J. Exp. Biol. (1971), **54**, 515–524
With 6 text-figures
Printed in Great Britain

COXAL MUSCLE RECEPTORS IN THE CRAB: THE RECEPTOR CURRENT AND SOME PROPERTIES OF THE RECEPTOR NERVE FIBRES

BY ALAN ROBERTS* AND B. M. H. BUSH
Departments of Zoology and Physiology, University of Bristol

(*Received* 6 *October* 1970)

INTRODUCTION

The coxal muscle receptors in the crab provide a useful preparation for the study of receptor function. The two largest sensory neurones innervating the receptor complex consist of fibres 2–5 mm long and 50 μm in diameter which run straight from the thoracic ganglion, containing their cell bodies, to branch just before reaching the muscle. One, the T fibre, has many branches which innervate the connective tissue at the proximal insertion of the receptor muscle. The second, the S fibre, has two principal branches running to connective tissue strands on either side of the receptor muscle (see Alexandrowicz & Whitear, 1957; Whitear, 1965). The S and T fibres are accessible for study as they are the principal constituents of a fine receptor nerve.

The basic responses of the S and T fibres of the crab coxal muscle receptor have been described in a previous paper (Ripley, Bush & Roberts, 1968). The aim there was to characterize receptor potential responses in terms of changes in receptor muscle length. Dynamic and static components were distinguished and, in general, the transformation of stimulus into receptor potential followed a pattern familiar in many receptors. What was unusual was that the receptor potential did not generate impulses (cf. Gwilliam, 1965; Millecchia & Mauro, 1969). It appears that the receptor potentials normally spread passively to the central nervous ganglion where they can excite motoneurones forming part of a stretch reflex (Bush & Roberts, 1968). This unusual behaviour suggested that a closer look at the properties of these large receptor cells would be interesting. We report here the results of experiments on the electrical properties of the receptor fibres and on the ionic dependence of their resting and receptor potentials. The details of the relations of the S and T fibre receptor potentials to the parameters of mechanical stimulation will be analysed elsewhere (B. M. H. Bush & A. Roberts, in preparation).

METHODS

All the experiments were carried out on isolated receptor organs of shore crabs (*Carcinus maenas*) with carapace widths from 30 to 50 mm. The isolated preparation consisted of the promotor muscle, containing the receptor muscle with its flanking connective tissue strands, and the receptor nerve innervating these structures and then running centrally to the thoracic ganglion. Other nerves were cut away. The promotor

* Partly supported by an S.R.C. fellowship.

muscle attachments and thoracic ganglion were pinned to a Polythene sheet in a small Perspex dish and illuminated obliquely from beneath to show up the individual neurones in the receptor nerve. For mechanical stimulation the distal insertion of the receptor muscle was dissected free and clamped in a slit at the end of a piece of Nylon monofilament. This was attached to a Pye-Ling V47 vibrator whose movement was controlled by an electronic function generator and monitored by an RCA 5734 mechano-electric transducer.

Recording and current-passing techniques using 3 M-KCl-filled glass capillary microelectrodes were conventional. In experiments where the saline composition was changed, a 3 M-KCl-agar-silver chloride indifferent electrode of large diameter but small pore size was used as recommended by Strickholm (1968). Recordings were displayed on an oscilloscope and sometimes also on a potentiometric pen recorder.

The crab saline had the following composition: Na 500 mM, K 12 mM, Ca 12 mM, Mg 20 mM, Cl 576 mM and HCO_3 1 mM. Osmotically equivalent substitutions were made using choline chloride, tris (hydroxymethyl aminomethane) chloride, sucrose and sodium acetate, sodium benzenesulphonate and sodium isethionate. The bath was continuously perfused with saline solutions except during tests on the effects of tetrodotoxin when the flow was stopped and the drug was added to the bath. Tetrodotoxin was supplied by Sankyo Co. Tokyo. Experiments were carried out at room temperatures between 17 and 22 °C.

