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Diffusion of drugs through stationary water layers as the rate limiting process in their action at membrane receptors

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Summary

1. Preparations bathed in a well stirred solution have been considered as heterogeneous systems in which the solid phase is enveloped by a thin layer of stationary liquid. Any substance applied into the bulk solution must pass through this layer by diffusion before reaching the receptors.

2. The rate of diffusion through the stationary layer can govern the time course of the cellular responses to applied drugs provided that (i) all receptors involved in the response are situated at an equal distance from the solution and (ii) interaction with the receptor and consequent cellular events are very rapid.

3. These conditions have been verified for two responses: the contraction of guinea-pig ileum by acetylcholine (ACh), carbamylcholine (CCh), histamine and KCl, and the depolarization of the rat isolated sympathetic ganglion by ACh in the presence of eserine. A method of analysis has been applied which allows a complete dose-response curve to be obtained from only two responses.

4. Diffusion half-times measured for pieces of ileum were $4{\cdot}13 \pm 0{\cdot}13$ s (S.E. of mean) for ACh, $3{\cdot}60 \pm 0{\cdot}05$ s for CCh and $1{\cdot}01 \pm 0{\cdot}05$ s for KCl. The equivalent thickness of the stationary layer calculated from these values was respectively 93 μm, 87 μm and 70 μm. The average diffusion half-time for ACh in sympathetic ganglia was $14{\cdot}19 \pm 1{\cdot}05$ s. This gives an equivalent thickness of 173 μm.

5. Diffusion half-times were increased by increasing the viscosity of the bathing solution without changing the concentration response relationship.

6. The time course of contractions of guinea-pig ileum are no longer diffusion limited in the presence of a competitive antagonist or when the temperature is lowered from 35° to 25° C.

Introduction

Diffusion processes have long been known to influence the kinetics of chemical reactions occurring at interfaces in heterogeneous systems (Noyes & Whitney, 1897; Nernst, 1904). When a solid is placed in a well stirred liquid, a stationary layer of liquid will envelop the solid and substances interacting with the solid must first diffuse through this layer. Brunner (1904) measured the thickness of the stationary layer for simple physico-chemical systems and found values of 18 to 52 μm.

Isolated tissues bathed in salt solutions resemble the heterogeneous systems already referred to. It is known that stationary layers influence the movement of ions and water through artificial membranes (Ginzburg & Katchalski, 1963) and various epithelia (Dainty & House, 1966; Diamond, 1966). Moreover, macromolecular interfaces, such as cell membranes, are readily able to stabilize water into ordered hydration shells since the surface contains many atoms, such as N and O, capable of forming hydrogen bonds. The ordering in the hydration shells decreases with the distance from the interface.

Many drugs, particularly neurotransmitters and their congeners, act on receptors occurring on cell membranes (Cook, 1926; del Castillo & Katz, 1955) and must pass through the stationary layer by diffusion to reach the receptors. If the drug-receptor interaction is rapid, then diffusion can be the rate limiting process and this will determine the time course of the reaction and maybe the response too.

In preparations where the geometry of the diffusion pathways to the surface receptors is not too complicated, it is shown that it is possible to predict the actual concentration of drug existing at the receptors at any time after its addition to the bulk phase. The procedure allows a complete concentration-response relationship to be established with only two responses. The method has general applicability and is particularly valuable in instances in which dose-response relationships cannot be obtained with conventional steady state methods. Such instances might be where the experimental protocol is difficult (for example microelectrode recording) or where responses can only be obtained infrequently. The method does however require that equilibrium responses can be obtained.

Methods

Pieces of guinea-pig ileum or strips of ileal longitudinal muscle (Ambache, 1954) were mounted in Tyrode solution of the following composition: NaCl, 136 mM; KCl, 2·6 mM; NaH_2PO_4 0·32 mM; $CaCl_2$ 1·0 mM; $MgCl_2$ 0·5 mM; glucose, 5 mM; $NaHCO_3$, 12 mM and bubbled with air (pH 8·4). In a few experiments a higher $CaCl_2$ concentration (2·0 mM) was used to suppress spontaneous activity, and in others Krebs solution gassed with 95% oxygen and 5% carbon dioxide was employed. The temperature was usually maintained at 35° C. Tension responses were measured with an isometric strain gauge, employing an RCA 5734 mechano-electronic transducer, and a permanent record was obtained on a pen recorder. External electrodes were used to record the depolarization of isolated rat superior cervical ganglia as described previously (Dunant, 1967). In both types of experiment the volume of the bathing fluid was large (50 ml) compared with the tissue. Bathing fluids were gassed vigorously and uniformly during the experiments. Drugs were added to the bathing fluid by rapid injection of a small volume (0·5–1·0 ml). The response transients were analysed as described in the theory section. The relative viscosities of Ringer solution with and without added sucrose or " Ficoll " (Pharmacia, M.W.: ca. 400,000) were compared using an Ostwald viscometer.

