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VARIATIONS IN THE PERMEABILITY PROPERTIES OF THE INHIBITORY POST-SYNAPTIC MEMBRANE OF THE CRAYFISH NEUROMUSCULAR JUNCTION WHEN ACTIVATED BY DIFFERENT CONCENTRATIONS OF GABA

BY A. TAKEUCHI AND NORIKO TAKEUCHI

From the Department of Physiology, School of Medicine, Juntendo University, Hongo, Tokyo, Japan

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SUMMARY

1. The membrane conductance of the crayfish muscle was measured with intracellular micro-electrodes. Increase in the membrane conductance induced by various concentrations of γ-amino butyric acid (GABA) was measured in the Br^-, NO_3^-, I^- and CNS^- solutions and compared with that in Cl^- solution. When the membrane was activated by lower concentrations of GABA, the resulting membrane conductance in NO_3^- and I^- solutions was larger than in Cl^- solution, while the membrane conductance activated by higher concentrations in NO_3^- and I^- solutions were smaller than in Cl^- solution. The membrane conductance activated by GABA was larger in Br^- solution and smaller in CNS^- solution than the membrane conductance activated in Cl^- solution, throughout the concentration range of GABA examined.

2. The reversal potential of inhibitory junctional potentials (i.j.p.s) was measured shortly after replacing Cl^- by foreign anions: Br^-, NO_3^-, I^- and CNS^-. In I^- solution the i.j.p.s produced by lower stimulation frequencies showed a reversal potential at a more hyperpolarized level than those produced by higher stimulation rates.

3. Shortly after replacing Cl^- by I^-, the GABA potential induced by iontophoretic application showed a triphasic potential change at the 'reversal' potential. The reversal level of the GABA potential produced by smaller doses was at a more hyperpolarized level than that produced by larger doses.

4. Possibilities of a concentration effect of GABA are discussed. It is suggested that the relative permeability of the crayfish inhibitory membrane to anions changes depending on the concentration of GABA.

INTRODUCTION

Permeability properties of the inhibitory post-synaptic membrane have been investigated on vertebrate central neurones and on a wide variety of invertebrate cells. In most of these synapses, the inhibitory membrane is highly permeable to anions (cf. Ginsborg, 1967). The relative permeability of the inhibitory membrane to anions differs with various anions in central neurones (Kelly, Krnjević, Morris & Yim, 1969), crayfish giant fibres (Ochi, 1969) and crayfish neuromuscular junctions (Takeuchi & Takeuchi, 1967, 1971; Motokizawa, Reuben & Grundfest, 1969). In previous studies, when measuring the relative conductance of the crayfish inhibitory membrane, it was assumed that the permeability properties of the membrane activated by GABA were independent of its concentration. Thus, the concentration of GABA was fixed at a value which produced about the maximal conductance change (Takeuchi & Takeuchi, 1967, 1969, 1971). In the present experiments the membrane conductance and the reversal potential of the inhibitory post-synaptic membrane were measured in different concentrations of GABA, while Cl^- in the bath solution was replaced by foreign anions, such as Br^-, NO_3^-, I^- or CNS^-. It was observed that the relative conductance of the inhibitory membrane to anions and the reversal potential of i.j.p.s or of the GABA potential changed, when the stimulation frequency or the concentration of GABA was altered.

METHODS

Experimental procedures were similar to those described in previous papers (Takeuchi & Takeuchi, 1967, 1971). The abductor muscle of the dactyl in the first walking leg of the crayfish (*Cambarus clarkii*) was used. Membrane potentials were measured differentially between an intracellular micro-electrode and a coarse KCl-filled micro-electrode which was placed near the muscle fibre. Intracellular micro-electrodes were filled with 0·6 M-K_2SO_4. The bath was connected to ground with a saturated KCl-calomel electrode. The pH of the bath solution was adjusted to 7·2 by Tris maleate buffer. In order to change the composition of anions in the bath solution, NaCl was replaced by equimolar Na salts of foreign anions. The membrane current–membrane potential (V–I) relationship was recorded on an X–Y recorder and the input conductance was measured from the slope of the V–I relation at the resting potential. The membrane conductance was calculated as reported previously (Takeuchi & Takeuchi, 1967). The bath solution was kept flowing during the experiment. The reversal potential was measured usually within 3 min after replacing the bath solution with test solutions.

RESULTS

Membrane resistance

Fig. 1 shows records of the membrane potential produced by constant, hyperpolarizing current pulses. After the muscle had been equilibrated with each of several solutions, GABA was added to the solution for the

period indicated. In the normal Cl^- solution, 1×10^{-5} M-GABA produced little change in the amplitude of the electrotonic potential. After equilibration with NO_3^- solution, addition of 1×10^{-5} M-GABA decreased the amplitude of electrotonic potential to about 80% of the control. In I^- solution, 1×10^{-5} M-GABA was still more effective and the electrotonic potential decreased to about 45% of that without GABA. Addition of 2×10^{-4} M-GABA decreased the electrotonic potential to about 18–27%

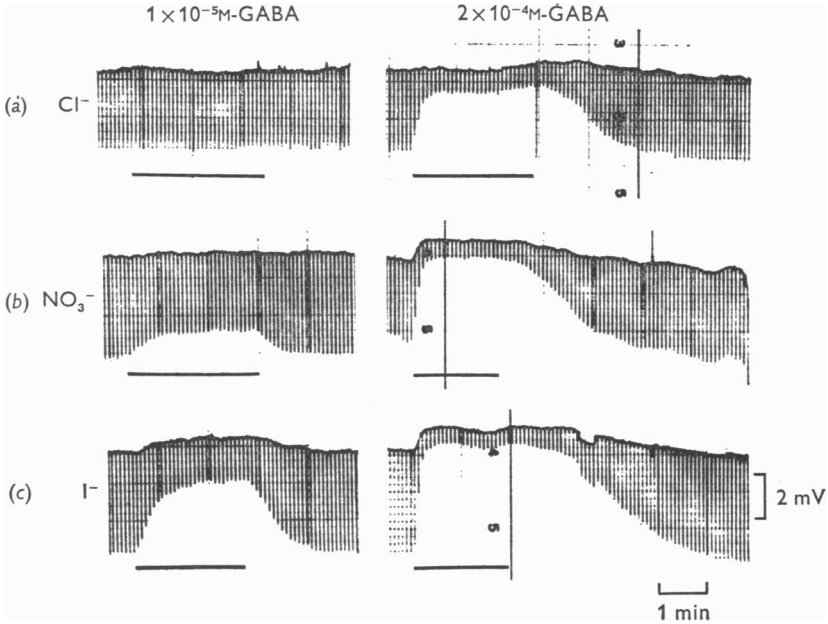

Fig. 1. Membrane potential change measured with a pen writer produced by constant, hyperpolarizing current pulses (*a*, 2×10^{-8} A; *b* and *c*, $1{\cdot}6 \times 10^{-8}$ A; duration, 1 sec). Records were obtained from the same muscle fibre after the muscle had been equilibrated with each solution. GABA was added to the external solution for the period indicated.

