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The association of serotonin with the alimentary canal of the African migratory locust, *Locusta migratoria*: distribution, physiology and pharmacological profile

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Abstract

The association of serotonin with the alimentary canal of *Locusta migratoria* was investigated using immunohistochemistry and high performance liquid chromatography (HPLC) coupled to electrochemical detection. Serotonin-like immunoreactive processes were differentially distributed between and within three regions of the alimentary canal; the foregut, midgut and hindgut. The midgut possessed the most serotonin-like immunoreactive processes, while the hindgut contained only a few immunoreactive processes. Using HPLC coupled to electrochemical detection the serotonin content was highest in the midgut followed by the foregut and hindgut. The physiological response of the midgut to serotonin as well as to the combination of serotonin and proctolin was also examined. It was found that the application of serotonin to the midgut leads to a dose-dependent reduction in tonus of the circular muscles. Serotonin was also able to inhibit a proctolin-induced contraction of the midgut in a dose-dependent manner. The physiological and pharmacological properties of serotonin agonists and antagonists on the midgut were also investigated. The results indicate that α-methyl 5-HT was the most effective agonist leading to a 108% relaxation at 10^{-9} M compared to that caused by the same serotonin concentration. Among several serotonin receptor antagonists tested, mianserin was the most potent. The application of mianserin at 10^{-5} M in combination with 5×10^{-6} M serotonin resulted in a 66% reduction of the serotonin-induced relaxation of midgut muscle. The serotonin antagonist cyproheptadine was less effective leading to a 39% reduction of the 5×10^{-6} M serotonin-induced relaxation. Ketanserin was a weak antagonist.

Keywords: Serotonin; Alimentary canal; Muscle contraction; HPLC; Immunohistochemisry

1. Introduction

The insect alimentary canal with its three distinct regions including the foregut, midgut, and hindgut, is involved in many physiological processes. For example, the midgut is the primary region of the alimentary canal that facilitates the breakdown and absorption of nutrient components (Terra and Ferreira, 1994). The insect foregut and midgut are mainly innervated by the stomatogastric nervous system (SNS) which includes the frontal ganglion, hypocerebral ganglion and ingluvial ganglion (see Hartenstein, 1997). The hindgut receives innervation from the most posterior abdominal ganglion via the proctodeal nerves (Maestro et al., 1998). Midgut endocrine-like cells have been investigated in a number of insect species (Brown et al., 1986; Žitňan et al., 1993; Veenstra et al., 1995). These endocrine-like cells are considered to play a vital role in the regulation of digestive processes including the control of gut motility and enzyme secretion (Lange and Orchard, 1998; Fusé et al., 1999). Morphological studies performed on the secretory granules of endocrine cells in the midgut of the cockroach *Blaberus craniifer* have shown the presence of at least 10 different endocrine cell types (Andries and Tramu, 1985). Serotonin (5-hydroxytryptamine or 5-HT), as a biogenic amine (or indolalkylamine), has a wide range of distribution in the central nervous system (CNS) and in peripheral tissues in both vertebrate and invertebrate species (see Nässel, 1988; Beltz, 1999; Gor-

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idis and Rohrer, 2002). Serotonin has also been regarded as an important neuroactive chemical in the nervous system of many insect species. While it is believed to have both central and peripheral roles only a few studies have managed to define these roles (see Nässel, 1988). Some pharmacological effects on insect heart (Collins and Miller, 1977), salivary glands (Berridge and Patel, 1968), and Malpighian tubules (Maddrell et al., 1991) suggest that serotonin may act as a neurohormone. Indeed serotonin-like immunoreactive neurohaemal structures have been described lying on the neural sheath of ganglia and peripheral nerves (see Nässel, 1988). An extensive serotonin-like immunoreactive neurohaemal complex covers peripheral nerves in a variety of insect species (Davis, 1987; Flanagan, 1984; Nässel and Elekes, 1985). In the blood-feeding bug, *Rhodnius prolixus*, these neurohaemal complexes release serotonin into the haemolymph, where serotonin acts as a neurohormone controling diuresis following a blood meal (Lange et al., 1988; Te Brugge et al., 2002). There is evidence that serotonin modulates activity within the insect brain. This modulatory activity may control the flow of information through conventional synapses, or it may regulate responses of target cells to classical transmitters in the brain (Tyrer et al., 1984; Wurden and Homberg, 1995; Bicker, 1999). Peripherally, serotonin increases the frequency and amplitude of contractions of the visceral muscles of the fore and hindgut of locust, *L. migratoria* and cockroach, *Leucophaea maderae* (Huddart and Oldfield, 1982; Cook and Holman, 1978). In vitro experiments have also shown that serotonin has a strong excitatory effect on the isolated midgut of the stick insect, *Carausius morosus* (see Luffy and Dorn, 1991). Studies on the effect of serotonin on the isolated foregut of *S. gregaria* have revealed weak contraction at low doses ($<10^{-8}$ M) and a dose-dependent relaxation of this tissue when applied at concentrations ranging from 2×10^{-8} to 3×10^{-6} M (Banner et al., 1987a,b).

The current study is primarily focused on the association of serotonin with the alimentary canal of the adult locust, *L. migratoria*. Immunohistochemistry is used to reveal the distribution of serotonin within the alimentary canal, and high-performance liquid chromatography (HPLC) coupled to electrochemical detection is used to quantify the serotonin content of the alimentary canal. In addition, muscle bioassays are performed to determine the physiological effects of serotonin and its agonists and antagonists on midgut circular muscle contraction.

