ON THE TWO COMPONENTS OF THE ACTION
POTENTIAL FROM SINGLE NODES
OF RANVIER IN POTASSIUM-RICH MEDIA

Saburo Hashimura*

Department of Physiology, Faculty of Medicine,
Kyushu University, Fukuoka

Since the discovery of the restoration of excitability of the nerve in potassium-rich media by application of anodal current (LORENTE DE NO, 1947; SATO and FUKUDA, 1953), particularly recently, many investigations on the behaviours of various excitable membranes in potassium-rich media have appeared (STÄMPFLI, 1958; MUELLER, 1958a, b and c; TASAKI, 1959; LÜTTGAR, 1959 and 1960; MEVES, 1960 and 1961; WRIGHT and OYAMA, 1961; SEGAL, 1958; MOORE, 1959; HAGIWARA et al, 1961). The fact that the excitable membranes can produce an active response in the potassium-rich solution which is completely deprived of sodium has been admitted by many investigators. On the nature of the activity in the potassium-rich solution considerations have been made either on the basis of ionic theory or on other theories. Those who stand on the ionic theory investigate the active response in the potassium-rich solution as an activity of potassium carrier system (LÜTTGAR, 1960; MEVES, 1960 and 1961; WRIGHT and OYAMA, 1961). On the contrary, some other investigators interpret the fact as manifestation of electromotance (MUELLER, 1958c) or of the two stable states (TASAKI, 1959; SPYROPOLLOS, 1961). Here the active response in potassium-rich media was investigated and it was found that the active response was composed of, at least, two mutually independent processes which depended on sodium and potassium ions respectively.

METHODS

Singly dissected myelinated nerve fiber of frog (Rana nigromaculata) or toad (Bufo vulgaris japonica) was mounted on a specially designed nerve chamber (Fig. 1). The test Ranvier's node (N1) was placed in the central gap which was constructed with two thin glass tubings arranged coaxially with a small distance of about one hundred microns. One end of the tubing was connected to a three-way cock channeled with the solution suppliers. Rotation of the cock could channel the tubing to either

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* 橋村三郎
FIG. 1. Sketch of the nerve chamber. The dashed part is a copper plate attached downside of the Ringer pool N₂ to compensate the capacitative leakage and is shown in broken line in Fig. 2. See text for further explanation.

FIG. 2. Schematic diagram of the electric circuit with the nerve fiber in place. N₁ is the test node. N₀ and N₂ are anaesthetized with cocaine. Two stimulator (Stim 1 and Stim 11) are arranged in parallel and the stimuli are applied through 500Ω resistor. One of the stimulator is used, in most case, as the anodal current source for membrane potential change. S₁ and S₂ are switches. The dotted line represents the compensation of capacitative leakage.
one of the solutions with which the test Ranvier's node could be completely surrounded. The maximum flow rate was about two milliliter per minute, so the environmental solution of the test Ranvier's node could be exchanged within a few seconds completely. The neighbouring two nodes (N₁ and N₂) were anaesthetized with cocaine.

The responses of the test node were recorded in conventional way (Fig. 2).

The normal Ringer solution contained 112 mM NaCl, 2 mM KCl, 2 mM CaCl₂ and 2 mM NaHCO₃. The potassium-rich test solutions were prepared by replacing sodium with the same amount of potassium or by adding solid potassium chlorid, the solution being hypertonic in the latter case.

The experiments were carried out from 1958 to 1960. No significant differences were observed between the frog and the toad nerves.

RESULTS

When potassium-rich solution (20 mM Na and 100 mM K) was applied for a certain period to the Ranvier's node, there took place depolarization and repolarization. The whole process was presented in Fig. 3A. Often there was seen irregular oscillation of the membrane potential during the membrane potential shifts. The lower row in Fig. 3 (C and D) was obtained at faster sweep speed, and showed the oscillation clearly. The medium solution was

Fig. 3. The whole time course of the depolarization and repolarization by application of potassium-rich solution. The potassium-rich solution contained 100 mM KCl and inversely reduced NaCl keeping the other ions constant. The upward arrow indicates the application of potassium-rich solution and the downward the Ringer. The slanting arrows in B indicate the application of anodal pulses. By the third anodal pulse, there can be seen the transitory membrane potential level. Also, one can note the temporarily increased depolarization after breaking of the anodal pulses. Note the exponential time course of the sweep.
flowing continuously throughout the experiment, so either the appearance of the oscillation or the slower time course of the recovery at the repolarization could be considered to possess some particular significance, on which, however, no further description will be given in this paper.

