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SUSTAINING OLFACTION AT LOW SALINITIES: MAPPING ION FLUX ASSOCIATED WITH THE OLFACTORY SENSILLA OF THE BLUE CRAB *CALLINECTES SAPIDUS*

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Summary

To test the hypothesis of a diffusion-generated, ionic/osmotic microenvironment within the olfactory sensilla (aesthetascs), flux gradients of Ca^{2+} and K^+ associated with the external surfaces of these sensilla were spatially mapped using self-referencing, ion-selective microelectrodes. Blue crabs (*Callinectes sapidus*) acclimated to low-salinity conditions (15 % sea water and fresh water) showed a net efflux of ions from the aesthetascs. The region of maximum flux associated with each aesthetasc conformed to that predicted from structural data and corresponded to the permeable region of the cuticle separating the olfactory dendrites from the external environment. Estimates of net flux from the entire tuft of aesthetascs for both Ca^{2+} and K^+ fell within the predicted range on the basis of comparisons with $^{22}Na^+$ flux measured previously and assuming a passive diffusion model of ion movement from the hemolymph to the sensillar lymph and, ultimately, to the external environment. The maximum concentrations of these ions measured deep within the tuft are discussed in the light of a potential across the aesthetascs that may limit ion efflux at low salinities.

Key words: self-referencing electrode, ion-selective electrode, aesthetasc, blue crab, *Callinectes sapidus*, Ca^{2+} flux, K^+ flux, low salinity adaptation, olfaction.

Introduction

A fundamental challenge to sustaining olfaction in aquatic organisms at low salinities is that of maintaining the appropriate ionic/osmotic transmembrane gradients required for neural function in olfactory dendrites while simultaneously ‘exposing’ these dendrites to the external environment (Gleeson et al., 1996, 1997). Insight on how this problem might be solved in vertebrates has been gained through studies in various fish and amphibian species. Indeed, recent advances in understanding the nature of ion currents involved in olfactory transduction, together with *in vivo* data on olfactory receptor responses in various ionic conditions, provide an important basis for deciphering the means by which suitable transmembrane gradients can be maintained in chemosensory systems challenged by low- or variable-salinity conditions (e.g. Suzuki 1978; Kurahashi and Yau, 1993; Shoji et al., 1994, 1996; Kleene and Pun, 1996; Reuter et al., 1998). One concept that has emerged from this work is that a Ca^{2+}-activated Cl^- current may play a major role in generating the receptor potential in freshwater conditions. This idea remains speculative, however, until more information on the nature and dynamics of the ionic environment within the mucus bathing the olfactory cilia can be obtained (Kurahashi and Yau, 1993; Kleene and Pun, 1996; Reuter et al., 1998).

In the euryhaline blue crab *Callinectes sapidus*, we propose that, at low salinities, a diffusion-generated microenvironment within the olfactory sensilla (aesthetascs) is responsible for sustaining the functional integrity of the olfactory dendrites (Gleeson et al., 1996, 1997). It has been shown previously that the outer dendritic segments of the aesthetascs are reduced in length at low salinities: in freshwater (FW)-acclimated crabs, the portion of each dendritic process separated from the external environment by the permeable cuticle of the aesthetasc, and therefore ‘exposed’ to the external milieu, is approximately 150 μm in length compared with over 500 μm in seawater (SW)-acclimated animals (Gleeson et al., 1996). It is hypothesized that passive diffusion of ions from the hemolymph to the sensillar lymph creates an extracellular ionic/osmotic environment within the aesthetasc that supports the structural and functional integrity of these ‘exposed’ regions of the outer dendritic segments (Gleeson et al., 1997). This idea is further supported by recent findings that provide evidence for a paracellular pathway by which the diffusion of ions between the hemolymph and sensillar lymph can occur (Gleeson et al., 2000).

In the present study, we spatially mapped the flux gradients of Ca^{2+} and K^+ associated with the aesthetascs using self-referencing, ion-selective microelectrodes in blue crabs acclimated to brackish and freshwater conditions. These

profiles provide evidence for a net efflux of ions from the aesthetascs at low salinities, revealing a region of maximum flux corresponding to that anticipated from the structure of aesthetascs. The flux levels for both Ca^{2+} and K^+, when compared with those measured previously for Na^+ (Gleeson et al., 1997), fell within a range consistent with that predicted for passive diffusion from the hemolymph along a pathway similar to that followed by Na^+. The concentrations of these ions within the aesthetasc tuft are considered in the light of a potential across the aesthetascs that may limit ion flux at low salinities.

Materials and methods

Animals

Mature, intermolt male specimens of *Callinectes sapidus* Rathbun were used in all experiments and sustained on a diet of shrimp throughout the holding periods in the laboratory. SW-acclimated animals were obtained locally from salt-marsh creeks and initially held for several days at the Whitney Laboratory in tanks with a flow-through supply of ambient sea water. Acclimation to 15 % sea water was accomplished by subsequently transferring these crabs to aquaria containing aerated 50 % sea water (i.e. ambient sea water diluted with deionized water) on day 1 and 25 % sea water on day 2. On day 3, the animals were moved to tanks with a flow-through supply of 13–18 % sea water, where they were held for at least 10 days. This flow-through water supply was generated by a fail-safe dilution system that mixed ambient sea water with dechlorinated (i.e. charcoal-filtered) tap water. The crabs were then transported to the Marine Biological Laboratory in Woods Hole, where they were held in aquaria containing aerated 15 % sea water that was changed every 2 days.

