Brit. J. Pharmacol. (1962), **18**, 563–571.

EFFECT OF β-DIETHYLAMINOETHYL 3,3-DIPHENYLPROPYLACETATE ON THE ACTION OF SUXAMETHONIUM AND OTHER NEUROMUSCULAR BLOCKING DRUGS

BY

D. DELLA BELLA, F. ROGNONI AND U. TEOTINO

From the Research Laboratories of the Laboratorio Bioterapico Milanese Selvi & Co., Milan, Italy

(*Received January* 29, 1962)

On the frog rectus abdominis muscle and on sciatic nerve-tibialis anterior muscle preparations, β-diethylaminoethyl 3,3-diphenylpropylacetate (SKF 525A) antagonized the actions of acetylcholine and potassium chloride as well as having an antiveratrine action. The blocking action at the skeletal neuromuscular junction of suxamethonium and its disulphonium analogue, decamethonium, tubocurarine and gallamine was enhanced by SKF 525A in the rabbit and in the isolated rat phrenic nerve-diaphragm preparation. The activity of suxamethonium and decamethonium in the cat was reduced. On the rat phrenic nerve-diaphragm preparation, pretreatment with SKF 525A abolished both the mutual antagonism between suxamethonium and tubocurarine and the antagonizing effect of tetraethylammonium against suxamethonium. The antagonism between tetraethylammonium and tubocurarine was unimpaired.

It is well known that β-diethylaminoethyl 3,3-diphenylpropylacetate (SKF 525A), a compound lacking any obvious direct pharmacological effects, can enhance and prolong characteristically the activity of a number of compounds differing widely in both molecular structure and pharmacological properties, such as various analgesic drugs (Cook, Navis & Fellows, 1954b), local anaesthetic agents (Horáková, Votava & Roth, 1959), hypnotic compounds (Cook, Toner & Fellows, 1954c ; Cook, Macko & Fellows, 1954a ; Axelrod, Reichenthal & Brodie, 1954), tubocurarine and mephenesin-like drugs (Macko, Cook, Toner & Fellows, 1953 ; Maas, Carey & Heming, 1953 ; Bovet, Bovet-Nitti, Bettschart & Scognamiglio, 1956 ; Bettschart, Scognamiglio & Bovet, 1956 ; Navis, Toner & Cook, 1953).

In order to throw light on the mechanism by which the activity of suxamethonium and other neuromuscular blocking drugs is influenced *in vivo* by β-diethylaminoethyl 3,3-diphenylpropylacetate, the present investigation was carried out.

METHODS

For the experiments with isolated neuromuscular preparations, the frog rectus abdominis muscle was prepared in the usual manner (bath vol., 10 ml.) and the rat phrenic nerve-diaphragm according to the method of Bülbring (1946) set up in a 100 ml. bath. Experiments *in vivo* were performed on adult female rabbits (average weight 2.5 kg) or on cats (weighing about

2 kg) anaesthetized with 80 mg/kg of chloralose given intravenously. Sciatic nerve-tibialis anterior muscle preparations were made in the conventional manner using a Brown-Schuster myograph. In some experiments in the cat, the tibialis artery was isolated and cannulated distally for retrograde intra-arterial injection according to the technique of Brown (1938). In the *in vivo* experiments and in those on the rat diaphragm, indirect stimulation of the motor nerve was effected by means of platinum electrodes connected to an electronic stimulator delivering rectangular pulses. Single supramaximal stimuli of 0.5 msec duration at a rate of 8 pulses/min were applied.

The following drugs were used: suxamethonium chloride, its disulphonium analogue, decamethonium iodide, tubocurarine chloride, gallamine iodide, tetraethylammonium bromide, β-diethylaminoethyl 3,3-diphenylpropylacetate (SKF 525A), acetylcholine chloride, veratrine sulphate, potassium chloride. Doses are expressed in terms of the salts.

