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OVERSHOOT AND BLOCK OF CONDUCTION BY LIPID-SOLUBLE ACETYLCHOLINE ANALOGUES*

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It was suggested, in 1941, in modification of the original hypothesis of neurohumoral transmission, that the release and action of acetylcholine are intracellular processes taking place within the conducting membrane along the entire length of the nerve fiber[1-3]. Acetylcholine was postulated to be responsible for the change in electrical polarity of the membrane which occurs during the passage of the nerve impulse. This view was substantiated during the following decade. In particular, the inseparability of acetylcholinesterase and conduction was demonstrated. Moreover, when the sequence of energy transformations occurring during nerve activity was established and acetylcholine was integrated into the metabolic pathways of conducting cells, it became apparent that the activity of acetylcholine precedes the other events, suggesting that it is the specific operative substance in nerve conduction in the sense applied by MEYERHOF to ATP in muscle contraction[4,5].

Later it was suggested that acetylcholine combines with a receptor substance (probably a protein which resembles acetylcholinesterase) 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 potentials—and receptor inhibitors—substances which combine 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

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derived from them by the replacement of a methyl group with a proton, are receptor inhibitors[4,5].

A major objection to the basic concept was the absence of any effect of acetylcholine or other quaternary ions upon conduction even when applied in high concentration, in contrast to the remarkable activity of these compounds at synaptic junctions. This want of effect was explained by impermeability of these quaternary ammonium ions. These lipid-insoluble compounds would hardly be expected to penetrate the lipid layers of axons and it was in fact demonstrated that acetylcholine and neostigmine do not penetrate into the axoplasm of the giant axon of Squid[6,7]. Tertiary derivatives, on the other hand, were shown to penetrate and those which are inhibitors of acetylcholinesterase such as eserine and 3-hydroxydimethylaniline dimethylcarbamate (tertiary analogue of prostigmine) block conduction[8]. These tertiary amines are also receptor inhibitors and block occurs without depolarization. Other lipid-soluble anticholinesterases such as diisopropylfluorophosphate (DFP) also block conduction.

Recently we have undertaken studies with lipid-soluble quaternary ammonium iodides. These compounds are derived from physiologically active chloroform-insoluble quaternary ammonium ions by replacing a methyl group with a longer chain; in this instance a dodecyl group. The resulting compounds are soluble in chloroform and in water.

The first such compound studied was pyridine-2-aldoxime dodeciodide (PAD), a lipid-soluble analogue of the nerve gas antidote pyridine-2-aldoxime methiodide (PAM). When used together, these compounds greatly extend the range of survival against the powerful nerve gas sarin[9].

In this paper we report the effect of PAD, of pyridine dodeciodide, and of β-acetoxyethyldimethyl dodecyl ammonium iodide*, a lipid-soluble analogue of acetylcholine, upon three conducting tissues: isolated single electroplax of the electric organ of *Electrophorus electricus*, crab nerve and lobster nerve. These effects are contrasted with the effect of the lipid-insoluble PAM, pyridine methiodide, and acetylcholine.

The electroplax is a flat rectangular cell through which microelectrodes are easily inserted. One face is a conducting membrane, highly innervated, containing some 50,000 synapses. Single cells are mounted between separate pools of solution[10,11].

Both quaternary and tertiary amines are effective: in general (with some few exceptions) the former block the electrical discharge with depolarization, but the latter block without affecting the membrane potential[12]. Both block the direct and indirect stimulus. The action of quaternary amines however, is restricted to the junction[10]. This can be demonstrated with curare. As with muscle, curare effects only the synapse and blocks only the response to indirect stimulation. In the presence of curare, the tertiary amines block the direct response but the quaternary ions are without effect. The two methiodides reported in this work in general follow the usual pattern—they block response to both direct and indirect stimulation. In the presence of curare they are without effect (Table I).

