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Sodium Channel Blockers: The Size/Solubility Hypothesis Revisited

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SUMMARY

The influence that drug size has on the rate of recovery of sodium channels in heart tissue has been reexamined. A drug dimension that looks at the end-on view of the molecule provides a substantially better explanation for the size dependence of repriming kinetics than does molecular weight. A quantitative model for the recovery time is provided that couples proton exchange kinetics with a drug size-dependent process that is related to recovery from inactivation. Drugs having a wider span at their aromatic end produce more slowing of the rate of recovery from inactivation.

Sodium channel blockers often slow down the rate of recovery of channels after depolarizations. A size/solubility hypothesis was provided several years ago that explained why some drugs, such as lidocaine, slow down this recovery process by only 10–20-fold (in cardiac tissue), whereas other drugs might slow it down by as much as 1000-fold. Slower recovery rates were observed for drugs that were larger or more hydrophilic (1). This earlier analysis used a descriptive mathematical expression to correlate recovery half-times (HT) for different drugs with their physicochemical attributes, namely molecular weight (MW), partition coefficient (P), and pK_a:

$$\log(\mathrm{HT}) = a(\mathrm{MW}) - b\log Q - c \qquad (1)$$

where distribution coefficient $\log Q = \log P - \log(1 + 10^{\mathrm{pK_a - pH}})$.

This quantitative structure-activity relation was successful in explaining the hundredfold range of different recovery times observed for 36 of 40 different drugs (reviewed in Ref. 2). However, several different functional expressions can be used besides Eq. 1. In the absence of a more mechanistic formulation, one can expect errors associated with predictions for new drugs. Predictions will most likely be in error when parameters outside the range used for the original regression fit are encountered, as was found for a drug having an unusually high pK_a (3).

A new analysis has recently been published that explained the combined pH dependence and voltage dependence of lidocaine's recovery kinetics. These studies were done, using voltage-clamp techniques, on isolated bullfrog atrial cells (4). As illustrated below, proton-exchange rates and a voltage-dependent reaction step related to recovery of drug-altered channels from inactivation were used to explain the changes in recovery times for lidocaine under differing conditions of pH and membrane potential:

Blocked charged

$$l_p \updownarrow k_p[\mathrm{H}]$$

Blocked neutral $\xrightarrow{(g\,T_h)^{-1}}$ Unblocked

This new study provided a mechanistically more satisfying formulation for the recovery time than the size/solubility hypothesis described above. The new expression for the recovery time constant is:

$$T = T_p + (g\ T_h) + (g\ T_h)T_p k_p[\mathrm{H}] \qquad (2)$$

where protonation rate (k_p) and deprotonation rate ($l_p = 1/T_p$) refer to proton exchange for drug bound to its channel receptor. The remaining factor ($g\ T_h$) represents a slower than normal (by g-fold) recovery from inactivation for drug-altered channels. The prior analysis (4) suggested that the slowing factor was 10 for lidocaine (4).

This paper will describe how each of the proton exchange parameters (k_p and l_p) and the slowing factor (g) might differ for different drug structures. Ban's laboratory (5) has provided important kinetic studies on a homologous series of drugs that will be helpful for analysis of the effect of drug size on the slowing factor g.

Methods

A number of homologous drugs have been studied for their ability to slow repriming of cardiac Na channels (5). These investigators used measurements of upstroke velocities recorded with intracellular electrodes in guinea pig papillary muscles. Extra premature stimuli were applied at varying times after action potentials in order to estimate recovery of myocardial sodium channels. Over several years they compiled recovery time measurements for more than 20 β-adrenergic blocking drugs of their block of cardiac Na channels. This group of drugs is particularly useful in the context here, because they all have pK_a values within the narrow range of 9.5 to 9.65, yet they display a 15-fold

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variation in recovery times. These results will be used below to examine the role of drug size in the recovery process.

In order to examine drug size more critically, ALCHEMY II software, obtained from Tripos Associates (St. Louis, MO) and implemented on an IBM AT computer, was used to generate appropriate three-dimensional models of drug molecules. Methods of classical molecular mechanics are used by this program to minimize strains associated with bond stretching, angle bending, and torsion, as well as van der Waal's interactions. The minimizer performs a conjugate gradient minimization on a force-field equation, which is dependent on the positions of the atoms in the structure.