RESULTS

(*a*) *Resting potentials*

The usual value of resting potential on penetration or withdrawal is between 50 and 60 mV in S and T fibres. There appears to be no large difference between these fibres, though in different preparations a wide range of resting potentials was recorded. Some of this variability was presumably due to damage done during preparation or penetration. However, some must be natural, and good receptor responses have been recorded from fibres with resting potentials of 40 to as much as 80 mV. Resting potentials and receptor responses were generally stable for a number of hours.

Ionic basis

The potassium concentration of the bath solution was changed by replacing various amounts of sodium with potassium. Potential changes resulting from these ion substitutions occurred in less than half a minute, indicating that there is no large diffusion barrier between the bath and the neurone's membrane. This inference is supported by the fact that tetrodotoxin effects were clear 40 s after introduction of the drug to the bath. The steady-state relation between external potassium concentration and membrane potential is illustrated in Fig. 1. The resting fibre membrane must be permeable to potassium. However, the maximum slope of 30 mV/ten-fold change in external potassium concentration indicates that other ions could be involved in determining the resting membrane potential. Complete replacement of external sodium ions by choline or tris (see below) produced changes in resting potential of up to about 5 mV, suggesting that the resting sodium permeability is low. Resting chloride permeability is probably also low compared to potassium permeability. Replacement of

chloride with sucrose, acetate, isethionate and benzene-sulphonate (reducing chloride concentration by 461 mM) gave different and inconsistent results, though all produced hyperpolarization.

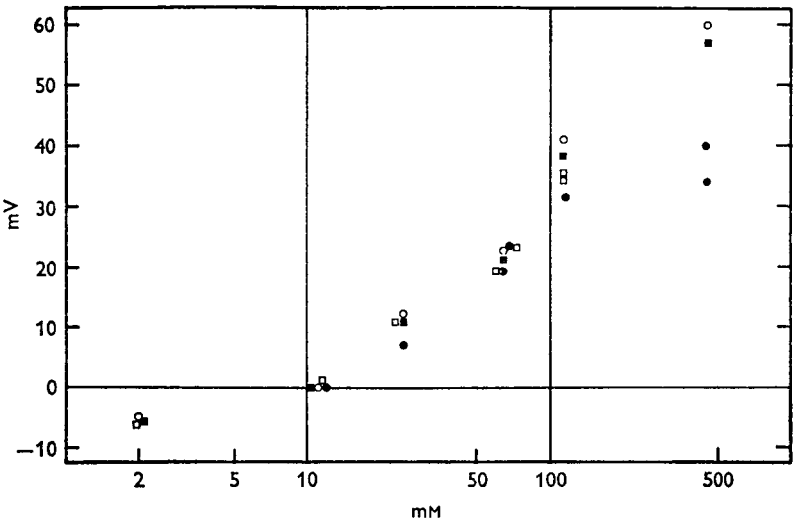

Fig. 1. Effect of changes in the external potassium concentration on the steady-state membrane potential. The resting potential in the normal saline is taken as zero and had values between 50 and 60 mV. The points plotted are from two S fibres (circles) and two T fibres (squares).

(*b*) *Electrical properties of receptor nerve fibres*

It is clear from our previous work (Ripley *et al.* 1968; Bush & Roberts, 1968) that the coxal muscle receptors do not usually generate impulses in response to muscle stretch, even though the receptor potential can depolarize the membrane to near zero. This suggested that the fibre was rather unresponsive to potential changes. We have investigated this by using microelectrodes to pass current into fibres while recording their responses with one or two other microelectrodes.

The S and T fibres behaved similarly. Input resistances ranged from 770 to 835 kΩ and tended to decrease during experiments indicating a gradual deterioration of our preparations. An almost ohmic voltage/current relationship was most common. An example from a T fibre is shown in Fig. 2. Some rectification was usually apparent when depolarization reached about 10 mV from zero potential. In the hyperpolarizing quadrant rectification was often only apparent after hyperpolarizations of 80 mV or more. In some S and T fibres intermediate levels of hyperpolarization were accompanied by increased input resistance. Further, some fibres showed a small, graded, active response to depolarizing current. In the example of Fig. 3 this had a threshold between 20 and 30 mV depolarized from the resting potential and increased the response to a step current pulse by up to 21 %.

