

# Imipramine Inhibition of Transient $K^+$ Current: An External Open Channel Blocker Preventing Fast Inactivation

Chung-Chin Kuo

Department of Physiology, National Taiwan University College of Medicine, and Department of Neurology, National Taiwan University Hospital, Taipei 100, Taiwan, Republic of China

**ABSTRACT** Rapidly inactivating  $K^+$  current ( $K_A$  current) is recorded from rat hippocampal neurons by whole-cell patch-clamp technique and suitable voltage protocols. It is found that imipramine, a commonly prescribed tricyclic antidepressant, is an open  $K_A$  channel blocker with a binding rate constant of  $5.6 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$  and an apparent dissociation constant of no more than  $6 \mu\text{M}$  if applied extracellularly in pH 7.4. The inhibitory effect is more pronounced in more alkaline extracellular solution, suggesting that the neutral form of imipramine is much more active than the charged form. In contrast, intracellular imipramine shows no inhibitory effect. Furthermore, the inhibitory effect of imipramine is antagonized by external but not internal  $K^+$ . These findings suggest an imipramine binding site located close to the external pore mouth. It is also found that the inactivation curve of  $K_A$  current is not changed by imipramine. Moreover, the recovery of  $K_A$  current after a step depolarization is accelerated in the presence of imipramine. These findings suggest insignificant binding of imipramine to the fast inactivated  $K_A$  channel. The selective binding of imipramine to only the activated but not the deactivated or inactivated states seems to suggest continual gating conformational changes in the external pore mouth of these neuronal  $K_A$  channels during membrane depolarization.

## INTRODUCTION

Imipramine is an important antidepressant agent frequently prescribed in psychiatric and other clinics. It contains a characteristic three-ring nucleus and is a member of the "tricyclic antidepressants." The therapeutic effect of tricyclic antidepressants against depression is most likely ascribable to their modulation of the aminergic (norepinephrine and serotonin) transmission in the central nervous system (for a review see Hollister, 1995). Nevertheless, these compounds have also been shown to inhibit  $K^+$  and other ionic currents (Isenberg and Tamargo, 1985; Ogata and Narahashi, 1989; Delpon et al., 1992; Ogata and Tatebayashi, 1993; Woollorton and Mathie, 1993, 1995). In this regard, it is interesting that the inhibition of  $K^+$  currents by imipramine seems to be different in different preparations. In sympathetic neurons, Woollorton and Mathie (1993) found that imipramine had a stronger inhibitory effect on the late, sustained part of whole-cell  $K^+$  currents than on the early peak of  $K^+$  currents, as if imipramine preferentially blocks the delayed-rectifier  $K^+$  current rather than the transient or A-type  $K^+$  current ( $K_A$  current). On the other hand, Delpon et al. (1992) integrated the first 50 ms of  $K^+$  current elicited upon depolarization instead of measuring just the peak current and argued that imipramine is a potent blocker of  $K_A$  current in cardiac cells.

The foregoing seemingly conflicting observations may be reconciled if imipramine is an open channel blocker of the

$K_A$  currents. In other words, if imipramine has high affinity toward only the open but not the closed or resting states of the channel, it would take some time for the current to be inhibited by the drug after the channel is opened upon depolarization. Thus imipramine would not decrease the early peak as much as the late  $K^+$  currents, but would have a significant effect against the early current if one integrates the first 50 ms of  $K^+$  current upon depolarization. The findings that imipramine shortens the time to peak and increases the decaying rate of the transient  $K^+$  currents also support the open channel blocking effect of imipramine (Delpon et al., 1992). Because different affinities of a drug toward the closed and open states of a channel are consequences of channel gating, the characterization of an open-channel blocking phenomenon in more detail would not only be of pharmacological interest, but would also shed light on the gating conformational changes of the channel. I therefore studied the mechanisms underlying imipramine inhibition of  $K_A$  current in hippocampal neurons with two goals. First, I would like to examine whether imipramine is an open channel blocker of the  $K_A$  current in mammalian central neurons, and to measure the affinity and kinetics of imipramine binding to the  $K_A$  channel to assess its pharmacological significance. Second and even more interesting, I would try to deduce some gating conformational changes of the channel by investigating the location and nature of the imipramine binding site. It is found in this study that imipramine preferentially blocks the open  $K_A$  channels with an apparent dissociation constant of no more than  $6 \mu\text{M}$  in pH 7.4, and the inhibitory effect of imipramine is stronger in more alkaline solution. The imipramine "receptor" is probably located close to the external pore mouth and consists of one aromatic and one aliphatic molecular determinant, implying gating conformational changes involving amino ac-

Received for publication 16 June 1998 and in final form 1 September 1998.

Address reprint requests to Dr. Chung-Chin Kuo, Department of Physiology, National Taiwan University College of Medicine, and Department of Neurology, National Taiwan University Hospital, No. 1, Jen-Ai Rd., 1st Section, Taipei 100, Taiwan, Republic of China. Tel.: 886-2-23123456, ext. 8236; Fax: 886-2-23964350; E-mail: cckuo@ha.mc.ntu.edu.tw.

© 1998 by the Biophysical Society

0006-3495/98/12/2845/13 \$2.00

ids containing such side-chain groups. Moreover, although imipramine is an open  $K_A$  channel blocker from the outside, imipramine binding and fast inactivation of the channel seem to be mutually exclusive. This further suggests different conformations near the external pore mouth between the open and fast inactivated states of central neuronal  $K_A$  channels.

## MATERIALS AND METHODS

### Cell preparation

Coronal slices of the whole brain were prepared from 7- to 14-day-old Long-Evans rats. The CA1 region was dissected from the slices and cut into small chunks. After treatment for 5–10 min at 37°C in dissociation medium (in mM, 82  $Na_2SO_4$ , 30  $K_2SO_4$ , 3  $MgCl_2$ , 5 HEPES, and 0.001% phenol red indicator, pH 7.4) containing 0.5 mg/ml trypsin (type XI; Sigma, St. Louis, MO), tissue chunks were moved to dissociation medium containing no trypsin but 1 mg/ml bovine serum albumin (Sigma) and 1 mg/ml trypsin inhibitor (type II-S; Sigma). Each time when cells were needed, two or three chunks were picked and triturated to release single neurons.

### Whole-cell recording

The dissociated neurons were put in a recording chamber containing Tyrode's solution (in mM, 150 NaCl, 4 KCl, 2  $MgCl_2$ , 2  $CaCl_2$ , and 10 HEPES, pH 7.4). Whole-cell voltage-clamp recordings were obtained using pipettes pulled from borosilicate micropipettes (OD 1.55–1.60 mm; Hilgenberg, Malsfeld, Germany), fire polished, and coated with Sylgard (Dow-Corning, Midland, MI). Except for the internal imipramine experiments in Fig. 4 and the low internal  $K^+$  experiments in Fig. 5, the pipettes were filled with the standard internal solution, containing (in mM) 75 KCl, 75 KF, 2.5  $MgCl_2$ , 5 HEPES, 5 EGTA, with pH adjusted to 7.4 by KOH. For the experiments studying the effect of internal imipramine in Fig. 4, 100  $\mu M$  imipramine was added to the standard internal solution. In Fig. 5 B, the "15 mM internal  $K^+$  solution" contained the same components as the standard internal solution, except that (in mM) 75 KCl + 75 KF was replaced by 15 KCl + 60 NMGC (N-methyl-D-glucamine chloride) + 75 NMGF (N-methyl-D-glucamine fluoride). A seal was formed and the whole-cell configuration was obtained in Tyrode's solution. The cell was then lifted from the bottom of the chamber and moved in front of an array of flow pipes (microcapillary, content 1  $\mu l$ , length 64 mm; Hilgenberg) emitting external recording solutions. Except for the "150 mM external  $K^+$ " experiments in Fig. 5, the external solutions were basically Tyrode's solutions (pH 5.8–8.2 for the experiments in Fig. 6, pH 7.4 for the other experiments) with or without different concentrations of drugs. The 150 mM  $K^+$  external solution used in Fig. 5 was made by changing the 150 mM NaCl + 4 mM KCl in Tyrode's solution to 150 mM KCl + 4 mM NaCl, with all other components kept the same. Imipramine, benzotropine, diphenhydramine, and tranlycypromine were dissolved in water, and other drugs were dissolved in dimethylsulfoxide to make a 100 mM stock solution, which was then diluted into Tyrode's or 150 mM  $K^+$  external solution to attain the final concentrations desired. The final concentration of dimethylsulfoxide (0.1% or less) was not found to have a detectable effect on  $K^+$  currents. All external solutions contained 1  $\mu M$  tetrodotoxin to block the sodium currents. The  $Ca^{2+}$  currents in these preparations tended to run down so quickly and completely (possibly because of the fluoride ions in the internal solution) that almost no  $Ca^{2+}$  currents were detectable ~3 min after establishment of the whole-cell configuration. Some sustained or "late"  $K^+$  currents also ran down in a few minutes, presumably because of the reduction of  $Ca^{2+}$ -dependent  $K^+$  currents when the intracellular space was dialyzed with EGTA. Thus I always waited for at least 5–10 min after the establishment of whole-cell configuration to start the recording. In this way organic or inorganic  $Ca^{2+}$  channel blockers are spared to avoid unnecessary drug-drug interaction or modulation of the  $K_A$  currents. Cur-

rents were recorded at room temperature (~25°C) with an Axoclamp 200A amplifier, filtered at 5 kHz with a four-pole Bessel filter, digitized at 100–300- $\mu s$  intervals, and stored using a Digidata-1200 analog/digital interface and pCLAMP software (Axon Instruments, Foster City, CA). Residual series resistance was generally smaller than 1 M $\Omega$  after partial compensation (typically more than 80%), and the product of residual series resistance and cell capacitance was generally less than 20  $\mu s$ . All statistics are given as mean  $\pm$  SEM.