RESULTS

Influence of β-diethylaminoethyl 3,3-diphenylpropylacetate upon neuromuscular block in the rabbit and in the cat induced by suxamethonium and other neuromuscular blocking drugs. Using a sciatic nerve-tibialis anterior muscle preparation, preliminary experiments confirmed the results obtained in the rabbit by Bettschart *et al.* (1956) on the influence of β-diethylaminoethyl 3,3-diphenylpropylacetate on "head-drop" and on the LD50 dose of suxamethonium. In Fig. 1 β-diethylaminoethyl 3,3-diphenylpropylacetate 10 mg/kg given intravenously prolonged and intensified the partial block induced by 200 μg/kg of suxamethonium. The administration of suxamethonium 100 μg/kg then caused a more marked block than that seen originally with double the dose. This hypersensitivity faded with successive administrations of suxamethonium. About 2 hr after the treatment with β-diethylaminoethyl 3,3-diphenylpropylacetate the response to suxamethonium had returned to that seen originally.

β-Diethylaminoethyl 3,3-diphenylpropylacetate in doses of 5 to 10 mg/kg by intravenous injections caused enhancement of the blocking effects induced by tubocurarine, decamethonium, gallamine and a suxamethonium disulphonium analogue.

In the cat the effect of treatment with β-diethylaminoethyl 3,3-diphenylpropylacetate on neuromuscular block following the drugs used in the rabbit was different. Suxamethonium block was antagonized by β-diethylaminoethyl 3,3-diphenylpropylacetate and the effect of this antagonism was still visible 60 min later. Both tibialis and soleus preparations responded in the same manner (Fig. 2). Similar results were obtained with the disulphonium analogue and with decamethonium. In contrast, a marked enhancement of the neuromuscular block, similar to that seen in the rabbit, was found with tubocurarine and gallamine.

In an attempt to explain this species difference, β-diethylaminoethyl 3,3-diphenylpropylacetate was incubated with rat blood or cat blood at a concentration of 25 to 50 μg/ml. for 2 hr at 38° C. The quantity of β-diethylaminoethyl 3,3-diphenylpropylacetate remaining in the blood was then assayed on the frog rectus abdominis muscle preparation, the sensitivity of which to acetylcholine was markedly reduced by the compound as described below. The concentrations of β-diethylaminoethyl 3,3-diphenylpropylacetate in the incubated blood had not altered, so that the species difference could not be attributed to enzymatic hydrolysis.

