

EFFECTS OF SEROTONIN, DOPAMINE AND ERGOMETRINE ON LOCOMOTION IN THE PULMONATE MOLLUSC *HELIX LUCORUM*

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Summary

The terrestrial snail *Helix lucorum* crawls using waves of muscular contraction (pedal waves) that spread along the sole of its foot. Crawling speed depends on wave generation frequency (step frequency) and the distance the snail moves forwards during each wave (step length). In a previous study, video recordings of a crawling snail showed that its sole length varied over a wide range and was directly correlated with mollusc speed. Speed depended on step length, which was directly related to sole length, rather than on step frequency, which remained rather constant. In the present study, the effects of dopamine, ergometrine (a blocker of dopamine receptors in molluscs) and serotonin injection on the linear relationship between sole length and locomotor speed in *Helix lucorum* were studied. In crawling snails, dopamine caused sole contraction, and locomotion slowed down or ceased. Ergometrine stimulated locomotion, which resembled rapid crawling with an extended sole, as

observed under normal conditions. Serotonin stimulated locomotion and accelerated crawling significantly without causing changes in sole length. The acceleration of locomotion induced by serotonin injection was due to pedal wave (step) elongation. It is proposed that, during each locomotor episode, dopamine controls snail speed by regulating sole length, which determines the amplitude of contraction of the muscle cells involved in pedal waves and, as a result, step length; serotonin determines the basic step length and shifts the linear relationship between sole length and mollusc speed upwards along the axis of mollusc speed. The efficiency of the serotonergic system depends on the physiological state of the mollusc (e.g. that characteristic of summer or winter).

Key words: locomotion, pedal wave, sole length, serotonin, dopamine, ergometrine, mollusc, snail, *Helix lucorum*.

Introduction

The process of crawling by means of muscular contractions (pedal waves) has been investigated in terrestrial snails (*Helix aspersa*, *H. pomatia*) and slugs (Lissman, 1945a; Lissman, 1945b; Jones, 1973; Jones, 1975; Denny, 1981). Fig. 1 represents a crawling *Helix lucorum* and the ventral part (sole) of its foot, which is involved in locomotion. Fig. 1B is taken from Denny (Denny, 1981) and shows a section of the foot of the slug *Ariolimax columbianus* which, like *Helix lucorum*, uses muscular waves for locomotion. The snail adheres to the substratum upon which it crawls. This adhesion is the result of a thin layer of mucus, which coats the sole and acts as a glue. The pedal mucus is produced either by the suprapedal mucus gland or by the ciliated epithelium, which transports the mucus along the sole. The ciliated epithelium overlies smooth muscle cells oriented at an angle to the sole surface (Rogers, 1969). The snail crawls as a result of contractions of these cells (pedal waves), and the contractions spread from rear to front and displace each point of the sole forwards a certain distance (step length). In *Helix lucorum*, 8–12 waves pass along the sole simultaneously. The rate of locomotion depends both on step length and on the frequency of muscle wave generation (step frequency).

Many pulmonates regulate their speed of locomotion by controlling the speed of the pedal waves (Crozier and Pilz, 1924; Jones, 1975; Denny, 1980). Studies on locomotion in intact *Helix lucorum* showed that, during a locomotor episode, the snail could change considerably the length of its sole, i.e. the tone of the smooth muscle cells underlying the sole (Pavlova, 1994). At a constant wave generation frequency, the speed of the mollusc and pedal wave progression were directly correlated with sole length. Thus, *Helix lucorum* can control its speed by its changing sole length and, therefore, step length because this depends directly on sole length.

The purpose of the present study was to analyse how the linear relationship between sole length and locomotion rate in *Helix lucorum* changes under the influence of neurotransmitters involved in the control of locomotion in molluscs. Two neurotransmitters, dopamine and serotonin (5-hydroxytryptamine, 5-HT), were used; these have both been found in the central nervous system of *Helix lucorum* (Ierusalimsky et al., 1997).

Dopamine inhibits swimming activity in the pteropod mollusc *Clione limacina* (Sakharov and Kabotyansky, 1986). Ergot alkaloids, such as ergometrine, are conventionally used

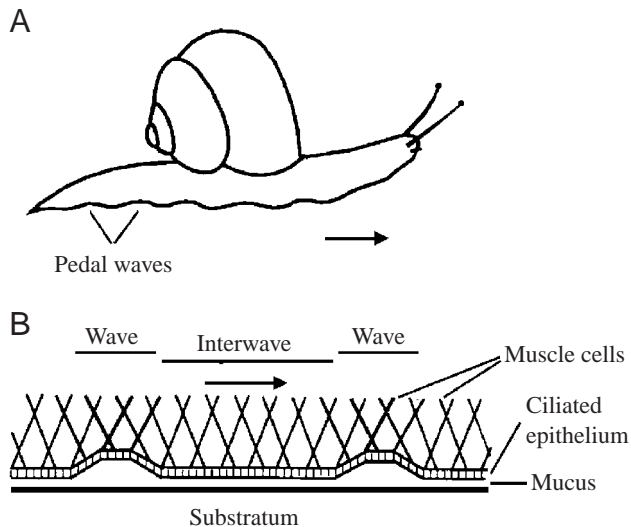


Fig. 1. Diagram of a crawling snail *Helix lucorum*. (A) The snail crawls using muscular pedal waves that spread along the sole of its foot. (B) Diagrammatic representation of a sagittal section of part of the ventral surface of the foot (sole) in contact with the substratum (for explanation, see text). B is taken from Denny (1981). Arrows indicate that the snail and pedal waves move in the same direction.

