J. Physiol. (1963), **167**, *pp.* 374–388
With 11 *text-figures*
Printed in Great Britain

SOME EFFECTS OF CHANGES IN IONIC CONCENTRATION ON THE ACTION POTENTIAL OF SYMPATHETIC GANGLION CELLS IN THE FROG

By J. G. BLACKMAN,* B. L. GINSBORG† and C. RAY‡
From the Department of Pharmacology, University of Edinburgh

(*Received* 14 *November* 1962)

The action potential of sympathetic ganglion cells in the frog, in whatever way it is generated, always includes a phase of after-hyperpolarization, or 'positive phase' (Nishi & Koketsu, 1960; Blackman, Ginsborg & Ray, 1963*a*). A plausible explanation for the existence of such a phase is that the absolute value of the resting potential is less than that of the potassium equilibrium potential and that the depolarization caused by the action potential produces a long-lasting increase in the permeability of the membrane to potassium ions. This explanation (cf. Eccles, 1955), which is identical with that given for the existence of the positive phase of the action potential in cephalopod axons (Hodgkin, 1958) has been subjected to tests, the results of which are described in this paper.

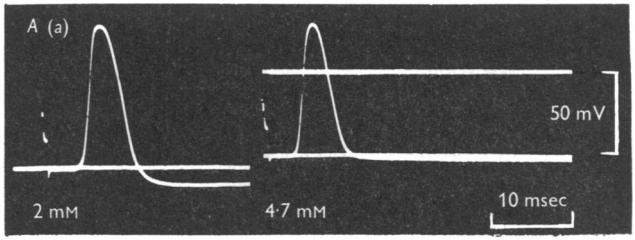
The methods have been described in the preceding paper (Blackman *et al.* 1963*a*).

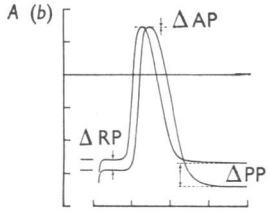
RESULTS

Effect of changes in K^+ concentration

Figure 1*A*, *B* illustrates the effect of increasing the K^+ concentration on the resting and action potentials, in response to antidromic stimulation, of two cells. In the experiment of Fig. 1*A* the initial bathing solution was of the usual composition (see Methods, Blackman *et al.* 1963*a*) and the concentration of K^+ was increased by adding an appropriate volume of isotonic KCl to the bath. In the experiment of Fig. 1*B* the Cl^- in the bathing solution was replaced by methyl sulphate, and $KMeSO_4$ was added to the bath. It is evident that the results are qualitatively consistent with the idea that the positive phase of the action potential reflects an increase in the permeability of the membrane to K^+ ions, since the membrane potential at the peak of the positive phase is considerably more sensitive to the effect of K^+ ions than is the resting potential. Similar results were obtained

* I.C.I. Fellow. Present address: University of Otago, Dunedin, New Zealand.
† Member of External Scientific Staff of the M.R.C.
‡ Colombo Plan Fellow. Present address: Central Drug Research Institute, Lucknow, India.





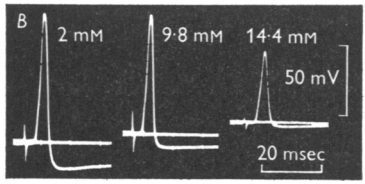


Fig. 1. Effect of increase in external K^+ concentration on the antidromic action potential of two cells. K^+ mM concentrations shown in figure. *A* (*a*) Cell 128, chloride-Ringer's fluid. Zero potential level obtained on removal of electrode from the cell; (*b*) Superimposed tracings of responses shown in (*a*), giving nomenclature used in Table 1. (*B*) Cell 137, methylsulphate-Ringer's fluid.

TABLE 1. Effect of changes in external K^+ concentration on the antidromic action potential (mV)

Cell no.	2 mM-K			4·7 mM-K			7·4 mM-K			
	a.p.	r.p.	p.p.	ΔAP	ΔRP	ΔPP	ΔAP	ΔRP	ΔPP	
125	99	48	29	8	3	13	—	—	—	
126	71	—	19	—	—	—	6	7	19	Normal bathing fluid
128	86	—	11	0	4	12	—	—	—	
159	82	—	12	—	—	—	15	6	14	
166	71	—	28	3	2	5	20	6	19	
173	94	—	20	0	4	8	—	—	—	
130	69	—	13	3	9	14	—	—	—	
*135	71	—	9	0	3	11	—	—	—	Methyl sulphate bathing fluid
*136	73	—	3	5	6	9	—	—	—	
*137	95	56	19	0	0	0	0	7	15	
Mean	—	—	—	3	4	9	10	6·5	17	

a.p. = amplitude of the 'spike' measured from the resting base line; r.p. = resting potential measured from the zero level; p.p. = peak amplitude of the 'positive phase' measured from the resting base line (see Fig. 1). ΔAP. ΔRP, ΔPP are defined as in Fig. 1 *A* (*b*).
*, Micro-electrode filled with 5 M-Na citrate.

from a number of cells, and they are given in Table 1. It can be seen, for example, that an increase in K^+ concentration from its normal value of 2 to 4·7 mM caused a reduction in the absolute value of the resting potential of 4 mV, on the average, whereas the equivalent reduction of the membrane potential at the peak of the positive phase was 9 mV. There was no obvious difference between the results when Cl^- was the principal anion and those in which methylsulphate was used to replace Cl^-. On the assumption that

