Dept. of Pharmacology,
Faculty of Vet. Med., Alexandria Univ.,
Head of Dept. Prof. Dr. B. Reiad.

THE DEPOLARIZING NEUROMUSCULAR BLOCKING ACTIVITY OF DIMINAZINE ACETURATE (BERENIL(R)) IN DIFFERENT ANIMAL SPECIES

(With 6 Figs.)

By

A.F. EL-SAWI and F.R. ALI*

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النشاط اللااستقطابي المانع للاتصال العصبي العضلـــــــــي

للدايمينازين استيورات في الحيوانات المختلفة

عبدالسلام الصاوى ، فتحي رضوان

تم في هذا البحث دراسة تأثير الدايمينازين استيورات على الاتصال العصبي العضلي وقد تبين من البحث أن هذا الدواء ينشط الانتفاضات العضلية المحدثة في العضلة العنقية ذات البطنين المعزولة من رقبة الكتكوت كما انه ينشط تأثير مادة الكارباكول عليها. كما تبين أن هذا الدواء يسبب تأثيرا مثبطا على عضلة الحجاب الحاجز في الفئران كما لوحظ أن هذا الدواء يسبب نشاطا في الانقباضات العضلية لعضلات الشفه العليا في الكلاب والأرانب وخنازير غينا وعلى العضلة القصبية الأمامية في الأرانب.. كما تبين أن دواء الدايمينازين استيورات يقوى التأثير المثبط لمادة السكسنايل كولين على عضلات الشفه العليا للكلاب . ويستخلص من هذه الدراسة أن دواء الدايمينازين استيورات يؤثر على الاتصال العصبي العضلي بتأثير لا استقطابي.

SUMMARY

The effect of diminazine aceturate (Berenil(R)) on the neuromuscular transmission was studied. On the chick biventor cervicis muscle, diminazine augmented the indirectly elicited muscle twitches and the contracture induced by carbachol on this avian muscle. On the rat diaphragmatic muscle, higher doses of the drug produced partial neuromuscular blockade which was potentiated by the addition of neostigmine. Diminazine aceturate augmented the indirectly elicited muscle twitches of the upper lip muscles of anaesthetised dogs, rabbits and guinea pigs followed by partial neuromuscular blockade, meanwhile, it induced paralysis of the tibialis muscle of rabbits. Diminazine potentiated the inhibitory effect of succinyl choline on the upper lip muscles of anaesthetised dogs. It could be concluded that Diminazine aceturate affected the neuromuscular transmission with a depolarizing action.

* Dept. of Forensic Medicine, Fac. of Vet. Med., Alex. Univ.

INTRODUCTION

Diminazine aceturate (Berenil)(R) is a trypanocidal and babesicidal drug belonging to the series of diamedines. WEIN (1943) and DAYKIN (1956) reported that diminazine administration resulted in arteriolar dilatation lowering the blood pressure. They added that low concentration of diamedines increased the tone and contractility of the rabbit intestine. FUSSGANER (1955) and ENIGK and REUSSE (1955) recorded that dogs administered diminazine (30 - 35 mg/kg b.wt.) showed tremors, nystagmus and ataxia. Higher doses of diminazine resulted in tonic spasms, incoordinated movements and eventually death. Moreover, LOSES and CROCKETT (1969) found that dogs administered diminazine at 15, 20 or 60 mg/kg b.wt. showed nervous symptoms including spastic paralysis and nystagmus followed by death. NAUDE, et al. (1970) studied the toxic effect of diminazine in dogs and cattle. With toxic doses they observed severe symptoms such as imbalance, rolling movements, extensor rigidity, opisthotonus, nystagmus and terminal paralysis in most of dogs. Mild symptoms were produced in cattle.

Studies and references concerning the effect of Diminazine aceturate on the neuromuscular transmission are so scarce. Moreover, few literature dealing with this subject was available. Therefore, the aim of the present work was to study the effect of Diminazine aceturate on the neuromuscular transmission both in vivo and vitro in different animal species.

