CONDUCTION OF NERVOUS IMPULSES IN

NaCl-DEFICIENT MEDIA

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Since Overton's finding on muscle (1), examination on the effect of Na - deficient media upon the nervous function has been a centre of interest (2- 7). The blocking concentration, or the minimal concentration required for con-duction, has been said to be 10-15% of that of the normal Ringer for nonmy-elinated fibres of the Crustacean (4) and 0.011 M for myelinated fibres of the frog (5), which are almost coincident with that obtained for muscle by Overton (about 10%). However, no precise determination has been made with res-pect to fibres of different types or sizes, although it seems certain that different fibres react in different manners to NaCl-deficiency (6, 7). According to Cres-citelli, B-fibres are most sensitive to lack of sodium; they are blocked earlier than A-fibres and many of C-fibres in one and the same NaCl-deficient fluid; further, there are some differences among fibres belonging to A-group itself. These facts suggest that not only fibre types but also individual fibres of each type resist to NaCl-deficiency in different grades. The present study aims to clarify this point and, at the same time, to carry out observations on some phenomena of conduction NaCl-deficient solutions.

I.METHOD

A sciatic-peroneal nerve was dissected out of a toad (Bufo vulgaris). The epineurium, an effective diffusion barrier (8), was removed by about 1.5 cm. from the bifurcation of the peroneal and fibural nerves towards the periphery, along the peroneal branch. The preparation was mounted on a separating cham-ber, and the test solution was applied on the desheathed part (fig. 1). In most of

FIG. 1. Experimental arrangement for nerve trunk.
N: Nerve trunk, D.N: Desheathed part, S: Stimulus, R: Recording apparatus, a, b, d, e: Normal Ringer, c: Test solution.

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the experiments, the number of fibres was reduced by an amount to simplify the records of the action current. A stimulating current pulse of a 0.5 msec. duration and of a maximal intensity was applied to a central portion of the nerve across a separating wall, and the action current was led off from about the tarsal joint. Fibres examined were those of A-group only. For recording, a 3-stage resistance-capacity coupled amplifier and a Braun tube oscilloscope were employed. The solutions tested were 1) isotonic NaCl solution, 2) normal Ringer solution variously diluted with isotonic sucrose solution, and 3) Ringer solution of which NaCl was partly substituted by cholin chloride.
Examinations were carried out also on single-fibres. The method will be described later.

H. PRELIMINARY EXAMINATIONS

1. Effect of time

Desheathing is very useful for shortening the "diffusion" time, but still we have to wait for some time for deciding the concentration effect. For approxi-mate estimation of this time, we measured the time necessary for complete block in a NaCl-free solution. It was about 30-40 minutes in a whole trunk and

variously shorter in trunks with reduced number of fibres; for example, about 4-5 minutes when reduced to ca 1/3.

2. Configuration of the records in relation to NaCl concentration
In passing through the bath of the test solution, there will occur velocity change in some fibres and block in some others. As the result, the configuration of the records will change correspondingly.
Chief phenomena observed are 1) decrease of the current in lower NaCl-concentrations, and 2) multiple peaks. The latter is not always an abnormal event, but often a single peak became double, or a double peak multiple, in low NaCl-concentrations only. The peak was mostly single when the number of fibres was reduced properly.
The gradual decrease in peak-height is never a simple matter, but the principal cause must be gradual decrease in number of fibres active at the lead-off electrode. For this reason, it was taken as an indication of block of some fibres in the present experiments.

3. Sensitivity of the recording system in relation to number of active fibres
For interpretation of the results obtained, we have to be aware of the sen-sitivity of the recording system in relation to the number of active fibres. By means of the present apparatus, the activity of "five" fibres could be caught with certainty, but that of a single fibre not. Therefore, the true critical NaCl-concentration may be somewhat smaller than that measured out.

III. EXPERIMENTAL RESULTS

1. The case of whole trunks

A nerve trunk was partly desheathed, keeping all fibres intact. First, normal

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Ringer solution was employed as control, and then test solutions, that is, Ringer solution diluted with isotonic sucrose solution in various grades. Each observa-tion was continued for more than 30', until a steady state was reached. The results obtained are shown in fig. 2 graphically. We see there that 1) some new fibres get blocked at every lowering of NaCl concentration, and 2) the peak q which was higher at the beginning became lower than p in the end. This shows, in agreement with Crescitelli (7), differences in manner of reaction of different fibre groups to low NaCl-concentrations.

FIG. 2. Action current-NaCl concentr.-relation (1). Whole trunk. Numerals in brackets indicate grades of dilution.