as dopamine receptor blockers in molluscs. Ergometrine inhibits both depolarizing and hyperpolarizing synaptic potentials evoked by dopamine in *Helix pomatia* (Juel, 1983) and completely abolishes the K^+ outward current induced by dopamine in the neurons of *Aplysia kurodai* (Sawada and Maeno, 1987). Ergot alkaloids stimulate swimming in the pteropod mollusc *Clione limacina* (Sakharov and Kabotyansky, 1986) and disinhibit muscle wave generation in the foot of *Helix pomatia* (Sakharov and Salanki, 1982).

Unlike dopamine, 5-HT stimulates and facilitates locomotion in the molluscs *Tritonia diomedea*, *Clione limacina*, *Lymnaea stagnalis* and *Aplysia brasiliiana* (Audesirk et al., 1979; Arshavsky et al., 1984; Syed et al., 1988; Parsons and Pinsker, 1989). This effect of 5-HT on locomotion in *Helix lucorum* has been described in a brief communication (Pavlova, 1996).

The present study describes the effects of dopamine, ergometrine and 5-HT on locomotion in *Helix lucorum*.

Materials and methods

Experiments were carried out on adult molluscs *Helix lucorum* (class Gastropoda, subclass Pulmonata, order Stylommatophora) weighing 7–32 g, which were collected in the Crimea. The snails were kept in a moist terrarium at room temperature (18–22 °C) and fed on lettuce leaves and carrots. The locomotor kinematics of each snail was observed individually by allowing the mollusc to crawl vertically up a transparent marked wall so that the entire sole surface was visible. Such locomotor episodes usually lasted for several dozens of minutes. In each episode, only locomotion at a steady rate (without apparent acceleration or deceleration) and with a constant step frequency was studied. Periods during

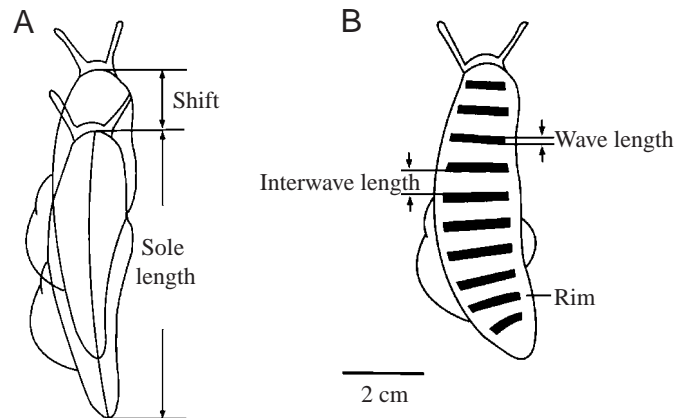


Fig. 2. Drawings of the sole of a snail crawling up a vertical transparent wall (projections transferred from photographs to graph paper). (A) Two projections from photographs taken 25 s apart. (B) Projection with alternating dark and light bands corresponding to pedal waves and interwaves, respectively.

which step frequency decreased by more than 6% were excluded from analysis. Such periods usually preceded the cessation of crawling and accounted for less than 30% of the total duration of a locomotor episode.

Locomotor episodes were videotaped at 25 frames s^{-1} to record mollusc speed, wave speed and sole length. To determine wave speed, the time required for the passage of a wave along the sole was measured. In some experiments, a photographic camera was used to record mollusc speed and sole length and to obtain clear images both of waves and of the intervals between them (interwaves). All images of the sole, waves and interwaves presented below were copied from these photographs. Photographs of a crawling mollusc were taken at constant intervals of 10–25 s. Contours of the sole were transferred from serial photographs onto graph paper and collated with respect to reference marks on the wall. Therefore, several consecutive projections of the sole on the same sheet of paper could be obtained (Fig. 2A). Sequences with two adjacent projections differing in length by no more than 3% were chosen; it was assumed that sole length in the crawling snail remained constant in these cases. The speed of the mollusc (the displacement of the rostral terminal point of the sole between projections) at each sole length was calculated. Dozens of different values of sole length and mollusc speed were obtained for each snail.