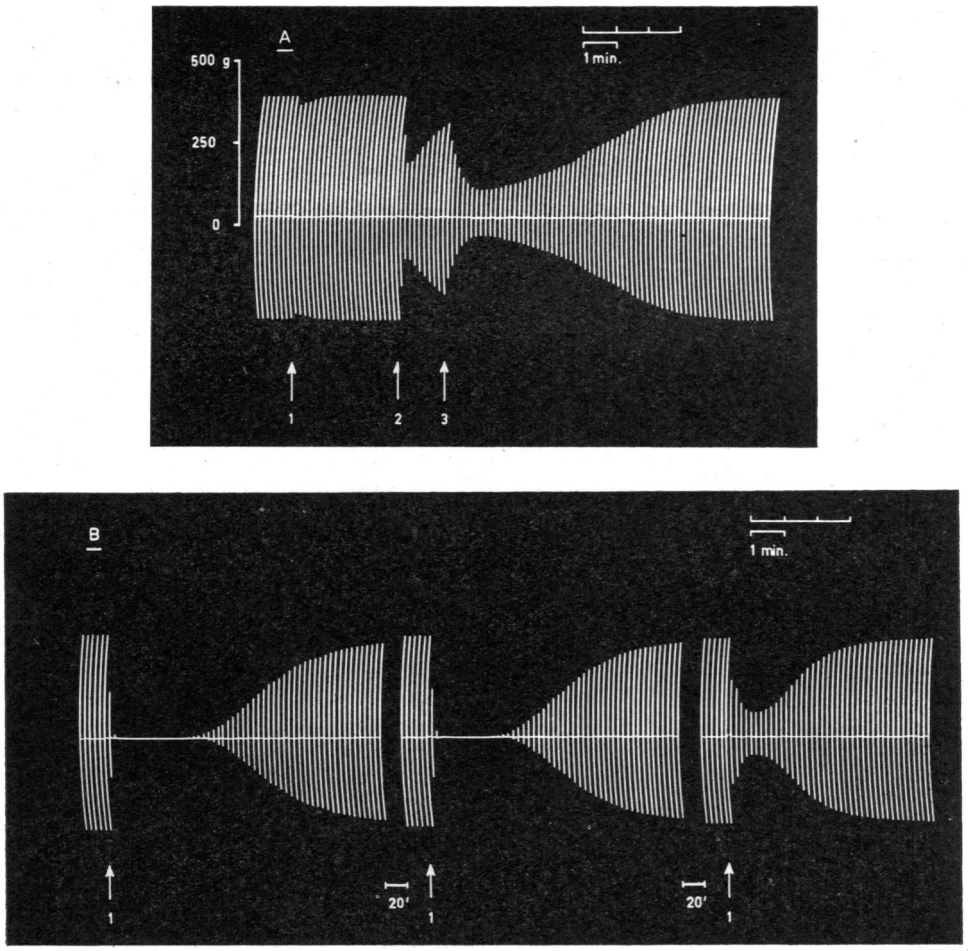

Fig. 1. Rabbit anaesthetized with chloralose; sciatic nerve-tibialis anterior muscle preparation. Indirect stimulation by single supramaximal stimuli. A, At (1) suxamethonium 100 μg/kg. At (2) suxamethonium 200 μg/kg. At (3) SKF 525A 10 mg/kg intravenously enhanced intensity of block. B, Continued from A, after treatment with SKF 525A. At (1) suxamethonium 100 μg/kg. The increased sensitivity of the preparation is clearly evident in the rabbit.

Using the cat tibialis anterior muscle prepared according to Brown (1938), doses as low as 10 to 20 μg of β-diethylaminoethyl 3,3-diphenylpropylacetate intra-arterially produced a marked reduction of the responses elicited by intra-arterial injections of both acetylcholine (1.5 μg) and potassium chloride (2 mg). The responses to the two stimulants had returned to their original values within 30 to 40 min. This antagonistic effect of β-diethylaminoethyl 3,3-diphenylpropylacetate was present, though less marked, when the compound was administered intravenously (Fig. 3).

Influence of β-diethylaminoethyl 3,3-diphenylpropylacetate on the response of the frog rectus abdominis muscle preparation. β-Diethylaminoethyl 3,3-diphenyl-

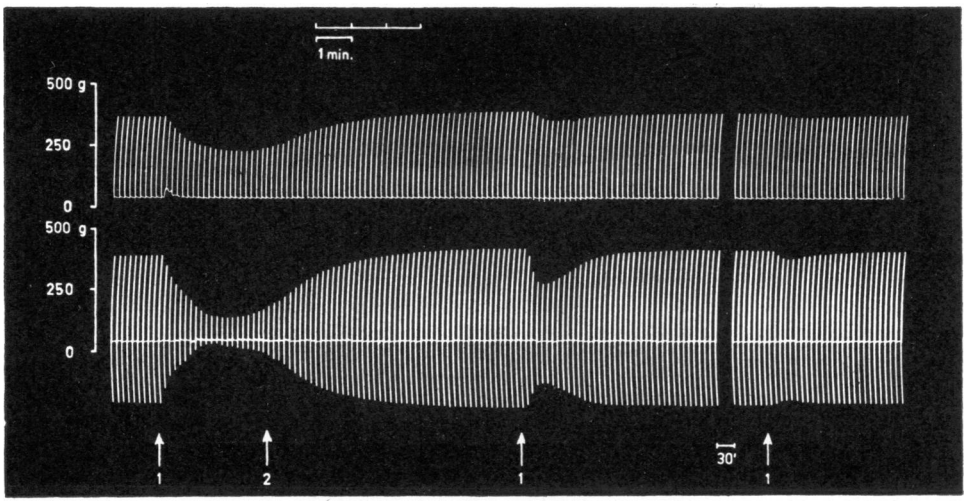

Fig. 2. Sciatic nerve-tibialis anterior muscle preparation (upper tracing) and soleus (lower tracing) of the cat. Chloralose anaesthesia. Indirect stimulation by single supramaximal stimuli. At (1) suxamethonium 50 μg/kg. At (2) SKF 525A 10 mg/kg intravenously. In the cat the response to suxamethonium is antagonized.