of the control in each solution. It was also observed that after addition of 2×10^{-4} M-GABA it took longer to wash out the effect of GABA in NO_3^- and I^- solutions than in Cl^- solution. These results suggest that GABA was more effective at lower concentrations in I^- and NO_3^- solutions than in Cl^- solution.

Dose–conductance relationship

The dose–conductance curves of GABA were measured after the muscle had been equilibrated with Br^-, NO_3^-, I^- or CNS^- solutions and are compared with those obtained from the same muscle fibres in Cl^- solution (Fig. 2). Experimental procedures were the same as reported in a previous

paper (Takeuchi & Takeuchi, 1967). Ordinates represent the increase in the inhibitory membrane conductance ($g_m L$), where g_m is the increase in membrane conductance per unit length and L, the half length of the muscle. Abscissae indicate the concentration of GABA added to the solution. In Br^- solution the membrane conductances activated by each

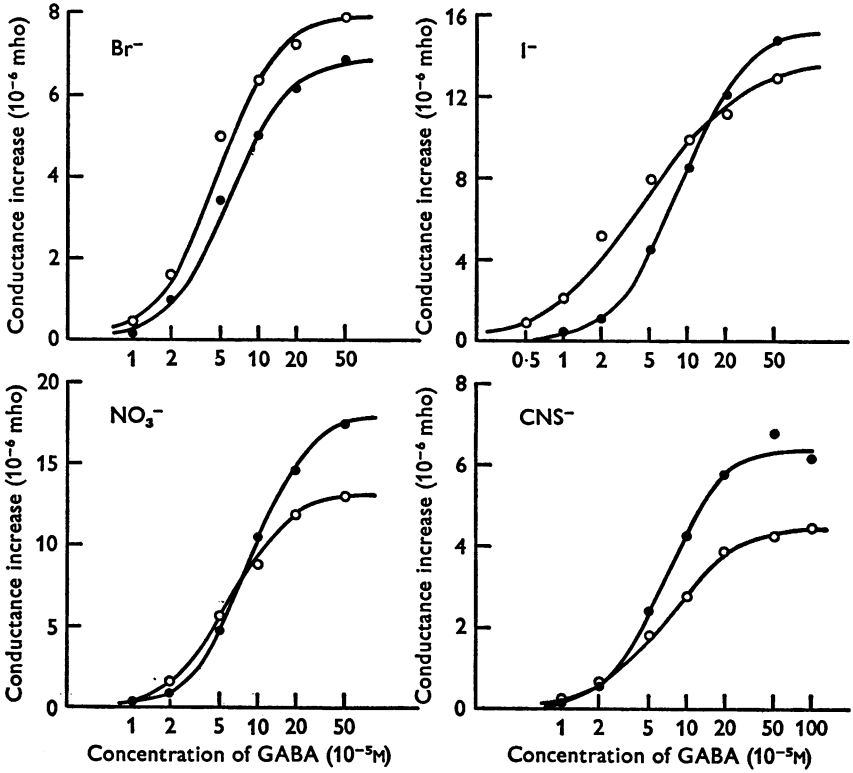

Fig. 2. Dose–conductance relationship of GABA in various solutions. Ordinates, the inhibitory membrane conductance ($g_m L$). Abscissae, concentration of GABA added to the solution. ●, obtained in Cl^- solution. ○, obtained from the same fibre after equilibration with foreign anions. Curves are drawn according to the eqn.: $y = y_{max}(1+K/A^n)^{-1}$, where A is the concentration of GABA. n and K are constants. Values of n are: in Br^-, ● and ○, 1·79; in NO_3^-, ●, 1·94, ○, 1·88; in I^-, ●, 1·67, ○, 1·08; in CNS^-, ●, 1·85, ○, 1·46.

concentration of GABA is larger than the one obtained in Cl^- solution. The shape of the dose–conductance curve was in general similar to that obtained in Cl^- solution, although the ratio of membrane conductance in Br^- solution to that in Cl^- solution was larger at lower concentrations of GABA. When Cl^- was replaced by I^-, the membrane conductance activated by lower concentrations of GABA was remarkably larger than that in Cl^-

solution, while the membrane conductance activated by higher concentrations was smaller. The inhibitory membrane conductance in I^- solution was three to four times larger than that in Cl^- solution when 2×10^{-5} M-GABA was applied. The large membrane conductance activated by lower concentrations of GABA in I^- solution was not due to a shift of the dose–conductance curve to lower concentrations, but the slope of the curve was less steep ($n = 1{\cdot}33 \pm 0{\cdot}07$ (mean $\pm$ S.E., six experiments), in I^- solution; $n = 1{\cdot}9 \pm 0{\cdot}03$ in Cl^- solution (Takeuchi & Takeuchi, 1969)). A similar but less remarkable change was also observed by substituting NO_3^- for Cl^-. The difference between the dose–conductance curves in NO_3^- solution and in Cl^- solution was small, but repeated experiments showed that at lower concentrations of GABA the activated membrane conductance was slightly larger in NO_3^- solution than in Cl^- solution. In CNS^- solution, the dose–conductance relationship was rather variable, but the membrane conductance activated by each concentration of GABA was almost the same or smaller than in Cl^- solution. The slope of the curve was slightly smaller than that in Cl^- solution.

