JOURNAL OF APPLIED POLYMER SCIENCE VOL. 7, PP. 1265–1271 (1963)

Sign Reversal of Permselective Membranes: A Possible Mechanism for Neural Conduction

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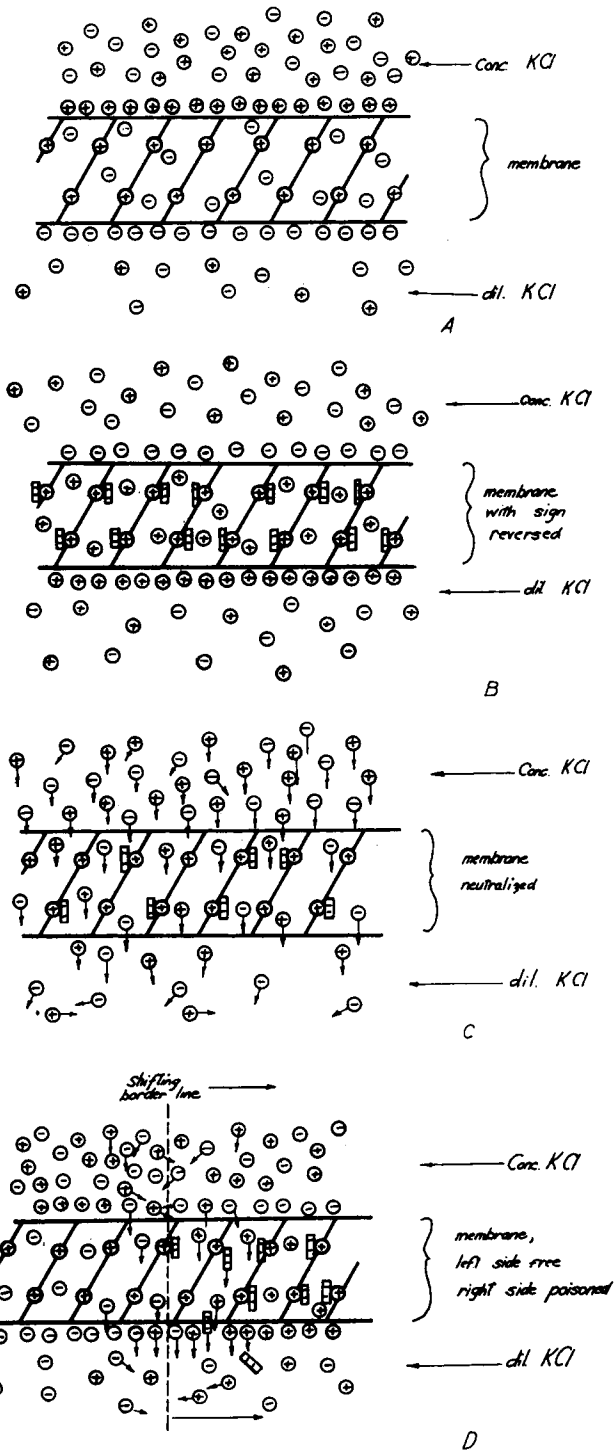
Some charged "permselective" membranes change the sign of their charge if they are in contact with solutions containing counterions of sufficiently high charge density. Owing to the high electric field strength at the surface of these counterions they are attracted so strongly to the fixed ions of the membrane that they become more or less permanently attached to them. If their electric charge is higher than the charge of the fixed ions they overcompensate for it and thus the resultant fixed charge within the membrane changes its sign.[1–3] We have found that they can be removed from the membrane by an electric current.

The same may happen if a covalent bond is formed between the fixed ions within the membrane and some compound with a group of opposite charge in its molecule. Thus, for example, a membrane containing fixed carboxyl, sulfonic acid, or phosphonic acid groups (a cation selective membrane) changes into an anion selective membrane if the acidic groups are esterified by an amino alcohol. For divalent phosphonic acids each OH group has to be esterified to change the total negative charge of fixed ions into a total positive charge. A similar covalent bond can be formed by amidating the acid groups with polyamines, in which case one amino group of the polyamine would give the amide link while the other or others would contribute their positive charge to change the sign of the originally cation-selective membrane. On the other hand, the anion-selective membranes produced by these reactions would change back to cation exchange membranes on hydrolysis of the ester or amido bonds.

If only half of the active sites within a membrane is changed into active sites of opposite sign but same charge, the membrane contains the same amount of positive and negative fixed charges and thus has lost its ion selectivity.