MATERIAL and METHODS

Diminazine aceturate (Berenil(R), Hoechst).

Alpha-chloralose (Prolabo), Carbachol (Arab Drug Co.) Ethyl Carbamate (Urethane, BDH), Neostigmine bromide (Sigma), Thiopentone sodium (Biochemie), Succinyl choline (Chole-succinyl, Cairo) and Tubocurarine (Sigma) were used in this study.

I- Experiments on isolated preparations:

1) Chick biventor cervicis muscle preparation:

The praparation was carried out according to the method described by GINSBORG and WARRINER (1960). The muscle was isolated and bathed in Kreb's solution, aerated with Carbogen at 37°C. The upper end of the muscle was attached to a Force-Displacement Transducer (FT -03C) connected to a Grass Polygraph (Model 7D). The nerve was stimulated by a means of a Grass stimulator (Model S48) using rectangular pulses of supramaximal voltage (10-20) and 1 msec. duration at a frequency of 0.1 Hz.

2) Rat phrenic nerve hemi-diaphragm preparation:

This was set up in 100 ml organ bath according to the method of bulbring (1946), using Kreb's solution at 37°C, aerated with Carbogen. The phrenic nerve was

stimulated at a frequency of 0.1 Hz with a rectangular pulses of 1 msec. duration at supramaximal voltage (10-30). The muscle was connected to a Force-Displacement Transducer (FT -03C) and contractions were recorded on a Grass Polygraph (Model 7D).

II- Experiments on intact animals:

The upper lip muscles of anaesthetised dogs, rabbits and guinea pigs were used according to the method described by EL-SAWI, et al. (1985) Anaesthesia in 5 mongrel mature healthy dogs was induced by thiopentone sodium (10 mg/kg b.wt.) given i.v as 5% solution in saline. Anaesthesia was then maintained by alpha-chloralose at a dose of 100 mg/kg b.wt. given as 1% solution in saline. Three rabbits and three guinea pigs were anaesthetised using ethylcarbamate at a dose of 1.75 gm/kg b.wt. injected intra -venously. The animals were heparinized using heparin (El-Nil) at a dose of 500 I.U/kg i.v. The trachea was cannulated using glass connula.

2) The tibialis anterior muscle:

Simultaneous recording of the responses of the anterior tibialis muscle together with the upper lip muscles of anaesthetised rabbit (in the same animal) was performed. The method described by BROWN (1938) was adopted for the tibialis muscle.

The dorsal buccal branch of the facial nerve and the lateral popliteal nerve were stimulated every 10 seconds interval with a pulse wave duration of 1 msec. at supramzximal voltage (1.5-6). Tracings were recorded on a smoked paper on the drum of the Kymograph.

RESULTS

I- Effect on isolated skeletal muscle preparations:

1) Effect on the chick biventor cervicis muscle:

Diminazine aceturate in doses of 10 or 20 ug/ml augmented the electrically evoked muscle twitches of the chick biventor cervicis muscle. The drug induced slight augmentation of the contracture induced by Carbachol (5 ug/ml), (Fig. 1).

2) Effect on the rat phrenic nerve hemidiaphragem preparation:

Diminazine aceturate (10 ug/ml) slightly augmented the indirectly evoked muscle twitches of the diaphragmatic muscle. Doses of 20 or 40 ug/ml of Diminazine induced partial neuromuscular blockade which was potentiated by the addition of neostigmine (0.3 ug/ml). Diminazine aceturate (20 ug/ml) potentiated the neuromuscular blockade induced by tubocurarine (1 ug/ml) on the diaphragmatic muscle, (Fig. 2 & 3).

