AGE-DEPENDENT VARIATION IN RESPONSE TO TUBOCURARINE IN THE ISOLATED RAT DIAPHRAGM

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SUMMARY
The EC$_{50}$ of tubocurarine was determined in phrenic nerve–hemidiaphragm preparations obtained from 35 Sprague-Dawley rats aged 0-46 days. We measured also the ratio of the fourth to the first twitch in the train-of-four (T4:T1) when the first twitch of the train was depressed to 50% of control. The preparation was not unduly sensitive to tubocurarine at 0 days and there was little evidence of T4:T1 fade. However, by age 11 days the preparation exhibited fade and a three-fold sensitivity to tubocurarine similar to that in the human neonate. We conclude that the phrenic nerve–hemidiaphragm preparation from 11-day-old rats should be a suitable model in which to investigate the biochemical and electrophysiological basis of the sensitivity seen in humans.

KEY WORDS
Age factors. Neuromuscular relaxants: tubocurarine.

There is increasing clinical evidence that human neonates are sensitive to the effects of non-depolarizing neuromuscular blocking drugs compared with adults. In 1982, Fisher and colleagues found that the steady-state plasma concentration of tubocurarine required to produce 50% depression of twitch height in neonates was one-third of that required in adults [1]. Later, Meakin and co-workers [2] and Meretoja, Wirtavuori and Neuvonen [3] reported a similar degree of sensitivity to atracurium and vecuronium in neonates when dose was based on surface area. However, age-dependent variation in response to non-depolarizing neuromuscular blockers has not been demonstrated in an animal model. Accordingly, we were interested to determine the concentration-response relationship for tubocurarine in phrenic nerve–hemidiaphragm preparations of rats from birth to 46 days.

MATERIALS AND METHODS
Thirty-five Sprague–Dawley rats aged from a few hours to 46 days were killed with an overdose of ether. Immediately after death, the left hemidiaphragm and phrenic nerve were removed and mounted under tension in a 100-ml organ bath containing modified Krebs solution [4]. The solution was maintained at 37°C and aerated with a mixture of 5% carbon dioxide in oxygen. The phrenic nerve was aspirated into a suction electrode and stimulated supramaximally at 2 Hz for 2 s every 10 s using a Devices 3072 stimulator. A UFI force–displacement transducer was used to measure the resulting twitch tension of the muscle, which was recorded on a Grass model 79 polygraph.

After an initial period of 20 min during which the train-of-four response was allowed to stabilize, increments of a $5 \times 10^{-7}$ mol litre$^{-1}$ solution of tubocurarine were added to the organ bath to provide calculated concentrations in the range $1-8 \times 10^{-7}$ mol litre$^{-1}$. Depression of the first twitch of the train (T1), expressed as a percentage of the control, was recorded after 15–30 min at each new concentration. Individual concentration–response curves were constructed, from which the concentration required to produce 50% depression of T1 (EC$_{50}$) was estimated using the line of best fit. In addition, the height of the fourth twitch, expressed as a percentage of the first in each train (T4:T1) was measured when T1 was 50% of control. The results from all 35 preparations were plotted against age in days.

At the conclusion of 24 experiments, tubocurarine was removed from the organ bath by repeated washing with modified Krebs solution and stable values of T1 were recorded. Comparison of the mean of these values with that of the controls obtained before the addition of tubocurarine to the bath provided an indication of the stability of the preparation.

Statistical significance was determined using Student's $t$ test and the null hypothesis was rejected when $P < 0.05$. Data are expressed as mean (SEM).

RESULTS
The mean heights of T1 before and after 24 experiments were 32.4 (1.4) and 29.3 (3.2) mm, respectively (no significant difference).

From 0 to 7 days, the EC$_{50}$ decreased progressively, from 5.6 to $1.2 \times 10^{-7}$ mol litre$^{-1}$. It then increased to 21 days, after which it became relatively
FIG. 1. Variation in the EC$_{50}$ of tubocurarine with age in the rat phrenic nerve–hemidiaphragm preparation.

FIG. 2. Variation in the T4:T1 ratio (at T1 50% of control) with age in the rat phrenic nerve–hemidiaphragm preparation.

stable (fig. 1). The mean EC$_{50}$ from 10 experiments performed on or after 21 days was 7.2 (0.2) $\times 10^{-7}$ mol litre$^{-1}$.

From 0 to 8 days the T4:T1 ratio was virtually 100%. It then decreased progressively to 25 days, after which it became relatively stable (fig. 2). The mean T4:T1 ratio from seven experiments performed on or after 25 days was 15 (3)%.

**DISCUSSION**

Our results indicate that the diaphragm of the newborn rat was not unduly sensitive to tubocurarine compared with that of the adult. Similarly, there was little evidence of T4:T1 fade, which characterizes curariform block in human neonates. However, these responses change during early extrauterine life, and by 11 days the rat diaphragm preparation exhibited a degree of fade and sensitivity to tubocurarine similar to those seen in the human neonate [1–3]. The differences in response to tubocurarine between the newborn rat and the human neonate probably reflect differences in the degree of maturity.

The biphasic relationship between EC$_{50}$ and age described in figure 1 may be explained by a combination of a change in the acetylcholine receptor and an increase in the availability of neurotransmitter after birth. The nicotinic acetylcholine receptor in mammalian muscles may exist in two forms [5, 6]. The first type, which occurs predominantly in fetal muscle, is characterized by a mean open time of 6 ms and a conductance of 35 picosiemens (pS). The second type, which is found mainly in mature muscle, has a mean open time of 1.5 ms and a conductance of 50 pS. In the rat, the fraction of slow gating receptors decreases steadily from virtually 100% immediately after birth to less than 20% 3 weeks later [7]. Because of the longer open times, each quantum of acetylcholine which reacts with a slow gating receptor produces a larger endplate current than one which reacts with a fast gating receptor. This could increase the safety factor in neuromuscular transmission in the youngest rats [8, 9], thereby reducing the efficacy of tubocurarine. Thus the gradual appearance of tubocurarine sensitivity during the first week of life may be explained by the steady reduction in the numbers of slow gating receptors. The subsequent disappearance of this sensitivity may be the result of an increase in the availability of neurotransmitter in enlarging and maturing nerve terminals [10].

There is evidence that somatic motor nerve terminals possess prejunctional acetylcholine receptors which function in a positive feedback mechanism to mobilize acetylcholine stored within the nerve endings [11, 12]. When these receptors are blocked by a non-depolarizing neuromuscular blocking drug such as tubocurarine, the supply of acetylcholine no longer keeps up with demand during repeated nerve stimulation, and fade occurs. The relationship between T4:T1 fade and age shown in figure 2 suggests that, in the rat, this mechanism may not be fully functional until the second week of extrauterine life. Possible reasons for this include a low density of prejunctional acetylcholine receptors, or inadequate stores of acetylcholine in the neonatal motor nerve terminal.

In conclusion, we have shown that the isolated phrenic nerve–diaphragm preparation from the 11-day-old rat exhibits fade and a degree of sensitivity to tubocurarine similar to that seen in the human neonate. It should therefore be a suitable animal model in which to investigate the biochemical and electrophysiological basis of the sensitivity seen in humans.

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**REFERENCES**


