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CHANGES PRODUCED BY SODIUM-FREE CONDITION IN THE RECEPTOR POTENTIAL OF THE NON-MYELINATED TERMINAL IN PACINIAN CORPUSCLES

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In order to study the effect of change in the ionic composition of the external fluid on the activity of the non-myelinated nerve terminal of Pacinian corpuscles, DIAMOND, GRAY and INMAN[4] adopted a method of perfusing a Pacinian corpuscle, because the lamellae surrounding the terminal act as a diffusion barrier and it is hardly possible to study the effect of modifying the solution when the solution outside a corpuscle being changed[5,7]. With the above technique DIAMOND *et al.*[4] have shown that perfusion of a Pacinian corpuscle with a Na-free solution abolished the nerve impulse from the corpuscle in less than 2 min after the purfusion and that the maximum receptor potential was reduced in 11-30 min to 10% of the initial value. From this experiment they have concluded that the receptor potential results from a transport of charge mostly by Na ions across the non-myelinated terminal membrane.

Recently SATO and OZEKI[13] and OZEKI and SATO[11,12] succeeded in recording the receptor potential and the all-or-nothing impulse from the non-myelinated nerve terminal of Pacinian corpuscles, of which the lamellae had been removed. The preparation employed by them consisted of the non-myelinated nerve terminal surrounded by the central core, measuring about 50 μ in diameter (see Plate 1 of OZEKI and SATO[11]). This preparation seemed to be suitable for studying the effect of ionic change in the solution on the activity of the terminal because of the absence of the diffusion barrier. Therefore the experiments were undertaken to investigate changes in the receptor potential at the non-myelinated nerve terminal after the saline solution surrounding the terminal had been replaced by Na deficient solutions, either choline or Li solution.

The results reported here are rather preliminary because it is thought that

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they should be published preferably together with the publication of the results of the experiments by NISHI[9] using a perfusion technique.

METHODS

The materials and methods were almost the same as those described by OZEKI and SATO[11, 12]. The outer lamellae surrounding the central core of the Pacinian corpuscle, isolated from a mesentery of an anesthetized cat, were removed with a pair of needles. The central core, measuring about 50 μ in diameter and containing the non-myelinated nerve terminal, was mounted on one of the saline pools and the axon connected with the terminal on the other, both saline pools being separated by a air gap of 0.2–0.3 mm in width. Potential changes generated at the terminal and axon were recorded across the air gap with a c.r.-coupled amplifier having a time-constant of 1 sec. Mechanical stimulation of the terminal was carried out with a fine glass stylus placed on the corpuscle at one end and attached to the Rochelle salt crystal at the other, the crystal being driven with a pulse generator. A mechanical compression lasting a few msec and having a moderate and constant strength was applied to the terminal throughout an experiment.

Replacement of the saline solution, in which the terminal was immersed, by a sodium-deficient solution was made with a small glass pipette, first by drawing out all the fluid in the pool and subsequently by filling the pool with a new solution.

The sodium deficient solutions were prepared by replacing all NaCl in the saline solution (mM: NaCl 154, KCl 5.6, $CaCl_2$ 2.16, $NaHCO_3$ 2.40) by either choline chloride or lithium chloride.

RESULTS

The difficulties in performing this kind of experiments are that the activity of the non-myelinated nerve terminal often deteriorated spontaneously and that the terminal was sometimes damaged either on mounting the preparation on the air-gap or in exchanging the solution. Therefore, it was necessary to check in each experiment whether the terminal, after being re-immersed in the saline solution, was capable of eliciting the receptor potential of similar magnitude to that obtained before immersion in a Na-deficient solution, and the experiments, in which the receptor potential had not been recovered after re-immersion in the saline solution, had to be discarded.

The results of three experiments on the effect of replacing NaCl by choline chloride on the receptor potential are demonstrated in FIG. 1. All the experiments were carried out on the terminals, in which impulse activity had been abolished spontaneously. In these three experiments the amplitude of the receptor potential suddenly increased immediately after replacement of the saline by choline solution and was gradually reduced to 25–50% of the original value within about 60–90 min. Upon re-immersion of the terminal in the saline solution the receptor potential amplitude was recovered again to a value which is greater than the original value. The sudden increase in the receptor potential

amplitude immediately after the replacement of the saline by choline solution and after restoration of the terminal into the saline solution is probably an artifact caused by a change in the resistance of the axon across the air gap during the exchange of solutions, because the receptor potential, increased after restoration of the terminal in the saline solution, was gradually reduced to the original value.