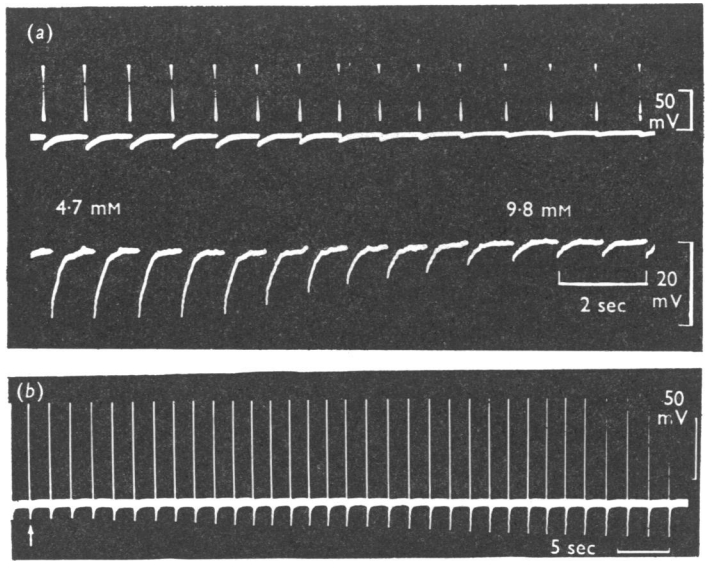


Fig. 2. Effect of altering the external K^+ concentration on the antidromic responses in two cells. (*a*) Cell 137, methylsulphate-Ringer's fluid. The responses were recorded at two different gains. The upper trace shows the full response and shows no change in the spike height. The lower trace (at higher gain) shows the change in the resting level and in the peak amplitude of the positive phase. $KMeSO_4$ (in isotonic solution) added to the bath 10 sec before first action potential shown. (*b*) Cell 173, chloride-Ringer's fluid. K^+-free solution run into the bath, starting at the arrow and continuing throughout the period shown.

methylsulphate is an impermeant anion (see Hutter & Noble, 1960), the contribution made to the membrane conductance by Cl^- ions must be small.

The effect of reducing the K^+ concentration from 2 mM to 0 was examined in two cells. No change occurred in the resting potential, but the absolute value of the membrane potential at the peak of the positive phase increased by 3 and 4 mV, respectively, in the two experiments.

As is shown in Fig. 2, the effects of changes in K^+ concentration occurred rapidly. The delay between the changes in membrane potential and addi-

tion of K^+ to the bath in Fig. 2(*a*) is probably to be accounted for, largely, by the time required for mixing in the bath. The slower change in Fig. 2(*b*) when the concentration of K^+ was reduced from 4·7 mM to 0, reflects the time required to effect the total replacement of the bathing solution.

The effects of changes of K^+ concentration on the positive phase of the action potential in response to orthodromic stimulation were essentially similar to those described above, if account is taken of the more complex form of the positive phase of the orthodromic response (Nishi & Koketsu, 1960; Blackman *et al.* 1963*a*). On some occasions an increase in the amplitude of the negative wave was observed on increasing K^+ (e.g. Fig. 3*A*), indicating that an increase in the intensity of the action of the transmitter had occurred. This may be related to an increase in output of acetylcholine from the presynaptic nerve such as probably occurs in the presence of enhanced K^+ in the mammalian ganglion (Brown & Feldberg, 1936) and at the amphibian neuromuscular junction (Takeuchi & Takeuchi, 1961).

At concentrations of K^+ between about 9 and 11 mM the response to orthodromic stimulation failed (e.g. Fig. 3*A*, *f*). A response to antidromic stimulation usually still remained at the critical concentration for orthodromic block, which indicates that the block occurs along the presynaptic pathway rather than in the post-synaptic cell body. At a slightly higher concentration the response to antidromic stimulation becomes greatly attenuated (Fig. 3*B*, *b*). Evidently, this cannot be due to failure of propagation along the post-synaptic axon, and it is also not due to the inability of the cell to produce an action potential. This is made clear by the records in Fig. 3*B*, *b*, in which it can be seen that although the response to antidromic stimulation is attenuated, the cell is still capable of producing an action potential as a result of spontaneous synaptic activity (see Blackman, Ginsborg & Ray, 1963*b*). Furthermore, the action potential generated by the cell is able to propagate along the post-synaptic axon, since it occludes the antidromic response, when its timing allows it to collide with an action potential travelling antidromically along the post-synaptic axon (Fig. 3*B b*, *c*). A probable explanation for the attenuation of the antidromic response is that the resistance of the cell is reduced as a result of the increased K^+ concentration and 'short-circuits' the action potential in the post-synaptic axon, which is generated across a relatively high resistance. The action potential resulting from spontaneous synaptic activity, however, is associated with the low internal resistance of the 'active' membrane of the whole cell body; it is therefore not attenuated by the inactive post-synaptic axon.

