Effects of Calcium Channel Agonists (BAY K 8644, CGP28392 and YC-170) on $^{45}$Ca Uptake by Rat Uterine Segments

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ABSTRACT—The characteristics of the stimulating effects of the calcium channel agonists BAY K 8644, CGP28392 and YC-170 on $^{45}$Ca uptake by rat uterine segments were investigated. BAY K 8644, CGP28392 and YC-170 caused about 150, 100 and 150% increase, respectively, in the $^{45}$Ca uptake induced by 20 mM KCl. The ED$_{50}$ values of BAY K 8644, CGP28392 and YC-170 were $1.8 \times 10^{-9}$, $2.5 \times 10^{-8}$ and $9.8 \times 10^{-9}$ M, respectively. These agonists had little effect on the $^{45}$Ca uptake induced by $10^{-6}$ M acetylcholine. They also did not affect the basal $^{45}$Ca uptake. Their enhancing effects were blocked by the Ca channel antagonist nitrendipine. We conclude that rat uterine segments have voltage-sensitive Ca channels that are stimulated by Ca channel agonists (BAY K 8644, CGP28392 and YC-170) under depolarizing conditions and that the characteristics of the stimulating effects of CGP28392 and YC-170 on $^{45}$Ca uptake by rat uterine segments are qualitatively the same as those of BAY K 8644.

Ca channels are important in excitation-secretion coupling, because increase in the cytoplasmic concentration of free Ca plays a key role in the regulation of Ca-dependent enzyme activity and hormone release (1). Some 1,4-dihydropyridine (DHP) derivatives are known to block the flow of Ca$^{2+}$ through voltage-sensitive Ca channels. Conversely, DHP derivatives such as BAY K 8644 (BAY), CGP28392 (CGP) and YC-170 (YC) (2–6) have recently been found to act as Ca-channel agonists and increase the Ca current in various excitable cells (7–11). These agonists and antagonists have been utilized in studying how voltage-sensitive Ca channels are physiologically (normally) regulated (7).

We previously reported the characteristics of Ca influxes through voltage- and receptor-operated Ca channels in rat uterine muscle (12). In this work, we examined the characteristics of the stimulating effects of BAY, CGP and YC on $^{45}$Ca uptake by rat uterine segments to elucidate how voltage-sensitive Ca channels in rat uterine muscle are physiologically regulated. A preliminary report of this work has appeared in abstract form (13).

MATERIALS AND METHODS

$^{17\beta}$-Estradiol-3-benzoate was purchased from Teikokuzoki Hormone Mfg. BAY, CGP, nitrendipine and YC were gifts from Bayer AG (through Prof. F. Hoffmeister and Dr. B. Garthoff), Ciba-Geigy AG (through Prof. H. Brunner and Dr. K. Scheibli), Yoshitomi Pharmaceutical Ind. and Yamanouchi Pharmaceutical Co. (through Dr. M. Asano), respectively. Ca agonists and antagonist were...
used, as solutions in 100% polyethylene glycol #200 (PG #200).

**Rat uterine segments**

Virgin female Wistar rats weighing about 150 g (8–10 weeks old) were used. They were ovarictomized without regard to the stage of the estrous cycle. From day 10 after ovaricotomy, they were given 10 μg of 17β-estradiol-3-benzoate by i.s. injection once every 12 hr for 48 hr and then sacrificed. The uterine segments were placed in modified Locke-Ringer solution at room temperature, bubbled with 5% CO₂ + 95% O₂. The modified Locke-Ringer solution consisted of: 135.6 mM NaCl, 5.4 mM KCl, 0.4 mM CaCl₂, 0.2 mM MgCl₂, 20 mM NaHCO₃, 0.2 mM KH₂PO₄, 0.6 mM Na₂HPO₄ and 2.8 mM D-glucose. They were then divided longitudinally into segments (8–12 mg tissue/segment, about 1.5 × 10 mm).