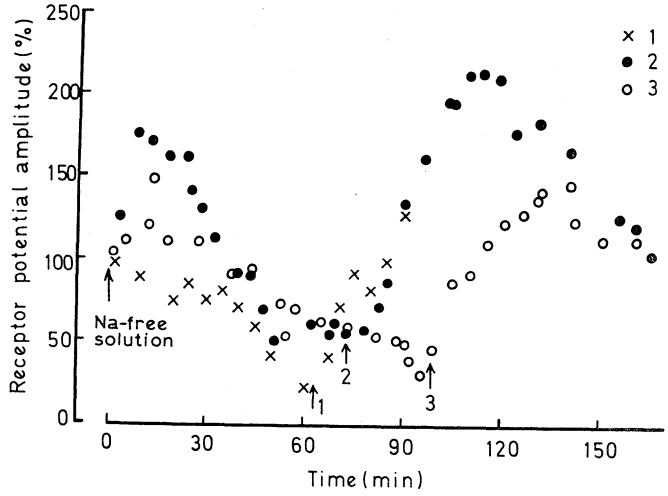

FIG. 1. Time course of change in the amplitude of the receptor potential after replacement of the normal saline immersing the non-myelinated terminal by choline solution. The vertical axis indicates the receptor potential amplitude relative to that before replacement of the saline solution, and the horizontal axis the time after the replacement. Each symbol indicates a series of experiments. At the lefthand arrow the saline was replaced by choline solution and at the subsequent arrows the choline solution was again replaced by the saline.

When the saline solution immersing the terminal was replaced by Li solution a gradual decrease of the receptor potential amplitude, preceded by its sudden augmentation, was observed, as shown in FIG. 2. However, the decline of the receptor potential in Li solution is small compared with that in choline solution: In FIG. 2 the receptor potential amplitude was reduced to 70% of the original value within about 60–90 min. After re-immersing the terminal in the saline solution, the receptor potential recovered roughly to the original value. This indicates that the decrease in the receptor potential amplitude is attributable to the effect of Li ions on the terminal but not to its spontaneous deterioration. The reduction on the receptor potential in Li solution would progress gradually if the terminal is kept immersed in Li solution, but it could not be determined in the present experiments how much the receptor potential is reduced maximally in the Li solution.

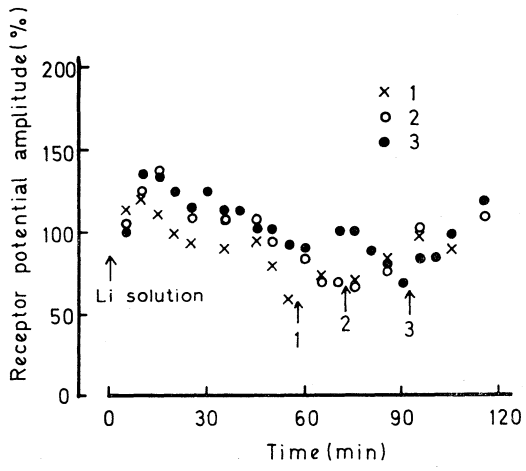

FIG. 2. Time course of change in the amplitude of the receptor potential after replacement of the normal saline by Li solution. Each symbol indicates a series of experiments. At the lefthand arrow the saline was replaced by Li solution and at the subsequent arrows the Li solution was again replaced by the saline.

