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Pancuronium, Unlike Other Nondepolarizing Relaxants, Retains Potency at Hypothermia

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The authors studied the pharmacodynamics of four nondepolarising relaxants, *d*-tubocurarine (*d*TC), pancuronium, metocurine, and gallamine, at 25° C, 31° C, and 37° C. The rat phrenic nerve-hemidiaphragm preparation with vascular perfusion was used for these investigations. For each drug at each temperature, a dose-response curve for twitch depression was constructed. ED_{50} values were calculated using probit-log dose regression. *d*TC, metocurine, and gallamine each demonstrated a near twofold increase in ED_{50} at 25° C compared with 37° C. No such relationship was apparent with pancuronium. In addition, the slopes of the dose-response curves were analyzed for effects due to temperature or drug. Slopes were not influenced by temperature; however, the slopes for metocurine and *d*TC were lower than those for pancuronium and gallamine. The authors conclude that in the rat, pancuronium retains potency at hypothermia, whereas the other relaxants decrease potency. In addition, metocurine and *d*TC exhibit less steep dose-response curves under these experimental conditions. (Key words: Hypothermia. Neuromuscular relaxants: gallamine; metocurine; pancuronium; *d*-tubocurarine. Temperature: hypothermia.)

TEMPERATURE MAY AFFECT the degree of muscular relaxation obtained from nondepolarizing neuromuscular blocking drugs. Some studies have shown a decrease in relaxant potency at decreased temperature,[1–3] while other studies have shown an increase in potency with decreased temperature.[4–7] In several studies, drug levels in plasma were changing or were unmeasured.[1–4,7] Thus, any pharmacodynamic changes of relaxant potency with temperature may be confounded by the effects of temperature change on drug pharmacokinetics.

In this study, we investigated relaxant pharmacodynamics at three different temperatures. The rat phrenic nerve diaphragm preparation,[8] modified to include vascular perfusion,‡ permits maintenance of constant concentrations of relaxant in the perfusate. Four nondepolarizing neuromuscular relaxants, *d*-tubocurarine (*d*TC), pancuronium, metocurine, and gallamine, were studied under these conditions of unchanging pharmacokinetics.

Materials and Methods

APPARATUS

Immediately following cervical section, the left phrenic nerve and hemidiaphragm of male Wistar rats were excised. A stereoscopic dissection microscope aided placement of a short polyethylene cannula into the left phrenic vein. The nerve was placed on silver wire stimulating electrodes. A nylon tie linked the costal margin to a Grass® FT03C strain gauge. Care was taken to prevent damage to muscle fibers at the costal margin and at the insertions to the central tendon. A thin polyethylene covering prevented evaporative heat and water loss from the muscle surface. Two feedback controlled heaters and a distant overhead heat lamp provided temperature stability to within 0.2° C as measured by a thermocouple in contact with the muscle (see fig. 1). Uniform heating of the muscle with this equipment was confirmed by surface temperature measurements.

The composition of the perfusion medium was sodium 143 mM, potassium 4.4 mM, calcium 1.25 mM, magnesium 0.61 mM, chloride 123.7 mM, bicarbonate 24.9 mM, sulfate 0.61 mM, phosphate 1.10 mM, glucose 11.1 mM, choline 0.01 mM, and insulin 200 mU per liter. Calcium and magnesium levels were those that kept concentrations of the ionized species in the normal range.[9] Insulin was added in concentrations slightly higher than usual mean levels for the rat,[10] to ensure availability of glucose for uptake by the muscle. Five per cent CO_2 and 95% O_2 were bubbled through the solution at 37° C, providing typical gas tensions of P_{O_2} = 563 mmHg, P_{CO_2} = 40 mmHg, and a typical *p*H = 7.37. All drugs were added to this solution. The perfusate was then drawn into sealed syringes and allowed to cool. A calibrated syringe pump delivered the perfusate to the cannulated vein at a rate between 0.4 and 0.8 ml/min.

