Ca\(^{2+}\)-ANTAGONISTIC EFFECTS OF FLURAZEPAM, A BENZODIAZEPINE DERIVATIVE, ON ISOLATED GUINEA PIG LEFT ATRIA

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Abstract—Effects of flurazepam, a benzodiazepine derivative, on Ca\(^{2+}\)-induced cardio-stimulant contractile activity in normal Tyrode’s solution and Ca\(^{2+}\)-mediated contraction in K\(^+\)-rich (19–22 mM) Tyrode’s solution were investigated in electrically driven left atrial preparations isolated from guinea-pigs. In normal Tyrode’s solution, flurazepam (1 X 10\(^{-6}\), 1 X 10\(^{-5}\) and 1 X 10\(^{-4}\) M) noncompetitively shifted the dose-response curves for CaCl\(_2\) downwards. In K\(^+\) (19 mM)-rich Tyrode’s solution, flurazepam (3 X 10\(^{-5}\) M) decreased contractile amplitude time-dependently; and after addition of CaCl\(_2\) (final: 8 mM), contractile amplitude was increased time-dependently. In K\(^+\) (19 mM)-depolarized preparations, flurazepam (3 X 10\(^{-5}\) M) competitively shifted the dose-response curve for CaCl\(_2\) rightwards. In the K\(^+\) (22 mM)-depolarized isoproterenol (3.8 X 10\(^{-6}\) M)-treated atrial preparation, flurazepam (3 X 10\(^{-5}\) M) consistently suppressed contraction. Flurazepam (9 X 10\(^{-5}\) M) suppressed atrial contraction in tetrodotoxin (TTX) (2 X 10\(^{-5}\) M)-added normal Tyrode’s solution, and CaCl\(_2\) (final: 8 mM) partially restored the contraction. These results suggest that flurazepam inhibits transmembrane Ca\(^{2+}\)-influx into the atrial muscle cell.

The antiarrhythmic effect of benzodiazepines has been reported by some investigators. Gillis et al. (1) found an inhibition of ventricular arrhythmia by chlordiazepoxide in cats. They suspected a depression of certain regions of the central nervous system as the site of this inhibition. Nevins et al. (2) reported that diazepam was not markedly effective against ouabain-induced ventricular arrhythmia in dogs. This relatively low potency of diazepam was attributed to the propylene glycol used as diluent because this diluent had been shown to be arrhythmogenic in cats (3). Muir et al. (4) stated that in dogs with coronary artery occlusion, diazepam reduced the frequency of ventricular ectopic activities by an unknown mechanism. Wo (5) also found that diazepam was effective against BaCl\(_2\)-induced ventricular arrhythmia in rabbits. This antiarrhythmic effect was suspected to be produced partly by a peripherally mediated mechanism that was probably concerned with its local anesthetic effect. On the other hand, Tuganowski and Wolanski (6) investigated the influence of diazepam on isolated rabbit left atrium and observed the decrease in depolarization velocity, prolongation of repolarization, and increase in effective refractory period in action potential. Liebeswar (7) found that flurazepam reduced the maximum rising velocity of the action potential in guinea-pig papillary muscle preparations. This reduction was attributed to the blockade of the fast inward Na\(^+\)-current by flurazepam. In an in vitro study using rat cardiac muscle, Sugimoto et al. (8) observed that diazepam prolonged the
refractory period by ca. 90% in the left atrial muscle and by ca. 50% in the papillary muscle. The maximum driving frequency was decreased in the left atrium, but not in the papillary muscle. Based on these results, it was suggested that diazepam acts more potently on the atrium than on the ventricle.

It is well known that Ca$$^{2+}$$-influx plays an important role in arrhythmia. Leslie et al. (9) reported that chlordiazepoxide inhibited depolarization-induced Ca$$^{2+}$$-influx into brain synaptosomes in mice. We demonstrated inhibitory effects of diazepam and flurazepam on Ca$$^{2+}$$-influx into intestinal smooth muscle cells (10). However, there has been no report on the effects of benzodiazepines on Ca$$^{2+}$$-influx in heart.

The present study was undertaken to examine the effects of flurazepam, a water soluble benzodiazepine derivative, on Ca$$^{2+}$$-influx in atrial preparations isolated from guinea pigs.