Theory

When a drug is added to the solution bathing an isolated tissue then the genesis of the response occurs in three stages: (i) diffusion of the drug through the stationary

layer, (ii) interaction with the receptor and (iii) the cellular consequences of this interaction. Diffusion through a plane sheet is given by:

$$\delta C / \delta t = D \, \delta^2 C / \delta x^2 \quad \ldots\ldots (1)$$

where C refers to concentration, t to time, x to the distance normal to the plane of diffusion at $x=0$, and D to the diffusion coefficient of the drug.

Consider diffusion of a drug with an assumed diffusion constant D through an unstirred (stationary) layer at $0<x<l$. The plane (cell surfaces), $x=0$, is assumed to be impermeable and the uptake of drug on to receptors is assumed negligible, so there is no flux across $x=0$, that is the boundary condition is $\left(\delta C / \delta x\right)_{x=0} = 0$. At $t<0$, $C=0$ in both the layer and the bulk solution. At $t=0$ the bulk concentration, $x>l$, is raised to Co. The solution is identical with that of diffusion into a sheet of thickness $2l$ from a concentration Co on both sides. The solution is given by equation 1 (Crank (1956), pp. 45, 58).

If the time course of response to a drug is diffusion limited then it is possible to calculate the concentration of drug existing at the cell surfaces any time after addition of the drug and to relate concentration to effect. However, to do this it is necessary to make four assumptions.

(i) Only a minute fraction of the cell surface is covered by receptors (for references see Miller & Lewis, 1969) and the amount of drug immobilized by the receptors is small compared with the amount reaching the surface, so that equation 1 will be applicable.

(ii) The cellular processes are so rapid that the time course is determined only by diffusion. Certainly drug receptor interactions are rapid compared to diffusion processes, however this may not be true of the events following drug receptor interaction.

(iii) All receptors are situated at the interface of the preparation or, alternatively, the stationary layer has a constant thickness, l. Most measurements seem to show that tissues reduce the effective diffusion of drugs by a factor of 1·5 to 4·0, while the theoretical argument assumes $\left(\delta C / \delta x\right)_{x=0} = 0$. It may therefore be that the cell surface must be regarded as the barrier rather than the interface between the tissue and bathing fluid. If the cell surface is the barrier then rather special geometry would be required to make l the same for all receptors.

(iv) The diffusion coefficient for a drug is assumed to be constant. Since the ordering of water in various strata of the unstirred layer may be different the diffusion coefficient may be different in different strata.

There is no direct method to measure l which will be used as an operational parameter. We can work, however, with the dimensionless parameter Dt/l^2 by employing a graphical procedure similar to that of Diamond (1966).

Since l is small compared with dimensions of most preparations (for example ileum) it is appropriate to use solutions of equation 1 for diffusion through a plane

