THE EFFECT OF LOWERED TEMPERATURE ON THE NEUROMUSCULAR BLOCKING ACTION OF SUXAMETHONIUM ON THE RAT DIAPHRAGM

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Received June 6, 1962

The neuromuscular blocking action of suxamethonium on the rat isolated phrenic nerve diaphragm preparation was observed at 20 and at 37°. The effect of lowered temperature is to intensify the neuromuscular block produced by suxamethonium and to render the depolarisation characteristics of the blockade more distinct. Competitive inhibition is not apparent at 20° in contrast to the competitive features of the block which have been reported to be present at 37°.

BIGLAND, Goetzee, Maclagan and Zaimis (1958), using nerve muscle preparations *in situ* in cats and dogs showed that cooling much prolonged the action of suxamethonium without affecting the nature of the blockade no matter how long the paralysis lasted. A tetanus was always well sustained and did not antagonise the block, neostigmine was ineffective or prolonged the block and tubocurarine antagonised it. Their results from experiments on the isolated rat diaphragm were similar to those obtained in the whole animal, namely cooling greatly increased both the magnitude and duration of the blockade.

Since the depolarising action of suxamethonium is complicated by some measure of competitive inhibition in the isolated rat diaphragm at 37° (Whittaker, 1962), it seemed useful to investigate further the nature of the neuromuscular blockade at lower temperatures.

EXPERIMENTAL

Method

Bülbring's preparation was used (1946). Supramaximal rectangular pulses of 0·1 to 0·3 msec. duration were applied to the phrenic nerve at 6/min. and the muscle contractions were recorded by a spring-loaded lever. The muscle was immersed in a bath of 75 ml. capacity containing the modified Tyrode solution used in previous work (Whittaker, 1962). The fluid was aerated with 95 per cent oxygen and 5 per cent carbon dioxide at temperatures of 20° and 37° $\pm$ 0·25°. Doses are of suxamethonium bromide, tubocurarine chloride, neostigmine bromide, edrophonium bromide and potassium chloride.

RESULTS

The Effect of Temperature on the Preparation

West (1947) found that the preparation maintained the original size of contractions for a longer period if the temperature of the bath fluid was lowered from 37 to 20°. On cooling from 37° the muscle tension first increased, reaching a maximum at 25 to 30°, then declined slightly;

below 20° the speed of contraction was greatly decreased (Burgen, Dickens and Zatman, 1949). These observations have been confirmed and also it was found that the effects of temperature change were reversible; that is the described tension changes took place either when the contractions were recorded first at 20° and then after raising the temperature to 37° or when the contractions were recorded first at 37° and then after lowering the temperature to 20°. For the experiments at lower temperature, 20° was selected because, as observed by West (1947), the preparation at 20° showed little sign of fatigue even after 8 hr.

The Effect of Varying Doses of Suxamethonium at 20°

Doses in the range of 40 to 100 μg. produced an initial potentiation of the diaphragm contractions followed by the onset of a slow neuromuscular block. As the dose increased in this range the extent and duration of the potentiation was reduced and, as would be expected, the onset of neuromuscular blockade was earlier. In general, doses greater than 100 μg. produced a complete block; the time needed for complete block shortening as the dose increased. When the indirectly excited maximal twitches were completely blocked, the muscle still responded to direct stimulation.

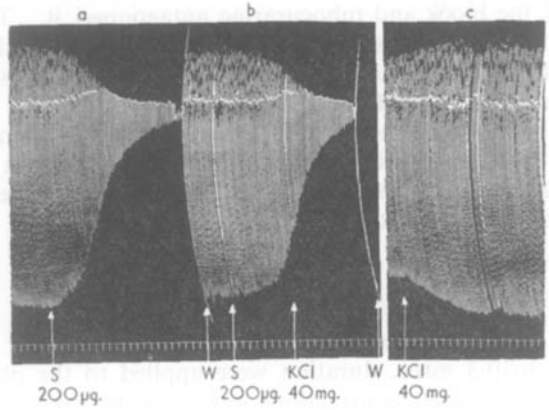

FIG. 1. The absence of antagonism by potassium chloride of the neuromuscular block produced by suxamethonium. Rat isolated diaphragm preparation stimulated through phrenic nerve, 6/min. At S, suxamethonium added to bath fluid. In (b) potassium chloride added to bath fluid 4 min. after suxamethonium. In (c) control with potassium alone is shown. At W, preparation washed with Tyrode solution. Temp. 20°. Time 30 sec.

