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EFFECTS OF PROPRANOLOL AND A NUMBER OF ITS ANALOGUES ON SODIUM CHANNELS

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Abstract—To assess the relative contributions that the sodium channel blocking activity of propranolol may play in a variety of its therapeutic applications, its effects were examined *in vitro* with a sodium channel specific $^{22}Na^+$ uptake system, using rat brain membranes. Propranolol inhibited $^{22}Na^+$ uptake in the rat brain membrane preparation by acting as a competitive inhibitor of the binding of the sodium channel opening agent veratridine, with an IC_{50} for this action of 6.5 μM. This is approximately one order of magnitude higher in concentration than that necessary for expression of the β-adrenergic antagonism of propranolol. The binding of propranolol and its action to block sodium channels were demonstrably different from those of the neurotoxins tetrodotoxin and saxitoxin. Propranolol had effects on sodium channels that are similar, although not identical to those of the local anesthetics procaine and lidocaine. The concentrations of propranolol and a number of its analogues which produced 50% inhibition of $^{22}Na^+$ uptake (IC_{50} values ranging from 4 to > 100 μM) were similar to the concentrations of these same analogues which were required to produce negative inotropic and antiarrythmic effects (ED_{40}) on isolated rabbit atria [D. O. Rauls and J. K. Baker, *J. med. Chem.* **22**, 81 (1979)]. These effects showed correlations of 0.945 and 0.936, respectively, with the $^{22}Na^+$ uptake inhibition. It is concluded from this information that a substantial proportion of the negative inotropic and antiarrythmic effects of propranolol is due to its action on sodium channels.

Propranolol is a non-selective β-adrenergic blocking drug currently in wide use for (among others) the treatment of hypertension, the prophylaxis of angina pectoris, and the control of certain types of cardiac arrythmias. In addition to its β-adrenergic blocking activity, propranolol exhibits CNS depressant and anticonvulsant effects which are most likely due to a local anesthetic-like non-adrenergic action [1]. The working hypothesis in this study was that the local anesthetic-like action of propranolol may be responsible to a greater or lesser extent for its efficacies in a variety of its therapeutic applications and side effects. A major component of the local anesthetic-like action of propranolol is its ability to block sodium channels [2].

Sodium channels are the plasma membrane components responsible for controlling sodium ion conductance in excitable cells. Normally sodium channels open and close in response to voltage changes across the membrane. However, it is becoming increasingly evident that these macromolecular membrane components are receptors for, and their function is influenced by, numerous agents. Veratridine and a number of other neurotoxins [3, 4] specifically open sodium channels while tetrodotoxin and saxitoxin specifically block them [4, 5]. In addition, a number of substances of lesser specificity, for example local anesthetics and propranolol, will block Na^+ channels [2, 6–8].

Osmotically sensitive membrane preparations from rat brain are able to take up $^{22}Na^+$ in response to specific sodium channel opening agents such as veratridine [9–11]. This $^{22}Na^+$ uptake is blocked by tetrodotoxin and saxitoxin and has been shown to be sensitive to a number of pharmacologically active agents such as local anesthetics which are known to affect sodium channels [9]. The $^{22}Na^+$ uptake technique used for this study exhibits the following important characteristics. The reequilibration rate of channel-opening toxins is slow in relation to the time frame of the assay and at low concentrations of channel-opening toxins the sodium channel dependent $^{22}Na^+$ uptake at equilibrium is dependent upon the concentration of channel opener [12]. These features allow analysis of the equilibrium $^{22}Na^+$ uptake data as a measure of the equilibrium binding of the channel-opening toxins [9]. Furthermore, the effects of various agents on this uptake system are those which are specific for the sodium channel, thus allowing a direct assessment of Na^+ channel action exerted by agents which normally exhibit diverse activities. Data obtained and analyzed by this technique using various neurotoxins and local anesthetics have shown good agreement with similar experimental results from conventional electrophysiological measurements [9].

Propranolol and a number of propranolol analogues of varying lipophilicity, previously characterized with respect to an antiarrythmic activity and a negative inotropic effect on isolated rabbit atria [13, 14], were analyzed with respect to their influence on sodium channels using the $^{22}Na^+$ uptake system.

MATERIALS AND METHODS

Materials. Male Wistar rats, 125–300 g, were purchased from Harlan Industries, Indianapolis IN. Veratridine and Dowex 50W-X8, 50–100 mesh,

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hydrogen form, were obtained from the Sigma Chemical Co., St. Louis, MO. *dl*-Propranolol HCl was a gift from Ayerst Laboratories, New York, NY. Propranolol analogues were prepared as previously described [13]. All other materials were as previously described [9].

