The Journal of Experimental Biology **199**, 699–709 (1996)
Printed in Great Britain © The Company of Biologists Limited 1996
JEB0166

THE UPTAKE AND RELEASE OF SEROTONIN AND DOPAMINE ASSOCIATED WITH LOCUST (*LOCUSTA MIGRATORIA*) SALIVARY GLANDS

DECLAN W. ALI AND IAN ORCHARD
Department of Zoology, 25 Harbord Street, University of Toronto, Toronto, Ontario, Canada M5S 1A1

Accepted 24 October 1995

Summary

The uptake and release characteristics of dopamine and serotonin in the salivary glands of the locust *Locusta migratoria* were examined. Cyclic AMP levels were determined in salivary glands in which the salivary nerve was stimulated under different experimental paradigms. Stimulation of the salivary nerve leads to time- and frequency-dependent elevations of cyclic AMP levels in the glands. The potent and specific D1 receptor antagonist SCH-23390 is capable of partially inhibiting the electrophysiologically induced elevations of cyclic AMP levels. The salivary glands appear to possess uptake transporters for serotonin and dopamine. [^{3}H]serotonin uptake is Na^+-dependent and is composed of high- and low-affinity components. [^{3}H]dopamine uptake is Na^+-independent and can be partially reduced by a challenge with high-K^+ saline and by a challenge with ice-cold saline. Uptake inhibitors are capable of blocking the uptake of radiolabelled serotonin and dopamine. There is a Ca^{2+}-dependent efflux of [^{3}H]serotonin and [^{3}H]dopamine from previously loaded salivary glands in response to stimulation of the salivary nerve and to treatment with a high-K^+ saline.

Key words: salivary glands, insect, dopamine, serotonin, cyclic AMP, uptake, release, locust, *Locusta migratoria*.

Introduction

Locust salivary glands are innervated *via* the salivary nerve, nerve 7b, which is a branch of nerve 7 that originates from the suboesophageal ganglion (Altman and Kien, 1979). Nerve 7b contains the axons of two neurones, SN1 and SN2, whose cell bodies are located within the suboesophageal ganglion (Altman and Kien, 1979). Dopamine has been shown to be present within SN1 (Gifford *et al.* 1991) and serotonin within SN2 (Tyrer *et al.* 1984; Gifford *et al.* 1991; Ali *et al.* 1993). It has therefore been postulated that dopamine and serotonin probably act as neurotransmitters in the salivary glands of the locust. However, in order to demonstrate that a particular chemical is a transmitter substance, a number of criteria must first be fulfilled. For instance, the chemical must be present within the presynaptic neurone: this has been shown for dopamine in SN1 (Gifford *et al.* 1991; Ali *et al.* 1993) and for serotonin in SN2 (Tyrer *et al.* 1984; Gifford *et al.* 1991; Ali *et al.* 1993). The presynaptic neurone must contain the enzymes necessary for synthesis of the proposed neurotransmitter: this is indicated by the positive tyrosine-hydroxylase-like immunoreactivity of SN1 and the salivary gland processes, suggesting the presence of this rate-limiting enzyme for the production of catecholamines within SN1 (Orchard *et al.* 1992; Ali *et al.* 1993), although currently there are no data to indicate the presence of enzymes responsible for the biosynthesis of serotonin within SN2. The chemical must mimic the postsynaptic actions of the natural transmitter: there is evidence to suggest that dopamine and serotonin alter salivary secretory rates (Baines *et al.* 1989) and elevate cyclic AMP levels in a dose-dependent manner (Ali and Orchard, 1994), while stimulation of the salivary nerve also alters secretory rates and elevates cyclic AMP levels in the glands (Ali and Orchard, 1994). Drugs that modify the postsynaptic actions of the natural transmitter should have similar effects upon the actions of the chemical under investigation: we have found that specific dopaminergic and serotonergic receptor antagonists are capable of blocking the dopamine- and serotonin-induced elevation of cyclic AMP levels in the glands and that receptor blockers are capable of partially blocking the nerve-7b-stimulated increase in cyclic AMP levels in the glands (Ali and Orchard, 1994). Other criteria that must be fulfilled include evidence that the chemical is released upon specific stimulation of the neurone and that there exists a mechanism for removal of the chemical. Therefore, if it is to be demonstrated that dopamine and serotonin are true functional neurotransmitters in this system, it must be shown that there is a removal mechanism for these amines associated with the salivary glands. Further evidence for the release of dopamine and serotonin from SN1 and SN2 is also necessary.

Vertebrates have two main mechanisms for removing and inactivating biogenic amines from the synaptic cleft; the first is a high-affinity uptake mechanism in which a transporter translocates the amine into the neurone, while the second is the

use of enzymes, such as monoamine oxidase (MAO) and catechol-*O*-methyl transferase (COMT) that initiate the metabolism of biogenic amines (Cooper *et al.* 1991). Insects do not appear to have large quantities of MAO and it is presumed that an uptake transporter is the primary method of removal of biogenic amines from the synaptic cleft (Evans, 1980). The presence of a high-affinity uptake mechanism for serotonin is well documented for the abdominal nerves of *Rhodnius prolixus* (Flanagan and Berlind, 1984; Orchard, 1989) and in cultured neurones from *Periplaneta americana* (L.) (Bermudez and Beadle, 1989). Similarly, high-affinity uptake mechanisms for octopamine appear to be present in the cockroach ventral nerve cord (Evans, 1978) and in the larval firefly light organ (Carlson and Evans, 1986). Several of these studies have also shown that, once taken up *via* a high-affinity mechanism, radiolabelled amines can be released from these preparations (Orchard, 1989; Carlson and Evans, 1986; Morton and Evans, 1984; Flanagan and Berlind, 1984).

The present study was carried out in order to investigate the characteristics of the release of dopamine and serotonin from SN1 and SN2, and to determine whether uptake transporters for dopamine and serotonin are present in the salivary glands of the locust.

Materials and methods

Insects

Adult male *Locusta migratoria migratorioides* (R. & F.) were taken 6–10 days post-ecdysis from a crowded colony maintained at 30 °C under a 12 h:12 h light:dark regime. Insects were fed daily on freshly grown wheat supplemented with bran.

Cyclic AMP measurements

Salivary glands were dissected under physiological saline (150 mmol l^{-1} NaCl, 10 mmol l^{-1} KCl, 4 mmol l^{-1} $CaCl_2$, 2 mmol l^{-1} $MgCl_2$, 4 mmol l^{-1} $NaHCO_3$, 5 mmol l^{-1} Hepes, pH 7.2, 90 mmol l^{-1} sucrose, 5 mmol l^{-1} trehalose) and assayed for cyclic AMP levels after different experimental treatments. Modified salines used in these treatments included Ca^{2+}-free, high-Mg^{2+} saline (4 mmol l^{-1} $CaCl_2$ replaced with 8 mmol l^{-1} $MgCl_2$), and high-K^+ saline (100 mmol l^{-1} NaCl replaced with equimolar KCl). Salivary glands were incubated in 0.5 mmol l^{-1} isobutyl methylxanthine (IBMX) for 10 min at room temperature along with the appropriate incubation saline with or without pharmacological reagents. At the end of the incubation period, the reaction was terminated by the addition of 500 μl of boiling 0.05 mol l^{-1} sodium acetate buffer, pH 6.2, followed by 5 min of boiling. The samples were stored at −20 °C until cyclic AMP determinations were performed. The samples were thawed, sonicated, centrifuged at 8800 ***g*** for 10 min, and the supernatant removed for cyclic AMP determination while the pellet was dissolved in 50 μl of 0.5 mol l^{-1} NaOH for protein determination. Cyclic AMP levels were determined by radioimmunoassay (Lange and Orchard, 1986) using a commercially available kit (New England Nuclear, Lachine, Quebec, Canada). The protein content of the glands was determined using the Bio-Rad protein assay (Bio-Rad, Richmond, CA, USA) based upon the method of Bradford (1976) using gamma globulin as standard.