The drug dimensions obtained using a similar program from the Xiris Corporation have already been used to discern which drugs can and cannot access the pathway to the sodium channel receptor that is available during maintained depolarizations (6). I have found that larger dimensions are obtained using ALCHEMY, which uses more precise bond angle geometries. In particular, the minimum dimension, or x dimension, is about 50% larger using ALCHEMY, with the other dimensions being roughly the same.

Results

How might different drugs differ in their recovery kinetics according to the new quantitative model suggested by Eq. 2? There are three parameters that must be considered in order to answer this question. Two parameters are concerned with rates of proton exchange, whereas the third deals with a process related to recovery from channel inactivation. Each of these parameters will now be considered.

Deprotonation rate. Drugs that have different pK_a values generally have similar protonation rates, related to collision encounter frequencies in the bulk aqueous phase, but different deprotonation rates (7). Thus, pK_a provides a measure of the strength of proton-drug bonding. A drug with a higher pK_a has more stable proton-drug bonding, so that it is less likely to attain adequate vibrational energies for deprotonation. Such a drug's deprotonation time constant, T_p, will, therefore, be slower. This deprotonation time, T_p, can be related to a given drug's aqueous phase pK_a, according to:

$$T_p = 10^{pK_a - X} \text{ sec} \quad (3)$$

The parameter X averaged 10.6 in the bulk aqueous phase for several weak acids listed in Table IV.1(a) in the study by Eigen and De Maeyer (7). However, within the sodium channel binding site under consideration here, this parameter appears to be somewhat smaller, having a value of about 9.9 at 24° according to voltage-clamp results obtained for mexiletine. Mexiletine's pK_a is 9.3 (8) and its deprotonation time constant is estimated to be 0.25 sec[1] for bullfrog atrial cells. Thus, deprotonation rates within the water-restricted channel may be several times slower than values measured for the bulk aqueous phase. However, pK_a may still provide a measure of relative rates of deprotonation at the channel receptor site for different drugs, because pK_a still describes the strength of the proton-drug bond. This is, Eq. 3 can be used to describe deprotonation times for different drugs at an equivalent channel receptor site, using $X = 9.9$ and bulk phase pK_a.

It is important to use the pK_a appropriate to the temperature of interest when using this expression for T_p. pK_a values are usually reported for 25°; they are 0.2–0.3 units lower at 37° and substantially higher at 10° (9).

Protonation rate. As noted in Ref. 7, the protonation rate k_p does not vary much in the bulk aqueous phase for different drugs. However, it is possible that collision encounter frequencies within the restricted microenvironment of the channel might be more sensitive to structural differences near the tertiary nitrogen than would be the case in the bulk phase. Given this precaution, k_p is estimated to be 1.3×10^9/M/sec for lidocaine, and for lidocaine-like drugs, in the cardiac Na channel at 24°. This estimate is based on the pK' [$pK' = \log(T_p k_p)$] of lidocaine in the channel of 7.1 (4), coupled with Eq. 3 which describes the deprotonation time of lidocaine at the channel receptor in terms of its pK_a (7.9) in the bulk phase. This estimate of protonation rate for cardiac channels at 37° is 2–3-fold faster than the k_p estimate provided by Schwarz *et al.* (10) for lidocaine in skeletal muscle at 13°. Protonation rates in aqueous buffers at 25° are at least another order of magnitude faster, averaging 4×10^{10}/M/sec in the work of Eigen and De Maeyer (7).

Recovery from inactivation. The voltage dependence of recovery times is explained by the $g\ T_h$ term in Eq. 2. That is, recovery times are slower at more depolarized potential levels for drugs like lidocaine that show primarily this "inactivation enhancement" behavior. The slowing factor g, which is estimated to be 10 for lidocaine (4), appears to increase as certain drug dimensions increase, as will be described below.

First, it is instructive to examine, in general terms, the voltage-dependent behavior predicted by Eq. 2. The deprotonation time T_p, which is thought to be relatively voltage independent (4), dominates recovery kinetics at extremely hyperpolarized potential levels. This deprotonation time, given by Eq. 3, can even provide rate-limiting kinetics for channel repriming at normal (resting) levels of membrane potential if a drug has a high enough pK_a (above 10).

Fig. 1 shows the expected relationship between recovery time and membrane potential for several possible combinations of parameters, according to Eq. 2. Amide-linked drugs such as lidocaine have unusually low pK_a values for amines (less than 8); for these drugs the T_p term on the left in Eq. 2 contributes less than 0.01 sec to the recovery time. Such drugs will tend, therefore, to have voltage dependencies of recovery times that nearly parallel those observed for recovery from inactivation (T_h), albeit on a slower time scale, as predicted by the other factors in Eq. 2.

