increased after perfusion with octopamine (Fig. 6). Although the activity decrease was never visible for more than 1 min, it is possible that the inhibition extends throughout the entire octopamine perfusion, but is mostly masked by the relatively stronger excitatory effect of octopamine. Consistent with this possibility is the finding that the SR was often slightly excited upon return of saline without octopamine (Fig. 6A). This biphasic response of the SR was observed in intact animals, perfused thorax preparations (Fig. 6A) and completely isolated SR preparations (Fig. 6B), indicating that it is a property of the SR itself. The occurrence of the biphasic response was not dependent on the concentration and was observed at high (Fig. 6A) as well as low (Fig. 6B) octopamine concentrations.

**Specificity of stretch receptor responses**

The specificity of the SR response to octopamine was examined by comparing the effects of a variety of agents known to have effects on insect octopamine receptors (Evans, 1981; Lange and Orchard, 1986; Orchard and Lange, 1985b; Whim and Evans, 1988). Comparing the effects of octopamine before and after the injection of antagonists showed that the SR was antagonized by the $\alpha$-adrenergic antagonist phentolamine, but not by the $\beta$-adrenergic antagonist DL-propranolol (Fig. 7).

The effects of several biogenic amines ($5 \times 10^{-5} \text{ mol}l^{-1}$) on the SR were

![Fig. 6. Sequential spike histograms demonstrating the biphasic modulation of the SR response by octopamine obtained in a perfused thorax preparation (A) and an *in vitro* preparation (B). (A) The SR response to passive wing movements first decreased and then increased after perfusion with $10^{-4} \text{ mol}l^{-1}$ DL-octopamine. Note the slight increase after return of saline without octopamine. (B) The SR response to passive wing movements was biphasically modulated after perfusion with $10^{-6} \text{ mol}l^{-1}$ DL-octopamine.](image-url)
examined (Fig. 8). The naturally occurring isomer of octopamine in the locust is d-octopamine (Goosey and Candy, 1980). d-Octopamine had similar effects on the SR to DL-octopamine \((P=0.6)\) (Fig. 8). Synephrine, the \(N\)-methylated derivative of octopamine, had a significantly weaker effect than octopamine, but a significantly stronger effect than tyramine, a monophenolic amine which has, in contrast to synephrine and octopamine, no hydroxyl group on the \(\beta\)-carbon of its chain. The effect of synephrine was similar to the effect of DL-metanephrine \((P=0.54)\), a derivative of the catecholamine \(L\)-adrenaline. No significant effect on the SR was found for the catecholamine dopamine.

The indolalkylamine 5-hydroxytryptamine (5-HT, serotonin) had, despite its quite different chemical structure from octopamine, a strong effect on the SR. In intact animals the effect of 5-HT was not significantly different from that of octopamine (Fig. 8) and a similar result was found in perfused thorax preparations. In these reduced preparations, \(10^{-5}\) mol \(1^{-1}\) octopamine increased the SR activity by \(31.1\pm19\%\) (s.d., \(N=10\)) and the same concentrations of 5-HT increased the SR activity by \(37\pm19\%\) (s.d., \(N=6\)). This raises the question of whether 5-HT and octopamine act at the same receptor or whether there are two

![Fig. 7. The effect of antagonists on the aminergic modulation of the SR response to sinusoidal wing movements. Data were obtained from intact animals. The effect of an amine on the SR response was measured for the same animal 30 min before and 2 min after the injection of a blocking agent. Histograms represent the mean of the given number of such experiments. Bars represent standard errors. Left: the mean effect of four DL-octopamine injections with different concentrations \([EFCs 5\times10^{-7} mol \cdot 1^{-1} (1), 5\times10^{-6} mol \cdot 1^{-1} (1), 5\times10^{-5} mol \cdot 1^{-1} (2)]\) on the SR response before and after injections of phentolamine \((EFC 5\times10^{-5} mol \cdot 1^{-1})\). Phentolamine considerably reduces the effect of DL-octopamine. Middle: The mean effect of three DL-octopamine injections \((EFC 5\times10^{-5} mol \cdot 1^{-1})\) before and after injections of DL-propranolol \((EFC 5\times10^{-5} mol \cdot 1^{-1})\). DL-Propranolol does not block the DL-octopamine effect. Right: the mean effect of three serotonin injections \((EFC 5\times10^{-5} mol \cdot 1^{-1})\) before and after injections of methysergide \((EFC 5\times10^{-4} mol \cdot 1^{-1})\). Methysergide does not block the serotonin effect.](image-url)
different receptors located on the SR. Two findings suggest that 5-HT acts as an agonist of the octopamine receptor, as has previously been described (Evans and O'Shea, 1978; Whim and Evans, 1988): (1) the 5-HT response was blocked by the α-adrenergic antagonist phentolamine (not shown), but was not blocked by the 5-HT antagonist methysergide (Fig. 7) and (2) 5-HT was in three cases ineffective when the SR was saturated after injection of high doses of octopamine (not shown).

