Ionic Permeability of the Inhibitory Postsynaptic Membrane of Lobster Muscle Fibers

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ABSTRACT Reversal potentials (E_{IPSP}) of the inhibitory postsynaptic potential and the membrane resting potentials (E_M) of lobster muscle fibers were determined with intracellular recording under a variety of ionic conditions. E_{IPSP} is solely dependent on the electromotive force of anionic batteries; i.e., on the electrochemical gradient for a "mobile" fraction of intracellular Cl (Cl_i) which is considerably smaller than the total intracellular Cl. The active inhibitory membrane is more permeable to certain "foreign" anions in the order $NO_3 > SCN > Br > Cl$. The membrane is impermeable to BrO_3, isethionate, and methylsulfate, but is slightly permeable to acetate and propionate. The level of Cl_i appears to be determined in part by some active (pump?) process and most of the anions studied appear to interfere with the steady-state level of Cl_i.

INTRODUCTION

Many of the inhibitory synapses that have been studied by various workers owe their electrogenesis mainly or solely to a conductance increase of the postsynaptic membrane for anions; i.e., the membrane becomes more permeable to Cl in the normal milieu. The inhibitory postsynaptic membrane of the lobster neuromuscular system also appears to become highly anion-selective during synaptic activity (Grundfest, Reuben, and Rickles, 1959; Gainer, Reuben, and Grundfest, 1967) and this was confirmed in the present work. However, some earlier experiments (Reuben, 1959, and unpublished data) suggested that various monovalent anions which were substituted for Cl could not be classified simply as permeant or impermeant by measuring the change in the inhibitory postsynaptic potential (IPSP). A systematic survey of the effects of a number of foreign anionic substituents was therefore undertaken and is reported here. A preliminary account of this work has appeared (Motokizawa et al., 1967).

The distribution of Cl as well as of the foreign anions across the cell boundary was estimated by continuous and simultaneous measurements of the membrane potential (E_M) and the emf of the inhibitory ionic battery (E_{IPSP}) after exposing the neuromuscular preparation to various anions, or upon altering the potassium concentration (K_o) of the saline. These measurements allowed conclusions to be drawn regarding the permeability characteristics of the nonsynaptic as well as of the inhibitory synaptic membrane components of the muscle fibers.

METHODS

Stretcher muscles of the walking legs of lobster (*Homarus americanus*) were prepared with their exciter and inhibitor axons (Grundfest et al., 1959). The preparation was fixed in a chamber and immersed in 30 ml of solution of the desired composition. The standard saline was modified from Dalton's (1958) formulation, by eliminating Mg and SO_4. It contained (in mM/liter) Na 477, K 10, Ca 25, and Cl 537. The pH (7.3–7.5) was adjusted with Tris buffer. All the Cl was eliminated only in certain cases; in most of the experiments all or part of the NaCl was replaced with an equivalent amount of the Na salt of the various "foreign" anions. The replacement of all the NaCl reduces the Cl to 11% of the standard medium. This degree of substitution of Br, NO_3, or SCN for Cl did not affect the responsiveness of the preparation to neural stimuli. Replacement of 89% of the Cl with the larger anions, and particularly with propionate or formate, induced block of neuromuscular transmission. This was probably due in part to a long lasting transient depolarization of the axons and consequent block of their spike electrogenesis that has been observed in lobster (Freeman et al., 1966) and crayfish (Asada, unpublished data). The block observed in the present experiments, however, was not always reversed with time during exposure to the foreign anion, whereas the axon recovered its ability to produce spikes. It is therefore likely that the foreign anions also affected the secretory transmissional processes of the terminals, since the inhibitory synaptic membrane could still be activated by γ-aminobutyric acid (GABA), though it was unresponsive to neural stimuli (see Fig. 17).

Transmission was maintained (or it recovered) when the concentration of the foreign anion did not exceed 50%, or sometimes 75%, so that most of the experiments dealing with the larger anions, described in the Results, and employing neural stimulation were limited to replacement of only 50 or 75% of the Cl. In experiments in which transmission was blocked when all or most of the Cl was replaced, the inhibitory synapses were activated with GABA.

The change from one solution in the bath to another was made by adding the new at one end of the chamber while withdrawing the old at the other by suction. Every 15 min during an experiment 10 ml of the solution in the chamber were withdrawn and replaced with fresh solution. The temperature in the bath ranged between 17° and 20°C.

Two microelectrodes, less than 200 μ apart, were inserted into one of the superficial fibers of the muscle and were usually kept in place during the experiment. One of the electrodes, filled with 3 M KCl, was used for registering the potentials. The

other was usually filled with 2 M K citrate and was used for applying currents. However, KCl-filled electrodes were also used occasionally for passing currents. No differences that might be ascribed to the presence of Cl could be detected, presumably because the volume of the muscle fibers is large compared with the amount of Cl that might be released from the electrode by leakage from the tip, or by brief applied inward currents. The stimulating and recording equipment was standard for the laboratory.

The basic data relate to the changes in the amplitudes of the inhibitory postsynaptic potentials (IPSP's) when the membrane potential of a muscle fiber was varied by an intracellularly applied current (Figs. 1 and 3–5). The excitatory postsynaptic potentials were usually also observed (Figs. 3–5). For convenience, the IPSP's were the maximal or nearly maximal summated potentials evoked by stimulating the inhibitor axon at 75 or 100 per sec. However, the present findings were also duplicated with single IPSP's (Fig. 1). The intercept on the abscissa (membrane potential, E_M) at which the IPSP appears to be zero gives the value of the reversal potential (E_{IPSP}) which is at, or close to the emf of the ionic battery for the inhibitory electrogenesis (Grundfest, 1961, 1966). In other illustrations (Figs. 2, 3, 7–10, 12, 14, 15, and 18) the E_{IPSP} values as so determined are plotted directly. In still others, the variations of E_M with applied currents are plotted before and during activation of the inhibitory synapses. Current-voltage (I-E) characteristics (Figs. 11, 13, and 16) were obtained by activating the inhibitory synapses either by neural stimulation or by GABA. Both modes of activation caused nearly identical changes in the I-E characteristic and in the inhibitory electrogenesis. However, transmission was blocked in the experiments of Figs. 17 and 18. For uniformity, therefore, all the illustrative examples of such measurements (Figs. 11, 13, 16–18) present data in which the inhibitory synapses were activated by GABA. This mode of activation also eliminated the possibility that the data might be complicated by the effects of the foreign anions on the secretory processes of transmitter release. The two I-E characteristics thus obtained are essentially straight lines that intersect at the reversal potential. They also provide data on the conductance of the muscle fiber membrane (Grundfest et al., 1959). All examples are representative of at least three similar experiments; some are samples from six or more such experiments.

RESULTS

A. *The Ionic Battery for the IPSP*

REVERSAL POTENTIAL (E_{IPSP}) OF SINGLE AND SUMMATED IPSP'S The average resting potential in the present work was about −75 mv, whereas it was about −70 mv in earlier studies that were made with a saline containing 15 mM/liter KCl (Grundfest et al., 1959; Gainer et al., 1967). The individual as well as the fused IPSP's consequently were somewhat smaller, but they still were negative to the resting potential (Fig. 1). The reversal potentials were identical, whether they were determined from measurements on single IPSP's (open circles) or on the summated, fused, and maximal IPSP's evoked by stimulating the axon at 100/sec (filled circles).

