A Carbamate-Type Cholinesterase Inhibitor 2-sec-Butylphenyl N-Methylcarbamate Insecticide Blocks L-Type Ca\(^{2+}\) Channel in Guinea Pig Ventricular Myocytes

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ABSTRACT—2-sec-Butylphenyl N-methylcarbamate (BPMC) is a carbamate-type cholinesterase (ChE) inhibitor with unique toxicological properties such as noncholinergic cardiovascular collapse. Effects of BPMC on L-type Ca\(^{2+}\) channel currents (I\(_{\text{CaL}}\)) were studied in isolated guinea pig ventricular myocytes using the whole-cell patch-clamp technique, since the examination of cardiovascular responses indicated its Ca\(^{2+}\) antagonistic action. BPMC induced bradycardic and hypotensive responses in vivo and inhibited contraction of isolated papillary muscles (IC\(_{50}\)/Gb\(_{4}\) = 1.3 × 10\(^{-4}\) M) in guinea pigs. BPMC produced reversible block of I\(_{\text{CaL}}\) in the concentration range of 10\(^{-4}\) – 10\(^{-3}\) M. At test potentials between -30 mV and +20 mV, BPMC at 3 × 10\(^{-4}\) M caused marked acceleration of decay rate of I\(_{\text{CaL}}\) with moderate reduction of peak I\(_{\text{CaL}}\) amplitude. BPMC (3 × 10\(^{-4}\) M) shifted the steady-state inactivation curve to the hyperpolarizing direction by 12.7 mV. Decay rate of Ba\(^{2+}\) currents (I\(_{\text{BaL}}\)) was also accelerated by BPMC. Fitting analysis of inactivation kinetics of I\(_{\text{BaL}}\) with a two-exponential equation revealed that BPMC accelerates the slow inactivation component. At concentrations for blocking peak I\(_{\text{BaL}}\) by ca. 30%, the inactivation kinetics of I\(_{\text{BaL}}\) were significantly accelerated by BPMC, but merely slightly accelerated by Ca\(^{2+}\) channel antagonists such as diltiazem, nifedipine, or verapamil. These results indicate that BPMC, in addition to the inhibition of ChE, blocks L-type Ca\(^{2+}\) channels by accelerating voltage-dependent inactivation.

Keywords: 2-sec-Butylphenyl N-methylcarbamate (BPMC), L-type Ca\(^{2+}\) channel, Inactivation, Non-cholinergic effect

Carbamate-type ChE inhibitors produce toxicological or pharmacological actions as a result of the synaptic accumulation of acetylcholine (ACh) in insects and mammals, including human beings (1). Among ChE inhibitors, 2-sec-butylphenyl N-methylcarbamate (BPMC) has been widely used as an insecticide in the agricultural field (2). We have reported that the compound produces unique toxicological properties in rabbits. That is, while injection of physostigmine, a typical ChE inhibitor, produces an atropine-sensitive hypertensive response followed by a cessation of spontaneous breathing, BPMC causes an atropine-insensitive reduction of blood pressure and death by cardiovascular collapse (3, 4). In addition, its toxicity is also characterized by a low degree of lethality compared to its anti-ChE activity (5). The hypotensive response to BPMC is attributed to a reduction of cardiac contractility and peripheral vascular resistance in vivo. BPMC also inhibits contraction of the isolated cardiac and aortic smooth muscles with similar magnitudes. In the isolated aorta, the inhibitory effects of BPMC on the high K\(^+\)-induced aortic contraction was accompanied by a decrease in cytosolic Ca\(^{2+}\) levels and reversed by the addition of an L-type Ca\(^{2+}\) channel agonist, Bay K 8644 (6). Based on these results, we hypothesized that BPMC may have an inhibitory effect on cardiac L-type Ca\(^{2+}\) channels.

