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THE NEUROMUSCULAR BLOCKING ACTION OF γ-OXALOLAUDONIUM BROMIDE

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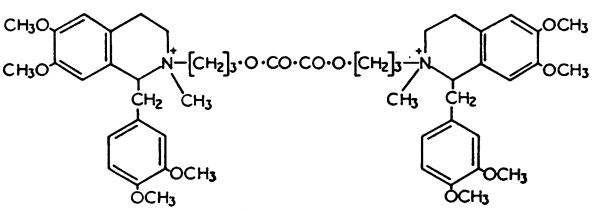
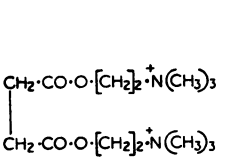
In small animals γ-oxalolaudonium caused flaccid paralysis; in the cat it produced a curare-like rather than a decamethonium-like block of neuromuscular transmission. The potency of γ-oxalolaudonium was only 1/30 to 1/40 that of suxamethonium, but the duration of paralysis was very short, being about one-half that of equiactive doses of suxamethonium. Successive doses of γ-oxalolaudonium were not cumulative and the paralysis could be antagonized by neostigmine. γ-Oxalolaudonium exhibited low toxicity especially in artificially ventilated animals, and it did not show ganglion-blocking or histamine-releasing activity to any large degree.

Laudexium is a synthetic drug whose mode and duration of action closely resemble those of tubocurarine. Suxamethonium is a neuromuscular blocking agent of brief duration which acts by depolarizing the motor end-plate region. It was thought that the brief duration of suxamethonium and the curare-like mode of action of laudexium might be combined in one compound by incorporating some



I



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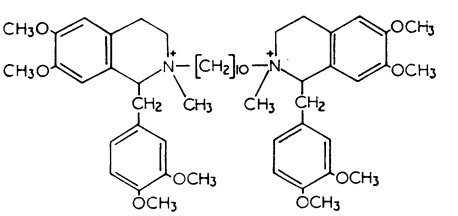

III

of the chemical features of suxamethonium into the laudexium molecule. Three series of compounds—symmetrical bis-esters, unsymmetrical bis-esters and symmetrical bis-amides—were therefore synthesized (Collier, Gladych, Macauley

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& Taylor, 1958a & b ; Gladych & Taylor, 1961). From the initial pharmacological work on these compounds already reported (Collier *et al.*, 1958a & b), one symmetrical bis-ester appeared to be worthy of further examination, and it is the more detailed pharmacology of this compound, designated γ-oxalolaudonium, that is now described. The chemical structure of γ-oxalolaudonium (I) and its relationship to suxamethonium (II) and laudexium (III) are shown above.

METHODS

Drugs. The following salts were used: γ-oxalolaudonium bromide, suxamethonium chloride, tubocurarine chloride, neostigmine methylsulphate, nicotine tartrate and histamine acid phosphate. Drugs were dissolved in normal saline and doses are given as weights of their respective salts.

Action in mice. Paralysing activity was determined in male albino mice weighing 18 to 22 g using the rotating drum technique of Collier, Hall & Fieller (1949). In acute toxicity tests deaths were recorded after 7 days.

Action in rabbits. Paralysis was determined by loss of righting reflex in white Himalayan rabbits of either sex and of body weight 2 to 3 kg. In acute toxicity tests deaths were recorded after 7 days. Blood pressure was recorded by mercury manometer from the carotid artery of rabbits anaesthetized with urethane (1 g/kg intravenously). Drugs were administered intravenously through a cannula in the jugular vein.

Action in chicks. Drugs were administered intravenously by the jugular vein to day-old chicks.

Action on the myoneural junction. In vitro experiments were performed on the isolated rectus abdominis muscle of the frog according to the procedure of Brittain, Chesher, Collier & Grimshaw (1959). *In vivo* experiments were performed on cats anaesthetized with chloralose (80 mg/kg intraperitoneally). A hind limb was set up in a vertical position on a Brown–Schuster myograph stand. Shielded electrodes were placed on the sciatic nerve and the nerve was ligated central to the electrodes. Twitches and tetani of the tibialis anterior muscle were elicited by supramaximal rectangular pulses of 0.35 to 1.0 msec duration. The muscle was attached to a flat spring myograph and the contractions were recorded on smoked paper. Blood pressure was recorded from the carotid artery with a mercury manometer. While the animal was artificially ventilated, drugs were injected intravenously through a cannula in either the femoral or jugular vein.

