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ELECTROGENIC Na^+ TRANSPORT IN A CRUSTACEAN COXAL RECEPTOR

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SUMMARY

1. The response of the coxal receptors of the crab *Scylla serrata* to step stretches consisted of a partial action potential, V_a, followed by a steady-state depolarization, V_s. The input resistance of the fibre was reduced during V_s.

2. In the absence of stimulation, the dendrites of the receptors depolarized when external Na^+ was substituted with choline or Li^+, and when the external K^+ concentration was increased or decreased. The dendrites also depolarized when ouabain was added to the saline.

3. The amplitude of both V_a and V_s was dependent on external Na^+. In cells which were depolarized by ouabain, the amplitude of V_s increased when the K^+ concentration of the saline was reduced.

4. V_s was followed by a small, but long-lasting, after-potential which was depolarizing when the membrane potential was between -70 and -60 mV. In cells depolarized by ouabain or by low K^+ saline, the after-potential became hyperpolarizing.

5. When trains of brief stretches (each 5 ms in duration) were used as stimuli, the cells responded with trains of V_a responses. During this tetanic stimulation the cells hyperpolarized; cessation of the stimulus train was followed by a long-lasting hyperpolarization (PTH).

6. PTH was abolished in Li^+ saline, in low K^+ saline, and in the presence of ouabain. In control or in low K^+ saline, PTH was not accompanied by a decrease in the input resistance of the fibres.

7. It is concluded that an electrogenic Na^+ pump (or equivalent process) contributes a substantial fraction of the membrane potential of the unstimulated coxal receptors. Pump activity could be increased by Na^+-loading the distal part of the cells with trains of V_a responses. By contrast, during the steady-state response to stretch, the pump was not activated.

INTRODUCTION

The fibres of the crustacean coxal receptor neurones (Alexandrowicz & Whitear, 1957) depolarize when exposed to artificial sea water (ASW) containing ouabain, and also when exposed to low K^+ ASW and to cold temperature (Mirolli, 1974; Mirolli, quoted in Bush, 1976). On the strength of analogy with the results obtained in other preparations (Gorman & Marmor, 1970), these data suggest that active

electrogenic Na^+ transport (Thomas, 1969, 1972) could be present in these sensory cells.

This suggestion is of potential interest because the occurrence of electrogenic Na^+ transport seems to be common among sensory neurones. In some mechanoreceptors of the crayfish (Nakajima & Takahashi, 1966) and of the leech (Van Essen, 1973) Na^+ loading consequent to intense spiking activity results in a prolonged hyperpolarization due to a transient increase of the activity of an electrogenic Na^+ pump. It has been convincingly argued that the pump-mediated hyperpolarization may contribute to sensory adaptation in these cells, although it is recognized that other factors are also involved in this phenomenon (same references; see also Nakajima & Onodera, 1969, and Sokolove & Cooke, 1971). It has also been suggested that electrogenic Na^+ transport may be important in sensory transduction proper. The most clear evidence for this conclusion has come from studies done on the non-spiking visual receptors of vertebrates (Zuckerman, 1973). However, in other non-spiking sensory cells the role of active Na^+ transport seems limited to the regulation of the internal Na^+ concentration (Brown & Lisman, 1972).

Although the basic response to mechanical stimuli of the crustacean coxal receptors is a graded potential (Bush & Roberts, 1971; Roberts & Bush, 1971), in some species the response to step stretches may be more complex involving also an initial active response which is a partial Na^+ spike (Roberts & Bush, 1971). As shown in the preceding paper (Mirolli, 1979), in the coxal receptors of *Scylla serrata* the ability to generate this partially active response is limited to the distal part of the cells where the graded potentials also originate. These neurones, therefore, seemed to afford a particularly favourable material for the study of the possible physiological significance of electrogenic Na^+ transport in the process of mechano-transduction. The results which are presented in this paper provide additional, and more complete, evidence that electrogenic Na^+ transport is indeed present in the coxal receptors. The effects of the pump can be demonstrated using the partially active response as a way of Na^+ loading the distal portion of the cells. However, as in the case of the *Limulus* photoreceptors, studied by Brown & Lisman (1972), electrogenic Na^+ pumping does not affect directly the magnitude of the *graded* receptor potentials (at least in the experimental conditions used in this study).

METHODS

Most of the techniques used have been presented in detail in the preceding paper (Mirolli, 1979). The composition of the standard saline used (ASW) was the same: its Na^+ content was changed by substituting NaCl with either LiCl or choline Cl; its K content was reduced by adding less KCl to the standard salt mixture, and was increased by substituting NaCl with KCl. The digitalis alkaloid added to the solution, indicated with the generic name of ouabain in the paper, was Strophanthin G (Sigma Co.).

