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Effects of the *NN'*-triethyl analogue of suxamethonium on neuromuscular transmission

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The effects of succinyltriethylcholine on neuromuscular transmission in the cat and chick, and on the output of acetylcholine from the isolated rat hemidiaphragm, are described. Succinyltriethylcholine exhibits an initial post-junctional non-depolarizing blocking action. A secondary prejunctional inhibitory action on acetylcholine output, is due to succinyltriethylcholine rather than to the hydrolysis products. The compound also exhibits a facilitatory action which may be due to anticholinesterase activity.

THE neuromuscular blocking action of the *NN'*-triethyl analogue of suxamethonium (succinyl-TEC; I) was briefly reported by Bovet (1951, 1959). Experiments on avians indicated that it was devoid of depolarizing activity, and, in the rabbit head-drop test, it was found to possess about 1/60 of the potency of suxamethonium.

$$Et_3N^+.CH_2.CH_2.O.CO.CH_2.CH_2.CO.O.CH_2.CH_2.N^+Et_3 \ 2I^-$$

I

It might be expected that succinyl-TEC, like suxamethonium, would possess short duration of action and, since relatively weak potency need not be a disadvantage in an otherwise useful drug, it was decided to re-examine the effects of the compound in more detail. In view of the prejunctional action of many ethonium compounds on acetylcholine synthesis and output (Bowman, Hemsworth & Rand, 1967), succinyl-TEC was also examined for this effect.

Experimental

CHEMISTRY

Bis-2-ethylaminoethyl succinate was prepared by esterifying 2-diethylaminoethanol (0·75 mole) with succinic acid (0·25 mole). The mixture was first refluxed in toluene (1 ml conc. sulphuric acid catalyst) for 8 hr after which a toluene/water azeotrope was distilled off. The reaction mixture was dissolved in ether and extracted with sodium bicarbonate solution. After drying the ether solution over anhydrous sodium sulphate, removal of the solvent left a yellow oil.

The quaternary ammonium salt was prepared by heating the above ester (0·03 mole) with ethyl iodide (0·06 mole) in acetone in a sealed tube for 48 hr. The precipitated quaternary salt gave a colourless crystalline solid, bis-2-triethylaminoethyl succinate iodide (m.p. 169·5–170°) when crystallized from absolute ethanol/methyl ethyl ketone. Found; C, 38·3; H, 6·7; I, 39·5; N, 4·4%. $C_{20}H_{42}I_2N_2O_4$ requires C, 38·2; H, 6·7; I, 40·4; N, 4·5%.

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In a second synthesis bis-2-chloroethyl succinate was prepared using the method of Walker (1950) from 2-chloroethanol and succinic acid. This was converted to the iodo-ester by refluxing with 15% sodium iodide in acetone solution. When the iodo ester was refluxed with triethylamine in acetone a solid separated out which on crystallization from alcohol yielded white crystals (m.p. 168–169°). Mixed melting point determination and infrared spectra confirmed the identical nature of the compounds obtained from the two syntheses.

PHARMACOLOGY

Pharmacological tests were made on the following preparations:

1. The tibialis anterior muscles of cats anaesthetized with chloralose (80 mg/kg injected intraperitoneally). Maximal twitches of a tibialis anterior muscle were elicited by stimulation of the sciatic nerve with rectangular pulses of 50 μsec duration and of about twice the strength required to evoke a maximal twitch. In most experiments, maximal twitches of both tibialis anterior muscles were elicited simultaneously, one being excited once every sec through a 1:1 isolation transformer, and the other once every 10 sec (Bowman & Rand, 1961; Bowman, Hemsworth & Rand, 1962). Drugs were injected intravenously through a cannula in a jugular vein or close-arterially by the technique of Brown (1938).

2. Observations were made after intravenous injection into young conscious chicks (Buttle & Zaimis, 1949).

3. The effects of succinyl-TEC on the acetylcholine output from the isolated innervated rat hemidiaphragm preparation were studied using a method identical with that described by Bowman & Hemsworth (1965).

