Brit. J. Pharmacol. (1961), 17, 372–379.

ACTION OF BRETYLIUM AND GUANETHIDINE AT THE NEUROMUSCULAR JUNCTION

BY

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(*Received July* 10, 1961)

Bretylium and guanethidine produced a block of neuromuscular transmission in the rat phrenic nerve diaphragm and cat sciatic gastrocnemius preparations, but had a potentiating effect on acetylcholine-induced contracture of the frog rectus. On the sciatic gastrocnemius preparation of the cat the compounds had a twofold action, consisting of an initial transient, and a delayed but more sustained, block of neuromuscular transmission. Bretylium and guanethidine had no immediate effect on the response of the muscle to direct stimulation, though a weak delayed depressant effect was observed. Intravenous injection of these compounds produced a flaccid paralysis in pigeons. Possible mechanisms of the neuromuscular blocking action of bretylium and guanethidine are discussed. Inhibition of acetylcholine release from motor nerve endings resulting from local anaesthesia by these compounds is suggested as a possible mechanism of their neuromuscular blocking action.

Two new antihypertensive drugs, bretylium and guanethidine, have recently been introduced in therapeutics. During the clinical use of these drugs, muscle weakness and fatigue have been reported as side-effects (Campbell & Montuschi, 1960; Evanson & Sears, 1960; Dollery, 1961; Page, 1961).

Boura & Green (1959) showed that bretylium blocks the contractile response of the rat phrenic nerve diaphragm preparation to indirect stimulation. They also reported respiratory paralysis and inhibition of the response of gastrocnemius muscle to indirect stimulation in cats anaesthetized with chloralose. Sensitivity to direct stimulation was found to be unaltered.

Vernikos-Danellis & Zaimis (1960), however, failed to observe any neuromuscular blocking action with either of the drugs in the tibialis anterior sciatic nerve preparation of cats, but demonstrated a very slow decrease in the maximal twitch tension of the muscle over a period of 2 to 3 hr. Electromyograms recorded from tibialis anterior muscles of these animals showed a myopathic pattern.

The present study was undertaken to investigate the neuromuscular blocking actions of bretylium and guanethidine.

METHODS

Rat phrenic nerve diaphragm preparation

The method was essentially similar to that described by Bülbring (1946). Rats weighing between 110 and 150 g were used. Unless otherwise specified all experiments were done at room temperature which ranged between 35 and 39° C. In all, 30 preparations were used.

For indirect stimulation the phrenic nerve was stimulated every 10 sec with single rectangular pulses of 4 V and 0.5 msec duration. For direct stimulation the tendinous portion of the muscle was connected to the writing lever by a thin copper wire which served as one electrode, the other electrode being fixed at the base of the fan-shaped diaphragm muscle. The muscle was stimulated every 10 sec with single rectangular pulses of 100 V and 5.0 msec duration.

In some experiments complete block to indirect stimulation was produced by tubocurarine (1×10^{-4}) before direct stimulation was begun.

In experiments designed to study the effect of lowering the temperature of the bath fluid on drug action, the temperature was maintained at the desired level ($\pm 1°$ C) and the muscle was allowed to acclimatize at each temperature for 20 min before the drugs were added.

Sciatic gastrocnemius preparation of cat

Eighteen cats weighing between 2 and 3 kg were anaesthetized with intravenous chloralose (90 mg/kg). Contractions of the gastrocnemius muscle were recorded isometrically as described by Burn, Finney & Goodwin (1952). For indirect stimulation the peripheral end of the cut sciatic nerve was stimulated every 10 sec with rectangular pulses of 5 V and 0.5 msec duration. For direct stimulation single rectangular pulses of 120 V and 5 msec duration were applied to the muscle every 10 sec by means of a bipolar electrode. Control responses to direct stimulation were taken after complete paralysis to indirect stimulation.

Retrograde injections were made into the cannulated contralateral common iliac artery. Heparin (10 mg/kg) was administered just after cannulation.

Frog rectus

Frogs were kept in a refrigerator at 10° C for about 24 hr, since muscles from such frogs gave more consistent responses to acetylcholine. The response of the muscle to acetylcholine chloride 0.6×10^{-6} to 1.2×10^{-5} was recorded for 90 sec every 10 min. When the response to a fixed dose of acetylcholine was reproducible the compounds under study were added to the bath for a specified period before the addition of acetylcholine and the change in the response to acetylcholine determined. Twelve preparations were used in all.