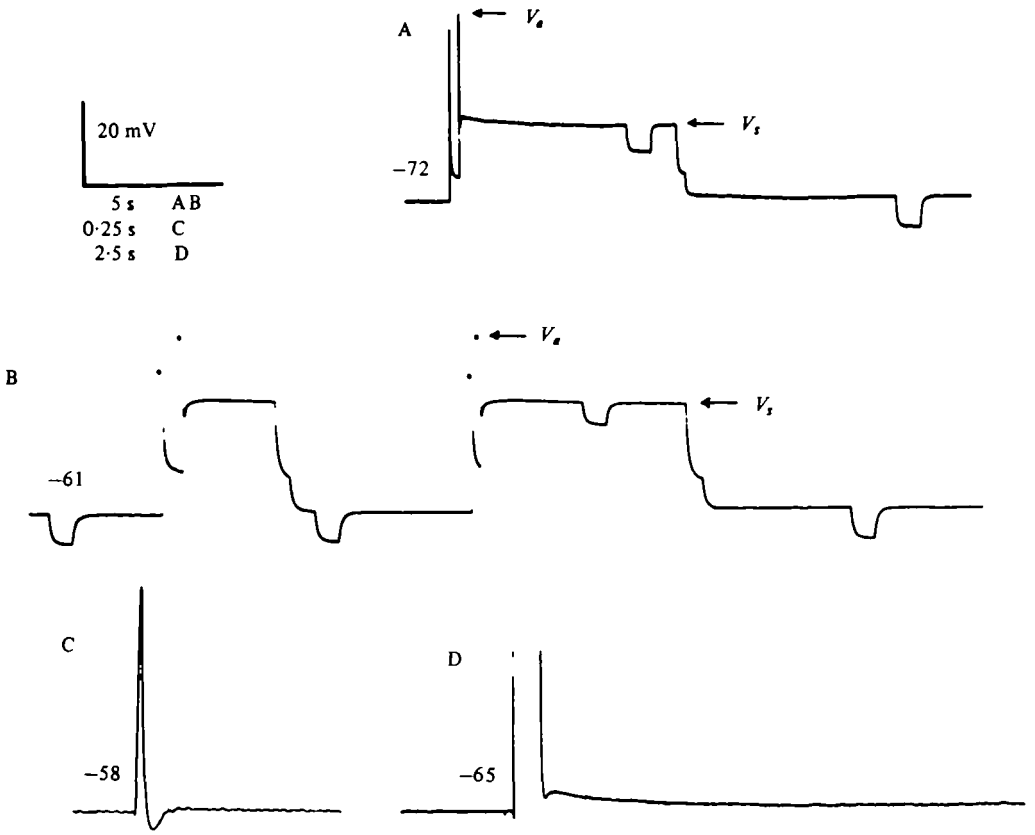

Fig. 1. Response of the S fibre to stretches of different durations. The responses shown were recorded in four different fibres at about 1 mm from the bifurcation. (A, B) Responses to long-lasting stretches applied in two consecutive and equal steps; each step resulted in a fast transient, V_a, followed by a steady-state plateau, V_s. Note the small, long-lasting depolarizing after-potential following the release of the stimulus. The input resistance, R_T, was measured in both A and B by injecting a current pulse with a second microelectrode inserted about 0·2 mm more proximally than the recording electrode. The magnitude of the injected current was 5 nA in A (R_T = 1·5 MΩ) and 2·8 nA in B (R_T = 2·5 MΩ); in both A and B R_T decreased during stretch. (C) Isolated V_a response to a 5 ms stretch pulse. The response was followed by a brief hyperpolarizing after-potential on release of the stimulus. (D) High gain recording of the after-potential following the release of a 0·5 s stretch; the peak of the response was off trace at the gain used. The depolarizing after-potential was preceded by a transient hyperpolarization appearing as a notch in the records. In this and in the following Figures the transmembrane potential of the fibres is indicated, in mV, at the beginning of each trace.

RESULTS

Response to stretch and associated conductance changes

As already described in the preceding paper (Mirolli, 1979), the response of the S fibre of *Scylla serrata* to step stretches consisted of a fast initial transient, V_a, and a steady-state plateau, V_s, both depolarizing (Fig. 1 A). V_s was followed, on release of the stimulus, by a long-lasting after-potential, which was depolarizing when the transmembrane potential of the fibre, V_m, was about −60 mV or more negative (same Fig.) and hyperpolarizing when V_m was more positive. V_a could be isolated

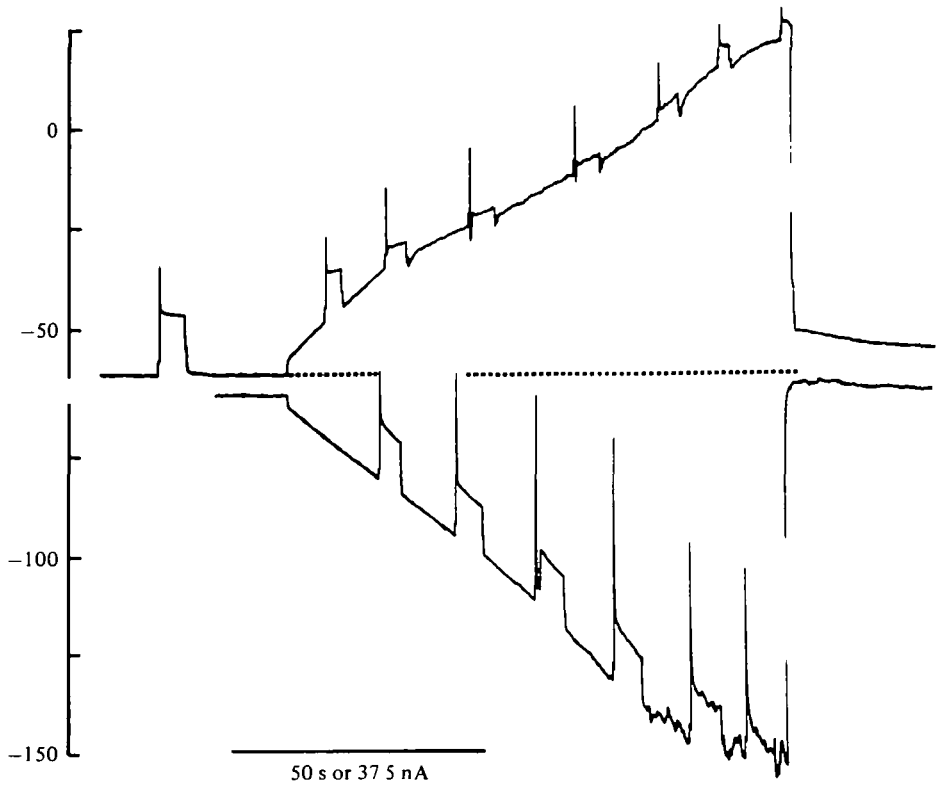

Fig. 2. Changes of the response to a constant stretch of the S fibre during current ramps. Recording and current injecting electrodes were 5·7 and 5·9 mm, respectively, from the bifurcation. The linear current ramp injected (not shown) was 0·75 nA per second. The input resistance at the beginning of the ramp was 2 MΩ. In the depolarizing quadrant the fibre exhibited pronounced rectification, and the steady-state response to stretch V_s was clearly not a monotonic function of the transmembrane potential, V_m. Instead, the amplitude of V_s decreased at first, reaching a minimum when the fibre was depolarized to about −10 mV, and then increased (with respect to this minimum) for more positive values of V_m. Note the hyperpolarizing release after-potential. In the hyperpolarizing quadrant the amplitude of V_s decreased, with progressively more negative values of V_m, but only by a small amount until, at about −150 mV, the fibre developed a high conductance state. On cessation of the depolarizing ramps the fibre remained depolarized, thus exhibiting hysteresis; a depolarizing hysteresis of smaller magnitude is also evident after the hyperpolarizing ramps.

from V_s by using very brief stretch pulses (3–5 ms in duration). Isolated V_a was, in general, followed by a short-lasting hyperpolarization (Fig. 1C), but this after-potential was not always present (Fig. 9B). A small transient hyperpolarization could also be present after a steady-state stretch (Fig. 1D).

In fibres which were, presumably, in good condition (V_m between about −60 mV and −70 mV, and input resistance, R_T, larger than 1·5 MΩ, see Mirolli, 1978), V_s showed only a slight decline in amplitude or 'sagging', even when stretches lasting over 10 min were used (Fig. 1A and B, see legend). The input resistance of the fibre decreased during the steady-state response to stretch (Fig. 1A and B). During the release after-potential following V_s, R_T was not significantly different from what it was in the unstretched receptor (Fig. 1B). No reliable measurements of the input resistance changes, if any, during V_a and its short after-potential were taken.

