A KINETIC STUDY OF EFFECTS OF PROPRANOLOL AND N-PROPYLAJMALINE ON THE RATE OF RISE OF ACTION POTENTIAL IN GUINEA PIG PAPILLARY MUSCLES

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Abstract—In isolated guinea pig papillary muscles driven at 1 Hz, propranolol in the concentrations of 10^{-6}, 2 \times 10^{-6} \text{ and } 5 \times 10^{-6} \text{ g/ml and N-propylajmaline (NPA) in the concentrations of } 2 \times 10^{-7}, 5 \times 10^{-7} \text{ and } 10^{-6} \text{ g/ml both depressed dose-dependently the maximum rate of rise of action potential (V}_{\text{max}} \text{) without affecting resting potential. With a rise in driving frequency from 0.25 to 5 Hz, V}_{\text{max}} \text{ was reduced progressively both in propranolol- and NPA-treated preparations but such was slight in the control. Comparison of effects of both drugs in a concentration which exerted a similar extent of depression of V}_{\text{max}} \text{ at 1 Hz revealed that propranolol usually exerted less depression at lower frequencies and a greater depression at higher frequencies than NPA. V}_{\text{max}} \text{ depressed under the influence of propranolol was recovered toward the pre-drug level in the first response after a pause in stimulation with rate constants of 0.081 to 0.116 sec}^{-1}. A kinetic model was constructed on the basis of assumption that drug molecules were taken up to the site where they affect the sodium carrying system at the very beginning of excitation and were released during diastolic intervals with the rate constant of the recovery. Two modifications of the model are presented to elucidate the effects of both drugs.

A series of antiarrhythmics like quinidine and procainamide has been referred to as class 1 drugs by Vaughan Williams (1), as they share properties which depress the maximum rate of rise of action potential (V}_{\text{max}} \text{) in cardiac tissues without changing the resting potential. This action, which also represents a depression of the inward sodium current (2, 3), is associated with a decrease in conduction velocity and phase-4-depolarization as well as an increase in the threshold of excitability and the effective refractory period (4). All these factors are considered to contribute to a clinical improvement of cardiac arrhythmia (4).

Moreover, it is known that some antiarrhythmics of this class depressed V}_{\text{max}} \text{ progressively with an increase in driving frequency (5−8). These agents are considered to be especially useful for protection of heart muscles by filtering out abnormal high frequency discharges (1, 6).}

It has been suggested that there are several factors which contribute to this frequency-dependent depression. Trautwein (9) pointed out that in experiments done by Johnson and McKinnon on quinidine (5), a lowering of resting membrane potentials might be responsible for the acceleration of the depression of V}_{\text{max}} \text{ by higher frequencies of stimulation.

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Chen et al. (7) and Chen and Gettes (8) attributed this depression of quinidine to inhibition of activity of sodium-, potassium-dependent ATPase and that of lidocaine to interference with reactivation kinetics of the sodium carrying system.

Also relevant to this subject are the findings of Heistracher et al. (10), Liebeswar et al. (11) and Heistracher (12). These authors showed that, in cardiac tissues, V\textsubscript{max} which was depressed under the influence of antiarrhythmics such as quinidine, procainamide, ajmaline, N-propylajmaline (10-12) and flurazepam (13) recovered toward the pre-drug level in the first response after a pause in driving stimuli. Each antiarrhythmic is characterized by its recovery time length which ranges from some 10 sec to some 10 min. They interpreted these and other findings on the basis of their assumption that electrical activity, especially its initial phase in muscle fibers is required for a drug to reach the site where it affects the sodium carrying system, and that the drug was released from this site during periods of electrical inactivity.

According to this assumption, one of the factors determining whether a drug exerts a frequency-dependent or frequency-independent depression of V\textsubscript{max} is the relative ratio of the rate constants of association and dissociation of the drug with the site.

The present study is mainly concerned with effects of propranolol and N-propylajmaline on V\textsubscript{max}, influences of changes in driving frequency on these effects and also influences of pauses in stimulation on the effects of propranolol. A kinetic model was constructed on the basis of the above interpretation. Both propranolol in various cardiac tissues (6, 14-22) and N-propylajmaline in Purkinje fibers (23) have been shown to possess class 1 action. However, the former was reported to be characteristic of its marked frequency-dependent effect (6, 16, 22) and the latter, of its slow recovery during periods of electrical inactivity (12).

An analogous model has been presented by Courtney (24) who studied a frequency-dependent or use-dependent inhibition of sodium current by a lidocaine-like local anesthetic, GEA 968, in voltage-clamped frog myelinated fibers. These findings were explained in terms of a combination of the drug with sodium channels which were opened by depolarization and a shift of the curve of the relationship between membrane potential and sodium inactivation to one of a hyperpolarizing direction. This author succeeded in simulating a temporal progress of inhibition and its removal, of sodium conductance by repetitive depolarizing and hyperpolarizing-depolarizing pulses by modifying the inactivation variable, h, of the Hodgkin-Huxley equation (25) in drug combined channels. The present kinetic model is an approach where the recovery time course and dose-dependency of the depression of V\textsubscript{max} is discussed.