L-type Ca\(^{2+}\) channels are major pathways for the entry of Ca\(^{2+}\) to cardiac and vascular smooth muscle cells, which is responsible for the initiation of myocardial contraction and the regulation of vascular smooth muscle tone. The Ca\(^{2+}\) channel shifts its gating state from the resting state to the open state on depolarization and then inactivates rapidly.
Inactivation of Ca$^{2+}$ channels is an important regulatory mechanism for intracellular Ca$^{2+}$ homeostasis, vascular smooth muscle tone (8), and action potential duration in cardiac myocytes (9). The inactivation is produced by distinct mechanisms such as voltage-dependent inactivation and Ca$^{2+}$-dependent inactivation (10). Although critical amino acids within L-type Ca$^{2+}$ channel $\alpha_{1C}$ subunit for the inactivation have been reported (11–13), the molecular mechanisms underlying inactivation of L-type Ca$^{2+}$ channels are not fully understood. Compounds that modulate the specific component of inactivation kinetics would be helpful as pharmacological tools for clarification of the regulatory mechanism of Ca$^{2+}$ channel gating.

In this study, we examined effects of BPMC on L-type Ca$^{2+}$ channel currents in isolated guinea pig ventricular myocytes. We found that BPMC blocks L-type Ca$^{2+}$ channels through acceleration of a voltage-dependent inactivation process.

MATERIALS AND METHODS

Recording of blood pressure

Male Hartley guinea pigs (300 – 500 g; Charles River Japan, Inc., Yokohama) were anesthetized with urethane (1.2 g/kg, i.p.). Blood pressure was monitored through polyethylene tubing inserted into the femoral artery via a pressure transducer (TP-200T; Nihon Kohden, Inc., Tokyo) and recorded with a polygraph (RM-6000, Nihon Kohden, Inc.). BPMC was intravenously injected through a polyethylene tubing inserted into the femoral vein. The experiments were conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacological Society and the Recommendations from the Declaration of Helsinki.

Measurement of papillary muscle contraction

The papillary muscle was rapidly isolated from guinea pigs anesthetized with sodium pentobarbital (50 mg/kg, i.p.). The isolated papillary muscle was placed in a Magnus tube filled with Tyrode solution (described below) at 37°C. Muscle tension preloaded with an initial passive tension of 1 g was recorded isometrically with a force-displacement transducer (TB-651T, Nihon Kohden, Inc.) connected to a polygraph (RMP-6000, Nihon Kohden, Inc.). Contraction of the papillary muscle was induced by an electrical stimulation (rectangular pulses, 2-Hz frequency, 5-ms duration, and supramaximal voltage).

Cell isolation

Single ventricular myocytes were enzymatically isolated from guinea pig heart according to the method described before (14, 15). The animals were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). The heart was isolated and perfused via a Langendorff apparatus at 37°C with the following solutions: Ca$^{2+}$-free Tyrode solution for 10 min, Tyrode solution containing collagenase (200 IU/ml; Yakult Co., Tokyo) for 8 – 10 min, and KB solution (described below) for 10 min. The isolated cells were stored in KB solution at 4°C. Experiments were performed within 12 h after cell isolation.

Electrophysiology

L-type Ca$^{2+}$ channel currents were recorded using the whole-cell configuration of the patch-clamp technique. Isolated cells were placed in an experimental bathing chamber (volume of 0.4 ml) mounted on the stage of an inverted microscope (IX70; Olympus Co., Tokyo) and perfused with the external solution at room temperature. Patch pipettes (2 – 5 MΩ) were pulled from borosilicate glass and polished with heat. Voltage-clamp protocols and data acquisition were performed with a patch-clamp amplifier (Axopatch 1-D or 200B; Axon Instruments, Inc., Foster City, CA, USA) driven by a computer using Clampex software (pCLAMP 6.0.3, Axon Instruments, Inc.) and interfaced with an A/D converter (DigiData 1200, Axon Instruments, Inc.). Recordings were started at least 5 min after rupture of the membrane patch to equilibrate the cytosol with the pipette solution. Current data were sampled at 2.5 kHz. Leak currents were electrically subtracted with the P/4 protocol of Clampex software. However, the analysis of inactivation kinetics was carried out without leak-subtraction to avoid the possible contamination of other time-dependent current components.

Solutions

Tyrode solution, containing: 135 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl$_2$, 10 mM MgCl$_2$, 5.8 mM glucose and 5 mM HEPES (pH adjusted to 7.4 with tris(hydroxymethyl)aminomethane), was used as a control solution (normal Tyrode solution). Nominally Ca$^{2+}$-free solution was prepared by omitting CaCl$_2$ from the normal Tyrode solution. KB solution contained the following components: 70 mM potassium glutamate, 20 mM KCl, 10 mM oxalic acid, 10 mM KH$_2$PO$_4$, 10 mM taurine, 5.8 mM glucose, 5 mM HEPES and 0.5 mM ethylene glycol bis(β-aminoethyl-ether)-N,N′,N″,N′′-tetraacetic acid (EGTA) (pH adjusted to 7.4 with KOH).