Pulses of 150-μs duration originated from a constant current generator. Current magnitude was set to twice that necessary to achieve maximal twitch tension. Resting muscle fiber length was set to optimize twitch tension and was fixed for the duration of the experiment.

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‡ Bierkamper G, Goldberg A: Vascular perfused rat phrenic nerve hemidiaphragm. J Electrophysiol Tech 6:40–46, 1978.

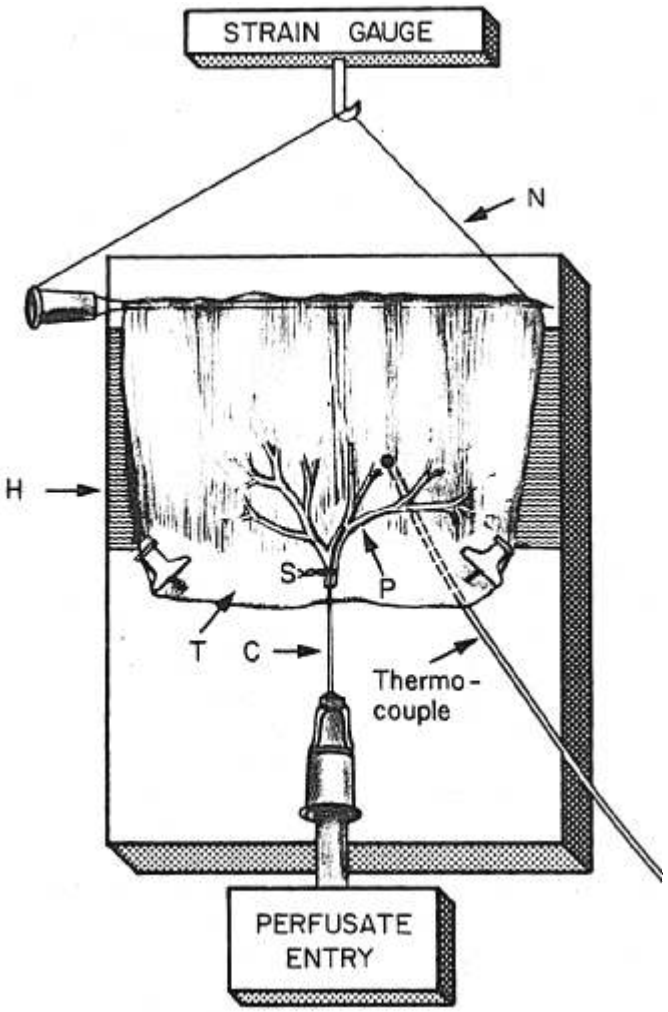

FIG. 1. Schematic of platform used in perfused diaphragm experiment. Muscle is shown as placed horizontally on the platform. The polyethylene cannula, C, supplies perfusate to the entire muscle via phrenic vein, P. The nylon tie, N, couples the strain guage to a needle that is passed through the costal margin and tendinous attachments of the diaphragm. The phrenic nerve and stimulation electrodes are on the under surface and not seen in this view.

EXPERIMENTAL PLAN

The preparation was set at the first working temperature. Drug-free perfusate was used to establish a stable baseline twitch tension. Three pulses at 0.1 Hz were delivered to the nerve under program control by a PET 4016 digital computer (Commodore Business Machines, Norristown, Pennsylvania 19403). Developed muscle tension passed from the transducer to an analog to digital converter (John Bell Engineering, Redwood City, California 94064), which was sampled by the PET. The average peak twitch tension for three pulses was calculated. When this average tension stabilized, it was designated as baseline tension. Then, perfusate containing a measured concentration of relaxant was infused at the same rate while triplicate tension measurements were repeated every 3 to 5 min until stabilization (8 to 12 min) and continued for a total of 20 min to ensure equilibration. Several different drug concentrations were infused in succession. Then temperature was changed, a minimum of 20 min was allowed to pass, and the entire process was repeated on the same diaphragm beginning with drug-free perfusate and baseline tension measurement. This series of experiments tested temperatures of 25° C, 31° C, and 37° C. Each diaphragm was studied at all three temperatures. A total of 20 diaphragms were studied in random order: five muscles with pancuronium, five with gallamine, five with metocurine, and five with *d*TC. The order of temperatures was varied randomly, as was the sequence of increasing or decreasing drug concentrations.

