Calcium Antagonistic Effects and the In Vitro Duration of Actions of KW-3049, a New 1,4-Dihydropyridine Derivative, in Isolated Canine Coronary Arteries

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Accepted February 3, 1988

Abstract—The newly-developed 1,4-dihydropyridine derivative KW-3049 was investigated for calcium antagonistic effects in isolated canine coronary arteries. KW-3049 relaxed the arteries contracted by KCl-depolarization with an IC50 of $7.4 \times 10^{-9}$ M, while the IC50 of nifedipine, verapamil and diltiazem were $9.1 \times 10^{-9}$ M, $1.7 \times 10^{-7}$ M and $3.1 \times 10^{-7}$ M, respectively. Comparison with negative inotropic activities examined in electrically-driven canine papillary muscles indicated that KW-3049 was more selective for vasorelaxing versus negative inotropic activities than nifedipine, verapamil and diltiazem. KW-3049 inhibited 45Ca-uptake induced by depolarization without affecting 45Ca-uptake in polarized arteries. Inhibitory effects of KW-3049 at $10^{-9}$ and $10^{-8}$ M on depolarization-induced contractions of arteries exhibited no recovery for up to 4 hr after washout of the tissues, whereas those of nifedipine, nitrendipine, verapamil and diltiazem at vasoinhibitory concentrations disappeared within 1 to 4 hr after washout. The uptake and efflux of [3H]-compounds of KW-3049, nitrendipine, verapamil and diltiazem were examined. The uptake of compound after 2 hr of incubation was the highest for nitrendipine. The efflux rate of KW-3049 was 1/10 or less than those of the other compounds examined. In summary, the present results in isolated coronary arteries demonstrate that KW-3049 is a potent, vasculoselective calcium antagonist whose effects persist long even after washout of tissues presumably due to its slow dissociation rate from arteries.

Calcium antagonists or calcium entry blockers in general have potent vasodilating actions, particularly characterized by the specific dilation of cardiac and cerebral vascular beds (1-3) and have widely been used for the treatment of cardiovascular disorders such as angina pectoris and hypertension (4-6). Although calcium antagonists share common pharmacological effects based on the inhibition of Ca2+-influx into cells (7-9), they differ slightly in their cardiac actions (10, 11).

KW-3049 is a newly-developed, 1,4-dihydropyridine derivative (Fig. 1) which is characterized by long-lasting antihypertensive and antianginal activities (12, 13). In the present study, we examined the vasculoselectivity of KW-3049 and the effects on stimulated Ca-entry into vascular smooth muscle of isolated canine coronary arteries. In addition, the uptake and efflux of [3H]-KW-3049 in coronary arteries were also investigated in relation to its long duration of Ca-

Fig. 1. Chemical structure of KW-3049, (±)-(R*)-2,6-dimethyl-4-((m-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylic acid (R*)-1-benzyl-3-piperidinyl ester, methyl ester hydrochloride.
antagonistic actions. The preliminary results have been presented at the 59th General Meeting of the Japanese Pharmacological Society (1986) (14).

Materials and Methods

Preparations and tension measurements:
Adult mongrel dogs of either sex weighing 5 to 25 kg were anesthetized by intravenous administration of pentobarbital sodium (30 mg/kg). After the thorax was opened, the heart was isolated and was promptly placed in oxygenated, physiological salt solution at room temperature. The circumflex branch of the left coronary artery was dissected from the connective tissue to make a spiral strip with a width of about 2 mm and a length of about 25 mm. The spiral strip was suspended in a 30-ml organ-bath filled with Krebs-Henseleit solution of the following composition: 117.6 mM NaCl, 5.4 mM KCl, 0.56 mM MgSO4, 2.5 mM CaCl2, 1.2 mM NaH2PO4, 24.99 mM NaHCO3 and 11.1 mM glucose. The solution was maintained at 37°C and ventilated with a gas-mixture of 95% O2 and 5% CO2. After the artery was equilibrated for 2 hr in the Krebs-Henseleit solution under the resting tension of 1.5 g, the changes in tension were isometrically determined by a force-displacement transducer (SB-1T, Nihon Kohden) (15) and recorded on a polygraph (Nihon Kohden) or a pen-recorder (Yokogawa).

