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CONDUCTION BLOCK PRODUCED BY ACETYLCHOLINE IN CETYLTRIMETHYLAMMONIUM-TREATED FROG NERVES*

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Abstract—After pretreatment with the synthetic cationic surfactant, cetyltrimethylammonium bromide, frog sciatic nerves were found to be very susceptible to axonal conduction block by externally applied acetylcholine, as evidenced by depression of the amplitude of the A-potential; the block was completely reversible at 0·02 M; at 0·1 M, acetylcholine block was largely though not completely reversible. Comparison with glucose and sucrose controls demonstrated that the reduction of the A-potential was not a response that could be attributed to a deficiency of sodium in the external medium; conduction block was clearly due to acetylcholine. The massive concentration of acetylcholine required to effect conduction block—together with the observations that comparable block was achieved with choline, tri- and tetra-methylammonium, and dimethylaminoethyl acetate—suggest that the effect of acetylcholine may have been nonspecific. The possibility, however, of an acetylcholine specificity cannot be ruled out, for the surfactant pretreatment may have subtly altered the normal physicochemical properties of the axonal membranes, and any previously existing specificity was modified. Furthermore, it appeared that neostigmine and eserine potentiated the depression of the A-potential by acetylcholine, suggesting that lesser external concentrations might achieve conduction block if acetylcholine were not so rapidly hydrolyzed.

THE lipid-insoluble quaternary ammonium ions of *d*-tubocurarine and neostigmine produce substantial depression of the amplitude of the compound action potential of frog sciatic nerves which have been pretreated with cetyltrimethylammonium bromide (CTMB) and other ionic surfactants. At concentrations between 0·0001 and 0·001 M, a conduction block is produced. In sharp contrast with these results, inordinately high concentrations (0·075 to 0·1 M) of acetylcholine and other lipid-insoluble choline esters are needed to cause a marked depression of the amplitude of the compound action potential.[1–3] Commenting on these observations Spyropoulos and Tasaki[4] have raised two relevant points: (1) the conduction block produced by acetylcholine may be due to a sodium-deficient environment, and (2) the abnormally high concentration needed to produce block may indicate that the action of acetylcholine is nonspecific. This report is concerned with the question: Is the conduction block brought about by acetylcholine in CTMB-pretreated nerves a sodium-lack phenomenon and, if not, to what extent is it specific to acetylcholine?

METHODS

Sciatic nerves from autumn and early-winter frogs (*Rana pipiens*) were used. Except during the brief recording procedures, the nerves were immersed in a phosphate-buffered Ringer's solution (BFR), the CTMB pretreatment bath, or one of the test

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solutions. The temperature of all solutions was kept constant at 20° and the pH maintained at 7·2 with an all-sodium Sörensen's phosphate buffer (M/15); 20% of the volume of all solutions was composed of the buffer. Thus no test solution contained less than 23 mEq Na^+/l. For series *j* thru m_1, 80% of the volume was made up of a stock Ringer's solution of the following composition: NaCl, 104·0 mM; KCl, 3·0 mM; $CaCl_2$, 0·8 mM; and glucose, 4·0 mM. In the series *a* thru *i*, distilled water was used in place of the Ringer's stock. Sucrose was added, when necessary, to achieve a final osmolarity of 0·235 for all solutions. The method of recording was the same as that employed previously.[1, 2]

Freshly dissected nerves were treated for 1 hr in BFR containing CTMB (10^{-3} M); they were then washed for 15 min with CTMB-free BFR. The blocking action of various test solutions, as evidenced by a decrease in the amplitude of the A-component of the compound action potential, was examined over a 40-min exposure interval. Nerves were then washed with BFR for 30 or 60 min and the action potential recorded (R_{30} or R_{60}) to test the reversibility of the block.

RESULTS

Previous findings were confirmed; after treating nerves with CTMB, acetylcholine (0·1 M) brought about very substantial conduction block within 10 min of exposure. On the other hand, solutions of 0·2 M glucose or sucrose, which were identical with the acetylcholine solution in terms of Na^+, did not depress the amplitude of the action potential. Table 1 shows the means of the results for the 10- and 40-min recordings of acetylcholine, sucrose, glucose, and NaCl-control series. A buffered NaCl solution (series *d*) rather than BFR was employed as a control since such a solution more nearly mimicked the ACh, sucrose, and glucose solutions in terms of the absence of

TABLE 1. CONDUCTION BLOCK BY ACETYLCHOLINE IN CTMB-TREATED NERVES*

Series	Experiment	No. of nerves	% Initial amplitude (±SD) After 10 min	After 40 min	Reversal R_{60}
a	0·1 M ACh†	24	41 (14)	21 (15)	84 (18)
b	0·2 M sucrose†	22	90 (31)	92 (31)	69 (22)
c	0·2 M glucose†	6	96 (27)	114 (18)	77 (7)
d	0·1 M NaCl	6	100 (6)	106 (73)	116 (20)

* Nerves were placed in BFR containing CTMB (10^{-3} M) for 1 hr, washed for 15 min in BFR and amplitude of action potential recorded; nerves were then placed in test solutions. Values given are means of percentages of initial amplitude of the action potentials at indicated times after exposure to test solution.

