AN EXPERIMENTAL ASPECT OF THE HYPERPOLARIZATION OF S. J. P.

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In a previous paper (1) the experiments were performed from the point of view that the hyperpolarization following the S. J. P. (small-nerve junctional potential) was an essential component of an S. J. P. In fact, most of S. J. P.'s obtained were followed by the phase of hyperpolarization, but in very infrequent cases no hyperpolarization of which causes remain obscure can be observed. As its cause someone considers the effect of injury. The upper S. J. P. with a hyperpolarization in Figure 1 was obtained, in spring, from a muscle which showed a typically intensive spike potential, however, the lower S. J. P. without a hyperpolarization, in Figure 1, was, in summer, recorded from a muscle from which a relatively lower spike potential was taken. But this correlation does not be exactly. Moreover, in that paper (1) it was described that the

Figure 1. S. J. P. with and without a hyperpolarization,
capable of observing in the normal muscle.

process for the depolarization of S. J. P. and that for its hyperpolarization existed independently. This fact may be supported by the description of Stephenson (2) that potassium accumulation and membrane potential change are not significantly correlated.

The present paper focuses on the process determining the hyperpolarization (always

outside-positive after potential) capable of observing in the S. J. P. Because the synaptic potential such as E. P. P. decays monophasically or recovers without a positive after potential, suggesting the passive recovery of the active membrane, S. J. P. may be not a simple synaptic potential.

It is probable that. S. J. P. is the synchronized activity of non-conducted responses at a few small-nerve junctions in a tonic fiber, however, if these unitary non-propagatable responses have no positive after potential it can not be considered that S. J. P. has a following hyperpolarization.

METHODS

Small-nerve junctional potential (S. J. P.) was obtained from the isolated sciatic-ili-ofibularis preparations of frogs by the microelectrode technique. The experiments were carried out in all seasons, however, the results in summer were poor.

The control S. J. P. without any treatment and S. J. P. treated afterwards by metabolic inhibitors were analysed from the point of the time course. In this time the former was followed by a hyperpolarization while the latter lost the phase of hyperpolarization. These analyses have been performed on the basis that the hyperpolarization is a proper manifestation peculiar to the S. J. P. of the frog's skeletal muscle.

RESULTS AND DISCUSSION

With respect to the response of de-and hyper-polarization of an S. J. P. to metabolic inhibitors, it was suggested that both phases were grounded by another metabolic basis (1). Simultaneously, it was thought that S. J. P. did not be a simple synaptic potential and its hyperpolarization might be associated with the cell activity near the cell membrane.

The previous report (1) indicates, as described in Table 1, the metabolic inhibitors such as sodium fluoride or monoiodoacetic acid etc. revealed the difference in their inhibition activity, especially upon the hyperpolarization of an S. J. P. In detail, it is supposed the hyperpolarization is established by the cellular metabolism considerably

TABLE 1
Responses of a hyperpolarization of S. J. P. to drugs.

acetylcholine	vagostigmine	sodium fluoride (5 mM)	monoiodoacetic acid (1 mM)
resists more than depolarization	resists more than depolarization	disappears more rapid than depolarization	remains unchanged longer than depolarization

affected by the action of sodium fluoride. Tosteson and Johnson (3) postulate that 10^{-2} M. NaF has little effect on glucose consumption and increases the free diffusion of K^+ , and 10^{-3} M. IAA inhibits glucose consumption and inhibits the K^+ influx moderately. The concentration used by them, however, is relatively higher than that by this author. If this experimental consideration is applied to these author's data, the

disappearance of a hyperpolarization by the use of sodium fluoride can be explained by the fact that the potassium equilibrium potential has not been able to be established by the increase of the free diffusion of K^+ . It is, also, evidently that a hyperpolarization is closely related to the metabolism. With regard to the metabolism-dependent hyperpolarization, though it was reported (4) the after potential is sensitive to the change of membrane potential the change of the hyperpolarization did not associate with the change of the resting membrane level in the author's observations.

On the other hand, the depolarization and the hyperpolarization of an S.J.P. which represent the post-synaptic events show an unequal sensitivity to the local application of the metabolic inhibitors. Since the resting potential level of a tonic muscle fiber, similar to a twitch fiber (5), situates more negative than the equilibrium potential for potassium ion, the presence of a following hyperpolarization exhibits the transient increase of potassium permeability toward the potassium equilibrium level. Therefore, it is supposed that the factors to affect the hyperpolarization presumably influence on the potassium permeability. Burke and Ginsborg (6) stated the slower time course of S.J.P. was due to the multiple innervation of whole surface of slow fiber membrane. The Figures described here demonstrate that the difference in the slower decaying time course is not due to the different types of the innervation but to the difference of the decay-determining factors.

