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PROPERTIES OF A SET OF INTERNAL RECEPTORS IN THE MEDICINAL LEECH: THE NEPHRIDIAL NERVE CELLS MONITOR EXTRACELLULAR CHLORIDE CONCENTRATION

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Summary

In the leech, *Hirudo medicinalis*, the functional properties of the peripheral nephridial nerve cells (NNCs) were investigated using long-term extracellular recordings from both intact and isolated preparations.

The NNCs respond selectively to changes in external Cl^- concentration: their electrical activity is highest in fluid containing normal (i.e. low) extracellular [Cl^-]. Their sensitivity to Cl^- is confined to the dendritic tree. Extracellular [Cl^-] is a control factor for ion homeostasis. It increases drastically after a blood meal, and after the animal has left the water or invaded brackish water. The NNCs continuously monitor the extracellular Cl^- concentration: their burst rate changes, without adapting, by a factor of 4 in the physiological range between 40 and 90 mmol l^{-1}.

Intracellular recordings from the NNC in isolated nephridial complexes suggest that a high Cl^- conductance in combination with active transport of Cl^- could be responsible for the observed Cl^- sensitivity.

Introduction

Fluctuations in osmolality and ionic composition of body fluids occur in marine organisms following changes of external salinity, which often persist for hours or days. Various strategies of neuronal adaptation are found in euryhaline osmoconformers (reviewed by Treherne, 1980). Similar, but transient, fluctuations in body fluid composition occur in blood-sucking animals after feeding. This is especially evident in the leech, *Hirudo medicinalis*. In its blood, the cation ($Na^+ + K^+$) concentration is 130 mmol l^{-1} and the Cl^- concentration only 36 mmol l^{-1} (Boroffka, 1968). This imbalance is compensated for by organic acids (Zerbst-Boroffka, 1970; Zebe *et al.* 1981; U. Hoeger & A. Wenning, unpublished results). Feeding on blood, which is hypertonic and contains Cl^- as the only anion, causes the Cl^- concentration in the blood of the leech to increase 2·5-fold within

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15–30 min, while the cation concentration (mainly Na^+) increases only slightly (Wenning *et al.* 1980).

As the segmentally arranged ganglia lie within the ventral blood vessel and the glial sheath is no barrier for ions, water and small molecules (Nicholls & Kuffler, 1964; Schlue & Deitmer, 1980), neurones are exposed to the low blood [Cl^-] and, for example after feeding, to all changes in blood ion concentrations and osmolality. So far, however, all investigations of function and electrical properties of leech neurones have relied on a Cl^--based saline hypertonic to leech blood (see Muller *et al.* 1981; Sawyer, 1986).

Within 48 h of fluid intake, the original osmotic and ionic blood concentrations are re-established by the increased salt and volume output of the 17 pairs of nephridia (Wenning *et al.* 1980). Since body water and extracellular ion concentrations are controlled separately, volume receptors, as well as salt receptors, have been postulated (Zerbst-Boroffka, 1978; Wenning *et al.* 1980). Several questions arise. What information is needed to re-establish the original ion concentrations after osmotic stress? Is neuronal activity affected by transient ion imbalances? How is information about salt and volume status obtained?

This study describes the functional properties of a set of 34 internal receptors, the nephridial nerve cells (NNCs) (Wenning, 1983). To assess their specificity, selected central neurones were also investigated under similar conditions.

Each NNC is associated with one nephridial complex. The cell body lies on the urinary bladder. Its dendrites extend into the nephridium and its axon into the corresponding segmental ganglion *via* the nephridial nerve (Wenning, 1983). The receptive endings lie in the extracellular space between urine-forming cells and blood capillaries (Wenning & Cahill, 1986). A pilot study using intact preparations suggested a sensitivity to extracellular ionic changes rather than to changes in body water (Wenning, 1986). To investigate the electrical activity of the NNCs in greater detail, isolated preparations were subjected to different bathing media and special attention was paid to external [Cl^-] and osmolality. Some of the results have appeared in abstract form (Wenning, 1985, 1988).

Materials and methods

Leeches (*Hirudo medicinalis* L.) were obtained from commercial suppliers and kept at 17–21 °C in water from lake Constance. Experiments were carried out at room temperature (20–22 °C).

Extracellular recordings

Isolated preparations (Fig. 1)

For extracellular recordings from the NNC, a leech was pinned through the suckers and opened dorsally. The head brain was severed, the animal cut into two and each half used for experiments. One excretory system, nephridium and bladder, was exposed. The dorsomedial part of the bladder was removed leaving the final canal, the NNC soma and its dendrites in the nephridium intact. The

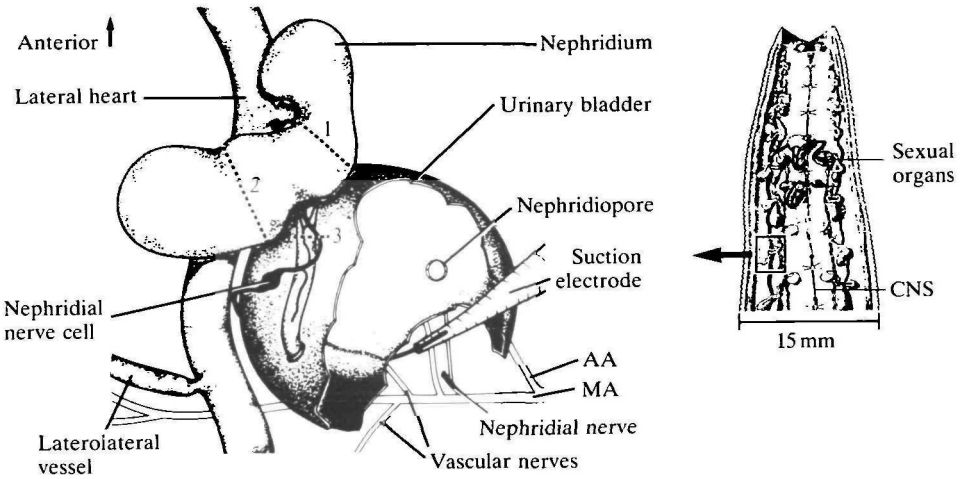

Fig. 1. Dorsal view of the anterior half of a dissected leech preparation (right; after Renner, 1984). The crop has been removed. The ventral nerve cord was severed near the brain. The isolated preparation used for extracellular recordings from the NNC is shown in detail (left). A suction electrode was applied to the peripheral stump of the nephridial nerve. Sequential cuts through the nephridium are indicated by dotted lines. Note that the NNC soma is not drawn to scale. AA, anterior nerve; MA, median anterior nerve.

median anterior nerve and its branches were visible through the ventral bladder wall. The nephridial nerve was exposed and a suction electrode applied to its peripheral stump.