A tube made of an ideal permselective membrane of either sign would be able to separate indefinitely two ionic solutions of different concentration (activity) on either side of the tube wall, while a potential difference would be established across it, due to incipient diffusion of the counterions across the membrane from the concentrated into the dilute solution. Owing to the fact that the co-ions are unable to cross the ideal membrane they cannot follow the counterions, and thus the established potential difference


Conc. KCl
membrane
dil. KCl
A
Conc. KCl
membrane with sign reversed
dil. KCl
B
Conc. KCl
membrane neutralized
dil. KCl
C
Shifting border line
Conc. KCl
membrane, left side free right side poisoned
dil. KCl
D

brings the process to an early halt. However, if there is an area of opposite charge (permselectivity) on this membrane, the co-ions of the original membrane are able to pass at the border of this area while its counterions pass across the original membrane at the other side of the borderline (Fig. 1*D*). This means that diffusion in the direction of the activity gradient becomes possible because of the presence of both sorts of membrane side by side, while it has been impossible across membranes of one sign. It could be said that a ring current flowed around the borderline, if the electric side of the phenomenon only were important. Actually, however, no material current really returns across the membrane, but two currents of oppositely charged ions move across it side by side in the same direction. The distribution of the currents at different distances from the borderline are determined by the relative resistances of membrane and solutions. In consequence of this diffusion of ions the activity gradient decreases in the vicinity of this borderline and with it the potential across the membrane also decreases. It becomes actually zero if the activities or the mobilities of cation and anion have become locally equal. Farther away from the borderline, within the area of inverted charge, the potential itself becomes reversed.

If a mechanism were found by which the diffusion of ions at the borderline reversed the charge of the original membrane, the position of the borderline itself would travel along the length of the tube up to its end, and the position of the area where ions traverse the membrane and where the potential difference becomes zero and then changes sign would travel with it. This phenomenon would be in many respects similar to the propagation of an impulse along a nerve fiber. Experimental evidence has been found by Gengerelli[4] that a magnetic field advances in front of the impulse, and this field could be produced by the aforementioned ring currents' progression along the borderline. A separate mechanism would have to be invoked which restores the original situation shortly after the disturbance has passed, that is, after the sign of the charge at a given point had been reversed for some time.

I do not propose any definite mechanism of this kind. However, I wish

Fig. 1. Crude representation of ionic distribution around normal and "poisoned" charged membranes: (⊕ or ⊖) mobile ions; (⊛) Original positive fixed charge in the membrane; ⊟ double-charged negative "poisoning" counterion; (⊖ or ⊕) ion diffusing along a concentration gradient. (*A*) Normal anion-seelective membrane. No diffusion across membrane after the potential difference has been established by passage of some negative ions. (*B*) Same membrane with reversed sign because of "poisoning" by double-charged counterions. No diffusion across the membrane after the potential difference has been established by passage of some positive ions. (*C*) Half of the positive sites of the normal membrane occupied by double-charged "poisoning" counterions. The fixed charge is just neutralized and the membrane is no longer ion-selective. KCl diffuses across. (*D*) Left side of the membrane cross section is normal, right side is "poisoned." Two streams of ions traverse the membrane in the vicinity of the border line, anions at the left and cations at the right. Ionic distribution is statistically equalized on both sides by lateral diffusion. Some poisoning ions are slowly washed through by diffusing KCl so that the borderline itself is slowly shifted to the right.

to call attention to some possibilities which might be partially responsible for such behavior.

Shifting of Borderline by Loosing "Poisoning" Ions

Suppose a tube to consist of a permselective membrane which has its sign reversed throughout its length by infiltration with counterions of high valency, as described in the first paragraph. Suppose that a difference in concentration of a salt exists between the inside and outside of the tube. A constant potential gradient results across the wall of the tube. To illustrate: Let the original membrane be cation-selective; the "poisoned" membrane of which the tube consists is therefore anion selective. Let the concentration of, say, KCl within be less than around it. The resting potential across the tube will then be negative within.

Now let us pass a localized electric current across a small area of the tube surface which sweeps the "poisoning" cations out of the membrane at this point. Its original cation selectivity is thus restored here. K^+ ions will begin to enter the tube across this area while Cl^- ions will move inwards and parallel to them, a little beyond the borderline where the membrane is still in its reversed state. It is not proven, but conceivable, that the flow of anions would sweep with it the bound "poisoning" cations of higher charge if they are not fixed by extremely strong forces. Once they are removed from the membrane, this area near the borderline is itself depoisoned and becomes cation-selective. The borderline has thus advanced somewhat into the "poisoned," originally anion-selective, area of the membrane, and the process can continue along the length of the tube.

Regeneration of the initial state involves (*a*) repoisoning of the depoisoned areas and (*b*) reëstablishment of the original concentration gradient across the tube wall.

