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THE ACTION OF GANGLIONIC BLOCKING DRUGS ON THE SYNAPTIC RESPONSES OF RAT SUBMANDIBULAR GANGLION CELLS

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1 The effects of tubocurarine, hexamethonium and trimetaphan on the synaptic currents of rat submandibular ganglion cells have been measured at 20°C by means of a two-microelectrode voltage-clamp system. The aim was to distinguish between the receptor-blocking and channel-blocking actions of those drugs, and to test for possible selectivity of action on the 'fast' and 'slow' acetylcholine-operated channels.

2 Tubocurarine had no effect on the amplitude of evoked synaptic currents (e.s.cs) or miniature synaptic currents (m.s.cs), except at concentrations exceeding 20 μM. The slow component of the e.s.c. was shortened by tubocurarine, this effect becoming more marked as the cell was hyperpolarized. The timecourse of m.s.cs, which have no slow component, was unaffected.

3 Hexamethonium (2–30 μM) caused a voltage-dependent reduction of e.s.c. amplitude, and voltage-dependent shortening of both fast and slow components of the e.s.c. M.s.cs were also shortened.

4 Trimetaphan (2–10 μM) reduced the amplitude of e.s.cs and m.s.cs. Neither component of the e.s.c. was shortened by trimetaphan; however, the slow component was reduced in amplitude more than the fast component, so that the overall duration of the e.s.c. appeared to be reduced. At higher concentrations (15–25 μM) trimetaphan clearly shortened the fast component.

5 It is concluded that tubocurarine acts selectively on the slow ionic channels, the association rate constant being $2.8 \times 10^6\ M^{-1}\ s^{-1}$ at −80 mV. Hexamethonium acts on both fast and slow channels, the association rate constants, at −80 mV, being respectively $5.3 \times 10^6\ M^{-1}\ s^{-1}$ and $1.3 \times 10^7\ M^{-1}\ s^{-1}$. With both drugs, the association rate constant increases if the cell is hyperpolarized, this effect being more pronounced with hexamethonium than with tubocurarine.

6 The marked voltage-dependent reduction of e.s.c. amplitude by hexamethonium cannot be accounted for by open channel block, and requires an additional mechanism, the nature of which is discussed.

7 Trimetaphan, at low concentrations, acts in a way consistent with receptor block, and shows a degree of selectivity for the slow component of the e.s.c.

8 In an appendix, the effect of temporal dispersion of the time of opening of ionic channels on the amplitude and time-course of the composite synaptic response is analysed. It is concluded that the shortening of the time-constant of the e.s.c. decay by hexamethonium cannot, by itself, account for the drug's effect on e.s.c. amplitude.

Introduction

There is now good evidence that many agents that block the action of neurotransmitters do so by affecting the functioning of the associated ionic channels rather than by directly blocking the receptors. Blackman (1959) speculated that hexamethonium might block ganglionic transmission in this way, following his demonstration that its blocking action was enhanced by hyperpolarization of the cell. Adams (1976, 1977) suggested that a variety of substances, including barbiturates and local anaesthetics, exert their effect at the motor endplate by acting on the acetylcholine (ACh)-operated ionic channels, and there is now very good evidence that many local anaesthetics produce this effect at the neuromuscular junction and at other cholinergic synapses (Adams, 1977; Ruff, 1977; Marty, 1978; Neher & Steinbach, 1978; Tiedt, Albuquerque, Bakry, Eldefrawi & Eldefrawi, 1979), an action which strikingly resembles their effect on the voltage-sensitive sodium channels of electrically excitable membranes (Strichartz,

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1973; Hille, 1977). It has more recently been shown that many drugs thought to act selectively on ACh receptors owe their blocking action in part to an effect on the ionic channels. Tubocurarine (Manalis, 1977; Katz & Miledi, 1978; Ascher, Marty & Neild, 1978; Colquhoun, Dreyer & Sheridan, 1979), gallamine (Katz & Miledi, 1978; Colquhoun & Sheridan, 1981) and decamethonium (Adams & Sakmann, 1978) fall into this category of drugs with a dual blocking action. In a study of the actions of ganglionic blocking agents on rat submandibular ganglion cells Ascher, Large & Rang (1979) showed that hexamethonium, decamethonium and tubocurarine appeared to act on the ion channel rather than on the receptor, whereas the action of two other blocking agents, trimetaphan and surugatoxin was compatible with a receptor blocking effect.

The model that has most commonly been proposed to account for the properties of channel blocking drugs, and which can successfully account for most of the kinetic observations that have been made, is the following:

$$nA + R \underset{}{\overset{}{\rightleftharpoons}} A_nR \underset{\alpha}{\overset{\beta}{\rightleftharpoons}} A_nR^* \underset{k^*_{-B}}{\overset{k^*_{+B}}{\rightleftharpoons}} A_nR^*B \quad (1)$$

In this scheme, A is acetylcholine, R the receptor in its resting (closed) state, R* the receptor in its active (open) state, and B is the blocking agent. A_nR^*B is thus the active but blocked state of the channel. The scheme envisages that B can only bind to open channels (R*); with B bound, therefore, the channel cannot close, and it can only return to its resting state via the open state, A_nR^*. Obviously, more elaborate kinetic schemes can easily be envisaged, in which, for example, B might be able to bind to R as well as R*, and the action of some compounds seems to require such additional reaction steps (Adams, 1977; Feltz, Large & Trautmann, 1977; Adler, Oliveira, Albuquerque, Mansour & Eldefrawi, 1979; Tiedt *et al.*, 1979; Adams & Feltz, 1980). However, in general, the action of many blocking agents seems to conform well to scheme (1), and the preferential (and voltage-dependent) binding to the open channel suggests that these drugs reach their site of action via the mouth of the channel, like sodium ions except that they become lodged in the channel instead of passing freely through.

The main purpose of this paper is to analyse the actions of three ganglionic blocking agents, tubocurarine, hexamethonium and trimetaphan, on the synaptic currents of submandibular ganglion cells. The normal characteristics of these synaptic responses, which resemble endplate currents in many respects, have been described in detail by Rang (1981). They decay exponentially following the peak, the rate constant being sensitive to the membrane potential and representing the rate of closure of the channels, governed by α in scheme (1). The main differences from the endplate are: (i) the decay is bi-exponential, apparently because there are two types of ACh-operated channel with different mean lifetimes; (ii) the mean lifetimes are much longer (about 10 ms and 50 ms respectively at −80 mV and 20°C, compared with about 2 ms for endplate channels). The normal characteristics thus conform with the left-hand part of scheme (1), except that there appear to be two separate species of R functioning in parallel.

According to scheme (1), the action of a channel blocking drug will be to leave the peak amplitude of the synaptic response unchanged, since the channels open *before* the blocking drug can work, but to alter decay kinetics. The normal exponential decay (rate constant $k_c = \alpha$) of the response, will be split into two components with rate constants, k_1 and k_2, respectively larger and smaller than k_c. Both the rate constants and the amplitudes of the two components are quite complex functions of k^*_{+B}, k^*_{-B} and [B] (see Adams, 1977; Colquhoun & Hawkes, 1977; Ascher *et al.*, 1978). If, however, the dissociation rate constant k^*_{-B} is small with respect to α (strictly speaking it must be small with respect to $\alpha + k^*_{+B}[B]$), the second component becomes very small, and the observed effect is a speeding of the normal rate of decay from $k_c = \alpha$ to $k_1 = \alpha + k^*_{+B}[B]$. Thus the increase in the rate constant, $\Delta k = k_1 - k_c$ can be used to estimate the rate constant for the binding of B to the channel

$$\frac{\Delta k}{[B]} = k^*_{+B} \quad (2)$$

In this paper an attempt is made to determine the site of action (receptor or channel) of three blocking agents, namely tubocurarine, hexamethonium and trimetaphan, from measurements of their effects on the amplitude and time course of synaptic currents at different membrane potentials, and to test whether they show appreciable selectivity for the 'fast' or 'slow' channels that are present in these cells. The choice of these three blocking agents was based mainly on the rapid reversibility of their effects. Preliminary experiments showed that other blocking agents, such as mecamylamine, pempidine and chlorisondamine, produce clear-cut effects, but the slowness with which they develop and the even slower recovery made these drugs very difficult to study quantitatively, and the results are not given here.

The results obtained confirm the general conclusions of Ascher *et al.* (1979), but reveal differing patterns of selectivity among the three antagonists for the 'fast' and 'slow' channels, and also reveal an additional, voltage-dependent action of hex-

amethonium that appears to be distinct from its channel-blocking effect.

Methods

The methods were essentially as described in previous papers (Ascher *et al.*, 1979; Rang, 1981). Submandibular ganglia, with an attached length of chorda tympani nerve were dissected from rats (150–200 g hooded rats of either sex) that had been killed with chloroform and bled out by injection of Krebs solution via the left ventricle. The ganglion was cleaned as much as possible and pinned out in the recording chamber (about 1 ml capacity) which was mounted on the stage of a Nomarski microscope (water immersion objective ×40). The chorda tympani was drawn into a suction electrode for stimulation. The chamber was continuously perfused with oxygenated Krebs solution at about 3 ml/min and the temperature was maintained at 20°C. When drug solutions were added or washed out, the flow rate was usually increased to 5–6 ml/min, and exchange of the bath contents was estimated to be complete in about 30 s. With the three blocking agents tested, the drug effect was usually fully developed within 3–4 min, and recovery took 5–7 min.

Records of synaptic currents (e.s.cs) were made with the two-microelectrode voltage-clamp system described previously, the records being stored on magnetic tape for later computer analysis. A low pass filter (cut-off frequency 500–700 Hz, roll-off 48 dB/decade) was used to cut out high frequency noise. The cut-off frequency was set so that the filter did not affect the rise-time or peak amplitude of the e.s.c. In the case of evoked synaptic currents, three or four consecutive signals, digitized at 2000 Hz, were usually averaged and then fitted by a non-linear least squares method to give estimates of the amplitudes and rate constants of the two exponential components. The signal-to-noise ratio in these records was large, and the only purpose of averaging was to minimize the effects of small random fluctuations in amplitude of individual responses.

