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Regional Differences in K Channels of Abdominal and Circumesophageal Segments of the Crayfish Medial Giant Axon

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Summary. Intracellular recordings reveal that the membrane of the circumesophageal region of the medial giant axon of crayfish responds to replacement of Cl with propionate differently from that of the abdominal region of the same axon. The connective hyperpolarizes in the propionate saline, whereas the abdominal region undergoes the transient depolarization that is expected when a permeant anion (Cl) is replaced with an impermeant one (propionate). The hyperpolarization of the connectives is accompanied by an increased conductance, a decreased length constant, and an increase in threshold current for intracellular stimulation. These effects are specific for the connectives and for propionate. They do not occur on replacing Cl with other large anions, isethionate, methane sulfonate, or glucuronate. The effects of propionate are independent of Na or Ca and result from an increased K conductance. The hyperpolarization induced by propionate is increased in a K-free saline, where the resting potential (E_M) is considerably positive to the emf of the K battery (E_K). It is abolished in elevated K_o when $E_M = E_K$.

In the course of a study of the electrophysiology of the crayfish medial giant axon (Yamagishi & Grundfest, 1971) we observed a striking difference in the response of the abdominal and circumesophageal portions of the membrane to replacement of Cl_o with propionate. Since the axon is permeable to Cl (Strickholm & Wallin, 1967), replacement of this anion with an impermeant one may be expected to induce a transient depolarization and, when Cl is reintroduced, a transient hyperpolarization (Hodgkin & Horowicz, 1959).

Propionate is an impermeant anion for many cells (*cf.* Reuben, Girardier & Grundfest, 1964) and it appears to be so for the abdominal portions of the medial and lateral giant axons as well. The circumesopha-

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geal portion of the medial axon, however, hyperpolarizes when Cl_o is replaced with propionate and it remains hyperpolarized at least up to 1 hr while in the propionate saline. Analysis indicates that propionate, but not other anions tested, specifically increases the K-permeability of the membrane of the circumesophageal region. A preliminary report appeared in 1972 (Grundfest & Yamagishi).

Materials and Methods

The crayfish (*Procambarus*) were obtained from California. A length of nerve cord (3.5–6 cm) which included the circumesophageal connective was removed and divided longitudinally. The half-cord was mounted in a lucite chamber in which the bathing solution could be changed within 1–2 min (Yamagishi & Grundfest, 1971). The sheaths around the giant axons in the region of impalement with microelectrodes were carefully removed under a dissecting microscope. Except for experiments like those shown in Figs. 1 and 2, the work was confined to the circumesophageal connective. The medial giant axons ranged in diameter from 120 to 160 μm in the connective and from 70 to 100 μm in the abdominal region. The axon was usually impaled with two microcapillary electrodes filled with 3 M KCl (resistance 15 to 30 M ohm). Membrane potentials were recorded differentially against a KCl-agar-AgCl-Ag bridge. Effective resistance was measured with intracellularly applied inward currents and spikes of the axons were evoked either by intracellular or extracellular stimuli. All the axons studied generated spikes 100 mV or more in amplitude. The temperature ranged between 19 ° and 23 °C.

The standard bathing medium contained (in mM) 205 NaCl, 5.4 KCl and 13.5 $CaCl_2$; pH was adjusted to 7.3 with Tris buffer (Girardier, Reuben, Brandt & Grundfest, 1963; Reuben *et al.*, 1964). The major experimental procedure was exchange of this medium for one in which some "foreign" monovalent anion replaced all the Cl. In some experiments K_o was varied and in others all Na_o was replaced with Tris or with Ca. The total osmolarity was adjusted to 464 mosmols.

Results

The Different Regional Effects of Replacing Cl with Propionate

For the experiments illustrated in Fig. 1, two recording microelectrodes were inserted in the abdominal and circumesophageal regions, respectively, as shown in the diagram. The preparation was 6 cm long, the insertion sites were 3.7 cm apart, and the axon was stimulated externally at its caudal extremity. The spikes which propagated rostrally into the two regions are shown in the records taken at the indicated times before and after replacement of Cl with propionate. The resting potential in the control (Cl) saline was −82 mV in the abdominal region and