Uptake of [^{3}H]amines

Salivary glands were dissected under physiological saline at room temperature and incubated in saline containing either 5-hydroxy[G-^{3}H]tryptamine creatinine sulphate (6.73×10^{11} Bq mmol $^{-1}$) or [2,5,6-^{3}H]dopamine (7.29×10^{11} Bq mmol $^{-1}$) (Amersham, Buckinghamshire, England). Tissues were routinely incubated in 1.85×10^4 Bq amine ml $^{-1}$ for 10 min, except in the time course studies and in release experiments in which longer incubation times were needed in order to assess the release of [^{3}H]amine above background levels. For [^{3}H]dopamine, tissues were incubated in 3.7×10^4 Bq amine ml $^{-1}$ for 10 min since the effects of various treatments on the uptake of [^{3}H]dopamine were more noticeable at concentrations above 1.85×10^4 Bq ml $^{-1}$. In experiments designed to test the effects of ions or uptake inhibitors on the uptake of [^{3}H]amine, tissues were washed for 30 min at room temperature in the ion-free medium (except for high-K^+ saline), or in the presence of the uptake inhibitor, prior to a 10 min incubation in [^{3}H]amine. The various ion-free salines consisted of the following: Na^+ replaced by Tris–HCl for Na^+-free saline; Ca^{2+} replaced by equimolar Mg^{2+} for Ca^{2+}-free saline; NaCl and KCl replaced by equimolar sodium acetate and potassium acetate for the reduced-Cl^- saline; 100 mmol l^{-1} Na^+ replaced by 100 mmol l^{-1} K^+ for the high-K^+ saline. Following incubations, tissues were washed several times for 30 min in saline in order to remove extraneous radioactivity, solubilized overnight in 0.5 ml of BTS-450 tissue solubilizer (Beckman, Mississauga, Ontario, Canada) and dissolved in 10 ml of Econofluor (New England Nuclear). Samples were left overnight to dark-adapt and counted on a Beckman LS60001C scintillation counter. Radioactivity was estimated at a counting efficiency of 43 % and correction for variations in quenching was made by reference to the external standard.

Release of [^{3}H]amines

For release experiments, samples were incubated in 1.85×10^4 Bq amine ml $^{-1}$, washed for 30 min and then placed in 100 μl of a series of solutions consisting of high-K^+ saline (100 mmol l^{-1} K^+ replacing 100 mmol l^{-1} Na^+) or Ca^{2+}-free, high-Mg^{2+} saline, with or without extra K^+, or placed in 200 μl of normal saline or Ca^{2+}-free, high-Mg^{2+} saline, for electrical stimulation of the salivary nerve. The incubation saline from all release experiments was collected after each 5 min of incubation and the radioactivity in the sample was measured. All incubation media were added directly to Ready Caps with Xtalscint (Beckman) and air-dried overnight. The counting efficiency of the Ready Caps was estimated to be 25 % for [^{3}H]serotonin and 16 % for [^{3}H]dopamine.

For HPLC analysis, tissues were incubated in 1.85×10^4 Bq amine ml $^{-1}$ for 1 h, washed for 30 min, extracted

into 100 μl of ice-cold HPLC buffer (75 mmol l^{-1} NaH_2PO_4, 0.3 mmol l^{-1} sodium octyl sulphate, 50 μmol l^{-1} EDTA, 8 % methanol, 5 % acetonitrile adjusted to pH 3.3 with orthophosphoric acid), sonicated, centrifuged at 8800 ***g*** and filtered through a 0.2 μm filter before injection. 10 μl of the extracted gland was added directly to Ready Caps and counted for radioactivity. The injected samples were spiked with 200 pg of serotonin or dopamine and run on HPLC with electrochemical detection as previously described (Ali *et al.* 1993; Elia *et al.* 1994). 1 ml fractions were collected and 200 μl samples were added to Ready Caps to count the radioactivity.

Neurophysiology

Salivary glands were dissected and placed in 200 μl pools of saline containing the appropriate reagents. Nerve 7b was gently sucked into a suction electrode and stimulated at 15 Hz for 5 min with 1 ms square-wave pulses, except for time course and frequency experiments. A second electrode was placed distally to monitor evoked potentials (Fig. 1). Voltages of 0.3–2 V were generally sufficient for recruitment of SN1 and SN2. For stimulation in a Ca^{2+}-free, high-Mg^{2+} saline, glands were washed in the saline for 5 min prior to stimulation. In experiments designed to test the effects of the dopamine receptor antagonist SCH-23390 on cyclic AMP levels, salivary glands were routinely preincubated for 5 min in 10 μmol l^{-1} SCH-23390 prior to 5 min of stimulation. Following stimulation, tissues and perfusate were added to 500 μl of boiling 0.05 mol l^{-1} sodium acetate buffer, pH 6.2, followed by 5 min of boiling. Cyclic AMP levels were determined as described above. Controls for these experiments consisted of salivary glands stimulated with a subthreshold voltage.

To estimate release of [^{3}H]amine, salivary glands were incubated for 1 h in [^{3}H]amine, washed for 30 min in normal saline or 25 min in normal saline and 5 min in Ca^{2+}-free, high-Mg^{2+} saline (for stimulation in a Ca^{2+}-free saline). The tissues were prepared for stimulation as described above and stimulated for 5 min. Each gland functioned as its own control, in which nerve 7b was stimulated with a subthreshold voltage for 5 min. The perfusate was collected on Ready Caps and fresh saline was applied to the glands. Nerve 7b was then stimulated with a supratheshold stimulus for 5 min. The perfusate was collected onto Ready Caps. Salivary glands were collected, solubilized and counted for radioactivity.

Chemicals

Imipramine, clomipramine, nomifensine, buproprion, quipazine, GBR-12909 and (+)-SCH-23390 were obtained from Research Biochemical Inc. (Natick, MA, USA). 5-Hydroxy-[G-^{3}H]tryptamine creatinine sulphate (6.73×10^{11} Bq mmol^{-1}) and [2,5,6-^{3}H]dopamine (7.29×10^{11} Bq mmol^{-1}) were obtained from Amersham, UK. Dopamine, serotonin and IBMX were obtained from Sigma Chemical Co. (St Louis, MO, USA).

Statistics

One-way analysis of variance (ANOVA) with a Duncan's multiple-range and Newman–Keuls statistical test were performed on groups of data within each experiment to ascertain which groups were significantly different from the controls ($P<0.05$). Values given in the text represent means ± S.E.M. and values of N are given in the figure legends.

Results

Cyclic AMP determinations

Stimulation of the salivary nerve, nerve 7b, which contains one dopaminergic axon (from SN1) and one serotonergic axon (from SN2) at 15 Hz for various times resulted in an accumulation of cyclic AMP in the salivary glands. The elevated levels of cyclic AMP reached a plateau after the first 2 min of stimulation (Fig. 2A), resulting in an increase of cyclic AMP over basal levels of 53.6±10 pmol mg^{-1} protein. Salivary glands preincubated in 10 μmol l^{-1} of the dopamine

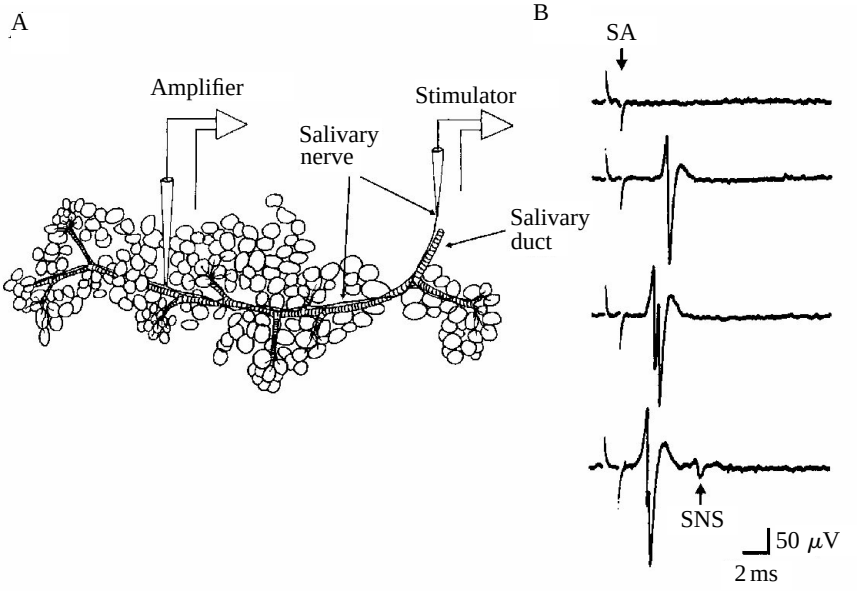

Fig. 1. (A) Schematic drawing of a salivary gland from *Locusta migratoria* prepared for neural stimulation and recording from the salivary nerve, nerve 7b. (B) Stimulation of nerve 7b (SA, arrow) is followed by action potentials from SN1 (second trace), SN1 and SN2 (third trace) and eventually from SN1, SN2 and the satellite nervous system (SNS) (fourth trace) as the stimulating voltage is gradually increased (from top trace to bottom trace).