Materials and Methods

Guinea-pigs of both sexes, weighing 350–500 g, were stunned. The heart was rapidly removed, and the left atrium was dissected free and suspended in an organ bath (50 ml) containing Tyrode’s solution. The bathing fluid was bubbled with a mixture of 95% O$$_{2}$$ and 5% CO$$_{2}$$ and was maintained at 33±0.5°C. These preparations were electrically stimulated at a constant rate (1 Hz) and constant duration (3 msec) with a square wave electronic bipolar stimulator (SEN-3201, Nihon Kohden Kogyo Co., Japan). The contractile response of the atrial preparation was recorded isometrically on an ink-writing oscillograph (RJG-4002, Nihon Kohden Kogyo Co., Japan). The contractile response of the atrial preparation was recorded isometrically on an ink-writing oscillograph (RJG-4002, Nihon Kohden Kogyo Co., Japan). The contractile response of the atrial preparation was recorded isometrically on an ink-writing oscillograph (RJG-4002, Nihon Kohden Kogyo Co., Japan). The contractile response of the atrial preparation was recorded isometrically on an ink-writing oscillograph (RJG-4002, Nihon Kohden Kogyo Co., Japan). The contractile response of the atrial preparation was recorded isometrically on an ink-writing oscillograph (RJG-4002, Nihon Kohden Kogyo Co., Japan). The contractile response of the atrial preparation was recorded isometrically on an ink-writing oscillograph (RJG-4002, Nihon Kohden Kogyo Co., Japan). The contractile response of the atrial preparation was recorded isometrically on an ink-writing oscillograph (RJG-4002, Nihon Kohden Kogyo Co., Japan). The contractile response of the atrial preparation was recorded isometrically on an ink-writing oscillograph (RJG-4002, Nihon Kohden Kogyo Co., Japan). The contractile response of the atrial preparation was recorded isometrically on an ink-writing oscillograph (RJG-4002, Nihon Kohden Kogyo Co., Japan). The contractile response of the atrial preparation was recorded isometrically on an ink-writing oscillograph (RJG-4002, Nihon Kohden Kogyo Co., Japan). The contractile response of the atrial preparation was recorded isometrically on an ink-writing oscillograph (RJG-4002, Nihon Kohden Kogyo Co., Japan). The contractile response of the atrial preparation was recorded isometrically on an ink-writing oscillograph (RJG-4002, Nihon Kohden Kogyo Co., Japan). The contractile response of the atrial preparation was recorded isometrically on an ink-writing oscillograph (RJG-4002, Nihon Kohden Kogyo Co., Japan). The contractile response of the atrial preparation was recorded isometrically on an ink-writing oscillograph (RJG-4002, Nihon Kohden Kogyo Co., Japan).

1) Studies on Ca$$^{2+}$$-induced contraction

The preparations were exposed to flurazepam (1×10^{-6}, 1×10^{-5} and 1×10^{-4} M) for 30 min in normal Tyrode’s solution. Ca$$^{2+}$$-induced contraction was elicited by cumulative addition of CaCl$$_{2}$$ (final concentration: 9 mM) to the bathing fluid. Maximum contraction of control preparations (in the presence of 9 mM CaCl$$_{2}$$) was taken as 100%. Results are expressed as the mean±S.E. The data obtained were statistically analyzed by Student’s t-test, and differences detected at P values less than 0.05 were considered significant.

2) Studies on Ca$$^{2+}$$-mediated contraction
   a) Studies on atria partially depolarized in K$$^{+}$$-rich (19 mM) Tyrode’s solution: The normal Tyrode’s solution was substituted with K$$^{+}$$-rich (19 mM) Tyrode’s solution, and contractile activities were continuously monitored. After the steady state was attained, flurazepam (3×10^{-5} M) was added. Ca$$^{2+}$$-mediated responses in the presence of flurazepam were checked by further addition of CaCl$$_{2}$$ (final: 8 mM).

b) Studies on dose-response curve: The preparations were exposed to flurazepam (3×10^{-5} M) for 30 min in K$$^{+}$$-rich (19 mM) Tyrode’s solution in a similar method to a). When the contraction was decreased, stimulus intensity was increased (threshold stimulation), and CaCl$$_{2}$$ (2–9 mM) was added cumulatively to the medium. Inhibitory effects of flurazepam (3×10^{-5} M) on the cardio-stimulant dose-response curve of CaCl$$_{2}$$ (2–9 mM) were observed.