**MATERIALS AND METHODS**

Guinea pigs of either sex weighing 250 to 600 g were sacrificed by a blow to the head. The heart was rapidly removed and placed in Tyrode solution. The papillary muscles were dissected from the ventricles and mounted in a Perspex chamber. Tyrode solution, aerated with 95% oxygen and 5% CO\textsubscript{2}, was constantly perfused through the tissue chamber. Temperature was maintained at 37°C. The pH of nutrient medium was approximately 7.3. The
composition of the Tyrode solution, in millimoles per liter, was: NaCl: 136.9; KCl: 5.4; CaCl\(_2\): 1.8; MgCl\(_2\): 1.05; NaHCO\(_3\): 11.9; NaH\(_2\)PO\(_4\): 0.42; glucose: 10.0.

Electrical stimulation was provided by a Grass-S4C or a Nihon-Kohden SEN-1101 stimulator. The stimuli were isolated from the ground by a stimulus isolation unit (SIU-4A or Nihon-Kohden SS101J) and delivered to the preparation via Ag-AgCl electrodes inserted into capillaries filled with Tyrode solution. The duration of each pulse was 1 msec and the stimulation frequency, 1 Hz.

Transmembrane potentials were recorded with glass microelectrodes, machine-pulled from Pyrex glass tubing, and filled with 3 M KCl. Suitable electrodes had electrical resistances of 10 to 30 M\(\Omega\) and tip potentials of less than 5 mV. The recording equipment consisted of a cathode follower connected to a direct coupled amplifier (Tektronix 3A 74 or M-701 microprobe system) and a dual beam oscilloscope (Tektronix RM 565 or Nihon-Kohden VC-9). The maximum rate of rise of action potential (\(V_{\text{max}}\)) was determined by electrical differentiation (RC-unit, 500 k\(\Omega\), 100 pF). Calibration by a sawtooth generator showed this apparatus to work linearly throughout the demanded range. In most of the experiments, the rising phase and the crest of this differentiated wave form were illuminated by an intensity modulator, similar to that described by Evans (26).

The recording was preceded by an equilibration period of at least one hour at the stimulation frequency of 1 Hz. The stimulus intensity was set at approximately 3 times the threshold voltage at this time. After 15 min of recording of a stable single cell activity, the stimulus frequency was changed in some preparations from 1 Hz to 0.5, 2, 3.1, 4 and 5 Hz (ascending order) and occasionally, further 4, 3.1, 2, 0.5 and 0.25 Hz (descending order) and finally to 1 Hz again at each time interval of approximately 3 min. This constitutes one series of the experiments on changes in stimulation frequency in control preparations. The Grass stimulator was triggered by a Tektronix type 162 waveform generator to provide these frequencies. After 30 min of recording of a single cell activity at 1 Hz of stimulation, the tissue chamber was perfused with a Tyrode solution containing propranolol hydrochloride (I.C.I. Pharmaceuticals) or N-propylajmaline bitartrate (NPA) (Fa. Giulini, West Germany). Concentrations used were 10\(^{-6}\), 2 \times 10\(^{-6}\) and 5 \times 10\(^{-6}\) g/ml (3.4, 6.8 and 16.9 \(\mu\)M) of propranolol and 2 \times 10\(^{-7}\), 5 \times 10\(^{-7}\) and 10\(^{-6}\) g/ml (0.39, 0.96 and 1.93 \(\mu\)M) of NPA. Each preparation was exposed to the lowest concentration and then successively to one or two of the higher concentrations of either drug at intervals of 1.2 to 1.5 hours. After more than 30 and 20 min of exposure to one concentration of propranolol and of NPA, respectively, the experiments on changes in stimulation frequency in ascending and descending orders were performed. Membrane parameter values (resting potential, \(V_{\text{max}}\), total amplitude and time to repolarization (90, 50 and 25\%)) were measured immediately before each frequency change. As there was no significant difference at least in the values of \(V_{\text{max}}\) and resting potential between the experiments in ascending order and those in descending order, the membrane parameter values in the descending order from 4 to 0.5 Hz were not included herein. In a majority of the preparations the microelectrode was maintained in the same cell before and after drug addition, and in a few preparations, it was dislodged during the
experiments. The experiments were continued by impalement of a nearby cell with the membrane parameter values similar to the previously recorded one. In the following sections, $V_{\text{max},1}$, $V_{\text{max},2}$, $V_{\text{max},3}$ or $V_{\text{max},4}$ represents the maximum rate of rise at 1 Hz or f Hz in control preparations (0) or drug-treated preparations (D) in this order.

RESULTS

Experiments on changes in frequency of stimulation

The most prominent effect of both propranolol and NPA was a dose-dependent depression of $V_{\text{max}}$. $V_{\text{max},i}$ immediately before the frequency change after drug addition relative to that before drug addition ($V_{\text{max},i}/V_{\text{max},i-1}$) was $0.83 \pm 0.04$ (N = 6), $0.71 \pm 0.08$ (N = 6) and $0.62 \pm 0.03$ (N = 5) at $10^{-6}$, $2 \times 10^{-6}$ and $5 \times 10^{-6}$ g/ml of propranolol and $0.79 \pm 0.03$ (N = 6) $0.65 \pm 0.04$ (N = 7) and $0.57 \pm 0.03$ (N = 5) at $2 \times 10^{-7}$, $5 \times 10^{-7}$ and $10^{-6}$ g/ml of NPA, respectively. Neither drug significantly affected the resting potential, total amplitude or time to repolarization (90, 50 and 25%) at this time.

Figs. 1, 2 and 3 show respectively representative action potentials recorded at various frequencies of stimulation in a control preparation and $10^{-6}$ g/ml propranolol- and $5 \times 10^{-7}$ g/ml NPA.