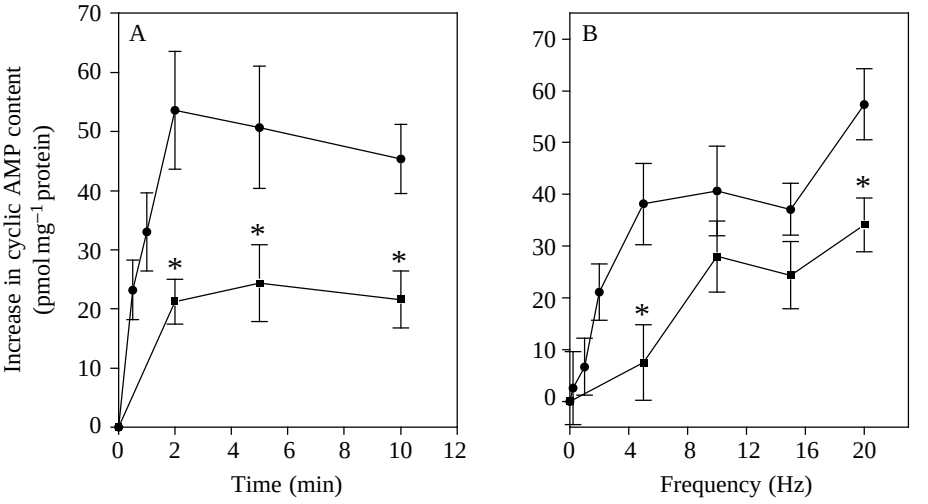

Fig. 2. The effect of neural activity upon the cyclic AMP content of the salivary glands. (A) Time course when nerve 7b is stimulated at 15 Hz with 1 ms square-wave pulses in normal saline (●) or in the presence of 10 μmol l^{-1} SCH-23390 (■). (B) Effect of frequency of stimulation when nerve 7b is stimulated for 5 min in normal saline (●) or in the presence of 10 μmol l^{-1} SCH-23390 (■). An increase in cyclic AMP content represents the increase above that in control tissues. Basal levels were 36.96±2.38 pmol mg^{-1} protein. All values represent means ± S.E.M. of 6–13 determinations. An asterisk marks values that are significantly different from control values (P<0.05).

receptor antagonist SCH-23390 for 5 min experienced smaller elevations of cyclic AMP levels, reaching approximately 22 pmol mg^{-1} protein above basal levels. Such high concentrations of the dopamine antagonist have previously been shown to block completely dopamine-induced elevations of cyclic AMP level in salivary glands, and so it is likely that the difference in cyclic AMP values between the two curves (Fig. 2A) is due to the effects of dopamine. The cyclic AMP levels in the presence of SCH-23390 are probably due to serotonin released by neural stimulation. Serotonergic receptor blockers were not used in these experiments since no blocker was completely effective at inhibiting the effects of serotonin (Ali and Orchard, 1994); thus, differences due to the serotonergic antagonist may not necessarily be an accurate reflection of the effects of serotonin.

To investigate the effects of the frequency of activity of SN1 and SN2 on salivary gland cyclic AMP levels, the salivary nerve was stimulated for 5 min at varying frequencies (Fig. 2B). Cyclic AMP levels were maximally elevated (approximately 40 pmol mg^{-1} protein) at and above a frequency of 5 Hz. Frequencies above 20 Hz were not used since it was difficult to maintain action potential activity above this frequency. In addition, the physiologically relevant level of activity of the salivary neurones rarely exceeds a frequency of 15 Hz (Baines *et al.* 1989), although a recent publication (Schactner and Bräunig, 1995) indicates an average frequency of activity for SN1 (during feeding) of 8–9 Hz and for SN2 of 6–7 Hz. SCH-23390 was capable of reducing the effects of neural stimulation upon cyclic AMP levels. This was particularly noticeable at low frequencies of stimulation (Fig. 2B).

Treatment with high-K^+ saline (50–100 mmol l^{-1}) resulted in elevations of cyclic AMP levels within salivary glands (Fig. 3). Maximum values of 68 pmol mg^{-1} protein were achieved in 100 mmol l^{-1} KCl. Ca^{2+}-free, high-Mg^{2+} saline abolished the effect of elevated [K^+] on cyclic AMP levels (Fig. 3). Ca^{2+}-free, high-Mg^{2+} saline also blocked the increase in cyclic AMP levels induced by electrical stimulation of the salivary nerve (Fig. 4A), suggesting that this saline either inhibits the release of amines from SN1 and SN2 or blocks the cyclic AMP transduction mechanism. To test the viability of the cyclic AMP transduction mechanism, we examined the ability of dopamine and serotonin to elevate cyclic AMP levels in tissues incubated in a Ca^{2+}-free, high-Mg^{2+} saline (Fig. 4B). Dopamine and serotonin (0.5 μmol l^{-1}) were able to elevate cyclic AMP levels in the glands, suggesting that the inhibition in stimulated preparations is probably due to a requirement for Ca^{2+} to mediate the release of dopamine and serotonin from SN1 and SN2.

Uptake of [^{3}H]serotonin and [^{3}H]dopamine

Salivary glands incubated in tritiated serotonin and dopamine (0.5 μmol l^{-1}) accumulated [^{3}H]amine over a period of 3 h in which maximum accumulation was attained after the first 60 min of incubation (Fig. 5). For [^{3}H]serotonin, a maximum accumulation of 2.58±0.25 pmol per salivary gland

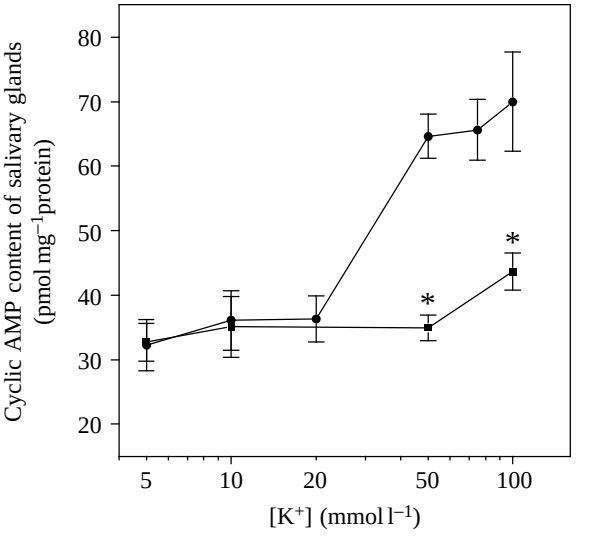

Fig. 3. The effect of [K^+] (●) on the cyclic AMP content of locust salivary glands. The effect of [K^+] in saline containing 0 mmol l^{-1} Ca^{2+}, 8 mmol l^{-1} Mg^{2+} is also shown (■). Values represent means ± S.E.M. of 6–12 determinations. An asterisk marks values that are significantly different from control values (P<0.05).

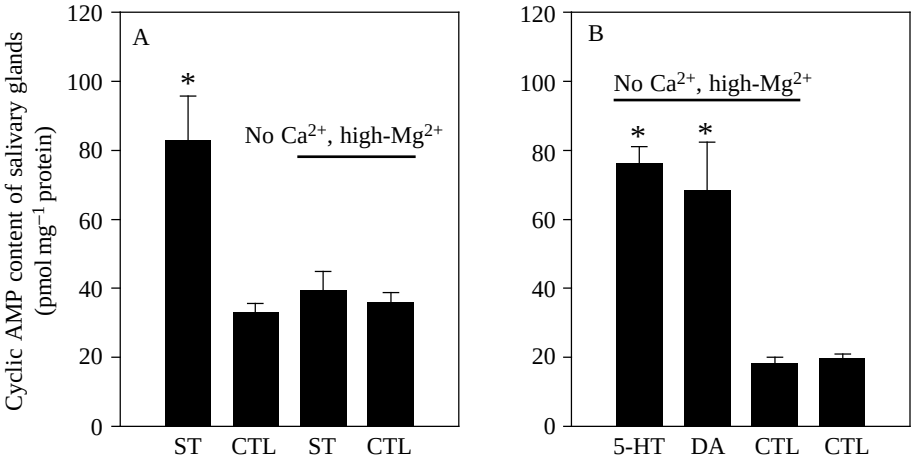

Fig. 4. The cyclic AMP content of locust salivary glands (in pmol mg^{-1} protein) is shown in response to (A) neural stimulation (15 Hz for 5 min) in normal saline and in 0 mmol l^{-1} Ca^{2+}, 8 mmol l^{-1} Mg^{2+} saline (bar) and in response to (B) 5×10^{-7} mol l^{-1} serotonin (5-HT) or dopamine (DA) in 0 mmol l^{-1} Ca^{2+}, 8 mmol l^{-1} Mg^{2+} saline (bar). ST, neural stimulation; CTL, control group. Values represent means + S.E.M. of seven determinations. An asterisk marks values that are significantly different from control values (P<0.05).

was achieved, whereas the maximum for [^{3}H]dopamine was 1.58±0.11 pmol per salivary gland.