2. Materials and methods

2.1. Animals

Experiments were conducted on mature adult female locusts, *L. migratoria*. These animals were raised in a crowded laboratory colony at the University of Toronto at Mississauga, Canada, kept on a 12:12 h light/dark regime at 30°C, and fed fresh wheat seedlings supplemented with bran and carrots.

2.2. Chemicals

Proctolin was obtained from Peninsula Laboratories (San Carlos, California, USA), and reconstituted in double distilled water to yield a stock solution of 1 mM peptide. The stock solution was aliquoted and stored in a −20 °C freezer. Working dilutions were made from the stock solutions in locust saline. All other Chemicals were purchased from Sigma Chemical Co. (St Louis, MO, USA). Serotonin agonists and antagonists were reconstituted in saline and fresh stocks were used for each preparation.

2.3. Immunohistochemistry

The immunohistochemical protocol was modified from that described by Donini et al. (2001) & Clark and Lange (2002). The head, legs and wings of females of *L. migratoria* were removed and a mid-ventral incision was made through the abdomen and thorax. The locust was pinned to a Sylgard-coated dish such that internal structures were exposed. Freshly prepared fixative consisting of 4% paraformaldehyde in Millonigs phosphate buffer (0.5% NaCl, 1.45% $Na_2HPO_4.2H_2O$, $NaH_2PO_4.H_2O$ at pH 7.3–7.4) was immediately applied for 5–10 min followed by washing in Tris–HCl buffer pH 7.4 (0.6% Tris-base, 0.8% NaCl). The alimentary canal, including foregut, midgut and hingut, was excised in Tris–HCl buffer, and a lateral incision was made so that the alimentary system would form a sheet that was held flat using minuten pins and left overnight at room temperature in fixative. The following day, the majority of fat and trachea were removed. The preparations were incubated in rabbit anti-serotonin antiserum (INCSTAR, Stillwater, MN, USA) diluted 1:1000 in Tris–HCl buffer containing 2% normal goat serum, 0.25% Triton-X 100, 3% skim milk powder and 0.25% bovine serum albumin, for 48 h at 4 °C. The secondary antibody used was affinity purified goat anti-rabbit antibody conjugated to Cy3 (diluted 1:600) (Bio/Can, Mississauga, Canada) for 48 h at 4 °C. Controls were treated similarly, with the exception that the primary antiserum was pre-absorbed with the serotonin BSA conjugate (DiaSorin, Stillwater, MN, USA) for 28–40 h at 4 °C at a resulting concentration of 10 μg/ml. Following the immunohistochemical procedure, preparations were laid flat on glass slides and mounted with methyl salicylate for viewing. Slides were viewed with a Nikon Optiphot 2 Epifluorescence Microscope (Nikon Corporation, Tokyo, Japan). Pictures were taken on the Nikon Microscope with the aid of a Nikon

Microflex UFX-DX photomicrographic attachment (Nikon Corporation, Tokyo, Japan).

2.4. HPLC

Detection and quantification of serotonin in the alimentary canal including the foregut, midgut and hindgut, was performed using HPLC coupled to an electrochemical detector (see Clark and Lange, 2002). The alimentary canal was dissected under physiological saline and placed in 100 μl of HPLC buffer consisting of 75 mM NaH_2PO_4, 0.3 mM sodium octylsulphate, 50 μM EDTA, 2.5% acetonitrile and 4% methanol adjusted to pH 2.75 with orthophosphoric acid. The mixture was sonicated, centrifuged at 14,000***g*** at 4 °C and filtered through a 0.22 μm centrifugal filter prior to injection. Samples were injected onto a Brownlee C_{18}-Spheri 5 HPLC column (4.6 mm × 22 cm), and separated using the mobile phase (HPLC buffer) at a flow rate of 0.7 ml min^{-1}. Serotonin was detected electrochemically using an ESA model 5100 detection system coupled to a model 5010A dual coulometric detector (ESA Inc., Bedford, MA, USA). The first detector was set at 0.075 V to act as a screen, while a guard cell inserted before the injection valve was set at 0.5 V to pre-oxidize the mobile phase. The second detector was set at 0.15 V and the output was recorded on a linear chart recorder. Serotonin was quantified using the external standard method and samples were spiked with serotonin to confirm the identity of the oxidizable substances and as a check for losses.

2.5. Physiological experiments

Midgut tissues from adult female locusts were dissected out in physiological locust saline [150 mM NaCl, 10 mM KCl, 4 mM $CaCl_2$, 2 mM $MgCl_2$, 4 mM $NaHCO_3$, 5 mM HEPES (pH 7.2), 90 mM sucrose, and 5 mM trehalose] via a mid-ventral incision after removal of the head, legs and wings. The luminal contents of the midgut were removed carefully and two pieces of thread were guided through the lumen. Both threads were loosely knotted with one loop attached to a Sylgard coated dish by using minuten pins, and the other at the opposite end was attached to a Grass Force Displacement Transducer (Grass Instruments Model FT03 Quincy, MA, USA) by a short piece of thread. This setup allowed for the monitoring of circular muscle contractions in the preparation. The midgut was kept moistened in a physiological saline bath where the circular muscle contraction was monitored on a 200 mm Flat-Bed, single channel chart recorder (VWR International, Mississauga, ON, Canada), connected to the force transducer through an amplifier. Serotonin and other chemicals were applied by removing half the volume of saline and replacing it with an equal volume of saline containing twice the final concentration of serotonin or other chemicals. The tissue was washed with physiological saline after the addition of chemicals and allowed to rest for approximately 5–10 min between applications. It was important to let the tissue rest between the applications to ensure that the muscle would respond in a consistent manner.