DISCUSSION

The results of the present experiments at least indicate the following two facts: i) Replacement of Na by choline or Li in the solution bathing the non-myelinated terminal reduces the receptor potential gradually and ii) the reduction is smaller in Li solution than in choline solution. The fact that the amplitude of the receptor potential is reduced markedly in Na-free solution is in agreement with the results by DIAMOND *et al.*[4], but the magnitude of the reduction in the present experiments is small and the time course of the reduction is slow compared with those observed by DIAMOND *et al.*[4], whose results indicate that the amplitude of the receptor potential decreases in 11–30 min. to a constant value of about 10% of the original value. In the experiments shown in FIG. 1 the receptor potential would have been reduced more if the replacement of the choline solution by the saline were made at a later stage. Therefore the receptor potential amplitude maximally reduced by sodium-deficient condition cannot be assessed in the present experiments, but the results in FIG. 1 indicate a slower time course of the reduction of the receptor potential than that obtained by DIAMOND *et al.*[4] The difference in the time course of the reduction is attributed to the difference in the method how the saline solution was replaced by Na-deficient solution. By the perfusion method adopted by DIAMOND *et al.*[4] and NISHI[10] the solution reaches the immediate outside of the terminal quickly, while by the method adopted in the present experiments the new solution does not reach so quickly

outside the terminal because the central core, surrounding the terminal and measuring about 50 μ in diameter[12], probably acts as a diffusion barrier.

The second fact revealed in the present experiments that the reduction of the receptor potential amplitude in Li solution is small and slow compared with that in choline solution indicates that Li ions can substitute for Na ions in generating the receptor potential. If the former cannot substitute for the latter, the receptor potential would have been reduced to the similar extent as observed in choline solution. However, ability of Li ions in generating the receptor potential is not considered to be the same as that of Na ions, because the receptor potential is reduced in amplitude in the former solution. Two possibilities for causing the reduction of the receptor potential in Li solution may be considered. The first is the gradual depolarization of the terminal membrane in Li solution, such as observed in the myelinated nerve[2], the skeletal muscle fiber[1,15] and the mammalian papillary muscle[3]. The other is a gradual increase in Li concentration inside the terminal, such as seen in the skeletal muscle fiber[1,8,15] and the papillary muscle[3].

The receptor potential of the stretch receptor of the crayfish varies in proportion to the membrane potential[14] and there is also evidence suggesting that similar relationship exists between the receptor potential of the non-myelinated terminal of Pacinian corpuscles and the resting potential[5,9]. In the frog sartorius muscle fiber the membrane is first hyperpolarized by about 3-4 mV immediately after the exposure of the muscle to Li RINGER's solution and this is followed by a gradual depolarization of a few mV 2 hr after the replacement of RINGER's solution by Li RINGER's solution which amounts to about 10 mV 5 hr after the replacement[15]. Therefore if the similar depolarization occurs at the membrane of the non-myelinated terminal after the exposure of the terminal to Li solution, the receptor potential would be reduced in amplitude. However, the magnitude of reduction in the receptor potential, expected to be produced by the depolarization, is rather small to account for the reduction of 30% of the original amplitude in 60-90 min after the replacement of the normal saline by Li solution: In the stretch receptor cell the depolarization of 10 mV causes a reduction of the receptor potential by about 20%. Therefore the reduction of the receptor potential in Li solution would be attributed mainly to a gradual increase in Li concentration inside the terminal. In the frog sartorius muscle fiber the internal Na concentration $[Na]_i$ decreases to less than one-third of the normal value and the internal Li concentration $[Li]_i$ rises to a value almost equal to $[Na]_i$ in the normal fiber within 1 hr after exposure of the muscle to Li RINGER's solution, and $[Li]_i$ increases gradually with time[15]. If such a marked increase in $[Li]_i$ occurs in the non-myelinated nerve terminal and if the non-myelinated terminal undergoes a transient increase in the permeability of the membrane to Na as well as Li ions, the impulse activity would be abolished as observed by NISHI[10],

and the receptor potential would be reduced in amplitude because both kinds of potentials are dependent not only on the external Na and Li concentration but also on the internal concentration of Na and Li[6].

SUMMARY

The receptor potential of the non-myelinated terminal of Pacinian corpuscles, of which the lamellae had been almost removed, was recorded in normal saline and Na-deficient solutions. After replacement of the saline solution immersing the terminal by choline solution the receptor potential amplitude is reduced gradually to 25-50% of the original value in 60-90 min, the time course of the reduction being much slower than that obtained by perfusion of the corpuscle. Replacement of the saline solution by Li solution caused a decrease of the receptor potential to 70% of the original value in 60-90 min. The difference in the time course of the reduction in the receptor potential in Li solution from that in choline solution indicates that Li ions can substitute for Na in generating the receptor potential. The decrease in the receptor potential in Li solution has been attributed to accumulation of Li ions inside the terminal.

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