Waves and interwaves were readily visible in the photographs as alternating dark and light transverse bands (Fig. 2B). The relationship between waves and interwaves observed simultaneously on the sole was estimated by measuring the total absolute lengths of all the waves (Σ_w) and interwaves (Σ_{iw}) and calculating their ratio (Σ_w/Σ_{iw}). Partially visible terminal waves and interwaves at the rostral and caudal ends were not taken into account. In some experiments, the proportion of muscle cells involved in contraction waves was estimated by calculating the percentage of the sole area covered by waves.

The variables described above were recorded twice: during a spontaneous locomotor episode and immediately after the injection of a test substance. Locomotion was recorded for 20–30 min following injection. Dopamine, ergometrine maleate (Sigma) and serotonin–creatinine sulphate complex (Calbiochem) were dissolved in *Helix* physiological saline of the following composition (in mmol l^{-1}): 80 NaCl, 4 KCl, 8 CaCl_2 , 5 MgCl_2 and 5 Tris, pH 7.8 (Chase, 1986). Freshly prepared solutions (0.2–0.6 ml) were injected into the dorsal side of the foot. Control snails were injected with the same volume of physiological saline.

Results

The frequency of pedal waves (steps) generated by snails varied from 32 to 46 min^{-1} but was relatively constant within each locomotor episode.

In crawling snails treated with dopamine at doses of 0.5–5.0 mg g^{-1} body mass, the sole shortened considerably within 1–2 min after injection. Its length occasionally decreased below the lower limit recorded in the same snail under normal conditions. At doses below 1.5–2.0 mg g^{-1} , locomotion slowed down for a short period (approximately 10 min), and the frequency of pedal wave generation decreased to 18–25 min^{-1} . At doses exceeding 1.5–2.0 mg g^{-1} , locomotion ceased for 10–25 min. In both cases, the normal pattern of locomotion was restored several dozens of minutes after dopamine injection. Dopamine also had side effects: the sole of injected molluscs looked dry, and the snails were difficult to detach from the substratum. Control injection of physiological saline was never accompanied by visible changes in locomotion.

Ergometrine at a dose of 0.8 mg g^{-1} body mass induced pedal wave generation in resting snails. This dose was used in later experiments because (i) pedal wave frequency after injection was similar to that recorded during a spontaneous locomotor episode, (ii) the snail attached to the substratum within a few minutes, and (iii) the induced locomotor episode lasted for dozens of minutes. At doses one order of magnitude lower, pedal wave generation ceased within 10 min. At doses one order of magnitude higher, the snail could not attach itself to the substratum for dozens of minutes. The data discussed below were recorded from the onset of induced locomotion until the frequency of pedal wave generation began to decrease noticeably and a slight decrease in mollusc speed and movement with an elongated sole indicated that the period of locomotor activity was ending. After ergometrine-induced locomotor episodes, snails always stopped with an elongated sole.

Injection of ergometrine always resulted in muscular wave generation and elongation of the sole. The molluscs demonstrated rapid crawling with a long sole. Both sole length and mollusc speed reached maximal values exceeding those recorded in normal conditions. Here and below, tests in which sole length and mollusc speed varied considerably under normal conditions are used in the illustrations and statistical

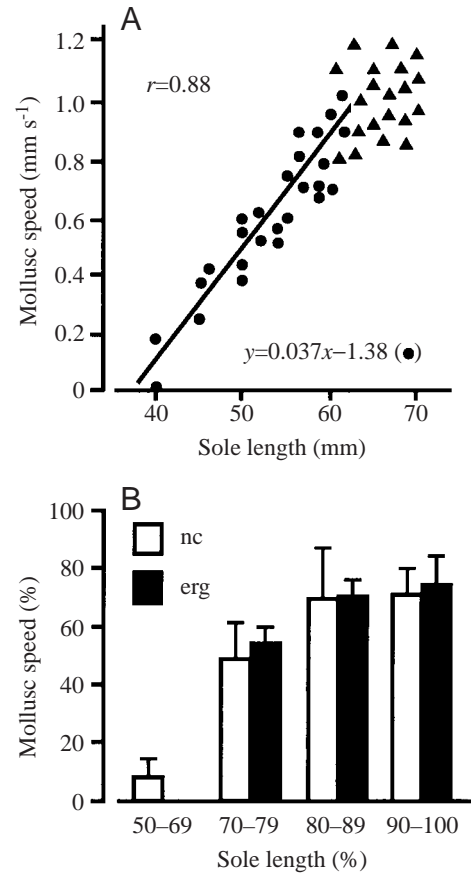


Fig. 3. Effects of ergometrine (0.8 mg g^{-1} body mass) on sole length and mollusc speed. (A) Values of sole length and mollusc speed were recorded during spontaneous (●) and ergometrine-induced (▲) locomotor episodes in one snail. (B) Mean values obtained from seven snails in normal conditions (nc) and after ergometrine injection (erg). In each snail, the maximum sole length and mollusc speed after ergometrine injection were taken as 100%. Values are means + s.d. There was no significant difference (*t*-test) in mollusc speed recorded at the same sole length before and after ergometrine injection ($P>0.59$).

analysis. Fig. 3A shows the relationship between sole length and mollusc speed before and after ergometrine injection. The linear relationship between sole length and mollusc speed under normal conditions was revealed by least-squares regression. However, this method was not used when the injection of a test substance (in this case, ergometrine) was followed by the gradual cessation of the locomotor response.

