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Actions of a New Muscle Relaxant (AH8165) on Neuromuscular Transmission

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The effects of a new muscle relaxant, AH8165, on miniature endplate potential (MEPP) amplitude and frequency, endplate sensitivity to acetylcholine, and muscle twitch tension were studied *in vitro* in the frog sartorius muscle. Nerve terminal effects were studied *in vivo* in the cat soleus muscle and its ventral root fibers. AH8165 stimulates the nerve terminal, as evidenced by increased MEPP frequency and the appearance of post-drug repetitive activity. In the same concentration range at which MEPP frequency is increased, MEPP amplitude, endplate sensitivity to acetylcholine, and twitch tension are decreased. This suggests that AH8165 produces muscle relaxation by blocking postsynaptic cholinergic receptors. (Key words: Neuromuscular relaxants, AH8165.)

SINCE THE INTRODUCTION of neuromuscular blocking agents into clinical medicine, there has been a continued search for relaxants possessing fewer side effects and more desirable blocking properties. AH8165 was synthesized in an attempt to achieve this goal. Animal experimentation has demonstrated this compound to be a short-acting nondepolarizing relaxant which is rapid in onset of effect and completely reversed by cholinesterase inhibitors.[1,2] Preliminary clinical trials in the United Kingdom[3] have shown its effect to be as rapid in onset as that of succinylcholine, of shorter duration than that of *d*-tubocurarine, and completely reversed by cholinesterase inhibitors.

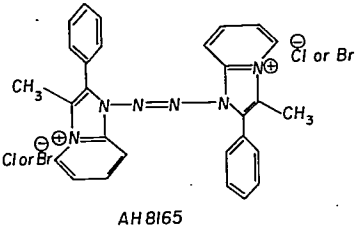

FIG. 1. Chemical structure of AH8165 (azobis-arylimidazo-(1,2-a)-pyridinium).

We examined the effects of AH8165 on selected aspects of neuromuscular transmission to elucidate the site and mechanism of action of this compound.

Materials and Methods

Crystalline AH8165 (azobis-arylimidazo-(1,2-a) pyridinium) (fig. 1) was dissolved in frog Ringer's solution and used for all *in-vitro* experiments.

Miniature endplate potential (MEPP) amplitude and frequency, as well as sensitivity of the endplate to iontophoretically applied acetylcholine, were studied using an *in-vitro* frog sartorius muscle preparation. The indirectly stimulated sciatic nerve–sartorius muscle preparation was used for muscle twitch tension studies.[4,5] The sciatic nerve–gastrocnemius muscle preparation of the frog with guinea-pig-ileum bioassay was utilized to examine alterations in acetylcholine release.[6] In the cat, *in vivo* post-drug repetitive activity (PDR), posttetanic repetition (PTR), and posttetanic potentiation (PTP) were evaluated in the soleus muscle

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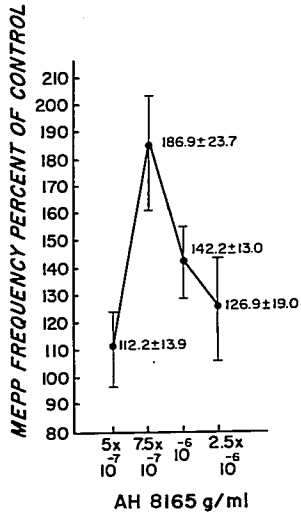

FIG. 2. Effects of AH8165 on MEPP frequency at concentrations from 5×10^{-7} to 2.5×10^{-6}. N = 10; values are means ± SE.

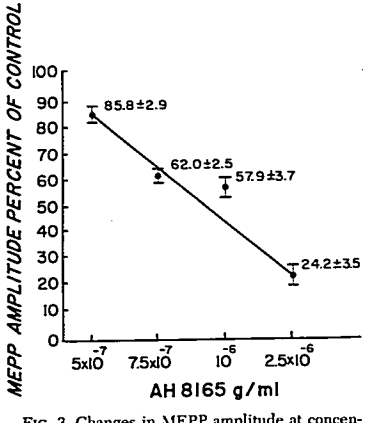

FIG. 3. Changes in MEPP amplitude at concentrations between 5×10^{-7} and 2.5×10^{-6} g/l AH 8165. N = 10; values are means ± SE.

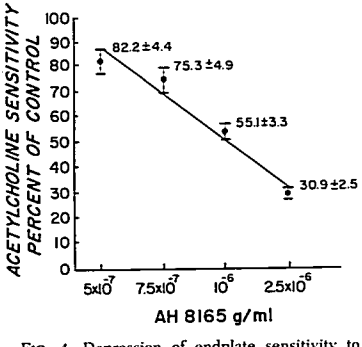

FIG. 4. Depression of endplate sensitivity to acetylcholine caused by AH8165. N = 8; values are means ± SE.