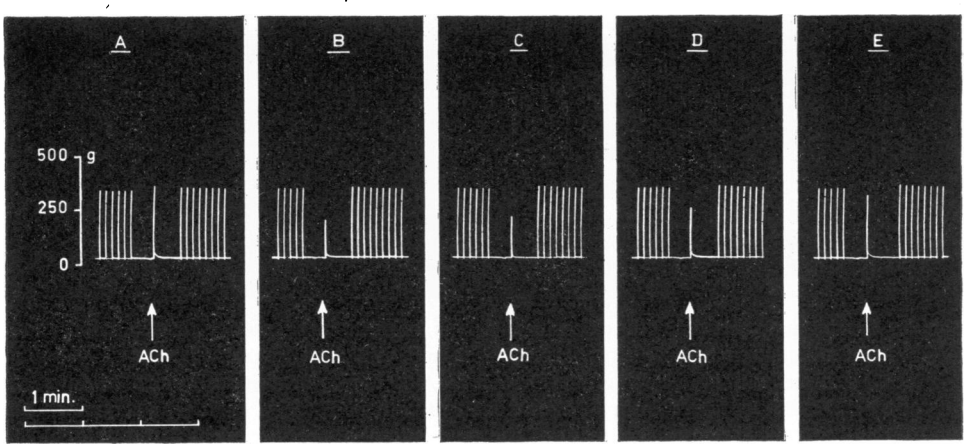

Fig. 3. Cat sciatic nerve-tibialis anterior muscle prepared for intra-arterial injection. Chloralose anaesthesia. Indirect stimulation with single supramaximal stimuli. A, At ACh, retrograde injection into tibialis anterior artery of acetylcholine 1.5 μg. Between A and B SKF 525A 10 mg/kg was given intravenously. In B, C, D, and E, responses to intra-arterial injections of acetylcholine (ACh) at 10, 20, 40 and 60 min intervals respectively, after the SKF 525A administration. The direct response to acetylcholine was reduced at first, but slowly recovered.

propylacetate antagonized very strongly the responses of this preparation to both acetylcholine and suxamethonium (Fig. 4). As in the cat, the action of potassium chloride was also antagonized (Fig. 5). Both duration and magnitude of the inhibiting effects were proportional to the concentrations of β-diethylaminoethyl 3,3-diphenylpropylacetate as well as to the time of contact with the responsive organ.

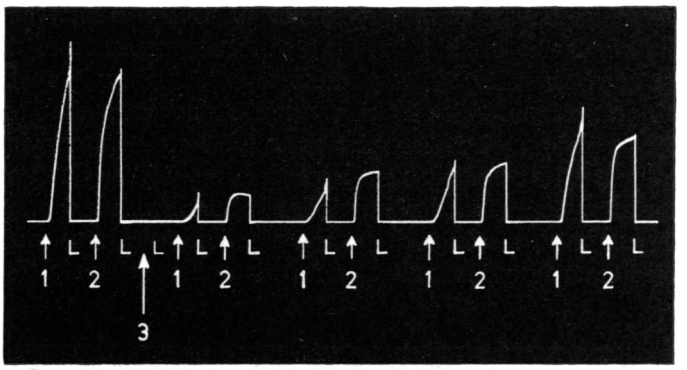

Fig. 4. Isolated frog rectus abdominis muscle preparation. At (1) suxamethonium 1.5 μg/ml., at (2) acetylcholine 0.6 μg/ml., both 2-min exposures; at (3) SKF 525A 2.5 μg/ml., 5-min exposure; at L, washing. 15 min between (1) and (2), 30 min between each pair of responses to the two stimulants. SKF 525A reduced the effect of both stimulants. Bath vol., 10 ml.