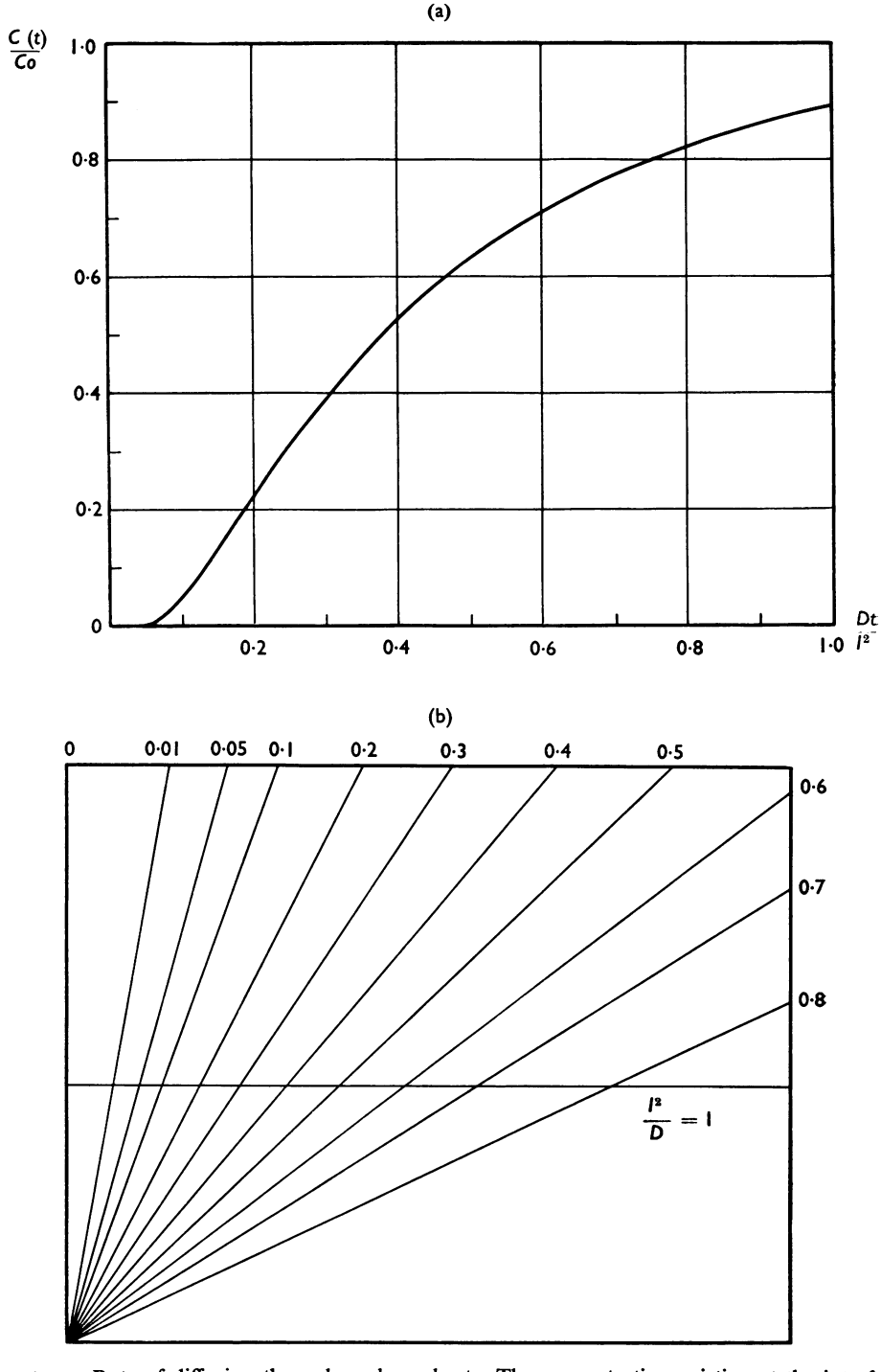

FIG. 1. a, Rate of diffusion through a plane sheet. The concentration existing at the interface at the time t, as a proportion of the concentration in the bulk solution ($C(t)/Co$) is plotted against the dimensionless parameter Dt/l^2. Values are drawn from the tables of Olson & Schultz (1942). b, Diagram showing different values of $C(t)/Co$ (oblique lines) for an infinite number of values of D/l^2. The horizontal line corresponds to a l^2/D ratio of 1. Dimension of the abscissa is time.

sheet. Numerical values of the relative concentration $C(t)/Co$ as a function of Dt/l^2 have been drawn from tables of Olson & Schultz (1942). $C(t)$ represents the drug concentration existing at time t at $x=0$. Figure 1a shows these relationships. Figure 1b consists of graphs of l^2/D against t for various values of $C(t)/Co$. For example from Fig. 1a when $C(t)/Co=0{\cdot}5$ we have $Dt/l^2=0{\cdot}38$. Therefore $l^2/D=2{\cdot}632\ t$ (seconds), which is the equation for the straight line plotted in Fig. 1b. It is seen that there must be a definite horizontal line corresponding to the correct l^2/D ratio for a particular experimental situation. This position can be discovered as follows. A record of a transient response to a supramaximal concentration of drug is obtained at a fast paper speed. In addition a submaximal response is recorded under equilibrium conditions. A horizontal line (AA′) is drawn on the transient response at the level of the submaximal equilibrium response. Fig. 1b, prepared on a piece of transparent paper, is placed over the transient response in such a way that the following criteria are achieved (see Fig. 2):

(i) The time of drug addition is made to correspond to zero on the time axis ($t=0$).

(ii) The lines representing the transient response, the level of the submaximal response and the correct concentration ratio $\frac{\text{Submaximal dose}}{\text{Supramaximal dose}}$ are made to intersect at one point (shown by the small circle in Fig. 2). At this point it is

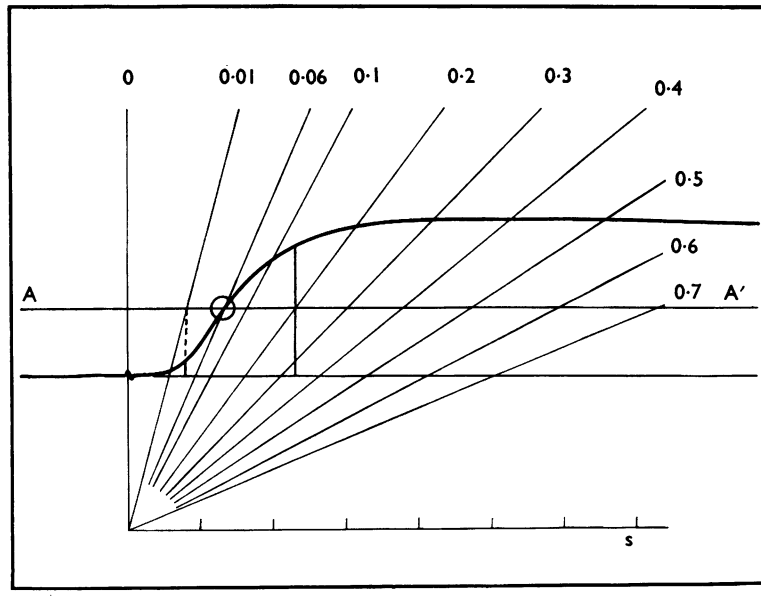

FIG. 2. Analysis of a transient record. The contraction of a piece of ileum was recorded as a function of time after addition of carbamylcholine (CCh, final concentration in the solution 10^{-5}M). An equilibrium response to 6×10^{-7}M CCh was also recorded. Its value is shown on the transient record by the horizontal line A—A′, intersecting the supramaximal transient response at a point marked by the circle. This concentration of CCh must also have been present at this interface at this time during the transient response. At this instant the ratio $C(t)/Co=0{\cdot}06$. When the transient was arranged with respect to Fig. 1b as described in the text, the time required to reach any other fractional concentration could be read off on the line A—A′. For example the time required for $C(t)/Co$ to be 0·5 (the diffusion half-time) was 4·7 s.

