

**SOME EFFECTS OF PROCAINE AT THE TOAD  
END-PLATE ARE NOT CONSISTENT WITH A SIMPLE  
CHANNEL-BLOCKING MODEL**

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SUMMARY

1. Miniature end-plate currents (m.e.p.c.s) were recorded extracellularly from toad sartorius muscle fibres exposed to solutions containing procaine at pH 5·4, 7·4 and 9·9.

2. The decay phase of m.e.p.c.s was analysed to determine whether the effects of procaine were consistent with a sequential channel-blocking model.

3. Averaged m.e.p.c.s measured in the presence of procaine were biphasic, decaying as the sum of two exponential components. However, about 10–15% of m.e.p.c.s decayed as single exponentials and were not biphasic.

4. At pH 9·9 the relative amplitudes of the fast and slow phases were generally consistent with the decay time constants, according to the predictions of the blocking model. Such a correlation was not found at pH 5·4 or 7·4. In addition, the rate of decay of m.e.p.c.s at pH 5·4 did not increase as predicted with procaine concentration.

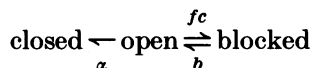
5. These results demonstrate that the sequential blocking model is unable to account for all of the effects of procaine on m.e.p.c. decay. In addition, the finding that some m.e.p.c.s are single exponentials, while most are biphasic, suggests a heterogeneity of receptor-channel complexes.

INTRODUCTION

At the neuromuscular junction, acetylcholine (ACh) interacts with post-synaptic receptors to open ionic channels, allowing current to flow across the muscle membrane. End-plate currents (e.p.c.s) evoked by nerve stimulation, and miniature end-plate currents (m.e.p.c.s) produced by the spontaneous release of individual quanta of ACh, normally decay with a single exponential time course which reflects the average lifetime of open channels (Anderson & Stevens, 1973).

A large number of substances alter this single exponential decay, often producing double exponential, or biphasic, decays. Such compounds, of which the local anaesthetic procaine is best known, are thought to enter and block ion channels which have previously been opened by ACh. Evidence in support of this idea has come from voltage-jump experiments (Adams, 1977) and single-channel recordings (Neher &

Steinbach, 1978), which suggest that the effects of procaine on the time course of decay of e.p.c.s and m.e.p.c.s are consistent with a simple, sequential blocking model:



where  $\alpha$ ,  $f$  and  $b$  are rate constants, and  $c$  is drug concentration. If so, the decay phase of e.p.c.s and m.e.p.c.s recorded in the presence of a blocking drug can be described as the sum of two exponentials:

$$I(t) = A_f \cdot \exp(-r_f t) + A_s \cdot \exp(-r_s t) \quad (1)$$

where  $I(t)$  is the current amplitude;  $A_f$  and  $A_s$  are the amplitudes of the fast and slow phases respectively; and  $r_f$  and  $r_s$  are the reciprocals of the time constants of the fast and slow phases, with  $r_f > r_s$ .

According to this model, the fast component of e.p.c. or m.e.p.c. decay would be associated with drug entry into open channels, which are consequently rendered non-conducting. A prolonged current tail would be produced as channels which had become blocked later returned to their original conducting state.

In addition to altering the decay phase of e.p.c.s and m.e.p.c.s, procaine also acts to depress the amplitude of responses to cholinergic agonists. In order to explain this effect, Adams (1977) has modified the sequential model to allow procaine to bind to closed channels, although this variation in no way affects the kinetic predictions of the model.

The sequential channel-blocking model has gained widespread acceptance, and is frequently invoked to explain the actions of drugs which alter the time course of post-synaptic currents. However, the effects of some drugs which appear at first to be channel blockers have been found, upon closer examination, to be inconsistent with this sequential model. Hexafluorodiethyl ether produces biphasic m.e.p.c.s at the toad end-plate, but at lower temperatures the slower phase decays faster than m.e.p.c.s recorded in the absence of the drug (Gage & Sah, 1982). Barbiturates may be channel blockers at the end-plate (Adams, 1976), but do not act in a similar fashion in *Aplysia*. ACh-induced relaxations (Wachtel & Wilson, 1983) and spontaneous synaptic currents (Adams, Gage & Hamill, 1982) measured in the presence of barbiturates have a double exponential time course, but the slower component has the same time constant as control. These observations are not consistent with the simple sequential model above. In fact, we report here that the simple channel-blocking model is also unable to explain some of the effects of procaine at the end-plate.