Right ventricular papillary muscles of about 1 mm in diameter and 3 to 5 mm in length were prepared from the heart of dogs weighing 5 to 8 kg. The papillary muscle was suspended in an organ-bath filled with oxygenated, Krebs-Henseleit solution at 32°C and was electrically stimulated (3 msec, 1 Hz, supramaximal V) under a resting-tension of 1 g. The contractile responses were isometrically determined as mentioned above.

Pharmacological examinations: Contraction of coronary arteries were evoked by adding 500 μl of 3 M KCl solution to the organ bath. When the high K (final 55 mM K+) solution induced a sustained contraction, an antagonist was cumulatively added to the organ-bath to obtain a concentration-relaxation curve. The negative inotropic effect of an antagonist was examined in electrically-driven, ventricular papillary muscles. When contractile responses were stabilized, an antagonist was cumulatively applied to the preparation to obtain a concentration-inhibition curve. In the above two dose-response studies, each concentration of drugs was added when the tension became stable after the addition of the previous concentration of drugs.

The in vitro duration of actions of an antagonist was examined by the following procedure. When contractile responses following 5 min exposure to 55 mM KCl became stable, the artery was incubated in drug-containing solution for 30 min and the inhibitory effect of the drug on 55 mM KCl-induced contraction was determined. Thereafter, the artery was washed 3 times with drug-free Krebs-Henseleit solution. At intervals of 15 or 30 min, 55 mM KCl-induced contractions followed by washout of the tissues (3 times) were repeated until 4 hr after elimination of drug-containing solution. The preliminary studies showed that in untreated arteries, the stable contractions were elicited by 55 mM KCl throughout the experimental period.

45Ca-uptake and 45Ca-efflux: According to the method described by Karaki and Weiss (16), La3+-resistant 45Ca uptake of the coronary artery was studied. The coronary arteries were suspended in physiological salt solution (PSS: 125 mM NaCl, 2.7 mM KCl, 2.0 mM CaCl2, 1.2 mM MgCl2, 11 mM glucose, 23.8 mM Tris HCl, pH 7.4) ventilated with 100% O2 at 37°C. After the arteries were equilibrated for 1 hr under a resting-tension of 1 g, they were pretreated with drug or solvent (final 0.03% polyethylene glycol-400)-containing PSS for 30 min. Thereafter, the arteries were incubated in one of the following solutions containing 0.5 μCi/ml 45Ca (New England Nuclear) for 30 min: PSS+solvent, PSS+drug, high K PSS+solvent and high K PSS+drug. After washing in PSS, the arteries were transferred to an iso-osmotic La3+-substituted solution (80.8 mM LaCl3, 11 mM glucose, 6 mM Tris maleate, pH 6.8 at 0°C) ventilated with 100% O2. After washing in 2 tubes of the La3+-substituted solution for 30 min each, the arteries were gently blotted with filter paper, weighed
and solubilized in 1 ml of Soluene®-350 (Packard) for 3 hr at 50°C. The amount of $^{45}$Ca was measured by a liquid scintillation counter using a scintillation cocktail consisting of 4.0 g DOP (2,5-diphenyloxazole) 0.1 g POPOP (1,4-bis[2-(5-phenyloxazolyl)]benzene), 700 ml toluene and 300 ml methanol. The amount of $^{45}$Ca-uptake was calculated by the following formula:

$$\text{dpm in muscle} \times \frac{\text{mole Ca/l medium}}{\text{wet wt. (kg)} \times \text{dpm/I medium}}$$

