Effects of Ca Channel Agonists on \( ^{45} \text{Ca} \) Uptake Differ Depending on the State of NG108-15 Cells

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ABSTRACT—We investigated the effects of various Ca channel agonists (Ca agonist) derived from 1,4-dihydropyridine on KCl-stimulated \( ^{45} \text{Ca} \) uptake by differentiated and undifferentiated neuroblastoma \( \times \) glioma hybrid NG108-15 cells with and without dibutyryl cAMP. Ca agonists Bay K 8644, YC-170 and CGP 28392 enhanced KCl-stimulated \( ^{45} \text{Ca} \) uptake in differentiated NG108-15 cells, but only slightly in undifferentiated NG108-15 cells. The rank order of the enhancing effects was roughly Bay K 8644 \( > \) YC-170 \( > \) CGP 28392. These results suggest that there is some difference between the mechanisms by which these Ca agonists affect KCl-stimulated \( ^{45} \text{Ca} \) uptake in differentiated and undifferentiated NG108-15 cells, although the nature of that difference is not clear.

Keywords: Ca agonist, Differentiation, \( ^{45} \text{Ca} \) uptake (KCl-stimulated)

It has been established that neuroblastoma \( \times \) glioma hybrid NG108-15 (NG108-15) cells differentiate when treated with certain reagents such as prostaglandin E\(_1\), theophylline, and dibutyryl cAMP (Bt\(_2\)cAMP) (1, 2). Differentiation of NG108-15 cells is indicated by changes such as neurite extension, increased intracellular acetylcholine, increased small or large vesicles and release of acetylcholine (1, 3). In addition to these marked morphological and biochemical changes, NG108-15 cells may also show other functional changes.

Recently, we reported that the magnitude or rate of KCl-stimulated \( ^{45} \text{Ca} \) uptake increased progressively during the differentiation of NG108-15 cells with Bt\(_2\)cAMP, but was not influenced during the differentiation of PC12h pheochromocytoma cells with nerve growth factor (4). We concluded that the enhancement of KCl-stimulated \( ^{45} \text{Ca} \) uptake by differentiated NG108-15 cells with Bt\(_2\)cAMP is due, at least in part, to an increase in the amount of specific \( ^{3} \text{H}(+) \)PN200-110 (PN) binding sites; i.e., the cells showed a quantitative increase in peripheral L-type voltage-sensitive Ca channels (VSCCs) (5). It was also observed that the Ca-channel agonist (Ca agonist) Bay K 8644 (Bay) enhanced KCl-stimulated \( ^{45} \text{Ca} \) uptake in differentiated NG108-15 cells (4, 6, 7). However, there has been little investigation regarding the enhancing effects of various Ca agonists on KCl-stimulated \( ^{45} \text{Ca} \) uptake by differentiated (treated with Bt\(_2\)cAMP) and undifferentiated (without Bt\(_2\)cAMP treatment) NG108-15 cells.

In the present study, we investigated the enhancing effect of various Ca agonists derived from 1,4-dihydropyridine (DHP) such as Bay, YC-170 (YC) and CGP 28392 (CGP) on KCl-stimulated \( ^{45} \text{Ca} \) uptake by differentiated and undifferentiated NG108-15 cells.

Differentiated and undifferentiated NG108-15 cells were obtained by culturing the cells for five days in the presence (Bt\(_2\)cAMP-treated: differentiated) or absence (untreated: undifferentiated) of 0.75 mM Bt\(_2\)cAMP, as previously reported (4). KCl-stimulated \( ^{45} \text{Ca} \) uptake by differentiated and undifferentiated NG108-15 cells was assayed according to the method of Ichida et al. (4). Differentiated and undifferentiated cells were incubated for 15 min at 37°C with 2 ml prewarmed (37°C) reaction solution. Reactions were started by the addition of \( ^{45} \text{CaCl}_2 \) (about 1 mCi/dish) with KCl (50 mM) or NaCl (50 mM) and were carried out for 30 sec at 37°C. Ca agonists were added to the dishes 5 min before the addition of \( ^{45} \text{CaCl}_2 \) with KCl or NaCl. The \( ^{45} \text{Ca} \) uptake reactions were stopped after the indicated times by aspiration of the reaction solution in the dishes. The dishes were then washed three times with 5 ml of ice-cold reaction (La\(^{3+}\))-solution (with the same composition as the reaction-mixture but with 1 mM LaCl\(_3\) instead of 1 mM CaCl\(_2\)). The cells in the dishes were then solubilized by adding of 1.5 ml sodium deoxycholate (2%), and aliquots of the resulting solution were used for measurements of radioactivity and concentration of protein.
KCl-stimulated $^{45}$Ca uptake was calculated as the difference between the uptakes in the presence of KCl and NaCl, as previously described (8, 9). The $^{45}$Ca uptake in the presence of NaCl was taken to be the basal $^{45}$Ca uptake.

Protein was measured according to the method of Smith et al. (10), using bovine serum albumin as a standard.

Statistical analyses were performed using the unpaired Student's $t$-test, and the statistical evaluation for multiple groups was performed by one-way analysis of variance. A level of $P<0.05$ was regarded as indicating a statistically significant difference.

