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# The prejunctional actions of some non-depolarizing blocking drugs

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## Summary

1. Trains of end-plate potentials (e.p.p.s) have been recorded from the isolated tenuissimus of the cat. The muscle was paralyzed either by transversely cutting the muscle fibres or by non-depolarizing blocking drugs.

2. The following parameters of transmitter synthesis, storage and release have been calculated: the quantal content of the first e.p.p. in the train, the size of the available store, fractional release, quantum size, and the rate of refilling of the available store.

3. Tubocurarine and benzoquinonium depressed the rate of refilling of the available store causing its depletion at high rates of stimulation. This was offset by an increase in fractional release, which in the case of tubocurarine was sufficient for the quantal content of the first e.p.p. to be unchanged.

4. Dimethyltubocurarine and pancuronium had a similar effect to tubocurarine on the rate of refilling of the store and depletion of the store at high rates of stimulation but did not increase fractional release. There was, therefore, a decrease in the quantal content of the first e.p.p.

5. Lignocaine depressed the rate of refilling of the store and depleted the store at high rates of stimulation. Fractional release was also depressed.

6. It is suggested that the non-depolarizing drugs have a weak local anaesthetic action retarding the influx of sodium into the nerve terminal which slows the rate of refilling of the store. This effect is due to the quaternary ammonium head. The presence of a phenolic group increases fractional release due either to an increased influx of calcium into the nerve terminal or to a potentiation of the actions of calcium.

## Introduction

In 1936, Dale, Feldberg & Vogt concluded that 'when transmission of excitation from the nerve to the perfused (cat skeletal) muscle is prevented by curarine, stimulation of the motor nerve fibres causes the usual release of acetylcholine'. This subsequently was understood to mean that curare alkaloids had only a postjunctional action, and did not change the amount of transmitter released by a nerve impulse, although Dale *et al.* (1936) did not make such a categorical statement and their methods of estimation of acetylcholine were not precise enough to do so. Lilleheil & Naess (1961) concluded that tubocurarine (Tc) in addition to its postjunctional effect also had a pre-junctional action which was responsible for the rapid decline of the end-plate potential (e.p.p.) amplitude during a tetanic stimulus

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in the rat isolated phrenic nerve-diaphragm. This conclusion was supported by Beani, Bianchi & Ledda (1964) who used isolated guinea-pig diaphragm, by Hubbard, Wilson & Miyamoto (1969) and Galindo (1971) who used isolated rat diaphragm, by Maeno & Nobe (1970) who used isolated sartorius muscle of the frog, and by Blaber (1970) and Jacobs & Blaber (1971) who used isolated cat tenuissimus. The conclusion that Tc has a presynaptic action has been challenged by Beránek & Vyskočil (1967) using isolated rat diaphragm, by Chang, Cheng & Chen (1967) using isolated guinea-pig diaphragm, by Bauer (1971) using isolated golden hamster diaphragm, and by Auerbach & Betz (1971) using isolated frog sartorius and rat diaphragm. Hubbard *et al.* (1969) suggested that the failure of Beránek & Vyskočil (1967) to observe a pre-junctional effect of Tc was due to their use of an increased magnesium concentration in the Ringer solution to paralyze the muscle.

Auerbach & Betz (1971) questioned the conclusions of Hubbard *et al.* (1969), who had prevented contractions of the diaphragm by transversely cutting the muscle fibres, on the grounds that this technique may have introduced an artifact if recordings were made more than 100–200 $\mu$m from the end-plate focus.

The present work re-examines the question of a pre-junctional action of tubocurarine. A cut-fibre preparation of the isolated tenuissimus of the cat has been used which is a much more stable preparation and has a higher resting potential than the cut fibre preparation of the isolated rat diaphragm. The artifact discussed by Auerbach & Betz (1971) is also avoided in the cut fibre preparation of the cat tenuissimus muscle, since, in order to record an e.p.p. with a rise-time of less than 1·0 ms, the microelectrode must be located within 20 $\mu$m of the end-plate.

A series of non-depolarizing blocking compounds has been used, which were sufficiently potent to paralyze the muscle without cutting the muscle fibres, in an attempt to define the structure-activity relationships of the pre-junctional action.