$^{45}$Ca efflux from the coronary artery was studied according to the method described by Church and Zsoter (17). After a pair of coronary arteries were prepared from the same dog, one was used as a control and the other was treated with KW-3049 ($10^{-7}$ M). Following the equilibrium in normal Krebs-Henseleit solution for 60 min, each artery was exposed to a radioactive loading solution containing 6 $\mu$Ci/ml $^{45}$Ca in Krebs-Henseleit solution for 90 min. Thereafter, the artery which was loaded with $^{45}$Ca was transferred successively to a series of 12 tubes filled with 5 ml of Ca-free Krebs-Henseleit solution including solvent (final 0.03% polyethylene-glycol-400) or KW-3049 ($10^{-7}$ M) at intervals of 5 or 10 min. At the end of the collection period, the artery was digested with Soluene®-350, and the residual $^{45}$Ca was counted. For determining the amount of $^{45}$Ca efflux into Ca-free Krebs-Henseleit solution, aliquots of 1 ml were taken from each efflux tube, and the amount of $^{45}$Ca was measured by a liquid scintillation counter using Bray’s solution which consisted of 4.0 g DOP, 60 g naphthalene, 100 ml methanol, 20 ml ethyleneglycol and 880 ml 1,4-dioxane.

Uptake and efflux of $[^3H]$-antagonists:

With a slight modification of the method described by Mras and Sperelakis (18), the uptake and efflux of calcium antagonists were investigated in the coronary arteries. The $[^3H]$-labeled antagonists presently examined include $[^3H]$-KW-3049 and commercially available $[^3H]$-compounds of nitrendipine, verapamil and diltiazem (New England Nuclear).

The coronary arteries were equilibrated in Krebs-Henseleit solution bubbled with 95% $O_2$ and 5% $CO_2$ at 37°C for 60 to 90 min under a resting tension of 1 g. Thereafter, the arteries were incubated in loading solution containing one of the calcium antagonists at the submaximal concentrations which relax K-depolarized arteries (KW-3049, $10^{-7}$ M; nitrendipine, $10^{-7}$ M; verapamil $2 \times 10^{-6}$ M; diltiazem, $2 \times 10^{-6}$ M) and corresponding 0.5 $\mu$Ci/ml of $[^3H]$-compound. After 2 hr of $[^3H]$-loading, the arteries were washed for 30 sec in Krebs-Henseleit solution, gently blotted with filter paper and weighed. The $[^3H]$-loaded arteries were solubilized in 1 ml of Soluene®-350, and the amount of $^3H$ was measured.

The tissue : medium (T/M) ratio was obtained by dividing the amount of drug taken up per kg wet weight of tissue by the concentration of drug per liter of incubation medium.

For efflux experiments, the arteries were incubated in a loading solution containing one of $10^{-7}$ M of KW-3049, $10^{-7}$ M of nitrendipine, $2 \times 10^{-6}$ M of verapamil or $2 \times 10^{-6}$ M of diltiazem plus trace amounts of the corresponding $[^3H]$-compounds (1 $\mu$Ci/ml) for 2 hr. The artery which was loaded with $[^3H]$-antagonists was transferred to a series of 11 tubes filled with 6 ml of Krebs-Henseleit solution at fixed intervals over 90 min. At the end of the efflux period, the artery was digested with Soluene®-350 and the residual $^3H$ was counted. The amount of $^3H$ efflux into Krebs-Henseleit solution was determined as previously described in the experiment of $^{45}$Ca-efflux. The data obtained were expressed either as desaturation curves or as rate coefficient curves.

Drugs used: KW-3049 (hydrochloride), nitrendipine and nisoldipine were synthesized in our laboratories. The other drugs used were nifedipine (Bayer), verapamil (hydrochloride, Sigma) and diltiazem (hydrochloride, Tanabe...
Pharmaceuticals). The dihydropyridine calcium antagonists such as KW-3049, nifedipine, and nisoldipine were dissolved in 10% polyethylene glycol-400, and verapamil and diltiazem were dissolved in distilled water. In the experiments of the present study, the concentration of polyethylene glycol-400 in the physiological salt solution reached a maximum of 0.03%, and the effects of solvents were negligible.

Statistics: Values are expressed as means±S.E. Determination of significant difference for 45Ca-uptake values was carried out by analysis of variance using Duncan’s test for individual comparisons.

Results

Vasodilating and negative inotropic effects: When the coronary arteries were treated with 55 mM KCl, the developed tension was 1.37±0.11 g (n=32). KW-3049, nifedipine, verapamil and diltiazem, which were cumulatively applied to the organ-bath, relaxed the arteries in concentration-dependent manners. The relaxing action of KW-3049 was similar to or slightly stronger in activity than that of nifedipine and 20 to 40 times as potent as those of verapamil and diltiazem, respectively. Relaxing actions evoked by nifedipine, verapamil and diltiazem occurred soon after the application of drugs, while that evoked by KW-3049 occurred slowly and reached a peak level at 60 to 120 min after the drug application.