2. The case of trunks with reduced number of fibres

As stated above, a long time is needed for completion of diffusion in a whole trunk, even if desheathed. The greatest reason must be that there are a great many fibres. As stated already, reduction of fibres to ca 1/3 shortened the time to ca and in addition simplified the wave form greatly. We planned then to re-examine the results, obtained above, under these better con-ditions. Cares were taken to avoid the influences due to injury currents origi-nating from the cut fibres.
An example of the results obtained with Ringer solution diluted with isotonic sucrose solution is shown in fig. 3 and table 1. Evidently, the action current diminishes its size stepwise, with stepwise lowering of NaCl-concentration. At the same time, the conduction time is lengthened gradually. The highest value or the upper limit of NaCl-concentration, at which the action current just begins to decrease, could not be determined precisely. The effect of diluted Ringer

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FIG. 3. Action current-NaCl concentr.-relation (2). Number of fibres reduced. a, m: control (normal Ringer). Temp. 16.0•Ž.

TABLE 1

solution seemed to be somewhat different from that of diluted isotonic NaCl solution, but it could not be worked out clearly.
A remarkable acquisition is that the action current decreases very little to begin with, and then suddenly enormously when the NaCl-content of the bathing fluid is lowered down to about 10% of the normal. This was a most usual and most common occurrence which we encountered. The value "10%" agrees with that hitherto reported as the blocking concentration (4, 5, 7) but in view of the

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present experiment, it is concerned with fibres which are relatively sensitive to Na* -deficiency, because there remain some, though not many, fibres which are still conductive.

3. The case of single myelinated fibres
The objects of this series of experiments are 1) to determine the critical NaCl concentration under conditions free from time-effect, 2) to measure the thresholds together with the action current, in fluids of various NaCl concen-trations, and 3) to explore the relation between fibre diameter and the critical NaCl concentration.

A single node exposed to a narcotic solution is said to get narcotized pro-mptly and show no progression of the effect (9). If this means an immediate contact of the applied fluid with the
nodal membrane, then the method will
be very useful in the present inves-
tigation, too, because it can eliminate
the time-effect which is disturbing.
The experimental arrangement is shown in fig. 4. For isolation of a fibre, Ichioka's (10) simple method was employed. A stimulus (a con-stant current pulse of threshold strength and of a 0.5 msec. duration) was applied to the trunk (fig. 4, A) or directly to the node N2 (fig. 4, B), and the membrane current from N2 was recorded. In trunk stimulation, the test solution was appiled to N1 and N2, while N3 was narcotized by
means of a 3% urethane-test solution.
A part of the trunk near N1 was des-
heathed by about 1 cm., aiming at a
quick affection by the test solution.
In direct stimulation, the test solution
was applied to N2, and a 3% urethane-test solution to N1 and N3. The solutions tested were the normal Ringer and isotonic NaCl solution diluted variously with isotonic sucrose solution.
The results obtained are as follows:
1) The case of trunk stimulation. First, it was confirmed that a test solu-tion manifests its full effect almost momentaneously, showing no progression during an hour after the application. Namely, an action current of a certain definite size appeared promptly as a function of the NaCl-concentration applied. An example of the results obtained is shown fig. 5, A. In •this case, the fibre was 20 it thick and the obtained critical concentration was so small as 2.2-

2.5% of the normal. Later, it was found that a 6 /2 thick fibre gives a value of 12%, which suggested that smaller fibres might generally give larger values.

FIG. 4. Experimental arrangements
for single fibres.
A: Trunk stim (test solution on Ni,
N2 and 3% urethane-test solution on Ni),
B: node stim (test solution on N2, and
urethane test-solution on Ni, Ni).

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A B

FIG. 5. Action current-
NaCl conctr.-relation (3).
Single fibre (20 it thick).
22.5•Ž.
A: trunk stim, B: node
stim, a, i: normal Ringer, b:
isot. sucrose/isot. NaCl=1 :1,
c: 4:1, d: 10:1, e: 20:1,f:

30:1, g: 40:1, h, j: 45:1.

This supposition was put under detailed experimental examinations, which re-sulted in showing that it is really so, as shown in fig. 6 and table 2, last column. The reason for this fact is not quite clear at present. The action potential might be smaller in thinner fibres, but a more probable reason may be that the threshold is higher in smaller fibres (see the next series of experiments).
Fig. 5 shows further that the action current gets weaker and the conduction time gets longer with lower NaCl-concentrations, as was confirmed with trunks. The former, together with the threshold elevation, makes the safety factor for propagation small, which makes the latter comprehensive as a natural sequence of it.

2) The case of node stimulation. The object of this series of experiments
lies in direct examination of the action current and of the threshold in low NaCl-concentrations. The results obtained with fibres of various diameters are

given in table 2, with other kinds of data. It is clear and certain that the action current decreases and the threshold gets high in fluids of low NaCl-concentra-tions. An example is illustrated in fig. 7. It seems highly probable that, at the critical concentration, the intensity of the action current gets equal to the threshold, to bring forth a block.

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TABLE 2

A.C.: action current

FIG. 6. Critical NaCl concentr.-fibre diameter-relation.

