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The Effects of Chloramphenicol Isomers on the Motor End-plate Nicotinic Receptor-Ion Channel Complex

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SUMMARY

Four enantiomers of chloramphenicol have been tested for their effects on end-plate current and miniature end-plate current decay and amplitude characteristics in the voltage-clamped cutaneous nerve-muscle preparation of the garter snake. All four enantiomers exhibited effects on end-plate current and miniature end-plate current decay at similar concentrations (0.2–1.0 mM), indicating that the measured effect was not related to the antibacterial action of the compounds in which D-*threo* chloramphenicol is known to be at least 50 times more powerful than the L-*threo* and D- and L-*erythro* isomers. The compounds slightly increased end-plate current but not miniature end-plate current amplitude, indicating that they produce an increase in end-plate current quantal content. This effect was verified by an analysis of end-plate current driving functions (see Appendix) for one of the chloramphenicol isomers. In addition to this presynaptic action, all four compounds converted end-plate current and miniature end-plate current decays from single to double exponential functions. This effect was both concentration and voltage dependent. For all four compounds, hyperpolarization resulted in a progressive decrease in τ_f and an increase in τ_s. The relative amplitudes of the fast and slow decay components were independent of membrane potential. The results are interpreted in terms of the drugs blocking the open form of the acetylcholine-activated receptor-ion channel complex. However, in addition to affecting decay characteristics, all four compounds increased the charge passed during both end-plate and miniature end-plate currents. This effect was concentration but not voltage dependent and is inconsistent with the predictions of the sequential model for open ion channel blockade. By using an extension of Ruff's analysis of the sequential model of open end-plate ion channel blockade, we have been able to show that the action of the chloramphenicols on end-plate current amplitude and time course can be explained by the combination of two distinct mechanisms. First is an open channel block conforming to the sequential model and with calculated channel blocking affinity constants ranging from 0.3–1.0 mM. The channel blocking actions of all four isomers were shown to be independent of membrane voltage. Second is an action to slow channel closing, resulting in prolonged open time and hence increased charge passed during the end-plate current. This effect was strongly concentration dependent, but not voltage dependent. We suggest that this action is due to the lipid-soluble chloramphenicols dissolving within the membrane and causing a change in the dielectric constant which leads to a hindering of the conformational change in molecular structure underlying channel closure. Failure to take any effect of channel blocking drugs on closing characteristics into account can lead to inaccurate estimates of the kinetics of the channel block. Despite the deviation of the actions of the compounds from the predictions of the sequential model for end-plate ion channel block, it is clear from the results that the channel binding site is unable to distinguish between D- and L- forms of either *threo* or *erythro* chloramphenicol. It is therefore concluded that the channel binding site is not stereoselective in nature.

In therapeutic doses, three classes of antibiotic agents have been found to interfere with neuromuscular transmission in man (see Refs. 1–3 for reviews). Recent work has shown that neomycin (4), polymyxin B (5, 6), lincomycin and clindamycin (7, 8), and, to a lesser extent, streptomycin (9) alter the decay rates of EPCs and MEPCs in a concentration-dependent manner. Such an action on decay rates has been interpreted in terms of the drugs blocking the open form of the end-plate receptor-coupled ion channel, although the contribution of the channel blocking action of these antibiotics, which occurs at very high concentrations, to their overall neuromuscular blocking activity has not been determined.

Lincomycin and clindamycin are known to exert their antibacterial action by binding to the 50 S subunit of bacterial ribosomes leading to an inhibition of the peptidyl transferase reaction (10). Binding at the 50 S ribosome appears to be a specific receptor-mediated process as the binding of erythromycin which also acts at this site can be completely inhibited by lincosamides (10). In order to ascertain if there are any common features in the binding of antibiotics to bacterial ribosomes and to end-plate ion channels, we have now investigated chloramphenicol, which binds to the 50 S ribosomal

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ABBREVIATIONS: EPC, end-plate current; MEPC, miniature end-plate current; ACh, acetylcholine.

site in a stereoselective manner, as only one of its four possible isomers (D-*threo* chloramphenicol) possesses any appreciable antibacterial activity (11). Study of the four isomers of chloramphenicol affords an opportunity to test the relationship between binding to the ion channel and binding to the bacterial ribosomal site and, in addition, should provide information on the stereoselectivity of the end-plate channel binding site.

The four compounds used were D- and L-*threo* chloramphenicol and D- and L-*erythro* chloramphenicol. Both D- and L-isomers of each of the *threo* and *erythro* forms of chloramphenicol were shown to possess end-plate ion channel blocking activities in the same concentration range. The results demonstrate the absence of true stereospecificity of the end-plate channel binding site and the lack of correspondence between channel blocking and antibacterial activities in this series of agents. All four compounds increased the charge passed during EPCs and MEPCs, suggesting that their end-plate ion channel blocking actions deviate from the predictions of the sequential model of open ion channel block. A kinetic analysis has shown that, in addition to blocking the channels, the drugs also affect the normal channel closing rate. The influence of this additional action of the drugs on the calculations of blocking and unblocking rate constants and affinity constants has been investigated.

Materials and Methods

Electrophysiological techniques. All experiments were carried out *in vitro* at room temperature (18–22°) on twitch muscle fibers of the costocutaneous muscle of the garter snake, *Thamnophis sirtalis*, using the two-microelectrode voltage clamp technique. In experiments in which EPCs were recorded, an approximately 20-mm length of motor nerve supplying the muscle was isolated. The muscles were mounted in physiological solution maintained at pH 7.1–7.2 containing (mM): NaCl, 159; KCl, 4.2; $CaCl_2$, 2; and HEPES buffer (*N*-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), 1.0. To eliminate muscle contraction during experiments in which nerve-evoked EPCs were recorded, a cut muscle preparation was used as described previously (8). In some experiments in which EPCs were too large in amplitude to allow adequate voltage clamping, the calcium concentration was lowered to 1.0–1.5 mM. No differences in EPC decay rates were observed in the different levels of calcium. MEPCs were recorded in uncut muscle preparations.

Microelectrodes were filled with 3 M KCl and had resistances of 3–8 Mohm. During evoked EPCs adequacy of voltage clamping was assessed from the voltage deviation, less than 1% of the driving force (holding potential-reversal potential) being regarded adequate. The reversal potential was assumed to be −5 mV (8). The motor nerve was stimulated at a frequency of 0.5 Hz with rectangular pulses of 0.05 msec duration and of strength greater than that required to elicit EPCs.

Collection and analysis of EPC and MEPC data. EPCs and MEPCs were passed through a low pass (DC, 5 kHz) filter and recorded on an FM tape recorder (Racal 4DS, DC, 5 kHz) for later analysis. Signals from the tape recorder were amplified and sampled every 40 μsec by an analog-to-digital converter interface unit (Cambridge Electronic Design 502) and analyzed by a laboratory minicomputer (PDP 11/23, Digital Equipment Corp.). Peak amplitude and decay characteristics of individual currents were measured by an EPC analysis program (12) and, subsequently, series of currents (10–20 for EPCs, 11–178 for MEPCs) were aligned at the midpoint of the rising phase, averaged, and reanalyzed. Rise times were calculated from the averaged signal as the time from 5–95% of the peak current. At maximum gain the rise time of the voltage clamp was 0.07 msec (10–90% peak).

In the presence of the chloramphenicol isomers, EPCs and MEPCs with biphasic decays were recorded. These biphasic currents were fitted to the sum of two exponentials by an iterative nonlinear least squares procedure (13) that fitted the currents from 95% of the peak to the baseline. There was no evidence for the existence of more than two exponentials on the decaying phase of EPCs recorded in the presence of the chloramphenicol isomers.

The total charge passed by the channels during EPCs or MEPCs was assessed from the area under the averaged currents. Fitted exponentials were integrated as follows for single exponentials:

$$\text{Area} = \int (Ie^{-t/\tau})dt = I\tau \tag{1}$$

and for double exponentials:

$$\text{Area} = \int (I_f e^{-t/\tau_f} + I_s e^{-t/\tau_s})dt$$
$$\text{Area} = I_f\tau_f + I_s\tau_s \tag{2}$$

In some experiments EPC and MEPC driving functions were derived as described in detail in the Appendix.

Drug application. The drugs used were D-*threo*, L-*threo*, D-*erythro*, and L-*erythro* chloramphenicol, supplied by Warner Lambert-Parke Davis, Pontypool, Gwent, Wales. The compounds were all soluble in the normal physiological solution used.

After a series of control measurements in normal physiological solution, the solution containing the chloramphenicol isomer (0.2–1.0 mM) was introduced into the muscle chamber at an approximate rate of 5 ml/min for 5 min. After a further minimum time of 5 min equilibration, measurements were made.

In most experiments on EPCs, three concentrations of drug were used at the same end-plate. Only one end-plate per muscle was used and only one drug per end-plate. All results in the text and tables are expressed as mean ± standard error.

Results

Effects of chloramphenicol isomers on the amplitude and time course of EPCs and MEPCs. In control physiological solution, EPCs and MEPCs decayed with time courses that could be fitted by a single exponential function:

$$I(t) = I(0)e^{-t/\tau} \tag{3}$$

where $I(0)$ is the peak current amplitude, $I(t)$ is the current amplitude at time t after the peak, and τ is the time constant of current decay. The decay time constant was dependent on membrane potential according to the relationship:

$$\tau(V) = \tau(0)e^{-V/H} \tag{4}$$

where $\tau(V)$ is the decay time constant at a membrane potential V, $\tau(0)$ is the decay time constant at a membrane potential of 0 mV, and H is the characteristic change in membrane potential required to produce an e-fold change in τ.

