Acta Medica Okayama

Volume 36, *Issue* 3 1982 *Article* 7

JUNE 1982

Inhibition of human motor endplate cholinesterase by anticholinesterase compounds.

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Abstract

The inhibition of human motor endplate cholinesterase by anticholinesterase compounds was studied using isolated muscle membrane preparation. Ambenonium was most potent, and edrophonium was least potent in inhibiting motor endplate cholinesterase. The slope of the regression line for inhibition of motor endplate cholinesterase was greatest for ambenonium, and smallest for neostigmine and edrophonium. These compounds were less potent inhibitors of plasma cholinesterase. Ambenonium was more specific, and other compounds were less specific inhibitors of motor endplate cholinesterase. In myasthenic patients, these compounds produced adequate inhibition of motor endplate cholinesterase even in the presence of relatively mild plasma cholinesterase inhibition.

KEYWORDS: human motor endplate, cholinesterase, anticholinesterase compounds, myasthenia gravis

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*PMID: 7113748 [PubMed - indexed for MEDLINE]

Acta Med. Okayama 36, (3), 229–232 (1982)

—— BRIEF NOTE ——

INHIBITION OF HUMAN MOTOR ENDPLATE CHOLINESTERASE BY ANTICHOLINESTERASE COMPOUNDS

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Received November 6, 1981

Abstract. The inhibition of human motor endplate cholinesterase by anticholinesterase compounds was studied using isolated muscle membrane preparation. Ambenonium was most potent, and edrophonium was least potent in inhibiting motor endplate cholinesterase. The slope of the regression line for inhibition of motor endplate cholinesterase was greatest for ambenonium, and smallest for neostigmine and edrophonium. These compounds were less potent inhibitors of plasma cholinesterase. Ambenonium was more specific, and other compounds were less specific inhibitors of motor endplate cholinesterase. In myasthenic patients, these compounds produced adequate inhibition of motor endplate cholinesterase even in the presence of relatively mild plasma cholinesterase inhibition.

Key words : human motor endplate, cholinesterase, anticholinesterase compounds, myasthenia gravis.

The effect of anticholinesterase compounds on cholinesterase (ChE) has been relatively poorly studied in human tissues, particularly skeletal muscles. There has been no quantitative study of their action on motor endplate ChE because of the lack of a suitable method. In this paper, the inhibitory action of anticholinesterase compounds on human motor endplate ChE was studied using isolated muscle membrane preparation.

The anticholinesterase compounds studied were ambenonium chloride, neostigmine methylsulfate, pyridostigmine bromide and edrophonium chloride. The muscle membrane preparation was isolated by a modification of our previous method (1). Human intercostal muscle was homogenized in a Polytron homogenizer with 50 mM $CaCl_2$ solution, and filtered through an 18 mesh plastic net. In order to prepare the muscle membrane fraction, the sarcoplasmic components of the homogenate were dissolved with buffered KCl solution, and removed by repeated washing with 25 mM glycine-NaOH buffer and 2.5×10^{-7} M NaOH solution. The isolated muscle membrane contained ChE activity only in the motor endplates as observed by the histochemical staining for ChE activity (2). The ChE activity of the membrane was determined by a modification of the

hydroxamic acid method (3), with an incubation at 37℃ for 30 min in a medium containing 100 mM NaCl, 10 mM $MgCl_2$, 30 mM sodium phosphate buffer at pH 7.5, and 4 mM acetylcholine bromide as substrate. Protein was measured by the method of Lowry *et al.* (4) with bovine serum albumin as the standard. The effect of anticholinesterase compounds on ChE was determined by incubation of the sample in the medium with the compound but without substrate for 15 min at room temperature prior to the addition of acetylcholine as substrate.

The ChE activity of human muscle membrane was 1.25 ± 0.18 μmoles of acetylcholine hydrolyzed in 30 min (mean ± 95% confidence limits in 15 samples). From the number of motor endplates in human muscle membrane, a single motor endplate was calculated to have hydrolyzed 0.40 ± 0.24 μmoles of acetylcholine in 30 min (mean ± 95% confidence limits in 15 samples), or 1.35 × 10^8 molecules of acetylcholine per msec.