Pigeons

Drug solutions were injected intravenously into pigeons to study the paralytic effect on skeletal musculature. The criterion of paralysis was inability of the pigeon to stand on its legs. The slopes of the log dose-probit effect curves, the median paralytic dose (PD50), and the standard error of the PD50 were calculated as described by Burn *et al.* (1952), employing at least 15 pigeons for each determination.

Drugs

Bretylium tosylate, guanethidine sulphate, physostigmine salicylate, d-tubocurarine chloride and decamethonium iodide were used throughout these experiments. The doses or concentrations refer to the salts.

RESULTS

Phrenic nerve diaphragm preparation

Bretylium and guanethidine (1×10^{-4} to 3.3×10^{-4}) inhibited the contractile response of the diaphragm muscle to indirect stimulation ; the degree of block was proportional to the dose of the drugs. The blocking action was manifest immediately after the addition of the drugs ; complete recovery was obtained within 30 to 40 sec of the removal of the compounds from the bath. The compounds had no effect on the response of the muscle to direct stimulation, which was effective even after the compounds had produced complete block to indirect stimulation (Fig. 1). Likewise, in experiments where curarized preparations were used bretylium and guanethidine had no action on the response of the muscle to direct stimulation.

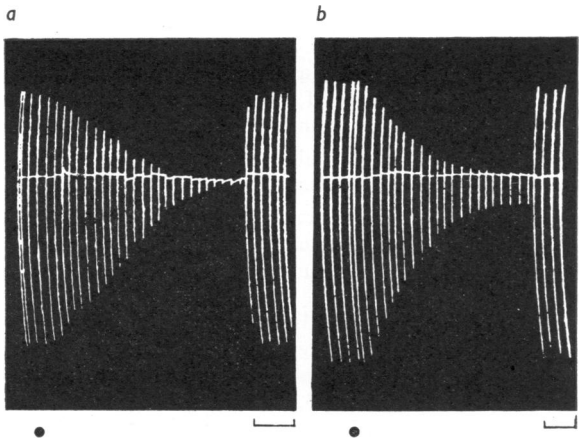

Fig. 1. Rat phrenic nerve diaphragm. Effect (at dots) of bretylium 3.3×10^{-4} (*a*) and guanethidine 3.3×10^{-4} (*b*) on the responses to indirect stimulation (4 V, 0.5 msec, 6/min). The period marked by bar indicates direct stimulation (100 V, 5 msec, 6/min) in presence of drugs.

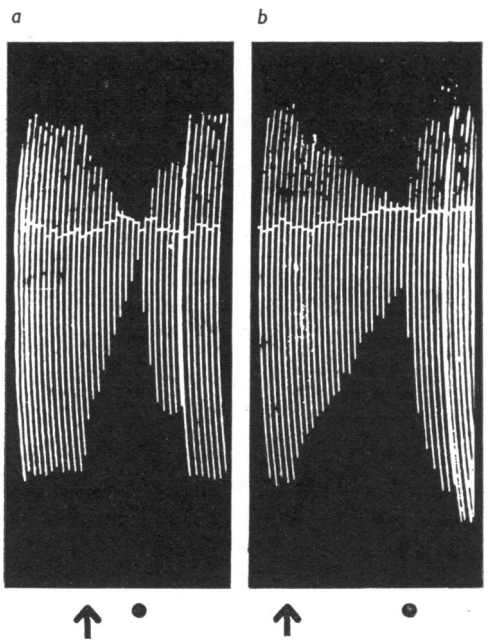

Fig. 2. Rat phrenic nerve diaphragm: indirect stimulation (4 V, 0.5 msec, 6/min). Effect (at dots) of potassium chloride (1.6 mg/ml.) on the blocking action of bretylium 3.3×10^{-4} (*a*) and guanethidine 3.3×10^{-4} (*b*) (at arrows).

Potassium chloride (1.6 mg/ml.) antagonized the blocking action of bretylium and guanethidine to indirect stimulation (Fig. 2). The block was not antagonized by physostigmine (3 μg/ml.).

Both tubocurarine and decamethoniun acted additively with bretylium or guanethidine (Fig. 3).

