PHARMACOLOGICAL EFFECTS OF FLURAZEPAM AND DIAZEPAM ON ISOLATED CANINE ARTERIES

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Abstract—The effects of flurazepam and diazepam, benzodiazepine derivatives, on contractions (or contractures) induced by Ca++, K+ or norepinephrine were examined in the isolated canine coronary artery and thoracic aorta. Ca++-Induced contraction was evoked by cumulative addition of CaCl2 to Ca++-depleted K+-depolarizing solution; K+; and norepinephrine-induced contractions were evoked by cumulative addition of KCl and norepinephrine, respectively, to the medium. Flurazepam and diazepam (1×10^-5, 3×10^-5 and 1×10^-4 M for coronary artery; 3×10^-5 and 1×10^-4 M for thoracic aorta) shifted the dose-response curves for KCl downwards in a non-competitive manner, and shifted the dose-response curves for CaCl2 to the right in a competitive manner. Ca++-Induced contracture was inhibited completely by addition of flurazepam or diazepam (1×10^-4 M), and the inhibition was reversed dose-dependently by addition of CaCl2. Flurazepam and diazepam (3×10^-5 and 1×10^-4 M) shifted the dose-response curves for norepinephrine both rightwards and downwards in the thoracic aorta. These findings suggest that flurazepam and diazepam inhibit Ca++-influx into the cells (Ca++-antagonistic effect), causing relaxation and inhibition of K+-, Ca++-, or norepinephrine-induced contraction (or contracture) of the vascular smooth muscle.

Benzodiazepines are known to have anticonvulsant, antianxiety and muscle relaxant actions resulting from the action on the central nervous system. The mechanism of their central actions have been studied with a special references to benzodiazepine receptors in relation to the GABA system.

As peripheral actions, benzodiazepines produce a dose-dependent decrease in the systemic blood pressure and an increase in the coronary blood flow in dogs (1–3), cats (4) and humans (5). With regard to the action of benzodiazepines on the cardiovascular system, Abel et al. (2) suggested that diazepam may act as a specific ganglion-stimulant, causing sympathetic and cholinergic vasodilatation. On the other hand, Daniell (3) proposed that benzodiazepines may have a direct effect on the cardiovascular system. However, the concentrations of benzodiazepine which are necessary to produce peripheral action are approximately 10 times higher than those necessary to produce anticonvulsant (6) and muscle relaxant actions (7). Recently, some investigators have found that the benzodiazepine receptor in the brain has two binding sites with high and low affinity (8, 9). Furthermore, $K_l$ values of the heart and kidney in inhibiting $[^3H]$diazepam binding are approximately 10 times lower than that of the brain (10). Thus, the functional role of the peripheral benzodiazepine binding sites remains unknown. Benzodiazepine binding sites on vascular smooth muscle have not been reported. Moreover, whether any of these binding sites are related to the peripheral actions of benzodiazepines is not yet known.
This paper describes some studies on the mechanism of the vasodilating effect of benzodiazepines and their effects on contractions (or contractures) of isolated canine coronary artery and thoracic aorta induced by K\(^+\), Ca\(^{++}\) and norepinephrine.

**Materials and Methods**

Mongrel dogs of either sex, weighing 8 to 13 kg, were used. The dogs were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and sacrificed by bleeding from the common carotid arteries. The heart and thoracic aorta were excised. The distal portion of the ventral interventricular branch of the left coronary artery (0.3–0.7 mm) was removed. The vessels were cut helically at 45° to the longitudinal axis into strips (coronary artery: about 25×2 mm). Further the thoracic aorta was cut into strips longitudinally (about 30×2 mm). The helical strips were fixed vertically between hooks in an 50 ml organ bath containing nutrient solution. The response of preparations was recorded isometrically on an ink-writing oscillograph (SR-651, Watanabe Inst. Corp., Japan) through a force-displacement transducer (SB-1T-H, Nihon Kohden Kogyo C., Japan). The resting tension was adjusted to 1.5 g. The bathing solution was bubbled with a mixture of 95% O\(_2\) and 5% CO\(_2\) and was maintained at 37±0.5°C (11).