**Measurement of ⁴⁵Ca uptake**

ACh- and KCl-stimulated ⁴⁵Ca uptakes were measured as reported previously (13) with slight modifications: The uterine segments prepared were blotted with filter paper, weighed and equilibrated for a minimum of 60 min in Ca-depleted Ringer solution. The Ca-depleted Ringer solution had the same composition as Ringer solution but without CaCl₂ and with 0.1 mM ethylene glycol bis (β-aminooethyl) N,N'-tetraacetic acid. The Ca-depleted Ringer solution was changed every 10 min during equilibration and bubbled with 5% CO₂ + 95% O₂ at 30°C (pH 7.4) during equilibration or experiments. The final volume of these reaction systems was 3 ml.

For measurements of KCl-stimulated ⁴⁵Ca uptake, the uterine segments were immersed in Ca-depleted high KCl Ringer (20 mM or 40 mM KCl) solution, which had the same composition as Ca-depleted Ringer solution but with KCl in place of NaCl, for 10 min at 30°C; and then CaCl₂ (1 mM) containing ⁴⁵CaCl₂ (about 0.5 μCi/tube) was added. As a control, Ca-depleted Ringer solution instead of Ca-depleted high KCl solution was used. For measurements of ACh-stimulated ⁴⁵Ca uptake, the uterine segments were immersed in the Ca-depleted Ringer solution of the muscle bath for 10 min at 30°C, and then ACh (10⁻⁶ M or 3 × 10⁻⁴ M) and CaCl₂ (final concentration, 1 mM) with ⁴⁵CaCl₂ (about 0.5 μCi/tube) were added to the muscle bath. As a control, instead of ACh, the same volume of Ca-depleted Ringer solution was added to the muscle bath.

For examination of the effects of Ca-channel agonists and antagonist, these compounds were added to the muscle bath 10 min before the addition of CaCl₂ with ⁴⁵CaCl₂. As a control, the same volume of PG #200 (1%) was added instead of Ca-channel agonists or antagonist. Experiments with these Ca-channel agonists and antagonist were carried out under dim light.

After addition of CaCl₂ with ⁴⁵CaCl₂, the mixture containing high KCl and ACh were incubated for 5 min and 2 min at 30°C, respectively. Then, the reactions were stopped by separating the uterine segments from the reaction medium. The uterine segments were then rinsed twice with 5 ml of ice-cold Ca-depleted Ringer solution (containing 10 mM LaCl₃) and placed in 10 ml of ice-cold Ca-depleted Ringer solution (containing 10 mM LaCl₃) for 40 min. Then 500 μl of NCS solubilizer (Amersham Co.) and 40 μl of distilled water were added, and the mixture was incubated for 2 hr at 50°C. The solubilized mixture was adjusted to pH 6.0 to 6.5 with acetic acid and left for about 15 hr at room temperature. Five milliliters of ACS II (Amersham Co.) were then added and the ⁴⁵Ca radioactivity was counted in a liquid scintillation counter (Packard Tri-carb 2050).

KCl- and ACh-stimulated ⁴⁵Ca uptakes were calculated as the differences between the uptakes in the absence and presence of 20 mM KCl, 40 mM KCl, 10⁻⁶ M ACh or 3 × 10⁻⁴ M ACh. The value of 20 mM KCl-, 40 mM KCl-, 10⁻⁶ M ACh- and 3 × 10⁻⁴ M ACh-stimulated ⁴⁵Ca uptake for 5 min (KCI) or 2 min (ACh) at 30°C were 129.1 ± 13.2 (n = 25), 327.0 ± 30.0 (n = 6), 102.7 ± 17.6 (n = 12) and 195.8 ± 22.5 (n = 6) pmol/mg of wet
Fig. 1. Effects of Ca-channel agonists on ACh- and KCl-stimulated $^{45}$Ca uptake. Panels A and B, C and D, and E and F show the effects of BAY, CGP and YC, respectively, on $^{45}$Ca uptake in the absence (closed circles) and presence (open circles) of 20 mM KCl or $10^{-6}$ M ACh. The control in panels A, C and E shows the $^{45}$Ca uptake with Ca-depleted Ringer solution instead of Ca-depleted high KCl (20 mM) solution as described under the Materials and Methods section. Points and bars are means ± S.E.'s of values for four to nine preparations. *P < 0.05, **P < 0.005: compared with $^{45}$Ca uptake induced by KCl in the absence of Ca-channel agonist. # P < 0.05: compared with $^{45}$Ca uptake in absence of 20 mM KCl or $10^{-6}$ M ACh.
Statistical analyses
Statistical analyses were carried out by Student's t-test and differences giving \( P < 0.05 \) were considered as significant.