A corollary of this explanation is that the cell body of the sympathetic ganglion cell is capable of actively sustaining an action potential. This

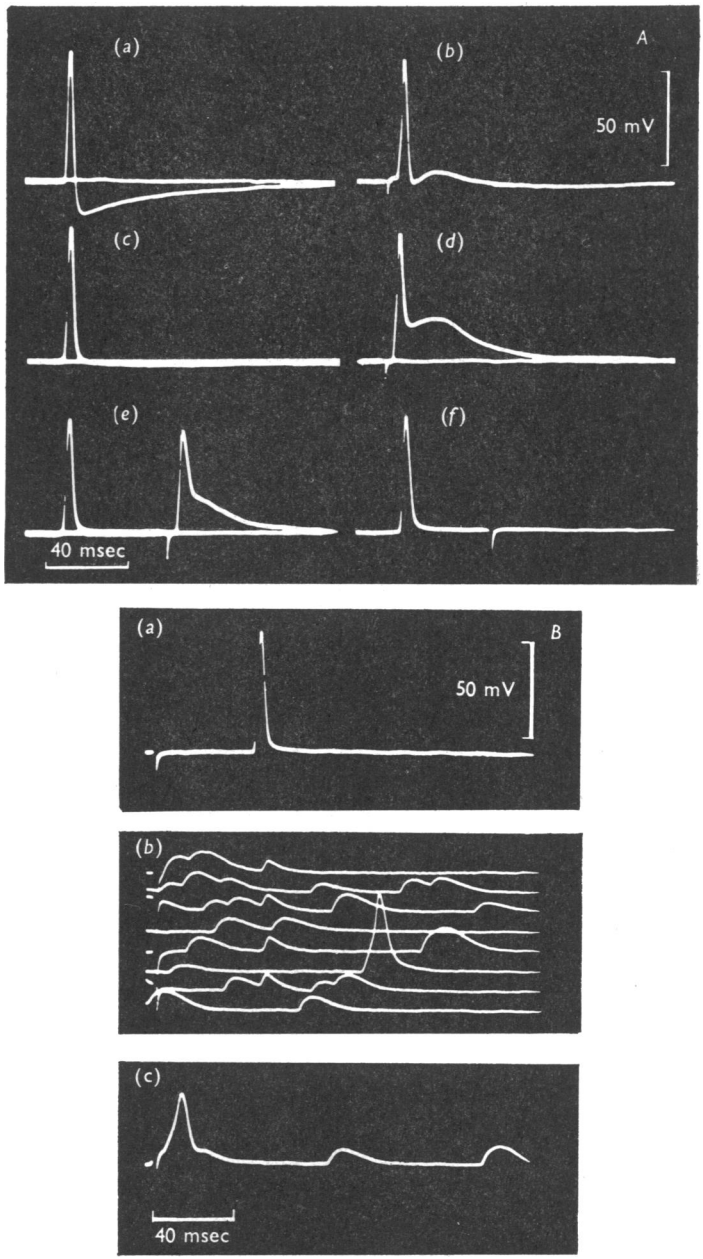


Fig. 3. For legend see opposite page.

conclusion has been reached on other grounds by Nishi & Koketsu (1960) (cf. Eccles, 1961).

In the experiment partly illustrated in Fig. 1*B*, an attempt was made to follow the changes in resting potential with larger changes in K^+ concentration, in a Cl^--free solution. The results are illustrated in Fig. 4. It is evident that the resting potential is dependent on the external K^+ concentration, but attempts to fit the experimental observations to an equation of the type discussed by Hodgkin & Horowicz (1959) were not particularly successful. The observations were, however, not very reliable, since changes in the leakage resistance around the tip of the micro-electrode may well have occurred, especially as a result of the mechanical disturbance involved in adding fluid to the bath.

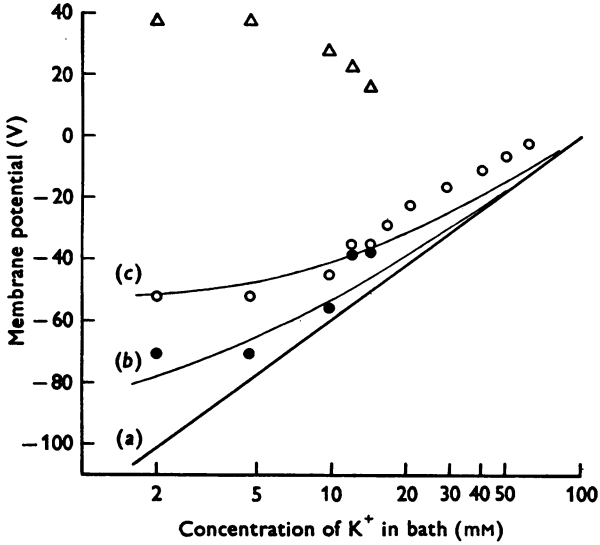


Fig. 4. Relation between $[K]_o$ and membrane potential. Cell 137, methylsulphate solution. △, Overshoot of action potential; ○, resting potential; ●, positive phase. (*a*) Theoretical 'Nernst' relation, $V = 59 \log ([K]_o/[K]_i)$ (*b*) and (*c*) $V = 59 \log ([K]_o + \alpha[Na]_o)/([K]_i + \alpha[Na]_i)$; $[K]_i$ is taken as 100 mM and $[Na]_i$ as 20 mM. α is the presumed ratio between the permeabilities of the membrane to Na and K: in (*b*) α was taken as 0·025 and in (*c*) as 0·1 (see text).

