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ANION PERMEABILITY OF THE SYNAPTIC AND NON-SYNAPTIC MOTONEURONE MEMBRANE

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It was discovered by Coombs, Eccles & Fatt (1955*a*) that the hyperpolarization of the inhibitory post-synaptic potential (IPSP) changed to a depolarization when Cl^- ions were injected either by diffusion or electrophoretically into cat spinal motoneurones. This effect was satisfactorily explained by assuming that, when activated, the inhibitory post-synaptic membrane is permeable to Cl^- ions. A similar effect was obtained for Br^-, NO_3^- and SCN^- ions, but not for HCO_3^-, $CH_3CO_2^-$, SO_4^{2-}, HPO_4^{2-} and glutamate ions. Since the ions in the former group are smaller in their hydrated form than those in the latter, it was postulated that in the activated state the inhibitory post-synaptic membrane has a sieve-like structure with a pore size large enough to allow the passage of SCN^- ions, yet small enough to block bicarbonate and acetate ions. A differential permeability of the inhibitory post-junctional membrane was also reported on crustacean muscle (Boistel & Fatt, 1958) and crustacean stretch receptor cell (Hagiwara, Kusano & Saito, 1960) for Cl^- ions on the one hand, there being impermeability to sulphate and large organic ions on the other. The first aim of the present investigation was to employ a more extensive anion series in order to test the postulate that the differential permeability is explicable by the pore size. Eleven anion species have now been established as penetrating and another sixteen as non-penetrating through the activated inhibitory post-synaptic membrane of cat spinal motoneurones. A preliminary report of some of these findings has been given (Araki, Ito & Oscarsson, 1961).

Coombs *et al.* (1955*a*) observed that the size of the IPSP, changed by injection of Cl^-, Br^-, NO_3^- and SCN^- ions, recovered exponentially with a time constant of about 30 sec. In the course of the present experiments it was found that the time course of the IPSP recovery varied according to the injected anion species. Since the recovery is caused by the removal

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of injected anions from the cell, its time constant should be related to the permeability of the cell membrane in general. Further investigation of these time constants has been the second aim of the present experiments.

METHODS

The experiments were performed on 25 cats under pentobarbitone sodium anaesthesia. The cord was transected at the L2 level. The nerves from posterior biceps-semitendinosus (PBST), gastrocnemius-soleus, plantaris, flexor digitorum plus flexor hallucis longus, and peroneal muscles were dissected and mounted on stimulating electrodes in a paraffin pool. The quadriceps nerve was stimulated by a buried electrode. The technique of intracellular recording was the same as that described by Eccles, Fatt, Landgren & Winsbury (1954). PBST motoneurones were particularly searched for, because they receive a large IPSP from Ia afferents in the quadriceps nerve (Brock, Coombs & Eccles, 1952). The motoneurones were identified by antidromic stimulation of the cut L7 and S1 ventral roots and by the synaptic potentials evoked by Group Ia volleys in various nerves (Eccles, Eccles & Lundberg, 1957*a*). A pure IPSP could be obtained by using a stimulus strength subthreshold for Ib fibres, which was particularly easy with the quadriceps nerve on account of the frequency of a good threshold separation of the Ia and Ib fibres (Bradley & Eccles, 1953). The afferent volleys were monitored with a surface electrode placed at the upper L7 dorsal root entry. The IPSP produced by the Renshaw cell pathway was occasionally used (cf. Coombs *et al.* 1955*a*) and was obtained by the ventral root stimulation just subthreshold for the axon belonging to the impaled motoneurone, or by a maximal ventral root stimulus when the antidromic invasion was blocked (Eccles, Fatt & Koketsu, 1954). In some cases polysynaptic IPSPs evoked in extensor motoneurones by Group Ib or II volleys, or by a volley from the peroneal nerve, containing cutaneous afferents (cf. Eccles, Eccles & Lundberg, 1957*a*, *b*; Eccles & Lundberg, 1959) were employed for qualitative observations of anion effects (Table 2).

Capillary glass electrodes were boiled in distilled water under reduced air pressure at about 60 °C. The water in the shaft of the electrode was then sucked out through a thin vinyl tube and substituted by the solutions listed in Table 1, which contain various anions as either potassium or sodium salts. Potassium salts were preferred, unless their solubility was very small as was the case with ClO_4^-, ClO_3^- and BrO_3^-, but not necessarily so because the effect of sodium ions upon the IPSP by diffusion from the electrode tip was always negligible. More than 24 hr after the filling with solutions micro-electrodes with resistance of 8–20 MΩ were selected; but with solutions containing relatively low concentrations of $KMnO_4$ or $Na_2C_2O_4$ resistances up to 50 MΩ were tolerated. Solutions of NaF, or $NaMnO_4$, or NaCN usually caused a blunting of the electrode tip, but among many electrodes prepared some remained with a tip diameter less than 1 μ and could be used successfully for impalement of motoneurones.

The micro-electrode was connected to a current generator through a 100 MΩ resistor, and anions were injected from the tip into the cell by passing currents of $3-10\times10^{-8}$A for 30–120 sec (cf. Coombs *et al.* 1955 *a*). The potentials were recorded through the same electrode that served for the injection. After application of the current there was usually a transient change of the tip potential of the electrode, amounting to 5–20 mV or more, either positive or negative, which diminished in most cases within 20–30 sec. This artifact potential was obtained in isolation by recording with the tip in the extracellular medium and by applying the same current that was used for injection. The intracellular resting potential was then derived by subtracting the artifact potential from the potential recorded inside the cell. This method of artifact compensation is not entirely satisfactory, as the shift of the electrode tip from the intracellular to the extracellular medium may cause a change in the artifact

potential, either for mechanical reasons or because of the different ionic composition of the two phases of the media. However, it was useful in showing how far the recorded potential was reliable in indicating the resting potential of the cell. Electrodes with large artifact potentials were rejected.

TABLE 1. Solutions used for filling micro-electrodes

Substance	Concentration (M)	Substance	Concentration (M)
KCl	3	NaN_3	5
KBr	4	$NaClO_3$	5
KI	3	$NaClO_4$	1, 3 and 5
KNO_3	3	$NaBF_4$	3
KNO_2	5	$NaHCO_2$	5
KSCN	5	$NaBrO_3$	2
$KHCO_2$	2	$NaHSO_3$	3·4
$KCH_3CH_2CO_2$	3	$Na_2C_2O_4$	0·3
$K_3Fe(CN)_6$	0·8	NaF	0·8
$K_4Fe(CN)_6$	0·8	$NaCH_3(CH_2)_2CO_2$	5
K-Citrate	2	Na-L-Glutamate	5*
$KMnO_4$	0·4	NaCN	3
$NaMnO_4$	3		

* pH was adjusted to 8.

Any potential appearing between the silver wire dipped in the solution of the micro-electrode and the indifferent electrode in contact with the back muscles of the cat was cancelled by an electromotive force inserted between the indifferent electrode and the earth, so as to prevent currents driving ions from the electrode. The current passed through the micro-electrode for injections was monitored by a differential d.c. recording of the potential drop which was set up across 100 MΩ resistor by the current. The effective resistance of the 100 MΩ resistor, as the input impedance, was raised to about 5000 MΩ by a series feedback device (Ito, 1960). The resting and action potentials were recorded through a cathode follower by a d.c. amplifier. The resting potential was registered by an ink-writing recorder while the action potential and the synaptic potentials, the latter being amplified by a condenser-coupled amplifier (time constant usually 20 msec, but 500 msec when recording Renshaw IPSP), were displayed by a cathode-ray oscilloscope. The changes of the synaptic potentials and of the action potential following the injection of anions were recorded by photographing sweeps repeated at a frequency of 5/sec. The photographic film was automatically advanced every 4 sec, giving records with about 20 superposed traces.