The usual protocol was to stimulate the nerve with a single supramaximal stimulus (0.2–0.5 ms duration) every 10 s throughout the experiment. A control series of e.s.cs was recorded at various membrane potentials (usually −40 mV to −100 mV in 20 mV steps with 3 responses at each potential). Potentials less negative than −40 mV often gave poorly-clamped records, because the resting impedance of the cells decreased sharply, and the current-passing electrode was liable to block. Potentials beyond −100 mV (and sometimes beyond −80 mV) appeared to damage the cells. In most experiments the potential was held at −40 mV or −60 mV and jumped to the test potential 100–200 ms before the stimulus, returning to the holding potential 300–400 ms after the stimulus. In other experiments the potential was adjusted manually and held during two or three stimuli. The same results were obtained by either method.

The blocking agent was applied with the membrane potential held at −40 mV or −60 mV. Equilibrium was reached in 3–4 min, and after 5–10 min the synaptic responses were recorded. With channel blocking drugs there was a possibility that cumulative block would develop with repeated stimulation at hyperpolarized potentials. In a few cells the response at −100 mV in the presence of hexamethonium tended to decline during the series of three stimuli, and subsequent responses at −40 mV were also reduced, but this effect was never large enough to introduce a serious error, and was often absent altogether. Where it did occur, the number of stimuli delivered at −100 mV in the presence of hexamethonium was reduced to one or two to minimize the error produced.

In most experiments the drug was washed out, and the control series repeated after each trial. Recovery was usually complete within 5 min. The effect of the drug was expressed in terms of the mean parameters of the control records taken before and after the test records. Where this was not possible, either because successive drug tests were made without intervening controls or because the impalement was lost, the control records immediately before or after the test records were used as a basis for comparison.

Miniature synaptic currents (m.s.cs) were recorded by adding 20 mM KCl to the medium, which increases their normally very low frequency to about 1/s. The low-pass filter setting used was 250–300 Hz (roll-off 48 dB/decade. This enabled the m.s.cs, whose amplitude at −80 mV was 100–150 pA, to be recorded above the noise level (20–30 pA peak-to-peak). The range of membrane potential over which m.s.cs could be recorded satisfactorily was usually −60 mV to −100 mV. Most experiments were done at −80 mV, and the voltage-dependence of the effects of the blocking agents was not examined.

M.s.cs were recorded on magnetic tape for 1–2 min. The signals were then captured and stored by a computer programme that used an amplitude discriminator to pick out m.s.cs above the baseline noise. An editing and averaging programme allowed artefacts to be discarded, and also allowed the rising phase of all the events to be precisely aligned before averaging. The average was built up of 30–40 m.s.cs and fitted (usually to a single exponential component) by the least squares method. As shown previously (Rang, 1981) m.s.cs usually contain only one exponential component, corresponding to the fast component of the e.s.c. In this series, a small number

of cells gave m.s.cs that appeared to have long tails in addition to the fast component. The resolution was not good enough to permit quantitative analysis, and these cells were excluded from the analysis.

The main difficulty in analysing m.s.cs is that their small size may cause them to be lost in the baseline noise, and there is a risk that the capturing programme will obtain a biassed sample. Furthermore, the low-pass filtering needed to reduce the baseline noise is likely to distort the rising phase and attenuate the peak amplitude of the m.s.cs. Though these problems ought not to affect calculation of the time constant, they introduce some unreliability into the estimation of the m.s.c. amplitude. An estimate of the possible error due to biassed sampling was obtained for some cells by recording control m.s.cs at different membrane potentials. Taking the reversal potential as -10 mV and assuming a linear current-voltage relationship for the peak amplitude (Rang, 1981) enables the expected effect of membrane potential on m.s.c. amplitude to be calculated. If the event capturing programme is introducing a bias, the measured mean m.s.c. amplitude should vary less than expected when the membrane potential is changed. In eight cells, for which recordings were made at more than one level of the resting potential, the mean e.s.c. amplitude at -60 mV was 0.82 ± 0.02 times the amplitude at -80 mV, whereas the expected factor was 0.71. On the other hand at -100 mV the measured amplitude factor was 1.26 ± 0.04, compared with the expected value of 1.29. This suggests that m.s.cs were reasonably faithfully recorded at -80 mV and -100 mV, but appreciably overestimated at -60 mV. By the same token, we would expect that any reduction in peak amplitude caused by the blocking agents would tend to be underestimated.

Solutions and drugs

The Krebs solution had the following composition (mM): NaCl 119, KCl 4.7, $CaCl_2$ 5, KH_2PO_4 1.2, $NaHCO_3$ 25, $MgSO_4$ 1.2 and glucose, 11. The solution was bubbled with 95% O_2: 5% CO_2. The calcium concentration was double that of normal Krebs-Henseleit solution as this improved the survival of cells after impalement with two electrodes.

Drugs used were (+)-tubocurarine chloride (Koch-Light), hexamethonium bromide (Koch-Light) and trimetaphan camsylate (Roche).

Results

Normal e.s.cs in this preparation (see Rang, 1981) rise rapidly to a peak (rise time 2 ms) and decay in two exponential phases. The fast phase usually amounts to 50–75% of the total amplitude and decays with a time constant, τ_f, equal to 5–10 ms. The time constant of the slow phase, τ_s is 30–60 ms, and both phases become slower as the membrane is hyperpolarized.

In investigating the action of the blocking agents we have measured the effect on the peak e.s.c. amplitude, on the relative amplitudes of the fast and slow phases, and on the time constants of these two phases.

Experimental records of e.s.cs elicited in the presence of the three blocking agents that have been studied in detail are shown in Figures 1–3. The analysis was based on measurements made on 3–8 cells studied at each concentration, except for a few concentrations that were tested on only one or two cells.

Effects on peak amplitude

Tubocurarine Tubocurarine at 20 μM or less had very little effect on peak amplitude of e.s.cs, irrespective of the membrane potential (see Figure 1). At 40 μM an appreciable inhibition occurred and this increased as the membrane was hyperpolarized, as can be seen in Figure 1. In this cell 40 μM tubocurarine reduced the peak amplitude by 20% at -40 mV and by 33% at -100 mV. Results obtained on a number of cells (Figure 10) indicate that the concentration giving a 50% block at -80 mV exceeds 40 μM, but it has not been determined at all accurately.

Hexamethonium Hexamethonium is much more effective at reducing the peak amplitude, and the inhibition also shows marked voltage dependence, as can be seen from Figure 2. The 50% blocking concentration at -80 mV was close to 10 μM (Figure 10). It is convenient to measure the inhibitory effect as

$$\Lambda = \frac{\text{Amplitude in absence of inhibitor}}{\text{Amplitude in presence of inhibitor}}$$

(see Ascher, *et al.*, 1978; 1979). The voltage dependence of Λ can be expressed by calculating $\Lambda(V) - 1$ for various membrane potentials. Figure 3 shows the results for various hexamethonium concentrations, normalized by expressing $\Lambda(V) - 1$ with respect to $\Lambda(-80) - 1$ for each experiment. Previous work on voltage-dependent channel blocking drugs (see Ascher *et al.*, 1978; 1979; Colquhoun *et al.*, 1979; Colquhoun & Sheridan, 1981) has suggested that their affinity for the blocking site is related exponentially to the membrane potential, the characteristic voltage (i.e. the potential change needed for an e-fold change in affinity) being generally in the range of 25 to 50 mV. The voltage range studied in the present experiments is not sufficient to show whether or not the relationship is strictly exponential, but Figure 3