We have determined cable constants (Table 1) by two techniques: (*a*) by recording receptor potentials with two microelectrodes about 2 mm apart in a fibre (1–3 in Table); and (*b*) by passing current pulses through one microelectrode and recording the resulting potential changes with a second, adjacent electrode (4 in Table), and in some cases with a third at a distance of about 2 mm away (5–8 in Table). These determina-

tions were made assuming that the S and T fibres could be treated as infinite cables. Since more measurements are needed to see whether this assumption is justified, the figures are only provisional. We hope to report further on this in a later paper.

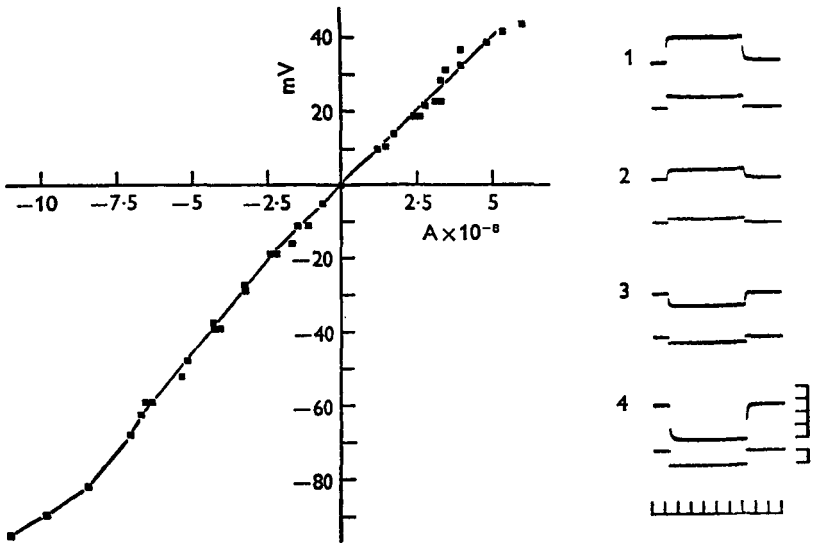

Fig. 2. Current/voltage relationship in a T fibre at steady state, measured using two microelectrodes approximately 50 μm apart and inserted into the mid-region of the fibre. Traces 1–4 show examples of the potential recordings (upper beam) and current pulses used in constructing the graph. Current was measured from the voltage drop across a 10 kΩ resistor in the earth line. Calibrations are 20 mV, 0·05 μA and 50 ms/division.