## RESULTS

### Imipramine speeds the decay of transient $K^+$ current but does not affect its activation phase

Fig. 1 A shows the dose-dependent inhibitory effect of imipramine on the macroscopic  $K^+$  currents elicited by a step depolarization. The decay of the currents is speeded by imipramine, and the inhibition of the late currents seems more pronounced than the inhibition of the early peak currents. As the current under observation here is a mixture of different kinds of  $K^+$  currents, there are two possible causes of the acceleration of current decay: imipramine could be an open channel blocker of some rapidly activating  $K^+$  currents, or it could be a selective inhibitor of some late currents. To have a purer population of currents, I try to isolate the  $K_A$  current from the delayed rectifier and other late  $K^+$  currents by a pulse protocol in Fig. 1 B (see also Numann et al., 1987; Wooltorton and Mathie, 1993). An advantage of using a voltage protocol rather than a pharmacological formula here is that unnecessary interactions among different drugs can be avoided. The  $K_A$  current isolated in this way activates fast, reaching its peak within 3–4 ms of depolarization to +100 mV, and then shows an inactivating time constant around 20 ms. (About 30% of the cells do show a longer inactivating time constant by this pulse protocol. However, only those that show a time constant less than 25 ms are used for further studies.) There is still a "sustained" component of such  $K_A$  current. This sustained component may consist of both truly noninactivating current through  $K_A$  channels (e.g., Timpe et al., 1988; Iverson et al., 1988) and the "contaminating" delayed rectifier or other late  $K^+$  currents. Imipramine not only speeds  $K_A$  current decay and inhibits the current, but also shows a dose-dependent shortening of the time to peak current (Fig. 1 B). The early activation phase of the currents, however, is almost unaffected by imipramine. All of these findings suggest that imipramine binds to and blocks the pore only when the  $K_A$  channel is open. Similar findings are also obtained in those cells showing prominent  $K_A$  current upon a simple depolarizing pulse (Fig. 1 C), arguing against any significant artifact associated with the pulse protocol used to isolate the  $K_A$  current.

### The rate of decay of transient $K^+$ current is linearly related to imipramine concentrations only when the concentrations are not high

If imipramine is an open channel blocker of  $K_A$  current, then the accelerated decay of the current would be mostly

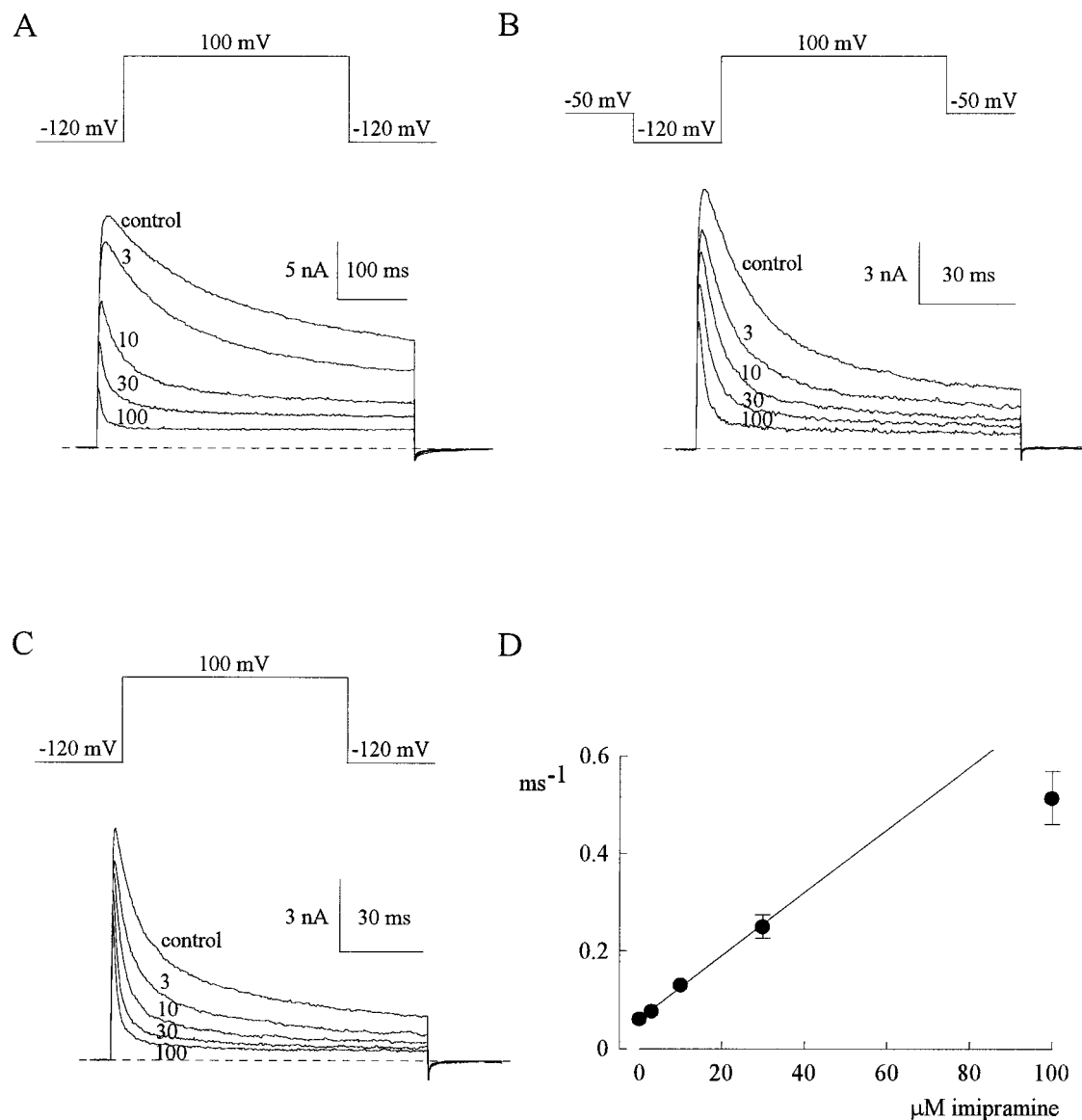


FIGURE 1 Dose-dependent inhibition of  $K^+$  currents by imipramine. (A)  $K^+$  currents in controls and 3, 10, 30, or 100  $\mu\text{M}$  imipramine (denoted by each trace) in a cell held at  $-120$  mV and stepped every 3 s to  $+100$  mV for 450 ms to elicit the current. Dashed line, zero current level. (B) The  $K_A$  current is isolated by a pulse protocol shown in the upper panel. Specifically, the cell is held at  $-50$  mV and then hyperpolarized to  $-120$  mV for 50 ms before it is stepped every 2 s to  $+100$  mV for 100–200 ms to elicit the current. (C) Some cells show prominent  $K_A$  current with just a simple pulse protocol. For example, this cell is held at  $-120$  mV and stepped every 2 s to  $+100$  mV for 115 ms to elicit the current. Note that the effects of 3–100  $\mu\text{M}$  imipramine are quite similar to those observed in B. (D) The inverses of the decaying time constants of the currents in B from six cells are (in  $\text{ms}^{-1}$ )  $0.055 \pm 0.006$  (control),  $0.069 \pm 0.007$  (3  $\mu\text{M}$ ),  $0.12 \pm 0.012$  (10  $\mu\text{M}$ ),  $0.21 \pm 0.02$  (30  $\mu\text{M}$ ),  $0.43 \pm 0.05$  (100  $\mu\text{M}$ ), and these values are plotted against imipramine concentration. The line is a linear regression fit to the first four mean values (0–30  $\mu\text{M}$  imipramine). The intercept and the slope are  $0.056 \text{ ms}^{-1}$  and  $5.6 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$ , respectively.

related to drug binding (the O to OB step in the simplified scheme in Fig. 2 A). When the activation step or C to O rate is made very fast by strong depolarization (e.g., to  $+100$  mV), the O to OB step may become rate limiting in the whole “reaction,” and the observed kinetics of current decay should be positively correlated with drug concentrations. Fig. 1 D shows this is indeed the case when imipramine concentrations are not high. The linear relationship at the low concentration range further suggests a one-to-one binding (simple bimolecular reaction) between imipramine and

$K_A$  channels. The slope of the regression line,  $5.6 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$ , can be viewed as the binding rate constant between imipramine and the open  $K_A$  channel. Interestingly, at concentrations 100  $\mu\text{M}$  or higher, the observed macroscopic binding rates of imipramine fall below the prediction by the regression line. This is actually consistent with rather than in contradiction to the notion that imipramine blocks open  $K_A$  channels via a simple bimolecular reaction. In the control condition, the activation of  $K_A$  current in the experiments in general has a time constant of 0.7–1 ms. Simplis-

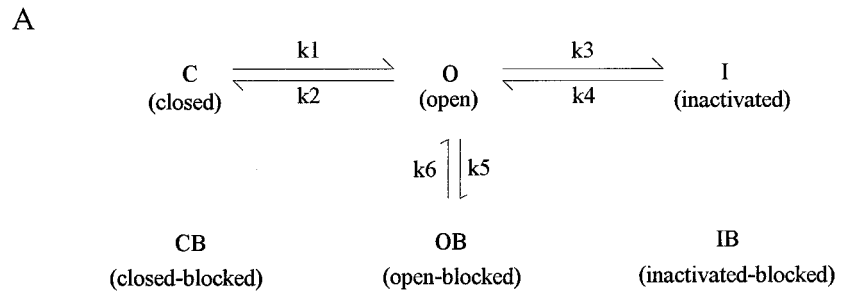
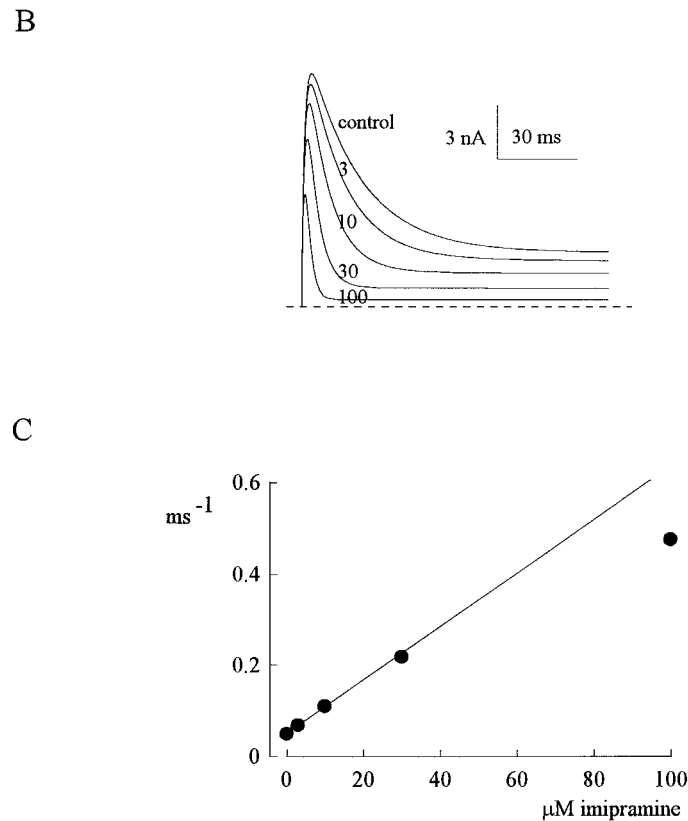