Before the start of experiments, the preparations were allowed to equilibrate in the bathing solution for 60 to 120 min. During the equilibration period, the solution was replaced every 15 to 20 min. The composition of the nutrient solution (Krebs-Henseleit solution) was as follows (mM): NaCl, 118; KCl, 4.96; CaCl\(_2\), 2.5; NaHCO\(_3\), 25; KH\(_2\)PO\(_4\), 1.18; MgSO\(_4\), 1.18; and glucose, 5.55.

1. **Studies on Ca\(^{++}\)-induced contraction:** Other bathing solutions were as follows: i) Ca\(^{++}\)-depleted solution, consisting of nutrient solution without 2.5 mM CaCl\(_2\); ii) for the coronary artery, Ca\(^{++}\)-depleted K\(^+\)-depolarizing solution, consisting of Ca\(^{++}\)-depleted solution in which all the NaCl and NaHCO\(_3\) were replaced by equimolar concentrations of KCl and KHCO\(_3\), respectively (12); for the thoracic aorta, Ca\(^{++}\)-depleted K\(^+\)-depolarizing solution, consisting of Ca\(^{++}\)-depleted solution in which NaCl and KCl were replaced by 42.69 and 80 mM, respectively (13). Ca\(^{++}\)-Induced contraction was elicited by cumulative addition of CaCl\(_2\) (1×10\(^{-5}\)–3×10\(^{-2}\) M) after the arterial strip had been suspended in Ca\(^{++}\)-depleted solution for 30 min (coronary artery) or 60 min (thoracic aorta) and in Ca\(^{++}\)-depleted K\(^+\)-depolarizing solution for an additional 10 min (coronary artery) or 15 min (thoracic aorta). The arterial strip was then returned to Ca\(^{++}\)-depleted solution. Flurazepam and diazepam were added 5 min before the addition of CaCl\(_2\).

Ca\(^{++}\)-Induced contracture in the thoracic aorta was elicited by the same method as Ca\(^{++}\)-induced contraction.

2. **Studies on contraction induced by K\(^+\) and norepinephrine:** Contractions were elicited by cumulative addition of KCl (coronary artery: 1–30 mM, thoracic aorta: 1–50 mM) and norepinephrine (1×10\(^{-8}\)–1×10\(^{-4}\) M) to normal Krebs-Henseleit solution (14). The arterial strip was then washed with Krebs-Henseleit solution. Flurazepam and diazepam were added 5 min before the addition of KCl and norepinephrine.

The drugs used were flurazepam hydrochloride (Roche), diazepam (Cercine®, Takeda Pharm.) and \(\beta\)-norepinephrine bitartrate (Sigma). They were added directly to the bathing solution.

Values of contraction are expressed as means±S.E. in mg, and the maximum control contraction induced by KCl, CaCl\(_2\) or norepinephrine was taken as 100%. The value of contraction expressed as mg or % in the presence of flurazepam or diazepam was compared with that of the respective control contraction in the absence of the drug. The
Student's *t*-test was used to determine statistical significance (unpaired test), and *P* values less than 0.05 were considered significant.

**Results**

1. **Effects on K⁺-induced contraction:** In the coronary artery, flurazepam and diazepam at $1 \times 10^{-5}$, $3 \times 10^{-5}$ and $1 \times 10^{-4}$ M caused a dose-dependent inhibition of K⁺-induced contractions, and shifted the dose-response curves for KCl downwards. Flurazepam at $3 \times 10^{-5}$ M and diazepam at $1 \times 10^{-5}$ M significantly reduced the K⁺-induced contractions at 30 mM KCl, and flurazepam at $1 \times 10^{-4}$ M and diazepam at $3 \times 10^{-5}$ and $1 \times 10^{-4}$ M also significantly reduced the K⁺-induced contractions (Fig. 1, A and B).

In the thoracic aorta, flurazepam and diazepam at $3 \times 10^{-5}$ and $1 \times 10^{-4}$ M caused a dose-dependent inhibition of the K⁺-induced contractions, and they shifted the dose-response curves for KCl downwards. Flurazepam at $1 \times 10^{-5}$ and $1 \times 10^{-4}$ M significantly reduced the K⁺-induced contraction at higher concentrations of KCl, and diazepam at $1 \times 10^{-4}$ M also significantly reduced the K⁺-induced contractions (Fig. 2, A and B).

