tween interneurones active during elevation and depression to produce a 2-phase symmetrical pattern. Second, intraburst spike frequency was similarly high in all instances (dragonfly: 280–480 Hz; mayfly: 250–350 Hz). Locust and cricket flight interneurones discharge at a frequency greater than 200 Hz during a burst whereas the flight motor neurones have an intraburst frequency of only around 70 Hz[19,20]. Third, all the flight systems exhibited an asymmetry such that a slowing of the rhythm (seen at the end of flight sequences) is produced by an increase in the duration of one particular phase of a cycle (e.g. fig. 2D) rather than by proportional increases in the durations of all phases of a cycle. This is evident in the deafferented flight motor pattern of locusts as a relatively constant elevator to depressor latency[25] which can be accounted for by the known interneuronal circuitry[18]. The possession of these three features results in recordings from each of the insects that can appear almost identical, and it also suggests a basic similarity in how the flight motor pattern is generated in the different insects.

The insects studied here differ markedly in the structure and function of their flight systems. In light of the differences and given that insect wings are thought to have a common evolutionary heritage[7], the simplest explanation for the similarity demonstrated here is that it results from a conservative evolution within the central nervous system[1]. Other explanations which are not ruled out by our results include divergence from a common stock followed by evolutionary convergence, or convergence after a polyphyletic evolution of flight and its neural control system (although the wings themselves may be monophyletic[7]). If the simplest explanation is correct and evolution of flight circuitry in the central nervous system is conservative, then it may be at the level of sensory modification of the centrally generated flight rhythm that one should search for adaptive features of organization.

Acknowledgments. The work reported here was supported by the Natural Sciences and Engineering Research Council of Canada and by the Faculty of Graduate Studies and Research at McGill University. We thank K. G. Pearson and G. S. Pollack for their comments on a preliminary draft of the manuscript, and V. R. Vickery for help in identifying the mayfly that was used.

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0014-4754/88/090735-04$1.50 + 0.20/0

Two serotonin-sensitive potassium channels in the identified heart excitatory neurone of the African giant snail, *Achatina fulica* Fèrussac

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Received 21 April 1988; accepted 25 May 1988

Summary. By the patch clamp experiments, two serotonin-sensitive K^+ channels (SL-channel and SS-channel) were demonstrated in the identified heart excitatory neurone (PON) of the African giant snail, *Achatina fulica* Fèrussac. The activities of both channels could be recorded in the steady state and those activities disappeared on application of 5-HT.
Key words. Potassium channel; serotonin; modulation; snail neurone; patch clamp.

In the identified heart excitatory neurone (PON) of the African giant snail, *Achatina fulica* Fèrussac, serotonin (5-HT) produces slow depolarization via a decrease of the K^+ conductance[1]. The properties of the 5-HT-sensitive K^+ current in PON are similar to those of the S-channel (serotonin-sensitive K^+ channel) current in sensory neurones of *Aplysia*[2]. Both currents are a background K^+ current and contribute to the resting and action potentials. In *Aplysia* neurones, a single S-channel current has already been identified[3–5]. In the present study, single channel recordings by the patch-clamp technique were made to clarify the characteristics of the 5-HT-sensitive K^+ channel in PON. We found that the 5-HT-sensitive K^+ current in PON was due to the activities of two different 5-HT-sensitive K^+ channels, and that one of them had similar properties to the S-channel.

The subesophageal ganglia were dissected out of the animal and the dorsal surface of the right parietal ganglion was desheathed to expose the nerve cells. The preparation was pinned to the bottom of a chamber and treated by 0.5% trypsin dissolved in the normal solution for 15–20 min. The composition of the normal solution was (in mM): NaCl 61, KCl 3.3, $CaCl_2$ 10.7, $MgCl_2$ 13, glucose 5, HEPES 10, pH 7.5. After the enzymatic treatment, the preparation was perfused with Tris solution (all NaCl of the normal solution being replaced with TrisCl) to block the bursting activity of PON (see below). The temperature of the perfusate was

