ACTION OF LIPID-SOLUBLE QUATERNARY AMMONIUM IONS ON THE RESTING POTENTIAL OF MYELINATED NERVE FIBERS OF THE FROG

WOLF D. DETTBARN

Department of Neurology, College of Physicians and Surgeons, Columbia University, New York, N.Y. (U.S.A.)

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SUMMARY

1. This paper presents an approach to the mechanism of action of lipid-soluble quaternary ammonium ions (PAD, PDI, noracetylcholine 12) by analyzing their effect on the resting potential of nerve fibers.

2. The degree of depolarization depends on the concentration of the compounds. The time course is slower than with $2 \cdot 10^{-2}$ *M* KCl. PAD is the most effective of the 3 compounds investigated.

3. Washing with Ringer's solution brings the potential back to the initial value after 30 min (10^{-4} *M* PAD, $5 \cdot 10^{-4}$ *M* noracetylcholine). When smaller concentrations of PAD are used, the depolarization is reversed in a shorter time.

4. The depolarization must be the result of a change in the relative permeability of the various active ions.

5. Physostigmine–Ringer's solution scarcely alters the resting potential. Physostigmine ($7 \cdot 10^{-3}$ *M*) inhibits the depolarization of 10^{-4} *M* PAD. Lower concentrations of physostigmine counteract the effect of PAD, but do not abolish it, while higher concentrations of PAD overcome the protective action.

6. The antagonism between PAD and physostigmine is typical for competitive agents.

7. It is suggested that the lipid-soluble quaternary ammonium ions act with a receptor and that this reaction effects a change in permeability.

INTRODUCTION

The lack of effect of acetylcholine and other quaternary ammonium ions on conduction in nerve, even when applied in very high concentrations, is in striking contrast to their actions at the neuromuscular junction and other functional regions. However, the conducting membranes are not permeable by these compounds. It has been demonstrated by NACHMANSOHN and co-workers[1,2] that neither acetylcholine nor neostigmine penetrates the giant axon of Squid. Recently a few quaternary ammonium ions which are lipid-soluble have been developed in NACHMANSOHN's laboratory. These compounds are derived from physiologically active, chloroform-

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insoluble quaternary ammonium ions by replacing one methyl group by a dodecyl radicle. The derived compounds are more soluble in chloroform than in water. SCHOFFENIELS, WILSON AND NACHMANSOHN[3] have shown that low concentrations of these lipid-soluble compounds block conduction in the axons of lobsters and crabs and depolarize the isolated electric cell of *Electrophorus electricus*. HINTERBUCHNER AND WILSON[4,5] showed in frog's rectus abdominis muscle that they produce a contracture which is reversible and repeatable even when the myoneural junction has been blocked by curare. All these observations point to the conclusion that lipid-soluble quaternary ammonium ions are acting on the conducting membrane. In the present paper myelinated nerve fibers have been examined to see if they are affected similarly by these agents.

Resting potentials were recorded by the technique described by STAEMPFLI[6]. By this method the exact time course of changes in the resting potential could be followed. The degree of depolarization with various concentrations of pyridine-2-aldoxime dodeciodide (PAD), pyridine dodeciodide (PDI) and of acetoxyethyldimethyl dodecyl ammonium iodide (noracetylcholine 12) has been measured and also the differences between the three compounds in duration of action and in reversibility. Inhibition of the action of these compounds by physostigmine has been demonstrated.

METHOD

The resting potential of one part of a nerve bundle in Ringer's solution was compared with the resting potential of another part of the same nerve bathed with test solution. The test solution was Ringer's solution with varied concentrations of PAD, PDI, or noracetylcholine 12. We used bundles of fibers from the *N. tibialis* of *Rana pipiens*. The potency of the tested compounds was judged by the magnitude of the depolarization. Results represent average values of at least 5 experiments.

