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Nicardipine inhibits N-type calcium channels in dbcAMP-differentiated neuroblastoma × glioma hybrid cells (NG 108-15 cells)

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KEY WORDS dihydropyridines; nicardipine; nifedipine; calcium channels; Fura-2; cyclic AMP; NG 108-15 cells

ABSTRACT

AIM: To investigate the possibility of dihydropyridine inhibition of N-type calcium channels. **METHODS:** Effects of nifedipine and nicardipine on the high K^+-induced intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) increase were studied by measuring $[Ca^{2+}]_i$ using the fluorescent indicator Fura-2. **RESULTS:** Pretreatment of cells with nifedipine 50 $\mu mol \cdot L^{-1}$ inhibited the high K^+-induced $[Ca^{2+}]_i$ transient by about 60 % ($n = 3$); however, pretreatment of cells with nicardipine 10 $\mu mol \cdot L^{-1}$ completely prevented the high K^+-evoked $[Ca^{2+}]_i$ increase in dibutyryl cyclic AMP (dbcAMP)-differentiated NG 108-15 cells ($n = 5$). The high K^+-induced $[Ca^{2+}]_i$ increase was mediated by L- and N-type voltage-sensitive calcium channels (VSCC) in NG 108-15 cells. **CONCLUSION:** Nicardipine at micromolar range inhibited both L- and N-type VSCC in dbcAMP-differentiated NG 108-15 cells whereas nifedipine mainly inhibited L-type calcium channels.

INTRODUCTION

Dihydropyridines have long been considered to be a group of inhibitors of L-type voltage-sensitive Ca^{2+} channels (VSCC). Recently, among this large category of drugs some dihydropyridines such as nimodipine and nitrendipine have been found to have also an effect on N-type VSCC[1-4]. Evidence has been accumulating that the selective blockade of N-type VSCC is consistently neuroprotective in rodent models of cerebral ischemia[5-9], indicating that components of calcium influx regulated by N-type VSCC are importantly implicated in neuronal degeneration. Therefore, it was of great importance to further explore dihydropyridine effect on N-type VSCC. In the present study, the effect of nifedipine and nicardipine on the high K^+-evoked intracellular calcium concentration ($[Ca^{2+}]_i$) rise, which is mainly mediated by L- and N-type VSCC[1,10], would be investigated in dibutyryl cyclic AMP (dbcAMP)-differentiated NG 108-15 cells. The main objective of this study was to observe whether or not nifedipine and nicardipine affect N-type VSCC in the favorable neuronal model, NG 108-15 cell line.

MATERIALS AND METHODS

Cell culture and $[Ca^{2+}]_i$ measuring method were described previously[1]. Briefly, NG 108-15 cells of low passages (provided by Universität Dresden Carl Gustav Carus) were cultured in Dulbecco's modified Eagle's medium (DMEM). Differentiation was induced for 5 d by incorporation of dbcAMP 0.96 $mmol \cdot L^{-1}$ into the medium and reduction of serum concentration to 1 %. Differentiated NG 108-15 cells were loaded with Fura-2 by incubation with Fura 2-AM 2.5 $\mu mol \cdot$

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L^{-1} at 37 ℃ for 30 min in DMEM. The fluorescence of cell suspensions at λ_{ex}/nm = 340 and 380 and λ_{em}/nm = 505 was measured with a dual-wavelength fluorometer (LS50B, Perkin Elmer). Sodium dodecyl sulfate (SDS, final concentration 0.2 %) and egtazic acid (final concentration 7.5 mmol·L^{-1}) were used to obtain R_{min} and R_{max}, respectively. $[Ca^{2+}]_i$ was calculated from the ratio (R) of the fluorescence at 340 nm to that at 380 nm (F_{340}/F_{380})[11]. Nifedipine and nicardipine were obtained from Research Biochemicals International (RBI). Data were treated with t-test.

RESULTS AND DISCUSSION

Nifedipine 50 μmol·L^{-1} inhibited the high K^+-induced increase by only about 60 %. High K^+ 50 mmol·L^{-1} induced a rapid increase in $[Ca^{2+}]_i$ from the basal level of (100 ± 28) nmol·L^{-1} to a peak value of (339 ± 99) nmol·L^{-1} (n = 60); Pretreatment of cells with nifedipine 50 μmol·L^{-1} sharply inhibited this increase from (81 ± 2) nmol·L^{-1} to a peak value of (175 ± 12) nmol·L^{-1}, (n = 3) (Tab 1, Fig 1).

Tab 1 also presented the effect of nicardipine on the high K^+ (50 mmol·L^{-1})-evoked

Tab 1. Inhibitory effect of nifedipine and nicardipine on the high K^+ (50 mmol·L^{-1})-induced $[Ca^{2+}]_i$ transient. Cells were preincubated with nifedipine or nicardipine for 2 min before basal $[Ca^{2+}]_i$ was measured. $^cP < 0.01$ vs control.