The simple sequential model places restrictions on the time course of m.e.p.c.s which have been altered by a channel blocker. Once values of  $r_f$  and  $r_s$  have been determined, the fractional amplitude of the slow phase,  $A = A_s/(A_f + A_s)$ , also becomes fixed. A theoretical value for  $A$  may be calculated from the prediction that the area under a m.e.p.c. should be unaffected by interaction of procaine with open channels. According to the sequential model, the probability that an open channel will close remains constant, and is not affected by periods of block. Thus the total open time of a channel is not altered by procaine, although it is distributed over a longer time span (Neher & Steinbach, 1978; Ruff, 1982). The total charge transferred

during the opening of a channel should therefore be unchanged by procaine, implying that the area under a biphasic m.e.p.c. produced by procaine should be the same as under a control m.e.p.c. produced by activation of the same number of channels:

$$\int_0^{\infty} I(0) \exp(-\alpha t) dt = \int_0^{\infty} \frac{A_f}{A_f + A_s} \cdot I(0) \cdot \exp(-r_f t) dt + \int_0^{\infty} \frac{A_s}{A_f + A_s} \cdot I(0) \cdot \exp(-r_s t) dt,$$

where  $I(0)$  is the peak current ( $t = 0$ ). This equation can be used to calculate the fractional amplitude of the slow phase in terms of the measured rate constants, since it follows that

$$A = \frac{r_s(r_f - \alpha)}{\alpha(r_f - r_s)}. \quad (2)$$

It should be noted that the fractional amplitude,  $A$ , is uniquely defined in terms of rate constants (or decay time constants) and is independent of drug concentration and binding constants.

Using these equations, we have found that the predictions of the blocking model are inconsistent with the characteristics of biphasic m.e.p.c.s produced by procaine.

#### METHODS

The methods used in these experiments have been described previously (Gage, Hamill & Wachtel, 1983). M.e.p.c.s were recorded extracellularly at the resting membrane potential ( $-70$  to  $-90$  mV) from end-plate regions of sartorius muscle fibres of the cane toad *Bufo marinus*. Extracellular electrodes were filled with a Ringer solution containing 200 mM-NaCl, 2.5 mM-KCl and 1.8 mM-CaCl<sub>2</sub>. Muscles were bathed in a solution containing 170 mM-NaCl, 2.5 mM-KCl, 1.8 mM-CaCl<sub>2</sub> and 2 mM-MES (2-(*N*-morpholino)ethanesulphonic acid), HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid), or CHES (cyclohexylaminoethanesulphonic acid) buffer adjusted to pH 5.4, 7.4 or 9.9, respectively. Experiments were performed at room temperature (17–19 °C).

Data were filtered at 1 Hz and 3.5 kHz and stored on FM tape for later analysis. The time constant of decay of m.e.p.c.s recorded in the absence of procaine,  $\tau_D$ , was determined by a least-squares fit to  $I(t) = I(0) \exp(-t/\tau_D)$ . Averaged m.e.p.c.s measured in the presence of procaine were expressed as the sum of two exponential components with time constants  $\tau_f$  and  $\tau_s$  ( $\tau_f^{-1}$  and  $\tau_s^{-1}$  respectively) according to eqn. (1). Rate constants and amplitudes were chosen to provide the best fit to the averaged m.e.p.c.s. Predicted values of the fractional amplitude of the slow phase ( $A$ ) were calculated from eqn. (2).

#### RESULTS

##### *Amplitudes of the two decay components*

Fig. 1 shows m.e.p.c.s recorded in experiments in the absence and presence of procaine at different pHs. In control solutions, the time constant of decay ( $\tau_D = 1/\alpha$ ) was usually slightly less at pH 5.4 and 9.9 than at pH 7.4. After 30 min exposure to 100  $\mu$ M-procaine ( $pK_a$  8.9), averaged m.e.p.c.s recorded at pH 5.4, 7.4 and 9.9 were obviously biphasic, as expected. However, the relative amplitudes of the two phases were different at each pH. At pH 5.4, the fast component was much smaller than at pH 7.4, and m.e.p.c.s generally appeared to be prolonged compared with control currents. The slow component measured at pH 9.9 was very small, and m.e.p.c. decay was faster than in control. These effects of pH on the relative amplitudes of the two

components were also confirmed in paired experiments involving five fibres in which the pH was changed while the total procaine concentration was maintained constant.