Unless stated otherwise, preparation and initial recordings were in physiological saline (Nicholls & Kuffler, 1964). Solutions (Table 1) were used in the following sequence: (1) physiological saline, (2) test saline (variations in ions and osmolality), (3) artificial blood, (4) physiological saline. Bath volume was about 20 ml and fluid exchange took 3–4 min.

Intact preparations (Fig. 2)

For extracellular recordings from the NNCs in leeches under osmotic stress, the usual methods of anaesthesia (cooling, alcohol) could not be applied because this would have interfered drastically with the animal's response. The animals were pinned ventral side up and either immediately covered with water (control) or subjected to different experimental procedures. For crop-loading experiments, fluid was infused within 1–2 min *via* a flame-polished glass tube inserted through the pharynx into the crop (for details see Zerbst-Boroffka, 1973). For injections into the bloodstream, the lateral heart tube was exposed in a posterior segment and solutions were injected with glass capillaries.

For preparation of the nephridial nerve, a polyethylene catheter filled with 3–5 μl of coloured water (Kiton Pure Blue, Chroma, Stuttgart) was inserted through the nephridiopore into the urinary bladder of a midbody segment. The

Table 1. *Composition of different bathing media for isolated preparations*

	Na^+	K^+	Cl^-	Ca^{2+}	Tris–maleate		Osmolality (mosmol $kg^{-1}H_2O$)
	(mmol l^{-1}) ± S.D. (*N*)					Organic anions	± S.D. (*N*)
Leech blood[1]	126 ± 13 (23)	5 ± 1 (23)	36 ± 6 (27)			Concentrations depend on physiological condition[4]	189 ± 16·5 (23)
Physiological saline[2]	115	4	122·6	1·8	10		240
Artificial blood I[3]	120	4	38·6	1·8	−/10*	15 succinate, 14 lactate, 10 fumarate, 10 $NaHCO_3$ 1·5 α-ketoglutarate, 5 citrate, 0·09 pyruvate	200
Artificial blood II[4]	120	4	38·6	1·8		30 malate, 4 succinate, 2 lactate, 4 fumarate, 0·3 citrate, 2 α-ketoglutarate, 10 $NaHCO_3$	200

All solutions were adjusted to pH 7·4 with NaOH.
* Without (−) and with 10 mmol l^{-1} Tris–maleate; [1] Wenning *et al.* 1980; [2] Nicholls & Kuffler, 1964; [3] Zerbst-Boroffka, 1970; [4] U. Hoeger & A. Wenning, unpublished results.

segment was then opened lateroventrally leaving the sphincter of the nephridiopore intact. The mixture of coloured fluid and urine provided visual contrast to the silver-white nerves. The catheter remained in place and allowed simultaneous monitoring of urine flow (Boroffka, 1968). For *en passant* recordings, the nephridial nerve was placed on a single hook electrode (tip electrolytically tapered) and lifted above the fluid level (Fig. 2B). The reference electrode was in the bath. If the first preparation failed, a second segment was used. In experiments where fluid had been injected into the bloodstream, recordings were carried out in a segment other than the operated one. After the experiments leeches were allowed to swim freely. All survived for at least 6 weeks.

Recordings were made under the following conditions: (1) control, 1–3 h after the animal had been pinned out; (2) hypotonic crop loading, 1–2 h after loading of the crop with 3 ml of 36 mmol l^{-1} NaCl, pH 7·4; (3) hypertonic crop loading, 1–1·5 h after loading of the crop with 3 ml of 145 mmol l^{-1} NaCl + 5 mmol l^{-1} KCl, pH 7·4; (4) 2 h after injection of 100 µl of 2 mol l^{-1} D-sorbitol into the bloodstream; (5) 2 h after injection of 100 µl of 0·2 mol l^{-1} D-glucose into the bloodstream.

Data analysis

Activity of the NNC was expressed as number of bursts per minute. For data analysis and statistics, each recording period (30–80 min) was divided into bins 5 min long. Either the mean frequency of bursts ± S.E.M. in a specific medium was used (Fig. 7), or the mean frequency of bursts in each bin was plotted *versus* time (Fig. 4). Data analysis also included interburst interval histograms (Fig. 6).