When an appropriate anodal current pulse was applied to the depolarized membrane, the membrane potential took a particular time course; i.e. the membrane was hyperpolarized undergoing a transitory membrane potential level (see Discussion) whose duration depended on the strength of the current. The height of the transitory membrane potential level was significantly higher than the normal resting level (Fig. 3B). After the membrane was repolarized by the applied anodal current beyond the transitory membrane potential level, an active response by either cathodal stimulus or breaking of the anodal current could be initiated. The response to the breaking of the anodal current is shown in Fig. 3B. In these cases, the response took place at a critical membrane potential, as reported by many investigators in various tissues.

The active response in potassium-rich media is very similar to the normal action potential (Fig. 4C and D). In this experiment the membrane potential was kept several ten millivolts higher than that of normal resting level only for the sake of technical easiness. In this case the concentration of sodium was normal and that of potassium was in excess, so the applied solution was correspondingly hypertonic, but the hypertonicity to such extent did not show

![Fig. 4. The action potentials in normal Ringer solution (A and B) and in potassium-rich medium (C and D). In B and D, the extent of hyperpolarization was changed stepwise. Note the insignificant change for the spike component. In C and D, the medium contained 112 mM Na and 60 mM K.](image-url)
much effect qualitatively. One can note that the contour of the active response in potassium-rich solution, though it is inexcitable without being repolarized by the anodal current, is quite similar to that of normal action potential except the later part. Hence, it will be called "K-excess action potential" hereafter. A significant difference between the normal action potential and the K-excess action potential is the time course of the later part, the prolonged tail.

When the membrane potential level was changed, no particular change was seen in the normal action potential (Fig. 4B) except the slight change of the spike height. In the case of K-excess action potential (Fig. 4D), however, the later part changed its time course and extent significantly, leaving the spike unchanged.

When the sodium concentration was changed keeping the excess potassium constant, the spike height decreased depending on the sodium concentration, but the later part of the K-excess action potential did not change qualitatively (Fig. 5A to D). On the other hand, when the sodium concentration was kept constant and the potassium concentration was changed, the spike height did not change, though no clear difference was seen in the prolonged tail either (Fig. 5E to H). These results suggest that the earlier part (spike) of the K-excess action potential is effected by sodium but the later prolonged part is not, and though it is not decisive in this experiment, these two components behave mutually independently.
When the interval of two successive stimuli was very short, the spike induced by the second stimulus decreased its height like the normal action potential in the relative refractory period (Fig. 6). On the contrary, the later part developed for some time and showed a significant summation.

**Fig. 6.** Effect of the second stimulus applied at various intervals after the first stimulus. Sodium and potassium concentrations were both 60 mM.

In Fig. 7, stimuli of constant strength but various durations were applied to the test node in various sodium and potassium concentrations. From A to D the sodium concentration was decreased and the potassium concentration was correspondingly increased. In A, when the duration of the stimuli was short, no active response took place, but when the duration was long enough the spike potential was initiated being followed by a slight prolonged tail. When the duration of the stimuli became longer the spike did not change but the prolonged tail became more and more significant. These phenomena are clearer in B to D. In B to D, the prolonged tail developed progressively, and finally it became regenerative; i.e. it developed even after cessation of the stimuli.
showing a tendency to reach a certain membrane potential level. The spike potential changed its height depending on the concentration of sodium, but its general character was quite similar to that of normal action potential. On the contrary, the prolonged tail developed independently of the sodium concentration or the initial spike component.