FIGURE 2 Simulation of the inhibitory effect of imipramine on  $K_A$  currents. (A) A simplified gating model for  $K_A$  channels depicting the open channel blocking effect of imipramine. After the channel is opened at depolarization, the pore may subsequently become nonconducting because of either channel inactivation (state I) or imipramine binding (state OB). Two other nonconducting states CB and IB, whose existence is discussed in more detail later, are temporarily disregarded here.  $k_1$  to  $k_6$  denote the rate constant of each reaction. (B) Simulated currents based on the scheme in A.  $k_1$ ,  $k_2$ ,  $k_3$ ,  $k_4$ , and  $k_6$  are (in  $s^{-1}$ ) 800, 1, 40, 10, and 18, respectively.  $k_5$  is  $6 \times 10^6 M^{-1} s^{-1}$  times imipramine concentrations (0, 3, 10, 30, or 100  $\mu M$ ). The opening probability (occupancy of the O state) at each time point is calculated by the Runge-Kutta method, and the final current at each time point is set as the product of opening probability and 15 nA (an arbitrarily defined value for the current when all channels are open). Dashed line: zero current level. (C) The inverses of the decaying time constants of the currents in B are plotted against imipramine concentrations. The line is a linear regression fit to the first four points (0–30  $\mu M$  imipramine). The intercept and the slope are 0.051  $ms^{-1}$  and  $5.6 \times 10^6 M^{-1} s^{-1}$ , respectively. Note that the point at 100  $\mu M$  imipramine deviates from the line as in Fig. 1 D.



tically this would imply a C to O rate around  $1000 s^{-1}$  in Fig. 2 A. According to the slope of the regression line, 100  $\mu M$  imipramine would yield an O to OB rate around  $\sim 600 s^{-1}$ . These numbers indicate that the O to OB step is no longer so “rate limiting” for the overall reaction. The observed rate of current decay would thus be slower than the “true” O to OB rate because of the slow supply of state O, the “reactant” of the O to OB step. These concepts are recapitulated by the simulated currents in Fig. 2, B and C.

### The affinity between imipramine and the open $K_A$ channel

As the interaction between imipramine and the open  $K_A$  channels in hippocampal neurons seems to be a simple bimolecular reaction, it is desirable to estimate the dissoci-

ation constant between the two molecules. However, if one measures the percentage of inhibition early in the step depolarization, the drug binding reaction has not reached its steady state. If one measures the percentage of inhibition late in the step depolarization, the current may be contaminated by the delayed rectifier or other late  $K^+$  currents. I therefore measure both the degree of inhibition at three time constants of current decay at each drug concentration (Fig. 3 A) and at 100 ms of depolarization (Fig. 3 B). Both yield a dissociation constant of  $\sim 6 \mu M$ . This value should be viewed as a high estimate of the dissociation constant (low estimate of the affinity) between imipramine and the open  $K_A$  channels in hippocampal neurons. This is because in Fig. 3 A the blocking reaction may still slightly fall short of the steady state, and in Fig. 3 B the measurement may be contaminated by the delayed rectifier current and a “truly”

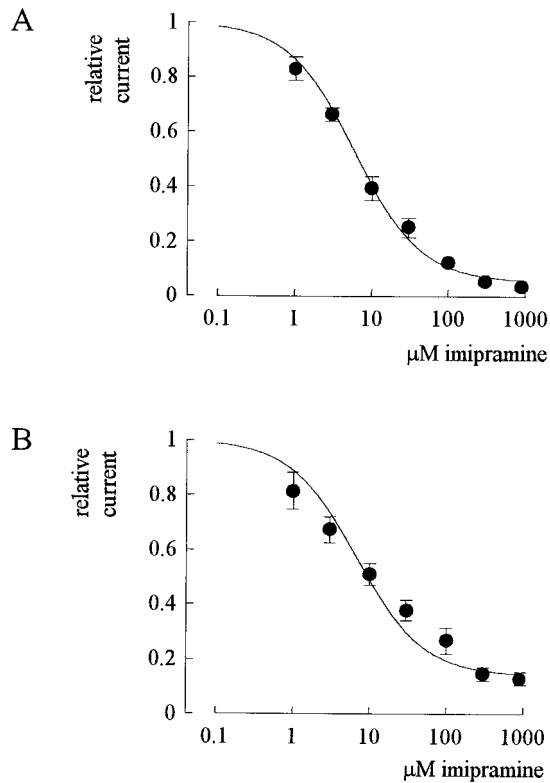


FIGURE 3 Affinity between imipramine and K<sub>A</sub> channels. K<sub>A</sub> current is recorded with the pulse protocol in Fig. 1 B. (A) The current at three time constants of current decay in the presence of imipramine is normalized to the control current at the same time point in six cells and is plotted against the concentration of imipramine, [imipramine]. The line is a best fit to the data points of the form: relative current = 0.05 + (1 - 0.05)/[1 + ([imipramine]/5.9 μM)]. (B) In the same cells as in A, the current at 100 ms of depolarization in the presence of imipramine is normalized to the control current at the same time point and is plotted against [imipramine]. The line is a best fit to the data points of the form: relative current = 0.14 + (1 - 0.14)/[1 + ([imipramine]/6.7 μM)].

noninactivating K<sup>+</sup> current. The delayed rectifier current seems to be less sensitive to imipramine (dissociation constant ~7 μM; see Woollorton and Mathie, 1993). The noninactivating K<sup>+</sup> currents may manifest themselves as small currents elicited by a test pulse to +100 mV directly from a holding potential of -50 mV and are essentially insensitive to imipramine (data not shown).

#### Imipramine has no effect on the K<sub>A</sub> current if applied internally

In previous experiments imipramine is applied externally (from the extracellular side). However, the uncharged form of imipramine might cross the membrane easily. Because both the internal and external solutions are at pH 7.4, and there is no continuous and rapid “wash” of the intracellular space, intracellular imipramine concentration may build up or even become as high as that in the external solution. Therefore based on only the previous experiments, one cannot tell whether the externally applied imipramine

blocks the open K<sub>A</sub> channel from the outside or from the inside. Taking advantage of the rapid and continuous solution change of the extracellular space in the experimental system, I examine the effect of internal imipramine by adding 100 μM imipramine to the pipette solution. Now the imipramine crossing the membrane and reaching the outside will be quickly washed away, and thus the internally applied imipramine should not build up any significant concentration in the external solution. Very similar to the findings with drug-free internal solution, in the first few minutes after breakthrough into the cell, the late currents become smaller (see Materials and Methods). However, there is no significant reduction of the early K<sup>+</sup> current throughout the experiments (up to ~90 min), and the current amplitude is always comparable to those observed with drug-free internal solution. Moreover, the speed of current decay and the inhibition produced by 10 μM external imipramine are very similar whether the internal solution contains 100 μM imipramine or not, confirming that the K<sub>A</sub> current observed in the presence of internal imipramine is not a residual current already under significant inhibition (Fig. 4 A–C). These findings suggest that imipramine blocks the K<sub>A</sub> channel pore from the outside rather than from the inside.

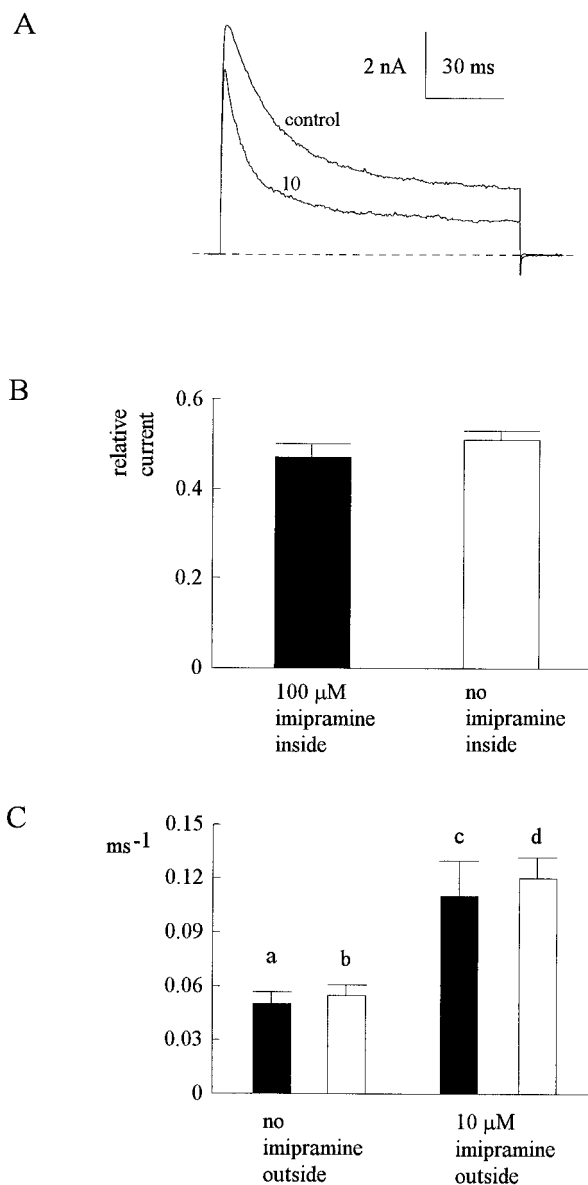
#### The inhibitory effect of imipramine on the K<sub>A</sub> current is antagonized by external but not internal K<sup>+</sup>

If imipramine blocks the pore of open K<sub>A</sub> channels, then it would be interesting to see whether there is any interaction between the blocking imipramine and other travelers in the pore, e.g., K<sup>+</sup> ions. The inhibition by imipramine seems to be unchanged if the internal K<sup>+</sup> concentration is decreased from 150 to 15 mM. However, the inhibition is significantly reduced when external K<sup>+</sup> is increased from 4 to 150 mM (Fig. 5, A and B). Fig. 5 C shows that the binding rate constants are also reduced in 150 mM external K<sup>+</sup>. These findings not only support the foregoing view that imipramine blocks the K<sub>A</sub> channel pore from outside, but also suggest that the imipramine binding site may be located quite superficially in the pore (see the Discussion).

