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Rate of onset and offset of neuromuscular block in the isolated rat diaphragm

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Introduction

Waud (1967) has suggested that the rate of action of tubocurarine applied directly to a motor endplate is limited by diffusion, although his conclusion is contrary to that reached earlier by del Castillo & Katz (1957). However, it is generally agreed that when tubocurarine is added to a solution bathing an isolated rat diaphragm the rate of onset and offset of neuromuscular blockade is likely to be limited by the rate of diffusion of the drug molecules to their site of action (Paton & Waud, 1964). Nevertheless, attempts to prove this have not been entirely successful (Holmes, Jenden & Taylor, 1951; Creese, Taylor & Tilton, 1959). It is also of interest that the rate of onset of blockade is commonly seen to increase with successive applications of the same concentration of tubocurarine (Chou, 1947; Godfrey, Mogey & Taylor, 1950; Holmes *et al.*, 1951). The effect is obvious even when the tissue is washed for prolonged periods between applications of tubocurarine (Fig. 1).

If the muscle is regarded as an infinite sheet of uniform thickness exposed on both sides to the drug solution at zero time then predicted curves can be constructed for the onset and offset of neuromuscular blockade by methods similar in principle to those used by Hill & Macpherson (1954). These curves (predicted on the basis of a diffusion model) can then be compared with those observed experimentally.

Methods

The rat phrenic nerve diaphragm preparation (Bulbring, 1946) was modified so as to take the form of a rectangular strip 1·5–2·0 cm wide. All the diaphragms used were from male Wistar albino rats (110–170 g). The rectangular form of the strip of diaphragm was maintained by tying the rib margin to the foot of a perspex holder and clipping the cut edge of tendon to a 2 cm length of fine glass tubing which also carried the upper platinum electrode for direct stimulation of the muscle. The muscle tension was recorded by means of an isometric transducer (Statham, Model G 10B-15-350) and U.V. recorder (S.E. Laboratories, Model 2005). Single maximal contractions in response to alternate direct and indirect stimulation were recorded at a total rate of four/min, the responses to direct stimulation acting as controls in the presence of a neuromuscular blocking agent (Fig. 1). Pulses applied to the phrenic nerve were of 0·1 ms duration at 10 V, and pulses applied directly to the muscle were of 0·5 ms duration at 100 V. All experiments were at 36 ± 0·5° C in Krebs solution (Krebs & Henseleit, 1932) thoroughly gassed with 95%

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O_2/5% CO_2. Blocking agents were added to the organ bath from a pipette and washed out by overflow.

The usual experimental procedure was to record the onset of an equilibrium partial blockade, then to determine a cumulative dose-response curve, and finally to record the offset of action of the same concentration of drug as had been used for onset. This procedure was begun 2·5 h after excision of the diaphragm and during this preliminary period the muscle had been treated with the neuromuscular blocking agent and allowed to recover completely after washout. The time required to measure equilibrium blockade at five concentrations (for a dose-response curve) was at least 1 h for gallamine, and 2 h for tubocurarine. When the offset of action of the drug had been recorded the thickness of the tissue was measured and the parameters for the diffusion of [^{14}C] sucrose through the muscle interspaces were determined by methods described in another paper (Brookes & Mackay, 1971). In order to estimate the values of the tortuosity factors predicted from the rates of onset and offset of neuromuscular block the diffusion coefficients of the tubocurarine