† Contained 23 mEq Na^+/l.

the usual small amounts of K^+, Ca^{2+}, and so forth. The acetylcholine-produced block is largely, though not completely, reversible. It seems reasonably certain that the conduction block is not due to a relatively low $[Na^+]_o$ but to an action of acetylcholine itself.

The effects of four agents were compared with the action of acetylcholine, at equimolar concentrations. All four of the agents are identical or very similar to the cationic nitrogen portion of acetylcholine: dimethylaminoethylacetate (DMAEA), the

tertiary analog of acetylcholine; tetramethylammonium; trimethylamine; and choline, the quaternary ammonium hydrolytic product of acetylcholine. The data, presented in Table 2, do not indicate that the effect produced by acetylcholine is specific; in fact, trimethylamine and choline achieved comparable conduction block and tetramethylammonium and DMAEA actually may have been slightly more effective than

TABLE 2. COMPARISON OF CONDUCTION BLOCK BY ACETYLCHOLINE AND RELATED AGENTS*

Series	Experiment	No. of nerves	% Initial amplitude (± SD) After 10 min	After 40 min	Reversal R_{60}
a	0·1 M ACh	24	41 (14)	21 (15)	84 (18)
e	0·1 M DMAEA	12	44 (14)	11 (11)	76 (18)
f	0·1 M $(CH_3)_4N^+$	16	27 (17)	18 (17)	72 (30)
g	0·1 M $(CH)_3)_3NH^+$	12	39 (9)	22 (13)	83 (16)
h	0·1 M choline	16	40 (13)	24 (16)	78 (22)

* Procedure the same as that in Table 1.

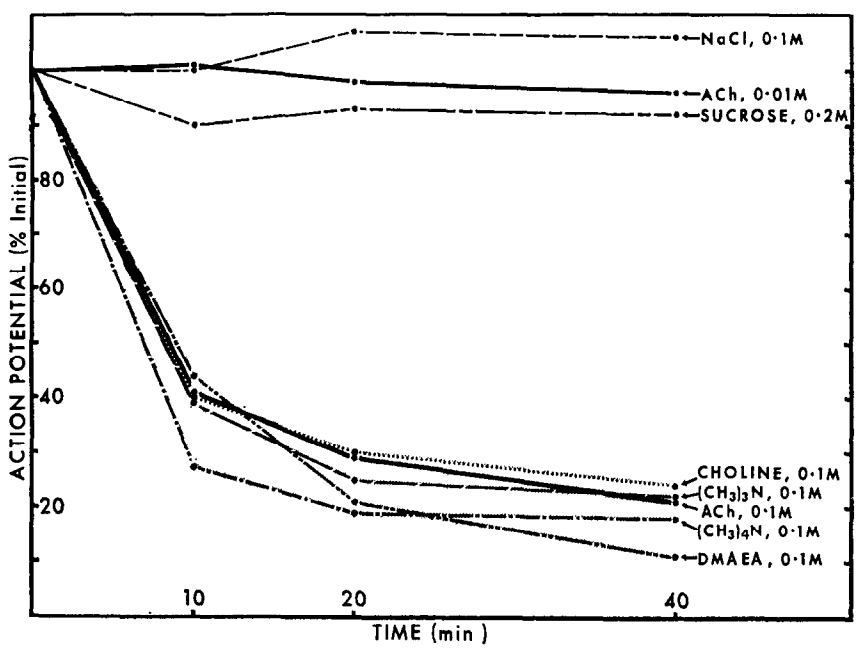

FIG. 1. Depression of the A-potential by acetylcholine and related agents. The ordinate shows the amplitude of the A-potential expressed as percentage of the initial magnitude; the abscissa is time in minutes. Note that the curves for choline, $(CH_3)_3N$, $(CH_3)_4N$, and DMAEA all fall within the ACh channel (±SD of the ACh curve.)

ACh. Fig. 1 shows that the blocking activity of the four agents structurally related to ACh is very little, if any, different from that of ACh itself.