#### The inhibitory effect of imipramine is more pronounced in more alkaline solution

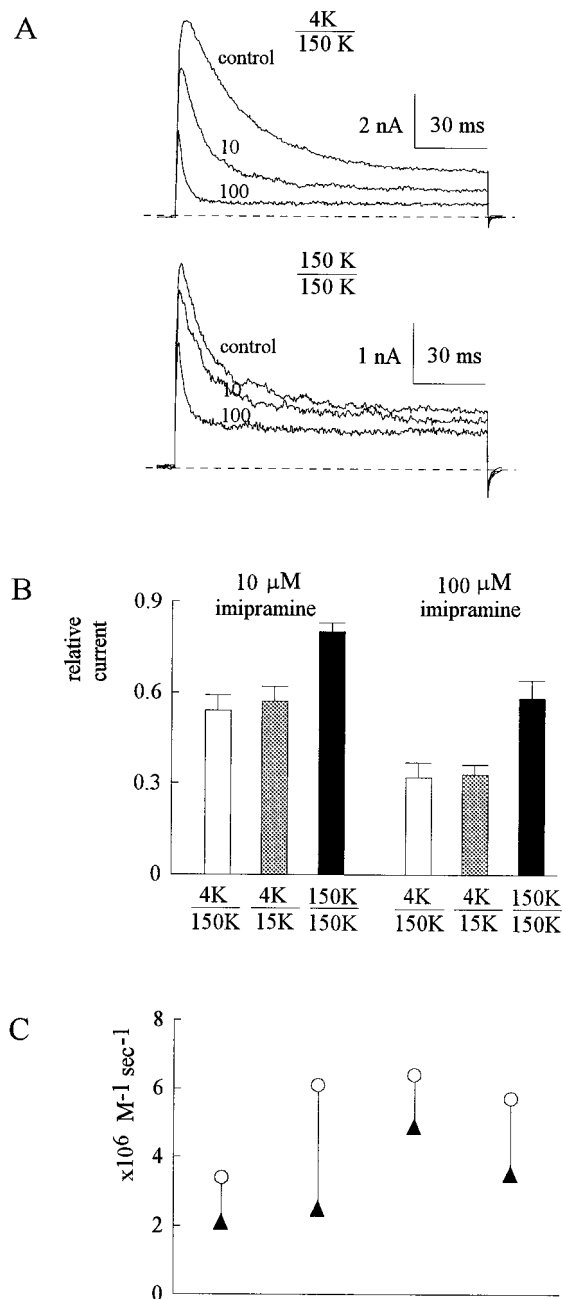
Imipramine has a pK<sub>a</sub> of 9.5, and thus in aqueous solution it can be protonated and becomes a charged molecule. Woollorton and Mathie (1995) have shown that the uncharged form of amitriptyline (another commonly prescribed tricyclic antidepressant also containing a tertiary amine side chain) is probably the active form responsible for the blockade of delayed rectifier K<sup>+</sup> currents. To see whether the neutral form of imipramine is also more active than the charged (protonated) form in blocking the K<sub>A</sub> current, the effect of imipramine is examined in solutions of pH 5.8, 6.6, and 8.2 (Fig. 6). The effect of imipramine in pH





**FIGURE 4** Internal imipramine has no effect on  $K_A$  currents. (A)  $K_A$  current is recorded with the same pulse protocol as that in Fig. 1 B, but here the internal solution contains  $100 \mu\text{M}$  imipramine. The currents are obtained 20 min after establishment of the whole-cell configuration in the presence of either 0 (control) or  $10 \mu\text{M}$  external imipramine (denoted by the traces). Dashed line: zero current level. (B) With  $100 \mu\text{M}$  internal imipramine, the relative current at 100 ms of depolarization in  $10 \mu\text{M}$  external imipramine (normalized to that in zero external imipramine, *black bar*) is  $0.47 \pm 0.03$  ( $n = 4$ ). As a comparison, the relative current with no imipramine inside is  $0.51 \pm 0.02$  (*white bar*, data from Fig. 3 B). (C) The decaying rates of  $K_A$  current in the absence of external imipramine are  $0.05 \pm 0.007$  ( $n = 4$ , *black bar a*) and  $0.055 \pm 0.006$   $\text{ms}^{-1}$  ( $n = 6$ , *white bar b*, data from Fig. 1 D) for  $100 \mu\text{M}$  and zero internal imipramine, respectively. In the presence of  $10 \mu\text{M}$  external imipramine, the decaying rates of  $K_A$  current are  $0.11 \pm 0.02$  ( $n = 4$ , *black bar c*) and  $0.12 \pm 0.012$   $\text{ms}^{-1}$  ( $n = 6$ , *white bar d*, data from Fig. 1 D) for  $100 \mu\text{M}$  and zero internal imipramine, respectively.

9.0 or higher is not documented because of apparent changes of the whole-cell  $K^+$  currents in those very alkaline solutions. (The "transient"  $K^+$  current becomes quite small,



**FIGURE 5** The inhibitory effect of imipramine on  $K_A$  current is antagonized by external  $K^+$ . (A) The inhibitory effect of 10 and  $100 \mu\text{M}$  external imipramine in Tyrode's solution (which contains 4 mM  $K^+$ , *upper panel*) is larger than that in 150 mM  $K^+$  external solution (*lower panel*). The sweeps are from the same cell, and the pulse protocol is the same as that in Fig. 1 B. The internal solution contains 150 mM  $K^+$  (standard internal solution). Dashed lines, Zero current level. (B) In each of the three different experimental conditions, i.e., external 4 mM  $K^+$ /internal 150 mM  $K^+$ , external 4 mM  $K^+$ /internal 15 mM  $K^+$ , and external 150 mM  $K^+$ /internal 150 mM  $K^+$ , the relative currents in  $10 \mu\text{M}$  imipramine are defined by the same method as that in Fig. 3 B and are  $0.54 \pm 0.05$  ( $n = 4$ ),  $0.57 \pm 0.05$  ( $n = 5$ ), and  $0.8 \pm 0.03$  ( $n = 4$ ), respectively. In  $100 \mu\text{M}$  imipramine, the relative currents are  $0.32 \pm 0.05$ ,  $0.33 \pm 0.03$ , and  $0.59 \pm 0.06$  in (external  $K^+$ /internal  $K^+$  in mM) 4/150, 4/15, and 150/150, respectively. (C) The data in external 4 mM  $K^+$ /internal 150 mM  $K^+$  and in external 150 mM  $K^+$ /internal 150 mM  $K^+$  in B are from the same cells. In all four of the cells, the binding rate constants are always smaller in 150 mM external  $K^+$  ( $\blacktriangle$ ) than in 4 mM external  $K^+$  ( $\circ$ ).

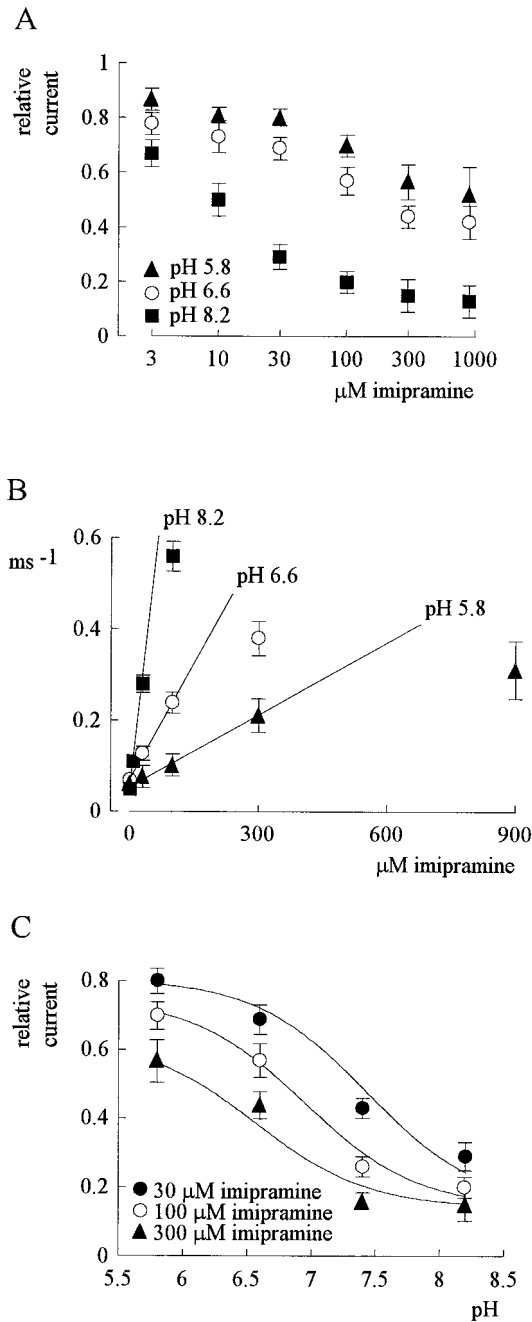


FIGURE 6 The inhibitory effect of imipramine is more pronounced in more alkaline solution. (A) Measurements similar to those in Fig. 3 B are repeated in external solutions of pH 5.8 ( $n = 6$ ), 6.6 ( $n = 6$ ), and 8.2 ( $n = 8$ ). The current at 100 ms of depolarization in the presence of imipramine is normalized to the control current at the same time point and is plotted against the (total) concentration of imipramine. The external Tyrode's solution is adjusted to the desired pH with HCl or NaOH. The pH 5.8 solution may contain 5 mM 2-[N-morpholino]ethanesulfonic acid as an additional buffer (the results are very similar whether the additional buffer is added or not, and data from both conditions are used indiscriminately for statistics). Only cells showing transient K<sup>+</sup> current with a decaying time constant less than 25 ms are used. Along with the data in pH 7.4 in Fig. 3 B, the inhibitory effect of imipramine on K<sub>A</sub> current is clearly stronger in more alkaline solutions. (B) Measurements similar to those in Fig. 1 D are repeated in external solutions of pH 5.8–8.2. The inverses of the decaying time constants are plotted against the (total) concentration of imipramine. The cell numbers are the same as those in A. The lines are linear regression

and its decaying time constant is usually quite longer than 25 ms, the cut line for identification and selection of K<sub>A</sub> currents in this study.) Fig. 6 A shows that the inhibition by imipramine is pH dependent and is stronger in more alkaline solutions. Consistent with this finding, the peak K<sub>A</sub> current is always reached earlier in higher pH with the same (total) concentration of imipramine (data not shown), and the macroscopic binding rate constant is smaller in lower pH (Fig. 6 B).