Fig. 3B shows the effect of ergometrine on sole length and mollusc speed in seven snails. The maximum sole length (taken as 100%) was calculated by averaging the three longest values of sole length recorded in each snail (in this case in the presence of ergometrine); the same procedure was performed for the mollusc speed data. The relative mollusc speed (as a percentage) was then calculated for sole lengths equal to 50–69, 70–79, 80–89 and 90–100% of the maximum under normal conditions and after ergometrine injection. Finally, the mean values of mollusc speed obtained for each sole length

category were averaged for all snails. This procedure allowed comparison of mollusc speed recorded at the same sole length before and after ergometrine injection. The data showed that the sole of a snail injected with ergometrine never shortened to 50–69 % of its maximum length, as occurred under normal conditions, and that crawling never slowed to values as low as those recorded in normal conditions. Statistical analysis (*t*-test) showed no significant difference in mollusc speed recorded at the same sole length before and after ergometrine injection ($P>0.59$).

Sole length in snails increased after ergometrine injection. The sole, which was fairly long at the onset of the induced locomotor episode, continued to elongate for 10–15 min. Fig. 4 shows the effects of ergometrine on sole length in eight snails. For each snail, the total respective numbers of sole length measurements under normal conditions and after ergometrine injection and the maximum sole length in the presence of ergometrine were taken as 100 %. The percentages of recorded sole length values that were 50–69, 70–79, 80–89 and 90–100 % of the maximum sole length were then calculated for spontaneous and ergometrine-induced locomotor episodes. All data were averaged in all snails. This procedure allowed comparison the numbers of different sole lengths recorded before and after ergometrine injection. The results show that the preferred sole length in snails crawling under normal conditions is 70–89 % of the maximum; after ergometrine injection, this increases to 80–100 %. Statistical analysis (*t*-test) of the results showed a significant difference in the number of measurements of sole length recorded before and after ergometrine injection. Most recordings were made under normal conditions, when the sole length was equal to 70–79 % ($P=0.009$) and after ergometrine injection, when the sole length was equal to 90–100 % ($P=0.0002$). Using an alternative method of statistical analysis (χ^2), the total number of measurements of sole length equal to 50–79 % and

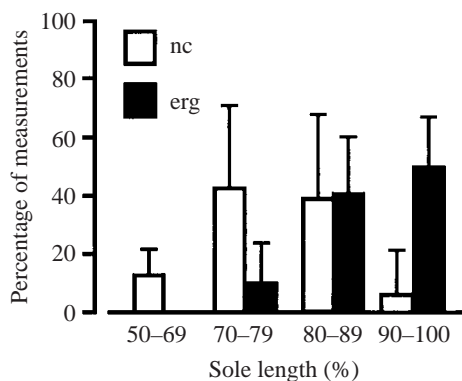


Fig. 4. Effects of ergometrine (0.8 mg g^{-1} body mass) on sole length in eight snails. In each mollusc, the respective total number of sole length measurements before (normal conditions, nc) and after ergometrine injection (erg) and the maximum sole length in the presence of ergometrine were taken as 100 %. Values are means + s.d. Statistical analysis (χ^2 -test) showed that ergometrine injection caused sole elongation ($N=259$, $\chi^2=65.3$, $P<0.0001$).

80–100 % of maximum sole length was calculated before and after ergometrine injection; ergometrine reliably elongated the sole ($N=259$, $\chi^2=65.3$, $P<0.0001$).

Ergometrine had no significant effect on the frequency of pedal wave generation. Wave frequency occasionally increased by 15 % during the first 10–15 min of the induced locomotor episode. In general, the time of wave progression along the shortened or elongated sole was approximately the same before and after ergometrine injection (Fig. 5A). Before injection, the wave speed was directly proportional to sole length. In the injected snail, both variables reached the highest values recorded under normal conditions or exceeded them. Mean values for five snails are shown in Fig. 5B. Percentage values were calculated as described above for Fig. 3B. The ergometrine-induced decrease in mean wave speed at 80–100 % sole length relative to values in normal snails reflects the initial decrease in the frequency of wave generation. Nevertheless, there was no significant difference between wave