RESULTS

In comparison with the depolarization caused by increased K-ion concentration ($2 \cdot 10^{-2}$ *M* KCl) which reached its end value in 30 sec, these compounds (10^{-3} *M*) decreased the resting potential more slowly. The maximum depolarization obtained after 10 min exposure was 35 mV with PAD, 31 mV with noracetylcholine 12, and 34 mV with PDI. The approach to the end value was slow. Switching over to Ringer's solution resulted after noracetylcholine 12 and PDI in a repolarization, which after 10 min was still far below the original value. After depolarization by PAD there was no repolarization. With $2 \cdot 10^{-2}$ *M* K-ion a depolarization of 26 mV was reached after the first 30 sec and this value remained constant during 10 min, as shown previously by DETTBARN[7], and DETTBARN AND STAEMPFLI[8]. When we compared the depolarizations after 2 min, we found a depolarization of 26 mV with $2 \cdot 10^{-2}$ *M* K-ion, of 20 mV with PAD, of 27 mV with noracetylcholine 12, and of 32 mV with PDI. The extent of the depolarization is related to the concentration of the compounds. PAD is the most effective. See Table I. The concentration influences the repolarization. With a concentration of 10^{-3} *M* little or no repolarization follows the return to Ringer's solution. Even after $5 \cdot 10^{-4}$ *M* PAD only a small amount of repolarization occurs (from —18 mV to —15 mV) while with noracetylcholine 12

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TABLE I

ABSOLUTE CHANGE OF THE MAGNITUDE OF THE DEPOLARIZATION IN RELATION TO DIFFERENT MOLAR CONCENTRATIONS OF PAD, NORACETYL (NORACETYLCHOLINE 12), PDI, KCl AND TO TIME

Concn. moles/l	*Compounds*	*Depolarization after 2 min mV*	*Depolarization after 10 min mV*
10^{-3}	PAD	—20.0	—35.0
	Noracetyl	—27.0	—31.0
	PDI	—32.0	—34.0
$5 \cdot 10^{-4}$	PAD	— 8.0	—18.0
	Noracetyl	— 5.0	—14.0
$3.5 \cdot 10^{-4}$	Noracetyl	— 1.0	—11.0
10^{-4}	PAD	— 6.0	—13.0
	Noracetyl	—	—
	PDI	—	—
$5 \cdot 10^{-5}$	PAD	— 3.0	—12.0
$3.5 \cdot 10^{-5}$	PAD	— 3.0	—
10^{-5}	PAD	— 2.0	— 3.0
$2 \cdot 10^{-2}$	KCl	—26.0	—26.0

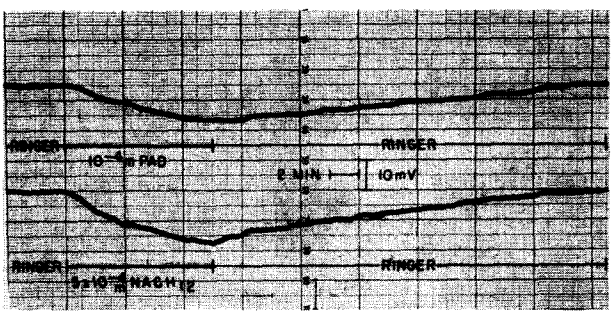

Fig. 1. Course of the repolarization in Ringer's solution after 10 min exposure to 10^{-4} *M* PAD (upper curve) and $5 \cdot 10^{-4}$ *M* NACh 12 (noracetylcholine 12) lower curve. Both are back to their initial values after 28 min. Nerve bundle of *Rana pipiens*.

the potential returned from —15 mV to —3 mV. With 10^{-4} *M* PAD the potential returned from 13 mV to 9 mV, PAD in concentration of $5 \cdot 10^{-5}$ *M* and 10^{-5} *M* causes complete reversion. The results mentioned above are observations after 10 min. Repolarization is completed for 10^{-4} *M* PAD and $5 \cdot 10^{-4}$ *M* noracetylcholine 12 after 30 min (Fig. 1).