The slowing factor. How does the slowing factor g depend on drug structure? Drug size is an obvious candidate, because larger drugs tend to show slower recovery kinetics (1). Ehring *et al.* (11) have already suggested, using a very different kind of analysis, that recovery from inactivation is slowed to a greater degree by larger drugs. A new way of looking at drug size will be introduced here in order to examine this issue in greater detail.

I would now like to test the hypothesis that the slowing factor g is larger for larger drugs. Twenty-six β-adrenergic blocking drugs have been studied in Ban's laboratory (5) for their ability to slow repriming of cardiac Na channels (see Methods). This is a particularly good group of drugs to compare in the context being raised here because they all have very similar pK_a values and, thus, very similar deprotonation times (<0.3 sec). Thus, any errors in estimates of proton exchange kinetics, as de-

[1] Four measurements of recovery times for mexiletine were provided in Ref. 4. These were fit to equation $T = T_p + a(T_h)$, as suggested by Eq. 2, in order to estimate $T_p = 0.25$ sec.

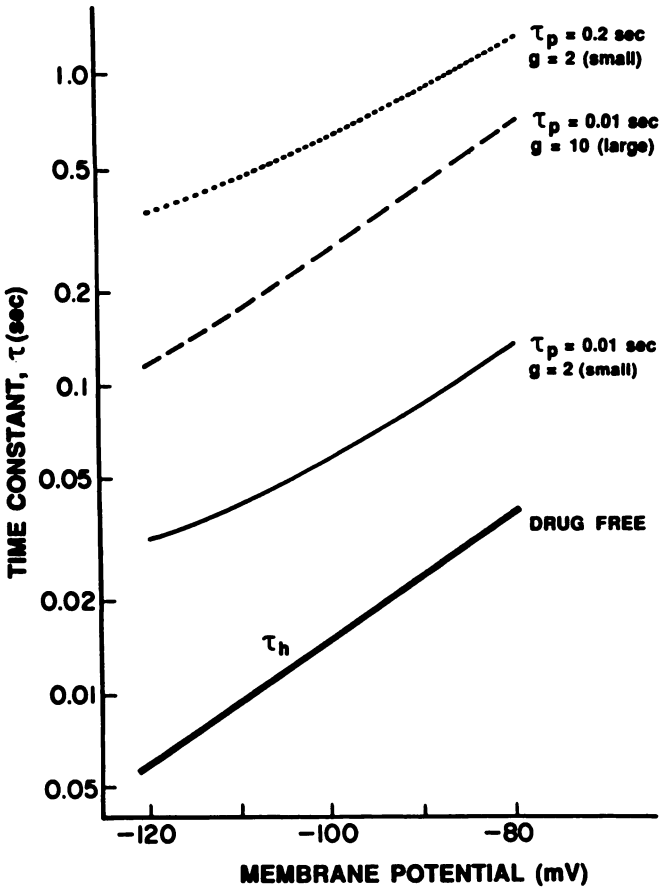

Fig. 1. A comparison of times needed for Na channels to recover from drug effect between action potentials. Eq. 2 predicts the recovery time as a function of the slowing factor g, the voltage-dependent time constant (T_h) governing recovery from channel inactivation, the deprotonation time constant T_p, and the protonation rate k_p (see text). Several possible behaviors for recovery times as a function of membrane potential are described here. In general, longer recovery times will be expected for larger drugs (larger g factor) (– – –) or for drugs having higher pK_a values (-----). In each instance, Eq. 2 predicts a slowing of recovery times at lower pH.

