USE DEPENDENT BLOCK OF SODIUM CHANNELS BY LOCAL ANESTHETICS IS BASED ON PI-PI AROMATIC INTERACTION

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The photo shows a student of P.G. Kostyuk, Prof. N.T. Parkhomenko.

Sodium current (I_{Na}) inhibition by prototypical local anesthetics (bupivacaine, lidocaine, and other "caines"), nonsedative anticonvulsants (phenytoin, carbamazepine and lamotrigine), antidepressant (amitriptyline), and anti-arrhythmic (amiodarone) have two modes and some have been operationally defined: use-dependent or phasic block, measured as a decrease in I_{Na} during repetitive activation and tonic block, measured as I_{Na} decrease in the preceding activation absence. The molecular mechanisms accounting for the voltage- and use-dependent block of sodium channels by these drugs, despite of intensive and profound investigations, have remained elusive.

In terms of a steady-state effect, these drugs show much higher affinity to the open (inactivated) than to the resting sodium channels and thus selectively bind to the former rather than to the latter (Bean et al., 1983; Matsuki et al., 1984; Butterworth and Strichartz, 1990; Kuo and Bean, 1994). In terms of kinetics, they have relatively slow binding rates to the receptor on inactivated Na^+ channel during depolarization, thus stabilization of the inactivated Na^+ channels do not reach the steady state under one short action potential (AP) but are gradually accumulated with repeated ones.

Estimation procedure for the kinetics of use-dependent block was described first by Courtney (1975, 1978). This approach, "as originally described, did not permit determination of the "true" binding rates, but did allow for quantitative estimates of (a) the fractional increase in blocked channels produced by each pulse in the depolarizing pulse train, and (b) the rate of recovery from use-depen-

dent block between pulses" (Chernoff, 1990). The model was re-derived by Chernoff. In the new model, "all possible binding reactions during a pulse are lumped into two terms: one (a*k) describes the fraction of drug-free channels that bind LA during the pulse; the other (a*1) describes the fraction of previously-bound channels which unbind LA during the pulse". Parameter estimation procedure is based on measurements of the decrease in peak I_{Na} during a train of repetitive depolarizations of nerve membrane. By calculating values for a*k and a**1 over a range of pulse durations, the model allows following the time course of binding and unbinding of LA during a depolarizing pulse (Chernoff, 1990).

With using this kinetic method as well as the steady state ("statistical") one, we undertook a comparative study of voltage and use-dependent block of sodium channels in dorsal rat ganglion neurons using new drug D57 with anesthetic action, which exerts a well pronounced use dependent effect.

Experimental procedures were the same as in the previous work (Parkhomenko et al., 2008). Kinetic parameters corresponding to Chernoff's model were estimated according to the author's protocol including some modifications of the first steps. Parameters obtained from the steady state data were calculated as in the paper of A.O. Grant et al. (2000). So, from the block at the steady state (b_{ss}), time constant of current relaxation (τ_b) and concentration of drug (D), we can calculate the rate constants of association and dissociation: $\tau_b = 1/(k_{on}D + k_{off})$, $b_{ss} = k_{on}D/(k_{on}D + k_{off})$, where k_{on} and k_{off} are the association and dissociation rate constants, respectively ($K_D = k_{off} / k_{on}$).

Data presented in Fig. 1 show a unique type of the dependence of the block parameters on the stimulation frequency at a holding potential of –80 mV for both models. Only difference for them is that in the kinetic model the rate constant increases by approximately fifteen times at 4.0 Hz as compared to that at 0.5 Hz, while for the steady state approach the increase is only by six times.

Taking into account most general opinion about the much more decrease in the dissociation rate as compared to the increase in the association rate in conditions when growth of block proceeds by use dependent process, it is possible to compare the data on the dissociation rate constants obtained according to these two different models. Data of Fig. 2 are very interesting in two aspects. Firstly, increase in dissociation rate constants obtained by these different methods appears to be changed by the same times, namely threefold, when stimulating frequency changed by 8 times. Secondly, increase in the dissociation rate constants obtained at both approaches is 2-5 times smaller than increase them for association.

Thus, both models give close data on the dependence of dissociation parameters but some different ones relating to association.

One of the approaches for estimation of possible molecular mechanism of use-dependent block pronounced by drugs may be calculation of binding energy of the latter to the sodium ionic channels. This energy is usually determined by