FIG. 7. Action current and threshold varying with NaCl-concentr.

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DISCUSSION

I. The critical NaCl-concentration
The desheathing shortens the time for diffusion a great deal, but still some
time is needed for its completion. Therefore, we have to continue each obser-
vation for a sufficiently long time, to determine the critical concentration with
certainty. In works on trunks in the present investigation, the time necessary
for complete block in a Na'-free solution was adopted as the standard. This is
practically admissible, but still there is a slight uncertainty as to whether the
time was enough long or not, because the test solutions always contain more
or less NaCl. It is desirable, therefore, to examine the matter by a method free
from the time effect, if possible. It was for this purpose that we tried experi-
ments on single fibres, where the effect of the applied solution manifests itself
promptly, as was stated previously and observed also by Huxley and Stampfli
(5).
Now, the results obtained on single fibres with an application time of about
1-2 minutes, showed clearly that the critical concentration in question is lower
in thicker fibres, further that the lowest value obtained (2-2.5%) on multi-fibre
preparations is nothing but that of the thickest fibres (about 20 ƒÊ). The fact
that the p-wave, which represents fibres of larger velocities, that is, of larger
diameters, did vanish only lately (fig. 2), became comprehensive, too, from this
view-point.
Hitherto, the critical concentration has been said to be about 10% of the
normal, which is about four times larger than the smallest obtained in the pre-
sent investigation. What is the reason for this discrepancy ? As was clarified
above, the fibre size plays an important role, but it is very improbable that the
previous works were done on thin fibres only. Is it then due to some methodical
difference ? For, in the present investigation, the NaCl-content of the test solu-
tion was normal to begin with, to be lowered gradually, while Crescitelli, who
too obtained about 10% as the lowest value for A-fibres. first made the fibres
non-conductive by applying a NaCl-free solution, and then raised the NaCl-
concentration by degree to determine the critical concentration just necessary
for restoration of conduction. We recognized experimentally that there is a
tendency that our method gives a little too low values and his a little too high
ones, but the difference was too small to be responsible for the observed facts.
Considering possible mistakes or inexactness in preparing the test solutions,
observations were repeated with freshly and carefully prepared solutions, but
the results were the same. The sources of the discrepancy might then lie in
the difference of the materials (frog in Huxley and Sffimpfli, and Crescitelli;
toad in the present experiment) or else in the difference of the time of appli-
cation of the test solutions. For, Crescitelli says that it is a "matter of minutes"
to make a whole trunk non-conductive by means of a NaCl-free solution, and,
perhaps based on this, he seems to have applied test solutions just for a few
minutes. In the present investigation, however, the application time for a whole
trunk was 30-40 minutes, which is about 10 times longer than his. It is greatly
regreted that the cause of the disagreement could not be clarified after all.
But at any rate, the value "2-2.5%" having been obtained without fail on multi-

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fibre as well as large single fibre preparations, the present author is convinced of its correctness. It should be added, however, that it is also certain that the action current diminishes rapidly towards a very low value if the NaCl-content is made lower than about 10% of the normal. This indicates that the majority of the fibres are blocked about in this concentration.

2. Fibre-grouping viewed from critical NaCl-concentration
As described above, fibres are blocked group-wise with lowering of the NaCl-concentration of the external medium. Otherwise expressed, there appears a sort of fibre-grouping with respect to the critical NaCl-concentration. If the concentration. were controlled more finely, the grouping would have been finer too. It is not clear at present, in what relationship this grouping stands to other kinds of grouping, but it seems probably the same in nature with that of a, (3, γ, etc., because they are both in close connexion with the fibre size.

3. Safety factor for propagation
In fluids of low NaCl-concentrations, the action current (C) gets weaker while the threshold (S) gets higher, until finally a block is reached. Now, if the safety factor (f) for propagation in the critical concentration is taken unity, then f in the normal state can be evaluated from the values of C and S obtained

in the normal and critical concentrations respectively. We see from table 2 that f of large fibres thus computed are generally larger than those of small fibres. The highest value obtained was 4.5. This is a little smaller than that of Tasaki (f>5), but the agreement may be said to be fairly good, if we con-sider the difference in the way of measurement.

SUMMARY

Employing sciatic-peroneal nerves (desheathed) of the toad or single myeli-nated fibres prepared from them as material, and taking the action current as the sign of propagation, measurements were carried out among others on the critical NaCl-concentration of the bathing fluid just necessary for conduction. The observations were done on A-fibres only.
The value obtained varied from about 10% to 2% of the normal, according
to the fibre size, small or large. The lowest value obtained in the case of nerve trunks turned out to be that of the largest fibres. We see from these observa-tions that the normal Ringer solution contains NaCl in such amounts as ap-proximately 10 to 50 times larger than the critical. The safety factor for pro-pagation, too, was found larger in larger fibres.

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