Legend to Fig. 3

Fig. 3. Effect of increase in external K^+ concentration on the antidromic and orthodromic responses from the same cell. Cell 135, methylsulphate-Ringer's fluid. *A*. (*a*) Antidromic response and (*b*) orthodromic response in 2 mM-K^+. (*c*) Antidromic and (*d*) orthodromic response in 4·6 mM-K^+. (*e*) 7·1 mM-K^+, antidromic and orthodromic responses on the same trace. (*f*) 9·6 mM-K^+, note the orthodromic response is blocked. *B*. Attenuation of antidromic action potential by high external K^+ concentration. (*a*) antidromic response in 9·6 mM-K^+ (cf. *A*,*f*). (*b*) 11·8 mM-K^+; note miniature synaptic potentials and small antidromic response on alternate traces with stimulus artifacts. (*c*) Antidromic response blocked by action potential produced by spontaneous synaptic activity (see text). The conduction velocity in the post-ganglionic axon was about 0·24 m/sec.

Effects of changes in Cl^- concentration

The fact that changes in K^+ concentration affect the membrane potential in the same way, whether Cl^- or $MeSO_4^-$ are the main external anions, suggests as has already been mentioned that Cl^- ions do not contribute greatly to the conductance of the membrane either in its resting state or at the peak of the positive phase. Further evidence which suggests that this is the case was obtained in experiments in which the membrane potential was observed during the replacement of a Cl^- bathing fluid by a bathing fluid in which $MeSO_4^-$ was substituted for Cl^-. In several experiments no immediate effect on the resting potential was observed (after making allowance for the change in junction potential at the bath electrode). If Cl^- ions contributed significantly to the membrane conductance, a transient depolarization would have occurred (see Hodgkin & Horowicz, 1959).

Effects of changes in Na^+ concentration

In a number of experiments the effects were studied of partial replacement of the NaCl in the bathing fluid (in the presence of 10^{-4} M tubocurarine) by choline chloride, or choline methyl sulphate (which was kindly made by Dr K. A. Scott) or by sucrose. It has been argued that one of the factors responsible for the appearance of the positive phase in the action potential is that the absolute value of the resting potential is less than that of the K^+ equilibrium potential. This in turn implies that the membrane permeability, in the resting state, is not perfectly selective to K^+; an obvious additional ionic species to which the membrane might be permeable is Na^+, and it would then be expected that the replacement of Na^+ by an impermeant cation would hyperpolarize the membrane. This effect, however, was never observed: in the better experiments, in which there was no reason to suspect that the electrode had been partially dislodged during the replacement of the bath, and after making allowance where necessary for changes in junction potential at the bath electrode, there was no change in the resting potential of the cell. This result, however, does not exclude the possibility that Na^+ permeability is responsible for the depressed value of the resting potential with respect to the K^+ equilibrium potential, since there are several alternative explanations for the absence of the expected hyperpolarization, as has been pointed out by Adrian & Freygang (1962) for the case of muscle.

The effect of replacing three-quarters of the NaCl by choline chloride is illustrated in the case of one cell in Fig. 5. It will be observed that the rate of rise and the amplitude of the response to antidromic stimulation were reduced in reduced Na concentration. The reduction in amplitude was, however, consistently found to be smaller than would be expected on the

basis that the amplitude of the 'overshoot' behaves like the e.m.f. of a Na^+ electrode (see Hodgkin, 1958).

Another effect observed in low Na^+ concentrations was presumably related to the greater difficulty in invasion of the cell body by the impulse travelling antidromically along the post-synaptic axon. The inflexion on the rising phase of the antidromic response sometimes seen in normal solution (Nishi & Koketsu, 1960) was accentuated, and the second of two fairly close

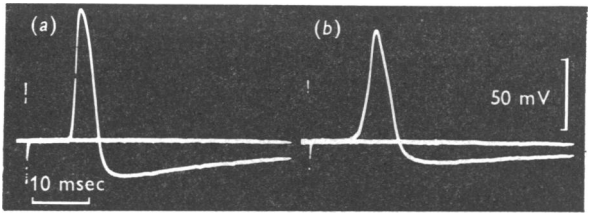


Fig. 5. Effect on 'antidromic action potential' of replacing $\frac{3}{4}$ of the external NaCl by choline chloride (in presence of 2×10^{-4} M tubocurarine). Cell 164. (*a*) Normal bathing solution; (*b*) 'low-Na^+' bathing solution (see text).

stimuli often failed (Fig. 6) producing only an attenuated response of the kind seen in high K^+ concentrations (e.g. Fig. 3*B*). In addition, fairly small reductions in resting potential, presumably due to spontaneous deterioration in the seal between membrane and electrode, caused large reductions in the response to antidromic stimulation (Fig. 7). The records illustrated in Fig. 7 suggest that the increase in permeability to K^+ is graded with the preceding depolarization, having thus the characteristics of 'delayed rectification' as observed in the squid axon (Hodgkin & Huxley, 1952) and the 'slow fibres' of the frog (Burke & Ginsborg, 1956)

Effects of trains of stimuli

Although the results summarized in Table 1 are qualitatively consistent with the conventional explanation for the existence of the positive phase, they are not in quantitative agreement with the idea that the membrane potential at the peak of the positive phase behaves like a K^+ electrode. The maximum change observed when K^+ was increased from 2 to 4·7 mM was 14 mV. The equivalent change in the e.m.f. of a K^+ electrode would have been $59 \times \log 2{\cdot}35$ mV or, about 22 mV. A similar sort of discrepancy has been observed in the case of the squid axon and a number of possible explanations for it have been given (see Frankenhaeuser & Hodgkin, 1956). One contributing factor might be that the K^+ concentration in the immediate neighbourhood of the cell might be higher than that in the bath, and this would lead to a false estimate of the change in e.m.f. of an equivalent K^+ electrode. The sympathetic ganglion cell of the frog is

closely invested over most of its surface by a capsular cell (Taxi, 1961; and unpublished observations) and this might be envisaged to restrict the diffusion of the K^+ which leaks from the cell, in the same way as the Schwann cell around the giant axon of the squid (Frankenhaeuser & Hodgkin, 1956). It might even be argued that the situation in the case of the impaled sympathetic ganglion cell is aggravated by leakage of K^+ into the cell from the micro-electrode (Nastuk & Hodgkin, 1950; Coombs, Eccles & Fatt, 1955), the additional K^+ subsequently having to be extruded initially into

