LOCAL ANESTHETIC ACTIVITY OF $\beta$-ADRENERGIC BLOCKING DRUGS IN THE CRAYFISH GIANT AXON, WITH REFERENCE TO CALCIUM ION

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Abstract—The actions of $\beta$-adrenergic blocking drugs, propranolol, pindolol, timolol, carteolol, sotalol and practolol, were examined regarding effects on crayfish giant axon in an attempt to determine the relationship among chemical structure and local anesthetic activity, and the interaction of these drugs with Ca$^{++}$. The activities of local anesthetics such as procaine and lidocaine served for comparisons. A conventional microelectrode technique was used to obtain the resting membrane and action potentials. All drugs except sotalol and practolol dose-dependently inhibited the $dV/dt$ and amplitude of the action potential with a slight decrease (less than 6 mV) in the resting membrane potential. The relative potencies of these drugs in the reduction of the $dV/dt$ were as follows: propranolol 13.3, pindolol 1.7, procaine 1.0, lidocaine 0.91, timolol 0.71 and carteolol 0.22. Sotalol and practolol had little activity. The chemical structure of the drugs for local anesthetic action was closely related to that of the lipophilic aromatic group; the most potent activity seen in the naphthyl group. Potentiation of the local anesthetic activity in low Ca$^{++}$ solution was obtained with pindolol, timolol, carteolol, sotalol and practolol. Reduction of the activity in high Ca$^{++}$ solution was observed with propranolol, pindolol, timolol, procaine and lidocaine. These results suggest that external Ca$^{++}$ competes with the local anesthetic action of the $\beta$-adrenergic blocking drugs, at the site of the action potential generating mechanism.

Fitzgerald (1, 2) classified $\beta$-adrenergic blocking drugs into five groups based on intrinsic sympathomimetic properties, local anesthetic activity and selectivity in different blockades of tissue $\beta$-receptors. According to this classification, the drugs in Groups I and II possess local anesthetic activity, while drugs in Groups III and IV have no such activity. Intrinsic sympathomimetic property is seen in drugs belonging to Group I and III and selectivity of action on different $\beta$-receptors is involved in the drugs in Group V. The presence or absence of local anesthetic activity of $\beta$-adrenergic blocking drugs is one of the important factors in the treatment of cardiac disorders.

The relationship between the chemical structure of local anesthetics and their local anesthetic activity has been well documented (3). Local anesthetics inhibit sodium conductance and to lesser extent potassium conductance (4, 5). However, one of the major factors to be determined is whether or not local anesthetics and $\beta$-adrenergic blocking drugs compete with Ca$^{++}$ for the membrane site where Ca$^{++}$ may play an important role in controlling a gating element. Recently, it has been demonstrated that sodium inactivation...
produced by procaine is reversed by adding Ca\textsuperscript{2+} as well as by prepulse and holding hyperpolarization (6-8). Thus, the present study was undertaken for the following purposes: 1) to examine the relationship between the chemical structure of \( \beta \)-adrenergic blocking drugs and their local anesthetic activity as compared to the activity of local anesthetics, procaine and lidocaine, of which considerable data are available, and 2) to determine whether \( \beta \)-adrenergic blocking drugs compete with Ca\textsuperscript{2+} for the site involved in local anesthetic action.

**MATERIALS AND METHODS**

The experiments were performed in vitro on the circumoesophageal giant axon of the crayfish *Procambarus clarkii*. The axon was excised and the surrounding sheath removed. The axon, with a diameter of 50–100 \( \mu \), was mounted in a chamber into which physiological solution was continuously perfused at the rate of 5 ml/min by a gravitated inlet and suction outlet. The bath volume was approx. 5 ml. Two glass capillary microelectrodes filled with 3 M KCl were inserted into the same axon with an inter-electrode distance of about 100 \( \mu \). One was used to record the membrane and action potentials, and the other to deliver the stimulating current. The stimulus, composed of a square wave pulse of 30 msec duration, was applied every 1.0 sec. Action potential was displayed on an oscilloscope (Nihon Kohden, VC-7) with a high impedance cathode follower and preamplifier (Nihon Kohden, MZ-3B). The rate of rise of action potential (dV/dt) was simultaneously recorded using a RC circuit with a time constant of 22 \( \mu \)sec for electrical differentiation. Resting membrane potential was continuously recorded on a rectigram (Nihon Kohden, PMP-3302). Reference electrodes consisting of an Ag-AgCl wire in an agar Ringer-filled glass tube were also placed in the chamber.