ANALYSIS

Each average tension measurement, when divided by the baseline average tension for the same temperature, yielded the tension ratio. Tension ratios for each drug and temperature were plotted on a probit scale against the log of drug concentration (see fig. 2). The probits, obtained from statistical tables, transform a sigmoidal dose-response curve to a straight line.[11] This technique applies to muscle tension data because of the "all-or-none" firing characteristics of individual muscle fibers.[12]

Linear regression analysis applied to each dose-response curve yielded values for slope and intercept with their associated errors. The ED_{50} values with their errors for each temperature in a given diaphragm were calculated by using the values for slope and intercept of the regression lines. The ED_{50} is that drug concentration producing a twitch ratio of 0.50 for the temperature studied.

For each of the four drugs, two-way repeated measures analysis of variance was used to test for an effect of temperature on ED_{50}. If a temperature effect was found, the Newman-Keuls test for comparison of multiple means was used to determine at which temperature the ED_{50} was different. Slopes of the dose-response curves were subjected to three-way repeated measures analysis of variance to test for effects due to drug or temperature. The Newman-Keuls method was used to test for differences in slopes among drugs. For all comparisons, P less than 0.05 was considered significant.

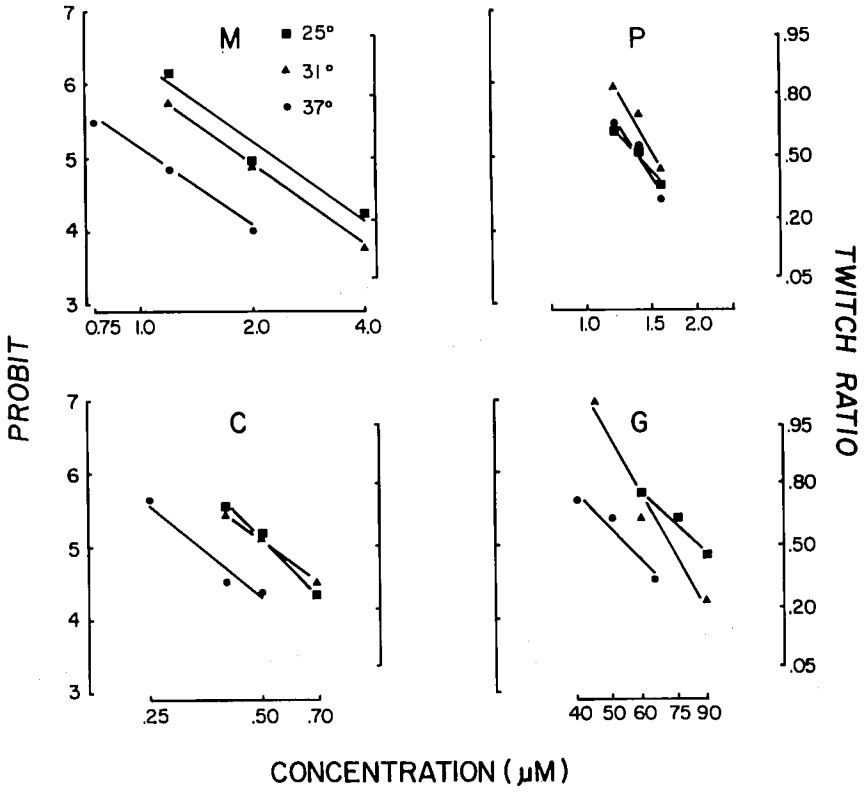

FIG. 2. Typical dose-response curves for each drug at each of three temperatures. Twitch ratio (right vertical axis) and its associated probit (left vertical axis) are plotted against drug concentration in μM. ■ = 25° C, ▲ = 31° C, and ● = 37° C. Best fit regression lines are indicated. M = metocurine, P = pancuronium, C = *d*TC, and G = gallamine.

