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DRUG BLOCKADE OF OPEN END-PLATE CHANNELS

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SUMMARY

1. The actions of amylobarbitone, thiopentone, methohexitone and methyprylone at voltage-clamped frog end-plates were studied.

2. In the presence of barbiturates the conductance change evoked by an iontophoretic carbachol application was reduced by a prepulse of carbachol. The extra inhibition evoked by a prepulse disappeared exponentially with a time constant of 150–200 ms.

3. Barbiturates produce an increased rate of decay of nerve evoked end-plate currents. The concentration and voltage dependence of the barbiturate e.p.c. decay rates tally with the hypothesis that the increased rate of decay is due to block of active receptor-channel complexes by barbiturates with a rate constant of $\sim 10^6$ M^{-1} s^{-1}.

4. Conductance changes produced by bath applied agonists were depressed by thiopentone, the effect becoming greater the higher the agonist concentration. This effect, and also the observation that the concentration of thiopentone required to depress the bath agonist response is much greater than the apparent dissociation constant for binding to active receptor-channel complexes calculated from kinetic measurements, suggest that the selectivity for binding to open receptor-channel complexes is very high.

5. Methyprylone, which is structurally similar to the barbiturates, is only a weak antagonist and shows no interpulse interaction. It was predicted that methyprylone should produce fast and slow components in the e.p.c. decay, and this prediction was verified.

6. In the presence of barbiturates large iontophoretic carbachol applications produce conductance changes which show fast and slow components. Under these conditions the effects of carbachol prepulses become complex. However the effects are qualitatively consistent with the notion that different components of the response are contributed by channels located at various distances from the iontophoretic pipette tip.

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7. All the data agree with a model in which the channel has three states: closed, open and blocked. Only open channels can block, and blocked channels can only open.

INTRODUCTION

Quilliam (1955) observed that barbiturates would block the contraction of frog ileofibularis muscle evoked by bath application of acetylcholine without affecting that produced by nerve stimulation. Cash (1970) then demonstrated that the surface depolarization of toe muscle induced by acetylcholine was also blocked by barbiturates at concentrations that had no effect on neuromuscular transmission. These findings were extended to single end-plates using intracellular recording (Adams, Cash & Quilliam, 1970). Barbiturates abolished iontophoretic acetylcholine potentials with little effect on either end-plate potentials or miniatures. This observation has been confirmed by Magazanik (1971) and by Seyama & Narahashi (1975). However using higher barbiturate concentrations some modification in the time course of the end-plate potential was noticed (Adams, 1974*a*). This is primarily due to the increased rate of decay of the underlying end-plate current (e.p.c.) (Adams, 1974*b*; Seyama & Narahashi, 1975). A quantitative analysis of barbiturate action is presented below.

METHODS

The experiments were performed on superficial end-plates of sartorius muscles from *Rana temporaria* or *R. pipiens*. For most experiments the Ringer had the following composition (mM) : Na, 112; Cl, 118·1; K, 2·5; Ca, 1·8; Tris, 5; adjusted to pH 7·4 with HCl. In other experiments the Ringer composition was (mM) : Na, 113·5; K, 1·9; Cl, 114·1; HCO_3 2·4; H_2PO_4 0·064, Ca 1·2; pH $\sim$ 8·0.

The muscle bath had a facility for rapid exchange of Ringer, and in all except iontophoresis experiments the perfusion was continuous. In any case, end-plates were kept under voltage clamp before, during and following solution changes. The effects described appeared quickly ($\sim$ 1 min). End-plates were located by iontophoretic mapping or, for end-plate current (e.p.c.) experiments, by mapping the focal extracellular field. To study e.p.c.s neuromuscular transmission was usually blocked using 2–4 μM tubocurarine. However very similar results were obtained in preparations blocked with 8–12 mM magnesium ion.

The voltage clamp amplifier consisted of a Tektronix 1A7A differential preamplifier feeding a $\pm$ 10 V operational amplifier. Membrane potentials were recorded differentially between a bath electrode and the voltage micro-electrode. The bath was held at earth potential using a second bath electrode connected to a current operational amplifier. Clamp current was measured in this way for dose-response experiments. However for e.p.c.s or iontophoretic responses the current was measured as the voltage drop across a 1 MΩ resistor between the clamp amplifier output and the current micro-electrode with F.E.T. voltage followers. These components were mounted close to the current micro-electrode, and the connecting wire was not shielded, to avoid capacitative losses to earth. Voltage micro-electrodes were filled with potassium chloride solution and current electrodes with potassium sulphate, acetate or citrate. Both electrodes had resistances $<$ 5 MΩ. Twin micropipettes

were used for iontophoresis, both barrels being filled with 1 M carbachol. In the absence of barbiturate, the response to a pulse to the second barrel was not affected by a pulse to the first barrel, unless both responses overlapped.

The following drugs were used: sodium amylobarbitone (Lilly); sodium thiopentone (Abbott); sodium methohexitone (Lilly); methyprylone (Roche); carbachol chloride (Koch-Light); choline chloride (B.H.D.); tetramethylammonium bromide (TMA, B.D.H.); tuborcurarine chloride (Burroughs-Wellcome). The experiments were conducted at 18–22° C.

RESULTS

Fig. 1 shows the effect of 1 mM amylobarbitone on the e.p.c. recorded in a voltage clamped fibre. The main kinetic effect is an increased rate of decay of the e.p.c. In this case there was little change in the initial rate of rise, though sometimes the rate of rise was reduced. However this effect is probably accounted for by the prolongation of the terminal action potential (Adams, 1974*a*; Thomson & Turkanis, 1973). The observation that the main effect develops only following the onset of receptor activation recalls Steinbach's (1968*b*) suggestion that certain antagonists can bind to and block only activated receptors.

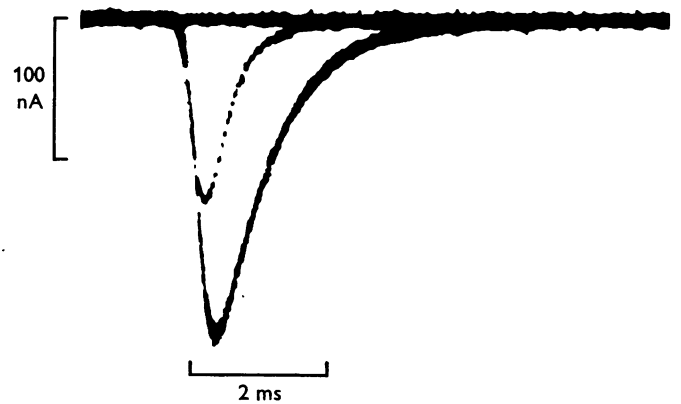

Fig. 1. Effect of amylobarbitone (1·2 mM) on the e.p.c. Neuromuscular transmission was blocked using 2 μM tubocurarine, and records in the absence and presence (smaller response) of amylobarbitone superimposed on a base line.