II- Effect on intact animals:

1) Effect on the upper lip muscles:

Diminazine aceturate in a dose of 2 mg/kg b.wt. i.v augmented the indirect muscle twitches of the upper lip muscles of dogs. A dose of 3 mg/kg b.wt. i.v induced partial neuromuscular blockade which was preceded by slight augmentation of muscle twitches. Diminazine aceturate (3 mg/kg i.v) augmented the inhibitory effect of succinyl choline (30 ug/kg i.v) on the upper lip muscles of dogs, (Fig. 4).

Rabbits treated by Diminazine aceturate (2 mg/kg b.wt. i.v) showed partial neuromuscular blockade of the indirectly elicited muscle twitches of the upper lip muscles, (Fig. 5). Guinea pigs showed a similar response to Diminazine aceturate (2 mg/kg b.wt i.v), (Fig. 6).

2) Effect on the anterior tibialis muscle:

Diminazine aceturate (2 mg/kg b.wt i.v) induced paralysis of the tibialis anterior muscle, meanwhile the drug induced partial neuromuscular blockade of the upper lip muscles of the same animal when the responses of the two preparations were recorded simultaneously, (Fig. 5).

DISCUSSION

The present work was undertaken in an attempt to demonstrate the possible effects of Diminazine aceturate on the skeletal neuromuscular junction. This trypanocide elicited two distinguished effects on response to indirect stumulation. At low level of concentration the drug augmented the muscle twitches, whereas at high level partial neuromuscular blockade was induced. These findings are compatible with those reported by ABDEL-MOTAL (1978).

The augmentation of muscle twitches induced by Diminazine aceturate could not be attributed to an anticholinesterase activity since the drug was not able to antagonize d.tubocurarine induced neuromuscular blockade in the rat diaphragmatic muscle. Whereas, acetylcholinesterase inhibitors are known to antagonize such blockade (ZAIMIS, 1953). So, it is established that drugs which act on the motor nerve terminals whereby decreasing acetylchline release, potentiate the neuromuscular blockade induced by competitive blockers (BRAZIL and CORRADO, 1957).

At the post-junctional site, the response of the chick biventor cervicis muscle was used by HISER, et al. (1975) as at test for the presence of depolarizing activity. Accordingly, Diminazine aceturate has a post-junctional stimulant activity as it augmented the indirectly elicited muscle twitches and the contracture induced by carbachol on the chick biventor cervicis muscle. Moreover, the drug exaggerated and prolonged the inhibitory effect of succinyl choline suggesting a synergistic effect.

It is apparent that Diminazine aceturate effect the neuromuscular transmission non comptitively, since neostigmine failed to antagonize its blockade on the rat diaphragmatic muscle. The blockade produced by depolarizing drugs is unaffected or increased by administration of an anticholinesterase drug (ZAIMIS, 1953). Moreover, it is well known that competitive neuromuscular blockers induced flaccid paralysis. In contrast, the forementioned literature showed that Diminazine aceturate produced spastic paralysis, extensor rigidity and other exaggerated neuromuscular symptoms.

In addition, the present study showed that the response of the upper lip muscles was markedly different from that of the tibialis muscle to Diminazine aceturate. This could be attributed to differences in the sensitivity of some skeletal muscles of the same animal to neuromuscular blockers, (ZAIMIS, 1976 and EL-SAWI, 1987).

It could be concluded that Diminazine aceturate has a neuromuscular blocking activity of the depolarizing type.