All four chloramphenicol isomers converted MEPC and EPC decays from single to double exponential functions. The initial decay rate was faster and the subsequent decay rate slower than that of control and could be described by the sum of two exponential functions:

$$I(t) = I_s e^{-t/\tau_s} + I_f e^{-t/\tau_f} \tag{5}$$

where I_f and I_s, and I_f and τ_s are the amplitudes and time constants respectively of the fast and slow components.

At the lower end of the concentration range used of all four chloramphenicols, i.e., the concentrations that produced only small effects on current decays, EPC amplitudes were increased in most experiments whereas MEPC amplitudes were unaffected or slightly reduced (Table 1), suggesting that the compounds were producing a small increase in quantal content. However, we considered this measure of quantal content to be

somewhat inaccurate due to the possibility of rapid end-plate channel block occurring during the rising phase of the EPC, which would truncate the peak amplitude and hence reduce any apparent increase in quantal content.

Accordingly, we used the EPC driving function, which is a measure of the rate of opening of the end-plate channels (see Appendix for details), to obtain a better index of quantal content. Its time course is dependent on the rate of release of transmitter and its subsequent binding to receptors and eventual breakdown. It should remain unaltered in the presence of a drug which acts only by block of the open end-plate channel, and hence any changes measured reflect changes in the amount of transmitter released, or changes in the number of available receptors, i.e., postjunctional block.

We have derived driving functions from control EPCs and MEPCs recorded in the presence of L-*erythro* chloramphenicol (0.5 mM), as an example. The drug markedly increased the size of the driving function (Fig. 1), indicating an increase in quantal content. To obtain a more quantitative measure of the increase in quantal content we have resynthesized a single exponentially decaying EPC from the drug-treated driving function by convoluting the driving function with an exponential function, as described in the Appendix. The resultant simulated EPC is shown superimposed on the measured control EPC in Fig. 1. It can be seen that the simulated EPC is approximately 72% greater in amplitude than the measured EPC, indicating a substantial increase in quantal content. In contrast, superimposition of the measured control and double exponential decaying drug-treated EPCs (Fig. 1) shows only a small increase in amplitude (13%), thus greatly underestimating the actual increase in quantal content produced by the drug. The drug did not increase the driving function from MEPCs.

TABLE 1
Effects of chloramphenicol isomers (0.5 mM) on EPC and MEPC amplitudes measured at −90 mV
The number of experiments is shown in parentheses. EPC and MEPC amplitudes, which were obtained in different experiments, are compared in the last column to give an approximate value for EPC quantal content.

Compound	EPC amplitude	MEPC amplitude	Quantal content
		% control	
D-*threo*	112 ± 12 (3)	73 ± 3 (8)	153
L-*threo*	104 ± 15 (3)	77 ± 3 (6)	135
D-*erythro*	116 ± 8 (6)	89 ± 7 (7)	130
L-*erythro*	117 ± 6 (7)	94 ± 7 (6)	124

Concentration and voltage dependence of the EPC isomers. At a given membrane potential, increasing the concentrations of the chloramphenicol isomer (0.2–1.0 mM) resulted in a progressive increase in τ_s and a decrease in τ_f of EPCs. Increasing concentrations of the isomers produced an increase in I_s relative to I_f and a progressive decrease in EPC rise time. The compounds had no effects on MEPC rise time. The effects of the *threo* isomers on EPCs are shown in Fig. 2 and summarized in Table 2. The effects of the *erythro* isomers were closely similar (Table 2).

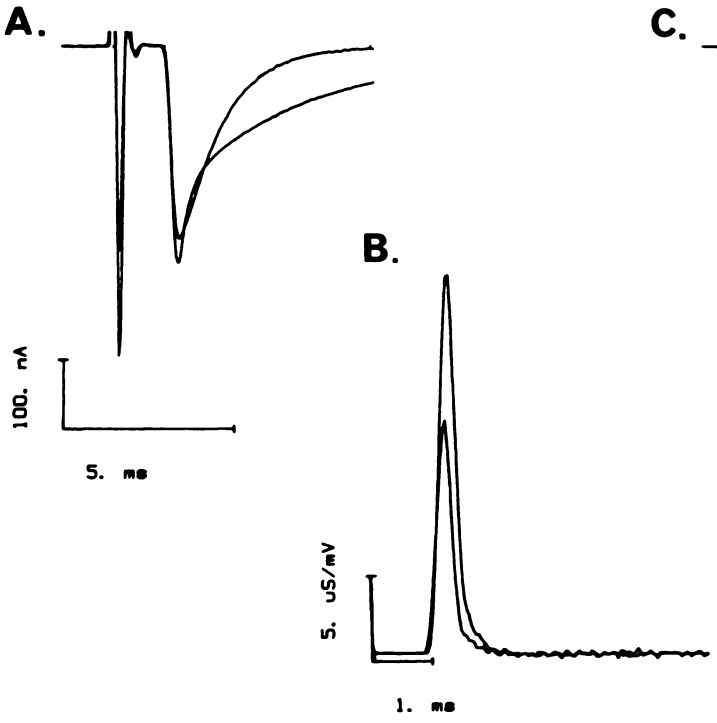

Fig. 1. Effect of L-*erythro* chloramphenicol on EPC driving function. A. Superimposed measured EPCs in the absence (*smaller trace*) and presence (*larger*, double exponentially decaying *trace*) of L-*erythro* chloramphenicol (0.5 mM). Records shown here are computer averages of 10–20 individually evoked EPCs. B. Superimposed driving functions generated from the averaged EPCs illustrated in A, showing the large increase in driving function produced by 0.5 mM L-*erythro* chloramphenicol (*larger trace*). The *smaller trace* represents the control driving function. C. Superimposed single exponentially decaying, simulated EPCs generated from the EPC driving functions shown in B. The smallest EPC is generated from the control driving function. It is precisely superimposable on the measured control EPC shown in A. The larger trace is generated from the driving function derived from EPCs in the presence of L-*erythro* chloramphenicol (0.5 mM). This EPC has been generated with the same decay time constant as the control EPC and therefore represents the effects of the drug on EPCs after subtracting all its postjunctional actions on end-plate channels. It can be seen that the increase in the simulated EPC amplitude produced by the channel blocking drug is much greater than that in the measured EPCs shown in A. *Calibration bars* as in A.

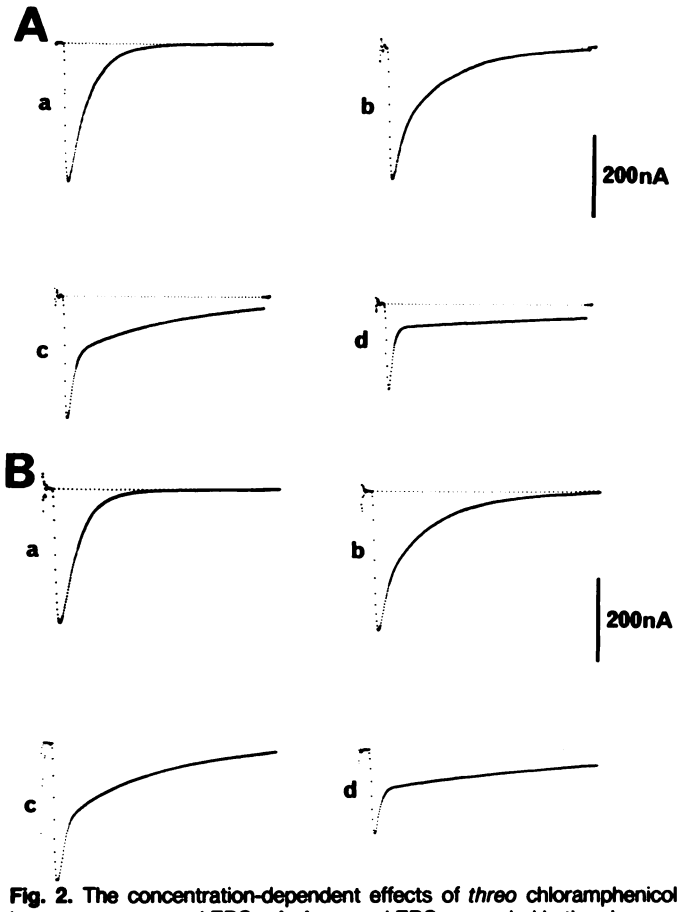

Fig. 2. The concentration-dependent effects of *threo* chloramphenicol isomers on averaged EPCs. A. Averaged EPCs recorded in the absence (a) and in the presence of D-*threo* chloramphenicol, 0.2 mM (b), 0.5 mM (c), and 1.0 mM (d). Records are from the same end-plate voltage clamped at −90 mV. B. Averaged EPCs recorded in the absence (a) and in the presence of L-*threo* chloramphenicol, 0.2 mM (b), 0.5 mM (c), and 1.0 mM (d). All records represent 20-msec samples of the EPCs. Both compounds converted the single exponential control EPC decay into a biphasic decay. with one component faster and the other slower than the control decay rate. Decays were slightly faster in the presence of the L-*threo* analog than in the D-*threo* analog. Mean values of τ for several end-plates are shown in Table 2.