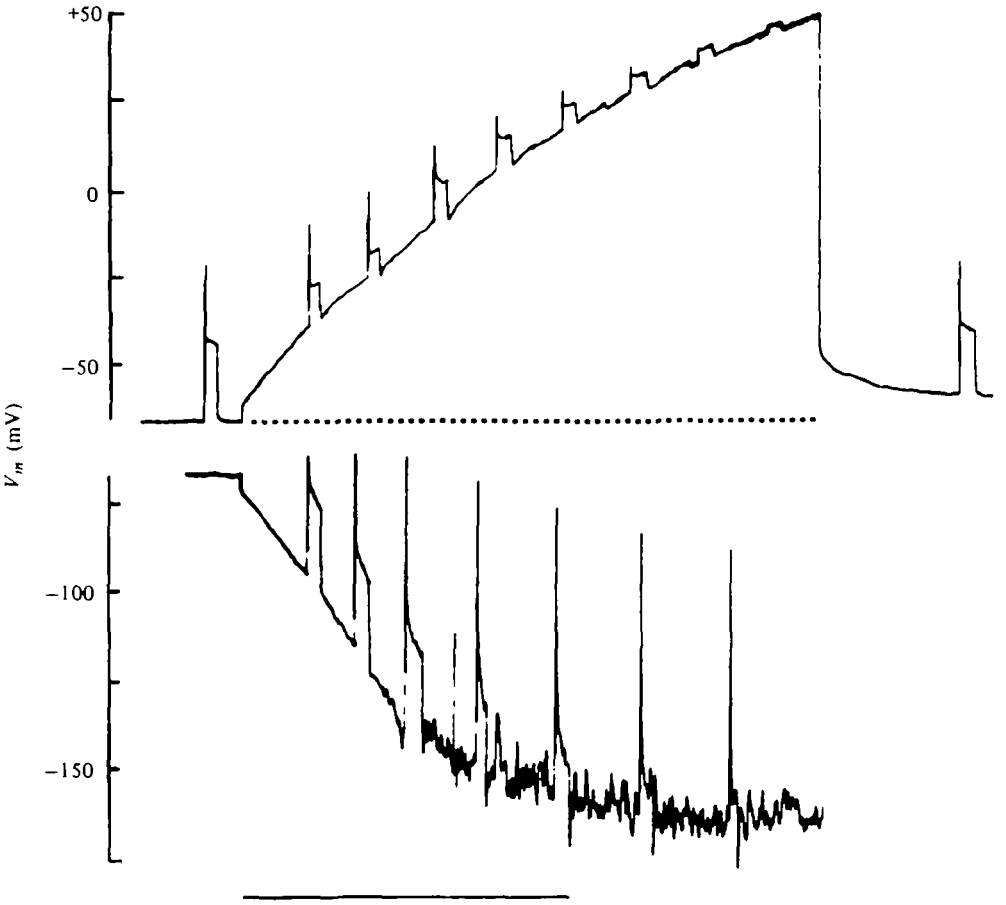

Fig. 3. Changes of the response to stretch of the S fibre during current ramps. Current and voltage recording electrodes were both about 0·9 mm from the bifurcation. The input resistance at the beginning of the ramp was 1·9 MΩ. V_s changed in the hyperpolarizing and depolarizing quadrants in a manner similar to that referred to in the legend to Fig. 2. Note that V_s was reduced for positive values of V_m but was not reversed, even when the fibre was depolarized to about +45 mV, and that the amplitude of both V_a and V_s was decreased during the depolarizing hysteresis following cessation of the ramp. Sagging of V_s and the hyperpolarizing after-potential following release of the stimulus are evident only between −50 and about +10 mV. Peak of the V_a responses were off-trace in the first part of the hyperpolarizing quadrant. Further comments in text.

Effects of injected current

When slow-rising current ramps were injected into the S fibre, the transmembrane potential and the input resistance changed in a complex manner (Mirolli, 1979). Equally complex were the changes in the response to stretch during the current ramps (Figs. 2 and 3).

In the hyperpolarizing quadrant, between the resting potential and about −140 mV, V_a appeared to be a monotonic function of the transmembrane potential, its amplitude increasing with more negative values of V_m (Figs. 2 and 3; see legend to Fig. 3). By contrast, the amplitude of V_s was either unchanged (Fig. 2) or only slightly

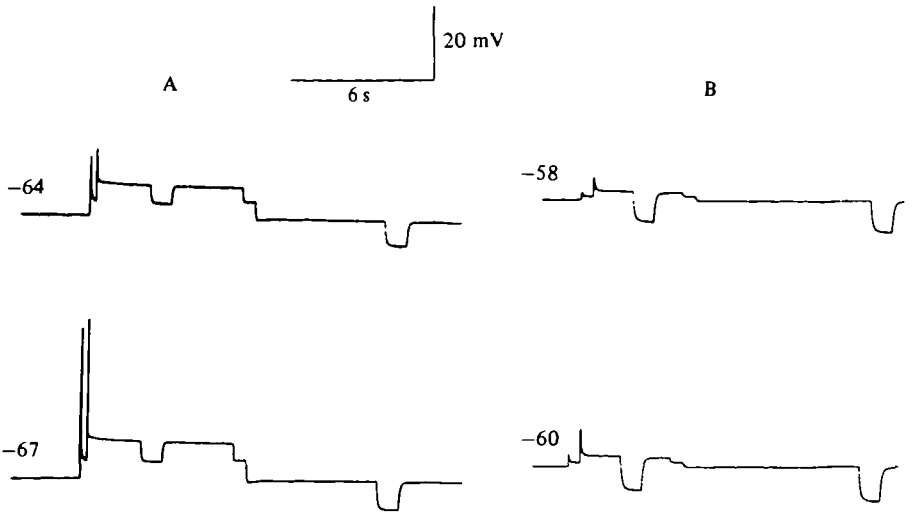

Fig. 4. Effect of choline ASW on the input characteristics and the response to stretch of the S fibre. Records obtained simultaneously at 7·7 mm (upper trace) and 0·8 mm (lower trace) from the bifurcation; a third electrode (trace not shown) was inserted at 1·0 mm from the bifurcation and used to inject a 4·6 nA current pulse. (A) Responses to current and to a test stretch in Na^+ ASW. (B) Responses to the same current and stretch stimuli in choline ASW, recorded about 7 min after the change of the saline. Note the marked effect of choline ASW on the amplitude of V_a and V_s which were both reduced, and on the input resistance of the fibre, which was increased. The ratios measuring the spatial decrement of the steady-state response to stretch and to injected current were, on average, about 0·88 in Na^+ ASW and increased to 0·96 in choline ASW. Further comments in text.

increased (Fig. 3); neither a sagging of V_s nor a release after-potential were evident (same Figures). With larger outward currents the fibre developed a high conductance state (Marmor, 1971 and 1975; Mirolli, 1979) during which the amplitude of V_s and, to a lesser extent, that of V_a, were reduced (same Figures).

In the depolarizing quadrant the amplitude of V_a decreased, somewhat irregularly, with progressively larger values of V_m. The amplitude of V_s decreased for values of V_m up to about -20 mV, increased between -20 mV and about $+10$ mV, and then decreased again for more positive values of V_m (Figs. 2 and 3). These non-monotonic changes in the amplitude of V_s were more evident when a depolarizing current was injected in the proximal part of the fibre (Fig. 2) than when the current electrode was near the bifurcation (Fig. 3). With the current electrode in a distal position (and thus close to the sensory ending), the fibres could be depolarized up to $+50$ mV, and in these cases the amplitude of V_s was greatly reduced suggesting that the transmembrane potential at the site of current injection was close to the reversal potential of the response (Fig. 3). In the depolarizing quadrant, between about -50 and $+10$ mV, V_s showed a distinct sagging and was followed by a hyperpolarizing release after-potential (Figs. 2 and 3). On cessation of both inward and outward current ramps the fibres remained partially depolarized for a considerable time (same Figures; see also Fig. 6 in previous paper). During this hysteresis the amplitude of both V_s and V_a was reduced (Fig. 3).