Furthermore such a mechanism readily accounts for the initial observation of the striking differential sensitivity to barbiturates of iontophoretically and neurally evoked acetylcholine depolarization. The iontophoretic response develops relatively slowly and involves repetitive activation of a local patch of receptors. Most of the receptors would become blocked during the rising phase of the response so that the peak is severely curtailed. Much higher concentrations are required to curtail the peak of

the e.p.c., since in this situation the receptors are activated almost synchronously and instantaneously. However in general it is very difficult to decide from electrophysiological data alone whether the barbiturates block activated receptors or open channels. Hence a more general formulation of the hypothesis is desirable. According to this the receptor-channel complex ('converter') has two states, inactive-closed (Q) and active-open (Q*). Barbiturates combine with Q* but not with Q, rendering the converter non-conducting. The first three sections below explore this hypothesis as a quantitative model of barbiturate action.

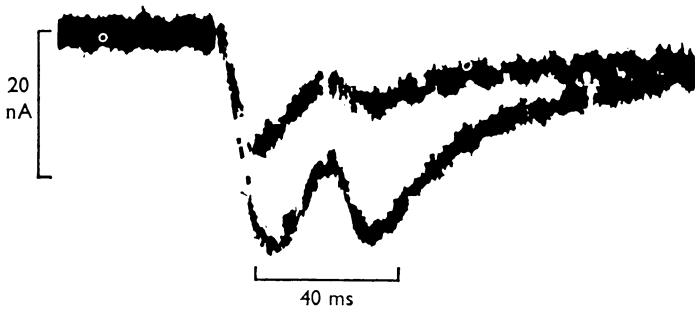

Fig. 2. Effect of amylobarbitone (80 μM) on double iontophoretic carbachol response. Two closely spaced equiactive carbachol pulses were applied to a voltage-clamped end-plate from separate barrels of a twin pipette. Superimposed on the control responses are the responses to the same pulses in the presence of amylobarbitone. Note that amylobarbitone depressed the second response more than the first.

Unblocking of converters

An iontophoretic pulse of carbachol will activate converters and allow barbiturate to block them. Therefore a second pulse of carbachol should elicit a smaller response if it follows very closely on the first, since fewer converters are available for activation. This prediction has been confirmed with amylobarbitone, thiopentone and methohexitone. To perform the experiment it was necessary to use twin carbachol pipettes so that a separate barrel could be used for each pulse. If two pulses are applied to a single barrel interaction effects occur which make the amplitude of the second response strongly dependent on the interpulse interval even in the controls, almost certainly due to a transient drug build-up in the pipette tip.

In Fig. 2 a double pulse of carbachol was applied to a voltage-clamped end-plate, and the expelling pulses adjusted to give equal responses. In the presence of amylobarbitone both responses were depressed, but the second more so than the first. This type of experiment can be used to measure the

rate of dissociation of blocker from converters, since as the interpulse interval is increased the converters have more time to unblock, and the second response is less depressed. This process can be represented $CQ^* \underset{b}{\rightarrow} Q$. It can be seen in Fig. 3 that as the interpulse interval is increased the depression of the second response does lessen, until finally a separation is

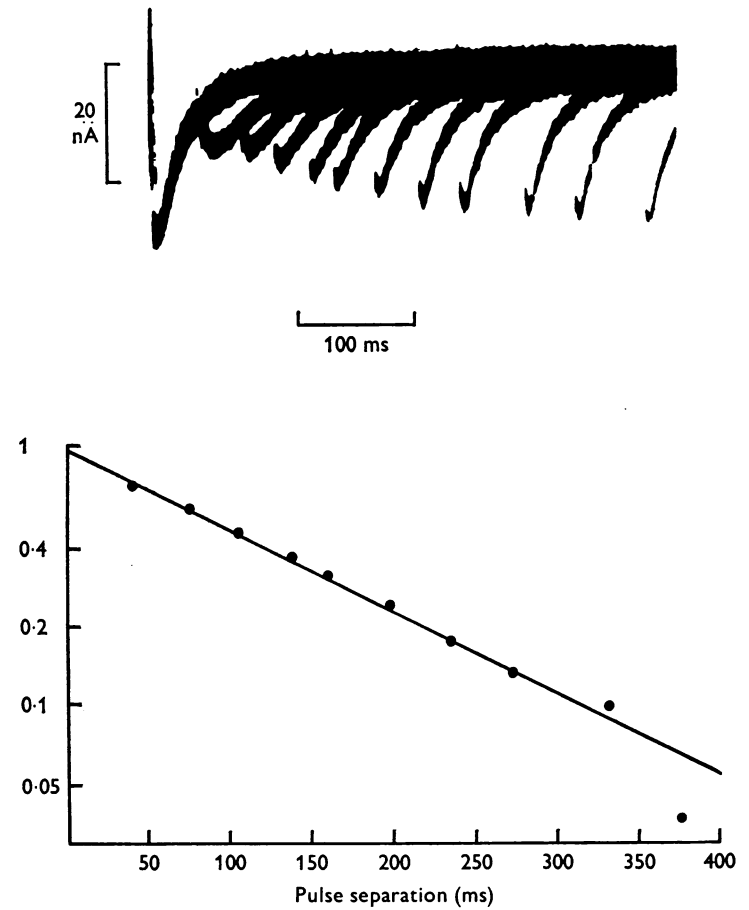

Fig. 3. Decay of interpulse interaction. The records were obtained by superimposing responses to a variably delayed carbachol pulse following a carbachol prepulse in the presence of 120 μM amylobarbitone. In the graph the fractional inhibition of the second response relative to its amplitude in the absence of a prepulse is plotted on a log scale against the interpulse delay. The straight line has a slope of 7·3 s^{-1}.

reached at which the presence or absence of the first pulse makes no difference to the amplitude of the second response. If I_∞ is the amplitude of the second response at long times (or in the absence of the first pulse) and I_t

the amplitude with a pulse separation t, then the fractional inhibition of the second response by the first, $(I_\infty - I_t)/I_\infty$, should decrease exponentially with a rate constant b. In semilog plots this appeared to be so (Fig. 3). These measurements were made for amylobarbitone, thiopentone and methohexitone and the data are presented in Table 1. The decay of the interpulse interaction was unaffected by membrane potential variation from -70 to -150 mV.