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**Fig. 1.** Action potentials recorded from guinea pig papillary muscle before drug application. Upper trace, action potentials. Lower trace, electrically differentiated action potentials. Stimulation rate, 1, 0.5, 2, 3.1, 4, 5 and 0.25 Hz in this order. Calibration at the lower right, 200 msec and 40 mV for action potentials and 200 V/sec for differentials. The flickering of the base line in the lower beam is an artifact arising from the intensity modulator (same in the following Figs.)

**Fig. 2.** Action potentials recorded from guinea pig papillary muscle exposed to $10^{-6}$ g/ml of propranolol for approx. 45 min. Calibration, see Fig. 1. C shows action potential and its differential at 1 Hz before propranolol.
g/ml NPA-treated preparations. Mean values of $V_{\text{max}}$ and resting potential, total amplitude and time to repolarization (25, 50 and 90%) are shown in the preparations treated with $10^{-6}$, $2 \times 10^{-6}$ and $5 \times 10^{-6}$ g/ml of propranolol and with $2 \times 10^{-7}$, $5 \times 10^{-7}$ and $10^{-6}$ g/ml of NPA in comparison with those in the control preparations in Figs. 4A and B and 5A and B. In the control preparations $V_{\text{max}}$ was slightly decreased by an increase, and was little affected by a decrease, in the frequency of stimulation. At 5 Hz of stimulation, the mean relative
change of $V_{\text{max}}$ to that at 1 Hz was 0.865 and at all other frequencies, it was between 0.9 and 1.1.

The shape of the curves relating $V_{\text{max}}$ to the frequency of stimulation became successively steeper in the control preparations, the preparations treated with low to high concentrations of NPA and those treated with low to high concentrations of propranolol, in this order.

Table 1 shows mean values of $V_{\text{max}0.1}/V_{\text{max}0.1}$ and $V_{\text{max}D.1}/V_{\text{max}D.1}$ at each concentration of both drugs. A relative change of $V_{\text{max}}$ at 1 Hz approximately 5 min after one series of the experiments to $V_{\text{max}}$ immediately before that was not significantly different from unity. This indicates that the effect of the drugs on $V_{\text{max}}$ remained in an almost steady state during one series of the experiments on frequency changes. $V_{\text{max}D.1}/V_{\text{max}D.1}$ was significantly different from $V_{\text{max}0.1}/V_{\text{max}0.1}$ at any frequency of stimulation in the presence of any concentration of propranolol and NPA. Some values at 0.25 and 0.5 Hz in the presence of NPA were recognized as exceptions.

As shown in this Table, the difference of the mean value of $V_{\text{max}D.1}/V_{\text{max}D.1}$ for $2 \times 10^{-7}$ g/ml of NPA versus $10^{-6}$ g/ml of propranolol, that for $5 \times 10^{-7}$ g/ml of NPA versus $2 \times 10^{-6}$ g/ml of propranolol, that for $10^{-6}$ g/ml of NPA versus $5 \times 10^{-6}$ g/ml of propranolol were all significant, with a few exceptions. There was no significant difference in $V_{\text{max}D.1}/V_{\text{max}0.1}$ between one concentration of NPA and the concentration, paired above, of propranolol.

Since $V_{\text{max}0.1}$ and $V_{\text{max}D.1}$ were not always measured in the same preparation, $V_{\text{max}D.1}/V_{\text{max}0.1}$ was calculated as $\frac{\langle V_{\text{max}0.1}/V_{\text{max}D.1}\rangle_{\text{A}} - \langle V_{\text{max}0.1}/V_{\text{max}D.1}\rangle_{\text{B}}}{\langle V_{\text{max}0.1}/V_{\text{max}D.1}\rangle_{\text{A}}}$.
TABLE 1. Relative changes of $V_{\text{max}}$ as affected by frequency change in control ($V_{\text{max}(0)} / V_{\text{max}(f)}$) and propranolol- and NPA-treated preparations ($V_{\text{max}(0)} / V_{\text{max}(f)}$).

<table>
<thead>
<tr>
<th>Frequency (Hz)</th>
<th>0.5</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>0.25</th>
<th>1(a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.023</td>
<td>0.954</td>
<td>0.920</td>
<td>0.907</td>
<td>0.865</td>
<td>0.599</td>
<td>0.988</td>
</tr>
<tr>
<td>10^{-6}</td>
<td>0.080</td>
<td>0.073</td>
<td>0.083</td>
<td>0.011</td>
<td>0.014</td>
<td>-0.013</td>
<td>±0.014</td>
</tr>
<tr>
<td>10^{-7}</td>
<td>1.210</td>
<td>0.776</td>
<td>0.646</td>
<td>0.587</td>
<td>0.481</td>
<td>1.316</td>
<td>0.968</td>
</tr>
<tr>
<td>10^{-8}</td>
<td>0.082</td>
<td>0.021</td>
<td>0.052</td>
<td>0.046</td>
<td>0.119</td>
<td>0.040</td>
<td>±0.056</td>
</tr>
</tbody>
</table>

Propranolol (g/ml)