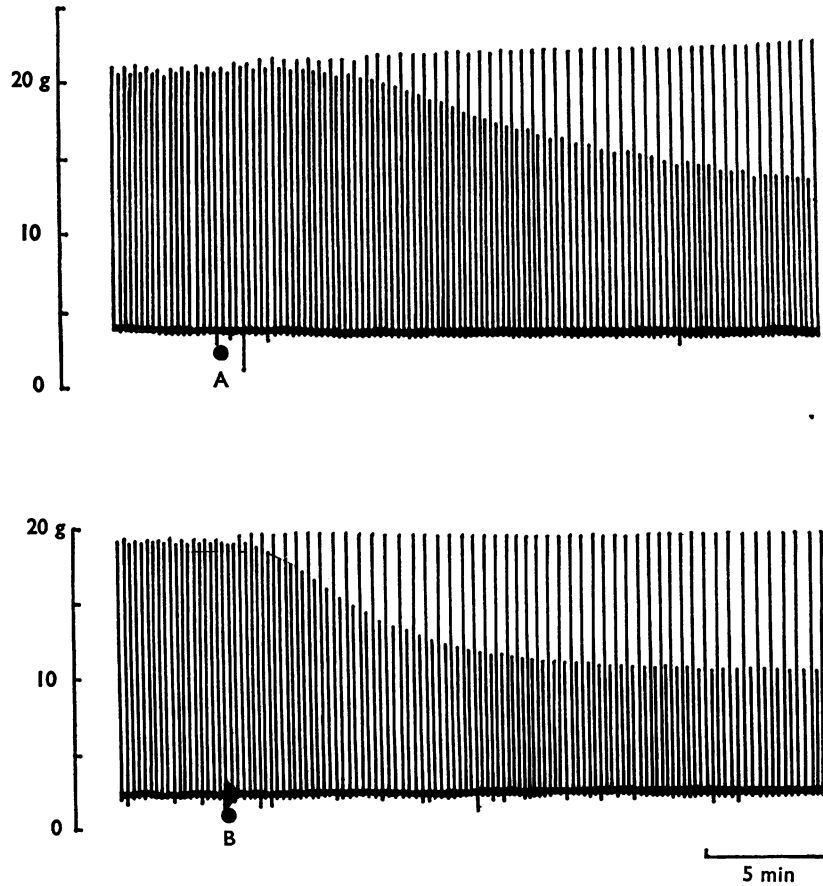

FIG. 1. Onsets of action of tubocurarine (0·75 μg/ml) applied to an isolated rat diaphragm 30 min (A) and 150 min (B) after dissection of the muscle. In each case the drug was allowed to act for 25 min and was then washed out of the tissue. The record shows maximal isometric contractions elicited by alternate direct and indirect stimuli applied to the diaphragm at a total rate of four/minute.

and gallamine cations in free solution at 36° C were taken to be $5{\cdot}06 \times 10^{-6}$ cm²/s and $5{\cdot}86 \times 10^{-6}$ cm²/s respectively (Brookes & Mackay, 1971).

Results and Discussion

The diaphragm is regarded here as an infinite plane sheet of thickness $2b$. The muscle fibres are assumed to act as impervious cylinders so that the diffusion path of the drug is lengthened by a factor k (the tortuosity factor). The solution of the diffusion equation for diffusion into or out of such a sheet, initially with uniform interstitial concentration C_0 and with both surfaces kept at a constant concentration C_1 (Crank, 1956), is:

$$\frac{C - C_o}{C_1 - C_o} = \frac{1-4}{\pi} \sum_{n=0}^{\infty} \frac{(-1)^n}{2n+1} \cos \frac{(2n+1)\pi x}{2b} \exp\left[-(2n+1)^2 \pi^2 \frac{D't}{4b^2}\right]$$

where $D' = D/k^2$ is the apparent 'non-steady state' diffusion coefficient of the substance in the diaphragm, and D is its diffusion coefficient in free solution. C is the interstitial concentration of the drug at position x and time t.

From the above equation the distribution of concentration of gallamine or tubocurarine within the diaphragm can be calculated for any chosen value of $D't/4b^2$. The experimentally determined dose-response curve was used to convert the distribution of concentration at any given time into a distribution of neuromuscular blockade at that time from which the corresponding overall degree of block was obtained by integration.

The mean tortuosity factor estimated from the onset of action of tubocurarine, 2·5 h to 3·0 h after dissection of the diaphragm, was $3{\cdot}1 \pm 0{\cdot}08$ (S.E. of mean of four results) and the mean value from the offset 3 h later was $2{\cdot}5 \pm 0{\cdot}07$. The mean value obtained by subsequently measuring the diffusion of [^{14}C] sucrose through the same diaphragms, beginning the diffusion experiment about 1 h after recording the offset of blockade by tubocurarine, was $2{\cdot}23 \pm 0{\cdot}18$. In all such experiments the fit of the observed and theoretical curves was as good as that shown in Fig. 2. The deviation between observed and predicted curves at the beginning of onset was found in all experiments and was not modified by previous equilibration of the

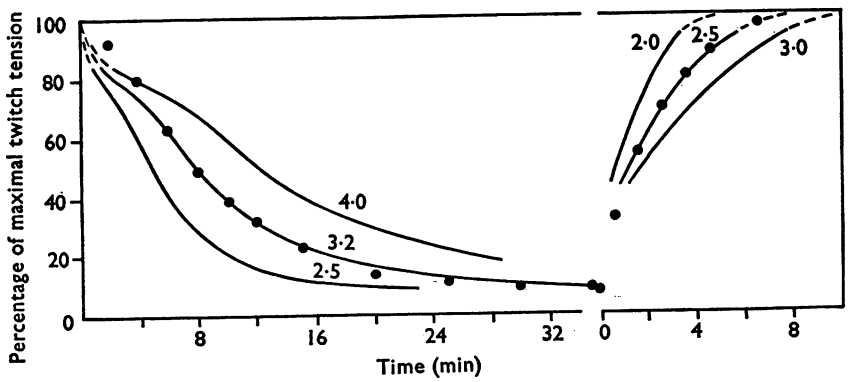

FIG. 2. Onset and offset of action of tubocurarine (0·9 μg/ml) applied to an isolated rat diaphragm. Filled circles show the experimental values of the percentage blockade. Full lines indicate values calculated on the basis of the diffusion model. The value of the tortuosity factor, k, used for each calculated curve is shown in the figure. The dashed section of the calculated curve indicates guessed extrapolation to zero blockade.

tissue with a concentration of tubocurarine (0·225 μg/ml) which was just less than that required to produce a detectable blockade.