Tables 3 and 4 show the influence of ACh in conjunction with neostigmine or eserine upon conduction. The addition of a small amount of neostigmine to 0·1 M

ACh seems to potentiate the depression of the amplitude of the action potential—series *i* in Table 3. ACh alone is ineffective at 0·01 M; the addition of eserine, however, appears to result in increasing conduction block as the eserine concentration is increased. Eventually the eserine itself causes some block, at or above 0·001 M (see series m_1 in Table 3, and series *n* in Table 4).

TABLE 3. INFLUENCE OF ESERINE AND NEOSTIGMINE ON CONDUCTION BLOCK BY ACETYLCHOLINE*

Series	Experiment	No. of nerves	% Initial amplitude (±SD) After 10 min	After 40 min	Reversal R_{60}
a	0·1 M ACh	24	41 (14)	21 (15)	84 (18)
i	0·1 M ACh				
	c̄ 3×10^{-5} M neostigmine	12	37 (15)	10 (13)	76 (20)
i_1	0·2 M sucrose				
	c̄ 3×10^{-5} M neostigmine	12	93 (26)	97 (44)	71 (27)
j	0·01 M ACh	12	101 (11)	96 (9)	104 (9)
k	0·01 M ACh				
	c̄ $6{\cdot}2 \times 10^{-5}$ M eserine SO_4	4	99	92	108
l	0·01 M ACh				
	c̄ $2{\cdot}5 \times 10^{-4}$ M eserine SO_4	4	92	84	96
m	0·01 M ACh				
	c̄ 5×10^{-3} M eserine SO_4	4	64	29	60
m_1	0·02 M sucrose				
	c̄ 5×10^{-3} M eserine SO_4	4	76	41	80

* Procedure the same as that in Table 1. Comparing series a with i at the 40-min interval, P is between 0·05 and 0·02.

TABLE 4. INFLUENCE OF ESERINE ON CONDUCTION BLOCK BY ACETYLCHOLINE AND RELATED AGENTS*

Series	Experiment	No. of nerves	% Initial amplitude (±sd) After 10 min	After 40 min	Reversal R_{60}
n	0·04 M sucrose*	12	91 (9)	81 (9)	93 (8)
o	0·02 M ACh*	12	80 (6)	75 (9)	101 (10)
p	0·02 M choline*	12	85 (5)	82 (4)	105 (8)
q	0·02 M $(CH_3)_3NH^+$*	12	89 (6)	81 (7)	102 (11)

* With 10^{-3} M eserine SO_4. Comparing series *o* with *n*, *p*, and *q* at the 10-min interval, "2-sided" P values are: *o* vs. *n*, 0·01 to 0·001; *o* vs. *p*, 0·02 to 0·050; *o* vs. *q*, 0·01 to 0·001.

DISCUSSION

Acetylcholine, at the high concentration of 10^{-1} M, very effectively depressed the amplitude of the A-potential of CTMB-pretreated frog sciatic nerves. Acetylcholine, at this massive concentration, was without comparable effect on untreated sciatic nerves. Lorente de Nó has reported that 0·1 M acetylcholine *in distilled water* is without effect for at least 5 hr on bullfrog sciatic nerves[5]; we venture that there were two principle reasons for this lack of effect: (1) the sheathed bullfrog sciatic nerves employed were abundantly endowed with various permeability barriers which precluded acetylcholine ions from reaching the neuronal membranes and participating in an

interaction that might have culminated in axonal conduction block, and (2) the intact epineurium trapped sufficient sodium ions to sustain conduction even in the absence of sodium in the external medium. Crescitelli has demonstrated that a Na^+-free medium brings about rapid, complete, and reversible conduction block in desheathed bullfrog sciatic nerves; he also showed that 11 mEq Na^+/l. was sufficient to maintain most A-fiber activity and that 22 mEq Na^+/l. was capable of maintaining conduction in all A fibers.[6, 7]

Since in our experiments the 0·1 M acetylcholine solutions contained 23 mEq Na^+/l., and because of the work of Crescitelli, it seemed unlikely that the acetylcholine action was a sodium-deficiency response. It is conceivable, however, that the CTMB-pretreated nerves were more sensitive to a relative sodium lack than were untreated, desheathed sciatic nerves of bullfrog. The results summarized in Table 1, although revealing little about the susceptibility of CTMB-treated nerves to Na^+ deficiency, show that the acetylcholine-produced depression of the A-potential is not a Na^+-lack phenomenon. A few of the sucrose-treated nerves exhibited appreciable conduction block (note the large standard deviation for series *b*); yet the severe contrast between the mean behavior of the 28 nerves exposed to glucose or sucrose and the 24 nerves treated with acetylcholine leaves no doubt that the reduction of the A-potential by acetylcholine was not a response that can be attributed primarily and solely to the relative deficiency of Na^+ in the external medium. An interaction involving acetylcholine somewhere within the sciatic complex appears to underlie the conduction block.