The responses corresponding to other concentrations were read off by measuring the height of the transient at the normal which intersects the line A—A′ at different values of $C(t)/Co$. In the figure the solid vertical lines represent responses corresponding to values of $C(t)/Co$ of 0·01 and 0·2 (in this example 10^{-7} and 2×10^{-6}M CCh).

assumed that the concentration of drug which prevailed at the receptors during the transient response was equal to that of the submaximal response. Knowing the time required for that particular concentration to be reached, one can easily read on the graph the times corresponding to other fractional concentrations. These times are given on the horizontal line (A—A′ on Fig. 2). The response to any concentration is read off by measuring the amplitude of the transient at the appropriate time. Thus a concentration-response curve can be built up, and, if the basic assumptions are true, it should be in good agreement with a relationship obtained by conventional methods.

The horizontal line intersects the line $C(t)/Co=0{\cdot}5$ at a time called the *diffusion half-time*. It is the time necessary for the substance to reach half of the final concentration at the interface, that is at the receptors. Again if basic assumptions are valid, the diffusion half-time should be constant for a given preparation.

Results

The tension responses of a piece of ileum have been expressed in Fig. 3a as a function of carbamylcholine (CCh) concentration. Four maximal transient responses and twelve submaximal equilibrium responses were recorded during this experiment in a random sequence. A concentration-tension relationship was calculated by the method described using a transient response to 10^{-5}M CCh and an equilibrium response to 4×10^{-7}M CCh. It is shown in the figure that good agreement was obtained between the derived relationship and that determined by conventional methods.

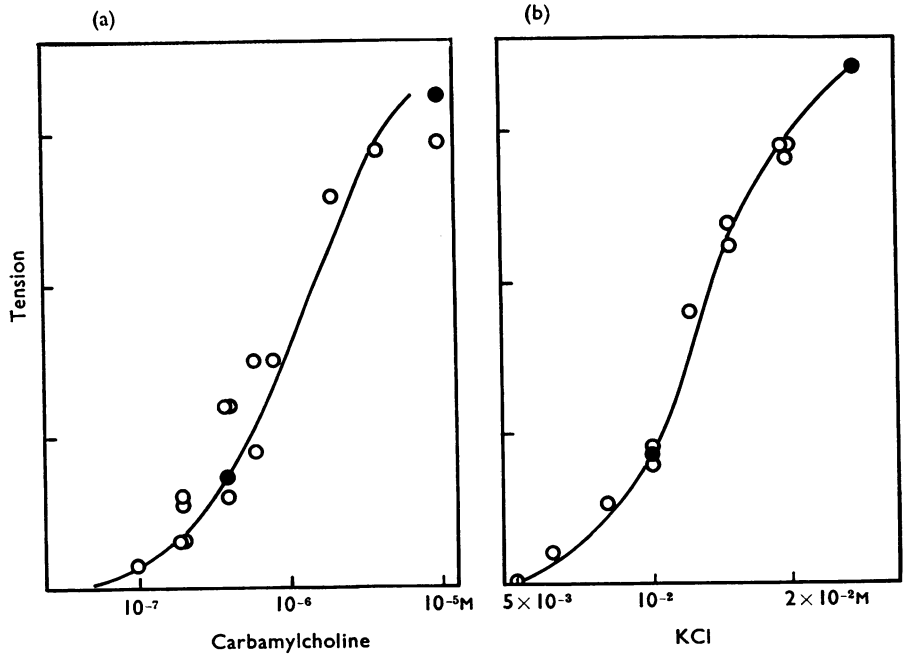

FIG. 3. Concentration-tension relationships for two different pieces of ileum for carbamylcholine (Fig. 3a, experiment No. 33) and for KCl (Fig. 3b, No. 32). The concentration of KCl does not include the 2·6 mM KCl present in the Tyrode solution. The curves represent the relationship obtained by analysis from the two responses shown by filled circles, while the open circles show the steady state responses not used in this analysis. Ordinates are changes in tension expressed in arbitrary units.

The diffusion half-time measured by this procedure was 3·2 s. Obviously other dose-response curves can be obtained by choosing any one of the four transient responses and any one of the twelve equilibrium responses. Thus forty-eight dose-response curves can be calculated each of them providing its diffusion half-time. This has been done and the forty-eight diffusion half-times are given in Table 1. The average diffusion half-time was 3·27 s (±0·07 S.E. of mean). Table 1 shows mean values obtained by considering each individual transient with all the equilibrium submaximal responses, and by considering responses to each submaximal CCh concentration with all four transient responses.

Statistical analysis showed that variance amongst the six means so determined is not bigger than the variance of all the individual values. (*F* distribution: $P>0{\cdot}05$ for vertical columns and for horizontal groups.) That is the dispersion arises mainly from individual values. This finding is important since, if you consider the different values obtained with a single transient and several equilibrium responses, any systematic deviation in the diffusion half-times would indicate a deviation of the derived from the steady state relationships. The analysis shows that the derived curves do not differ from the curve determined by the conventional manner.