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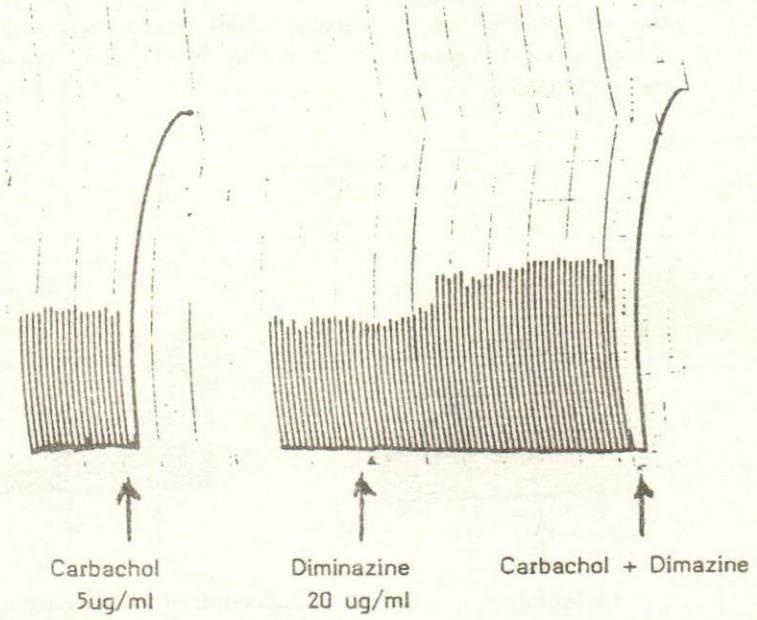

Fig. (1): Effect of Diminazine Aceturate on electrically evoked muscle twitches and carbachol induced contracture of the chick biventor cervicis muscle.

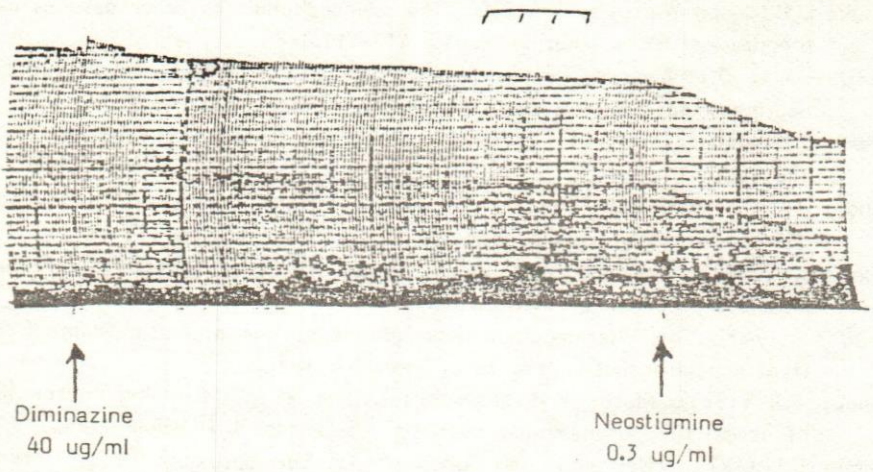

Fig. (2): Effect of Diminazine aceturate on electrically evoked muscle twitches of the rat phrenic nerve hemidiaphragm preparation. Indirect muscle twitches were licited by supramaximal voltag of 1 msec. duration at 0.1 Hz. Time marke 1 minute.

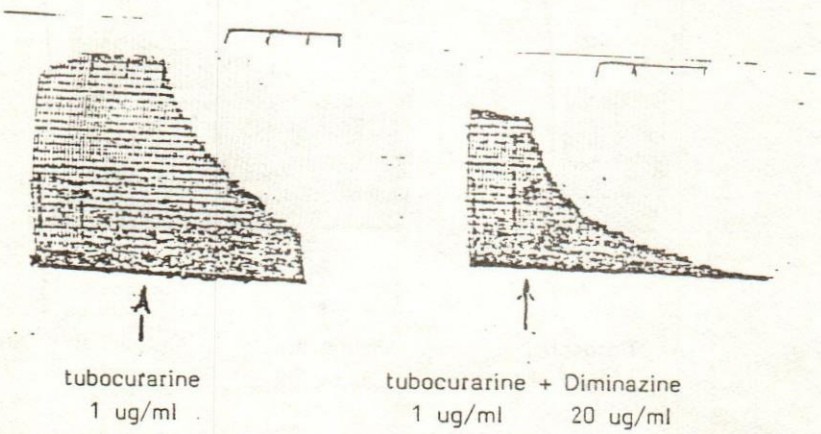

Fig. (3): The effect of Diminazine on the neuromuscular blockade induced by tubocurarie on the rat diaphragm
Time interval : 1 minute.