Two sets of data tend to support the concern raised by Spyropoulos and Tasaki that the blocking action of ACh is nonspecific: (1) several agents which are structurally related to ACh brought about the same degree of depression of the A-potential as acetylcholine itself, and (2) the enormous concentrations of ACh required to block conduction seem inconsistent with any significant specificity of action. A nearly isotonic solution of ACh was needed to achieve substantial conduction block in CTMB-treated nerves; a 0·01 M solution of ACh was without effect (see Fig. 1 and contrast series *a* with series *j* in Table 3). Since conduction block was not produced by 0·01 M ACh, it seems that the external application of ACh to surfactant-treated nerves either does not culminate in an effective interaction of ACh with the hypothetical membrane receptor protein,[8] (assumed that sufficient ACh molecules achieve access to an adequate number of receptor sites), or that an effective concentration of ACh in the immediate vicinity of the membrane receptor sites was not reached, or that the postulated receptors do not exist. Since curare, dimethylcurare, or neostigmine at concentrations of less than 0·001 M were quite effective in blocking conduction in surfactant-pretreated nerves[2] it might seem unlikely that inadequate dispersion or disruption of permeability barriers underlay the failure of ACh to block conduction at "low" concentrations. Nevertheless the production of a "barrier-free" system in the frog sciatic nerve comparable to a synaptic region also seems unlikely. It is probable that the surfactant pretreatment reduces barriers differentially with respect to different compounds. This view is supported by work done in this laboratory (results unpublished) in which the uptakes of different agents, such as sucrose, sodium, iodide, and albumin are quite differently effected by CTMB and other surfactants. Thus differences in penetrability, reactive potency, stability, and so on may tend to mask a specificity of terminal action. It should be pointed out that cobra venom, like CTMB,

does not cause an inordinate degree of sensitivity of the giant squid axon with respect to conduction block by ACh.[9] Cottonmouth moccasin venom, however, does appear to render axons quite sensitive to ACh block.[10] Another consideration is that ACh molecules may reach the axonal membrane but are hydrolyzed rapidly enough as they approach the regions where successful interactions might occur that an effective concentration is not reached. The possibility that this actually may occur is suggested by the results presented in Tables 3 and 4. The effect of 0·1 M ACh was potentiated and slightly accelerated by the addition of a small amount of neostigmine (cf. series *a* and *i* in Table 3); this amount of neostigmine, 0·00003 M, by itself was without effect on the CTMB-treated nerves. The progressive addition of eserine to 0·01 M ACh led to increased conduction block; eventually, at 0·005 M eserine, a situation was reached in which eserine itself produced depression of the amplitude of the A-potential. Thus, the apparent lack of specificity of conduction block might be the result of a substantial diminution of the ACh concentration in the neighborhood of the hypothetical receptor sites. Another consideration is that the lack of specificity might be the result of a modification of the physicochemical binding properties of the receptor protein as a result of the CTMB treatment.

It must be noted that others have observed conduction block, or membrane depolarization, or both from the external application of ACh. Tourtellotte, *et al.*[11] noted reversible conduction block by ACh (2 to 10 mM) in the optic nerve of *Limulus*, and Ochs and Mukherjee[12] reported depolarization of nerve-free areas of frog sartorius fibers with ACh (10^{-6} to 10^{-5} g/cc). The interpretations and methodology of Ochs and Mukherjee, it should be pointed out, have been challenged by Katz and Miledi[13] who were unable to repeat the results. Armett and Ritchie[14] found that ACh (0·1 to 1·6 mM) depolarized the membrane and reduced the size of the spike potential of C-fibers in the desheathed vagus nerve of the rabbit. More recently Rosenberg and Ehrenpreis[9] have reported reversible conduction block by curare (1·4 to 5·6 mM) and ACh (22 to 90 mM) in the squid giant axon after pretreatment with cobra venom or cetyltrimethylammonium chloride, or both. The former objection to a cholinergic theory of impulse conduction that lipid-insoluble quaternary ammonium ions are without effect on conduction is certainly no longer supported, as shown by a large amount of experimental data obtained from a variety of biological systems under greatly varying conditions.

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