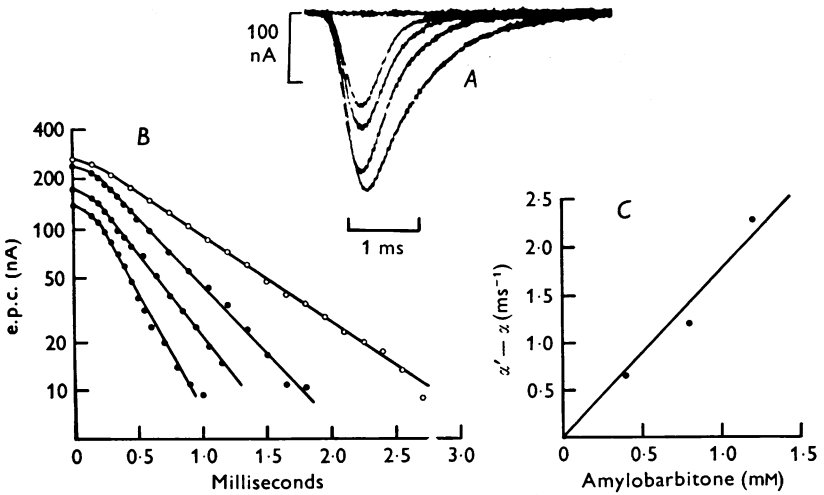

Fig. 4. Analysis of amylobarbitone action on the e.p.c. decay. *A*, superimposed e.p.c.s in the presence of various concentrations of amylobarbitone (0, 0·4, 0·8 and 1·2 mM). The preparation was blocked using 2 μM tubocurarine. *B*, semilog plots of e.p.c. decays shown in *A*. Open circles: controls (no amylobarbitone); filled circles: plus amylobarbitone. *C*, the difference of the control and test slopes in *B* is plotted against amylobarbitone concentration. The slope of the straight line is $1{\cdot}8 \times 10^6$ M^{-1} s^{-1}.

Blocking of converters

During the decay phase of the e.p.c. the converters turn off exponentially with a rate constant α (Magleby & Stevens, 1972*a*), which may reflect a receptor conformation change, the intrinsic channel closing rate, the dissociation of acetylcholine or its diffusion from the cleft, though this does not matter for the present argument. In the presence of barbiturate the converters can either turn off normally or become blocked so that the new rate constant for the e.p.c. decay, α', will be approximately $\alpha + cf$, where c is the barbiturate concentration and f the forward rate constant for reaction with open converters (see Discussion). Hence a comparison of e.p.c. tails in the presence and absence of barbiturate will yield cf. In Fig. 4 are shown e.p.c. tails in the presence of increasing concentrations of amylo-

barbitone, and these tails are then plotted semilogarithmically. As expected the plots are straight but the slopes are increased by barbiturate and the difference of the control and test slopes $\alpha'-\alpha$ yields cf, which is shown plotted against the amylobarbitone concentration (Fig. 4*c*). The relationship is approximately linear, suggesting a second order reaction, and f can be obtained from the slope of the line. In any one experiment it

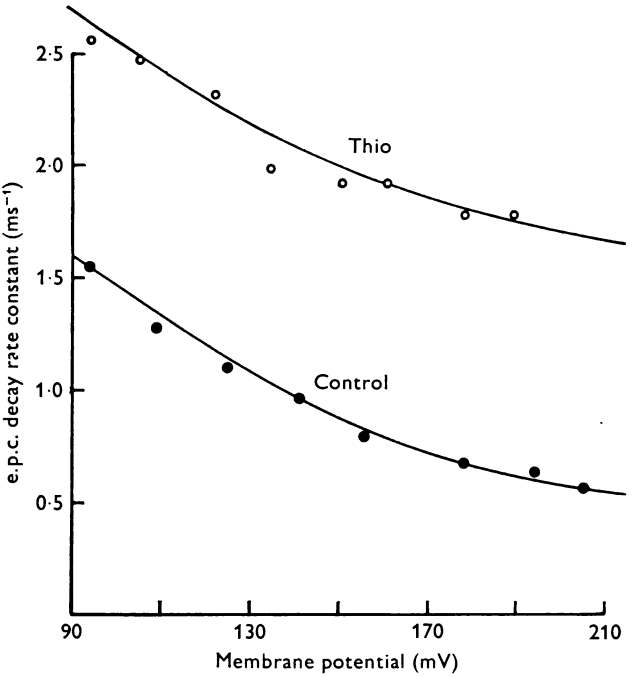

Fig. 5. Effect of thiopentone on voltage dependence of e.p.c. decay rate constants. Filled circles: controls; open circles: plus 0·76 mM thiopentone. The vertical displacement of the two continuous curves with this concentration corresponds to a second order rate constant of $1{\cdot}4 \times 10^6$ M^{-1} s^{-1}. Preparation blocked with 3 μM tubocurarine.

was not possible to obtain more than three points, so the linear relation was not entirely convincing. However comparing different experiments there was no over-all tendency for the points to curve up or down.

Hyperpolarization decreases α (Kordaš, 1969; Magleby & Stevens, 1972*a*). However the voltage sensitivity of the decay of e.p.c.s was decreased in the presence of barbiturates, since now many of the converters 'close' by being blocked, rather than following the voltage dependent path. Fig. 5 shows an experiment in which the voltage dependence of both α and α' were measured. The points are simply displaced in the presence of

thiopentone by an amount that should equal *cf*. Using the known concentration of thiopentone *f* can again be calculated.

Values of *f* obtained in experiments with amylobarbitone, thiopentone and methohexitone are given in Table. 1 These values are all about 10^6 M^{-1} s^{-1}. This similarity suggests that the structural variation between these molecules does not importantly influence *f*, which is probably mainly governed by diffusion of the barbiturate molecule to a rather inaccessible intrachannel site (see Discussion). It is also interesting to note that these values for *f* are very similar to that determined by Schwarz, Ulbricht & Wagner (1973) for tetrodotoxin binding to frog node. The great difference in the affinity of tetrodotoxin and barbiturates stems entirely from the much lower rate of dissociation in the former case.

TABLE 1. Kinetic parameters from e.p.c. and double pulse experiments. Under *f* are given the forward rate constants for blocking of open converters obtained from e.p.c. data as described in the text. Under *b* is given the reverse rate constant determined from double pulse iontophoresis experiments as described in the text. **b* for methyprylone is rate constant calculated as described in the text. All *f* experiments and some *b* experiments in Tris Ringer. Means ± S.E. Number of experiments in brackets. In analysing the methyprylone data curve stripping was not used, so the estimates are only approximate

Drug	*f* 10^6 M^{-1} s^{-1}	*b* s^{-1}	*b*/*f* μM
Thiopentone	1·4 (1)	6·03 ± 1·27 (3)	4·3
Amylobarbitone	1·00 ± 0·27 (4)	7·3 (1)	7·3
Methohexitone	1·2 (1)	4·72 ± 1·74 (3)	3·9
Methyprylone	2·0 (2)	*682 (2)	341

A method for determining *b* was described above. Hence it is possible to estimate K_c^*, the equilibrium constant for binding to the open converter, from the ratio *b*/*f*. Estimates for amylobarbitone, thiopentone and methohexitone are given in Table 1, and lie in the range 1–10 μM. Clearly the apparent binding affinity is quite high, and the very high concentrations needed to affect the e.p.c. amplitude arise because turn-on and turn-off of converters in this situation is very rapid compared with *b*.