For each concentration used, membrane hyperpolarization resulted in a progressive increase in τ_s of EPCs but had no major effect on τ_f. Hyperpolarization resulted in no change in the ratio of I_s/I_f. Similar results were observed on MEPCs at 0.5 mM, a concentration high enough to produce biphasic MEPCs at all holding potentials studied yet low enough to avoid marked depression of MEPC amplitude. The results (Table 3) showed that, as for EPCs, the effect of D- and L-*threo* chloramphenicol were closely similar and that hyperpolarization again resulted in no change of I_s/I_f ratio. Similar results were obtained with D and L-*erythro* chloramphenicol (Table 4).

Plots of τ versus membrane potential for both EPCs and MEPCs showed that τ_f became less dependent on membrane voltage than in control with increasing concentrations of each isomer. In contrast, although increasing concentrations of each isomer progressively increased τ_s of EPCs, the voltage dependence of τ_s remained similar to or tended to become slightly greater than that of control (Fig. 3).

Evidence of deviation from the sequential model of channel block. The concentration- and voltage-dependent effects of drugs described above are normally interpreted in terms of the drugs blocking the open form of the receptor-ion channel complex (14–16). However, we made observations that suggested that the channel blocking action of the compounds might not fit precisely with the predictions of the sequential model of block, in which the blocked form of the channel returns to the open form before finally closing or being reblocked (15–17). According to this model the total charge passed during an EPC or MEPC should be the same in the presence of a channel blocker as in its absence (18). In our experiments we observed that, although there was little change in peak EPC amplitude in the presence of the *threo* chloramphenicols, the slow phase of decay contributed a particularly large component to the EPC. To assess this we measured the total charge passed by the channels during EPCs and MEPCs by integrating the fitted exponentials.

The charge passed was normalized by dividing by the driving force. Such integration showed that in the presence of both the *threo* and *erythro* isomers the total charge passed during the EPC was substantially greater than in control (Fig. 4). The

TABLE 2

The concentration-dependent effects of the four chloramphenicol isomers on EPC amplitude and time course

Values shown are for end-plates voltage-clamped at −90 mV.

Drug concentration	N	Rise time	Peak I	I_s/I_f	τ_f	τ_s
(mM)		msec	% control		msec	
D-*threo*						
0	6	0.36 ± 0.02	100		1.72 ± 0.09[a]	
0.2	5	0.35 ± 0.04	104 ± 11	1.39 ± 0.10	0.72 ± 0.02	4.2 ± 0.2
0.5	3	0.31 ± 0.02	112 ± 12	0.67 ± 0.05	0.47 ± 0.01	9.9 ± 0.5
1.0	4	0.28 ± 0.02	90 ± 14	0.31 ± 0.01	0.36 ± 0.01	28.1 ± 2.6
L-*threo*						
0	5	0.37 ± 0.02	100		1.36 ± 0.05[a]	
0.2	5	0.33 ± 0.03	97 ± 4	1.21 ± 0.11	0.60 ± 0.04	4.1 ± 0.2
0.5	3	0.28 ± 0.02	104 ± 15	0.82 ± 0.07	0.41 ± 0.02	9.0 ± 0.7
1.0	4	0.28 ± 0.06	59 ± 15	0.71 ± 0.15	0.35 ± 0.02	18.0 ± 1.1
D-*erythro*						
0	14	0.42 ± 0.01	100		1.62 ± 0.07[a]	
0.3	6	0.37 ± 0.03	102 ± 5	2.19 ± 0.27	0.87 ± 0.16	3.2 ± 0.2
0.5	6	0.33 ± 0.03	116 ± 8	1.62 ± 0.17	0.51 ± 0.04	5.4 ± 0.3
1.0	6	0.32 ± 0.01	112 ± 8	0.70 ± 0.04	0.38 ± 0.01	10.9 ± 0.4
L-*erythro*						
0	8	0.38 ± 0.02	100		1.47 ± 0.07[a]	
0.2	5	0.34 ± 0.02	111 ± 3	1.84 ± 0.35	0.98 ± 0.11	2.7 ± 0.1
0.5	7	0.31 ± 0.02	117 ± 6	1.57 ± 0.21	0.38 ± 0.03	4.3 ± 0.3
1.0	4	0.29 ± 0.02	107 ± 9	0.90 ± 0.11	0.26 ± 0.02	9.4 ± 0.7

[a] Control value of τ.

TABLE 3
The voltage-dependent effects of D- and L-*threo* chloramphenicol (0.5 mM) on EPC and MEPC amplitude and time course

Compound	V_m	Peak I	I_s/I_f	τ_f	τ_s
	mV	*% control*			*msec*
			EPCs		
D-*threo* (n = 3)	−70	119 ± 13	0.74 ± 0.12	0.47 ± 0.01	7.8 ± 0.5
	−90	112 ± 12	0.67 ± 0.05	0.47 ± 0.01	9.9 ± 0.5
	−110	102 ± 11	0.72 ± 0.07	0.47 ± 0.01	12.4 ± 0.8
	−130	96 ± 6	0.77 ± 0.09	0.49 ± 0.01	15.6 ± 1.1
L-*threo* (n = 3)	−70	109 ± 19	0.90 ± 0.05	0.41 ± 0.01	7.0 ± 0.6
	−90	104 ± 15	0.82 ± 0.07	0.41 ± 0.02	9.0 ± 0.7
	−110	98 ± 13	0.90 ± 0.07	0.43 ± 0.01	11.7 ± 0.9
	−130	95 ± 14	1.03 ± 0.13	0.52 ± 0.05	15.9 ± 1.4
			MEPCs		
D-*threo* (n = 8)	−70	76 ± 3	0.53 ± 0.07	0.27 ± 0.04	3.3 ± 0.3
	−90	73 ± 3	0.50 ± 0.05	0.28 ± 0.04	4.7 ± 0.3
	−110	68 ± 3	0.52 ± 0.07	0.27 ± 0.01	5.9 ± 0.3
	−130	67 ± 6	0.46 ± 0.06	0.24 ± 0.03	7.6 ± 0.4
L-*threo* (n = 6)	−70	80 ± 5	0.44 ± 0.09	0.21 ± 0.04	6.6 ± 0.5
	−90	77 ± 3	0.43 ± 0.07	0.23 ± 0.02	7.8 ± 0.5
	−110	82 ± 4	0.46 ± 0.07	0.24 ± 0.03	9.6 ± 0.9
	−130	84 ± 5	0.50 ± 0.08	0.26 ± 0.04	13.2 ± 1.0

TABLE 4
The voltage-dependent effects of D- and L-*erythro* chloramphenicol (0.5 mM) on EPC and MEPC amplitude and time course

Compound	V_m	Peak I	I_s/I_f	τ_f	τ_s
	mV	*% control*			*msec*
			EPCs		
D-*erythro* (n = 6)	−70	130 ± 10	1.70 ± 0.22	0.49 ± 0.04	3.9 ± 0.2
	−90	116 ± 8	1.62 ± 0.17	0.51 ± 0.04	5.4 ± 0.3
	−110	120 ± 9	1.44 ± 0.14	0.52 ± 0.04	7.1 ± 0.4
	−130	114 ± 7	1.46 ± 0.17	0.57 ± 0.06	9.0 ± 0.4
L-*erythro* (n = 7)	−70	119 ± 8	1.78 ± 0.29	0.41 ± 0.04	3.3 ± 0.2
	−90	117 ± 6	1.57 ± 0.21	0.38 ± 0.03	4.3 ± 0.3
	−110	108 ± 8	1.47 ± 0.14	0.38 ± 0.04	5.8 ± 0.5
	−130	105 ± 6	1.49 ± 0.14	0.39 ± 0.03	7.7 ± 0.5
			MEPCs		
D-*erythro* (n = 7)	−70	97 ± 9	0.82 ± 0.27	0.29 ± 0.08	3.8 ± 0.5
	−90	89 ± 7	0.78 ± 0.23	0.27 ± 0.06	4.5 ± 0.4
	−110	92 ± 8	0.75 ± 0.19	0.29 ± 0.06	5.7 ± 0.5
	−130	92 ± 7	0.87 ± 0.20	0.26 ± 0.06	7.4 ± 0.7
L-*erythro* (n = 6)	−70	94 ± 6	0.92 ± 0.36	0.17 ± 0.04	3.4 ± 0.4
	−90	94 ± 7	1.15 ± 0.52	0.17 ± 0.02	3.4 ± 0.2
	−110	94 ± 5	1.08 ± 0.39	0.21 ± 0.05	4.2 ± 0.1
	−130	92 ± 5	1.16 ± 0.46	0.20 ± 0.04	5.7 ± 0.3

increase in charge passed as a result of the chloramphenicols was concentration dependent. Despite the increase in charge passed, the relationship between charge passed and membrane potential remained similar to that of control EPCs. D-*threo* and both D- and L-*erythro* chloramphenicols produced an approximate doubling of the charge passed at 0.2 mM. The charge increased about 3 times at 0.5 mM and 5 times at 1.0 mM. L-*threo* chloramphenicol produced qualitatively similar results, except that, as a result of the reduction of peak EPC amplitude at 1.0 mM, at this concentration the total charge passed was less than at 0.5 mM.