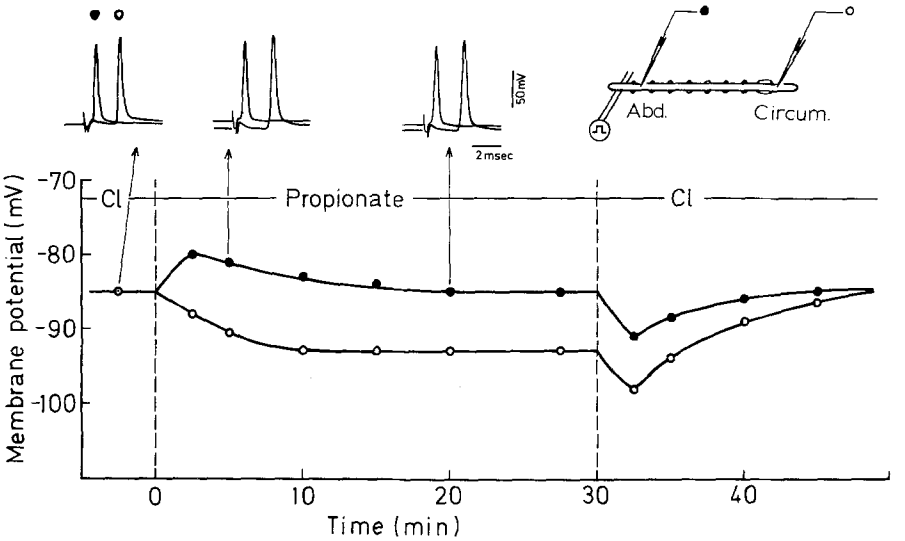

Fig. 1. Different regional effects on replacing Cl with propionate. Simultaneous recording from abdominal (●) and circumesophageal (○) regions 3.7 cm apart (diagram, upper right). Spikes evoked by external stimulation are shown in upper left. The resting potentials (−82 and 85 mV) were made coincident for the control condition (Cl) in the graphs and recordings. Second recording shows nearly maximal separation of traces as abdominal region depolarized while the connective hyperpolarized. Third recording was made when the resting potential of the abdominal region had returned to its control level. Cl was restored after 30 min. Both regions exhibited transient hyperpolarization and return to initial levels within about 20 min

−85 mV in the circumesophageal, but to simplify presentation of the data, the potentials were made to coincide in the records and graphs of Fig. 1.

Substitution of Cl by propionate did not affect the spike amplitude or form, indicating that the kinetics of the electrogenesis were essentially unchanged, but the conduction velocity decreased from 21.8 to 18.5 m/sec. The resting potential, however, underwent markedly different changes in the two regions. The abdominal region depolarized transiently to a peak of 5 mV and the potential returned to the initial value after 15–20 min. The potential changed more slowly in the connective, but the response was a hyperpolarization of almost 10 mV which persisted until Cl was again introduced. Then, a transient hyperpolarization was always observed in the abdominal region, but the hyperpolarization which occurred in the circumesophageal connective in this and other experiments was sometimes small or absent (Fig. 2). The initial transient hyperpolarization on restoring Cl indicates that some intracellular Cl is lost during the exposure of the axon to the propionate saline. It should

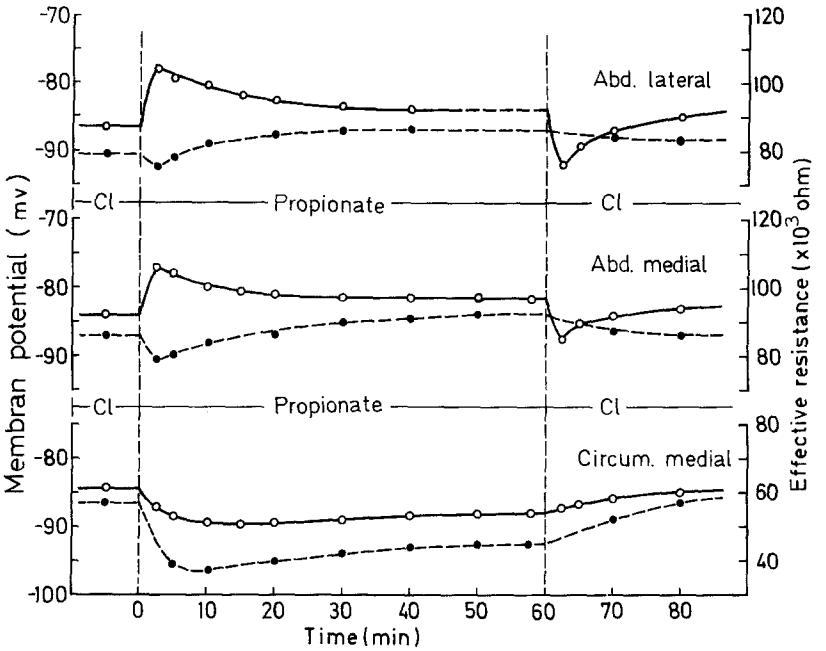

Fig. 2. Comparison of effects of replacement of Cl with propionate (and reverse) on membrane potential (open circles) and input resistance (filled circles) of the abdominal lateral and medial axons and of the circumesophageal region of the latter axon. Data points are averages of 4, 7, and 9 experiments, respectively

be noted that the changes in potential at the two sites could not have interacted by electrotonic spread. The length constant (λ) of this preparation is about 2 mm (*cf.* Fig. 3; Yamagishi & Grundfest, 1971), so that the recording electrodes were about 20λ apart.