The converter-barbiturate equilibrium

A number of experiments were performed using bath application of thiopentone (38–190 μM) combined with bath application of various agonists (carbachol, choline and TMA). One simple test of the mechanism postulated above was to compare the effectiveness of thiopentone as an antagonist of agonists of different efficacies adjusted in concentration to give the same response level, since if the responses are equal the converters

are activated to the same extent and hence the depression by an open converter blocker should be equal. In the experiment shown in Fig. 6 this was clearly the case, since the choline and TMA responses were equally depressed by thiopentone, while tubocurarine depressed the choline response less than the TMA response, presumably because the agonist *occupancy* is much less in the latter case. However this test does not distinguish the proposed mechanism from simple non-competitive antagonism, so it was necessary to perform dose–response experiments.

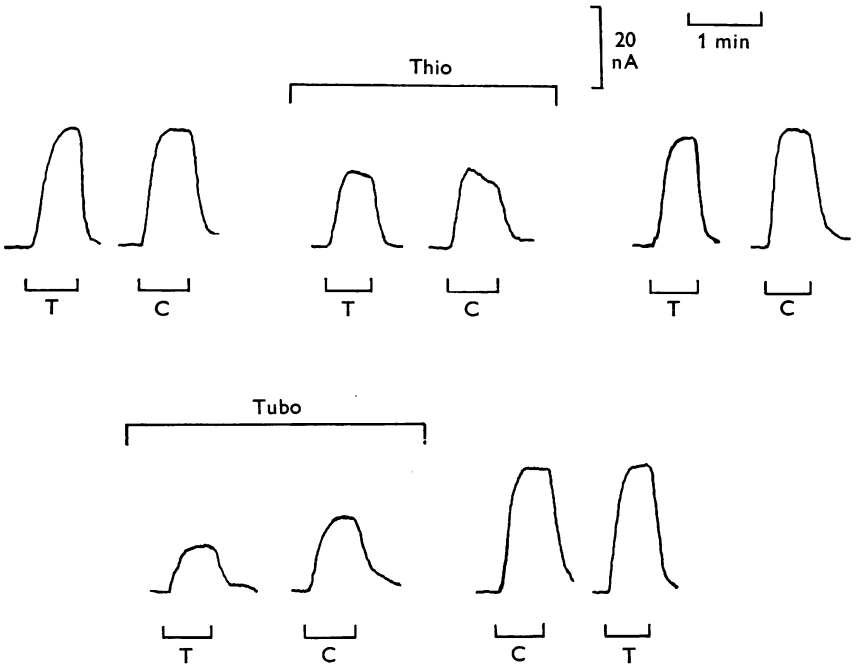

Fig. 6. Comparison of thiopentone and tubocurarine action on end-plate currents evoked by bath-applied TMA (T, 60 μM) and choline (C, 3·5 mM). The agonist concentrations were adjusted to give equal responses. Note that thiopentone (120 μM) depresses both responses equally whereas tubocurarine (0·5 μM) depresses the TMA response more than the choline response. Recoveries are also shown.

Suppose that an antagonist C can bind to and block both active and inactive converters at a site different from the agonist binding site. This might be represented

$$\begin{array}{ccc} C+Q & \overset{L}{\rightleftharpoons} & Q^*+C \\ \Big\uparrow\!\Big\downarrow K_c & & \Big\downarrow\!\Big\uparrow K_c^* \\ CQ & \rightleftharpoons & CQ^* \end{array} \qquad (1)$$

where the value of the equilibrium constant L is determined by the nature and concentration of the agonist. The fraction of active converters y is given, at equilibrium, by

$$y = \frac{1}{L(c+K_c)/K_c + (c+K_c^*)/K_c^*} \quad (2)$$

where c is the antagonist concentration. If the antagonist binds preferentially to inactive converters $K_c^* \gg K_c$ and the agonist dose–conductance curve will be more depressed at the foot than at the top. Qualitatively a similar pattern is also produced by a classical competitive antagonist,

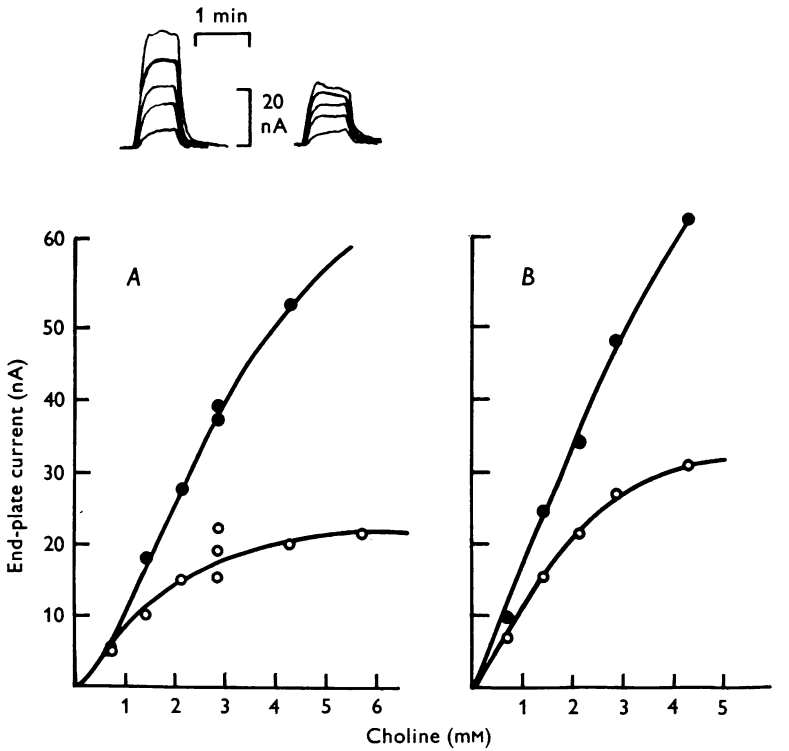

Fig. 7. Modification of the choline dose–response curve by thiopentone (80 μM). The records show superimposed tracings of responses to bath applications of various concentrations of choline in the absence (left) and the presence of thiopentone. The graphs show dose–response data from this experiment (A) and another (B). In each case the control curves are shown as filled symbols.

though the classical dose-ratio prediction will not be obtained since $(1/(L+1))$ is not a linear function of the agonist concentration (Rang, 1973; Jenkinson & Terrar, 1973; Adams, 1975*b*). If $K_c^* = K_c$ the antago-

nist is strictly non-competitive and the fractional depression of the dose–conductance curve depends on the antagonist but not the agonist concentration. If $K_c^* \ll K_c$, as is proposed for barbiturates, the antagonist will depress the dose–conductance curve much more near the top than near the foot.

In a series of seven experiments using thiopentone with choline, carbachol or TMA as agonist this prediction was qualitatively confirmed (Fig. 7). In these experiments the thiopentone concentration required to halve the equilibrium clamp current was much higher than the dissociation constant calculated as b/f. This was expected since the clamp current evoked by bath agonist application was always less than 250 nA, suggesting that only a very small fraction of the available converters were activated (Anderson & Stevens, 1973).