Membrane preparation and $^{22}Na^+$ uptake measurements. $^{22}Na^+$ uptake measurements with some minor modifications as detailed below were performed on rat brain membranes prepared as previously described [12] using the column method described previously [9]. A whole brain from a freshly killed rat was rinsed with ice-cold standard buffer (SB) (0.32 M sucrose, 10 mM Tris–HCl, 1 mM KN_3, pH 7.5) and homogenized in 30 ml of SB at 300 rpm, 10 up-and-down strokes with a 0.125 mm clearance glass–Teflon homogenizer. All membrane preparation steps were performed at 0–5°. The homogenate was diluted to 50 ml with SB and centrifuged for 10 min at 300 *g*. The supernatant fraction was recentrifuged at 20,000 *g* for 20 min. The pellet from the second centrifugation was resuspended using the glass-Teflon homogenizer (5 strokes at 100 rpm in 25 ml of SB containing 1 mM ouabain) and allowed to stand for 1 hr.

Three microliters of a stock solution of propranolol or a propranolol analogue in SB or 3 μl of SB for control was added to 0.3-ml aliquots of the membrane preparation and incubated at 25° for 10 min. All assay steps were performed at 25°. Then 3 μl of a veratridine stock solution in ethanol or 3 μl of ethanol for control was added and incubated for an additional 20 min. Next 0.3 ml of 0.3 μCi/ml $^{22}Na^+$ as NaCl in SB was added and incubated for an additional 5 min. At this time the sample was passed through a 2-ml bed volume cation exchange column prepared as previously described [9] with the exception that Dowex 50W-X8 50–100 mesh resin was substituted in place of AG 50W-X8 100–200 mesh resin. This change had no effect on the outcome of the assay. This change was necessitated when a new batch of AG 50W-X8 resin failed to pass the membrane particles. The 50–100 mesh resin was found to pass 95+% of the membrane particles based on protein analysis using the method of Lowry *et al.* [15]. The $^{22}Na^+$ contents of the column effluents were determined with a gamma radiation detector. Propranolol analogues 1 and 2 (Table 1) were prepared in ethanol rather than SB. Control blanks for experiments with these analogues received 3 μl of ethanol.

RESULTS

Analysis of the effects of propranolol on $^{22}Na^+$ uptake. Propranolol was found to inhibit veratridine-stimulated $^{22}Na^+$ uptake in the brain membrane system described. This inhibition showed an IC_{50} of 6.5 μM (Fig. 1). Propranolol concentrations below 50 μM had no detectable effect on $^{22}Na^+$ uptake in the absence of veratridine (control); however, propranolol concentrations of 50 μM and greater significantly depressed the control uptake (Fig. 1). This depression of control uptake at elevated concentrations was observed previously with the local anesthetics lidocaine and procaine and is believed to be due to disruption of membrane integrity by these agents [9]. All of the propranolol analogues were tested at their highest concentrations to ensure that there was no contribution from any effect on control uptake which would influence the results.

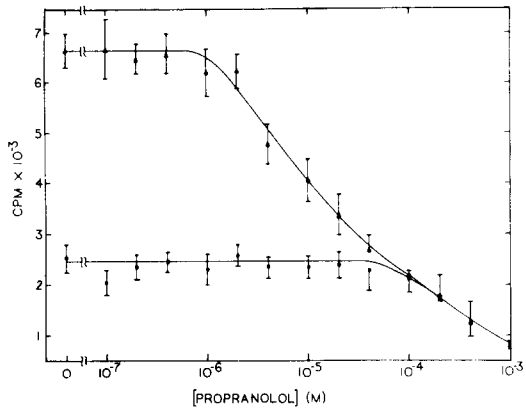


Fig. 1. Dose–response curve for propranolol inhibition of veratridine-stimulated $^{22}Na^+$ uptake. Key: (●) control; and (▲) 5×10^{-6} M veratridine. The data points are the mean ± S.D. of three independent determinations.