The graphs of Fig. 2 show averaged data for the changes in potential of the two regions (open circles; abdominal, n=7; connective, n=9) and data of 4 experiments on the lateral giant axon. The effective resistance was also measured in these axons (filled circles). The abdominal segments of both medial and lateral axons depolarized transiently, while the effective resistance, after a brief initial decrease, rose in both cases from 79 to 86 kΩ in the lateral axon and from 86 to 91 kΩ in the medial. The hyperpolarization which developed in the connective was associated with a decrease in effective resistance, from 57 to 38 kΩ initially, and to a steady value of 45 kΩ after 50 min in the propionate saline. On reintroducing Cl the effective resistance returned to its initial value in all 3 cases.

The decrease in effective resistance of the circumesophageal connective when propionate replaces Cl is confirmed by two other types of

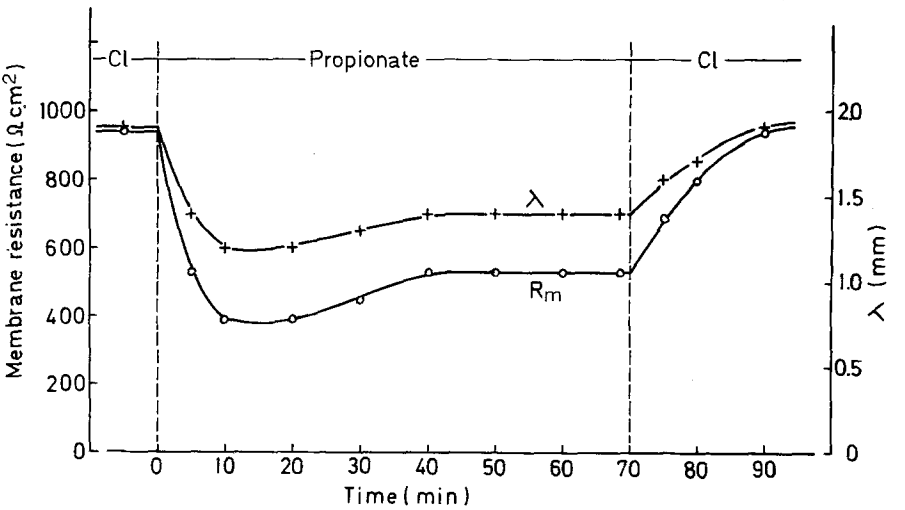

Fig. 3. Calculated in length constant (λ) and membrane resistance (R_M) of circumesophageal connective on replacing Cl with propionate. Initial values were again obtained after returning axon to Cl-saline

data. The length constant (λ) of the membrane decreased from 1.9 mm in the Cl saline (Fig. 3) to 1.2 mm initially on replacing Cl with propionate and then rose to a steady value of 1.4 mm. In the axon of Fig. 3 the membrane resistance of the circumesophageal region, which was 940 Ω cm^2 in the Cl saline, decreased to 390 Ω cm^2 10 min after replacing Cl with propionate and attained a steady value of 530 Ω cm^2 after 50 min in propionate. The change in both λ and R_M were reversed when Cl was reintroduced.

The membrane resistance differs markedly in the circumesophageal and abdominal regions (average 870 Ω cm^2 and 1600 Ω cm^2, respectively), but we have not analyzed the ionic components of the total conductance. The membrane resistance of the lateral giant axon is about 3000 Ω cm^2 (Watanabe & Grundfest, 1961), but the responses to propionate are similar to those of the abdominal segment of the medial fiber (Fig. 2).

Further evidence that the membrane conductance increased in the propionate saline is provided by the change in the threshold to evoke a spike. For these measurements the stimulating and recording microelectrodes were only about 50 μm apart. The threshold depolarization of the axon of Fig. 3 was 21 mV in Cl and 19 mV in propionate. However, in the control saline a current of 48 μa was required to elicit a spike, but immediately after replacement of Cl with propionate the threshold current rose to 119 μa, and decreased to 94 μa after 50 min in propionate. These changes are consistent with and presumably account for the slowed conduction of the nerve impulse.