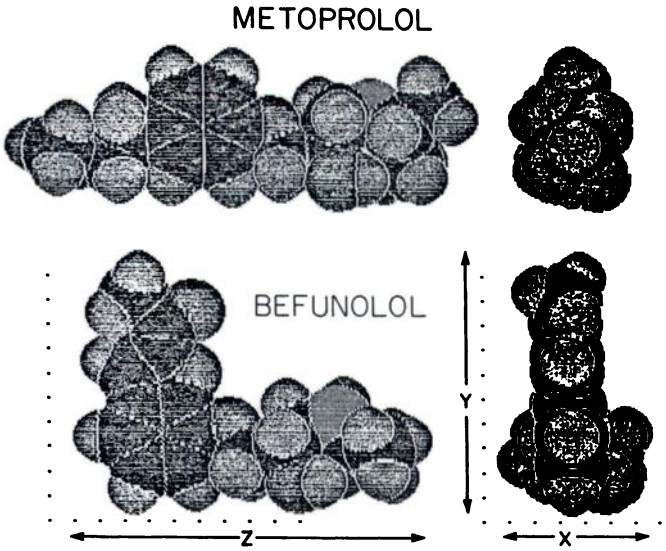

Fig. 2. Two projected views of blocking drugs modeled using the ALCHEMY II system. Metoprolol and befunolol are equally large when viewed via the maximally exposed views on the *left*. However, metoprolol presents a much smaller end-on profile (*right*); which appears to be an important determinant of how much a drug can slow down the rate of recovery from channel inactivation. The dimensions x and y are identified from the end-on profiles at the right, with the spanning width at the aromatic end, called y_a, being used in the analysis that follows.

scribed above, would influence Eq. 2 in a consistent manner. This circumstance, and the very similar tertiary amine structures of these drugs, should make it easier to determine whether systematic changes in the slowing factor g occur as a result of changes in the size of substitutions at the aromatic end of the molecule.

Fig. 2 gives examples of how these end-on dimensions are obtained from plots of ALCHEMY II output. The minimum dimension x is determined primarily by the flexible side chain that contains the tertiary amine group. This x dimension averaged 6.0 Å for the *N*-tert butyl group of the compounds and 5.8 Å for the others (isopropyl amino). The major difference in the end-on dimensions of this series of compounds is, therefore, attributable to differences in their span, y_a, at the aromatic end. These are compiled in Table 1, along with total strains associated with the energy minimization.

Ban *et al.* (5) had already noted a good correlation between recovery times and molecular weight in their study. However, they also noted that five drugs displayed recovery times that were too fast (Fig. 3A, *closed circles*); these are all relatively "thin" molecules, like metoprolol in Fig. 2. These five anomalous drugs all fall into line in the correlations shown in Fig. 3B,

TABLE 1
Recovery times and sizes of 26 adrenergic blockers

	Molecular weight	τ	Aromatic dimensions		Model strain
			x_a	y_a	
		sec	Å		kcal/mol
Acebutolol	336	5.2	6.3	9.0	5
Pindolol	249	4.5	3.1	9.0	15[a]
Bufetolol	324	4.1	5.0	12.0	10[a]
Befunolol	275	2.9	3.8	10.7	13[a]
D32-T[b]	252	2.4	4.1	7.8	<1
Propranolol	260	2.1	3.1	9.2	<1
Carteolol-T	294	2.1	4.8	9.2	<2
Oxprenolol	267	2.0	5.1	9.2	<2
D2-T	240	1.9	4.0	7.8	<1
Penbutolol-T	292	1.8	5.3	10.2	<2
Bupranolol-T	270	1.8	3.8	8.8	<1
Ko1313	232	1.7	4.2	9.0	<1
Indenolol	247	1.7	4.0	9.0	8[a]
D24-T	245	1.7	4.1	7.8	<1
Bunitrolol-T	249	1.5	4.2	9.0	<1
Timolol	317	1.5	6.6	8.0	19[a]
Alprenolol	250	1.3	4.5	9.6	<1
D25	236	0.9	4.1	9.1	<1
D4-T	236	0.9	4.0	6.5	<1
D3-T	236	0.8	4.0	7.8	<1
Ko707	236	0.7	4.1	9.1	<1
Ko592	223	0.6	4.0	7.8	<1
Pamalotol	308	0.5	4.6	6.4	<1
Sotalol	272	0.45	5.2	6.3	6
Metoprolol	268	0.3	4.7	7.0	<2
Atenolol	267	0.3	4.0	7.0	<1

[a] High angle strain within five-member ring.
[b] -T, *N*-tert butyl derivatives (5).

which uses the end-on dimension y_a, the span at the aromatic end of the molecule, as the measure of drug size. This approach takes their exceptional thinness into account. Only pindolol and acebutolol are anomalous now; they display slower recovery