To investigate the Na^+-dependence of the uptake mechanism, glands were incubated for 10 min at room temperature in either normal or Na^+-free (Na^+ replaced by Tris–HCl) saline over a concentration range of 0.05–50 μmol l^{-1} [^{3}H]serotonin (Fig. 6A) and dopamine (Fig. 6C). The Na^+-specific uptake shown in Fig. 6A (filled triangles) is calculated by subtracting the Na^+-sensitive uptake from the Na^+-insensitive uptake over the complete concentration range. At lower concentrations (0.05–0.5 μmol l^{-1}) the Na^+-specific uptake represents approximately 67 % of the total uptake, whereas at higher concentrations (0.5–50 μmol l^{-1}) the Na^+-specific uptake represents approximately 32 % of total values. An Eadie–Hofstee plot of the Na^+-specific uptake of [^{3}H]serotonin (Fig. 6B) suggests the presence of two separate uptake mechanisms with different affinities for serotonin. The high-affinity component appears to have a maximum rate of uptake of 1.24 pmol salivary gland^{-1} 10 min^{-1} and an apparent K_m of 0.74 μmol l^{-1}. The low-affinity component has a maximum rate of uptake of 7.75 pmol salivary gland^{-1} 10 min^{-1} and an apparent K_m of 30 μmol l^{-1}.

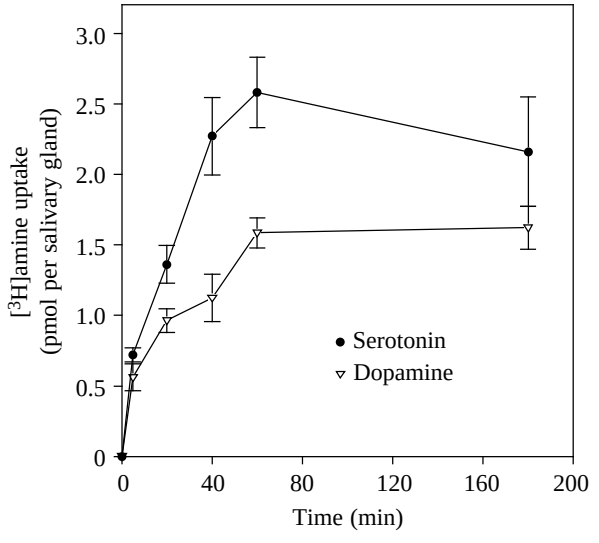

Fig. 5. Time course of uptake of serotonin (●) and dopamine (▽) by locust salivary glands from saline containing 5×10^{-7} mol l^{-1} amine. Values represent means ± S.E.M. of five determinations.

Interestingly, the uptake of [^{3}H]dopamine was not affected by a Na^+-free saline, as shown in Fig. 6C. To investigate further the uptake mechanisms of dopamine and serotonin, we examined the ability of different ions to affect the uptake of [^{3}H]amine.

Ice-cold saline, Na^+-free saline and a combined Na^+- and Ca^{2+}-free saline were capable of reducing the uptake of [^{3}H]serotonin in locust salivary glands. The total accumulation of radiolabel in tissues incubated in 0.5 μmol l^{-1} [^{3}H]serotonin for 10 min was 703±29 fmol per salivary gland compared with that of glands incubated in ice-cold saline of 67±16 fmol per salivary gland, in Na^+-free saline of 250±29 fmol per salivary gland and in a Na^+- and Ca^{2+}-free saline of 256±46 fmol per salivary gland. Ca^{2+}-free saline had no effect on the ability of glands to accumulate [^{3}H]serotonin. Similarly, a reduced-Cl^- (12 mmol l^{-1} Cl^- instead of 172 mmol l^{-1} Cl^-) and a high-K^+ (100 mmol l^{-1} K^+) saline had no significant effect on the uptake of [^{3}H]serotonin in a 10 min incubation period (Fig. 7A). The effects of ions on [^{3}H]dopamine uptake were different from those on serotonin uptake. An ice-cold saline reduced the [^{3}H]dopamine (1 μmol l^{-1}) uptake from control levels of 1190±119 fmol per salivary gland to 579±62 fmol per salivary gland, a reduction of 51 % (Fig. 7B). The only other saline capable of affecting the uptake was high-K^+ saline, which reduced the accumulation to 667±101 fmol per salivary gland, representing an inhibition of 44 % from control levels.

The effects of a number of uptake inhibitors on the uptake of [^{3}H]serotonin are shown in Fig. 8A. The specific mammalian serotonin uptake inhibitor quipazine, the tricyclic antidepressants imipramine and clomipramine, and the dopamine uptake inhibitors GBR-12909 and nomifensine, were capable of blocking the uptake of [^{3}H]serotonin, whereas the other dopamine uptake inhibitor, buproprion, was ineffective (Fig. 8A). At a concentration of 50 μmol l^{-1}, quipazine was the most effective uptake inhibitor, inhibiting the serotonin uptake by 79 %, while imipramine was as

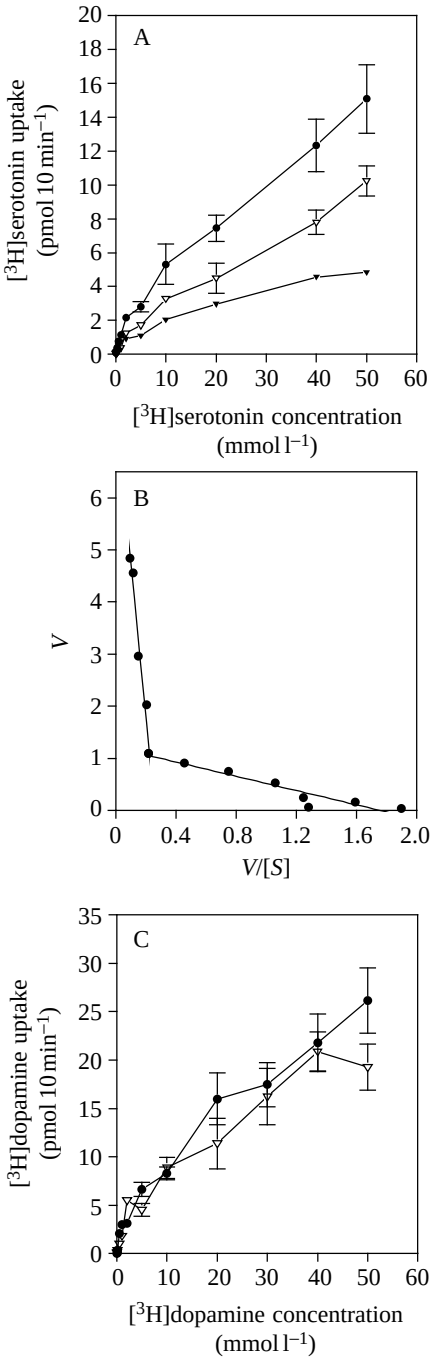

Fig. 6. (A,C) The rate of uptake of serotonin and dopamine into the salivary glands (V in pmol 10 min^{-1}) is plotted against serotonin ([S] in mmol l^{-1}) and dopamine concentration in the bathing medium. The filled circles represent total uptake in normal saline and the open triangles represent the Na^+-insensitive uptake in Na^+-free saline. The Na^+-sensitive uptake component, represented by the filled triangles in A, was obtained by subtracting the Na^+-insensitive component from the total uptake rate at each serotonin concentration. Points represent means ± S.E.M. of five determinations. (B) An Eadie–Hofstee plot for the Na^+-specific uptake component of serotonin into the salivary glands. Lines were drawn using a first-order linear regression for each uptake component. High-affinity component: r^2=0.91, P<0.05; low-affinity component: r^2=0.97, P<0.05.