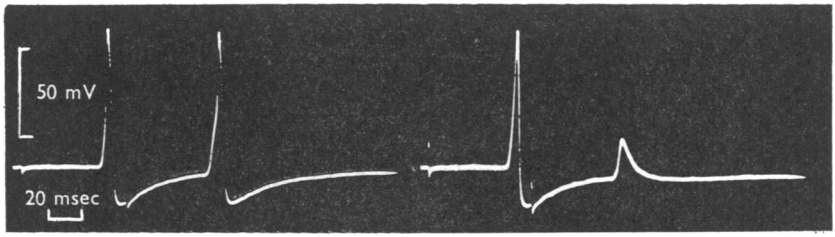


Fig. 6. Reduction in rate of rise of antidromic action potential and occasional failure of response in 'low-Na^+' solution. Cell 143, methylsulphate bathing solution, 77 mM-Na $MeSO_4$ replaced by 141 mM sucrose. Both traces show responses to two successive antidromic stimuli (see text).

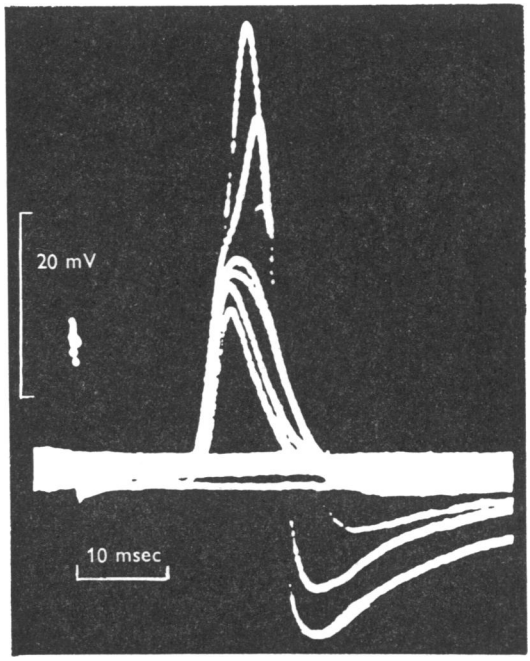


Fig. 7. Change in response to antidromic stimulus with change in resting potential, in low Na. Cell 144, bath solution as in Fig. 6 (see text).

the narrow space between the cell and the capsule. The influence of K^+ leaking from the micro-electrode, however, appears to be small, since as can be seen from Table 1 there was no consistent difference between the sensitivity to K^+ of the positive phase of action potentials recorded from cells impaled with electrodes filled with KCl and from cells impaled with electrodes filled with Na citrate. Leaving aside the effect of leakage from the micro-electrode, if K^+ accumulates in the immediate neighbourhood of

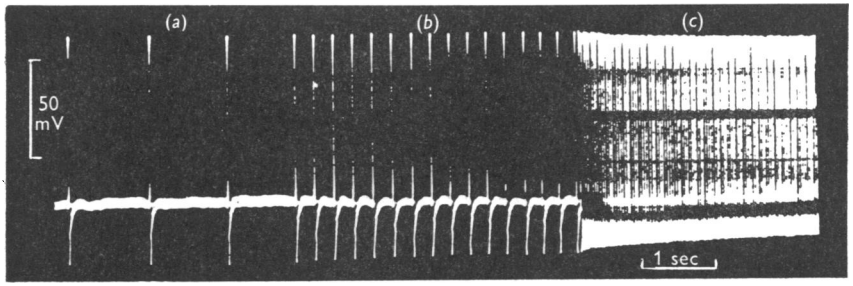


Fig. 8. Effect on antidromic response of increase in rate of stimulation. Cell 170. (*a*) 1/sec, (*b*) 4/sec, (*c*) 29/sec. Note the small decline in amplitude of the overshoot in (*c*) (cf. Frankenhaeuser & Hodgkin, 1956).

the cell it would be expected (Frankenhaeuser & Hodgkin, 1956) that during a train of repetitive responses the K^+ concentration would build up and reduce the membrane potential (i.e. its absolute value) at the peak of the successive positive phases. That this effect does occur is illustrated in Fig. 8 (see also Table 2). The high noise level makes accurate measurements impossible, but it seems that the time course of the absolute value of the membrane potential (v) at the peak of the positive phase during the train of stimuli may be fitted approximately by the relationship

$$v = v_\infty + (v_0 - v_\infty) \exp(-t/\tau)$$

where v_0 refers to the value of the potential in a response to an isolated stimulus and v_∞ to a stimulus during the train at equilibrium. In five different cells the value of τ varied from about 0·5 sec to 1 sec. Values for $v_0 - v_\infty$ are given in the final column of Table 2 in a number of experiments; v_∞ generally refers to the value of v 10 sec after the beginning of the train. In support of the idea that the depression of the absolute value of the membrane potential at the peak of the positive phase is, in fact, due to K^+ accumulation around the cell, are the following observations (see Frankenhaeuser & Hodgkin, 1956). The time constant, τ, appeared to be independent of the stimulation rate; the amplitude of the depression increased with increased frequency of stimulation; and for a given frequency, the amplitude appeared to be a maximum at the normal external concentration of K^+.

