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Molecular Pharmacology of the Effects of Lidocaine and Related Drugs on Na^+ channels

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Introduction

Use-dependency of the effects of class I antiarrhythmic drugs and local anesthetics on Na^+ channels in excitable cells is an important property for understanding the basic mechanism of drug-channel interactions as well as for an intelligent use in clinical setting (Pilat and Heistracher 1964; Heistracher 1971; Hondeghem and Katzung 1977; Oshita et al. 1980; Starmer et al. 1984; Butterworth and Strichartz 1990; Hille 1992; Barber et al 1992; Nuss et al. 1995). There exist at least two kinds of these channel blockers, i.e., open channel blockers and inactivation channel blockers among class I three subgroups . The former enhances the blocking activities during the rising phase of action potentials (APs), but the latter, during the plateau, whereas both groups of drugs reduce the activities during the diastolic intervals (Kodama et al. 1990; Kojima and Ban, 1988; Hamamoto et al. 1994). Although only one receptor site for use-dependence has been assumed to be within the ion-conducting pore in the Na^+ channel accessible from the intracellular side (Hondeghem and Katzung 1977; Starmer et al. 1984; Hille, 1992), many findings, particularly on inactivation channel blockers, do not favor such a one -receptor hypothesis.

A physico-chemical approach was made by our group in 1980's to the study of the drug -Na^+ channel interactions (see Ichiyama et al. (1986) and references therein) to find a correlation between those properties such as molecular weight (M.W.) and log n-octanol/water partition coefficients (log P) and use -dependent properties for Na^+ channel blockers. We studied more than 20 β-adrenoceptor blocking agents and other structurally related drugs on the maximum upstroke velocity (Vmax) of APs, a rough measure of Na^+ currents, in guinea-pig papillary muscles. Significant correlations were found between time-independent reduction of Vmax and log P, between time constants of the recovery (τrec) of the reduction of Vmax in premature action potentials and M.W. and between the zero-time intercept (the time of the end of AP duration of the conditioning response) of slow reactivation relative to the predrug Vmax value (which represents approximately a fraction of channels blocked per AP) and log P as well as M.W., all at the concentration of 100 μM. A distinct advantage for these drugs is that all these drugs possess similar pKa values (9.5-9.65), therefore, if the rate of loosing proton at the site of block (Starmer and Courtney, 1986) predominates as the rate limiting process, all these drugs would leave the channels at the same rate. On this line, Courtney (1990) demonstrated that molecular size, i.e., spanning at their aromatic end (end-on spans) was a better index to correlate with τrec than the M.W. for the above and other series of drugs of which deprotonation rates were less than 0.3 sec and end-on spans, less than 10-11 Å. While these drugs as well as amide -linked drugs including lidocaine are all tertiary amines and may act as inactivation chan-

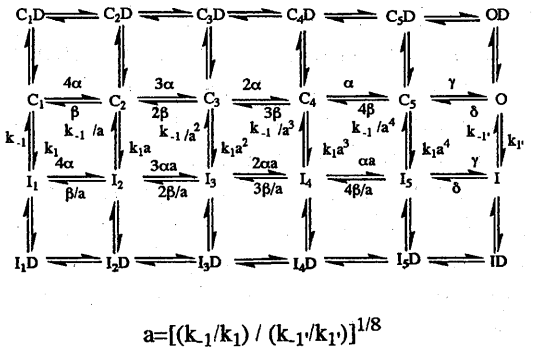
nel blockers (Courtney 1990), more bulkier or hydrophilic drugs such as disopyramide, quinidine, some phenothiazine derivatives and GEA 968 act exclusively or preferentially as open-channel blockers. Although several exceptions (see Bolotina et al. 1992 and references therein) were noted to this hypothetical rule, these findings give a clue to estimating the dimensions of receptor sites which appear to exist at least in two forms. The two groups of drugs also differ in their access route to the receptor sites in nerves and hearts. That is, both groups act only from the intracellular site in neuronal tissues but those drugs such as penticainide (Gruber et al. 1991), lidocaine and its quaternary derivatives, QX-314 and QX-222 (triethyl and trimethyl N derivatives of lidocaine) act from the extracellular as well as from the intracellular sides in cardiac tissues (see Baumgarten et al. 1991, Qu et al. 1995 and references therein).

The blocking effects on cardiac tissues of lidocaine at therapeutic concentrations of 15 -30 μM correspond to the effects at 100-250 μM in myelinated nerve fibers under voltage -clamp conditions (see Courtney and Strichartz, 1987). The steady state level of block by disopyramide but not by lidocaine was affected by the elevation of the external Na^+ concentration (Barber et al. 1992).