Thus a complete dose-response relationship has been obtained by analysing two responses with a method based only on diffusion equations. This implies that the proposed assumptions are rather satisfactorily obeyed for this preparation. It would seem that diffusion through the stationary layer is the main rate limiting factor governing the development of tension in response to externally applied carbamylcholine.

Similarly, results have been obtained using guinea-pig ileum (or strips of longitudinal muscle) with other substances: acetylcholine, histamine, tetramethylammonium and KCl. Two examples are shown in Figs. 3b and 4b. The results are summarized in Table 2. In a given preparation the diffusion half-time was shorter for more rapidly diffusing drugs, as was to be expected. For instance in a preparation tested with CCh the diffusion half-time was 3·78 s, while KCl gave a diffusion

TABLE 1. *Distribution of diffusion half-times obtained from ileum No.* 33.

Equilibrium responses to carbamylcholine	Transient responses to carbamylcholine: 2×10^{-6}M	4×10^{-6}M	10^{-5}M	10^{-5}M	Means
10^{-7}M	3·4	3·0	3·2	2·7	3·08
2×10^{-7}M	3·0	2·9	3·2	2·8	3·26
	3·0	2·9	3·2	2·8	
	3·3	3·8	3·7	3·8	
	3·2	3·3	3·6	3·3	
4×10^{-7}M	3·3	4·2	4·0	3·8	3·37
	2·6	3·3	3·2	2·8	
	3·3	4·2	4·0	3·8	
	2·4	3·1	3·1	2·8	
6×10^{-7}M	2·1	3·0	3·0	2·8	3·23
	2·9	4·1	4·1	3·8	
8×10^{-7}M	2·4	3·4	3·6	3·4	3·20
Means	2·91	3·46	3·49	3·22	3·27±0·07 S.E. of mean

The dose response relationship of the same preparation is shown in Fig. 3a. Each number represents the diffusion half-time (in s) calculated by using one transient record with one submaximal equilibrium response.

half-time of 1·18 s. In general, diffusion half-times were more constant in different pieces of ileum rather than in different strips of longitudinal muscle. The average diffusion half-times for whole ileum were $4{\cdot}13 \pm 0{\cdot}13$ s (S.E. of mean) for ACh, $3{\cdot}60 \pm 0{\cdot}05$ s for CCh and $1{\cdot}01 \pm 0{\cdot}05$ s for KCl.

Role of viscosity

Since viscosity affects (i) the diffusion coefficient of the drug and (ii) the thickness of the stationary layer, it was to be expected that changes in the viscosity of the bathing medium would affect the diffusion half-time but not the dose-response relationship.

The viscosity of the Tyrode solution has been changed by adding sucrose (30 to 60 mM) or " Ficoll " (0·25 mM) (in **Methods**). Table 2 (below) shows diffusion half-times for CCh and KCl for preparations bathed in normal and more viscous media. When viscosity was increased the level of the responses was the same but the time-course was slower. Diffusion half-times were always significantly longer ($P<0{\cdot}0005$). The curves obtained in high viscosity solutions by transient analysis were identical with those obtained by conventional methods.

The increase in diffusion half-times caused by sucrose were large compared to the minor changes in viscosity. It is probable that this effect results mainly from an increase in l rather than a change in the diffusion constants. This effect was not apparent with Ficoll. No explanation can be provided for the sucrose effect.

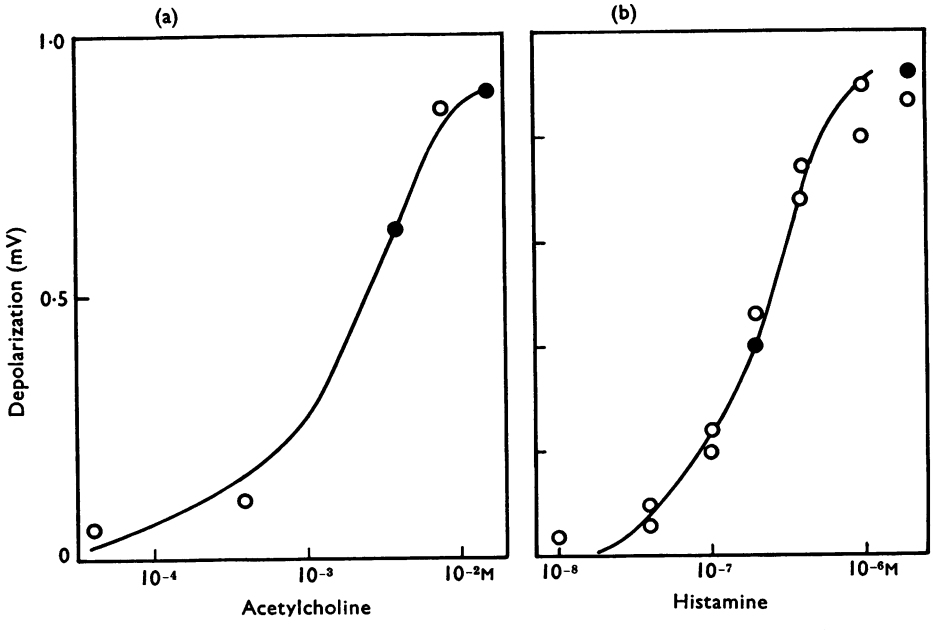

FIG. 4. a, Concentration-depolarization relationship for a denervated superior cervical ganglion of the rat by acetylcholine (experiment No. 228). b, Concentration-tension relationship of piece of whole ileum for histamine (experiment No. 8). Changes in tension are expressed in arbitrary units. In both graphs, the line is the dose-response relationship calculated by transient analysis using the two responses shown by solid circles. Open circles are equilibrium values of other responses.