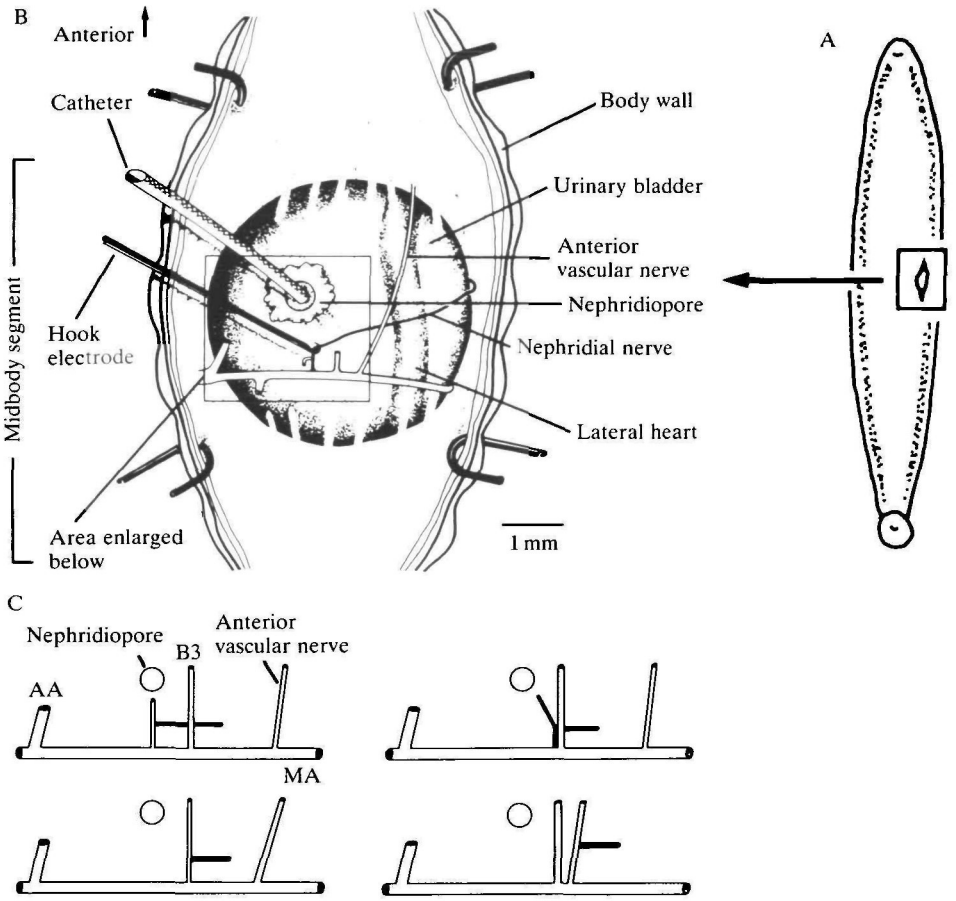

Fig. 2. (A) Ventral view of an intact preparation used for extracellular recordings from the NNC. The area indicated is shown enlarged in B. (B) In a right midbody segment, the nephridial nerve was exposed and placed over a hook electrode. The nephridia, circulation and the urinary bladder were left intact. The catheter allowed simultaneous urine flow measurements. The area indicated is enlarged in C. (C) Most common branching patterns of the nephridial nerve (black) from the median anterior nerve (MA) are shown in relation to nerve B3 (Ort *et al.* 1974), the anterior vascular nerve, and the nephridiopore (circle). Note that the nephridial nerve can also branch from B3 or the anterior vascular nerve.

Intracellular recordings

For intracellular recordings from the NNC, the cell, together with the dorsal wall of the urinary bladder, a nephridium and a piece of lateral heart tube, was dissected out. Protease treatment (Sigma, type XIV; 6–10 min) of the NNC soma facilitated impalement. For intracellular recordings from central neurones, single ganglia or ganglion chains were isolated (segments 1–7 and 3–7). The following neurones were studied: Retzius cells (R), Leydig cells, the cutaneous mechanoreceptors T and N, and the heart excitor motoneurones (HE). All were identified

by position and electrical activity. For intracellular recording from the T cell during stimulation of its sensory endings in the skin, a ganglion with part of the body wall was isolated (Nicholls & Baylor, 1968). A piezoelectric crystal was used for stimulation. Preparations were mounted in a Sylgard-lined recording chamber and viewed with a dark-field condenser as described in Muller *et al.* (1981).

Recording was begun in the same medium as that used for dissection (physiological saline or artificial blood), which was then replaced by high- or low-Cl^- medium, sometimes followed by the first medium again, each for a period of about 30 min. Recording was resumed 10 min after medium change.

Glass microelectrodes were filled with 4 mol l^{-1} potassium acetate + 20 mmol l^{-1} KCl and had resistances of 40–45 MΩ. A Ag–AgCl electrode was used as reference. Neurones were reimpaled in each recording period. To compensate for the changes in junction potential of the different salines, microelectrodes were rebalanced between penetrations. Data were stored on an FM tape recorder (Racal Recorders Ltd, England) at 9·5 cm s^{-1} (cut-off frequency: 1 kHz) and transferred to a chart recorder for the preparation of figures.

Terminology

Numbering of segments and nomenclature of nerves and neurones follow Muller *et al.* (1981). The term 'external [Cl^-], is used instead of 'Cl^- concentration of the bathing medium' or 'Cl^- concentration of leech blood'.

Results

Extracellular recordings

Branching of the nephridial nerve from the anterior segmental nerve varied (for common variations see Fig. 2C). The nephridial nerve is well anchored in the connective tissue of the urinary bladder and contains only a few axons (Wenning & Cahill, 1986). Even in *en passant* recordings, the only action potentials detected were those of the spontaneously active NNC (Wenning, 1983).

The NNC was tested for mechanoreceptive functions using different preparations. Neither inflation of the bladder *via* the catheter nor movements of the animal (see Fig. 5A) in intact preparations had any effect on NNC activity. Heart tube contractions rhythmically deformed the nephridial complex and, consequently, the soma and dendrites of the NNC, but did not change its activity. Wherever the nephridial nerve accompanied the anterior vascular nerve (Fig. 2C), signals from the HE neurone and from the NNC were both recorded (see Fig. 5D), but no correlation was observed. In isolated preparations, mechanical stimulation of the nephridia, the surrounding tissue or the heart were without effect.

Isolated preparations

In physiological saline (122·6 mmol l^{-1} Cl^-, Table 1), the mean spontaneous activity of the NNC was 0·67 bursts min^{-1} (see Fig. 7*k*). Interburst intervals varied considerably (see Fig. 6). Intraburst frequency was initially 6–7 Hz. When th

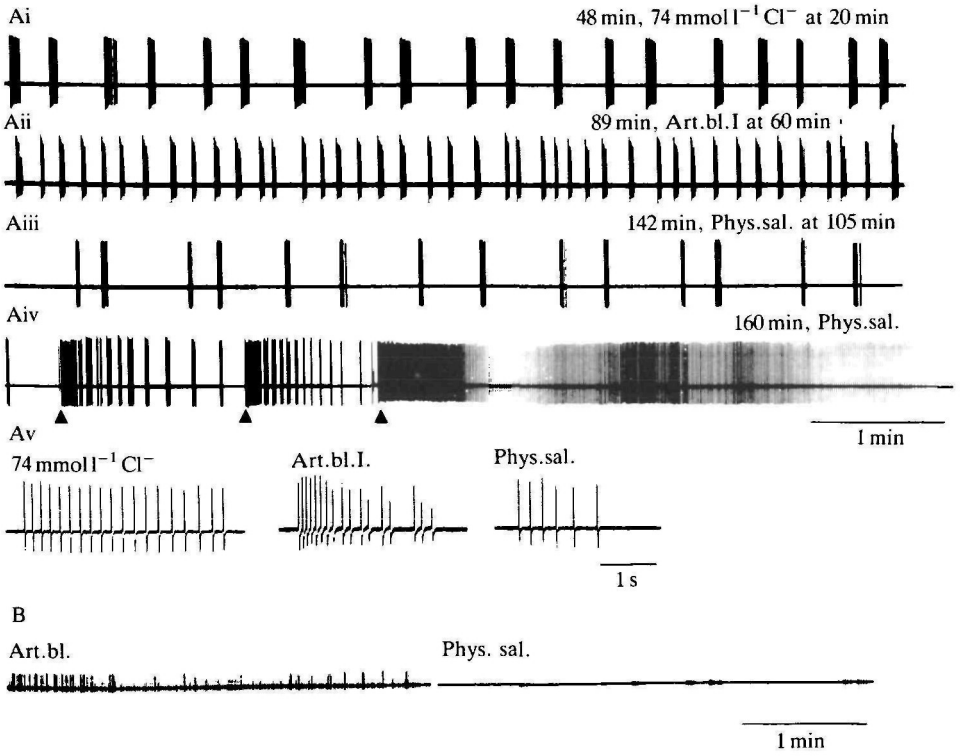

Fig. 3. Portions of extracellular recordings from the NNC in two isolated preparations. (Ai) Recorded 48 min after the beginning of the experiment. Activity of the NNC 28 min after changing the bath to 74 mmol l^{-1} external Cl^-. (Aii) Recorded 89 min after beginning of the experiment. Cell activity 29 min after changing the bath to artificial blood I (Art.bl.I.). (Aiii) Recorded 142 min after beginning of the experiment. Cell activity 37 min after change to physiological saline (Phys.sal.). (Aiv) Recorded 160 min after beginning of the experiment. Parts of the nephridia were cut successively (arrows; sequence indicated on Fig. 1) leading to massive discharges. (Av) Intraburst frequencies vary with different external Cl^-. (B) In this preparation the nephridium was removed, leaving only dendritic stumps of the NNC on the central canal. Note the marked response to low (left) and high (right) external $[Cl^-]$.