RELATIVE INDEPENDENCE OF E_M AND E_{IPSP} A compilation of measurements on 103 different muscle preparations bathed in a standard saline (Fig. 2) shows that the hyperpolarization caused by the IPSP's was essentially independent of the resting potential. Except in a few fibers, E_{IPSP} was negative to E_M over a range of variation of E_M from −62 to −86 mv. In many

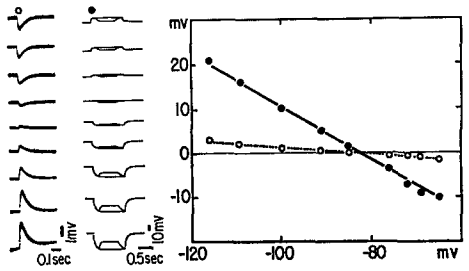

FIGURE 1. Determination of the reversal potential (E_{IPSP}) for single IPSP's (records, left column; open circles in graph), and for summated IPSP's evoked by stimulating the inhibitor axon at 100/sec. The three uppermost traces show the responses during depolarizations by outward currents. The change in E_M produced by the applied current is seen in the right column, which was recorded at a slower sweep speed. The next lower records were made without an applied current and the remainder were taken with an inward applied current. The value of E_M at which the IPSP becomes zero is the reversal potential. E_{IPSP} (ca. −82 mv) was identical for both sets of measurements. Resting potential −76 mv.

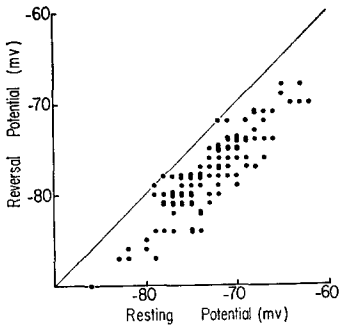

FIGURE 2. Scatter diagram for the reversal potentials and resting potentials of muscle fibers in 103 different preparations in the standard saline. The values expected if the two potentials were coincident are given by the 45° line.

fibers E_{IPSP} was hyperpolarizing by as much as 8 mv. The mean E_{IPSP} (± SE) was −78 mv ± 0.4 mv.

INDEPENDENCE OF E_{IPSP} FROM E_K The likelihood that the emf of the K battery (E_K) contributes to the inhibitory electrogenesis was ruled out by experiments like those of Fig. 3. The preparation, initially (1) in the standard saline, was exposed (2) for 10 min to a solution that was K-free, then (3) for 10 min to one in which KCl had been increased to 20 mM and, finally (4), again to a K-free solution. The exposure times were kept brief so that little

redistribution of ions could have taken place (Dunham and Gainer, 1968). The exciter axon was stimulated by trains of pulses and the EPSP's obtained are shown in the upper line of records. Stimulation of the inhibitor axon at 100/sec yielded the IPSP's shown in the middle line of records. Despite changes in sign as well as in amplitude, the latter electrogenesis was inhibitory for the EPSP's (lower line of records).

The deletion or increase of KCl changed the resting potential markedly; the respective E_M values are indicated by arrows on the abscissa of the main

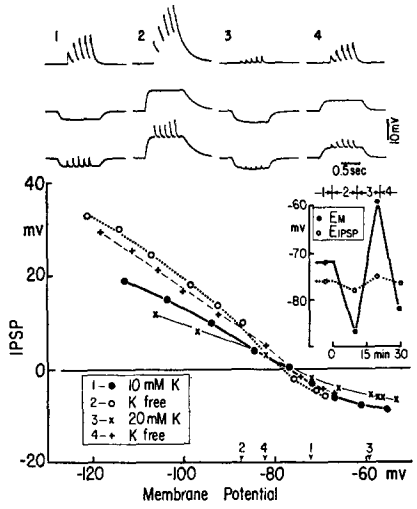

FIGURE 3. Independence of E_{IPSP} from changes in E_M induced by changing the level of K in the bathing medium. Records, trains of EPSP's, of summated IPSP's, and of both together recorded with the preparations in the different media specified in the inset box. Note the increased amplitudes of EPSP's in K-free saline (2) and the reduced amplitudes in 20 mM KCl. The resting potentials for the different conditions are shown as filled circles in the inset graph and as arrows on the base line of the main graph. The latter shows the magnitudes of the summated IPSP's when the membrane potential was changed with an applied current. E_{IPSP}, represented by the crossing of the zero value and by the open circles of the inset graph, was relatively independent of the large changes in E_M.

graph, and as the filled points on the inset graph. The main graph shows the changes in amplitudes and sign of the IPSP's during changes in membrane potential effected by intracellularly applied currents. In all four sets of measurements the IPSP's reversed sign at a membrane potential of about −76 mv. The independence of the changes of E_M and E_{IPSP} is shown directly in the inset graph of Fig. 3.

The changes in external K also caused marked changes in membrane resistance and these as well as the change in electrochemical driving force, $E_M - E_{EPSP}$, are reflected in the changes in amplitude of the EPSP's (upper

records). The resistance rose when K was removed and the EPSP's increased in amplitude as the load (conductance of the nonsynaptic membrane) on the synaptic generator was decreased. The resistance decreased greatly on elevating K to 20 mM and the EPSP's were then markedly diminished. The effect of the high K persists for some time and the resistance was still relatively low when the preparation was reexposed to the K-free saline for only 10 min.

These changes in resistance are reflected also in the different slopes of the lines to the left of the reversal potential in the main graph. The relation be-

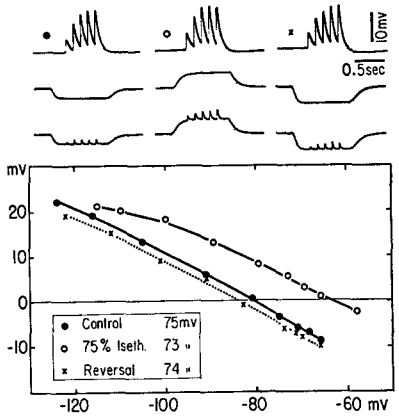

FIGURE 4. Change in E_{IPSP} with change in the level of external Cl. Records show the EPSP's, IPSP's, and both together in the control saline, on reducing Cl_o to 25% (substituting with isethionate) and upon restoration of the Cl. The amplitudes of the EPSP's were essentially unaffected, indicating that the membrane resistance was nearly unchanged on removing Cl. The resting potentials in the different media are shown in the inset box. The amplitudes of the IPSP's are plotted in the graph against the membrane potential (as changed by an applied current). The reversal potential shifted toward depolarization by nearly 20 mv when Cl was reduced. This change was reversed upon returning the preparation to the standard saline.

tween IPSP's and the membrane potential was essentially linear in this region for all the different ionic conditions. To the right of the reversal potential, however, there was a marked curvature. This distortion of the synaptic potentials was also observed in crayfish muscle (Ozeki et al., 1966) and as in the latter case, it is probably due to an increased conductance of the nonsynaptic membrane that is induced by depolarizing K activation.

DEPENDENCE OF E_{IPSP} ON THE Cl GRADIENT Whereas E_{IPSP} is quite unaffected by changes in the K gradient that markedly alter E_M (Fig. 3), E_{IPSP} is affected by changes in the Cl gradient, although E_M may remain almost unchanged (Figs. 4 and 5). In the experiment of Fig. 4 the Cl was reduced by 75% (from 537 mM to 134 mM) with substitution of Na isethionate for NaCl. E_M was altered by only a few millivolts (from −75 to −73 mv).

The membrane resistance also remained essentially unaltered, increasing by only 10%. However, the curve relating membrane potential and amplitude of IPSP's was shifted toward the right by almost 20 mv. The IPSP's recorded at the resting potential (−73 mv) became depolarizing. E_{IPSP} shifted from about −80 mv to approximately −62 mv.