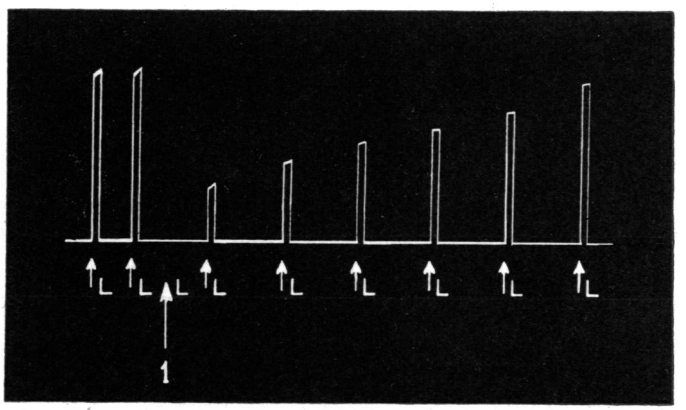

Fig. 5. Isolated frog rectus abdominis muscle preparation. At small arrows, 2 mg/ml. KCl, 2-min exposure. At (1) SKF 525A 5 μg/ml., 5-min exposure. At L, wash-out. 30 min between responses. SKF 525A compound reduced the contracture produced by KCl. Bath vol., 10 ml.

Furthermore, β-diethylaminoethyl 3,3-diphenylpropylacetate inhibited the increased sensitiveness to potassium ions following treatment with either veratrine or tetraethylammonium (Della Bella *et al.*, 1959).

Influence of β-diethylaminoethyl 3,3-diphenylpropylacetate on the phrenic nerve-diaphragm preparation of the rat. Concentrations of 10 μg/ml. of β-diethylaminoethyl 3,3-diphenylpropylacetate decreased the amplitude of contraction to indirect stimulation gradually, resulting in a 50% reduction after 15 min. Recovery occurred after prolonged washing. Higher concentrations caused reductions, the degree and rate of development of which were proportional to the magnitude of the doses used. The contractions in response to direct stimulation of the muscle were unimpaired when the indirect response was reduced by β-diethylaminoethyl 3,3-diphenylpropylacetate.

β-Diethylaminoethyl 3,3-diphenylpropylacetate (5 to 10 μg/ml.) enhanced the blocking activity of suxamethonium, but the greatest augmentation of the action of suxamethonium appeared after β-diethylaminoethyl 3,3-diphenylpropylacetate had been removed from the bath. This striking " tardive " enhancement was dependent on both the concentrations of β-diethylaminoethyl 3,3-diphenylpropylacetate used and on the duration of its contact with the preparation. If 10 μg/ml. of β-diethylaminoethyl 3,3-diphenylpropylacetate was left in contact with the preparation for 10 min which was then thoroughly washed, the increased sensitivity to suxamethonium lasted from 3 to 4 hr and only then waned very slowly (Fig. 6). The