The time course of the decay of an S.J.P. which is naturally followed by a hyperpolarization has been analyzed. As shown in Figure 2, the decay time course of an S.J.P., originally without a hyperpolarization, declines very steeply and reaches the zero line of a relative size of the amplitude (the ordinate) with requirement of about 36 msec. This time duration is relatively shorter than the decay time duration of an S.J.P. with the hyperpolarization, suggesting the lack of an activity which causes the hyperpolarization. In Figures, the numbers in the abscissa show the time from the

Figure 2. The time course of the decay of S.J.P. originally with and without a following hyperpolarization. S.J.P. with and without a hyperpolarization, capable of observing in the normal muscle.

peak of an S.J.P., and the ordinate demonstrates a relative size of the amplitude in the decay of an S.J.P. at various moments. Therefore, it is presumed the decay of an S.J.P. followed by the hyperpolarization is composed of the relatively rapid decaying phase (the initial part of the decay curve) and the relatively slow phase which is thereafter observed (the terminal phase of the decay curve).

An interesting fact is obtained by tracing the decaying time course of the S.J.P. which is normally or which is treated by the metabolic inhibitors etc. This analysis may inform what determines the decay of S.J.P. As known from Figures 3—6, the decay in S.J.P., which lost its hyperpolarization, observed from a time course, inclines slower than the decay in S.J.P. with the hyperpolarization. The decay of an S.J.P. with a hyperpolarization is slightly more rapid than that of an S.J.P. which has become without a hyperpolarization. This fact indicates that the factor to determine the time course of the decay of S.J.P. is different in the S.J.P. with hyperpolarization and in that without hyperpolarization. When the hyperpolarization of an S.J.P. is extinguished the decay time course, first, moves in all parts of the decaying curve to approach toward the abscissa with the centrality of the same point itself where is a crossing of the original decay of an S.J.P. with the hyperpolarization and the abscissa, and then moves slightly in parallel upwards.

In other words, it is distinct that to make the hyperpolarization extinctive is to lose the function which makes the K^+ transport toward equilibrium potential more posi-

Figure 3. The time course of the decay of S.J.P. before and after the treatment of sodium fluoride. S.J.P. before the treatment is characterized by a following hyperpolarization.

Figure 4. The decaying time course of an S.J.P. which has a following hyperpolarization, and the decaying time course when this S.J.P. has lost its hyperpolarization by the monoiodoacetic acid.

Figure 5. The time course of the decay of a control S.J.P. followed by a hyperpolarization and the change of the time course of the decay of this S.J.P. when its hyperpolarization is lost by the treatment of acetylcholine.

Figure 6. The time course of the decay of S.J.P. with a hyperpolarization in normal state and that of S.J.P. of which hyperpolarization is disappeared by treating with vagostigmine.

tive. Namely, the decay of S. J. P. with a hyperpolarization is probably established by the cooperation of two processes, a passive K^+ transport process and an active process to make it more intensively. And the latter process is perhaps maintained by the constant energy supply due to a glycolysis because the glycolysis-inhibitor extinguishes the hyperpolarization. The former passive process remains as far as the depolarization can be taken place.

Moreover, as known from Figures, a difference exists between the decay of S.J.P. which has no hyperpolarization and the decay of S.J.P. of which hyperpolarization has been lost.

All muscle responses are expressions in varying degrees of both tonic and tetanic components. And it is supposed that the contraction of a fast muscle fiber is caused by the propagated contraction taking place in whole fiber surface while that of a slow fiber is due to the simultaneous synchronized activation of whole surface. If the role of action potential of every muscle fiber is to generate the contraction of the contractile actomyosin it can be thought that the spike potential or S.J.P. similarly causes the contraction of actomyosin. If, as reviewed by Gelfan (7), the existence of different contractile proteins is assumed in the slow and fast fibers, the spike or S.J.P. must be respectively required for generating the contraction of each protein. However, assuming that the same contractile protein is contained in both muscle fibers, the overshoot is not always necessary for this contraction. Therefore it is supposed that only the ionic process across the membrane during the synaptic potential, E.P.P. and S.J.P., may be related to the contraction. Some author (8) discussed on the relationship between the muscle contraction and the critical amount of resting potential decrease necessary for it. If these considerations being accurate, it is the question that is there any functional role in the hyperpolarization of an S.J.P. Of course, the fact that the resting potential of a slow fiber is less than that of a twitch fiber indicates the difference in membrane characteristics in both fibers. This is, also, supported by a fact of the difference in responsibility of E.P.P. and S.J.P. to the treatment of DNP. Schulte and Henatsch (9) reported that the electrical responses in "twitch Typ" and in

"tonic Typ" are caused by "Alpha Endorgane" and "Gamma Endorgane" respectively. This postulation presumably reveals that two types of responses in two muscle fibers are only due to the style of each junction.

In future, the gap between the electrical phenomena at the membrane surface of muscle fibers and the contraction of actomyosin must be investigated.

SUMMARY

The hyperpolarization of an S.J.P. obtained from an isolated frog's iliofibularis by the intracellular microelectrode study has been analysed.

It has been clarified that the metabolism which is especially inhibited by the sodium fluoride is closely attributable to the following hyperpolarization of an S.J.P.

The decay of an S.J.P. originally without a hyperpolarization is different from that of an S.J.P. which has lost its hyperpolarization. The decay of the former declines along a steep inflected curve, while the decay of an S.J.P. originally with a hyperpolarization declines relatively slowly. The decay of an S.J.P. which is originally followed by the hyperpolarization is established by two processes, a passive process which is physicochemically, and an active metabolism-dependent process, especially inhibited by the sodium fluoride.

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