The differences in m.e.p.c. decay could not be predicted from changes in the concentration of the ionized form of procaine, which is thought to be the active species. Procaine is almost totally in a charged form at pH 7.4, and the amount of charged procaine in the solution should essentially be the same at pH 5.4 as at 7.4. The shape of m.e.p.c.s recorded at pH 9.9 was not consistent with a simple lowering of ionized procaine concentration, but probably reflects another site of action (Gage *et al.* 1983). Even so, changes in the concentration of the active form of the drug are not sufficient to explain the shape of m.e.p.c.s at pH 5.4 and 9.9. The relative

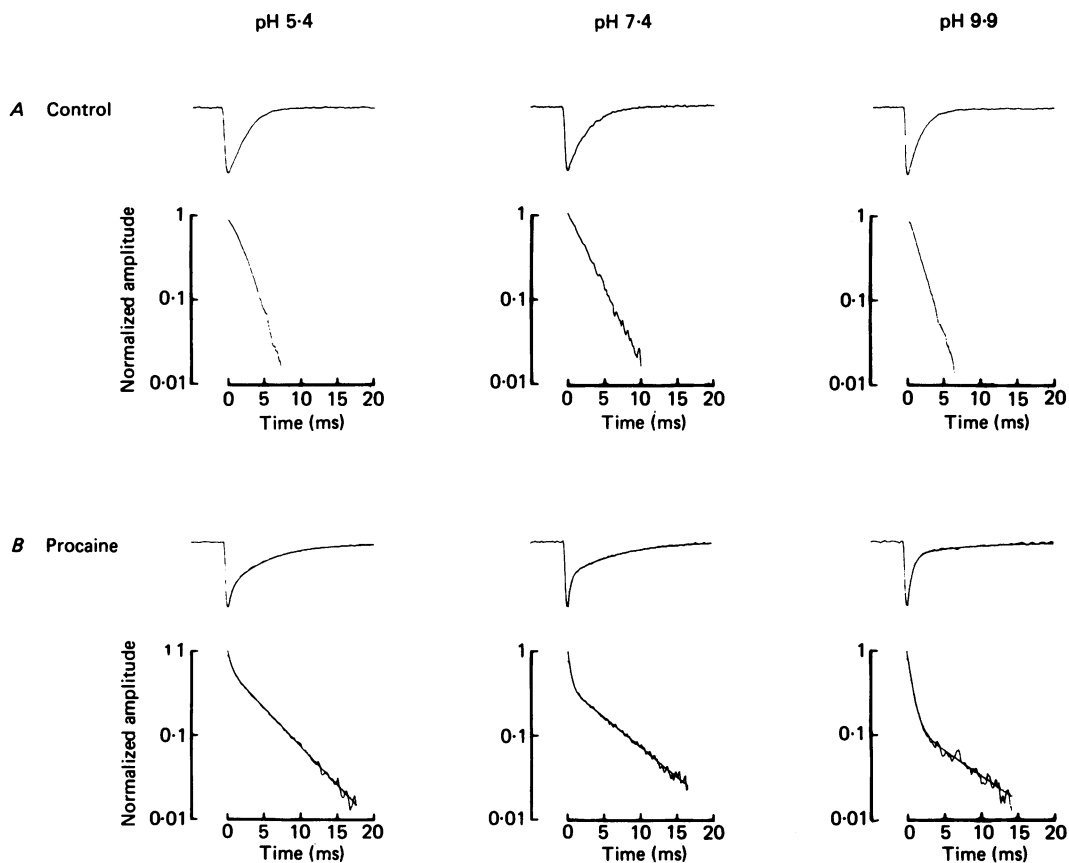


Fig. 1. Effect of pH on biphasic m.e.p.c.s produced by procaine. *A*, averaged m.e.p.c.s recorded extracellularly at pH 5.4, 7.4 or 9.9 in control solution. Semilogarithmic plots of the decay phases are shown below the currents. *B*, averaged m.e.p.c.s recorded 30 min after application of 100  $\mu$ M-procaine at each pH, and semilogarithmic plots of their decay phases. Fitted curves described by the parameters shown in Table 1 are superimposed on the procaine data. Each record is the average of twenty-six to ninety-eight m.e.p.c.s. Currents have been normalized to the same peak height. Results represent three separate experiments.

amplitudes of the fast and slow phases are defined only in terms of the decay rates, and cannot be independently modified by changes in effective drug concentration or changes in the affinity of procaine for its binding site(s).