In two typical experiments with carbachol 75 μM thiopentone reduced $\sim$ 100 nA currents to 0·56 of their original value. Using a single channel conductance of 25 pMho and 10^7 converters per end-plate 100 nA corresponds to $L = 200$, so that the depression predicted from eqn. (2) assuming completely selective binding to open converters is only 0·92. However if the selectivity K_c/K_c^* ratio were 25 : 1 then the predicted depression becomes 0·56, so that the total selectivity for Q* assumed in other sections seems justified. It can be noted that eqn. (2) predicts that in the extreme limit $L \to \infty$, corresponding to very small agonist concentrations, non-competitive antagonism will be seen for finite selectivity. Non-competitive antagonism of carbachol depolarizations by barbiturates has been reported by Lee Son, Waud & Waud (1974). The potency sequence for various barbiturates as antagonists of iontophoretic carbachol conductance responses in frog muscle (barbitone < phenobarbitone < hexobarbitone < pentobarbitone < amylobarbitone $\sim$ thiopentone < methohexital; Adams, 1974*a*) is similar to their sequence obtained using guinea-pig muscle. Since Lee Son *et al.* did not use voltage clamp it is likely that their experiments were restricted to very high L values. If their ED_{50} measures K_c and b/f measures K_c^*, this supports the contention that $K_c^* \ll K_c$. The observation that high concentrations of barbiturates are required to depress miniature end-plate potentials (Adams, 1974*a*) or end-plate currents (Figs. 1, 4; Seyama & Narahashi, 1975) is in line with this view.

The effect of pH

Possible variation in the potency of amylobarbitone with changing pH was studied in three experiments. The potency of amylobarbitone was assessed by running a standard 80 μM solution through the bath while eliciting carbachol iontophoretic currents in a voltage-clamped end-plate (see Adams, 1975*c*). The amylobarbitone effect was calculated as the ratio of the average of the response amplitude before and after the exposure to the plateau amplitude during exposure. In a separate control experiment with thiopentone it was found that this quantity was approximately linearly related to the thiopentone concentration in the range of effects studied (Fig. 8). This procedure was then repeated in a Ringer of different pH (Tris buffer brought to the required pH with HCl). It was clear that the

effect of amylobarbitone was decreased by increasing the pH (Fig. 8). The results agreed with a curve calculated assuming that only the neutral species was active, and that the pK of amylobarbitone is 7·9 (Sober, 1973). In these experiments the action of amylobarbitone developed as rapidly as that of tubocurarine, although the recovery was somewhat slower.

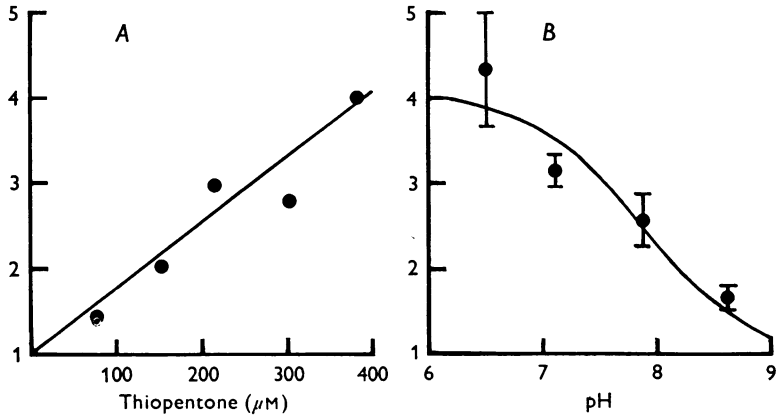

Fig. 8. Assessment of pH dependence of amylobarbitone action. In both graphs the ordinate shows the ratio of the amplitudes of iontophoretic carbachol currents elicited in voltage-clamped end-plates without and with barbiturate present in the bath. Thus 1 corresponds to zero barbiturate effect and numbers greater than one to a depression. The left graph shows the variation in this parameter with concentration of thiopentone (unfortunately similar data for amylobarbitone is not available). The experiment was performed in pH 8 Ringer (see Methods) and this explains the relatively low potency observed. The right graph shows collected results from three experiments (error bars give ± S.E. of means) in which the amylobarbitone effect was estimated in Ringer of varying pH. The continuous curve shows the relation expected if only the unionized form depresses the response, the maximum effect with all the amylobarbitone (80 μM) unionized set equal to 4 and the pK equal to 7·9.

Methyprylone

Methyprylone is closely related to the barbiturates (being barbitone with a ring nitrogen replaced by a methylated carbon) but was reported by Cash (1970) to be devoid of any selective antagonism of exogenous acetylcholine. Also it is a very weak antagonist of iontophoretically evoked carbachol conductance changes (Fig. 9). However it produced characteristic changes in the e.p.c. tail. The initial rate of decay was accelerated and when this effect was analysed in the usual way (Fig. 10) a forward rate constant similar to the barbiturates was obtained (Table 1). This suggests that the low potency of methyprylone is due primarily to an

increase in b rather than a decrease in f. This was confirmed by observing that there was little change in the time course of iontophoretic responses during methyprylone action (Fig. 9*A*), suggesting that equilibrium exists throughout such responses, and that there was no detectable interpulse interaction (Fig. 9*B*). It was therefore interesting to note that the e.p.c.

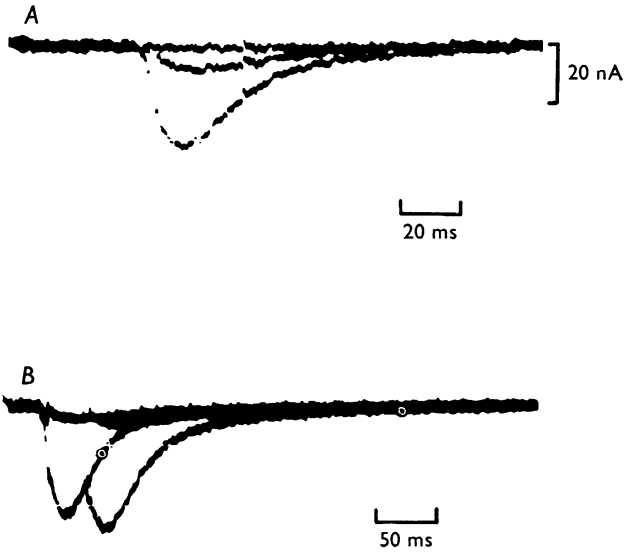

Fig. 9. Effects of methyprylone (2·7 mM) on carbachol iontophoretic responses. *A*, superimposed base line, control response and response in the presence of methyprylone (smaller response). *B*, four responses were superimposed: responses to a carbachol pulse with and without a carbachol prepulse in the presence and absence of methyprylone. The responses to the second carbachol pulse in the presence of methyprylone shows up as a thickened base line. However despite the short pulse separation and the great depression of the response in the presence of methyprylone, no additional effect of the prepulse could be discerned.

tail in the presence of methyprylone shows a second slow component (Fig. 10) which was slower than the decay of the e.p.c. before methyprylone treatment. Although the decay of the control e.p.c. was slowed by hyperpolarization (Kordaš, 1969) the slow component of the methyprylone e.p.c. tail always remained slower than the control, since it was also slowed by hyperpolarization (Fig. 11). It should be noted that although the methyprylone e.p.c. resembles that recorded in the presence of procaine (Kordaš, 1970; Maeno, Edwards & Hashimura, 1971; Deguchi & Narahashi, 1971; Adams, 1974*a*) hyperpolarization did not produce an increased initial rate of decay nor was there an anomalous decrease in the amplitude

of the slow component. Instead, the slow component amplitude increased with hyperpolarization rather more than did the peak amplitude.