Contractile force of electrically stimulated, ventricular papillary muscles was 0.84±0.09 g (n=24). KW-3049, nifedipine, verapamil and diltiazem, when applied cumulatively, induced concentration-dependent falls of contractile force. The cardioinhibitory action of KW-3049 was less potent than those of nifedipine and verapamil and almost equipotent to that of diltiazem. As was the case in the relaxation of coronary arteries, the inhibitory effect of KW-3049 on papillary muscle appeared more gradually than those of the other antagonists.

Comparison of the ratios of relaxing potency of KCl-depolarized arteries and negative inotropic potency in papillary muscles indicates that the selectivity for coronary arteries is the highest for KW-3049, followed by nifedipine, diltiazem and verapamil (Table 1).

45Ca-uptake and 45Ca-efflux: KW-3049 at 10^-7 M, nifedipine at 10^-7 M, verapamil at 10^-6 M and diltiazem at 10^-6 M had no effect on 45Ca-uptake into polarized, canine coronary arteries (Table 2).

45Ca-uptake was 124.4±8.4 moles/kg (n=28) in the control group (polarized arteries) and 207.6±15.0 moles/kg (n=26) in the 55 mM KCl (high K) treated group. Pretreatment with KW-3049 at 10^-9 to 10^-7 M for 30 min inhibited concentration-dependently the 45Ca-uptake following exposure to high K solution (Fig. 2). Nifedipine at 10^-7 M, verapamil at 10^-6 M and diltiazem at 10^-6 M also significantly inhibited the increase in 45Ca-uptake by high K.

Figure 3 shows the effect of KW-3049 on

| Table 1. Comparison of the relaxing actions on the canine coronary arteries contracted by 55 mM KCl with the inhibitory actions on contractile force of canine ventricular papillary muscles |
|---------------------------------|-----------------|-----------------|------------------|
| **Compound**                   | **Coronary artery** IC50 (M) (1) | **Papillary muscle** IC50 (M) (2) | **Ratio** (2)/(1) |
| KW-3049                        | (7.4±2.4) × 10^-9 | (5.5±2.7) × 10^-6 | 743.2 |
| Nifedipine                     | (9.1±1.5) × 10^-9 | (4.7±2.7) × 10^-7 | 51.6 |
| Verapamil                      | (1.7±0.3) × 10^-7 | (1.1±0.4) × 10^-6 | 8.5 |
| Diltiazem                      | (3.1±0.6) × 10^-7 | (7.0±3.2) × 10^-6 | 22.6 |

IC50 values which relax coronary arteries (1) or decrease developed tension of papillary muscles (2) by 50% and the ratio of (2) to (1) are listed. Each value is the mean±S.E. of 8 (coronary artery) or 6 (papillary muscle) examinations.
Table 2. Effects of KW-3049, nifedipine, verapamil and diltiazem on La3+-resistant 45Ca-uptake into polarized, canine coronary arteries

| Treatment      | (N)a | 45Ca-Uptake (μmoles/kg) |
|----------------|------|-------------------------|
| Control        | (28) | 124.4± 8.4b             |
| KW-3049 (10^-7 M) | (5)  | 127.1± 9.2              |
| Nifedipine     | (6)  | 115.4±11.2              |
| Verapamil      | (5)  | 141.1±14.4              |
| Diltiazem      | (6)  | 113.4±12.7              |

a No. of arteries examined. b Values are presented as the mean±S.E.

Fig. 2. Effects of KW-3049 (KW), nifedipine (NIF), verapamil (VER) and diltiazem (DIL) on La3+-resistant 45Ca-uptake into canine coronary arteries. The values are presented as the mean±S.E. of the number indicated in each column.

Fig. 3. Effect of KW-3049 (10^-7 M) on 45Ca-efflux from coronary arteries. Desaturation (upper panel) and corresponding rate coefficient (lower panel) curves from 4 paired muscles are presented. Each point represents the mean±S.E.