RESULTS

Penetrating anions

The injection of NO_2^-, I^-, BF_4^-, ClO_3^- and HCO_2^- ions by a hyperpolarizing current of $3-10\times10^{-8}$A for 30–120 sec readily induced a reversal of the IPSP. For example, Fig. 1 illustrates the reversal and recovery of the Ia IPSP(A) and of the Renshaw IPSP(B) by the injection of NO_2^- ions. As pointed out by Coombs *et al.* (1955*a*), the reversed IPSP is not a mirror image of the original hyperpolarization; it attains its peak earlier and decays more quickly than the latter (Fig. 1). When the reversed IPSP turns back to a hyperpolarization in the course of recovery, there is usually a diphasic stage; a small depolarization being followed by a hyperpolarization (Fig. 1A, at 88–92 sec), as already reported and explained by

Coombs *et al.* (1955*a*). The earlier summit of the reversed IPSP is particularly noticeable for the Renshaw IPSP (Fig. 1B, at 52–56 sec). The change in the size of the IPSP by injection of NO_2^- ions in another PBST motoneurone is plotted in Fig. 3A. Because of the above-mentioned change in

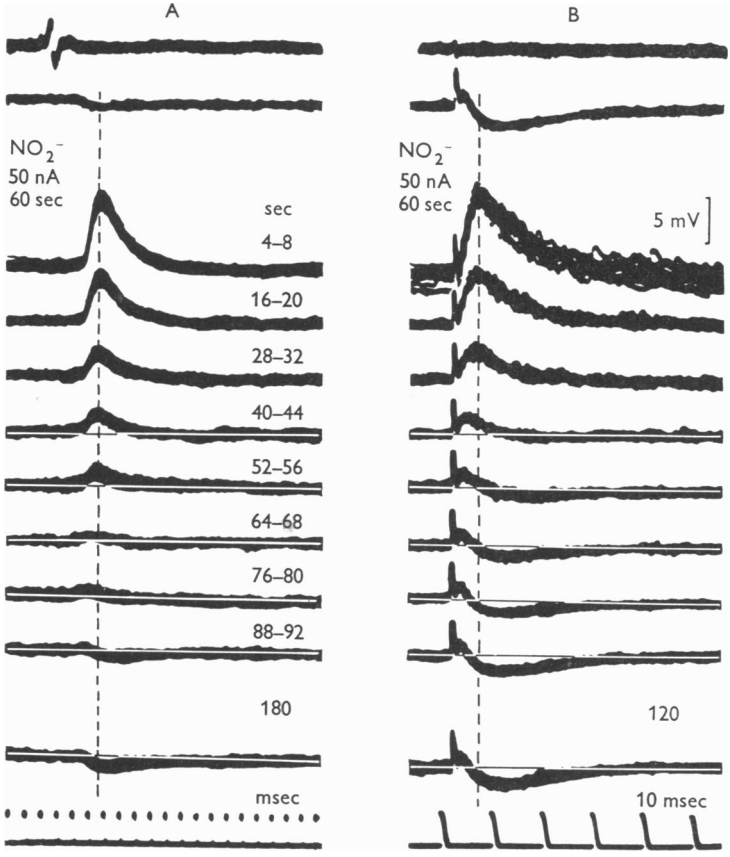

Fig. 1. Effects of electrophoretic injection of NO_2^- ions into motoneurones. A, Ia IPSP in a PBST motoneurone evoked by quadriceps Ia volley. B, Renshaw IPSP in a motoneurone, the innervation of which was not identified, induced by a maximal L7 ventral root stimulation. Records in the top row show incoming volleys recorded from the surface of the cord (upper trace) and the control IPSPs thereby evoked before the injection (lower traces). Records from the second to the ninth row illustrate IPSPs at the indicated time (identical in A and B) after the injection of NO_2^- ions by the passage of a current of 5×10^{-8}A for 60 sec. The bottom records are IPSPs at the end of recovery, its approximate time being shown in records. In this and in successive figures IPSPs are illustrated with positivity upwards. Reference base lines are drawn in traces at the fifth to tenth row, and vertical interrupted lines are through peaks of reversed IPSPs, indicating the points in the time course of IPSPs where their sizes are measured (see text). Note different time scales for A and B. All records were formed by the superposition of about twenty faint traces.

the time course, the size of the IPSP was not measured by its peak value, but by the amplitude at a fixed time after its beginning, corresponding to the peak of the largest depolarizing IPSP, as shown by the interrupted line in Fig. 1. Hence the plotted size of the hyperpolarizing IPSPs in Figs. 3–6 is smaller than its peak value, the discrepancy being slight for Ia IPSP (Fig. 1A), but considerable for Renshaw IPSP (Fig. 1B) (see p. 424). In the case of Fig. 3A the IPSP turned to a depolarizing potential spon-

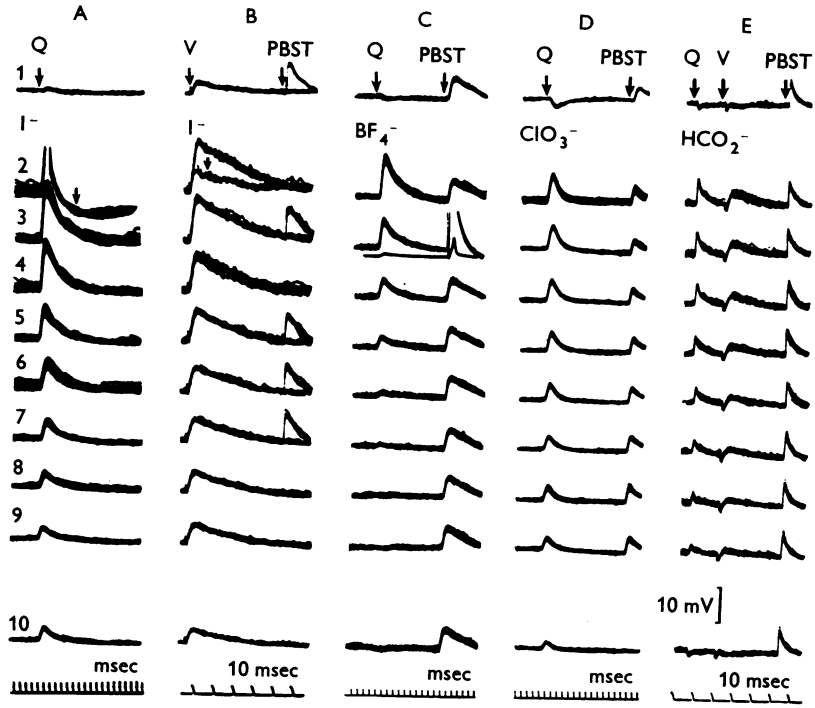

Fig. 2. Changes in IPSPs produced by electrophoretic injections of I^- (A and B), BF_4^- (C), ClO_3^- (D) and HCO_2^- (E) ions. Ia IPSPs (A, C, D and E) and Renshaw IPSPs (B and E) were obtained by quadriceps volleys (Q) and by L7 ventral root stimuli (V), respectively, in PBST motoneurones. Records in the first row are controls before the injections. A current of 5×10^{-8}A for 60 sec produced the injection. The time at which successive records from the second to the ninth row were taken was 0–4, 20–24, 40–44, 80–84, 100–104, 120–124, 140–144 and 160–164 sec in A, and the same as in Fig. 1 for others. Bottom traces are IPSPs at 180 sec in A, B and C, at 160 sec in D and at 360 sec in E after injections. In records at the second row of A and B the reversed IPSPs elicited spike potentials and traces of their after-hyperpolarization are marked by arrows. Monosynaptic EPSPs produced by volleys in the posterior biceps-semitendinosus nerve (PBST) are displayed at the end of sweeps by continuous (C, D and E) or intermittent (B) stimulation. In the third row of C a spike potential was obtained by an antidromic stimulation in place of the EPSPs, being shown in an extra trace with a gain of about one tenth of that for other records. Note two kinds of time scales for A, C, D and B, E.

taneously after impalement of the cell, apparently due to the NO_2^- ions that diffused out of the electrode (cf. Coombs *et al.* 1955*a*). The final value of the IPSP after the recovery is usually slightly different from the one before the injection; it remained often as a depolarizing potential (see Figs. 3A; 2A, B, D).

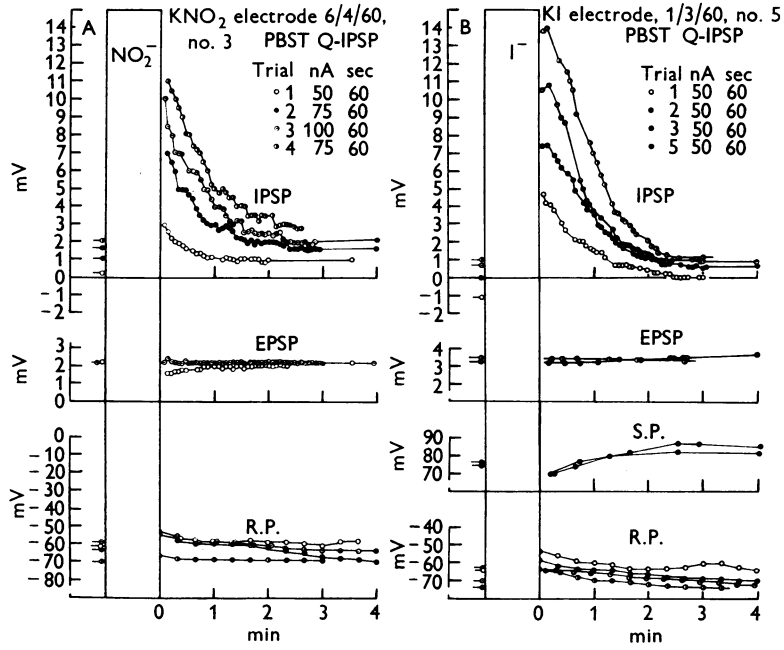

Fig. 3. Plot of sizes of IPSPs, EPSPs, antidromically evoked spike potentials (S.P.) and resting potentials (R.P.) before and after injections of NO_2^-(A) and I^-(B) ions. Plots of four trials into one and the same cell are superposed, the order of which are shown in the figure with the strength and duration of currents employed for injections. In A of this figure as well as in B of Fig. 4 spike potentials were not obtained, owing to the spontaneous block of antidromic invasion.