The Effect of Potassium Chloride on the Neuromuscular Block Produced by Suxamethonium at 20°

When suxamethonium alone was added to the bath fluid almost complete block was produced in 10 min. (Fig. 1a) and potassium chloride added 4 min. after the suxamethonium had little effect on the block (Fig. 1b). The increase in response of the diaphragm when potassium chloride was added to the bath fluid in the absence of suxamethonium is shown in Fig. 1c.

The Effect of Anticholinesterases on the Neuromuscular Block Produced by Suxamethonium at 20°

Neostigmine. The addition of suxamethonium 80 μg. alone to the bath fluid produced 84 per cent reduction in the contraction height in 12 min. (Fig. 2a). However, when neostigmine was added 4 min. after the suxamethonium the block was quickly intensified and a complete block resulted within 10 min. (Fig. 2b).

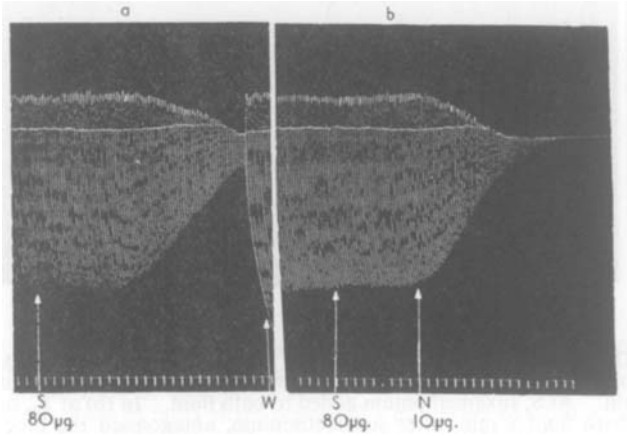

FIG. 2. The intensification by neostigmine of the neuromuscular block produced by suxamethonium. Rat isolated diaphragm preparation stimulated through phrenic nerve, 6/min. At S, suxamethonium added to the bath fluid. In (b) at N, neostigmine added to the bath fluid 4 min. after suxamethonium. At W, preparation washed with Tyrode solution. Temp. 20°. Time 30 sec.

Edrophonium. The addition of edrophonium to the bath fluid 8 min. after suxamethonium also caused intensification of the neuromuscular block.

The Effect of Tubocurarine on the Neuromuscular Block Produced by Suxamethonium at 20°

Suitable doses of suxamethonium were added to the bath fluid and followed at various time intervals by varying doses of tubocurarine. It was found that tubocurarine produced a marked though transitory antagonism of the suxamethonium block, which was then followed by a slowly progressive block in the presence of suxamethonium and tubocurarine (Fig. 3b). A further dose of tubocurarine added to the bath fluid did not antagonise the combined block but in general caused further intensification (Fig. 3b).

The Effect of Varying Doses of Suxamethonium at 37°

Varying amounts of suxamethonium were added to the bath fluid and allowed to act for 15 min. in some experiments, for 30 min. in others, or for shorter periods if a complete neuromuscular block was produced. The preparation was washed several times and allowed to recover.

Doses in the range 40 to 100 μg. when added to the bath fluid had little effect on the contractions of the diaphragm, in contrast to the initial potentiation of response before neuromuscular block produced by these doses at 20°. Doses in the range of 100 to 800 μg. occasionally produced a slight initial potentiation of contractions which was followed by a slow