The physiological solution was of the following composition: NaCl, 205 mM; KCl, 5.4 mM; CaCl\textsubscript{2}, 13.5 mM; MgCl\textsubscript{2}, 2.6 mM; NaHCO\textsubscript{3}, 2.3 mM. When the Ca\textsuperscript{2+} concentration was reduced to 1/10 and 1/50 of the normal solution, Tris (hydroxymethyl) aminomethane was added to maintain constant the osmolality of the solution. In the high Ca\textsuperscript{2+} concentration, the osmolality was adjusted by decreasing the concentration of NaCl. All solutions were bubbled with a mixture of 95 % O\textsubscript{2} and 5 % CO\textsubscript{2} for about 30 min, and the final pH was adjusted to 7.4 by adding either 1N HCl or 1N NaOH. The solution with an alkaline pH (pH = 8.5) was maintained by addition of 1 N NaOH.

The following drugs were used: procaine hydrochloride (Fujisawa) pKa: 8.95; lidocaine hydrochloride (Fujisawa) pKa: 7.72; dl-propranolol hydrochloride (Sigma) pKa: 9.45; dl-pindolol maleate (Sandoz) pKa: 9.30; 1-timolol maleate (Nippon Merck-Banyu) pKa\textsubscript{1}: 6.3, pKa\textsubscript{2}: 8.8; dl-carteolol hydrochloride (Otsuka) pKa: 9.75; dl-sotalol hydrochloride (kindly provided by Dr. T. Ishizaki, Bristol-Banyu) pKa\textsubscript{1}: 10.00, pKa\textsubscript{2}: 8.46; and dl-practolol hydrochloride (Otsuka) pKa: 9.5. These drugs were dissolved in physiological solution and were continuously perfused into the bath.
RESULTS

1) Effects of procaine and lidocaine: Figure 1-a and -b demonstrate typical effects of the eight drugs tested on the action potential and the rate of rise (dV/dt) of the potential recorded from the crayfish giant axon. Local anesthetics, procaine and lidocaine, dose-dependently decreased the dV/dt and amplitude of the action potential, as shown in Table 1. Exposure of the axon to both drugs (10 mM) for 30 min resulted in over 95% inhibition of the dV/dt of the action potential. The resting membrane potential was slightly depolarized, however, the reduction was less than 7% of the control with a dose of 10 mM (Table 2). Procaine and lidocaine had no effect on the action potential threshold. Washing the preparation with physiological solution produced a recovery of the action potential in 30 min.

2) Effects of β-adrenergic blocking drugs: When 0.05 mM propranolol was included in the bath for 30 min, there were no alterations of the dV/dt and amplitude of the action

Fig. 1-a. Effects of procaine 10 mM (A), lidocaine 10 mM (B), propranolol 0.5 mM (C) and pindolol 5 mM (D) on the action potential of crayfish giant axon for 30 min, and the recovery 30 min after washing. Upper traces of each figure are the action potential, and lower traces the dV/dt of the potential. Calibration: 50 mV for the action potential and 500 V/sec for the dV/dt. Time scale: 1 msec.
potential (Table 1). An increase in the concentration of this drug produced a dose-dependent reduction of the action potential, and exposure of the axon to 0.5 mM propranolol completely blocked the action potential generating mechanism within 30 min. However, the resting membrane potential and action potential threshold were not significantly altered with doses up to 0.5 mM propranolol (Table 2). A complete restoration of the action potential was usually detected 30 min after washing the axon with physiological solution (Fig. 1-a).

Pindolol and timolol produced inhibitory effects on the dV/dt and amplitude of the action potential, but the potency of these drugs was much less than that obtained with propranolol. A complete and 89% inhibition of the dV/dt were observed 30 min after the treatment of the axon with 5 mM pindolol and 10 mM timolol, respectively (Table 1). The resting membrane potentials were depolarized by 6.2% and 7.2% after 30 min exposure of

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FIG. 1-b. Effects of timolol 10 mM (E), carteolol 20 mM (F), sotalol 20 mM (G) and practolol 20 mM (H) on the action potential of crayfish giant axon for 30 min, and the recovery 30 min after washing. Upper traces of each figure are the action potential, and lower traces the dV/dt of the potential. Calibration and time scale: See Fig. 1-a.
the preparation to 5 mM pindolol and 10 mM timolol, respectively (Table 2).