The difference in the mode of actions of drugs in these tissues in these studies would finally be attributed to the differences in the structure among Na^+ channel isomers. On this line, this review describes the kinetic mode of actions of Na^+ channel blockers, especially lidocaine and quaternary derivatives which have been extensively studied using the native cardiac tissues and translation~expression systems such as *Xenopus laevis* oocytes (hereafter referred to as oocytes) in which recombinant cardiac Na^+ channels are expressed. It constitutes a step toward understanding the sites of action of drugs within the Na^+ channel in molecular terms.

Electrophysiological studies of kinetics of Na^+ channel and its blockers in cardiac tissues

Voltage clamp experiments, which record the macroscopic currents to maintain the membrane potential at a constant level, patch clamp experiments, which record currents flowing through individual ionic channels (single channel recording), and experiments of recording gating currents, which are associated with movement of electrically charged regions of the channel macromolecule that underlie the transitions between conducting and non-conducting forms, have been made to investigate electrophysiologic effects of Na^+ channel and other channel blocking agents (see Patlak 1991; Armstrong 1992). The works on Vmax, which is not directly proportional to Na^+ current (Tsien et al. 1984), but still gives important information at physiological medium and temperature, are not included here. Figure 1 shows a modified version of the model presented by Kuo and Bean (1994) for their experiments on the Na^+ channels in rat hippocampal cells. This model, modified from Hodgkin-Huxley equation (French and Horn 1983), incorporates many important recent experimental findings such as activation of four identical, independent primary voltage sensors (C_1-C_5), followed by an additional opening steps ($C_5 \rightarrow O$) required before opening, and inactivation (I), which has little



$$a=[(k_{-1}/k_1) / (k_{-1'}/k_{1'})]^{1/8}$$

Figure 1. A gating model for Na^+ channels. Modified from Kuo and Bean (1994). See text for details.

intrinsic voltage-dependence. In this figure, activation and deactivation rate constants are voltage-dependent ($\alpha = 20\ \exp(V/40)$; $\beta = 3\ \exp(-V/40)$; $Y = 50\ \exp(V/100)$; $\delta = 0.2\ \exp(-V/25)$), whereas the dissociation and association of C_1 to O with an inactivation particle lead to the inactivation state, i.e., the formation of I_1 to I_5 and I ($k_1 = 0.004$, $k_{-1} = 4.5$, $k_1' = 4$, $k_{-1}' = 0.008$). These processes are assumed here as voltage-independent, although in cardiac tissues it may not be so (Hanck et al.1994; Hurwitz et al. 1992). According to the principle of microscopic reversibility, which means that in every closed cycle, the product of rate constants going clockwise around any cycle must equal the product going counterclockwise (Hille 1992), the rates with respect to the transition between Ci, Ii, I and O are determined. With the above values of rate constants, $a = 5.233$ is given from the equation shown in Fig. 1. Therefore, the on rate from C_1 to C_5 are 0.004, 0.02, 0.109, 0.573 and 3. The off rates are from I_1 to I_5 are 4.5, 0.19, 0.036, 0.007, 0.001. That is, the inactivation particle binds voltage-independently to the channel with higher affinities as the channel is more activated ($C \rightarrow O$) and with lower affinities as the channel is more deactivated ($O \rightarrow C$). The model can elucidate well the voltage-dependence of activation and inactivation, the shorter initial delay and τrec with increasing hyperpolarization through an increase in C_1 and very small ionic currents during recovery through $I \rightarrow O$ pathway. The effects of phenytoin are explained in this model as an inactivation channel blocker, competing with the inactivation particle. When the rate constants were slowed to $k_1 = 0.00001$, $k_{-1} = 0.023$, $k_1' = 0.001$, $k_{-1}' = 0.00004$, voltage-dependent τrec from phenytoin block was well reproduced (Kuo and Bean 1994). The gating current, Igij, resulting from channels associated with forward and backward P_i to P_j transition (probability of the channel occupying the i and j states, respectively) is expressed as:

$$Igij = (qij + qji)\ (kij\ Pi - kji Pj)$$

(Sheets and Hanck 1995), where qij and qji and kij and kji, represent, respectively, the valences and rate constants associated with the i to j and j to i transitions. In models by Patlak (1991) and Sheets et al. (1995), the last step before opening, i. e., C_5, was not considered.

