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A NOTE ON AN UNUSUAL EFFECT OF GALLAMINE AND TUBOCURARINE

BY

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Paton & Zaimis (1952) contrasted the effects of depolarizing muscle relaxants with those of competitive blocking agents resembling tubocurarine. Decamethonium caused potentiation of twitches with repetitive discharge, whereas competitive antagonists did not have this effect. However, Riker & Wescoe (1951) reported that gallamine, in doses smaller than, and up to, the minimal effective curariform dose, may produce a slight potentiation of the muscle response to indirect stimulation. A similar observation is reported here on rat phrenic nerve-diaphragm preparations together with an examination of the possible mechanism.

METHODS

Phrenic nerve-diaphragm preparations from 300-g rats were set up in Krebs solution (in g/l.: NaCl 6.92, KCl 0.354, $CaCl_2$ 0.282, KH_2PO_4 0.162, $MgSO_47H_2O$ 0.294, $NaHCO_3$ 2.1 and glucose 2.0) gassed with 95% oxygen and 5% carbon dioxide in a 25-ml. jacketed bath. The temperature was controlled at 37 or 25° C thermostatically. Recordings were made on a smoked drum using a Starling spring-loaded heart lever. The phrenic nerve was stimulated at 12 shocks/min with supramaximal rectangular pulses of 0.75 msec duration. Supramaximal stimulation was achieved by setting duration and frequency of stimuli appropriately and then increasing the voltage to 5 V above that which produced the greatest muscle twitch. Gallamine and tubocurarine were added to the bath.

The acetylcholine output of the preparation in the presence of gallamine was measured by the method described by Matthews & Quilliam (1964), using the anaesthetized rat's blood pressure for the assay.

Monophasic action potentials were recorded using a double-chambered bath designed by Niedergerke (1956). A 3.0-mm-wide strip of diaphragm from an immature (100 g) rat was placed with its nerve supply in the bath and the costal margin immersed in isotonic potassium chloride solution in the lower chamber. The strip passed between two greased plates with a 1.0-mm hole at their centre into the upper chamber, filled with Krebs solution equilibrated with 95% oxygen and 5% carbon dioxide, and its apex was attached to a valve transducer (R.C.A. 5734). The double chamber was maintained at 37° C in a thermostatically controlled water bath. Monophasic action potentials were carried by chlorided silver electrodes attached to the upper and lower chambers of the bath to a preamplifier and were photographed with the isometric twitch tension from a Cossor oscilloscope. The phrenic nerve was stimulated at 12 shocks/min with rectangular pulses and the pulse duration or voltage was increased until repetitive firing of the muscle action potential occurred. After reducing the stimulus until repetition ceased, recordings were taken from the muscle in normal Krebs solution, again after adding gallamine (3 to 4 μg/ml.) or tubocurarine (0.08 to 0.16 μg/ml.) to the bath and finally after washing and returning to normal Krebs solution. Similarly, records were taken of phrenic nerve action potentials.

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RESULTS

In sixteen out of twenty experiments, gallamine (20 to 40 μg/ml.) increased twitch tension during phrenic nerve stimulation (Fig. 1). When the bath temperature was reduced to 25° C, gallamine in the same dose was without effect (Fig. 2). Halving the calcium chloride concentration increased the twitch tension resulting from nerve stimulation. Under these

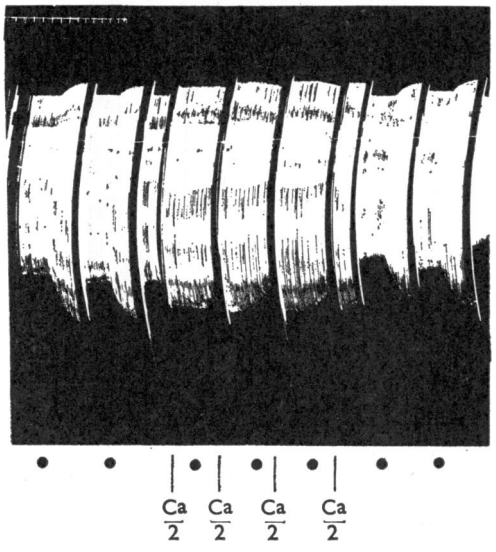

Fig. 1. Effect of halving the calcium concentration of the medium on the action of gallamine (40 μg/ml.) on a rat phrenic nerve-diaphragm preparation suspended in 25 ml. of Krebs solution gassed with 95% O_2 and 5% CO_2 at 37° C. The phrenic nerve was stimulated by supramaximal shocks, duration 0.75 msec at 12/min. At the dots, 1.0 mg of gallamine triethiodide in 0.05 ml. of distilled water was added to the bath. The bath was washed out at 5-min intervals over a period of 30 min after each response. At Ca/2 normal Krebs solution was replaced by solution containing half the calcium chloride concentration. Time marks, 30 sec.