To further quantify this deviation from the sequential model, we used the equations described by Ruff (15), from which it is possible to predict the rates of the fast and slow components of decay. Thus, Ruff showed that:

$$a_s = (a + F + GC - ((a + F + GC)^2 - 4aF)^{1/2})/2 \quad (6)$$

$$a_f = (a + F + GC + ((a + F + GC)^2 - 4aF)^{1/2})/2 \quad (7)$$

where a is the control decay rate ($1/\tau$), a_s is the decay rate of the slow phase ($1/\tau_s$), and a_f is the decay rate of the fast phase ($1/\tau_f$). F and G are the channel unblocking and blocking rate constants, respectively, and C is the drug concentration. The equations can be rearranged to give the rate constants:

$$F = \frac{(a_f a_s)}{a} \text{ or } \frac{\tau}{\tau_f \tau_s} \quad (8)$$

and

$$GC = \frac{1}{\tau_f} + \frac{1}{\tau_s} - \frac{1}{\tau} - F \quad (9)$$

Ruff (15) also derived an equation predicting the ratio of amplitudes of the exponential decays expected from drugs conforming to the sequential model:

$$\frac{I_s}{I_f} = \frac{(F - a_s)}{(a_f - F)} \quad (10)$$

where I_f and I_s are the amplitudes of the fast and slow exponentials, respectively. By substituting the value of F from Eq. 8 into Eq. 10 we get:

$$\frac{I_s}{I_f} = \frac{\tau - \tau_f}{\tau_s - \tau} \quad (11)$$

From Eq. 11 and the measured decay time contants we have calculated theoretical amplitude ratios and compared them with

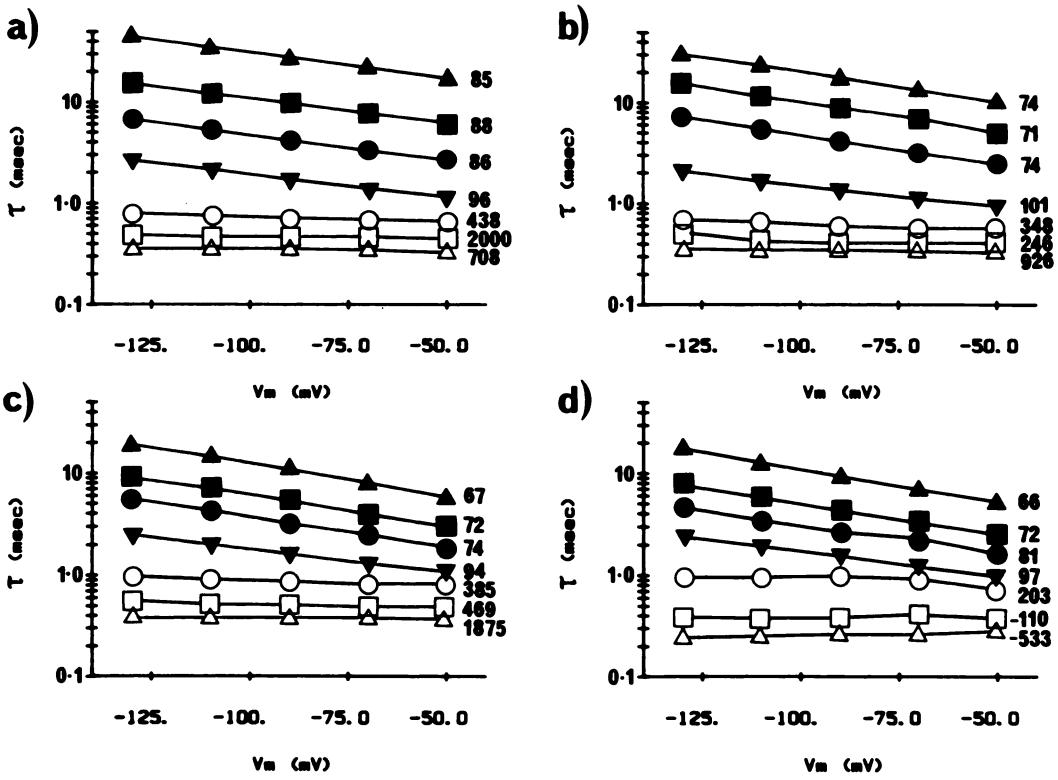

Fig. 3. Semilogarithmic plots of EPC time constants against membrane potential in the presence of the four chloramphenicol isomers. Plots of τ_f (*open symbols*) and τ_s (*solid symbols*) for EPCs against membrane potential (*Vm*) were recorded in the presence of D-*threo* (a), L-*threo* (b), D-*erythro* (c), and L-*erythro* (d) chloramphenicol at concentrations of 0.2 mM (○, ●)[2] 0.5 mM (□, ■), and 1.0 mM (△, ▲). The control decay time constant is shown as *inverted triangles*. *H*-values, denoting the voltage dependence of τ, are shown (in mV) associated with each plot. Results represent the mean of 3–8 measurements.

the measured values. The values for EPC amplitude ratios from three cells voltage clamped in three concentrations of D-*threo* chloramphenicol are shown in Fig. 5 and the values for MEPC amplitude ratios over a range of holding potentials in all four isomers (0.5 mM) are shown in Fig. 6. It can be clearly seen from Figs. 5 and 6 that neither the measured nor the predicted amplitude ratios are voltage dependent and that the predicted values are markedly less than the measured values for both EPCs amd MEPCs. Thus we conclude from this deviation of the amplitude ratios from the predictions of the sequential model that the four chloramphenicol isomers do not fit a strict interpretation of the sequential model for channel block.

Calculation of rate constants. One interpretation of the observed deviation from the predictions of the model is that more than one mechanism of drug effect is involved. Thus, the overall effect could be caused by a conventional type of channel block that conforms to the model plus a second mechanism which results in the deviation. As the observed deviation resulted in an increase in charge passed, one possibility for the effect is an action on the channel open time, i.e., the closing rate. As Eq. 10 makes no assumption as to the closing rate, blocking and unblocking rate constants can be calculated using information obtained solely in the presence of the drug. To do this it is necessary to rearrange Eq. 10 to:

$$F = \frac{I_s\tau_s + I_f\tau_f}{\tau_f\tau_s(I_f + I_s)} \qquad (12)$$

Substituting this value for F in Eq. 8 results in:

$$\tau_{calc} = \frac{I_s\tau_s + I_f\tau_f}{I_f + I_s} \qquad (13)$$

Thus, using measured values of amplitudes and time contants in the presence of drugs producing double exponential decays it is possible to arrive back at a value for τ, i.e., the reciprocal of the channel closing rate in the presence of drug. The value of τ from Eq. 13 is designated τ_{calc}. Calculation of G can be made by substitution of values for F from Eq. 12 and τ_{calc} from Eq. 13 in Eq. 9.

Values for F, G, and K_B, the affinity constant for channel block (F/G), calculated from double exponential decays of MEPCs in the presence of 0.5 mM of each chloramphenicol isomer, are shown in Fig. 7, plotted against membrane potential. None of the calculated rates for any of the four isomers showed a significant dependence on membrane potential. F, G, and K_B values were also calculated from EPC data. Like the values derived from MEPCs, these were independent of membrane potential. In addition, there was no concentration dependence of the rate and affinity constants. A comparison of values from MEPCs and EPCs at 0.5 mM of each of the compounds is shown in Table 5. The results indicate that there are no major differences between the D- and L-isomers of either the *threo* or *erythro* forms of chloramphenicol. In general the *erythro* isomers possessed faster unblocking rates than those of the *threo* isomers. As the *threo* isomers had faster blocking rates than the *erythro* isomers, the calculated affinities for the *erythro* compounds were lower than those for the *threo* isomers.

Effects of the drugs on channel closing. Using the assumptions made in the previous section, i.e., that the drugs produce open channel block conforming to the sequential model, plus a secondary action, we have calculated from Eq. 13 the channel closing rates ($1/\tau_{calc}$) required to make the measured amplitude and decay time constant values fit the sequential model. These values for MEPCs have been compared to the control value from the same end-plates as ratios of τ_{calc}/τ (Table 6). The results show that all four chloramphenicol

[2] In the case of D-*erythro* chloramphenicol the lowest concentration used was 0.3 mM.