The findings in Fig. 6, A and B, strongly imply that the neutral form of imipramine is more active than the charged form. Unfortunately, in Fig. 6 A the K<sub>A</sub> currents in pH 5.8 and 6.6 are so incompletely inhibited, even with 900 μM (total) imipramine, that it is not feasible to have a reliable fit to determine the dissociation constant in these acidic solutions. An alternative method is therefore used to make a rough estimate of the relative strengths of different forms of imipramine (Fig. 4 C). Assuming both neutral and charged forms of imipramine bind to the same receptor, and the maximum inhibition produced by imipramine in our preparation is 86% (0.14 relative current with saturating concentrations of imipramine in Fig. 3 B), we may have

$$\text{relative current} = 0.14 + 0.86 / (1 + [I]/K_{d,I} + [IH^+]/K_{d,IH^+}) \quad (1)$$

where [I] and [IH<sup>+</sup>] are the concentrations of neutral and charged forms of imipramine, respectively, and  $K_{d,I}$  and  $K_{d,IH^+}$  are the dissociation constants of the neutral and charged form of imipramine, respectively. Given a pK<sub>a</sub> value 9.5 for the protonated imipramine, we have

$$[IH^+] = (1 / (1 + 10^{-(9.5 + \text{pH})})) * [\text{imipramine}] \quad (2)$$

and

$$[I] = (10^{-(9.5 + \text{pH})} / (1 + 10^{-(9.5 + \text{pH})})) * [\text{imipramine}] \quad (3)$$

where [imipramine] represents the total concentration of imipramine. Fig. 6 C plots the relative current in the presence of 30, 100, and 300 μM imipramine against the pH of the solution. The relative current could be reasonably approximated by Eqs. 1–3 with  $K_{d,I}$  fixed at 0.2 μM and  $K_{d,IH^+}$  set at 500, 1200, and 2000 μM for the data in 30, 100, and 300 μM imipramine, respectively. Although these values

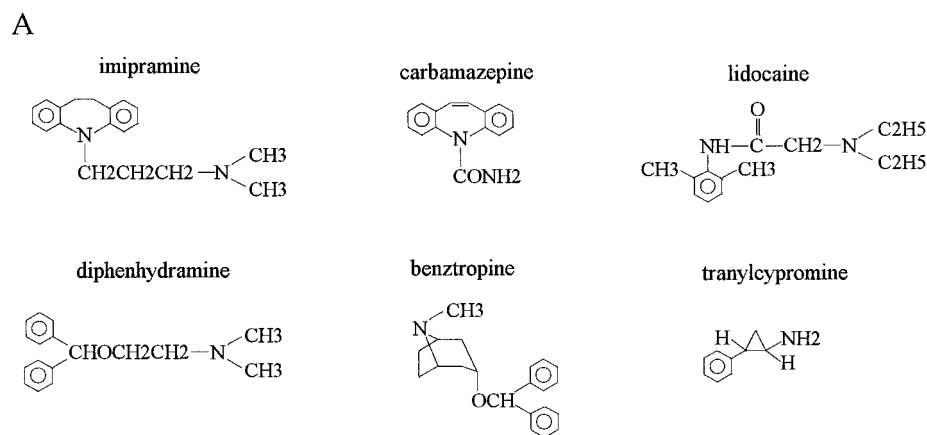
fits to the data obtained in 0–300 μM imipramine, 0–100 μM imipramine, and 0–30 μM imipramine for pH 5.8, 6.6, and 8.2, respectively. The intercepts of the lines are 0.07, 0.08, and 0.04 ms<sup>-1</sup>, and the slopes are  $0.48 \times 10^6$ ,  $1.35 \times 10^6$ , and  $8.0 \times 10^6$  M<sup>-1</sup> s<sup>-1</sup> for pH 5.8, 6.6, and 8.2, respectively. It is interesting to note that the linearity of the data tends to reach a higher concentration range in more acidic solutions, which is consistent with the faster macroscopic binding rates in more alkaline solutions. (C) The relative current in the presence of 30, 100, or 300 μM imipramine is plotted against the pH of the solution; data are from A and Fig. 3 B. The lines are drawn by Eqs. 1, 2, and 3, with the dissociation constant between the neutral form of imipramine and the K<sub>A</sub> channel set to 0.2 μM and the dissociation constant between the protonated form of imipramine and the channel set to 500–2000 μM (see text for details).

should only be regarded as rough estimates of  $K_{d,I}$  and  $K_{d,IH^+}$ , it seems reasonable to conclude that the dissociation constant between the neutral form of imipramine and the  $K_A$  channel is in the submicromolar range, whereas the affinity between the charged form of imipramine and the channel is very much lower.

### Similar inhibitory effect on the $K_A$ current by other drugs

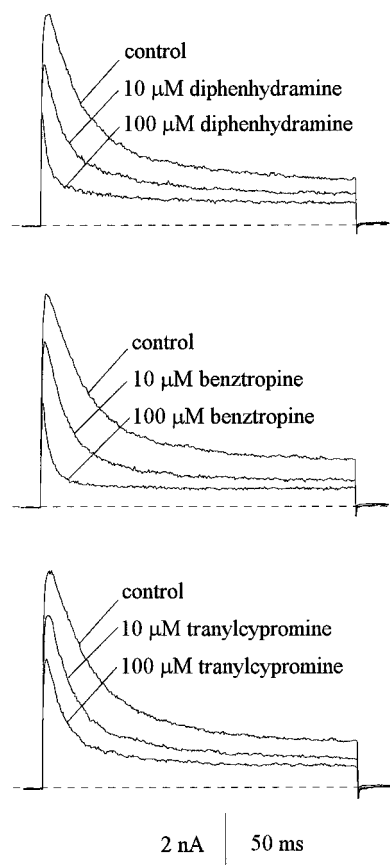
Imipramine contains a tricyclic nucleus and a three-carbon amine side chain. To explore the major structural determi-

nants for imipramine binding to the open  $K_A$  channel, I studied the effect on the  $K_A$  current of several other compounds (Fig. 7 A). Diphenhydramine is an antihistamine with two phenyl groups and a "side chain" comparable in size to that of imipramine. Benztropine is an anticholinergic with two phenyl groups and a cyclic side chain. Although neither diphenhydramine nor benztropine has a tricyclic structure, they both show a strong inhibitory effect on the  $K_A$  current very similar to that of imipramine (Fig. 7 B). The inhibited currents also display the same key features, such as shortened time to peak and accelerated current decay. Thus two separate phenyl groups may be as effective as the



B

FIGURE 7 Inhibition of  $K_A$  current by other drugs. (A) Structure of some of the drugs tested. (B)  $K_A$  current is obtained in one cell by the same protocol as in Fig. 1 B. Diphenhydramine, benzotropine, and tranlycypromine at 10–100  $\mu\text{M}$  all produce significant inhibition of the current. Dashed lines, Zero current level.





tricyclic structure in supporting drug binding to the open  $K_A$  channel. On the other hand, neither 100  $\mu\text{M}$  carbamazepine, an anticonvulsant and antidepressant with a tricyclic structure but only a very short amide side chain, nor 100  $\mu\text{M}$  lamotrigine, a new anticonvulsant with two aromatic rings but no side chain, shows any significant inhibitory effect on the  $K_A$  current (data not shown). These findings suggest an important role of the side chain. Most interestingly, tranylcypromine, an antidepressant with only one phenyl group and a "triangular" side chain, shows significant inhibition of the  $K_A$  current (although the effect is less potent than those of imipramine, diphenhydramine, or benztropine; Fig. 7 B). Yet both 100  $\mu\text{M}$  benzamide, a simpler compound with a phenyl group plus a very short amide side chain, and 100  $\mu\text{M}$  *n*-butylamine, a four-carbon amine with no aromatic groups, fail to inhibit  $K_A$  current significantly (data not shown). All together the findings in Figs. 6 and 7 suggest that there may be two essential structural parts to the drug-channel interaction, the phenyl group and the three- to four-carbon aliphatic (amine) chain, with the latter most favorably in the neutral form.

### Imipramine does not bind to the inactivated $K_A$ channel

The  $K_A$  current is a transient current because the channel tends to be rapidly inactivated when it is open. Being a consequence of channel activation, the inactivated state may share some common conformations with the open state. It would therefore be interesting to see whether imipramine, an open channel blocker, also binds to the inactivated channels. If imipramine also has significant affinity toward the inactivated  $K_A$  channels, then the inactivation curve should be shifted by imipramine because of the occupancy of state IB (Fig. 2 A). This argument is similar to the cases of local anesthetics (e.g., lidocaine) or anticonvulsants (e.g., phenytoin) that have high affinities toward the inactivated  $\text{Na}^+$  channels (Bean et al., 1983; Kuo and Bean, 1994). Fig. 8 shows the inactivation curve of  $K_A$  current in hippocampal neurons. This curve, however, is not shifted in the presence of 10–30  $\mu\text{M}$  imipramine, suggesting little occupancy of state IB in up to 30  $\mu\text{M}$  imipramine. Compared to the affinity between imipramine and the open state (dissociation constant no more than 6  $\mu\text{M}$  in pH 7.4), the affinity between imipramine and the inactivated state seems much smaller.