## Methods

The preparation and technique used have been described previously (Blaber & Christ, 1967; Blaber, 1970). The isolated tenuissimus muscle was stimulated once a minute at a frequency of 200 Hz for 0·5 seconds. Intracellular recordings were made with glass microelectrodes (resistance 10–15 M$\Omega$), care being taken that all end-plate potentials (e.p.p.s) had a rise-time of less than 1·0 millisecond.

The e.p.p.s were recorded on film and the amplitudes measured after magnification with a photographic enlarger. This information, together with the membrane potential of the muscle fibre at the time of recording the train and the equilibrium potential (−12·8 mV (Blaber, 1972)) formed the data for a computer programme (CDC 6,600).

The parameters calculated were: the quantal content of the first e.p.p. in the train, the size of the available store, fractional release (mean probability of release), quantum size, and the rate of refilling of the available store (mobilization plus synthesis of transmitter). The method was based on that of Elmqvist & Quastel (1965), and fully described by Blaber (1970). For each drug, end-plates were sampled from several muscles. Statistical differences were calculated with an unpaired double-tailed *t* test.

Drugs used in this study were: (+)-tubocurarine chloride (Koch-Light Laboratories), dimethyltubocurarine iodide (Eli Lilly & Co.), lignocaine hydrochloride (Pharmaceutical Manufacturing Co.), benzoquinonium chloride (Sterling-Winthrop Research Institute), and pancuronium bromide (Organon Laboratories Ltd.).

## Results

The results of all experiments are given in Table 1. The control values obtained from cut-fibre preparations are somewhat higher than previously reported for tenuissimus muscle (Blaber, 1970; Jacobs & Blaber, 1971) and may have been due to the extra care taken in the location of the microelectrode in the end-plate.

TABLE 1. *Effect of non-depolarizing blocking drugs on transmitter synthesis and release*

| | Quantal content of first e.p.p. (quanta)† | Available store (quanta)† | Fractional release† | Quantum size (mV)† | Rate of refilling of available store (quanta/ms)† | *n* |
|---|---|---|---|---|---|---|
| Control* | 363±43 | 3,078±229 | 0·12±0·01 | 0·108±0·010 | 22·9±1·7 | 32 |
| Tubocurarine* (0·72 μM) | 617±89§ | 2,274±282 | 0·22±0·02§ | 0·045±0·007§ | 19·8±2·3 | 18 |
| Tubocurarine (3·6 μM) | 343±16 | 1,838±120§ | 0·20±0·01§ | 0·012±0·001§ | 16·5±0·9§ | 27 |
| Dimethyltubocurarine (0·83 μM) | 240±16§ | 1,665±88§ | 0·14±0·01 | 0·011±0·001§ | 16·2±0·8§ | 47 |
| Lignocaine* (42 μM) | 137±10§ | 1,923±132§ | 0·07±0·01§ | 0·086±0·011 | 13·6±0·7§ | 26 |
| Benzoquinonium (2·0 μM) | 246±24‡ | 1,119±69§ | 0·21±0·01§ | 0·015±0·001§ | 11·9±0·6§ | 26 |
| Pancuronium (0·28 μM) | 176±11§ | 1,672±103§ | 0·10±0·01 | 0·014±0·001§ | 14·7±0·8§ | 30 |

* Cut fibre preparations; † ±S.E.M.; ‡ Difference statistically significant from control ($P$=0·05); § Difference statistically significant from control ($P$=0·01).

### *Tubocurarine*

The effects of the low concentration of Tc (0·72 μM) confirm those reported by Blaber (1970). The quantal content of the first e.p.p. was increased to 170% of the control value. This was due to an increase in the fractional release, since there was no significant effect on the rate of refilling or on the size of the available store. This concentration of Tc is not sufficient to paralyze the muscle and these experiments were conducted with cut-fibre preparations. The quantal size was reduced to 41% of the control value.

When the muscles were paralyzed with a higher concentration of Tc (3·6 μM) there was no change in the quantal content of the first e.p.p. This was due to the increased fractional release being offset by a reduced rate of filling of the available store and a reduction in the size of the available store to 59% of the control value. The quantum size was reduced to approximately 2%, assuming the control quantum size to be equal to the amplitude of the miniature end-plate potentials (0·6 mV) (Blaber & Christ, 1967).