Changes in Ia IPSP and Renshaw IPSP by injection of I^- ions are shown in Fig. 2. Just after the injection (2nd row in Fig. 2A, B), the depolarizing IPSPs reached the critical level for generation of spikes, which was often observed after injections of the effective ions (cf. Coombs *et al.* 1955*a*). The time course of the IPSP recovery after four injections of I^- ions is shown in Fig. 3B. In Fig. 3 as well as in Figs. 4 and 5 the reproducibility of the effect of injections of any particular anion may be appreciated by the superposed plots of four successive trials into one and the same cell. Effects of BF_4^-, ClO_3^- and HCO_2^- ions upon the IPSP are qualitatively the same as NO_2^- and I^- ions, as seen in Fig. 2C, D, E, but the time course of the IPSP recovery is particularly slow for ClO_3^- and HCO_2^- ions (see Figs. 4B and 5A and Table 4).

The effect of injections of the above anions was easily reproducible and similar to the effect of the previously tested Cl^-, Br^-, NO_3^- and SCN^- ions. The latter ions have been re-tested in the present experiments, and the number of trials of injections is listed in Table 2. As observed by Coombs *et al.* (1955*a*) and also as revealed by some of the anions in the present experiments (Table 2), all types of inhibition, whether Ia IPSPs, Renshaw IPSPs or polysynaptic IPSPs, were affected in an essentially similar

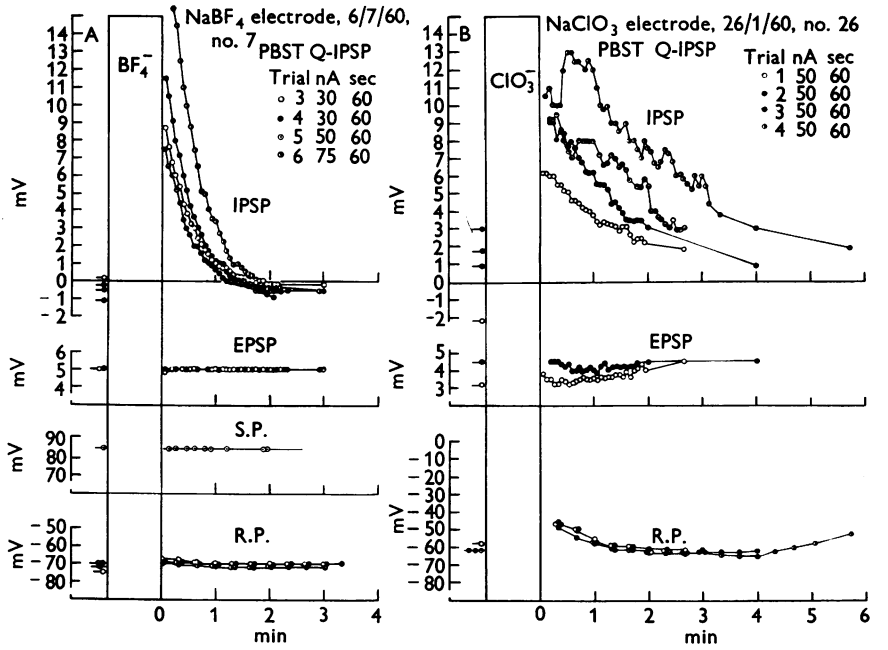

Fig. 4. Plot of intracellular potentials before and after injections of BF_4^- (A) and ClO_3^- (B) ions. Conventions and symbols as for Fig. 3.

manner. The change of the IPSP size caused by the injection of these anions is given in Table 3 by the algebraic difference between the IPSPs before and just after the injection. The figures in the five PBST motoneurones of this table are the largest among cells examined with each anion (Table 2). It appears in Table 3 that the change of the IPSP is particularly large with NO_3^- and SCN^- ions, while it is relatively small with ClO_3^- and HCO_2^-. However, since the IPSP size, or strictly speaking the IPSP conductance (cf. Coombs *et al.* 1955*a*), depends on the density of inhibitory innervation, and hence varies considerably in the population of PBST cells, it is not possible to compare the effect of injections simply by the IPSP size. The values in Table 3 and comparison of Figs. 3, 4 and 5 suggest that no quantitative difference has been shown to exist between the effects of these various anions in reversing the IPSP.

N_3^- ions are also effective in converting the IPSP to a depolarizing potential, but, since the injection of this ion species causes a reduction of the membrane potential, the pattern of changes of the IPSP is somewhat different from those observed with the anions of Table 3. Even injections of the anions of Table 3 often caused damage to the cell, presumably owing to the mechanical shift of the electrode tip, relative to the cell, that was caused by the swelling of the cell during the injection (see p. 424).

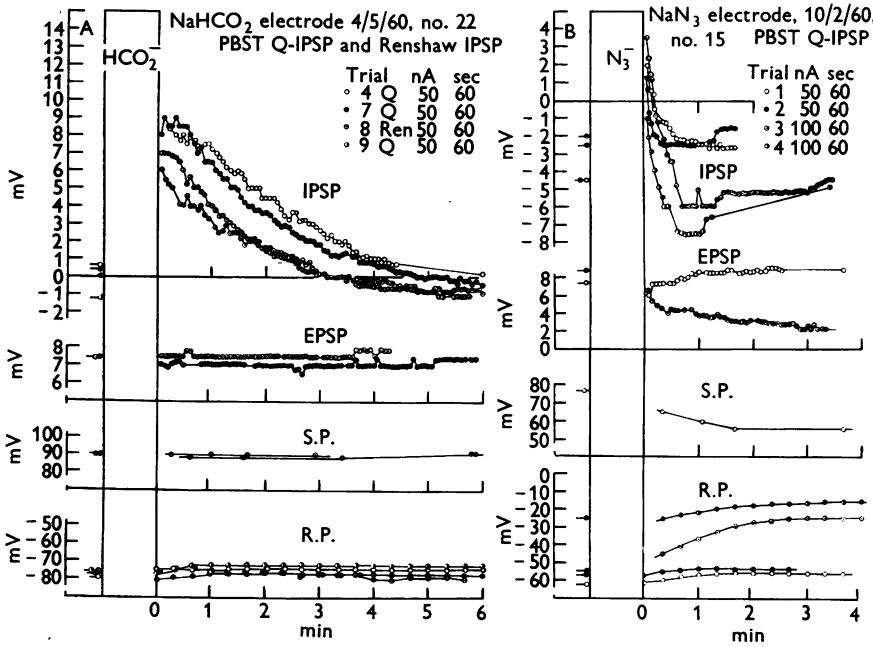

Fig. 5. Plot as in Fig. 3 of intracellular potentials for injections of HCO_2^- (A) and N_3^- (B) ions. Note that in trial 8 of A the size of Renshaw IPSP is plotted in place of Ia IPSPs in other trials.

When the cell was in this way destroyed or damaged irreversibly, these results were not included in Table 2. With N_3^-, however, the damage of the cell seemed to be induced by the ion, owing possibly to its well known effect as a metabolic inhibitor (cf. Ling & Gerard, 1949; Gerard & Doty, 1950). For example, in the experiments of Fig. 5B, the first two injections by 5×10^{-8} A affected the membrane potential little, while the following two trials with larger amounts of injected ions caused a remarkable reduction of the membrane potential. Since a depolarizing IPSP is depressed or even reversed by reduction of the resting potential (Coombs *et al.* 1955*a*), the induced change in the IPSP might have been much larger in the later two trials than in the first two, if there had been no reduction of the membrane potential. This reduction of the membrane potential was usually pro-

gressive, and correspondingly the reversed IPSP was converted rapidly to a hyperpolarizing potential and was later reduced again, as is seen in Fig. 5B. There were 51 injections into 20 cells, in all of which a depolarization of the membrane was observed to some degree. However, when a large reduction of the membrane potential was avoided by a relatively small dose of injected ions, the small change so induced in the IPSP recovered in an approximately exponential way. Hence it is concluded