In vitro duration of Ca-antagonistic actions: The inhibitory effects on the KCI-induced contractions of coronary arteries after the elimination of drug and washout of tissues were examined as an index of the in vitro duration of Ca-antagonistic actions.

The contractions evoked by the addition of 50 mM KCl (final 55.4 mM KCl) before treatments with drugs were 1.19±0.07 g (n=69). Figure 4 shows the time-response curves for the inhibitory actions of various concentrations of KW-3049, nifedipine, nitrendipine, verapamil, diltiazem and nisoldipine on KCI-induced contractions after washout of the arteries. Pretreatments with KW-3049 at 10^-10, 10^-9 and 10^-8 M for 30 min inhibited KCI-induced contractions by 10.9±2.9%, 39.9±4.4% and 84.1±3.5% (n=5), respectively. When KCI-induced contractions of the
arteries treated with KW-3049 were examined, no recovery of contractions was observed up to 240 min despite repeated washout of the tissues. In the coronary arteries treated with 10⁻⁹ and 10⁻⁸ M of KW-3049, there was a tendency of increasing inhibitory actions over the inhibitory responses in the presence of the drug, 15 to 30 min after washout of the tissues.

Pretreatments with nifedipine, verapamil, diltiazem and nitrendipine inhibited the KCl-induced contractions in concentration-dependent manners. However, the KCl-induced contractions of arteries treated with these agents recovered gradually by washout of the tissues and almost reached to the pretreatment levels within 1 to 4 hr. The inhibitory effects of nisoldipine were relatively long-acting and at its higher concentration (10⁻⁷ M), the depressed contractions did not recover to the pretreatment levels 4 hr after washout of the tissues. Thus, the effect of KW-3049 was the most long-acting among the 6 drugs examined.

Uptake and efflux of [³H]-Ca-antagonists: Table 3 shows the uptake of [³H]-Ca-antagonists into coronary arteries after 2 hr of incubation in drug-containing solution. The T/M ratio of all of the Ca-antagonists examined was higher than 1.0, indicating that these drugs are appreciably taken up and condensed in coronary arteries. Comparison of the T/M ratios of each drug demonstrates that nitrendipine is most highly taken up, followed by KW-3049, verapamil and diltiazem.

Figure 5 shows the efflux curves for [³H]-KW-3049, [³H]-nitrendipine, [³H]-verapamil and [³H]-diltiazem in coronary arteries. All the drugs examined were released into drug-free solutions, and the efflux curves for each drug were composed of 2 or more compartments. Comparisons of desaturation curves as well as rate coefficient curves for each drug clearly indicate that the dissociation of KW-3049 from the arteries is much slower than those of nitrendipine, verapamil and...
At 60 min after the start of efflux, the rate coefficient of KW-3049, nitrendipine, verapamil and diltiazem were 0.10±0.01%, 0.96±0.08% min⁻¹, 1.24±0.15% min⁻¹ and 1.64±0.14% min⁻¹, respectively, showing that the dissociation rate of KW-3049 is about 1/10 or less than those of the other three drugs.

Discussion

A blockade of potential dependent Ca-channels is a common property of many organic Ca-antagonists (8, 9, 19). KW-3049 at 10⁻⁹ to 10⁻⁷ M inhibited depolarization-induced ⁴⁵Ca-uptake in coronary arteries without affecting ⁴⁵Ca-uptake in polarized muscles. This type of inhibition was also the case with the prototype of Ca-antagonists such as verapamil, nifedipine and diltiazem. These data indicate that KW-3049 is one of the Ca-antagonists. This is in accordance with the previous electrophysiological observation (20) that KW-3049 exhibits inhibitory actions on cardiac slow channels. It has also been reported (21) that KW-3049 selectively and competitively binds to ³H-nitrendipine binding sites of rat cardiac membranes with high affinity. Therefore, it seems reasonable to assume that KW-3049 exerts Ca-antagonistic effects via a mechanism like those of other dihydropyridine-type Ca-antagonists.