effective as a Na^+-free saline, reducing the uptake of radiolabel from control levels of 801±36 fmol per salivary gland in normal saline to 260±24 fmol per salivary gland, representing a 68 % inhibition of total uptake and a 90 % inhibition of Na^+-specific uptake. Clomipramine (50 μmol l^{-1}) was also an effective inhibitor, blocking the specific uptake of [^{3}H]serotonin by 89 %. GBR-12909 blocked 89 %, and nomifensine 74 %, of the Na^+-specific uptake, whereas buproprion had no effect on the uptake of serotonin in locust salivary glands. The tricyclic antidepressants and the dopamine uptake inhibitors were all capable of reducing the uptake of [^{3}H]dopamine (1 μmol l^{-1}) in glands incubated in 50 μmol l^{-1} inhibitor (Fig. 8B). The most potent inhibitor was imipramine, which inhibited the total uptake by 70 %. Clomipramine had a similar potency and reduced the uptake from 1563±208 to 534±123 fmol per salivary gland, which represents an inhibition of 66 %. The selective dopamine uptake inhibitor GBR-12909 was as effective as imipramine and clomipramine and reduced the uptake levels by approximately 67 %. The other dopamine uptake inhibitors, buproprion and nomifensine, were slightly less effective, reducing the uptake levels to 965±174 and 966±222 fmol per salivary gland, respectively. The selective serotonin uptake inhibitor quipazine had no effect on the dopamine uptake rate in locust salivary glands. The effects of imipramine and GBR-12909 were not significantly different from that of ice-cold saline, in which the uptake of [^{3}H]dopamine was reduced to 808±91 fmol per salivary gland (Fig. 8B).

Release of [^{3}H]amine

To investigate the possibility that accumulated amines are cycled into a releasable pool, tissues previously incubated in tritiated amines were incubated for 5 min in various solutions consisting of either 100 mmol l^{-1} K^+ or Ca^{2+}-free, high-Mg^{2+} saline with or without 100 mmol l^{-1} K^+. Salivary glands were capable of releasing previously accumulated [^{3}H]serotonin in response to high-K^+ saline, as shown in Figs 9 and 10. When salivary glands were previously loaded for 1 h in [^{3}H]serotonin, they were capable of releasing approximately 36 fmol of tritium above background levels when incubated in a high-K^+ saline (the 45–50 min period compared with the 40–45 min period), while a second consecutive 5 min wash in 100 mmol l^{-1} K^+ induced an efflux of 34 fmol of tritium (the 50–55 min period compared with the 40–45 min period; Fig. 9A). The total release of tritium was 3.7 % of the final content of the glands (1.86±0.13 pmol per salivary gland). Glands preincubated for 1 h in [^{3}H]dopamine released approximately 80 fmol of tritium above background levels in the first 100 mmol l^{-1} K^+ wash. The second consecutive 5 min 100 mmol l^{-1} K^+ wash was unable to induce an efflux of tritium above background levels: 95.3±10.2 fmol per salivary gland in the 50–55 min period compared with 98.4±21.2 fmol per salivary gland in the 40–45 min period. The total release of tritium was 8.5 % of the final ^{3}H content of the glands (0.94±0.17 pmol per salivary gland).

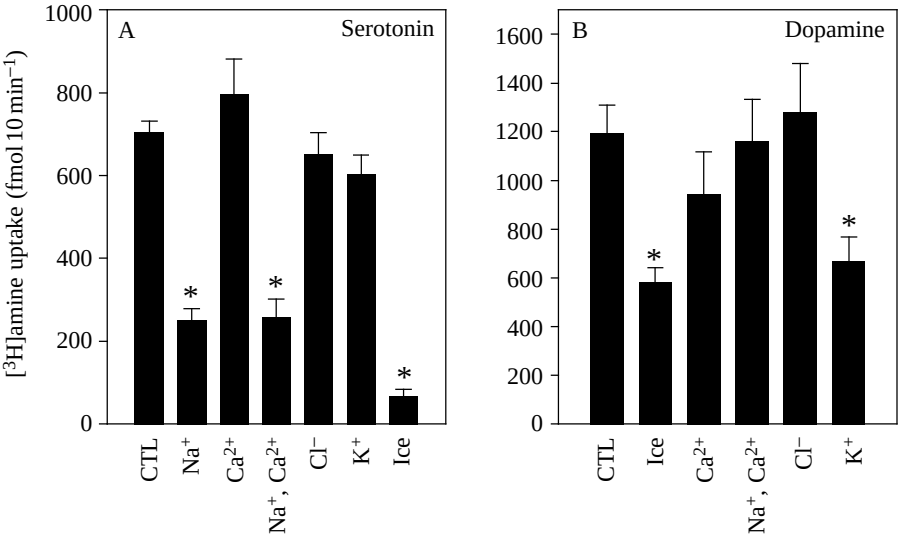

Fig. 7. Effect of ions and an ice-cold saline on the accumulation of [^{3}H]amine by salivary glands from saline containing 5×10^{-7} mol l^{-1} serotonin (A) or 10^{-6} mol l^{-1} dopamine (B). Controls (CTL) represent the accumulation of [^{3}H]amine in normal saline at room temperature for 10 min. Na^+, Na^+-free; Ca^{2+}, Ca^{2+}-free; Na^+, Ca^{2+}, Na^+- and Ca^{2+}-free; Cl^-, reduced-Cl^- (reduced from 172 to 12 mmol l^{-1}); K^+, 100 mmol l^{-1} K^+; Ice, ice-cold saline. Values represent means + S.E.M. of six determinations. An asterisk marks values that are significantly different from control values (P<0.05).

In a similar experiment, when salivary glands were preincubated for 3 h in [^{3}H]serotonin, washed, and then incubated for 5 min in various solutions, approximately 120 fmol per salivary gland of radiolabel, above background levels, was released during the 45–50 min period (Fig. 10). A second incubation in high-K^+ saline also induced about 120 fmol per salivary gland of release. Ca^{2+}-free, high-Mg^{2+} saline was capable of preventing the release of radiolabel, as shown during the 35–40 min incubation period in Fig. 10A. The total release of 240 fmol per salivary gland was approximately 11.2 % of the final ^{3}H content of the glands (2.16±0.39 pmol per salivary gland). Similar results were obtained for glands incubated in [^{3}H]dopamine. A high-K^+ saline was effective at inducing a release of radiolabel of approximately 66 fmol per salivary gland above basal levels (Fig. 10B). Ca^{2+}-free, high-Mg^{2+} saline abolished the effects of 100 mmol l^{-1} K^+, as shown in Fig. 10B. The release of radiolabel during the complete 10 min incubation period in 100 mmol l^{-1} K^+, was 8 % of the final [^{3}H]dopamine accumulated by the salivary glands (1.62±0.15 pmol per salivary gland).

To examine the ability of SN1 and SN2 to release [^{3}H]serotonin and [^{3}H]dopamine from previously loaded stores, salivary glands were incubated in 0.5 μmol l^{-1} [^{3}H]amine, washed for 30 min and then nerve 7b was electrically stimulated. The perfusate from stimulated preparations contained 39 fmol per salivary gland of [^{3}H]serotonin and 41 fmol per salivary gland of [^{3}H]dopamine, above the levels of the non-stimulated preparations (Fig. 11A,B). These values represent 2.8 % of the total [^{3}H]serotonin content and 4.2 % of the total [^{3}H]dopamine content of the salivary glands. Similar experiments in a Ca^{2+}-free, high-Mg^{2+} saline showed no significant difference between the efflux levels of stimulated and unstimulated preparations for both amines (Fig. 11A,B).

An HPLC analysis of loaded salivary glands revealed that approximately 100±16 % of the ^{3}H label accumulated from [^{3}H]dopamine co-eluted with dopamine, while 94±9 % of the label accumulated from [^{3}H]serotonin co-eluted with serotonin. These results suggest that the amines are not metabolised within the first 1.5 h after uptake is initiated (1 h incubation period plus 30 min wash).