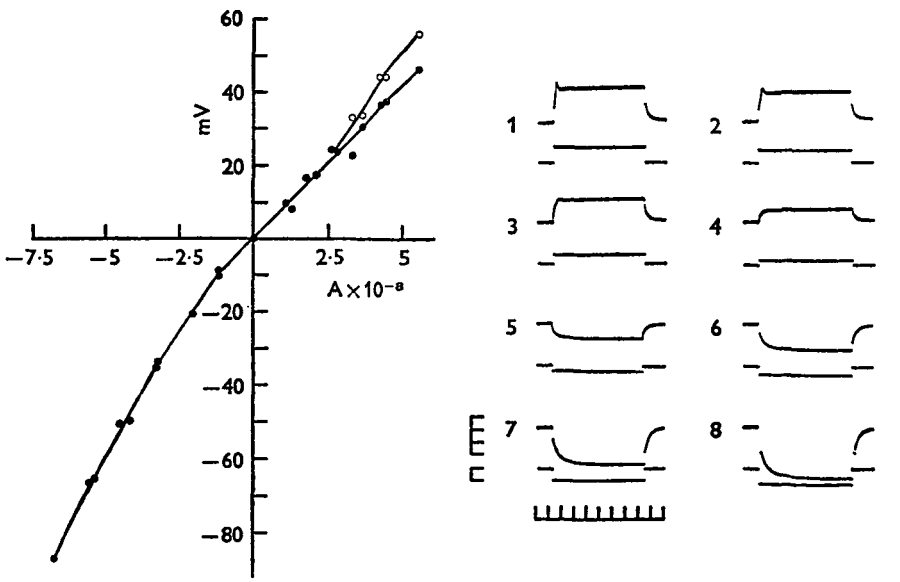

Fig. 3. Current/voltage relations of an S fibre at steady state (filled circles) and at the peak of the 'on' transient (open circles) where an active response is clear. Traces 1–8 are examples of the responses used in making the graph. The details are the same as in Fig. 2. Calibrations are 20 mV, 0·05 μA and 20 ms/division.

Table 1. *Cable constants*

	1	2	3	4	5	6	7	8
Fibre ...	S	S	T	T	T	S	S	S
Diameter (μm)	55	48	45	33	40	40	67	67
Length (mm)	3·5	3·5	3·5	—	4·5	4·5	4·0	5·2
Space constant (mm)	3·1	4·1	3·3	—	1·7	3·8	5·0	3·6
Time constant (ms)	—	—	—	7·3	3·2	6·7	11·0	6·5
Membrane potential (−mV)	50	45	45	50	50	55	60	55

(*c*) *Receptor potentials*

Having looked at resting membrane properties and electrical responses of the sensory neurones, we can now examine receptor potentials evoked by muscle stretch and attempt to define what ions are carrying current. The response to a pull has two main components, dynamic and static, and these are most obvious in the response to a fast, 'step' pull on the receptor muscle (see Figs. 4, 5). Consequently this type of stimulus has been used in the experiments which follow. Initially, an attempt is made to identify any active, sodium-dependent spike potentials by using tetrodotoxin. After this, the effects of changes in the concentration of sodium in the bathing solution will be considered.

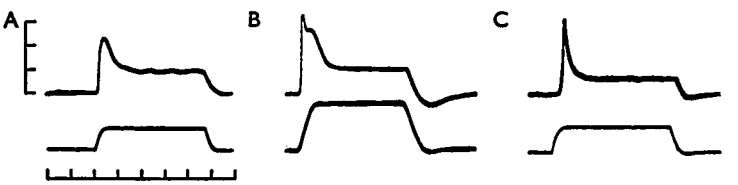

Fig. 4. Examples of T fibre receptor potentials (upper beam) in response to fast 'step' pulls on the receptor muscle and showing different degrees of spikiness in the dynamic component. The stimulus is indicated in the lower beam from the transducer on the puller. Increased stretch moves this beam up. Calibrations are 20 mV and 20 ms/division.

Effects of tetrodotoxin

A fairly common feature of the near-maximal response of either fibre to a fast, 'step' pull is a graded spike on the rising phase of the receptor potential (e.g. Figs. 4, 5). Very occasionally, similar spikes can occur later in the response, where they are probably evoked by slight oscillations of potential resulting from receptor muscle movements (e.g. Fig. 5). These late spikes have been seen in only a few preparations, including two of *Potamon perlatus*, the South African river crab.

The effect of 10^{-8} M tetrodotoxin on the early and later spike components is shown in Fig. 5. The later spikes are abolished and the early ones are reduced while the rest of the response remains unaffected. This suggests that some part of both types of spike depends on a transient sodium current, but that the early spike may have two components one of which is not blocked by tetrodotoxin.

These results, and those from current injection (see Fig. 3), point to a certain degree of depolarization-sensitive sodium activation in some part of the fibre and possibly the dendrites. This excitability can lead to local, graded spikes which are not

propagated and are of a slower time course than the impulses in other crustacean axons (duration 2–5 ms). Where graded spikes were absent, tetrodotoxin had no effect on the receptor potentials.