maintained at 24 °C by a thermoelectric device. The single channel currents of the cell body of PON were recorded in the cell-attached or the inside-out configuration of the improved patch-clamp method[6]. All recordings were made in the steady state. The feedback resistor of the current-voltage converter was 10 GΩ. The resting potential of the neurone was usually recorded simultaneously by the microelectrode. The patch-pipette had an outer tip diameter of 1–3 µm and the tip was fire-polished. The pipette was usually filled with Tris solution or 85 mM K^+ solution. The composition of the 85 mM K^+ solution was (in mM): KCl 85, $MgCl_2$ 13, HEPES 10, pH 7.5. When the pipette was filled with 85 mM K^+ solution, a liquid-junction potential (about 9 mV, the pipette negative) was developed and the measured potential was corrected for such potential after the experiment. In some experiments, the pipette was filled with the normal solution to ascertain whether $Tris^+$ has any action on the activities of the channels. The results were not different from those obtained by the pipette filled with Tris solution. When the single channel currents were recorded in the inside-out configuration, the bath solution was changed to 130 mM K^+ solution, the composition of which was (in mM): KCl 100, KOH 30, glucose 5, EGTA 5, HEPES 10, pH 7.5. 5-HT was applied by bath perfusion.

Since PON is the bursting neurone[7], the resting potential is not stable. Accordingly, the patch potential of the cell-attached patch cannot be clamped if the recordings are made in the normal solution. Thus, all experiments were made under Na^+-free condition (Tris solution), in which the bursting activity of PON was completely blocked and the resting potential was stable at an average value of -63.3 ± 6.7 mV (mean ± SD, n = 20). Figure 1 shows single channel activities of the two distinct 5-HT-sensitive channels recorded by the pipette filled with Tris solution.

The channel shown in figure 1 A, which has the larger unitary current, is tentatively called the SL-channel (serotonin-sensitive large channel), and the channel in figure 1 B is called the SS-channel (serotonin-sensitive small channel) in this paper. In the present experiments, successful single channel recordings were made in 6 patches for the SL-channel and 17 for the SS-channel out of 46 patches from 26 cells. Both SL- and SS-channels were functioning in the steady depolarized state and showed flickering kinetics; i.e. a fast transition between the open state and the closed one. When 5-HT was applied by bath perfusion, the activities of both channels disappeared with some delay. Similar results were obtained in all the tested preparations for the SL-channel (n = 4) and in 8 preparations out of 11 for the SS-channel. The recovery of channel activity was not usually observed during the recording period; the recovery was seen in one patch for the SL-channel and two patches for the SS-channel. The irreversibility of 5-HT action is probably because of the high concentration of 5-HT being used. Because the limit of stable single channel recording was 10–20 min in most experiments, rather high concentration of 5-HT was used to ensure its effect. The activities of both channels recorded in the inside-out patch where the intracellular surface was perfused with Ca^{2+}-free solution containing EGTA (130 mM K^+ solution) were not different from those in the cell attached patch (data not shown), suggesting that these channels are not Ca^{2+}-dependent.

I–V relationships of these two channels are illustrated in figure 2. The I–V relationship of the SL-channel was nonlinear and showed clear outward-rectification when the pipette solution contained 3.3 mM K^+; i.e. Tris or the normal solution (fig. 2 A 2, circles). The data could be fitted with the

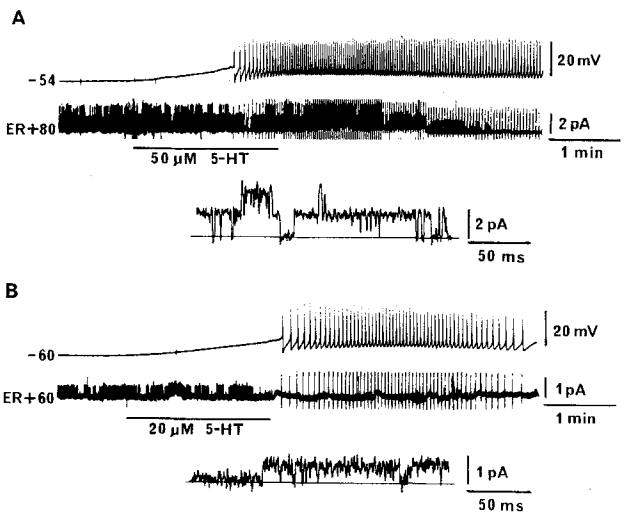

Figure 1. Two 5-HT-sensitive channels. *A* SL-channel. *B* SS-channel. In both *A* and *B*, upper trace is the membrane potential of PON, middle trace is activity of the ion channel in the cell-attached patch, and lower trace is unitary currents before application of 5-HT at the expanded time scale. The pipette was filled with Tris solution in both experiments. Left-side figure in each upper trace indicates the resting potential of PON before application of 5-HT. ER: the resting potential. The patch potential was 80 mV more positive than the resting potential in *A* and 60 mV more positive in *B*. Straight line in each lower trace shows the closed level of the channel.