The occupancy of state IB can also be checked from a kinetic point of view. If there is significant occupancy of state IB in the presence of imipramine, then the recovery from "inactivation" in such a condition (recovery mainly from state IB) should be slower than the recovery in the control condition (recovery entirely from state I). This is not the case in Fig. 9, where the recovery from "inactivation" is not slower, but is even faster, in the presence of imipramine. The faster recovery in imipramine further supports the concept that imipramine binding and channel inactivation are mutually exclusive in these neuronal  $K_A$  channels (see the Discussion).

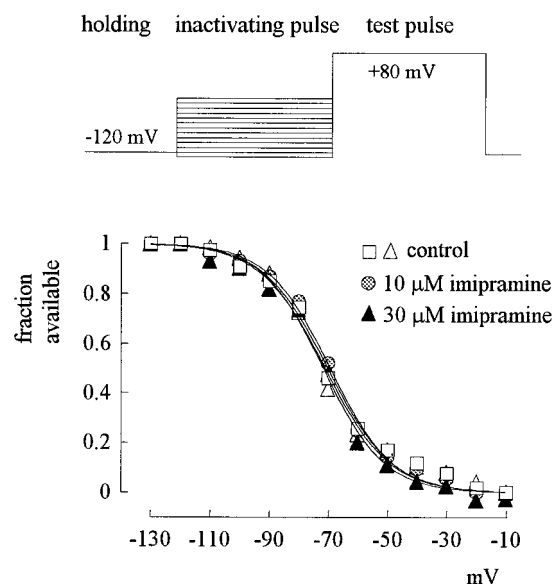


FIGURE 8 The inactivation curve of  $K_A$  current. The cell is held at  $-120$  mV and stepped every 5 s to the inactivating pulse ( $-130$  to  $-10$  mV) for 450 ms before being stepped to a test pulse at  $+80$  mV for another 450 ms. The difference between the peak and the end current during the test pulse after each inactivating pulse is normalized to that after an inactivating pulse at  $-120$  mV to yield the available fraction of  $K_A$  current, which is plotted against the voltage of the inactivating pulse. The lines are fits of a Boltzmann function  $1/(1 + \exp[(V - V_h)/k])$ , with  $V_h$  values of  $-71.1$ ,  $-69.4$ ,  $-71.7$ , and  $-70.2$  mV and  $k$  values of 10.9, 10.5, 10.3, and 10.8 for the four sets of data in control (before imipramine), 10  $\mu\text{M}$  imipramine, 30  $\mu\text{M}$  imipramine, and control (after imipramine), respectively.

## DISCUSSION

### Imipramine: an open $K_A$ channel blocker

There are different kinds of  $K^+$  currents in mammalian central neurons (for a review see Aronson, 1992). In this study I have focused on the  $K_A$  or fast transient  $K^+$  current, which activates rapidly within a few milliseconds and then inactivates within a few tens of milliseconds. In the presence of imipramine, the peak of  $K_A$  current was reduced in amplitude, yet was reached earlier (than in control) and was followed by an accelerated decay. Moreover, the speed of current decay was linearly correlated with imipramine concentration when the concentration was not high. These findings suggest that imipramine blocks open  $K_A$  channels, possibly through a one-to-one binding reaction. Furthermore, in the continuous presence of imipramine, although the current has decreased to a low steady-state level at the end of a test pulse (for example, in the experiments in Fig. 1 B), the same high peak current is always present at the next test pulse. This means that the imipramine molecule bound to the open channel pore during the test pulses must unbind during the intervening hyperpolarization (see below for more discussion on the unbinding processes). Overall, it is concluded that imipramine has a much higher affinity for the open channel than for the closed or resting channels.

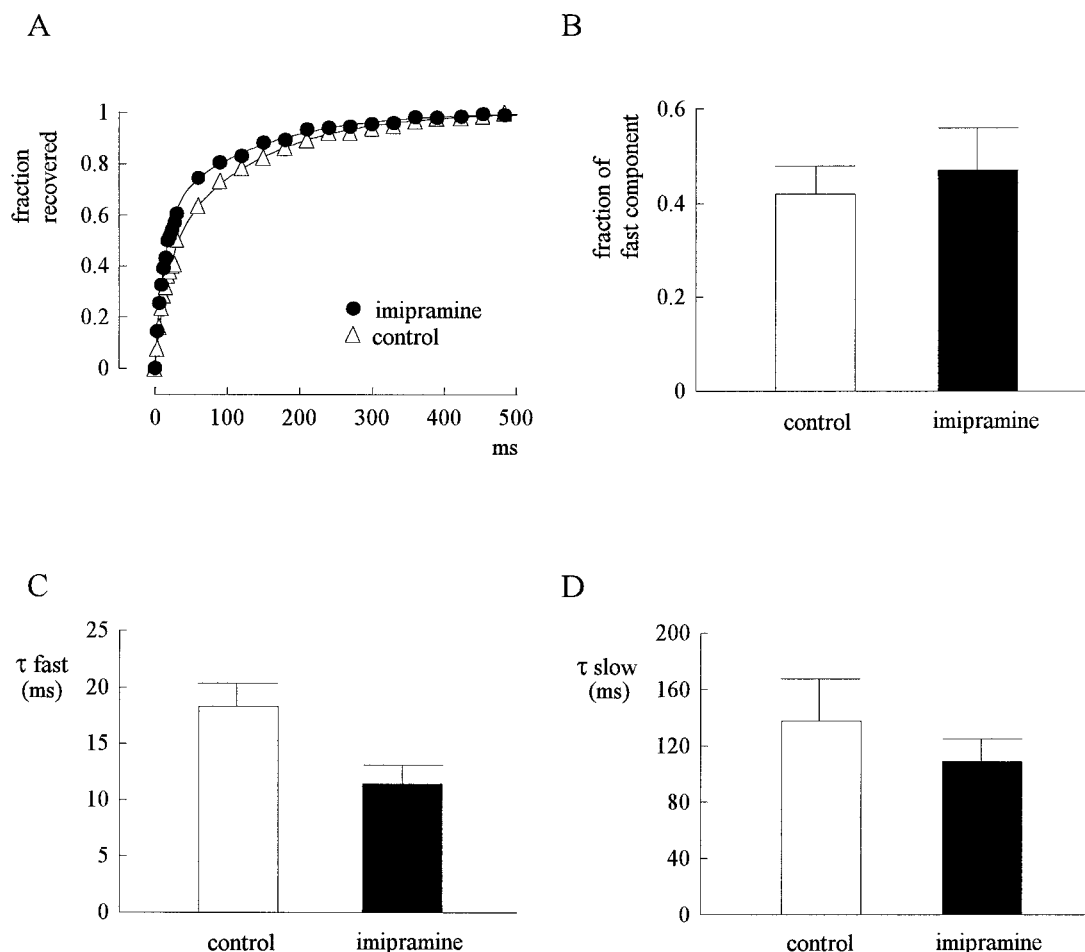


FIGURE 9 Recovery of K<sub>A</sub> current from inactivation and imipramine block at -120 mV. (A) The cell is held at -120 mV and stepped every 5 s to an inactivating pulse at +80 mV for 450 ms. The cell was then stepped back to a recovery gap potential at -120 mV for variable length before being stepped again to a test pulse at +80 mV for another 450 ms. The difference between the peak and the end current during the test pulse is normalized to that during the inactivating pulse to yield the fraction recovered, which is then plotted against the duration of the gap recovery potential. The lines are two-exponential fits to the two sets of data and are of the form: fraction recovered =  $1 - 0.44\exp(-t/17.4 \text{ ms}) - 0.56\exp(-t/128 \text{ ms})$  (in control,  $t$  denotes duration of the gap potential in ms) and fraction recovered =  $1 - 0.51\exp(-t/11.3 \text{ ms}) - 0.49\exp(-t/109 \text{ ms})$  (in 30  $\mu\text{M}$  imipramine). (B) The fraction of the fast component of recovery from four cells is  $0.42 \pm 0.06$  (in control) and  $0.47 \pm 0.09$  (in 30  $\mu\text{M}$  imipramine). (C) The fast time constant of recovery from the same four cells is  $18.3 \pm 2.1 \text{ ms}$  (in control) and  $11.4 \pm 1.5 \text{ ms}$  (in 30  $\mu\text{M}$  imipramine). (D) The slow time constant of recovery from the same four cells is  $139 \pm 30 \text{ ms}$  (in control) and  $110 \pm 16 \text{ ms}$  (in 30  $\mu\text{M}$  imipramine).

### The active form of imipramine

Using a permanently charged analog of amitriptyline (*N*-methylamitriptyline), Wooltorton and Mathie (1995) argued that the unprotonated (uncharged) form of tricyclic antidepressants represents the active form inhibiting late K<sup>+</sup> currents. Consistent with this argument, I found that the inhibitory effect of imipramine on K<sub>A</sub> current is pH-dependent and becomes more pronounced in more alkaline solutions (Fig. 6 A). A rough estimate of the dissociation constant between the neutral form of imipramine and the K<sub>A</sub> channels is  $\sim 0.2 \mu\text{M}$ , whereas the affinity between the protonated form and the channel probably is 1000-fold lower (Fig. 6 C). The much higher affinity of the neutral form of imipramine implies that ionic bonds probably do not play a significant role in the imipramine-K<sub>A</sub> channel interaction.

This may shed light on the structure of the imipramine receptor in central neuronal K<sub>A</sub> channels (see below).