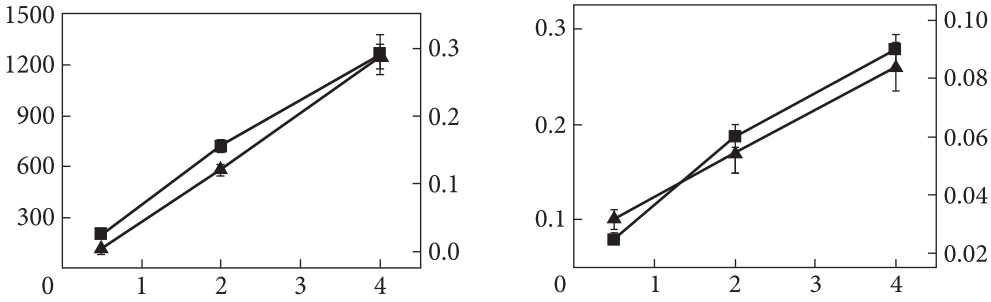

Fig. 1. Dependence of the rate constants for binding of D57 with ion channels on the frequency of 6 msec stimulating pulses to 10 mV at the neuron holding potential –80 mV. Constants calculated using statistical (left scale) and kinetic model of Chernoff (right scale). For left, k_{on}, M/sec (triangles) and right — a*k, rate of drug association with sodium channels (squares), respectively. Abscissa shows frequency of impulses (Hz)

Fig. 2. Dependence of the rate constants for dissociation of D57 from ion channels on the frequency of 6 msec stimulating pulses at the neuron holding potential –80 mV. Constants calculated using statistical (left scale) and kinetic model of Chernoff (right scale). For left — k_{off} as sec^{-1} (triangles) and right — a**l, rate of drug dissociation from sodium channels (squares), respectively. Abscissa shows frequency of impulses (Hz)

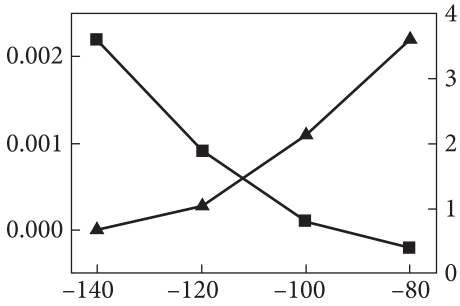

Fig. 3. The dependence of dissociation constants (K_D — triangle — left scale) and binding energy (kcal/M — square — right scale) of D57 with sodium ion channels on different membrane potentials of neurons. Abscissa shows holding potential of neurons

the equation $\Delta G = 0.58 \cdot \ln (K_{in}/K_{il})$ kcal / mol, where K_{in}/K_{il} is the fractional change in the block (dissociation) constant of sodium currents while changing the potential and frequency of pulses.

Data in Fig. 3 show that binding energy of D57 to sodium channels is in the range 0.5-3.5 kcal/M and seems to be relatively small comparing with the energies of covalent or ionic bonds. Such values may represent the energies of electrostatic, hydrophobic or hydrogen bonding forces. Aromatic-aromatic types of electrostatic interaction (2-5 kcal/mol) are ones of the principal noncovalent forces governing the fundamental process of molecular recognition among molecules in biology and chemistry (Sinnokrot et al., 2002, Powell et al., 2007).

Assumption of namely electrostatic aromatic-aromatic interaction being crucial for use dependent local anesthetics (LA) action based also on the structure of well-established site in sodium channels where it is realized. In different sodium channels LA pronounce use dependence only if amino acid residue of

phenylalanine is present in S6 segment of domain D4 of sodium channel α-subunit at the position Phe-1759 following the heart NaV1.5 isoform numbering, corresponding to Phe-1579 in skeletal NaV1.4 and Phe-1764 in brain NaV1.2 (Ragsdale *et al.* 1994; Wright *et al.* 1998; Yarov-Yarovoy *et al.* 2001; Yarov-Yarovoy *et al.* 2002, McNutly MM et al. 2007). Participation of tyrosine, other aromatic amino acid residue, is much smaller and some controversial.

Thus, it is evident that local anesthetic molecule having two different parts, amine and aromatic, interacts with aromatic residue in sodium channel polypeptide chain. If it interacts via the amine group, the more probable stechiometry is 1:1. However, much better approximation of experimental data were obtained by using the Hill type equation with more than two coefficients, instead of the Bolzman type with 1:1 molecular interaction (Leuwer et al., 2004, Parkhomenko et al., 2008). So, this discrepancy may be "limited" if one assumes interaction of anesthetic molecules with sodium channels via its aromatic moiety and additionally via stacking. Because of namely such interaction allows more than one LA molecule to be bound to one aromatic residue of amino acid of sodium channel.

The major trends in the aromatic-aromatic interaction energy can be rationalized using a simple model based on electrostatic interactions between the π-faces of the two aromatic rings. However, electrostatic interactions between the substituents of one ring and the π-face of the other make an additional contribution, due to the slight offset in the stacking geometry. This property makes aromatic stacking interactions particularly sensitive to changes in orientation as well as to the nature and location of substituents (Cockroft S et al., 2007).

The presence of substituents on the aromatic ring, irrespective of their electron withdrawing or donating nature, leads to an increase in the binding energy, and the displaced-stacked conformations are more stabilized than the T-shaped conformers. Taking into account that substituents as well as the conformational effects are correlated with the electrostatic interaction, this implies that the substituent effect may be important in apolar media, in particular, for assembling processes (Lee EC et al., 2007).

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