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Title: IN VITRO NEUROMUSCULAR EFFECT OF ACETAMINOPYRIDINE-N-OXIDE

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Introduction. 4-Aminopyridine (4-APYR) antagonizes non-depolarization block in humans[1]. as well as in vitro.[2]. Due to poor ionization, 4-APYR easily penetrates the blood-brain barrier, resulting in central stimulating symptoms.

We synthesized a derivative of 4-APYR, acetamino pyridine-N-Oxide (ANO) which has greater polarity than 4-APYR and studied the in vitro neuromuscular effects and antagonism of d-Tc by ANO in rat preparations.

Methods. Rat phrenic nerve-hemidiaphragm preparation were suspended in an organ bath in mammalian Krebs' solution aerated with 95%O_2-5%CO_2 at 37°C. Supramaximal square wave stimuli of 0.1 and 2.0 msec were applied at 0.1 Hz for indirect and direct stimulation, respectively.

The isometric twitch tension was recorded and the effect of 4-APYR and ANO on twitch tension was determined, following the production of a 90% neuromuscular block.

To determine the effect of ANO on presynaptic Acetyl choline (Ach) release, the preparations were stimulated indirectly.

After 120 min equilibration in eserinized Krebs' solution, Ach release was determined during 20 min collection periods before and after the addition of ANO (4μg/ml).

Ach concentrations were determined with a biological guinea pig ileum assay.

Results. ANO (1.5μg/ml) increased the indirect and direct twitch tension to 155.1 ± 8.3 and 130.8 ± 3.0% of the control, respectively (Fig.1).

The ED50 of 4-APYR and ANO administered to reverse the 90% d-Tc induced neuromuscular block is summarized in Table 1.

The equipotent ratio (ED50 of 4-APYR/ED50 of ANO) between these two compounds was 2.4.

In the presence of 4 μg/ml ANO, Ach release in the rat preparations was increased by about 38% from 5.62 ± 0.25 to 7.76 ± 0.51 ng/g/min ($p<0.05$).

Discussion. Our in vitro findings indicate that the antagonistic effect per mg ANO is 2.5 times greater than that of 4-APYR.

The pharmacological action of ANO may be ascribable to an increase in presynaptic Ach release and to augmentation of the contractile strength of the muscle.

References.

1. Stoyanov E, Vulchev P, Shturbova M, et al: Clinical electromyomechanographic and electromyographic studies in decurarization with Pymadine. Anaesth Resusc Intensive Ther 4:139-143, 1976

2. Foldes FF, Agoston S, van de Pol F, et al: The in vitro neuromuscular effects of 4-Aminopyridine and its interaction with neuromuscular blocking agents (Abstr). American Society of Anesthesiologists Annual Meeting, 1976, pp 179-180

Table 1.
Antagonism of the d-Tubocurarine Induced Neuromuscular Block by 4-Aminopyridine (4-APYR) and Acetaminopyridine-N-Oxide (ANO)

Antagonists	ED50 ng/ml
4-APYR	199.5 ± 25.67*
ANO	81.8 ± 10.74

Mean ± SEM of 6 experiments in which a 90% block was produced.
* Significant difference between the two drugs at $p<0.05$ by the student's t-test.