For more general treatments of drug effects possible drug-channel interactions are interconnected here, but for a first approximation only $C_1 \leftrightarrow C_1D$ and $I \leftrightarrow ID$ interactions are usually considered as resting and inactivation blocks.

If the association and dissociation rates ($C_i \leftrightarrow C_iD$, $O \leftrightarrow OD$, $I_i \leftrightarrow I_iD$, $I \leftrightarrow ID$) are common in all processes and the transition rates between drug-bound states ($C_iD \leftrightarrow C_{i+1}D$, $C_5D \leftrightarrow OD$, $I_iD \leftrightarrow I_{i+1}D$, $I_5D \leftrightarrow ID$) are equal to those between drug-free states ($C_i \leftrightarrow C_{i+1}$, $C_5 \leftrightarrow O$, $I_i \leftrightarrow I_{i+1}$, $I_5 \leftrightarrow I$), then it represents an alternative way of expression of guarded receptor hypothesis (Starmer et al. 1984), and if they differ in each step it represents that of expression of modulated receptor hypothesis (Hondeghem and Katzung 1984, Hille, 1992). Kinetic analyses must be done not only on the state transition of drug-free channels but also on that of drug-bound channels.

A study on macroscopic Na^+ current by Bean et al. (1983) in rabbit Purkinje fibers demonstrated that the longer the depolarizing conditioning pulse (Vc) the stronger was the block in the test response (Vt) to a certain extent of the duration of Vc, leading to the conclusion that the dissociation equilibrium constant, Kd, of lidocaine for ID = 10 μM and is much less than Kd for C_1D = 440 μM (assuming a 1:1 binding reaction including all the cases below).

Studies on the effects of lidocaine and/or QX-314 by means of single channel recording were made by several groups including Nilius et al. (1987) (5 μM lidocaine; inside-out patch), McDonald et al. (1989) (extracellular 50 μM lidocaine), Baumgarten et al. (1991) (100 μM lidocaine and 500 μM QX-314) and Bennett et al. (1995) (inside-out patch) in the cell-attached pipette solution in mammalian cardiac ventricular and Purkinje cells and revealed the following. The open channel probability but not single channel conductance during Vt was reduced depending on the voltage of Vc, suggesting a formation of ID and OD, which were non-conductive or less conductive than control. Late openings or reopenings were preferentially depressed dur-

ing Vt or on the repetition of Vt. Also, the open time during Vt was shorter than that during Vc. Both findings suggest that C_iD to OD is faster (shorter latency) than C_i to O and the conductive OD has a shorter open time, i.e., OD → C_5D or OD → ID is faster than O → C_5 or O → I (McDonald et al. 1989). No correlations between channel opening during Vc and that during Vt suggest that for opening of channel during Vt the formation of C_iD is more determinant than that of OD during Vc (McDonald et al. 1989). The open time during Vt was increased with an increase in the hyperpolarization level of Vc, suggesting that the reaction, $C_1 \rightarrow C_1D$ proceeded less than that $C_i \rightarrow C_iD(i>1)$. Kd for resting state is 20 μM to 136 μM, whereas that for inactivated state is 2.5 μM in inside -out patch (Nilius et al. 1987). When fast inactivation was removed by α-chymotrypsin, the same Vc caused little or no block.

Gingrich et al. (1993) demonstrated that QX-314 blocked the channel (formation of C_iD and OD) when applied intracellularly (Kd=4.4μM) at the site 70% of the electric field from the cytoplasmic entrance within the pore in preparations in which fast inactivation was removed by papain in guinea-pig ventricular cells. External application upward of 10 mM was ineffective.

Barber et al. (1992) did not find a decrease in open time and conducting OD in their patch clamp experiments in rabbit cardiac preparations. They attributed the differences from the results by McDonald et al. (1989) and Baumgarten et al. (1991) to species differences, more inclusive data set and difference in the treatment of overlapping events in single channel recording.

A gating current study of intracellular QX -222 in canine Purkinje fibers (Hanck et al. 1994) suggested that OD was conductive but overall voltage-dependence of gating was reduced. No currents due to the movement of QX-222 were detected.