A detailed analysis of these effects is given in the Discussion. However it can be noted here that the present model predicts a slow component in

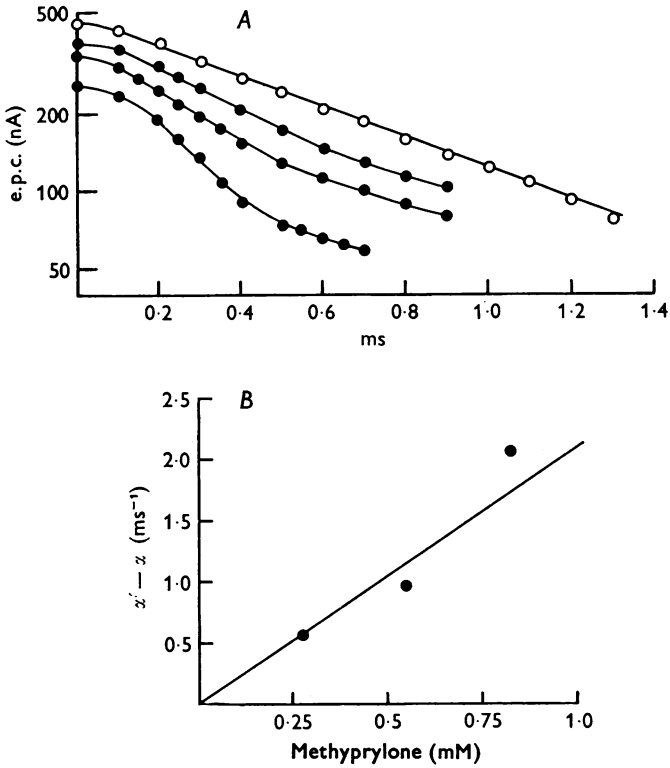

Fig. 10. The e.p.c. decay in the presence of methyprylone in a preparation blocked with 2 μM tubocurarine. *A* shows e.p.c. decays plotted semilogarithmically in the absence (open circles) and presence (filled circles) of various concentrations of methyprylone. *B* shows the slopes of the fast component of the e.p.c. tails plotted in *A* (after subtracting the slope of the control) as a function of methyprylone concentration. The slope of the line drawn is $2{\cdot}2 \times 10^6$ M^{-1} s^{-1}.

the e.p.c. tail in the case of a rapidly dissociating converter blocker. The transient appearance of open converters following dissociation of blocker accounts for the slow tail. In the case of the barbiturates the dissociation occurs so slowly that at any moment only a negligible number of converters are open.

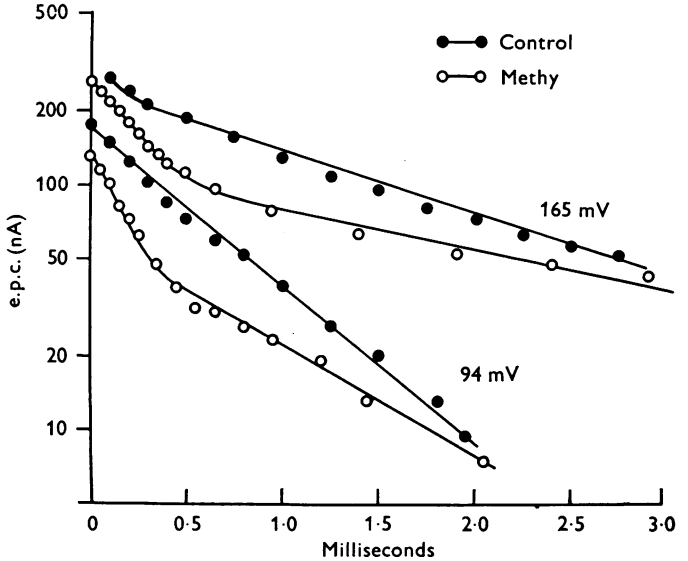

Fig. 11. Effect of membrane potential on methyprylone e.p.c. tails. Filled circles: controls (+4 μM tubocurarine); open circles: +methyprylone (0·8 mM; tubocurarine now 2 μM). The e.p.c.s were elicited at two different membrane potentials. Hyperpolarization slows the control decays and also the fast and slow components of the methyprylone e.p.c. decay. However the fast component remains faster than the control, and the slow component slower.

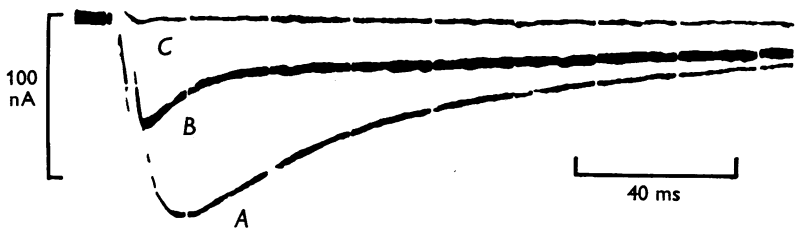

Fig. 12. Effect of amylobarbitone (120 μM) on response to a large iontophoretic dose of carbachol. *A*, control; *B*, amylobarbitone; *C*, base line.

Further experiments with iontophoresis

If a very large pulse of carbachol is applied in the absence of barbiturate, giving a peak response ~ 100 nA, then in the presence of amylobarbitone, thiopentone or methohexitone (Fig. 12) the iontophoretic response shows a fast and then a slow phase. In the presence of barbiturates low strength carbachol pulses evoke a simple response and as the dose is increased (Fig. 13) the peak of the response appears earlier and a slow tail emerges.