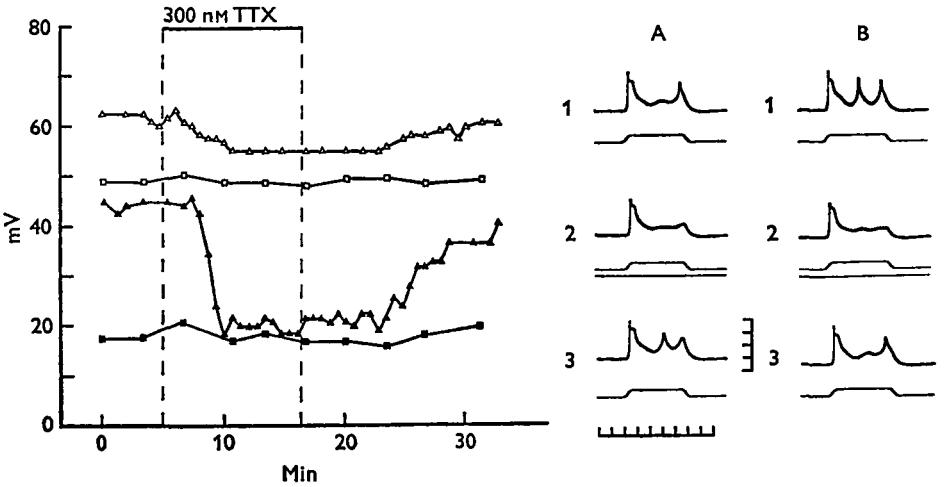

Fig. 5. Effect of tetrodotoxin on the responses of a T fibre with marked active components. (A) and (B) show examples of receptor potentials in two successive applications of tetrodotoxin. In both, (1) is in normal saline, (2) with an extra trace under the stimulus mark, is at the maximum effect of $0{\cdot}3 \times 10^{-6}$ M TTX, and (3) is after recovery in normal saline. In each picture the upper beam shows the receptor potential and the beam below this comes from the transducer on the pulling device. The time course and magnitudes of the changes under tetrodotoxin are indicated in the graph where the following are plotted: peak of the initial dynamic component (open triangles), dynamic plateau immediately after this peak (open squares), maximum of any later spikes (closed triangles) and maximum of static component (closed squares). Calibrations for (A) and (B) are 20 mV and 20 ms/division.

Effects of low external sodium concentration

The experiments with tetrodotoxin have shown that some small part of the receptor potential recorded in the S or T fibre can be due to transient sodium current. The replacement of all sodium in the bathing solution by large organic cations (tris and choline) was carried out to see whether that part of the receptor potential unaffected by tetrodotoxin was, none the less, generated partly or wholly by sodium current, as is the case for most other receptors studied.

Washing with sodium-free solution produced a small change in resting potential and a slow decrease of the receptor potential to a steady reduced level (see Fig. 6). This reduced response was only reached 20–30 min after the change to sodium-free solution. However, once attained, the reduced response was maintained, in sodium-free solution, for up to an hour (cf. Fulpius & Baumann, 1969) which was the longest period tested.

In four experiments in which two T and three S fibres were studied, sodium-free solutions brought about an average reduction of receptor potentials to 31% of their recovered value. The average S fibre reduction was to 26%, the T to 36%. Choline produced a slightly greater reduction in the dynamic and static components of the two

fibres than tris (see Fig. 6). At present these differences are unresolved. We conclude from these experiments that a large proportion of the total receptor currents in S and T fibres is normally carried by sodium ions.