bathing medium was changed to artificial blood I (38·6 mmol l^{-1} Cl^-), both the mean frequency of bursts (see Fig. 7*e*) and that of action potentials within a burst (Fig. 3A) increased. When the NNC was not spontaneously active, it began firing in artificial blood. Whether the NNC fired at low (Fig. 4, left) or high (Fig. 4, right) frequency in physiological saline, the relative increase of its activity in artificial blood was similar. Intermediate Cl^- concentrations, obtained by appropriate mixing of artificial blood with physiological saline or of physiological saline with distilled water, caused intermediate activity (Figs 3Ai, 7*f*,*g*,*h*,*i*). The new discharge pattern persisted throughout the recording time in the respective medium (Figs 3, 4). The responses to changes in external $[Cl^-]$ were reversible (Fig. 4).

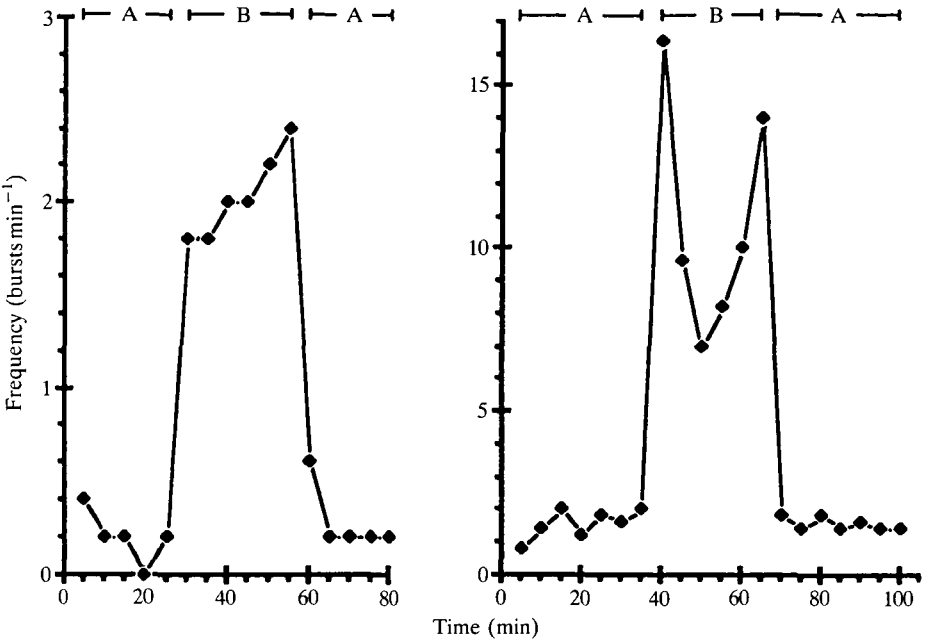

Fig. 4. Frequency of bursts (bin length 5 min) in extracellular recordings from the NNC in isolated preparations. Note the difference in the frequency of bursts in the two preparations in physiological saline (-A-) and its similar increase upon change to artificial blood (-B-).

The specificity of the NNCs' response to Cl^- was tested by using other anions. Activity also increased when physiological saline was exchanged for: (1) artificial blood containing different organic anions (artificial blood II; Table 1); (2) solutions in which organic anions were replaced by SO_4^{2-}; and (3) artificial blood buffered with Tris–maleate. When SO_4^{2-} test saline was exchanged for artificial blood, activity increased further (data not shown). The response of the NNC was not affected by the pH of the medium (tested between 7·1 and 7·8).

The sensitivity of the NNCs to osmolality was tested using two different methods. Osmolality was increased to 240 mosmol kg^{-1} H_2O by adding 40 mmol l^{-1} D-sorbitol to artificial blood I. Activity increased in four of six preparations and increased further after a change to artificial blood in five preparations (data not shown). Osmolality was lowered by diluting physiological saline with distilled water (4:1), resulting in a test saline of 190 mosmol kg^{-1} H_2O and 90 mmol l^{-1} Cl^-. The frequency of bursts remained low (Fig. 7*i*).

The sensitivity of the NNC appeared to be confined to the dendrites. Severing of the nephridial lobes (as indicated on Fig. 1) led to marked, but transient, discharges (Fig. 3Aiv). However, some dendrites on the final canal were still intact (Fig. 1) and, after 5–10 min, firing resumed and showed a strong response to changes in external [Cl^-] (Fig. 3B). After severing the dendrites near the soma, propagated action potentials were no longer recorded.

Intact preparations

The specificity of the NNC was further examined using long-term extracellular *en passant* recordings in intact preparations under defined physiological conditions. The following parameters were tested. (1) Blood *volume* which increased after crop loading (Zerbst-Boroffka, 1978; Hildebrandt & Zerbst-Boroffka, 1988). (2) Blood *osmolality* which increased after hypertonic crop loading and after injection of hypertonic D-Sorbit solution (100 μl, 2 mol l^{-1}) into the bloodstream but decreased after hypotonic crop loading. (3) Blood *ions* (Na^+, K^+, Cl^-) which did not change after injection of either D-Sorbit or isotonic D-glucose solutions but decreased after hypotonic crop loading and increased (especially Cl^-) after hypertonic crop loading (Zerbst-Boroffka, 1973; Wenning *et al.* 1980).