From a comparison of the values shown in the final column of Table 2 and those in column 7 of Table 1 it may be seen that with one exception (cell 166), stimuli at the rate of 30/sec do not increase the external K^+ concentration by more than 2·7 mM. On the assumption that repolarization after the peak of the action potential is accomplished by the efflux of K^+ from the cell, the minimum net efflux is probably of the order of $(24 \times 10^{-6}) \times (100 \times 10^{-3}) \times 10^{-5}$ mole/cm²/action potential or 720 pmole/cm²/sec at a stimulation rate of 30/sec. This assumes a membrane capacity of 24 μF/cm² (Nishi & Koketsu, 1960) and a voltage swing of 100 mV. No information is available on the resting leakage of K^+ from ganglion cells, but if it is of the same order as that which occurs from cephalopod axons (50 pmole/cm/sec; Hodgkin & Keynes, 1955), the K^+ concentration around the cell, at rest, will not differ appreciably from the bulk concentration in the bathing fluid.

TABLE 2. Reduction in the absolute value of the membrane potential corresponding to the peak of the positive phase during trains of antidromic stimulation

Cell no.	$[K]_o$ (mM)	Amplitude of: Action potential in response to isolated stimulus (mV)	Amplitude of: Positive phase of response to isolated stimulus (mV)	Frequency of stimulation (stimuli/sec)	Change in membrane potential at peak positive phase (mV)
153	2	66	14	18	†0·5
159*	2	82	12	6 30	0·8 3·0
164	2	92	15	20	2·7
166*	2 7·4	71 45	28 15	6 32 6 32	†2·5 9·5 1·2 3·0
170	2	76	25	5 29	†3·5 7·5
173*	2 4·7 0	94 88 89	20 11 31	6 13 21 6 10 6 13	3·0 5·0 6·0 †1·5 3·0 2·5 4·0
183	2	85	23	10	3·0

* See also Table 1.

† Mean values from two or more trains of responses.

The effects of trains of stimuli on the positive phase of the action potential in response to orthodromic stimuli are more difficult to interpret, because the amplitude of the positive phase is affected by the concurrent activity of the transmitter (see Blackman *et al.* 1963*a*), and the intensity of this activity fluctuates during repetitive activity (see Blackman, Ginsborg & Ray, 1963*c*). In cells in which transmitter activity was not very intense, however, it could be seen that the positive phase of the orthodromic action potential behaved in much the same way as that of the antidromic action potential during repetitive stimulation (Fig. 10).

A feature of the records shown in Figs. 9 and 10 which invites comment is the behaviour of the membrane potential *between* responses. Evidently, its absolute value *increased*, and the normal resting potential was not restored

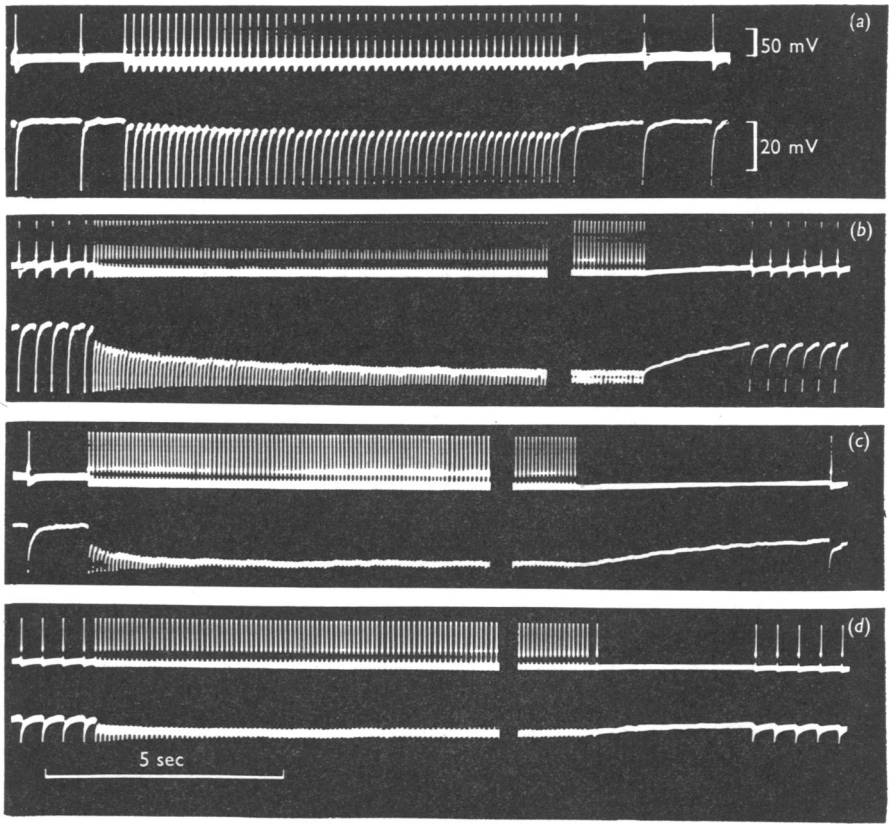


Fig. 9. Effect on positive phase of 'antidromic action potential' of increase in rate of stimulation in different external concentrations of K^+. Cell 173 (cf. Table 2). In each record upper and lower traces recorded simultaneously. K^+ concentrations are 0 in (*a*) and (*b*); 2 mM in (*c*); 4·7 mM in (*d*).