The inhibitory effect of physostigmine on the action of PAD. For reasons which we will discuss later, we suppose that physostigmine would inhibit the action of PAD and the other compounds. We used only PAD for these experiments. First the bundle is rinsed with physostigmine for 10 min and then for the same time with a combination of physostigmine and PAD. When physostigmine was added to the nerve bundle in $7 \cdot 10^{-3}$ *M*, addition of a markedly depolarizing concentration of 10^{-4} *M* PAD had no effect. Lower concentrations of physostigmine counteract the effect of PAD, but

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TABLE II

MAGNITUDE OF DEPOLARIZATION AND REPOLARIZATION BY COMBINED APPLICATION OF VARIED CONCENTRATIONS OF PHYSOSTIGMINE AND PAD

Concentration, moles/l		*Depolarization mV*	*Repolarization mV*
Physostigmine	*PAD*		
10^{-3}	10^{-4}	—11.0	—6.0
$4 \cdot 10^{-3}$	10^{-4}	— 8.0	—4.0
$7 \cdot 10^{-3}$	10^{-4}	— 0.0	+ 2.0
$7 \cdot 10^{-3}$	$5 \cdot 10^{-4}$	—16.0	—8.0
$7 \cdot 10^{-3}$	10^{-3}	—22.0	—7.0

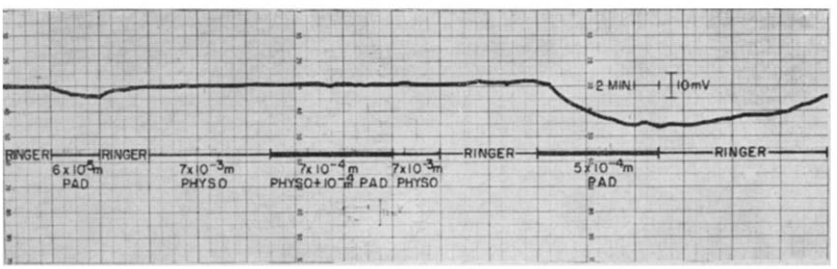

Fig. 2. Comparison of the effects of different concentrations of PAD on the resting potential with and without physostigmine. Note depolarization of $6 \cdot 10^{-5}$ *M* PAD; 10^{-4} *M* PAD in combination with $7 \cdot 10^{-3}$ *M* physostigmine has no effect. A stronger concentration of PAD $5 \cdot 10^{-4}$ *M* in the absence of physostigmine has a markedly depolarizing action. Nerve bundle of *Rana pipiens*.

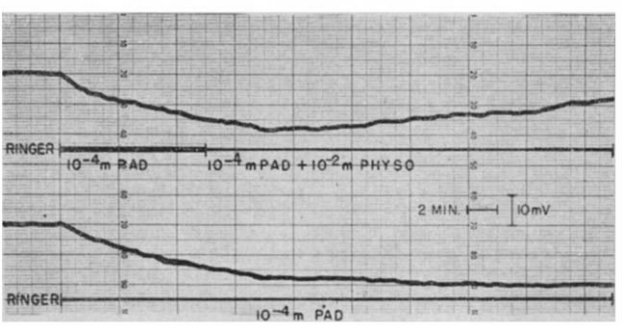

Fig. 3. After washing the nerve bundle 10 min with 10^{-4} *M* PAD, the bathing solution was replaced by 10^{-4} *M* PAD and 10^{-2} *M* physostigmine (Physo). As one can see, the depolarization is decreased after 28 min to —8 mV (upper curve). The lower curve, the same but without physostigmine. No change of depolarization occurs, so that the decrease in depolarization is attributed to the presence of physostigmine. Nerve bundle of *Rana pipiens*.

do not abolish it. Higher concentrations of PAD overcome the protective action of physostigmine. See Table II.