**Effects of peptides on the stretch receptor response**

It is well known that the peptides AKH I and AKH II are released during flight (Orchard and Lange, 1985a; Orchard, 1987). AKH I and II, as well as other peptides with similar structures, have neuromodulatory functions in crustaceans and insects (O'Shea et al. 1984; Nusbaum and Marder, 1988). It was therefore of interest to test for modulation of the SR by AKH I and II, or by other peptides, since many insect muscles that are modulated by biogenic amines are also modulated by peptides (Orchard and Lange, 1987; Evans and Myers, 1986). We examined a possible modulation by AKH I and AKH II at physiological concentrations of $5 \times 10^{-8}$ mol l$^{-1}$. The mean effects in intact animals were $-2.4 \pm 4.6\%$ (s.d.) for AKH I ($N=5$) and $3.3 \pm 11.9\%$ (s.d.) for AKH II. The modulatory action of these peptides was also examined in perfused thorax preparations at, for peptides, very high concentrations of $10^{-6}$ mol l$^{-1}$. The mean effects at these high concentrations were $-11 \pm 10.9\%$ (s.d., $N=4$) for AKH I and $21 \pm 17\%$ (s.d.,

![Fig. 8. The action of biogenic amines on the SR responses to sinusoidal wing movements. Data were obtained from intact animals. The effects of amine injection on the SR response are given as a mean percentage increase. Estimated final concentrations for all compounds were $5 \times 10^{-5}$ mol l$^{-1}$, the numbers indicate the sample size; the bars represent standard errors.](image)
Thus, the effect of these peptides in intact and perfused thorax preparations is very weak, but AKH I tends to decrease the SR response, whereas AKH II tends to increase it. However, these effects were not significant at physiological concentrations \((5 \times 10^{-8}\text{ mol}^{-1})\) and probably also not significant at higher concentrations \((10^{-6}\text{ mol}^{-1})\). No significant modulation of the SR response was found for three other peptides, FMRFamide \((-6\pm17\% \text{ s.d.}, N=5)\), YGGFLRFamide \((3.7\pm6\% \text{ s.d.}, N=5)\) and proctolin \((8.7\pm8.7\% \text{ s.d.}, N=5)\), which were each examined at a concentration of \(5 \times 10^{-8}\text{ mol}^{-1}\).

**Effects of DUM cells on the stretch receptor**

The dose–response curve obtained for the octopamine effect (Fig. 5) suggests that the concentration of octopamine occurring in the haemolymph during flight is sufficient to enhance the response of the SR significantly. However, one source of haemolymph octopamine may be the octopaminergic DUM cells. The possibility that octopamine released from DUM cells has an effect on the SR was examined by stimulating N1, N3 and N5 contralateral to the SR. All three nerves contain axons from DUM cells. Stimulation of nerve N1 modulated the SR response, as shown in Fig. 9. Before N1 stimulation, the SR responded constantly with 1 spike per wing depression (Fig. 9A, five oscilloscope sweeps superimposed). After 15 s of N1 stimulation the same wing movements often evoked 2 spikes per wing depression (Fig. 9B). A modulation of the SR also occurred following N3 and N5 stimulation (Fig. 10). The effect on the SR evoked by DUM cell stimulation was shorter lived than that caused by octopamine injection, ranging from 2 to 3 min (Fig. 10). We found no significant difference in the average increase caused by N3 and N5 stimulation (Fig. 11). However, the effect caused by N1 stimulation was significantly less than the effect caused by N5 and N3 stimulation (Fig. 11). An explanation for this difference might lie in the different number of DUM cells associated with each nerve. Only one DUM cell, DUMDL, leaves through nerve