FW-acclimated crabs were obtained from Lake George, a freshwater section of the St Johns River in Florida, and transported to the Marine Biological Laboratory, where they were held in tanks containing aerated water approximating the ion concentrations present in Lake George (Gleeson et al., 1997). This water was made by diluting ambient sea water to 1.6 % with deionized water and adding sufficient $CaCl_2$ to yield a calcium concentration of approximately 1.26 mmol l^{-1}. The water was changed every other day.

Ion measurements

All measurements using ion-selective microelectrodes were performed at the BioCurrents Research Center at the Marine Biological Laboratory in Woods Hole, MA, USA. A stable whole-animal preparation was developed for measuring ion flux associated with the antennule (Fig. 1). Crabs were secured to a rigid platform using Velcro straps, and the left antennule (held in place by a clamp anchored to the platform) was

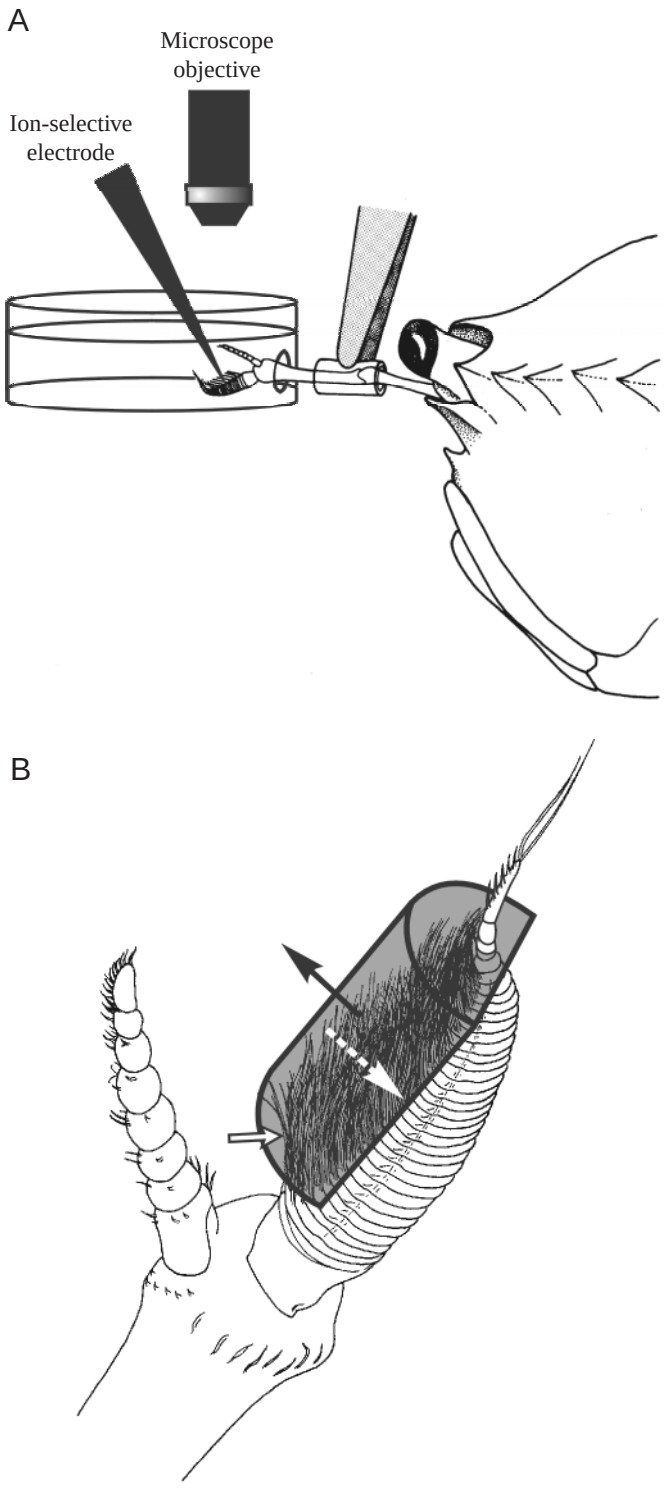

Fig. 1. (A) Schematic diagram of the preparation used for ion measurements in the vicinity of the aesthetasc tuft using self-referencing, ion-selective microelectrodes (modified from Gleeson et al., 1997). (B) Drawing of the antennule of the blue crab illustrating survey lines extending away from (filled arrow) and into (broken arrow) the aesthetasc tuft on the outer flagellum along which the ion flux measurements shown in Figs 2–4 were made. The open arrow illustrates the electrode orientation used for measurements made perpendicular to the first row of aesthetascs. The shaded half-cylinder covering the tuft (2 mm in length, with a radius of 0.5 mm) approximates the spatial dimensions of a typical aesthetasc tuft. The surface area of this cylinder was used to estimate ion flux from the entire tuft and, dividing by 700, the net flux per aesthetasc (modified from Gleeson, 1980).

inserted into a reservoir (also secured to the platform) containing approximately 2 ml of water with ionic concentrations appropriate for the acclimation conditions of the animal. A layer of Sylgard lining the bottom of the reservoir permitted the aesthetasc-bearing outer flagellum to be clamped in place with small pins such that flicking was prevented. The whole assembly was placed under an upright compound microscope (Zeiss, Axioskop) enclosed in a Faraday box. Ion gradient measurements were collected using a computer-controlled ion-selective microelectrode (Ion View software BRC) while simultaneously monitoring the electrode's position *via* a video camera.