Molecular biology of Na^+ channel

The Na^+ channels of the mammalian brain are heterotrimeric complexes of glycosylated proteins consisting of α (260 kDa), β1 (36 kDa; noncovalently associated with α subunit; a single membrane-spanning segment (Isom et al. 1995a)), and β2 (33 kDa; connected with α subunit through disulfide linkage) subunits (see Catterall 1995). α -Subunits alone can generate Na current in the normal or in a distorted form in oocytes and other cell lines. Many experiments have been made on site-direced mutagenesis, chimera formation, specific binding of channel blockers such as guayanyl toxins (tetrodotoxin and saxitoxin), tetraethyl ammonium and charybdotoxin, antibody binding, electrophysiological studies, comparison of homologous sequences with respect to Na^+ channels *per se* or in K^+ channels (Noda and Numa 1987; Guy and Durell 1994; Catterel 1995). By reviewing these results the following may be concluded (Figs. 2-3). The α subunit is the pore-forming subunit; it contains four homologous domains (I to Ⅳ). Each domain consists of six transmembrane segments which spans the membrane in α-helical conformation (S1 to S6). The positively charged S4 segment acts as the voltage sensor when the transmembrane potential changes to move the activation gates (postulated as the segments linking S4' s to S5's (Durell and Guy 1992)) from the closed to open state. The segment linking domain Ⅲ and Ⅳ (Ⅲ-Ⅳ linker) forms the Na^+ channel's fast inactivation gate. IFM motif of the Ⅲ-Ⅳ linker (1485, 1486, 1487; gothic letters in Fig. 2) has an affinity for a region somewhere within the Na^+ channel pore, acting as an inactivation particle. The structure is likened to the hinged-lid structures of allosteric enzymes (West et al. 1992). Ⅳ S6 segment which have been implicated in the binding of channel blockers (Ragdale et al. 1994) is not directly involved in the interaction of IFM motifs (McPhee et al. 1995). The short segment between S5 and S6 (called as P segment, SS1-SS2 or H5) forms β (Lipkins and Fozzard 1994) or helical hairpins (Durell and Guy 1992) that transverse the outer part of the transmembrane region and constitutes the selectivity filter for ion permeation, a tetrodotoxin-binding portion of the pore, and the activation gate.

Although cRNA encoding α subunits from brain, skeletal muscle, and heart alone is sufficient to encode functional Na^+ channels