Action on ganglia. The ganglionic blocking activity of drugs was investigated using the isolated ileum of the guinea-pig by the method of Feldberg (1951).

Release of histamine. Liberation of histamine was assessed by weal formation in human skin (Collier & Macauley, 1952).

RESULTS

Action in mice, rabbits and chicks

γ-Oxalolaudonium when administered intravenously to mice in effective doses (1 to 2 mg/kg) caused a short-lasting flaccid paralysis neither preceded nor followed

TABLE 1

PARALYSING ACTIVITY AND ACUTE TOXICITY OF γ-OXALOLAUDONIUM BROMIDE INJECTED INTRAVENOUSLY INTO MICE

Index	No. of experiments	Mean value mg/kg	Range of values mg/kg
ED50	10	1·61	1·38–1·95
LD50	6	5·31	4·76–6·46

by excitation. The results of these experiments together with studies on the intravenous acute toxicity of γ-oxalolaudonium are summarized in Table 1. Information

from experiments of this type led to the belief that the drug might be unstable in aqueous solution. A 0.5% solution of γ-oxalolaudonium was prepared in saline, and suitable dilutions were administered intravenously to mice immediately, 24 hr and 96 hr after preparation. The paralysing activity and acute toxicity of γ-oxalolaudonium were determined, and the results of these experiments, summarized in Table 2, show that the drug was unstable in aqueous solution, and therefore in all other experiments solutions of γ-oxalolaudonium were freshly prepared.

TABLE 2

INSTABILITY OF γ-OXALOLAUDONIUM BROMIDE IN AQUEOUS SOLUTION

Time in hr after preparation of solution	No. of mice	ED50±s.e. mg/kg	LD50±s.e. mg/kg
0	45	1·4±0·06	5·5±0·04
24	24	4·0±0·15	—
96	36	9·3±0·70	22·4±0·80

In rabbits, intravenous γ-oxalolaudonium (1 to 2 mg/kg) caused immediate loss of the righting reflex which lasted from 2 to 4 min and was associated with respiratory difficulty. After recovery of the righting reflex the head-drop posture was evident for a very short time. The intravenous LD50 of the drug lay between 2 and 5 mg/kg.

The mode of action of γ-oxalolaudonium was investigated in chicks. While intravenous suxamethonium, 0.05 mg/kg, caused rigid extension of the legs and retraction of the head, intravenous γ-oxalolaudonium, 12.5 mg/kg and above, caused a flaccid paralysis similar to that described for tubocurarine by Buttle & Zaimis (1949).

Action on myoneural junction

In vitro *experiments.* γ-Oxalolaudonium, 1.33×10^{-6}, failed to stimulate the isolated frog rectus abdominis preparation but antagonized its response to suxamethonium.

In vivo *experiments.* In the cat, γ-oxalolaudonium, 3 to 4 mg/kg intravenously, caused 90 to 100% paralysis of the indirectly evoked maximal twitches of the tibialis anterior muscle without initial stimulation. These experiments, which are summarized in Table 3, show that the drug has a short-lasting neuromuscular

TABLE 3

PARALYSING ACTIVITY OF γ-OXALOLAUDONIUM ON THE MAXIMAL TWITCHES OF THE CAT TIBIALIS ANTERIOR MUSCLE IN RESPONSE TO INDIRECT STIMULATION

Duration is expressed as the time between treatment and recovery of the twitch tension to 75% of its value before treatment