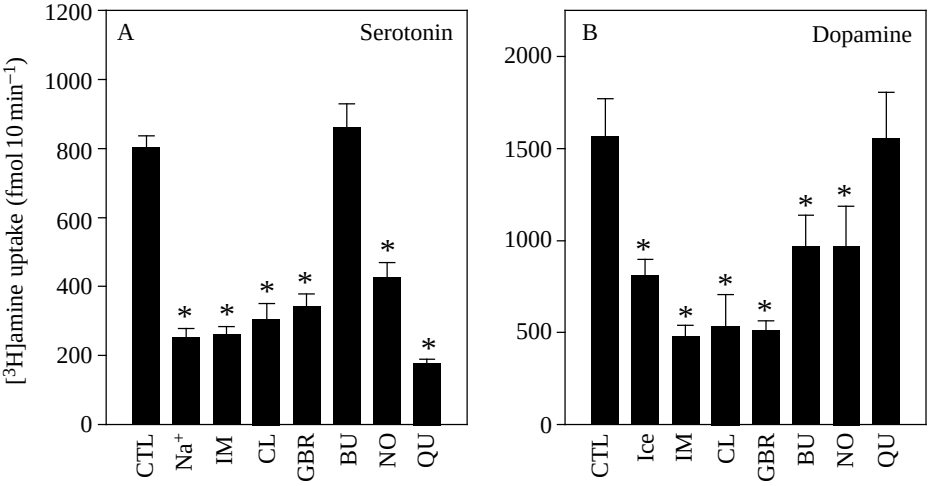

Fig. 8. Effects of uptake inhibitors (5×10^{-5} mol l^{-1}) on the accumulation of [^{3}H]amine by salivary glands from saline containing 5×10^{-7} mol l^{-1} serotonin (A) or 10^{-6} mol l^{-1} dopamine (B). Control (CTL) represents uptake of amine in normal saline for 10 min. Na^+, Na^+-free saline; Ice, ice-cold saline; IM, imipramine; CL, clomipramine; GBR, GBR-12909; BU, buproprion; NO, nomifensine; QU, quipazine. Values represent means + S.E.M. of six determinations. An asterisk marks values that are significantly different from control values (P<0.05).

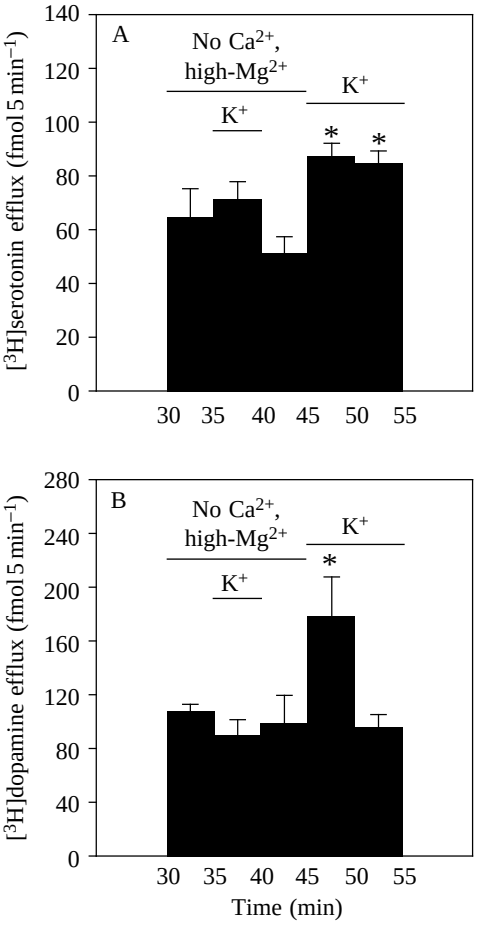

Fig. 9. Depolarization-induced release of serotonin (A) and dopamine (B) from salivary glands. Salivary glands were incubated in 5×10^{-7} mol l^{-1} [^{3}H]serotonin or [^{3}H]dopamine for 1 h, washed in saline several times for 30 min and then incubated for 5 min in various solutions. There was a significant increase in the efflux of tritium induced by 100 mmol l^{-1} K^+ saline when Ca^{2+} was present (45–50 min period) but not in the absence of Ca^{2+} (35–40 min period). A second consecutive exposure to 100 mmol l^{-1} K^+ saline released an equivalent amount of tritium to the first exposure in glands incubated in serotonin. No tritium was released from glands incubated in dopamine during the second exposure to 100 mmol l^{-1} K^+. Values represent means + S.E.M. of six determinations. Final amine contents of salivary glands were 1.86±0.13 pmol of [^{3}H]serotonin and 0.94±0.17 pmol of [^{3}H]dopamine for A and B, respectively. An asterisk marks values that are significantly different from control values (P<0.05).

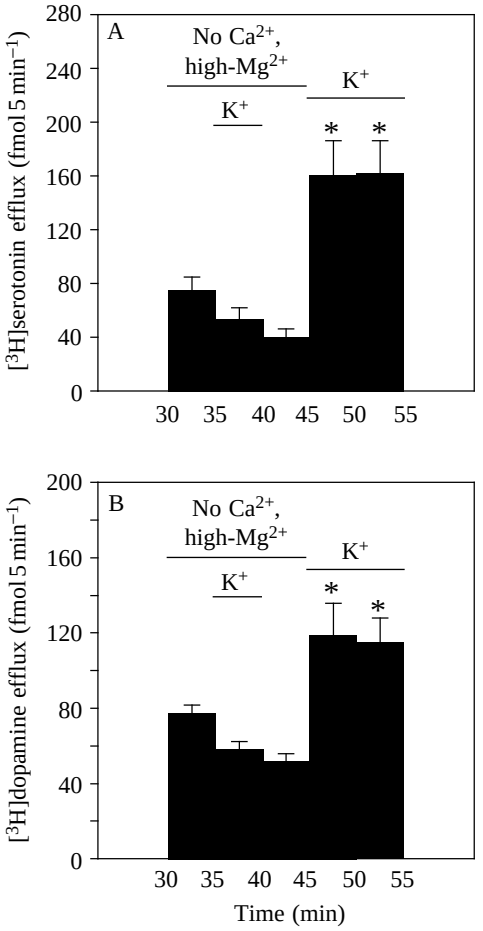

Fig. 10. Depolarization-induced release of serotonin (A) and dopamine (B) from salivary glands. Salivary glands were incubated in 5×10^{-7} mol l^{-1} [^{3}H]serotonin or [^{3}H]dopamine for 3 h, washed in saline several times for 30 min and then incubated for 5 min in various solutions. There was a significant increase in the efflux of tritium induced by 100 mmol l^{-1} K^+ saline when Ca^{2+} was present (45–50 min period) but not in the absence of Ca^{2+} (35–40 min period). A second consecutive exposure to 100 mmol l^{-1} K^+ saline released an equivalent amount of tritium from glands incubated in both serotonin and dopamine. Values represent means + S.E.M. of eight determinations. Final amine contents of salivary glands were 2.16±0.39 pmol of [^{3}H]serotonin and 1.62±0.15 pmol [^{3}H]dopamine in A and B, respectively. An asterisk marks values that are significantly different from control values (P<0.05).

Discussion

There have been a number of studies demonstrating that the biogenic amines dopamine and serotonin are associated with the salivary glands of *Locusta migratoria* (Baines *et al.* 1989; Baines and Tyrer, 1989; Gifford *et al.* 1991; Ali *et al.* 1993; Ali and Orchard, 1994), where they have a number of effects, including increasing salivary secretory rates and elevating cyclic AMP levels. Receptors for serotonin and dopamine have also been shown to be associated with salivary gland preparations (Lafon-Cazal and Bockaert, 1984; Ali and Orchard, 1994). We have carried out the present study to confirm that dopamine and serotonin are indeed natural neurotransmitters in locust salivary glands. We therefore examined the release and inactivation mechanisms for these amines in the glands.