The sequence of the relative membrane conductance activated by lower concentrations of GABA (in concentrations below about 5×10^{-5} M) was approximately in the order $I^- > Br^- > NO_3^- > Cl^- \geqslant CNS^-$. This sequence was different from that activated by higher concentrations of GABA (Takeuchi & Takeuchi, 1967).

I.j.p.s in foreign anions

I.j.p.s were recorded with a micro-electrode inserted at the mid-position of the muscle fibre, and the membrane potential was changed by passing current through a second microelectrode inserted within about 50 μ from the recording electrode. Summated i.j.p.s set up by trains of stimuli in various solutions are shown in Fig. 3. *A* and *B* were obtained from two different muscle fibres. In Cl^- solution, i.j.p.s showed a slight depolarization at the resting potential (second trace from the right). As the membrane was depolarized, the amplitude of i.j.p.s decreased and its sign was reversed at about 2 mV from the resting level. The time course of i.j.p.s was not changed by changing the membrane potential, i.e. the depolarizing i.j.p.s are the mirror image of the hyperpolarizing i.j.p.s (Fig. 4*a*).

When i.j.p.s were recorded shortly after replacing the external Cl^- by I^-, i.j.p.s changed from a depolarizing direction to a hyperpolarizing direction (Takeuchi & Takeuchi, 1971). The amplitude was large and it reached its peak value relatively rapidly. The peak amplitude was either maintained at this level or it declined slowly (Figs. 3 and 5). The falling phase of the summated i.j.p.s was usually prolonged. In the case of Fig. 3*A*, the time from peak to half amplitude was about 40 msec in Cl^- solution and 160 msec

in I^- solution. The 'half-time' of the electrotonic potential produced by square current pulses was short compared to the time course of the falling phase of i.j.p.s, it being about 10–20 msec in both Cl^- and I^- solutions (see

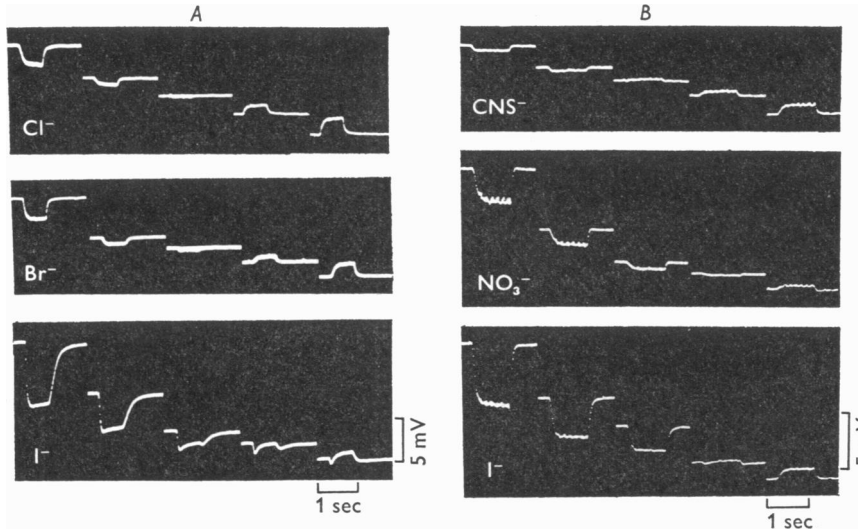

Fig. 3. I.j.p.s at various membrane potentials in Cl^-, Br^-, NO_3^-, I^- and CNS^- solutions. *A* and *B* were obtained from two different muscles. Stimulation rates were 73/sec in *A* and 60/sec in *B*. I.j.p.s obtained at the resting potential are shown on the left side of each record, except in Cl^- solution where the second trace from the right was recorded at the resting potential. The vertical displacements of the records are equal to the shift in membrane potential caused by the applied current.

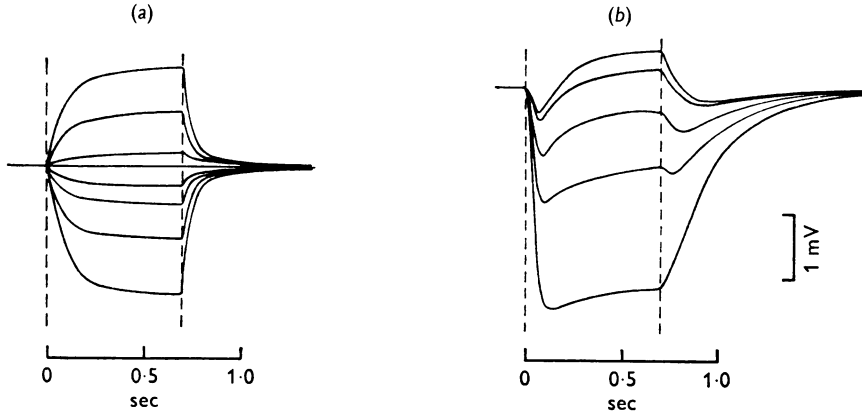

Fig. 4. Superimposed tracings of summated i.j.p.s recorded at various membrane potentials. *a*, recorded in Cl^- solution. *b*, shortly after replacing Cl^- by I^-. Repetitive stimulation was applied at 73/sec for 690 msec. Hyperpolarizing i.j.p.s are shown as downward direction.

also Fatt & Katz, 1953; Fatt & Ginsborg, 1958). Since lower concentrations of GABA were more effective in I^- solution than in Cl^- solution, the prolonged falling phase in I^- solution may reflect small amounts of residual transmitter which are too low to cause the potential change in the Cl^- solution. However, other possibilities, e.g. the effect of I^- on the reabsorption of GABA, are not excluded.

When the membrane was hyperpolarized by supplying current, the amplitude of i.j.p.s was decreased and their time course changed in I^- solution (Fig. 3*A*). The rising phase became steeper and the peak was flattened or declined. Near the reversal potential a triphasic potential change was observed, i.e. a sharp downward deflexion was followed by an upward deflexion and it terminated with another downward deflexion. As the membrane was hyperpolarized further, the i.j.p.s showed a smooth upward curve and no decline in its peak amplitude was observed (Fig. 5). When the muscle was soaked for long time in I^- solution, the reversal potential shifted back to approximately the same value as that in Cl^- solution and the triphasic potential was not observed at the reversal potential.