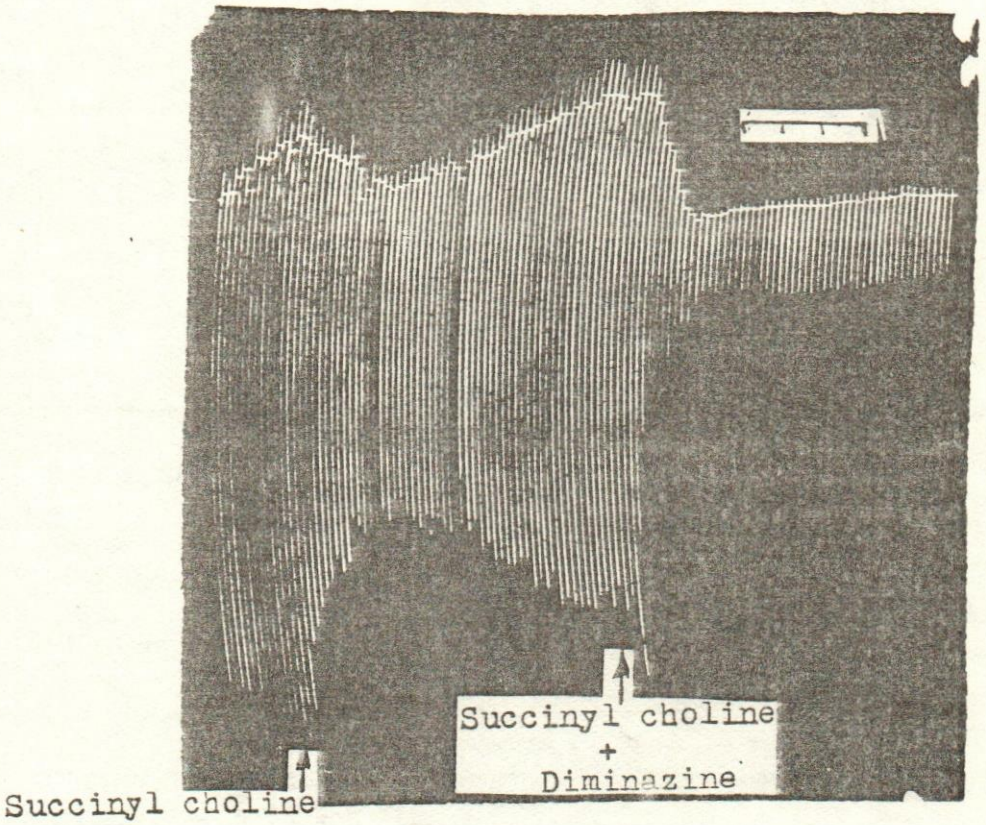

Fig. (4): Interaction of Diminazine aceturate (3 mg/kg i.v) with Succinyl choline (30 ug/kg i.v) one the upper lip muscles of anaesthetised dog. Indirect muscle twitches were elicited by 1.5 - volts repeated every 10 seconds interval.

Time marks : 1 minute.