A similar shift was also obtained in the experiment shown in Fig. 5. After replacing Cl with isethionate E_M shifted in the positive direction by about 3 mv (arrows 1 and 2), while E_{IPSP} shifted by about 17 mv. The fiber was then exposed to the same low Cl saline but now also made K-free. E_M shifted

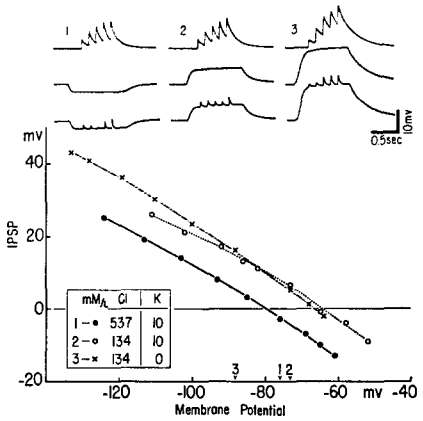

FIGURE 5. Another experiment in which Cl was reduced by 75% (substituting with isethionate), but the membrane potential was also altered by removal of K. The large increase in EPSP's (upper line of records, column 3) shows that the resistance increased considerably upon the removal of K. The relation between E_M and IPSP's was not affected, however, and E_{IPSP} remained at about −65 mv in the low Cl saline. Arrows on the abscissa indicate the resting potential for each of the experimental conditions.

by about 17 mv in the negative direction (arrow 3), but E_{IPSP} remained at the more positive value.

ROLE OF THE CATIONS Ca plays no significant role in the inhibitory electrogenesis. In a typical experiment E_{IPSP} shifted from about −87 mv to −77 mv on replacing 50% of the Cl with isethionate. A fourfold change in Ca_o did not materially affect E_{IPSP} although the fiber was depolarized when Ca was reduced. The inhibitory electrogenesis is not affected by removal of 50% of the Na from the bathing medium (Reuben, 1959, and unpublished data). E_{IPSP} and the responses to GABA of crayfish muscle fibers are likewise unaffected by removal of all Na (Ozeki and Grundfest, 1967; Takeuchi and Takeuchi, 1967).

E_{IPSP} AND Cl GRADIENT In summary, it appears justifiable to conclude that the inhibitory electrogenesis is due solely to the gradient for Cl. Furthermore, since E_{IPSP} is generally negative to E_M in lobster muscle fibers it ap-

pears that the intracellular concentration of Cl is less than that which it might be predicted to be in electrochemical equilibrium. The relation between changes in Cl_o and the change in E_{IPSP} is shown in Fig. 6. The 58 mv slope that is predicted from the Nernst relation is indicated by the broken line. The observed changes in E_{IPSP} deviated markedly from expectation as Cl_o decreased. The form of the deviation resembles that observed in the change of E_M with K_o in many cells (Hodgkin and Katz, 1949; Hodgkin, 1951).

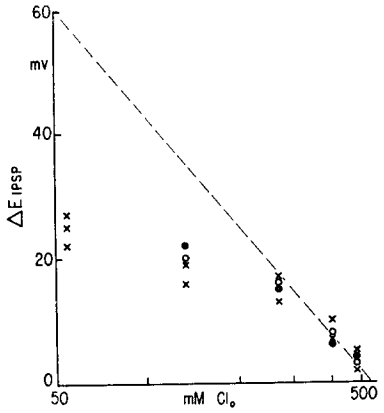

FIGURE 6. Dependence of E_{IPSP} on external Cl. Ordinate, change in the reversal potential relative to that in the control saline upon reduction of Cl_o (substituting with isethionate). Each symbol represents a different preparation. Duplicate symbols at one value of Cl_o indicate measurements on different muscle fibers in one preparation. Broken line shows the slope expected for the Nernst relation (58 mv/decade change in Cl_o).

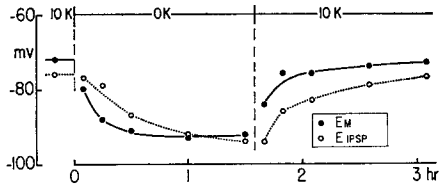

FIGURE 7. Changes in E_{IPSP} during redistribution of Cl. At time zero the preparation was subjected to a K-free medium while Cl_o remained constant (537 mM). E_M responded with hyperpolarization more rapidly than did E_{IPSP} and the IPSP's were depolarizing. After about 1.5 hr the IPSP's again became negative with respect to E_M. When K was reintroduced, E_M again changed more rapidly, but after about 1.5 hr in the control medium the original relation between E_M and E_{IPSP} was again obtained.

EFFECTS OF Cl REDISTRIBUTION Fig. 7 exhibits the changes in E_M and in E_{IPSP} during and after 1½ hr exposure of the fiber to a K-free medium. E_M changed more rapidly than did E_{IPSP} so that the IPSP's at first became depolarizing relative to the new resting potential. However, after about 1 hr in the K-free saline E_{IPSP} again became negative to E_M, indicating that the intracellular Cl was gradually reduced so as to restore the relation between E_M and E_{IPSP} that was observed in the standard medium. On restoring K to the bathing solution the change in E_{IPSP} again lagged behind the change in E_M, but after about 1½ hr the relation between E_M and E_{IPSP} that was observed initially was again attained.

Muscles that are kept in the K-free saline for a longer time become strongly hyperpolarized. After 20 hr in the K-free saline E_M ranged between -102 and -120 mv with a mean of -109 mv (Gainer et al., 1967, Table 2). Nevertheless, the IPSP's of such preparations still are more negative than E_M (Gainer et al., 1967, Fig. 2), reenforcing the conclusion that Cl is regulated in some manner so as to maintain E_{Cl} negative to E_M, presumably by a pumping mechanism.

B. *Relative Permselectivity of the Inhibitory Synaptic Membrane for Various Anions*

While the electrophysiological estimates of relative permeabilities of different ions may be subject to considerable error (cf. Freeman et al., 1966; Grundfest,

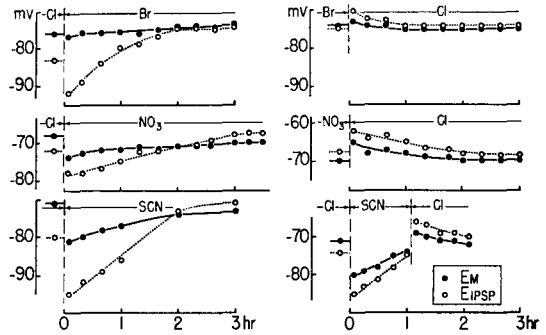

FIGURE 8. Changes in E_M and E_{IPSP} induced upon changing the anion from 100% Cl to 89% Br, NO_3, or SCN and back. Left, the change indicated was made at time zero. The membrane hyperpolarized slightly when Br was substituted for Cl and more so when the substitution was made with NO_3 or SCN. E_{IPSP} also shifted to a more negative value. These changes were transient and after E_M had returned nearly to the original level, E_{IPSP} was positive to E_M or only slightly negative. Right, upon reversal from exposure to Br or NO_3 (continuation of the experiment for the same fibers) there was a transient depolarization of E_M. E_{IPSP} also shifted toward positivity, but it remained so for at least 3 hr. Similar data but for another experiment with SCN are shown in the lowest graph.

1967; Gainer and Grundfest, 1968) they do provide a qualitative comparison.

In the experiments of this section various anions were substituted for some or most of the Cl in the control medium, and the time course of changes in E_M and E_{IPSP} was followed for an appropriate time. In most of the experiments the time course of reversal of these changes was also followed after returning the preparation to the control saline. There appeared to be three classes of effects that were associated with different groups of anions. Within each group there were some minor differences as well, but these will not be stressed at the present time.