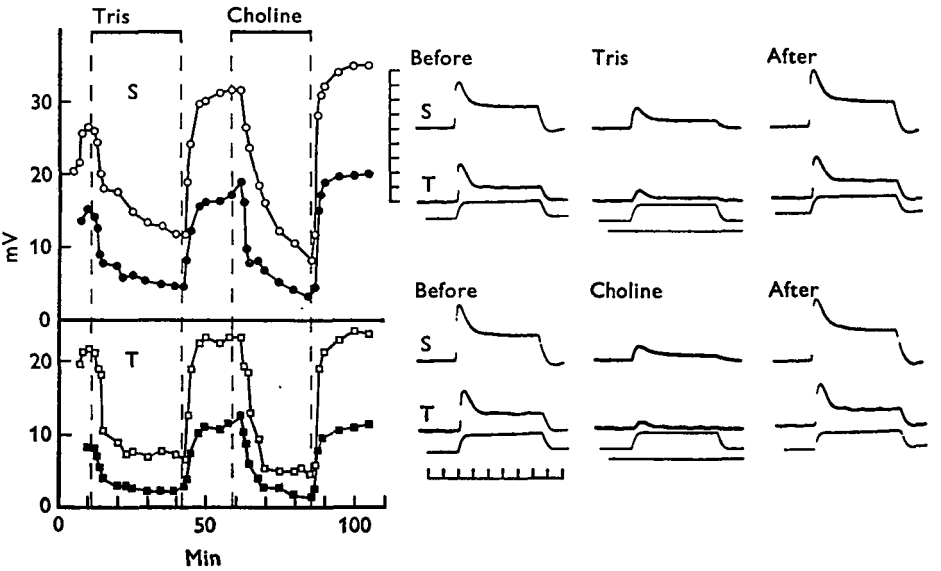

Fig. 6. Effect on receptor potentials of replacing external sodium with tris or choline. Responses of S and T fibres were recorded. S fibre receptor potentials are shown in the top trace of the records and the upper of the two graphs to the left, plotted as circles. T fibre receptor potentials are shown in traces just above the stimulus trace and are plotted as squares. Open symbols on the graphs are the peak of the dynamic component, closed ones give the amplitude of the static component measured just before the stimulus was released. In the records an extra beam below the stimulus mark indicates that sodium is replaced. Calibration for the records are 10 mV and 20 ms/division.

DISCUSSION

The coxal muscle receptors in the crab appear to share many membrane properties with the other primary sensory cells that have been studied, including several photoreceptors. These shared properties are as follows.

(*a*) *The resting potential* is potassium dependent, but the low slope of the curve relating membrane potential to external potassium concentration suggests either that other ions, perhaps chloride or sodium, are also involved, or that there is a region of the receptor neurone's membrane which is inaccessible to changes in bathing solutions. (Double penetrations of the cells have indicated for the present experiments that microelectrode entry does not usually cause depolarization, so an explanation in terms of a shunt due to damage seems unlikely.)

(*b*) *The receptor potential* is generated principally by a sodium current. The dynamic and static components of the receptor potential show a similar sodium dependency. However, when sodium is removed, the receptor potential is not abolished and the ionic mechanism for the remaining response has still to be clarified. Other ions, such as calcium, could carry receptor current in the absence of sodium or even normally. Alternatively there could be some sequestered sodium store which, in conjunction with

the sodium pump, could maintain a small response even when the general extracellular sodium concentration was reduced. A system like this or some other limiting diffusion pathway could account for the very slow decline of the receptor potential after sodium removal.

(*c*) *Active membrane response* appears to make only a small contribution to the recorded receptor potentials as is clear from experiments with tetrodotoxin and current injection. The location of membranes giving rise to the active responses is not evident from the present experiments, and could be within the receptor dendrites or fibre, or both.

These topics are discussed, often at greater length, in studies on the vertebrate muscle spindle (Ottoson, 1964; Calma, 1965), Pacinian corpuscle (Diamond, Gray & Inman, 1958) the crayfish stretch receptor (Edwards, Terzuolo & Washizu, 1963; Obara & Grundfest, 1968; Obara, 1968), the honeybee retinula cell (Fulpius & Baumann, 1969), the *Limulus* ventral photoreceptor (Millecchia & Mauro, 1969) and the barnacle eye (Brown *et al.* 1970). In the last two studies voltage-clamp techniques, possible on more spherical receptor cells, have allowed investigation of the receptor current. They have confirmed that dynamic and static components of the receptor potential depend on changes in membrane conductance (Fuortes, 1959; Rushton, 1959), and that the receptor current is mainly carried by sodium ions. Normally, but especially in the absence of sodium, the role of other ions, particularly calcium, is still uncertain.