Tracings of i.j.p.s obtained at various membrane potentials shortly after substituting I^- for Cl^- are superposed in Fig. 4*b*. It shows that the downward deflexions of the triphasic potential correspond to the rising and falling phases of the summated i.j.p.s.

When Cl^- was replaced by Br^-, NO_3^- or CNS^-, the reversal potential for the i.j.p.s also shifted towards the hyperpolarized level up to about 10 mV (Takeuchi & Takeuchi, 1971). However, i.j.p.s reversed their sign almost uniformly and triphasic potentials, as observed in the I^- solution, were not detected (Fig. 3).

There are several possibilities to be considered in explaining the triphasic potential changes of i.j.p.s in I^- solution.

(*a*) The reversal potential of i.j.p.s obtained here may not indicate exactly the true equilibrium potential, because the inhibitory junctions are distributed diffusely over the muscle fibre and the membrane potential was changed by passing current through a micro-electrode inserted in the middle of the fibre (Burke & Ginsborg, 1956). The space constant of the crayfish muscle is long compared to the muscle length and the difference between the reversal potential and the equilibrium potential may be small. However, there remains a possibility that the reversal potential changes, even though the equilibrium potential is constant, when the conductance of the inhibitory junctional membrane varies by the action of different concentrations of the transmitter.

The electrotonic potential of the crayfish muscle fibre is described by the equations of the short cable with infinite resistance at both ends (Orkand, 1962). When a constant current I_0 is applied at the mid-position ($x = 0$), the electrotonic potential at $x = 0$ is given by

$$V_0 = \frac{1}{2} I_0 r_i \coth \frac{L}{\lambda} \qquad (1)$$

λ is the space constant which is equal to $\sqrt{(r_m/r_i)}$ where r_m is the membrane resistance $\times$ unit length and r_i is the resistance of the core per unit length. L is the half length of the fibre. When the inhibitory nerve is stimulated, i.j.p.s are superimposed upon the electrotonic potential. If it is assumed that the transmitter opens additional channels in the membrane which are distributed evenly over the muscle fibre and that the

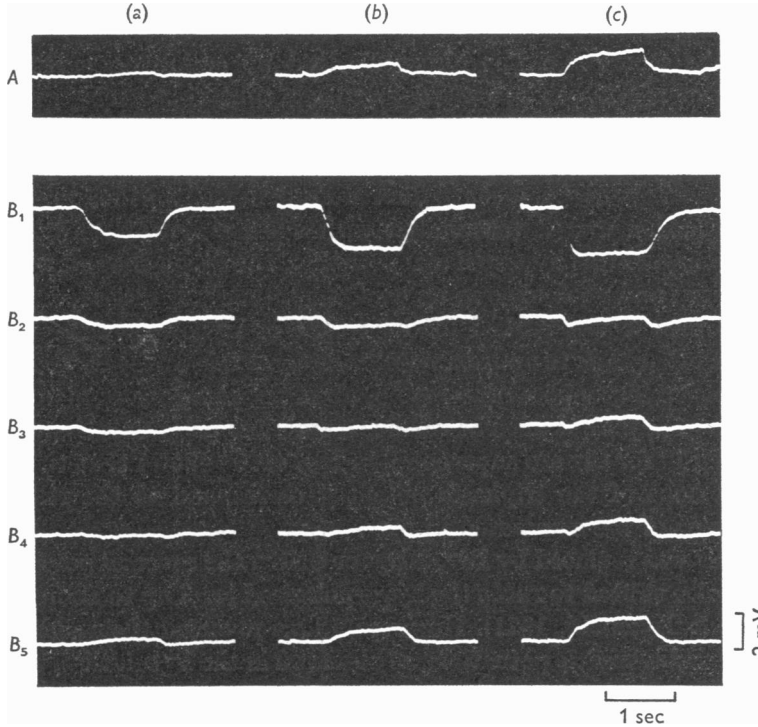

Fig. 5. I.j.p.s recorded in Cl^- and I^- solutions, with different frequencies of stimulation. Stimulation rates are 38/sec (*a*), 61/sec (*b*) and 85/sec (*c*). Records in *A* were obtained in Cl^- solution, after hyperpolarizing the membrane by 3·5 mV. The reversal potential was at 4·7 mV depolarized level. Records in *B* were obtained at various membrane potentials shortly after replacing Cl^- by I^-. B_1 was recorded at the resting potential (74 mV); B_2, 78·8 mV; B_3, 79·5 mV; B_4, 80·4 mV; B_5, 81 mV.

interval for which the junctions are active is long compared to the time constant the membrane, the amplitude of superimposed i.j.p.s (V_s) recorded at $x = 0$ is given by

$$V_s = V_o \left(\frac{1}{\sqrt{(1+k)}} \frac{\coth \sqrt{(1+k)} L/\lambda}{\coth L/\lambda} - 1 \right) + \frac{k}{1+k} E_o, \qquad (2)$$

$$E_o = E_s - E_m$$

$$k = r_m/r_s,$$

E_s is the equilibrium potential of the activated junctional membrane with respect to the bath solution; E_m, the resting potential with respect to the bath solution; E_o,

the equilibrium potential measured from the resting potential; r_s, the resistance of the activated junctional membrane × unit length. In the present experiments, a train of stimuli was applied for about 1 sec and the second assumption may not be very inadequate.

When $V_s = 0$, the electrotonic potential (V_0) is equal to the reversal potential (V_R). V_R/E_0 is calculated from eqn. (2), if L/λ and k are known. L/λ was about 0·3 in Cl^- solution at the resting potential and it increased to about 0·5 when the membrane was hyperpolarized by about 10 mV. (L/λ in I^- solution was less than these values). With $L/\lambda = 0{\cdot}5$ and $0 < k < 10$, V_R/E_0 lies between 1·07 and 1·08. (When L/λ is 0·3, V_R/E_0 is less than 1·03 for all values of k.) Since k was usually less than 10 (cf. Fig. 2 of Takeuchi & Takeuchi, 1967) and E_0 would be about 10 mV when Cl^- of the bath solution was replaced by I^-, the change in the reversal potential when k was altered at E_0 = constant would be less than 0·1 mV. This value is much smaller than the observed change in the reversal potential in I^- solution (about 2 mV in the case of Fig. 6).