3) Studies on isoproterenol-induced contractions

As described in method 2) b), atrial preparations were exposed to K$$^{+}$$-rich Tyrode’s solution containing 22 mM K$$^{+}$$ with an equimolar reduction of Na$$^{+}$$. By this treatment, the preparations were depolarized and became unexcitable within a few minutes. Then isoproterenol (3.8×10^{-6}M) was added.
to the bathing fluid to induce the contractions; and afterwards, flurazepam (3×10⁻⁶ M) was added.

4) Studies on tetrodotoxin (TTX)-treated atrial preparations

Atrial preparations were made as described in method 1). Tetrodotoxin (2×10⁻⁵ M) was added to the bathing fluid. After the contractions were decreased, flurazepam (3×10⁻⁵ and 9×10⁻⁵ M) was added; and afterwards, CaCl₂ (final: 8 mM) was added to the bathing fluid.

The drugs used were flurazepam hydrochloride (Roche), isoproteronol hydrochloride (Sigma) and tetrodotoxin (Sankyo).

Results

1) Effects of flurazepam on Ca²⁺-induced contraction

When CaCl₂ was cumulatively added (2-9 mM), concentration-dependent contractions developed. Flurazepam at 1×10⁻⁶, 1×10⁻⁵ and 1×10⁻⁴ M significantly inhibited 8 and 9 mM; 6, 8, 9 mM; and 6, 7, 8, 9 mM Ca²⁺-induced contractions, respectively (P<0.05, 0.01; 0.01, 0.05; 0.01, 0.01, 0.01 and 0.05, respectively). Flurazepam shifted non-competitively the Ca²⁺-induced contraction downwards (P<0.05, 0.01; 0.01, 0.05; 0.01, 0.01, 0.01 and 0.05, respectively) (Fig. 1).

2) Effects of flurazepam on Ca²⁺-mediated contraction

a) Effects on atrium partially depolarized in K⁺-rich (19 mM) Tyrode’s solution: As shown in Fig. 2, flurazepam (3×10⁻⁶ M) decreased time-dependently Ca²⁺-mediated contractile responses. The cardiodepressant effect of flurazepam was overcome by increasing the Ca²⁺ concentration from 1.8 to 8 mM in K⁺-rich Tyrode’s solution time-dependently (Fig. 2).

b) Effect on dose-response curve: When CaCl₂ was cumulatively added, dose-

![Fig. 1. Dose response curves for demonstration of the effects of flurazepam on Ca²⁺-induced contractions of electrically driven isolated guinea-pig left atrium. After cumulative addition of CaCl₂ to Tyrode’s solution, Ca²⁺-induced contractions were recorded. Points and vertical bars are the mean±S.E. The number of experiments are shown in parentheses. The mean maximum contraction (9 mM) was 1117±113.4 mg tension (N=10). *: P<0.05, **: P<0.01. Significantly different from control values.](image)

![Fig. 2. Effects of flurazepam (3×10⁻⁶ M) or Ca²⁺-mediated contractile activities of guinea-pig left atrium in K⁺-rich (19 mM) Tyrode’s solution. After addition of flurazepam (after 30 min), 8 mM CaCl₂ (final concentration) was added directly to the K⁺-rich Tyrode’s solution. Similar results were obtained in 25 experiments.](image)
dependent contractions developed. Flurazepam (3x10^-5 M) shifted competitively the Ca\(^{2+}\)-mediated contraction's dose-response curve rightwards and inhibited significantly both at CaCl\(_2\) concentrations of 3 and 5 mM (P<0.05 and 0.05, respectively) (Fig. 3).

3) Effects of flurazepam on isoproterenol-induced contraction

As shown in Fig. 4, contractions of electrically driven left atrium disappeared completely after depolarization in K\(^+\)-rich (22 mM) medium. Isoproterenol (3.8x10^-6 M) induced contraction in thus depolarized preparations. This isoproterenol-induced contraction was consistently blocked by flurazepam (3x10^-5 M). (Fig. 4).