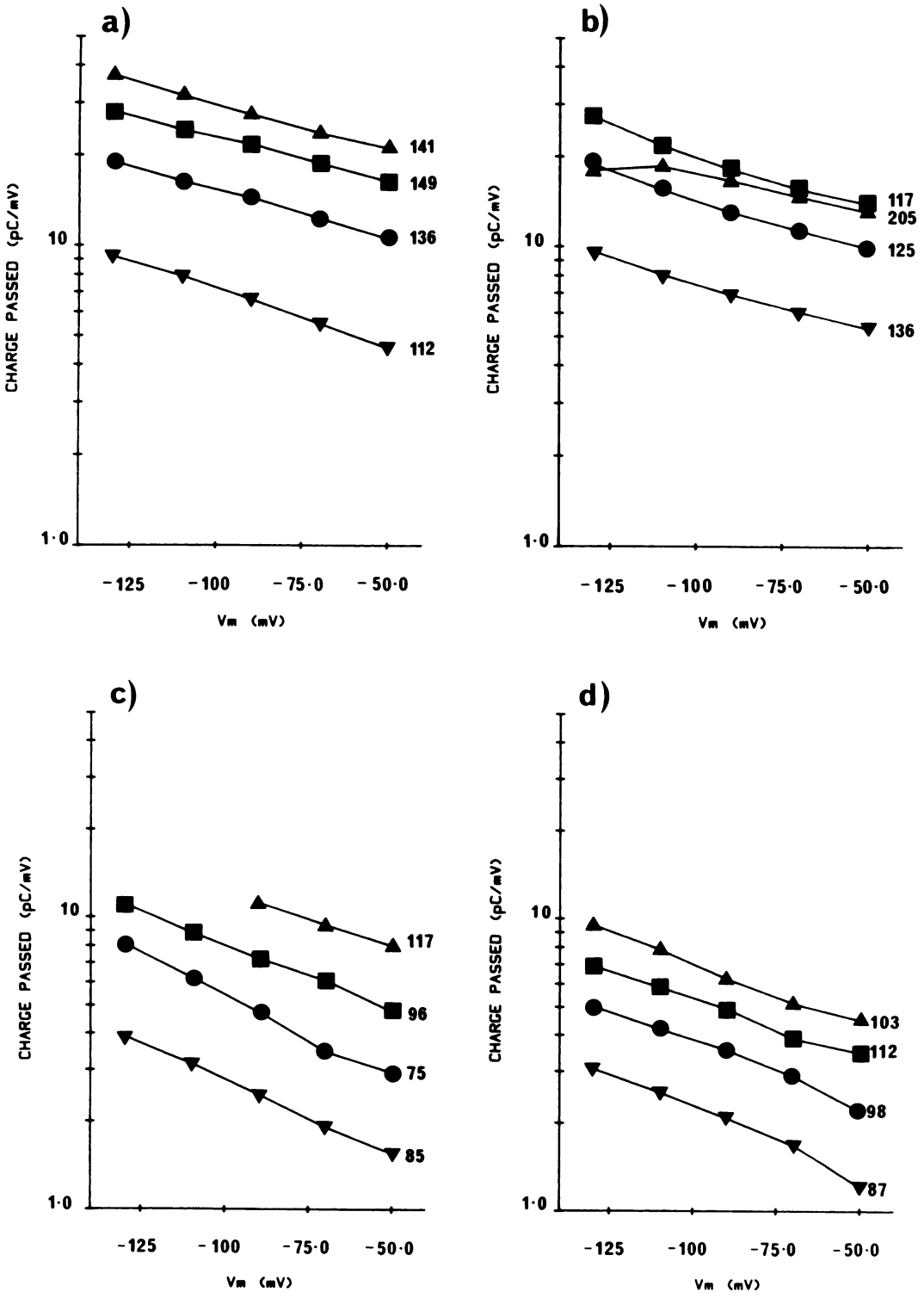

Fig. 4. Semilogarithmic plots of charge passed during EPCs against membrane potential in the presence of the four chloramphenicol isomers. Plots of charge passed derived from integration of EPCs against membrane potential (*Vm*) in the presence of D-*threo* (a), L-*threo* (b), D-*erythro* (c), and L-*erythro* (d) chloramphenicol at concentrations of 0.2 mM (●),[2] 0.5 mM (■), and 1.0 mM (▲). Control charge passed is shown as *inverted triangles*. Values denoting the voltage dependence of charge passed are shown (in mV) associated with each plot. Note the similarity of the control voltage dependence values to those for the voltage dependence of τ shown in Fig. 3. Note also the concentration-dependent increase in charge passed in the presence of D-*threo* and D- and L-*erythro* chloramphenicol, but the lesser increase with 1.0 mM L-*threo* chloramphenicol compared to that produced by 0.5 mM of the same isomer. This highest concentration of L-*threo* chloramphenicol produced a reduction in peak EPC amplitude, hence reducing the charged passed. Results represent the mean of 1–4 measurements.

isomers produce an increase in this ratio, indicating a reduction in channel closing rate. Thus, for example, D-*threo* chloramphenicol at 0.5 mM produces an approximately 50% reduction in channel closing rate at all membrane potentials studied. The reduction in closing rate was rather less with the other three compounds, but with all four compounds the effect was clearly independent of membrane potential (Table 6).

With the available EPC data it was possible to investigate the concentration dependence of this effect on channel closing. Fig. 8 shows semilogarithmic plots of τ and τ_{calc} versus membrane potential in the presence of three concentrations of each isomer. From these plots it can be seen that all four chloramphenicol isomers produce a concentration-dependent increase in τ, i.e., a decrease in channel closing rate, without affecting the overall voltage dependence of τ. Thus, H, the characteristic change in membrane potential required to produce an e-fold change in τ, remains similar to control values (Fig. 8).

Influence of effects of channel closing rate on measurement of blocking and unblocking rate constants. Our experiments have shown that the chloramphenicol isomers

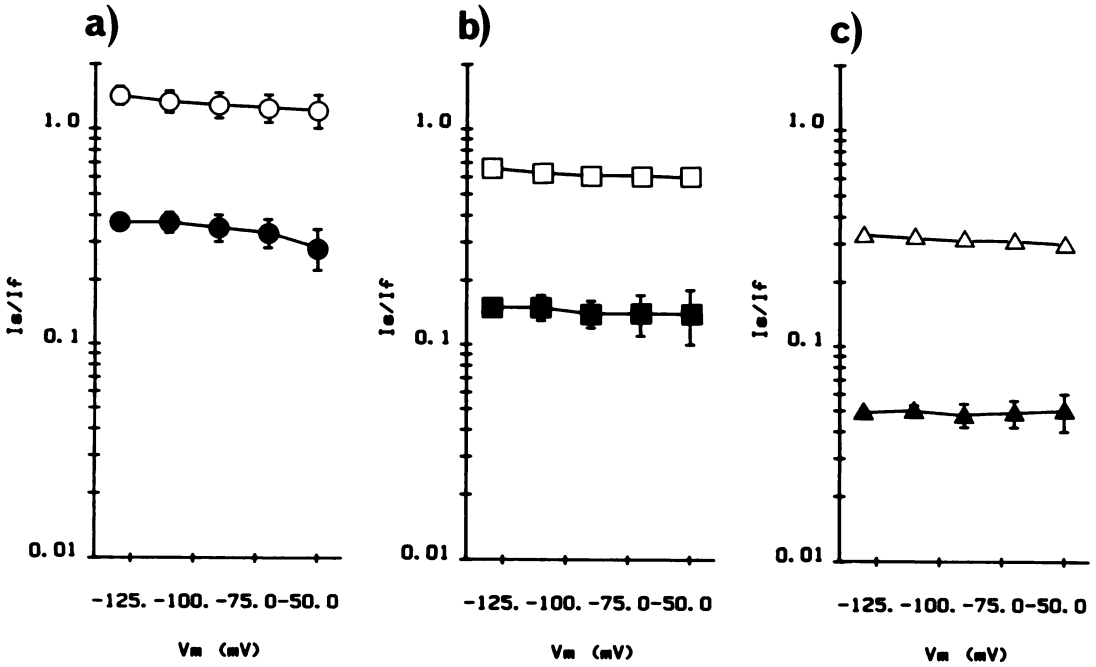

Fig. 5. Concentration-dependent effects of D-*threo* chloramphenicol on measured and predicted amplitude ratios for biphasic EPCs. Semilogarithmic plots were drawn of amplitude ratio (I_s/I_f) against membrane potential (*Vm*) in the presence of 0.2 mM (a), 0.5 mM (b), and 1.0 mM (c) D-*threo* chloramphenicol. The *upper plot* (*open symbols*) in each *panel* is the measured amplitude ratio and the *lower plot* (*solid symbols*) is that predicted from the sequential model using the measured values of τ, τ_f, and τ_s. Note the lack of voltage dependence of I_s/I_f and the increased percentage deviation from the predictions of the sequential model with increasing concentration of drug. Results represent the mean and SE of 3 measurements. *Error bars* have been omitted where they fall within the symbol.

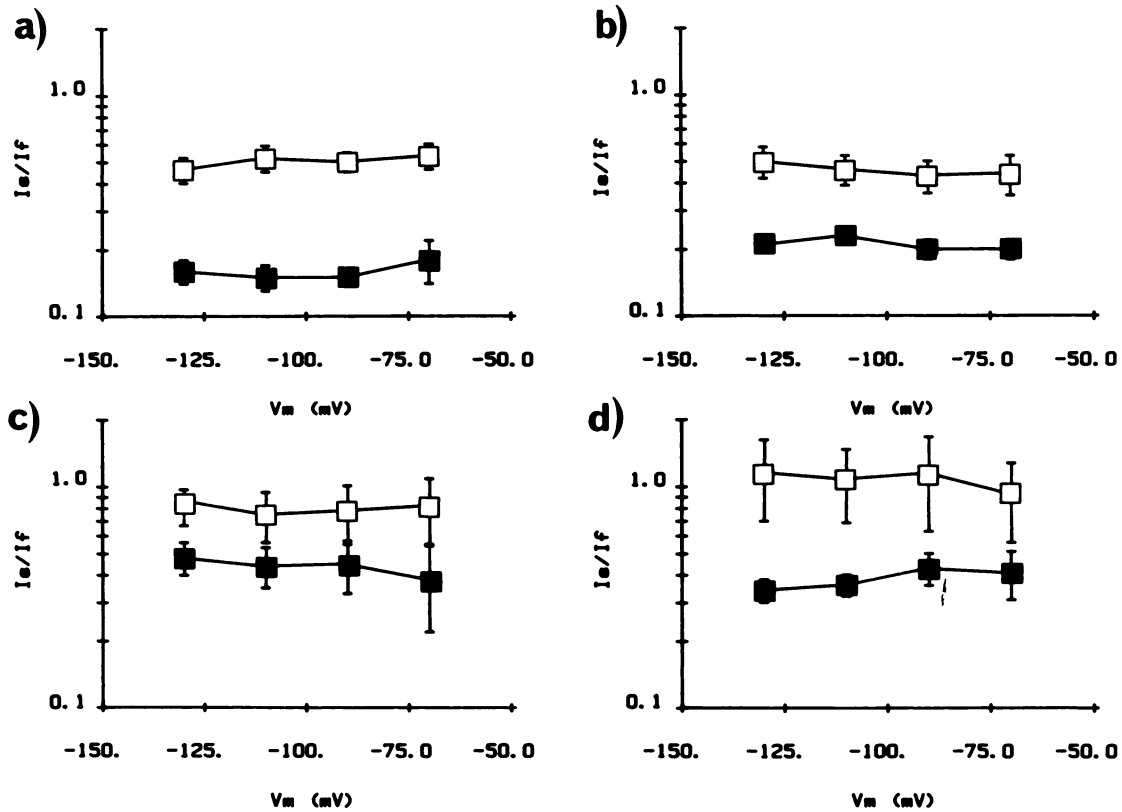

Fig. 6. Effects of the four chloramphenicol isomers on measured and predicted amplitude ratios for biphasic MEPCs. Semilogarithmic plots were drawn of amplitude ratio (I_s/I_f) against membrane potential (*Vm*) in the presence of 0.5 mM D-*threo* (a), L-*threo* (b), D-*erythro* (c), and L-*erythro* (d) chloramphenicol. The *upper plot* in each *panel* (*open symbols*) is the measured amplitude ratio and the *lower plot* (*solid symbols*) is that predicted from the sequential model using the measured values of τ, τ_f, and τ_s. Note that the deviation from the predictions of the sequential model is shown by all four isomers. Results represent the mean and SE of 6–8 experiments. *Error bars* have been omitted where they fall within the symbol.