Inhibition of depolarization-induced Ca-uptake is usually well correlated with the relaxing or antispasmodic effects of many kinds of drugs on the depolarized smooth muscles (19, 22, 23). In the present study, KW-3049 at concentrations which inhibited depolarization-induced ⁴⁵Ca-uptake relaxed the depolarized coronary arteries. These observations as well as the lack of an effect on ⁴⁵Ca-efflux suggest that the potent relaxing actions of KW-3049 in coronary arteries are

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**Table 3. Uptake of calcium antagonists into canine coronary arteries**

| Drug       | Medium conc. (µmoles/l) | Tissue conc. (µmoles/kg) | T/M Ratio |
|------------|-------------------------|--------------------------|-----------|
| KW-3049    | 0.1                     | 2.59±0.50                | 25.9±5.0  |
| Nitrendipine | 0.1                  | 3.62±0.54                | 36.2±5.4  |
| Verapamil  | 2                       | 19.0±1.9                 | 9.5±1.0   |
| Diltiazem  | 2                       | 11.6±1.2                 | 5.8±0.6   |

The coronary arteries were incubated in solution containing ³H-antagonists for 2 hr. * Concentrations employed are those which relax coronary arteries submaximally. ** The tissue : medium (T/M) ratio was obtained by dividing the amount of drug taken up per kg wet weight of tissue by the concentration of drug per liter of incubation medium. • Each value is presented as the mean±S.E. of 4 examinations.
mediated via an inhibition of potential-dependent Ca-channels.

The class of Ca-antagonists includes many compounds with diverse chemical structures; and their pharmacological effects, particularly their cardiac actions, differ among individual agents (10, 11, 24, 25). KW-3049 produced relaxation of isolated coronary arteries at similar concentrations of nifedipine, whereas the negative inotropic actions of KW-3049, as examined in isolated ventricular papillary muscles, were less potent than that of nifedipine. This type of vasculoselectivity was highest for KW-3049 among the 4 Ca-antagonists presently examined. Such selectivity as observed for KW-3049 may be one of the advantages in clinical applications since patients with lowered ventricular performance could be expected to be less susceptible to the incidence of cardiac failure. With regard to the other cardiac actions such as the effects on sinus node and AV-conduction, it has already been reported (20) that KW-3049 is similar in nature to nifedipine, and the inhibition by KW-3049 is less prominent than that of verapamil.

Recently, there have been some reports concerning the Ca-antagonists whose inhibitory effects in isolated vascular smooth muscles are hardly recovered even after washout of the tissues. This type of Ca-antagonist includes nisoldipine (26), amlodipine (27) and flunarizine (28). In the present study, we have examined, using the isolated coronary arteries, the time-course of changes in inhibitory actions of depolarization-induced contractions after washout of the tissues. The data showed that the inhibitory effects of KW-3049 persisted for a long time even following 4 hr of washout. In contrast, the other 5 Ca-antagonists examined were relatively short-lasting in their inhibitory effects, although the effect of higher concentration (10^{-7} M) of nisoldipine was hardly eliminated by washing. Comparison of the T1/2 values at concentrations of drugs which inhibit depolarization-induced contractions to similar degrees indicated that the in vitro duration of the actions of KW-3049 was the longest, followed by nisoldipine, nitrendipine, verapamil, nifedipine and diltiazem. It is of interest to note that the above order of the in vitro durations of actions is well in accordance with the order of durations of antihypertensive effects in conscious, spontaneously hypertensive rats after the intravenous administrations (A. Karasawa, unpublished observations). Pang and Sperelakis (29) demonstrated that nitrendipine was highly taken up into monolayer cultures of rat aortic smooth muscle cells, while the uptake of diltiazem was less in quantity. This was confirmed in the present experiments using coronary arteries. The order of uptake (T/M ratio) into coronary arteries after 2 hr of incubation in drug-containing solutions was: nitrendipine>KW-3049>verapamil>diltiazem. Since the in vitro duration of actions of KW-3049 was much longer than that of nitrendipine, it could be concluded that a higher amount of uptake does not necessarily result in a longer duration of actions in vitro. The pharmacological observations and the high uptake ratio of drugs suggest that the drugs exist at Ca-channels as well as at non-specific binding sites. In this sense, the equilibrium or diffusion of drugs between Ca-channels and the other binding sites such as lipid bilayer (30) must be also taken into consideration. The precise distribution of drugs in coronary arteries awaits further investigations.