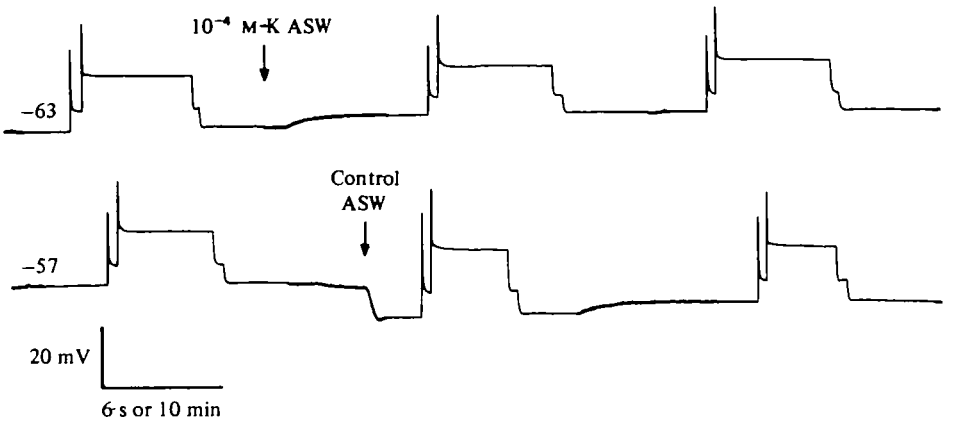

Fig. 5. Effect of low K^+ ASW on the membrane potential and on the response to stretch of the S fibre. Upper and lower traces are consecutive; stretch stimulus was constant throughout the experiment. The first arrow indicates the change of saline from control ASW to 10^{-4} K ASW; the second arrow, the return to control ASW. The two time calibrations correspond to the two different speeds at which the chart recorder was run during the experiments; the portion of the records obtained at slower speeds can be recognized from the thicker trace.

Effect of external sodium and potassium

The input properties and the response to stretch of the S fibre depended on the external concentration of both these cations. When choline was substituted for Na^+, the immediate effect on the transmembrane potential varied from preparation to preparation (data not shown). Ultimately, however, the fibres depolarized while their input resistance, R_T, increased (Fig. 4). The amplitude of both V_a and V_s was reduced (same Fig.), an observation confirming the results obtained by Roberts & Bush (1971) in *Carcinus* receptors. The reduction in the amplitude of the response to stretch was accompanied by a reduction in the conductance increase associated with V_s; at the same time the ratios measuring the spatial decrement of the response to stretch and of the response to injected current (Mirolli, 1979) both increased (Fig. 4, see legend). Thus, substitution of Na^+ with choline affected not only the input characteristics of the region of the fibre sensitive to stretch but also the cable properties of the dendrite of the S fibre.

When Na was substituted with Li^+, the fibre depolarized (Fig. 9B) while the amplitude of V_a and V_s was slightly reduced. Li ASW, *per se*, did not have a noticeable effect on R_T, on the after-potential, or on the cable properties of the dendrite (data not shown).

The fibres also depolarized in low K^+ ASW. In some preparations the depolarization observed was minor, amounting to about 5 mV (Fig. 5); the amplitude of both V_a and V_s was slightly decreased, but only transiently (same Fig.). In other preparations the depolarization resulting from exposure to low K^+ saline was more pronounced, and the amplitude of both V_a and V_s was substantially reduced (Fig. 6, see legend). In these cases sagging was more pronounced, and the release after-potential became hyperpolarizing (same Fig.). In low K^+ R_T was always increased (Figs. 6 and 9C) as were the ratios measuring the spatial decay of the response to stretch and to injected current (Fig. 6, see legend). These effects suggest that the concentration of external K^+ affects the cable properties of the dendrites of coxal receptors.