One of the primary concerns of the present experiments has been to establish the ionic currents underlying the receptor potentials in the coxal muscle receptors. A further step is to relate these findings to the detailed structure of the sensory endings. In many receptors, for example, there appear to be specializations of the receptor membrane morphology which result in a larger surface area. In the crab coxal receptor this is achieved by a system of small T-branches extending from the main dendrites and embedded in strands of electron-dense material (Whitear, 1965). This encasing of what may be the site of mechanoelectric transduction might well influence ion movement in ways as yet unclear. One effect may be to limit diffusion of (e.g. sodium) ions, as suggested above.

In general, the active responses of these sensory fibres are more reminiscent of those found in crustacean muscle fibres than of those in other neurones. We have certainly seen no indications of all-or-none, propagated impulses in the S, T or even the smaller diameter 'D' fibre in the same nerve (innervating the 'depressor receptor': see Alexandrowicz & Whitear, 1957). Rather, the membranes of these receptors commonly give only small, graded, local active responses similar to those found in more electrically passive (and slowly contracting) crustacean muscle fibres (see Atwood, 1967). Sometimes more spiky membrane responses do occur in the receptor neurones; for examples see Fig. 4 (B, C). These resemble graded spikes occurring naturally (Atwood, Hoyle & Smyth, 1965) or in the presence of alkali-earth ions (Werman & Grundfest, 1961) in crustacean muscle. They may result partially from particular stimulus configurations or conditions in the receptor muscle. Rarely in *Carcinus*, but perhaps more commonly in *Potamon*, there is a definite larger active response superimposed on the basic receptor potential in the form of a variable number of graded spikes, which are abolished by tetrodotoxin (Fig. 5). The variability in active response, though hard

to pin down, may be significant functionally as it will affect the sharpness of voltage transients, which tend to be filtered out during transmission to the CNS by the resistive and capacitative elements of the fibre membrane.

It is possible that the variability in active response may be related to the normal physiological cycles of crabs. Many excitable membranes show very complex relationships between membrane properties and the concentrations of divalent cations present, particularly of calcium. One expression of this is the inverse relation in barnacle muscle fibres between their electrical excitability and the extracellular divalent ion concentration (Hagiwara & Takahashi, 1967). However, electrical excitability is only one measure of very general effects, including for example effects on resting potassium permeability and the resting potential, or on activation and inactivation processes during spike potentials. What strikes one is the degree to which membrane properties can be modified by prevailing conditions, especially when it is known that the relative concentrations of divalent cations in crustacean blood can show considerable fluctuations depending, for instance, on the time of year and the stage in the moult cycle (Andrews, 1967). Such variation may underly the differences in amount and nature of the active response seen in the present experiments on the coxal muscle receptors. Any functional interpretation of this variability must wait for further experiments on the reflexes.

SUMMARY

1. The resting membrane potential of S and T receptor fibres is usually -50 to -60 mV and is potassium dependent.

2. The neurone membrane may also be permeable to other ions or some part of it may be inaccessible.

3. The electrical responses of the fibres are nearly ohmic for 40 mV on either side of the resting membrane potential.

4. In some fibres there is a little depolarizing electrogenesis.

5. Receptor potentials were evoked by 'step' pulls on the receptor muscle. In response to fast pulls there is often a variable spike component, abolished by tetrodotoxin and presumably therefore sodium dependent.

6. Ion substitution experiments indicate that the rest of the receptor potential is also mainly sodium dependent. However, in the absence of sodium a small receptor response remains. Possible reasons for this are discussed.

We thank the U.S.P.H.S. for some of the equipment bought from the research grant NB 06666 to B. M. H. B.

REFERENCES

ALEXANDROWICZ, J. S. & WHITEAR, M. (1957). Receptor elements in the coxal region of Decapod Crustacea. *J. mar. biol. Ass. U.K.* **36**, 603–28.