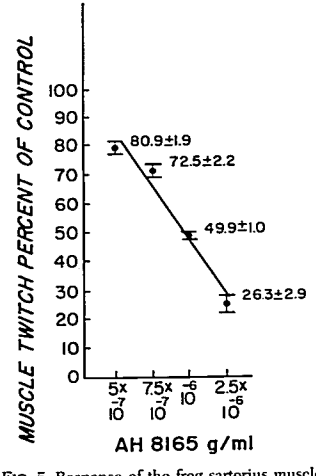

FIG. 5. Response of the frog sartorius muscle to indirect stimulation in the presence of AH8165. N = 8; values are means ± SE.

and its motor nerve following the method of Standaert and Riker.[7]

Statistical comparisons were made using Student's t test. In all cases the level of probability was ≤ 0.05.[8]

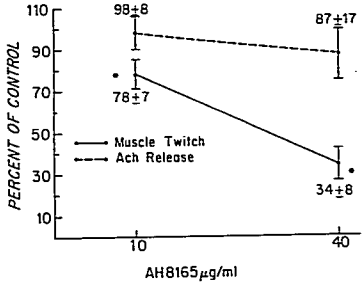

FIG. 6. Effect of AH8165 on acetylcholine release and contraction height in the indirectly stimulated frog sciatic nerve–gastrocnemius muscle preparation. N = 6; values are mean ± SE. *$P \leq 0.005$.

Results

AH8165 in a concentration range between 10^{-11} g/ml and 5×10^{-7} g/ml produced no significant change in MEPP frequency. At 7.5×10^{-7} g/ml, MEPP frequency was 187 per cent of control; it returned to about control value at 2.5×10^{-6} g/ml (fig. 2). In a dose range of 10^{-11} to 10^{-8} g/ml, MEPP amplitude was relatively unchanged. At 5×10^{-7} g/ml, MEPP amplitude decreased to 86 per cent of control; 2.5×10^{-6} g/ml decreased it to 24 per cent of control (fig. 3). Over the same concentration ranges, sensitivities of the endplate to iontophoretically applied acetylcholine were 82 and 31 per cent of control, respectively (fig. 4), while twitch tensions of the indirectly stimulated preparation were 81 and 26 per cent of control (fig. 5). Resting membrane potentials were unaltered over the range of concentrations used.

At the two concentrations examined, 10 μg/ml (10^{-5} g/ml) and 40 μg/ml (4×10^{-5} g/ml) acetylcholine release was not significantly changed from control levels by AH 8165 at a time when muscle contraction was decreased to 78 and 34 per cent of the control values, respectively (fig. 6). Recordings from the ventral root fibers supplying the cat soleus muscle showed that AH8165 in a concentration of 0.001 μg/kg administered intra-arterially produced a transient increase in antidromic action potentials (PDR) concomitant with increases in muscle contraction and contracture of the muscle (fig. 7). At concentrations of 0.01 to 10 μg/kg, the agent did not affect the contraction strength but reduced PTP and PTR (fig. 8), both of which recovered in a 15-minute period, while 10–100 μg/kg reduced PTP, PTR and contraction strength.

Discussion

That AH8165 in low concentrations (10^{-11} to 10^{-7} g/ml) caused no change in MEPP amplitude or frequency while in higher concentrations it produced significant increases in MEPP frequency suggests a presynaptic drug action.[9,10] Further evidence of the nerve terminal activity of AH8165 is shown by the presence of PDR and alterations in PTP and PTR in low concentrations.

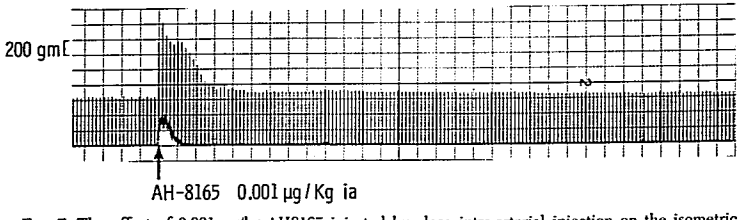

FIG. 7. The effect of 0.001 μg/kg AH8165 injected by close intra-arterial injection on the isometric contraction tension of a cat soleus muscle stimulated supramaximally through its motor nerve. Nerve stimulated once every 2.5 sec. The arrow indicates point of injection.