In control animals, a characteristic NNC burst consisted of 3–7 action potentials with decreasing amplitude and frequency, beginning at 14 Hz (Fig. 5A). The mean frequency of bursts was 7·5 min^{-1} (Fig. 7*d*). After hypertonic crop loading (high blood osmolality and $[Cl^-]$, little effect on $[Na^+]$), it decreased significantly, and the initial intraburst frequency dropped to 2–3 Hz (Figs 5C, 7*j*). After hypotonic crop loading (blood osmolality and $[Na^+]$ low, little effect on $[Cl^-]$), frequency of bursts and intraburst frequency (initial rate 12 Hz) remained high (Figs 5B, 7*a*). Injection of D-Sorbit solution into the bloodstream caused blood osmolality to increase from approx. 190 (control) to 281 ± 15 mosmol kg^{-1} H_2O ($N = 6$) without changing the concentration of blood ions. The mean frequency of NNC bursts was then 6·8 min^{-1} (Fig. 7*b*); in the example shown (Fig. 5E), it was 9·4 min^{-1}. Injection of isotonic glucose solution did not change blood ion concentrations or osmolality ($N = 6$), and the NNCs' activity remained high (Figs 4D, 7*c*). A second unit, occasionally recorded after hypotonic crop loading, and, more rarely, after hypertonic crop loading, was characterized by bursts of up to 30 action potentials of decreasing amplitude and increasing frequency (Fig. 5B). Final urine flow, measured simultaneously, did not differ from that of non-operated animals.

As in isolated preparations, the NNC also responded selectively to changes in external $[Cl^-]$ in intact preparations, but not to changes in blood osmolality, blood cations or blood volume.

Intracellular recordings

Changes of external $[Cl^-]$ resulted in changes of the membrane potential (E_M) of the NNC, as seen in intracellular recordings from its soma in isolated nephridial complexes (Fig. 8). In physiological saline, E_M was −56·4 ± 8·7 mV (S.D.) and the size of soma spikes was 20–30 mV. In artificial blood, the values were −38·8 ± 9·1 mV (S.D.) and <10 mV, respectively (Table 2). Effects were fully reversible (Fig. 8).

For comparison, the effect of low (= normal) external $[Cl^-]$ on the spontaneous activity of some centrally located neurones was tested either in single ganglia or in ganglion chains. For Retzius cells, E_M remained the same in physiological saline or artificial blood (Table 2), but spontaneous activity greatly increased in low-Cl^-

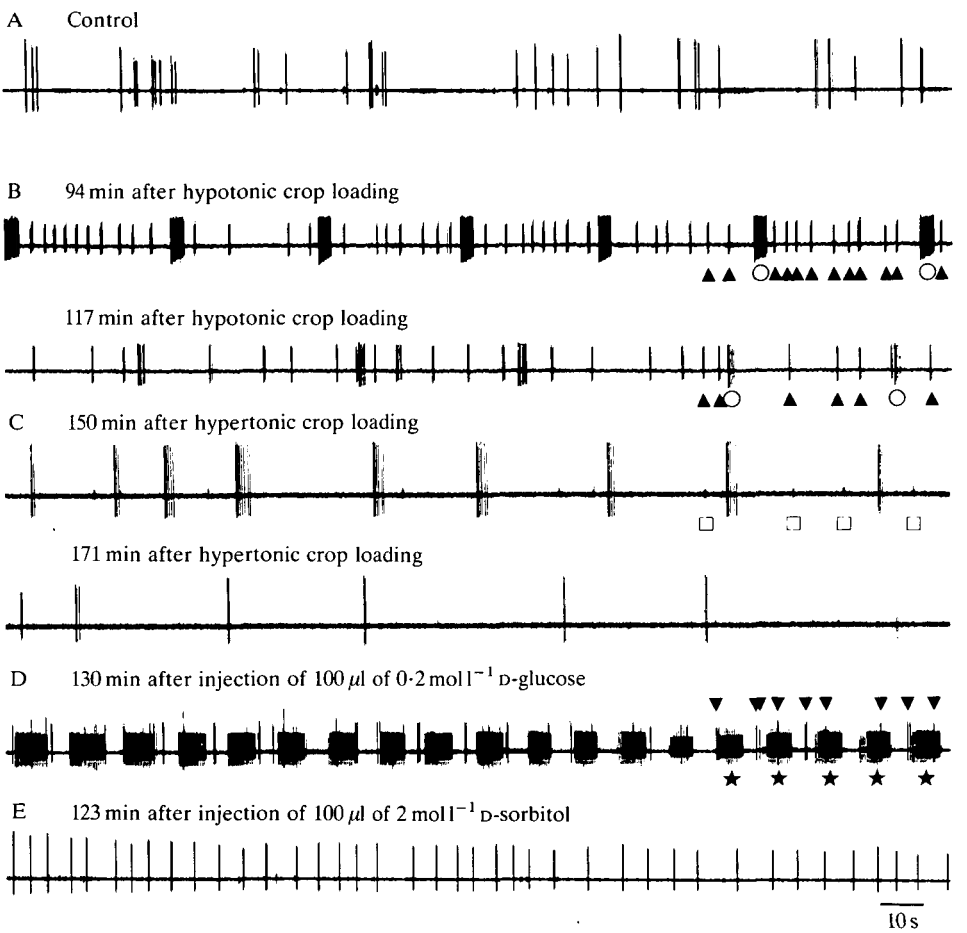

Fig. 5. Sections of extracellular *en passant* recordings from the nephridial nerve in intact preparations under five experimental conditions with different effects on blood ions and osmolality. (A) Control: animal movements caused fluctuations in the fluid level around the recording site. Note the concomitant fluctuations in the signal to noise ratio of the NNC. (B) Hypotonic crop loading causing an increase in blood volume, a decrease in osmolality, and a slight decrease in [Cl^-]. Upper trace (94 min after crop loading): note the appearance of a second unit (○) in addition to the NNC (▲); lower trace (23 min later, almost 2 h after crop loading): the frequency of bursting of the NNC and second unit have decreased. (C) Hypertonic crop loading causing an increase in blood volume, osmolality and [Cl^-]. Upper trace (150 min after crop loading): note the low frequency of bursts of the NNC and a second small unit (□). Lower trace (171 min after crop loading): activity has further decreased. (D) 2 h after injection of isotonic D-glucose solution into the lateral heart, blood ions and osmolality are normal. Frequency of bursts of the NNC (▼) does not change. In this preparation, heart motor neurone output was also recorded (★, heart rate: 5·4 min^{-1}). (E) 2 h after injection of D-sorbitol into the lateral heart, blood osmolality has increased without affecting blood ions. In the experiment shown, the mean frequency of NNC bursts was 9–10 bursts min^{-1}.

conditions, and action potentials occurred in bursts (Fig. 9). Leydig cells exhibited more spontaneous activity in low external [Cl^-] than in high external [Cl^-]. E_M, the frequency of spontaneous action potentials in T and N cells, and the response