2. **Effects on Ca⁺⁺-induced contraction:** In the coronary artery, flurazepam and diazepam at $1 \times 10^{-5}$, $3 \times 10^{-5}$ and $1 \times 10^{-4}$ M caused a dose-dependent inhibition of the Ca⁺⁺-induced contractions, and they produced a parallel displacement of the dose-response curves to the right. Flurazepam and diazepam at $3 \times 10^{-5}$ M significantly reduced the Ca⁺⁺-induced contractions at concentrations of $1 \times 10^{-3}$-$3 \times 10^{-3}$ M CaCl₂, and flurazepam and diazepam also significantly reduced the Ca⁺⁺-induced contractions (Fig. 3, A and B).

In the thoracic aorta, flurazepam and diazepam at $3 \times 10^{-5}$ and $1 \times 10^{-4}$ M caused a dose-dependent inhibition of the Ca⁺⁺-induced contractions, and they produced a
parallel displacement of the dose-response curves to the right. Flurazepam and diazepam at $3 \times 10^{-5}$ M significantly reduced the Ca$^{++}$-induced contractions at concentrations of $1 \times 10^{-3}$ to $3 \times 10^{-3}$ M CaCl$_2$, and flurazepam and diazepam at $1 \times 10^{-4}$ M also significantly reduced the Ca$^{++}$-induced contractions (Fig. 4, A and B). These results suggest that flurazepam and diazepam antagonize Ca$^{++}$-induced contractions in a competitive manner.

In the thoracic aorta, Ca$^{++}$-induced contracture with $3 \times 10^{-5}$ M CaCl$_2$ was inhibited completely by flurazepam and diazepam at $1 \times 10^{-4}$ M, and the inhibition was reversed dose-dependently by addition of CaCl$_2$ ($1 \times 10^{-2}$ and $3 \times 10^{-2}$ M) (Fig. 5, A and B).

3. Effects on norepinephrine-induced contraction: Flurazepam and diazepam at $3 \times 10^{-5}$ and $1 \times 10^{-4}$ M caused a dose-dependent inhibition of norepinephrine-induced contractions in the thoracic aorta and they shifted the dose-response curves both rightwards and downwards. Flurazepam and diazepam at $1 \times 10^{-4}$ M significantly reduced the norepinephrine-induced contractions (Fig. 6, A and B).

Discussion

In in vivo experiments in anaesthetized dogs, diazepam (1–4 mg/kg, i.v.) produces a transient (1–3 min) dose-dependent decrease in blood pressure (10–36 mmHg) and more sustained (2–15 min) increase in coronary blood flow of between 13 and 62 ml/min (15). Dose-dependent decrease in the blood pressure and increase in the coronary blood flow were also observed with flurazepam as well as diazepam at doses of 1–10 mg/kg (i.v.) in open-chest dogs (data not shown). Moreover in in vitro experiments, when
contraction of various isolated canine arteries had been induced by 25 mM KCl (11), both flurazepam and diazepam caused relaxation, and the ED50 values were between 1.9 x 10^{-5} M and 2.2 x 10^{-4} M (data not shown). These results suggest that flurazepam and diazepam, the benzodiazepine derivatives, have the ability to relax the isolated vascular smooth muscle. Moreover these concentrations of flurazepam and diazepam to relax isolated arteries are in good agreement with those used in in vivo experiments (16).

In the isolated smooth muscle, high K+ solution sustains cell membrane depolarization (17-19) and evokes muscle contraction (K+-induced contracture) resulting from increases in Ca^{2+} influx (19-22) through activation of Ca channels (opening or widening) (23) of the muscle cell membrane. In the vascular smooth muscle, K+-induced

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**Fig. 5.** Effects of excess CaCl₂ on relaxation of isolated canine thoracic aorta induced by flurazepam and diazepam. Contracture was induced with 3 x 10^{-3} M CaCl₂ in Ca²⁺-depleted K⁺-depolarizing solution. After the tension had reached a steady state, flurazepam (A) and diazepam (B) were added at concentration of 1 x 10^{-4} M. 1 x 10^{-2} or 3 x 10^{-2} M CaCl₂ were added after development of flurazepam- and diazepam-induced relaxation.