When 5–20 mM carteolol were included in the bath for 30 min, the dV/dt of the action potential was decreased by 40–60% (Table 1). However, complete blockade of the action potential was not observed.

Table 1. Changes in the rising rate, amplitude and threshold of action potential after 30 min exposure of the crayfish giant axon to procaine, lidocaine, propranolol, pindolol, timolol, carteolol, sotalol and practolol

| Drugs    | mM   | n | Rising rate (V/sec) | Amplitude (mV) | Threshold (mV) |
|----------|------|---|---------------------|----------------|----------------|
|          | (%)  |   | (% change)          |                |                |
| Procaine | 0    | 23| 609 ± 17*           | 94.3 ± 2.3     | 31.7 ± 1.1     |
|          | 1    | 6 | 387 ± 33            | 81.3 ± 7.1     | 32.2 ± 2.4     |
|          | 2.5  | 6 | 280 ± 39            | 60.0 ± 7.5     | 33.7 ± 1.8     |
|          | 5    | 6 | 136 ± 28            | 45.3 ± 9.7     |                |
|          | 10   | 5 | 27 ± 17             | 12.0 ± 7.4     |                |
|          | 0    | 20| 675 ± 25            | 91.4 ± 1.7     | 30.6 ± 1.3     |
|          | 1    | 4 | 500 ± 46            | 89.0 ± 5.2     | 37.5 ± 1.5     |
| Lidoaine | 2.5  | 6 | 352 ± 53            | 67.3 ± 3.0     | 29.0 ± 2.1     |
|          | 5    | 6 | 121 ± 54            | 30.7 ± 13.7    |                |
|          | 10   | 4 | 34 ± 95             | 13.5 ± 5.7     |                |
|          | 0    | 27| 663 ± 24            | 95.8 ± 2.1     | 30.5 ± 1.4     |
|          | 0.05 | 4 | 682 ± 74            | 91.3 ± 5.0     | 31.0 ± 4.2     |
| Propranolol | 0.1 | 7 | 461 ± 57            | 82.3 ± 6.4     | 30.6 ± 3.1     |
|          | 0.25 | 8 | 134 ± 53            | 35.6 ± 11.2    |                |
|          | 0.5  | 8 | 26 ± 20             | 15.4 ± 10.3    |                |
|          | 0    | 20| 555 ± 18            | 99.3 ± 2.3     | 25.2 ± 1.2     |
|          | 0.5  | 4 | 426 ± 31            | 96.0 ± 1.8     | 32.5 ± 3.2     |
| Pindolol | 1    | 5 | 327 ± 56            | 74.0 ± 6.7     | 27.2 ± 1.9     |
|          | 2.5  | 5 | 68 ± 26             | 34.8 ± 9.6     |                |
|          | 5    | 6 | 0 ± 100             | 0              |                |
|          | 0    | 21| 592 ± 15            | 99.5 ± 2.5     | 29.2 ± 1.8     |
|          | 1    | 4 | 450 ± 67            | 86.0 ± 10.0    | 30.5 ± 4.1     |
| Timolol  | 2.5  | 7 | 341 ± 32            | 75.7 ± 4.0     | 30.0 ± 3.0     |
|          | 5    | 5 | 213 ± 44            | 66.0 ± 5.0     | 33.6 ± 4.9     |
|          | 10   | 5 | 64 ± 39             | 28.6 ± 17.5    |                |
|          | 0    | 16| 568 ± 34            | 101.6 ± 3.2    | 27.1 ± 1.1     |
|          | 2.5  | 4 | 386 ± 37            | 81.8 ± 8.4     | 26.0 ± 2.5     |
| Carteolol| 5    | 4 | 335 ± 24            | 77.5 ± 9.1     | 27.3 ± 3.8     |
|          | 10   | 4 | 296 ± 46            | 76.3 ± 7.4     | 26.8 ± 1.9     |
|          | 20   | 4 | 227 ± 53            | 74.3 ± 7.0     |                |
|          | 0    | 8 | 631 ± 33            | 98.4 ± 3.2     | 27.4 ± 1.9     |
| Sotalol  | 10   | 4 | 557 ± 35            | 99.0 ± 5.3     | 28.8 ± 3.2     |
|          | 20   | 4 | 561 ± 40            | 88.0 ± 2.6     | 34.5 ± 8.8     |
| Practolol| 0    | 8 | 753 ± 10            | 100.8 ± 1.7    | 28.5 ± 0.6     |
|          | 10   | 4 | 756 ± 14            | 96.8 ± 2.6     | 29.5 ± 0.5     |
|          | 20   | 4 | 716 ± 27            | 99.3 ± 1.1     | 29.3 ± 2.0     |

n: Number of preparations.
*: Each value represents the mean ± standard error.
potential did not occur even with 20 mM carteolol, while the resting membrane potential was slightly (5.3%) depolarized. Sotalol and practolol up to 20 mM had no apparent effect on the action potential. The resting membrane potential and action potential threshold remained unaffected during treatment of sotalol and practolol (Table 2).