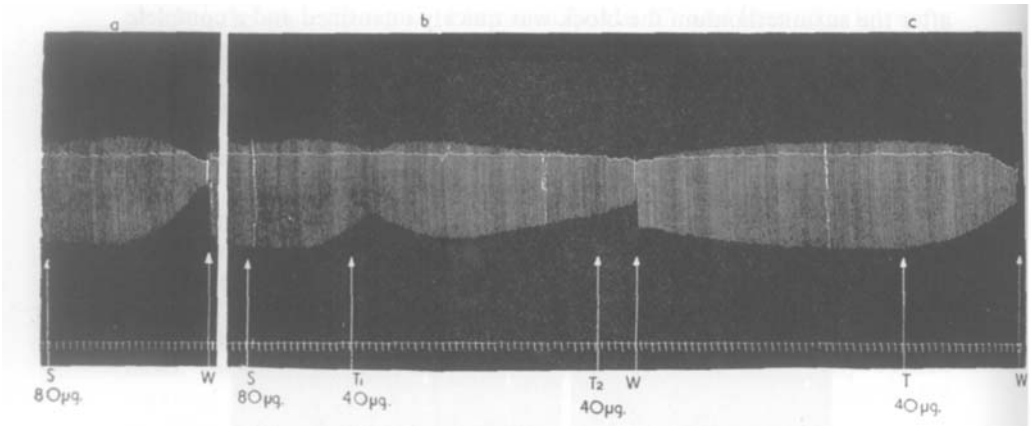

FIG. 3. The antagonism by tubocurarine of the neuromuscular block produced by suxamethonium. Rat isolated diaphragm preparation, stimulated through phrenic nerve, 6/min. At S, suxamethonium added to bath fluid. In (b) at T_1, tubocurarine added to bath fluid 8 min. after suxamethonium, antagonised the block. A subsequent dose of tubocurarine (T_2) added to bath fluid failed to produce further antagonism. (a) and (c) are control results with suxamethonium and tubocurarine alone. At W, preparation washed with Tyrode solution. Temp. 20°. Time 30 sec.

block. In most experiments however the initial potentiation of the response was absent, the neuromuscular block showed an initial fairly sharp onset, then progressed to a steady level and later declined slowly (Fig. 4). For complete block doses greater than 800 μg. were required.

DISCUSSION

The rat diaphragm preparation is more sensitive to suxamethonium at 20° than at 37°. This is in accordance with the findings of Bigland and others (1958). Moreover the neuromuscular block produced by suxamethonium at 20° appears to be due to end-plate depolarisation, as indicated by an initial stimulation before the onset of the block, the lack of effect of potassium on the block, intensification by the anticholinesterases, neostigmine and edrophonium and antagonism of the block by tubocurarine.

Whittaker (1962) reported that at 37° there were competitive features in the neuromuscular block produced by suxamethonium in the rat diaphragm preparation, the block being antagonised by potassium and intensified by tubocurarine.

Several workers have shown that suxamethonium is hydrolysed mainly by plasma cholinesterase in two stages (i) fairly rapidly to succinylmonocholine and choline and (ii) much more slowly to succinic acid and choline (V. P. Whittaker and Wijesundera, 1952; Tsuji, Foldes and Rhodes, Jr.,

1955). Also the breakdown of suxamethonium *in vitro* by both true and pseudocholinesterases has been reported (Low and Tammelin, 1951). Stovner (1958) studied the action of succinylmonocholine and succinyldicholine in nerve-diaphragm preparations of rats and kittens and in nerve muscle preparations *in situ* in rabbits and cats, and concluded that the neuromuscular block produced by succinylmonocholine was

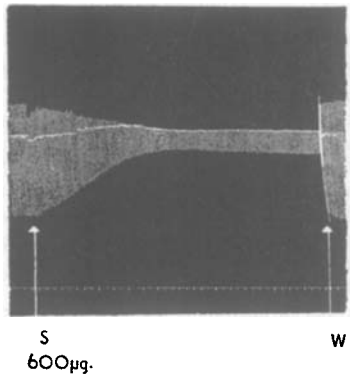

FIG. 4. The neuromuscular block produced by suxamethonium (S) at 37°. Rat isolated diaphragm preparation stimulated through phrenic nerve, 6/min. Initial potentiation of response is absent. The neuromuscular block consists of an initial phase of fairly sharp onset and a prolonged phase at a steady level. At W, preparation washed with Tyrode solution. Time 30 sec.

more competitive in nature than the block produced by succinyldicholine. Therefore Whittaker (1962) suggested that the competitive features of the suxamethonium block in the rat diaphragm preparation at 37° might be related to the enzymatic hydrolysis of suxamethonium and the formation of succinylmonocholine. The increased effect of suxamethonium at 20° and the absence of competitive features from the neuromuscular block may be due partly to the reduced cholinesterase activity at the lower temperature. However, the effects cannot be due solely to inactivation of the enzyme since Bigland and others (1958), have shown that cooling affects a neuromuscular block produced by decamethonium more, or at least as much as, a block produced by suxamethonium and it is known that decamethonium is not destroyed enzymatically in the same manner as suxamethonium (Zaimis, 1950).

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