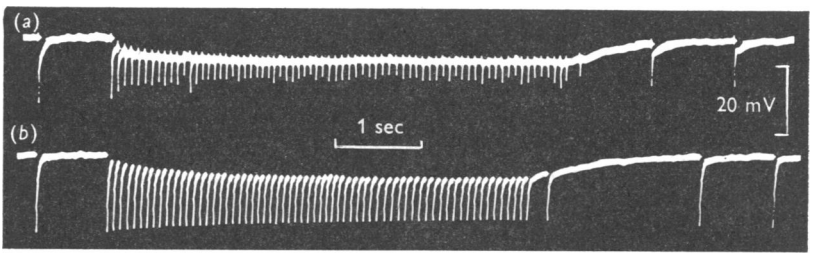


Fig. 10. Effect of repetitive stimuli (at about 12/sec) on the 'positive phases' of (*a*) the orthodromic responses and (*b*) the antidromic responses. Cell 183. Only the positive phase of each action potential is seen in the recording conditions used (high gain and a slowly moving film). Note the decrease in the peak amplitude of the positive phase, the increase in the absolute value of the resting level between stimuli, and the slow return of the resting potential at the end of the higher rate of stimulation (see text).

for some time after the end of the train of stimuli. The phenomenon might be explained either by a depression in Na^+ permeability or a prolongation of the phase of increased K^+ permeability of the membrane, and it clearly requires further investigation. The magnitude and time course of the effect appears to be similar whether the stimulation is antidromic or orthodromic (Fig. 10). The effect shown in Figs. 9 and 10 does not invariably occur in so

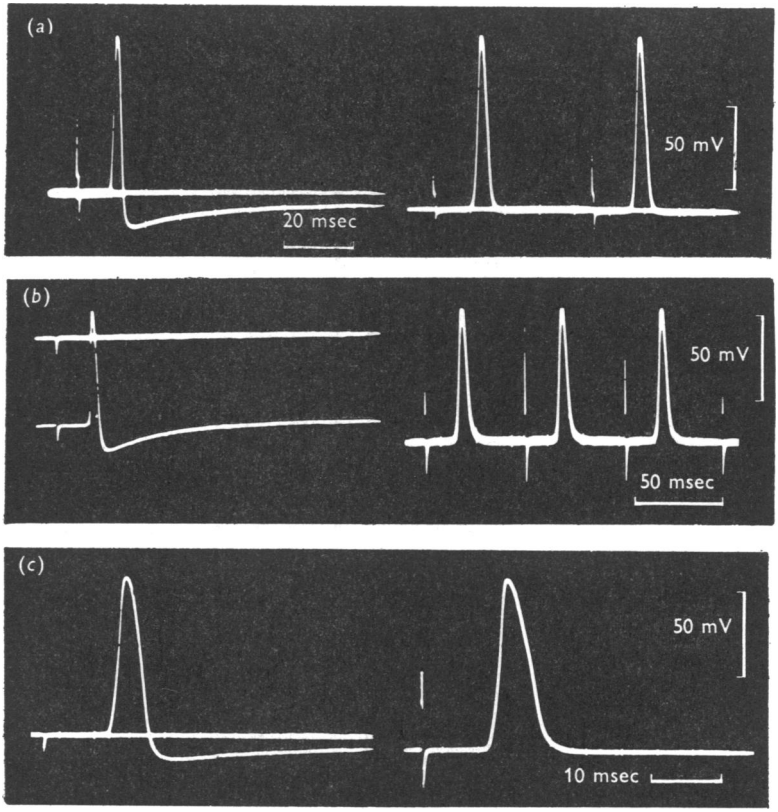


Fig. 11. Effect of rate of stimulation on 'antidromic action potential' at equilibrium (see text). In each record, left-hand trace is response to isolated stimulus, right-hand trace is response during repetitive stimulation. (*a*), Cell 173 (cf. Fig. 9*c*), stimulation rate *ca.* 23/sec; (*b*), cell 153 (zero line obtained on withdrawal of electrode), *ca.* 20/sec; (*c*) cell 164, *ca.* 20/sec.

marked a way (e.g. Fig. 8) (and it was not seen in four experiments in which the bathing solution contained 10^{-4} M tubocurarine) but in a number of experiments it was sufficiently great to abolish the positive phase of the action potential (Fig. 11). Although the time course of the development of the increase in membrane potential between stimuli is similar to that of the

depression of the membrane potential at the peak of the positive phase, there seems no reason, at present, to suppose that the phenomena are related.