TABLE 2. *Experiments on pieces of whole ileum and strips of longitudinal muscle performed with various drugs.*

Expt. number	Pieces of whole ileum. Acetylcholine		Carbamylcholine		Other drugs		
6	4·09±0·15	(18)			9·66±0·52	(27)	Histamine
7	4·02±0·28	(44)					
8	5·45±0·34	(24)					
10	4·26±0·25	(8)					
12	3·33±0·05	(12)					
13	3·83±0·32	(12)					
15	3·22±0·23	(15)					
32			3·78±0·03	(54)	1·18±0·04	(19)	KCl
33			3·27±0·07	(48)			
34			3·38±0·08	(30)	0·83±0·06	(18)	KCl
	Strips of longitudinal muscle						
9	3·08±0·12	(24)					
16	6·77±0·31	(9)					
18	5·89±0·66	(24)			3·36±0·24	(36)	TMA
19	4·23±0·11	(18)					
21	3·88±0·10	(8)					
22	5·40±0·22	(48)					
23	3·63±0·29	(3)					
24	6·92±0·18	(20)					
26	3·41±0·15	(10)					
17			5·21±0·19	(57)			
28			4·35±0·48	(4)			
31			2·33±0·09	(68)			
35					0·75±0·05	(20)	KCl
	Effects of viscosity changes	Relative viscosity					
33	Whole ileum. Tyrode	*1·00*	3·27±0·07	(48)			
	Tyrode + 30 mM—Sucrose	*1·02*	4·73±0·23	(12)			
	Tyrode + 0·25 mM—"Ficoll"	*4·65*	5·69±0·47	(10)			
31	Strip. Tyrode	*1·00*	2·33±0·09	(68)			
	Tyrode + 60 mM—Sucrose	*1·04*	6·89±0·70	(16)			
35	Strip. Tyrode	*1·00*			0·75±0·05	(20)	KCl
	Tyrode + 40 mM—Sucrose	*1·03*			1·10±0·07	(9)	KCl

The values are the diffusion half-times in s±S.E. of mean. The number of measurements are given in brackets.
Lowest section of the table: Diffusion half-times in s±S.E. are given for three preparations with and without a change in viscosity of the solution. Italic numbers are values of the relative viscosity as measured with an Ostwald viscometer. In each case, the increase in diffusion half-time is very significant ($P<0·0005$).

Depolarization of sympathetic ganglion

It is shown later in this paper that transient analysis fails to give the true dose-response relationship in conditions where the speed of the cellular events is rate-limiting. These difficulties might be overcome if some parameter, such as changes in permeability, conductance or potential, which are more direct consequences of the drug-receptor interaction, are recorded instead of a mechanical contraction.

Experiments have been performed on depolarization responses of isolated sympathetic ganglia by acetylcholine. An example of depolarization-concentration curve is given in Fig. 4a for a denervated ganglion. Again, agreement was found between derived and equilibrium relationships, but here the diffusion half-times were much longer than in guinea-pig ileum. Their average value in thirteen preparations was $14{\cdot}19 \pm 1{\cdot}05$ s (S.E.).

Diffusion in presence of a competitive antagonist

Transient analysis of responses to ACh or CCh was attempted with guinea-pig ileum and strips in the presence of increasing concentrations of a competitive antagonist. Diffusion half-times and dose-response relationships were found unchanged with very low concentrations of antagonist (10^{-11} or 10^{-13}M). Above this concentration the dose-response curve was displaced and the time course of responses was slowed. Apparent diffusion half-times suddenly became greater. Figure 5 illustrates such an experiment performed on a muscle strip with ACh and atropine. Transient analysis