RESULTS

The 20 mM KCl-stimulated \(^{45}\)Ca uptake was increased dose-dependently to maxima of about 150, 100 and 150%, respectively, by BAY (Fig. 1A), CGP (Fig. 1C) and YC (Fig. 1E). The concentrations of BAY, CGP and YC for half-maximum effects (ED\(_{50}\) values) were \( 1.8 \times 10^{-9} \), \( 2.5 \times 10^{-8} \) and \( 9.8 \times 10^{-9} \) M, respectively. However, these Ca-channel agonists did not affect the basal \(^{45}\)Ca uptake (in the absence of 20 mM KCl or \( 10^{-6} \) M ACh; Fig. 1, A, B, C, E and F) or \( 10^{-6} \) M ACh-stimulated \(^{45}\)Ca uptake (Fig. 1, B, D and F). The augmenting effects of CGP and YC on KCl-stimulated \(^{45}\)Ca uptake decreased at higher concentrations of \( 10^{-6} \) M. The concentration of 20 mM KCl and \( 10^{-6} \) M ACh used have induced about 30% of the maximal response for KCl and a half-maximal response for ACh, respectively (data not shown).

As shown in Fig. 2 (A, B and C), the augmenting effects of BAY, CGP and YC were completely inhibited by \( 10^{-8} \) M nitrendipine. The rest of the KCl (20 mM)-stimulated \(^{45}\)Ca uptake (\(^{45}\)Ca uptake stimulated by 20 mM KCl in absence of Ca agonists) was partially inhibited by ten times higher concentration (\( 10^{-7} \) M) of nitrendipine, but \( 10^{-7} \) M nitrendipine did not affect the basal \(^{45}\)Ca uptake in the presence of any agonist. Although we could not examine the inhibitory effect of various concentrations of nitrendipine on 20 mM KCl and \( 10^{-6} \) M ACh-stimulated \(^{45}\)Ca uptakes without these Ca agonists because the proportions of \(^{45}\)Ca uptake stimulated by 20 mM KCl (as shown in Fig. 1, A, C and E) and \( 10^{-6} \) M ACh (as shown in Fig. 1, B, D and F) to basal \(^{45}\)Ca uptake were very small (but KCl- and ACh-stimulated \(^{45}\)Ca uptake showed significance comparing with basal \(^{45}\)Ca uptake), we

Fig. 2. Effect of nitrendipine on KCl-stimulated \(^{45}\)Ca uptake induced by the Ca-channel agonists BAY (panel A), CGP (panel B) and YC (panel C). Columns and bars are means and S.E.’s for four to five preparations. *\( P < 0.05 \): compared with basal \(^{45}\)Ca uptake (in absence of KCl). **\( P < 0.05 \): compared with \(^{45}\)Ca uptake induced by 20 mM KCl (Control). ***\( P < 0.002 \): compared with \(^{45}\)Ca uptake induced by 20 mM KCl in presence of BAY. □: Ringer, ■: 20 mM KCl.
could show the inhibitory effect of various concentrations of nitrendipine on 40 mM KCI- or 3 × 10^-4 M ACh-stimulated 45Ca uptake without Ca channel agonists as shown in Fig. 3 (A and B). The KCl- and ACh-stimulated 45Ca uptakes were almost completely inhibited by 10^-5 M nitrendipine, but not by 10^-6 M nitrendipine. The IC50 values of nitrendipine on KCl- and ACh-stimulated 45Ca uptake were 3 × 10^-6 M and 5.0 × 10^-6 M, respectively. Nitrendipine at 10^-5 M did not affect the basal 45Ca uptake.