For recording Ca$^{2+}$ currents through L-type Ca$^{2+}$ channels ($I_{Ca(L)}$), Ca$^{2+}$ at 1.8 mM was used as a charge carrier. The inward rectifier K$^+$ channel currents were blocked by BaCl$_2$ at 0.2 mM. To record Ba$^{2+}$ currents through the L-type Ca$^{2+}$ channel ($I_{Ba(L)}$), the external solution was made by substituting equimolar BaCl$_2$ for CaCl$_2$ in the Tyrode solution. The patch pipettes were filled with the solution containing 110 mM CsCl, 30 mM tetraethylammonium chloride, 5 mM MgATP, 1 mM NaCl, 2 mM CaCl$_2$,
0.2 mM cyclic AMP, 10 mM 1,2-bis(2-aminophenoxy)-ethane-N,N,N',N'-tetraacetic acid (BAPTA) and 10 mM HEPES (pH adjusted to 7.2 with CsOH).

Drugs
BPMC or nifedipine was dissolved in dimethylsulfoxide and then mixed with external solution. The final concentration of dimethylsulfoxide was 0.1% or less so that dimethylsulfoxide itself did not affect L-type Ca\(^{2+}\) channel currents. Diltiazem or verapamil was dissolved in external solution. For intravenous injection, BPMC was cumulatively injected at doses of 0, 12.5, 50, and 200 \(\mu\)mol/kg in a volume of 1 ml/kg. BPMC was emulsified in physiological saline containing 1% Tween 80 with a Polytron homogenizer (Kinematica GmbH, Luzern, Switzerland) immediately before the experiment. BPMC, diltiazem hydrochloride, and verapamil hydrochloride were purchased from Wako Pure Chemical Industries, Ltd. (Osaka). Nifedipine was obtained from Sigma Chemical Co. (St. Louis, MO, USA).

Data analyses
Data analysis was performed with computer programs Clampfit (pCLAMP 6.0.3, Axon Instruments, Inc.) or Origin (ver. 4.0; Microcal Software, Inc., Northampton, MA, USA).

Steady-state inactivation curves were fitted to a Boltzmann’s equation with Origin,

\[
I(\%) = 100 \left(1 + \exp\left(\frac{V - V_{0.5}}{k}\right)\right),
\]

where \(V\) is the conditioning depolarization, \(V_{0.5}\) is the mid potential, and \(k\) is the slope factor of the curve.

The time course of decay of \(I_{\text{Ba}(L)}\) was fitted to a two-exponential equation with Clampfit,

\[
I = A_{\text{fast}}\exp(-t/\tau_{\text{fast}}) + A_{\text{slow}}\exp(-t/\tau_{\text{slow}}) + C,
\]

where \(t\) is the time, \(A_{\text{fast}}\) or \(A_{\text{slow}}\) is the amplitude of fast or slow component, respectively, and \(C\) is the amplitude of the noninactivating current component. \(\tau_{\text{fast}}\) or \(\tau_{\text{slow}}\) is the time constant of decay of fast or slow component, respectively. The relative \(A_{\text{fast}}\) or \(A_{\text{slow}}\) was calculated as \(A_{\text{fast}}/(A_{\text{fast}} + A_{\text{slow}} + C)\) or \(A_{\text{slow}}/(A_{\text{fast}} + A_{\text{slow}} + C)\), respectively.

All data are presented as the mean \(\pm\) S.E.M. Statistical significance was assessed by the paired or Student’s \(t\)-test (16).