Dose of γ-oxalolaudonium bromide mg/kg	1·0	2·0	3·0	4·0
No. of experiments	3	1	14	2
Mean % reduction of twitch response±s.e.	13·0±6·1	25·5	91·0±2·2	100
Mean duration of effect (min)±s.e.	—	1·1	3·3±0·2	7·5±0·2

blocking action. Experiments in which γ-oxalolaudonium was compared with suxamethonium showed that γ-oxalolaudonium had only 1/30 to 1/40 of the potency of suxamethonium, although its duration was very short, being only about one-half that of equiactive doses of suxamethonium (Fig. 1). Moreover, successive equal

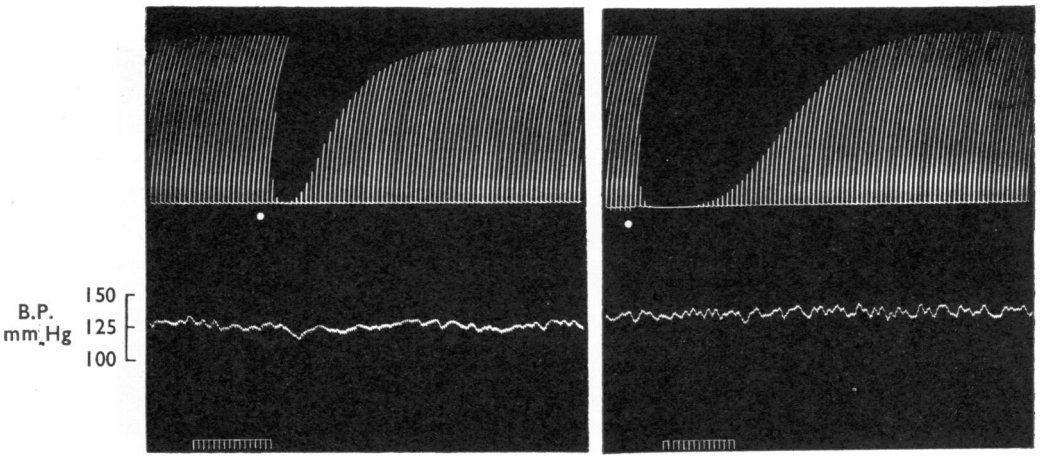

Fig. 1. Cat, chloralose. Upper tracing, maximal twitches of the tibialis anterior muscle elicited by indirect stimulation. Lower tracing, carotid blood pressure. Time scale, 10 sec intervals. Left-hand panel, 3 mg/kg γ-oxalolaudonium; right-hand panel, 150 μg/kg suxamethonium; both drugs injected intravenously.

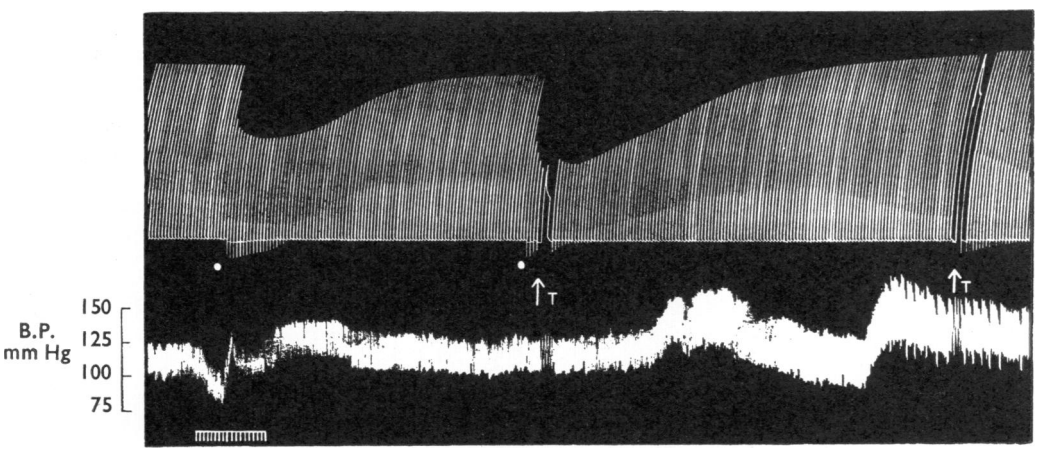

Fig. 2. Cat, chloralose. Upper tracing, maximal twitches of the tibialis anterior muscle elicited by indirect stimulation. Lower tracing, carotid blood pressure. Time scale, 10 sec intervals. At dots 50 μg/kg suxamethonium injected intravenously. ↑T indicates tetanus of 10 sec duration and frequency 30/sec.

doses of γ-oxalolaudonium did not last longer in effect than the initial dose, although successive equal doses of suxamethonium showed cumulative effects.