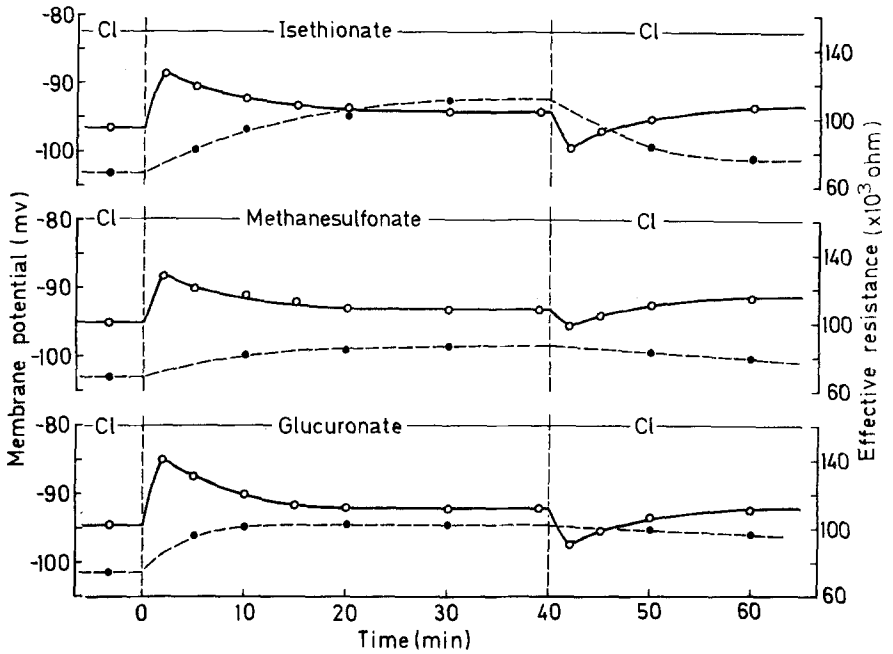

Fig. 4. Changes in resting potential (open circles) and effective resistance (filled circles) on replacing Cl with isethionate, methanesulfonate and glucuronate K-free salines. Averages of 5 or more experiments

Effects of Other Anions

The experiments illustrated in Figs. 4 and 5 demonstrate that among the anions tested only propionate evoked the hyperpolarization associated with a decrease in effective resistance of the membrane in the circumesophageal region. The axons were equilibrated in K-free salines, a condition that increases the hyperpolarization induced on replacing Cl with propionate (Fig. 6) and also improves survival of the axons (Yamagishi & Grundfest, 1971). The effects of three large anions (Fig. 4) are those expected for impermeant species – transient depolarization and an increase in effective resistance. Replacement of Cl with Br, NO_3, or acetate evoked hyperpolarization which was associated with little or no change in effective resistance, but when a change did occur (with Br, Fig. 5) the resistance increased.

Ionic Basis for the Effects of Propionate

The increased conductance which is associated with hyperpolarization on replacing Cl with propionate implies that the change in membrane

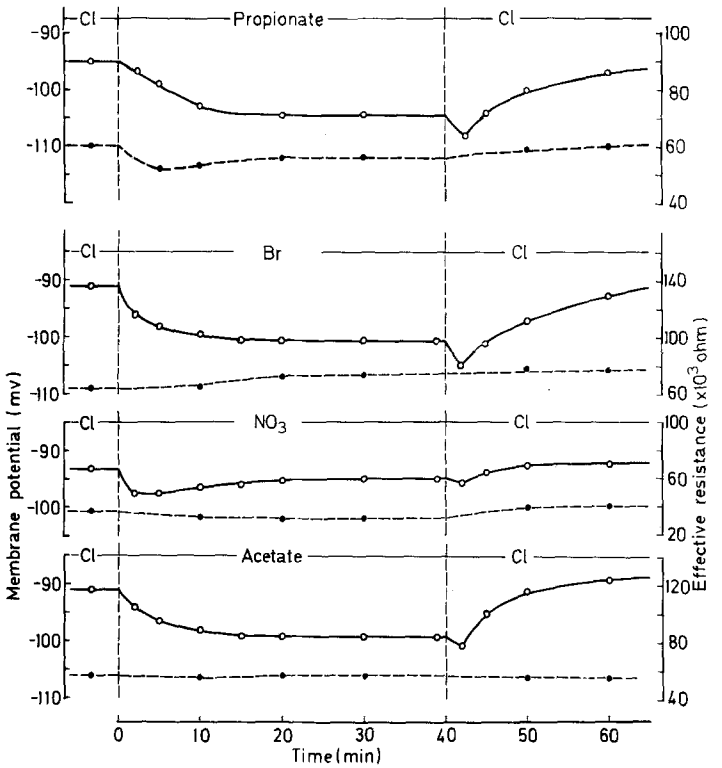

Fig. 5. Changes in resting potential (open circles) and effective resistance (filled circles) on replacing Cl with propionate (10 experiments), Br (5 experiments), NO_3 and acetate (4 experiments each). K-free salines were used in all cases