One of remarkable changes in Fig. 7 is the much prolonged tail which is quite similar to the plateau phase in many kinds of action potential. The configuration of the plateau was clearly demonstrated in Fig 8. In these experiments environmental sodium was got rid of and was replaced by potassium. When the stimulus was weak, there appeared the prolonged tail in K-excess action potential to various extents, but when the strength was slightly increased, there took place a regenerative response in all-or-none manner with a certain threshold (Fig. 8A). The regenerative all-or-none manner response decreased its height gradually showing remarkable plateau phase and finally reached the initial membrane potential level (Fig. 8B to D). The duration of this response could be controlled by the membrane potential level, and if the membrane potential level was properly adjusted (lower membrane potential), it continued up to several seconds or more. The maximum height of the response was plotted against potassium concentration and was presented in Fig. 9. Because the height was measured under various extent of hyperpolarizing current to keep the membrane active, the actual height must be higher by the i-r drop by the anodal current, though no correction was carried out in this case. The plotted results approximately coincided with the membrane depolarization by the application of excess potassium (dotted line in the figure, quoted from Huxley and Stämpfli, 1951). Though it was not shown precisely, these phenomena could be observed regardless of the presence or absence of sodium ions. So it
FIG. 9. The maximum potassium action potential height plotted against potassium concentration in logarithmic scale. The dotted line was quoted from Huxley and Stampfl (1951). The maximum height was measured under the anodal current application without correcting the i-r drop by the current.

can be concluded that this regenerative response can take place independently of the spike which has intimate relation with sodium ions.

In Fig. 10 nearly the same experiment was carried out. In A, there can be seen the spike component and the prolonged tail. In B, the nerve membrane

![Diagram](image_url)

**Fig. 10.** Effect of cocaine on the K-excess action potential. B was cocainized. Note the disappearance of the spike component in B.

was treated with small amount of cocaine and the spike component disappeared, but prolonged tail remained unchanged. This experiment confirms the fact that the spike and the prolonged tail can be initiated mutually independently.

It is well known that appropriately depolarized membrane initiates anodal
break excitation readily. In the nerve treated with excess potassium the membrane was depolarized, and therefore it is probable that an anodal break response is initiated with ease in such preparation. Such experiments were presented in Fig. 11. When excess of potassium concentration was 20 mM, there took place anodal break excitation after appropriate current application (Fig. 11A). If the duration of the anodal current was too short, the off response could not take place and only graded potential was seen. When the duration became longer the graded potential developed and finally there appeared the action potential. The longer the anodal current, the higher the spike height of the anodal break excitation became, finally reaching a maximum steady height (Fig. 11A). When the extent of the potassium excess was greater, the situation was somehow different. With 40 mM potassium application (Fig. 11B),

![Images of anodal break excitation](A to C) and the effect of stimulus strength on the spike height (D) in potassium-rich media. Ionic compositions were in A, 20 mM K and 100 mM Na; in B and C, 40 mM K and 80 mM Na; in D, 60 mM K and 60 mM Na, respectively.

if enough strength of anodal pulse was applied, there could take place anodal break excitation easily. In this case, the time course of the anodal break excitation was quite different from that of the former experiment (Fig. 11A), particularly at the later part; i.e. the spike height increased and reached a maximum, but later it became lower and finally the spike disappeared. In proportion to these phenomena, one can note the peculiar time course of the hyperpolarization, hyperpolarizing response, and the slower rising phase of the anodal break response. It is apparent that, when the hyperpolarizing response
appeared and developed, the rising phase of the anodal break excitation became slower and in proportion to it the spike height became lower (Fig. 11B). In Fig. 11C, the duration of the anodal current was kept constant and the strength of the current was increased progressively. When the current was weak, only local response was seen (Fig. 11C 1), but increased intensity of the current produced an all-or-none spike (Fig. 11C 3). Further increase of the current intensity brought about the disappearance of the spike component leaving behind the slow component (Fig. 11C 4). In the hyperpolarized membrane, when the cathodal stimuli were decreased stepwise, the steep spike component appeared progressively later and became smaller in size and finally disappeared (Fig. 11D). In every case, one can note the correlation between the slowly developing component and the decrease of the spike height.