Results

Twenty rats weighing 300 to 500 g were studied. Baseline tension was 12.2 g ± 4.5 (SD) at 37° C, increasing to 14.3 g ± 5.6 at 31° C and to 17.3 g ± 6.6 at 25° C. Because muscle tension changes with temperature, even in the absence of a drug,[2,13,14] twitch ratios always were calculated using tensions measured with drug (numerator) and without drug (denominator) at the same temperature. Figure 2 demonstrates typical dose-response curves: each panel shows data from one of the five diaphragms studied with the drug indicated. The r values for the least squares regression lines were all greater than 0.95.

Table 1 displays the mean ED_{50} values for the four drugs and three temperatures studied. For *d*TC, metocurine, and gallamine, the ED_{50} values are higher at decreased temperatures, and ED_{50} values at 25° C and 31° C are not different significantly at the 0.05 level. The ED_{50} values at 37° C, however, differ significantly from those at 25° C and 31° C: for each of these three drugs, the value at 37° C is lower than those at 25° C and 31° C. Pancuronium ED_{50} values, however, do not follow the same pattern. The ED_{50} at 31° C differs significantly from those at 25° C and 37° C, but this change in ED_{50} is less than 12% at 31° C *vs.* 37° C or 25° C. In contrast, the three other drug ED_{50} values vary nearly twofold at 25° C *vs.* 37° C. To verify the presence of an effect of temperature with some drugs but not others, we performed a two-way analysis of variance, testing for a drug-temperature interaction. It was highly significant ($P < 0.005$).

Table 2 shows the mean values of slopes of the dose-response curves for each of the four drugs at each of the three temperatures. There was no effect of temperature on the dose-response curve slopes. Accordingly, the slopes were pooled to yield a mean slope for each drug. However, the slopes differ significantly according to the drug studied. The mean slopes for each drug were tested for differences by the Newman-Keuls method. The slopes for metocurine and *d*TC were less steep than those of pancuronium and gallamine. Pan-

TABLE 1. Drug ED_{50} Values (μM) at Each Temperature. Each entry is the Mean ± SD for Five Measurements

Drug	25°C	31°C	37°C
*d*TC	0.52 ± 0.03	0.53 ± 0.07	0.36 ± 0.05‡
Pancuronium	1.33 ± 0.09	1.48 ± 0.09†	1.36 ± 0.05
Metocurine	0.23 ± 0.03	0.19 ± 0.01	0.11 ± 0.02*
Gallamine	93 ± 17	78 ± 13	53 ± 3.0*

* $P < 0.01$ for ED_{50} at 37°C different from ED_{50} at 25°C and $P < 0.05$ for ED_{50} at 37°C different from ED_{50} at 31°C.
† $P < 0.05$ for ED_{50} at 31°C different from ED_{50} at 25°C and 37°C.
‡ $P < 0.01$ for ED_{50} at 37°C different from ED_{50} at 25°C and $P < 0.01$ for ED_{50} at 37° different from ED_{50} at 31°C.

TABLE 2. Drug Dose-response Slope (μM^{-1}) for Each Temperature. Each Value is the Mean of Five Measurements

Drug	25° C	31° C	37° C	Mean ± SD*
*d*TC	−4.82	−5.06	−4.84	−4.91 ± 1.20†
Pancuronium	−6.76	−7.94	−6.88	−7.19 ± 1.37
Metocurine	−3.46	−3.16	−3.14	−3.25 ± 0.74‡
Gallamine	−6.76	−8.10	−7.54	−7.47 ± 2.14

* Mean ± standard deviation for all slopes (N = 15) for a given drug.

† $P < 0.05$ for mean slope different from those of pancuronium and gallamine.

‡ $P < 0.01$ for mean slope different from those of pancuronium and gallamine.

curonium and gallamine slopes did not differ from each other; also metocurine and *d*TC slopes did not differ from each other.