2.6. Cyclic AMP determination

Cyclic AMP content of the locust midgut muscle was examined. For this purpose midguts from adult female locusts were dissected out in physiological locust saline, the gastric caeca were removed and the remaining midgut tissue was cut into six equal pieces. These midgut tissues were incubated for 10 min in 100 μl of saline containing varying concentrations of serotonin and 3-isobutyl-1-methylxanthine (IBMX) (Research Biochemicals INC., Natick, MA, USA). Experiments were terminated by the addition of 500 μl of boiling 0.05 M sodium acetate buffer (pH 6.2) to the tubes and then boiled for a period of 5 min. The samples were sonicated for approximately 15 s using a Branson Sonifier 250 (VWR International, Mississauga, Ontario). The homogenates were centrifuged at 10,000***g*** for 10 min. Cyclic AMP levels were assayed with a suitable aliquot of supernatant using a modified radioimmunoassay kit (Perkin Elmer Life Sciences Inc., Boston, MA, USA). Protein assay was conducted by dissolving the protein pellet in 100 μl of 1 M NaOH for 2 h in a 55 °C water bath. The Bio-Rad (Mississauga, Canada) Protein Assay System was employed for protein determination using a bovine gamma globulin standard (see Bradford, 1976).

3. Results

Immunohistochemical analysis revealed the distribution of serotonin-like immunoreactive processes throughout the alimentary canal of the locust, *L. migratoria*. Serotonin-like immunoreactive processes are evident on all regions of the alimentary canal including the foregut, midgut and hindgut (Fig. 1). Serotonin-like immunoreactive processes are, however, unevenly distributed on the alimentary canal. There is a much denser network of serotonin-like immunoreactive processes on the midgut in comparison to that of foregut and hindgut. In addition, immunohistochemical studies performed on the midgut revealed that serotonin-like immunoreactive processes are also distributed in a differential manner in this tissue (Fig. 2). There is a greater density of serotonin-like immunoreactive processes in the posterior region (Figs. 1C and 2) than in the middle or anterior regions (Fig. 1D, E and 2). The serotonin-like immunoreactive processes over the gastric caeca were found to be of relatively high density (Fig. 1B and 2). The serotonin-like immunoreactive processes in the midgut with processes running in vertical and horizontal parallel lines

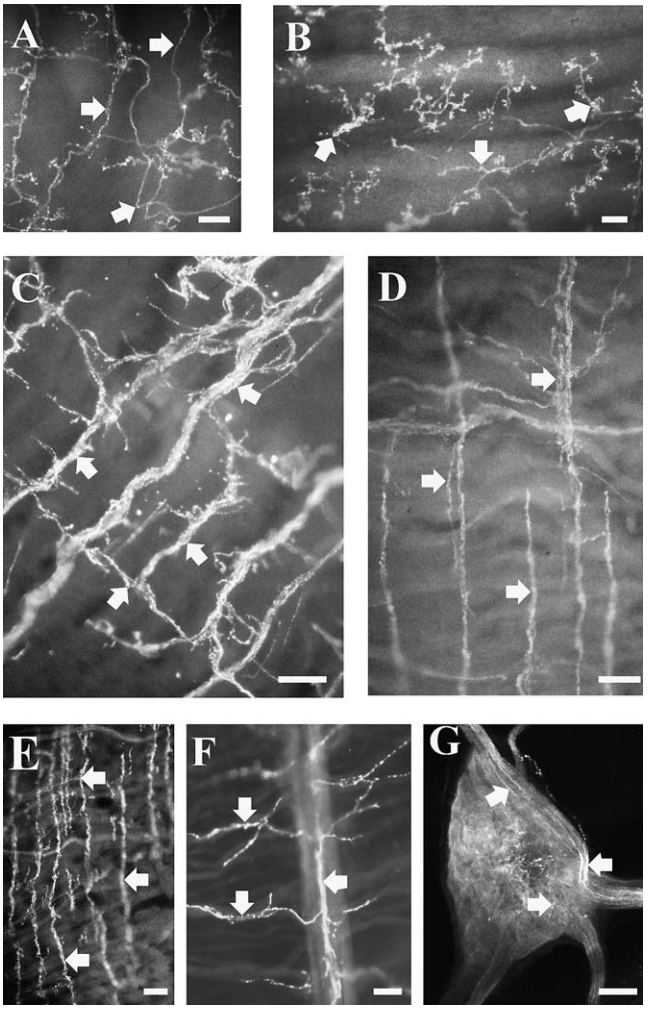

Fig. 1. Micrographs of serotonin-like immunoreactive processes on the alimentary canal of adult female locust, *L. migratoria.* (A) Micrographs of immunoreactive processes (indicated by arrows) associated with the foregut. (B) Micrographs of immunoreactive processes associated with the gastric caeca (indicated by arrows). (C)–(E) show serotonin-like immunoreactive processes (indicated by arrows) in the posterior, middle, and anterior regions of the midgut respectively. (F) Micrograph of immunoreactive processes (indicated by arrows) associated with the hindgut. (G) Whole mount preparation of the ventricular ganglion; Serotonin-like immunoreactive processes were found to be entering this ganglion, arborizing and exiting the ganglion and innervating various regions of the alimentary canal (indicated by arrows). Scale bars on figures represent 30 μm.