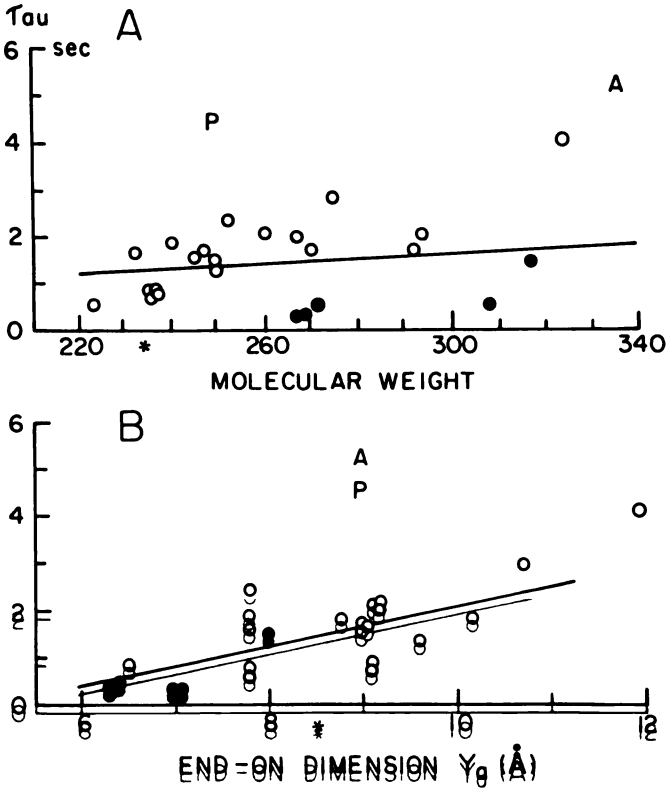

Fig. 3. Comparison of original size measure (molecular weight) and new size measure as predictors of recovery kinetics. A, ● five thin drugs (atenolol, metoprolol, sotalol, pamatolol, and timolol) that display faster recovery kinetics than would be expected based on molecular weight. B, y_o dimension can be used to bring these five exceptions into line. Excluding pindolol (*P*) and acebutolol (*A*) (which are too slow in either scheme), the overall regression fit improves from $r \equiv 0.38$ in A to 0.78 in B ($n \equiv 24$). *, lidocaine's position on each abscissa.

kinetics than would be predicted based upon either molecular weight or end-on dimension y_o.

What kinds of slowing factors, g, are represented in Fig. 3? First note that, according to the best fit lines in Fig. 3, minimum time constants (T_r) should occur for those drugs having end-on dimensions of $y \leq 6$ Å, where $g \equiv 1$.[2] As the spanning width (y_o dimension) increases beyond this, the recovery time should increase, as predicted by Eq. 2, via increases in the g factor. If lidocaine's slowing factor of 10 can be applied to β-adrenergic blockers of similar dimensions, then $g \equiv 10$ should occur for y_o near 8.5 Å in Fig. 3B (*asterisk*). A slowing factor of 10 would be equivalent to a voltage shift of inactivation recovery kinetics of 23 mV, according to the gating model and interpretation of Hondeghem and Katzung (12).

Discussion

Proton exchange rates for drugs bound to their sodium channel receptor appear to be somewhat slower than rates appropriate to the bulk aqueous phase. Deprotonation rates are 4–5-fold slower, and this may be attributable to damping of overall molecular vibrations for drugs bound in the receptor environment. Estimates of protonation rates, which are consistent with earlier independent estimates (10), are estimated to be even slower (20–40 times) within the protected channel microenvironment. The apparent pK_a (pK') of a drug at its receptor site can, therefore, be substantially lower (0.5 to 1 pK units) at its receptor site than it is in the bulk aqueous phase.

These proton exchange rates can provide rate-limiting kinetics for the net "unblocking" process observed with drug treatment, as suggested by Schwarz *et al.* (10). The unblocking process is envisioned as a deprotonation step followed by a recovery from channel inactivation that is slower than normal, as Khodorov *et al.* (13) have already suggested. Finally, I have added a novel interpretation of how specific drug dimensions might influence the rate of recovery from apparent channel inactivation. The resulting quantitative model integrates these several concepts in order to explain the 1000-fold variability in repriming rates observed for cardiac sodium channels exposed to drugs (2).

Apparent exceptions to the rule. There are fewer exceptions to the new size rule (Fig. 3B) than to the former size rule, which was simply based on molecular weight. Some other exceptions to the earlier size/solubility hypothesis have also been noted. The aprindine derivative A777 represented one of the first clear exceptions to the former rule (14). pK_a differences between these two drugs can be used to explain 2–3-fold differences in their recovery times, with aprindine being slower; this comes close to the 3.3-fold difference that was measured.