The former may be imagined to proceed simply by a back-diffusion of the "poisoning" ions into the membrane where they are preferentially fixed to the active sites. Back-diffusion would be especially quick if the volume within the tube were so small that the ions could not escape far from the tube wall. Reëstablishment of the concentration gradient means active transport of the salt against its thermodynamic gradient. We do not know how this is done, but we do know that it often is done across biological membranes and that it consumes energy. It is a fundamental problem of biochemistry and I do not profess to propose its solution. Admitted that it exists, it is able to recharge the tube. The proposed hypothesis is limited to the propagation of the original electric impulse along it.

Experiments that would produce this propagation effect could be performed on artificial membranes. It would probably be advisable to work with as thin membranes as possible. It would also be very important to find out the optimal charge density within the membrane and to find the most desirable poisoning ion, one having an electric field at its surface just strong enough to assure sign reversal of the membrane but not so strong that the stream of anions would be unable to sweep it from the membrane.

All this will certainly involve much trial and error and it cannot even be said that it must end in the desired effect of impulse propagation. All that can be said is that there seems to be no prima facie reason why the effect should not be found. There is no hope, of course, for the regeneration of the concentration gradient. The propagation effect, if found, would constitute a crude physicochemical model of neural conduction.

Choline Esterified into Acidic Sites Reversing Sign of Membrane

The model we have just dealt with does not contain the elements we know to be present in neural tissue. These are acetylcholine, choline, phospholipids, and the enzymes cholinesterase and choline acetylase. Also, at least one receptor compound for acetylcholine (or choline?) seems to exist.

How can these elements be incorporated into a somewhat similar theory of neural conduction? To some extent, at least, they can be incorporated in the following manner.

Suppose the wall of the active nerve membrane contains acid groups such as carboxyl or phosphoric acid groups bound to a protein or a lipoprotein or whatever it may be. Such a membrane wall would be cation-selective per se. Suppose these acid groups are esterified in the resting state of the nerve by choline (an alcohol with a strong quaternary ammonium group in its molecule). This esterified membrane has lost its acidic, cation-active sites, these having been replaced by strongly basic anion-active groups.

It is known that there is a solution rich in NaCl outside the neural tube, whereas there is a solution of potassium and colloidal anions within. Nothing is known about the mechanism that upholds this double gradient of alkali ions across the membrane. It is, however, known that it is maintained in a dynamic equilibrium. Be it as it may, potassium ions are held within a Donnan equilibrium against colloidal anions whereas Cl^- ions will be able to penetrate across the positive cholinester membrane. A negative charge will be established within the tube.

Hydrolysis of the ester linkage between choline and the acidic membrane reverses the situation: the hydrolyzed surface would become cation-selective and in the neighborhood of the borderline between hydrolyzed and intact membrane Na^+, K^+, and Cl^- ions will traverse the nerve wall. Such concentration changes during excitation of a nerve actually have been observed.

All this is, of course, only a small part of the still obscure story. The process must begin at a nerve ending or synapse by activation of the ester-splitting enzyme, and this activation must progress along the nerve together with the hydrolysis itself so that new adjoining areas always become hydrolyzed. I don't know why and how this could be so. Perhaps the splitting enzyme has to be bound simultaneously to positive and negative sites and therefore attaches itself at the borderline. Then the free choline which, for all we know, might have been immediately transformed into acetylcholine, has to be built in again into the membrane by esterification of the acidic sites and eventually the "sodium pump" has to reëstablish the

K^+–Na^+ steady state around the nerve. Evidently, much more remains to be explained than has been by this hypothesis. The postulation of choline bound to acidic sites of the wall instead of acetylcholine bound in a similar place is less disturbing and is even more plausible from the point of view of organic chemistry. The esterase may be just as effective in hydrolyzing such "choline membranates" as in hydrolyzing acetylcholine. Maybe it acts as a transferase between fixed acidic sites and acetate. In all events, experimental evidence should be criticized strongly before any function is attributed to acetylcholine. The possibility should be considered that choline is stored within the nerve membrane esterified to its acidic groups.

The opposite picture may also be envisaged: the resting state of the membrane being acidic and the choline being liberated from acetylcholine and built into the acidic sites during the impulse. There would be some difficulty, however, in explaining the sign of the resting potential.

The new assumption boils down to the following. A nerve membrane known to contain phospholipids could well change from cationic to anionic selectivity if its acidic sites were esterified with choline and then hydrolyzed back, or vice versa. Some cholinesteratic reactions could well be hydrolysis of membrane-bound choline and not necessarily of acetylcholine.

It seems worth while to go on thinking along these lines and to devise experiments that could decide for or against this hypothesis. Perhaps radioactive acetate could be employed as tracer to show where acetylcholine is really present in the system and where it is not.