4. The isolated transmurally stimulated oesophagus preparation of the young chick mounted in Krebs-Henseleit solution of the following composition g/litre: NaCl, 6·95; KCl, 0·34; $CaCl_2$, 0·28; KH_2PO_4, 0·162; $MgSO_4$, 0·294; $NaHCO_3$, 2·1; dextrose, 2·0, which was bubbled with 5% carbon dioxide in oxygen and at 32°.

The drugs used were: choline chloride, α-chloralose, acetylcholine chloride and atropine sulphate (British Drug Houses), neostigmine methylsulphate and edrophonium chloride (Roche). The doses quoted, including those of succinyl-TEC, refer to the base or to the cation.

Results

NEUROMUSCULAR BLOCKING ACTION

Chicks. On intravenous injection into young conscious chicks succinyl-TEC (10–20 mg/kg) produced flaccid paralysis, confirming Bovet's (1951, 1959) finding that the blocking action was of a non-depolarizing nature.

Cat tibialis anterior muscle. In the cat tibialis anterior muscle preparation stimulated indirectly once every 10 sec, doses of 10–15 mg/kg of

succinyl-TEC injected intravenously produced a brief (2–3 min) period of twitch augmentation. Larger doses (20–50 mg/kg) produced neuromuscular block of a duration intermediate between that of tubocurarine and suxamethonium, taking about 10–15 min to full recovery from a 90–100% block of twitch height. A short period of twitch augmentation was occasionally observed before the blocking action of the compound, and frequently, after the block, the twitches recovered to an amplitude greater than that before the block. This post-block augmentation of twitches, when observed, usually lasted for 5–15 min, and was always preceded by a two-stage recovery from the block; an initial slow steady recovery up to about 75% of the original twitch height was followed by a secondary rapid recovery leading into the twitch augmentation (Figs 1 and 4).

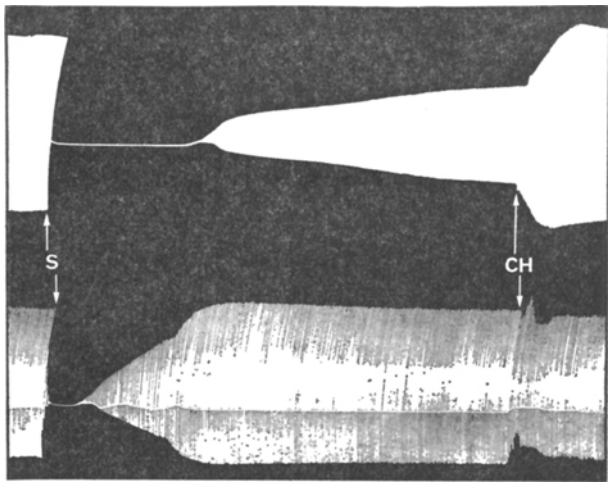

FIG. 1. Cat 2·65 kg, chloralose anaesthesia. Maximal twitches of right and left tibialis anterior muscles elicited indirectly by 1 shock/sec and 1 shock/10 sec respectively. At S, 50 mg/kg succinyl-TEC, and at CH 5 mg/kg of choline were injected intravenously.

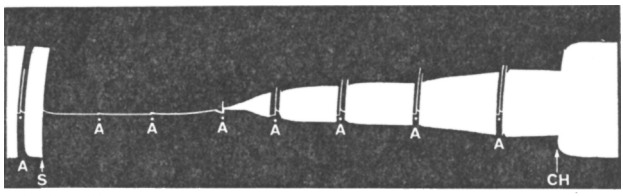

FIG. 2. Cat 2·85 kg. Maximal twitches of a tibialis anterior muscle elicited indirectly once every second. At A, electrical stimulation was temporarily stopped and 7·5 μg of acetylcholine was injected close-arterially. At S, 50 mg/kg of succinyl-TEC and at CH 5 mg/kg of choline were injected intravenously. The horizontal bar corresponds to a period of 10 min.