Inhibitor/ μmol·L^{-1}	n	basal $[Ca^{2+}]_i$/ nmol·L^{-1}	Peak $[Ca^{2+}]_i$ by K^+/nmol·L^{-1}	Inhibition /%
Control	60	100 ± 28	339 ± 99	
Nifedipine				
10	14	108 ± 28	254 ± 7^c	36
50	3	81 ± 2	175 ± 12^c	60
Nicardipine				
10	5	97 ± 22	no transient	100

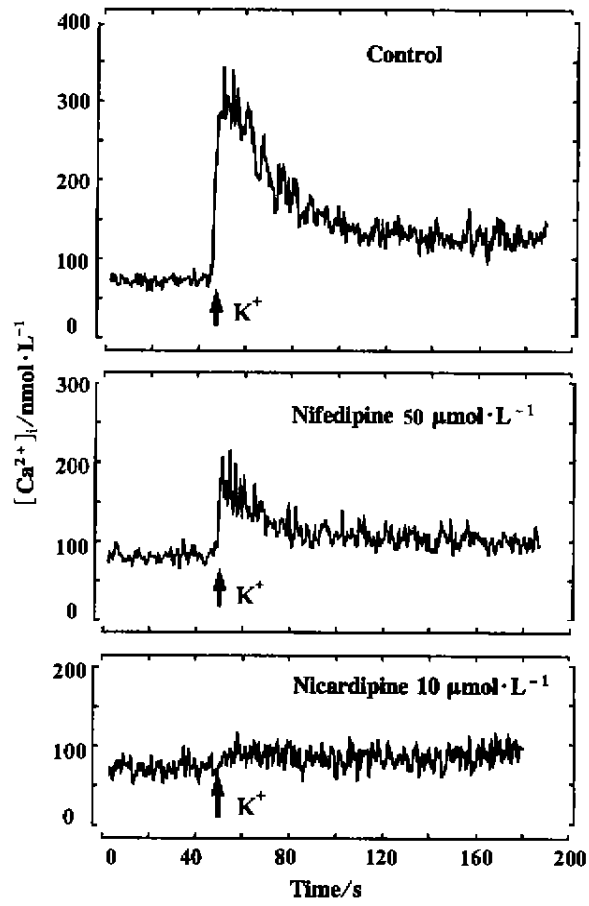

Fig 1. Effects of nifedipine 50 μmol·L^{-1} and nicardipine 10 μmol·L^{-1} on the high K^+ (50 mmol·L^{-1})-induced $[Ca^{2+}]_i$ increase in dbcAMP-differentiated NG 108-15 cells. Cell suspension (2 mL of 6×10^5 cells) was transferred into a thermostatted stirred cuvette and allowed to stay for 2 min. The basal level was measured for 50 s before the extracellular K^+ concentration was elevated to 50 mmol·L^{-1} (arrows).

$[Ca^{2+}]_i$ increase in dbcAMP-differentiated NG 108-15 cells. Pretreatment of cells with nicardipine (10 μmol·L^{-1}), high K^+ did not cause any $[Ca^{2+}]_i$ transient. Fig 1 exhibited the inhibitory curve of nicardipine effect on this high K^+ (50 mmol·L^{-1})-evoked $[Ca^{2+}]_i$ increase. Preincubation of cells with nicardipine 10 μmol·L^{-1} for 2 min completely prevented the high K^+-evoked $[Ca^{2+}]_i$ transient (n = 5). Only a slight gradual $[Ca^{2+}]_i$ rise was observed

after K^+ addition. This might be due to the Na^+-Ca^{2+} exchange during the cell membrane depolarization by K^+[12]. Nimodipine and nitrendipine inhibited N-type calcium channels because both agents inhibited the high K^+-evoked $[Ca^{2+}]_i$ transient[1]. However, they prevented completely the increase at 4 times higher concentration (50 $\mu mol \cdot L^{-1}$) than nicardipine, and they inhibited this increase only about 60 % at 10 $\mu mol \cdot L^{-1}$[1]. Most interestingly, nimodipine at 10 $\mu mol \cdot L^{-1}$ together with ω-conotoxin GVIA 7.2 $\mu mol \cdot L^{-1}$, a selective N-type calcium channel blocker, completely prevented this high K^+-induced increase. This implied that nimodipine at 10 $\mu mol \cdot L^{-1}$ affected mostly L-type channel while at higher concentration inhibited also N-type calcium channel[1] (Fig 2).

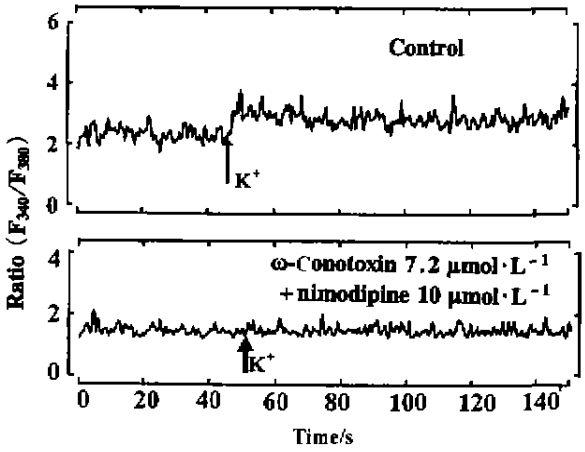

Fig 2. The complete blockade of the high K^+-evoked Ca^{2+} signal by ω-conotoxin GVIA 7.2 $\mu mol \cdot L^{-1}$ together with nimodipine 10 $\mu mol \cdot L^{-1}$ in dbcAMP-differentiated NG 108-15 cells. Buffer contains 1 $g \cdot L^{-1}$ bovine serum albumin. Cells were preincubated with ω-conotoxin GVIA 7.2 $\mu mol \cdot L^{-1}$ for 20 min and nimodipine 10 $\mu mol \cdot L^{-1}$ (added at 18th min), then basal level was recorded before K^+ addition (arrows). This graphic represents 4 experiments.