Self-referencing, ion-selective microelectrodes were used to map precisely the concentration gradients (and, by calculation, the flux profiles) associated with the outer flagellum of the antennule for both Ca^{2+} and K^+. The theory and details of the general technique are described elsewhere (Smith et al., 1999). In the present study, electrodes were fashioned from glass micropipettes (WPI catalog no. TW 150-4) pulled to a patch electrode shape. For Ca^{2+} measurements, the electrodes were back-filled with 100 mmol l^{-1} $CaCl_2$ and front-filled with a short column (30 μm) of Ca^{2+}-selective liquid membrane (Fluka Calcium Ionophore I; Cocktail A). The reference electrode bridge contained 3 mol l^{-1} KCl in 3% agar. In the K^+ measurement experiments, the electrodes were back-filled with 100 mmol l^{-1} KCl and front-filled with a column (150 μm) of K^+-selective liquid membrane (Fluka Potassium Ionophore; Cocktail B). The reference electrode bridge contained 3 mol l^{-1} NaCl in 3% agar. The Nernstian response characteristics of each electrode were checked in a series of standards before and after each experiment.

Solutions used in the antennular reservoir for K^+ measurements were made from artificial seawater (ASW; concentrations in mmol l^{-1}: NaCl, 423; KCl, 8.9; $CaCl_2$, 12.9; $MgCl_2$, 23.1; $MgSO_4$, 25.6; $NaHCO_3$, 2.1) diluted with deionized water to 15% or 1.6%. The latter dilution is herein referred to as artificial fresh water (AFW). For Ca^{2+} experiments, similar dilutions were made using Ca^{2+}-free ASW and subsequently adding $CaCl_2$ to yield solutions containing 100 μmol l^{-1} Ca^{2+} unless otherwise specified.

Data analysis

All flux and concentration values presented in the figures are means ± S.D. for at least five data points acquired sequentially over a period of 60 s at each electrode position. Calculations of flux were made as in Smith et al. (1999); these are essentially based on the Fick equation ($J=D\Delta C/\Delta r$) where J is flux, D is the diffusion coefficient and ΔC is the difference in concentration (from the calibrated Nernst slope of the electrode) between the poles of the electrode's excursion distance (Δr). The excursion distance in these experiments was 50 μm, and diffusion coefficients of 8.0×10^{-6} cm^{-2} s^{-1} for Ca^{2+} and 2.0×10^{-5} cm^{-2} s^{-1} for K^+ were assumed.

Results

Outward fluxes of both Ca^{2+} and K^+ from the aesthetasc tuft were clearly evident in FW-acclimated animals (Fig. 2) and were comparable with those observed previously in crabs acclimated to 15% sea water (Gleeson et al., 2000). Under the conditions of these experiments, both ions exhibited measurable flux gradients extending several hundred micrometers from the tuft. This was found in all the crab antennule preparations (four for Ca^{2+} and five for K^+) probed for outward flux of ions in AFW.

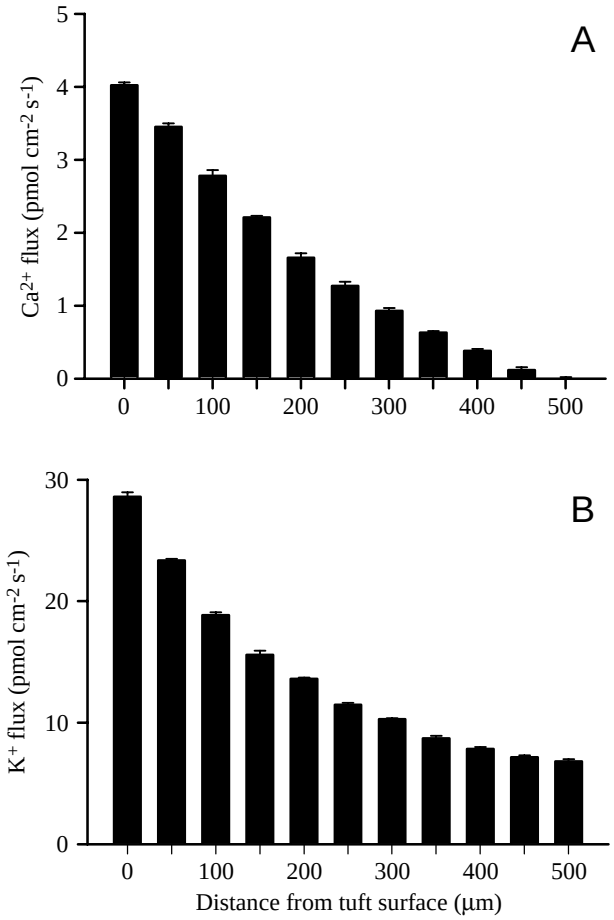

Fig. 2. Outward flux of Ca^{2+} (A) and K^+ (B) from the aesthetasc tuft of blue crabs acclimated to fresh water. The profiles are representative of data generated in all antennule preparations probed for outward flux of ions in artificial fresh water (four for Ca^{2+} and five for K^+). Measurements were made sequentially at 50 μm increments along lines extending outwards from the ventral surface of the tuft (tips of the aesthetascs) as shown by the filled arrow in Fig. 1B. Values are means + S.D., $N\geqslant5$.