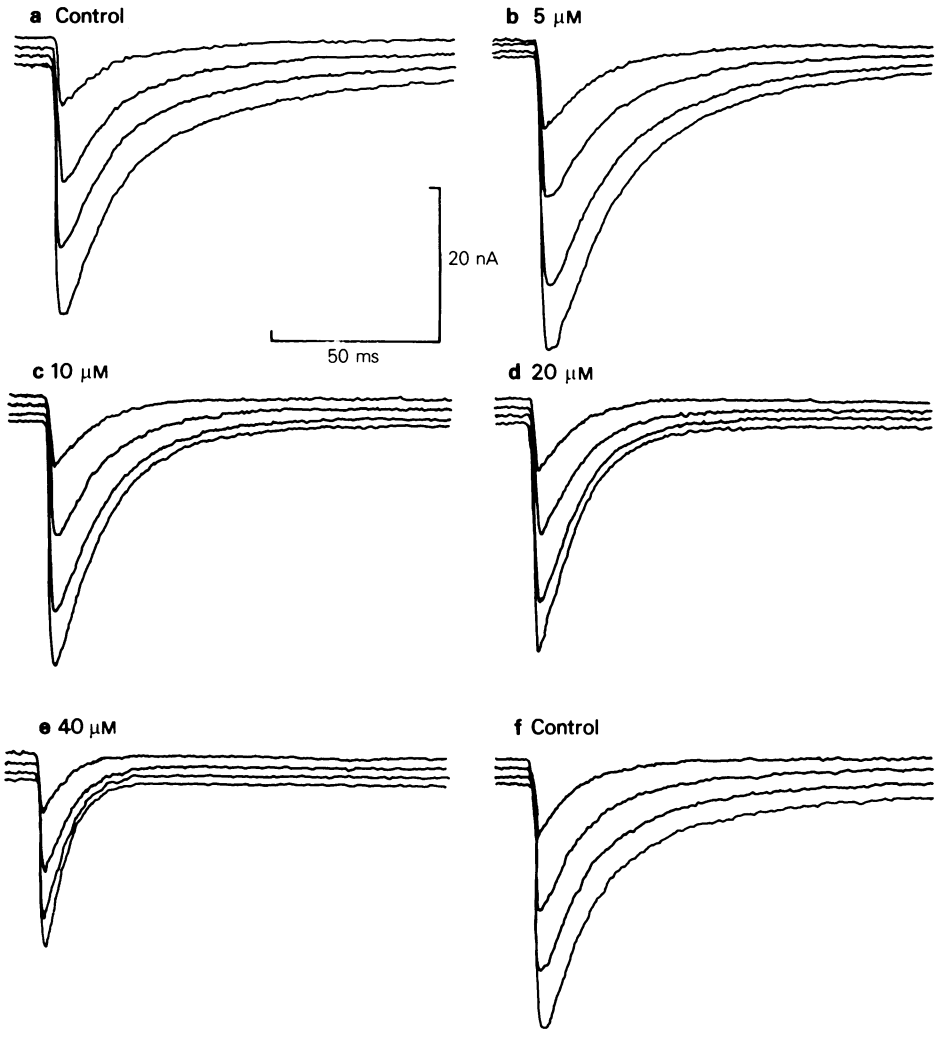

Figure 1 The effect of tubocurarine on e.s.cs. Each panel shows e.s.cs recorded at −40 mV (smallest response), −60 mV, −80 mV and −100 mV (largest response). Each tubocurarine concentration was allowed to act for 5–10 min before the reading was made, and the final control series (f) was recorded after washing the preparation for about 20 min. Note that no decrease in amplitude occurs up to 20 μM tubocurarine, though considerable shortening of the e.s.c. is evident.

shows an e-fold change in $\Lambda - 1$ with a potential change of about 50 mV. This is less steep than the voltage dependence of the blocking action of hexamethonium against equilibrium responses to bath-applied agonists, where $\Lambda - 1$ increases more than 3 fold with a 30 mV hyperpolarization, equivalent to an e-fold change in 26 mV (Ascher *et al.*, 1979). On the simplest assumptions, one would predict that a drug that acts purely by blocking open channels should not affect the amplitude of synaptic currents. Thus, if the acetylcholine concentration rises and falls rapidly (with respect to the rate of channel closing or channel block) and the release of quanta is synchronous, the peak conductance will be achieved before any block can occur. The reduction of the peak amplitude by hexamethonium therefore implies either that a mechanism other than open channel block is operating, or that these kinetic assumptions do not hold fully.

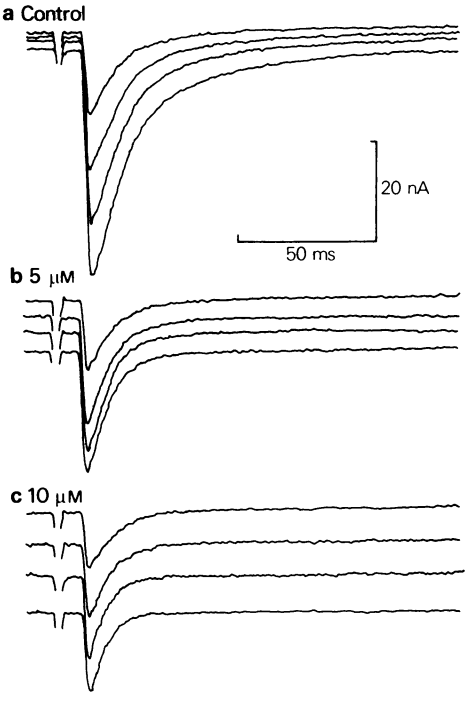

Figure 2 The effect of hexamethonium on e.s.cs. Each panel shows e.s.cs recorded at −40 mV (smallest response), −60 mV, −80 mV and −100 mV. In (b) and (c) the baseline has been displaced slightly between sweeps for clarity. Each hexamethonium concentration was applied for 5–10 min before the recording was made. Note that as well as shortening the e.s.c., hexamethonium reduces the amplitude markedly, this effect being much greater at −100 mV than at more depolarized potentials.

Trimetaphan Trimetaphan reduced the peak amplitude of e.s.cs in a simple non-voltage-dependent manner (Figures 3 and 4) the concentration required for 50% inhibition being about 2 μM.

Effects of blocking agents on the time course of e.s.cs

All these blocking agents shortened the time-course of e.s.cs, though in different ways, as is shown in Figures 5, 7 and 9.

Tubocurarine Tubocurarine, at concentrations exceeding 5 μM, caused the normal bi-exponential decay to become a single exponential. With 10 μM tubocurarine, the time constant was usually intermediate between τ_f and τ_s; at higher tubocurarine concentrations it became shorter (see Figures 1 and 5). Whereas in unblocked preparations τ_f and τ_s both increase as the cell is hyperpolarized (see Rang, 1981), in the presence of tubocurarine τ stays essentially unchanged, or decreases, with hyperpolarization (Figure 5). At 5 μM tubocurarine, the decay remained bi-exponential and analysis showed that the relative amplitude of the two components was essentially unchanged. It seemed likely that the disappearance of the second component with higher tubocurarine concentrations resulted from a selective speeding of the slow component; once the difference between τ_f and τ_s is less a factor of about two, the separation of the two components becomes indistinct, and they tend to be counted as one. The lack of effect of tubocurarine on m.s.cs (see later section) is consistent with this interpretation, since m.s.cs correspond to the fast component of the e.s.c. On the assumption that the overall time constant in the presence of tubocurarine represents the speeded-up τ_s, and that this speeding results from channel block, the results can be used to calculate the rate of association of tubocurarine with the open slow channels, k^*_{+s}, as described in the introduction. The calculated value of k^*_{+s} for a series of experiments as a function of membrane potential is shown in Figure 6. Where several tubocurarine concentrations were tested on one cell, the relationship between $1/\tau$ and concentration was usually linear, as predicted. In some cases there was a tendency for $1/\tau$ to increase less than predicted as tubocurarine concentration was increased, which could be the result of the failure to distinguish between τ_f and τ_s in the presence of tubocurarine. Thus, at low tubocurarine concentrations the calculation of a composite rate constant would tend to overestimate the speeding of the slow component. At high tubocurarine concentrations, when the 'slow' component was equal to or even

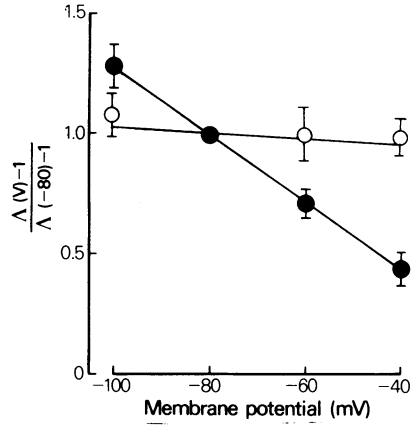

Figure 3 Voltage-dependence of the effect of hexamethonium (●) and trimetaphan (○) on the e.s.c. amplitude. Results for hexamethonium and trimetaphan concentrations from 2–30 μM are plotted by calculating $[\Lambda(V)-1]1/[\Lambda(-80)-1]$ for each experiment (see text). Each point represents the mean for measurements on 4–10 cells; vertical lines indicate s.e.mean.

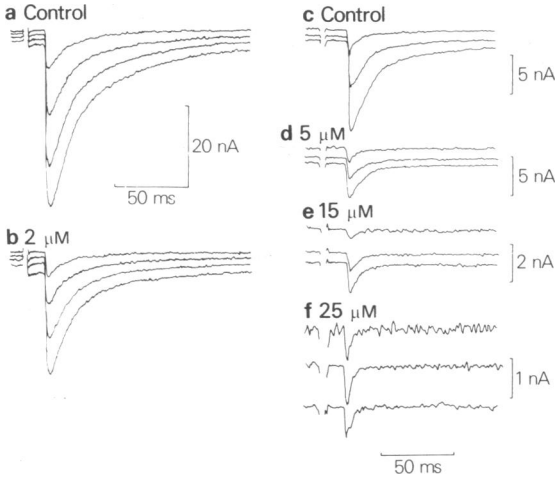

Figure 4 The effect of trimetaphan on e.s.cs.: (a) and (b) were recorded from one cell (records at −40 mV, −60 mV, −80 mV and −100 mV); (c)–(f) are from another cell (records at −40 mV, −60 mV and −80 mV). Each drug concentration was applied for 5–10 min before the recording was made. The main effect of trimetaphan is on e.s.c. amplitude, with little voltage-dependence, though at higher concentrations shortening of the e.s.c., and voltage-dependence, are evident.

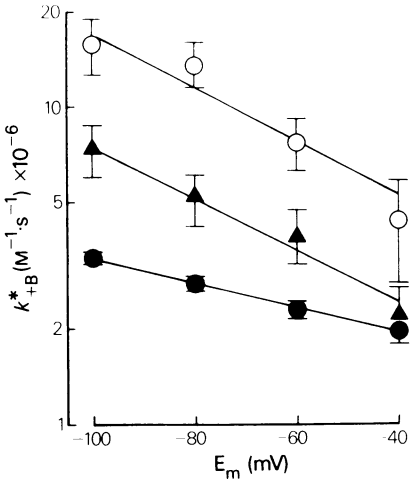

Figure 6 The voltage-dependence of the association rate, k^*_{+B}, for tubocurarine (●) combining with the slow channels, and for hexamethonium combining with the slow (▲) and the fast (○) channels. Each point represents the mean of 3–8 measurements; vertical lines show s.e.mean. The lines are drawn by eye and their slopes correspond to an e-fold change in k^*_{+B} with 115 mV change in membrane potential for tubocurarine, and 56 mV for hexamethonium.

faster than the unchanged fast component, the composite rate constant would be a true estimate or an under-estimate of τ_s, thus distorting the relationship between $1/\tau$ and tubocurarine concentration as found. Taking the mean of all the estimates of the association rate constant k^*_{+s} for the slow channel without attempting to correct for this source of error gives the value $2.8 \times 10^6\ M^{-1}\ s^{-1}$ at −80 mV (Figure 6). The slope of the line drawn in Figure 6 corresponds to a characteristic voltage of 115 mV.