HIGHLY PERMEANT ANIONS Substitution with Br, NO_3, or SCN for Cl caused a transient hyperpolarization of the muscle fibers (Fig. 8). It was

smallest in the Br saline and largest in SCN, but even in the latter case replacement of 89% of the Cl with the foreign anion led to an initial change in E_M of about only 10 mv. At first, E_{IPSP} became considerably more negative than in the Cl saline, but gradually shifted toward more positive values. After some 2–4 hr in the presence of the foreign anion E_{IPSP} became slightly positive to E_M. When the preparations were returned to the Cl saline the membrane underwent a transient depolarization. E_{IPSP}, which was also positive to E_M, changed very slowly and was still positive to E_M after several hours in the standard saline. The slow drift of E_{IPSP} toward positivity relative to E_M and the persistence of this positivity after reintroducing Cl indicate that factors

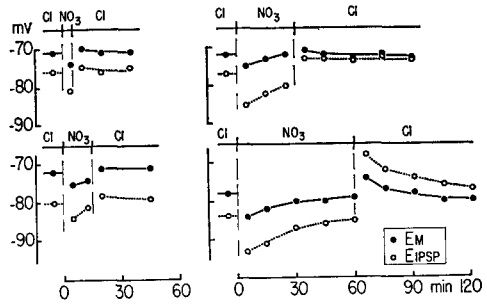

FIGURE 9. Changes in E_M and E_{IPSP} during and after exposure of a muscle to a high concentration of NO_3 for various times. Each graph represents a different experiment. Broken vertical lines indicate the interval during which Cl was reduced by 89% with substitution of NO_3. Exposure to NO_3 for 15 min or less did not materially change E_{IPSP} on subsequent replacement of the Cl, but exposure for 30 min reduced E_{IPSP} in the Cl saline markedly and after a 1 hr exposure to NO_3, E_{IPSP} in the Cl saline became strongly positive relative to E_M and remained positive for 2 hr. Compare with the longer exposure in Fig. 8.

other than diffusion affect the distribution of the anions, but these aspects have not been pursued in the present work.

However, clear-cut evidence was obtained for the intracellular accumulation of the foreign anions, and this is shown by the experiments of Figs. 9 and 10, in which NO_3 was the foreign anion. In the experiment of Fig. 9 the concentration of NO_3 in the medium was kept constant, 89% of the Cl having been replaced. The exposure to NO_3 was varied from 5 min to 1 hr. During exposures of only 5–15 min an instantaneous shift in both E_M and E_{IPSP} was observed, but there was no significant change in the relation between E_M and E_{IPSP} after the NO_3 had been replaced with Cl. With longer exposures to NO_3 the relation between E_M and E_{IPSP} in the Cl medium was altered. Following a 30 min exposure to NO_3 E_{IPSP} was only slightly negative to E_M and still remained so when measurements were ended 1 hr after return of the preparation to the Cl saline. The shifts in E_M upon reintroducing Cl following

the longer exposures to NO_3 were considerably smaller than the shifts in E_{IPSP}. The effects of a still longer exposure of the preparation to NO_3 saline are shown in Fig. 8.

In the experiments of Fig. 10 four preparations were each exposed for 1 hr,

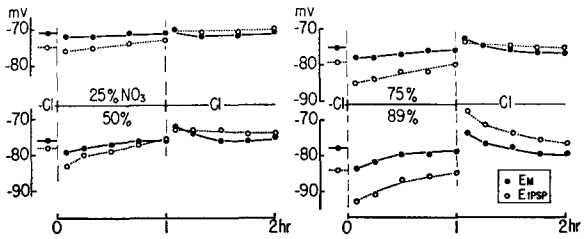

FIGURE 10. Effects of E_M and E_{IPSP} during and after exposure of muscle fibers to different concentrations of NO_3. Each graph represents a different preparation. Preparation in lower right is the same as that in the lower right of Fig. 9. Both E_M and E_{IPSP} became transiently more hyperpolarizing when the concentration of NO_3 was increased. While E_M returned to the original level 1 hr after restoring full Cl, E_{IPSP} continued to be positive to E_M.

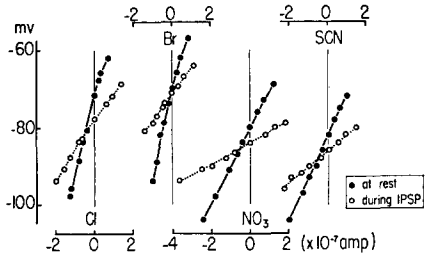

FIGURE 11. Current-voltage characteristics of muscle fibers at rest and during inhibitory postsynaptic electrogenesis in a standard saline and on substituting Br, NO_3, or SCN for 89% of Cl. The inhibitory synapses were activated by GABA. The curves in Cl and Br were obtained on one preparation, but the control curves for the preparations treated with NO_3 and SCN were omitted to simplify the figure. Measurements were made about 1 hr after the change to the foreign anion and the crossing of the two characteristic lines was negative to E_M. E_{IPSP} of the neurally evoked IPSP's is also negative to E_M at this time (Fig. 8). The slopes of both lines were not markedly different in the Cl and Br media, indicating that Br was about as permeant as Cl. The slopes were markedly altered in the NO_3 and SCN media, indicating that the membrane, both at rest and during inhibitory activity, was more permeable to these anions than to Cl.

each to a different concentration of NO_3. The transient change in E_M and the shift of E_{IPSP} after Cl had been reintroduced were larger when the preparation had been exposed to higher concentrations of NO_3. However, even during exposure of the fibers to lower concentrations of NO_3, E_{IPSP} shifted toward positivity and in all the cases shown E_{IPSP} became positive to E_M after restoration of the Cl. It is likely, therefore, that entry of NO_3 into the muscle

fibers is comparatively rapid. Additional effects, such as the possibility that NO_3 interferes with Cl movement through the inhibitory membrane or that the intracellular concentration of NO_3 remains high in the Cl saline are not ruled out, however.

The transient changes in E_M and the shifts of E_{IPSP} to greater negativity

TABLE I

EFFECTS OF SUBSTITUTING VARIOUS ANIONS FOR Cl

Substitutions as indicated in second column. Changes were measured in resting potential (ΔE_M), reversal potential (ΔE_{IPSP}), effective resistance of the resting cell (ΔR_M), and calculated change in conductance during synaptic activity (G_I in Cl = 100%). Minus signs indicate hyperpolarization or decrease of R_M relative to the control values. The ions tested are divided into four groups, highly permeant, slightly permeant, impermeant, respectively; formate is placed separately because of its "anomalous" and irreversible effects. Top row, average; middle row, range; lowest row (in parentheses), number of experiments.

Anion	Conc	ΔE_M	ΔE_{IPSP} Initial	ΔE_{IPSP} After		ΔR_M	G_I
	%	mv	mv	mv	min	%	
Permeability > Cl							
NO_3^-	89	−4	−7	−2	60	−15	154
		−2 to −6	−4 to −9	−1 to −3		−9 to −20	122 to 178
		(4)	(3)	(3)		(3)	(3)
Br^-	89	−2	−6	2	60	12	124
		−1 to −3	−4 to −7	1 to 3		5 to 20	110 to 136
		(3)	(3)	(3)		(4)	(3)
SCN^-	89	−9	−13	−4	60	−27	108
		−6 to −12	−11 to −14	−1 to −6		−20 to −37	96 to 128
		(4)	(3)	(3)		(3)	(3)
Slightly permeant							
CH_3COO^-	50	1	7	−11	90	9	20
		0 to 2	4 to 9	−9 to −13		8 to 11	17 to 25
		(3)	(3)	(3)		(3)	(3)
$C_2H_5COO^-$	50	2	8	−26	90	13	9
		2 to 3	6 to 10	−24 to −29		4 to 21	5 to 13
		(5)	(4)	(5)		(3)	(3)
Impermeant							
BrO_3^-	50	−2	9	3	90	15	43
		−1 to −3	5 to 12	0 to 5		7 to 30	34 to 52
		(4)	(4)	(3)		(3)	(3)
$CH_3SO_4^-$	50	1	9	6	90	13	50
		—	8 to 11	5 to 8		5 to 26	44 to 53
		(3)	(3)	(3)		(3)	(3)
$C_3H_4OHSO_3^-$	50	1	15	10	90	23	59
		0 to 2	13 to 17	10 to 11		10 to 33	43 to 72
		(5)	(4)	(3)		(3)	(3)
$HCOO^-$	50	1	8	29	30	−4.5	293
		0 to 2	7 to 9	16 to 22		−3 to −5	255 to 300
		(3)	(3)	(3)		(3)	(3)