The dodeciodides are strikingly different. They block both direct and indirect

* To preserve in the name of this compound its relationship to acetylcholine we propose to call it noracetylcholine dodeciodide. This terminology indicates that a methyl group has been replaced by a dodecyl group.

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TABLE I

EFFECTS OF LIPID-SOLUBLE AND LIPID-INSOLUBLE QUATERNARY NITROGEN DERIVATIVES ON THE ELECTRICAL CHARACTERISTICS OF A SINGLE ISOLATED ELECTROPLAX

The following compounds were tested: pyridine-2-aldoxime dodeciodide (PAD); pyridine-2-aldoxime methiodide (PAM); pyridine dodeciodide (PDI); pyridine methiodide (PMI); acetylcholine (ACh) and noracetylcholine dodeciodide (norACh DI). The response to both direct and indirect stimulation was recorded with extracellular electrodes, the resting potential (RP) with an intracellular microelectrode. *d*-Tubocurarine, where indicated, was added at $1 \cdot 10^{-4}$ *M* concentration 15 min before application of the compound tested; in these cases the response to indirect stimulation at 0 time (15 min after addition of curare) was abolished, that to direct stimulation unaffected.

Compound	*M-concn.*	*Time of exposure min*	*Effect on el. activity*		*RP (inside electrode) mV*
			direct	*indirect*	
PAD	$3 \cdot 10^{-3}$	0	—	—	— 80
		9	none	abolished	— 80
		14	abolished	abolished	— 74
		30	abolished	abolished	+ 33
PAD + Curare	$3 \cdot 10^{-3}$	0	—	—	— 84
		5	none	—	— 75
		15	abolished	—	+ 30
PAM	$1.5 \cdot 10^{-2}$	0	—	—	no measurement
		10	none	abolished	
		30	none	abolished	
PDI	$5 \cdot 10^{-3}$	0	—	—	— 70
		8	abolished	abolished	— 56
		20	abolished	abolished	+ 20
		30	abolished	abolished	+ 32
PDI + Curare	$5 \cdot 10^{-3}$	0	—	—	— 78
		10	abolished	—	— 70
		30	abolished	—	+ 40
PMI	$2 \cdot 10^{-2}$	45	abolished	abolished	no measurement
PMI + Curare	$2 \cdot 10^{-2}$	60	none	—	no measurement
norACh DI	$1 \cdot 10^{-3}$	0	—	—	— 72
		10	abolished	abolished	— 60
		30	abolished	abolished	+ 12
		50	abolished	abolished	+ 26
norACh DI + Curare	$1 \cdot 10^{-3}$	0	—	—	— 78
		10	abolished	—	— 68
		30	abolished	—	+ 20
ACh	$2 \cdot 10^{-5}$	0	—	—	— 80
		7	abolished	abolished	— 65
		27	abolished	abolished	— 35
ACh + Curare	$3 \cdot 10^{-5}$	0	—	—	— 81
		120	none	—	— 82

response, but even in the presence of curare they block the direct spike (Fig. 1). This shows that they penetrate into and affect the conducting membrane. The first effect is a broadening of the spike (Fig. 2), then the spike declines and finally disappears. But the most remarkable phenomenon is that they reverse the electrical polarity of the

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membrane—they produce an overshoot which is so characteristic of the physiological process (Table I)[13,14]. This is the first report of any compound which reverses the polarity of any membrane conducting or otherwise.

With crab and lobster nerves, these lipid-soluble quaternary ammonium ions produce block of conduction (Table II). With crab nerve, only 10^{-4} *M* PAD (equivalent to 18 γ/ml of acetylcholine) solution is required to produce block. Lobster

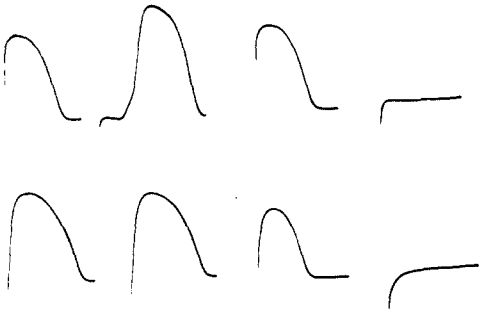

Fig. 1. Electroplax. From left to right. Top row: direct spike, indirect spike; 10 min after 10^{-4} *M* curare: direct spike, indirect spike (blocked). Bottom row: direct spike 6, 10, 12 and 19 min after $3 \cdot 10^{-3}$ *M* PAD.