Ion measurements extending into the tuft from its ventral surface (i.e. along lines from the tips of the aesthetascs to their bases), under both AFW and 15% ASW conditions, revealed an increasing flux gradient with depth followed by a precipitous drop in the vicinity of the basal regions of the aesthetascs. Representative profiles of this rise and fall in flux, as the electrode was advanced into the tuft, are illustrated in Fig. 3 for Ca^{2+} and Fig. 4 for K^+. This general pattern of flux was a consistent finding for both Ca^{2+} and K^+ in all antennule

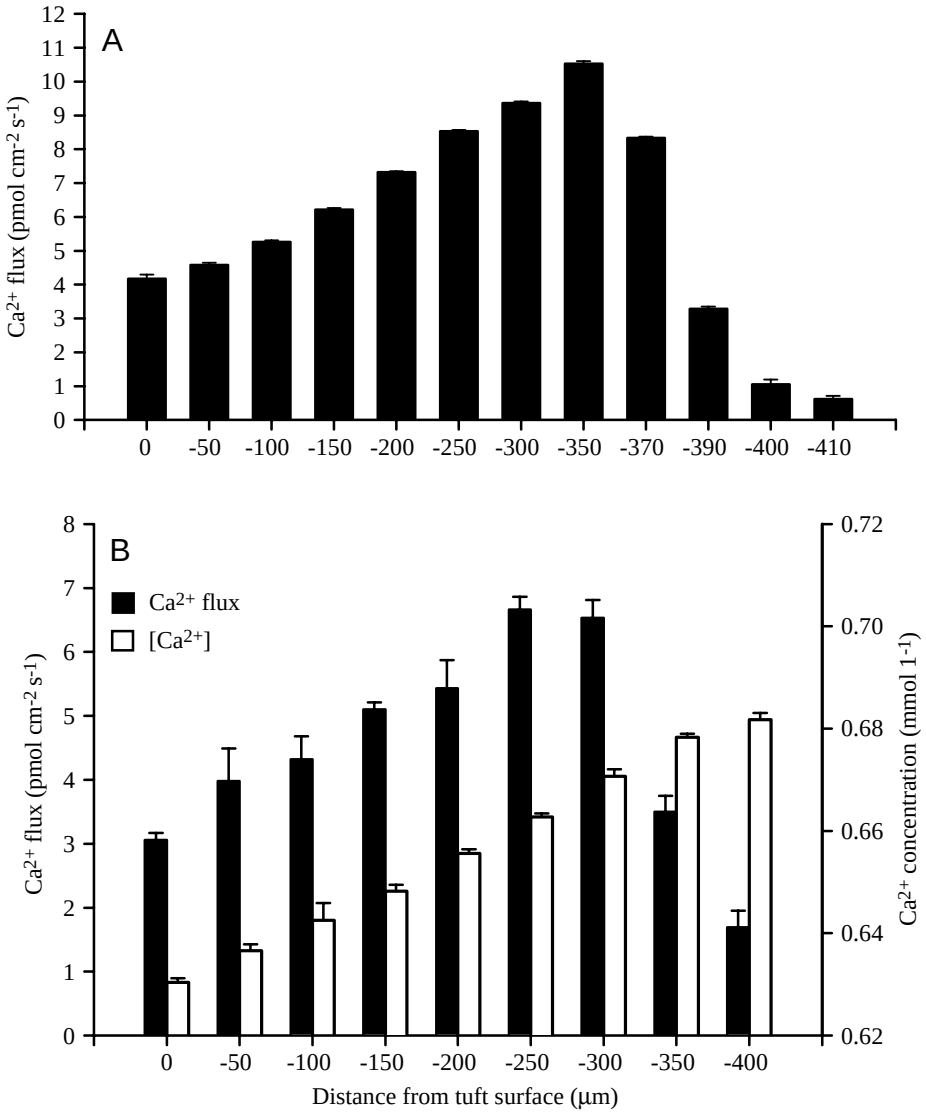

Fig. 3. (A,B) Ca^{2+} flux and concentration at different positions within the aesthetasc tuft. The profiles are representative of data derived from seven antennule preparations. Measurements were made sequentially along lines extending inwards from the ventral surface of the tuft (i.e. from the tips of the aesthetascs to their bases), as shown by the broken arrow in Fig. 1B. (A) Ca^{2+} flux in artificial fresh water (AFW). (B) Ca^{2+} flux and concentration in AFW containing 500 μmol l^{-1} Ca^{2+}. Values are means + S.D., $N \geqslant 5$.

preparations measured [i.e. a total of seven preparations for Ca^{2+} (five in AFW, one in 15% ASW and one in AFW containing 500 μmol l^{-1} Ca^{2+}) and a total of nine preparations for K^+ (seven in AFW and two in 15% ASW)]. Observations of the electrode position during these experiments, together with subsequent analysis of photo images and video recordings, indicated that the location of the rise to maximum flux, followed by a drop, closely corresponded to the distal terminus of the constricted region of aesthetascs (Gleeson et al., 1996). This association between maximal flux and the distal border of the constricted region was further supported by measurements of K^+ flux using a horizontal scanning orientation adjacent to the first row of aesthetascs (Fig. 1B, open arrow). Profiles at this orientation also showed a flux maximum distal to the constricted region.