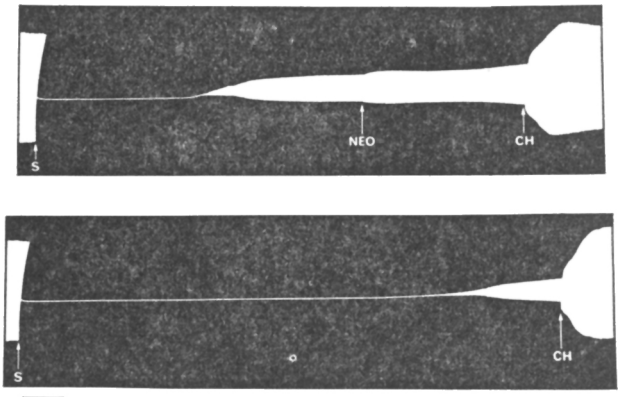

FIG. 3. Cat 2·65 kg. Maximal twitches of a tibialis anterior muscle elicited indirectly once every second. The lower trace is a continuation of the upper trace, the time between the two being 60 min. In both traces at S, 50 μg/kg of succinyl-TEC, and at CH, 5 mg/kg of choline were injected intravenously. In the upper trace at NEO, 100 μg/kg of neostigmine was injected intravenously. Note the transient effect of neostigmine on the secondary phase of block, and compare with the complete reversal produced by choline. In the lower trace 100 μg/kg of neostigmine was injected 5 min before the succinyl-TEC. Note the prolongation of the initial stage of block when the lower trace is compared with the upper. The horizontal bar corresponds to 10 min.

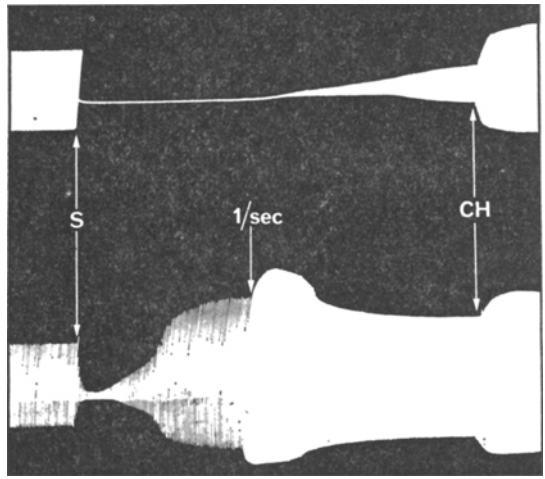

FIG. 4. Cat 2·5 kg. Maximal twitches of the right and left tibialis anterior muscles elicited indirectly by 1 shock/sec and 1 shock/10 sec respectively. At the marked arrow the stimulation rate of the right muscle was increased to 1/sec. At S, 40 mg/kg succinyl-TEC, and at CH, 5 mg/kg of choline were injected intravenously. Note the two-stage recovery from the initial blocking action of succinyl-TEC in the more slowly stimulated muscle, and the post-block twitch augmentation.

Neostigmine and edrophonium (100 μg/kg intravenously or 5 μg close-arterially) failed to hasten recovery from the blocking action of succinyl-TEC. The depth of block produced by succinyl-TEC was not reduced

by previous administration of neostigmine (100 μg/kg, i.v.), but the duration of action of the compound was prolonged. During partial block produced by succinyl-TEC, tetanic tension, elicited by stimulating the sciatic nerve at 50/sec for 5 sec, although depressed, did not wane during the period of repetitive stimulation. Immediately after the tetanus there was a reduction in the depth of block when single shock indirect testing was resumed.

When tested on cat tibialis anterior muscles stimulated at 1/sec the first dose of succinyl-TEC (40–50 mg/kg) usually produced a block which, though deeper than that in the more slowly stimulated muscle, recovered at about the same rate. Other neuromuscular blocking drugs are also more effective at higher rates of stimulation (Preston & Maanen, 1953; Wislicki, 1958). Subsequent doses of succinyl-TEC produced a biphasic block of the more rapidly stimulated muscle. The twitches remained completely blocked for a period of 15–30 min. They then began to recover, either very slowly and continuously over a period of 1–2 hr, or partially, to become constant at a reduced level for about 30 min before full recovery occurred. Contractions produced by close-arterially injected acetylcholine were blocked during the initial block of the twitches. Responses to acetylcholine then recovered at a faster rate than did the twitches, so that, during the second phase of block the responses to acetylcholine returned to their original height while the twitch height remained partially depressed (Fig. 2). This result is in sharp contrast to that recorded when the block is entirely due to a post-junctional action, such as with tubocurarine (see for example, Bowman & others, 1962). When tubocurarine is the blocking agent used, twitches evoked by nerve stimulation return to control levels much more quickly than do contractions produced by injected acetylcholine. The results therefore indicate that whereas the initial phase of block produced by succinyl-TEC is post-junctional in origin, the second phase is pre-junctional.

