Title : IN VITRO 4-AMINOPYRIDINE-ANTICHOLINESTERASE INTERACTION

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Introduction. It has been reported that 4-aminopyridine (4-APYR) reliably antagonizes nondepolarization block in man and is free of unwanted side effects[1]. In contrast, Miller et al[2] found that 4-APYR is a poor antagonist of profound neuromuscular (n.m.) block in man. They observed, however, that satisfactory reversal of the n.m. block could be achieved by combinations of relatively small doses of 4-APYR and anticholinesterases both in rats[2] and man[3]. The present study was undertaken to compare in vitro the antagonism of nondepolarizing block by 4-APYR, neostigmine or pyridostigmine alone or by various combinations of these two types of agents.

Methods. The experiments were carried out on the rat phrenic nerve-hemidiaphragm preparation suspended in an organ bath in mammalian Krebs' solution, aerated with 95% O_2 - 5% O_2, at 37°C. The hemidiaphragms were exposed to a resting tension of 10 g and were indirectly stimulated at the rate of 0.1 Hz with supramaximal square wave pulses of 0.1 msec duration. The isometric twitch tension was recorded with a FT03 transducer on a Grass polygraph. The effect on the twitch tension of 4-APYR, neostigmine or pyridostigmine or those of combinations of 4-APYR with the two anticholinesterases was determined in some experiments in the absence of relaxants and in others after an about 90% n.m. block was produced by d-tubocurarine (d-Tc) or pancuronium.

Results. In the absence of relaxants the preliminary administration of a marginally effective concentration of 4-APYR decreased the concentrations of neostigmine and pyridostigmine required to increase the twitch by 50% of control by a factor of about 10 ($p<0.001$). The preliminary administration of neostigmine or pyridostigmine had similar effect on the ED50 of 4-APYR. The ED50 values of the compounds administered alone or preceded by another type of antagonist, for the reversal of an about 90% d-Tc induced n.m. block are summarized in table 1. The data indicate that the preliminary addition of a marginally effective concentration of 4-APYR significantly decreases the ED50 of neostigmine or pyridostigmine and that the preliminary administration of either of the two anticholinesterases has a similar effect on the ED50 of 4-APYR. Similar results were obtained with pancuronium.

Discussion. Our in vitro findings on the mutual increase of the antagonist effects of 4-APYR and anticholinesterases are in agreement with the in vivo observations of Miller et al[2,3]. This mutual "potentiation" of the antagonist effect may be explained by the different mechanism of action of the two types of antagonists. 4-APYR increases presynaptic acetylcholine (ACh) release and it augments the contractile strength of the muscle[4]. The primary effect of neostigmine or pyridostigmine is inhibition of the hydrolysis of ACh released by the nerve impulse. Because of the 4-APYR induced increased ACh release, the ACh concentration required for the competitive displacement of n.m. blocking agents from the cholinergic receptors of the postjunctional membrane, can be achieved with relatively low concentrations of anticholinesterases. The restoration of muscle strength is further enhanced by the direct beneficial effect of 4-APYR on the contractility of the muscle fibers.

References.

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Table 1. Antagonism of the d-Tubocurarine Induced Neuromuscular Block by 4-Aminopyridine, Neostigmine, Pyridostigmine and by Combinations of these Agents.

Antagonists	ED50 ng/ml
4-Aminopyridine	192.5±22.87[1]
Neostigmine	3.1± 0.17
Pyridostigmine	27.5± 1.16
4-APYR[2] →Neostigmine	1.0± 0.12*
4-APYR →Pyridostigmine	6.1± 0.61*
Neostigmine[3] →4-APYR	110.0±10.00†
Pyridostigmine[3] →[4] 4-APYR	99.1±14.97†

[1] Means±SEM of 4 to 5 experiments in which 90% n.m. block was produced by d-tubocurarine. [2] 1 ng/ml; [3] 6 ng/ml; [4] 100 ng/ml. * and † indicate significance at the $p<0.001$ and 0.02 levels respectively between the ED50 of neostigmine or pyridostigmine alone or preceded by 4-aminopyridine and the ED50 of 4-aminopyridine alone or preceded by neostigmine or pyridostigmine.