<table>
<thead>
<tr>
<th>Frequency (Hz)</th>
<th>0.5</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>0.25</th>
<th>1(a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 x 10^{-7}</td>
<td>1.045</td>
<td>0.884</td>
<td>0.849</td>
<td>0.797</td>
<td>0.746</td>
<td>1.024</td>
<td>0.959</td>
</tr>
<tr>
<td>5 x 10^{-7}</td>
<td>0.017</td>
<td>0.020</td>
<td>-0.022</td>
<td>±0.026</td>
<td>±0.027</td>
<td>±0.042</td>
<td>±0.028</td>
</tr>
<tr>
<td>10^{-6}</td>
<td>1.079**</td>
<td>0.882**</td>
<td>0.825**</td>
<td>0.747**</td>
<td>0.647*</td>
<td>1.121**</td>
<td>0.956</td>
</tr>
<tr>
<td>5 x 10^{-6}</td>
<td>0.024</td>
<td>0.022</td>
<td>-0.023</td>
<td>±0.025</td>
<td>±0.028</td>
<td>±0.045</td>
<td>±0.021</td>
</tr>
<tr>
<td>10^{-5}</td>
<td>1.081</td>
<td>0.808**</td>
<td>0.738**</td>
<td>0.693**</td>
<td>0.543</td>
<td>1.102*</td>
<td>0.874</td>
</tr>
<tr>
<td></td>
<td>±0.034</td>
<td>±0.027</td>
<td>±0.049</td>
<td>±0.041</td>
<td>±0.047</td>
<td>±0.098</td>
<td>±0.070</td>
</tr>
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*P<0.05 **P<0.01 in the following test: $V_{\text{max}(0)} / V_{\text{max}(f)}$ in the presence of one concentration of NPA was compared with that in the presence of one concentration of propranolol ($2 \times 10^{-7}$ g/ml versus $10^{-6}$ g/ml, $5 \times 10^{-7}$ g/ml versus $2 \times 10^{-6}$ g/ml and $10^{-5}$ g/ml of the former versus $5 \times 10^{-6}$ g/ml of the latter, respectively). (a) approximately 5 min after switching back to 1 Hz at the end of one series of the experiments on changes in driving frequency. Number of preparations: shown in parentheses for control, 6 for $10^{-6}$ and $2 \times 10^{-6}$ g/ml propranolol and $2 \times 10^{-7}$ g/ml NPA, 7 for $5 \times 10^{-7}$ g/ml NPA and 5 for $5 \times 10^{-6}$ g/ml propranolol (except 5 Hz) and $10^{-5}$ g/ml NPA. In one preparation exposed to $5 \times 10^{-6}$ g/ml propranolol no 1:1 responses were elicited by the maximum stimulation feasible at 5 Hz.

where Av denotes the mean of the values in parentheses. ($V_{\text{max}(0)} / V_{\text{max}(f)}$)_{AV} are shown in the initial part of this Result section and ($V_{\text{max}(0)} / V_{\text{max}(f)}$)_{AV} and ($V_{\text{max}(0)} / V_{\text{max}(f)}$)_{AV}, in Table 1. These values were calculated according to the above formula and then subtracted from unity. The results are shown in Figs. 6A and B. The points represent assumed concentrations of the drug-receptor complex and the lines were drawn according to the formulation shown in Appendix (see also Discussion). These Figs. demonstrate clearly that propranolol in any concentration reduced $V_{\text{max}}$ more frequency-dependently than did NPA.

Total amplitude, time to repolarization (25, 50 and 90%) and resting potential reduced progressively with a rise in driving frequency in the drug-treated preparations in a similar way to those in the control preparations (Figs. 4A and B and 5A and B). However, in the preparations treated with $5 \times 10^{-6}$ g/ml of propranolol the reduction of total amplitude at 3.1 and 4 Hz and the shortening of time to repolarization (25 and 50%) at 0.25 Hz were more marked than those in the control preparations. These membrane parameter values recovered to the level prior to the series of experiments in approximately 5 minutes after being switched back to 1 Hz. An exception was a shortening of time to 50% repolarization...
FIG. 6. Effects of propranolol (A) and NPA (B) on $\dot{V}_{\text{max}}$ at various frequencies of stimulation in guinea pig papillary muscles (relative values to the maximal effect, corrected for changes of $V_{\text{max}}$ in control preparations). Same preparations as in Fig. 4. A: Curves were drawn from upper according to eq. (10) to fit the experimental mean values of $10^{-5} \text{ g/ml}$ (●) $(k_{D} \text{Jt}_{1} = 0.013$ (solid line) and $0.013-0.07e^{-5.0t}$ (dotted line)), those of $2 \times 10^{-6} \text{ g/ml}$ (△) $(k_{D} \text{Jt}_{1} = 0.027$ (solid line) and $0.027-0.02e^{-5.0t}$ (dotted line)) and those of $5 \times 10^{-6} \text{ g/ml}$ (▲) $(k_{D} \text{Jt}_{1} = 0.046$ (solid line)). $k_{2} = 0.1$ in all curves. B: Curves were drawn from upper according to eq. (10) to fit the experimental mean values of $2 \times 10^{-7} \text{ g/ml}$ (●) $(k_{D} \text{Jt}_{1} = 0.0023$ (solid line) and $0.0023-0.0022e^{-0.8t}$ (dotted line)), those of $5 \times 10^{-7} \text{ g/ml}$ (△) $(k_{D} \text{Jt}_{1} = 0.0046$ (solid line) and $0.0046-0.0045e^{-0.8t}$ (dotted lines)) and those of $10^{-6} \text{ g/ml}$ (▲) $(k_{D} \text{Jt}_{1} = 0.0062$ (solid line) and $0.0062-0.0058e^{-0.8t}$ (dotted line)). $k_{2} = 0.005$ in all curves. $t$ represents here the diastolic interval ($t_{1} - t_{f}$).