Ion measurements within the aesthetasc tuft also revealed an increase in concentration for both Ca^{2+} and K^+ as a function of depth from the tuft surface. For these measurements, the concentration at each position of the electrode was calculated from the mean value of potentials recorded at both ends of the electrode's 50 μm excursion axis. Fig. 3B illustrates a profile for Ca^{2+} concentration compared with the corresponding flux levels at different depths within the tuft. Note that, whereas flux drops sharply between −300 and −400 μm from the tuft surface, Ca^{2+} concentration rises to a peak in this region. The profile of increasing concentration to a maximum in the basal portion of the tuft was a consistent finding for both Ca^{2+} and K^+ in AFW and 15% ASW conditions. Peak concentration (mean ± S.E.M.) measured in the basal region of the aesthetasc tuft in AFW conditions was 0.21±0.03 mmol l^{-1} (N=5) for Ca^{2+} and 0.43±0.06 mmol l^{-1} (N=6) for K^+.

Flux gradients surveyed at non-aesthetasc locations on the outer flagellum of the antennule tended to be very shallow if present. Considered together with concentration measures in these regions, as well as the orientation of flux gradients associated with the tuft, it is likely that these minor levels of apparent flux derived from that associated with the aesthetascs (Fig. 5).

Ca^{2+} and K^+ fluxes on a per aesthetasc basis were calculated by using flux measurements at the surface of the tuft and

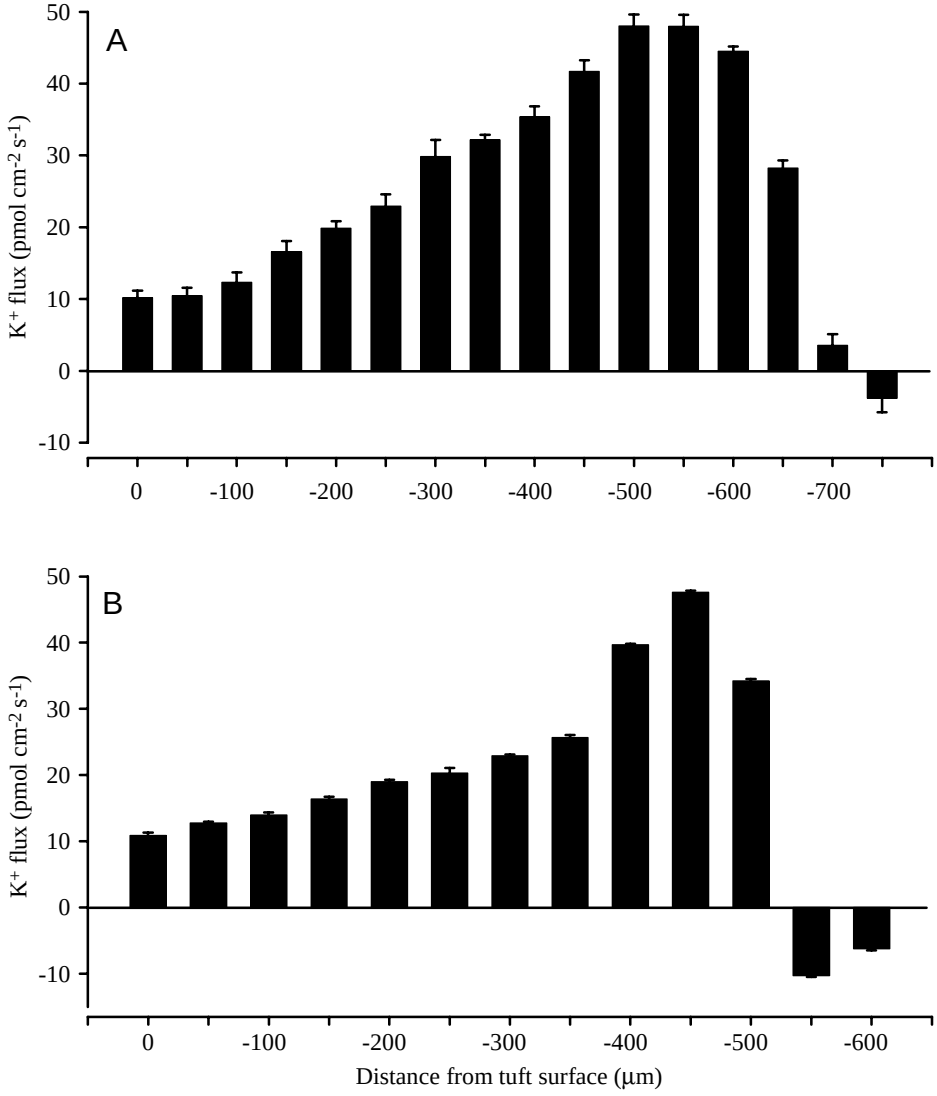

Fig. 4. (A,B) K^+ flux at different positions within the aesthetasc tuft. The profiles are representative of data derived from nine antennule preparations. Measurements were made as in Fig. 3. (A) K^+ flux in 15% artificial sea water. (B) K^+ flux in artificial fresh water. Values are means + S.D., $N \geq 5$.