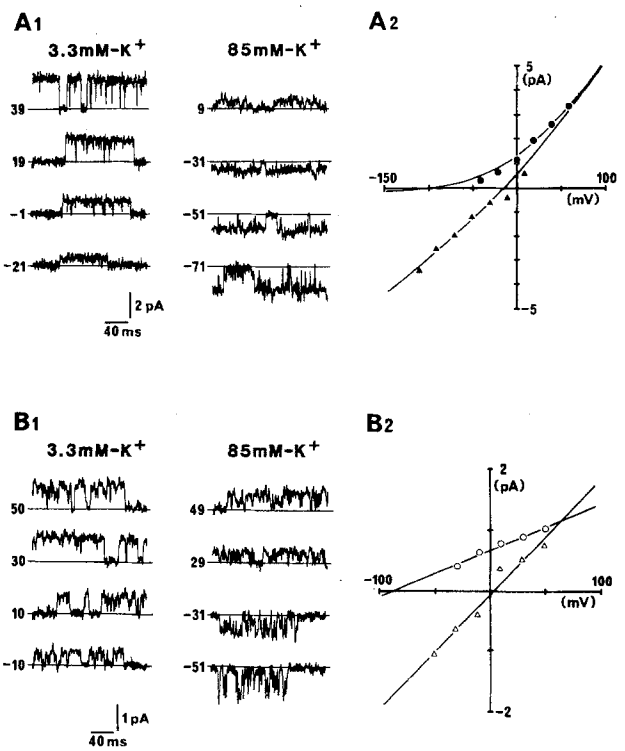

Figure 2. *A 1* Unitary currents of SL-channel with the pipette containing 3.3 mM K^+ or 85 mM K^+. Left-side figure in each trace indicates the patch potential. *A 2* I–V relationships of SL-channel. The same data as shown in A 1. Circle; 3.3 mM K^+. Triangle; 85 mM K^+. The smooth line for the data in 3.3 mM K^+ was the regression line of the GHK-equation by the least square method. P_K and $[K^+]_{in}$ were estimated to be 9.2×10^{-14} cm³/s and 147 mM. The smooth line for the data in 85 mM K^+ was drawn by the GHK-equation using the same P_K and $[K^+]_{in}$. *B 1* Unitary currents of SS-channel with the pipette containing 3.3 mM K^+ or 85 mM K^+. Left-side figure in each trace indicates the patch potential. *B 2* I–V relationships of SS-channel. The same data as shown in B 1. Circle; 3.3 mM K^+. Triangle; 85 mM K^+. The straight lines were drawn by the least square method. Single channel conductance; 7.5 pS in 3.3 mM K^+ and 18.9 pS in 85 mM K^+.

Goldman-Hodgkin-Katz equation (GHK-equation)[8]. In the experiment of figure 2 A 1, the permeability to K^+ (P_K) and $[K^+]_{in}$ were estimated to be 9.2×10^{-14} cm³/s and 147 mM by the least square method. Thus, the K^+ equilibrium potential (E_K) was calculated to be -97.3 mV. The mean $\pm$ SD of P_K and E_K were $9.4 \pm 0.1 \times 10^{-14}$ cm³/s and -96.6 ± 0.75 mV (n = 4). The slope conductance at 0 mV was 23.2 ± 5.2 pS (n = 4). In two patches, the reversal of the SL-channel current was obtained when the pipette was filled with 85 mM K^+ solution (fig. 2 A). The reversal potential of them was -7.9 mV and -4.2 mV, and the slope conductance at 0 mV was 34.0 pS and 27.3 pS. The I–V relationship under such conditions was almost linear (fig. 2 A 2, triangles) and could be fitted quite reasonably with the GHK-equation using the values of P_K and $[K^+]_{in}$ obtained by the fitting of the data in 3.3 mM K^+. These results indicate that the SL-channel is a K^+ channel and its outward-rectifying property is due to the constant-field rectification. Although detailed kinetic analyses have not yet been done, the inspection of single channel data revealed that the SL-channel was almost voltage-independent. The activity of the channel was not markedly changed over a wide range of voltages, and the single channel current could be recorded at the hyperpolarized potential as well as the depolarized potential if the driving force for K^+ was appropriate (see the data in 85 mM K^+ in fig. 2).