on replacing Cl with Br, NO_3, or SCN indicate that the muscle fiber, both at rest and during the IPSP, is somewhat more permeable to these foreign anions than it is to Cl. This conclusion is supported by data on the I-E characteristics (Fig. 11). The slopes of the characteristics indicate that the effective resistance of the resting fiber as well as that during inhibitory activity became smaller in NO_3 and SCN than they were in Cl or Br. The changes in conductance produced by activation of the inhibitory synapses were calculated from the data of the I-E characteristics. These values are expressed relative to the conductance that the synapses contribute in the Cl saline (G_I, Table I). For the three highly permeant anions the order was NO_3 > SCN > Br > Cl.

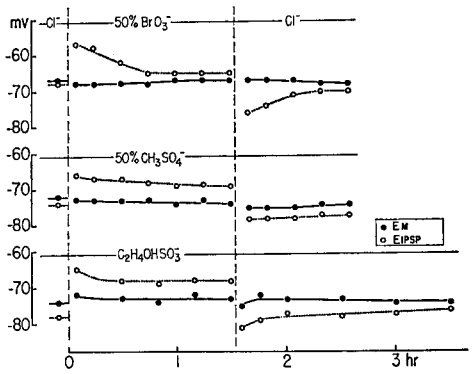

FIGURE 12. Time courses of changes in E_M and E_{IPSP} on substitution of BrO_3^-, $CH_3SO^-_4$, and isethionate for 50% of the Cl in the bathing medium. The exposure to the foreign anion was for 1.5 hr in each experiment, after which the preparation was returned to the control saline. Note that E_{IPSP} remained positive to E_M as long as the foreign anion was present, but changed toward hyperpolarization when Cl was restored.

IMPERMEANT ANIONS In marked contrast to the effects of Br, NO_3, and SCN were those observed when the foreign anion substituting for Cl was BrO_3, CH_3SO_4, or isethionate (Fig. 12). As noted in the Methods, one of these anions usually substituted for only 50% of the Cl. The transient change in E_M was very small and might be in either direction, but when isethionate was the foreign anion the change in E_M was always a slight depolarization. E_{IPSP} shifted from relative negativity to positivity and the initial change ranged between about 10 and 15 mv. The relative positivity of E_{IPSP} decreased during the first 30 min to 1 hr, but E_{IPSP} remained positive to E_M thereafter for the duration of the measurements. The longest exposure to this category of foreign anion was for 3 hr (Fig. 14, upper graph). The conductance of the postsynaptic membrane did not increase as much in the presence of these three anions as it did when Cl was present (Fig. 13, Table I).

When the foreign anion was removed and Cl was restored (Fig. 12),

E_{IPSP} became hyperpolarizing, usually showing an excess hyperpolarization initially and gradually returning toward the control level. The changes caused by removal of the foreign anion were approximately symmetrical with those observed when this anion was introduced. The data of Figs. 12 and 13 indicate that the three anions (BrO_3, CH_3SO_4, and isethionate) are impermeant during activation of the inhibitory synaptic membrane. However, they also indicate that there is only a limited redistribution of Cl following its depletion in the bathing medium.

Absence of redistribution of Cl is shown further by experiments like that shown in the upper graph of Fig. 14. After the muscle fiber had been equilibrated for 1 hr in the 50% isethionate medium containing 10 mM/liter K, the preparation was bathed in a medium from which the K had been re-

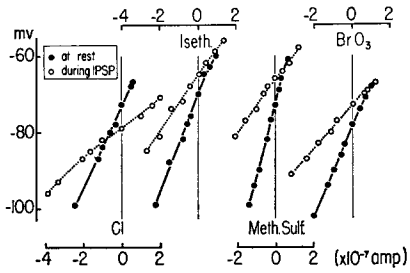

FIGURE 13. Current-voltage characteristics of the resting membrane and during the IPSP's in Cl, and after replacing 50% of the Cl with isethionate, methylsulfate, and bromate. Each graph with a foreign anion represents a different preparation. The IPSP's were evoked by applying GABA. For the membrane at rest the slope of the characteristic changed relatively little. The slopes were smaller for the active membrane in the presence of the foreign anion than in Cl. Note that the crossing of the two characteristics occurred only with outward (depolarizing) currents in the presence of the foreign anion, in contrast to the data of Fig. 11, for permeant foreign anions.

moved. The membrane hyperpolarized by more than 20 mv, attaining a steady state in about 1½ hr in the K-free medium. It is noteworthy that the time course of the hyperpolarization of the fiber was slow even though there appeared to be no change in Cl_i, such as might be expected from the data of Fig. 7. E_{IPSP} also was not affected during depolarization that was caused by increasing K from 10 to 20 mM and back. In one such experiment E_{IPSP} changed from −78 to −65 mv on replacing 50% of the Cl with isethionate. E_M changed from −74 to −61 mv upon increasing K to 20 mM, but E_{IPSP} changed only to −63 mv. When the preparation was returned to the saline containing 10 mM K, E_M returned to −73 mv while E_{IPSP} remained essentially unchanged.

POORLY PERMEANT ANIONS When propionate or acetate was substituted for Cl there was a small transient depolarization of E_M which was followed

by a small hyperpolarization (Fig. 15). On restoring the Cl an initial small hyperpolarization was followed by a fairly rapid return to the original membrane potential. E_{IPSP} also became depolarizing initially when the foreign anion was introduced. However, it reverted to hyperpolarization after 20–30

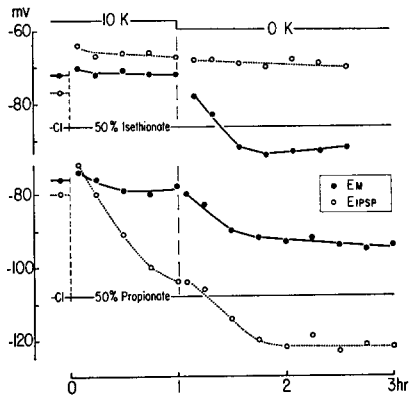

FIGURE 14. Differential effects on E_M and E_{IPSP} induced by changing K in the bathing medium when the foreign anion was impermeant (isethionate, upper graph) or slightly permeant (propionate, lower graph) for the active inhibitory membrane. E_{IPSP} was not affected in the former case when E_M became hyperpolarized by some 20 mv upon removal of K. In the propionate saline E_{IPSP} had become hyperpolarizing relative to E_M before K was reduced and the hyperpolarization was increased by some 15 mv in step with the change of E_M. Further description in text.