DISCUSSION

The spike component of the active response in potassium-rich solution containing sodium ions has a time course comparable with that of normal action potential. Its dependence on sodium concentration and other characters are quite similar to that of normal action potential. In the ionic theory, it has been shown that the sodium carrier system is thrown into inactivation by depolarization and that the restoration of the membrane potential brings about the reactivation of the carrier system (HODGKIN and HUXLEY, 1952a and b). Therefore, it can be considered that the restoration of the excitability in potassium-rich solution is mainly resulted from reactivation of the sodium carrier system by the anodal current application, and the spike component of the K-excess action potential coincides with it.

An interesting fact in this experiment is the existence of a slow component which follows the spike and can take place independently.

As has been shown in the result, this slow component depends on potassium ions in the environment. With appropriate strength of the stimulus, it can be initiated in all-or-none manner, and its initiation is quite similar to that of normal action potential in which the local response swings to the all-or-none action potential at the critical firing membrane potential level. Hence, because of the similarity of the phenomena between the sodium carrier system in the normal action potential and the slow component of the K-excess action potential, it can be considered that the potassium carrier system takes the role for the slow component. The prolonged tail in the K-excess action potential can be investigated as the local response of the potassium carrier system or its abortive form.

When an anodal current pulse is applied to the KCl-treated Ranvier's node, there can be seen the transitory membrane potential level. The membrane changes its resistance discontinuously at this membrane potential level (MOORE,
1959; LÜTTGÄU, 1960; HASHIMURA, unpublished data), and the critical membrane potential level beyond which the membrane can initiate the K-excess action potential coincides with this level. Therefore, it can be considered that the potassium carrier system inactivated by depolarization can be reactivated enough at this membrane potential level and that the slow time course of the transition means the slowness of the reactivation process. LÜTTGÄU considered three states for potassium carrier system (LÜTTGÄU, 1960), and inactivated and reactivated states here presented correspond to his Inaktiver Zustand and Ruhezustand respectively.

Decrease of the spike height in the anodal break excitation with longer anodal pulse can be investigated as follows. As the sodium carrier system can be brought into reactivation even by a short anodal current pulse because of its faster time course, there can take place the spike with the appropriate anodal current pulse being its height in proportion with the extent of reactivation. On the other hand, the potassium reactivation is very slow, and after the membrane potential recovers beyond the transitory membrane potential level, the reactivation of potassium carrier system takes place too, and the potassium begins to take the role simultaneously. Then, if the threshold membrane potential level for the potassium business is lower than that for sodium, the potassium business may precede the sodium's. In the voltage-clamp experiment there has been shown the lower threshold for potassium current (WRIGHT and OOOYAMA, 1961). So, there can occur inactivation of sodium carrier system during the slow depolarization by potassium action potential, finally resulting complete inactivation of the former. This consideration can be proved clearly by the concomitance of the slowly rising phase and decrease or disappearance of the spike component after the transitory membrane potential level is reached as shown in Fig. 11. The facilitation of the sodium business by faster depolarization before the occurrence of sodium inactivation (Fig. 11D) confirms these consideration.

The lower threshold for the potassium business, though it was not shown directly in this paper, and its all-or-none character lead to the deduction that the potassium business can take place before the sodium business in certain experimental condition. Hence, it is clear that the potassium carrier system can take place independently from the sodium carrier system. On the other hand, the sodium business can take place during the potassium's is in inactivation (Fig. 11 for example). Therefore, it is safe to say that these two processes are mutually independent though in the usual experimental condition they take place in turn. So, the initiation of the slow component even after the block of the sodium carrier system by cocaine application can be understood easily.
SUMMARY

1. With the single Ranvier's node of frog and toad, the active response in potassium-rich media (nominated here as "K-excess action potential") was studied.
2. The active response in the potassium-rich media could be separated in two components with their difference in characters for the time course, refractory, dependence on sodium and potassium ions, sensitivity to cocaine, threshold membrane potential level for firing and the time course of reactivation.
3. These two processes were the faster component and the slower one, and coincided with the sodium carrier system and potassium carrier system in the ionic theory, respectively.
4. It was presented that both of sodium and potassium carrier system behaved similarly in many respects except the significantly slow time course of the latter.
5. From the experimental facts it was concluded that these two components were mutually independent processes.
6. Some results on the anodal break response were presented and the interpretation was made in the above stated conclusion.

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TWO COMPONENTS OF ACTION POTENTIAL