In another experiment we showed the depolarizing effect of PAD before and after the application of physostigmine, but during the application of physostigmine the depolarization was abolished (Fig. 2). Fig. 3 shows the result of further experiments on the counteraction of physostigmine. The nerve bundle was exposed for 10 min to 10^{-4} *M* PAD and was depolarized 16 mV. After 10 min the bathing solution

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was replaced by a solution of the same concentration of PAD, but now in a combination with 10^{-2} *M* physostigmine. At first the depolarization increased still further from —16 mV to —18 mV but after 30 min the depolarization decreased to —8 mV. To exclude the possibility that after application of 10^{-4} *M* PAD alone for 30 min the depolarization would decrease, we gave 10^{-4} *M* PAD for 30 min. After 10 min the depolarization was —13 mV, after 20 min —16 mV, and after 30 min —19 mV. The decrease of the depolarization in the previous experiment can be attributed to the physostigmine (see Fig. 3).

DISCUSSION

A full discussion of the action of PAD, noracetylcholine 12 and PDI on the resting potential is postponed to a subsequent paper. STAEMPFLI[9] tested one of these compounds, noracetylcholine 12, on the Ranvier node of frog nerve. He found that at a concentration of 10^{-3} *M* the compound produced a complete and irreversible depolarization of the membrane, in 10^{-4} *M* a large prolongation of the action potential and after longer application an almost completely reversible depolarization; at a concentration of $3 \cdot 10^{-5}$ *M* the amplitude and duration of the action potential were increased. The effect on the membrane takes place within seconds, only slightly less rapidly than that observed with KCl. From this he believes that the site of action must be at the membrane, for the permeation and action of noracetylcholine 12 inside the cell would need a longer time, and he attributes the almost irreversible depolarization to an inside effect.

The depolarization must be the result of a change in the relative permeabilities of the various active ions.

For the interpretation of the effects it is crucial to know whether or not they are produced by a specific action like that suggested by NACHMANSOHN[10] and WILSON AND NACHMANSOHN[11] for the effect of acetylcholine. It has been suggested that acetylcholine combines with a receptor substance, probably a protein, which resembles cholinesterase and that this reaction brings about a change in conformation which alters the membrane permeability. This suggestion introduces the possibility of receptor activators, substances which combine reversibly with the receptor and evoke a change in membrane potential and receptor inhibitors—substances which act reversibly with the receptor but are unable to evoke activity. In general, it has been noted that while simple quaternary ammonium ions are receptor activators, the tertiary ammonium ions, which are derived from them by replacement of a methyl group by a proton are receptor inhibitors. If PAD depolarizes the membrane by reacting with the suggested acetylcholine receptor, if it is a receptor activator, a receptor inhibitor should by competition prevent the combination of the activator with the receptor and thereby prevent depolarization. Physostigmine, as is well known, has a high affinity for the acetylcholine system. It is a potent cholinesterase inhibitor, it is also a receptor inhibitor. We have seen that when physostigmine is added, the usually markedly depolarizing action of 10^{-4} *M* PAD is without effect, while higher concentrations of PAD overcome this protective action. Also depolarization produced by PAD can be overcome by adding physostigmine. These facts suggest that there is a competition of both compounds for the same receptor (DETTBARN, WILSON AND NACHMANSOHN[12]).

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STRUCTURAL DIFFERENCES IN THE NUCLEIC ACIDS OF SOME TOBACCO MOSAIC VIRUS STRAINS*

II. DI- AND TRI-NUCLEOTIDES IN RIBONUCLEASE DIGESTS

K. K. REDDI

Virus Laboratory, University of California, Berkeley, Calif. (U.S.A.)

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SUMMARY

The nucleic acids isolated from three strains of tobacco mosaic virus were digested with pancreatic ribonuclease at 23° for twelve hours at pH 7.6. The di-nucleotides, adenylyl-cytidylic acid, adenylyl-uridylic acid and guanylyl-uridylic acid and a tri-nucleotide, adenylyl-guanylyl-cytidylic acid were isolated from such digests and estimated. There was a significant difference in the amounts of these occurring in each of the digests. From the results it was concluded that the way the individual nucleotides are arranged in each of the nucleic acids of TMV, HR and M is different.

INTRODUCTION

The protein and nucleic acid components of tobacco mosaic virus (TMV) strains were analyzed with a view to establishing a chemical basis for the distinctive biological

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