RESULTS

Effects of BPMC on cardiovascular function

Prior to the electrophysiological experiments, effects of BPMC on cardiovascular functions were studied in guinea pigs. Intravenous injection of BPMC produced a dose-dependent decrease in blood pressure during the injection of 12.5 \(\mu\)mol/kg or more (Fig. 1). The depressor response persisted for 20 – 30 min at 50 \(\mu\)mol/kg. At this dose, heart rate also decreased during the injection. The injection of 200 \(\mu\)mol/kg caused a rapid and progressive decrease in blood pressure and heart rate, resulting in death within 10 min after the injection. BPMC also inhibited the electrically evoked contraction of isolated papillary muscles in a concentration-dependent manner with an IC\(_{50}\) value of \(1.3 \times 10^{-4}\) M (Fig. 2). These cardiovascular profiles of BPMC were in good agreement with our previous reports (3, 5).
Effects of BPMC on I_{Ca(L)}

I_{Ca(L)} was evoked by depolarizing test pulses (100-ms duration) from a holding potential of −50 mV to a test potential of −10 mV. On depolarization, I_{Ca(L)} reached to the peak amplitude within 5 ms and then gradually decayed with a substantial current left at the end of the test pulse (Fig. 3A). Application of BPMC at $3 \times 10^{-4}$ M suppressed I_{Ca(L)}, which was almost fully recovered by wash out. The block of I_{Ca(L)} by BPMC was characterized not only by a decrease in peak I_{Ca(L)}, but also by a marked acceleration of decay kinetics (Fig. 3B). While $3 \times 10^{-4}$ M BPMC inhibited the peak I_{Ca(L)} by ca. 40%, it completely inhibited I_{Ca(L)} at the end of the test pulse (pulse-end, Fig. 3C). Thus, BPMC inhibited pulse-end I_{Ca(L)} more potently than peak I_{Ca(L)}. IC_{50} values of BPMC for the block of peak I_{Ca(L)} and pulse-end I_{Ca(L)} were $5.2 \times 10^{-4}$ M and $1.3 \times 10^{-4}$ M, respectively.

Effects of BPMC on inactivation kinetics of I_{Ca(L)}

Effects of BPMC on the current-voltage relationships of I_{Ca(L)} were examined by giving test pulses up to +20 mV in 10 mV steps at 0.1 Hz from a holding potential of −50 mV. The results are summarized in Fig. 4. Application of $3 \times 10^{-4}$ M BPMC inhibited both the peak and pulse-end currents between −30 mV and +20 mV (Fig. 4: B and C). BPMC again inhibited the pulse-end currents more effectively than the peak currents, which was more prominent at more depolarized voltages.

Effects of BPMC on the steady-state inactivation curve of I_{Ca(L)} were examined by the double-pulse protocol (Fig. 5). Pre-pulses for 5 s from −60 mV to +10 mV in

![Figure 2](image)

**Fig. 2.** Effects of BPMC on contraction of the isolated papillary muscle in guinea pigs. Contraction was induced by electrical stimulation (rectangular pulses, 2-Hz frequency, 5-ms duration, and supramaximal voltage). The ordinate represents the percentage of muscle tension compared with the value measured before the application of BPMC. Each point and vertical bar represents the mean ± S.E.M. of 4 experiments.

![Figure 3](image)

**Fig. 3.** Effects of BPMC on I_{Ca(L)} in guinea pig ventricular myocytes. The currents were elicited by voltage-clamp steps (100-ms duration) from a holding potential of −50 mV to a test potential of −10 mV at 0.1 Hz. A: A typical set of current traces representing 4 experiments. Dotted lines indicate zero current level. B: Traces normalized to peak currents. C: Concentration-response curve. Open and closed circles represent peak and pulse-end currents, respectively. The ordinate represents the percentage of currents normalized to the value measured before the application of BPMC. Each point and vertical bar represents the mean ± S.E.M. of 3 or 4 experiments. Significantly different from peak currents, **P<0.01.
10 mV steps preceded 50-ms apart from the test pulses (0 mV, 100 ms). Peak currents were normalized to the maximal peak $I_{Ca(L)}$. Application of BPMC at $3 \times 10^{-4}$M shifted the voltage-dependence of the inactivation curve toward the hyperpolarizing direction. The $V_{0.5}$ value was decreased from $-25.7 \pm 2.3$ mV in the control to $-38.4 \pm 1.6$ mV in the presence of BPMC ($P<0.05$, $n=3$) without significant changes in the slope factor ($k$ for control and BPMC was $4.5 \pm 0.3$ mV and $3.8 \pm 0.2$ mV, respectively, $n=3$), indicating a parallel shift of the curve to the hyperpolarizing direction by $12.7$ mV. After washout of BPMC, the $V_{0.5}$ value recovered to $-30.5 \pm 1.4$ mV with a $k$ of $4.9 \pm 0.1$ mV ($n=3$).