If the time course of i.j.p.s produced by nerve endings which distributed near the end of the muscle fibre is different from that near the middle of the fibre, the triphasic potential might appear at the reversal potential. In this case, if the recording and current electrodes were inserted at the end of the muscle fibre, the direction of the triphasic potential would be reversed. However, the triphasic potential of the same direction was observed both at the end and middle of the fibre in the I^- solution.

(*b*) When the membrane potential of a fibre is brought to the reversal potential, other fibres still produce i.j.p.s and may cause the potential change at the fibre under investigation. However, if the recording electrode was placed on the surface of muscle fibre, no appreciable potential change was detected and this possibility may be neglected.

In Figs. 3 and 5, i.j.p.s in I^- solution are large compared with those in Cl^- solution. The input conductance of the post-synaptic membrane during i.j.p.s was estimated from the V–I relation. In Fig. 5, the increase in the input conductance during stimulation at 85/sec was about $1{\cdot}2 \times 10^{-6}$ mho in Cl^- solution and $2{\cdot}2 \times 10^{-6}$ mho in I^- solution. Although the conductance increase produced by GABA is variable from fibre to fibre, this value corresponds to relatively low concentrations of GABA (probably about $2\text{–}3 \times 10^{-5}$ M). The membrane conductance activated by this range of GABA concentration was several times larger in I^- solution than in Cl^- solution. Therefore, the larger i.j.p.s in I^- solution in the present experiments may be attributable, at least partly, to the relatively greater conductance of the inhibitory membrane produced by GABA at low concentration, although other actions of I^-, e.g. increase in the transmitter release from the nerve endings, is not excluded.

Reversal potential of i.j.p.s in I^- solution

In the records of Fig. 5 i.j.p.s were set up at stimulation frequencies of 38 (*a*), 61 (*b*) and 85/sec (*c*) and the reversal potentials were determined at these different stimulation rates. Records in Fig. 5*A* were obtained in Cl^- solution by hyperpolarizing the membrane by about 3·5 mV (The reversal potential was at 4·7 mV depolarized level.) I.j.p.s recorded at various

membrane potentials shortly after substituting I^- for Cl^- are shown in Fig. 5*B*. I.j.p.s in B_1 were recorded at the resting potential. The i.j.p.s at 38/sec (*a*) show a gradual increase in their amplitude. As the stimulation frequency was increased, the peak of the summated i.j.p.s became flat and the falling phase prolonged. The peak amplitude tended to decline at 85/sec (*c*).

When the membrane was hyperpolarized in I^- solution, i.j.p.s produced at 38/sec simply reversed their sign at about 81 mV (B_5) and no appreciable changes in their time course was observed. I.j.p.s produced at higher stimulation rates, however, showed changes in their time course during hyperpolarization of the membrane. In B_2 and B_3, the i.j.p.s at 38/sec (*a*) showed a simple downward deflexion, but those produced at higher stimulation rates showed triphasic potential changes. When the membrane was hyperpolarized further, the stimulation at 38/sec produced little potential change, and i.j.p.s produced by higher stimulation rates showed a simple upward deflexion (B_5). No decline was observed in the peak amplitude of the depolarizing i.j.p.s.

Amplitudes of i.j.p.s produced by stimulation rates of 85/sec were measured at two points, one during the rising phase and the other during the plateau of i.j.p.s and plotted against the membrane potential (Fig. 6*A*). Filled and open circles indicate the amplitude measured at 0·1 and 1 sec, respectively, after the start of repetitive stimulation. In Cl^- solution, lines drawn through points measured at 0·1 and at 1 sec cross the membrane potential axis at the same point. However, shortly after substituting I^- for Cl^-, the line interpolated through the filled circles crosses the axis at a point about 2 mV more hyperpolarized than that through the open circles. In Fig. 6*B*, the peak amplitude of i.j.p.s stimulated at various frequencies are plotted against the membrane potential. In Cl^- solution all three lines cross the axis at the same point. Shortly after replacing Cl^- by I^-, the reversal potential of i.j.p.s produced by a stimulation rate of 38/sec (filled circles) occurred at a level about 2·2 mV more hyperpolarized than that produced by 85/sec (open circles).

In the normal Cl^- solution, the reversal potential is almost at the same level as the resting potential, while after replacing Cl^- by I^- the reversal potential shifted towards a hyperpolarized level relative to the resting potential. However, the results in Fig. 6*A* and *B* appear not to be due to the difference between the reversal potential and the resting potential in I^- solution. In Fig. 6*C*, the reversal potential was changed by altering the concentration of the external Cl^-. When the Cl^- was replaced by methylsulphate, the reversal potential shifted by about 13 mV in the depolarized direction relative to the resting potential. After equilibration with low Cl^- solution, readmission of the normal Cl^- solution shifted the reversal