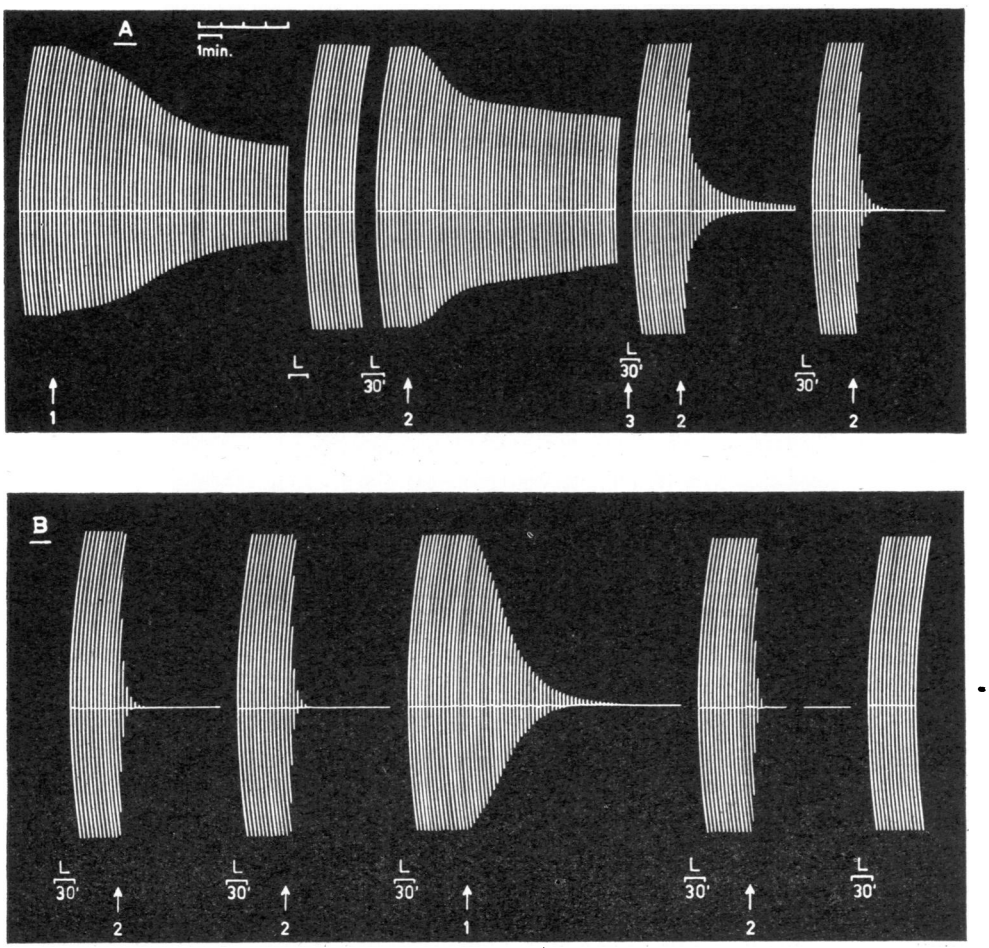

Fig. 6 (A and B). Isolated rat phrenic nerve-diaphragm preparation. Indirect stimulation with single supramaximal stimuli. At (1) tubocurarine 0.4 μg/ml. At (2) suxamethonium 0.8 μg/ml. At (3) 5-min exposure to SKF 525A 10 μg/ml. Wash-out at L. Both tubocurarine and suxamethonium effects were potentiated for some hours after the SKF 525A treatment although the preparation was washed thoroughly. The second dose of suxamethonium (2) was recorded 10 min after treatment with SKF 525A. Bath vol., 100 ml.

preparation also showed increased sensitivity to tubocurarine, the disulphonium analogue, decamethonium and gallamine block.

In view of these results we thought it interesting to examine whether pretreatment with β-diethylaminoethyl 3,3-diphenylpropylacetate modified the characteristic mutual antagonism between suxamethonium and tubocurarine and the antagonistic action of tetraethylammonium and of calcium ions against these drugs. Pretreatment with β-diethylaminoethyl 3,3-diphenylpropylacetate caused a clear synergism instead of the usual antagonism between tubocurarine and suxamethonium (Fig. 7A) and similarly tubocurarine increased a block produced by suxamethonium.