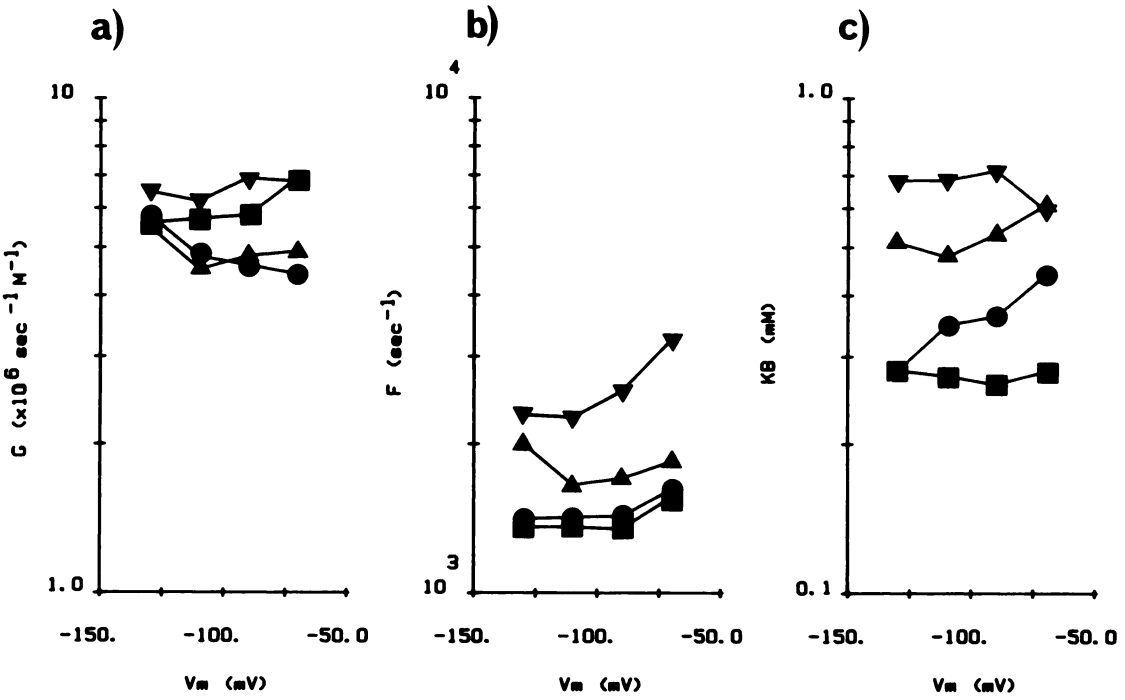

Fig. 7. Plots of blocking and unblocking rate constants and affinity constants against membrane potential for the four chloramphenicol isomers. Semilogarithmic plots were drawn of blocking rate constant, *G* (a), unblocking rate constant, *F* (b), and affinity constant, K_B (c) against membrane potential (*Vm*) for D-*threo* (●), L-*threo* (■), D-*erythro* (▲), and L-*erythro* (▼) chloramphenicol. Results are derived from experiments performed on MEPCs in the presence of 0.5 mM of each isomer. It can be seen that none of the values is obviously dependent upon membrane potential. Results represent the mean of 6–8 experiments.

TABLE 5

Blocking (*G*) and unblocking (*F*) rate constants and affinity constants (K_B) measured in the presence of 0.5 mM of each of the four chloramphenicol isomers

Results are shown for EPCs and MEPCs and have been averaged from results measured at four holding potentials (−70, −90, −110, and −130 mV). EPC- and MEPC-derived values, which were obtained in different experiments, are compared in the final column.

	EPC	MEPC	MEPC/EPC
	G values ($\times 10^6$ $M^{-1}sec^{-1}$)		
D-*threo*	2.12 ± 0.05	4.90 ± 0.44	2.31
L-*threo*	2.17 ± 0.11	6.00 ± 0.48	2.76
D-*erythro*	1.33 ± 0.08	4.92 ± 0.55	3.70
L-*erythro*	1.86 ± 0.20	6.60 ± 0.81	3.55
	F values (sec^{-1})		
D-*threo*	930 ± 30	1470 ± 80	1.58
L-*threo*	1150 ± 30	1410 ± 40	1.23
D-*erythro*	1310 ± 60	1800 ± 130	1.37
L-*erythro*	1690 ± 70	2590 ± 370	1.53
	K_B values (mM)		
D-*threo*	0.45 ± 0.03	0.36 ± 0.03	0.800
L-*threo*	0.55 ± 0.03	0.28 ± 0.02	0.509
D-*erythro*	1.05 ± 0.06	0.53 ± 0.07	0.506
L-*erythro*	1.06 ± 0.08	0.67 ± 0.12	0.632

TABLE 6

The decrease in channel closing rate, expressed as the ratio of τ_{calc}/τ, for MEPCs measured in the presence (τ_{calc}) and absence (τ) of each of the four chloramphenicol isomers (0.5 mM)

Results are shown for four membrane potentials. A value of 2 for the ratio represents a 2-fold slowing of the channel closing rate. Values represent mean and SE.

Compound	τ_{calc}/τ			
	−70 mV	−90 mV	−110 mV	−130 mV
D-*threo*	1.83 ± 0.13	2.05 ± 0.13	2.12 ± 0.19	2.13 ± 0.26
L-*threo*	1.63 ± 0.11	1.63 ± 0.08	1.58 ± 0.14	1.78 ± 0.14
D-*erythro*	1.58 ± 0.22	1.33 ± 0.20	1.36 ± 0.19	1.45 ± 0.26
L-*erythro*	1.67 ± 0.16	1.49 ± 0.14	1.64 ± 0.23	1.75 ± 0.22

apparently increase τ and, hence, calculation of rate constants from Eqs. 8 and 9 will lead to errors. We have thus investigated the extent to which the changes in channel closing rate affect the values of *F*, *G*, and K_B obtained using either the measured or calculated value for the control decay time constant. As the values for *F*, *G*, and K_B calculated from EPCs and MEPCs were previously found to be independent of membrane potential, the values from four membrane potentials (−70, −90, −110, and −130 mV) have been averaged for the comparison. The results for MEPCs in the presence of 0.5 mM of each of the four chloramphenicol isomers are shown in Table 7. As predicted from Eq. 8, the increase in channel open time results in a proportional increase in *F*. *G* was little affected, showing only an approximately 10% decrease with the change in closing rate. The affinity constant K_B was therefore increased mainly in proportion to τ_{calc} and to *F*. For D-*threo* chloramphenicol, for example, K_b calculated using the measured τ value was 50% of that calculated using the τ_{calc} value, i.e., a 2 times overestimate of affinity.

Discussion

Like the lincosamide antibiotic lincomycin (7, 8), the local anesthetic agents QX222 and procaine (15–17), the tropane alkaloid scopolamine (19), and the neuromuscular blocking agent gallamine (20), all four chloramphenicol isomers tested changed EPC or MEPC decays from single exponential to double exponential functions in a concentration-dependent