![Fig. 9. The effect of contralateral N1 stimulation on the SR response to sinusoidal wing movements. Data were obtained from intact animals. Upper traces: extracellular recording from the forewing SR. Lower traces: stimulus, as described for Fig. 1. (A) The SR response before N1 stimulation. Five traces were superimposed. (B) The SR response after N1 stimulation (train: 15 s, individual pulse: 2 ms, frequency: 10 Hz). One oscilloscope sweep is shown.](image-url)
1, whereas at least six DUM cells leave through nerve 3 (one DUM3, two DUM3,4,5 and three DUM3,4) and four DUM cells leave through nerve 5 (two DUM3,4,5 and two DUM5) (numbers according to Watson, 1984). The effects were blocked by injecting phentolamine into the haemolymph (final concentration, $5 \times 10^{-4}$ mol$^{-1}$) (Fig. 11), suggesting that they were mediated by octopamine. Although the data strongly suggest that the effect on the SR was caused by stimulation of DUM cells projecting into the nerve which was stimulated contralaterally, they do not prove this. The stimulation of a whole nerve excites not only DUM cells exiting contralaterally but also various sensory afferents.

![Fig. 10](image-url)  
**Fig. 10.** A sequential spike histogram demonstrating the effect of N3 and N5 stimulation on the SR response to sinusoidal wing movements. Data were obtained from intact animals. For stimulation parameters, see Fig. 8. Bin width: 2 s. (A) The effect of stimulating N5 contralateral to the SR. (B) The effect of stimulating N3 contralateral to the SR. The effect of nerve stimulation was much shorter in duration than the effect caused by octopamine injection (Fig. 3A).

![Fig. 11](image-url)  
**Fig. 11.** Histograms demonstrating the effect of contralateral nerve stimulations on the SR response to sinusoidal wing movements as well as the effect of phentolamine on the modulatory action of N5 stimulation. Data were obtained from intact animals. The effects of nerve stimulation are given as a mean percentage increase in the SR response. The numbers indicate the number of stimulation experiments; the bars represent standard errors. For stimulation parameters, see Fig. 8. Right-hand blocks: the modulatory effect of N5 stimulation was measured 30 min before and 2 min after injection of $10 \mu l$ of $10^{-3}$ mol$^{-1}$ phentolamine.
which, in turn, excite interneurones and other DUM cells within the CNS. This could lead to a release of octopamine from other undefined sources.

Activity of DUM cells during flight

The results described in the previous sections suggest that DUM cell activity contributes to an enhancement of the activity of the SR. For these neurones to contribute to an increase in octopamine level during flight, they must obviously be activated at this time. Although this possibility has often been discussed (e.g. Whim and Evans, 1988; Malamud et al. 1988), it has not previously been directly demonstrated. We investigated the activity of the DUM cells during flight by intracellular recording. Since we were mainly interested in the effects of DUM cells on the forewing SR, recordings were obtained in the mesothoracic ganglion. The structure of three types of mesothoracic DUM cells (Fig. 12) is very similar to the structure of DUM cells previously described for the metathoracic ganglion (Watson, 1984). It was therefore possible to identify these neurones by their anatomical structure. Two of these DUM cell types are known to innervate flight muscles. The DUMDL innervates the dorso-longitudinal muscle 112 (Hoyle et al.
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1980), and approximately five individual cells of the DUM3,4 type innervate the subalar muscle (M99) and four cells of the same type innervate the posterior tergocoxal muscle (M90) (Kutsch and Schneider, 1987). The peripheral target of the DUM3 type is so far unknown.