The response of the muscle to tetanic stimulation of the motor nerve has been used as an indication of the mode of action of neuromuscular blocking substances (Paton & Zaimis, 1949). During partial paralysis by suxamethonium, tetanic tension was fairly well sustained (Fig. 2); following partial paralysis by γ-oxalolaudonium, however, tetanic stimulation produced only a poorly sustained response (Fig. 3).

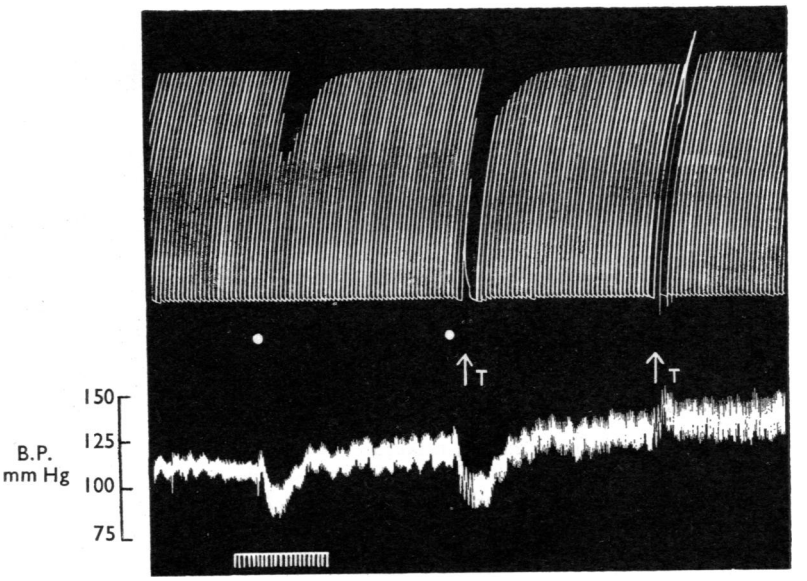

Fig. 3. Cat, chloralose. Upper tracing, maximal twitches of the tibialis anterior muscle elicited by indirect stimulation. Lower tracing, carotid blood pressure. Time scale, 10 sec intervals. At dots 2 mg/kg γ-oxalolaudonium injected intravenously. ↑T indicates tetanus of 10 sec duration and frequency 30/sec.

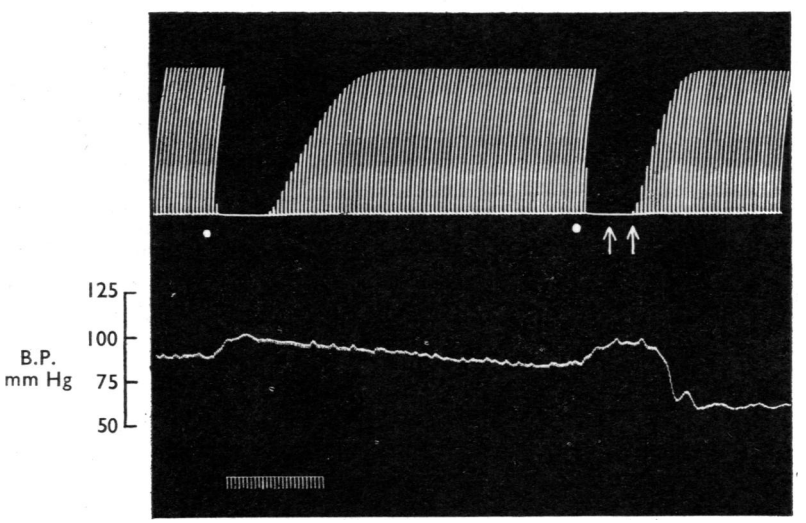

Fig. 4. Cat, chloralose. Upper tracing, maximal twitches of the tibialis anterior muscle elicited by indirect stimulation. Lower tracing, carotid blood pressure. Time scale, 10 sec intervals. At dots 10 mg/kg γ-oxalolaudonium, at arrows 30 μg/kg neostigmine. Both drugs injected intravenously.