Similar experiments were carried out using gallamine instead of tubocurarine. The onset of partial blockade by gallamine (150–200 μg/ml) was recorded 2–2·5 h after dissection of the diaphragm and offset was recorded about 1·5 h later. The dose-response curve obtained during the interval was corrected to allow for a slow progressive increase in the sensitivity of the tissue to gallamine during the experiment (Brookes, 1967). The tortuosity factors required to give the best fit between the predicted and observed curves were $2{\cdot}5 \pm 0{\cdot}11$ (S.E. of mean of four results) for onset and $2{\cdot}35 \pm 0{\cdot}10$ for offset. These are to be compared with the value $2{\cdot}73 \pm 0{\cdot}14$ for subsequent diffusion of [^{14}C] sucrose through the same diaphragms.

In all of these experiments there was an initial deviation between observed and predicted onset curves similar to that observed with tubocurarine. The fact that such an initial deviation was seen with both tubocurarine and gallamine (at quite different concentrations) and was not affected by previous equilibration with a sub-blocking concentration of tubocurarine, argues against it being the result of tissue binding of the drug.

The tortuosity factors required to obtain a good fit of the predicted and observed rates of onset and offset of blockade are in good agreement with those estimated from diffusion experiments with [^{14}C] sucrose through the same diaphragms. This agreement suggests that the diffusion model is basically correct. It seems likely that the initial onset of blockade is consistently slower than that predicted by the diffusion model (see Fig. 2) because of assumptions inherent in the model. For instance, the assumption that the region within which the neuromuscular junctions are distributed coincides exactly with the region across which diffusion occurs is likely to be a source of error. If it is assumed that the first layer of neuromuscular junctions occurs at a depth about 50 μm inside the diaphragm then the initial deviation between predicted and observed onset curves is very much reduced. Another factor which may contribute to such an initial deviation is the presence of unstirred films of solution at the surface of the diaphragm.

The results presented here show that in these experiments the onset and offset of neuromuscular blockade by tubocurarine and gallamine are diffusion controlled. Experiments with [^{14}C] sucrose have shown that the tortuosity factor for rat diaphragm decreases with time after excision of the muscle (Brookes & Mackay, 1971). It therefore seems likely that the increased rate of onset of blockade seen with successive applications of the same concentration of tubocurarine, is largely due to this decrease in tortuosity factor.

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REFERENCES

Brookes, N. (1967). Ph.D. thesis, University of Leeds.

Brookes, N. & Mackay, D. (1971). Diffusion of labelled substances through isolated rat diaphragm. *Br. J. Pharmac.*, **41**, 367–378.

Bulbring, E. (1946). Observations on the isolated phrenic nerve diaphragm preparation of the rat. *Br. J. Pharmac. Chemother.*, **1**, 38–61.

Castillo, J. del & Katz, B. (1957). A study of curare action with an electrical micro method. *Proc. R. Soc.* B, **146**, 339–356.

Chou, T. C. (1947). A method of estimating curare-like activity on the isolated phrenic nerve diaphragm preparation of the rat. *Br. J. Pharmac. Chemother.*, **2**, 1–7.

CRANK, J. (1956). *The Mathematics of Diffusion*, p. 45. Oxford: Clarendon Press.

CREESE, R., TAYLOR, D. B. & TILTON, B. (1959). In: *Curare and Curare-like Agents*, ed. Bovet, D., Bovet-Nitti, F. & Marini-Bettolo, G. B., p. 386. Amsterdam: Elsevier.

GODFREY, E. I., MOGEY, G. A. & TAYLOR, D. L. (1950). An automatic assay apparatus. *Br. J. Pharmac. Chemother.*, **5**, 381–388.

HILL, A. V. & MACPHERSON, L. (1954). The effect of nitrate, iodide and bromide on the duration of the active state in skeletal muscle. *Proc. R. Soc.*, B, **143**, 81–102.

HOLMES, P. E. B., JENDEN, D. J. & TAYLOR, D. B. (1951). The analysis of the mode of action of curare on neuromuscular transmission; the effect of temperature changes. *J. Pharmac. exp. Ther.*, **103**, 382–402.

KREBS, J. A. & HENSELEIT, K. (1932). Untersuchungen über die Harnstoffbildung im Tierkörper. *Hoppe-Seylers z. physiol. Chem.*, **210**, 33–66.

PATON, W. D. M. & WAUD, D. R. (1964). A quantitative investigation of the relationship between the rate of access of a drug to receptor and the rate of onset or offset of action. *Arch. exp. Path. Pharmak.*, **248**, 124–143.

WAUD, D. R. (1967). The rate of action of competitive neuromuscular blocking agents. *J. Pharmac. exp. Ther.*, **158**, 99–114.

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