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Synopsis

An electrochemical mechanism is sought for to explain the sudden transient change of potential across a nerve membrane during neural conduction. Ion-permselective membranes change the sign of their permselectivity if the charge of their fixed ions is overcompensated for by highly charged counterions which become strongly bound to these fixed sites by electrostatic forces. Imagine a membrane with its sign thus reversed beyond a given borderline: cations could traverse on one side in the vicinity of this line and anions on the other, while no ions would traverse pure membrane faces themselves. Thus concentrations would be equalized at the borderline by passive diffusion, while the sign of membrane potential would change from one side to the other of the border. A mechanism for automatic propagation of the border along the membrane is suggested. Sign reversal of membranes can also be caused by covalent bonding of oppositely charged groups to a charged membrane site, for instance by esterification of a carboxyl group or of a phosphoric acid group of a lipoproteid nerve membrane with the amino alcohol choline. Hydrolysis of such an ester would cause the sign of the membrane to revert to its original direction. Such acetic sites and choline abound on nerve membranes, together with enzymes, which are known to split and synthesize choline esters.

Their electrochemical function in neutral conduction might well be this changing of sign of membrane permselectivity.

Résumé

On a examiné un mécanisme électrochimique pour expliquer le changement transitoire soudain du potentiel au travers d'une membrane nerveuse durant la conduction neurale. Les membranes sélectivement perméables aux ions, changent le signe de leur permsélectivité (sélectivité de perméation) si l'échange de leurs ions fixés est surcompensée par des contresions fortement chargés qui deviennent fortement liés à ces sites fixes par des forces électrostatiques. Imaginons une membrane dont le signe est donc inversé au delà d'une ligne marginale donnée, les cations trasverseraient d'un côté au voisinage de cette ligne et les anions de l'autre, tandis qu'aucun ion ne passe au travers des faces mêmes de la membrane pure. Donc, les concentrations s'égaliseraient à la ligne marginale par diffusion passive pendant que le signe du potentiel de la membrane changerait d'un côté à l'autre de cette marge. On suggère un mécanisme relatif à la propagation automatique de cette marge au long de la membrane. L'inversion de signe des membranes peut également être causée par la liaison covalente des groupements de charge opposée à un site chargé de la membrane: par example en estérifiant un groupement carboxylique ou un groupement acide phosphorique d'une membrane nerveuse lipoprotéique avec l'alcool aminé choline. L'hydrolyse d'un tel ester inverserait le signe de cette membrane en son sens initial. De tels sites acides et la choline abondent sur les membranes nerveuses en même temps que des enzymes qui sont connues comme scindant et synthétisant les esters de la choline. Leur fonction électrochimique dans la conduction neurale pourrait bien être ce changement de signe de la permsélectivité de la membrane.

Zusammenfassung

Ein elektrochemischer Mechanismus zur Erklärung der plötzlichen, kurzzeitigen Potentialänderung an einer Nervenmembran während der Nervenleitung wird gesucht. Ionen-permselektive Membranen wechseln bei Überkompensation der Ladung ihrer fixierten Ionen durch hochgeladene, durch elektrostatische Kräfte fest an diese fixierten Plätze gebundene Gegenionen das Vorzeichen ihrer Permselektivität. Bei einer Membran deren Vorzeichen auf diese Weise jenseits einer gegebenen Grenzlinie umgekehrt wird, könnten Kationen auf einer Seite dieser Linie durch die Membrane gelangen und Anionen auf der anderen, während durch die reinen Membranflachen selbst keine Ionenwanderung erfolgt. Damit würden die Konzentrationen an der Grenzlinie durch passive Diffusion ausgeglichen werden, während das Vorzeichen des Membranpotentials sich von der einen Seite der Grenze zur anderen ändern würde. Ein Mechanismus für die automatische Wanderung der Grenze durch die Membrane wird vorgeschlagen. Vorzeichenumkehr von Membranen kann auch durch kovalente Bindung entgegengesetzt geladener Gruppen an einen geladenen Membranpunkt verursacht werden. Zum Beispiel durch Veresterung einer Carboxylgruppe oder einer Phosphorsäuregruppe einer Lipoproteid-Nervenmembran mit dem Aminoalkohol Cholin. Hydrolyse eines solchen Esters würde das Vorzeichen der Membran in die ursprüngliche Richtung umkehren. Solche saure Gruppen und Cholin sind gemeinsam mit Enzymen zur Spaltung und Bildung von Cholinestern an Nervenmembranen genügend vorhanden; ihre elektrochemische Funktion bei der Nerveneitung kann sehr wohl in dieser Vorzeichenänderung der Membranpermselektivität bestehen.

Received January 15, 1962