In inhibition of human motor endplate ChE, ambenonium was most potent, followed by neostigmine and pyridostigmine, and edrophonium was least potent (Table 1). The slope of the regression line for percent inhibition of ChE in probit versus log molar concentration of anticholinesterase compounds is inversely proportional to the difference between the effective and toxic doses, which is one of the reasons for its safety margin. The slope was greatest in ambenonium, followed by pyridostigmine, and smallest in neostigmine and edrophonium. The inhibitory activity for human motor endplate ChE relative to the activity for human plasma ChE indicates relative specificity, and was greatest for ambenonium, followed by edrophonium and neostigmine, and smallest for pyridostigmine.

Following an intramuscular injection of 0.7 mg neostigmine in 5 patients with myasthenia gravis, the maximum inhibitlon of plasma ChE was a mean of 11.8% (Table 2). This degree of inhibition was achieved in vitro by a neostigmine concentration of 34.1 nM. If the conditions in vivo are similar to the conditions in vitro, motor endplate ChE at this concentration of neostigmine would

TABLE 1. INHIBITION OF CHOLINESTERASE ACTIVITY OF HUMAN MOTOR ENDPLATE AND PLASMA BY ANTICHOLINESTERASE COMPOUNDS[a]

	Edrophonium	Pyridostigmine	Neostigmine	Ambenonium
pI_{50}				
Motor endplate	5.17±0.10 (10)	6.15±0.04 (13)	7.33±0.09 (16)	8.52±0.09 (11)
Plasma	3.54±0.07 (17)	5.65±0.06 (18)	6.73±0.05 (33)	5.00±0.03 (21)
Slope				
Motor endplate	1.25±0.33 (10)	1.43±0.13 (13)	1.25±0.20 (16)	1.65±0.29 (11)
Plasma	1.37±0.14 (17)	1.60±0.13 (18)	1.56±0.12 (33)	1.12±0.05 (21)
Activity ratio				
endplate/plasma	42.1	3.1	3.9	3289

a Mean±95% confidence limits (number of estimations).

have been inhibited by 41.7%. Oral administration of 150 mg pyridostigmine to 5 myasthenic patients produced a maximum plasma ChE inhibition of 14.3% (Table 3). This inhibition corresponds to a plasma pyridostigmine concentration of 497 nM, and therefore to a motor endplate ChE inhibition of 40.3%.

The results suggest that these compounds produce enough inhibition of

TABLE2. INHIBITION OF PLASMA CHOLINESTERASE IN PATIENTS WITH MYASTHENIA GRAVIS FOLLOWING INTRAMUSCULAR INJECTION OF 0.7 MG NEOSTIGMINE METHYLSULFATE, AND ESTIMATED PLASMA NEOSTIGMINE CONCENTRATION AND MOTOR ENDPLATE CHOLINESTERASE INHIBITION

Patient	Inhibition of plasma cholinesterase (%)[a]	Estimated plasma neostigmine concentration (nM)	Estimated inhibition of motor endplate cholinesterase (%)[a]
1	19.8	52.6	52.5
2	14.2	37.7	45.3
3	14.4	38.3	45.7
4	7.8	22.6	34.6
5	6.3	19.2	31.4
Mean	11.8	34.1	41.7
95% confidence limits	6.2 — 20.1	17.3 — 50.9	31.3 — 52.8

a Percent values were calculated after the probit transformation.

TABLE 3. INHIBITION OF PLASMA CHOLINESTERASE IN PATIENTS WITH MYASTHENIA GRAVIS FOLLOWING ORAL ADMINISTRATION OF 150 MG PYRIDOSTIGMINE BROMIDE, AND ESTIMATED PLASMA PYRIDOSTIGMINE CONCENTRATION AND MOTOR ENDPLATE CHOLINESTERASE INHIBITION

Patient	Inhibition of plasma cholinesterase (%)[a]	Estimated plasma pyridostigmine concentration (nM)	Estimated inhibition of motor endplate cholinesterase (%)[a]
6	9.6	341	32.3
7	17.2	569	44.3
8	12.1	414	36.7
9	21.2	708	49.7
10	13.5	458	39.1
Mean	14.3	497	40.3
95% confidence limits	9.5 — 20.6	320 — 676	32.1 — 48.9

a Percent values were calculated after the probit transformation.

motor endplate ChE and facilitate neuromuscular transmission at a does which causes relatively mild plasma ChE inhibition.

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