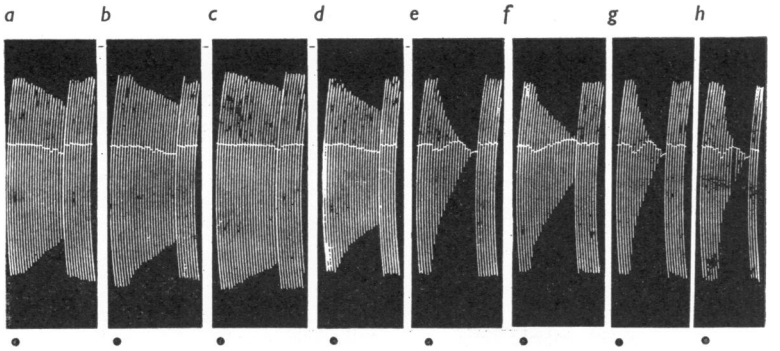

Fig. 3. Rat phrenic nerve diaphragm: indirect stimulation (4 V, 0.5 msec, 6/min). Effect of (at dots): (*a*) tubocurarine, 0.4×10^{-6}; (*b*) decamethonium, 3.3×10^{-5}; (*c*) bretylium, 2.3×10^{-4}; (*d*) guanethidine, 3.3×10^{-4}; (*e*) bretylium, 2.3×10^{-4}, plus tubocurarine, 0.4×10^{-6}; (*f*) bretylium, 2.3×10^{-4}, plus decamethonium, 3.3×10^{-5}; (*g*) guanethidine, 3.3×10^{-4}, plus tubocurarine, 0.4×10^{-6}; (*h*) guanethidine, 3.3×10^{-4}, plus decamethonium, 3.3×10^{-5}.

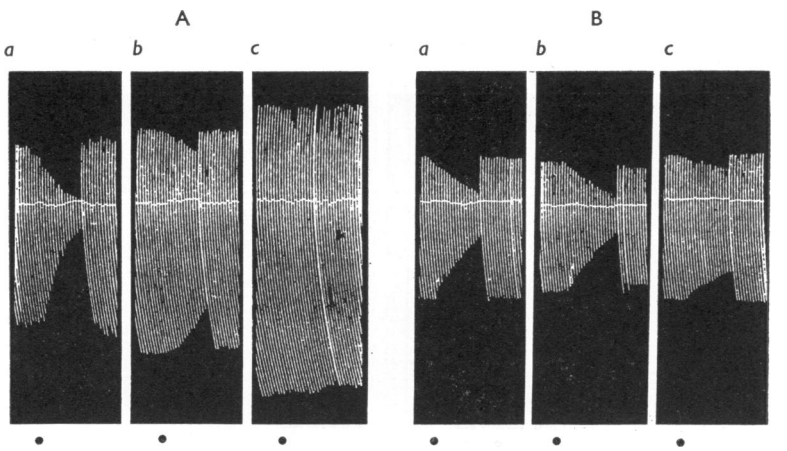

Fig. 4. Rat phrenic nerve diaphragm: indirect stimulation (4 V, 0.5 msec, 6/min). Effect (at dots) of bretylium 2.3×10^{-4} (A), and guanethidine 3.3×10^{-4} (B) at 36° C (*a*), 30° C (*b*), and 25° C (*c*).

The blocking action of bretylium and guanethidine was decreased when the temperature was lowered from 36° C to 25° C (Fig. 4).

Sciatic gastrocnemius preparation of cat

Bretylium and guanethidine (5 to 40 mg) injected intra-arterially produced a transient block, of varying degrees, of the response of the muscle to indirect stimulation; the drugs, however, had no immediate effect on the response of the muscle to direct stimulation (Fig. 5). In all but three experiments the initial transient block was followed after 15 to 60 min by a delayed block which progressed to completion within 30 to 290 min (Fig. 6). No recovery was seen even after 2 to 3 hr. After the response of the muscle to indirect stimulation was completely blocked, direct