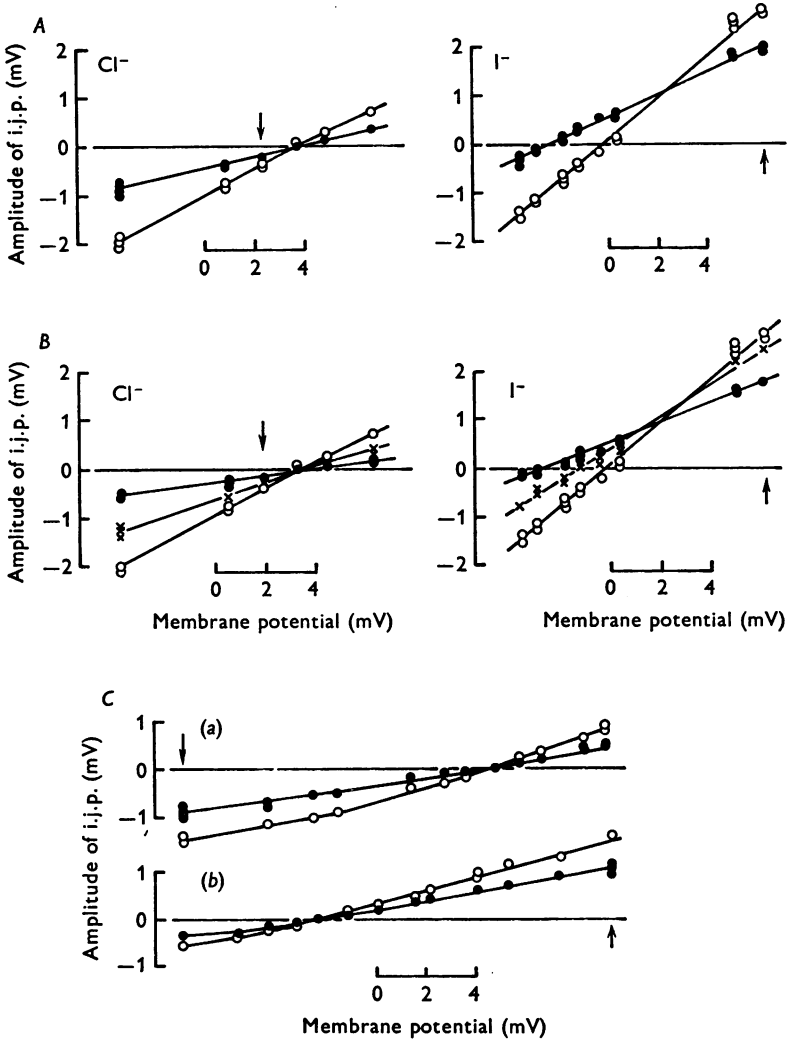

Fig. 6. Reversal potential of i.j.p.s. Abscissae, the membrane potential. Ordinates, the amplitude of i.j.p.s. Minus indicates the depolarizing direction. Arrows indicate the resting potential. *A*, amplitude is measured at 0·1 sec (●) and 1 sec (○) after the start of repetitive stimulation (85/sec). *B*, amplitude of i.j.p.s recorded at 1 sec after the start of repetitive stimulation of three different frequencies. ●, 38/sec; ×, 61/sec; ○, 85/sec. Same muscle fibre as *A*. *C*, amplitude of i.j.p.s recorded when the concentration of Cl^- in solution was changed by substituting methylsulphate for Cl^-. Amplitude is measured at 0·1 sec (●) and 1 sec (○) after the start of repetitive stimulation (83/sec). *a*, recorded immediately after decreasing the external Cl^- concentration from 250·5 to 84·5 mM. The reversal potential shifted by about 13 mV to the depolarizing direction. *b*, the normal Cl^- solution was readmitted after the muscle had been equilibrated with low Cl^- solution. The reversal potential shifted by about 12 mV to the hyperpolarizing direction. Arrows indicate the resting potential.

potential by about 12 mV in the hyperpolarized direction. In both cases no difference was observed between the reversal potentials measured at 0·1 and 1 sec after the start of the stimulation.

These results indicate that shortly after replacing Cl^- by I^- the reversal potential is changed during the course of repetitive stimulation or at different stimulation frequencies. The amount of transmitter released may increase during the course of repetitive stimulation and the maximal amount depend on the stimulation frequency. Therefore, it is suggested that the change in the reversal potential of i.j.p.s in I^- solution may be due to varying concentrations of the transmitter.

GABA potential in I^- solution

GABA was applied iontophoretically and the recording electrode was inserted close to a GABA sensitive spot (Takeuchi & Takeuchi, 1965). Only GABA sensitive spots located within about 300 μ from the end of muscle fibre were used. The reversal potential was measured by passing current through a current electrode which was inserted at about 300 μ from the end of muscle fibre. Under such conditions the membrane between the point at which the current electrode was inserted and the end of fibre would be practically equipotential when the membrane was hyperpolarized. (Since λ was about 1·5–2·5 mm, the potential difference would be within 2%.) GABA potentials recorded in Cl^- solution and those obtained shortly after replacing Cl^- by I^- are shown in Fig. 7*A* and *B* respectively. The GABA potentials were produced by a smaller amount released in *a* and by a larger one in *b*. Upper traces monitor the current pulse for the GABA injection. The membrane potential was changed by passing current through a current electrode and the GABA potential was measured at various membrane potentials. (The membrane potential is indicated by the vertical displacement of the lower trace from the upper trace which corresponds to 75·2 mV in *A*, 72·4 mV in B_1 and 87·5 mV in B_2–B_4.) GABA potentials in *A* were recorded after hyperpolarizing the membrane by 2·4 mV. The reversal potential was at about 3·8 mV in the depolarized direction. When Cl^- was replaced by I^-, application of GABA produced a large hyperpolarization and its time course was prolonged (B_1). Prolongation was especially great when a large dose of GABA was applied. As the membrane was hyperpolarized, the amplitude of the GABA potential decreased and its peak became flat. In B_2 the smaller dose of GABA produced a hyperpolarization while application of the larger dose at the same membrane potential produced first an upward deflexion followed by a slow deflexion in the opposite direction. In B_3, application of a small dose produced little potential change and a larger dose at a slightly depolarized level produced an upward deflexion followed by a small downward de-

flexion. Close examination of B_2 and B_3 shows that immediately after application of the large dose of GABA, a small downward deflexion preceded the upward deflexion. When the muscle was more hyperpolarized, the GABA potential showed a simple depolarization (B_4).

In Fig. 8 the amplitude of the GABA potential is plotted against the membrane potential. Lines drawn through the points produced by a small