Bt$_2$cAMP and $^{45}$CaCl$_2$ (4–50 Ci/g calcium) were purchased from Yamasa Shoyu Co. (Chiba) and New England Nuclear (Boston, MA, USA), respectively. Bay K 8644, YC-170 and CGP 28392 were gifts from Bayer AG (Leverkusen, FRG), Yamanouchi Pharmaceutical Co.

**Fig. 1.** Effects of Ca agonists (DHP derivatives) on KCl-stimulated $^{45}$Ca uptake by Bt$_2$cAMP-treated and untreated NG108-15 cells. Bay K 8644 (panel A), YC-170 (panel B) and CGP 28392 (panel C) were added to 0.75 mM Bt$_2$cAMP-treated (○) or untreated (●) NG108-15 cells. NG108-15 cells were used 5 days after addition of Bt$_2$cAMP and $^{45}$Ca uptake with KCl or NaCl (30 sec at 37°C) was measured. *$P<0.05$: compared with KCl-stimulated $^{45}$Ca uptake in the absence of a Ca channel agonist. Points and bars are means±S.E.M. of values in 5 to 6 separate cultures.
The Ca agonists were dissolved in 50% Polyethylene glycol (PEG) 200. Other compounds were dissolved in the reaction solution. The Ca agonists were added in volumes of less than 1% of that of the reaction system. All agents were prepared on the day of use and were neutralized when necessary. Assays with DHP derivatives were performed under a safety light.

The enhancing effects of the Ca agonists Bay, YC and CGP on KCl-stimulated 45Ca uptake by Bt2cAMP-treated and untreated NG108-15 cells is shown in Fig. 1 (A – C). The enhancing effects of these Ca agonists on KCl-stimulated 45Ca uptake by untreated NG108-15 cells was hardly detectable, whereas the KCl-stimulated 45Ca uptake by Bt2cAMP-treated NG108-15 cells was significantly enhanced by these Ca agonists in a dose-dependent manner. The maximum enhancing effects of Bay, YC and CGP were about 4.0, 3.5 and 1.8 times, respectively; and their ED50 values were 36.0, 282.9 and 74.9 nM, respectively. It was also observed that these Ca agonists did not affect basal 45Ca uptake by Bt2cAMP-treated or untreated NG108-15 cells (data not shown).

The present study appears to be the first to report a difference between differentiated and undifferentiated NG108-15 cells regarding the enhancing effect of various Ca agonists such as Bay, YC and CGP on KCl-stimulated 45Ca uptake. These Ca agonists enhanced KCl-stimulated 45Ca uptake by Bt2cAMP-treated NG108-15 cells, but did not greatly enhance the uptake by untreated NG108-15 cells (Fig. 1, A – C). These results suggest that there is some difference between the mechanisms by which these Ca agonists affect KCl-stimulated 45Ca uptake in differentiated and undifferentiated NG108-15 cells, for example, these differences may be due to one or more of the following possibilities: a) The binding sites (receptors) of these Ca agonists were increased or become expressed. b) The factor(s) that acts to increase the binding sites of these Ca agonists was activated. c) The pathway between Ca agonists binding and KCl-stimulated 45Ca uptake was formed or becomes more strongly connected, although the nature of that difference is not clear.

The rank order of the enhancing effects of the Ca agonists Bay, YC and CGP on KCl-stimulated 45Ca uptake was as follows: Bay > YC > CGP. This is qualitatively the same result as that found for KCl-stimulated 45Ca uptake by the rat uterus, as reported by Ichida et al. (9). Freedman and Miller (6) and Noronha-Blob et al. (7) observed that Bay was approximately an order of magnitude more potent than CGP, but they did not investigate the effect of YC. Therefore, the present report describes the systematic study of the enhancing effects of these Ca agonists. Our results for Bay and CGP were more similar to those of Noronha-Blob et al. (7) than to those of Freedman and Miller (6), since Noronha-Blob et al. (7) observed that Bay is more potent than CGP in terms of enhancement rate and ED50 value for 45Ca uptake. These differing reports may be a function of the differing reaction times adopted for the 45Ca uptake. We used a reaction time of 0.5 min, while Noronha-Blob et al. (7) adopted a reaction time of 1 min. In contrast, Freedman and Miller (6) used a reaction time of 10 min. In this case, it seems likely that agonist-stimulated 45Ca uptake in the presence of high levels of KCl contain a higher ratio of 45Ca efflux by Ca2+/Mg2+ ATPase and/or Na+/Ca2+ exchange than is the case with a reaction time of 0.5 or 1 min; the reason for this was shown in our previous study (4). These results suggest that the proposed rank order of the enhancing effects of the Ca agonists tested in the present study may be reasonable.

We observed that the Ca agonists Bay, YC and CGP have little enhancing effect on basal 45Ca uptake by Bt2cAMP-treated and untreated NG108-15 cells under non-depolarizing conditions (5 mM KCl, data not shown), as observed previously (6, 7, 9). Our observations suggest that the enhancing effect on KCl-stimulated 45Ca uptake in differentiated NG108-15 cells was voltage-dependent.

Further study is required to clarify the mechanisms that account for the differential response associated with various Ca agonists between the cell types tested.

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