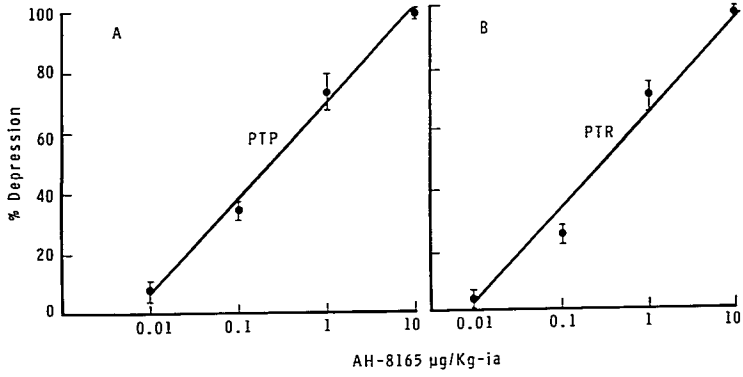

FIG. 8. Effect of AH8165 on posttetanic potentiation of the muscle twitch (PTP) and posttetanic repetitive activity recorded at the ventral root (PTR). N = 10; values are means ± SE. $P \leq 0.05$.

In higher concentrations nerve-terminal activity is depressed, as indicated by the decreases in both PTR and PTP. The nerve-terminal depression, as demonstrated in the ventral root recordings, could not be confirmed by MEPP frequency data. This is probably related to the fact that at higher drug concentrations MEPP amplitude decreased to below the noise level of the recording system.

Katz[9] and Katz and Thesleff[10] discussed the concepts that MEPP frequency is related principally to presynaptic drug activity, while MEPP amplitude is indicative of postsynaptic drug action. Our results demonstrated both pre- and postsynaptic activity of AH8165. Stimulation of the nerve terminal was evidenced by increased MEPP frequency. This stimulation persisted well into the range that produced equal depression of MEPP amplitude, sensitivity of the endplate to iontophoretically applied acetylcholine, and twitch tension. These data suggest that receptor blockade is the primary mechanism of action of AH8165 at the neuromuscular junction.

The postjunctional site of action of AH 8165 is further supported by the acetylcholine-release studies. In these studies we found no significant change in the amount of acetylcholine released by indirect stimulation, even though contraction height was markedly reduced. It was necessary to use higher drug concentrations in this preparation compared with the other studies performed to produce a decrease in contraction height. The need for higher drug concentrations may be the result of diffusion difficulties caused by the thickness of the gastrocnemius muscle compared with the sartorius, and/or different stimulation patterns used in this preparation (interrupted tetanic stimulation).

The short duration of action of this drug is evidenced by rapid recovery from its effects in the *in-vivo* cat preparation.

AH8165 has about one-tenth the potency of *d*-tubocurarine in blocking twitch tension of the indirectly stimulated frog sartorius muscle. This difference in potency does not pertain to other aspects of neuromuscular transmission, such as MEPP amplitude, endplate sensitivity to acetylcholine, or acetylcholine release. This discrepancy may reflect differences in degrees of action of the two drugs at the pre- and postsynaptic elements.

The data presented in this study suggest that AH8165 is a short-acting neuromuscular blocking agent with a predominant site of action at the postsynaptic membrane. On the basis of data obtained in animal studies and

limited clinical trials, the drug deserves further clinical investigation.

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Drugs and Their Use

BETA BLOCKADE AND ATRIAL TACHYCARDIA The authors studied nine children in whom supraventricular tachycardia refractory to conventional therapy was treated with propranolol. In three children normal sinus rhythm was restored and in three others frequency of paroxysmal arrhythmias was decreased. In one there was a reduction of the ventricular response to the ectopic rhythm, and in two patients propranolol had no effect. The doses of propranolol ranged from 0.5 to 4.0 mg/kg per day, given orally. There were few side-effects.

Little information is available concerning the use of propranolol as an antiarrhythmic agent in children. The drug is known to depress automaticity of the sinus and subsidiary pacemakers, slow conductivity of the sinoatrial and atrioventricular junctions, and increase the refractory period of the atrioventricular node. The treatment of choice in supraventricular tachycardia of childhood is digoxin. However, in 15 per cent of patients the condition is refractory to this drug, as well as to quinidine, cardioversion, and other modes of therapy. The authors claim that propranolol can be useful in these situations. The aim of treatment in these patients is restoration of a physiologic ventricular rate, rather than suppression of the ectopic rhythm. *(Dworkin, P., Bell, B., and Mirowski, M.: Propranolol in Supraventricular Tachycardias of Childhood. Arch Dis Child 48:382–385, 1973.)* ABSTRACTER'S COMMENT: These encouraging results plus personal experience indicate that propranolol is a useful adjunct in the treatment of childhood supraventricular arrhythmia refractory to conventional therapy.