form a ladder-like network. The rectangular shapes formed by these lines in the posterior midgut were smaller in area than those found in the middle or anterior midgut. Serotonin-like immunoreactive processes on the locust foregut are shown in Fig. 1A. These processes were distributed in an uneven pattern on this tissue. The density of these processes was low compared to those in midgut. There were less ladder-like shapes formed by the processes on the foregut; only in the middle region of this tissue were lines that formed some irregular rectangular patterns. The serotonin-like immunoreactive processes were also found on the hindgut but in very low density. There was no evidence of rectangular patterns in this region of the alimentary canal. These processes were single processes with short arborization unevenly distributed on the hindgut (Fig. 1F). The ventricular (ingluvial) ganglion located in the foregut region was also found to be immunoreactive (Fig. 1G). Serotonin-like immunoreactive processes were found to be entering this ganglion, arborizing and then leaving the ganglion and innervating various regions of the alimentary canal. No endocrine-like cells associated with the alimentary canal were found to be immunoreactive.

The association of serotonin with the three regions of the alimentary canal including the foregut, midgut, and hindgut of the locust was investigated and quantified using HPLC coupled to electrochemical detection (Table 1). The alimentary canal contained an oxidizable substance with the same retention time as serotonin. Spiking the sample with serotonin increased the area of the oxidizable peak with no evidence of a shoulder, thereby confirming its probable identity as serotonin. The serotonin content of the various regions of the alimentary canal is shown in Table 1. The highest content of serotonin is found in the midgut (minus gastric caeca) followed by the foregut, hindgut and then gastric caecae.

The response of the circular muscle of the midgut of *L. migratoria* to the biogenic amine serotonin was also examined. When applied in vitro, serotonin reduced the tonus of the muscle in a dose-dependent manner (Fig. 3A) with a threshold of 5×10^{-9} M and a maximal effect at 5×10^{-6} M (Fig. 3B). This decrease in tonus was reversible upon washing with physiological saline.

The inhibitory effect of serotonin on proctolin-

Table 1
Serotonin[a] in the alimentary canal of the locust, *Locusta migratoria*

Tissue	*n*	pg/tissue[a]	pg/mg protein
Foregut	6	2255.7±186.4	1340.3±127.1
Gastric caeca	5	1549.8±189.4	650.3±85.3
Midgut[b]	6	3824.8±293.4	2103.1±229.1
Hindgut	7	1782.5±180.6	1244.9±182.7

[a] As measured by HPLC coupled to electrochemical detection.
[b] Midgut minus gastric caeca.

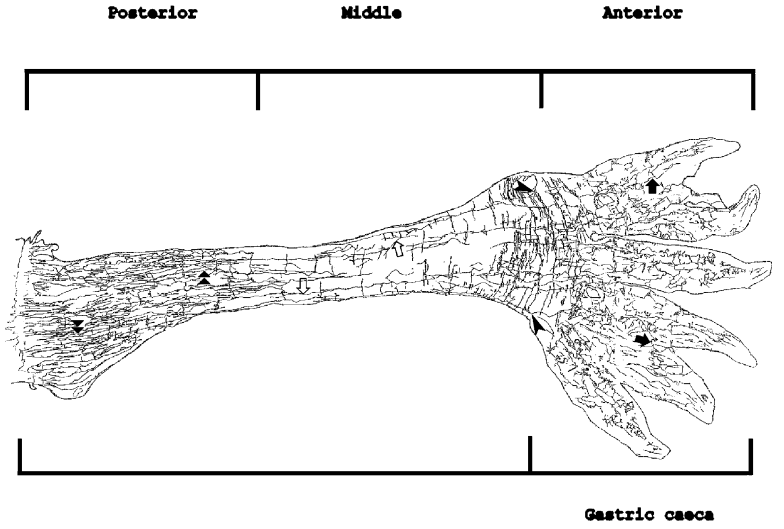

Fig. 2. Composite camera lucida drawing of the serotonin-like immunoreactive processes on the surface of the locust midgut. Midgut tissue displays a differential distribution pattern of serotonin-like immunoreactive processes, with a greater density of processes in the posterior region (double arrowheads). While anterior region of the midgut shows less density of the processes (arrowheads) compare to those on the posterior region, the middle region shows limited density of these processes (open arrows). An extensive network of serotonin-like immunoreactive processes is also present on the gastric caeca (Filled arrows). Composite is representative of 10 different preparations.

induced contractions of the midgut circular muscles is shown in Fig. 4. Application of proctolin to the midgut at 10^{-7} M resulted in a sustained increase in tonus (Fig. 4A), which was reversible upon washing with physiological saline. When serotonin was applied to the midgut concurrently with 10^{-7} M proctolin, there was a dose-dependent decrease in the amplitude of the proctolin-induced contractions with threshold at 10^{-12} M and maximal effect at 10^{-6} M serotonin (Fig. 4B).