assuming that these levels were uniform across the area defined by a tuft-sized half-cylinder (Fig. 1B). The measured flux multiplied by the surface area of this half-cylinder and divided by 700 (the approximate number of aesthetascs in an average tuft; Gleeson, 1982) yielded the per aesthetasc flux. In AFW, mean net outward flux for Ca^{2+} from the ventral surface of the tuft was found to be 3.0±0.32 pmol cm^{-2} s^{-1} (mean ± S.E.M., N=5). Similarly, mean net outward flux for K^+ in AFW was 15.5±2.7 pmol cm^{-2} s^{-1} (mean ± S.E.M., N=6). From these values, the per aesthetasc flux for Ca^{2+} in freshwater conditions was calculated to be 0.259 fmol aesthetasc^{-1} s^{-1}; for K^+ it was 1.26 fmol aesthetasc^{-1} s^{-1}.

Discussion

Our findings corroborate previous studies implicating a net outward diffusive flux of ions from the aesthetasc sensilla in low-salinity conditions and, hence, lend further support to the hypothesis of a dynamically sustained ionic/osmotic microenvironment within the aesthetascs. Flux gradients for both Ca^{2+} and K^+ were clearly associated with the tuft, exhibiting maximum values in proximity to the distal terminus of the constricted region of aesthetascs. The location of this maximum, together with estimates of the net efflux for these two ions, is consistent with a model in which ion movement from the hemolymph to the sensillar lymph, and ultimately out of the aesthetasc, occurs by passive diffusion through a paracellular pathway.

The estimates of net flux from the aesthetascs for Ca^{2+} and K^+ in low-salinity conditions, as calculated from measurements using ion-selective microelectrodes, are within the ranges of those expected assuming a passive diffusion model and based on the net flux measured previously for Na^+ (using radioactive ^{22}Na) as a benchmark (Gleeson et al., 1997). If the movement of ions from the hemolymph to the external medium by way of the aesthetascs occurs by simple diffusion, then the flux levels for any two ions should be such that the ratio of their net flux is comparable with the ratio of the concentration gradients driving the movement of each ion. Differences in diffusion coefficients and valence (if a potential exists across the system) are also factors to consider in such comparisons.