DUM cells of the morphological type DUMDL, DUM3, DUM3,4 and DUM5 were found to be phasic-tonically activated during flight. The phasic response of the DUM cells always occurred before flight activity was initiated, whether flight was evoked by light-on (Fig. 13B), light-off, frontal wind (Fig. 13C–F), hissing sounds, or by touching the abdomen or the cerci. However, a phasic response was also visible if a sensory stimulus did not evoke flight, as shown for the response to light-on (Fig. 13A). It is therefore very likely that the phasic response at the onset of flight was caused by the flight-initiating sensory stimulus itself. During flight, the DUM cells were tonically active. This tonic activity seemed to be caused by the flight activity itself, since it only appeared during flight (Fig. 13A,B). Both recordings were obtained from the same DUM3,4 cell and the sensory stimulus was in both cases light-on. The tonic activity occurred only if light also evoked flight. DUM3,4 was tonically active even though no further stimulus, such as wind, was given during this flight sequence. The tonic activity occurred in deafferented preparations (Fig. 13B,C,E,F) as well as in intact tethered flying locusts (Fig. 13D). We often observed that the tonic flight-related response of the DUM cells habituated if flight was evoked at short intervals. The tonic response was usually very strong if flight was evoked for the first time (Fig. 13C) and weaker after flight had been evoked several times (Fig. 13B,D,E,F). The DUM cell activity ceased in such cases, often before flight termination (Fig. 13D,E,F).

Discussion

Modulation of the stretch receptor by octopamine

The role of octopamine as a neuromodulator has been extensively studied in insects. Most studies have focused on the peripheral effects of octopamine on various insect muscles, such as the extensor tibiae muscle (Evans and Siegler, 1982; Hoyle and Field, 1983), the dorso-longitudinal muscle (Whim and Evans, 1988; Klaassen and Kammer, 1985), the second tergocoxal muscle (Malamud et al. 1988) and the oviduct visceral muscle (Orchard and Lange, 1985a). Some studies have also dealt with the central effects of octopamine (Sombati and Hoyle, 1984a,b; Stevenson and Kutsch, 1987, 1988; Kinnamon et al. 1984; Claassen and Kammer, 1986). In this study we report, for the first time in insects, that octopamine modulates a primary afference, the wing SR, in the periphery. The response of the wing SR to sinusoidal movements was enhanced in a dose-dependent manner when octopamine was injected into the haemolymph of almost intact animals (Figs 2, 3) or when the SR was perfused with octopamine in isolated thorax preparations (Fig. 4). In about 20% of the preparations the SR was modulated in a biphasic manner (Fig. 6) and the potentiation of the SR response,
Fig. 13. Activity of mesothoracic DUM cells during flight rhythmic activity. Upper traces: intracellular recording, lower traces: extracellular recordings from flight muscles. (A) The response of DUM3,4 (upper trace) to light-on. Rhythmic flight activity was not evoked by this stimulus, as indicated by the EMG from the subalar muscle 129 (lower trace). (B) The response of the same DUM3,4 (upper trace) to the same light stimulus as used in A. Rhythmic flight activity was evoked, as monitored by the EMG from muscle 129 (lower trace). The recording was obtained from a deafferented animal. (C) The activity of DUM3,4 (upper trace) during wind-evoked rhythmic flight activity, as indicated by the simultaneously recorded EMG from muscle 129 (lower trace). The recording was obtained from a deafferented animal. (D) The activity of DUM3,4 (upper trace) in a tethered flying locust, monitored by the simultaneously recorded EMG from muscle 127 (lower trace). (E) The activity of a DUM5 neurone (upper trace) during rhythmic flight activity, monitored by the EMG of muscle 129 (lower trace) in a deafferented preparation. (F) The activity of DUMDL (upper trace) during flight rhythmic activity, monitored by the nerve recording from muscle 112 (lower trace). The recording was obtained in a deafferented preparation. Calibration bar: A, (horizontal) 500 ms/(vertical) 40 mV; B, 500 ms/40 mV; C, 2 s/40 mV; D, 500 ms/30 mV; E, 1 s/50 mV; F, 500 ms/15 mV.
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as described above, was preceded by a brief inhibition (less than 1 min). Biphasic modulatory effects have previously been described for locusts (Robb et al. 1989; Cuthbert and Evans, 1989). In contrast to these reports, however, the occurrence of the biphasic response in the SR does not seem to be concentration-dependent. Further investigations are in progress to find the parameters responsible for the occurrence of the biphasic response in the SR.