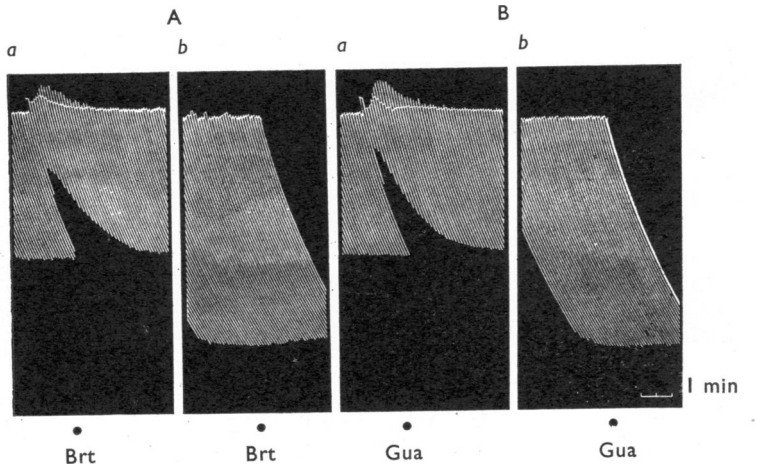

Fig. 5. Sciatic gastrocnemius preparation of cat. Chloralose anaesthesia. (*a*) Indirect stimulation (5 V, 0.5 msec, 6/min), and (*b*) direct stimulation (120 V, 5.0 msec, 6/min). Effect (at dots) of intra-arterial injections of bretylium 10 mg in A and guanethidine 10 mg in B.

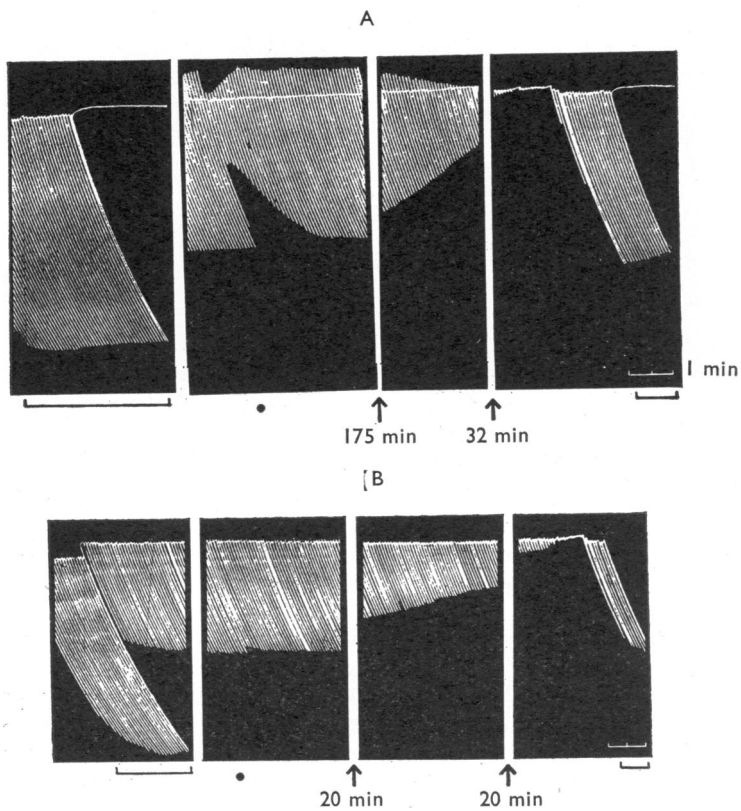

Fig. 6. Sciatic gastrocnemius preparation of cat. Indirect stimulation (5 V, 0.5 msec, 6/min). Effect of intra-arterial injections (at dots) of bretylium 40 mg (A) and guanethidine 20 mg (B). The period marked by bar indicates direct stimulation (120 V, 5 msec, 6/min).

stimulation was still effective, though less so than at the beginning of the experiment (Fig. 6). The delayed block was not antagonized by physostigmine (200 μg). Only in the initial stages could the block be temporarily reversed by potassium chloride (50 mg). Calcium chloride (50 mg) temporarily antagonized the delayed block produced by bretylium but not that elicited by guanethidine. Neuromuscular blocking action of tubocurarine (200 μg) was enhanced both in magnitude and in duration when tubocurarine was preceded by a small amount of bretylium (5 mg) while guanethidine (5 mg) increased only the duration of the block. The neuromuscular blocking action of decamethonium (30 μg) was not modified by either of the drugs.

Frog rectus

Bretylium and guanethidine (0.6×10^{-6} to 3×10^{-4}) had no inhibitory action on acetylcholine-induced contracture of the frog rectus. On the other hand, the compounds potentiated the stimulant action of acetylcholine (Fig. 7).