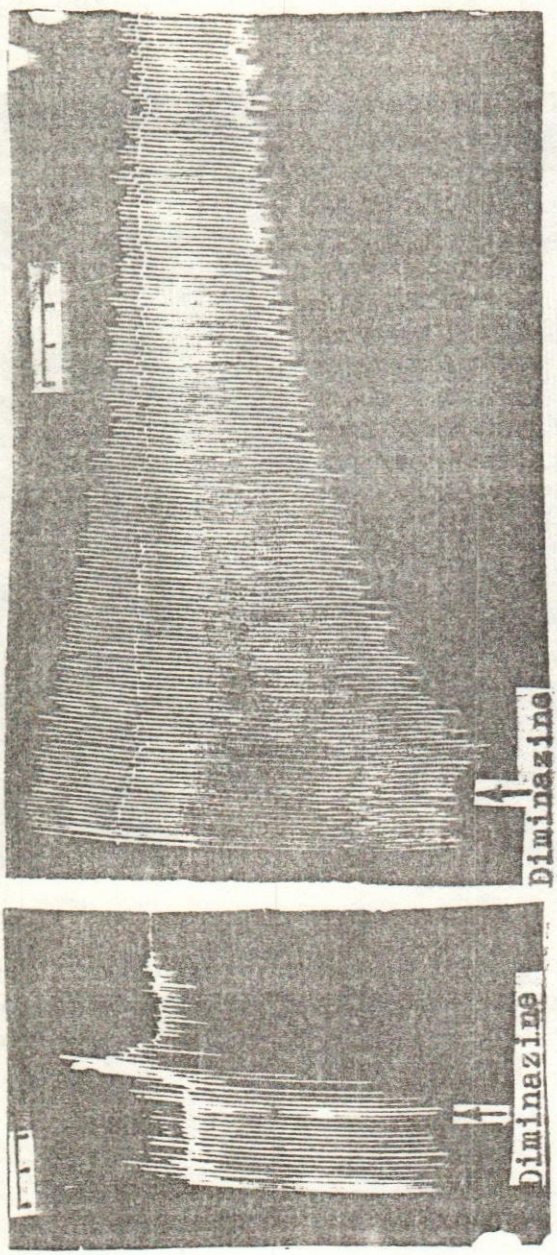

Fig. (5): Simultaneous recording of the rabbits tibialis muscle (A) and the upper lip muscles (B) to Diminazine aceturate (2 mg/kg i.v). Indirect muscle twitches were elicited by 1.5 - Volts repeated at 10 seconds interval.

Time marks : 1 minute.

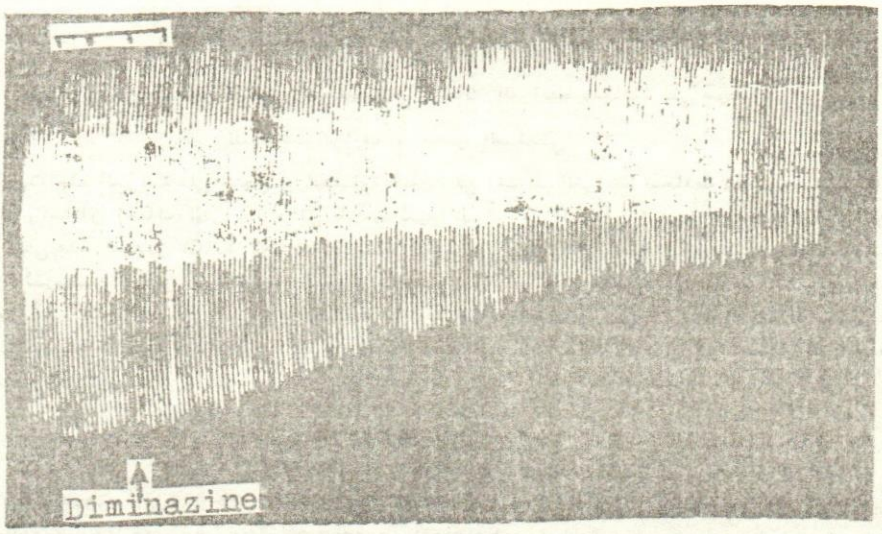

Fig. (6): Effect of Diminazine aceturate (2 mg/kg i.v) on the indirectly elicited muscle twitches of the upper lip muscles of anaesthetised guinea pigs.

Time marks : 1 minute.