MAXrNsLF vLLvPRpGTpSdSFRRfFTRESLAAIEKqRMiAEKeQkARk
34 35 (I)
GxGXxSxAT xTrSL pQkEqSeREkGdLeQP dEdEEnAgPRkPQnLsDLQeASgKKsLPDfLiY
69 66 (I)
GNdPiPRQ pELmIvGsEPLEDLDPFyYSiTnQkKTFIVLNKGKTaIF
104 101 (I)
RFSATNsALYViLStPFHnPViRRkAlAViK**ILVHSLFSN mMvLIM**
139 136 (I ;**S1**)
CTILTNCVFMAtQmHsDnPPPdWTK**YnVEYTFTAgIYTFESL**
174 171 (I;**S1,S2**)
ViKILARGA FCL HeAdFTFLRDn[d]**PWNWLDFStVIVI tMfAYTvTE**
209 206 (I,**S2,S3**)
FVDn[d]LGNVS**ALRTFRVLRALKTISVISpGL**KTIVGAL
244 241 (I;**S3,S4**)
IQSVKKLAsD**VMViLTVFCLSVFALIGLQLFMGNL**RHn
279 276 (I;**S5**)
KCVlRqXwXpXpXdXnXsXtXfXeXiXnXiXtXsNfFTnEnLsNlGdTwNGStVaEfAnDrGtLv
300 311 (I)
VnWmN*E* fSnLwDV*L* eYLiN*S* eDPkA*E* sNhYfLyLfKlNeGTqT*S* nDVaLLCGNSSDAGTq
335 346 (I)
CPEGYRiCLvKAGErNPDnHyGYTSFDStFAsWAFLAsLFRLM
370 381 (I)
TQDCfWERnLYQQlTLRSaAGKIt**YMIFFMvLVIFLGSFYL**
405 416 (I;**S6**)
ViNLILAVVAMAYEEQNQATIlAeETaEEqKEKaReFQEqAmMlE
440 451 (I;**S6**)
MqLKKEqHqEXeALqTaIaRaGaVaDaTsVaSeRsSrSdLfEsMgSaPgLgAiPgV*TN* fNSsXeXs 472
486
XsXsXvXaXsXkXlXsXsXkXsXeXkXeHlEkRmK*R* rSrKRkRxKRqL*M* kSeSqGaTgED*E* eG*C*
eGkD*E* eD 493 521
RaLvPrKSDaSEDGsPiRXkXkXgXfXq[r]XfXsXlAeL*M* gNsQ*H* rLStLyTeH*R* kGrLfSRsTpSh
520 556
MqR*K* sPlRlSSiRGSIlFTsFpRRXnXsXrXaXsXlXfXnXfXkXgXrXvRkDQ*L* iGSEAn
542 591
DFADDENhSTAfG*R* eEdSnEdSHrR*H* TdSLLfVPWhPrLhRgH*R* eP*T* rSrApQsGnQvPs
577 626
G*S* qPaGsA*T* rSaAsXrXgXiPGtY*H* lVA pLmN*H* GKR*K* mNhSTaVDCNGVVSLLvGAgGpDs
609 661
A*P* ElAtTsSpPvGSqY*H* lLLxRxPxMxVxLxDxRxPxPxXxXxDxTpTeTgPtStEtEPtGeGiPrQk
642 683
MrLrTsP*S* sQsAyPhCvA*V* sDmGdFlElEPdGpAsRQRALmSAmVaSViLTSnAtLmEEL
677 718
EESH*R* rR*H* qKCPPCWNyRkF*L* AQnH*R* mYcLIWEdCCPkLpWMlSkIvKQhK*G* vVKn
712 753
F*L* lVVMDPFA*T* vDLTaITMiCIVLNTLFMALmEHYNpMTA*S* eEqFEs
747 788 (II;**S1**)
Es**MvLQsVGNLVFTGIFTAEMTfFlKIIALm**DPYYYFQQe**GW**
782 823 (II;**S2**)
NIFDSgIfIVIsLSLMELGLSaRnMvG*S* eNgLS**VLRSFRLLRVF**
817 858 (II;**S3,S4**)
KL[F]AKSWPTLNTmLIKIIGNSVGALGN**LTLVLAIIVF**
852 893 (II;**S4,S5**)
IFAVVGMQLFGKNsYSkELcRvH*D* cR*X* kI*X* SDnSdGcLeLPRWHMMhDF
887 928 (II;**S5**)
FHAsFLIIvFRIvLCGEWIETMWDCMEVSaGQSt**ImCLLtVF**
922 963 (II;**S6**)
ImLmVMVIGNLVVLNLFLALLLSSFSAsDNLTaAPtDEdDG*R* n
957 998 (II;**S6**)
EMNNLQLiALvAgRImQRkGLiRdFVKRTkTiWrDeFCiCqGk*IL* aLfRvR*H* RkPq
992 1033
K*Q* KaPlAdAeLiAkTA pH*Q* lS*G* eQdLPxSxCxIxT*A* x*AT* xPxR*Y* xSxPxPxPxPxExVxExKxV
xPxPx*AT* xRxKx 1027 1045
ExTxR*Q* xFxExExD*G* x*KE* xR*Q* xPnGnQkGkTdPsGcDiS*P*
EnPhVtCtViPeIAg[D]VkAdElSnDyTl*ED* kDQg 1062 1071
EnEgDtEtEsNgSiLgGsTsEvEEkSySvKvQd*X* *Q* xESQdV*P* yVmSGfG*W* iH*P* nE*R* nP*G* *YP* sQ
l*ED* t*PS* vRtA*T* vWp
1097 1106