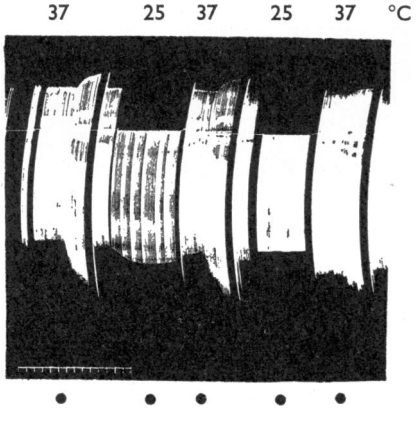

Fig. 2. Effect of reducing bath temperature on the action of gallamine on a rat phrenic nerve-diaphragm preparation under conditions similar to those described in Fig. 1. The temperature was reduced to 25° C where shown above the tracing. Gallamine was added to the bath fluid at the dots as described for Fig. 1. Time marks, 30 sec.

conditions gallamine reduced twitch tension whereas the same dose of gallamine caused augmentation in normal Krebs solution both before and after exposure to low calcium solution (Fig. 1). In the presence of physostigmine (5 μg/ml.), gallamine, which in normal

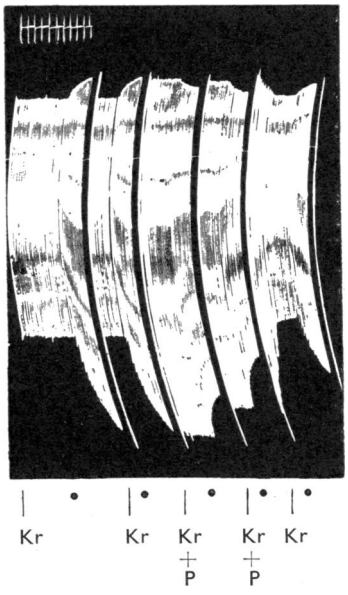

Fig. 3. The effect of physostigmine (5.0 μg/ml.) on the response of the rat phrenic nerve-diaphragm preparation. Conditions were as for Fig. 1. Gallamine was added to the bath at the dots, as described for Fig. 1. At Kr normal Krebs solution was added to the bath. At Kr+P Krebs solution containing physostigmine (5.0 μg/ml.) was added to the bath 20 min before the addition of gallamine. Before the last response to gallamine, the bath was washed out at 5-min intervals during a period of 60 min to remove all the physostigmine. Time marks, 30 sec.

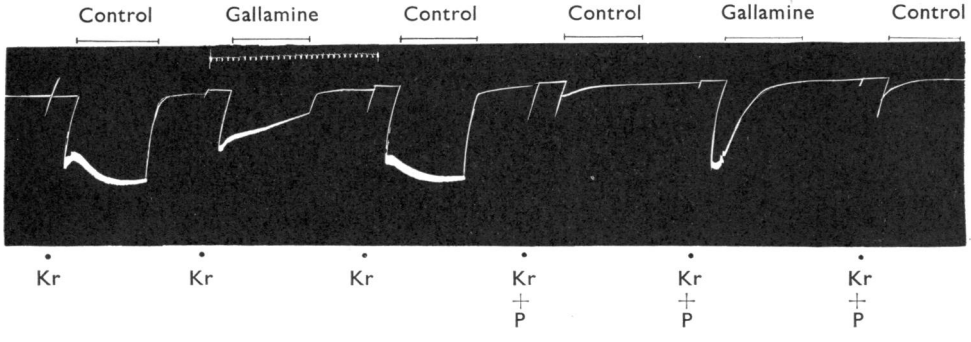

Fig. 4. The action of physostigmine on the effect of gallamine on tetanus of the rat phrenic nerve-diaphragm preparation. Conditions were as for Fig. 1, except that the phrenic nerve was stimulated with supramaximal stimuli of 0.75 msec duration at 25 shocks/sec for periods of 14 sec (indicated by lines above the record), in between each tetanus the bath was washed out. Control=response without gallamine. Gallamine=response in presence of gallamine (40 μg/ml.) added 3 min before tetanus. Kr=Krebs solution. Kr+P=Krebs solution containing physostigmine (5 μg/ml.). After the addition of physostigmine 20 min elapsed before the preparation was tetanized. Time marks, 1 sec.