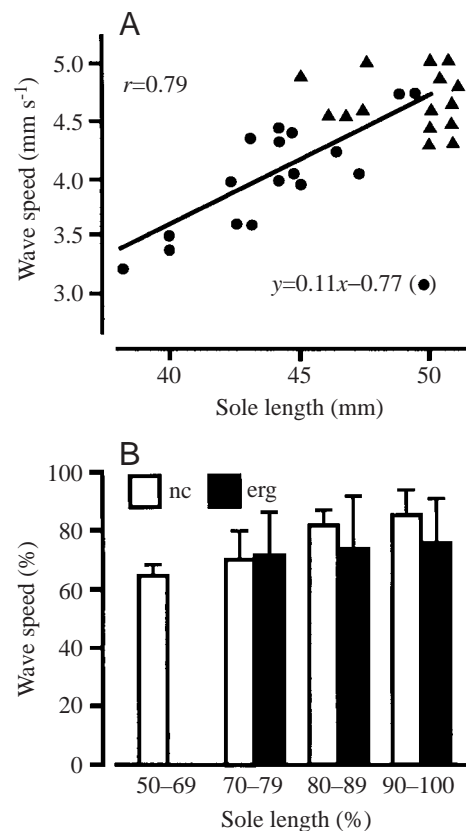


Fig. 5. Effects of ergometrine (0.8 mg g^{-1} body mass) on sole length and pedal wave speed. (A) Values of sole length and wave speed recorded before (●) and after (▲) ergometrine injection. (B) Effects of ergometrine on sole length and wave speed in five snails [measurements before (nc) and after (erg) ergometrine injection]. In each mollusc, maximum values of sole length and wave speed (after ergometrine injection) were taken as 100 %. Values are means + s.d. There was no significant difference (*t*-test) between wave speed recorded at the same sole length before and after ergometrine injection ($P>0.15$).

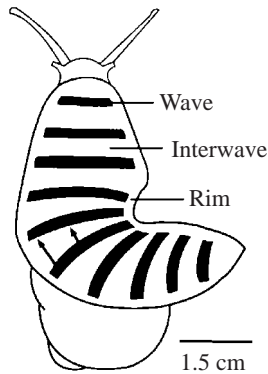


Fig. 6. Projection of the sole of a snail changing its direction of movement. Under normal conditions, the speed of the mollusc depends linearly on interwave length. The length of the arrows is proportional to interwave length and the speed of locomotion at the corresponding points on the sole. In addition, the number of waves passing through any point on the sole per unit time is the same, the periods of wave passage along an elongated and shortened sole are equal and wave speed is directly proportional to sole length.

speed recorded at the same sole length before and after ergometrine injection ($P > 0.15$).

In molluscs crawling with an elongated or shortened sole under normal conditions, the absolute length of pedal waves on the sole (Σ_w) remained unchanged, whereas that of interwaves (Σ_{iw}) changed in proportion to sole length. Correspondingly, the Σ_w/Σ_{iw} ratio was also correlated with sole length and varied from a minimum of 0.3 to a maximum of 0.9. Thus, mollusc speed was correlated with interwave length. This correlation was apparent in molluscs changing their direction of movement (Fig. 6). The elongated (outer) side of the sole moved more rapidly than the shortened (inner) side because the radius of its trajectory was longer; thus, both the rate of displacement (speed) of each point on the sole and interwave length gradually decreased along the transverse axis from the elongated to the shortened side. After ergometrine injection, the relationship between mollusc speed and

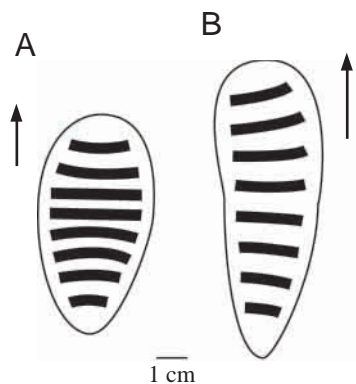


Fig. 7. Effects of ergometrine (0.8 mg g^{-1} body mass) on interwave length. Drawings from photographs of the sole made before (A) and after (B) ergometrine injection. Arrows show the direction and speed of the mollusc.

interwave length remained the same. Fig. 7 shows the sole of a mollusc photographed before and after ergometrine injection to illustrate this relationship.

Injections of 5-HT (optimal dose 2 mg g^{-1} body mass) also resulted in pedal wave generation. This dose was selected according to the same criteria as the dose of ergometrine (see above) and was used in most experiments. The typical pattern of qualitative changes induced by 5-HT differed from that in experiments with ergometrine: mollusc speed always increased significantly (approximately twofold), but sole length remained virtually unchanged. All data on injected molluscs were obtained during rapid locomotion induced by 5-HT, i.e. in the period when snail speed exceeded that recorded at the same values of sole length under normal conditions. Fig. 8A shows sole length and mollusc speed recorded before and after 5-HT injection. In some experiments, an injected mollusc could move rapidly even with a greatly shortened sole. Under normal conditions, molluscs with such a sole length remained motionless.