The effects of pH on the amplitudes and time constants of the two phases are not consistent with the relationships imposed by eqn. (2) above. The predictions of the blocking model are listed in Table 1 for comparison with the experimental observations shown in Fig. 1. From measured values of  $\tau_f$ ,  $\tau_s$  and  $\tau_D$ , the blocking model predicts that  $A$  at pH 5.4 should be 0.29, but the measured value was about twice that predicted.

TABLE 1. Time constants of the decay phases of the averaged m.e.p.c.s shown in Fig. 1.  $\tau_D$  is the time constant of decay of m.e.p.c.s recorded in the absence of procaine. Averaged m.e.p.c.s measured in the presence of 100  $\mu\text{M}$ -procaine were expressed as the sum of two exponential components with time constants  $\tau_f$  and  $\tau_s$  ( $\tau_f^{-1}$  and  $\tau_s^{-1}$  respectively) according to eqn. (1). Fitted curves (shown in Fig. 1) were often larger in amplitude than the measured m.e.p.c.s during the first 200–300  $\mu\text{s}$  following the peak, so that  $A_f + A_s$  was actually greater than the true peak height. This difference presumably arises because  $\tau_f$  was not insignificant compared to the rise time of the m.e.p.c.s, and some channels had already closed before others had opened. If the amplitude of the fast component were determined by subtracting  $A_s$  from the measured peak height, the discrepancies between measured and predicted values of  $A$  would become even greater, especially at pH 5.4 where the relative amplitude of the fast component is already smaller than predicted

	pH 5.4	pH 7.4	pH 9.9
$\tau_D$ (ms)	1.9	2.7	1.8
$\tau_f$ (ms)	0.46	0.39	0.68
$\tau_s$ (ms)	5.4	7.0	8.4
$A$ (measured)	0.64	0.43	0.17
$A$ (predicted)	0.29	0.35	0.14

### Single exponential decay

Another observation which is not explained by the blocking model involves the effects of procaine on some individual m.e.p.c.s. Although the decay of averaged m.e.p.c.s recorded at pH 5.4 was biphasic, the decay of about 10–15% of individual currents appeared to have only a single component, which resembled the slow phase of biphasic m.e.p.c.s. A fast component may have been present, but if so, it was too small to be detected. The single exponential m.e.p.c. shown in Fig. 2A, which was recorded in 100  $\mu\text{M}$ -procaine at pH 5.4, had a time constant of decay of 5.4 ms, which was longer than any  $\tau_D$  recorded in the same fibre before application of procaine: only five out of thirty-two m.e.p.c.s analysed in control solution at pH 5.4 had time constants greater than 2.4 ms, and the largest  $\tau_D$  was 2.6 ms.

At pH 9.9, some m.e.p.c.s recorded 30 min after the application of procaine also appeared to have a single exponential decay. Fig. 2B illustrates a m.e.p.c. with a decay time constant of 0.86 ms, smaller than the lowest  $\tau_D$  of 1.2 ms recorded in control solution. When the muscle was exposed to procaine at pH 9.9 for more than about 40–50 min, almost all m.e.p.c.s decayed exponentially with only a single detectable time constant which was much smaller than control. The slow component

of decay (if it existed) must have become so small that it could not be measured, and even averaged currents could not be resolved into two components. This finding was observed both with extracellular recording and in voltage-clamped fibres ( $n = 6$ ), and thus is not due to depolarization of the fibre to a membrane potential where  $\tau_D$  is normally smaller and where the effects of procaine may be less apparent.