Serotonergic receptor agonists were tested for their ability to reduce basal tonus of midgut circular muscle similar to that seen with serotonin. The most potent serotonin agonist, α-methylserotonin, induced relaxation equivalent to 108% that of serotonin when applied at 10^{-9} M (Fig. 5). When locust midguts were challenged with 10^{-8} M, 10^{-7} M, 10^{-6} M and 10^{-5} M concentrations of α-methylserotonin, they induced 69.4%, 74.5%, 84% and 30% the level of the serotonin-induced relaxation at the same concentrations respectively (Fig. 5B). 2,5-Dimetoxy-4-iodoamphetamine (–DOI) and 2-methyl 5-HT were among serotonin agonists examined during this experiment. These agonists induced little or no relaxation in the midgut circular muscle in comparison to the serotonin-induced relaxation (data not shown).

The effectiveness of several vertebrate serotonergic antagonists on the serotonin-induced relaxation of the locust midgut was also investigated (Fig. 6). The most potent serotonin antagonist was mianserin that inhibited the serotonin-induced relaxation by 66% when applied at 10^{-5} M with 5×10^{-6} M serotonin. The second most potent response on the locust midgut was induced by cyproheptadine, which inhibited the serotonin-induced response by 39%. Ketanserin led to a slight inhibition of the serotonin-induced relaxation on locust midgut. No antagonist tested was able to completely inhibit the serotonin-induced relaxation of the locust midgut circular muscle. The serotonin antagonist, metoclopramide had no effect on the serotonin-induced response in midgut.

Radioimmunoassay was used to determine the intracellular cyclic AMP level following the incubation of midgut tissue with serotonin. There was no change in cyclic AMP content at serotonin concentrations ranging from10^{-6} to 10^{-4} M in the presence of 5×10^{-4} M IBMX. There was a slight increase in cyclic AMP content at the highest dose tested, 10^{-3} M (145% of that of control) although this was not statistically significant (data not shown).

4. Discussion

In locust, the visceral muscles of the foregut are innervated by the stomatogastric nervous system (see Nässel, 1988). The hindgut of this insect is innervated by the proctodeal nerves from the terminal abdominal ganglion (Klemm et al., 1986). Both foregut and hindgut display myogenic rhythms modified by neural innervation. There are a number of neurotransmitter candidates that may be involved in controlling the fore and hindgut muscle (see Osborne et al., 1990). Immunohistochemical studies have revealed the innervation of the anterior foregut of *S. gregaria* by serotonin-like immunoreactive nerve fibers (Klemm et al., 1986). There is a ladder-like network of serotonin on the exterior muscle layer of the foregut and midgut in this insect. The immunohistochemical studies performed in the current study

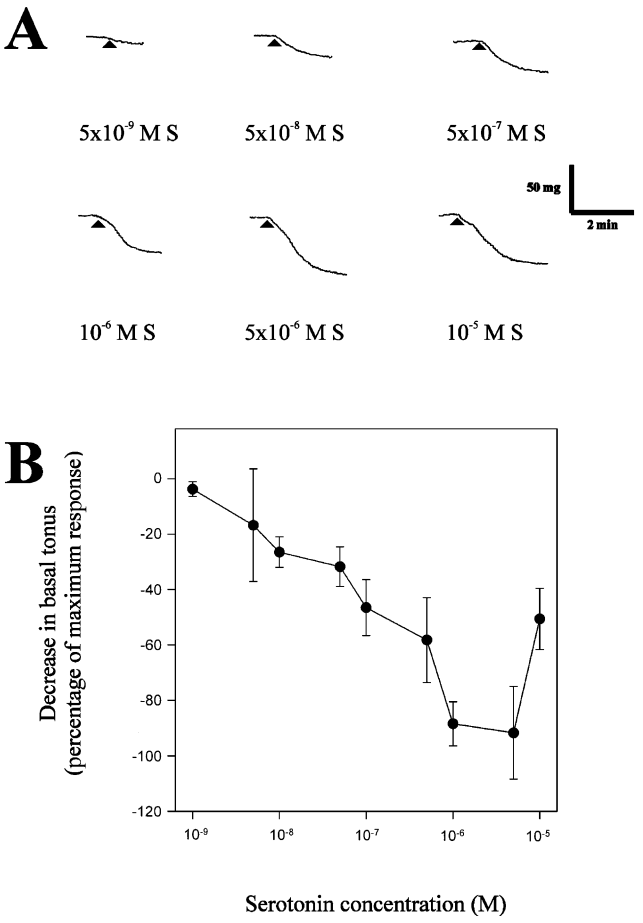

Fig. 3. The physiological effect of serotonin on the basal tonus of the circular muscles of the midgut of the locust, *L. migratoria*. (A) Serotonin (S) was applied to the midgut at varying concentrations (10^{-9} to 10^{-5} M) at the upward triangles. Midgut circular muscle relaxations were monitored by a force transducer and recorded on a chart recorder. (B) Dose–response curve of the effects of serotonin on the basal tonus of the midgut circular muscles. Percent change in basal tonus is measured relative to the maximum change in basal tonus of each preparation. All points are means ± SE of eight preparations