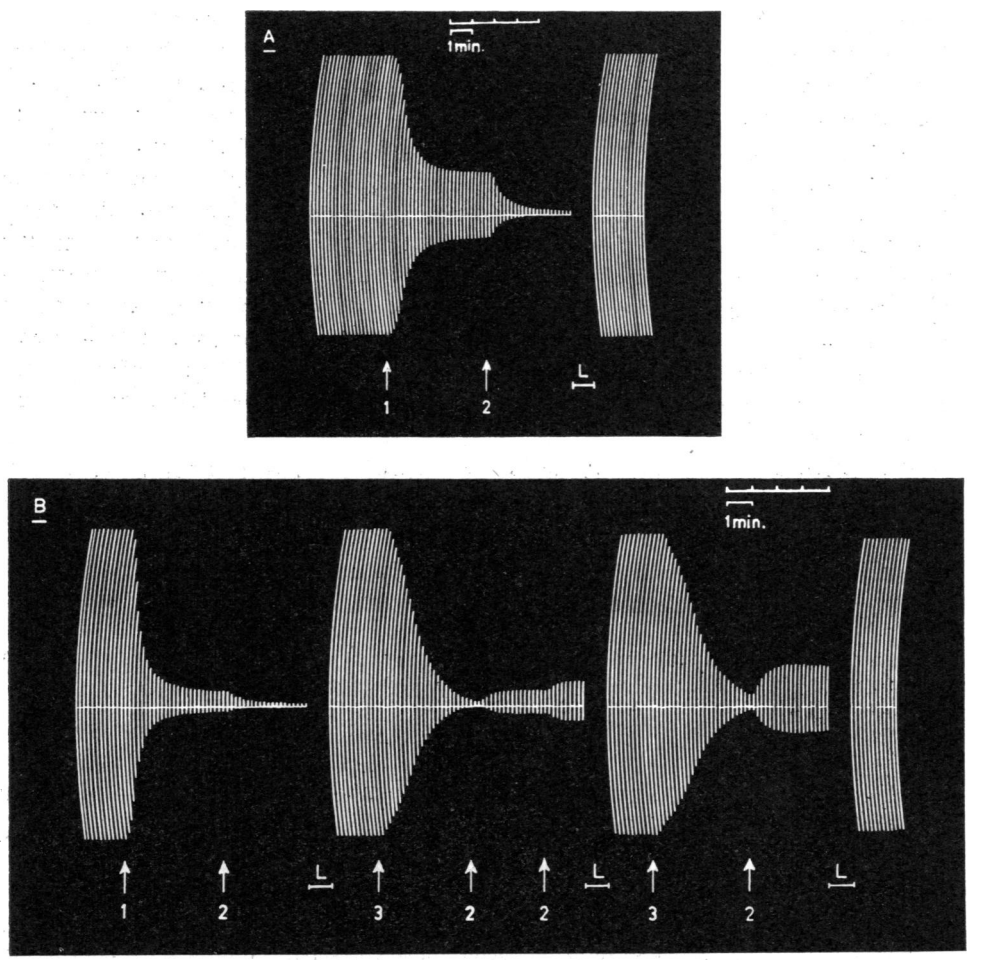

Fig. 7. Isolated rat phrenic nerve-diaphragm preparation. Indirect stimulation with single supramaximal stimuli. The preparation was pretreated with 10 μg/ml. SKF 525A for 5 min and then washed. Bath vol., 100 ml. A, At (1) tubocurarine 0.3 μg/ml. At (2) suxamethonium 0.4 μg/ml. Effects are synergistic instead of antagonistic as in normal preparation. B, Same preparation. At (1) suxamethonium 0.6 μg/ml. At (2) tetraethylammonium 50 μg/ml. At (3) tubocurarine 0.5 μg/ml. Tetraethylammonium did not antagonize suxamethonium block, and the antagonism of tubocurarine block was reduced reversibly.

While β-diethylaminoethyl 3,3-diphenylpropylacetate antagonized the anti-tubocurarine action of tetraethylammonium transiently, it completely and permanently abolished the anti-suxamethonium activity of tetraethylammonium. Sometimes, as in Fig. 7B, tetraethylammonium even increased paralysis. The antagonistic action of calcium ions towards tubocurarine and suxamethonium block was greatly reduced but not abolished.

DISCUSSION

Our findings and those of Harris & Milton (1961) suggest that β-diethylaminoethyl 3,3-diphenylpropylacetate can have a direct action at the neuromuscular junction. For instance, in the cat we found that β-diethylaminoethyl 3,3-diphenylpropylacetate administered either intra-arterially or intravenously reduced the direct responses of tibialis anterior to acetylcholine and to potassium. β-Diethylaminoethyl 3,3-diphenylpropylacetate also reduced the responses of the frog rectus abdominis to acetylcholine, potassium and suxamethonium. An action of β-diethylaminoethyl 3,3-diphenylpropylacetate at the neuromuscular junction could account for the modifications of the effect of blocking drugs. Our experiments show that these modifications vary with species and with the type of compounds tested. In the rabbit the blocking activity of the depolarizing drugs such as suxamethonium, its disulphonium analogue and decamethonium as well as that of tubocurarine and of gallamine was enhanced by β-diethylaminoethyl 3,3-diphenylpropylacetate whereas, in the cat, only the latter two compounds were more active while the effects of the others were antagonized. The responses in the cat diaphragm follow the pattern seen in the rabbit.