Furthermore the amplitude of the slow tail is increased more by hyperpolarization than is the amplitude of the peak response (Fig. 14) so that the amplitude of the slow tail may exceed that of the first fast peak, with a

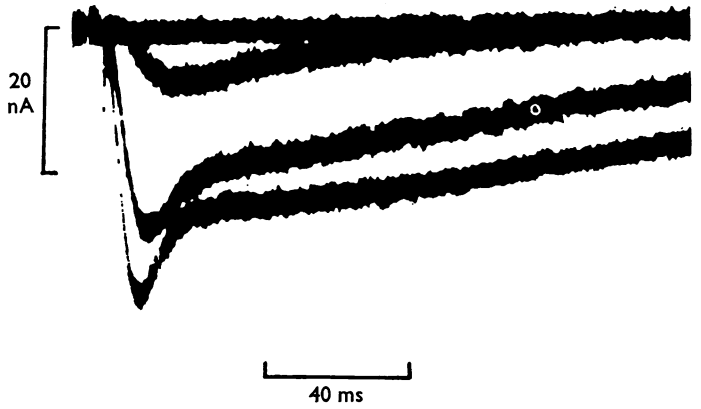

Fig. 13. Responses evoked by increasing iontophoretic doses of carbachol in the presence of amylobarbitone (120 μM).

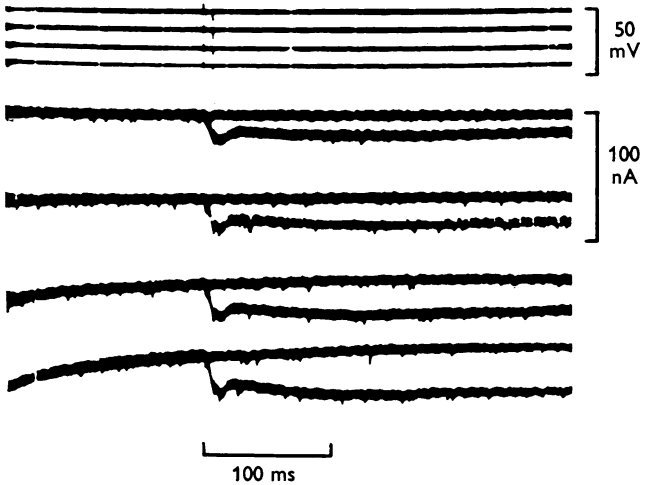

FIG. 14. Voltage dependence of iontophoretic carbachol responses in the presence of amylobarbitone (80 μM). Responses were elicited at the resting potential (upper traces) and during short hyperpolarizations. They are superimposed on base lines (clamp current in the absence of carbachol).

dip between. The end of the response in the presence of barbiturate may differ little from the end of the control response (Fig. 12). The effect of a prepulse is to reduce the amplitude of the peak component while the slow component remains constant or even increases (Fig. 15).

Unfortunately it is not possible yet to give a complete explanation of these effects. However the most likely cause is that agonist is continuing to act throughout the responses, and the net result stems from the interplay of kinetic factors and the complex diffusional geometry of iontophoretic drug application. Prolonged agonist action was suggested by the long time course of the control responses and also by the observation that

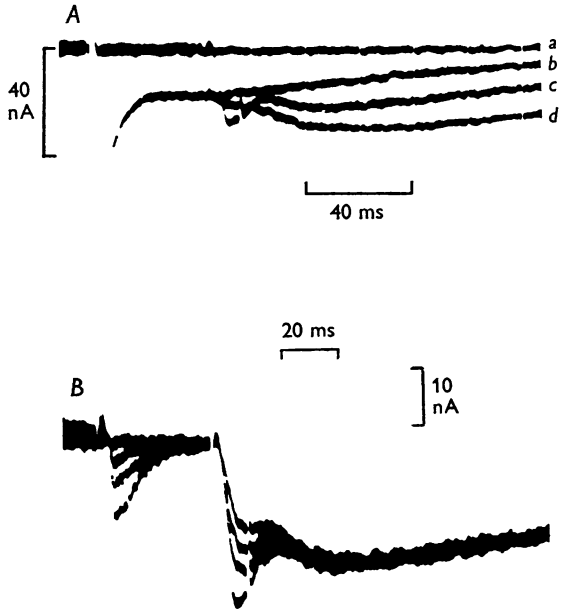

Fig. 15. Double pulse iontophoretic carbachol responses in the presence of amylobarbitone (120 μM). *A*, shows effect of a large prepulse on a large test pulse. *a*, base line; *b*, prepulse alone; *c*, test pulse alone; *d*, prepulse + test pulse. *B*, shows responses to a constant dose test pulse with increasing strength prepulses.

the interpulse interaction decayed more slowly following a large prepulse than following a small prepulse (Fig. 16). Continued agonist action during the interpulse period would slow recovery since channels that unblock may reblock and other channels may open and then block, so that a simple exponential recovery, with rate constant *b*, will no longer be observed.

Qualitatively the situation might be as follows. The first peak arises because carbachol reaches the receptors under the pipette tip very quickly, and the almost synchronous activation of converters produced a response before the antagonist has had time to act. This is why the prepulse reduces the fast component of the second response. The carbachol concentration at greater distances does not reach such high levels, and is much

slower in reaching a peak, and here the barbiturate-converter interaction can be regarded as almost an equilibrium situation. As the concentration at a near receptive area rises the response rapidly reaches its maximum, since at equilibrium the maximum response is achieved with lower concentrations in the presence of barbiturate (see Fig. 7). The concentration at an area a little farther out rises more slowly, and the same maximum is reached a little later, at a time when the contribution of the first area is beginning to fall. Thus as successive waves of area are activated the

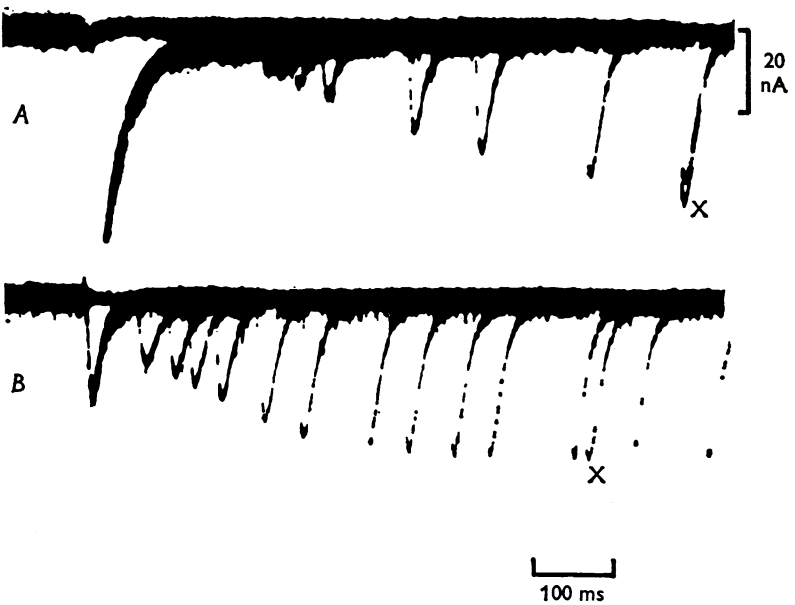

Fig. 16. Decay of carbachol interpulse interaction in the presence of methohexitone (70 μM) using large (*A*) and small (*B*) prepulses. The responses marked X were elicited without any prepulse.

over-all response remains relatively constant (observe the flat traces in Fig. 13). Finally at the end of the response only very distant areas are being activated.. These are subject to a very low agonist concentration (though spread over a wide area) and the response is very little inhibited by barbiturate, since one is at the foot of the dose–response curve. The relative constancy of the hump in the presence of variable prepulses (Fig. 15) occurs because the hump involves membrane which is not activated, and therefore not blocked, by the prepulse. The effect of hyperpolarization on the hump amplitude would then be a reflexion of the equilibrium voltage dependence of carbachol responses (Rang, 1973; Dionne & Stevens, 1975;

Adams, 1974*a*, 1976). The relative lack of effect on the initial peak amplitude arises because reducing α increases the probability of block by barbiturate.