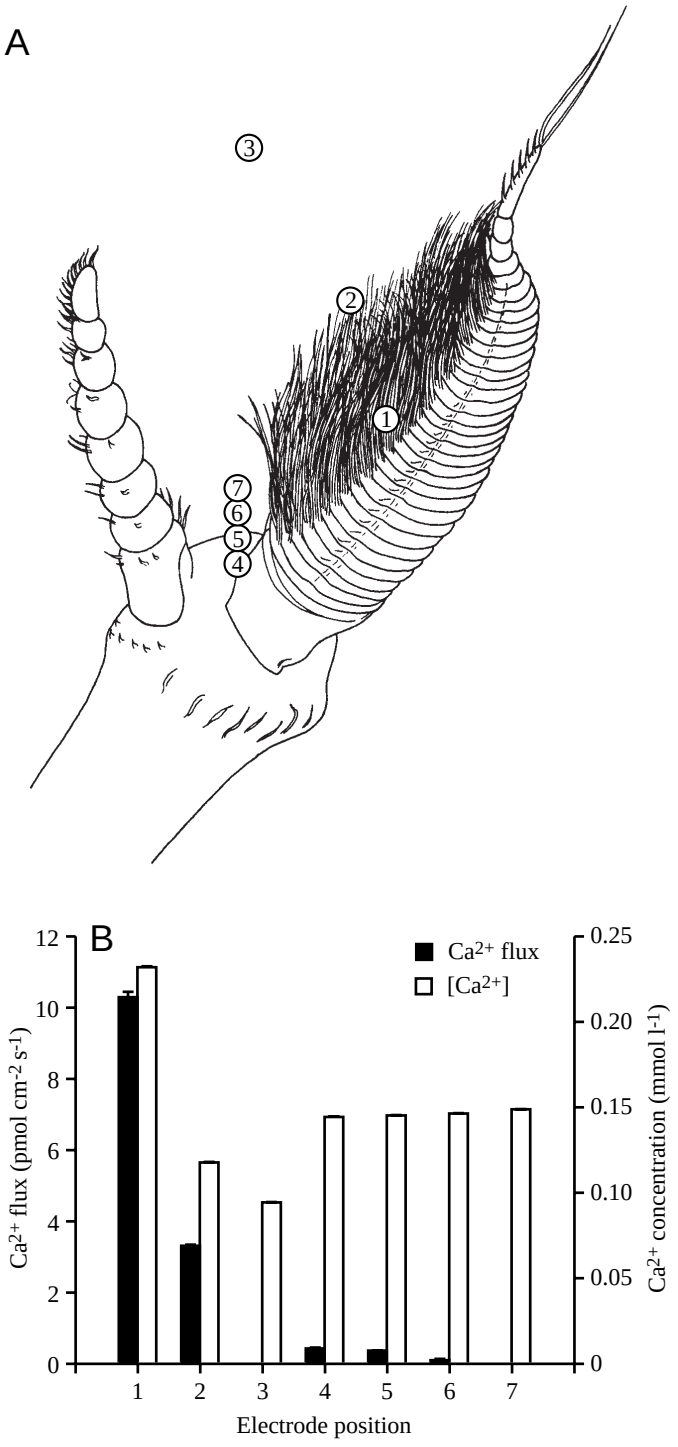

Fig. 5. (A) Schematic diagram of a blue crab antennule illustrating approximate electrode positions in B (modified from Gleeson, 1980). (B) Comparison of flux and concentration measurements for Ca^{2+} at selected locations within and adjacent to the aesthetasc tuft. The antennular reservoir contained 15 % artificial sea water. Electrode positions were as follows: (1) within the center of the tuft; (2) on the ventral surface of the tuft (the tips of the aesthetascs); (3) 1000 μm away from the ventral surface of the tuft; (4) on the surface of the most proximal segment of the outer flagellum (the non-aesthetasc region); (5–7) 50, 100, 150 μm away, respectively, from electrode position 4 along a survey line parallel to the aesthetascs. Values are means + S.D., $N \geqslant 5$.

In freshwater conditions, the net outward flux for Ca^{2+} was calculated to be 0.259 fmol aesthetasc^{-1} s^{-1} for a concentration gradient between the hemolymph and external medium of 9.8–10.2 mmol l^{-1} [based on a hemolymph concentration of 10.3 mmol l^{-1} (Gleeson et al., 1997) and external medium concentrations of 0.5 and 0.1 mmol l^{-1}, respectively]. Under similar conditions, the net flux of Na^{+} was found to be 42.17 fmol aesthetasc^{-1} s^{-1} when the concentration gradient between the hemolymph and external medium was 424.6 mmol l^{-1} (Gleeson et al., 1997). The flux ratio (i.e. Ca^{2+}/Na^{+}), corrected for differences in the diffusion coefficients (i.e. multiplied by 1.88), was 0.012, whereas the ratio of the concentration gradients was 0.023–0.024. These ratios are of similar magnitude, suggesting that Ca^{2+} diffuses passively from the hemolymph, as proposed for Na^{+}. The lower flux ratio may in part reflect the differential effects of a trans-antennule potential on ion flux due to valence differences between these ions (Gleeson et al., 1997). In addition, any differences in the permeability of the ions as they diffuse from the hemolymph to the sensillar lymph and out to the external medium could influence the flux ratio.

The net outward flux for K^{+} in freshwater conditions was calculated to be 1.26 fmol aesthetasc^{-1} s^{-1} for a concentration gradient between the hemolymph and external medium of 4.46 mmol l^{-1} [based on a hemolymph concentration of 4.60 mmol l^{-1} (Gleeson et al., 1997) and external medium concentration of 0.14 mmol l^{-1}]. The K^{+}/Na^{+} flux ratio, corrected for differences in the diffusion coefficients (i.e. multiplied by 0.75), was 0.022. This compares with a concentration-gradient ratio of 0.011. Again, the flux and concentration gradient ratios are of the same order of magnitude, suggesting that Na^{+} and K^{+} follow a similar diffusion pathway from the hemolymph. Here, too, permeability differences between the two ions could alter the flux ratio.

Flux profiles extending into the aesthetasc tuft from its ventral surface revealed an increase to a peak level just distal to the constricted region of the aesthetascs, followed by a steep drop or even a reversal of flux direction in some cases. This pattern is consistent with what might be predicted from the structure of aesthetascs (Gleeson et al., 1996) considered together with the dense arrangement of these sensilla on the outer flagellum. The constricted region is characterized externally by a slight taper in the aesthetasc that corresponds internally to a zone in which an auxiliary cell sheath surrounds the tightly packed outer dendritic segments of the olfactory receptor neurons. The distal border of this constricted region is approximately 120 μm from the base of the aesthetasc; beyond this point, there is no sheath around the dendrites, and the cuticle of the sensillum is very thin and permeable. As an electrode is advanced into the tuft, we propose that the rise in flux levels within the external medium reflects an approach

towards the major source of ions diffusing from the aesthetascs. As suggested by the structure of the aesthetasc, together with dye studies (Gleeson et al., 1996), ions passing into the sensillar lymph from the hemolymph can presumably diffuse to the external medium only through cuticle that is distal to the constricted region; and, indeed, the maximum flux levels measured within the tuft were consistently found at a position that corresponds closely with the distal terminus of the constricted region. Immediately proximal to this point, ion concentrations in the tuft were high and relatively constant, with flux levels approaching zero. This accumulation of ions in the basal region of the tuft probably derives, at least in part, from the tight clustering of aesthetascs together with diffusion that is constrained by the surface of the outer flagellum.