**Fig. 6.** Dose-response curves for effects of flurazepam (A) and diazepam (B) on norepinephrine-induced contraction of isolated canine thoracic aorta. Norepinephrine-induced contraction was elicited by cumulative addition of norepinephrine to the bathing medium. Flurazepam and diazepam were added 5 min before the addition of norepinephrine. Points and vertical bars are means±S.E. of values. Symbols indicate control values (●, N=8) and values with flurazepam and diazepam at 3 x 10^{-5} M (▲, N=4) and 1 x 10^{-4} M (■, N=4), respectively. The mean values for maximum contraction in the series with flurazepam and diazepam were 1143±62.9 and 1225±111.4 mg, respectively, (N=8). *P<0.05, **P<0.01: Significantly different from the control values.
contracture can be observed with an increase
in Ca\textsuperscript{++} influx into the cells (24). It has been
demonstrated that K\textsuperscript{+}-induced contraction
is evoked dose-dependently in isolated canine
basilar and coronary arteries (11, 14) and
rabbit thoracic aorta (25), and it was shown
in the present study that flurazepam and
diazepam (1 x 10\textsuperscript{-5}, 3 x 10\textsuperscript{-5} and 1 x 10\textsuperscript{-4} M)
shifted the dose-response curves for KCl
downwards in a non-competitive fashion in
the isolated coronary artery (Fig. 1, A and B)
and thoracic aorta (Fig. 2, A and B). Taking
these findings into consideration, it is sug-
gested that flurazepam and diazepam may
inhibit the transmembrane influx of Call.

Because cumulative addition of CaCl\textsubscript{2}-
induced contraction of isolated arteries in
Ca\textsuperscript{++}-depleted K\textsuperscript{+}-depolarizing solution (12,
13), it is possible that the Ca\textsuperscript{++}-induced
contraction depends on the influx of Ca\textsuperscript{++}
into vascular smooth muscle cells from the
external medium (13, 26). Ca\textsuperscript{++}-Induced
contraction was inhibited dose-dependently
by flurazepam or diazepam (parallel displace-
ment of the dose-response curves to the
right) in the coronary artery (Fig. 3, A and B)
and thoracic aorta (Fig. 4, A and B), and the
flurazepam or diazepam (1 x 10\textsuperscript{-4} M)-induced
relaxation of the thoracic aortic strips was
reversed dose-dependently by addition of
excess CaCl\textsubscript{2} (1 x 10\textsuperscript{-2} and 3 x 10\textsuperscript{-2} M) (Fig.
5, A and B). Accordingly, all these results
support the idea that flurazepam and diazepam
may antagonize transmembrane influx of Ca\textsuperscript{++},
as well as verapamil and diltiazem do (13).

On the other hand, in the norepinephrine
(high concentrations)-induced contraction of
rabbit aorta, there is no close relationship
between contraction and Ca\textsuperscript{++} influx as
detected by diltiazem (27). Flaim and Craven
(28) also demonstrated that both diltiazem
and verapamil inhibited \textsuperscript{45}Ca uptake from
the extracellular space during activation of the
receptor-operated Ca channel with nore-
epinephrine in rabbit aorta. Flurazepam and
diazepam (3 x 10\textsuperscript{-5} and 1 x 10\textsuperscript{-4} M) shifted
the dose-response curves for norepinephrine
rightwards and downwards (Fig. 6, A and B).
In the vascular smooth muscle, Ca\textsuperscript{++}-
antagonists which block inward Ca\textsuperscript{++} trans-
port are more effective in inhibiting high-K\textsuperscript{+}-
induced contractions than in inhibiting nor-
epinephrine-induced contractions (29, 30).
Flurazepam and diazepam were also more
effective in inhibiting K\textsuperscript{+} and Ca\textsuperscript{++}-induced
contractions than in inhibiting norepinephrine-induced contractions. Thus, the
action of flurazepam and diazepam in causing
inhibition of contraction (or contracture)
seem to be due to inhibition of transmembrane
influx of Ca\textsuperscript{++}, resembling the action of Ca\textsuperscript{++}-
antagonists such as verapamil and diltiazem
(13, 27).

In summary, these results indicate that
flurazepam and diazepam, benzodiazepine
derivatives, may exert a Ca\textsuperscript{++}-antagonistic
effect, resulting in relaxation and inhibition
of the K\textsuperscript{+-}, Ca\textsuperscript{++}-, or norepinephrine-induced
contraction (or contracture) of the vascular
smooth muscle.

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