potential involves an inside-negative battery. A likely candidate for this effect is the K battery (E_K) and the 3 types of experiments shown in Fig. 6 confirm that surmise.

1. The effect of propionate on the membrane potential depends on $[K]_o$ (Fig. 6*A*). The hyperpolarization that is observed in the control saline (5.4 mM K_o) becomes even larger when propionate replaces Cl in a K-free saline and E_K must become more negative. It decreases and reverses to transient depolarization when $[K]_o$ is increased and E_K becomes less negative.

2. The relation between E_M and $[K]_o$ more closely approaches that for a K-electrode (Fig. 6*B*) when propionate (filled circles) replaces Cl (open circles).

3. The change in conductance as a function of $[K]_o$ is greater in propionate (Fig. 6*C*, filled circles) than in Cl (open circles). The difference is particularly evident when $[K]_o$ is at or near that of the control saline.

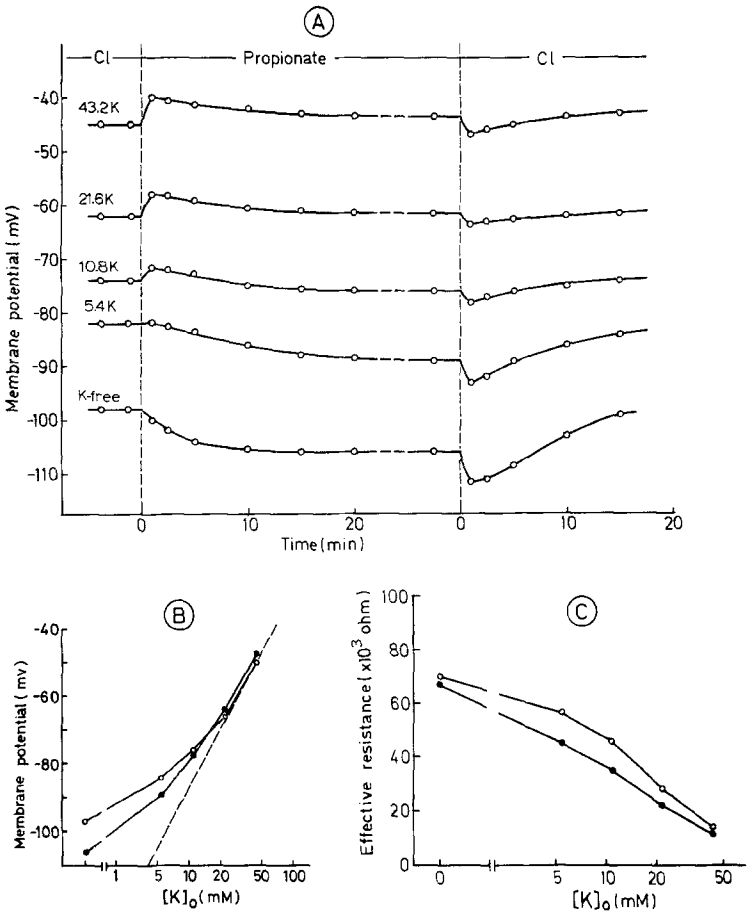

Fig. 6. Effects of $[K]_o$ on changes induced by replacing Cl_o with propionate. Data points are averages of 4 experiments. (*A*) Time course of changes in E_M for solutions containing different levels of $[K]_o$. (*B*) E_M as a function of $[K]_o$ in Cl saline (open circles) and in propionate (filled circles). Latter measurements made 20 min after replacing Cl with propionate. Broken line is drawn (with 58 mV/decade K_o slope) tangent to data points at $[K]_o$= 43.2 mM. (*C*) Change in effective resistance as a function of $[K]_o$, in Cl (open circles) and in propionate (filled circles). Latter measurements made 20 min after replacing Cl with propionate