The I–V relationship of the SS-channel was linear (fig. 2 B 2). When the pipette solution contained 3.3 mM K^+, the extrapolated reversal potential was -86.4 ± 12.3 mV and the single channel conductance was 8.5 ± 1.7 pS (n = 13). When the pipette was filled with 85 mM K^+ solution, the SS-channel current reversed at about 0 mV (0.0 ± 3.4 mV, n = 4) and the single channel conductance was increased to 17.1 ± 2.4 pS (n = 4). These results indicate that the SS-channel is also a K^+ channel. In contrast to the SL-channel, the SS-channel was voltage-dependent. Its activity was not seen until the patch potential was depolarized from the resting potential even though the pipette was filled with 85 mM K^+ solution and the opening probability was clearly increased by the depolarization.

The SL-channel seems to be the homologous channel to the S-channel of *Aplysia* neurones[3–5]. Both the SL-channel of PON and the S-channel of *Aplysia* function over a wide range of voltages near the resting potential and do not inactivate with prolonged depolarization. The I–V relationship of a single S-channel current also shows the outward rectification and can be fitted by the GHK-equation. Although the slope conductance at 0 mV of the SL-channel (about 23 pS) is less than that of the S-channel (about 50 pS), the published values for the P_K of the S-channel (1.31×10^{-13} cm³/s [3], 1.66×10^{-13} cm³/s [4] and 8.7×10^{-14} cm³/s [5]) are quite similar to the value obtained for the SL-channel of PON (9.3×10^{-14} cm³/s). The difference of the slope conductance may reflect the difference in species.

The SS-channel was the voltage-dependent K^+ channel and its single channel conductance was about 8.5 pS in the normal K^+-gradient. This value and the flickering kinetics of the SS-channel are comparable to those of the delayed rectifier K^+ channel identified in the squid giant axon[9] and the frog skeletal muscle[10]; however, detailed kinetic analyses of the SS-channel still need to be done.

The action of 5-HT on SL- and SS-channels is considered to be mediated by the second messenger system(s), as 5-HT applied by bath perfusion cannot reach the ion channels in the cell-attached patch[6]. In *Aplysia*, several pieces of evidence suggest that the modulation of the S-channel by 5-HT is mediated by a cyclic AMP-dependent protein kinase[11].

The 5-HT-sensitive K^+ current of PON in the whole-cell clamped condition did not reverse in the normal K^+-gradient and the reversal of this current was not seen until $[K^+]_{out}$ was raised 5-fold[1] or more. These results reported previously could be explained by the constant-field rectification of the SL-channel current and the voltage dependency of the SS-channel.

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0014-4754/88/090738-03$1.50 + 0.20/0

Ultrastructure and biochemistry of the pineal organ in deep-sea lanternfishes (Myctophidae)

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Received 17 May 1988; accepted 30 May 1988

Summary. Pineal structural and biochemical adaptations in lanternfishes included: 1) few photoreceptor outer segment discs; 2) conventional synapses between photoreceptors and pineal neurons; and 3) low levels (0–60 pg/pineal) of serotonin compared to those (> 1.0 ng/pineal) in the goldfish pineal organ. These findings suggest reduced photosensory and/or neuroendocrine functions in these deep-sea fishes.
Key words. Pineal; serotonin; photoreceptors; synapses; lanternfishes; goldfish.

The pineal gland of poikilothermic vertebrates contains well-differentiated photoreceptors that also have neuroendocrine functions. Indole biosynthetic pathways leading to the conversion of serotonin (5HT) to melatonin exhibit circadian rhythms with peak enzymatic activities occurring during the dark phase[1]. This functional relationship to photoperiodic