TABLE 2. The number of cells and trials of injections

Ion	No. of cells: Ia IPSP	Renshaw IPSP	Polysyn. IPSP	No. of injections
I^-	8	0	0	25
NO_2^-	5	3	0	21
BF_4^-	7	0	1	27
ClO_3^-	7	2	0	19
HCO_2^-	13	1	2	38
Cl^-	5	0	0	13
Br^-	6	1	0	16
SCN^-	5	1	0	18
NO_3^-	8	1	0	21
N_3^-	11	7	2	51
ClO_4^-	6	0	0	8
BrO_3^-	8	1	0	17
HSO_3^-	6	0	2	13
$C_2O_4^{2-}$	1	0	2	7
$CH_3CH_2CO_2^-$	3	0	2	8
n-Butyrate	0	0	6	10
$Fe(CN)_6^{3-}$	5	0	2	13
$Fe(CN)_6^{4-}$	1	0	3	4
F^-	4	0	9	15
HCO_3^-	5	1	0	23
L-Glutamate	0	2	4	14
Citrate	3	0	0	4

TABLE 3. Changes of the IPSP by anion injection (mV)

Ion	Motoneurones: *a*	*b*	*c*	*d*	*e*
Cl^-	9·0 (1)	7·9 (3)	7·4 (3)	6·8 (2)	4·8 (1)
Br^-	11·6 (3)	11·1 (4)	7·5 (1)	7·0 (2)	6·5 (2)
I^-	11·2 (3)	11·0 (3)	8·2 (4)	7·8 (4)	7·2 (1)
NO_3^-	14·4 (3)*	12·8 (4)	10·0 (1)	4·4 (1)	4·0 (1)
NO_2^-	10·1 (2)	8·8 (1)	7·4 (2)	7·2 (2)	7·1 (4)
SCN^-	14·8 (5)	12·8 (3)	11·8 (3)	10·1 (2)	8·4 (2)
BF_4^-	11·9 (6)	8·4 (5)	7·9 (3)	7·3 (3)	5·7 (6)
ClO_3^-	7·3 (4)	6·9 (1)	6·4 (2)	6·1 (1)	6·0 (1)
HCO_2^-	7·3 (1)	7·2 (2)	7·0 (8)	3·6 (1)	2·9 (2)

Figures are mean values of the algebraic differences of the IPSP sizes before and just after the injection, the number of trials being indicated in parentheses. For each anion the results on five motoneurones are shown in *a*, *b*, *c*, *d* and *e*. A different set of 5 motoneurones is of course employed for displaying the effects of each anion. Currents for the injection are 5×10^{-8} A during 60 sec in all but one case (*a* for NO_3^- ions, *). In this case 3×10^{-8} A was used in all of three injections. In another trial by 5×10^{-8} A in the same cell gave an IPSP as large as 20 mV.

that the effect of N_3^- ions is the same as that of other anion species of Table 3.

ClO_4^- ions are also effective, though the injection is technically difficult. During injection of this ion species through an electrode filled with $NaClO_4$ at a concentration from 5 to 1 M, the resistance of the electrode usually rose up to several thousand MΩ, with the consequence that it was impossible to record the IPSP and the resting potential. Moreover, when this block of the electrode happened early during the current application, the injection of anions was obstructed. Though this type of blockage sometimes occurred with electrodes filled with other anions, it was much commoner with the $NaClO_4$ electrodes, particularly when a hyperpolarizing current was passed in the intracellular position. Probably K^+ ions enter the electrode and precipitate in the tip as $KClO_4$, which has a low solubility (26 m-mole/100 g saturated solution at 40 °C; see Seidell, 1940). Indeed, a similar block was produced by a hyperpolarizing current when the electrode tip was in a 150 mM-KCl solution. Despite these difficulties it was possible to observe the reversal of the IPSP after injection into six cells listed in Table 2. The recovery of the IPSP followed an exponential curve reasonably well, though in only one case could the resting potential be properly controlled subsequent to the injection.

The effect upon the IPSP of the above anions, i.e., the increase of the IPSP in the depolarizing direction and its exponential recovery, leads immediately to the conclusion that they penetrate the activated inhibitory post-synaptic membrane, and therefore are labelled by plus signs in Table 5.

Non-penetrating anions

The effect of injection of BrO_3^-, HSO_3^-, $C_2O_4^{2-}$, $CH_3CH_2CO_2^-$, $CH_3(CH_2)_2CO_2^-$, $Fe(CN)_6^{3-}$ and $Fe(CN)_6^{4-}$ ions was similar to that described for SO_4^{2-} ions (Coombs *et al.* 1955*a*). The number of trials for each anion is shown in Table 2. After their injection the IPSP remained nearly the same or was slightly increased in size. The increased IPSP recovered gradually in about 1 min, as is illustrated for BrO_3^- ions in Fig. 6A. The increase of the hyperpolarization of the IPSP may be accounted for partly by the slight reduction of the resting potential which often followed the injection, and partly by the depletion of Cl^- ions from the cell by the hyperpolarizing current.

The ineffectiveness of the injection of the above anions immediately leads to the conclusion that the activated inhibitory post-synaptic membrane is impermeable to them (cf. Coombs *et al.* 1955*a*), except for HSO_3^- ions. Since the second hydrogen of H_2SO_3 has a relatively large acid dissociation constant, a conversion of HSO_3^- to SO_3^{2-} would occur in the cell, its degree being determined by the pH of the internal medium of the cell

that would be kept constant by a buffer action of the medium. According to the recent estimates of this dissociation constant, $10^{-6.99}$ to $10^{-7.205}$ (cf. Bjerrum, Schwarzenbach & Sillén, 1958), the ratio of $[SO_3^{2-}]:[HSO_3^-]$ in the cell would be expected to be close to unity at pH 7. Although there is no direct information on the pH of the internal medium of cat motoneurones, recent investigations on invertebrate nerve and muscle fibres (Caldwell, 1958) and on frog muscle fibres (Kostyuk & Sorokia, 1960) indicate that the pH in the cell is close to 7 under normal conditions. With the possible extreme of variation in pH, $\pm 0{\cdot}4$ (Caldwell, 1958; cf. also

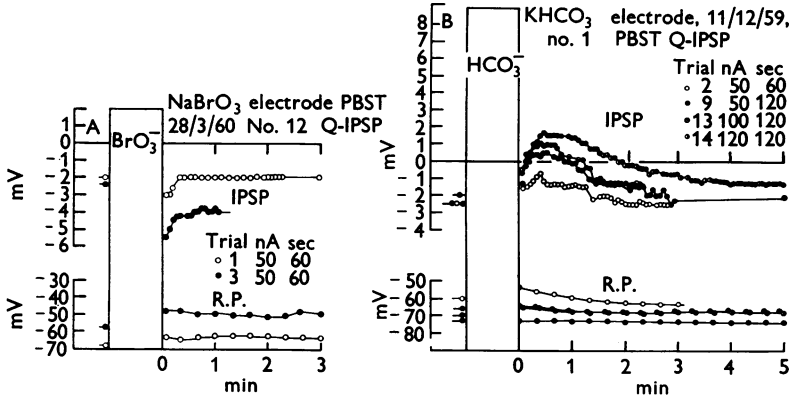

Fig. 6. Plot of IPSPs and resting potentials for injections of BrO_3^- (A) and HCO_3^- (B). Conventions as in Fig. 3.

Spyropoulous & Tasaki, 1960), the ratio of $[SO_3^{2-}]:[HSO_3^-]$ would decrease or increase by a factor of 2·5. Consequently, after relatively large injections by the passage of current of $5-10\times10^{-8}$A for 60–120 sec, the concentration of both HSO_3^- and SO_3^{2-} in the cell would be expected to be high enough to exhibit any influence that they might have upon the IPSP. The ineffectiveness of injection of HSO_3^- ions may thus be taken as indicating that neither HSO_3^- nor SO_3^{2-} ions pass through the activated inhibitory post-synaptic membrane. Similar considerations apply to the equilibrium between $H_2PO_4^-$ and HPO_4^{2-}, for which the acid dissociation constant is evaluated near 10^{-7} (cf. Bjerrum *et al.* 1958). The results obtained by Coombs *et al.* (1955*a*) by injecting HPO_4^{2-} can then be extended to $H_2PO_4^-$; hence the activated post-synaptic membrane is permeable to neither of these ions. For other anions (except HCO_3^-, see below) dealt with in this section, the values of the acid dissociation constants ensure their almost complete dissociation in the cell following injection.

F^- ions were also ineffective in converting the IPSP into a depolarizing potential, though a complication was introduced by their depolarizing action upon the resting membrane (Shanes & Brown, 1942). For example,

in four cells one injection by a current of 5×10^{-8} A for 60 sec sufficed to reduce the membrane potential down to -10 to -30 mV. However, in seven cases out of nine cells, where a relatively small current (3×10^{-8} A for 60 sec) was employed for injection of F^- ions, the resting potential was maintained at more than -50 mV. In these cases the IPSP was increased in the hyperpolarizing direction, indicating that the equilibrium potential of the IPSP was not significantly altered by injections. This situation closely resembled that with acetate ions, the ineffectiveness of which was proved on a more quantitative basis by the aid of IPSP–membrane–potential curve (Coombs *et al.* 1955*a*). In general, injection of penetrating anions by currents of 3×10^{-8} A induces a noticeable change of the IPSP. It can, therefore, be concluded that the activated inhibitory post-synaptic membrane is impermeable to F^- ions.