We have previously shown that stimulation of nerve 7b of isolated salivary gland preparations leads to increases in cyclic AMP levels that can be partially inhibited by dopaminergic (10 μmol l^{-1} SCH-23390) and serotonergic (10 μmol l^{-1} spiperone) receptor antogonists, from which we infer that dopamine and serotonin are released from nerve terminals and

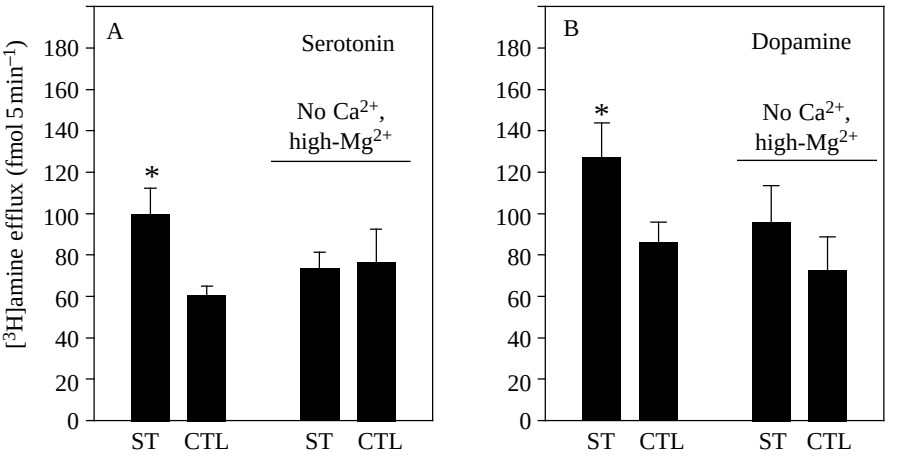

Fig. 11. Neurally stimulated release of serotonin and dopamine. Salivary glands were incubated in 5×10^{-7} mol l^{-1} [^{3}H]serotonin (A) or [^{3}H]dopamine (B) for 1 h, washed for 30 min in saline or 15 min in normal saline and 15 min in 0 mmol l^{-1} Ca^{2+}, 8 mmol l^{-1} Mg^{2+} saline, and then nerve 7b was electrically stimulated for 5 min. Controls (CTL) consisted of glands stimulated with a subthreshold stimulus while stimulations (ST) consisted of the same glands stimulated with a suprathreshold stimulus. Efflux of tritium was also quantified in 0 mmol l^{-1} Ca^{2+}, 8 mmol l^{-1} Mg^{2+} saline (bar). Values represent means + S.E.M. of 5–8 determinations. Final amine contents of the salivary glands were 1.41±0.05 pmol of [^{3}H]serotonin and 0.97±0.13 pmol [^{3}H]dopamine in A and B, respectively. An asterisk marks values that are significantly different from control values (P<0.05).

subsequently interact with receptors coupled with adenylate cyclase (Ali and Orchard, 1994). In the time course experiments using stimulation at 15 Hz, it seems clear that approximately 50 % of the elevated cyclic AMP level is due to the release of dopamine since this is the amount blocked by the dopamine receptor antagonist SCH-23390. The remainder of the elevated level of cyclic AMP is probably due to the release of serotonin, although this could not be definitively shown because we have not found a serotonergic antagonist which is 100 % effective against serotonin (Ali and Orchard, 1994). SCH-23390, in contrast, is effective at completely blocking relatively large dopamine-induced elevations of cyclic AMP level. The frequency experiments (Fig. 2) showed that SCH-23390 is more effective at blocking neurally evoked increases in cyclic AMP levels when both neurones are active at lower frequencies, suggesting a greater involvement of dopamine than of serotonin at lower frequencies. The activities of the neurones are quite different between the feeding and non-feeding states of the animal (Baines *et al.* 1989), and it would appear that the optimal release of dopamine and serotonin occur at different frequencies. This may not be a surprising result if, as recently postulated by Schactner and Bräunig (1995), dopamine and serotonin play different roles in the formation and secretion of saliva. Specifically, dopamine may induce the secretion of water and ions, while serotonin may induce the secretion of enzymes and proteinaceous components in the saliva (Schactner and Bräunig, 1995).

The stimulation-induced elevation of cyclic AMP levels is Ca^{2+}-dependent; however, Ca^{2+}-free saline does not interfere with the ability of dopamine and serotonin to bind to their receptors and to initiate the cyclic AMP transduction mechanism, implying that Ca^{2+} is needed for dopamine and serotonin to be released from SN1 and SN2. A high-K^+ saline, which is known to depolarise neurones and induce the release of substances at synaptic and neurohaemal sites, is also capable of elevating cyclic AMP levels in a Ca^{2+}-dependent fashion. Furthermore, both neural stimulation and a high-K^+ medium can induce the release of [^{3}H]dopamine and [^{3}H]serotonin from previously loaded locust salivary glands in a Ca^{2+}-dependent fashion. Taken together, these results suggest that dopamine and serotonin are released from SN1 and SN2, respectively, upon electrical stimulation of these neurones.

The primary means of removing biogenic amines from the synaptic cleft in the locust is *via* a re-uptake mechanism which transports the amine into the presynaptic terminal. We sought to examine the possible presence of uptake mechanisms for dopamine and serotonin associated with locust salivary glands. Over a 3 h period, glands incubated in [^{3}H]amine accumulated radiolabel linearly over the first hour of incubation. The re-uptake of amines has been shown on a number of occasions to be dependent upon Na^+ (see Kanner, 1994, for a review) and therefore a Na^+-free saline should reduce, if not abolish, the uptake of [^{3}H]serotonin and [^{3}H]dopamine. The uptake of [^{3}H]serotonin in locust salivary glands is also Na^+-dependent and appears to have two different uptake rates depending on the substrate concentration. The high-affinity component occurs at lower concentrations and has an apparent K_m of 0.74 μmol l^{-1} while the lower-affinity component has a K_m of 30 μmol l^{-1}. The K_m of the high-affinity component (0.74 μmol l^{-1}) is comparable to that in a lobster serotonergic neurone of 0.66 μmol l^{-1} (Livingstone *et al.* 1981) and in *Rhodnius prolixus* of 0.22 μmol l^{-1} (Orchard, 1989). The high-affinity uptake of [^{3}H]serotonin into cultured *Periplaneta americana* neurones has a K_m of 0.23 μmol l^{-1} (Bermudez and Beadle, 1989), and that of octopamine in cockroach ventral nerve cord of 0.48 μmol l^{-1} (Evans, 1978). The low-affinity uptake mechanism in *Locusta migratoria* salivary glands has a higher K_m (30 μmol l^{-1}) than the low-affinity mechanisms reported by Bermudez and Beadle (1989) in cockroach cultured neurones (2.14 μmol l^{-1}) and that reported by Evans (1978) in cockroach ventral nerve cord (19.8 μmol l^{-1}). It is possible that both high- and low-affinity mechanisms in locust salivary glands occur at synaptic sites. However, there are also some serotonergic neurohaemal areas lying on nerve 7b

(Bräunig, 1987, 1988). Thus, it is possible that the neurohaemal areas are capable of accumulating [^{3}H]serotonin and of releasing it in response to a high-K^+ challenge. Since we are primarily concerned with SN1 and SN2, our electrophysiological stimulation paradigms excluded the recruitment of these neurohaemal areas. As shown in Fig. 1, we were able preferentially to recruit activity from SN1 and SN2 with a voltage subthreshold to that required for the recruitment of the neurohaemal areas. This allowed us to investigate the efflux of radiolabel due to activation of the salivary neurones.

In vertebrates, uptake transporters occur on neurones as well as on glial cells, and one of our concerns is the cellular location of the uptake transporter. This remains to be definitively determined, since a high-K^+ efflux may occur from a number of cell types; however, the efflux of radiolabel from neurally stimulated preparations strengthens our postulation that at least some of the uptake of radiolabel occurs in the salivary neurones. That the release of [^{3}H]amine is Ca^{2+}-dependent also suggests a Ca^{2+}-mediated release from nerve terminals.

The uptake mechanism appears to be dependent primarily on Na^+ since a Ca^{2+}-free, reduced-Cl^- or a high-K^+ saline had no significant effect on the uptake of [^{3}H]serotonin. This ion-dependence differs from that for the uptake of [^{3}H]serotonin in cultured neurones of *Periplaneta americana* (Bermudez and Beadle, 1989) in which a Cl^--free medium and a high-K^+ saline reduced the uptake of [^{3}H]serotonin. In locust salivary glands, as in cultured neurones from *Periplaneta americana*, ice-cold medium reduced the uptake of radiolabel, presumably because the uptake mechanism is energy-dependent. The ability of specific uptake transporters to reduce the uptake of [^{3}H]serotonin (Fig. 8) serves to strengthen the evidence for the presence of a serotonergic transporter. It is interesting that of the three dopamine transport inhibitors, two (GBR-12909 and nomifensine) inhibit the uptake of [^{3}H]serotonin, whereas the third (buproprion) does not. It may be worth noting that imipramine and clomipramine are tricyclic antidepressants whereas nomifensine has a bicyclic structure and appears to be more closely related to the tricyclics than to buproprion. Thus, it appears that the salivary gland serotonin uptake transporter has some pharmacological differences from those reported from vertebrates.