**Effects of BPMC, diltiazem, nifedipine and verapamil on $I_{Ba(L)}$.**

Effects of BPMC on the inactivation kinetics of L-type $Ca^{2+}$ channel currents were compared with those of diltiazem, nifedipine, or verapamil. In order to analyze the kinetics of voltage-dependent inactivation, $Ba^{2+}$ was used as a charge carrier. After the block of $I_{Ba(L)}$ by each compound had reached the equilibrium state, $I_{Ba(L)}$ was measured by giving a test pulse from a holding potential of $-50$ mV to the test potential at $-10$ mV for 500 ms. Concentrations of drugs required for producing equivalent block of $I_{Ba(L)}$ to ca. 30%, with test pulses for 100 ms given at 0.1 Hz, were $3 \times 10^{-5}$ M for BPMC, $3 \times 10^{-6}$ M for dilt-
Diltiazem, 3x10^{-6} M  
Verapamil, 10^{-6} M

Fig. 6. Effects of BPMC, diltiazem, nifedipine, and verapamil on \( I_{\text{Ba(L)}} \). \( I_{\text{Ba(L)}} \) was elicited by a long test pulse (for 500 ms) from a holding potential of -50 mV to the test potential of -10 mV. After the control measurement, each drug was applied to confirm ca. 30% inhibition of the peak \( I_{\text{Ba(L)}} \) with test pulses for 100 ms given at 0.1 Hz. Then, the long test pulse was applied in the presence of the drug. Typical current traces representing 4 to 5 experiments are shown in the upper panel and normalized traces are shown in the lower panel. Each trace was obtained before (open circles) and during (closed circles) the application of the respective drugs. Dotted lines indicate zero current level.

**Table 1.** Effects of BPMC and Ca\(^{2+}\) channel blockers on inactivation kinetics of \( I_{\text{Ba(L)}} \)

| Drugs      | n  | Peak current (pA) | Pulse-end current (pA) | \( \tau_\text{fast} \) (ms) | \( \tau_\text{slow} \) (ms) | \( A_\text{fast} \) (%) | \( A_\text{slow} \) (%) | \( A_\text{fast}+A_\text{slow} \) (%) |
|------------|----|------------------|------------------------|-----------------|-----------------|----------------|----------------|-----------------------------|
| BPMC 5     | Before | -5870 ± 860     | -3096 ± 545            | 24 ± 4          | 478 ± 27        | 28 ± 2        | 30 ± 3         | 59 ± 3                      |
| 3x10^{-4} M | During | -4003 ± 609**   | -475 ± 56**            | 27 ± 3**        | 168 ± 8**       | 42 ± 2*       | 48 ± 1**       | 90 ± 2***                    |
| Ratio (%)  |       | 68 ± 3          | 16 ± 3                 | 115 ± 5         | 35 ± 2          |               |                |                             |
| Diltiazem 5 | Before | -5784 ± 523     | -3404 ± 357            | 30 ± 2          | 492 ± 62        | 29 ± 5        | 20 ± 3         | 49 ± 2                      |
| 3x10^{-4} M | During | -3924 ± 381***  | -2051 ± 208***         | 36 ± 2**        | 466 ± 57*       | 32 ± 5        | 25 ± 3**       | 56 ± 2**                    |
| Ratio (%)  |       | 68 ± 1          | 60 ± 1                 | 124 ± 4         | 95 ± 1          |               |                |                             |
| Nifedipine 5 | Before | -5519 ± 470     | -2869 ± 342            | 27 ± 2          | 452 ± 35        | 31 ± 3        | 26 ± 3         | 57 ± 3                      |
| 10^{-4} M  | During | -3843 ± 423***  | -1710 ± 210**          | 31 ± 3***       | 433 ± 26        | 34 ± 2*       | 32 ± 3**       | 66 ± 2**                    |
| Ratio (%)  |       | 69 ± 2          | 60 ± 2                 | 117 ± 4         | 96 ± 2          |               |                |                             |
| Verapamil 4 | Before | -6535 ± 308     | -3510 ± 149            | 31 ± 2          | 488 ± 33        | 29 ± 3        | 28 ± 2         | 56 ± 4                      |
| 10^{-4} M  | During | -4425 ± 243***  | -2140 ± 57**           | 37 ± 4**        | 439 ± 30**      | 32 ± 2*       | 31 ± 1         | 62 ± 2*                     |
| Ratio (%)  |       | 68 ± 1          | 61 ± 2                 | 119 ± 2         | 90 ± 1          |               |                |                             |