In their investigation Coombs *et al.* (1955*a*) could not exclude the possibility that HCO_3^- ions were slightly effective in changing the IPSP in the depolarizing direction. This ion species was therefore carefully tested on 23 occasions. The change induced in a PBST cell, in which eleven successful injections were made, is illustrated in Fig. 6B. In the first series of five injections a small reduction of the IPSP was observed, and as a typical and unique feature for this ion species the reduction was progressive with a maximum at about 30 sec after the injection, followed by a gradual recovery to the original size. In the later series the IPSP turned into a depolarizing potential following a similar time course to that in the initial series, but it never exceeded 4 mV. In all other cells only a small reduction of the IPSP was observed, without its reversal. These relatively small changes in IPSP cannot be explained by the conversion of HCO_3^- ions to H_2CO_3, for this would effectively remove only one fourth of the injected HCO_3^- at the pH of about 7 (see above and Bjerrum *et al.* 1958). Furthermore, the characteristic delayed maximum of action on the IPSP suggests that HCO_3^- ions are not effective by themselves and that the change of the IPSP is caused by some secondary process following the injection. One possibility to consider is a reduction of HCO_3^- to HCO_2^- ions in the cell, which is possibly facilitated by the high concentration of the former. The conversion of carbon dioxide to formic acid is catalysed by an enzyme which so far has only been reported to occur in certain bacteria (cf. Sumner & Myrbäck, 1952).

Since a light reduction of the hyperpolarizing IPSP following the injection of glutamate ions was described by Coombs *et al.* (1955*a*), this ion species also was re-examined with relatively large injections. However there was neither reversal nor any significant reduction of the IPSP. Thus, despite the very small effect previously reported, this ion species may be placed in the group of non-penetrating anions.

The sodium or potassium citrate electrodes have already been used for investigations on crustacean muscle (Boistel & Fatt, 1958) and on motoneurones (Kuno, 1959; Araki, Eccles & Ito, 1961) as indifferent for the IPSP. As would be expected, the injection of citrate ions gave results corresponding precisely to those given by the above non-penetrating anions.

In summary, sixteen anions, labelled by minus signs in Table 5, are specified as unable to penetrate the activated inhibitory post-synaptic membrane.

Untestable anions

With fourteen anion species, which have the hydrated size in the range of the present interest, injections were either unsuccessful or not tried because of the following reasons. They are labelled by *X* in Table 5.

(*a*) *Solubility of potassium salts.* The difficulty of injections due to low solubility of the potassium salts was described above (p. 419) for ClO_4^- ions. In this respect ReO_4^- and picrate ions were rejected as untestable, because their potassium salts have a solubility as small as 7·67 m-mole/100 g water at 40 °C and 2·89 m-mole/100 g water at 30 °C, respectively (cf. Seidell, 1952).

(*b*) *Oxidizing agents.* Blocking was regularly observed with electrodes containing MnO_4^- ions as either the sodium or potassium salt. A possible explanation is offered by their powerful oxidizing effect (cf. Latimer, 1959) which might cause coagulation of protein around the tip of the electrode. This kind of block happened even with electrodes having a large tip 2–3 μ across and made all attempts to inject these small ions (see Table 5) unsuccessful. Moreover, if injected successfully, it may be expected that the cell is damaged seriously by this ion species, as $KMnO_4$ solution of concentration as small as 50 mM (0·6%) suffices to fix the tissue for electron microscopy (Luft, 1956). Similar difficulty due to the oxidation can be expected also with ClO_3^- ions, which, therefore, were not tested in the present experiments. With this ion species a further trouble would be introduced by a possible contamination of Cl^- ions. On the other hand, with BrO_3^- there was neither blocking of the electrode nor any sign of liberation of Br^- due to an oxidation process, as described above (p. 419), the effect of which would be readily detected as a change of the IPSP. The time-course of this oxidation reaction may be too slow to affect the IPSP in the time scale of the present experiments. Since the redox potentials for ClO_3^- and ClO_4^- ions are less negative than for BrO_3^-, the reduction of these ions, producing Cl^- ions, would not play any role in the observed IPSP change (pp. 412, 419).

(*c*) *Equilibrium with hydrogen ions.* Fourteen injections of CN^- ions were tried into six cells, but any effect upon the IPSP, which might be expected from their small hydrated size (Table 5), could not be observed. However, since HCN has a very small acid dissociation constant (near 10^{-9}, cf. Bjerrum *et al.* 1958), even large injections cannot be expected to cause any appreciable concentration of free CN^- ions in the cell (see p. 420). Therefore, it was not possible to determine if the flux of CN^- ions could contribute to the production of the IPSP. Similarly, CO_3^{2-} ions are untestable because of their small acid dissociation constant (near 10^{-10}, cf. Bjerrum *et al.* 1958). On the other hand, the dissociation constant for the second hydrogen of $H_2C_2O_4$ is large (more than 10^{-5}, Bjerrum, Schwarzenbach & Sillén, 1957); hence $HC_2O_4^-$ ions injected into the cell would be converted almost completely into $C_2O_4^{2-}$, so the former is untestable. Finally, seven anions, $(CN)_2N^-$, $(CN)_3C^-$, $S_2O_6^{2-}$, $(NO_3)_3C^-$, $CH_2FCO_2^-$, $CH_2CNCO_2^-$ and $CH_2ICO_2^-$, are excluded, because there are no available data about their acid dissociation constants (cf. Bjerrum *et al.* 1957, 1958).

Recovery of the IPSP after anion injection

With nine penetrating anions, Cl^-, Br^-, I^-, NO_2^-, NO_3^-, SCN^-, BF_4^-, ClO_3^- and HCO_2^-, the presumed exponential time course of the IPSP recovery was tested by logarithmic plotting of the IPSP sizes, which were measured as the algebraic difference from the values obtained at the end of the recovery. As is seen in Fig. 7C, the points obtained in three trials of NO_2^- injections into one and the same cell fall along the same straight line. In the cell of Fig. 7B the magnitude of the IPSP increased with each

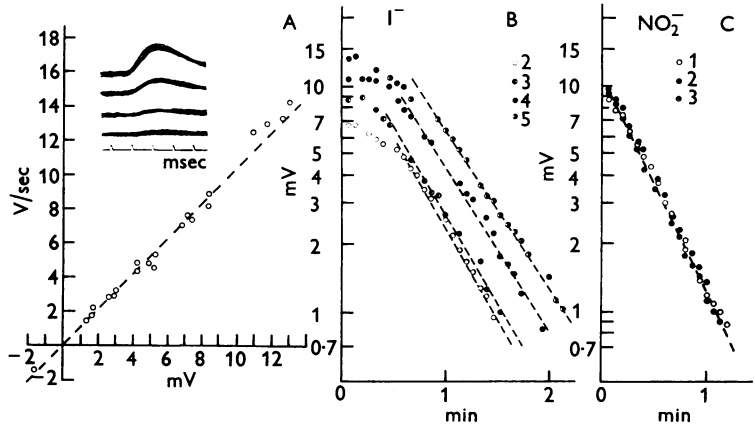

Fig. 7. A, illustrating a parallel change between the size and the steepest slope of rise of Ia IPSP by the injection of NO_3^- ions. Specimen records of recovering IPSPs are shown in the figure with a sweep velocity fast enough to measure the slope of rise. B, logarithmic plot of the IPSP size in the course of recovery after the injection of I^- ions. Note that ordinates are algebraic differences of IPSP sizes in the course of, and at the end of, the recovery. Plots are obtained from four trials in the cell that is illustrated in Fig. 3B. C, similar to B but for the injection of NO_2^- ions in a different cell from that shown in Fig. 3A.

injection of I^- ions, and for as long as 20–40 sec after the injection the slope of the recovery was much less steep than later. Nevertheless, after the late onset the recovery curves approximated to parallel straight lines. These curves of the IPSP recovery can be assumed to express the decrease of the intracellular concentration of injected ions as the ions diffuse out across the cell membrane into its environment (cf. Coombs *et al.* 1955*a*).

Over a wide range of values the size of the IPSP is proportional to the steepest slope of its rising phase (Fig. 7A) and this slope provides a measure of the maximum intensity of the inhibitory post-synaptic current (Curtis & Eccles, 1959). Thus the differences between the sizes of the IPSPs during the course and at the end of recovery, plotted in Fig. 7B and C, can be taken as indices of the intensities of the inhibitory post-synaptic current due to the efflux of injected ions throughout the various stages of recovery, which would in turn be proportional to the intracellular concentration of injected ions at that time. The size of the

Renshaw IPSP is measured at a fixed time (Fig. 1B) and exhibits changes resembling those of the Ia IPSP (Fig. 8C and D), when obtained in the same cell. Therefore it can be taken likewise as an index of the internal concentration of injected ions, despite the more complex nature of its generation.