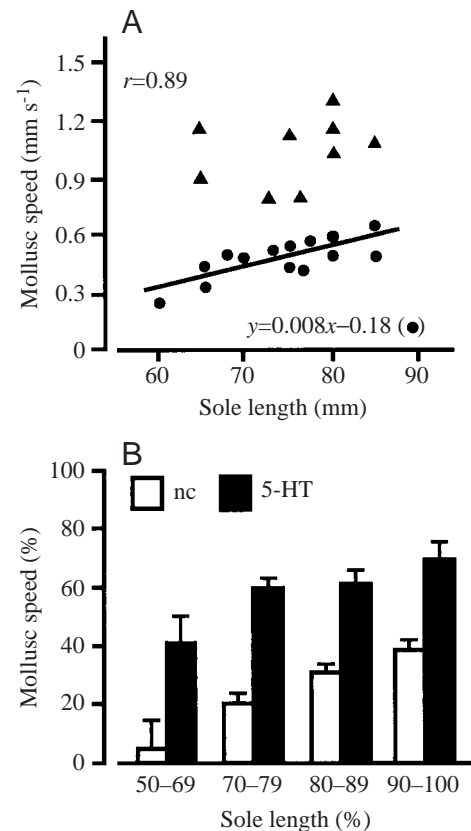


Fig. 8. Effects of serotonin (5-HT; 2 mg g^{-1} body mass) on mollusc speed and sole length. (A) Values of sole length and mollusc speed recorded before (●) and after (▲) 5-HT injection. (B) Effects of 5-HT on mollusc speed and sole length in five snails [measurements before (nc) and after (5-HT) serotonin injection]. In each mollusc, maximum values of sole length recorded before or after 5-HT injection and mollusc speed recorded in the presence of 5-HT were taken as 100%. Values are means + s.d. Both a t -test and χ^2 -test showed that mollusc speed was significantly increased after 5-HT injection ($P < 0.004$ and $N = 195$, $\chi^2 = 69.9$, $P < 0.0001$).

Fig. 8B presents data on the effects of 5-HT on sole length and mollusc speed in five snails. These were the snails that demonstrated a wide variation in sole length during the induced locomotor episode. In each snail, the three highest values of sole length recorded before or after 5-HT injection and of mollusc speed recorded after 5-HT injection were averaged and taken to be equal to 100%. Mean values of mollusc speed were then calculated at 50–69, 70–79, 80–89 and 90–100% of maximum sole length and averaged in all snails to allow a statistical comparison of changes in mollusc speed due to 5-HT injection. Mollusc speed was significantly increased in the presence of 5-HT (t -test, $P < 0.004$). To perform a χ^2 -test, the mollusc speed data were divided into four groups: the occasions (before 5-HT injection) when the speed was (i) less than and (ii) equal to the highest speed recorded under normal conditions and the occasions (after 5-HT injection) when the speed was (iii) less than and (iv) equal to or higher than this value (the highest speed recorded under normal conditions). The total number of values in each group obtained for five snails was calculated and compared. This analysis also showed a significant increase in mollusc speed after 5-HT injection ($N=195$, $\chi^2=69.9$, $P < 0.0001$).

Injections of 5-HT did not lead to statistically significant changes in sole length. Fig. 9 shows this effect of 5-HT in five snails. The respective total numbers of sole length measurements under normal conditions and after 5-HT injection and the maximum value of sole length under normal conditions were taken as 100%. The mean number of measurements of different sole lengths was calculated for each snail and the values averaged for all molluscs. The result shows no significant difference between the set of sole lengths recorded before and after 5-HT injection ($P > 0.85$). The preferred sole length in crawling snails, both before and after 5-HT injection, is 80–89% of the maximum recorded value.

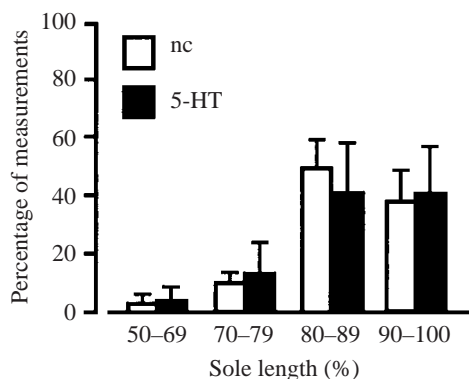


Fig. 9. Effects of serotonin (5-HT; 2 mg g^{-1} body mass) on sole length. In each snail, the respective total number of sole length measurements before (nc) and after (5-HT) serotonin injection and the maximum sole length recorded under normal conditions was taken as 100%. Values are means + s.d. from five snails. There was no significant effect (t -test) of 5-HT injection for any category of sole length ($P > 0.85$).

There was no significant effect of 5-HT on wave generation frequency and the time of wave progression along the sole: wave frequency increased by 18% only when pre-injection values were low ($30\text{--}35 \text{ min}^{-1}$). Fig. 10A shows that similar values of sole length and wave speed were obtained before and after 5-HT injection. A similar result was found for mean values from three snails (Fig. 10B).