### *Dimethyltubocurarine*

Paralysis of the muscle by dimethyltubocurarine (0·83 μM) also reduced the quantum size to approximately 2% of the amplitude of the miniature end-plate

potentials in unblocked muscle. Therefore, dimethyltubocurarine was more than four times as potent as Tc in its post-junctional effects. In addition, dimethyltubocurarine reduced the quantal content of the first e.p.p. to 66% of the control value. This was due to its having a similar effect to the higher concentration of Tc on the rate of refilling of the available store and on the size of the available store. However, the fractional release was unchanged from that of the control.

*Lignocaine*

Lignocaine (42 $\mu$M) did not cause any significant change in the quantum size and therefore did not exert any post-junctional blocking action. In this series of experiments the cut-fibre preparation had to be used to prevent muscle contractions. The quantal content of the first e.p.p. was reduced to 38% of the control value. This was due to decreases in the rate of refilling of the available store, in the size of the available store, and in the fractional release. Higher concentrations of lignocaine caused nerve block and the disappearance of the e.p.p. in an all-or-none fashion ; at a concentration of 42 $\mu$M there were no failures of e.p.p.s.

*Benzoquinonium*

Benzoquinonium (2·0 $\mu$M) reduced the quantum size to approximately 2% of the size of the m.e.p.p. in unblocked muscle, and therefore was approximately twice as potent as Tc in its post-junctional blocking action. The quantal content of the first e.p.p. was reduced to 68% of the control ; benzoquinonium had a greater effect than Tc in reducing the rate of refilling of the available store but had a similar effect in increasing the fractional release.

*Pancuronium*

Pancuronium (0·28 $\mu$M) also reduced the quantum size to 2% of the amplitude of the m.e.p.p. in unblocked muscle and therefore had a post-junctional blocking action approximately thirteen times that of Tc. The quantal content of the first e.p.p. was reduced to 48% of control due to reductions in the rate of refilling of the available store and in the size of the available store. There was no effect on fractional release.

**Discussion**

Tubocurarine in the low dose (0·72 $\mu$M) was found to increase the fractional release of transmitter, thus increasing the quantal content of the first e.p.p. Other workers have also shown that tubocurarine increases fractional release (Hubbard *et al.*, 1969 ; Blaber, 1970 ; Galindo, 1971).

A concentration of 3·6 $\mu$M Tc, which by itself paralyzed the muscle, also increased the fractional release. This was offset by a decreased available store of transmitter due to a decreased rate of refilling of the store, and as a result there was no change in the quantal content of the first e.p.p. Therefore at lower rates of stimulation the muscle paralysis would be due entirely to the post-junctional action. At high frequency stimulation the slow rate of refilling of the store leads to depletion and is responsible for the increased potency of Tc at higher stimulus frequencies (Blackman, 1963). The increased rate of decline in amplitude of the e.p.p.s during a tetanic stimulation in the presence of Tc is readily observed. In a cut-fibre

preparation a frequency of 200 Hz is required in order to observe a decline in e.p.p. amplitude (see Fig. 3, Blaber, 1970), whereas when the muscle is paralyzed with Tc the decline in amplitude of the e.p.p.s is readily observable at 25 Hz and is very pronounced at 100 Hz (see Fig. 3A, Jacobs & Blaber, 1971).

The controversy regarding the possible pre-junctional action of Tc is therefore due to various factors. If the store of transmitter is to be depleted leading to a reduced output of transmitter, Tc concentrations considerably in excess of those required just to paralyze the muscle would have to be used. In addition, very high frequencies of stimulation are needed. In those experiments reporting that Tc does not reduce transmitter output (Beránek & Vyskočil, 1967 ; Chang *et al.*, 1967 ; Bauer, 1971 ; Auerbach & Betz, 1971) it is possible that these conditions were not met, or that a Ringer solution was used which contained a high magnesium concentration which would reduce transmitter release and therefore prevent depletion. The concentration of Tc and stimulus frequency required to observe a pre-junctional action of Tc have not been specified for other species and it is not possible to make any direct comparisons. However, although Tc may not change transmitter release, the factors which affect release have been changed and the nerve terminal is now more vulnerable to the action of a second drug. Jacobs & Blaber (1971) have shown that sodium fluoride, and Blaber (1972) has shown that edrophonium, can increase transmitter release in the presence of Tc but not when given alone.