Several explanations may be offered for the initial slow rate of recovery that is seen in Fig. 7B, for example. As a rule, following the injection of anions of Table 3, the resting potential was reduced by 5–15 mV and then recovered to a steady level within 30–60 sec (Figs. 3 and 4). This reduction of the resting potential will diminish the size of the depolarizing IPSP and this effect will decline as the resting potential recovers. Thus the decrease of the IPSP size in the initial period of the recovery would have been more rapid if the membrane potential had been kept constant. Furthermore, the lower resting potential would diminish the efflux of anions through the whole cell membrane and consequently the rate of the decrease in concentration of injected ions. Another factor to consider is the change of the cell volume. During the passage of the hyperpolarizing current for injecting ions it can be expected that cations, presumably largely K^+ ions, are carried across the surface membrane into the cell and consequently that water enters the cell to keep the osmotic pressure constant (cf. Coombs *et al.* 1955*a*). Conversely, anions escaping from the cell during the recovery phase should be accompanied by an equivalent quantity of K^+ ions together with an appropriate quantity of water. The cell volume would thus decrease during the ion escape, which would slow the rate of reduction of the anion concentration in the cell.

As is shown in Fig. 7B and C, the rate of decrease of the IPSP size is fairly constant when recovering from about 5 to 1 mV. The time constant of the anion escape was thus derived from the rate of the IPSP decrease in this region. As there were many occasions when a random fluctuation of the resting potential considerably affected the size of the IPSP, the cases showing an IPSP recovery under a well-controlled resting potential were limited to 2–6 cells for each anion species. As will be realized from the description above (pp. 417–419), it was not possible to obtain any reliable values in the rate of recovery of the IPSP following injections of N_3^- and ClO_4^- ions. The time constants thus measured show a considerable range for any particular ion species (Table 4); nevertheless, there appear to be significant differences between the various anions. These differences correspond approximately with those in the half time obtained by Coombs *et al.* (1955*a*) with four anions: Br^-, 10–20; SCN^-, 15–20; Cl^-, 10–35; NO_3^-, 23–35 sec.

In the constant-field theory (Goldman, 1943; Hodgkin & Katz, 1949; cf. also Coombs *et al.* 1955*a*) the time constant of the anion escape is related not only to the permeability coefficient of the membrane but also to the ratio of the cell volume to the surface area, and to the membrane potential. However, the cells employed in the calculations of Table 4 had large resting potentials (from -60 to -80 mV) and tolerated the penetration and injection for a considerable time, which suggests that they were all large cells. Thus the modification of the time constant arising from variations in the volume-to-surface ratio of the cell and in the membrane potential would be of little significance. Consequently, the time constant of the IPSP recovery can be assumed as reciprocally proportional to the

permeability coefficient through the whole surface area of the motoneurone. The relative values of the latter are shown in Table 4. When determining the time constant, the IPSP was observed at a repetition rate of 5/sec, and therefore it may be questioned how far the above-determined permeability coefficients involve those of the activated inhibitory post-synaptic membrane. However, the following calculations would indicate that the leakage of ions through the activated inhibitory patches is negligible.

TABLE 4. Time constant of IPSP recovery

Anion species	No. of cells	No. of trials	Mean time constant (sec)	Range (sec)	Relative ability to penetrate
Br^-	4	10	21	19–22	1·24
SCN^-	5	16	21	17–24	1·24
Cl^-	3	8	26	24–30	1·00
NO_2^-	6	15	28	22–41	0·93
BF_4^-	5	19	33	18–47	0·79
I^-	4	14	39	32–45	0·67
NO_3^-	3	11	42	40–44	0·62
ClO_3^-	2	4	63	60–66	0·41
HCO_2^-	2	6	90	88–92	0·29

The relative ability to penetrate is obtained from reciprocals of time constants. The value for Cl^- ions is taken as unity.

The peak value of the inhibitory post-synaptic current generating each Ia IPSP can be derived approximately from the steepest rising slope of the IPSP (cf. Curtis & Eccles, 1959), on the assumption that the total effective capacity of the motoneurone membrane is $2{\cdot}5 \times 10^{-9}$F (Coombs, Curtis & Eccles, 1959). The difference of the inhibitory current at a stage of the recovery following injection from that after the complete recovery can be assumed to be due to the injected ions at the former stage. The efflux of injected ions through the activated inhibitory patches is then obtainable by averaging the inhibitory current over the 2 msec of the activation for each Ia IPSP (Curtis & Eccles, 1959, Fig. 2), and further over the 200 msec of the repetition period. The integral of the average efflux over the whole course of the recovery yields the total quantity of ions ejected through the inhibitory patches. For example, this is calculated as 0·056 p-equiv in the case of Fig. 7A and 0·072 p-equiv in that of Fig. 8A. On the other hand, the passage of a current of 5×10^{-8}A for 60 sec could carry about 30 p-equiv of ions into the cell, and at least one fourth of them would remain at the end of the injection (Coombs *et al.* 1955*a*), and would then diffuse out of the cell during the course of the recovery. Consequently the activated patches of the inhibitory post-synaptic membrane would contribute to no more than 1% of the total efflux of injected ions from the cell.

Support for the above calculation is provided by the following experiment. In four successive injections of HCO_2^- ions into one and the same PBST cell having a large Renshaw IPSP together with the Ia IPSP, the IPSP recovery was determined with different combinations of synaptic potentials on sweeps repeated at five/sec: (1) Ia IPSP + EPSP; (2) Renshaw IPSP + EPSP; (3) Ia IPSP + Renshaw IPSP; (4) Ia IPSP + Renshaw ISPP + EPSP. In the series (1) and (2) the efflux of HCO_2^- ions through

the inhibitory patches should have been somewhat different because of the different durations of the active phase and of the different number of patches involved, and further, in the series (4) the ionic efflux through the inhibitory post-synaptic membrane should be the sum of those in (1) and (2). Among these series, however, there is no significant difference in the rate of the IPSP recovery, which is indicated by the slope of interrupted lines in Fig. 8 A, B and D, the time constant ranging from 80 to 88 sec. Similarly, comparison of the series (3) with (4) reveals that the EPSP repeated with the same rate as the IPSP does not influence the time course

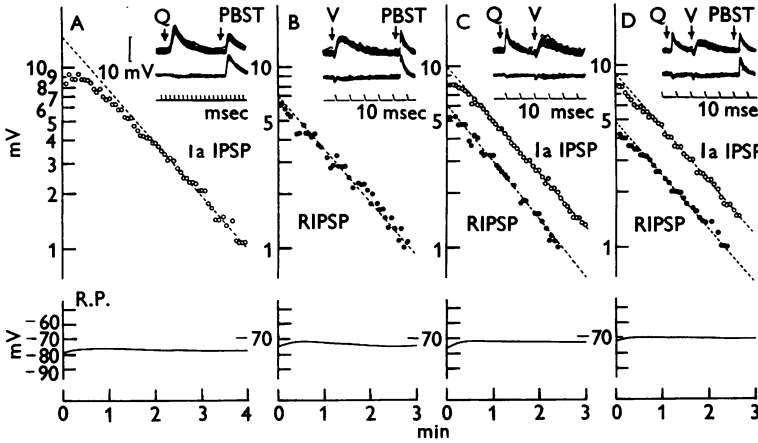

Fig. 8. Comparison of the time course of the IPSP recovery under operation of Ia IPSP, Renshaw IPSP and EPSP in different combinations. 7th (A), 8th (B), 9th (C) and 10th (D) trials of HCO_3^- injections into the same PBST motoneurone as shown in Fig. 5A. ○, size of Ia IPSP from quadriceps afferent (Q); ●, size of Renshaw IPSP (RIPSP) induced by a L7 ventral root stimulation (V), which was just subthreshold for the axon belonging to the cell. Note that ordinates are differential sizes of IPSPs in logarithmic scale as in Fig. 7B and C. Specimen records were obtained just (upper traces), and about 6 min (lower traces), after injections by the passage of current of 5×10^{-8}A for 60 sec. EPSPs were evoked from posterior biceps-semitendinosus afferents (PBST).

of the IPSP recovery. In other cases a spike potential was combined with the IPSP without any appreciable modification of the IPSP recovery. Besides the artificially induced synaptic potentials the motoneurones receive a random bombardment through its synapses. Indeed, Fig. 2 (A, B and E) shows a remarkable increase of the noise in the base line following the injection of ions, which apparently indicates the presence of random IPSPs. As is shown in Table 4, the time constant of the IPSP recovery varied over a considerable range for each anion species, but it was not possible to correlate it to the noise level in each cell. The contribution of the leaking current through randomly activated subsynaptic membrane

thus does not seem significant in modifying the IPSP recovery beyond the error of measurement in the present experiments. It can be concluded that the activated inhibitory post-synaptic membrane forms an insignificant part of the surface membrane of the motoneurones for which the relative permeability to anions is determined in Table 4.