Amiodarone is an example of a drug having a very high molecular weight (645) but intermediate recovery kinetics (15). Amiodarone is a long, relatively thin drug having an aromatic end-on dimension of $y_o \equiv 10.2$ Å; it would be expected to display an intermediate recovery time of several seconds (Fig. 3B), rather than the tens of seconds expected for a drug having such a large molecular weight (2).

BW A256C, reported on by Donoghue *et al.* (3), has a small, lidocaine-like, end-on span of $Y_o \equiv 8.9$ Å. However, it has a very high pK_a, so it would be expected to show recovery times of near 100 sec (via T_r terms in Eq. 1), as they observed, despite its small dimensions.

Real exceptions to the rule. Some stereoisomers differ in their blocking effects on Na channels. In particular, Yeh (16) and Clarkson (17) found that the local anesthetic RAC109-I produced greater frequency-dependent blocking effects and substantially slower recovery kinetics than did its enantiomer RAC109-II. The size/solubility hypothesis, as originally expressed in terms of molecular weight, pK_a, and lipid solubility, could not explain this important observation. This revised model can do no better, because stereoisomers will be true mirror images of each other and have the same y_o dimension. Thus, there must still be important steric aspects involved in the drug-receptor interaction.

Pindolol and acebutolol remain as the only exceptions to the new (y_o) size rule, as applied to this homologous series of 26 blocking drugs. Examination of these structures reveals an interesting point regarding pindolol. The aromatic structure of pindolol (Fig. 4) is identical to that of the amino acid tryptophan. An interesting speculation is that a tryptophan residue plays an important role in the Na channel inactivation machinery with which these drugs are thought to interact. Indenolol is very similar to pindolol but lacks the NH group (Fig. 4); its recovery time is simply predicted by its y_o dimension (Fig. 3B).

Larger drugs, such as penticainide and disopyramide (18), may not be able to interact with the inactivation gating mech-

[2] The minimum recovery time observed for this series of drugs, 0.3 sec, provides an independent estimate of deprotonation time T_r for these drugs at their channel receptor. According to Eq. 3, the parameter $X \cong 9.5 - \log(0.3) \cong 10$.

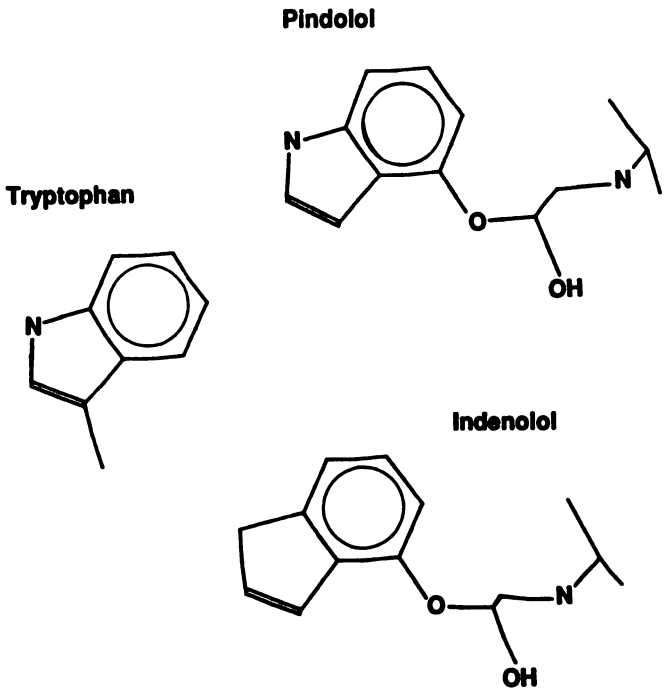

Fig. 4. Pindolol shows unusually slow recovery kinetics according to the revised size concept, as well as unusually strong blocking potency, given its lipid partitioning ability (Fig. 11 in Ref. 5). Pindolol closely resembles the tryptophan side chain. Indenolol is similar but lacks the nitrogen in the 5-ring; its recovery time is predicted by its y_a dimension.

anism in the same way as lidocaine. These drugs have end-on spans, y_a, in excess of 11 Å and show voltage-dependent behaviors of their recovery times that are opposite to that displayed for lidocaine. These bulkier drugs and quaternary drugs show "activation trapping" (18–19) rather than "inactivation enhancement" behaviors as observed for lidocaine. The quantitative formulation described here should only be applied to smaller tertiary (not quaternary) drugs that display predominantly inactivation enhancement behaviors, like lidocaine.

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