Choline (5 mg/kg, i.v.) rapidly and permanently reversed the secondary phase of block produced by succinyl-TEC in the more rapidly stimulated muscle (Figs 1–4). When injected during the initial phase, choline prevented the onset of the secondary phase of block. Neostigmine (100 μg/kg intravenously), injected during the secondary block, produced only a small increase in the twitch tension (Fig. 3), an effect similar to that observed during block produced by hemicholinium or triethylcholine (Reitzel & Long, 1959; Bowman & Rand, 1961).

The dependence of the secondary block on the rate of stimulation was further demonstrated in an experiment in which the twitches of the slowly stimulated muscle were allowed to recover completely after a dose of 50 mg/kg of succinyl-TEC. The stimulation rate was then increased to equal that for the more rapidly stimulated muscle when there was an initial increase in twitch tension, followed, after about 3 min, by a rapid decrease in tension until a partial but constant degree of block was maintained. Choline reversed the blocks in both muscles (Fig. 4).

Biphasic blocks similar to those described above were produced by

succinyl-TEC in cats which had been pretreated with neostigmine (0·1–0·2 mg/kg), the only difference being that the duration of the complete block of the more rapidly stimulated muscle was prolonged (Fig. 3). In an experiment in which 60 mg/kg of succinyl-TEC was incubated with 10 ml cat blood for 90 min at 37°, intravenous injection of the incubation mixture produced only a slightly smaller effect than did a control dose of the compound injected 2 hr earlier. The secondary choline-reversible phase of block was noted in both cases.

RELEASE OF ACETYLCHOLINE

Succinyl-TEC reduced the amount of acetylcholine released during nerve stimulation; the results are shown in Fig. 5. Succinyl-TEC added

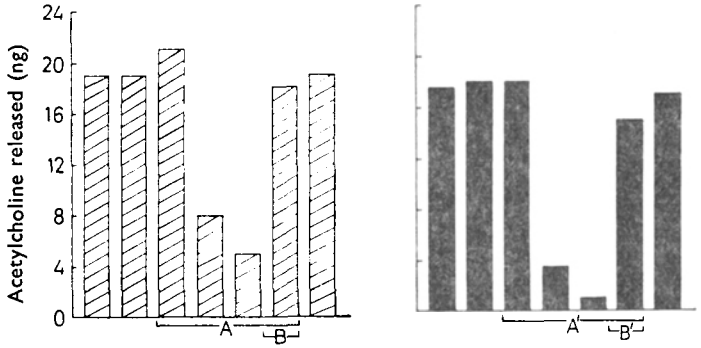

FIG. 5. Each column represents the acetylcholine (as cation) released during a 15 min period of stimulation at 1 shock/sec. The period between each 15 min collection period was 43 min. The first two columns in each histogram represent the release during control collections of acetylcholine before the addition of drugs. The third, fourth, fifth and sixth columns represent the acetylcholine released in the presence of succinyl-TEC (A, 150 μg/ml; A′, 250 μg/ml) which was initially added 2 min before the collection period represented by the third column. Choline (B, 50 μg/ml; B′, 75 μg/ml) was also present during the collection period represented by the sixth column, having been initially added 40 min before the collection period began. The last column represents the release after washing out both the succinyl-TEC and the choline. Each column represents the mean of results obtained on three diaphragms.