Among the penetrating anion species HCO_2^- could possibly be destroyed by oxidation in the cell. The hydrogenlyase found in certain bacteria catalyses reversibly the conversion of formic acid into CO_2 and H_2 (cf. Sumner & Myrbäck, 1952). The relative ability of HCO_2^- ions to penetrate through the cell membrane would then be smaller than that indicated by the relative value of the time constant of IPSP recovery. However, as this ion species is at the extreme upper end of the present sequence of time constants, this possibility does not detract from the assumed sequence of abilities to penetrate in the reversed order of the time constants (Table 4). An enzyme which oxidizes SCN^- to CN^- ions was reported in erythrocytes of man and certain other species (cf. Goodman & Gilman, 1955), but there are no data available about nerve cells.

Influence of anions on the EPSP and spike potential

Monosynaptic EPSPs were recorded by sampling intermittently or continuously in the sweeps that were used for displaying the IPSPs (Fig. 2). Figures 3 (A, B), 4 (A, B) and 5 (A) show that the EPSP remains unchanged or is transiently decreased in size following injection of anions which produces a large change of the IPSP. This behaviour of the EPSP is readily explained by a transient change in the resting potential. According to Coombs, Eccles & Fatt (1955*b*), the size of the EPSP is reduced with a decrease of the resting potential when the latter is relatively low, while when the resting potential is high it remains almost constant against small alterations of the resting level. A large decrease of the EPSP by injection of toxic anions (Figs. 5B) can similarly be accounted for by a heavy reduction of the resting potential.

The depression of the spike potential, as illustrated in Figs. 3A and 5B, can also be attributed to a reduction in the resting potential. No anion species influenced the spike potential without a change in the resting potential.

DISCUSSION

Table 5 lists forty-one anion species dealt with in Results in the order of their hydrated size relative to K^+ as calculated from the limiting conductance. The latter values in water at 25° C (except for BF_4^-) are available in compilations by Landolt-Börnstein (1936), Harned & Owen (1943) and Robinson & Stokes (1959). These anions are now classified into three categories; penetrating +, non-penetrating −, untestable *X*. It is notice-

able that the separation into two groups of penetrating and non-penetrating ions is attained sharply by their effectiveness on the IPSP, and that there are no transitional ions forming an intermediate zone. The possibility that HCO_3^- ions fall in this zone is excluded above (p. 421), because their small effect could be attributed to a secondary process induced by their injections in the cell.

Since Boyle & Conway's (1941) discussion, it has generally been accepted that the permeability of the muscle membrane is selective to small hydrated ions. Large hydrated ions, either cations or anions, pass the membrane only with difficulty (see also Hutter & Noble, 1960). The pore-structure hypothesis of the membrane was thus proposed; the exterior and interior media of the cell communicate through pores, and small hydrated

TABLE 5. Effects on the IPSP and some physical properties of anions

Ion	IPSP effect	Relative hydrated size	Hydration energy (kcal/g ion)	Naked ion size (Å)	Ion	IPSP effect	Relative hydrated size
CN^-	*X*	0·93	.	.	$(CN)_3C^-$	*X*	1·58
Br^-	+	0·94*	63	1·95	$(NO_3)_3C^-$	*X*	1·60
I^-	+	0·96*	49	2·16	HCO_3^-	—	1·65*
Cl^-	+	0·96*	67	1·81	$CH_2FCO_2^-$	*X*	1·66*
NO_2^-	+	1·02*	(64)	(1·32)	$CH_2CNCO_2^-$	*X*	1·69*
NO_3^-	+	1·03*	(55)	(1·98)	$CH_3CO_2^-$	—	1·80*
N_3^-	+	1·07*	.	.	$CH_2ICO_2^-$	*X*	1·81
ClO_4^-	+	1·09*	(54)	(2·92)	$HC_2O_4^-$	*X*	1·83†
SCN^-	+	1·11*	(45)	.	SO_4^{2-}	—	1·84*
BF_4^-	+	1·12‡	.	(2·88)	$C_2O_4^{2-}$	—	1·94*
ClO_3^-	+	1·14*	(61)	(2·16)	$H_2PO_4^-$	—	2·04
MnO_4^-	*X*	1·17	.	.	SO_3^{2-}	—	2·04
.....					$CH_3CH_2CO_2^-$	—	2·05*
BrO_3^-	—	1·32*	(68)	(2·38)	CO_3^{2-}	*X*	2·12*
F^-	—	1·33*	94	1·36	$Fe(CN)_6^{3-}$	—	2·19
ReO_4^-	*X*	1·34*	.	.	$CH_3(CH_2)_2CO_2^-$	—	2·25*
HCO_2^-	+	1·35*	.	(2·03)	$C_6H_2(NO_2)_3O^-$	*X*	2·41*
$(CN)_2N^-$	*X*	1·35	.	.	HPO_4^{2-}	—	2·58
ClO_2^-	*X*	1·41	.	.	$Fe(CN)_6^{4-}$	—	2·66*
HSO_3^-	—	1·47	.	.	Citrate	—	3·08
$S_2O_6^{2-}$	*X*	1·58	.	.	L-Glutamate	—	——

Relative hydrated sizes were calculated by Stokes's law from limiting conductances in water at 25 °C (*Robinson & Stokes, 1959; † Harned & Owen, 1943; others, Landolt-Börnstein, 1936) except that for BF_4^- (‡) which was derived from the Stokes radius in liquid sulphur dioxide relative to that of ClO_4^- in the same solvent (Lichtin & Pappas, 1957). Hydration energy for halide ions is obtained from Kortüm & Bockris (1951). Other values of hydration energy in parentheses were calculated from lyotropic numbers (Voet, 1937) with quoted values for halide ions as standard. Naked ion sizes are shown for halide ions by their crystal radii (Pauling, 1960) and for oxy-anions by $0{\cdot}25\,nr$ (in parentheses), where n is the number of arms extending from the central atom and r their length, i.e. the interatomic distance between the central atom and the surrounding oxygen atoms plus van der Waals radius of the oxygen atom (Coutour & Laidler, 1957). Values of $0{\cdot}25\,nr$ for BF_4^- and HCO_2^- ions were calculated in a similar way from the data given by Pauling (1956), employing for HCO_2^- the average length of two C–O and one C–H ligands as r.

ions thereby pass the membrane, while those larger than the pore size are all non-penetrating. No interference of ions with the pore wall is implied in the theory. The applicability of this hypothesis to the inhibitory post-synaptic membrane (Coombs *et al.* 1955*a*) can now be criticized on the basis of an extensive anion series. In Table 5 there are twelve anions with hydrated size finely graded around unity of K^+ ions, from 0·93 of CN^- to 1·17 of MnO_4^-, while a further increase of the hydrated size is obtained with a jump from 1·17 for MnO_4^- to 1·32 for BrO_3^-, as the dotted line in Table 5 indicates. With the exception of CN^- and MnO_4^-, which could not be successfully tested, all ions above the line in Table 5 were able to penetrate the inhibitory subsynaptic membrane. On the other hand, ability to penetrate was displayed for only one species of anion, HCO_2^-, below this line, i.e., with hydrated sizes of 1·32 or higher. Evidently, HCO_2^- ions present the only inconsistency with the pore-structure hypothesis of the activated inhibitory post-synaptic membrane. This exception, however, might be accounted for by consideration of the following factors. One possibility is that the relative hydrated size of HCO_2^- ions is smaller at 38° C (the cat temperature) than at 25 °C. For example, the relative size of Na^+ ions against K^+ ions decreases from 1·47 at 25 °C to 1·43 at 35 °C, though the relative sizes of Cl^-, Br^-, I^- and NO_3^- are known to be much the same at 35 °C as at 25 °C (cf. Robinson & Stokes, 1959). There are, however, at present no data about the temperature effect on the limiting conductance of HCO_2^- ions. Another possibility is that the HCO_2^- ion has an ellipsoid rather than a spherical shape and hence may pass through pores smaller than the average diameter of these ions. It may be recalled that the relative permeability to Cl^-, Br^- and I^- ions through an artificial ion-exchange membrane is in the order of their polarizability, which makes these ions behave like rods (cf. Shanes, 1958). If the results can thus be reconciled with the pore-structure hypothesis, the size of pores in the activated inhibitory post-synaptic membrane can be determined as smaller than the hydrated size of BrO_3^- ions, yet larger than that of ClO_3^- ions. Though there is considerable discrepancy between the results of different methods of measuring the degree of hydration of ions or the absolute size of hydrated ions (see Bockris, 1949; Bell, 1958; Stern & Amis, 1959), values of 3·25 and 2·85Å will be adopted for the sizes of hydrated BrO_3^- and ClO_3^- ions respectively (Kielland, 1937). These values can be regarded as the upper and lower limits of the effective pore radius in the activated inhibitory post-synaptic membrane of cat spinal motoneurones.