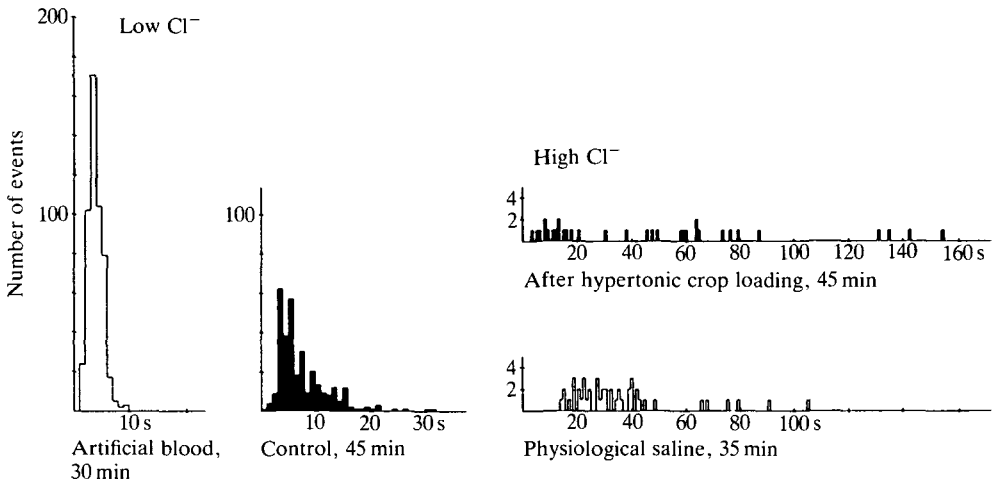

Fig. 6. Interburst interval histograms of isolated (open columns) and intact (filled columns) preparations. Recording time is indicated for each experiment. In low-Cl^- conditions (artificial blood, control), most burst intervals are < 10 s, whereas in high external [Cl^-] (hypertonic crop loading, physiological saline), they vary between 5 and 150 s. Note that at the same external [Cl^-], interburst intervals are similar in isolated and intact preparations.

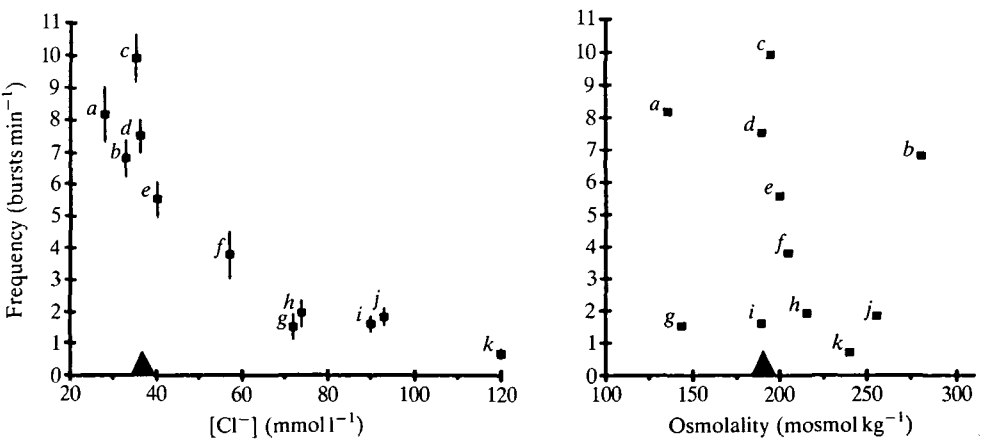

Fig. 7. Mean frequency of NNC bursts (±S.E.M. of the respective recording period) plotted against external [Cl^-] and osmolality. Arrowheads indicate normal blood [Cl^-] and osmolality. *a*, intact, after hypotonic crop loading ($N = 8$); *b*, intact, after injection of 2 mol l^{-1} D-sorbitol solution ($N = 9$); *c*, intact; after injection of 0·2 mol l^{-1} D-glucose solution ($N = 9$); *d*, intact, control ($N = 8$); *e*, isolated, artificial blood ($N = 29$); *f*,*g*,*h*,*i*, isolated, intermediate Cl^- concentrations ($N = 4$–9); *j*, intact, after hypertonic crop loading ($N = 8$); *k*, isolated, physiological saline ($N = 39$).

Table 2. *Intracellular recordings from selected neurones in different external [Cl^-]*

Neurones	Membrane potential (mV) ± S.D. (*N*)		Spontaneous activity (bursts min^{-1}) ± S.D. (*N*)	
	Physiological saline	Artificial blood	Physiological saline	Artificial blood
Nephridial nerve cell	−56·4 ± 8·7 (10)*	−38·8 ± 9·1 (10)*	Often tonically active	Always tonically active
Retzius	−54·2 ± 4·3 (24)	−53·4 ± 7·8 (19)	+	+++
Leydig	−47·4 ± 12·2 (5)	−40·7 ± 8·3 (3)	+	++
N	−47·5 ± 7·8 (13)	−52·8 ± 6·2 (12)	No difference	
T	−52·2 ± 6·6 (18)	−53 ± 5·6 (18)	No difference	
HE	−45·1 ± 4·2 (18)	−40·4 ± 6·2 (18)	4·6 ± 1·3 (9)	10·6 ± 1·5 (6)

* *t*-test $P < 0{\cdot}001$.

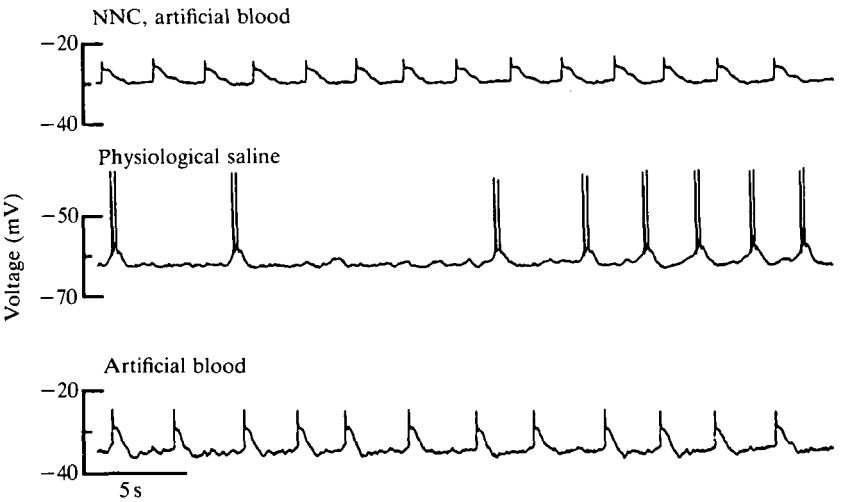

Fig. 8. Serial intracellular recordings of spontaneous activity of the same NNC in artificial blood, physiological saline and again in artificial blood. Note the difference in E_M and size of the soma spikes in different external [Cl^-].

of T neurones to mechanical stimulation with a piezoelectric crystal did not change in media of different [Cl^-] (Table 2; Fig. 9). Burst output from the HE neurones, which determine heart rate (Calabrese & Peterson, 1983), doubled in artificial blood, and the intraburst frequency increased (Table 2). Individual inhibitory postsynaptic potentials (IPSPs) in the HE cells, originating from the heart interneurones (HN; Thompson & Stent, 1976), disappeared in artificial blood. The inhibitory input of the HN persisted as shown by the rhythmic changes of the E_M in the HE (Fig. 9).