to the bath fluid 2 min before a collection period, showed no immediate depressant effect on acetylcholine release, but at the lower dose level used (150 μg/ml) slightly increased the acetylcholine output during the first collection period after its administration. During the second collection period after succinyl-TEC administration, the acetylcholine output was reduced by 58–81%, depending upon the dose used, and during the subsequent collection period, the acetylcholine output was reduced by 74–94%. When choline (50–75 μg/ml) had been present together with the succinyl-TEC for a period of 40 min, the acetylcholine output returned to over 90% of the control output. After washing out all the drugs from the bath the acetylcholine output returned to the control level. Control experiments confirmed the finding of Bowman &

Hemsworth (1965) that in the absence of succinyl-TEC the acetylcholine output remained constant over a period of 8 hr.

CHICK OESOPHAGUS

Succinyl-TEC (35–50 μg/ml) added to the fluid bathing an isolated transmurally stimulated chick oesophagus preparation produced a gradual increase in the size of the contractions elicited by electrical stimulation. When left in contact with the tissue for more than 5–10 min, succinyl-TEC produced spasm of the tissue, which was only relieved by cessation of stimulation and washing.

The response to added acetylcholine (5 μg/ml) was increased after succinyl-TEC, and the compound reversed the block of contractions produced by atropine (0·1 μg/ml).

Discussion

The results confirm Bovet's (1951, 1959) observation that succinyl-TEC is without depolarizing action, and they show that its effects on neuromuscular transmission are the result of both facilitatory and blocking actions. Because of its complexity of action, the relatively prolonged duration of its effect when compared with suxamethonium, and the inability of neostigmine to antagonize it, succinyl-TEC would not be of value as a neuromuscular blocking agent in surgical anaesthesia.

Facilitation of neuromuscular transmission produced by succinyl-TEC was evidenced by an increase in twitch tension which preceded and followed the blocking action of the compound. Two mechanisms of action probably contribute to the facilitatory action. Measurements of acetylcholine output showed that succinyl-TEC initially increased transmitter release, an action shared by other ethonium ions including tetraethylammonium (Collier & Exley, 1963) and triethylcholine (Bowman & Hemsworth, 1965). In addition succinyl-TEC probably possesses anticholinesterase activity as shown by its ability to augment responses of the isolated chick oesophagus to acetylcholine, and to produce a slowly developing spasm of the tissue. Several other ethonium ions have also been shown to possess anticholinesterase activity (Blaschko, Bülbring & Chou, 1949; Karczmar, 1957; Koelle, 1957; Arnold, Soria & Kirchner, 1954). These facilitatory components of action of succinyl-TEC may account for the maintenance of tetanic tension during partial neuromuscular block. An anticholinesterase action of the compound may explain the inability of neostigmine or edrophonium to antagonize the initial postjunctional blocking action of the compound. The non-depolarizing drug, benzoquinonium, also possesses anticholinesterase activity and its blocking action is similarly resistant to antagonism by anticholinesterase drugs (Hoppe, 1951; Bowman, 1958, 1966).

The secondary blocking action of succinyl-TEC exhibited the characteristics of prejunctional block arising from inhibition of acetylcholine synthesis. Thus, like that produced by hemicholinium, triethylcholine and related compounds (Schueler, 1960; Bowman & others, 1967), the

secondary block produced by succinyl-TEC occurred selectively in the more rapidly stimulated muscle and was reversed by choline. Its prejunctional nature was indicated by the fact that, during the block, contractions produced by acetylcholine were similar to the controls. Experiments on the rat diaphragm preparation confirmed that succinyl-TEC decreases the output of acetylcholine from the stimulated nerve and that this effect is reversed by choline.

Since succinyl-TEC hydrolyses to produce succinic acid and triethylcholine, it was considered that the secondary prejunctional block may have been due to triethylcholine, as was the case in a series of *NNN*-trisonium esters tested by Marshall (1968). However, destruction of succinyl-TEC on incubation with blood was very slow, as would be expected of a compound showing the properties of an inhibitor rather than of a substrate for cholinesterase. Furthermore, pretreatment with neostigmine did not change the type of block produced by succinyl-TEC and these results indicate that the effects are mainly due to the parent compound rather than to the products of hydrolysis.

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