To determine the nature of the inhibition of veratridine-stimulated $^{22}Na^+$ uptake by propranolol, five propranolol concentrations were analyzed with

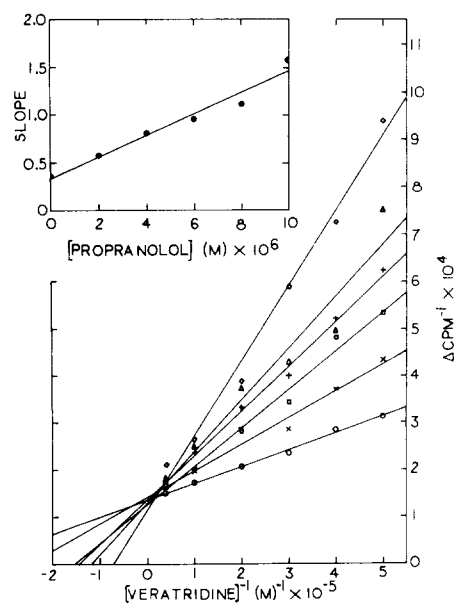
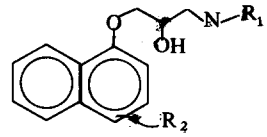


Fig. 2. Double-reciprocal plots of veratridine-stimulated $^{22}Na^+$ uptake and its inhibition by propranolol. Key (○) no propranolol; (×) 2×10^{-6} M propranolol; (□) 4×10^{-6} M propranolol; (+) 6×10^{-6} M propranolol; (△) 8×10^{-6} M propranolol; and (◇) 1×10^{-5} M propranolol. Data points are the reciprocals of the means from three independent determinations of the difference between the stimulated and control uptake. Data were fitted to the double-reciprocal plot by linear least squares. The inset is a replot of the slopes of the double-reciprocal lines vs the concentration of propranolol. Data were fitted using linear least squares. The units of the slope are $M \cdot cpm^{-1} \times 10^9$.

Table 1. Properties of propranolol analogues



Compound*	R_1	R_2	$-\log IC_{50}$ ($-Na$ channel inhibition)†	$-\log ED_{40}$ (contractility)‡	$-\log ED_{40}$ (MDF)‡	log P‡
1	$CH(CH_3)_2$	2,4-Br_2	5.19 ± 0.44	5.53 ± 0.04	5.10 ± 0.15	2.71 ± 0.01
2	$CH(CH_3)_2$	4-Cl	4.68 ± 0.60	5.26 ± 0.12	5.20 ± 0.06	2.00 ± 0.01
3	$CH(CH_3)_2$	H	5.19 ± 0.48	5.21 ± 0.10	5.43 ± 0.07	1.08 ± 0.01
4	$CH(CH_3)_2$	4-OH	4.38 ± 0.46	4.39 ± 0.20	4.60 ± 0.03	0.39 ± 0.01
5	$CH(CH_3)_2$	4-NH_2	4.82 ± 0.57	4.92 ± 0.20	5.02 ± 0.12	−0.27 ± 0.02
6	$CH(CH_3)_2$	4-SO_2NH_2	<4	3.35 ± 0.02	3.58 ± 0.10	−0.51 ± 0.02
7	$(CH_2)_3CH_3$	H	5.40 ± 0.49	5.38 ± 0.08	5.64 ± 0.08	2.08 ± 0.02
8	$(CH_2)_3OCH_3$	H	5.15 ± 0.48	5.40 ± 0.26	5.45 ± 0.15	1.28 ± 0.01
9	$(CH_2)_3NH_2$	H	4.52 ± 0.71	4.47 ± 0.04	4.50 ± 0.10	−0.19 ± 0.01
10	$(CH_2)CONH_2$	H	4.64 ± 0.41	4.79 ± 0.06	4.89 ± 0.10	0.31 ± 0.09
11	$(CH_2)_3CO_2H$	H	<4	3.02 ± 0.24	3.41 ± 0.28	0.21 ± 0.01
12	$(CH_2)_3N^+(CH_3)_3Cl^-$	H	4.40 ± 0.29	3.06 ± 0.06	3.08 ± 0.16	−1.89 ± 0.09

* Numerical designations of the various analogues are the same as used in the text.

† Confidence limits for the data were determined by the method of Litchfield and Wilcoxon [16].

‡ P is the octanol–water partition coefficient, and MDF is the mean driving force; data were taken from Rauls and Baker [13] and Baker *et al.* [14].

each of six concentrations of veratridine. The propranolol and veratridine concentrations were chosen from dose–response curves to lie within the range of maximum slope. A double-reciprocal analysis of these data is presented in Fig. 2. It was concluded from these data that propranolol showed a competitive mode of inhibition in which only a single affinity for propranolol is involved.