Effects of Na and Ca on the Responses to Propionate

We had demonstrated previously (Yamagishi & Grundfest, 1971) that the membrane of the circumesophageal connective at rest includes ionic batteries for Na and Ca as well as K and Cl. Fig. 7 shows two types of experiments which indicate that Na and Ca play little or no role

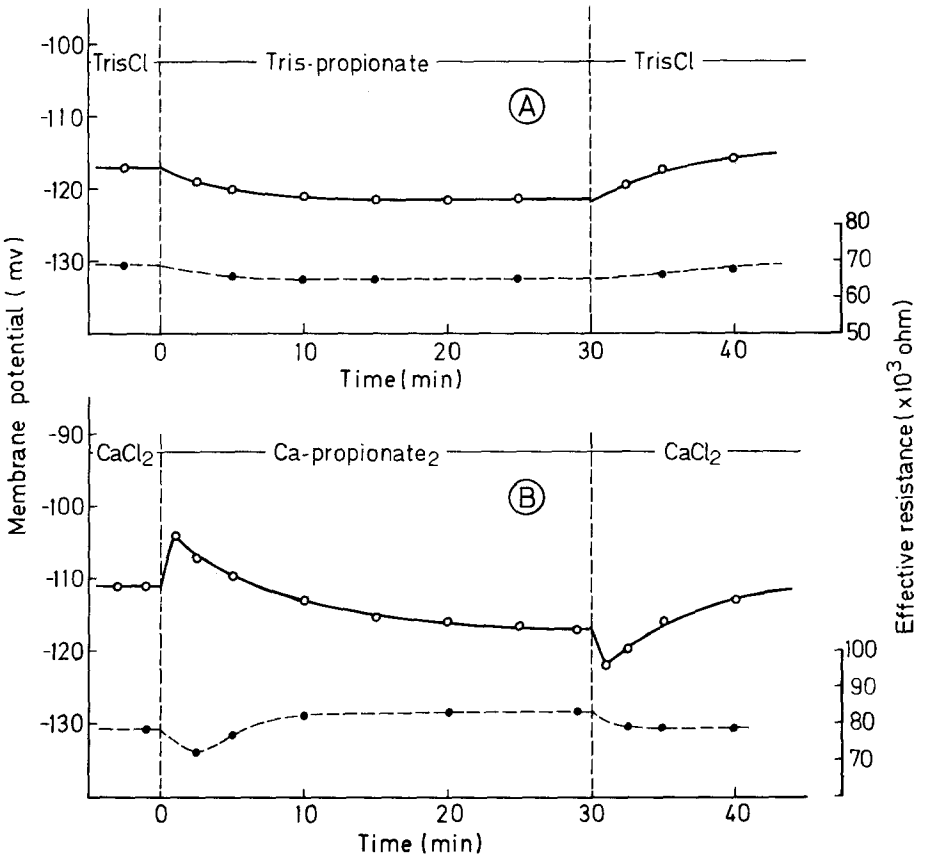

Fig. 7. Changes in membrane potential (open circles) and effective resistance (filled circles) on replacing Cl with propionate in K-free and Na-free salines. (*A*) Na replaced with Tris. (*B*) Na replaced with Ca

in the hyperpolarization and conductance increase that occur when Cl is replaced with propionate.

For these experiments the salines were K-free. When Na is completely substituted for with Tris Cl replacement of Cl with propionate (Fig. 7*A*) has essentially the same effect as does replacement of NaCl with Na propionate. The resting potential which is high in the Na-free saline is increased further on replacing Cl with propionate.

When the initial bathing medium is $CaCl_2$ (Fig. 7*B*) replacement of Cl with propionate also induces a hyperpolarization, but after an initial depolarization. The effective resistance is decreased only transiently in tempo with the initial depolarization. The steady state hyperpolarization is associated with an increase in effective resistance.

Discussion

The data of Figs. 3 and 6 provide an adequate and consistent explanation of the specific effect on the resting potential of the circumesophageal connective of replacing the Cl of the normal saline with propionate. The substitution causes an increase in the K conductance of the membrane in this segment, but not in the abdominal region of the same axon, nor in the lateral giant axon. Thus, the data disclose the existence of a specific variety of K-permselective channels which are restricted to a specific region of membrane. We shall reserve discussion of the properties of these specialized channels until later.