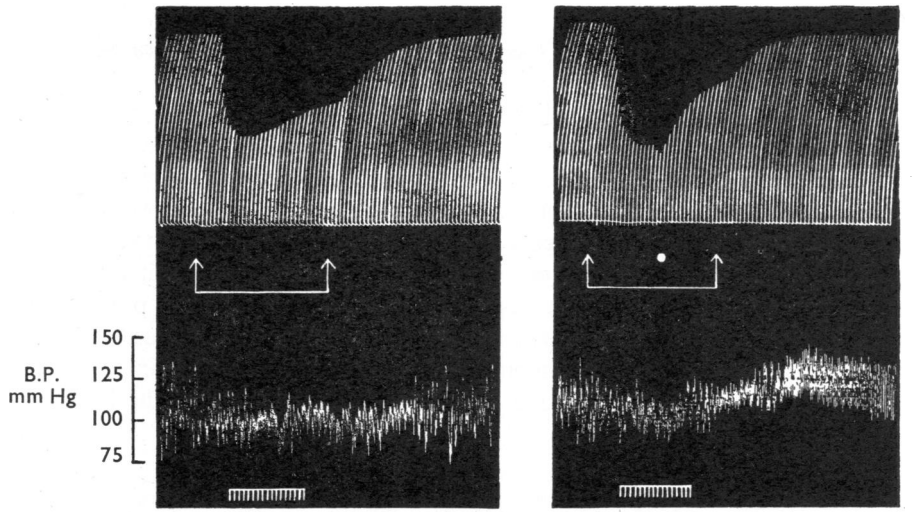

Fig. 5. Cat, chloralose. Upper tracing, maximal twitches of the tibialis anterior muscle elicited by indirect stimulation. Lower tracing, carotid blood pressure. Time scale, 10 sec intervals. During the period marked by arrows and horizontal bar a 1% solution of γ-oxalolaudonium was infused intravenously 0.5 ml./min. At dot, 20 μg/kg neostigmine was injected intravenously.

Since the results so far described indicated that γ-oxalolaudonium produced a curare-like block of neuromuscular transmission, further experiments were carried out to confirm this indication in two ways. In one, neostigmine was administered after complete block by γ-oxalolaudonium (Fig. 4); in the alternative method, neostigmine was administered during a partial paralysis induced by a constant infusion of γ-oxalolaudonium (Fig. 5). It is evident from these experiments that neostigmine antagonized the neuromuscular block induced by γ-oxalolaudonium.

Acute toxicity in artificially ventilated animals

Since the predominant toxic effect of γ-oxalolaudonium in normal animals was respiratory arrest, the toxicity of γ-oxalolaudonium was studied in artificially ventilated animals. Three rabbits tolerated successive intravenous doses of 5, 10 and 20 mg/kg, but of these only one animal survived a further dose of 40 mg/kg. Of three cats, two survived an intravenous dose of 40 mg/kg γ-oxalolaudonium. In

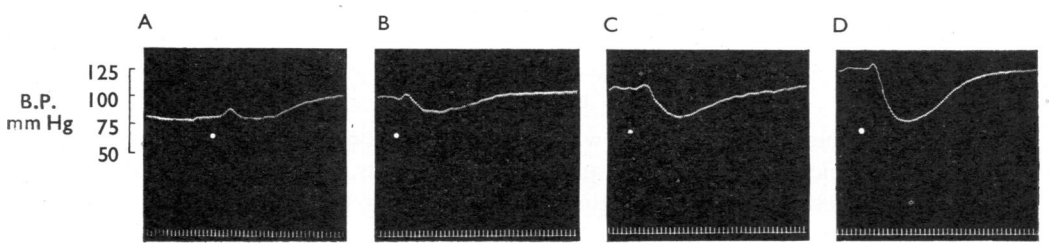

Fig. 6. Rabbit, urethane. Carotid blood pressure. Time scale, 10 sec intervals. At A, B, C and D, γ-oxalolaudonium injected intravenously at doses of 5, 10, 20 and 40 mg/kg respectively.

both rabbits and cats, 10 mg/kg and above produced a temporary fall in blood pressure, the results of one such experiment in the rabbit being illustrated in Fig. 6.