$P<0.05$ in the presence of $5 \times 10^{-5} \text{ g/ml}$ of propranolol.

Experiments on pauses in stimulation

The experiments on pauses in stimulation were carried out in preparations exposed to each concentration of propranolol. The driving stimuli were interrupted for 1 sec to 2 min in a random order at intervals of 30 sec to 5 min. The length of the interval depended on that of interruption. Depending on the length of pause, $V_{\text{max}}$ and total amplitude depressed under the influence of propranolol recovered toward the predrug level. The largest $\dot{V}_{\text{max}}$ (referred to as $\dot{V}_{\text{max,D,t}}$) and total amplitude after each pause of $t$ sec were observed in the first response. This was followed by a successive reduction to the steady-state prepause level. On the other hand, the largest $V_{\text{max}}$ and total amplitude in the first response occurred after pauses of more than 20 sec in the preparations exposed to any concentration of propranolol. The largest $\dot{V}_{\text{max}}$ in the first response usually appeared after a pause of the duration between 20 and 30 sec and is referred to as $\dot{V}_{\text{max,D,20}}$. This amounted to 0.75 to 1.1-fold as large as $V_{\text{max}}$ at 1 Hz before drug addition, when observed in the same cell.
During a pause, resting potential was changed by less than 3 mV, usually to the hyperpolarizing direction.

Fig. 7 shows representative experiments on pauses in stimulation in a preparation exposed to $2 \times 10^{-6}$ g/ml of propranolol. In these periods $V_{\text{max}}$ was already depressed by 30% of the predrug value.

Fig. 8 shows semilogarithmic plots of the difference between $V_{\text{max,D,t}}$ and $V_{\text{max,t}}$ against time length of pauses in representative experiments. These values were obtained during the periods in which the effect of the drug on $V_{\text{max,D,t}}$ was in an almost steady state. The data are represented approximately by linear lines. Rate constants i.e. reciprocals of time constant of recovery thus estimated were $0.081 \pm 0.005$ for $10^{-6}$ g/ml ($N=4$), $0.103 \pm 0.009$ for $2 \times 10^{-6}$ g/ml ($N=7$) and $0.116 \pm 0.014$ for $5 \times 10^{-6}$ g/ml ($N=4$) of propranolol. Though these constants tended to increase with the increase in concentration there was no significant difference between each two out of three.

**DISCUSSION**

The antiarrhythmic property of propranolol has been attributed to its depressant
action on \( V_{\text{max}} \) in cardiac tissues in addition to its \( \beta \)-blocking action (14, 17, 18). The depressant action of the drug on \( V_{\text{max}} \) was observed in the rabbit atrium (14, 16, 17), the guinea pig atrium and ventricle (6, 20, 22) and the canine Purkinje fiber and ventricle (15) at concentrations over 0.15 g/ml. Moreover, Pitt and Cox (16) in the rabbit atrium, Freeman and Turner (22) in the guinea pig atrium and Tritthardt et al. (6) in the papillary muscles of guinea pigs demonstrated \( V_{\text{max}} \) to be reduced more prominently at higher frequencies of stimulation than at lower ones. These findings were confirmed in the present experiments in the papillary muscles of guinea pigs. NPA was also reported to reduce \( V_{\text{max}} \) in the Purkinje fibers of sheep and calves at \( 5 \times 10^{-5} \) g/ml or more (23).

Resting potential progressively decreased with a rise in frequency to an extent much the same as seen in control, propranolol-treated and NPA-treated preparations.

After long exposure, the action potential duration was slightly shortened by the highest concentration of propranolol at the lower frequencies of stimulation. On the contrary, the duration was not affected by NPA. Several authors also demonstrated little effect of these concentration ranges of propranolol on action potential duration in the ventricular muscles of dogs (15, 19), guinea pigs (20) and humans (19). On the other hand, NPA at \( 5 \times 10^{-4} \) g/ml was reported to shorten the action potential duration in Purkinje fibers of sheep and calves (23). In the present experiments, \( V_{\text{max}} \) which was depressed under the influence of propranolol recovered toward the pre-drug level with the rate constants of 0.081 to 0.106 sec\(^{-1}\). Such time courses of recovery were shown to be relatively dose-independent for quinidine, ajmaline (11) in the canine or sheep Purkinje fibers and flurazepam (13) in the guinea pig papillary muscles. To determine the relationship between the frequency-dependent depression of \( V_{\text{max}} \) and the time-dependent recovery of the depression of \( V_{\text{max}} \), the author applied the kinetic model shown in the Appendix. Here a depression of \( V_{\text{max}} \) is assumed to be proportional to the concentration of the drug-receptor complex. The proportionality constant (the intrinsic activity (27)) was taken as unity with an expectation that the full occupation of the receptor would make \( V_{\text{max}} \) zero. As shown in the Appendix, the rate constant of recovery of the depression of \( V_{\text{max}} \) is equal to that of dissociation of the drug-receptor complex, \( k_2 \). Liebeswar et al. (11) showed that the recovery of the depression of \( V_{\text{max}} \) occurred only when the membrane potential was more negative than \(-60\) mV in voltage-clamped Purkinje fibers. In the present study, the dissociation is assumed to start at the time of \( 90\% \) repolarization, because the difference between the time of repolarization to \(-60\) mV and that to \( 90\% \) repolarization is considered to be within 10 msec and not to affect markedly the calculated results. Substitution of 0.1 as \( k_2 \) which is an approximate mean for all concentrations of propranolol and time to \( 90\% \) repolarization shown in Fig. 5A as \( t_f \) at \( f \) Hz (\( f = \frac{1}{t_f} \)) of stimulation \( (t_1 = 1, 4, 2, 0.5, 0.32, 0.25 \text{ and } 0.2) \) gives \( B \) in eq. (10).