SiQaVlSgEA TsTA dSfSeEnAlGE nAtSeTA eSfQsAsDeWsQR dQmEQ QeKsTA
kEPkQlAnPaGtCsGsEx 1132 1140
TxPxExDxSxYC xSEGSTAvDMiTgNaTpADE eLxLgEQIpPeDaLeGpEDeVsKlDePE
1167 1168
DaCFTEGdCVRRkCfPkCCMA qViDsTiTeQeSA gPkGKVlWWRnLRKTCYRH k
1202 1203
IVEHSnWFETFIIvFMILLSSGALAFEDIYLiEEqRKTI
1237 1238 (III;**S1**)
KVt**LmLEYADKMvFTYViLEMLLKWVAYGF**KqKmYFTNA
1272 1273 (III;**S2,S3**)
WCWLDFLIVDVSLVSLVtANTaLGFyAsEMlGPaIKSLRTL
1307 1308 (III;**S3,S4**)
RALRPLRALSRFEGMRVVVNALVlGAIPSIMN**VLLV**
1342 1343 (III;**S4,S5**)
CLIFWLIFSIMGVN[L]LFAGKFGyRhCINQyTEtGDeLmPxLfNdYv
1377 1377 (III;**S5**)
TsIvVNNKySEQ CEqSaFL lNiVL eTsGnEqLtYaWrTwKXnVKVNFDNVGAlGY
1411 1412
LAsLLQVATFKGWMDIMYAAVDSRGnYvEElQPQkWyEDY NL
1446 1447 (III;**S6**)
YMYIlYFVVI iFIIFGSFFTLNLFIGVIIDNFMQQKKK
1481 1482 (III;**S6**)
LfGGQDIFMTEEQKKYYNAMKKLGSKKPQKPIPRPLa
1516 1517
NKYfQGFmIvFD**IfVTKQAvFDViTsIMFiLICLNMVTMMVET**
1551 1552 (IV;**S1**)
DDQSPqEKmV*I* tN**ILAyKwINLLvFViAvIlFTGECIvVlKML lAiAsLRH**
1586 1587 (IV;**S2**)
YYFT**NiSgWNIFDFVVVILSIVGTmVfLSaDeIlI**QeKYFFvSP
1621 1622 (IV;**S3**)
TLF**RVIRLARIGRILRLIRkGAKGIRTLLFALMMSL**
1656 1657 (IV;**S4**)
PALFN**IGLLLFLVMFIYSaIFGMAsNFAYV**KWrEAvGID
1691 1692 (IV;**S5**)
DMFNFQeTFAgNSMLiCLFQITTSA GWDGLLSaPILNTsG
1726 1727
PPYdCDPNTeLkPdNhSpNgGsSXvRkGN*D* dCGSnPAsV**GIlfFFTvTsYIII**
1760 1762 (IV;**S6**)
SFLlvVVNMYIAIvILENFSVATEESTaEPLSEDDFDeM
1795 1797 (IV;**S6**)
FYEIvWEKFDPEdATQFIEYfL*S* cAV kLSDFADaALSdEpPLRlIA
1830 1832
KPNQkIvSqLINaMDLPMVSGDRIHCMlDILFAFTKRVLGES
1865 1867
GEMDALKrIQMEEKrFMAAsNPSKIvSYEPITTTLRkR
1900 1902
KHqEEVSATM iVIQRAFyRRHyLLQkRqSkVL KHkAvSFsLiFyRkQkQdAkG
1935 1937
GX kSxGxLxS*E* eDEDgAtPEiRkEGdLiIAtYdMV kMlN*S* GE eNFxSxRxRP xSAG tPL*P*
eSkStSdSv
1970 1965
ItSpSTStFsPPSYDSVTRkApTeSkDeNkLfPQ eVkRdAG kSDeYxSxRH
kSeEdDkLgAk 2005 1998
DFiPxPxSxPxDxRxDxXxXxXxXxXxXxXxXxXxRESIV
2019 2005

Figure 2. Amino acid sequence of Na^+ channel for rat heart I (rHI), deduced from its cloned cDNA (upper case), as compared to that for human heart I (hH1; Gellens et al. 1992; italic upper case + one space when different from the rHI), that for rat brain II (lower case when different from the rHI). rat brain II A are shown in lower case in brackets (Auld et al., 1990). Bold letters show transmembrane segments S1-S6 in each domain I-IV. X or x represents gaps (numbered according to Rogart et al. 1989). Intracellular segments are underlined. The residues referred to in the text are written in gothic letters.

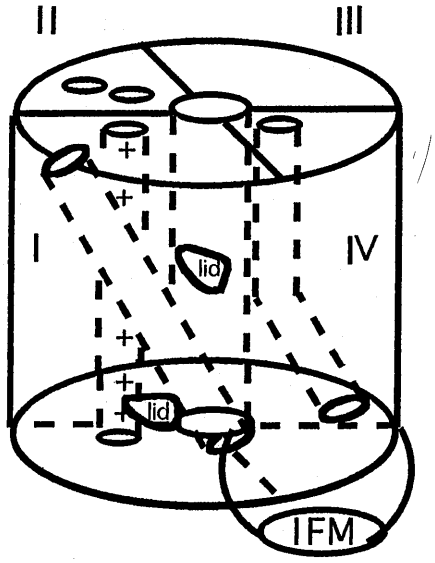


Figure 3. A speculative structural model for Na^+ channels. Four domains I-IV are centered around the channel pore. For simplicity only S4 (a dotted lined cylinder with + signs) in domain I is shown which is in contact with a hydrophilic part of another segment. Modified from Butterworth and Strichartz (1990). Lidocaine (lid) molecules block the channel not only within the pore but also in a cavity between helices to restrict their movement.

in oocytes, their rates of inactivation are abnormally slow and their voltage dependence of inactivation is shifted to more positive membrane potentials in comparison with the situation in native tissues. It appears that coexpression of $\beta 1$ subunits, a glycoprotein and non-covalently associated with α subunit, is required for the normal functional expression by stabilizing the channel complex in the plasma membrane in neuronal and human heart (Isom et al. 1995a; Makielski et al. 1996). $\beta 2$ subunits, containing a single transmembrane segment and a small intracellular domain, are only observed in neuronal Na^+ channels and functioning as important regulators of sodium channel expression and localization in neurons (Isom et al. 1995b).