Action on ganglia

In view of the hypotensive action of very large intravenous doses of γ-oxalolaudonium in anaesthetized animals, the drug was examined for ganglionic blocking activity. The responses of the isolated guinea-pig ileum to nicotine were compared in the absence or in the presence of γ-oxalolaudonium. In a concentration of 2.5×10^{-5}, γ-oxalolaudonium did not increase muscle tone, nor did it induce spontaneous movements of the tissue, nor modify the response of the preparation to nicotine. In contrast, tubocurarine in a concentration of 4×10^{-6} reduced the nicotine response by 49% (Fig. 7). It is evident from this experiment that γ-oxalolaudonium did not exhibit a ganglionic blocking action on this preparation at the largest dose tested.

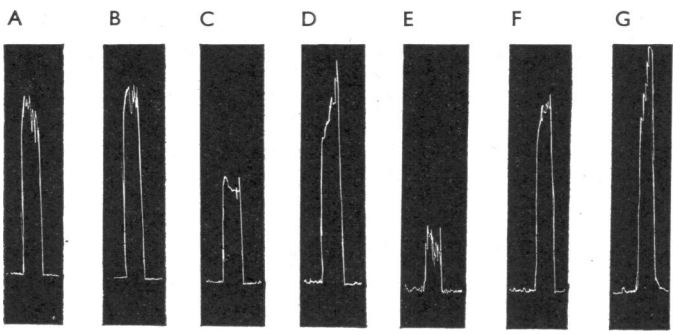

Fig. 7. Isolated guinea-pig ileum in 20 ml. bath. Contractions in response to 40 μg of nicotine. At A and G, no treatment; at B, D and F, 80, 160 and 500 μg γ-oxalolaudonium respectively; at C and E, 80 and 160 μg of tubocurarine respectively. Four histamine responses between each nicotine effect have been omitted from the tracing.

Release of histamine

As tubocurarine and laudexium are known to release histamine, the possible effect of γ-oxalolaudonium in this respect was investigated in human subjects. In two subjects, intradermal injection of 100 μg γ-oxalolaudonium caused local weal and flare attributed to release of histamine. Reference to previous skin tests with established neuromuscular blocking agents (Collier & Macauley, 1952) shows that γ-oxalolaudonium had less than half the potency in releasing histamine of laudexium, which in turn is less active than tubocurarine.

DISCUSSION

The experiments described show that γ-oxalolaudonium caused a very brief paralysis in chicks, mice, rabbits and cats. Five facts indicate that its mode of action resembles that of tubocurarine and differs from that of decamethonium or suxamethonium: (1) γ-oxalolaudonium did not stimulate the isolated rectus abdominis of the frog, but antagonized the action of suxamethonium on this preparation ; (2) it did not produce muscle fasciculations nor potentiate the maximal twitch

of the tibialis ; (3) during partial paralysis by γ-oxalolaudonium, tetanic stimulation produced a poorly sustained contracture ; (4) neostigmine antagonized the neuromuscular blockade due to γ-oxalolaudonium ; and (5) in chicks, γ-oxalolaudonium produced a flaccid paralysis.

In γ-oxalolaudonium the large heterocyclic end-groups of laudexium are linked by an ester chain somewhat resembling that of suxamethonium. The brevity and curare-like action of γ-oxalolaudonium show that, by combining some of the chemical features of laudexium and of suxamethonium, a compound can be obtained having some of the desirable features of each drug. Unfortunately this combination was attended by a marked loss of potency, which renders doubtful the clinical value of γ-oxalolaudonium.

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