ANDREWS, P. (1967). Über den Blutchemismus des Flusskrebses *Orconectes limosus* und seine Veränderung im Laufe des Jahres. *Z. vergl. Physiol.* **57**, 7–43.

ATWOOD, H. L. (1967). Crustacean neuromuscular mechanisms. *Am. Zool.* **7**, 527–51.

ATWOOD, H. L., HOYLE, G. & SMYTH, T. Jr. (1965). Mechanical and electrical responses of single innervated crab-muscle fibres. *J. Physiol., Lond.* **180**, 449–82.

BROWN, H. M., HAGIWARA, S., KOIKE, H. & MEECH, R. M. (1970). Membrane properties of a barnacle photoreceptor examined by the voltage clamp technique. *J. Physiol., Lond.* **208**, 385–415.

BUSH, B. M. H. & ROBERTS, A. (1968). Resistance reflexes from a crab muscle receptor without impulses. *Nature, Lond.* **218**, 1171–3.

CALMA, I. (1965). Ions and the receptor potential in the muscle spindle of the frog. *J. Physiol, Lond.* **177**, 31–41.

DIAMOND, J., GRAY, J. A. B. & INMAN, D. R. (1958). The relation between receptor potentials and the concentration of sodium ions. *J. Physiol., Lond.* **142**, 382–94.

EDWARDS, C., TERZUOLO, C. A. & WASHIZU, Y. (1963). The effect of changes of the ionic environment upon an isolated crustacean sensory neuron. *J. Neurophysiol.* **26**, 948–57.

FULPIUS, B. & BAUMANN, F. (1969). Effects of sodium, potassium, and calcium ions on slow and spike potentials in single photoreceptor cells. *J. gen. Physiol.* **53**, 541–62.

FUORTES, M. G. F. (1959). Initiation of impulses in visual cells of *Limulus*. *J. Physiol., Lond.* **148**, 14–28.

GWILLIAM, G. F. (1965). The mechanism of the shadow reflex in Cirripedia. II. Photoreceptor cell response, second-order responses, and motor cell output. *Biol. Bull. mar. biol. Lab., Woods Hole* **129**, 244–56.

HAGIWARA, S. & TAKAHASHI, K. (1967). Surface density of calcium ions and calcium spikes in the barnacle muscle fibre membrane. *J. gen. Physiol.* **50**, 583–601.

MILLECCHIA, R. & MAURO, A. (1969). The ventral photoreceptor cells of *Limulus*. II. The basic photoresponse. III. A voltage-clamp study. *J. gen. Physiol.* **54**, 310–51.

OBARA, S. (1968). Effects of some organic cations on generator potential of crayfish stretch receptor. *J. gen. Physiol.* **52**, 363–86.

OBARA, S. & GRUNDFEST, H. (1968). Effects of lithium on different membrane components of crayfish stretch receptor neurons. *J. gen. Physiol.* **51**, 635–54.

OTTOSON, D. (1964). The effect of sodium deficiency on the response of the isolated muscle spindle. *J. Physiol., Lond.* **171**, 109–18.

RIPLEY, S. H., BUSH, B. M. H. & ROBERTS, A. (1968). Crab muscle receptor which responds without impulses. *Nature, Lond.* **218**, 1170–1.

RUSHTON, W. A. H. (1959). A theoretical treatment of Fuortes's observations upon eccentric cell activity in *Limulus*. *J. Physiol., Lond.* **148**, 29–38.

STRICKHOLM, A. (1968). Reduction of response time for potential in salt bridge reference electrodes for electrophysiology. *Nature, Lond.* **217**, 80–1.

WERMAN, R. & GRUNDFEST, H. (1961). Graded and all-or-none electrogenesis in arthropod muscle. II. The effects of alkali-earth and onium ions on lobster muscle fibres. *J. gen. Physiol.* **44**, 997–1027.

WHITEAR, M. (1965). The fine structure of crustacean proprioceptors. II. The thoracico-coxal organs in *Carcinus*, *Pagurus*, and *Astacus*. *Phil. Trans. R. Soc. Ser.* B **248**, 437–56.