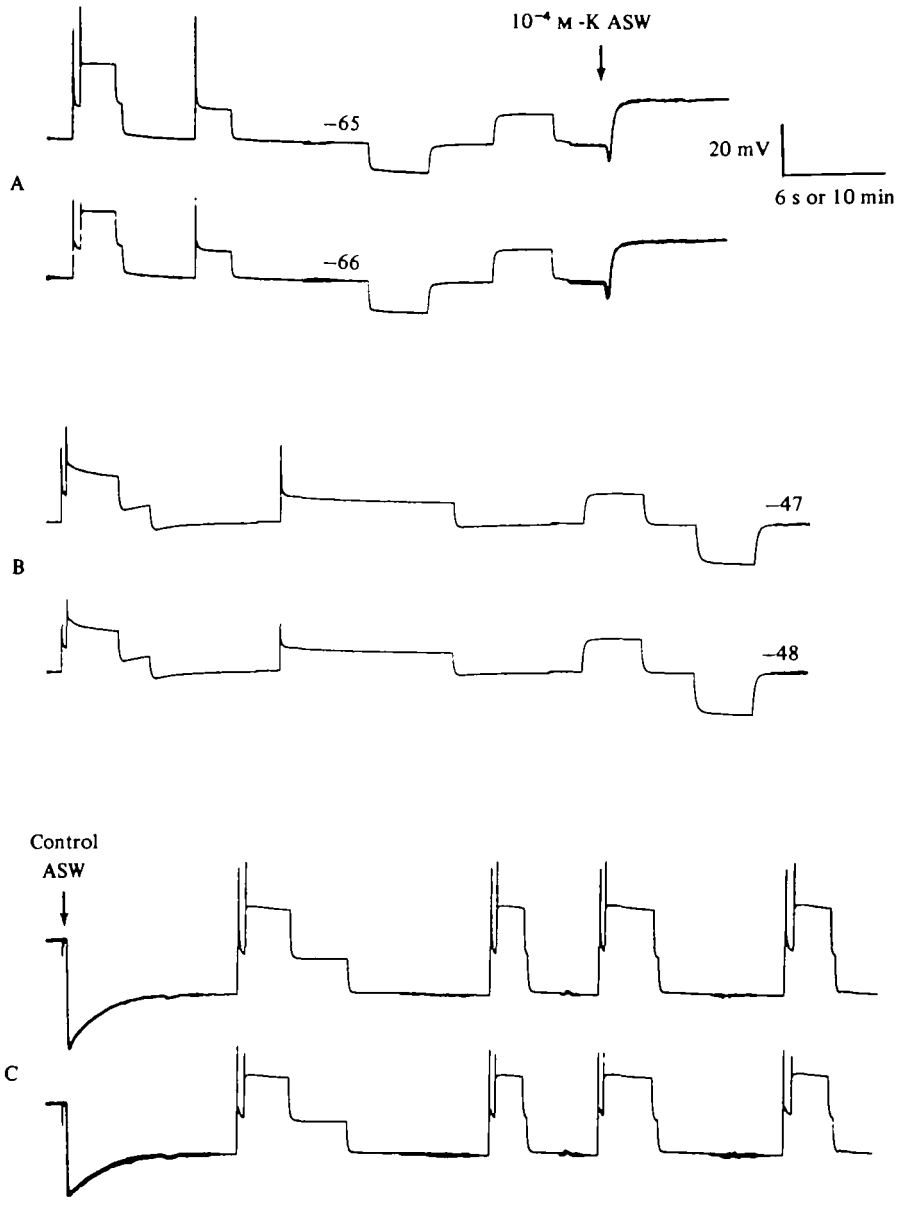

Fig. 6. Effect of low K^+ ASW on the membrane potential, the response to a constant stretch, and the input properties of the S fibre. Records obtained simultaneously at 0·7 (upper trace) and 5·5 mm, (lower trace) from the bifurcation; a third electrode, inserted 5·8 mm from the bifurcation (trace not shown), was used to inject a 4·5 nA current pulse. The traces shown in A, B, and C are consecutive. The saline was first changed in A (arrow) from control ASW to to 10^{-4} M-K ASW, and then back to control ASW (arrow in C). Note the fast time course of the depolarization following the change to low K^+ saline, the increase in R_T and the decrease in the response to stretch in low K^+ ASW (current and stretch stimuli were the same in control and in low K^+ ASW). The ratios for the decrement of the response to stretch and to injected current were about 0·90 in control ASW and 0·92 in low K^+ ASW. The transient hyperpolarization seen in A, immediately after the change to low K^+ saline, is possibly an artifact due to an air bubble in the saline.

On return to control ASW the fibre hyperpolarized to well below the value of V_m prior to exposure to low K^+ ASW (Figs. 3 and 4). During this hyperpolarization the amplitude of V_s increased significantly (same Figs.). In saline containing a higher complement of K^+ than control ASW, the fibre also depolarized, and R_T, V_a and V_s were decreased (data not shown). As in *Carcinus* coxal receptors (Roberts & Bush, 1971), the slope of a semi-log plot of V_m versus $[K^+]_o$ was small, between 20 and 30 mV per decade change in $[K^+]_o$. The initial changes in V_m, when external K^+ was reduced or increased, occurred with a relatively short time constant (about 30 s; Figs. 5 and 6), which was comparable to the estimates obtained by Abbott, Moreton & Pichon (1975) in their study of the K^+ movements across the crustacean peripheral epineurium. The fast time course of the effect of external K^+ on V_m suggests that the glial sheath covering the coxal receptor fibres offers little resistance to passive movement of K^+ (see Mirolli & Gorman, 1973).

Effects of ouabain

Exposure to ouabain in concentrations ranging from 10^{-3} to 10^{-3} M depolarized the S fibre (Fig. 7). This depolarization was not additive with that resulting from low K^+ ASW (same Fig.) or Li^+ ASW (data not shown), suggesting that these three procedures affected a common process, though not necessarily this process alone.

The time course of the depolarization produced by ouabain was dependent on the concentrations used. In 10^{-4} M ouabain the fibres depolarized approximately 10 mV in about one-half hour. Following this early phase the fibres depolarized at a somewhat slower rate, but in any case there was a complete loss of the transmembrane potential within several hours. During the early phase of the ouabain-induced depolarization (up to -55 to -50 mV), the input resistance was not appreciably changed (Fig. 7A); the amplitude of the response to stretch was also not systematically affected (data not shown). However, the release after-potential became distinctly hyperpolarizing (Fig. 7B).

During the progressively larger depolarization of the fibre, seen during long exposures to ouabain, the input resistance decreased as did the amplitude of both V_a and V_s. At the same time the sagging of V_s became extremely pronounced, and the release after-potential became a long-lasting hyperpolarization (Fig. 7D, see legend).

When the preparations were first treated with ouabain to depolarize them, the amplitude of V_s was not reduced but increased in low K^+ ASW (Figs. 7B–E). The effects of low K^+ ASW on the input resistance and on the release after-potential, previously described, were not modified by exposure to ouabain (same Fig., see legend).

Effect of tetanic stimulation

The depolarization resulting from exposure to ouabain and to Li^+ ASW suggests that the transmembrane potential of the S fibre is determined, in part, by a Na^+ pump. Moreover, the depolarizing effect of low K^+ ASW is consistent with the hypothesis that the pump is electrogenic. This evidence, however, is only indirect,

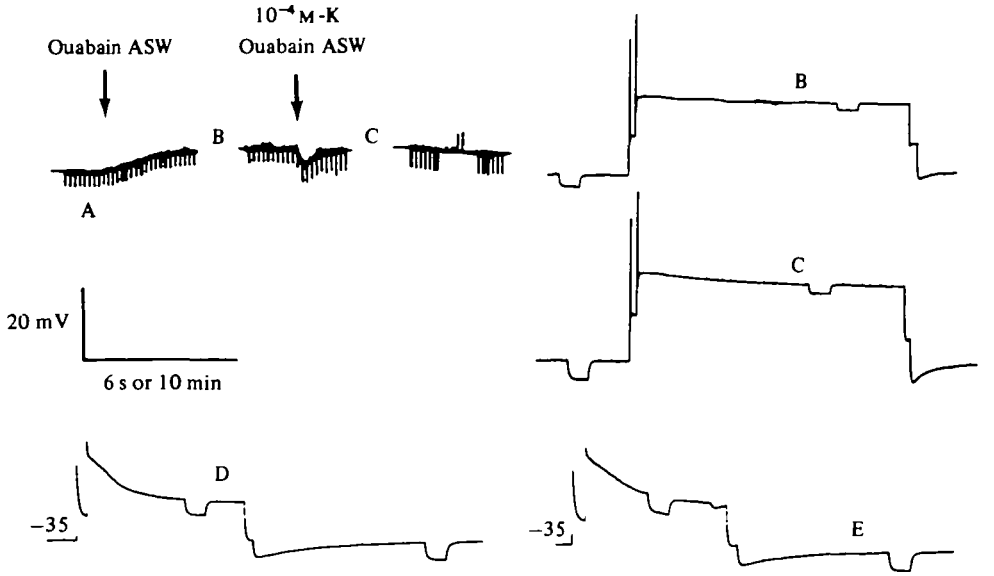

Fig. 7. Effects of ouabain ASW and low K^+ ouabain ASW on the transmembrane potential and input properties of the S fibre. (A) Changes in V_m and in R_T resulting from the change of the saline first from control ASW to 10^{-3} M ouabain ASW (first arrow), and then to 10^{-4} M-K ASW, 10^{-3} ouabain ASW (second arrow). The input resistance was monitored every 20 s by injecting a 2·5 nA current pulse; both current and recording electrodes were about 1·2 mm from the bifurcation (only voltage trace shown). The responses to injected current appear as sharp downward inflections in the record taken at a slow speed (see calibration at end of legend). The fibre depolarized by about 8 mV in ouabain ASW, but its input resistance (1·7 MΩ) did not change significantly. By contrast, reduction of external K^+ in presence of ouabain resulted in no significant change of V_m, while R_T increased from 1·7 to 2·4 MΩ. (B, C) Records from the same experiment, but taken on a faster time base at the points indicated on trace A to show the effect of low K^+ saline. In addition to the changes in R_T, already mentioned, note the marked increase in the amplitude of the response to the same test stretch and of the hyperpolarizing after-potential following the release of the stimulus. (D, E) Responses to a 4 nA current pulse and to a constant test stretch recorded in another cell depolarized to −35 mV after about 30 min in ouabain ASW. Record D was taken before and record E after the K^+ content of the ouabain saline was changed from $1·1 \times 10^{-2}$ M to 10^{-4} M. The responses recorded from the same cell in control saline are shown in Fig. 1 B. Comparison of Fig. 1 B and 7 D show the pronounced sagging, reduction in the amplitude of V_s and V_t as well as R_T observed after long exposure to ouabain; also noticeable is the hyperpolarizing after-potential. Comparison of Fig. 7 D and E shows that the amplitude of the responses to stretch and to injected current as well as that of the hyperpolarizing after-potential are all increased in low K^+ saline. Calibration: (A) 20 mV, 10 min; (B–E) 20 mV, 6 s.