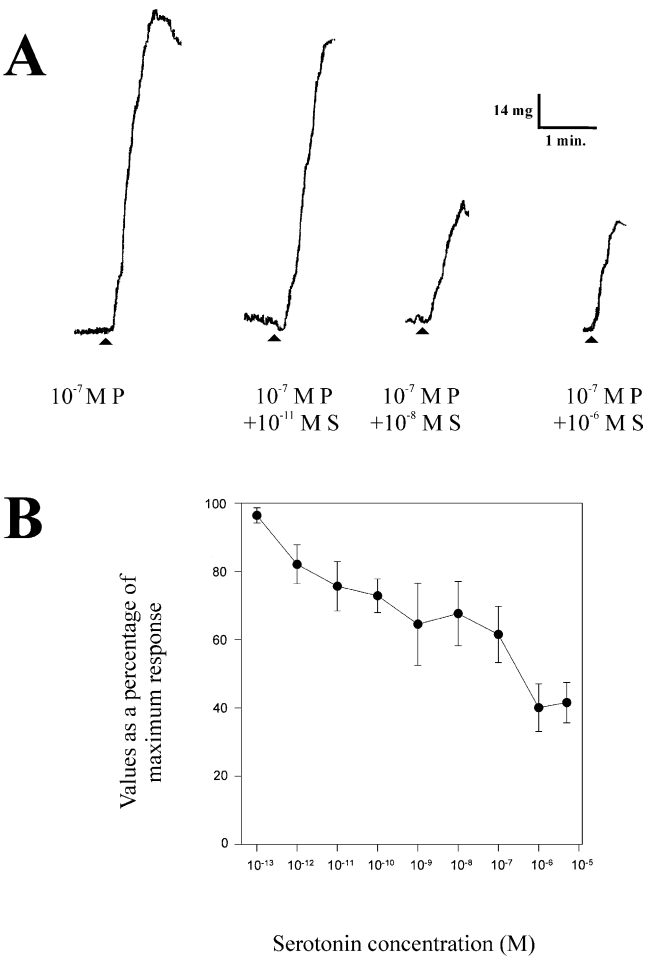

Fig. 4. The physiological effect of serotonin on proctolin-induced contractions of the midgut muscle of the locust, *L. migratoria.* (A) 10^{-7} M proctolin (P) was applied to the midguts alone and in the presence of varying concentrations of serotonin (S) at the upward solid triangles. (B) Dose response curve showing the inhibitory effects of serotonin on 10^{-7} M proctolin-induced contractions of the midguts. Symbols represent percentage of maximum contraction relative to the standard 10^{-7} M proctolin-induced contraction. Symbols represent means ± SE of seven independent preparations.

demonstrate a differential density of serotonin-like immunorective processes on the locust alimentary canal. These processes are abundant in the midgut tissue and form a ladder-like network. The presence of immunoreactive processes was also noticed on the foregut but with less density and in an irregular pattern. The hindgut had the fewest serotonin-like immunoreactive processes. The ventricular ganglion was also found to contain serotonin-like immunoreactive processes in the present study. Studies on the origin of the serotonin-like immunoreactive processes on the gut of *S. gregaria* have shown that approximately 80 serotonin-like immunoreactive cell bodies present in the frontal ganglion send their processes into the recurrent nerve (Klemm et al., 1986; see also Nässel, 1988). The serotonin-like immunoreactive fibres then enter into the neuropil of the occipital (hypocerebral) ganglion where two to four serotonin-like immunorective cell bodies were also found. The serotonin-like immunoreactive fibres continue from the occipital ganglion into the oesophageal nerves and then enter the ventricular ganglion where they arborize and after branching via peripheral nerves innervate the intestine. No serotonin-like immunoreactive endocrine-like cells were observed in the midgut in the present study. In support of the immunohistochemical data is the presence of serotonin in the different regions of the alimentary canal quantified by HPLC coupled to electrochemical detection. The tissue content of serotonin varies in a similar manner to that seen by immunohistochemistry, with the greatest content of serotonin in the midgut and the least in the hindgut.

The insect alimentary canal is suitable for physiological studies in light of substantial information obtained histochemically and immunohistochemically on the innervation and the identification of candidate neuro-

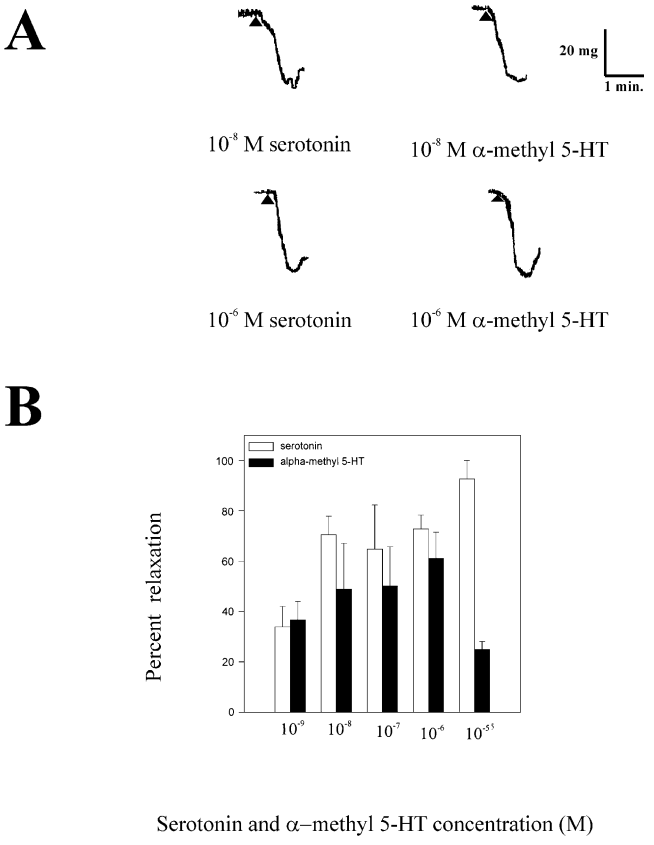

Fig. 5. The physiological effects of serotonin and the agonist α-methyl 5-hydroxytryptamine on midgut relaxation of the locust, *L. migratoria.* (A) serotonin (S), and α-methyl 5-hydroxytryptamine (α-methyl 5-HT) were applied to the midguts at 10^{-8} to 10^{-6} M at the upward triangles. Midgut relaxations were monitored by a force transducer and recorded on a chart recorder. (B) Bars indicate percentage of maximum relaxation relative to a maximum serotonin-induced response. Histogram bars represent mean ± SE (n = 5 to 6).