In the present experiments, 80% of the [3H]-KW-3049 taken up was still present in coronary arteries even 90 min after the arteries were exposed to drug-free solutions. The rate coefficient of efflux of KW-3049 was about 1/10 or less than those of nitrendipine, verapamil and diltiazem, indicating that KW-3049 is released at much slower speed from the coronary arteries. It seems that these results could clearly explain the pharmacological observations that the inhibitory effects of KW-3049 on depolarization-induced contractions continued even after the repeated washout of the tissues. Furthermore, this property of KW-3049 might, at least partly, play a role in its long lasting effects in vivo such as coronary vasodilating and antihypertensive actions (12).

References
1 Hashimoto, K., Taira, N., Chiba, S., Hashimoto, K., Jr., Endoh, M., Kokubun, M., Kokubun, H., Iijima, T., Kimura, T., Kubota, K. and Oguro, K.:
Cardiohemodynamic effects by Bay a 1040 in the dog. Arzneimittelforschung 22, 15-21 (1972)

2 Pargie, H., Rowland, E. and Krikler, D.: Role of calcium antagonists in cardiovascular therapy. Br. Heart J. 46, 8-16 (1981)

3 Tanaka, K., Gotoh, F., Murakami, F., Amano, T., Ohashi, H. and Suzuki, N.: Effects of nimodipine (Bay e 9736) on cerebral circulation in cats. Arzneimittelforschung 30, 1494-1497 (1980)

4 Pepine, C.J., Feldman, R.L., Hill, J.A., Conti, C.R., Mehta, J., Hill, C. and Scott, E.: Clinical outcome after treatment of rest angina with calcium blockers: Comparative experience during initial year of therapy with diltiazem, nifedipine and verapamil. Am. Heart J. 106, 1341-1347 (1983)

5 Ellrodt, G., Chew, C.Y.C. and Singh, B.N.: Therapeutic implication of slow-channel blockade in cardiocirculatory disorders. Circulation 62, 669-679 (1980)

6 Aoki, K., Kondoh, S., Mochizuki, A., Yoshida, T., Kato, S. and Takikami, K.: Antihypertensive effect of cardiovascular Ca2+ antagonist in hypertensive patients in the absence and presence of beta adrenergic blockade. Am. Heart J. 96, 219-226 (1978)

7 Fleckenstein, A.: Specific pharmacology of calcium in myocardium, cardiac pacemakers, and vascular smooth muscle. Annu. Rev. Pharmacol. Toxicol. 17, 149-166 (1977)

8 Cauvin, C., Loutzenhiser, R. and Van Breemen, C.: Mechanisms of calcium antagonist-induced vasodilation. Annu. Rev. Pharmacol. Toxicol. 23, 373-396 (1983)

9 Nayler, W.G. and Poole-Wilson, P.A.: Calcium antagonists: definition and mode of action. Basic Res. Cardiol. 76, 1-15 (1981)

10 Hof, R.P. and Scholtsyik, G.: Effects of the calcium antagonist PY 108-068 on myocardial tissues in vitro and on reflex tachycardia in vivo. J. Cardiovasc. Pharmacol. 5, 176-183 (1983)

11 Janis, R.A. and Triggie, D.J.: New developments in Ca2+ channel antagonists J. Med. Chem. 26, 775-785 (1983)

12 Shuto, K., Karasawa, A., Kubo, K., Nakamizo, N. and Watanabe, M.: Anti hypertensive and antianginal activities of KW-3049 (a novel calcium antagonist). Abstract of 9th International Congress of Pharmacology (IUPHAR), London, 89BP (1984)

13 Karasawa, A., Kubo, K., Shuto, K. and Nakamizo, N.: Antianginal effects of KW-3049, a new dihydropyridine Ca-antagonist. Japan. J. Pharmacol. 39, Supp. 291P (1985)

14 Karasawa, A., Kubo, K., Shuto, K., Oka, T. and Nakamizo, N.: Calcium antagonistic effects of KW-3049, a new 1,4-dihydropyridine derivative in isolated canine coronary arteries. Japan. J. Pharmacol. 40, Supp. 96P (1986)