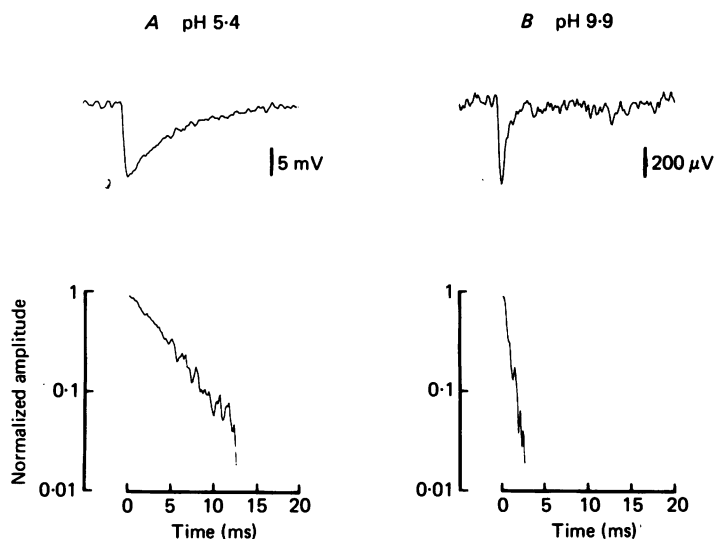


Fig. 2. Examples of single exponential m.e.p.c.s recorded 30 min after application of  $100 \mu\text{M}$ -procaine at pH 5.4 (A) or 9.9 (B). The semilogarithmic plots shown below each m.e.p.c. appear linear. The time constants of decay were 5.4 ms at pH 5.4 and 0.86 ms at pH 9.9. M.e.p.c.s are from the same experiments shown in Fig. 1, in which averaged currents were clearly biphasic.

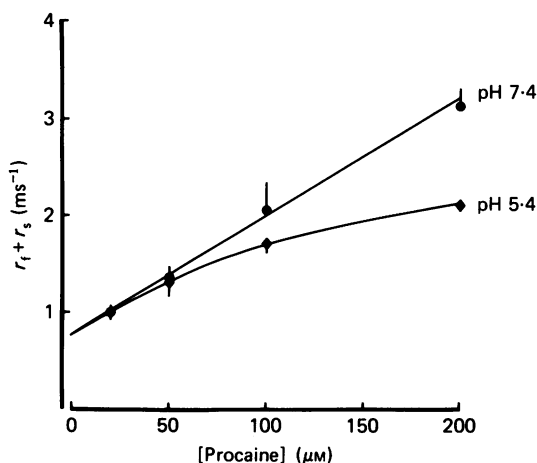


Fig. 3.  $r_f + r_s$  as a function of procaine concentration at pH 7.4 (●) and pH 5.4 (◆).  $r_f$  and  $r_s$  are the reciprocals of the time constants of the fast and slow phases ( $\tau_f$  and  $\tau_s$ ) measured from averaged, extracellularly recorded m.e.p.c.s. Each point is the mean of three experiments. Error bars represent 1 s.e. of means. Lines have been drawn by eye.

Single exponential m.e.p.c.s were seen in most experiments, and were recorded from the same end-plate regions as biphasic m.e.p.c.s. The blocking model does not explain the existence of such apparently single exponential currents with time constants very different from control values.

#### *Effects of drug concentration*

Fig. 3 illustrates another observation which is not consistent with the sequential model. Rate constants for the fast and slow components were measured at four different procaine concentrations at pH 5.4 and pH 7.4. Similar data could not be collected at pH 9.9 because the effects of procaine increased progressively with time, probably indicating that the procaine concentration at its site of action had not reached a steady-state value (Gage *et al.* 1983). The blocking model above predicts that  $r_f + r_s$  should increase linearly with procaine concentration, since  $r_f + r_s \cong fc + \alpha + b$  (for  $ab \ll (\alpha + b + fc)^2$ ) (Gage & Sah, 1982; Adams, 1976). Assuming that  $\alpha$  and  $b$  are independent of drug concentration, a plot of  $r_f + r_s$  versus  $c$  should have a slope  $f$  and intercept  $(\alpha + b)$ . As shown in Fig. 3, the relationship between  $r_f + r_s$  and  $c$  is reasonably linear at pH 7.4, yielding values of  $f = 1.3 \times 10^7 \text{ s}^{-1} \text{ mol}^{-1}$  and  $b = 300 \text{ s}^{-1}$ , in agreement with Adams (1977). At pH 5.4, however,  $r_f + r_s$  does not increase as predicted with drug concentration.