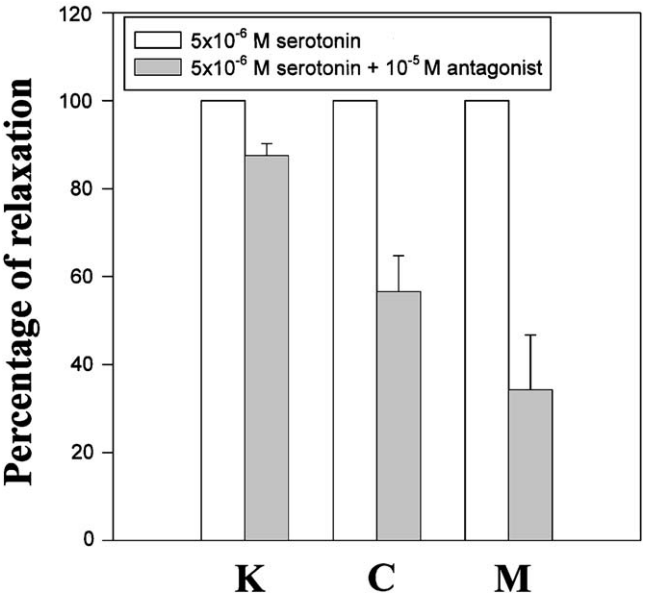

Fig. 6. The effects of different antagonists on serotonin-induced relaxation of the midgut circular muscle of the locust, *L. migratoria.* 5×10^{-6} M serotonin was applied to the midguts alone and in the presence of 10^{-5} M ketanserin (K), cyproheptadine (C), and mianserin (M). Bars indicate percentage of maximum relaxation relative to a 5×10^{-6} M serotonin induced relaxation. Histogram bars represent mean ± SE (n = 4–5).

transmitters. For example, studies have shown that serotonin may have an excitatory effect on insect visceral muscle contraction (see Osborne et al., 1990). Thus Davey (1962) and Colhoun (1963) showed that serotonin leads to the contraction of the isolated hindgut muscle in *P. americana*. In addition, physiological studies conducted on the foregut and hindgut of *L. migratoria* revealed the stimulation of contraction of these muscle tissues by serotonin (Freeman, 1966; Huddart and Oldfield, 1982). However, other studies indicate that serotonin leads to the relaxation of the isolated foregut of *S. gregaria* (Banner et al., 1987a,b). Serotonin, at concentrations ranging from 10^{-8} to 10^{-5} M, caused a dose-dependent relaxation of foregut muscle with maximum response at 3×10^{-6} M, whereas at lower doses (10^{-9}–10^{-8} M) serotonin caused a slight stimulation of contraction (Banner et al., 1987b).

The dose-dependent relaxation of foregut muscle induced by serotonin is similar to that of serotonin on midgut circular muscle in the current paper. Serotonin reduced the tonus of the locust midgut circular muscle in a dose-dependent manner. Maximum relaxation occurred at 5×10^{-6} M serotonin, while 10^{-5} M serotonin was less effective. This latter effect may be due to serotonin receptor desensitization or possibly cross-reaction with a different receptor. Furthermore, in the present study, serotonin was capable of dose-dependently inhibiting a proctolin-induced contraction of locust midgut.

In the present study it was demonstrated that serotonin leads to relaxation of midgut circular muscle. Based on this finding it could be speculated that serotonin may be involved in the control of peristalsis in this tissue and therefore coordinating the movement of food along the gut. In addition, it is possible that serotonin is coordinating other activities that may be considered feeding related. Thus, serotonin has been found to be a diuretic factor in locusts (see Coast et al., 2002). Multiple neuroactive chemicals are likely to be involved in the overall control of feeding-related activities, and while individual chemicals may coordinate multiple aspects, there is sufficient flexibility to allow individual tissues to be activated at times distinct from others (see Coast et al., 2002).

Serotonin acts through multiple receptors to mediate a variety of functions both in the invertebrates and vertebrates (see Tierney, 2001). Physiological studies have confirmed the existence of at least five serotonin receptor families with different second messenger coupling

properties. Most receptor studies and characterizations have been performed on vertebrate systems; whereas a few studies have been performed on the pharmacology of serotonin receptors in insects (see Tierney, 2001). Insect tissues in which serotonin receptors have been characterized include salivary glands of the blowfly, *Calliphora erythrocephala* (Berridge and Heslop, 1981) and locust, *L. migratoria* (Ali and Orchard, 1994), Malpighian tubules of the blood-feeding bug, *R. prolixus* (Maddrell et al., 1991), the foregut of the locust, *S. gregaria* (Banner et al., l987a,b), the corpus cardiacum and ventral nerve cord of the cockroach, *P. americana* (Gole et al., 1987), and the mandibular closer muscles of the cricket, *Gryllus domestica* (Baines and Downer, 1991).