DISCUSSION

The results which have been described appear to be consistent with the hypothesis stated in the introduction for the origin of the positive phase. A long-lasting positive after-potential also follows the 'spike' of nerve fibres, especially after repetitive stimulation, and a different mechanism for this after-potential in C fibres has been proposed by Ritchie & Straub (1957) and Greengard & Straub (1958). They have suggested that after activity, the initial increase in $[K]_o$ which accumulates immediately around the nerve fibre because of a diffusion barrier, and is responsible for a negative after-potential, is converted into a deficit by rapid metabolic retrieval by the cell; the resting potential then increases as a consequence of the abnormally low $[K]_o$. For several reasons this does not appear to be a plausible explanation for the present results; one finding obviously inconsistent with this explanation is that, unlike the case for the mammalian C fibre, reduction of $[K]_o$ to zero (see e.g. Fig. 2*b*) causes little if any increase in resting potential, although it does cause an increase in the positive phase.

The results have not made clear why the resting potential differs from the K equilibrium potential so greatly as to allow the existence of a positive phase of as much as 20 mV. One explanation which has not been excluded is that the difference is entirely due to the leak around the micro-electrode. The effect of the 'shunt' caused by such a leak on the membrane potential may be large, because the resistance of the cell is high (*ca.* 20 MΩ; Nishi & Koketsu, 1960). This is unlikely, however, to be the sole cause, because action potentials recorded from mammalian ganglia with external electrodes also include a positive phase.

SUMMARY

1. The effects have been studied of changes in the external concentration of K^+, Cl^- and Na^+ on the action potential, recorded with an intracellular electrode, of sympathetic ganglion cells in the frog (*Rana pipiens*).

2. The absolute value of the membrane potential at the peak of the positive phase is reduced to a greater extent than it is in the resting state by an increase in K^+. The substitution of methyl sulphate for Cl^- has no effect on the membrane potential. These facts are consistent with the idea that the positive phase is due to 'delayed rectification'.

3. During trains of stimuli there is a reduction in the amplitude of the positive phase of successive action potentials, due in part probably to an accumulation of K^+ in the immediate neighbourhood of the cell.

REFERENCES

ADRIAN, R. H. & FREYGANG, W. H. (1962). The potassium and chloride conductance of frog muscle membrane. *J. Physiol.* **163**, 61–103.

BLACKMAN, J. G., GINSBORG, B. L. & RAY, C. (1963*a*). Synaptic transmission in the sympathetic ganglion of the frog. *J. Physiol.* **167**, 355–373.

BLACKMAN, J. G., GINSBORG, B. L. & RAY, C. (1963*b*). Spontaneous synaptic activity in sympathetic ganglion cells of the frog. *J. Physiol.* **167**, 389–401.

BLACKMAN, J. G., GINSBORG, B. L. & RAY, C. (1963*c*). On the quantal release of the transmitter at a sympathetic synapse. *J. Physiol.* **167**, 402–415.

BROWN, G. L. & FELDBERG, W. (1936). The action of potassium on the superior cervical ganglion of the cat. *J. Physiol.* **86**, 290–305.

BURKE, W. & GINSBORG, B. L. (1956). The electrical properties of the 'slow' muscle fibre membrane. *J. Physiol.* **132**, 586–698.

COOMBS, J. S., ECCLES, J. C. & FATT, P. (1955). The specific ionic conductances and the ionic movements across the motoneuronal membrane that produce the inhibitory post-synaptic potential. *J. Physiol.* **130**, 326–373.

ECCLES, J. C. (1961). The mechanism of synaptic transmission. *Ergebn. Physiol.* **51**, 299–430.

ECCLES, R. M. (1955). Intracellular potentials recorded from a mammalian sympathetic ganglion. *J. Physiol.* **130**, 572–584.

FRANKENHAEUSER, B. & HODGKIN, A. L. (1956). The after-effects of impulses in the giant nerve fibres of *Loligo*. *J. Physiol.* **131**, 341–376.

GREENGARD, P. & STRAUB, R. W. (1958). After potentials in mammalian non-myelinated nerve fibres. *J. Physiol.* **144**, 442–462.

HODGKIN, A. L. (1958). Ionic movements and electrical activity in giant nerve fibres. *Proc. Roy. Soc.* B, **148**, 1–37.

HODGKIN, A. L. & HOROWICZ, P. (1959). The influence of potassium and chloride on the membrane potential of single muscle fibres. *J. Physiol.* **148**, 127–160.

HODGKIN, A. L. & HUXLEY, A. F. (1952). The components of membrane conductance in the giant axon of *Loligo*. *J. Physiol.* **116**, 473–496.

HODGKIN, A. L. & KEYNES, R. D. (1955). The potassium permeability of a giant nerve fibre. *J. Physiol.* **128**, 61–88.

HUTTER, O. F. & NOBLE, D. (1960). The chloride conductance of frog skeletal muscle. *J. Physiol.* **151**, 89–102.

NASTUK, W. L. & HODGKIN, A. L. (1950). The electrical activity of single muscle fibres. *J. cell. comp. Physiol.* **35**, 39–73.

NISHI, S. & KOKETSU, K. (1960). Electrical properties and activities of single sympathetic neurons in frogs. *J. cell. comp. Physiol.* **55**, 15–30.

RITCHIE, J. M. & STRAUB, R. W. (1957). The hyperpolarization which follows activity in mammalian non-medullated fibres. *J. Physiol.* **136**, 80–97.

TAKEUCHI, A. & TAKEUCHI, N. (1961). Changes in potassium concentration around motor nerve terminals, produced by current flow, and their effects on neuromuscular transmission. *J. Physiol.* **155**, 46–58.

TAXI, J. (1961). Étude de l'ultrastructure des zones synaptiques dans les ganglions sympathiques de la grenouille. *C.R. Acad. Sci., Paris*, **252**, 174–176.