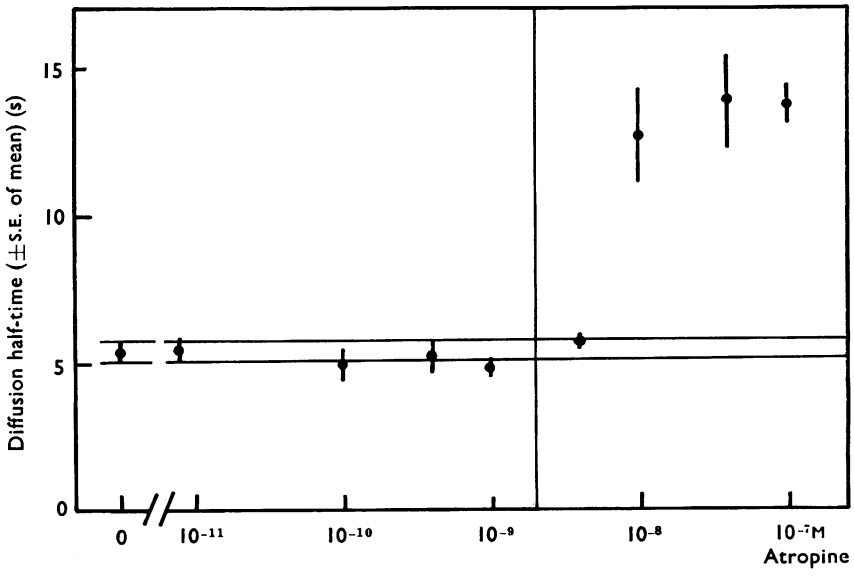

FIG. 5. Graph showing diffusion half-times for the responses of the strip of ileum (experiment No. 99) to acetylcholine (ACh) in the presence of increasing concentrations of atropine. Each point represents the mean of a number of determinations (from eight to forty-eight). The S.E. are shown. Horizontal lines indicate the S.E. of the mean response in the absence of atropine (forty-eight measurements). The vertical line is the apparent dissociation constant of atropine calculated for this experiment from the " dose-ratio " value (see Paton, 1961 ; that is the value of $\left[\frac{[\text{Atropine}]}{\text{Dose ratio of ACh}-1}\right]$.)

ceased to be valid at this stage since the apparent diffusion half-times were no longer constant above the critical concentration of antagonist ; that is, diffusion of antagonist through the stationary layer was no longer the main rate limiting stage in the response.

In every preparation, this critical concentration of antagonist corresponded to its apparent dissociation constant calculated by the dose-ratio method (Paton, 1961).

Conditions where transient analysis failed

It would be hazardous to extend this method of analysis to any preparation without care. For example, transient analysis gave satisfactory results on the guinea-pig ileum at 35° to 37° C. Diffusion is related to the absolute temperature, but the mechanical contraction itself is much more temperature dependent. The relationship is no longer held at 25° C, perhaps because the mechanical response became rate-limiting. We verified that derived and equilibrium relationships were not in agreement at that temperature for ileum muscle.

Contraction of the frog rectus abdominis muscle by ACh was also tested. Transient analysis based on the simple assumptions expressed above failed, especially with big muscles. The agreement between derived and equilibrium relations became less as the muscle thickness increased. Thus diffusion through the preparation towards deep receptors also determined the time course of the tension response.

ACh application to the dorsal muscle of the leech causes a contraction of very slow time course. In this muscle too, transient analysis was invalid. Figure 6 shows examples of the bad fit obtained when transient analysis was applied under unfavourable conditions.

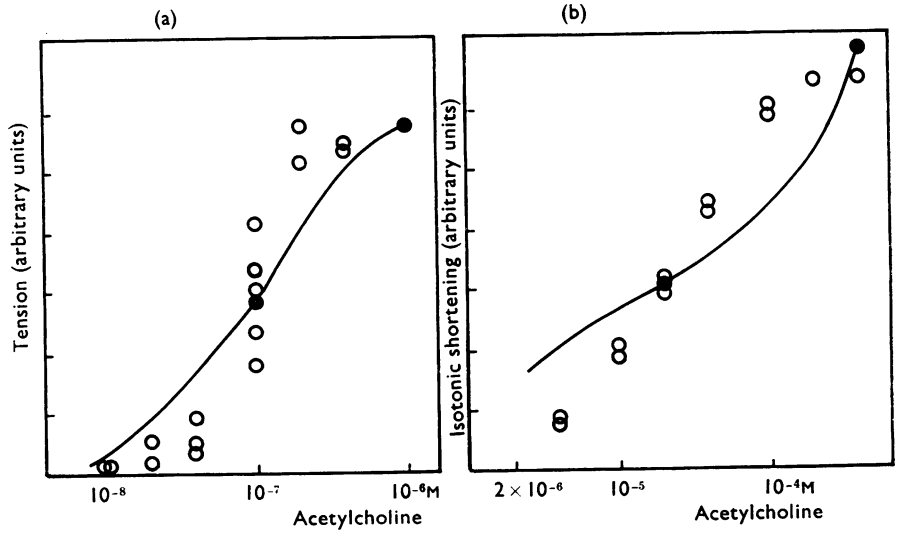

FIG. 6. Concentration-tension relationships for guinea-pig ileum at 28° C (a) and for frog rectus muscle at 20° C (b) to acetylcholine. The dots represent the experimental points and the lines represent the curves derived by transient analysis.

Discussion

Validity of the method

There have been a number of assumptions made in this work which now need justification. First it has been assumed that the mixing time of the drug in the bulk solution bathing the tissue was negligible. This is reasonable since when KCl was added to the bath it was found that the time required to record a conductance change across a pair of electrodes mounted in place of the tissue was always less than 0·05 s, even with solutions whose viscosity had been raised.

Second, the drug-receptor interaction has been regarded as being infinitely rapid, compared with the speed of diffusion. The life-time of the ACh-receptor complex is expected to be very short (Paton, 1961 ; Burgen, 1966) relative to the times measured in this work. A more prolonged interaction with antagonist could explain why transient analysis is no longer valid in atropinized ileum, since the time course of the response would be governed by both (i) diffusion of ACh towards the receptors and (ii) the rate of receptor liberation by dissociation of bound atropine.