The length of pedal waves increased after 5-HT injection. To enhance this effect and thus reduce relative measurement error, the dose of 5-HT was increased to 19 mg g^{-1} . Fig. 11 shows the sole of a mollusc under normal conditions and after 5-HT injection. Although the pedal waves seemingly fused after the injection, decreasing in number from 11–12 to 6–7, the sole area they occupied increased from 27.2 ± 5.8 to 43.3 ± 5.3 % (means \pm s.d., $N=3$). In other words, the relative number (proportion) of muscle cells simultaneously involved in pedal waves increased considerably. The area of the sole rim remained unchanged (approximately 25% of the sole), whereas that of interwaves decreased. The Σ_w/Σ_{iw} ratio,

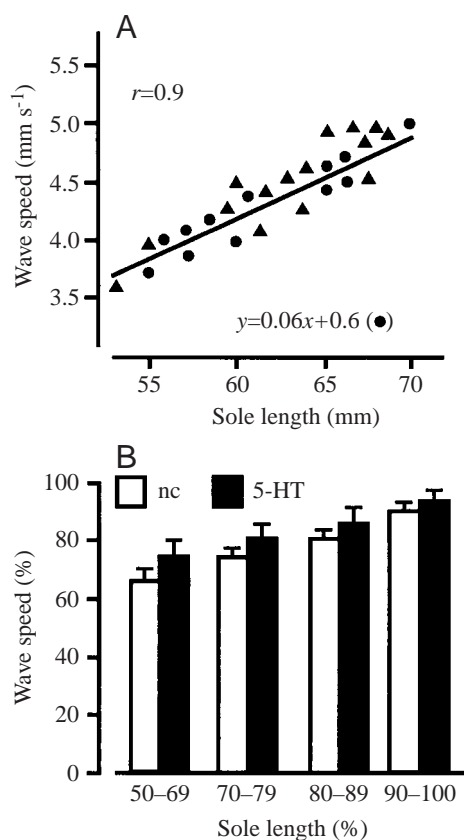


Fig. 10. Effects of serotonin (5-HT; 2 mg g^{-1} body mass) on wave speed. (A) Values of sole length and wave speed recorded before (\bullet) and after (\blacktriangle) 5-HT injection. (B) Mean sole length and wave speed before (nc) and after (5-HT) serotonin injection in three molluscs. In each snail, maximum sole length and wave speed recorded under normal conditions were taken as 100%. Values are means + s.d. There was no significant difference (t -test) between wave speed recorded at the same sole length before and after 5-HT injection ($P > 0.18$).

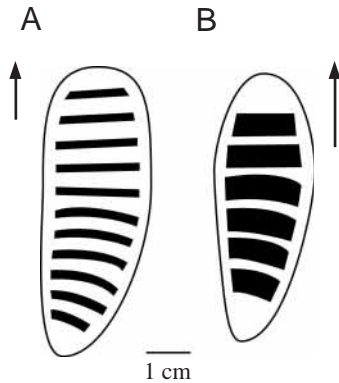


Fig. 11. Effects of serotonin (19 mg g^{-1} body mass) on pedal wave length. Drawings from photographs of the sole made before (A) and after (B) 5-HT injection. Arrows show the direction and speed of snail locomotion.

always less than 1.0 under normal conditions, could exceed this value after 5-HT injection, even reaching 1.7. During the induced locomotor episode, the number of waves on the sole gradually increased, whereas wave length and the Σ_w/Σ_{iw} ratio slowly decreased to mean values observed under normal conditions.

Both ergometrine and 5-HT had similar side effects. (i) At the onset of the induced locomotor episode, the foot looked swollen, the edge of the sole was slightly raised along its periphery, and the snail was easily detached from the substratum. (ii) Darkening or tactile stimulation of the tentacles was not followed by well-developed body contraction and retardation or cessation of locomotion, as is usually observed in the normal animal. (iii) The snails secreted increased amounts of mucus. (iv) Increased doses caused rhythmic ($2\text{--}3 \text{ min}^{-1}$) contraction and relaxation of the columellar muscles, displacing the shell rostrocaudally along the foot. In experiments with ergometrine, columellar muscle contraction was correlated with retardation or cessation of locomotion, with the sole remaining fairly long. (v) After the cessation of locomotion, the columellar muscles remained relaxed.

In control experiments, injections of physiological saline either had no effect or sometimes induced pedal wave generation lasting for several minutes; saline injections were never accompanied by changes in mollusc speed and sole length or by any of these side effects.

Discussion

The results described above demonstrate that both dopamine and 5-HT are involved in locomotor control in *Helix lucorum*. However, the effects of these neurotransmitters and their mechanisms are different.

Serotonin triggers locomotion, increasing mollusc speed (Fig. 8) *via* an increase in the proportion of muscle cells involved in each pedal wave and, thus, *via* an increase in step length (c.f. Fig. 11A,B) but not in step frequency (Fig. 10). 5-HT has no effect on sole length (Fig. 9) and apparently shifts

the linear relationship between sole length and mollusc speed upwards along the speed axis.