A reasonable fit to the experimental data for \( 5 \times 10^{-6} \) g/ml of propranolol was obtained by using eq. (10) with a proper value of \( A \) under the condition of \( k_2 \) for \( k_2 \). This is shown as the lowest solid line in Fig. 6A (\( \ln A = k_2[t_1]_0 = -0.046 \)). However, for \( 10^{-6} \) and \( 2 \times 10^{-6} \) g/ml, theoretical curves which fitted well at the lower driving frequencies always fell off downward at the higher frequencies. In Fig. 6A, the upper two solid lines are the
ones that fitted relatively well to the experimental data for $10^{-6} \text{g/ml } (-\ln \Lambda = 0.013)$ and for $2 \times 10^{-6} \text{g/ml } (-\ln \Lambda = 0.027)$. In order to improve a fit for the two lower concentrations, correction terms which were exponential functions of diastolic intervals ($t_1-Jt$) were introduced here. With the values which were obtained by subtraction of these correction terms from the above values, i.e. $k_1[D]Jt_1 = 0.013 - 0.07 \exp \{-5.9(t_1-Jt)\}$ for $10^{-6} \text{g/ml}$ and $0.027-0.02 \exp \{-5(t_1-Jt)\}$ for $2 \times 10^{-6} \text{g/ml}$, better fits were now obtained as shown as the dotted lines in Fig. 6A.

By dividing $k_1[D]Jt_1$ at the infinite diastolic interval by the molar concentrations of propranolol in the bath fluid, $3.85 \times 10^3$, $3.99 \times 10^3$, and $2.72 \times 10^3 \text{M}^{-1}$ for $10^{-6}$, $2 \times 10^{-6}$, and $5 \times 10^{-6} \text{g/ml}$ respectively obtained as $k_1Jt_1$. It is not known at present whether the biophase for the action of propranolol is in the intracellular or in the extracellular space, but if we take into account the slow cellular accumulation of this drug which required more than 3 hours (28), it is possible that the concentrations of propranolol in the biophase, defined here as [D], may not have been completely equilibrated with that in the bath fluid. Accordingly, if enough time were allowed for equilibration, $k_1Jt_1$, would be expected to be more constant. $Jt_1$, the initial phase of action potential, may represent a time period in which the inward sodium current increases to its peak value. This may be an order of 1 msec or somewhat less (unpublished observation, 2). According to the Hodgkin and Huxley theory (25), the sodium conductance increases as a result of a rapid rise in the m-variable and a slow fall in the h-variable in this period. If propranolol exerted little effects on these variables, as was demonstrated in voltage-clamped frog atria (21), $Jt_1$ would remain constant under the influence of the drug. From the above considerations, the changes of $k_1[D]Jt_1$ with diastolic intervals might be ascribed to those of $k_1$, the rate of association of the drug-receptor complex, although the secondary change of $Jt_1$ cannot be ruled out.

It is well known that a sudden change in frequency of stimulation induces an increase in sodium ions and a decrease in potassium ions in the cells of cardiac tissue (29). Calcium ions also increase in the cell, presumably in the sarcotubular system (29). This is particularly interesting, because an increase in calcium ions in the bath solution is known to antagonize a depressant effect of procaine or lidocaine on $V_{\text{max}}$ in frog ganglion cells (30) and that of procaine on sodium conductance in lobster axons (31). Weidmann (3) demonstrated that the curve relating $V_{\text{max}}$ to membrane potential in Purkinje fibers was shifted to the hyperpolarizing direction in the presence of cocaine, quinidine or procainamide and to the depolarizing direction by an increase in external calcium concentrations. Thus, it is conceivable that calcium ions increasing in the biophase at higher frequencies of stimulation may have antagonized the depressant action of propranolol, particularly at lower concentrations of the drug. In the present analysis, diastolic intervals were adopted instead of stimulation intervals, since the former are assumed to be more closely related to the cellular redistribution of ions after excitation.

Another possibility is that $k_2$, as a function of the membrane potential, may increase to a comparable degree with $k_1[D]$ with excitation of the cell. Therefore, $k_2$ (referred to as $k_2'$ hereafter) in eq. (1) and hence in eq. (4) and in $A$ is no longer the same as $k_2$ in eq. (3) and
hence in eq. (5) and in B. Indeed, Courtney, when reporting his findings on voltage-clamped myelinated fibers (24) stated that the repetitive opening of the sodium channels by depolarizing pulses facilitated both the binding and removal of the drug molecules of GEA968, with the channels. Reasonable fits were obtained from eq. (9) on the basis of this assumption with parameter values, $k_1[D] + k_2' = 0.026$, $0.048$ and $0.060$ and $[R_i]/r = 1.5$, $1.3$ and $1.1$ for $10^{-6}$, $2 \times 10^{-6}$ and $5 \times 10^{-6}$ g/ml of propranolol, respectively (Fig. 9A). Dividing by molar concentration of propranolol, $[D]$, $k_1 J_t$ values are $5.09 \times 10^3$, $5.47 \times 10^3$ and $3.23 \times 10^3$ M$^{-1}$ for $10^{-6}$, $2 \times 10^{-6}$ and $5 \times 10^{-6}$ g/ml of the drug, respectively. When the value, $0.001$ sec, was given to $J_{t_1}$, $k_2'$ was estimated to be $8.7$, $11.2$ and $5.5$ for each of these concentrations. These values are fairly constant except for the highest concentration of propranolol.