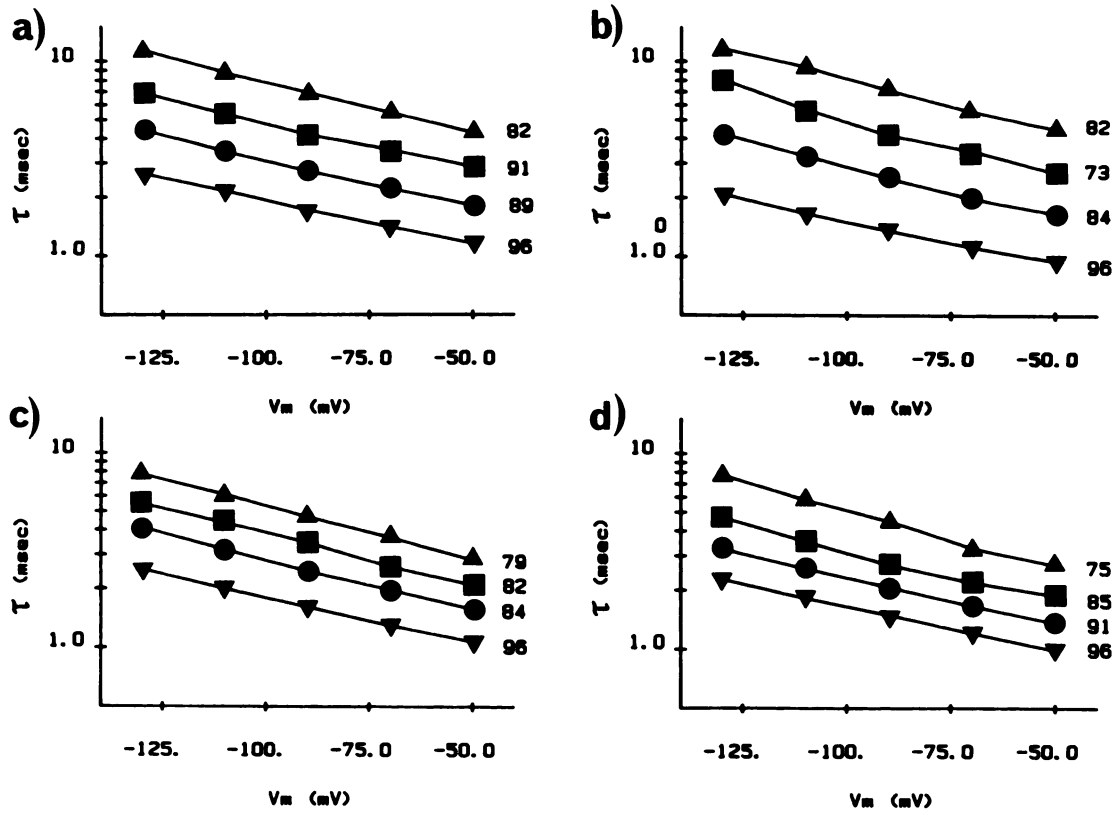

Fig. 8. Effects of the four chloramphenicol isomers on end-plate channel closing. Semilogarithmic plots were drawn of τ measured in the absence of drug and τ calculated in the presence of the four chloramphenicol isomers: D-*threo* (a), L-*threo* (b), D-*erythro* (c), and L-*erythro* (d) against membrane potential (*Vm*). The control decay time constant is shown in each *panel* as *inverted triangles*. τ_{calc} is shown in the presence of 0.3 mM D-*erythro* and 0.2 mM of the other three isomers (●), and in the presence of 0.5 mM (■) and 1.0 mM (▲) of all four of the isomers. Note the concentration-dependent increase in τ_{calc}, i.e., a decrease in the channel closing rate. *H*-values, denoting the voltage dependence of τ_{calc}, show little change in voltage sensitivity with increasing drug concentration. Results are from EPCs and represent the mean of 4–14 experiments.

TABLE 7

Comparison of blocking (*G*) and unblocking (*F*) rate constants and affinity constant (K_B) calculated from MEPCs recorded in the presence of each of the four chloramphenicol isomers (0.5 mM) using either measured control values of τ or τ values calculated from the predictions of the sequential model

The ratio of measured/calculated values indicates the effects of the action of the drugs (to slow channel closing) on the calculation of the constants. Values represent the mean and SE.

Drug	G_{meas}	G_{calc}	G_{calc}/G_{meas}
	A. Blocking rate constants (*G*)		
	$\times 10^6\ M^{-1}sec^{-1}$		
D-*threo*	5.17 ± 0.47	4.90 ± 0.44	0.98 ± 0.03
L-*threo*	6.59 ± 0.46	6.00 ± 0.48	0.89 ± 0.01
D-*erythro*	5.25 ± 0.53	4.92 ± 0.55	0.90 ± 0.03
L-*erythro*	7.26 ± 0.72	6.60 ± 0.81	0.86 ± 0.06
Drug	F_{meas}	F_{calc}	F_{calc}/F_{meas}
	B. Unblocking rate constants (*F*)		
	sec^{-1}		
D-*threo*	780 ± 60	1470 ± 80	2.03 ± 0.09
L-*threo*	870 ± 40	1410 ± 40	1.66 ± 0.06
D-*erythro*	1460 ± 160	1800 ± 130	1.43 ± 0.11
L-*erythro*	1800 ± 340	2590 ± 370	1.64 ± 0.09
Drug	K_{Bmeas}	K_{Bcalc}	K_{Bcalc}/K_{Bmeas}
	C. Affinity constants (K_B)		
	mM		
D-*threo*	0.18 ± 0.02	0.36 ± 0.03	2.12 ± 0.11
L-*threo*	0.14 ± 0.01	0.28 ± 0.02	1.89 ± 0.19
D-*erythro*	0.32 ± 0.03	0.53 ± 0.07	1.83 ± 0.51
L-*erythro*	0.28 ± 0.04	0.67 ± 0.12	2.21 ± 0.48

manner. Such concentration-dependent changes in decay characteristics have been interpreted in terms of the sequential model of open channel block:

$$\mathrm{ACh} + R \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} \mathrm{AChR} \underset{A}{\overset{B}{\rightleftharpoons}} \mathrm{AChR^*} + C \underset{F}{\overset{G[C]}{\rightleftharpoons}} \mathrm{AChR^*}\,C$$

(closed) (open) (blocked)

where R is the receptor and C the channel blocking drug, k_{+1} and k_{-1} are the association and dissociation rate constants for the transmitter interaction with the receptor, B and A are the channel opening and closing rate constants, and G and F are the blocking and unblocking rate constants for C.

It thus appears that, in common with the above substances, the chloramphenicol isomers used are acting in our system predominantly by open channel block. However, our observations suggest that such a mechanism for these compounds might be an oversimplification of the situation. According to the sequential model, the amount of charge passed through an end-plate channel should be the same both in the absence and presence of a channel blocking substance (18). The results with the chloramphenicols clearly showed an increase in charge passed with all four isomers.

We considered that the EPC results might have been complicated by the increase in EPC amplitude at the lower concentrations used. The fact that this was not accompanied by a corresponding increase in MEPC amplitude ruled out the possibility of an anticholinesterase action and indicated, rather, that the increased amplitude was due to an increase in evoked release. This notion is supported by the increase in EPC driving function observed in the presence of L-*erythro* chloramphenicol. Comparison of the simulated single exponential EPC derived from this driving function with the measured double exponential EPC indicated that the increase in quantal content was substantially larger than that predicted from simple comparison of control and drug-treated EPC amplitudes. This increase in quantal content explains the decreased rise times of EPCs in the presence of the chloramphenicols, which is made up of a faster rate of rise curtailed by channel block. That the increased EPC driving function was due to an increase in quantal content rather than an anticholinesterase action was confirmed by the fact that the chloramphenicol isomer did not increase the size of the driving function of MEPCs. Nevertheless, we consider that the increase in quantal content is not the main reason for the increased charge passed, as we observed an increase, albeit smaller, in charge passed during MEPCs. Thus, it is necessary to postulate that the chloramphenicol isomers do not act simply by blocking the end-plate channel but must possess at least one other mechanism.

By using an extension of the predictions of the sequential model for channel block provided by Ruff (15), we have been able to produce evidence that is consistent with two separate actions of the compounds on end-plate channels, namely, an open channel blocking action that is superimposed on an action to reduce the channel closing rate. We believe that the combination of these actions leads to the observed deviation from the simple predictions of the sequential model.

We have approached the problem of differentiating these actions using the assumption that a component of action of the compounds is due to open channel block obeying the sequential model. Based on this assumption it has been possible to calculate unblocking rate constants from the amplitudes and time constants of the drug-induced double exponentially decaying EPCs and MEPCs. These calculations did not make use of control decay time constant values. When the control decay time constant was calculated back from the double exponential decays these calculated values were longer than the measured control values. We thus conclude that the compounds prolong channel open time, in addition to blocking the channel.

Our observations and calculated values are consistent with these two actions alone. Thus, the longer opening channels in the presence of the chloramphenicols retain their voltage dependency and increase in open time is related to drug concentration. These characteristics of the chloramphenicols are closely similar to those of the alcohols which have been proposed to increase channel open time by changing the dielectric constant of the postjunctional membrane, thus altering the ability of the channel to revert to its closed form (21). Like the alcohols, the chloramphenicols are lipid soluble (22) and, hence, we consider that their physicochemical characteristics would be consistent with an action on the channel environment. It is noteworthy that the concentrations of chloramphenicol isomers required to produce prolongation of open time are low compared to those of other compounds previously shown to possess this property. Thus, we found that chloramphenicol prolonged open time at concentration of 0.2–1.0 mM, whereas ethanol, for example, requires around 0.2 M to produce similar effects (21). However, the log P values for the octanol/water partitioning of chloramphenicol is 1.5, i.e., a partition coefficient of 14:1, which represents a higher lipid solubility than most other substances that prolong channel open time, e.g., ethanol, 0.7:1 (23). The closely similar relationships between concentration and open time for the D- and L-forms of each stereoisomeric pair is consistent with the similarity of physicochemical properties of stereoisomers.

It is clear from our results that any calculation of rate constants for compounds with the above combination of activities must take into account the concentration-dependent increase in open time and, hence, increased τ, rather than the control open time. As F is related to τ by τ_o/τ_f, any concentration-dependent increase in τ would lead to an apparent concentration dependence of F if calculations were performed using control τ values.