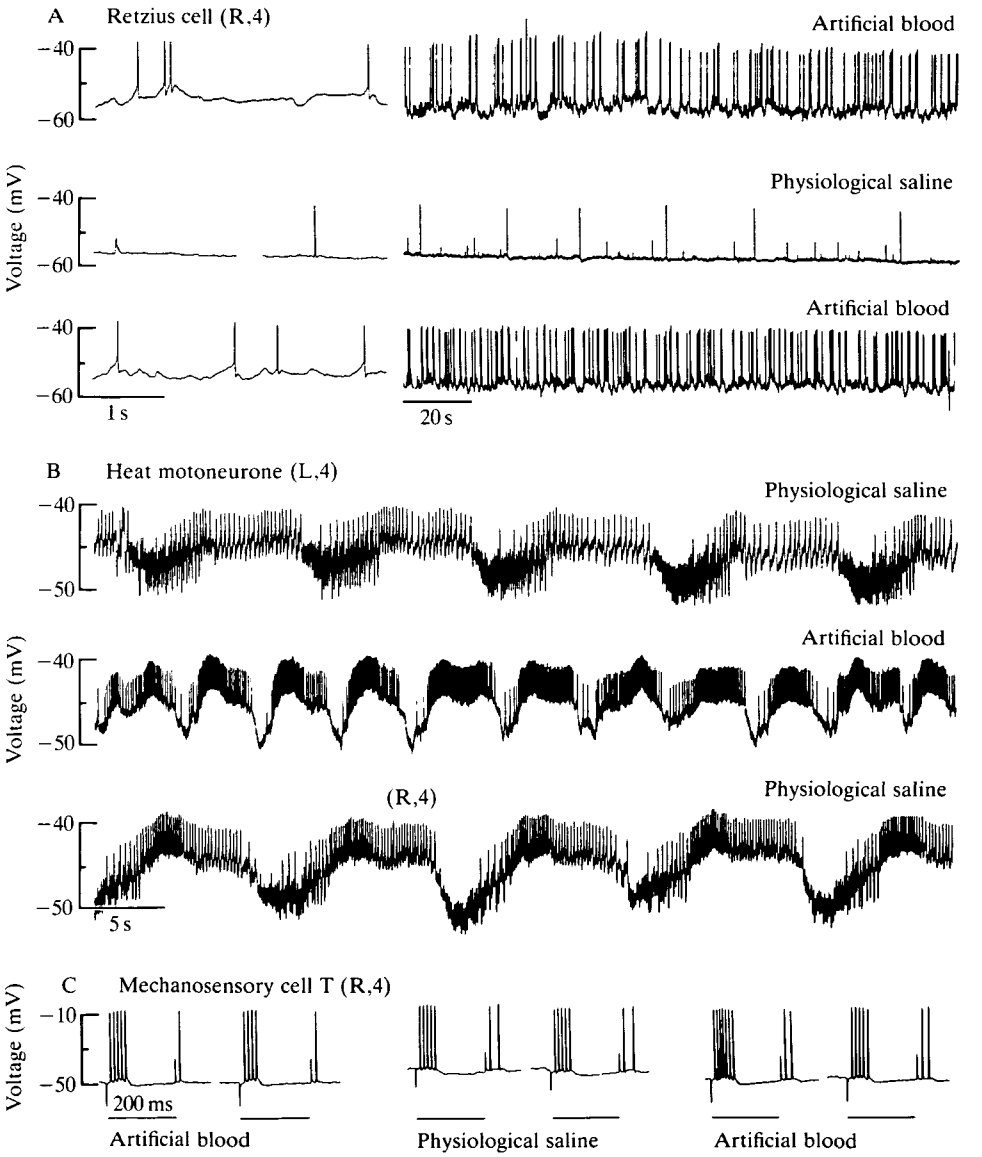

Fig. 9. Intracellular recordings of spontaneous activity of central neurones in high and low external [Cl^-]. (A) Serial recordings from one Retzius cell (R,4) of isolated ganglion chains (ganglia 3–7) in artificial blood, followed by recordings in physiological saline and again in artificial blood. The shape of action potentials changes in different external [Cl^-]. Note that effects are fully reversible. (B) Serial recordings from two heart motoneurones (L,4 and R,4) of one isolated ganglion chain (ganglia 3–7) in physiological saline, followed by artificial blood and again physiological saline. Note that effects are fully reversible and that IPSPs, but not cycling of E_M, disappear in artificial blood. (C) Serial intracellular recordings from one mechanosensory neurone (R,4). The respective body wall flap was attached to the ganglion and the skin stimulated with a piezoelectric crystal (stimulus length, 200 ms; indicated by the solid line and artefacts). Responses to the first and second stimulus are shown. Recordings began in physiological saline, followed by artificial blood and again physiological saline. Sensitivity does not change with external [Cl^-]. Action potentials were attenuated owing to filtering; the actual size was 63 mV.

Discussion

Internal receptors monitoring parameters such as osmolality, ion concentrations or volume of the body fluids are generally postulated for maintenance of salt and water homeostasis. Their locations, and some of their properties, are known in vertebrates. Osmoreceptors in the hypothalamic region and blood volume receptors in the right atrium and the veins (see Baylis, 1987) are examples. Receptors sensitive to increased blood [Na^+] were recorded from in the hypothalamic region in Pekin ducks (Gerstberger *et al.* 1988). The only invertebrate osmoreceptors identified are external ones, such as those in the osphradium of the marine snail *Aplysia* (Stinnakre & Tauc, 1969; Theler *et al.* 1987), but their functional properties are unknown. Reports on internal non-mechanoreceptors in invertebrates are rare. A noteworthy exception is a study of enteroceptors in the oesophagus of crustaceans (Altner *et al.* 1986). This dearth of information hinders a comparative evaluation of the internal receptors described in this study.