Our experiments show that the effects of β-diethylaminoethyl 3,3-diphenylpropylacetate conform neither to those typically exhibited by depolarizing blocking agents nor to those seen with tubocurarine-like drugs. Further work will be necessary before the mechanism of this interesting action of β-diethylaminoethyl 3,3-diphenylpropylacetate at the skeletal neuromuscular junction can be elucidated.

The authors would like to thank Smith, Kline & French Laboratories, Philadelphia, for a generous gift of β-diethylaminoethyl 3,3-diphenylpropylacetate (SKF 525A).

REFERENCES

AXELROD, J., REICHENTHAL, J. & BRODIE, B. B. (1954). Mechanism of the potentiating action of β-diethylaminoethyldiphenylpropylacetate. *J. Pharmacol.*, **112**, 49–54.

BETTSCHART, A., SCOGNAMIGLIO, W. & BOVET, D. (1956). Potenziamento degli effetti della succinilcolina ad opera del β-dietilamminoetildifenilpropilacetato (SKF 525-A). Contributo allo studio del meccanismo delle apnee prolungate provocate dalla succinilcolina. *Rend. Ist. Sup. Sanità (Roma)*, **19**, 721–731.

BOVET, D., BOVET-NITTI, F., BETTSCHART, A. & SCOGNAMIGLIO, W. (1956). Mécanisme de la potentialisation par le chlorhydrate de diéthylamino-éthyldiphénylpropylacétate des effets de quelques agents curarisants. *Helv. Physiol. Pharmacol. Acta*, **14**, 430–440.

BROWN, G. L. (1938). The preparation of the " tibialis anterior " (cat) for close arterial injections. *J. Physiol.*, **92**, 22*P*.

BÜLBRING, E. (1946). Observations on the isolated phrenic nerve diaphragm preparation of the rat. *Brit. J. Pharmacol.*, **1**, 38–61.

COOK, L., MACKO, E. & FELLOWS, E. J. (1954a). The effect of β-diethylaminoethyldiphenylpropylacetate hydrochloride on the action of a series of barbiturates and C.N.S. depressants. *J. Pharmacol.*, **112**, 382–386.

COOK, L., NAVIS, G. & FELLOWS, E. J. (1954b). Enhancement of the action of certain analgetic drugs by β-diethylaminoethyldiphenylpropylacetate hydrochloride. *J. Pharmacol.*, **112**, 473–479.

COOK, L., TONER, J. J. & FELLOWS, E. J. (1954c). The effect of β-diethylaminoethyldiphenylpropylacetate hydrochloride (SKF No. 525-A) on hexobarbital. *J. Pharmacol.*, **111**, 131–141.

DELLA BELLA, D. & ROGNONI, F. (1959). Tetraetilammonio e trasmissione neuromuscolare. *Boll. Soc. It. Biol. Sper.*, **35**, 1864–1868.

HARRIS, E. D. & MILTON, A. S. (1961). Inhibitory actions of β-diethylaminoethyl diphenyl-n-propyl acetate (SKF 525-A) on muscle contraction. *Proc. Soc. Exper. Biol. and Med.*, **107**, 157–160.

HORÁKOVÁ, Z., VOTAVA, Z. & ROTH, Z. (1959). Anesthetic activity and influence of Antihistamin-Spofa, promethazine, chlorpromazine and substance "SKF-525-A" on the action of local anesthetic (in Czech). *Ceskoslov. Farm.*, **8**, 355–361.

MAAS, A. R., CAREY, P. L. & HEMING, A. E. (1953). Effect of β-diethylaminoethyldiphenylpropyl acetate hydrochloride on the metabolism of 3-(o-toloxy)-1,2-propanediol. *Fed. Proc.*, **12**, 345–346.

MACKO, E., COOK, L., TONER, J. J. & FELLOWS, E. J. (1953). Potentiation of certain spinal cord depressants and central nervous system stimulants by β-diethylaminoethyldiphenylpropylacetate hydrochloride (SKF No. 525-A). *Fed. Proc.*, **12**, 346.

NAVIS, G. J., TONER, J. J. & COOK, L. (1953). Effect of β-diethylaminoethyldiphenylpropylacetate hydrochloride (SKF No. 525-A) on the activity of d-tubocurarine chloride. *Fed. Proc.*, **12**, 354.