3) Comparative potencies of the drugs: Figure 2 is a Lineweaver-Burk plot of the inhibitory effect of the drugs tested on the dV/dt of the action potential. A similar value of 1/maximum effect was obtained with procaine, lidocaine, propranolol, pindolol and timolol. Carteolol showed a different value for 1/maximum effect from those of the other drugs. Figure 3 represents the chemical structure of the eight drugs tested, 50% inhibition value of dV/dt of the action potential and their potency calculated from the inhibition value. The 50% inhibition value of dV/dt was as follows: propranolol 0.15 mM, pindolol 1.2 mM, procaine 2.0 mM, lidocaine 2.2 mM, timolol 2.8 mM and carteolol 9.0 mM. Propranolol was the most potent in inhibiting the rate of rise, followed by pindolol >procaine >lidocaine >timolol >carteolol; while sotalol and practolol were devoid of the action.

4) Effects of low and high calcium concentration: When the axon was exposed to 1.35 mM and 0.27 mM Ca++ solution for 30 min, for which, concentrations were 1/10 and 1/50 of the normal physiological solution respectively, the resting membrane potential gradually depolarized in accordance with lowering Ca++ (Table 3). The dV/dt and amplitude of the action potential were unaltered in 1.35 mM Ca++ solution, but were significantly

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**TABLE 2. Changes in the resting membrane potential after 30 min exposure of the crayfish giant axon to procaine, lidocaine, propranolol, pindolol, timolol, carteolol, sotalol and practolol**

| Drugs      | mM  | n  | Resting membrane potential (mV) | (% change) |
|------------|-----|----|---------------------------------|------------|
| Procaine   | 0   | 4  | 81.0±1.0*                       | -6.8       |
|            | 10  | 4  | 75.5±1.7                        |            |
| Lidocaine  | 0   | 3  | 81.3±2.7                        |            |
|            | 10  | 3  | 76.7±2.9                        |            |
| Propranolol| 0   | 7  | 84.0±1.5                        |            |
|            | 0.5 | 7  | 79.4±1.5                        |            |
| Pindolol   | 0   | 3  | 86.7±1.8                        | -6.2       |
|            | 5   | 3  | 81.3±1.3                        |            |
| Timolol    | 0   | 4  | 80.3±2.1                        | -7.2       |
|            | 10  | 4  | 74.5±3.2                        |            |
| Carteolol  | 0   | 4  | 85.5±1.0                        | -5.3       |
|            | 20  | 4  | 81.0±1.3                        |            |
| Sotalol    | 0   | 4  | 85.5±1.5                        | -0.6       |
|            | 20  | 4  | 85.0±1.0                        |            |
| Practolol  | 0   | 4  | 86.5±1.5                        | 0          |
|            | 20  | 4  | 86.5±1.5                        |            |

n: Number of preparations.
*: Each value represents the mean±standard error.
(P<0.01) reduced in 0.27 mM Ca++ solution. High Ca++ concentration (67.5 mM) did not affect the resting membrane potential, but did increase the action potential threshold to 40.0 mV from 26.1 mV. The dV/dt and amplitude of the action potential was decreased in 67.5 mM Ca++ solution, probably due to a reduction in the sodium concentration (Table 3).

The inhibitory effects of the drugs on the dV/dt of the action potential in the axon exposed to low (1.35 mM) and high (67.5 mM) external Ca++ concentration were compared with effects in the case of normal Ca++ concentration (13.5 mM) in an attempt to determine whether or not the drugs interacted with Ca++. Decreasing the concentration of Ca++ had no effect on the activities of procaine, lidocaine and propranolol, rather potentiated the action of pindolol, timolol, carteolol, sotalol and practolol on the dV/dt of the action potential (Fig. 4), with no apparent enhancement of depolarizing effects on the resting membrane potential. A complete blockade of the action potential occurred 30 min after exposure to 1 mM pindolol, 2.5 timolol and 10 mM carteolol in the low Ca++ solution, while these same concentrations of the drugs in the normal Ca++ solution produced an inhibition of the dV/dt only up to 40-50%. In addition, 10 mM sotalol and 10 mM practolol produced

![Fig. 2. Lineweaver-Burk plots of the inhibition rate (% inhibition/min) of the dV/dt of action potential against doses (mM) of the drugs. ○: procaine, ■: lidocaine, △: propranolol, ▲: pindolol, □: timolol, and ■: carteolol.](image-url)
over 50% reduction of the dV/dt in the low Ca++ solution.