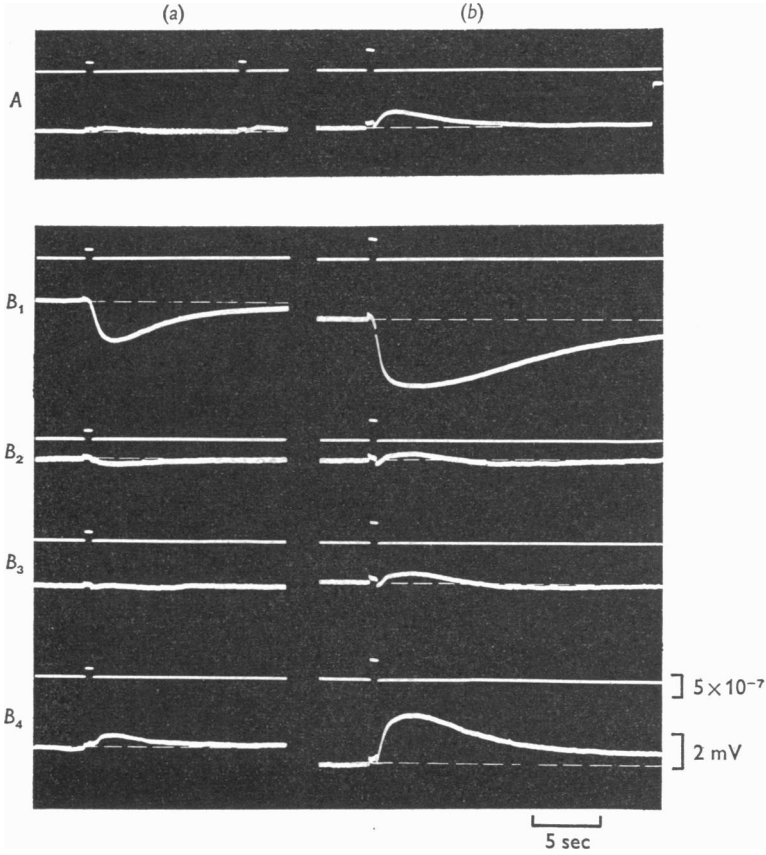

Fig. 7. GABA potentials in Cl^- and I^- solutions. Upper traces monitor the current pulses for GABA injection. The membrane potential was changed by passing current through a current electrode and the GABA potential was measured at various membrane potentials which are indicated by the vertical displacement of the lower traces from the upper traces. Upper traces correspond to the membrane potentials of 75·2 mV (A), 72·4 mV (B_1) and 87·5 mV (B_2–B_4). Small dose was applied in *a* and large dose in *b*. Records in A were obtained in Cl^- solution by hyperpolarizing the membrane by 2·4 mV. The reversal potential was at 3·8 mV depolarized level. Records in B were obtained at various membrane potentials shortly after replacing Cl^- by I^-. B_1 shows the GABA potentials recorded at the resting potential.

dose (filled circles) and by a large dose of GABA (open circles) cross the membrane potential axis at the same point in Cl^- solution. However, shortly after replacing Cl^- by I^-, the reversal potential of the GABA potential produced by small dose of GABA occurred about 1 mV in the hyperpolarized direction as compared with the large dose.

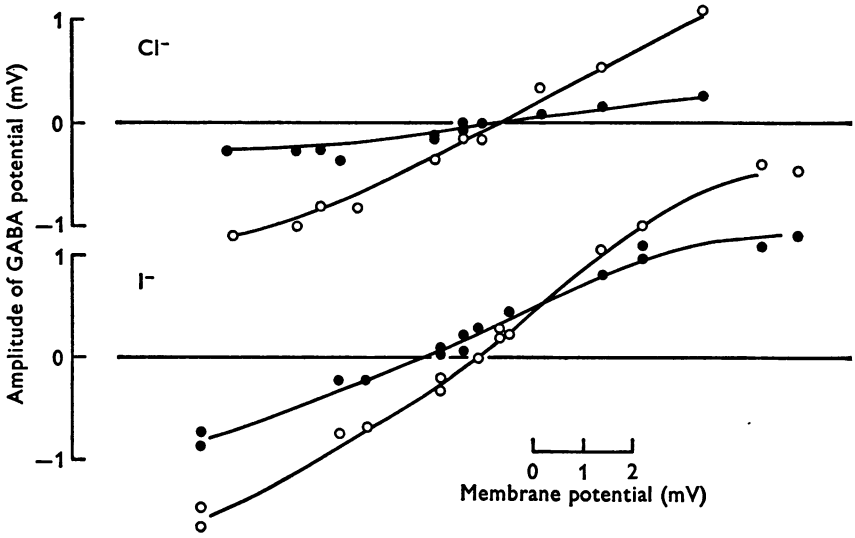

Fig. 8. Reversal potential of GABA potential in Cl^- and I^- solutions. Abscissae, the membrane potential. Ordinates, the amplitude of the GABA potential measured at 1 sec after the application of GABA. Minus indicates the depolarization. ●, amplitude of GABA potential produced by small injection current ($3{\cdot}4 \times 10^{-8}$ C). ○, by large current ($7{\cdot}2 \times 10^{-8}$ C).

When Cl^- was replaced by Br^-, NO_3^- and CNS^-, the reversal potential of the GABA potential shifted to a hyperpolarized level. At the reversal potential, potential changes, of the kind observed in I^- solution, were not detected, although in CNS^- solution, small downward deflexions were sometimes observed preceding the upward deflexions near the reversal potential.

DISCUSSION

The present results indicate that when the external Cl^- was replaced by some foreign anions, GABA produced a conductance increase at a lower concentration, while higher concentrations produced relatively smaller increases than that in Cl^- solution. The most remarkable effect was observed when Cl^- was replaced by I^-. In this solution concentrations of GABA as low as 5×10^{-6} M induced a conductance increase which was 5–10% of the maximal value. This concentration of GABA produced no

appreciable conductance increase in the Cl^- solution. The analysis of the dose–conductance curve indicates that the action of I^- is neither a parallel shift of the curve to lower concentrations nor a general increase in the responsiveness of the membrane. The slope of the dose–conductance curve was decreased in I^- solution. The change in the dose–conductance curve may be attributable to (*a*) anions acting on the receptors and changing their mode of combination with GABA or (*b*) the relative permeability of the inhibitory membrane to anions varying when the concentration of GABA is change. If the conductance increase is induced by a stepwise combination of two molecules of GABA to a receptor, the decrease in the slope of the dose–conductance curve may be explained by the decrease of the dissociation constant of the first reaction relative to the second reaction (eqn. (4) of Takeuchi & Takeuchi, 1969).

When the external Cl^- was replaced by permeable foreign anions such as Br^-, NO_3^-, I^- or CNS^-, the reversal potential shifted to a hyperpolarized level compared with that in the Cl^- solution (Takeuchi & Takeuchi, 1971). In I^- solution the reversal potential shifted to a more hyperpolarized level when activated by lower concentrations of GABA or transmitter than when activated by higher concentrations. An explanation of this result may be that the shift in the reversal potential on varying the GABA concentration is due to a depletion by inward diffusion across the membrane of I^- immediately outside the muscle in the junctional region. Such a process would operate only transiently following the replacement of the external solution with one containing I^-, until I^- came into equilibrium. Since the membrane conductance was measured after equilibration with the external solution, the change in the dose–conductance curve in I^- solution is not attributable to the above process. Alternatively, if the change in the reversal potential on varying the GABA concentration is due to the fact that the relative permeability of I^- compared with Cl^- is higher when activated by lower concentration of GABA than when activated by higher concentrations, the change in the dose–conductance curve in I^- solution may also be explained by a change in the permeability properties of the synaptic membrane.