### Location of the imipramine binding site

The binding rate of imipramine to the open K<sub>A</sub> channel is slowed by external but not internal K<sup>+</sup>. External K<sup>+</sup> ion may decrease the on rate of imipramine either by binding to the doorway of the imipramine receptor or by competing for the same receptor with imipramine. If the doorway of the imipramine receptor is blocked by K<sup>+</sup>, then based on the rationales of simple bimolecular reaction one would expect a similar decrease in both the binding and unbinding rates. Thus the steady-state inhibition of K<sub>A</sub> current may not be significantly changed. On the other hand, if K<sup>+</sup> and imip-

ramine are competing for the same site, one probably should observe reduced inhibition in high external K<sup>+</sup> because the binding rate of imipramine would be decreased but the unbinding rate may be relatively unaltered. The reduced inhibition of K<sub>A</sub> current by imipramine in high external K<sup>+</sup> (Fig. 5) would therefore imply a common binding site for external K<sup>+</sup> and imipramine.

Unlike external K<sup>+</sup>, internal K<sup>+</sup> does not seem to compete with imipramine for a binding site in the pore. This implies that although the binding site is in the pore (because imipramine is an open channel blocker), it can hardly be occupied by internal K<sup>+</sup> ion in our experiments. In view of the fact that the experimental K<sup>+</sup> currents are always outward, the site then must be separated from the extracellular solution by a very small energy barrier (in terms of the movement of K<sup>+</sup> ions) but separated from the intracellular solution by high energy barriers. The K<sup>+</sup> ion in the site then could have a much stronger tendency to go outside than inside to make the outward currents. This would suggest that this imipramine and K<sup>+</sup> ion binding site in the pore is located close to the external rather than the internal side. The finding that only extracellular but not intracellular imipramine produces significant inhibition of the K<sub>A</sub> current is consistent with such concepts. In Fig. 6 B the measured macroscopic binding rate constant approaches 10<sup>7</sup> M<sup>-1</sup> s<sup>-1</sup> in the solution of pH 8.2. This is a fairly fast binding rate constant for a macromolecule such as imipramine, and the binding rate constant for the neutral form of imipramine conceivably may be even faster. This would also support a superficial location of the drug binding site.

### Nature of the imipramine binding site

It is found that 10–100 μM diphenhydramine, benztrapine, and tranylcypromine all inhibited K<sub>A</sub> current with features similar to those of imipramine. On the other hand, 100 μM carbamazepine, lamotrigine, benzamide, and *n*-butylamine did not have a significant effect on K<sub>A</sub> currents. It seems that the tricyclic structure is not essential. Instead, a planar phenyl group plus a three-carbon amine side chain in appropriate conformation may represent the minimum requirement for effective binding to the open K<sub>A</sub> channel pore. On the other hand, the more potent inhibition by imipramine, diphenhydramine, and benztrapine than by tranylcypromine further suggests that a second phenyl group in an appropriate position and/or the terminal tertiary amine group (which yields a much higher hydrophobic bonding constant than the primary amine group; Zimmerman and Feldman, 1989) may contribute to a stronger binding.

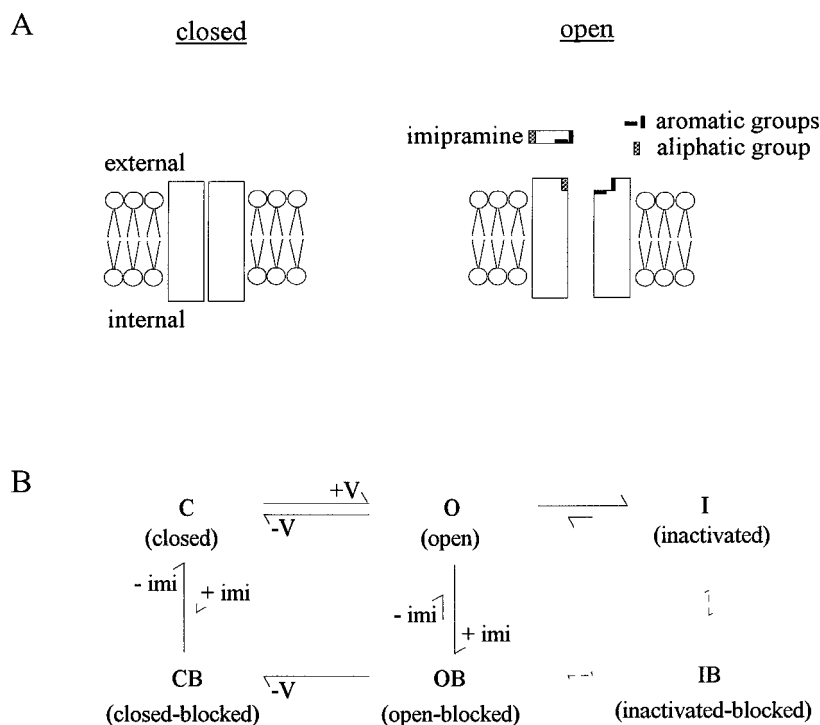
The structure of imipramine does not favor significant hydrogen bond formation. Furthermore, the finding that the neutral form of imipramine is much more effective than the charged form argues against a significant ionic bond between imipramine and the K<sub>A</sub> channel. Excluding ionic and hydrogen bonds, noncovalent binding between drug and biomacromolecules is mostly a hydrophobic bond (which

represents a freeing of water molecules and a gain in entropy) or a (induced) dipole-induced dipole bond (London forces or Debye forces; for a review see Zimmerman and Feldman, 1989) in nature. A rough estimate of the dissociation constant between the neutral form of imipramine and the open K<sub>A</sub> channel is ~0.2 μM (Fig. 6 C), which may be translated into a binding energy of ~15.5RT or ~9.2 kcal/mol. This figure is somewhat larger than the usual bond energy for a hydrophobic bond or an induced-dipole bond (1–7 kcal/mol; Zimmerman and Feldman, 1989). This is consistent with the notion that at least two such bonds are responsible for the binding of imipramine. Because effective hydrophobic and induced-dipole bonds both require close proximity between the binding counterparts, the planar benzene ring would tend to form a bond with the other planar benzene ring, and the aliphatic group would tend to cluster with other aliphatic groups. I therefore propose that the receptor for imipramine in the open K<sub>A</sub> channel pore consists of one or two aromatic sites and one aliphatic site (Fig. 10 A). The aromatic site should be composed of aromatic amino acids. The aliphatic site is possibly a short slot that may accommodate uncharged propylamine-like side chains. In this regard it is interesting that external K<sup>+</sup> competes with imipramine for some binding ligands. Although K<sup>+</sup> ion is unlikely to compete with imipramine for the aliphatic site, K<sup>+</sup> ion has been proposed to interact favorably with the electron-rich face of an aromatic ring (cation-π interaction; Miller, 1993; Kumpf and Dougherty, 1993).

### Implications for the gating conformational changes of central neuronal K<sub>A</sub> channels

The inactivation curve of K<sub>A</sub> current is not shifted in 30 μM imipramine (Fig. 8), and recovery from inactivation is faster in the presence of imipramine (Fig. 9). The latter is also consistent with previous reports that external K<sup>+</sup> could speed the recovery of inactivated K<sub>A</sub> channels (Demo and Yellen, 1991; Gomez-Lagunas and Armstrong, 1994; Levy and Deutsch, 1996; Kuo, 1997), given the finding that external K<sup>+</sup> competes with imipramine for binding to the open channel pore (Fig. 5). All together these findings suggest insignificant binding of imipramine to the inactivated state of K<sub>A</sub> channels (little occupancy of state IB in Fig. 10 B). There are at least two types of inactivation in K<sub>A</sub> channels. N-type inactivation results from binding of an inactivating particle to the internal pore mouth of the activated channel (Armstrong and Bezanilla, 1977; Armstrong, 1981; Hoshi et al., 1990; Zagotta et al., 1990), and C-type inactivation involves conformational changes at the external pore mouth (Hoshi et al., 1991; Choi et al., 1991; Lopez-Barneo et al., 1993; Ogielska et al., 1995; Liu et al., 1996). The negligible binding of imipramine, an external open channel blocker, to the inactivated state suggests that the conformation of the external pore mouth is different in the two gating states. The inactivated state under consideration here thus seems to have the character of C-type inactivation.

FIGURE 10 Schematic illustration of some major conclusions. (A) When the  $K_A$  channel is opened, some conformational changes happen at the external pore mouth to form a receptor for imipramine. The receptor may consist of one aliphatic site and one to two aromatic sites. (B) A semiquantitative simplified gating scheme of the  $K_A$  channel incorporating the molecular actions of imipramine. The channel is opened by depolarization (+V) and closed by hyperpolarization (-V). Imipramine binds much more readily to the open state than to the closed state; hence there is a much larger "+imi" arrow from the open state than from the closed state. The existence of the IB state is negligible, and the arrows connecting IB and I or IB and OB are thus drawn as dashed lines. Although imipramine binds to the channel at depolarization via the C-O-OB route, the unbinding of imipramine at hyperpolarization seems to happen significantly through the CB state, i.e., via the OB to CB to C route. The "-imi" arrow from CB to C is thus larger than that from OB to O. Also, CB and OB are connected by only a "-V" arrow to emphasize that state CB is significantly present only during the recovery from imipramine block at hyperpolarization.



In this regard it is interesting to note that the fast time constant of recovery in control is just  $\sim 18$  ms (Fig. 9). This is consistent with the reported time constants of recovery from N-type inactivation (Demo and Yellen, 1991; Gomez-Lagunas and Armstrong, 1994; Kuo, 1997), yet is much shorter than many reported time constants of recovery from C-type inactivation (Hoshi et al., 1991; Rasmusson et al., 1995; Levy and Deutsch, 1996; but in some alternatively spliced Shaker channel variants the recovery from C-type inactivation may be quite fast, see Hoshi et al., 1991). Thus at the end of the inactivating pulse in Fig. 9, many neuronal  $K_A$  channels are in a "fast" inactivated state. This state could be an N-type inactivated state that is also conformationally different from the open state in the external pore mouth or a C-type inactivated state that recovers very quickly at  $-120$  mV. In either case the finding that imipramine selectively binds to only the activated but not the deactivated or inactivated states is suggestive of interesting gating conformational changes in the external pore mouth of these neuronal  $K_A$  channels. It seems that the external pore mouth of the channel continues to change its conformation during membrane depolarization. The receptor for imipramine is produced at first but would subsequently be destroyed.