### DISCUSSION

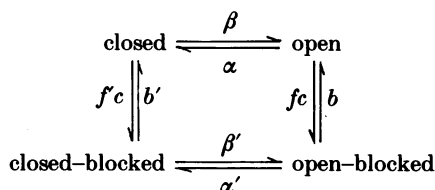
#### *Blocking models*

Although the blocking model provides a reasonable description of the effects of procaine on the time course of m.e.p.c.s at pH 7.4, inadequacies become apparent at lower or higher pH values. The aberrant relative amplitudes of the fast and slow components (Fig. 1 and Table 1), the existence of some m.e.p.c.s which have only a single exponential decay component (Fig. 2), and the non-linear dependence of  $r_f + r_s$  on drug concentration (Fig. 3) are not consistent with the model. Although these discrepancies become apparent only at pH values which are not likely to occur under physiological conditions, they nevertheless represent a failure of the sequential blocking model, and cast doubt on the validity of the blocking model at normal pH.

Some other models which represent variations of the sequential model are also unable to explain the influence of pH on the effects of procaine. For example, procaine could conceivably have other effects on channel lifetime that are distinct from its blocking action. If procaine were to change  $\alpha$  in addition to blocking channels, then the differences between measured and predicted values of the amplitude ratios could be reconciled. However, this would not explain the data in Fig. 3, in which  $r_f + r_s$  does not increase linearly with drug concentration. If procaine were to decrease  $\alpha$  at pH 5.4, then  $r_f + r_s$  ( $\cong fc + \alpha + b$ ) would also appear to decrease, but any decrease in  $\alpha$  could not possibly be large enough to account for the low value of  $r_f + r_s$  at pH 5.4.

A more general type of blocking model, which is also able to resolve the discrepancy between measured and predicted values of the amplitude ratio, again is not consistent with the data in Fig. 3. Adams (1976, 1977) used a cyclic model, in which procaine can also bind to closed channels, to explain depression of the

amplitude of steady-state responses to suberyldicholine in the presence of procaine. However, he assumed that blocked channels could open and close only very slowly, and thus the cyclic model was not kinetically different from the sequential one. On the other hand, if blocked channels can open and close at an appreciable rate, and if this rate were dependent on pH, then a cyclic model may be more useful for explaining some of the effects of procaine. However, this cyclic model predicts that  $r_f + r_s \cong fc + \alpha + b + \alpha'$ , and that  $r_f + r_s$  should again be a linear function of  $c$ . This prediction is not fulfilled.



#### *Heterogeneity of m.e.p.c.s*

In the presence of procaine, m.e.p.c.s recorded at pH 5.4, 7.4, and 9.9 were generally biphasic. However, not all m.e.p.c.s appeared identical, and m.e.p.c.s with single exponential decays, as well as m.e.p.c.s with biphasic decays, were seen at the same end-plate. At pH 5.4, m.e.p.c.s sometimes had single exponential decays with time constants larger than control, and did not show the fast component of biphasic decay. At pH 9.9, single exponential m.e.p.c.s decayed rapidly, and had no slow decay component. The finding that the decay of some m.e.p.c.s was a single exponential, while other m.e.p.c.s had a biphasic decay, suggests that the effects of procaine are not the same at all receptor-channel complexes.

Any model that is proposed to explain the effects of procaine must account for the interactions between pH, procaine and receptor-channel complexes. Alterations in pH may have numerous effects on the membrane and on end-plate channels. For example, the extent of ionization of membrane groups associated with the channel may change with the external pH, and thus the affinity of ACh for its receptors may depend on pH. Procaine may also modify the actions of ACh or the effects of pH on the actions of ACh. In addition procaine may bind at more than one site, depending on pH, and may be effective at both the outside and inside surfaces of the membrane (Gage *et al.* 1983). The sequential blocking model does not explain all of the effects of procaine on the decay of m.e.p.c.s, perhaps because it does not take into account multiple effects of procaine on a sequence of reactions. These must be considered in a more general model for the effect of local anaesthetics on ion channels.

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*Note added in proof.* Neher has recently measured ACh-induced single-channel currents in the presence of the lignocaine derivative QX222, and concluded that the simple sequential model does not apply at higher concentrations of the drug.



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