and there is at least one experimental fact which is, apparently, in contradiction with the hypothesis of electrogenic Na^+ transport. Since the response to stretch is Na^+-dependent (Fig. 4), one would expect that the fibre would hyperpolarize on release of the stimulus if an electrogenic pump were present, irrespective of the value of V_m. But this is not what is observed. Instead, as shown in the previous sections, the release after-potential following V_s was hyperpolarizing only when the fibres were depolarized. Moreover, the amplitude and time course of the release after-potential appeared to be unrelated to the duration and amplitude of the response to stretch, and thus to the amount of Na^+ presumably entering the fibres during steady-state stretch.

These considerations do not necessarily apply to the fast initial transient which

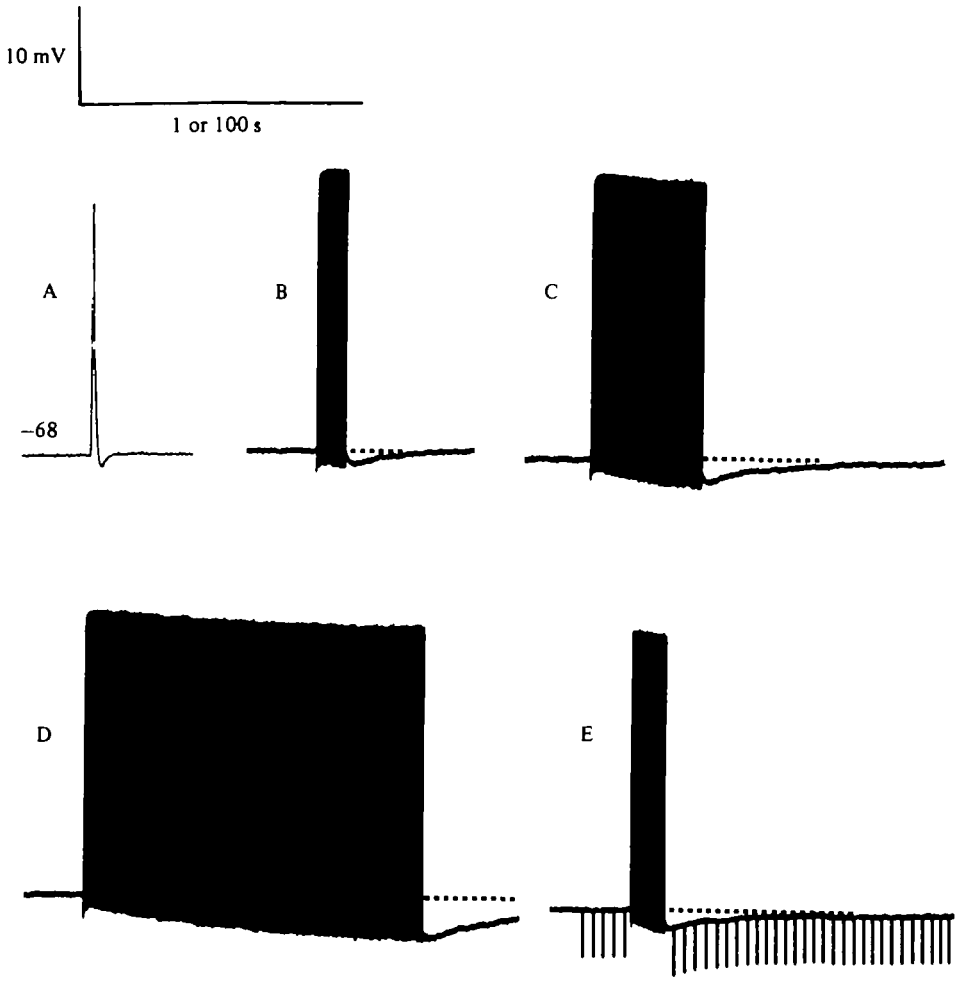

Fig. 8. Post-tetanic hyperpolarization (PTH) in the S fibre. (A) Isolated partial spikes (V_a response) recorded at 2 mm from the bifurcation. (B–E) PTH developed during and after trains of partial spikes resulting from high frequency (40 s^{-1}) mechanical stimulation of the sensory endings. Each stimulus was a 5 ms stretch. PTH increased in direct proportion to the train duration. (E) A repetitive current pulse of 3 nA and 0·5 s duration was injected in the fibre by a second microelectrode about 1 mm proximal to the recording point. R_T was about 1·6 MΩ and did not change during the hyperpolarization following the stimulus. Calibration: 10 mV for all; A 1 s, B–E 100 s.

is also Na^+-dependent (Fig. 4; see also Roberts & Bush, 1971). As already shown, V_a could be isolated from the steady state phase of the response by means of brief stretches (Fig. 1 C). By using trains of brief stretch stimuli, the S fibre could be driven to respond with a succession of partial spikes up to the frequency of 40 per second for several minutes (Fig. 8). In these experimental conditions the fibre hyperpolarized during stimulation; cessation of the stimulus was marked by a long-lasting post-tetanic hyperpolarization (PTH) whose magnitude and duration were proportional to the number of partial spikes elicited (Fig. 8, see legend). During PTH the input resistance of the fibre was not changed (same Fig.)