The values represent the mean ± S.E.M. n, number of cells; \( \tau_\text{fast} \), time constant of fast inactivation component; \( \tau_\text{slow} \), time constant of slow inactivation component; \( A_\text{fast} \), amplitude of fast inactivation component; \( A_\text{slow} \), amplitude of slow inactivation component. \( A_\text{fast} \) and \( A_\text{slow} \) are shown as relative amplitude (\( A_\text{fast} + A_\text{slow} + C = 100 \%) \), see Materials and Methods. Significantly different from the value measured before the application of each drug, *P<0.05, **P<0.01 and ***P<0.001.
DISCUSSION

The present study demonstrated that BPMC inhibits I_{Ca(L)} of the isolated guinea pig ventricular myocytes. We have reported that BPMC produces noncholinergic cardiovascular collapse, which is ascribed to the inhibition of cardiac and vascular smooth muscle contraction in rabbits (5). Based on our previous results with isolated rabbit aorta, we hypothesized that the suppression of cardiac and vascular smooth muscle contraction involves inhibition of Ca^{2+} entry through L-type Ca^{2+} channels (6). The present study clearly demonstrates that BPMC blocks L-type Ca^{2+} channels with marked acceleration of inactivation kinetics, which means that the amount of Ca^{2+} entry through L-type Ca^{2+} channels is significantly reduced by BPMC.

BPMC caused the acceleration of the decay rate of I_{Ca(L)} at lower concentrations than did the decrease in peak I_{Ca(L)}. Inactivation of L-type Ca^{2+} channels is a complex phenomenon involving both voltage- and Ca^{2+}-dependent mechanisms (7, 10). BPMC enhanced inactivation kinetics of both I_{Ca(L)} and I_{Ba(L)} in a similar manner, indicating that the Ca^{2+}-dependent inactivation process is excluded from the target of this compound. BPMC accelerated the slow inactivation component and its fraction of I_{Ba(L)}. The slow component mostly involves the voltage-dependent inactivation (17). Furthermore, BPMC shifted the steady-state inactivation curve to the hyperpolarizing direction. These results indicate that BPMC enhances the voltage-dependent inactivation process.

Decay rate of I_{Ca(L)} is also accelerated when Ca^{2+} antagonists, such as phenylalkylamines, bind to open channels during depolarization (18). On the other hand, benzothiazepines and dihydropyridines bind to the inactivated state and accelerate the gating shift from open state to the inactivated state (7, 19). In this study, the decay rate of the slow inactivation component of I_{Ba(L)} was slightly larger with verapamil than that by diltiazem or nifedipine. In spite of the same experimental procedure, BPMC accelerated the slow inactivation component much more significantly than did verapamil. The result indicates that BPMC accelerates the gating shift to the inactivated state much more significantly than do other Ca^{2+} antagonists. It has been reported that inhibitory effects of fendiline, L-type Ca^{2+} channel antagonist that preferentially binds to open channels, on cardiac I_{Ca(L)} and aortic contraction are enhanced by addition of Bay K 8644 that prolongs the open channel state (20). In contrast, inhibitory effects of BPMC on aortic contraction was antagonized by adding Bay K 8644 (6). When BPMC was applied without test pulses at a holding potential of ~90 mV for 3 min, the peak and end-pulse amplitudes of I_{Ca(L)} were suppressed to 71% and 33% of the control, respectively (data not shown). These results suggest that BPMC gains access to its binding site even when the Ca^{2+} channel is in the resting state. Thus BPMC may block Ca^{2+} channels in a mechanism distinct from that of open channel blockers.