Discussion

This study in the rat showed increased ED_{50} values for *d*TC, metocurine, and gallamine at 25° C and 31° C *vs.* 37° C. In 1951, Holmes *et al.*[13] demonstrated in the rat diaphragm a doubling of ED_{50} for *d*TC as temperature was lowered from 40° C to 25° C, then a decreasing ED_{50} from 25° C to below 10° C. Twitch ratios have been studied in cats[2] and humans[1] in whom one leg was cooled. Colder limbs exhibited larger twitch ratios with *d*TC and smaller ratios with succinylcholine.[1] Effects of hypothermia on muscle blood flow and drug delivery were not measured. Our results with *d*TC are similar.

The present study demonstrated different temperature effects on *d*TC potency and pancuronium potency; the latter changed minimally at 31° C and was unchanged at 25° C *vs.* 37° C. Miller *et al.* also found different temperature effects on these drugs in the cat. Using constant infusions of *d*TC[4] or pancuronium,[7] they concluded that hypothermia augments the neuromuscular block from each drug. Subsequent investigations using serum assays for *d*TC[3] and pancuronium[6] allowed separation of these effects into pharmacokinetic and pharmacodynamic factors. After accounting for changes in drug serum concentrations, they concluded *d*TC potency was decreased by cold,[3] in agreement with our findings. In addition, pancuronium behaved differently from *d*TC in their studies and in ours. In their work,[6] pancuronium ED_{50} decreased at 29° C *vs.* 38° C, while we observed a minimal increase in ED_{50} at 31° C and no effect at 25° C *vs.* 37° C. This discrepancy in results has several possible explanations. First, the pancuronium assay used by Miller *et al.*[6] measures both unchanged drug and metabolites. Decreased metabolism of pancuronium at 29° C[6] would yield larger concentrations of active drug in the colder animals, and thus decreased measured ED_{50} values. However, change in metabolic rate cannot account entirely for the difference in ED_{50} at 38° C and 29° C in that study. Second, marked species variation in response to relaxants is well-known.[15] Miller's group studied the cat; we used rat muscle. Third, measurements were made at only discrete temperatures in each study. Perhaps competing processes with pancuronium generate a multiphasic temperature response; sampling at two or three temperatures might generate different responses depending on the temperatures selected.

Farrell *et al.*[5] studied mouse diaphragms at 24° C and 37° C. Both *d*TC and pancuronium ED_{50} values were decreased at lower temperatures. They used Kreb's solution, which contains twice the physiologic concentrations of ionized calcium and magnesium for mammals. Foldes[9] has pointed out that concentrations of these cations lower than those in Kreb's solution yield normal concentrations in a bathing solution that lacks protein binding of serum. Could temperature change be altering calcium- or magnesium-dependent processes to different extents based on ionized cation levels? This might account for the agreement of our results with physiologic preparations,[1-3] while those of Farrell *et al.*[5] differ. Alternatively, species difference could account for the discrepancy.

The basis for change in relaxant potency with hypothermia remains speculative. Explanations include sluggish muscle membrane depolarization, diminished cholinesterase activity, increased acetylcholine release, and decreased receptor affinity.[14] This investigation supports no particular theory. However, the distinctive effect of pancuronium suggests that further studies should explore any unique pharmacologic properties of this drug; for example, it is a cholinesterase substrate.[14]

This investigation is unique in comparing the actions of all four commonly used relaxants in the same model. Pancuronium retained potency at 25° C *vs.* 37° C, whereas *d*TC, metocurine, and gallamine all decreased potency. For each of the four drugs, the dose-response curve slopes were not changed by decreasing temperature. This agrees with the results of Holmes *et al.*[13] with *d*TC. The present study reveals more shallow dose-response curves for metocurine and for *d*TC compared with those of pancuronium and gallamine. We are not aware of other studies of metocurine in the rat. Whether this result is due to differences in receptor affinities (presynaptic, postsynaptic, or elsewhere), or other pharmacodynamic factors is not known and awaits further study.

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