There have been a number of studies on serotonin antagonists in various insect visceral muscles. Banner et al. (1987b) showed that the serotonin receptor mediating tissue relaxation had properties common with mammalian 5-HT_2 receptors. Studies on the serotonin receptors of the blow fly salivary gland revealed the presence of two different serotonin receptors, a 5-HT_1 receptor acting through calcium channel and a 5-HT_2 receptor acting via cyclic AMP (Berridge and Heslop, 1981). In this regard, many serotonin agonists and antagonists have been used to determine the receptor properties of this biogenic amine. Ketanserin (at 10^{-5} M concentration) is reported to be a competitive antagonist of serotonin in the foregut of *S. gregaria* (Banner et al., 1987b). Whereas, mianserin was found to be a more potent antagonist of serotonin-induced relaxation of this tissue (Banner et al., 1987b). One of the reasons for difficulties in physiological characterization of serotonin receptors in different insect tissues may be due to the fact that most pharmacological agents have been developed in the vertebrate systems, and their agonistic or antagonistic activities defined on vertebrate tissues, while vertebrates and invertebrates most probably utilize different receptors in their cellular communications (see Osborne et al., 1990).

The pharmacological effect of serotonin agonists and antagonist was also investigated in the present study. The agonists employed in this study included α-methyl 5-hydroxytryptamine (5-HT_2 receptor agonist), 2, 5-Dimethoxy-4-iodoamphetamine or –DOI (5-HT_2 /5HT_{1c} receptor agonist), and 2-methyl 5-hydroxytryptamine (5-HT_3 receptor agonist). α-methyl 5-hydroxytryptamine was the most potent agonist and led to relaxation of midgut circular muscle comparable to the serotonin-induced response, whereas the other two agonists produced little or no relaxation. Mianserin (5-HT_2 receptor antagonist), cyproheptadine (5-HT_2 /5HT_{1c} receptor antagonist), ketanserin (5-HT_2 /5HT_{1c} receptor antagonist), and metoclopramide (5-HT_3 receptor antagonist) were the antagonists used in the present study. Mianserin was the most potent serotonin receptor antagonist while metoclopramide did not produce any antagonistic effect on the serotonin-induced relaxation. The pharmacological profile of serotonergic antagonists and agonists in the present study suggests the presence of a serotonin receptor similar to vertebrate 5-HT_2 receptor. Banner et al. (1987b) showed that the serotonin receptor mediating tissue relaxation had properties common with mammalian 5-HT_2 receptors. Such physiological effects of serotonin can be mediated through receptors linked to adenylate cyclase.

Serotonin-induced elevations in cyclic AMP content have been noted in the salivary glands of *C. morosus* (Asimakopoulos and Orchard, 1998), *C. erythrocephala* (Litosch et al., 1982) and *L. migratoria* (Ali and Orchard, 1994) and the anterior midgut of *R. prolixus* (Barrett et al., 1993). The increase in cyclic AMP levels can be blocked by serotonin antagonists; indicating that the activation of adenylyl cyclase is via a serotonin receptor. In vertebrates the 5-HT_2 receptor tends to be linked to the phosphatidylinositol second messenger system (Peroutka, 1988). However, many insect serotonergic receptors appear to be linked to adenylate cyclase and yet show characteristics of 5-HT_2 receptor (Berridge and Heslop, 1981; Gole et al., 1987; Barrett et al., 1993; Baines and Downer, 1991). The present study, however, did not reveal a significant increase in the cyclic AMP levels in the midgut tissue in response to serotonin. Although there was a slight increase in cyclic AMP content at 10^{-3} M, this was not significant. There is thus the possibility that the effect of serotonin on locust midgut is mediated via a different second messenger pathway. As discussed earlier, numerous studies have shown the diversity of serotonin receptors in insects and their different intracellular signaling pathways. An adenylate cyclase activity was reported in the homogenates from the thoracic ganglia of cockroach, *P. americana* in response to serotonin (Nathanson and Greengard, 1974). In studies on the salivary glands of the blowfly, inositol triphosphate was reported as an intracellular messenger in response to serotonin (Litosch et al., 1982). The molecular biological studies on *Drosophila melanogaster* have led to the identification of four different serotonin receptors with different second messenger coupling properties (see Tierney, 2001). One of these receptors was positively coupled to activation of adenylate cyclase; two of them inhibited adenylate cyclase in response to serotonin, and activated phospholipase C. Experiments on the fourth cloned receptor have not yet revealed specific transduction properties but it is postulated that this receptor is similar to the vertebrate 5-HT_2 receptor which activates phospholipase C (see Tierney, 2001). Studies on isolated somata from locust thoracic ganglia have also led to the identification of three different serotonin receptor types with different pharmacological properties (Bermudez et al., 1992). Results indicating that serotonin receptors could potentially couple to two different second messenger systems

have been the reason for some conflicting reports about the physiological effects of this biogenic amine in insects (Roeder, 2002).

The results of the present investigation indicate that serotonin is present in the innervation associated with the alimentary canal of *L. migratoria*. Also, serotonin leads to a dose-dependent relaxation of midgut circular muscle. Furthermore agonist and antagonist studies are indicative of the possibility that the relaxation of locust midgut muscle in response to serotonin is modulated via a receptor similar to a vertebrate 5-HT_2 receptor which is not positively coupled to adenylate cyclase.

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