To obtain a better assessment of the requirements for, and the nature of, the binding interaction of the propranolol molecule with the sodium channel, a number of propranolol analogues were tested for their effects on veratridine-stimulated $^{22}Na^+$ uptake using the rat brain membrane preparation. A dose–response relationship at 5 μM veratridine was determined for the inhibitory action of each propranolol analogue. The slopes of the dose–response relationships for the propranolol analogues were found to conform closely with that for the dose curve with propranolol, while the IC_{50} values varied depending on the analogue (Table 1). The assumption is made, based on this comparison, that there are no significant differences in the mechanisms of action of the various analogues from that of propranolol. The structures and apparent IC_{50} values for each of the analogues tested are tabulated in Table 1. The numerical designations for the various analogues used in the text are the same as used in Table 1. Also included in Table 1 are results of previously published [13, 14] data for these analogues. The fact that various biological and chemical analyses had already been performed on this series of analogues rendered them especially valuable for this study since it allowed a direct correlation between some of the biological and chemical properties of the analogues and their specific action on sodium channels. These correlations are shown in Figs. 3–5.

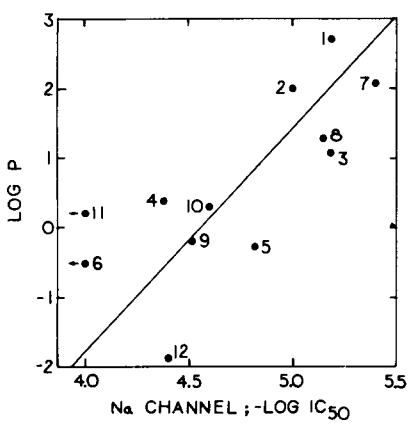


Fig. 3. Correlation of $-\log IC_{50}$ for Na^+ channel inhibition vs the log of the octanol–water partition coefficient (log P) for the various propranolol analogues. The numbering of the analogues is the same as in Table 1. Analogues 6 and 11 were excluded from the correlation analysis. The remaining analogues showed a linear correlation coefficient of 0.804. Data for log P taken from Rauls and Baker [13].

Figure 3 shows the correlation plot of sodium channel inhibitory activity of the various analogues versus the octanol–water partition coefficient [13]. Analogues 6 and 11, which are the only analogues capable of carrying a negative charge, were very poor Na^+ channel inhibitors, if at all, and were excluded from the calculations. A correlation coefficient of 0.804 was obtained for the remaining analogues. Basically, the more lipophilic the analogue the more effective it was at inhibition of Na^+ channels.

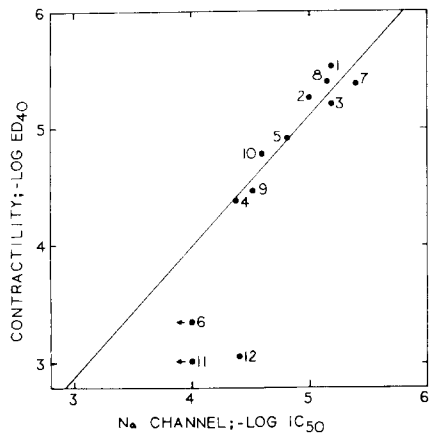


Fig. 4. Correlations of $-\log IC_{50}$ for Na^+ channel inhibition vs $-\log ED_{40}$ for contractility of the rabbit atrial preparations for the various propranolol analogues. The numbering of the analogues is the same as in Table 1. Analogues 6, 11 and 12 were excluded from the correlation analysis. The remaining analogues showed a linear correlation coefficient of 0.945. Data for log ED_{40}; contractility taken from Rauls and Baker [13].

Figure 4 shows the correlation of Na^+ channel inhibitory activity of the various analogues versus their negative inotropic effects as determined by reduction of contraction strength on isolated rabbit atria [13]. In this correlation, analogues 6, 11 and also 12 were excluded from the analysis. The remaining analogues showed a linear correlation coefficient of 0.945. Further, the values of the effective concentrations for the two activities were closely aligned. Analogue 12 is the only analogue which carries a fixed positive charge. This analogue showed approximately the activity expected, based on lipophilicity considerations for Na^+ channel inhibition, but much lower activity than expected for the negative inotropic and antiarrhythmic effects [13].