DISCUSSION

The barbiturate end-plate current

In the analysis presented above it was assumed that barbiturates block open but not closed converters. The following scheme represents events postulated to occur during a barbiturate e.p.c., assuming the dissociation of barbiturate to be negligibly slow (A = acetylcholine).

$$\begin{matrix}\text{release}\searrow \\ \text{removal}\swarrow\end{matrix} \quad A + Q \overset{K}{\rightleftharpoons} AQ \underset{\alpha}{\overset{\beta}{\rightleftharpoons}} AQ^* \xrightarrow{f.c} CAQ^*. \qquad (3)$$

It is assumed that the time course of [AQ] can be reasonably represented by a double exponential driving function (see Magleby & Stevens, 1972*b*, Fig. 4). It is further assumed that only a negligible fraction of the total of end-plate channels are opened during an e.p.c. This is likely because there is very little interaction between successive barbiturate e.p.c.s (Adams, 1974*b*). Then $d[AQ^*]/dt + (f.c + \alpha)[AQ^*] = \beta B[\exp(-pt) - \exp(-\omega t)]$ where B, p and ω are constants used to describe the time course of [AQ]. Integrating this equation one obtains

$$[AQ^*] = \beta B[r.\exp(-pt) - s.\exp(-\omega t) + (p-\omega)(r.s)\exp(-(f.c+\alpha)t)] \quad (4)$$

where $r = 1/(f.c+\alpha-p)$, $s = 1/(f.c+\alpha-\omega)$.

As the barbiturate concentration becomes very high the e.p.c. will approach in form the driving function. The analysis of the barbiturate e.p.c. presented in the Results in effect assumes that the release function is exceedingly rapid. This admittedly crude model shows clearly that as the time course of [AQ] is prolonged by eliminating acetylcholine hydrolysis the e.p.c. tail should not remain simply exponential. With $\alpha > p$ the e.p.c. tail should show an early fast component, which is not seen (Magleby & Stevens, 1972*b*; Gage & McBurney, 1975). A simple way out of this difficulty is to suppose that even in the absence of cholinesterase inhibitors $\alpha > p$. However then barbiturates could not produce their characteristic effect on the e.p.c.

Receptor or channel?

The experiments reported above lend strong support to the notion that the barbiturates employed block active but not inactive converters. However none of these experiments allows one to decide whether the block is of converters in their receptor or channel aspect. However the second alternative is more attractive. Firstly, simple occlusion of the channel offers a realistic physical picture of the blocking action. An action via the receptor would presumably have to be mediated by an 'allosteric' effect. Secondly, barbiturates are known to block other conductance systems (lobster axon: Blaustein, 1968; frog muscle resting conductance: Thesleff,

1956). Thirdly, barbiturates specifically block chemically operated cation-selective conductance systems irrespective of transmitter but not anion-selective systems (Barker & Gainer, 1973; Barker, 1975). Fourthly, and perhaps most important, procaine seems to act very like methyprylone (Kordaš, 1970; Deguchi & Narahashi, 1971; Adams, 1975*a*) with the additional feature of voltage-dependent binding rate constants. This suggests that the binding site is within the membrane electric field, and therefore probably within the channel. In the remainder of this Discussion it will be assumed that barbiturates block open but not closed end-plate channels. Similar ideas in relation to axon have been developed by Armstrong (1971), Woodhull (1973) and Strichartz (1973). Armstrong's (1971) analysis was found of particular value.

Gage, McBurney & Schneider (1975) and Gage & Hamill (1975) have recently persuasively argued that the changes in m.e.p.c. decay produced by alcohols and volatile anaesthetics are due to a change in the physical environment (dielectric constant or viscosity) of the converter. This explanation is unlikely to apply to the shortening of the e.p.c. by barbiturates, since it does not explain the iontophoretic interpulse interaction, the modification of the dose-response curve or the differential effect on iontophoretic and nerve-evoked potentials. Furthermore it seems unlikely to apply to the diphasic e.p.c. tails produced by methyprylone or hexanol, since such a mechanism could produce either a lengthening or a shortening, but not both.

Methyprylone

The following reaction scheme shows events postulated to occur during the methyprylone e.p.c. tail, assuming an impulse function for [AQ] (X = closed channel; X* = open channel; CX* = blocked channel)

$$\begin{array}{l} \mathrm{X}^* \underset{b}{\overset{f.c}{\rightleftharpoons}} \mathrm{CX}^*. \\ {\scriptstyle\alpha}\downarrow \\ \mathrm{X} \end{array} \qquad (5)$$

The slow tail arises from the repetitive blocking-unblocking cycles that a channel can undergo before finally closing. The equation for X* is $[\mathrm{X}^*]'' + (f.c+b+\alpha)[\mathrm{X}^*]' + \alpha b[\mathrm{X}^*] = 0$ and using as initial conditions $[\mathrm{X}^*]_0$ and $[\mathrm{CX}^*]_0 = 0$ the solution is $(r_1 \neq r_2)$

$$[\mathrm{X}^*] = \frac{[\mathrm{X}^*]_0}{r_2 - r_1}\left[(r_2 + f.c + \alpha)\exp(r_1 t) - (r_1 + f.c + \alpha)\exp(r_2 t)\right]$$

with

$$r_1, r_2 = \left[-(f.c+\alpha+b) \pm \sqrt{\{(f.c+\alpha+b)^2 - 4\alpha b\}}\right]/2. \qquad (6)$$

Experimentally r_1 and r_2, corresponding respectively to the positive and negative signs before the radical in equation (6), show up as the rate constants for the decay of the slow and fast components of the e.p.c. tail. If $r_1 \ll r_2$ then $f \approx (r_2+\alpha)/c$. The value of b can then be calculated using

the relation $b = -r_1 (f.c+\alpha+r_1)/(r_1+\alpha)$ (Table 1). The voltage dependence of the methyprylone e.p.c. tail then arises in a natural way from the voltage dependence of α (Magleby & Stevens, 1972*b*) which appears in all the expressions for tail amplitudes and decay constants. It is not necessary to suppose that any of the barbiturate or methyprylone binding reactions are strongly voltage dependent. This agrees with the finding that the neutral molecule is the blocking species.

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