The electrochemical basis of the propionate induced hyperpolarization of the connective stems from the fact that the resting membrane of the medial axon is a mixed electrode system (Yamagishi & Grundfest, 1971). By virtue of the contributions of other batteries E_M is somewhat positive to E_K. Increasing the conductance for K would then enhance the effectiveness of the K battery, thereby causing hyperpolarization. Replacement of Cl with propionate does increase the K conductance of the membrane of the connective, and hyperpolarization occurs when E_K is negative to E_M. With $[K]_o \geqq 10$ mM, replacement of Cl with propionate results only in a transient depolarization (Fig. 6*A*), and this is consistent with the findings of Fig. 6*B* and 6*C*. The resting conductance of the membrane is increased as K_o is increased (Fig. 6*C*) and the membrane approaches the condition of a K-electrode (Fig. 6*B*), even when Cl is the external anion.

Increased K conductance in the presence of propionate is also evidenced under conditions where the membrane potential is modified by changing $[Na]_o$ or $[Ca]_o$, both of which contribute to the resting potential (Yamagishi & Grundfest, 1971). Replacement of Na with Tris, thereby reducing the inside positivity of E_{Na}, leads to marked hyperpolarization. Thus, E_M, was nearly -120 mV in Tris Cl (Fig. 7*A*), while it was about -100 mV in the K-free NaCl saline (Fig. 6*A*). Nevertheless, hyperpolarization occurred in both cases on replacement of Cl with propionate, and in both cases this was associated with only a small decrease in resistance.

Increase of Ca in the absence of Na and Cl induces depolarization of the abdominal region when an impermeant anion replaces Cl (Yamagishi & Grundfest, 1971, Figs. 8 and 9). However, in the present work on the circumesophageal segments replacement of Cl with propionate (Fig. 7*B*) caused only an initial transient depolarization which was mir-

rored by an equally transient hyperpolarization on replacing the propionate with Cl. The steady-state potential was negative relative to that in Cl. The effective resistance, which decreased initially, probably as a result of the transient depolarization, increased slightly in Ca propionate. The experiments shown in Fig. 7 were done in K-free salines and the effective resistance was high (Fig. 6*C*). The small effect of propionate on the resistance in the K-free medium suggests that the number of K channels which can be opened by propionate is then at a minimum. Nevertheless, replacement of Cl with propionate improves the K-electrode characteristic of the membrane (Fig. 6*B*), in keeping with the effect of propionate on the resistance (Fig. 6*C*).

The resistance also changes little when the axons are bathed in salines containing more than 20 mM K (Fig. 6*C*). In this condition the membrane becomes predominantly a K-electrode, irrespective of the presence or absence of propionate (Fig. 6*B*). Propionate no longer induces hyperpolarization, since the membrane potential now approaches E_K also in the presence of Cl. The transient effects of replacing Cl with propionate (Fig. 6*A*) are those associated with the redistribution of Cl. Although the transient depolarization is masked by hyperpolarization when the axons are in K-free or in 5.4 mM K saline, redistribution of Cl does occur and this is evidenced by the transient hyperpolarization that usually occurs when propionate is replaced with Cl (Fig. 6*A*).

The number of K-permselective channels that contribute to the total electrically excitable K conductance (G_K) depends markedly on $[K]_o$ in some cells but not in others. As measured in the voltage clamped frog node, G_L and G_K remain essentially constant as K_o is varied from zero to 100 mM. The transition from the low conductance resting state (G_L) to K activation (G_K) is controlled solely by the membrane potential (Müller-Mohnsen, 1967; *cf.* Grundfest, 1975, Fig. 3). In eel electroplaques, however, G_K is strongly K-dependent. In K-free saline virtually all the G_K channels are closed, while the G_L channels are unaffected (Nakamura, Nakajima & Grundfest, 1965; Ruiz-Manresa, 1970; *cf.* Grundfest, 1975, Fig. 4). The number of open G_K channels increases steeply as K_o is increased so that in the presence of 50–100 mM K_o G_K becomes 2 to 4-fold higher than in the control saline (5 to 6 mM K_o). G_L is increased only slightly. Despite the depolarization induced by high K_o, the G_K channels do not close, and an electrical driving force of some 40–50 mV must be superimposed to induce the characteristic depolarizing inactivation of these G_K channels (Nakamura *et al.*, 1965). An intermediate condition has been reported in a starfish egg. About half the K channels are

controlled by increasing K_o, the rest by changing the membrane potential (Hagiwara & Takahashi, 1974).

Our data permit a few deductions regarding the properties of the propionate-activated K-conductance system. Although the electrophysiological evidence of the presence of these channels in the circumesophageal connective—and not in the abdominal region—is readily demonstrated, the number of the propionate-activated K channels cannot be very large. The resting conductance of the membrane is about 1 mmho/cm^2 and doubles in propionate. Thus, relative to the total number of K channels that are opened during the spike the number of propionate sensitive channels must be insignificant. At least some of the propionate sensitive channels appear to close in time (e.g., Fig. 3) perhaps as a manifestation of desensitization, but most remain open for 1 h or more.