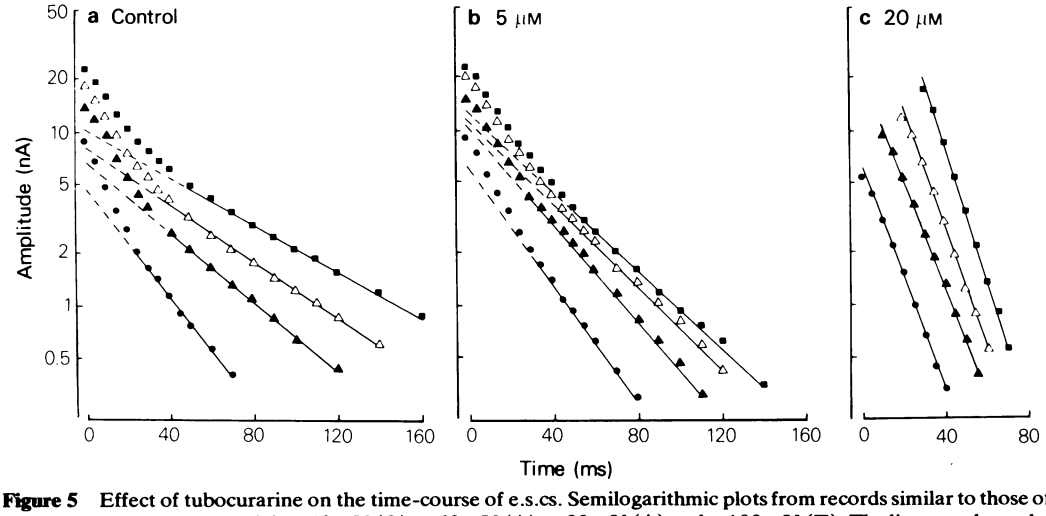

Figure 5 Effect of tubocurarine on the time-course of e.s.cs. Semilogarithmic plots from records similar to those of Figure 1. Membrane potentials −40 mV (●), −60 mV (▲), −80 mV (△) and −100 mV (■). The lines are drawn by eye and show the slow component of the response. In (c) successive plots are displaced horizontally by 10 ms for clarity. At 5 μM tubocurarine the slow component becomes faster and less voltage-dependent; at 20 μM only a single component is discernible, and its voltage-dependence is reversed.

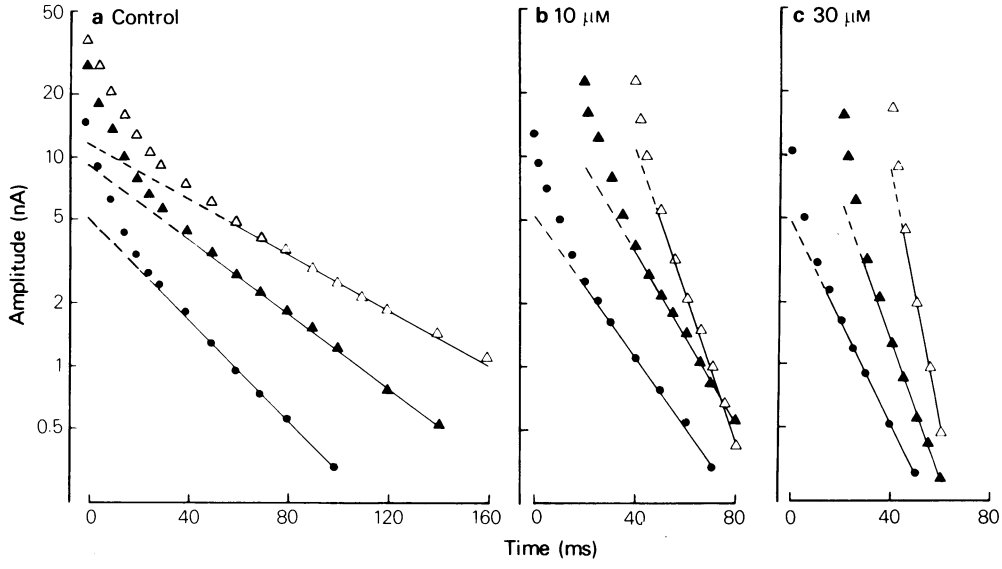

Figure 7 Effect of hexamethonium on the time course of e.s.cs at −40 mV (●), −60 mV (▲) and −80 mV (△). In (b) and (c), successive plots are displaced horizontally by 20 ms for clarity. The lines are drawn by eye through the slow component. Note that the e.s.cs remain bi-exponential (compare with Figure 5), and that the time constants decrease with hyperpolarization in the presence of hexamethonium.

Hexamethonium Hexamethonium also shortens the time course of e.s.cs in a voltage-dependent manner (Figures 1 and 7), but its action differs from that of tubocurarine in two respects: (a) it produces a voltage-dependent reduction of amplitude (Figure 2), (b) the time course remains bi-exponential, both components being speeded up by the drug (Figure 7). At concentrations up to 10 μM the reduction in amplitude was confined to the fast component, though at higher concentrations the slow component was also affected. At hexamethonium concentrations exceeding 10–20 μM, the reduction in amplitude of the fast component meant that the two components of the e.s.c. were no longer easily distinguishable, and the decay phase was fitted by a single exponential, the time constant being taken to be an altered value of τ_s. As with tubocurarine, the speeding effect was voltage-dependent, and Figure 6 shows the values of k^*_{+s} and k^*_{+f} for a series of experiments. k^*_{+f} was calculated from the results at low hexamethonium concentrations, where the fast component remained distinguishable. It can be seen that k^*_{+s} is rather higher for hexamethonium than for tubocurarine (5.3×10^6 M^{-1} s^{-1} at −80 mV) and its voltage dependence is also steeper (characteristic voltage = 56 mV). The association rate constant for the fast channel is larger still (1.3×10^7 M^{-1} s^{-1} at −80 mV) with a similar voltage-dependence (Figure 6).

To investigate the origin of the reduction in peak e.s.c. amplitude, which is not predicted by the simple channel-blocking mechanism, the effect of hexamethonium on the rising phase of the e.s.c. was studied. If the reduction in peak amplitude results from the asynchronous opening of channels during the rising phase of the e.s.c., so that some become blocked before others have opened, then the early part of the rising phase should be unaffected by hexamethonium. If, on the other hand, a different mechanism is operating to reduce the effectiveness of ACh in opening channels, the block should be evident throughout the rising phase. In several cells, e.s.cs were recorded with rather low resistance electrodes and adjustment of the clamp gain and capacitance compensation to optimize the frequency response so as to obtain as faithful a record as possible of the rising phase. Typical records are shown in Figure 8, from which it can be seen that the block appears to be present during the earliest part of the rising phase, suggesting that open channel block cannot fully account for the action of hexamethonium.

Trimetaphan The action of trimetaphan clearly differed from that of the other blocking agents, in that it affected the amplitude much more, and the time course much less (Figure 3 and Figure 9). At 5 μM trimetaphan the peak e.s.c. amplitude was reduced by about 60%, but both components were still present and neither time constant was appreciably changed (Figures 9 and 10). In contrast to hexamethonium, the decrease in amplitude was not voltage-dependent. It was consistently found, however, that the amplitude of the slow component, a_s,

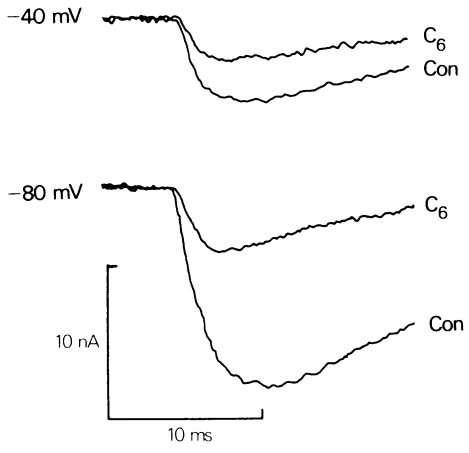

Figure 8 Effect of hexamethonium (C_6, 10 μM) on the rising phase of e.s.cs recorded at −40 mV and −80 mV. The earliest part of the rising phase shows inhibition by hexamethonium, suggesting that a mechanism other than open channel block is operating (see text).

was reduced more than that of the fast component, a_f. Thus, at 5 μM trimetaphan, a_f was reduced to 0.49 ± 0.04 (8 cells) of the control value whereas a_s was reduced to 0.19 ± 0.03 (8 cells). If this results from receptor block, with trimetaphan showing some degree of selectivity for the 'slow' receptors, we would expect the inhibition of the peak amplitude to follow the relationship

$$\frac{a_p(\text{drug})}{a_p(\text{control})} = \frac{K_s}{x + K_s} \cdot \frac{a_s(\text{control})}{a_p(\text{control})} + \frac{K_f}{x + K_f} \frac{a_f(\text{control})}{a_p(\text{control})} \qquad (1)$$

where K_s and K_f are equilibrium constants for trimetaphan binding to the 'slow' and 'fast' receptors and x is the trimetaphan concentration. The results appear to fit this equation satisfactorily with $K_s = 1.2$ μM and $K_f = 4.8$ μM, i.e. a 4 fold preference of the drug for 'slow' receptors. It should be noted, however, that equation (1) assumes that the antagonism is 'non-competitive' in the sense that antagonist occupancy does not decrease from its equilibrium value during the time that ACh acts. If there is some degree of re-equilibration during the time for which the transmitter acts, equation (1) will overestimate the true equilibrium constants. Indeed, the difference between K_f and K_s obtained from the results could, in principle, represent a difference in the dissociation

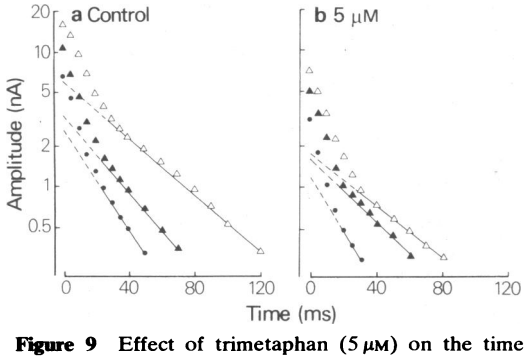

Figure 9 Effect of trimetaphan (5 μM) on the time course of e.s.cs recorded at −40 mV (●), −60 mV (▲) and −80 mV (△). The lines are drawn by eye through the slow component. Trimetaphan markedly reduces the amplitude of the slow component, but does not appreciably alter its time constant.