The inactivation process of L-type Ca^{2+} channel is a critical determinant of the temporal precision of Ca^{2+} signals and serves to prevent long term rise in the intracellular Ca^{2+} levels. In cardiac excitation-contraction coupling, Ca^{2+} influx through L-type Ca^{2+} channels plays critical roles, one as the trigger of the Ca^{2+}-induced Ca^{2+} release process, and another as the source for SR Ca^{2+} load. Thus acceleration of the inactivation process by BPMC would result in the reduction of the amount of Ca^{2+} influx via L-type Ca^{2+} channels and SR Ca^{2+} load, which leads to suppression of cardiac contraction.

Pharmacological tools that selectively modify the voltage-dependent inactivation process would help to investigate the molecular basis for inactivation mechanisms. Recently, it has been reported that 2,3-butandione monoxime produces a block of I_{Ca(L)} associated with a marked acceleration of decay rate (21, 22). The acceleration of inactivation kinetics by this compound occurs in a manner similar to that of BPMC, although mechanisms underlying the block of I_{Ca(L)} remain to be determined (23, 24). However, the chemical structure of 2,3-butandione monoxide differs from that of BPMC, and tenfold higher concentration of this compound is required to inhibit I_{Ca(L)} (21). That is, IC_{50} values for 2,3-butandione monoxide and BPMC are 5.8 × 10^{-3} M and 5.2 × 10^{-4} M, respectively. Although further studies are required to investigate whether they produce the acceleration through a common mechanism, BPMC may become an important tool for investigating the inactivation process.

BPMC and Ca^{2+} channel antagonists increased r_{fast} with similar magnitudes. It is reported that the fast inactivation component is promoted by divalent cations going through Ca^{2+} channels (17). Since these compounds blocked the peak amplitude of I_{Ba(L)} to a similar degree, the increase in r_{fast} may be due to the reduction of peak I_{Ba(L)} amplitude. However, BPMC enhanced A_{fast} more significantly than other Ca^{2+} channel antagonists, which infers that BPMC modifies the fast inactivation component.

BPMC produces unique pharmacological action not only as a Ca^{2+} channel blocker but also as a ChE inhibitor. Intravenous injection of physostigmine, a typical ChE inhibitor, produces hypertension followed by respiratory arrest via inhibition of ChE activity in the medulla oblongata (4). Since the hypertension is inhibited by the microinjection of muscarinic blockers into the ventral medulla (25, 26), the response appears to be elicited by the accumulation of ACh in the medulla (27). Intravenous injection of BPMC also inhibited ChE activity in the medulla (4). However, BPMC elicited a dose-dependent decrease in blood pressure and cardiovascular collapse, resulting in death by
noncholinergic inhibition of cardiac and vascular smooth muscle contraction (3, 5). BPMC inhibited both cardiac contraction and IC_{50} in vitro to the same degree. Likewise, BPMC inhibits contraction of the isolated thoracic aorta of rabbits with an IC_{50} value of 1.8 \times 10^{-4} M (6), which was close to the IC_{50} value of the negative inotropic effect on papillary muscles of guinea pigs (Fig. 2). These results support the idea that BPMC suppresses cardiovascular tone through the block of peripheral L-type Ca^{2+} channels and thereby produces the cardiovascular collapse.

BPMC produces weaker respiratory arrest and a lower degree of lethality compared to its anti-ChE activity (5). Ca^{2+} antagonists such as verapamil have been reported to inhibit the activity of the vasomotor area of the medulla (28). Thus BPMC may have dual effects on vasomotor properties in cardiovascular function. The block of the L-type Ca^{2+} channel is involved in its unique toxicological properties in cardiovascular function. The block of the L-type Ca^{2+} channel was characterized by the marked acceleration of the voltage-dependent inactivation process. These results indicate that BPMC modifies the inactivation process of cardiac L-type Ca^{2+} channels through a novel mechanism distinct from those of Ca^{2+} channel antagonists.

In conclusion, the blocking action of BPMC on the L-type Ca^{2+} channel is involved in its unique toxicological properties in cardiovascular function. The block of the L-type Ca^{2+} channel was characterized by the marked acceleration of the voltage-dependent inactivation process. These results indicate that BPMC modifies the inactivation process of cardiac L-type Ca^{2+} channels through a novel mechanism distinct from those of Ca^{2+} channel antagonists.

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