The concentrations of ions bathing the outer dendritic segments within aesthetascs in low-salinity conditions are unknown; however, net flux estimates permit some speculation as to the turnover of ions in this fluid environment. Such calculations were made previously for Na^+ in FW-acclimated animals (Gleeson et al., 1997); these determinations assumed that the approximate volume (12 pl) of sensillar lymph bathing the outer dendritic segments within an aesthetasc had a Na^+ concentration equal to that of hemolymph. The time required to replace the total Na^+ in this volume was calculated to be between 30 and 120 s. Similar determinations for Ca^{2+} and K^+, based on net flux per aesthetasc determined in the present study, yield 477 s and 44 s, respectively.

If, in fact, the cuticle above the constricted region of aesthetascs is freely permeable to Ca^{2+} and K^+, one might expect that the concentrations within the external medium immediately adjacent to these sensilla would correspond closely with those of the sensillar lymph bathing the dendrites internally. However, maximum concentrations measured within the tuft under low-salinity conditions, particularly in AFW, tended to be well below those found in the hemolymph and were indicative of ion levels that would cause rapid vesiculation of the outer dendritic segments in isolated aesthetascs (Gleeson et al., 1996). It is conceivable that the putative ionic/osmotic microenvironment created by the sensillar lymph has dimensions that simply do not extend beyond the cuticle, and measurements made in the external medium therefore provide little insight on its nature. However, a factor that could play an important role in limiting ion diffusion into the external medium is a potential across the cuticle. Indeed, a trans-antennule potential of −59 mV (hemolymph with respect to external medium) was measured previously in a similar freshwater-antennule preparation (Gleeson et al., 1997). It is not known where the potential drop occurs in the system but, if associated with the cuticle of the aesthetascs, such a potential could account for the relatively low concentrations of Ca^{2+} and K^+ in the external medium surrounding the sensilla. Three lines of evidence might support this idea. (i) Under AFW conditions, the equilibrium potentials for Ca^{2+} and K^+ correspond closely to the −59 mV trans-antennule potential measured previously. These values are −49.0 mV for Ca^{2+} and −59.7 mV for K^+ [calculated from the Nernst equation (at 25 ° C) and based on concentrations in the basal region of the aesthetasc tuft of 0.21 mmol l^{-1} and 0.43 mmol l^{-1} and hemolymph concentrations of 10.3 mmol l^{-1} and 4.6 mmol l^{-1} for Ca^{2+} and K^+, respectively]. (ii) In preliminary studies examining the effects of an artificial flick (i.e. the aesthetasc tuft is vigorously flushed with fresh medium such that the fluid within the tuft is completely exchanged within 1 s), a consistent finding has been that the concentrations of Ca^{2+} and K^+ in the basal region of the tuft immediately fall to ambient bathing medium levels, but then rapidly return to an apparent equilibrium concentration (equal to the pre-flick concentration) over the course of 5–10 min. (iii) The reduction in the unidirectional efflux of Na^+ from aesthetascs in freshwater conditions *versus* seawater conditions can be almost entirely accounted for by the more negative trans-antennule potential of the freshwater condition (Gleeson et al., 1997).

If the trans-antennule potential is partially or wholly associated with the cuticle of the aesthetascs, a question of particular interest is how it might be established. One possibility is the generation of a Donnan potential created by anions too large to diffuse across the cuticle from the sensillar lymph to the external medium. In this scenario, the diffusion of cations to the external medium would be limited by the potential gradient established across the cuticle. Such a potential is generated, for example, in the periplasmic space of enteric bacteria by highly anionic polysaccharides too large to diffuse through the porin proteins of the cell wall (Kennedy, 1982, 1987). These polymers give rise to a Donnan potential that results in cations accumulating at higher concentrations within the periplasm than in the medium (Stock et al., 1977; Sen et al., 1988). A second possible mechanism by which a potential might be generated across the aesthetasc is through selective permeability of the cuticle. Smith and Linton (1971) discovered that the gill cuticle of *C. sapidus* is more permeable to Na^+ than it is to Cl^-. Since that report, several studies examining the ionic permeabilities of gill and non-gill cuticle in crustacean species of varying ion- and osmoregulatory capacities have established the importance of the differential ion selectivity of gill cuticle for ion regulators (Avenet and Lignon, 1985; Lignon and Lenior, 1985; Lignon, 1987a,b; Lignon et al., 1988; Lignon and Péqueux, 1990). It is possible that the cuticle of the blue crab's aesthetasc, which has a thickness and structure similar to that of gill cuticle (Copeland and Fitzjarrell, 1968; Compère et al., 1989), also exhibits ion selectivity. In this scenario, if the absolute permeability to Cl^- is low, in freshwater conditions, the movement of Na^+ and other cations into the external medium would be constrained by the development of a diffusion potential. In essence, the outward flux of cations would be limited by the permeability of the cuticle to Cl^-.

In summary, our results provide further evidence for a net outward flux of ions from the aesthetascs at low salinities as well as more detailed information on the location of the ion-flux source. The findings support the hypothesis of a diffusion-generated microenvironment within the aesthetascs of the blue crab at low salinities (Gleeson et al., 1997). The possible

existence of a potential across the cuticle of the aesthetascs and its role in limiting ion losses, hence helping to sustain the ion concentrations within these sensilla, is an intriguing question that clearly deserves further study.

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