*Dimethyltubocurarine*

Dimethyltubocurarine produced effects similar to those of Tc except that there was no increase in fractional release. In the dimethyltubocurarine molecule, the phenolic groups adjacent to the quaternary nitrogens have been masked by methylation. It is possible that the phenolic group is responsible for the increase in fractional release. This is supported by the observations of Gallagher & Blaber (1970, 1972) that phenol and catechol increase the fractional release without changing the rate of refilling of the store or the size of the available store. Other compounds which increase the fractional release (mean probability of release) of transmitter and which contain a phenolic structure are noradrenaline (Kuba & Tomita, 1971) and edrophonium (Blaber, 1972).

Blaber (1970) showed that reduction of the calcium ion concentration reduced the fractional release without affecting the refilling of the store or the size of the available store. Katz and his co-workers (del Castillo & Katz, 1954 ; Katz & Miledi, 1965, 1968) have also concluded that calcium is the main factor influencing the probability of transmitter release. It is possible, therefore, that phenolic compounds increase transmitter release either by increasing the influx of calcium ions into the nerve terminal or by potentiating in some way the activities of the calcium ions.

*Lignocaine*

Lignocaine produced a similar effect to that of dimethyltubocurarine except that the fractional release was also significantly decreased. Lowering the sodium ion concentration in the Ringer solution decreases the rate of refilling of the store and the size of the available store but produces an increase in the fractional release (Blaber, 1970). It was assumed that the increase in fractional release was due to the removal of sodium ions competing with calcium ions for entry into the nerve

terminal (Birks & Cohen, 1965; Kelly, 1965; Gage & Quastel, 1966; Birks, Burstyn & Firth, 1968). It might be expected that local anaesthetics which prevent the entry of sodium ions (Taylor, 1959) would also reduce the rate of refilling of the store and the size of the available store. However, since sodium ions have not been removed, there would not be an increased influx of calcium ions or an increased fractional release. The unexpected decrease in fractional release produced by lignocaine may be an additional action due to its greater lipid permeability but only further experiments comparing several local anaesthetics would reveal this.

It is tentatively suggested that Tc and dimethyltubocurarine reduce the rate of refilling of the store and the size of the available store by a weak local anaesthetic action; they do not block nerve conduction presumably because of their low potency and also they do not exist in a non-ionized form and cannot therefore penetrate the myelin sheath. Tc, in higher concentrations than used in the present experiments, has been shown to prevent ephaptic stimulation of the first node by muscle action currents (Werner, 1961).

Decamethonium has been found to have the opposite effect to Tc and to increase the available store by increasing the rate of refilling of the store (Blaber, 1970).

It is proposed that the effect on the rate of refilling and size of the store is mediated by an effect through sodium ion influx due to the quaternary ammonium group.

*Benzoquinonium and pancuronium*

Benzoquinonium does not have a phenolic group adjacent to the quaternary nitrogen but was shown to increase fractional release. It is possible that the quinone structure can exist in the hydroxyl form and that the molecule is flexible enough for this to occupy the appropriate site. The effect of benzoquinonium on refilling and size of the available store was greater than that of Tc which would support the conclusions of Blaber & Bowman (1963) and Christ & Blaber (1968) that benzoquinonium has a greater pre-junctional action than Tc.

Pancuronium has a steroid nucleus and no phenolic groups. The presynaptic effects resembled those of dimethyltubocurarine, supporting the concept that the phenolic group is responsible for the effect on fractional release and the quaternary ammonium group for the effect on the rate of refilling of the store. These results support the report of Gergis, Dretchen, Sokoll & Long (1972) and Dretchen, Sokoll, Gergis & Long (1972) that pancuronium reduces transmitter release at concentrations that produce muscle blockade, but not that of Galindo (1972) that pancuronium has a weaker presynaptic action than Tc. However, the effects may depend on the concentration since Gergis *et al.* (1972) and Dretchen *et al.* (1972) state that at low concentrations pancuronium increases transmitter release. The mechanism for this is unknown.

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