In contrast, with an increase in the concentration of Ca++ there was a reduction of the inhibitory effects of procaine, lidocaine, propranolol, pindolol and timolol on the dV/dt of action potential, while no reduction was observed with carteolol (Fig. 5).

5) Effects of alkaline pH on the action of carteolol: Since carteolol has a pKa of 9.75, the highest value in the eight drugs tested, only 0.44% of the drug exists as an uncharged form in pH 7.4 of the normal physiological solution, according to calculations using a
Henderson-Hasselbalch's equation. Therefore, the effect of carteolol was examined in alkaline pH 8.5, where the uncharged form was calculated to increase to 5.32%. Alterations of the action potential and resting membrane potential were not observed in the axon placed in alkaline pH 8.5 for 40 min. When the axon was exposed to pH 8.5 for 10 min and then to 20 mM carteolol in pH 8.5 for 30 min, there was no enhancement of the inhibitory effects on the action potential and resting membrane potential. The dV/dt of the action potential was decreased to 311 ± 27 V/sec (S.E., n = 3) from 720 ± 14 V/sec 30 min after exposure to 20 mM of carteolol in pH 8.5. This inhibitory effect (-57%) on the dV/dt was similar to that (-60%) obtained with 20 mM of carteolol in a solution of pH 7.4.

Fig. 4. Time course of % change in the dV/dt of action potential after exposure of the axon to the drugs in the normal and low calcium solution. A: procaine 2.5 mM, B: lidocaine 2.5 mM, C: propranolol 0.1 mM, D: pindolol 1 mM, E: timolol 2.5 mM, F: carteolol 10 mM, G: sotalol 10 mM, and H: practolol 10 mM. Approximately 50% inhibition of dV/dt was obtained with the concentration of each drug except sotalol and practolol in normal calcium solution in 30 min. ●—●: exposure in normal calcium (13.5 mM) solution, and ○—○: exposure in low calcium (1.35 mM) solution. ( ): Number of preparations.
FIG. 5. Time course of % change in the dV/dt of action potential after exposure of the axon to the drugs in normal and high calcium solution. A: procaine 10 mM, B: lidocaine 10 mM, C: propranolol 0.5 mM, D: pindolol 5 mM, E: timolol 10 mM, and F: carteolol 20 mM. Complete inhibition of dV/dt was obtained with the concentration of each drug except carteolol in normal calcium solution in 30 min. ● - ●: Exposure in normal calcium (13.5 mM) solution, and ○ - ○: exposure in high calcium (67.5 mM) solution. ( ): Number of preparations.

DISCUSSION

In the crayfish giant axon, propranolol was the most potent of all the drugs used in blocking the action potential generation, and sotalol and practolol had little local anesthetic action. Complete blockade of the action potential generating mechanism did not occur with carteolol even in a concentration as high as 20 mM. The order of local anesthetic activities of procaine and propranolol in the crayfish axon was similar to that obtained in the lobster axon (9). There was little decrease in the resting membrane potential within 6 mV during the application of higher doses of procaine, lidocaine, propranolol, pindolol, timolol and carteolol, while no changes in the resting membrane potential were observed during the treatment with sotalol and practolol in concentrations as high as 20 mM. A decrease in the resting membrane potential, as induced by the addition of KCl up to 6 mV produced only a 5% reduction of the dV/dt of the action potential (data not included). Therefore, the inhibition of dV/dt by the treatment of β-adrenergic blocking drugs such as propranolol etc. is attributed to a decrease in sodium conductance, but not to a decrease in the resting membrane potential.