Electrophysiological studies of Na^+ channel blockers in *Xenopus* oocytes and other translation system:a consideration on the structure-functional relationship

Utilization of heterologously expressed recombinant Na^+ channel is giving more and more important information in the field of the study of the channel gating as well as drug-channel interaction. Application of various chemical agents and enzymes (drugs) such as pronase and guayanyl toxins to modify channel gatings or to remove inactivation would produce unintended drug-drug interactions and drug-channel interaction which would complicate the analysis even if the primary intent is attained. Experiments on site-directed mutagenesis of specific amino acids provide alternative ways to remove inactivation for determining the site of action. Useful preparations which produce only the ionic and gating currents of the channels under study or even only the gating currents alone have been provided with appropriate density to avoid overlapping of events of single channel activities (Pusch et al. 1991).

Figure 3 is a speculative model to elucidate the relation of structure and function of Na^+ channel. The activation occurs by a sequential voltage-dependent movements of the four domains (each domain acts as a gate) from no activation (C_1 in Fig. 1) to activation of all four domains (C_5), followed by open state (O). At each state of activation it interacts voltage-independently with the inactivation particle (presumable IFM motif) shown in the lower part of Fig. 1. This process is represented as I and I_1 to I_5 in Fig. 1. During this process, S6, assumed to be the channel pore forming segment, is thought to break into two (Guy and Durell 1992), and shown as a bended cylinder here.

Ragdale et al. (1994) demonstrated that a local anesthetic, etidocaine, interacts directly with amino acid residues relevant to the ion conducting channel pore. They made mutations which selectively modified drug binding to resting or to open and inactivated channels in rat brain IIA Na^+ channels (rIIA) expressed in oocytes (shown in brackets in

Fig. 2 for the difference from rII; Auld et al. 1990). For the wild type, the Kd value for etidocaine was 1 μM for ID and 300 μM for C_1D. Mutation F1764A (gothic letters in Fig. 2), which is near middle of Ⅳ S6 of a subunit and probably facing the pore, almost abolished the use- and voltage-dependent effects (Kd for C_1=1mM; for I=100μM). Mutation N1769A, which is probably oriented away from the channel pore, increased the resting block, C_1D, by 15 fold and was interpreted as suggesting a formation of partially inactivated channel conformation. Mutation I1760A (gothic letters in Fig. 2), near the extracellular end of Ⅳ S6, seems to create an access pathway for etidocaine and QX-314 molecules to reach the receptor site from the extracellular side.

Qu et al. (1995) also demonstrated that when mutation L1752F, T1755V, T1756S (gothic letters in Fig. 2) was brought to rat heart 1 (rH1) Na^+ channel expressed in oocytes, in which extracellular and intracellular QX-314 caused use-dependent effects, the extracellular route became ineffective and delayed the recovery from use-dependent block like in rIIA. Mutation T1755V alone caused almost the same degree of the effects as the above three mutation. Mutation F1762A (corresponding to F1764A in rIIA) reduced the binding of QX-314 applied both extracellularly and intracellularly. Mutation I1758A (corresponding I1760A in rIIA) failed to produce a functional channel.

Bennett et al. (1995) showed that in oocytes expressing recombinant human heart (hH1) Na^+ channels, lidocaine acted as an inactivation channel blocker and the block increased with prolongation of Vc (τblock= 589 ms;Kd=36 μM;Vc=-50 to +20 mV) and diminished with prolongation of the Vc-Vt interval (τrec=0.4 to 2 sec at-120 to -90 mV). Such voltage-dependence was considered to be due to voltage-dependence of affinity of the channel-states or binding of the charged form of lidocaine. After removal of fast inactivation by α-chymotrypsin or mutation IFM1485, 1486, 1487QQQ, lidocaine produced only a tonic block at Kd=400 μM, probably acting as an open channel blocker. These authors interpreted the results as suggesting that the major component of block was the formation of ID, but not that of C_iD. However, it is possible that with the loss of inactivation gate the pathway of $I_i \rightarrow C_i$ is also lost. The binding site was assumed either to be distinct from the open-channel blocking sites within the pore (S6) or to be within the pore (S6) but directly or indirectly retard unhinging the inactivation particle.