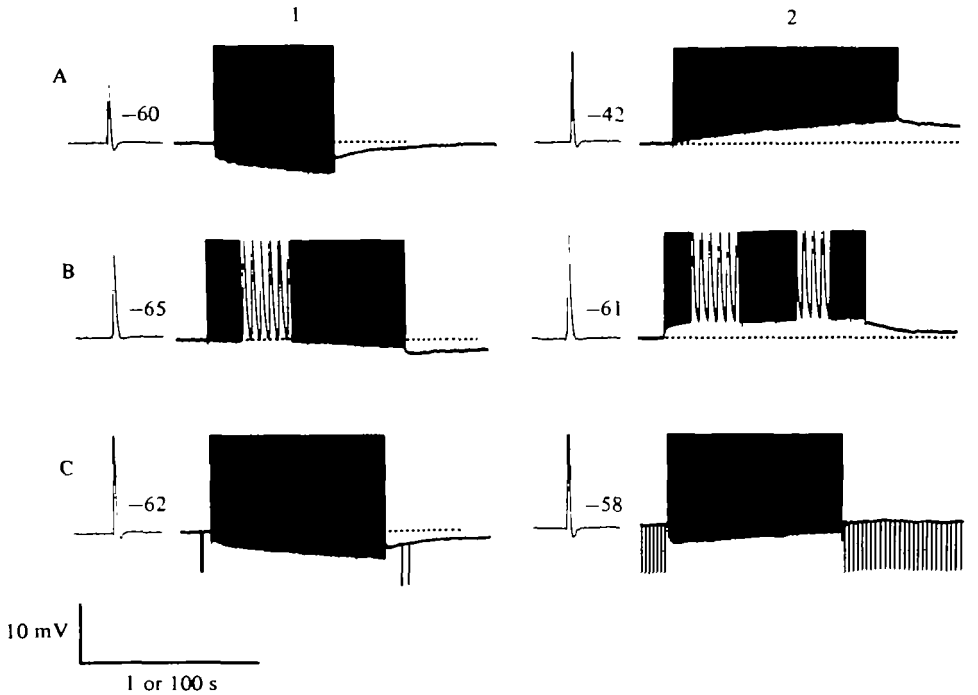

Fig. 9. Effect of ouabain, external Li^+ and low external K^+ on PTH in the S fibre. A_1, B_1 and C_1 show the isolated V_a and PTH obtained in control saline in three different preparations; A_2 the effect of ouabain ASW, B_2 the effect of Li^+ ASW, and C_2 the effect of 5×10^{-5} M-K^+ ASW. In B_1 and B_2 the speed of the chart recorder was increased during tetanic stimulation to show the train of V_a. In C_1 and C_2 the input resistance was measured with repetitive current pulses of 5 nA and 0·5 s duration. R_T was about 1·4 MΩ in control ASW, about 1·6 MΩ in low K^+ ASW, and did not change during the hyperpolarization following tetanic stimulation. In low K^+ ASW (C_2) the fibre was slowly depolarizing, and the small depolarization seen after the tetanus could be accounted for by the slow drift in the membrane potential. Stimulus parameters and calibration as in Fig. 8.

The amplitude of the hyperpolarization seen on cessation of the stimulus was not dependent, in any obvious manner, either on the magnitude of the transmembrane potential (Figs. 8 and 9) or on the presence of the short-lasting hyperpolarization which could follow V_a (Fig. 9 A_1, B_1, C_1). However, the amplitude and time course of the hyperpolarization seen during the stimulus itself was dependent on the short after-potential, and when this was not present, the hyperpolarization during tetanus was small and developed slowly (Fig. 9, B_1). In the presence of ouabain and in Li^+ ASW, PTH was changed to an equally long-lasting depolarization; during the period of stimulation the fibre depolarized by an amount which seemed qualitatively correlated with the presence or absence of the short-lasting after-potential following V_a (Fig. 9, A_2, B_2). In low K^+ ASW, PTH was abolished but not changed to a depolarization (Fig. 9, C_2, see legend). R_T did not change after the tetanus in low K^+ ASW (same Fig.).

Observations with the T fibre

In general, the results obtained with the T fibre paralleled those already described for the S fibre. Thus the response to stretch was characterized by an increase in the

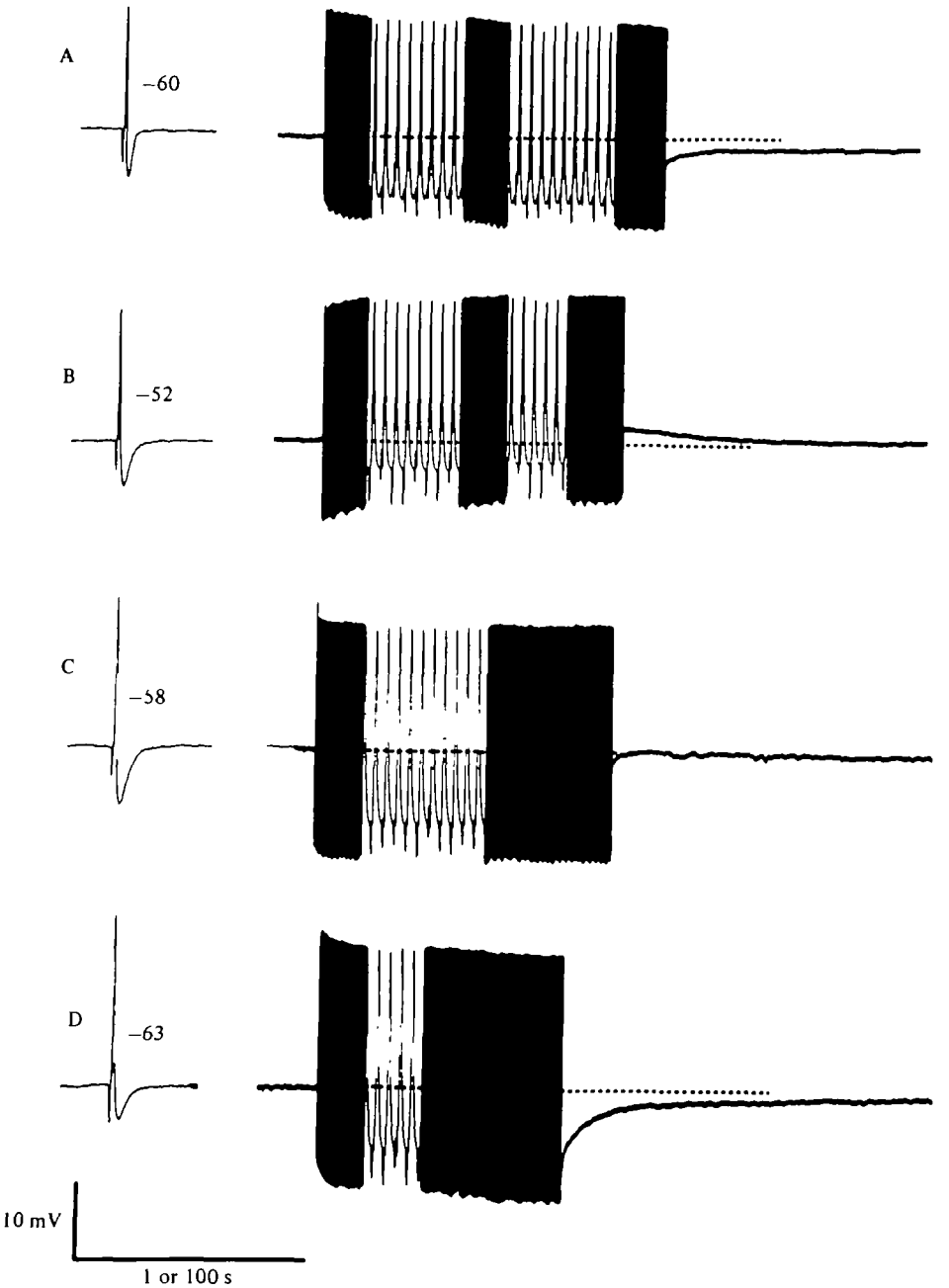

Fig. 10. PTH in the T fibre. Each row shows an isolated V_a response and the PTH developing during and after high frequency mechanical stimulation (40 s^{-1}). Recording point was about 1·5 mm from the endings. (A) In control ASW; (B) in Li^+ ASW; (C) in 5×10^{-5} M-K ASW; (D) in control ASW during recovery from exposure to low K^+ASW. Note that PTH is increased in control ASW after low K^+ ASW while the short hyperpolarization, following V_a, is decreased. The small downward deflexions seen just before each V_a response (arrow in A) are stimulus artifacts. Stimulus parameter and calibration as in Fig. 8.

input conductance and was dependent on external Na^+ and K^+. In the T fibre the isolated V_a component was always followed by a conspicuous hyperpolarizing after-potential whose amplitude was larger in low K^+ ASW and when the fibres were depolarized (Fig. 10A–C). Stimulation with trains of brief mechanical stimuli resulted in a long-lasting hyperpolarization which was abolished and changed to a depolarization in Li^+ ASW (Fig. 10B) and in the presence of ouabain (not shown). As in the S fibre, PTH was abolished but not changed to a depolarization after tetanus in low K^+ ASW, while during the stimulus the fibre hyperpolarized more than it did in control ASW (Fig. 10C). On return to control ASW after exposure to low K^+ ASW, the amplitude of the short-lasting hyperpolarization following V_a was reduced, but PTH was increased (Fig. 10D).