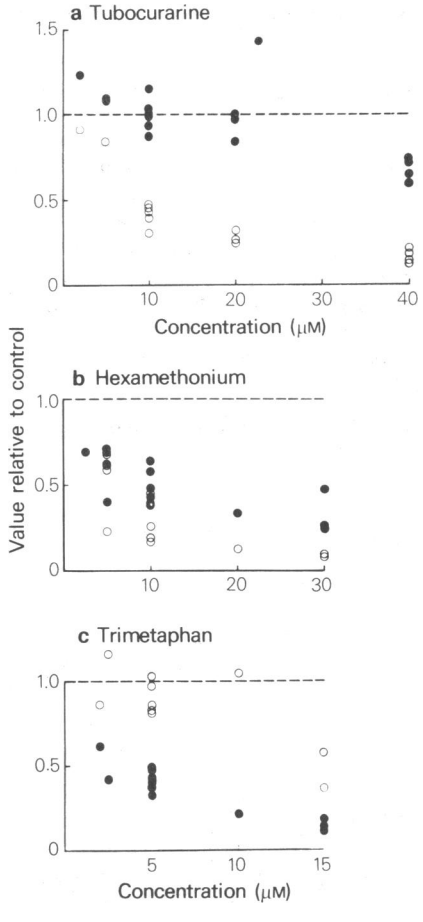

Figure 10 Effects of tubocurarine (a), hexamethonium (b) and trimetaphan (c) on the amplitude (●) and slow time constant, τ_s (○) of e.s.cs recorded at −80 mV. Each point represents a test in one cell, and the amplitude and slow time constant are expressed relative to the control values in that cell. Note that tubocurarine reduces τ_s without affecting amplitude, whereas trimetaphan shows the opposite pattern. Hexamethonium reduces both amplitude and τ_s.

rate constant of trimetaphan bound to 'fast' and 'slow' receptors rather than a true difference in affinity, a question that would need to be resolved by noise analysis or voltage-jump experiments (Rang, 1981).

At higher concentrations (15–25 μM) trimetaphan clearly reduced the time constant of the fast component (see Figure 3). Thus, in one cell 15 μM trimetaphan reduced τ_f (−80) to 57% of the control value, and 25 μM trimetaphan reduced it to 38% of the control. Unlike the effect of tubocurarine and hexamethonium, however, this effect showed no voltage-dependence, the fractional reduction in τ_f being the same at −40 mV and −80 mV.

The effects of the three blocking agents on the amplitude and time constant of e.s.cs are summarized in Figure 10.

Effects of blocking agents on miniature synaptic currents

It was of interest for two reasons to investigate how blocking agents affect miniature synaptic currents (m.s.cs). First, m.s.cs comprise only the fast component of the e.s.c. (Rang, 1981), and thus provide a rather simpler situation for analysis than e.s.cs. Secondly, non-synchronous release of transmitter, which may complicate the analysis of e.s.cs (see discussion) should not be a problem with m.s.cs. On the other hand, m.s.cs are very small in this preparation (about 100 pA at −80 mV), and the signal-to-noise ratio is unfavourable, so the analysis was less detailed than for e.s.cs. Most cells were studied at −80 mV which provided an adequate m.s.c. amplitude with better recording stability than more hyperpolarized potentials.

Tubocurarine Tubocurarine (10 μM) had no apparent effect on m.s.c. amplitude and slightly prolonged $\tau_{m.s.c.}$ (Table 1, Figure 11). At 5 μM (1 cell) tubocurarine increased m.s.c. amplitude by 27% (apparently a real effect, since it disappeared when the drug was washed out) without affecting $\tau_{m.s.c.}$. The ability of tubocurarine to enhance the sensitivity of ganglion cells to ionophoretically applied carbachol under certain conditions was noted in an earlier study (Ascher *et al.*, 1979). At 20 μM tubocurarine (2 cells) neither amplitude nor time constant were affected. This confirms the conclusion reached earlier that tubocurarine does not block or shorten the fast component.

Hexamethonium Hexamethonium (5–10 μM) appeared to reduce m.s.c. amplitude only very slightly, but markedly reduced $\tau_{m.s.c.}$ (Figure 11, Table 1). On the assumption that this speeding represented channel blocking by hexamethonium, the association rate

Table 1 Effects of tubocurarine and hexamethonium on miniature synaptic currents (m.s.cs)

Drug	*Concn* (μM)	*Control amplitude* (pA)	*Control time constant* (ms)	*Amplitude relative to control*		*Time constant relative to control*		k^*_{+B} $M^{-1}\,s^{-1} \times 10^{-6}$
Tubocurarine	5	121	13.0	1.27		1.03		
	10	149	11.8	0.94		1.08		
		116	11.7	1.05		0.91		
		52	7.9	0.96	1.01	1.16	1.10	
		152	7.2	0.91	± (5)	1.23	± (5)	
		137	7.1	1.18	0.04	1.11	0.05	
	20	197	6.3	0.95		1.05		
		83	9.8	0.87		0.99		
Hexamethonium	5	115	8.2	1.02		0.68		11.4
		80	15.6	1.04		0.68		6.0
		169	6.3	1.06	0.98	0.60	0.66	21.0
		88	18.4	0.82	± (6)	0.66	± (6)	5.5
		101	11.4	0.96	0.04	0.68	0.01	8.3
		106	12.1	0.97		0.64		9.3
	10	66	8.3	0.74		0.60		8.0
		61	7.9	0.98		0.76		3.9
		70	14.9	0.91	0.88	0.48	0.53	7.2
		81	11.2	0.97	± (5)	0.43	± (5)	11.7
		89	9.5	0.81	0.05	0.37	0.07	17.9
Mean ± s.e.mean		107 ± 9.0 (19)		10.5 ± 0.8 (19)				

Each line represents results from one cell, obtained by averaging 30–40 m.s.cs.

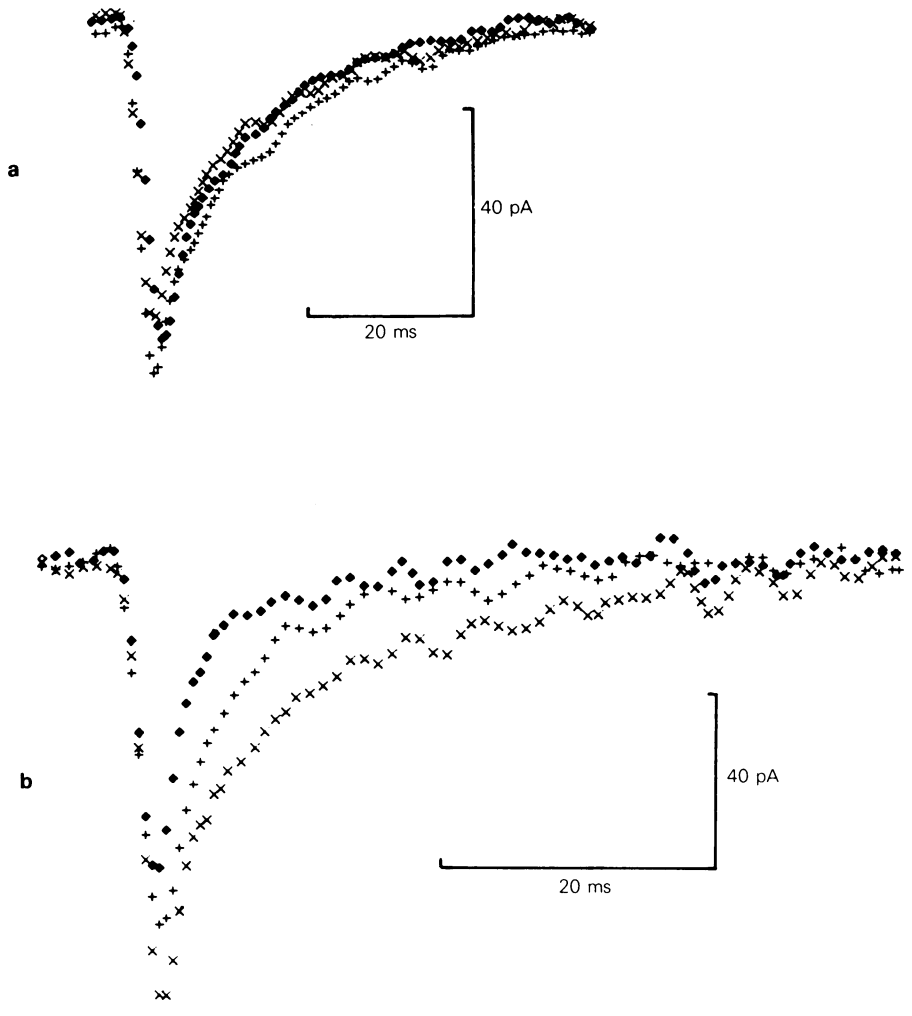

Figure 11 Computer-averaged m.s.cs, showing the effects of tubocurarine (a) and hexamethonium (b). Each plot represents the average of 20–30 m.s.cs. (a) Controls (×); tubocurarine 10 μM (+); tubocurarine 20 μM (◆). (b) Controls (×); hexamethonium μM (+); hexamethonium μM (◆). Tubocurarine has no effect beyond a small increase in m.s.c. amplitude at 10 μM. Hexamethonium slightly reduces the amplitude and markedly reduces the time constant of m.s.cs.

constant calculated from equation (1) was found to be $(10.0 \pm 1.19) \times 10^6\ M^{-1} s^{-1}$ (10 measurements in 6 cells), a value close to that found for the fast component of the e.s.c. $(13.3 \pm 2.0) \times 10^6\ M^{-1} s^{-1}$.