The faster recovery in the presence of imipramine (Fig. 9) also has implications for the existence of state CB (Fig. 10 B). If recovery from state I has a fast time constant of  $\sim 18$  ms, recovery from state OB must proceed with a speed no slower than  $\sim 90$   $s^{-1}$  (the inverse of  $\sim 11$  ms) to make a fast time constant of  $\sim 11$  ms for the macroscopic recovery in the presence of imipramine (Fig. 9 C). However, given a macroscopic binding rate of  $5.6 \times 10^6$   $M^{-1} s^{-1}$  and a

dissociation constant of no more than  $6$   $\mu M$  between imipramine and the open  $K_A$  channel, the OB to O rate in Fig. 10 B cannot be faster than  $\sim 33$   $s^{-1}$ . Thus there must be a recovery route other than OB to O during repolarization. It seems that the most straightforward choice in the simplified scheme is the OB to CB to C route. In other words, although there is little steady-state occupancy of state CB, this state may be significantly but transiently occupied during recovery from imipramine blockade. The unbinding of the blocking imipramine molecule during repolarization thus is reminiscent of the recovery from inactivation in  $Na^+$  channels, where the inactivated channel must deactivate before it can recover from inactivation (Kuo and Bean, 1994). Part of the recovery from inactivation in Shaker  $K^+$  channels may also proceed via such a "deactivation first" route (Kuo, 1997).

### Clinical implications

The plasma concentration of imipramine is usually  $0.5$ – $1$   $\mu M$  under therapeutic conditions, yet it may be higher than  $3$   $\mu M$  in overdose (Amsterdam et al., 1980; Benet et al., 1996). However, there may be as much as 90% plasma protein binding for imipramine, and thus the free imipramine concentration in the cerebrospinal fluid may be on the order of  $0.1$   $\mu M$  in therapeutic conditions. This value is close to the half-inhibitory concentration ( $IC_{50}$ ) of imipramine on specific ligand binding to the 5-HT<sub>2</sub> ( $\sim 472$  nM) and  $\alpha 1$ -adrenergic receptors ( $\sim 58$  nM; U'Prichard et al., 1978; Enna and Kendall, 1981), yet it is much smaller than the dissociation constant between imipramine and the open  $K_A$  channels measured here in pH 7.4 ( $\sim 6$   $\mu M$ ; Fig. 3) or



the dissociation constant between imipramine and the delayed rectifier channels reported elsewhere ( $\sim 7 \mu\text{M}$ ; Wooltorton and Mathie, 1993). Thus the antidepressant effect of imipramine is most likely ascribable to its action on the serotonergic and adrenergic systems, and the inhibition of neuronal K<sup>+</sup> current by imipramine probably would be of clinical significance only in overdose and/or conditions with extreme alkalosis. Some serious side effect of imipramine in toxic situations, such as seizures and cardiac arrhythmias, may be related to the drug's K<sup>+</sup> channel blocking effect and the subsequent changes in cellular excitability.

This work was supported by a grant from the National Science Council, Taiwan, R.O.C. (NSC 86-2314-B-002-004-M10).

## REFERENCES

- Amsterdam, J., D. Brunswick, and J. Mendels. 1980. The clinical application of tricyclic antidepressant pharmacokinetics and plasma levels. *Am. J. Psychiatr.* 137:653–663.
- Armstrong, C. M. 1981. Sodium channels and gating currents. *Physiol. Rev.* 61:644–683.
- Armstrong, C. M., and F. Bezanilla. 1977. Inactivation of the sodium channel. II. Gating current experiments. *J. Gen. Physiol.* 70:567–590.
- Aronson, J. K. 1992. Potassium channels in nervous tissue. *Biochem. Pharmacol.* 43:11–14.
- Bean, B. P., C. J. Cohen, and R. W. Tsien. 1983. Lidocaine block of cardiac sodium channels. *J. Gen. Physiol.* 81:613–642.
- Benet, L. Z., S. Fie, and J. B. Schwartz. 1996. Design and optimization of dosage regimens: pharmacokinetic data. In Goodman and Gilman's The Pharmacological Basis of Therapeutics, 9th Ed. J. G. Hardman, L. E. Limbird, P. B. Molinoff, and R. W. Ruddon, editors. McGraw-Hill, New York. 1707–1792.
- Choi, K. L., R. W. Aldrich, G. Yellen. 1991. Tetraethylammonium blockade distinguishes two inactivation mechanisms in voltage-activated K<sup>+</sup> channels. *Proc. Natl. Acad. Sci. USA.* 88:5092–5095.
- Delpont, E., J. Tamargo, and J. Sanchez-Chapula. 1992. Effects of imipramine on the transient outward current in rabbit atrial single cells. *Br. J. Pharmacol.* 106:464–469.
- Demo, S. D., and G. Yellen. 1991. The inactivation gate of the Shaker potassium channel behaves like an open-channel blocker. *Neuron.* 7:743–753.
- Enna, S. J., and D. A. Kendall. 1981. Interaction of antidepressants with brain neurotransmitter receptors. *J. Clin. Psychopharmacol.* 1:12S–16S.
- Gomez-Lagunas, F., and C. M. Armstrong. 1994. The relation between ion permeation and recovery from inactivation of ShakerB K<sup>+</sup> channels. *Biophys. J.* 67:1806–1815.
- Hollister, L. E. 1995. Antidepressant agents. In Basic and Clinical Pharmacology, 6th Ed. B. G. Katzung, editor. Prentice-Hall International, London. 448–459.
- Hoshi, T., W. N. Zagotta, and R. W. Aldrich. 1990. Biophysical and molecular mechanisms of Shaker potassium channel inactivation. *Science.* 250:533–538.
- Hoshi, T., W. N. Zagotta, and R. W. Aldrich. 1991. Two types of inactivation in Shaker potassium channels: effects of alterations in the carboxy-terminal region. *Neuron.* 7:547–556.
- Isenberg, G., and J. Tamargo. 1985. Effect of imipramine on calcium and potassium currents in isolated bovine ventricular myocytes. *Eur. J. Pharmacol.* 108:121–131.
- Iverson, L. E., M. A. Tanouye, H. A. Lester, N. Davidson, and B. Rudy. 1988. A-type potassium channels expressed from Shaker locus cDNA. *Proc. Natl. Acad. Sci. USA.* 85:5723–5727.
- Kumpf, R. A., and D. A. Dougherty. 1993. A mechanism for ion selectivity in potassium channels: computational studies of cation-p interactions. *Science.* 261:1708–1710.
- Kuo, C.-C. 1997. Deactivation retards recovery from inactivation in Shaker K<sup>+</sup> channels. *J. Neurosci.* 17:3436–3444.
- Kuo, C.-C., and B. P. Bean. 1994. Sodium channels must deactivate to recover from inactivation. *Neuron.* 12:819–829.
- Levy, D. I., and C. Deutsch. 1996. Recovery from C-type inactivation is modulated by extracellular potassium. *Biophys. J.* 70:798–805.
- Liu, Y., M. E. Jurman, and G. Yellen. 1996. Dynamic rearrangement of the outer mouth of a K<sup>+</sup> channel during gating. *Neuron.* 16:859–867.
- Lopez-Barneo, H., T. Hoshi, S. H. Heinemann, and R. W. Aldrich. 1993. Effects of external cations and mutations in the pore region on C-type inactivation of Shaker potassium channels. *Receptors Channels.* 1:61–71.
- Miller, C. 1993. Potassium selectivity in proteins: oxygen cage or in the face? *Science.* 261:1692–1693.
- Numann, R. E., W. J. Wadman, and R. K. S. Wong. 1987. Outward currents of single hippocampal cells obtained from the adult guinea pig. *J. Physiol. (Lond.).* 393:331–353.
- Ogata, N., and T. Narahashi. 1989. Block of sodium channels by psychotropic drugs in single guinea-pig cardiac myocytes. *Br. J. Pharmacol.* 97:905–913.
- Ogata, N., and H. Tatebayashi. 1993. Differential inhibition of a transient K<sup>+</sup> current by chlorpromazine and 4-aminopyridine in neurons of the rat dorsal root ganglia. *Br. J. Pharmacol.* 109:1239–1246.
- Ogielska, E. M., W. N. Zagotta, T. Hoshi, S. H. Heinemann, J. Haab, and R. W. Aldrich. 1995. Cooperative subunit interactions in C-type interaction of K channels. *Biophys. J.* 69:2449–2457.
- Rasmusson, R. L., M. J. Morales, R. C. Castellino, Y. Zhang, D. L. Campbell, and H. C. Strauss. 1995. C-type inactivation controls recovery in a fast inactivating cardiac K<sup>+</sup> channel (Kv1.4) expressed in Xenopus oocytes. *J. Physiol. (Lond.).* 489:709–721.
- Timpe, L. C., T. L. Schwarz, B. L. Timpel, D. M. Papazian, Y. N. Jan, and L. Y. Jan. 1988. Expression of functional potassium channels from Shaker cDNA in Xenopus oocytes. *Nature.* 331:143–145.
- U'Prichard, D. C., D. A. Greenberg, P. P. Sheehan, and S. H. Snyder. 1978. Tricyclic antidepressants: therapeutic properties and affinity for a-norenergic receptor binding sites in the brain. *Science.* 199:197–198.
- Wooltorton, J. R. A., and A. Mathie. 1993. Block of potassium currents in rat isolated sympathetic neurons by tricyclic antidepressants and structurally related compounds. *Br. J. Pharmacol.* 110:1126–1132.
- Wooltorton, J. R. A., and A. Mathie. 1995. Potent block of potassium currents in rat isolated sympathetic neurons by the uncharged form of amitriptyline and related tricyclic compounds. *Br. J. Pharmacol.* 116:2191–2200.
- Zagotta, W. N., T. Hoshi, and R. W. Aldrich. 1990. Restoration of inactivation in mutant Shaker potassium channels by a peptide derived from ShB. *Science.* 250:568–571.
- Zimmerman, J. J., and S. Feldman. 1989. Physical-Chemical Properties and Biological Activity. In Principles of Medicinal Chemistry, 3rd Ed. W. O. Foye, editor. Lea and Febiger, Malvern, PA. 7–37.