Wang et al. (1996) compared block by lidocaine in human cardiac (hH1) Na^+ channels with that in human skeletal muscle (hSkM1) Na^+ channels expressed in a transformed human kidney cell line. A whole-cell voltage clamp study revealed that the degree of the use-dependent block produced by lidocaine at 25 μM in hH1 was much the same with that produced by 100 μM in hSkM1. An additional block occurs during channel opening in hSkM1 but not in hH1. Their simulation study showed that ID was formed in both isomers, whereas OD was also formed in hSkM1.

Recently, Yang et al. (1996) demonstrated that by cystein scanning mutagenesis Ⅳ S4, voltage sensor, moved across the membrane. of which thickness was only 4.5 to 11 Å, but not 40 Å, that shickness of the membrane. This indicates that the channel protein must have deep hydrophilic crevices or vestibules, the bottom of which is so close to either the external or internal surface. The crevices were estimated to be positioned more than 6 Å apart from the ion conducting pore. This finding stimulates interest in speculating that extracellular or intracellular lidocaine would enter into such crevices and reduce the voltage-dependence of gating by restricting the movement of S4, as shown in Fig. 3. Such a radical change in the higher structure of Na^+ channel may produce unexpectedly large differences in the channel gating and drug -channel interactions between isomers which differ only in a few residues.

Another point worth noting is the role of β1 and β2 subunits. Coexpression of rat brain and skeletal muscle Na^+ channel α subunits with β1 subunits in oocytes makes the rates of activation and inactivation and shifts in the voltage dependence of activation and inactivation, more similar to those found in native tissues (Isom et al. 1992; Bennett et al. 1993). Although contradictory findings have

been reported on the role of $\beta 1$ in cardiac Na^+ channels (see Makielski et al. 1996), in studying coexpression of human hE1 α subunit with rat $\beta 1$ in oocytes, Makielski et al. (1996) found that positive shift of the steady state inactivation curve by 3-7 mV only when Vc was 10 sec or longer, otherwise the effects were minimal. They observed remarkable reduction of the sensitivity to lidocaine block in the presence of $\beta 1$ for the resting state (Kd for C_1D changed from 500 to 1400 μM) but not for the inactivated state (Kd=10 μM). From the closer Kd values without $\beta 1$ to those of native channels they suggested that native adult cardiac channels *in vitro* may not show a $\beta 1$ effect. Since the effect of a $\beta 1$ construct with cytoplasmic tail removed was the same as that of $\beta 1$, these authors favored a view that $\beta 1$ increased Kd for C_1 through indirect effects on α subunit.

On the contrary, Nuss et al. (1995) reported Kd for C_1D=486 μM in hE1 α+rat $\beta 1$ channels and 1168 μM in rat skeletal muscle($\mu 1$) α+rat $\beta 1$ channels, whereas Kds for ID were similar (12-16 μM) in the two preparations in oocyte expression system. The authors noted that the above estimates were too simple for the estimation of the relative affinities among different preparations. The differences of $\mu 1$ from cardiac Na^+ channels in Ⅳ S4 are V1757, S1758 and V1766 (same as rat brain II in Fig. 2; gothic letters; the last one was not referred to by the authors), the mutation of which to alanine had small to moderate effects on etidocaine sensitivity (Ragdale et al. 1994). The above results indicates that the sensitivity to lidocaine in the expression system appears less than that in native tissues. There is a possibility that hydrophobic channel blockers such as lidocaine may go through the lipid bilayer membrane to channel receptor sites (Chester et al. 1989) and, therefore, $\beta 1$ and $\beta 2$ subunits may stand in the access pathway of these channel blockers. Mutation of Ⅳ S6 amino acids oriented away from the channel pore may not only change the affinity of drugs that are within the channel pore (Ragdale et al. 1994) but also those that enter from membrane phase and reach the surrounding area of the pore.

Molecular pharmacology of Na^+ channel now requires more elaborate kinetic studies including a slow and a fast inactivation gating mechanism in the expression system (Tomaselli et al. 1995) as well as in native tissues (Sada et al. in preparations for chick embryonic heart) in addition to the molecular genetic studies.

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