Octopamine can cause the release of the peptide AKH from the isolated corpora cardiaca (Orchard and Lange, 1985a; see Orchard, 1987). Thus, one question is whether the SR is directly modulated by octopamine or indirectly by any AKH released by octopamine. Several findings, however, indicate that the SR is directly modulated by octopamine and not by AKH. The SR was modulated by octopamine in a perfused thorax preparation, in which, owing to the absence of the central nervous system (CNS) and the gut, the release of AKH or any other hormone can unequivocally be excluded (Fig. 4). The finding that the dose–response curves and the EC50 values obtained in the intact and the perfused animals were almost identical indicates that the SR modulation observed in the intact animal is due to octopamine and not to AKH or any other hormone (Fig. 5). We also found no significant modulation of the SR when AKH I or AKH II was injected in physiological concentrations of $5 \times 10^{-8}$ mol l$^{-1}$ into the intact animal. Finally, we found that the octopaminergic modulation of the SR response was blocked by phentolamine (Fig. 7), an $\alpha$-adrenergic antagonist known to block octopamine receptors, but which has no effect on AKH receptors on fat body (Orchard et al. 1982). All these findings indicate that the modulation of the SR response in both intact and perfused thorax preparations is due to octopamine and not to AKH.

The data obtained in the intact animal are very useful for estimating the functional significance of the octopaminergic modulation under natural conditions. These data indicate, for example, that the $2 \times 10^{-7}$ mol l$^{-1}$ octopamine circulating in the haemolymph during the first few minutes of flight (Goosey and Candy, 1980) would have access to the SR and enhance its response to passive wing movements (Fig. 5). The SR response was also modulated if the SR was stimulated at a frequency equivalent to the flight frequency (18 Hz) (Fig. 4). The results also suggest that spill-over from DUM cells projecting into nerves 1, 3 and 5 may contribute to the effects of haemolymph octopamine, since contralateral stimulation of DUM axons in these nerves resulted in a similar enhancement of the SR response to passive wing movement (Figs 9, 10, 11). Whilst the duration of the SR effect caused by DUM cell stimulation was shorter than that caused by octopamine injection, this is to be expected in view of the likelihood that DUM cell stimulation only increases the concentration of octopamine in the immediate vicinity of the SR. This local increase in concentration, and concomitant action on the SR, would be expected to decrease relatively quickly as the octopamine was diluted by the haemolymph. The octopamine injected into the haemolymph, in contrast, probably reaches the SR only after dilution by the haemolymph. This octopamine concentration would not be diluted further, but must be degraded
naturally. A further demonstration that the effects observed may be physiological, and functionally relevant, is given by the observation that DUM cells are indeed activated during flight (Fig. 13), and so probably do modulate the SR during this behaviour.

The sensitivity of the SR to octopamine (threshold about $5 \times 10^{-8}$ mol l$^{-1}$, Fig. 5) and the specificity of the SR response to various antagonists and agonists (Fig. 8) were similar to those reported previously for other insect octopamine receptors (Whim and Evans, 1988; Evans, 1981; Malamud et al. 1988; Orchard and Lange, 1985b, 1986; Hidoh and Fukami, 1987). The interpretation of the present results, however, is limited because the specificity studies for D-octopamine, synephrine, metanephrine, tyramine and dopamine were obtained in intact animals in which the drugs were transported via the haemolymph to the SR. In the haemolymph they would be exposed to various enzymes and uptake systems which might inactivate the drugs at different rates. Thus, the different effects of these five drugs might not merely reflect the specificity of the octopamine receptor, but might also reflect different inactivation rates in the haemolymph and differential access to the receptor sites (Evans, 1980, 1981). Although an interpretation of the receptor specificity is limited, some comparisons can be made. The SR was as sensitive to D-octopamine, the naturally occurring isomer of octopamine, as it was to DL-octopamine at $5 \times 10^{-5}$ mol l$^{-1}$, indicating that D-octopamine is the most active isomer in DL-octopamine at this near maximal concentration. D-Octopamine has previously been shown to be the more active isomer in a locust muscle (Evans, 1981). The N-methylated analogue of octopamine, synephrine, acted as an agonist, but was less potent than octopamine. Although this differs from the findings on some octopaminergic receptors (Evans and O'Shea, 1978; O'Shea and Evans, 1979; Orchard and Lange, 1985b; Carlson, 1968) in which synephrine was either more potent than octopamine or equipotent, it is similar to results on others (Harmar and Horn, 1977; Orr et al. 1985). Serotonin was the only agonist tested that was equipotent to octopamine. The question remains as to whether serotonin is really an agonist of the octopamine receptor or whether it acts at another amine receptor. Although the data obtained in this study suggest that serotonin acts as an agonist of the octopamine receptor they certainly cannot exclude the possibility that there are two amine receptors located on the SR. The main reason for this uncertainty lies in the limited information on the pharmacology of insect 5-HT receptors. The fact that methysergide had no effect on the SR response in our experiments (Fig. 7) could, for example, mean that this antagonist does not recognize insect 5-HT receptors. Similarly, the effects of gramine and mianserin are also not useful indicators, since they affect octopamine receptors (Evans, 1980; Orchard and Lange, 1986). Thus, the question of whether the SR has one (octopamine) or two (octopamine and 5-HT) types of receptors remains unresolved. Dopamine did not mimic the effects of octopamine on the SR, as has been observed for octopamine receptors on various muscles (Orchard and Lange, 1985b; Whim and Evans, 1988). Finally, the action of octopamine upon the SR was more sensitive to the $\alpha$-adrenergic receptor antagonist phentol...
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mine than to the \( \beta \)-adrenergic receptor antagonist DL-propranolol (Fig. 7), which is typical of all insect octopamine receptors.