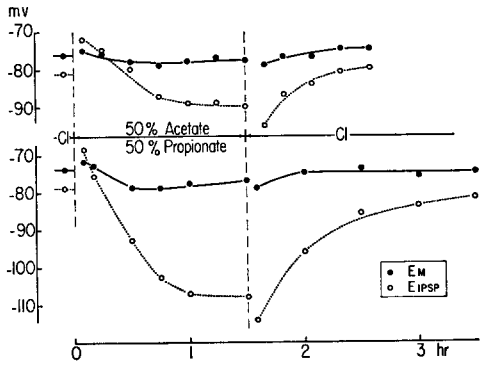

FIGURE 15. Effect on E_M and E_{IPSP} of substituting acetate (upper graph) or propionate (lower graph) for 50% of the Cl of the standard medium. The initial shift of E_{IPSP} toward positivity was rapidly reversed and after about 1 hr E_{IPSP} became steady at a value considerably more negative than that of E_M. See also the lower graph in Fig. 14. The change in E_{IPSP} was reversed slowly on restoring full Cl.

min and slowly attained a larger inside-negative value than in the control saline. In the experiments of Fig. 15 the negativity of E_{IPSP} remained at about this value until the Cl was restored, when a brief initial hyperpolarization of the reversal potential accompanied the transient hyperpolarization of the

muscle fiber. E_{IPSP} then gradually returned toward its original level thereafter. On substitution of acetate for Cl, the change of E_{IPSP} to a greater negativity was smaller than with propionate, but the general features of the change and of the effect of restoring Cl were the same. A conspicuous dif-

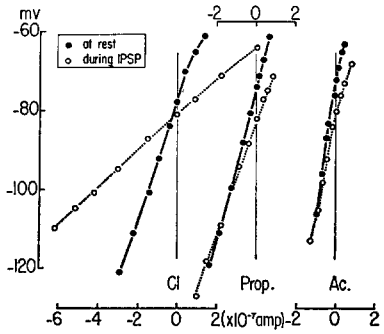

FIGURE 16. Current-voltage characteristics for resting membrane and during IPSP's (induced by applying GABA) in the presence of Cl and about 1 hr after 50% of the Cl was replaced with propionate or acetate. The control shown is that for the preparation that was subsequently tested with acetate. The slopes of the characteristics of the resting fibers were not markedly changed by the presence of the foreign anion, but during the IPSP's the characteristics became markedly steeper than in the Cl, indicating that the inhibitory membrane was much less permeable to the anions than during activity to Cl. Note also that the crossings of the characteristics occurred only when large inward currents were applied. Compare with the data of Figs. 11 and 13.

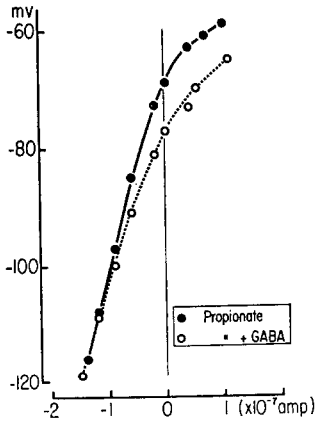

FIGURE 17. Current-voltage characteristics for a preparation that had been kept for 15 hr in a Cl-free propionate saline. Open circles and broken line show the changes induced on applying GABA (10^{-3} g/ml). The inhibitory membrane component remained capable of activation by GABA and the reversal potential (crossing of the two lines) was > -115 mv.

ference in the effects of these anions as opposed to the impermeant anions in the preceding section is the slight change in conductance that occurred when the inhibitory postsynaptic membrane was activated (Figs. 16 and 17, Table I). The conductance of the nonsynaptic membrane was not altered significantly.

The large change in E_{IPSP} and the very low increase in conductance during the IPSP's persisted when a preparation was kept in a Cl-free (100% propionate) saline for 12–24 hr. The I-E characteristics before and after the addition of GABA (Fig. 17) were essentially like those shown in Fig. 16, except for some increase in conductance to depolarizing currents in the experiment of Fig. 17. E_{IPSP} was above about -115 mv, but a precise measurement could not be obtained because the two lines have nearly identical slopes.

It was noted in connection with Fig. 7 that E_{IPSP} shifts to large negative values when the muscle is kept in a K-free saline containing the full complement of Cl. This finding has been interpreted as indicating an increase of the inward Cl gradient across the synaptic membrane as a result of efflux of KCl from the muscle fibers. When isethionate was substituted for part of the Cl in the medium, the redistribution of Cl was hindered, since E_{IPSP} did not shift

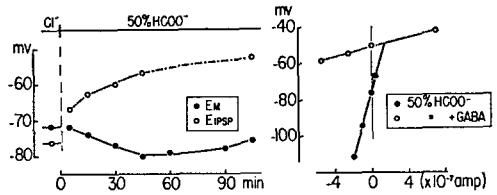

FIGURE 18. Changes induced by substitution of 50% formate for Cl. Left, the fiber hyperpolarized gradually while E_{IPSP} became relatively more positive. Transmission was blocked irreversibly after about 45 min. The last open circle represents the depolarization elicited on applying GABA. Right, current-voltage characteristics measured at this time. The conductance became very high in the presence of GABA.

when the membrane was hyperpolarized following removal of K_o (Fig. 14, upper graph). A similar experiment, but with propionate substituting for Cl is shown in the lower part of Fig. 14. E_{IPSP} had become about -105 mv when the bathing medium contained 10 mM K. It increased to about -120 mv when K was removed from the bathing solution. This finding indicates, therefore, that propionate does not block the redistribution of Cl whereas isethionate apparently does block it.

FORMATE The nature of the effects of this anion (Fig. 18) is more difficult to specify. When 50% of the Cl was replaced with formate E_{IPSP} shifted rapidly to relative positivity. However, it continued to shift to still less negative values during continuous exposure to formate, while E_M shifted to more negative values. After about 45 min in this medium the preparation no longer responded to neural stimuli, but the inhibitory synaptic membrane could be activated with the application of GABA. This caused a depolarization from a resting potential of about -80 mv to about -55 mv. During activation by GABA the effective resistance of the muscle fiber decreased markedly, from about 2×10^5 ohms to approximately 2×10^4 ohms. However, the large

change may have been due, at least in part, to the depolarization. When lobster muscle fibers are depolarized to about −50 mv, the change in conductance of the electrically excitable membrane components, which is due mainly to depolarizing K activation, becomes notable by flattening of the I-E characteristic (Ozeki et al., 1966; Gainer et al., 1967; Werman and Grundfest, 1961).

SECONDARY EFFECTS OF ACETATE E_{IPSP} remains strongly negative to E_M following prolonged exposure to propionate (Fig. 17). This is not the case when acetate is the foreign anion. E_{IPSP} declines slowly from its maximum negative value and when the preparation is exposed to the acetate saline for 10 hr E_{IPSP} becomes positive relative to E_M. It seems necessary to postulate that acetate is slowly transported into the fiber, accumulating there in sufficient quantity to reverse E_{IPSP} relative to E_M. However, we have not as yet pursued the analysis of this matter.

DISCUSSION

The emf of Inhibitory Electrogenesis The ionic battery for inhibitory postsynaptic electrogenesis in normal ionic conditions appears to be due solely to a gradient for Cl. Changes in the concentrations of the cations that are normally found in the bathing medium are without effect on E_{IPSP} (K_o, Figs. 3 and 5; Ca_o, p. 443; Na_o, Reuben, 1959) whereas changes in Cl_o alter E_{IPSP} rapidly (Figs. 4 and 5).

The change in E_{IPSP} with decrease in Cl_o does not follow the relation that is predicted from the Nernst equation for the emf of a single ionic battery (Fig. 6). The observed findings resemble rather those for the change of E_M with K_o in various cells (Hodgkin, 1951; Grundfest, 1967). The deviation of E_M from the Nernst relation has been ascribed (Hodgkin and Katz, 1949) to the contributions of other ionic batteries, as predicted from the constant field equation (Goldman, 1943). However, the present measurements describe the change solely in the emf of the inhibitory battery (ΔE_{IPSP}) and contributions of cationic batteries to this emf have been ruled out.