DISCUSSION

In this work, we found that the characteristics of the effects of the three Ca-channel agonists, BAY, CGP and YC, on 45Ca uptake by rat uterine segments are as follows:

(i) Under nondepolarizing conditions (5 mM KCl), they have little effect on 45Ca uptake (Fig. 1, A, B, C, D, E and F), as observed previously by others in rat cardiac cells and NG 108-15 cells (14, 15). We also examined whether they did affect the 45Ca uptake by ACh which was used to stimulate 45Ca uptake in rat uterine segments as reported previously (13, 16). They do not appreciably increase ACh (10^-6 M)-stimulated 45Ca uptake (Fig. 1, B, D and F). These observations suggest that their augmenting effects are voltage-dependent and that the mechanisms of the stimulating effects of high KCl and ACh on 45Ca uptake via Ca channels are partially different.

(ii) The half-maximal dose of BAY for its stimulating effect on 45Ca uptake was about 10^-9 M (Fig. 1A). This ED50 value of BAY was one order smaller than those of CGP and YC and shown in Fig. 1 (C and E). On the other hand, BAY and YC had greater stimulating effects than CGP as shown in these Figs. These results were consistent with those of others for 45Ca uptake by rat heart cells and NG 108-15 cells (14, 15), although the concentration of KCl used was different from our experiment and other experiments reported by Ichida et al. (12) and Ariyoshi et al. (13). In contrast, Ruzycky et al. (17) reported that the ED50 value of BAY for its effect on the contractile response of estrogen-dominated uterine smooth muscle under depolarizing conditions (40 mM KCl) was 3.24 × 10^-8 M, which is about 30 times higher the value obtained in this work.

(iii) 10^-8 M Nitrendipine, a DHP Ca-channel antagonist, completely inhibited the stimulating effects of all these Ca-channel agonists (Fig. 2, A, B and C), as reported previously by others (14, 15), but basal 45Ca uptake was not inhibited by nitrendipine at ten times higher concentration (10^-7 M).

The 45Ca uptake stimulated by 3 × 10^-4 M ACh or 40 mM KCl was inhibited by nitrendipine, as shown in Fig. 3 (A and B). However, we could not investigate the inhibitory effect of nitrendipine on the 45Ca uptake stimulated by a lower concentration of ACh such as 10^-6 M, because the proportion of 45Ca uptake stimulated by 10^-6 M ACh to basal 45Ca uptake was very small as shown in Fig. 1 (B, D.
and F). Therefore, our results do not fully exclude the possibility that the augmenting effect of DHP Ca-channel agonists are not voltage-dependent and that the mechanisms of the stimulating effects of high KCl and ACh on $^{45}$Ca uptake via Ca channels are qualitatively similar.

IC$_{50}$ values of nitrendipine on KCl (40 mM) and ACh ($3 \times 10^{-4}$ M)-stimulated $^{45}$Ca uptake (Fig. 3, A and B) were about one to two orders higher than the results from other investigators (18). The reason for the difference between the two results of nitrendipine is not known so far, but it seems likely that the reason may reflect a difference in the conditions for estradiol derivative treatment or a difference in the constituents of the medium used (especially, the concentration of MgCl$_2$).

From our findings, we conclude that rat uterine segments have voltage-sensitive, Ca channels whose activity is increased by Ca-channel agonists (BAY, CGP and YC) under depolarizing conditions and that the characteristics of the stimulating effects of CGP and YC on $^{45}$Ca uptake on these segments are qualitatively the same as those of BAY.

REFERENCES