DISCUSSION

The main conclusion of this paper is that an electrogenic process linked to a ouabain-sensitive Na^+ transport contributes a substantial fraction of the transmembrane potential of the relaxed crustacean coxal receptors, at least in the experimental conditions studied. This conclusion rests on a number of experimental data which can be briefly summarized as follows:

(*a*) The conductance to Na^+ (at room temperature) was quite large, even when the sensory endings were completely relaxed.

(*b*) The fibres depolarized in the presence of ouabain, when Li^+ was substituted for external Na and when external K^+ was reduced to below the normal complement present in standard sea water.

(*c*) Trains of partial spikes, whose amplitude depended on an inward Na^+ current, resulted in a long-lasting hyperpolarization (PTH) in standard sea water.

(*d*) PTH was changed to a depolarization in the presence of ouabain or in Li^+ ASW and was abolished in low K^+ ASW.

PTH could not be due to a depletion of external K^+ resulting from the activity of a neutral Na^+/K^+ pump because the fibres depolarized in low K^+ saline. Activity of a neutral Na^+/K^+ pump could also lead to a hyperpolarization, in fibres whose distal part was Na^+-loaded by trains of V_a, if Na^+ entry were accompanied by an increase in internal Ca^{++}; this could lead to an increase in K^+ conductance which, in turn, could drive the transmembrane voltage toward the K^+ equilibrium potential (E_K) (Jack, 1977). However, during PTH there was no evidence of a decrease in the input resistance of the fibres. In addition, when Na^+/K^+ transport was blocked, PTH was abolished or reversed in spite of the fact that the potential of the fibres was more positive, and thus farther from E_K (Figs. 9 and 10, see legend).

These data are adequately explained on the basis of an electrogenic Na^+/K^+ pump (Gorman & Marmor, 1970, 1974; Thomas, 1969, 1972). Other possible mechanisms, not necessarily mutually exclusive with an electrogenic Na^+ pump, should also be kept in mind. As Jack (1977) has suggested in his recent review, increased internal Na^+ could be exchanged for Ca^{2+}, and this process could be electrogenic. The stoichiometry of the process, when operating in the direction Na^+ out, Ca^{2+} in, is not known (Jack, 1977); also completely unknown is the Ca^{2+} metabolism in the coxal receptors and, in general, in mechanoreceptors. At present, therefore, this interesting possibility cannot be properly evaluated.

The experiments in which the sensory endings were tetanically stimulated were patterned after those done by Nakajima & Takahashi (1966), in their study of the electrogenic processes responsible for PTH in the crayfish abdominal stretch receptors, and the results are qualitatively similar. There are, however, some quantitative differences. In the coxal receptors (both S and T fibres) tetanus resulted in a depolarization when active Na^+ transport was blocked by ouabain or Li^+. This depolarization (which was not evident in the crayfish stretch receptor; Nakajima & Takahashi, 1966) is probably due to an accumulation of K^+ in the extracellular layer bounding the sensory endings, since it was not seen when Na^+ transport was blocked by low K^+ ASW (Figs. 7 and 8). In the crayfish receptors the PTH magnitude, measured immediately after relatively short tetani (about 3 s in duration), was large, about 7·5 mV (see Figs. 1 and 6 in Nakajima & Takahashi, 1966). By contrast, in the coxal receptors, even considering the depolarization seen after block of the pump, PTH amounted to only 1–2 mV, for tetani of comparable duration (Fig. 6). This difference is not surprising since, in the coxal receptors, PTH resulted from partial spikes, limited to the most distal portion of the dendrite (Mirolli, 1979), whereas in the crayfish receptors PTH depended on trains of full-sized action potential generated, presumably, by the entire cell.

No evidence for electrogenic Na^+ transport could be obtained when steady-state responses to stretch (V_s) were used as tests, in spite of the fact that responses of amplitude as large as 20–30 mV could be obtained. Since tension was not measured in these experiments, the possibility that the time course of the long-lasting after-potential following V_s reflected, at least in part, the characteristics of the elastic elements of the receptor organ cannot be excluded. However, aside from its origin, it is clear that the after-potential does not depend on electrogenic Na^+ transport. Its polarity and amplitude were not dependent on the presence of ouabain or on Li^+; moreover, when external K^+ was reduced, the V_s after-potential was either reduced or became hyperpolarizing, and so behaved exactly opposite to the PTH.

The difference between the results obtained by Na^+ loading the cells with trains of V_a and those obtained by using long-lasting steady-state stretches is the more striking when one considers that both V_a and V_s originate in the same region of the cell. A reasonable explanation for these results could be that less Na^+ enters the fibre during V_s than during a tetanic train of V_a of comparable duration. Another possibility is that the site of the conductance changes responsible for the steady state response to stretch is spatially separated from the regions, in the distal part of the fibre, where the Na^+ pump is located, whereas the site of origin of V_a is not. Krauhs & Mirolli (1975) were led to consider, on purely morphological grounds, the possibility of a segregation of the sites of the stretch-dependent conductance changes and of an electrogenic pump in the S fibre because the terminal fingers, the presumed locus of electromechanical transduction, do not contain mitochondria.

The amplitude of the steady-state response to stretch was slightly decreased in low K^+ ASW and also when Na^+ transport was blocked by ouabain. However, the reduction of the response was comparable with the reduction observed when the fibres were depolarized by injected current. Moreover, in conditions of equal polarization the response was systematically larger, albeit by a small amount, in low K^+ ASW than in control ASW, a result which can be explained most simply on the

basis of the increased input resistance and length constant of the fibre brought about by low external K^+.

Thus, if only the data obtained in fibres whose transmembrane potential was between about -70 and -50 mV are considered, the following conclusions seem justified. The response to steady-state stretch does not depend on electrogenic Na^+ transport except to the extent that this process may be required to maintain a steady-state ionic distribution across the membrane, as seems to be the case in other receptor neurones (Brown & Lisman, 1972). The amplitude of the response to stretch does not depend on external K^+ except in so far as external K^+ determines the input properties of the fibre.

Beyond this range of V_m, however, the behaviour of the steady-state response to stretch is puzzling. The non-monotonic dependence of V_s on the transmembrane potential, observed when the fibres are hyperpolarized or depolarized by injected current, cannot be explained without assuming some *ad hoc* voltage and time dependence of the conductance of the fibres. The ionic basis and the site of origin of these conductance changes remains to be determined.

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