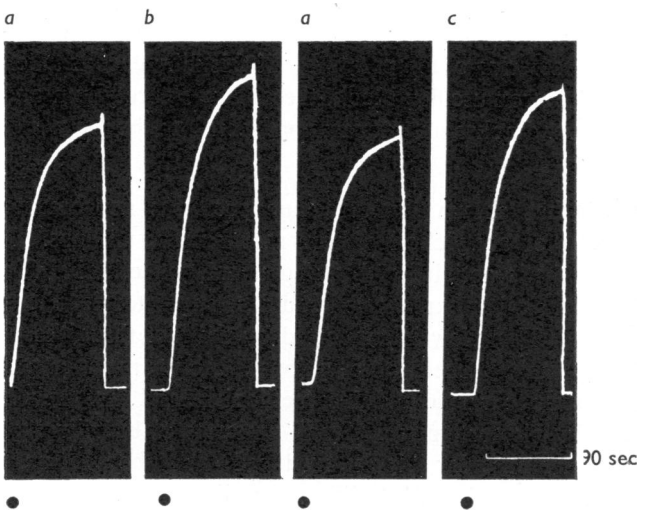

Fig. 7. Frog rectus. Effect of acetylcholine 0.6×10^{-6} (at dots). (*a*) Control responses; (*b*) and (*c*) 1 min after bretylium 0.6×10^{-6} and guanethidine 5×10^{-6} respectively.

Pigeons

Transient flaccid paralysis was observed in pigeons after the intravenous administration of bretylium and guanethidine. The PD50 values for bretylium and guanethidine were 23.1 ± 1.06 mg/kg and 10.86 ± 1.04 mg/kg respectively.

DISCUSSION

The results of our experiments show that both bretylium and guanethidine block transmission across the neuromuscular junction. On the sciatic gastrocnemius preparation of the cat the compounds had a twofold action, consisting of an initial transient, and a delayed but more sustained, block of neuromuscular transmission. The initial blocking action was short-lived and was not studied in detail. The

delayed blocking action and the blocking action on the rat phrenic nerve diaphragm preparation were studied in detail and are discussed.

Neuromuscular blocking agents in common use can be divided into two groups on the basis of the mechanism by which they act ; those which block by competition and those which block by depolarization. Each of these two types of neuromuscular block are pharmacologically well characterized (Paton & Zaimis, 1952 ; Brücke, 1956). An analysis of the neuromuscular blocking action of bretylium and guanethidine on the two nerve-muscle preparations used indicates that the block produced by these compounds belongs to neither of these two types. Antagonism of the blocking action of these compounds by potassium in rat phrenic nerve diaphragm and in the initial stages in sciatic gastrocnemius preparation indicates a possible similarity with the non-depolarizing type of block. However, the blocking action of these compounds is not antagonized by doses of physostigmine which completely antagonize tubocurarine block. Holmes, Jeden & Taylor (1951) and Bigland, Goetzee, Maclagan & Zaimis (1958) have shown that the neuromuscular blocking action of tubocurarine decreases and that of the decamethonium type of drugs increases with lowering of temperature. If this difference is taken as a criterion of the two types of neuromuscular block, the action of bretylium and guanethidine on the phrenic nerve diaphragm preparation seems to be of a non-depolarizing type.

Straughan (1961) has shown that procaine produces neuromuscular block by inhibiting the release of acetylcholine from motor nerve endings. In view of the reported local anaesthetic activity of bretylium and guanethidine (Boura & Green, 1959 ; Green, 1960), a similar explanation could be suggested for the blocking action of these compounds. This view is supported by the fact that, like procaine (Dixit, Gulati & Gokhale, unpublished observations), bretylium and guanethidine act additively with both tubocurarine and decamethonium and that their blocking action is not antagonized by physostigmine. Small amounts of bretylium or guanethidine potentiated the neuromuscular block produced by tubocurarine in the sciatic gastrocnemius preparation of cat, but did not modify the action of decamethonium, an observation consistent with an inhibition of release of transmitter from motor nerve endings. Moreover, absence of an inhibitory effect on acetylcholine-induced contracture of the frog rectus indicates a presynaptic site of blocking action of these drugs. With bretylium the local anaesthetic action is of considerable duration (Boura & Green, 1959), and this might explain the long duration of the delayed block seen in the sciatic gastrocnemius preparation of the cat.

Since the response of the gastrocnemius muscle to direct stimulation was not as fully effective as at the beginning of the experiment, after complete block to indirect stimulation was produced by bretylium or guanethidine, it is possible that these compounds also produce a delayed direct depression of muscle contractility.

The authors gratefully acknowledge the generous gifts of bretylium tosylate and decamethonium iodide by Burroughs Wellcome & Co., and guanethidine sulphate by Ciba (Basle). Thanks are also due to Dr J. D. Pathak, Dean, Medical College, Baroda, for providing facilities to carry out the work.

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