The same analyses were also made on the frequency-dependent effect of NPA on $V_{\text{max}}$, though it is possible that $J_{t_1}$ could be altered by the drug through its effects on the h-variable. In fact, Heistracher and Pillat (23) demonstrated an irregular contour of shift of the curve relating $V_{\text{max}}$ to membrane potential to the right by the drug in Purkinje fibers. No systematic study was made on the time course of recovery of the depression of $V_{\text{max}}$ in the preparations treated with NPA, but only its slow time course of recovery was confirmed in the present experiments (Fig. 10). Since such a time course of recovery was shown to be relatively concentration-independent, as mentioned above, it was estimated from Heistracher’s experiments on the effect of $2 \times 10^{-6}$ g/ml of NPA (10). Thus a semilogarithmic plot of his data

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**Fig. 9.** Same plots of effects of propranolol (A) and NPA (B) on $V_{\text{max}}$ at various frequencies of stimulation as in Fig. 6. A: Dotted lines were drawn from upper according to eq. (9) to fit the experimental mean values of $10^{-6}$ g/ml (●) ($(k_1[D]+k_2')J_{t_1}=0.026$ and $[R_i]/r=1.5$), those of $2 \times 10^{-6}$ g/ml (△) ($(k_1[D]+k_2')J_{t_1}=0.048$ and $[R_i]/r=1.3$) and those of $5 \times 10^{-6}$ g/ml (▲) ($(k_1[D]+k_2')J_{t_1}=0.060$ and $[R_i]/r=1.1$). $k_2'=0.1$ in all curves. B: Dotted lines were drawn from upper according to eq. (9) to fit the experimental mean values of $2 \times 10^{-7}$ g/ml (●) ($(k_1[D]+k_2')J_{t_1}=0.013$ and $[R_i]/r=3.4$), those of $5 \times 10^{-7}$ g/ml (△) ($(k_1[D]+k_2')J_{t_1}=0.022$ and $[R_i]/r=2.3$) and those of $10^{-6}$ g/ml (▲) ($(k_1[D]+k_2')J_{t_1}=0.023$ and $[R_i]/r=1.75$).
yielded 0.005 sec$^{-1}$ as the value of $k_2$. The value was small enough in comparison with $(t_1 - t_d)$, and therefore, a different value of $k_2$ in the same order did not appreciably alter the calculated results in regard to fit to the experimental data.

With 0.005 as $k_2$, 1 as the intrinsic activity and time to 90% repolarization shown in Fig. 5A as $\Delta t$, $E_{D,t}/E_{m,t}$ values calculated according to eq. (10) were found to be relatively frequency-independent, as far as the same order of $A$ as that of propranolol was concerned. However, with such an $A$ value, $E_{D,t}/E_{m,t}$ values were much larger, and with smaller $A$ values they were more frequency-dependent than the experimental data. Hence the calculation according to eq. (10) did not yield any reasonable fits.

It is assumed here also that NPA may be antagonized by ions such as calcium regarding the effect on $V_{max}$ at high frequencies of stimulation. Curves shown in Fig. 6B were calculated according to eq. (10) on the basis of the assumption that the $k_1[D]t_1$ values were constant (solid lines: 0.0023, 0.0046 and 0.0062 for $2 \times 10^{-7}$, $5 \times 10^{-7}$ and $10^{-6}$ g/ml, respectively) and that they were exponential functions of diastolic intervals (dotted lines $0.0022 \exp \{ -0.8(t_1 - t_d) \}$ for $2 \times 10^{-7}$ g/ml, $0.0045 \exp \{ -0.84(t_1 - t_d) \}$ for $5 \times 10^{-7}$ g/ml and $0.0058 \exp \{ -0.8(t_1 - t_d) \}$ for $10^{-6}$ g/ml was each subtracted from the above constant). Such large correction factors required for reasonable fits do not seem to provide any support to this view.

On the other hand, calculation according to eq. (9) yielded fairly satisfactory fits to the data except for a few points at high frequencies of stimulation. Parameter values used were $(k_1[D] - k_2') \times t_1 = 0.013$, 0.022 and 0.023 and $[R_t]/r = 3.4$, 2.3 and 1.75 for $2 \times 10^{-7}$, $5 \times 10^{-7}$ and $10^{-6}$ g/ml of NPA, respectively. Dividing by the molar concentrations of NPA, $[D]$, $k_1$,$t_1 = 10.0 \times 10^3$, $10.0 \times 10^3$ and $6.9 \times 10^3$ and with 0.001 sec as $\Delta t$, $k_2' = 9.4$, 12.5 and 10.0 were obtained for each of the three concentrations of NPA, in this order. Again, the values are fairly constant in this model. Thus, the frequency-dependent effects of propranolol and NPA on $V_{max}$ were tentatively elucidated in the present model in terms of the rate constants of the drug molecules to bind and to leave from the receptor site, and further detailed studies on the voltage-dependence of these rate constants are in progress.

APPENDIX

Assume that both association and dissociation of the drug-receptor complex,
D + R ⇔ DR, occur at the initial phase of action potential (time interval, \(Jt_1\)), no reaction
occurs during maintenance of action potential to 90% repolarization level, \(Jt_2\) (\(Jt_1 \leq Jt_2\))
and only dissociation, during the rest of time, in a preparation exposed to a drug whose
concentration in the biophase is [D]. Stimulation frequency is \((1/t_1)\) Hz.