Aminergic modulation of sense organs

The finding that a primary sensory receptor is directly modulated by a biogenic amine is new for insects. However, in the last few years some evidence has accumulated that indicates that primary afferents of other invertebrates are modulated by both biogenic amines and peptides. In crustaceans, various mechanoreceptors are modulated by octopamine and serotonin, as well as by the pentapeptide proctolin (Pasztor and Bush, 1987, 1989; Pasztor and Macmillan, 1988). A sense organ in the crab stomatogastric ganglion is modulated by serotonin (Katz et al. 1988) and in the mollusc *Aplysia* a mechanosensory neurone is modulated by dopamine, acetylcholine, serotonin and FMRFamide (Billy and Walters, 1988). Thus, the finding that sensory receptors are modulated by biogenic amines or peptides seems to be a widespread phenomenon in invertebrate groups. In addition to a direct modulation of the sense organ, there is evidence that sensory transmission is modulated within the CNS. The central responses to wing stimulation in the moth (Claassen and Kammer, 1986), to mechanical stimulation of the leg in the locust (Sombati and Hoyle, 1984b) and to olfactory stimuli in the honeybee (Mercer and Menzel, 1982) are enhanced after injection of octopamine into the CNS. In crustaceans, octopamine presynaptically increases the responses of the lateral giant escape reaction to sensory stimulation (Glanzman and Krasne, 1983).

It is well established that sense organs, in particular proprioceptors, play an important role in patterning motor activity in both vertebrates (eg. Rossignol et al. 1988) and invertebrates (e.g. Wolf and Pearson, 1988). In vertebrates, the activity of proprioceptors is modulated by efferent control (Prochazka et al. 1978). The degree of modulation is variable and depends on the animal’s activity state and behaviour (Prochazka et al. 1977). In insects, proprioceptors are not modulated by efferent control but, based on the results presented in this paper, the interesting hypothesis can be proposed that insect proprioceptors are modulated by hormones which are released according to the animal’s activity state and behaviour. The data presented have demonstrated that the activity of the SR is modulated by octopamine at concentrations which occur in the haemolymph following stress (Davenport and Evans, 1984) or at the onset of flight (Orchard and Lange, 1985a). The SR plays an important role in the control of the wing beat frequency (Reye and Pearson, 1987; Wolf and Pearson, 1988) and so an increase in the activity of the SR caused by an aminergic modulation should have an influence upon wing beat frequency. However, further behavioural experiments are necessary to demonstrate that this increase in the SR activity caused by octopamine during flight is also sufficiently high to alter the flight motor pattern.

The finding that the SR is modulated by octopamine raises the issue of whether modulation of proprioceptors is a general phenomenon for insects. Preliminary experiments suggest that this is the case. Other proprioceptors have also been
found to be modulated by octopamine: for example, the tegula (Ramirez et al. 1989) and the chordotonal organ (J. M. Ramirez, unpublished observation) in the locust wing, as well as some proprioceptors in the cockroach leg (J. M. Ramirez, in preparation).

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