4) Effects of flurazepam on tetrodotoxin-treated atria

After TTX (1x10^-5 M)-treatment for 10 min., amplitudes of contractions were decreased. Addition of flurazepam decreased the contractions presumably by the inhibition of Ca\(^{2+}\)-influx. After the contractions were...
decreased by TTX (2x10^{-5} M)-treatment, flurazepam (9x10^{-5} M) inhibited the con-
tractions. Addition of Ca^{2+} (final: 8 mM) partially restored these contractions. (Fig. 5).

Discussion
Flurazepam (1x10^{-5} and 1x10^{-4} M) noncompetitively inhibited Ca^{2+}-induced
contraction in normal Tyrode's solution. Inhibitory effects of flurazepam on Ca^{2+}-
induced contraction at high doses of Ca^{2+} are more prominent than those at low doses.
In general, competitive antagonism between Ca^{2+} and Ca^{2+}-antagonists in normal Tyrode's
solution is not seen in all cases.
Perhexiline is considered as a Ca^{2+}-antago-
nist and Vaughan Williams (11) reported that perhexiline (5.73 μM) reduced the slope as
well as the position of the dose-response curve of Ca^{2+} in isolated rabbit atria. In other
words, perhexiline elicited stronger inhibitory effects on high doses of Ca^{2+} than those at
low doses of Ca^{2+}. The response to flurazepam in the guinea-pig atrial preparation in our
experiments is somewhat similar to the response to perhexiline in the rabbit atrial
preparation. Therefore, similar Ca^{2+}-antagonistic mechanisms are involved in the
cases of flurazepam and perhexiline.
Ca^{2+}-induced contraction in normal
Tyrode's solution does not merely depend
upon extracellular Ca^{2+} concentration.
It was reported that in cardiac muscle, fast Na^{+}-current can be abolished by either
partial K^{+}-depolarization or tetrodotoxin.
In the present study, the cardiostimulant
dose-response curve of CaCl_2 in the presence
of flurazepam in the K^{+}-depolarized atrial
preparation shifted in parallel to the right
compared to the control curve, indicating that flurazepam is a competitive antagonist of
Ca^{2+}.
Fleckenstein (12) described that in
partially K^{+}-depolarized guinea-pig papillary
muscle preparation, Ca^{2+}-deficiency de-
creases the contractile force; and after readmission of Ca^{2+}, the contractile force is
restored time-dependently, and the Ca^{2+}-
antagonists (verapamil, D600, nifedipine,
diltiazem, perhexiline, fendiline) imitate the
effects of simple Ca^{2+} deficiency.
In our experiments, flurazepam decreases
the contractile amplitude time-dependently,
and the decreased contractions were restored
by raising the extracellular Ca^{2+} concentration
in both partially K^{+}-depolarized and TTX-
treated atrial preparations. These effects of
flurazepam imitate the effects of Ca^{2+}-
antagonists and simple Ca^{2+} deficiency.
In K^{+} (22 mM)-depolarized preparations, contractions are abolished, and then fast
Na^{+}-channels are thought to be inactivated.
Isoproterenol (3.8x10^{-6} M) restores con-
tractile activity. Then these contractions are
supposed to be mediated by slow inward Ca^{2+}
currents. Under this condition, the numbers
of Ca^{2+}-channels are suspected to be in-
creased (13). Therefore, the decrease in
contractile amplitude with flurazepam could
be elicited by the inhibition of Ca^{2+} influx
into atrial muscle cells.
All these findings indicate that flurazepam
inhibits the trans-membrane Ca^{2+}-influx in
isolated atrial muscle.
The exact site of action remains to be
clarified. Flurazepam inhibits 3H-nitrendi-
pine-binding to dog heart cell membrane
competitively (14), and so a possibility could
be that flurazepam acts on the Ca^{2+}-channel.
The clinical anti-arrhythmic effective dose
of flurazepam is much lower than the Ca^{2+}-
antagonistic dose of flurazepam in an isolated
atrial preparation. However flurazepam has
multiple sites of action. Namely, in vivo, all
the effects of flurazepam on CNS, coronary
artery, Na^{+}-influx and intracellular Ca^{2+}
binding sites contribute to the anti-arrhythmic
effect. Therefore, even a weak Ca^{2+}-
antagonistic effect may intensify the above-
mentioned effects, and then flurazepam could
probably evoke the anti-arrhythmic action in vivo.

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References