Then, at the time interval of \(Jt_1\) to \(Jt_1 + Jt_1\) (\(j=0, 1, 2, \ldots\))
\[
\frac{d[DR]}{dt} = k_1[D][R] - k_2[DR] \tag{1}
\]
at the time interval of \(Jt_1 + Jt_1\) to \(Jt_1 + Jt_2\) (\(Jt_1 = Jt_1 + Jt_2\))
\[
\frac{d[DR]}{dt} = 0 \tag{2}
\]
and at the time interval of \(Jt_1 + Jt_1\) to \((j + 1)Jt_1\)
\[
\frac{d[DR]}{dt} = -k_2[DR] \tag{3}
\]
where [DR] and [R] are respectively a concentration of the drug-receptor complex and that
of the receptor.

From eq. (1),
\[
[DR] = C \exp \left\{ -(k_1[D] + k_2)Jt_1 \right\} + r \tag{4}
\]
and from eq. (3)
\[
[DR] = C \exp(-k_2t_1) \tag{5}
\]
where \(C\) is the constant of integration and \(r = k_1[D][R]/(k_1[D] - k_2)\). Here \([R] = [R] + [DR]\) which represents the total receptor concentration.

[DR] can be taken as zero when \(t=0\) which is a time point after a sufficiently long
period of pause in stimulation. Substitution of this initial condition \(([DR]=0\) when \(t=0\))
in eq. (4)
\[
[DR]_{j=0} = r(1 - \exp \left\{ -(k_1[D] + k_2)Jt_1 \right\}) = r(1 - A) \tag{6}
\]
where \(A = \exp \left\{ -(k_1[D] + k_2)Jt_1 \right\}\). Here and now on \([DR]_{j=0} = [DR]\) holds between time
interval of \(Jt_1 + Jt_1\) to \(Jt_1 + Jt_2\) according to eq. (2)

Again substituting the above initial condition in eq. (3)
\[
[DR]_{j=1} = r(1 - A)\exp \left\{ -k_2(t_1 - Jt_1) \right\} = r(1 - A)B \tag{7}
\]
where \(B = \exp \left\{ -k_2(t_1 - Jt_1) \right\}\).

In the same way eqs. (1), (2) and (3) yield alternatively:
\[
[DR]_{j=1} = r(1 - A)(1 - AB) \tag{8}
\]
\[
[DR]_{j=2} = r(1 - A)(1 - 2AB) \tag{9}
\]
\[
[DR]_{j=3} = r(1 - A)(1 - 3AB - A^2B^2) \tag{10}
\]
In the steady state i.e. \(n \rightarrow \infty\)
\[
[DR]_{\infty} = rB(1 - A)/(1 - AB) \tag{11}
\]
and
\[
[DR]_{\infty + Jt_1} = r(1 - A)/(1 - AB) \tag{12}
\]
Now, if we assume that a depression of \(V_{\text{max}}\) is proportional to [DR] (\(\alpha\), a
proportionality constant, i.e. intrinsic activity (27)), an effect in the steady-state at the
frequency of stimulation of \(fHz\), \(E_{D, f}\), \((f=1/t_1)\) relative to the maximum effect, \(E_{m, f}\),
i.e. complete suppression of \( \dot{V}_{\text{max}} \) is from eq. (8)

\[
E_{D,\text{f}}/E_{m,\text{f}} = (\dot{V}_{\text{max}0,\text{f}} - \dot{V}_{\text{max}D,\text{f}})/\dot{V}_{\text{max}0,\text{f}} = \alpha[D\text{R}]_{\text{f}} + \beta [R\text{f}] - \alpha r(1-A)/(1-AB) \cdot \text{R}_{\text{f}}
\]

\[ \text{eq. (9)} \]

If \( k_{1[D]} \gg k_{2} \), then \( \rho = \exp\{-k_{1[D]}J_{1}\} \), \( r = [R] \) and \( E_{D,\text{f}}/E_{m,\text{f}} = \alpha(1-A)/(1-AB) \)

\[ \text{eq. (10)} \]

When the driving stimuli at 1 Hz are interrupted for \( t \) sec, in the first response after the pause:

\[
\dot{V}_{\text{max}0,\text{f}} - \dot{V}_{\text{max}D,\text{f}} = \alpha[D\text{R}]_{\text{f}} + \beta [R\text{f}] = \alpha r(1-A)(1+B'/A-BA)/(1-AB)
\]

\[ \text{eq. (11)} \]

where \( \dot{V}_{\text{max}0,\text{f}} - \dot{V}_{\text{max}D,\text{f}} \) is \( \dot{V}_{\text{max}} \) in this first response and \( B' = \exp\{-k_{2}(t-J_{1})\} \). On the other hand, \( \dot{V}_{\text{max}0,\text{f}} - \dot{V}_{\text{max}D,\text{f}} \) is proportional to \( [D\text{R}]_{\text{f}} \) in eq. (6) \( \dot{V}_{\text{max},\text{f}} \) is the largest \( \dot{V}_{\text{max}} \) in the first response after a sufficiently long period of a pause in stimulation when the recovery is at the maximum level and the difference of this value from eq. (11) is an exponential function of \( (t-J_{1}) \) with a time constant of \( 1/k_{2} \).

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