The Distribution of Cl The Cl gradient that causes the emf is not maintained as a consequence of a simple partial equilibrium in which $K_i/K_o = Cl_o/Cl_i$ (Donnan ratio: Boyle and Conway, 1941; Hodgkin, 1951). When Cl_o is at its full value, E_{IPSP} is approximately 5 mv negative to E_M. This is true in the absence of K_o (Fig. 7) when $E_K \gg E_M$ as well as in the presence of 10 mM K_o (Figs. 1 and 2) when $E_M \cong E_K$. Therefore the gradient for Cl must be so maintained that there is a deficit in Cl_i as compared with the distribution based on the Donnan ratio. The value of Cl_i calculated from the Nernst relation for an E_{IPSP} of −78 mv is about 24 mM. There is, however, a marked discrepancy between this type of electrophysiological estimate and

the analytical data for Cl_i. The total measured amount is almost four times as much, ca. 90 mM/kg cell water (Dunham and Gainer, 1968).

The discrepancy between the Cl_i estimated from the electrophysiological measurements and from the analytical data becomes still more marked in fibers that had been equilibrated in a K-free medium. After exposure of the muscle to such a medium for only 1.5 hr (Fig. 7) E_{IPSP} became -94 mv and after soaking for 12 or more hr in this saline E_{IPSP} was approximately -120 mv. If it is assumed that an estimate of Cl_i can be made from the electrophysiological data by applying the Nernst relationship, Cl_i must have decreased to 13 mM/liter in the experiment of Fig. 7 and to about 3 mM/liter when E_{IPSP} had changed to -120 mv.

Direct analyses, however, give very different values for Cl_i (Dunham and Gainer, 1968, Table 1). In freshly excised muscles Cl_i was about 70 mM/kg cells. After equilibration for 24 hr in salines containing 15 mM or 45 mM K, Cl_i was about 85 mM/kg cells. After equilibration for the same time in the K-free saline, Cl_i increased somewhat, to about 90 mM/kg cells. It is noteworthy, however, that the apparent loss of 20 mM Cl_i that was calculated from the present electrophysiological data is matched in the analytical data by a loss of K_i and a gain of Na_i (Dunham and Gainer, 1968, Table 1). In fresh muscle K_i was 124 mM/kg cells; after equilibration for 24 hr in the K-free saline K_i had fallen to about 106 mM/kg cells. Na_i was 83 mM initially and 106 mM after equilibration.

The electrophysiological measurements estimate Cl_i in units of millimoles per liter of free cell water. Conversion of the analytical data for Cl_i in fresh tissue from millimoles per kilogram of cells to millimoles per kilogram of cell water raised the value of Cl_i from 71 mM to 89 mM (Dunham and Gainer, 1968, Table 1). Thus, the discrepancies between the analytical data and the electrophysiological estimates would appear to be even larger than those described above.

As determined analytically, Cl_i appears to be distributed in two compartments (Dunham and Gainer, 1968). A fraction of about 30 mM remains after prolonged equilibration of the muscle in a Cl-free (propionate) medium. Since it is not exchangeable with ^{36}Cl, this fraction is regarded as immobile, or bound. The exchangeable Cl_i varies with Cl_o, but in the presence of 10 meq/liter K_o there is a nearly constant ratio with $Cl_o/Cl_i \approxeq 10$. Thus, the analytical and tracer data predict that E_{Cl} should be about -58 mv, whereas E_{IPSP}, which in the control medium reflects E_{Cl}, is about -78 mv.

If it is assumed that the analytical data for Cl_i are accurate within stated limits (Dunham and Gainer, 1968), it seems necessary to postulate that the gradient of Cl across the inhibitory synaptic membrane, which is calculated from the electrophysiological measurements, is not the same as that which prevails in the whole fiber. In fibers equilibrated in a K-free saline for a long

time the difference must be still greater. The electrophysiological measurements indicate a loss of some 20–25 meq/liter Cl_i, presumably from the subsynaptic regions, while the total Cl_i as measured by analysis remains approximately constant (Dunham and Gainer, 1968). Thus, to compensate for the Cl lost from the subsynaptic region there must presumably be a gain of Cl_i in other regions of the fiber. It is noteworthy that the fibers equilibrated in the K-free medium lose about 20 meq/liter K and gain a similar amount of Na (Dunham and Gainer, 1968) as if there were an efflux of 20 mM KCl from the subsynaptic region and an influx of 20 mM NaCl that was distributed in the rest of the muscle fiber. Investigations employing a variety of techniques (cf. Ernst, 1963, for extensive literature; Reuben et al., 1964) had indicated that K is not distributed homogeneously within muscle fibers. The present data also suggest that there must be a considerable degree of inhomogeneity in the ionic constituents of lobster muscle. Some of this inhomogeneity might be contributed by the highly structured myofibrillar components.

Changes in E_M during Exchange of Cl_o with a Foreign Anion The exchange of Cl_o with various foreign anions causes remarkably small changes in E_M in comparison with the large changes that are produced by varying K_o, indicating that the "transport number" (t_{Cl}) for Cl (Hodgkin and Horowicz, 1959) is small. Earlier measurements on whole lobster muscle preparations, with Cl replaced by propionate, had yielded a value of $t_{Cl} = 0.21$ (Gainer and Grundfest, 1968). The experimental arrangement of the present work did not permit measurement of the early changes in E_M. However, it is evident from the data of Figs. 8–10, 12, 14, and 15 that the changes in E_M that are caused by substituting various anions for Cl depend to some degree upon the substituting ions.

Initial Changes in E_{IPSP} Since activation of inhibitory synapses is due to an increase in conductance for anions, the change in E_{IPSP} should be a fairly accurate reflection of the permeability of the membrane to that anion. The initial negative shift of E_{IPSP} to greater negativity when Br, NO_3, or SCN was substituted for Cl_o (Figs. 8–10) thus indicates that these ions are more permeant than is Cl, *through the active inhibitory postsynaptic membrane.* The same criterion would indicate that propionate and acetate, as well as BrO_3, methylsulfate, and isethionate are impermeant or are less permeant than is Cl.

Time-Variant Changes in E_{IPSP} Since a change in E_{IPSP} denotes a change in the emf of the anionic battery, or batteries, it is independent of possible changes that the foreign anion might have caused in the output of inhibitory transmitter from the presynaptic nerve terminals, or from changes in the sensitivity of the postsynaptic membrane to the inhibitory transmitter.

The slow shift of E_{IPSP} from a large initial negativity, relative to E_M, toward and into relative positivity (Figs. 8–10) is probably due to the entry of Br,

NO_3, and SCN into the fibers. It follows that the mechanism by which the gradient of Cl_i is normally regulated in order to maintain E_{IPSP} negative to E_M must be incapable of maintaining a similar gradient for foreign anions more permeant than Cl. The original chloride gradient (indicated by E_{Cl}, or the original value of E_{IPSP}) was not attained in 3 hr after the permeant anion was again replaced with Cl (Fig. 8). This is in marked contrast to the rapid return of E_{IPSP} to relative negativity after removal of the impermeant anions, BrO_3, methylsulfate, or isethionate (Fig. 12), or the poorly permeant propionate and acetate (Fig. 15). Thus after the highly permeant foreign anions have entered the cell, their elimination appears to be slow.

When E_M is changed by reducing K_o, Cl_i of the subsynaptic space is redistributed to maintain E_{IPSP} negative to E_M (Fig. 7). However, E_{IPSP} remained positive to E_M for more than 1 hr during exposure of the preparation to BrO_3, methylsulfate, or isethionate (Figs. 12 and 14). E_{IPSP}, furthermore, remained essentially unaffected by a change of E_M to about -90 mv by removal of K_o in the presence of these anions (Fig. 14). It is likely, therefore, that these impermeant anions interfere with the efflux of Cl in the nonsynaptic membrane. This would also account for the very small changes in E_M that were observed on removal of Cl and on its restoration (Fig. 12). The conductance of the active synaptic membrane remains high (Fig. 13), indicating that there is considerable Cl in the subsynaptic regions to carry current across the synaptic membrane. The rapid return of E_{IPSP} to the control values on restoring full Cl (Fig. 12) suggests that the interference is a relatively simple effect, perhaps by block of the nonsynaptic Cl channels by the impermeant anions. Isotopic data would be required for a definite characterization of the mode of interference with Cl redistribution that is indicated by these findings (cf. Harris, 1958).