What factors determine the efficiency of the serotonergic system and, hence, a higher or lower mollusc speed at the same values of sole length? Dyakonova (Dyakonova, 1988) showed that certain identifiable neurons in the central nervous system of *Helix lucorum* are depolarised in the presence of 5-HT during the summer but lose their sensitivity to 5-HT in the winter. Ganglionic samples from satiated leeches *Hirudo medicinalis* contain 28% less 5-HT than those from unfed leeches (Lent et al., 1991). Hungry pond molluscs are characterised by increased locomotor activity (Bovbjerg, 1965).

Thus, it may be assumed that the physiological state of a snail (e.g. summer/winter, satiated/hungry) determines the proportion of muscle cells involved in the contraction waves (i.e. the basic step length) for each locomotor episode *via* the efficiency of the serotonergic system. For example, the plot of the linear relationship between sole length and mollusc speed could be displaced upwards in a hungry summer snail and downwards in a satiated winter one. It is noteworthy that the number of waves passing along the sole simultaneously also depends on the effect of 5-HT and is inversely correlated with step length and mollusc speed (Fig. 11).

The findings that dopamine causes a decrease in sole length and mollusc speed whereas ergometrine exerts the opposite effects (Figs 3, 4) suggest that dopamine participates in the control of the speed of crawling by regulating sole length during a locomotor episode. In snails injected with ergometrine, as during a normal locomotor episode, the increased speed of crawling was due to an increase in step length rather than in step frequency (Figs 5, 6).

The mechanism of an increase in step length upon sole elongation in response to ergometrine obviously differs from that described for the effect of 5-HT, which causes an increase in wave length (Fig. 11), whereas sole elongation leads to an increase in interwave length (Figs 6, 7). It is possible that, when the sole elongates, the amplitude of contraction of muscle cells involved in pedal waves increases proportionately and that this mechanism causes the linear relationship between sole length and mollusc speed. Thus, dopamine regulates mollusc speed by shortening the sole and causing a proportional decrease in step length *via* a decrease contraction amplitude of muscle cells involved in pedal waves.

Is dopamine a transmitter that switches locomotion off? The probable answer is no. The decrease in the frequency of pedal wave generation and retardation or cessation of locomotion, which always occurred against a background of sole shortening in the normal animal and after dopamine injection, was also observed in molluscs with a fairly long sole during columellar muscle contraction caused by ergometrine injection and under normal conditions (Pavlova, 1994). This is evidence for the existence of an inhibitory transmitter that can exert its effect in molluscs with any sole length.

Some data suggest the existence of yet another transmitter which, unlike dopamine, elongates the sole and proportionately

increases mollusc speed. Its effect manifests itself when dopamine receptors are blocked by ergometrine. In experiments with ergometrine, the sole was usually longer than under normal conditions (Figs 3–5, where sole length in the presence of ergometrine was taken as 100%). A peptide transmitter producing an effect associated with direct relaxation of longitudinal foot muscles and involved in locomotor control has been found in *Aplysia californica* (Hall and Lloyd, 1990). A similar peptide is produced in the central nervous system of *Helix lucorum* (Poteryaev et al., 1997). A balance between dopamine and such a transmitter could be responsible for the regulation of sole length and, hence, the speed of crawling in *Helix lucorum* during a locomotor episode.

All the neurotransmitters involved in the control of locomotion (5-HT, dopamine and the probable peptide transmitter) can have direct effects on the smooth muscle cells of the sole. Smooth muscle, unlike skeletal muscle, can be in three active states: rhythmic contraction, tonic contraction and relaxation. Each state is controlled by a different transmitter. Some individual molluscan muscle fibres are known to be innervated by two or more excitatory neurons, each releasing a different neurotransmitter (Carew et al., 1974; Muneoka and Twarog, 1983).

Both 5-HT and ergometrine stimulated mucus secretion. The role of mucus in attachment to the substratum and locomotion was studied in the slug *Ariolimax columbianus* (Denny, 1980). Observations on other slug species have demonstrated that the ciliated epithelium drives mucus over the sole both laterally and caudally (Barr, 1926; Barr, 1928). This is expected to aid in filling the entire space between the sole and the substratum with mucus and, thus, provides conditions for attachment to the substratum by suction. In limpets, for example, mechanisms of attachment by adhesion and by suction are equally important (Smith, 1991).

Apparently, mucus and the ciliated epithelium enable molluscs to attach to the substratum, and the functioning of smooth muscle cells determines the speed of locomotion. Transmitters controlling locomotion coordinate muscle cell functioning, ciliated epithelium activity, mucus secretion and viscosity to ensure the maximum efficiency of muscle contractions. Both 5-HT and ergometrine at large doses disturb this coordination; molluscs cannot attach themselves to the substratum, and pedal waves spread along the 'hanging' sole.

Information about the factors that affect the frequency of wave generation is scarce. It is known, however, that the frequency of pedal waves in the terrestrial slug *Limax* increases at higher ambient temperatures, and that this is accompanied by a decrease in step length (Crozier and Pilz, 1924; Trueman, 1983).

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