The existence of pores such as assumed by Boyle & Conway (1941) has recently been demonstrated in the erythrocyte membrane by quite independent techniques (Solomon, 1960), the radius of pores being determined at 3·4–4·2Å. These pores are presumed to be charged positively,

which would account for the large difference between the permeabilities to Cl^- and K^+ ions with similar hydrated sizes (Table 5). On the activated inhibitory post-junctional membrane of the crustacean muscle Boistel & Fatt (1958) already adopted a similar assumption for explaining the differential permeability between Cl^- and K^+ ions. On the other hand, according to the recent comparative measurements of cation permeability in the muscle membrane (Conway & Moore, 1945; Lubin & Schneider, 1957; Sjodin, 1959; Mullins, 1959; Mullins & Moore, 1960) there is a large variation in the relative permeabilities to K^+, Rb^+, Cs^+ and Tl^+ in spite of their similar hydrated size. Since this is inexplicable by the pore-structure hypothesis, the theory has been modified by assuming that ions interact with the pore wall (Shanes, 1958; Mullins, 1959, 1960). The relative permeability would then be governed by a combination of factors such as hydration energy, naked ion size and statistical distribution of pore size. Unfortunately, as pointed out above (p. 426), it is not possible to evaluate accurately the relative effect of anions in the present experiments. Moreover, even when two different anions are injected into one and the same cell through a double-barrelled micro-electrode, the comparison of their effects is fraught with uncertainties, because the accumulation of anions during the injection would vary on account of the different rates of the ionic losses from the cell during the injection and also because of the possible differences in the transport numbers for the different ions being injected. Consequently it would be premature to discuss the possibility of interaction of ions with the pore wall in the activated inhibitory post-synaptic membrane. However, it should be pointed out that the exceptional behaviour of HCO_2^- ions in relation to the pore-structure hypothesis cannot be accounted for by assuming an interaction between ions and the pore wall after the manner suggested by Shanes (1958) or by Mullins (1959, 1960).

According to Mullins (1959, 1960) the permeability to ions is determined by the matching of their size in a dehydrated state with pore size, for which a Gaussian distribution is assumed tentatively; ions either too large or too small in their naked size fail to invade the membrane by replacing their hydration with the solvation obtained from the pore wall. However, it cannot be explained in this way that in the series of naked ion sizes (Table 5) the membrane is as permeable to small HCO_3^- ions as to large ClO_4^- or BF_4^-, but is impermeable to BrO_3^- which lies in between. By Shanes's (1958) hypothesis the impermeability to BrO_3^- or F^- ions should be attributed to their large hydration energy, because another limiting factor, the naked ion size, is smaller for them than for penetrating ClO_4^- and BF_4^-. For HCO_2^- ions the hydration energy is not given in Table 5, but the larger hydrated size, in comparison with BrO_3^-, in spite of the smaller naked size suggests a higher degree of hydration, and so a larger hydration energy. This is in conflict with the fact that HCO_2^- ions readily pass through the activated inhibitory post-synaptic membrane.

On the other hand, the relative anion permeability of the whole surface area of motoneurones was estimated from the relative values of the time

constant of the IPSP recovery (Table 4). Comparable figures are available from investigations on muscle membrane. Conway & Moore (1945) found that Cl^-, Br^- and NO_3^- ions penetrate the resting membrane in the ratio of 1:0·67:0·5. Abbott (quoted by Hill & MacPherson, 1954) reported a much slower escape of radioactive I^- from the muscle (time constant, 90 min) than of radioactive Cl^- (time constant, 10 min). Recently, Harris (1958) gave the following figures for time constants of escape of anions from muscle to normal Ringer's solution: Cl^-, 8–16 min; Br^-, 10–13 min; SCN^-, 46 min; I^-, 60 min; NO_3^-, 80 min; while Simon, Johnstone, Shankly & Shaw (1959) obtained a faster rate of escape of I^- ions than Harris (1958) found. Discrepancies between these authors are apparently due to the difference in the external ionic composition employed and in the method of loading muscles in solutions containing various anions. The present sequence of permeability (Table 4) is in reasonable agreement with the sequence presented by Harris (1958) except for the high permeability displayed for SCN^- ions. It is uncertain whether this discrepancy for SCN^- ions is due to the difference between the mammalian spinal motoneurones and frog muscles either in respect of the membrane itself or of the metabolic process destroying SCN^- ions within the cell (see p. 427).

With the series of nine anions of Table 4, it is interesting to discuss the validity of various hypothesis for explaining the order of their relative permeability in the whole surface of the motoneurone. First, from the point of view of Boyle & Conway's theory (1941), the slow penetration of ClO_3^- and HCO_2^- ions is in keeping with their large hydrated size. However, as pointed out by Shanes (1958), the much lower permeability for I^- relative to Cl^-, Br^- or even NO_2^- cannot be explained on the same basis, because their hydrated sizes are very close to each other (Table 5). Secondly, for the polyatomic ions NO_2^-, NO_3^-, and ClO_3^- the permeability is inversely related to the naked ion size, but this relation is not obeyed by the ions HCO_2^- and BF_4^- (Table 5). This is not explicable even by assuming a normal distribution of the pore size (Mullins, 1959, 1960). The third factor to consider is the hydration energy of ions. There has been a series of attempts to correlate the anion effects on the muscle, which would in turn relate to the anion permeability, with the well-known lyotropic series (Chao, 1934, 1935; Hill & MacPherson, 1954; Harris, 1958; Hutter & Padsha, 1959). The importance of the adsorbability of ions to the membrane, in terms of the lyotropy, has thus been emphasized, as influencing their penetration through the membrane (see Harris, 1956). The lyotropic series of anions follows the reversed order of their hydration energy (Voet, 1937), which is given in Table 5; $Cl^- > NO_2^- > Br^- > ClO_3^- > NO_3^- > I^- > SCN^-$. Even though the most serious inconsistency by SCN^- ions is excluded by the possibility of their chemical destruction (see p. 427), the

penetration of ClO_3^- which is slower than NO_3^- or even I^- does not agree with this sequence.

In short, it is not possible to account for the present sequence of relative permeability by any one of these factors, but perhaps an appropriate combination of them may be more successful. By Shanes's (1958) hypothesis deviations at the position of HCO_2^- as well as of BF_4^- in the relation between the relative permeability and the naked ion size (see above) could be adjusted by taking the hydration energy into account. As previously mentioned, the hydration energy of HCO_2^- should be large, at least more than that for BrO_3^-, so that the slow penetration in spite of the small naked size may be expected. In comparison with ClO_3^-, BF_4^- would have a smaller degree of hydration, and so a smaller hydration energy, because of the larger naked ion size against the presumed similar hydrated size (see legend of Table 5). This could then be the cause of the relatively quick penetration of BF_4^-. However, an exact weighted relation between the hydration energy and the naked ion size in determining the permeability would be necessary for establishing this hypothesis.

SUMMARY

1. Single micro-electrodes filled with solutions containing various anions were inserted into cat motoneurones in the lower lumbar segments and used both for electrophoretic injection of anions and for intracellular recording.

2. The effect of injections was appraised by changes in IPSPs, especially Ia IPSPs in PBST motoneurones, but also Renshaw IPSPs or polysynaptic IPSPs.

3. As revealed by the reversal and subsequent recovery of IPSPs, the activated inhibitory post-synaptic membrane was found to be permeable to seven anion species, I^-, NO_2^-, BF_4^-, ClO_3^-, ClO_4^-, HCO_2^- and N_3^-, in addition to those previously reported, Cl^-, Br^-, SCN^- and NO_3^-.

4. Eleven new anion species were included in the group of anions unable to penetrate the activated inhibitory post-synaptic membrane, while fourteen anions remained untestable.

5. The time constants of the IPSP recovery after injections were assessed for nine penetrating anions, by which a sequence was determined for the order of their relative ability to penetrate the whole cell membrane; $Br^- = SCN^- > Cl^- > NO_2^- > BF_4^- > I^- > NO_3^- > ClO_3^- > HCO_2^-$.

6. No anion species was found to affect EPSPs and spike potentials primarily, any effect appearing to be secondary to a depression of the membrane potential.

7. The ability of anions to penetrate the activated inhibitory post-synaptic membrane was related to their relative hydrated sizes and was

reconcilable with the pore-structure hypothesis of the activated inhibitory post-synaptic membrane.

8. The sequence of the relative permeability to anions through the whole cell membrane was compared with that obtained in the muscle membrane. The applicability of various hypotheses was discussed.

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