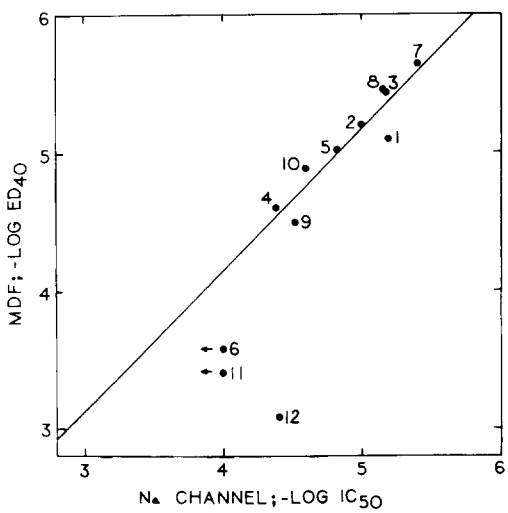


Fig. 5. Correlation of $-\log IC_{50}$ for Na^+ channel inhibition vs $-\log ED_{40}$ for the mean driving force (MDF) of the rabbit atrial preparation for the various propranolol analogues. The numbering of the analogues is the same as in Table 1. Analogues 6, 11, and 12 were excluded from the correlation analysis. The remaining analogues showed a linear correlation coefficient of 0.936. Data for $-\log ED_{40}$; MDF taken from Rauls and Baker [13].

Figure 5 shows the correlation between Na^+ channel inhibitory activity of the various analogues and their antiarrhythmic activities as determined by measuring the mean driving frequency at which the isolated rabbit atrium could no longer follow the stimulus [13]. Again, as with the previous correlation, analogues 6, 11 and 12 were excluded from the analysis. The remaining analogues showed a linear correlation coefficient of 0.936, and the absolute values of the effective concentrations for the two activities were again closely aligned.

DISCUSSION

In the intact nerve or muscle, sodium channels respond to positive going, or depolarizing, changes in the transmembrane voltage when a certain threshold level is attained by opening of the activation gate. This opening provides for a rapid flux of Na^+ into the cell, providing for further rapid depolarization and reversal of membrane potential. In the normal sequence of events, the sodium channel then inactivates via closing of the inactivation gate. Increasing potassium conductance and other events in the membrane then serve to restore the resting membrane potential. Repolarization of the membrane allows the activation gate to close and the inactivation gate to reopen, restoring the channel to its resting configuration [17].

At 0 membrane potential (the approximate conditions of the $^{22}Na^+$ uptake assay used for this study) [9], the activation gate should be open and the inactivation gate closed. Under these conditions the channel cannot pass Na^+. Veratridine binds to sodium channels and causes both the activation gate and the inactivation gate to be held open in a manner independent of membrane potential [9, 10]. Thus, since veratridine causes the gates to be held open and allows the channel to remain in a conducting configuration, its binding must occur in such a way that no occlusion of the pore results. Propranolol shows a single affinity competitive inhibition of veratridine activation of sodium channels. There are two basic possibilities for this type of competitive mechanism. The first is that the competitive inhibitor binds at the binding site of veratridine, thus displacing veratridine but failing to itself produce the activation. Propranolol is known to block sodium currents in electrically stimulated channels [2]. Thus, according to this mechanism, if propranolol binds at the veratridine binding site, it is unlikely that it blocks sodium conductance by directly occluding the pore.

The second possibility for a single affinity competitive mechanism is a situation in which the inhibitor (propranolol) binds only to a channel which is in a non-conducting configuration, i.e. gates closed, while the activator (veratridine) binds at a distinct site which exists only when the gates are open and thus prevents them from closing. Thus, the veratridine binding site will not exist when propranolol is bound, and the propranolol binding site will not exist

when veratridine is bound. The information presented here does not allow one to distinguish between these two possible mechanisms, but it does demonstrate that propranolol inhibition of Na^+ conductance is due to an effect on channel gating rather than simple occlusion of the pore.

The correlation obtained with lipophilicity versus sodium channel inhibition for the various analogues (Fig. 3) indicates two basic possibilities. The first is that the propranolol binding interaction with the sodium channel is to a substantial degree determined by lipophilicity. The second is that the binding site for propranolol may reside on the inside surface of the cell membrane and thus the analogue will be expected to penetrate the lipid bilayer prior to reaching its binding site. The data presented here do not allow one to distinguish between these two possibilities.

It seems reasonable to conclude from existing information and the information presented here that a substantial proportion of the selected antiarrythmic effects and negative inotropic effects of propranolol discussed here are due to its actions on sodium channels.

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