As for the velocity of cellular events, Katz & Miledi (1965) were able to show that depolarization of the frog sartorius occurs in less than 150 μs after close application of ACh by microiontophoresis. Assuming that this time-order applies also to ileum and sympathetic ganglia then the change in membrane properties consequent to the drug-receptor interaction will not be rate limiting. In the guinea-pig ileum the coupling of mechanical events to membrane changes must be rapid at 37° C. The correspondence of the equilibrium and derived dose-response curve argues *a posteriori* for this, but it must be remembered that equilibrium and derived relations were in agreement only at temperatures of 35°–37° C.

Finally, all receptors were considered to be situated at an equal distance from the bulk solution, the length of the diffusion pathway being the same for all receptors in a given preparation. How far is that assumption valid ? The longitudinal muscle covers the ileum as a thin and regular network. After removal of the mesentery there is no obvious barrier to diffusion between solution and receptors other than the stationary layer. In addition the thickness of the muscle itself is very small. Thus all the receptors concerned in these experiments would occur practically at the interphase.

Whole ileum was found to be a more favourable preparation for transient analysis than strips of isolated longitudinal muscle. Strips always twist or fold after removal from the ileum, so that diffusion pathways may become more tortuous. Table 2 shows that diffusion half-times were longer and less constant in strips than in ileum.

The contraction of frog rectus muscle by applied ACh is an obvious example of a situation where receptors occur at various distances from the bulk phase, particularly for the thick muscles. Diffusion through the tissue and the existence of two different kinds of muscle fibres makes the analysis too complicated (see Hill, 1909).

The superior cervical ganglion of the rat is covered by a sheath of connective tissue. Diffusion through this can easily account for the prolonged half-times found in this preparation. Although all the ganglion cells are not situated at the surface of the preparation, external electrodes record only the potential changes occurring near the surface because of the electrical conditions in the deeper regions.

Nature and thickness of the stationary layer

It is not necessary to know the exact nature and thickness of the stationary layer for the method of analysis described in this paper, but it is of interest to have some idea about its *equivalent thickness*, that is the thickness of the stationary layer if it were an homogeneous sheet of solution similar to the bulk phase. The relation of the equivalent thickness, l, to the diffusion half-time, t, can be obtained from Fig. 1:

$$\text{When } \frac{C(t)}{Co} = 0{\cdot}5, \text{ then } \frac{Dt}{l^2} = 0{\cdot}38.$$

D is known for dilute solutions for KCl ($1{\cdot}84 \times 10^{-5}$cm^{2}s^{-1}) and for ACh (about 8×10^{-6}cm^{2}s^{-1}; Del Castillo & Katz, 1955). Diffusion coefficient of CCh has been taken as the same as that of ACh on molecular weight considerations.

Thus, the equivalent thickness, l, for ileum was calculated as 93 μm (from ACh data), 87 μm (CCh) and 70 μm (KCl). The average thickness in sympathetic ganglia was 173 μm (ACh). The differences found in different ganglia may be related to differences in connective sheath thickness, which usually measures about 100 μm.

These values agree with those obtained on physico-chemical systems (Brunner, 1904) and on other biological preparations (Diamond, 1966; Dainty & House, 1966). The latter authors measured the stationary layer on frog skin. They reported that it is reduced when the stirring of the solution was increased, but it remains to some extent even if the stirring rate is very high.

The experiments with antagonists have shown that, at concentrations of antagonist greater than that at which half the receptors are occupied (apparent Ke) then the time course of the response to ACh is no longer diffusion limited. As the concentration of atropine is raised, the apparent diffusion half-time increases suddenly at a critical level of concentration and then remains constant. One is reminded of the results Paton & Rang (1965) obtained on the uptake of labelled atropine by strips of guinea-pig ileum. They found no measurable uptake at low concentrations but at a concentration of about 10^{-9}M atropine the uptake increased rapidly. Just above this concentration neither the amount taken up nor the rate of uptake were increased by raising the concentration.

It should be mentioned that Paton and Waud (1964) developed an analytical method very similar to that used in the present work. They, however, considered only the diffusion of drugs through the tissue for studying the relationship between the rate of access to receptors and the rate of onset of action.

The increase in diffusion half-times found in solutions of increased viscosity provide further support for the view that the time course of the response is diffusion limited. Substances of high molecular weight like "Ficoll" increase greatly the viscosity of a solution as measured "macroscopically", but they are less effective in modifying diffusion velocity than smaller molecules such as sucrose (see Burgen, 1966). Since viscosity affects both the stationary layer and diffusion coefficients, these results fail to give precise information.

The aim of this present study was a better knowledge of a very widely used experimental condition. It has been shown that for a number of tissues and drugs the time course of responses is diffusion limited. Moreover, this analytical method provides a rapid and easy way to obtain concentration-response relationships which ~~of the response occurs in three stages: (i) diffusion of the drug through the stationary~~

→ see next

mental technique is difficult. It is necessary however always to show in the first instance that the derived relationship agrees with that obtained with equilibrium conditions.

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