When the action of transmitter is analysed, it is assumed, in many cases, that the response is proportional to the number of receptors occupied by the transmitter. However, if the permeability properties change depending on the GABA concentration, the possibility arises that the conductance increase produced by GABA may not be linearly related to the number of receptors bound to GABA.

Changes in the reversal potential by varying the concentration of GABA were not observed in foreign anions other than I^-. However, a tendency for such a change was sometimes observed in Br^- or CNS^- solution. There-

fore, a difference in the reversal potential may exist in other anions when the concentration of GABA is altered, but it may have been too small to be detected under the present experimental condition.

I.j.p.s at various membrane potentials in I^- solution were calculated, assuming that the reversal potential changes during the course of stimulation. If the hyperbolic function is expanded and the second term is neglected, eqn. (2) is simplified to

$$V_s = \frac{k}{1+k}(E_0 - V_0). \tag{3}$$

In the present experiments this simplification may introduce only a small error. In eqn. (3), the reversal potential is equal to the equilibrium potential, while in eqn. (2) it may be about 7–8 % larger than the equilibrium potential (see p. 349).

Considering $k = r_m/r_s$ and putting $g = 1/r_s$ and $G = 1/r_m$, eqn. (3) is written as

$$V_s = (E_0 - V_0)(1 + G/g)^{-1}. \tag{4}$$

The conductance of the inhibitory membrane (g) relative to that of the non-synaptic membrane (G) is given by

$$g/G = \left(\frac{E_0 - V_0}{V_s} - 1\right)^{-1}. \tag{5}$$

The reversal potentials of the summated i.j.p.s at various times from the start of the stimulation were measured and are shown in the lower, right part of Fig. 9. g/G was calculated from the i.j.p.s recorded at the resting potential, using eqn. (5) (upper right, record in Fig. 9). I.j.p.s at various membrane potentials were calculated from the g/G and the reversal potential, using eqn. (4). (In these cases the reversal potential (V_R) was used instead of E_0. This may decrease the peak value of g/G by about 10 % but introduce no large error in calculation of the amplitude of i.j.p.s.)

The i.j.p.s calculated and those observed are presented in Fig. 9. Although the assumptions are simple, the agreement between calculated and observed i.j.p.s is fairly good.

If the relative permeability coefficient P_I/P_{Cl} was calculated from the shift of the reversal potential in this special case, it changed from about 1·34–1·23 during the course of the summated i.j.p.s. Relatively low values of the coefficient may be attributable to the fact that the reversal potential was measured some time after replacing Cl^- by I^-.

Changes in the reversal potential caused by different concentrations of GABA or transmitter were relatively small and only detectable in I^- solution. However, this observation may be of some meaning in considering the mechanism of the transmitter action. The currently held views assume that the transmitter combines with receptors which are located on the outer surface of the membrane and opens ionic channels which are preformed pores in the membrane (cf. Eccles, 1964; Ginsborg, 1967). The sieve-like effect of the pore to discriminate the ions by their hydrated ion

sizes is not compatible with observations on the inhibitory junction of the crayfish muscle, i.e. the anion selective properties and the interaction between different anions (Takeuchi & Takeuchi, 1967, 1971). To accommodate the pore structure to the above observations the pore must be charged positively. Alternatively the combination of GABA with receptors may activate or set free the carriers in the membrane which selectively bind anions. In either case, the anion selectivity of the pore or of the carrier may be changed when the concentration of GABA is varied. A simple

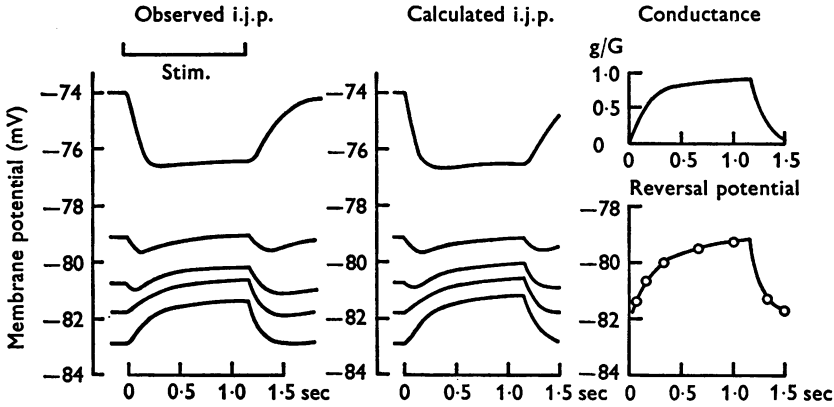

Fig. 9. Time course of i.j.p.s at various membrane potentials. Left, tracings of i.j.p.s recorded shortly after replacing Cl^- by I^-. Stimulation was applied at 85/sec for the period indicated. Uppermost trace was obtained at the resting potential. Middle, amplitude of i.j.p.s is calculated from g/G and the reversal potential using eqn. (4). Upper right, relative conductance of the inhibitory membrane (g/G) was calculated from the i.j.p.s recorded at the resting potential and the reversal potential using eqn. (5). Lower right, reversal potentials at various times from the start of stimulation were measured from the observed i.j.p.s.

explanation may be that GABA combines with the receptor and alters the charge profile of the membrane (by some ways, e.g. as suggested by Watkins (1965)), resulting in a change of the effective field strength of the membrane. In the ion exchange membrane the selectivity pattern is determined by the field strength of the charge (Eisenman, 1965; Diamond & Wright, 1969). With halide anions, the selectivity is in the order of $I^- > Br^- > Cl^-$ in low field strength. As the field strength increases it changes to $Br^- > Cl^- > I^-$ through $Br^- > I^- > Cl^-$. These are the sequences of the relative membrane conductance activated by GABA, when applied in increasing concentrations.

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