Trimetaphan Trimetaphan (2 μM) was tested on three cells. In each case it reduced the amplitude considerably. In two cells the amplitude appeared to be reduced by about 50%, but at this level it was not possible to measure the amplitude or time constant of m.s.cs with any certainty. In the third cell, the amplitude was reduced by 24% and the time constant was unchanged.

Discussion

The results obtained are summarized qualitatively in Table 2. When tested at low concentrations, the three blocking agents produce clearly distinct effects. Trimetaphan shows no voltage-dependence and its action is to reduce the amplitude of the synaptic response without affecting its time course. This is consistent with an action on the receptors, though dose-ratio measurements at equilibrium would be needed to provide positive evidence for a competitive interaction. It is of interest that the slow phase of the e.s.c. is more sensitive to trimetaphan than the fast

Table 2 Summary of results of effects of tubocurarine, hexamethonium and trimetaphan on synaptic responses of rat submandibular ganglionic cells

	Tubocurarine (up to 20 μM)	*Hexamethonium* (up to 30 μM)	*Trimetaphan* (up to 10 μM)
Amplitude			
Reduction of fast component	0†	+	+
Voltage-dependence		+	0
Reduction of slow component	?*	slight*	+
Voltage-dependence	?	?slight	0
Time course			
Reduction of τ_f	0	+	0
Voltage-dependence		+	
$k^*_{+f}(-80\,mV)\,M^{-1}s^{-1}$		1.3×10^7	
Reduction of τ_s	+	+	0
Voltage-dependence	+	+	
$k^*_{+s}(-80\,mV)\,M^{-1}s^{-1}$	2.8×10^6	5.3×10^6	

* Speeding of τ_s makes separation of components uncertain at concentrations exceeding 5 μM.
† Increase in amplitude in some cells at 2–5 μM.

phase, since this suggests that the receptors may be different. The estimated concentration producing 50% inhibition of the slow component (1.2 μM), was one quarter of the concentration needed to produce an equal block of the fast component. This might suggest a four fold difference in affinity for the two types of receptor, but this inference would be justified only if it were known that no dissociation of the blocking agent occurs during the rising phase of e.s.c. Nothing is known of the rate of dissociation of trimetaphan, and it is possible that the fast component of the e.s.c. is relatively less blocked because trimetaphan dissociates from these receptors to a greater extent than from the 'slow' receptors within the rise-time of the e.s.c. This could, indeed, occur without there being any difference between the actual dissociation rate constants of the trimetaphan-receptor complexes, for the agonist concentration is one factor that determines the rate of equilibration when an agonist and a competitive antagonist are acting simultaneously (see Rang, 1966). Thus, if the local ACh concentration responsible for the fast component were higher than that responsible for the slow component, it would displace trimetaphan from the receptors more rapidly and the peak inhibition would be less. The differential block of the slow component does not, therefore, show that different receptors are involved in the two components, though that is a possibility. If a drug were found with the opposite selectivity in its action, the evidence for a difference between the receptors would be more compelling.

The results show that trimetaphan at higher concentrations (10 μM and above) shortens the time constant as well as reducing the amplitude of the e.s.c. τ_s could not be reliably measured at concentrations exceeding 5 μM because of the very low amplitude of the slow component, but it did not appear to be reduced at 5 μM. The shortening of τ_f at higher concentrations of trimetaphan, in contrast to the effect of hexamethonium was not appreciably voltage-dependent. The mechanism is not clear, but since trimetaphan is a cationic sulfonium compound it seems unlikely that it would show a non-voltage-dependent channel blocking action. To distinguish between a channel blocking action and an indirect effect on channel lifetime it would be useful to measure the concentration-dependence of the kinetic effect, and also to study the effect with agonists whose mean channel lifetimes differ.

The blocking action of tubocurarine, at least in concentrations up to 20 μM, is confined to the slow component of the e.s.c. Its rather striking lack of effect on m.s.cs, which correspond to the fast component of the e.s.c., confirms this conclusion. The time constant of the slow component is reduced by tubocurarine in a voltage-dependent manner. The most likely interpretation is that this results from block of open channels, according to scheme 1, which agrees with the conclusion of Ascher *et al.* (1979). The absence of the predicted additional slow component of the e.s.c. in the presence of a channel-blocking drug (see Introduction) is not surprising, for Ascher *et al.* (1979) estimated that the rate of dissociation of tubocurarine, k^*_{-B}, was only $0.2\,s^{-1}$ at −80 mV, so the slow component would be a low amplitude, long-lasting tail which could not be easily detected. The association rate k^*_{+s} for tubocurarine acting on the slow channel was $2.8\times10^6\,M^{-1}s^{-1}$ at −80 mV. This agrees fairly well with our earlier and less direct estimate of $5\times10^6\,M^{-1}s^{-1}$ (Ascher *et al.* 1979) and is somewhat less than its estimated rate

constant ($8 \times 10^6 M^{-1} s^{-1}$ at 80 mV and 9°C) for blocking endplate channels (Colquhoun *et al.*, 1979). The fast channels in the ganglion do not, it seems, bind tubocurarine, and in comparing the present results with those of Ascher *et al.*, it should be remembered that no distinction between fast and slow channels was made in the earlier study.

Hexamethonium produced effects basically similar to those of tubocurarine, but with certain important differences. (1) The blocking action affected both slow and fast channels, with a rather higher rate of binding to fast than to slow channels. (2) The voltage-dependence of the association rate constant was steeper than with tubocurarine (e-fold increase in k^*_{+B} in 115 mV for tubocurarine, compared with 56 mV for hexamethonium; see Figure 7). (3) Hexamethonium produced a marked voltage-dependent decrease in e.s.c. amplitude, which tubocurarine did not do, except at high concentrations.

The effect of hexamethonium in blocking the fast channels is presumably an important factor in causing overall block of ganglionic transmission, since the fast channels contribute more than half of the peak synaptic current (Rang, 1981). The rate constant for blocking the fast channels was about $1.3 \times 10^7 M^{-1} s^{-1}$ at −80 mV, which is very similar to the rate of block of ACh-operated channels at the motor endplate by cationic blocking agents (Ruff, 1977; Feltz *et al.*, 1977; Neher & Steinbach, 1978; Adler, Albuquerque & Lebeda, 1978; Colquhoun *et al.*, 1979; Colquhoun & Sheridan, 1981). The association rate constants for hexamethonium and tubocurarine combining with ACh-operated channels of *Aplysia* neurones ($7.5 \times 10^5 M^{-1} s^{-1}$ and $4.4 \times 10^5 M^{-1} s^{-1}$ respectively at 12°C and −80 mV; Ascher *et al.*, 1978) are somewhat lower than those reported here, but the temperature difference will partly account for this.

The voltage-dependence of the association rate of drugs with endplate channels has been measured by various authors. In general, the characteristic voltage (i.e. the potential change giving an e-fold change in k^*_{+B}) lies in the range 50–100 mV (Neher & Steinbach, 1978; Colquhoun *et al.*, 1979; Colquhoun & Sheridan, 1981) though much shallower voltage-dependence of the channel blocking rate was found for atropine (Adams & Feltz, 1980). The simplest interpretation of these differences, on the basis of the channel blocking model, is that the position of the energy barrier within the channel, which has to be surmounted by a drug combining with the blocking site, varies for different drugs. On this basis, the energy barrier for tubocurarine would lie closer to the mouth of the channel than the barrier for hexamethonium.

Effects of various drugs on the peak amplitude of synaptic currents, which are not easily accounted for in terms of the simple channel blocking model shown in scheme (1), have been described by several authors, and no simple explanation can be offered. Thus, procaine (Adams, 1977), atropine (Feltz *et al.*, 1977), quinacrine (Tsai, Oliveira, Albuquerque, Eldefrawi & Eldefrawi, 1979; Adams & Feltz, 1980), tetraethylammonium (Adler, Oliveira, Albuquerque, Mansour & Eldefrawi, 1979), piperocaine (Tiedt *et al.*, 1979) all act in this way, and, except with quinacrine, the effect is highly voltage-sensitive, as it is with hexamethonium. With piperocaine, Tiedt *et al.* (1979) showed that the effect on amplitude took a few seconds to change in response to a step in membrane potential, whereas the shortening of the e.p.c. time constant was immediate. This and other evidence led them to postulate a second site of action, distinct from the ionic channel but showing similarly voltage-dependent binding, to account for the reduction of amplitude, and similar conclusions were reached by the other authors quoted above in relation to atropine, quinacrine and tetraethylammonium.