Properties of the NNC

That the sensory NNCs do not function as mechanoreceptors is indicated by the lack of correlation of their activity with blood pressure, blood volume or stretch in the bladder wall (see also Wenning, 1986). They are sensitive neither to changes of pH nor to changes of the organic acids which constitute 70 % of blood anions (Zerbst-Boroffka, 1970; U. Hoeger & A. Wenning, unpublished results). The NNC continues signalling when the nephridia are removed (see also below). The concomitant injury to the dendrites in the nephridia (see fig. 4 in Wenning & Cahill, 1986) leads to massive but transient discharges (Fig. 3A).

The frequency of bursts and the intraburst frequency of the NNC correlate strongly with changes of external [Cl^-] and do not correlate with external osmolality (Fig. 7). Furthermore, at the same external [Cl^-], the activity of the NNC is similar in recordings from intact and isolated preparations (Fig. 7). Recordings from intact preparations show the NNCs to be insensitive to the increase in blood volume after crop loading, as the frequency of bursts is high after hypotonic (Figs 5B, 7*a*) but low after hypertonic crop loading (Figs 5C, 7*j*). Isolated preparations permit monitoring of the NNC activity upon acute changes of the bathing medium. Furthermore, osmolality and ions can be changed separately beyond physiological limits. In such experiments the NNC responds within minutes and only to changes of [Cl^-] in the bath. The changed activity is maintained throughout the recording time (Figs 4, 6).

With the circulation intact, numerous blood vessels provide efficient solute and fluid exchange through the nephridial capillary network (Boroffka & Hamp, 1969; Hildebrandt, 1988). This complex blood supply does not permit perfusion of isolated nephridia. In isolated preparations, complete and rapid fluid exchange is therefore hindered by the unavoidable disruption of circulation. The NNC is more sensitive to changes in external [Cl^-] when the nephridium is removed (Fig. 3B). This potentiation might be due to exposure of the remaining dendrites on the final canal (Fig. 1).

When recorded intracellularly, E_M of the NNC (Table 2) and, consequently, the size of the soma spikes change with external $[Cl^-]$ (Fig. 8). This is not due to damage caused by repeated impalements of the rather flat neurone, as the effect is fully reversible (Fig. 8) and experiments were begun in high as well as low external $[Cl^-]$. Obviously, the E_M of the NNC depends on $[Cl^-]$ and is not governed exclusively by $[K^+]$. When extrapolated for a 10-fold increase of external $[Cl^-]$, E_M drops by 47 mV compared with the theoretical value of 58 mV for a perfect Cl^- electrode. Thus, the sensitivity of the NNC for Cl^- is likely to result from a high resting Cl^- conductance, in combination with an active transport of Cl^-. Investigations under voltage-clamp conditions along with ion substitution experiments will further clarify the underlying mechanism of Cl^- sensitivity.

The peripheral NNC qualifies as an ion receptor as it is continuously active in a pattern related to external $[Cl^-]$. Is Cl^- sensitivity a feature unique to these neurones and is this indeed their physiological role?

Effects of changes in external $[Cl^-]$ on central neurones

In contrast to the E_M of the NNC, that of the central neurones does not change with external $[Cl^-]$ (Table 2) and the contribution of Cl^- to their E_M seems to be negligible. Experiments on other sensory neurones (e.g. the cutaneous mechanoreceptive T cells, Nicholls & Baylor, 1968) show that neither their sensitivity to mechanical stimuli nor the rate of spontaneous action potentials changes with external $[Cl^-]$ (Fig. 9). The spontaneous activity of neurosecretory cells (Leydig, Retzius) and the cycle rate of the heartbeat central pattern generator (CPG) – an example of a different somatic function – were affected by changes in external $[Cl^-]$ (Table 2).

Retzius cells showed burst-like activity in low external $[Cl^-]$ and single action and synaptic potentials in high $[Cl^-]$. The underlying intracellular mechanisms of bursting and non-bursting patterns were recently studied by Dean & Leake (1988), but these experiments were performed under high-Cl^- conditions.

The cycle rate of the isolated heartbeat CPG doubles after a change from high-Cl^- to low-Cl^- conditions (Table 1). In physiological saline, it corresponds to the heart rate of intact preparations (Krahl & Zerbst-Boroffka, 1983; Arbas & Calabrese, 1984). Why, at the same low external $[Cl^-]$ is the output of the isolated heartbeat CPG twice as high as the heart rate of intact leeches? The HE cells still receive rhythmic inhibition (Fig. 9), which disappears at 3·8 mmol l^{-1} external Cl^- (Calabrese, 1979). Whether the observed frequency change of the heartbeat CPG is due to osmotic changes or, as in cardiac follower cells of *Limulus* (Prior & Pierce, 1981), to ionic changes needs further examination. Evaluation of the functional significance of this phenomenon, however, is difficult, as the heart rate of undisturbed leeches is unknown.

In the isolated leech CNS, hypertonic and high-Cl^- physiological saline provide 'healthy' (= quiet) recordings. High external $[Cl^-]$ might augment inhibitory input, and thus facilitate studies of neuronal interrelationships. It would be interesting and desirable, however, to study physiological functions (swimming,

heartbeat, feeding) in an environment more realistic for the leech CNS. This would help to distinguish osmotic and/or ionic effects on central neurones from effects of the lack of input in isolated ganglia.

Physiological role of the NNCs

As noted by Shaw (1961) for Na^+ regulation in crustaceans, ion regulatory mechanisms in invertebrates are not so much concerned with the maintenance of 'normal' blood ion concentrations, as with the prevention of changes beyond certain limits. In the leech, ion regulatory mechanisms might prevent too drastic an increase of blood [Cl^-] after feeding, when ion concentrations and osmolality increase drastically (Zerbst-Boroffka, 1973; Wenning *et al.* 1980). Monitoring extracellular [Cl^-] is advantageous for an animal with a low blood [Cl^-] and a high-Cl^- diet. In the physiological range from 40 to 90 mmol l^{-1} Cl^-, the burst output of the NNC varies by a factor of 4 (Fig. 7). Thus, the NNCs, with their dendrites in the highly vascularized nephridia, might well serve as internal Cl^- receptors. Their activity changes with, but does not adapt to, the ionic changes which persist for several hours upon osmotic stress. In contrast, neurones of marine organisms do adapt to salinity changes by various mechanisms (small axon diameter, internal structural support, rigid glial sheath, ion redistribution), ensuring normal electrical functioning (Treherne, 1980).

In the leech, salt and water homeostasis is maintained by nephridial activity, and the information relayed by an ion receptor such as the NNC might affect salt output by causing adjustment of final urine concentration. Identification of postsynaptic targets of the NNC could yield more information about their specific physiological role in acute salt and water regulation.

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