Fig. 2. Electroplax. Superimposed spikes before and 12 min after exposure to $3 \cdot 10^{-3}$ *M* concentration of PAD. Cal.: 1 msec. External electrodes.

TABLE II

EFFECT OF PYRIDINE-2-ALDOXIME DODECIODIDE (PAD) AND NORACETYLCHOLINE DODECIODIDE (norACh DI) ON THE ACTION POTENTIAL OF NERVE AXONS

Isolated fibers from the meropodite of the claws and walking legs of *Libinia emarginata* (spider crab) and *Homarus americana* (lobster) were used; from the latter either the whole bundle or a few strands of fibers were taken. Acetylcholine is well known to be without effect, PAM in $4 \cdot 10^{-3}$ *M* was without effect after 60 min exposure. *t* = time in min until complete block of action potential.

Species		*Compound*	*M concn.*	*t*
Spider crab		PAD	$1.7 \cdot 10^{-3}$	1
			$4 \cdot 10^{-4}$	5
			$1 \cdot 10^{-4}$ *	17
Lobster		PAD	$1 \cdot 10^{-3}$	10
			$5 \cdot 10^{-4}$	35
	Whole bundle	norACh DI	$2.5 \cdot 10^{-3}$	20
	Strands of fibers		$2.5 \cdot 10^{-3}$	10
			$2.5 \cdot 10^{-4}$ **	30

* Equivalent to 18 γ/ml acetylcholine chloride.
** Equivalent to 45 γ/ml acetylcholine chloride.

nerve requires higher concentrations, possibly because it does not fan out into such fine filaments as crab nerve. All three dodeciodides produce block. This is the first report that any quaternary ammonium ion blocks axonal conduction.

In summary, the lipid-soluble analogue of acetylcholine, noracetylcholine dodeciodide, and other lipid-soluble quaternary ammonium ions block conduction

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at low concentrations and produce an overshoot, which is a striking feature of conduction. Although we are just in the beginning of the work, this first demonstration of a direct action in combination with all the other available evidence appears at this writing to be a striking confirmation of the theory that acetylcholine is intrinsically connected with conduction.

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SUMMARY

Lipid-soluble quaternary ammonium derivatives, analogues of acetylcholine, have been prepared to test whether they would penetrate the conducting membrane and produce effects suggested to be caused during the physiological process by the action of internally released acetylcholine.

1. The following compounds were prepared: (i) pyridine-2-aldoxime dodeciodide (PAD), a lipid-soluble analogue of the nerve gas antidote pyridine-2-aldoxime methiodide (PAM); (ii) β-acetoxyethyldimethyl dodecyl ammonium iodide, referred to as noracetylcholine dodeciodide in order to preserve in the name its relationship to acetylcholine, and (iii) pyridine methiodide dodeciodide.

2. The effect of these compounds on conduction was tested with crab and lobster nerve and with the single isolated electroplax of *Electrophorus electricus* and compared with the lipid-insoluble analogues of the three compounds.

3. It was shown that all three lipid-soluble compounds block conduction in relatively low concentration. PAD, for instance, abolishes conduction in 10^{-4} *M* concentration, equivalent to 18 γ/ml acetylcholine chloride.

4. The compounds produce the reversal of polarity of the conducting membrane, the overshoot, which is so characteristic of the physiological process. This is in contrast to the failure of the lipid-insoluble quaternary ammonium derivatives to affect conduction in any way.

In combination with all the other data available, these observations are considered to be a striking support for the essential role attributed to acetylcholine in conduction.

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