In principle, the simple channel block model could account for the voltage-dependent decrease in peak amplitude if the opening of channels during the rising phase of the e.s.c. occurred over a time interval that was appreciable in relation to the mean open time of the channel. Shortening the mean open time by addition of the blocking agent would then decrease the peak amplitude by reducing the degree of 'overlap' in the opening of the population of channels. Asynchrony in the opening of channels could arise either because the released transmitter continues to act for an appreciable time, or because the release of individual quanta by the nerve impulse is asynchronous. Since the ganglion cell has about 50–100 boutons terminaux it is possible that delay in the spread of the presynaptic action potential could lead to temporal dispersion in transmitter release. The effect of temporal dispersion of channel opening on the characteristics of synaptic currents is analysed more fully in the Appendix, where two extreme possibilities are discussed. In the first model, the temporal distribution of channel opening times is assumed to be Gaussian, with standard deviation σ and a maximum at $t = 0$. This might be a reasonable description of the dispersion of transmitter release from different boutons. The second possibility assumes an exponential distribution of channel opening times, maximum at $t = 0$ and declining exponentially with time constant τ_r, a reasonable model for the persistence of transmitter action. In either case the mean channel lifetime is taken as τ, and the factor that determines the shape and amplitude of the synaptic response is the ratio σ/τ (for the Gaussian model) or τ_r/τ (for the exponential model). As expected, dispersion of the channel opening times results in a slowing of the

rise-time of the synaptic response, and the rate of rise of the normal synaptic response gives a rough idea of what value of σ/τ or τ_r/τ might be appropriate. At -80 mV the rise-time of the synaptic response between 10% and 90% of the peak amplitude, $t_{0.1-0.9}$, was about 2 ms, and τ_f was about 10 ms, so $t_{0.1-0.9}$ was close to 0.2 τ_f. As can be seen from the Appendix (Figure 13) this would correspond to $\sigma/\tau \simeq 0.1$ (for the Gaussian distribution of opening times) or $\tau_r/\tau \simeq 0.2$ (for an exponential distribution). From this can be calculated the expected effect on peak amplitude of reducing τ, if the amplitude of each individual event stays constant. At -80 mV 10 μM hexamethonium reduced τ_f on average to 51% of the control value. Thus, σ/τ would increase from 0.1 to 0.2, and this would reduce the peak amplitude to $0.66/0.81 = 84\%$ of the control value (see Figure 13). On the basis of the exponential distribution, the same calculation predicts a reduction to 81% of the control amplitude. The actual reduction was 50% (Figure 10). A similar discrepancy exists for the other hexamethonium concentrations studied. Thus, though the dispersion effect may well contribute somewhat to the reduction in e.s.c. amplitude, it cannot account for the whole effect. One piece of evidence, nevertheless, that seems to point to such a mechanism is that m.s.c. amplitude appears to be much less affected by hexamethonium than e.s.c. amplitude, which would be expected if temporal dispersion of individual quanta were appreciable. However, as has been stressed, the measurement of m.s.c. amplitude is not highly reliable, and this evidence should not carry too much weight. Similarly, other hypotheses based on the apparent insensitivity of m.s.c. amplitude to hexamethonium (e.g. that hexamethonium has a presynaptic action, or that the receptors acted on by potassium-evoked quanta behave differently from those contributing to the e.s.c.) are too far-fetched to rest on this evidence alone.

It therefore seems likely that hexamethonium has an action in addition to its channel blocking effect, that causes the voltage-dependent reduction in e.s.c. amplitude. Support for such an action of hexamethonium also comes from our earlier study of carbachol responses of submandibular ganglion cells (Ascher *et al.*, 1979). In voltage-jump experiments it was found that the 'instantaneous' current reached within a few milliseconds after a hyperpolarizing jump in the presence of hexamethonium was smaller than expected from the magnitude of the inward current flowing immediately before the jump, implying that a rapid increase in the blocking action of the drug had occurred, preceding the slow relaxation associated with the channel blocking reaction. Furthermore, the equilibrium level of block produced by hexamethonium showed less dependence on agonist concentration than was predicted from an exclusive action on open channels, and Ascher *et al.* (1979) calculated that the drug might also block closed channels or receptors, the dissociation constant for this effect being roughly 5 μM at -80 mV, which is close to the EC_{50} for reduction of e.s.c. amplitude at this potential (Figure 10). Furthermore, Ascher *et al.* (1979) found that, whereas the blocking action of tubocurarine increased progressively during a response to ionophoretic carbachol application lasting a few seconds (as expected for open channel block), this was not evident with hexamethonium even though the kinetic parameters for open channel block were very similar to those of tubocurarine. Thus, a substantial component of the voltage-dependent blocking action of hexamethonium appeared to exist before the agonist acted, as has now been found with synaptic currents.

This 'secondary' action of hexamethonium could, of course, be a receptor blocking effect, but its steep voltage-dependence makes this a little unlikely. At the neuromuscular junction, competitive antagonists show little or no voltage-dependence in their action (Jenkinson, 1960; Colquhoun *et al.*, 1979; Colquhoun & Sheridan, 1981) and to show voltage-dependence it would be necessary for the binding of the antagonist to show more voltage-sensitivity than that of the agonist, which is an improbable, though not impossible, condition. If, in the ganglion, the voltage-dependent effect of hexamethonium is attributed to receptor block, a different site of action would need to be postulated for the non-voltage-dependent blocking effect of trimetaphan and surugatoxin. It seems more economical to postulate that these drugs are acting on receptors, but not hexamethonium.

Could the 'secondary' hexamethonium effect be due to an action on closed channels? The speed with which the block adjusts to changes in membrane potential requires that the rates of association and dissociation are much faster than for open channel block, from which recovery in the absence of agonist appears to take tens of seconds (A.M. Gurney & H.P. Rang, unpublished results). Other examples of channel blocking drugs so far studied, including the interaction of hydrophilic local anaesthetics with axonal sodium channels (see, for example, Hille, 1977), have shown that they associate and dissociate much faster when the channel is open than when it is closed, and it would be far-fetched to postulate the reverse state of affairs with hexamethonium. One speculative hypothesis that would account for most of the observed phenomena is to suppose that hexamethonium can affect closed channels by binding close to the mouth of the channel. The superficial situation of this site would account for its rather shallow voltage-dependence (Figure 4) compared with the open-channel site, and would also explain

the fast onset and offset rate constants. When the channel opens, the hexamethonium might move to a site deeper within the membrane (hence showing steeper voltage-dependence) from which dissociation is much slower. A testable prediction of this model is that it should be possible to saturate the superficial sites with hexamethonium and then apply agonist to allow the hexamethonium molecules to reach the deeper sites without any ionic current flowing through the channels. If, on the other hand, the secondary action actually prevents channels from being opened by agonist, then it should also prevent open channel block from developing.

The conclusion that hexamethonium acts on the channel rather than the receptor clearly conflicts with the evidence of van Rossum (1962), McIsaac & Millerschoen (1963) and Flacke & Fleisch (1970) who observed a parallel shift of the log dose-effect curve for ACh or other ganglion stimulants in the presence of hexamethonium. The reason for the discrepancy may be that these studies involved indirect measurements of ganglion stimulating activity, and the membrane potential of the ganglion cells was not controlled. The voltage dependence of hexamethonium's action means that agonist-induced depolarization will tend to oppose its blocking effect, thus mimicking a competitive interaction.

APPENDIX

THE AVERAGE TIME COURSE OF NON-SYNCHRONOUS EXPONENTIAL EVENTS: THE EFFECT OF TEMPORAL DISPERSION OF CHANNEL OPENING TIMES ON CHARACTERISTICS OF SYNAPTIC CURRENTS

D. COLQUHOUN & H.P. RANG

If we suppose that the overall response consists of the sum of a large number of small non-synchronous exponential events, then the time course of the overall response can be predicted as shown below. Each event might represent a single m.s.c., with the temporal dispersion reflecting the variation in time of release of individual quanta. Alternatively, each event might correspond to a single channel, which is legitimately represented as an exponential rather than a square wave, provided that channel lifetime is exponentially distributed. In this case the temporal dispersion would reflect that variation in the time at which individual channels open which would be expected if, for example, the transmitter persisted for an appreciable time in the synaptic cleft.

It may be noted that a formally identical problem arises when roughly exponential events, such as miniature postsynaptic currents are averaged. This is commonly done, to increase precision, after a number of separate events have been recorded. There is no unambiguous method of deciding how to line up the events before they are averaged, so there will effectively be some scatter in the time origin of the events. The consequent distortion in the averaged event can be predicted by the method to be described.

A single event will be represented by an exponentially decaying curve with time constant τ, amplitude y_0 and an instantaneous rising phase, i.e.

$$\begin{aligned} y(t) &= y_0 \exp[-(t-t_0)/\tau] \quad \text{for } t > t_0 \\ y(t) &= 0 \qquad\qquad\qquad\quad \text{for } t < t_0 \end{aligned} \tag{A1}$$

where

- y_0 is the amplitude of a single event,
- τ is the time constant,
- t is time,
- t_0 is the time at which the event occurred (which is taken to be a variable).

The average value of y as a function of t is given by

$$\mathrm{E}[y(t)] = \int_{t_0 \to -\infty}^{t_0 = t} y_0 \exp[-(t-t_0)/\tau] f(t_0) \mathrm{d}t_0 \tag{A2}$$

where $f(t_0)$ is the probability density function of the variable t_0. Since each event contributes nothing until $t > t_0$, it is appropriate to integrate over the range $t_0 = -\infty$ to $t_0 = t$, rather than from $-\infty$ to $+\infty$.

Two forms of the probability density function, $f(t_0)$ will be considered, a symmetrical Gaussian distribution and an exponential distribution, which represents an extreme positively-skewed distribution. Physiologically, something between the two is probably more realistic, but these two examples represent useful limiting cases.