Taking the above into account, calculations of rate constants showed that, for both EPCs and MEPCs, all four chloramphenicol isomers possessed blocking and unblocking rate constants that were independent of membrane potential as were channel blocking affinity constants. Lack of voltage sensitivity has also been noted with the barbiturates (14) and with the non-charged local anesthetic benzocaine (24). Overall, the channel blocking kinetics of the chloramphenicols bear a striking resemblance to those of the barbiturate methyprylone (14). At the frog neuromuscular junction, methyprylone produced biphasic EPC decays and has blocking and unblocking rate constants which are similar to those of the chloramphenicols, and which are similarly independent of membrane voltage. Again, like those of methyprylone, the blocking rate constants of the chloramphenicols were relatively slow compared to those of the charged compounds tubocurarine (25), gallamine (20), and the local anesthetics procaine (26), QX222, and QX314 (18). In addition, as would be predicted from compounds exhibiting marked biphasic EPC decays, the unblocking rate constants were fast, resulting in a very low affinity for the channel binding site.

We were unable to show any relationship between antibac-

terial activity and channel blocking activity for the chloramphenicols. Thus, the clinically used isomer, D-*threo* chloramphenicol, possesses at least 50 times the antibacterial activity of the other three isomers tested (11). In contrast, at the end-plate the D- and L-forms of either *threo* or *erythro* chloramphenicol possessed very similar unblocking rate constants. The *F* values for the *erythro* compounds were somewhat faster than those for the *threo* compounds, thus largely explaining the small observed differences in EPC decay characteristics. The blocking rate constants were similar for all four compounds.

It should be stressed that the lowest concentration used in our experiments was approximately 10 times higher than the maximum blood level achieved during parenteral antibacterial therapy. We conclude that there is no relationship between the binding site on the 50 S subunit and the end-plate ion channel. However, the homologous series of four isomers which are stable and are known to possess unique conformations (11, 27) afforded a series of tools with which to examine the stereoselectivity of the ion channel.

It is clear, despite the deviation of the compounds from the predictions of the sequential model for end-plate channel block, that there are no major differences between the effects of the D- and L-forms of either *threo* or *erythro* chloramphenicol, although there appear to be some small differences between the effects of the *threo* and *erythro* forms. These differences in pharmacological activity between the *threo* and *erythro* forms of chloramphenicol may be related to chemical differences between the pairs. Thus, Jardetsky (27), in a nuclear magnetic resonance study, showed substantial differences in conformation between D-*threo* and D-*erythro* chloramphenicol after treatment of the substances with deuterium oxide. From this study Jardetsky proposed that the *threo* and *erythro* isomers each existed in a single unique conformation that was determined by hydrogen bonding between the hydroxyl groups attached to C1 and C3. Thus, although it might not be expected that the optical isomers of each pair of isomers would show differences in activity, some difference between the pairs might be expected. This is consistent with our observations.

A similar lack of stereospecificity to that shown by the chloramphenicols has been noted in (+)- and (−)-perhydrohistrionicotoxin (28), which are thought to act mainly on closed end-plate channels. In contrast, at the voltage-dependent sodium channel, local anesthetics act in a stereospecific manner (29). Our results suggest that end-plate ion channel blockade is not a stereospecific process. One proviso to our conclusion is that the compounds that were used for the study possessed extremely low affinity for the channel binding site. At a variety of receptor sites it has been shown that stereoselectivity decreases as affinity decreases (30), and, hence, it would be of interest to study the effects of open channel blockers with higher affinities than those of the chloramphenicols.

Acknowledgements

We are grateful to Warner Lambert-Parke Davis for the gifts of the chloramphenicol isomers. We are greatly indebted to Professor J. M. Midgley for invaluable advice concerning the stereochemical nature of the compounds. We also acknowledge Harry Breminer for construction of the electronic apparatus, and Janice Meehan and Avril McColl for help in preparing the manuscript. We thank the Organon Scientific Development Group for support for John Dempster.

Appendix

EPC Driving Function Analysis

EPCs have slower rising phases (0.3–0.5 msec in garter snake costocutaneous muscle) than those of MEPCs (0.2–0.3 msec), presumably due to the stimulus-evoked release of transmitter quanta being not totally synchronous. In the presence of a channel blocking drug, measurement of quantal content from the ratio of EPC and MEPC peak currents is therefore somewhat inaccurate due to the possibility of rapid channel block occurring during the rising phase of the EPC, to a much greater extent than it would during MEPCs. This would act to truncate the EPC peak current and hence would render inaccurate estimations of quantal content from EPC amplitudes.

Accordingly, to obtain a better index of quantal content, we chose to calculate the EPC driving function. The driving function, $W(t)$, is a measure of the rate of opening of the end-plate channels. Its time course is dependent on the rate of release of transmitter and its subsequent binding to receptors and eventual breakdown. However, the driving function is independent of the time course of EPC decay, if it is calculated from a correct theoretical expression for the time course of channel closure. Any changes in the driving functions can therefore only be due to changes in the amount of transmitter released, or changes in the number of available receptors, i.e., postjunctional block. EPCs and MEPCs were subjected to a deconvolution process similar to that described previously (6, 7). In this process, the measured EPC time course [EPC(t)] can be considered to be the results of an input signal [$W(t)$] passed through a filter function representing the exponential decay characteristics of the end-plate channels. By creating frequency domain representations of this signal using the fast Fourier transform (*FFT*), $W(t)$ can be obtained from:

$$W(t) = \frac{\mathrm{FFT}^{-1}\dfrac{[\mathrm{FFT}(\mathrm{EPC}(t)]}{[\mathrm{FFT}(e^{-t/\tau})]}}{(V_h - V_r)} \qquad (14)$$

where V_h is the holding potential and V_r is the reversal potential for the end-plate conductance, −5 mV (8). $W(t)$ is scaled by $(V_h - V_r)$ to obtain a value that is independent of the driving force. τ is the time constant of channel closure, obtained from an exponential curve fitted to the decay of EPC(t). $W(t)$ is calculated using a 512-point FFT from averaged EPC records (10–20 EPCs) which contain 512 points sampled at 40-μsec intervals. MEPC driving functions were obtained in a similar way except that 80–100 MEPCs were averaged.

Calculation of the driving function in the presence of a channel blocking drug is more difficult. Whereas the decay of the control EPC can be specified by the one time constant (τ), the biphasic EPC decay requires three parameters, the fast and slow decay time constants, τ_f and τ_s, and the ratio of amplitudes of the two exponentials I_s/I_f. I_s and τ_s are obtained without error by curve fit, but the fitted I_f and τ_f are distorted by the driving function, I_f being reduced in size and τ_f being increased. If the three values derived directly from the curve fit are used in the deconvolution, the resulting driving function has a faster decay than in control, smaller area, and a noticeable negative tail which is inconsistent with a driving function generated solely by the opening of conductance channels. To obtain a better approximation to the driving function, a semiqualitative approach, similar to that of Connors *et al.* (31), was used. A new value for τ_f was chosen which, when convoluted with the control driving function of the same cell and a single exponential decay, produced a simulated EPC with a time constant equal to the fitted τ_f. Starting with the values obtained from the curve fit, a series of driving functions was calculated with decreasing I_s/I_f until a driving function was created which did not have a negative tail.

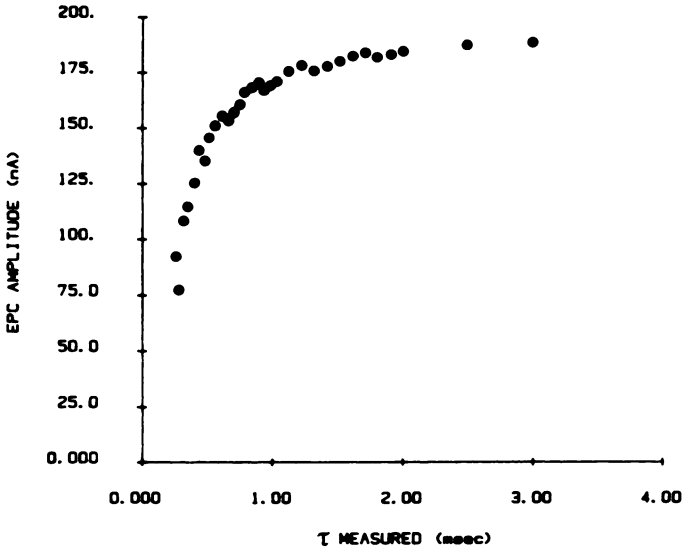

Fig. 9. Driving function-induced errors in measurement of amplitudes from simulated single exponential EPCs. The amplitude of simulated EPCs is shown as a function of their measured decay time constant. The simulated EPCs were generated by convoluting a single driving function with a series of exponential decays ranging from 0.3 to 3.0 msec. The driving function was obtained by deconvolution of control EPCs recorded at a holding membrane potential of −50 mV. It is apparent that, for $\tau < 0.75$ msec, the EPC peak current is strongly dependent on the decay time constant.

Using the driving function from the drug EPC, a simulated single exponential EPC, $EPC_s(t)$, can be calculated by convoluting with the same single exponential decay function as the control EPC.

$$EPC_s(t) = FFT^{-1}[FFT(W(t))FFT(e^{-t/\tau})] \qquad (15)$$

Comparison of the simulated control and drug EPCs allows us to observe changes in quantal content/receptor block effects in isolation from the ion channel blocking effects of the drug (see Fig. 1). In addition, by generating series of simulated single exponential EPCs with differing decay time constants from a single driving function, we have shown that EPC peak amplitude is strongly dependent on the given τ value. Thus, when τ is less than 0.75 msec, the amplitudes of the simulated EPCs are reduced by up to 60% ($\tau = 0.3$ msec) when compared to those with given τ values of 1 msec and greater (Fig. 9).

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