Similar experiments using [^{3}H]dopamine gave different results. The time course of [^{3}H]dopamine uptake by locust salivary glands showed an initial high rate followed by a levelling off. Interestingly, however, dopamine uptake by the salivary glands is not dependent upon Na^+ (Fig. 7). The uptake of radiolabel is also not dependent upon Cl^- and Ca^{2+}, but may depend upon ATP and a K^+ gradient, as indicated by the reduced uptake in ice-cold and in high-K^+ saline. Since high-K^+ saline also depolarizes the neurones, it is likely that the reduced uptake in 100 mmol l^{-1} K^+ may be due to a more positive membrane potential. The tricyclic antidepressants and the dopamine uptake inhibitors were all capable of reducing the uptake of [^{3}H]dopamine, although the specific mammalian uptake inhibitor quipazine was ineffective. The most potent uptake inhibitors actually had a greater effect than ice-cold saline on uptake rates, suggesting that ice-cold saline does not fully inhibit the energy-dependent uptake or that the uptake mechanism is not solely dependent upon energy.

The release of [^{3}H]serotonin and [^{3}H]dopamine from the salivary glands, in response to either stimulation of the salivary nerve or a high-K^+ challenge, indicates that the [^{3}H]amine associated with the glands is placed in a releasable pool. The trafficking of [^{3}H]amine into releasable pools seems to occur *via* different time-dependent methods for each amine. A larger percentage of the total [^{3}H]serotonin content of the glands is released from 3 h preincubated glands than from 1 h preincubated glands, although in each case equal quantities of tritium are released during the first and second exposures to high-K^+ saline. Tissues preincubated in [^{3}H]dopamine, however, release the same percentage of tritium regardless of the preincubation time. Also, the complete release of tritium occurs during the first 5 min exposure to high-K^+ saline for tissues preincubated for 1 h, but it occurs in equal quantities during the first and second exposures to high-K^+ saline for tissues preincubated for 3 h.

Some uptake transporters were able to inhibit the uptake of dopamine into locust salivary glands, which indicates that, in spite of being Na^+-independent, there is probably a dopaminergic transporter associated with SN1 that has some unique characteristics. In vertebrates, the uptake of catecholamines appears to take place *via* at least two types of mechanism with different ionic dependence, uptake$_1$ and uptake$_2$, although recently evidence has been presented for the uptake of [^{3}H]catecholamines *via* a mechanism distinct from these two (Martel *et al.* 1994). These uptake mechanisms have been characterised for noradrenaline in particular, in which the uptake$_1$ mechanism is Na^+-dependent whereas uptake$_2$ is not dependent upon extracellular changes in Na^+ or Ca^{2+} concentration over short periods (Bönisch *et al.* 1985). If, however, Na^+ and Ca^{2+} are omitted from the preincubation medium for relatively long periods (90 min), uptake$_2$ becomes affected.

The present results suggest that the dopamine transporter in locust salivary glands is unique but not necessarily unusual since it may have minor similarities with the uptake$_2$ system in vertebrates. Locust salivary glands may therefore provide us with an excellent opportunity to study the physical and biochemical properties of biogenic amine uptake transporters in insects.

This work was supported by the Natural Sciences and Engineering Research Council of Canada in the form of a grant to I.O. and a postgraduate scholarship to D.W.A.

References

ALI, D. W. AND ORCHARD, I. (1994). Characterization of dopamine and serotonin receptors on the salivary glands of the locust, *Locusta migratoria*. *Biogenic Amines* **10**, 195–212.

ALI, D. W., ORCHARD, I. AND LANGE, A. B. (1993). The aminergic

control of locust (*Locusta migratoria*) salivary glands: evidence for dopaminergic and serotonergic innervation. *J. Insect Physiol*. **39**, 623–632.

ALTMAN, J. S. AND KIEN, J. (1979). Suboesophageal neurons involved in head movements and feeding in locusts. *Proc. R. Soc. Lond. B* **205**, 209–227.

BAINES, R. A. AND TYRER, N. M. (1989). The innervation of locust salivary glands. II. Physiology of excitation and modulation. *J. comp. Physiol*. A **165**, 407–413.

BAINES, R. A., TYRER, N. M. AND MASON, J. C. (1989). The innervation of locust salivary glands. I. Innervation and analysis of transmitters. *J. comp. Physiol*. A **165**, 395–405.

BERMUDEZ, I. AND BEADLE, D. J. (1989). High-affinity uptake of [^{3}H]serotonin in cultured neurones of the cockroach *Periplaneta americana* (L.). *Archs Insect Biochem. Physiol*. **12**, 253–266.

BÖNISCH, H., BRYAN, L. J., HENSELING, M., O'DONELL, S. R., STOCKMAN, P. AND TRENDELENBURG, U. (1985). The effect of various ions on uptake$_2$ of catecholamines. *Naunyn-Schmiedeberg's Arch. Pharmac.* **328**, 407–416.

BRADFORD, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. *Analyt. Biochem*. **72**, 248–254.

BRÄUNIG, P. (1987). The satellite nervous system – an extensive neurohemal network in the locust head. *J. comp. Physiol*. A **160**, 69–77.

BRÄUNIG, P. (1988). Neurosecretory cells of the locust suboesophageal ganglion. *Symp. biol. hung*. **36**, 161–171.

CARLSON, A. D. AND EVANS, P. D. (1986). Inactivation of octopamine in larval firefly light organs by a high-affinity uptake mechanism. *J. exp. Biol*. **122**, 369–385.

COOPER, J. R., BLOOM, F. E. AND ROTH, R. H. (1991). *The Biochemical Basis of Neuropharmacology*, 6th edn. Toronto: Oxford University Press.

ELIA, A. J., ALI, D. W. AND ORCHARD, I. (1994). Immunochemical staining of tyrosine hydroxylase(TH)-like material in the salivary glands and ventral nerve cord of the cockroach, *Periplaneta americana* (L.). *J. Insect Physiol*. **40**, 671–683.

EVANS, P. D. (1978). Octopamine: a high affinity uptake mechanism in the nervous system of the cockroach. *J. Neurochem*. **30**, 1015–1022.

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

FLANAGAN, T. R. J. AND BERLIND, A. (1984). Serotonin modulation of the release of sequestered [^{3}H]serotonin from nerve terminals in an insect neurohemal organ *in vitro*. *Brain Res*. **306**, 243–250.

GIFFORD, A. N., NICHOLSON, R. A. AND PITMAN, R. M. (1991). The dopamine and 5-hydroxytryptamine content of locust and cockroach salivary neurones. *J. exp. Biol*. **161**, 405–414.

KANNER, B. I. (1994). Sodium-coupled neurotransmitter transport: structure, function and regulation. *J. exp. Biol.* **196**, 237–249.

LAFON-CAZAL, M. AND BOCKAERT, J. (1984). Pharmacological characterization of dopamine-sensitive adenylate cyclase in the salivary glands of *Locusta migratoria* L. *Insect Biochem*. **14**, 541–545.

LANGE, A. B. AND ORCHARD, I. (1986). Identified octopaminergic neurons modulate contractions of locust visceral muscle via adenosine 3′,5′-monophosphate (cyclic AMP). *Brain Res.* **363**, 340–349.

LIVINGSTONE, M. S., SCHAEFFER, S. F. AND KRAVITZ, E. A. (1981). Biochemistry and ultrastructure of serotonergic nerve endings in the lobster: serotonin and octopamine are contained in different nerve endings. *J. Neurobiol*. **12**, 27–54.

MARTEL, F., AZEVEDO, I. AND OSSWALD, W. (1994). Uptake of ^{3}H-catecholamines by rat liver cells occurs mainly through a system which is distinct from uptake$_1$ or uptake$_2$. *Naunyn-Schmiedeberg's Arch. Pharmac.* **350**, 130–142.

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

ORCHARD, I. (1989). Serotonergic neurohaemal tissue in *Rhodnius prolixus*: synthesis, release and uptake of serotonin. *J. Insect Physiol*. **35**, 943–947.

ORCHARD, I., LANGE, A. B. AND BROWN, B. B. (1992). Tyrosine hydroxylase-like immunoreactivity in the ventral nerve cord of the locust (*Locusta migratoria*), including neurones innervating the salivary glands. *J. Insect Physiol*. **38**, 19–27.

SCHACTNER, J. AND BRÄUNIG, P. (1995). Activity pattern of suboesophageal ganglion cells innervating the salivary glands of the locust *Locusta migratoria. J. comp. Physiol.* A **176**, 491–501.

TYRER, M. N., TURNER, J. D. AND ALTMAN, J. S. (1984). Identifiable neurons in the locust central nervous system that react with antibodies to serotonin. *J. comp. Neurol*. **227**, 313–330.