15 Karasawa, A., Shuto, K., Kubo, K., Kasuya, Y., Hashikami, M. and Shigenobu, K.: Hypotensive action of a new benznidazololinone derivative, theo-1-(2-hydroxy-2-(3,4,5-trimethoxyphenyl)-1-methylethyl)-4-(1,3-dihydro-2H-benzenidazo-2-one-1-yl) piperidine (KF-4942): prazosin-like mode of action. Arch. Int. Pharmacodynam. Ther. 261, 278-290 (1983)

16 Karaki, H. and Weiss, G.B.: Alterations in high and low affinity binding of 45Ca in rabbit aortic smooth muscle by norepinephrine and potassium after exposure to lanthanum and low temperature. J. Pharmacol. Exp. Ther. 211, 86-92 (1979)

17 Church, J. and Zsoter, T.T.: Calcium antagonistic drugs. Mechanism of action. Can. J. Physiol. Pharmacol. 58, 254-264 (1980)

18 Mars, S. and Sperelakis, N.: Comparison of [3H]-bepridil and [3H]verapamil uptake into rabbit aortic rings. J. Cardiovasc. Pharmacol. 4, 777-783 (1981)

19 Godfraid, T.: Mechanism of action of calcium entry blockers. Fed. Proc. 40, 2866-2871 (1981)

20 Kodama, I., Yoshitake, I., Nezasa, Y., Ikeda, N., Toyama, J. and Yamada, K.: Effects of KW-3049, a newly developed calcium antagonist, on rabbit sinus nodes and guinea pig ventricular muscles. Japan. Circ. J. 51, 1325-1334 (1987)

21 Ishii, I., Nishida, K., Oka, T. and Nakamizo, N.: Receptor binding properties of KW-3049, a new 1,4-dihydropyridine calcium channel blocker. J. Pharmacol. Exp. Ther. 240, 123-129 (1986)

22 Takayanagi, I., Karasawa, A. and Kasuya, Y.: Relaxation of depolarized guinea pig taenia caecum induced by some antispasmodics. Eur. J. Pharmacol. 50, 137-143 (1978)

23 Hof, R.P., Scholtsyik, G., Loutzenhiser, R., Van Breemen, C.: Mechanisms of calcium antagonist-induced vasodilation. Annu. Rev. Pharmacol. Toxicol. 23, 373-396 (1983)

24 Millard, R.W., Lathrop, D.A., Grupp, G., Ashraf, H., Grupp, I.L. and Schwartz, A.: Differential cardiovascular effects of calcium channel blocking agents: potential mechanisms. Am. J. Cardiol. 49, 499-506 (1982)

25 Singh, B.N. and Phil, D.: Pharmacological basis for the therapeutic applications of slow-channel blocking drugs. Angiology Aug, 492-515 (1982)
26 Kazda, S. and Towart, R.: The duration of action of calcium antagonists in vitro: a comparison of nifedipine and nisoldipine (Bay K 5552). Br. J. Pharmacol. 76, 255P (1982)

27 Burges, R.A., Carter, A.J., Gardiner, D.G. and Higgins, A.J.: Amlodipine, a new dihydropyridine calcium channel blocker with slow onset and long duration of action. Br. J. Pharmacol. 84, 281P (1985)

28 Van Nueten, J.M., Van Beek, J. and Janssen, P.A.J.: Effect of flunarizine on calcium-induced responses of peripheral vascular smooth muscle. Arch. Int. Pharmacodyn. Ther. 232, 42–52 (1978)

29 Pang, D.C. and Sperelakis, N.: Uptake of calcium antagonistic drugs into muscles as related to their lipid solubilities. Biochem. Pharmacol. 33, 821–826 (1984)

30 Rhodes, D.G., Sarmiento, J.G. and Herbette, L.G.: Kinetics of binding of membrane-active drugs to receptor sites. Diffusion-limited rates for a membrane bilayer approach of 1,4-dihydropyridine calcium channel antagonists to their active site. Mol. Pharmacol. 27, 612–623 (1985)