Gaussian distribution of t_0

Suppose that t_0 has a mean equal to zero, and standard deviation σ. The Gaussian distribution is then represented by

$$f(t_0) = \frac{1}{\sigma\sqrt{2\pi}} \exp(-t_0^2/2\sigma^2) \tag{A3}$$

Equation A2 thus becomes

$$\mathrm{E}[y(t)] = \frac{y_0}{\sigma\sqrt{2\pi}} \mathrm{e}^{-t/\tau} \int_{t_0=-\infty}^{t_0=t} \exp\left(\frac{t_0}{\tau} - \frac{t_0^2}{2\sigma^2}\right) \mathrm{d}t_0 \tag{A4}$$

which gives, on integration

$$\begin{aligned} \mathrm{E}[y(t)] = 0.5\, y_0 \exp(-t/\tau + \sigma^2/2\tau^2) \\ [1 + \mathrm{erf}(t/\sigma\sqrt{2} - \sigma/\tau\sqrt{2})] \end{aligned} \tag{A5}$$

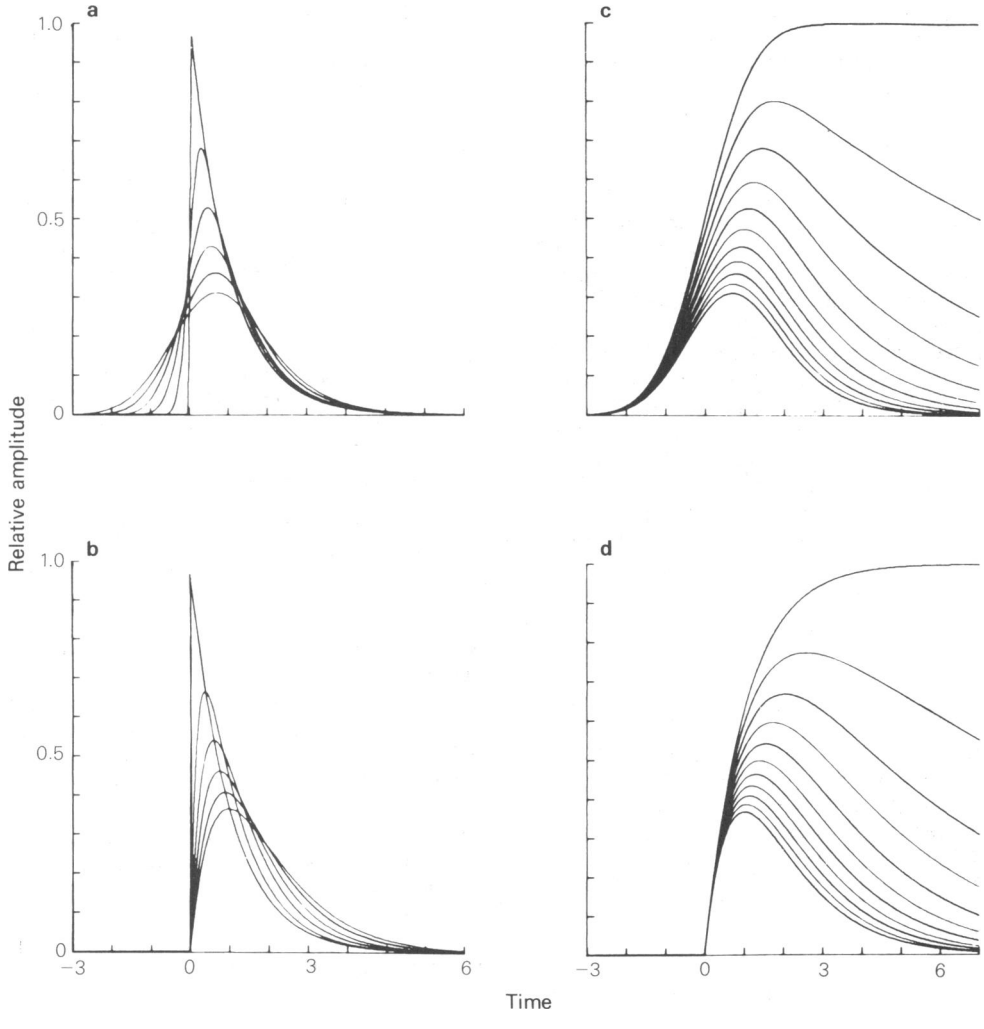

Figure 12 Theoretical curves representing the time course of overall synaptic responses when there is temporal dispersion of the time of occurrence of individual event (t_0) each of which rises instantaneously to a peak at t_0 and decays exponentially with time constant τ. In (a) and (c), the distribution of t_0 is assumed to be Gaussian, with a peak at time zero and standard deviation σ, and the curves are obtained by evaluating equation (A5). In (b) and (d), the distribution of t_0 is assumed to be exponential, with peak at time zero and time constant τ_r, and the curves are obtained by evaluating equation (A8). The degree of dispersion of t_0 with respect to the decay time constant τ is described by $R = \sigma/\tau$ for the Gaussian distribution and $R = \tau_r/\tau$ for the exponential distribution. In (a) and (b), the sharpest curve respresents $R = 0$, and the succeeding curves represent $R = 0.2, 0.4, 0.6, 0.8$ and 1.0. In (c) and (d) the top curve represents $R = 0$ and succeeding curves represent increments of 0.1, the lowest curve representing $R = 1.0$.

In (a) and (b) the time scale is normalized to the decay time constant τ and the curves show the effect of increasing the scatter in t_0 on the overall response. In (c) and (d) the time scale is normalized to σ or τ_r, and the curves show the effect of reducing the decay time constant in the presence of a fixed degree of scatter in t_0.

Exponential distribution of t_0

For this model,

$$f(t_0) = \frac{1}{\tau_r} \exp(-t_0/\tau_r) \qquad \text{(A6)}$$

where τ_r is, for example, the mean interval between the arrival of the nerve impulse at $t = 0$ and the occurrence of the event (τ_r is the 'time constant' of the exponential probability distribution, and also its standard deviation).

Equation A2 then becomes

$$E[y(t)] = \frac{y_0}{\tau_r} e^{-t/\tau} \int_{t_0=0}^{t_0=t} \exp\left[t_0\left(\frac{1}{\tau} - \frac{1}{\tau_r}\right)\right] dt_0. \qquad \text{(A7)}$$

Note that because no event occurs before $t_0 = 0$, this is now the lower limit of the integral.

Equation (A7) gives, on integration

$$E[y(t)] = \frac{y_0}{(1-\tau_r/\tau)} [\exp(-t/\tau) - \exp(-t/\tau_r)] \qquad \text{(A8)}$$

Interpretation of results

The predicted time course of the overall response is given by equations (A5) and (A8). They are plotted in Figure 12 for a range of values of σ/τ for the Gaussian distribution, or (analogously) τ_r/τ for the exponential distribution. The response is plotted against time normalized in the form $T = t/\tau$ in Figure 12(a,b), or against time normalized as $T = t/\sigma$ in Figure 12(c,d).

The graphs in Figure 12(a,b) are appropriate when the time constant, τ, of the fundamental event is regarded as fixed, and one wishes to consider the effect of varying σ (or τ_r). This would be appropriate for prediction of, for example, the effect of various degrees of uncertainty in the time origin on the averaging of a constant exponential event. The curves with the sharp peaks in Figure 12(a,b) show the undistorted exponential event (equation A1), seen when there is no scatter ($\sigma = 0$ or $\tau_r = 0$). Increasing scatter (i.e. increasing σ/τ or τ_r/τ) decreases the amplitude and increases the rise time (the area is unaffected).

The graphs in Figure 12(c,d) are appropriate when, as in the present work, the scatter (σ or τ_r) of the time origin is regarded as fixed (it is supposed to be a characteristic of the transmitter release process), and one wishes to see the effect of varying the time constant, τ, of the fundamental synaptic response. Reducing the time constant for the decay of each event (i.e. increasing σ/τ or τ_r/τ) again reduces the peak amplitude, as expected.

The magnitude of the effect on peak response is rather similar for the Gaussian and exponential models, as shown in Figure 13(a).

Increasing the scatter of the time of occurrence of individual events also prolongs the rise-time of the response, and Figure 13(b) shows the interval between 10% and 90% of peak amplitude, expressed relative to the decay time constant, τ, for the two models.

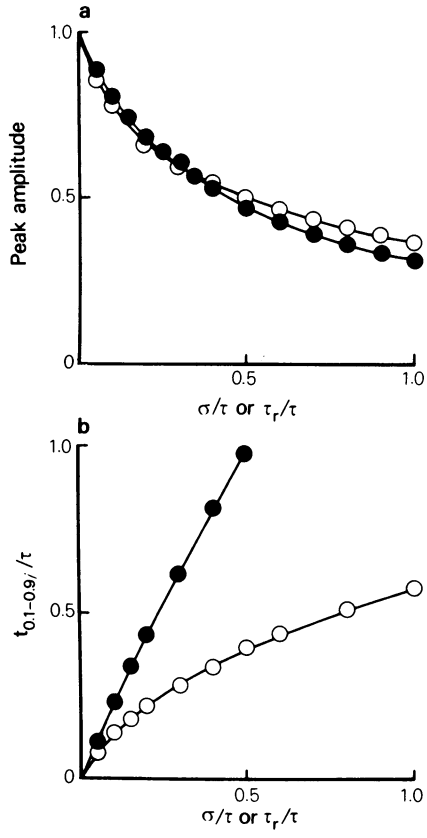

Figure 13 (a) Peak amplitude plotted as a function of R for the Gaussian (●) and exponential (○) models. The peak amplitude is expressed relative to the amplitude that would be recorded if the events were strictly synchronous (i.e. if $R = 0$). (b) Rise-time, expressed as the time taken for the signal to rise between 10% and 90% of its peak amplitude ($t_{0.1-0.9}$) as a function of R for the Gaussian (●) and exponential (○) models.

For normal e.s.cs $t_{0.1-0.9}$ was about 0.2 τ_f, corresponding to $R \simeq 0.1$ for the Gaussian model or $R \simeq 0.2$ for the exponential model.

Experimentally (see Results) $t_{0.1-0.9}/\tau$ was about 0.2 under control conditions, which would correspond to $\sigma/\tau \simeq 0.1$ for the Gaussian model or $\tau_r/\tau \simeq 0.2$ for the exponential model. The expected effect on e.s.c. amplitude of reducing τ (without changing the number or amplitude of the individual events can be ascertained from Figure 13. Thus, for the Gaussian model, the control amplitude ($\sigma/\tau = 0.1$) is 0.81; halving the decay time constant ($\sigma/\tau = 0.2$) reduces the amplitude to 0.68 (i.e. by 16%). A similar calculation for the exponential model shows that halving τ would reduce the amplitude by 19%.

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