We have already noted that the propionate-sensitive K conductance increases when E_M is in the range of -80 to -120 mV and, depending on the relation of E_M to E_K the hyperpolarization may reach 5 to 10 mV. As a first approximation, therefore, the propionate-activated gates for K channels are voltage insensitive (electrically inexcitable, Grundfest, 1957, 1966). Another indication of this condition is the persistence of the hyperpolarization when the K channels are opened. The after-hyperpolarization that results from the increase in G_K of squid giant axons lasts only a few msec (Hodgkin & Huxley, 1952), the K-conductance being quenched by the hyperpolarization. The resting membrane conductance even decreases thereafter (Shanes, Grundfest & Freygang, 1953). The quenching effect is less striking in the case of K channels of frog slow muscle fibers, *Tenebrio* muscle fibers and *Ascaris* oesophageal cells (Grundfest, 1967, 1971), but in all these the hyperpolarization lasts seconds at most, rather than hours as in the case of the propionate activated channels.

These various characteristics indicate that the gates which control these K channels are electrically inexcitable and presumably are activated by a conformational change of the membrane surface similar to those which are believed to be caused when pharmacological agents react with "receptor sites" that gate the permselectivity changes in synaptic membranes. In the present case gates (or receptor sites) are affected by one rather simple anion and not by other rather similar anions. It would therefore seem fruitful to analyze in considerable detail the physicochemical structure of these several anions. The fine structure of their electroenergetics might yield clues to the structure of the propionate sensitive K-gating sites on the membrane surface.

Rather specific and as yet unexplained action of propionate has been observed in other systems as well. Although it is generally an impermeant anion, e.g., for muscle fibers of lobster, crayfish and frog (Reuben *et al.*, 1964; Reuben, Lopez, Brandt & Grundfest, 1963) and for squid and lobster axons (Freeman, Reuben, Brandt & Grundfest, 1966) propionate is slightly permeant in the activated inhibitory membrane of lobster muscle fibers (Motokizawa, Reuben & Grundfest, 1969). Propionate, but not methylsulfate, prolongs the electrically evoked tension of crayfish muscle fibers (Dudel & Rüdel, 1969, and confirmed by Reuben, Brandt & Grundfest, 1974). Dudel & Rüdel suggested that this effect of propionate resulted from depolarization of the *SR* on replacing Cl of the bathing medium with propionate. Reuben *et al.* (1974) found, however, that propionate also prolonged the tension that was evoked by injecting Ca into fibers which were fully depolarized by the presence of high K_o. They explained the effect as a specific action of propionate, slowing the extrusion of Ca across the cell membrane.

Interactive effects of cations on other cations are, of course, well known (Reuben, Brandt, Girardier & Grundfest, 1967; Orentlicher & Ornstein, 1971; Reuben *et al.*, 1974). The effects described above both in muscle and in the circumesophageal connective show an action of a specific anion on membrane permeability to cations, Ca and K, respectively[1]. This fact need not be surprising since gates, particularly those that are electrically inexcitable, may be different structures from the permselective channels which the gates control. Thus, a given agent, e.g., the cationic acetylcholine, can control channels which are permselective for Na and K, K alone, or Cl alone.

Note Added in Proof

Barker and Levitan (1975) have observed hyperpolarization and increased K-conductance in neurons of *Navanax* on applying rather low concentrations of various complex organic compounds, including salicylate. They ascribe these effects to an increase in the anionic field strength of

1 The very large increase in conductance of skate electroplaques which results from depolarizing Cl activation (Cohen, Bennett & Grundfest, 1960; Hille, Bennett & Grundfest, 1965, and *unpublished*) is blocked by Ba. The *I-E* characteristic in the depolarizing quadrant, which normally exhibits a remarkable time-variant curvature, then becomes linear (ohmic), indicating that the K-permselective "leak" channels are unresponsive to electrical stimuli as well as to pharmacological inactivation by Ba (V. Grundfest, 1966. Fig. 21).

the cell membrane. Of the three large anions used in the present work, only propionate increased K-permeability and only in the circumesophageal region of the crayfish medial giant axon. It is unlikely, therefore, that the effect of propionate results from a change in the field strength of the membrane. It would be useful, however, to test on the axon the agents studied by Barker and Levitan. If these should affect the axonal membrane would the effect be general, or confined to the propionate sensitive region?

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