The initial changes in E_{IPSP} induced by replacing Cl with acetate or propionate (Figs. 14 and 15) are in the direction that is to be expected if these foreign anions are essentially impermeant. However, the conductance of the activated synaptic membrane was greatly decreased in the presence of propionate or acetate (Figs. 16 and 17). Further, E_{IPSP} was still strongly inside-negative and the conductance low after all the Cl_o had been replaced with propionate for a long time (Fig. 17).

The totality of the data of Figs. 14–17 and Table I thus leads us to conclude that the active inhibitory synaptic membrane of lobster muscle fiber is slightly permeable to acetate and propionate. The synaptic electrogenesis, measured by E_{IPSP}, is then caused by two ionic batteries, E_{Cl} and E_x (where x is the slightly permeant foreign anion). When the latter was substituted for Cl_o, E_{IPSP} initially became less negative, since Cl_i was still at, or near, its original level (Dunham and Gainer, 1968). As the mobile Cl_i diminished, E_{Cl} must have become more negative. E_{IPSP} shifted toward greater negativity (Figs.

14–17), and the Cl conductance of the active synaptic membrane decreased (Figs. 16 and 17). E_{IPSP} was altered to greater negativity by removal of K_o (Fig. 14), since E_{Cl} had now become even more inside-negative (Fig. 7). In fact, as may be expected, the change in E_{IPSP} was greater than that in E_M (cf. Figs. 7 and 14). In contrast was the result when isethionate was substituted for Cl. Removal of K_o caused an even larger hyperpolarization of E_M, but E_{IPSP} was only slightly affected. Clearly propionate must not interfere with the K-induced Cl redistribution whereas isethionate eliminates this redistribution.

The reversibility of the change in E_{IPSP} in the experiments of Fig. 15 indicates that there was no significant accumulation of propionate or acetate during the 1.5 hr exposure to the foreign anion. The time course of the changes in E_{IPSP} was nearly the same on removal of these anions as on their introduction and may be ascribed to the redistribution of Cl. When the preparation was maintained for a long time in a Cl-free propionate saline (Fig. 17), E_{IPSP} remained strongly inside-negative indicating that little, if any, propionate had entered the fibers. Thus, it seems likely that the permeability for propionate is confined to the active synaptic membrane. Osmometric data on crayfish muscle fibers (Reuben et al., 1964) and analytical measurements on both crayfish (Dunham et al., 1964) and lobster (Dunham and Gainer, 1968) also indicate that propionate is an impermeant anion for the resting cell. When the preparation is kept for a long time in an acetate saline, however, E_{IPSP} reverses to positive values relative to E_M. It is likely, therefore, that acetate enters the muscle fiber slowly, causing a change in E_x that would be reflected in the change of E_{IPSP}.

We cannot, at present, account for the effect of $HCOO^-$ (Fig. 18). The resting conductance appears to be slightly decreased by this anion. However, the very large slow shift of E_{IPSP} to relative positivity apparently represents an accumulation of formate within the cell. The large increase in conductance during activation of the inhibitory synapses by GABA may be partly due to depolarizing K activation. However, because formate tends to produce irreversible block of neural activation the data obtained with this foreign anion are limited.

Comparisons with Data on Other Inhibitory Synaptic Membranes The intracellular anion composition was altered in various neurons by iontophoretic injections to test whether or not these ions were permeant through the active inhibitory membrane (Araki et al., 1961; Ito et al., 1962; Asada, 1963; Kerkut and Thomas, 1964; Kelly et al., 1968). Reversal of the IPSP's was taken to indicate that the anions were permeant. In general, all hydrated anions with radii < 1.25 times that of the hydrated K ion could move through the synaptic membrane during inhibitory electrogenesis. As was also found in the present work, BrO_3^- was impermeant for cat motoneurons while HCO_2^-,

which is of approximately the same size, was permeant (Ito et al., 1962). BrO_3^- is also permeant in the snail neurons (Kerkut and Thomas, 1964). Acetate and propionate, which are permeant, but only poorly so in lobster (Figs. 15–17), were found to be impermeant for the neurons. Kelly et al. (1968) found, however, that in the cat anions as large as glutamate "contribute to the membrane current of cortical neurons during inhibition."

Takeuchi and Takeuchi (1967), as we did also in the present work, substituted the foreign anion for part or all of the Cl in the medium bathing crayfish muscle fibers. However, by applying GABA they studied in the main only the effects on the conductance of the fibers. On the basis of this criterion they rated the degree of permeability in the order: $Br^- > Cl^- > SCN > NO_3^- > HCOO^- > BrO_3^-$. Methylsulfate, glycerophosphate, and propionate were used as large anions, and were considered to be impermeant. In the lobster, NO_3^- and SCN^- appear to be more permeant than Br^- and all more so than Cl^- on the basis of their effects on E_{IPSP} as well as on conductance during synaptic activity (Figs. 8–11, Table I). In the presence of BrO_3^- the conductance increase caused by GABA in crayfish muscle fibers was some 20–40% of that in the Cl saline (Takeuchi and Takeuchi, 1967, Fig. 6), and it was therefore concluded that the inhibitory synaptic membrane of crayfish muscle is somewhat permeable to this anion. We also observed an increased conductance (by some 50% of the control, Fig. 13, Table I) in lobster muscle fibers in the presence of 50% BrO_3^-. However, the measurements of E_M and E_{IPSP} that were done on the same preparations (Fig. 12) dictated the conclusion that the synaptic membrane does not become permeable to BrO_3^- any more than to $CH_3SO_4^-$ or isethionate.

In the presence of these anions lobster muscle fibers apparently become incapable of losing intracellular Cl to the extent that would be required to restore E_{IPSP} toward relative negativity with respect to E_M . However, when the synaptic membrane is activated, Cl^- can move down its electrochemical gradient and can provide the observed increase in conductance. The direction of ion movement, an efflux of Cl^-, is clearly indicated by the relative positivity of E_{IPSP} . The absence of an accumulation of BrO_3^- is shown by the fact that E_{IPSP} reverses immediately to relative negativity (Fig. 12), whereas exposure to permeant anions causes a persistent change in E_{IPSP} relative to E_M (Figs. 9–11).

The data of Figs. 15–17 demonstrate the usefulness of combining measurements on conductance with those on the inhibitory electrogenesis. When propionate or acetate replaced Cl, the conductance increase during activation of the inhibitory synapses was almost negligible. Nevertheless, the electrogenesis showed that E_{IPSP} had shifted toward a large relative hyperpolarization. This shift persisted for a long time even after all Cl had been replaced with propionate (Fig. 17), thus providing definitive evidence that the in-

hibitory electrogenesis was due to the emf of the propionate battery. On the other hand, the reversal of E_{IPSP} to relative depolarization after long exposure of the muscle fibers to acetate indicates that this anion, unlike propionate, entered the cell in appreciable amounts, presumably mainly or entirely, through the nonsynaptic membrane.

Crayfish muscle, however, does appear to be impermeable to propionate not only at rest (Reuben et al., 1964) but also during activation of the inhibitory synapses (unpublished data by Girardier, Reuben, and Grundfest; cf. Grundfest, 1962, Fig. 22; Takeuchi and Takeuchi, 1966, Fig. 2). When the muscle fibers are equilibrated in a Cl-free, propionate saline for many hours they do not respond to GABA with a measurable increase in conductance, nor is there a significant change in the membrane potential, such as occurs in lobster muscle fibers.

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