Local anesthetics are composed of a hydrophilic amino group, an intermediate chain
and a lipophilic aromatic group (Fig. 3). It is generally accepted that the local anesthetic activity is mainly dependent upon pKa, distribution coefficient and lipid solubility of the drugs, since the drug enters the membrane in an uncharged form and acts on the inside and/or within the membrane as a charged form (7, 10–12). The pKa is mostly associated with the amino group, distribution coefficient with the intermediate chain and lipid solubility with the aromatic group (3). Since the β-adrenergic blocking drugs we examined possess these groups and are closely related to the structure of the local anesthetics, procaine and lidocaine, it is reasonable to take into account the factors mentioned above when comparing the local anesthetic potency of β-adrenergic blocking drugs. The pKa of drugs tested herein ranged from 7.72 (lidocaine) to 9.75 (carteolol). According to Henderson-Hasselbalch’s equation, the uncharged form of carteolol in physiological solution is 0.44%, this being the lowest of the drugs examined. In contrast, propranolol revealed potent local anesthetic activity regardless of the high value of pKa (9.45), the uncharged form being 0.88% in physiological solution (pH 7.4). Therefore, the effect of carteolol was tested in alkaline pH 8.5, where the amount of the uncharged form was estimated to increase to 5.32%, to determine whether or not the lack of potency of carteolol may be due to a small amount of the uncharged form (0.44%) in pH 7.4, as compared with propranolol. Carteolol, however, did not produce an enhancement of local anesthetic action on the axon placed in the alkaline pH 8.5. It is unlikely, therefore, that the differences in local anesthetic potency between propranolol and the other β-adrenergic blocking drugs are due to different abilities to penetrate the membrane. Furthermore, the local anesthetic activity is partly related to the distribution coefficient of the local anesthetics, which is mainly determined by the chemical structure of the intermediate chain (3). All β-adrenergic blocking drugs tested, except sotalol, have a similar structure in the intermediate chain (Fig. 3). Accordingly, it is suggested that differences in the local anesthetic potency between propranolol and the other β-adrenergic blocking drugs are attributed to differences in the structure in the aromatic group. It is pointed out that the amino group, which is responsible for lipid solubility, is one of the important sites bound to the membrane (3). In particular, the substitution of the phenyl radical of the aromatic ring is of great importance for local anesthetic action. This view is supported by the results obtained with sotalol and practolol, each having a long chain in the phenyl radical resulting in loss of the anesthetic activity. Thus, it could be concluded from the present results that the local anesthetic activity of β-adrenergic blocking drugs is closely related to the aromatic group and the most potent activity is obtained with the naphthyl group. The potency decreased with substitution of the aromatic group as follows: indol > 4-morpholino-1,2,5-thiadiazol > 3,4-dihydrocarbostyril > methane-sulfonanilide or acetanilide (Fig. 3). These results are in agreement with the findings of Hellembrecht et al. (13) who assessed the local anesthetic activity of β-adrenergic blocking drugs on extracellular action potential of frog sciatic nerve.

It is considered that Ca++ may be involved as a gate controlling element (14–16). However, it remains controversial whether or not Ca++ competes with local anesthetics on the site of action of the membrane. For instance, Hille (7), Blaustein and Goldman (16) and
Seeman et al. (17) concluded that Ca++ competes with procaine and lidocaine for the site involved in the gating. In contrast, Narahashi et al. (18) demonstrated that changing the concentration of Ca++ had no effect on the local anesthetic activity of procaine, as determined in internal perfusion experiments on the squid axon. The present study with β-adrenergic blocking drugs demonstrated that the local anesthetic activities of pindolol, timolol and carteolol were potentiated by decreasing the external Ca++ concentration and the activities of pindolol and timolol were reduced by increasing Ca++. These results suggest that Ca++ may compete with pindolol, timolol and carteolol for the binding site on the membrane, thereby these β-adrenergic blocking drugs produce moderate or weak local anesthetic action in the normal Ca++ concentration. In addition, sotalol and practolol in low Ca++ solution produced local anesthetic action on the axon, while they had little such action in normal Ca++ concentration. These results suggest that sotalol and practolol may bind the site which is related to blocking of the action potential. When the axon was exposed to procaine, lidocaine and propranolol, partial prevention was observed with an increase in levels of Ca++, while the potentiation of local anesthetic action was not obtained when the concentrations of Ca++ were lowered. Similar results were seen in the case of lobster axon exposed to procaine and propranolol, and a pronounced competition with Ca++ has been observed with pronethalol which had weaker local anesthetic activity than either procaine or propranolol (9). Our results are in line with the biochemical findings by Lee (19) that propranolol interacts with phospholipids. Thus it would seem apparent that external Ca++ competes with the local anesthetic action of β-adrenergic blocking drugs, at the site of the action potential generating mechanism.

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