Krebs solution increased twitch tension, produced a reduction (Fig. 3). No change in acetylcholine output in the presence of gallamine (40 to 160 μg/ml.) could be detected by assay using the blood pressure of the anaesthetized rat. These experiments required tetanic stimulation and the addition of physostigmine (5 μg/ml.) to the Krebs solution. Under these conditions gallamine (40 μg/ml.) increased the force and duration of the tetanic response whereas these were both reduced by gallamine in the absence of physostigmine (Fig. 4).

Gallamine (4 μg/ml.) induced repetitive firing of the action potentials of the diaphragm of the immature rat (Fig. 5) but was without effect on the action potential of the phrenic nerve at a concentration of 3 to 80 μg/ml.

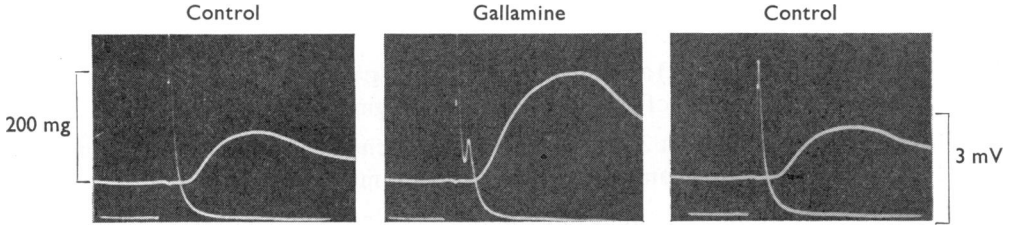

Fig. 5. The effect of gallamine on the rat phrenic nerve-diaphragm preparation suspended in a Niedergerke bath containing 30 ml. of Krebs solution equilibrated with 95% oxygen and 5% carbon dioxide at 37° C. The upper trace shows the isometric twitch tension with a 200-mg calibration. The lower trace shows the monophasic muscle action potential with a 3-mV calibration. The left-hand mounting is for normal Krebs solution, the middle mounting is after adding gallamine (4 μg/ml.) and the third is after washing and returning the preparation to normal Krebs solution.

Tubocurarine (0.08 to 0.16 μg/ml.) also increased twitch tension and produced repetitive firing during nerve stimulation.

One experiment was made in order to determine the effect of low doses of gallamine and tubocurarine, administered by close intra-arterial injection, on the soleus and tibialis anterior muscles of the cat anaesthetized with chloralose. The sciatic nerve was stimulated by supramaximal rectangular pulses of 1.0 msec duration at a frequency of 10 shocks/min. Under these conditions, gallamine (50 μg) and tubocurarine (10 μg) caused an increase in twitch tension of the tibialis anterior muscle but not of the soleus. If, however, a pulse duration of 0.5 msec was used, the same doses of gallamine and tubocurarine did not cause a stimulant effect.

DISCUSSION

It would appear from the evidence presented that gallamine, although normally a neuromuscular blocking agent, can in suitable concentrations produce an increase in twitch tension, thus confirming the observation of Riker & Wescoe (1951). In addition, it has been shown that tubocurarine has the same action in low concentrations. Records of the muscle action potentials demonstrate that the greater force of contraction is due to repetitive firing of the muscle fibres, which agrees with the findings of Payton (personal communication) on frog sartorius muscle. There was no repetitive firing of phrenic nerve action potentials. Therefore repetitive firing is presumably initiated at the muscle or neuromuscular junction.

The failure of gallamine to increase twitch tension at 25° C and the reduction in size of the twitch produced by concentrations which normally cause potentiation when calcium is reduced suggest that repetitive firing may result from increased release of acetylcholine. This is compatible with the reduction of twitch tension in the presence of physostigmine. The insensitivity of the assay preparation may account for the failure to detect an increased acetylcholine release.

The increase in force and duration of tetanus produced by gallamine in the presence of physostigmine is probably due to the prevention by the muscle relaxant of the depolarization block caused by the anticholinesterase; by preventing the block it produced the unexpected result for, in the absence of physostigmine, gallamine decreased both the force and duration of the tetanic response.

SUMMARY

1. Gallamine (20 to 40 μg/ml.) and tubocurarine (0.16 μg/ml.) increased the twitch tension obtained by nerve stimulation of phrenic nerve-diaphragm preparations at 37° C.

2. This effect did not occur at 25° C, and when the calcium chloride concentration of the Krebs solution was halved gallamine (40 μg/ml.) only diminished twitch tension.

3. In the presence of physostigmine (5 μg/ml.), gallamine (40 μg/ml.) reduced the twitch tension.

4. Gallamine (3 to 4 μg/ml.) and tubocurarine (0.08 to 0.16 μg/ml.) produced repetitive firing of the muscle action potentials, but no repetition from the phrenic nerve.

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