It has been long established that the activity of VSCC increases after treatment of NG 108-15 cells with dbcAMP[13,14]. Recently, it has been further determined that dbcAMP treatment of the cells strongly developed L- and N-type VSCC in NG 108-15 cells, and L- and N-type calcium channels were mainly involved in the high K^+-evoked $[Ca^{2+}]_i$ increase because this increase could be completely inhibited by the use of nifedipine (50 $\mu mol \cdot L^{-1}$) and ω-conotoxin GVIA (10 $\mu mol \cdot L^{-1}$) simultaneously[10]. Our results of nifedipine at 50 $\mu mol \cdot L^{-1}$ were similar to the reported ones[10]. This confirmed that nifedipine had an effect mainly on L-type calcium channels. Moreover, in our laboratory, as mentioned above, we have also confirmed that the high K^+-induced $[Ca^{2+}]_i$ transient was indeed composed of L- and N-type Ca^{2+} channels-mediated components in dbcAMP-differentiated NG 108-15 cells (Fig 2). Therefore, nicardipine 10 $\mu mol \cdot L^{-1}$ inhibited both L- and N-type calcium channels in NG 108-15 cells. This was the first report of nicardipine effect on N-type VSCC in NG 108-15 cells.

CONCLUSION Nicardipine inhibited N-type calcium channels in dbcAMP-differentiated NG 108-15 cells whereas nifedipine affected mainly L-type calcium channels in this cell line.

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REFERENCES

1 Li SN, Bräter M, Andreas K. Nimodipine and nitrendipine inhibit N-type calcium channels in dibutyryl cAMP-differentiated neuroblastoma × glioma hybrid (NG 108-15) cells. Neurosci Lett 1997; 230: 85 - 8.

2 Mansvelder HD, Stoof JC, Kits KS. Dihydropyridine block of ω-agatoxin IVA- and ω-

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conotoxin GVIA-sensitive Ca^{2+} channels in rat pituitary melanotropic cells.
Eur J Pharmacol 1996; 311: 293 – 304.

3 Diochot S, Richard S, Baldy-Moulinier M, Nargeot J, Valmier J. Dihydropyridines, phenylalkylamines and benzothiazepines block N-, P/Q- and R-type calcium currents.
Pflügers Arch 1995; 431: 10 – 9.

4 Jones SW, Jacobs LS. Dihydropyridine actions on calcium currents of frog sympathetic neurons.
J Neurosci 1990; 10: 2261 – 7.

5 Valentino K, Newcomb R, Gadbois T, Singh T, Bowersox S, Bitner S, *et al*. A selective N-type calcium channel antagonist protects against neuronal loss after global cerebral ischemia.
Proc Natl Acad Sci USA 1993; 90: 7894 – 7.

6 Zhao Q, Smith ML, Siesjö BK.
The omega-conopeptide SNX-111, an N-type calcium channel blocker, dramatically ameliorates brain damage due to transient focal ischemia.
Acta Physiol Scand 1994; 150: 459 – 61.

7 Bowersox SS, Singh T, Luther RR.
Selective blockade of N-type voltage-sensitive calcium channels protects against brain injury after transient focal cerebral ischemia in rats.
Brain Res 1997; 747: 343 – 7.

8 Buchan AM, Gertler SZ, Li H, Xue D, Huang ZG, Chaundy KE, *et al*. A selective N-type Ca^{2+}-channel blocker prevents CA1 injury 24h following severe forebrain ischemia and reduces infarction following focal ischemia.
J Cereb Blood Flow Metab 1994; 14: 903 – 10.

9 Yamada K, Teraoka T, Morita S, Hasegawa T, Nabeshima T. Omega-conotoxin GVIA protects against ischemia-induced neuronal death in the Mongolian gerbil but not against quinolinic acid-induced neurotoxicity in the rat.
Neuropharmacology 1994; 33: 251 – 4.

10 Chueh SH, Kao LS, Liu YT. Enhanced calcium signalling events in neuroblastoma × glioma hybrid NG108-15 cells after treatment with dibutyryl cyclic AMP. Brain Res 1994; 660: 81 – 7.

11 Grynkiewicz G, Poenie M, Tsien RY.
A new generation of Ca^{2+} indicators with greatly improved fluorescence properties.
J Biol Chem 1985; 260: 3440 – 50.

12 Nachshen DA, Kongsamut S.
'Slow' K^+-stimulated Ca^{2+} influx is mediated by Na^+-Ca^{2+} exchange: a pharmacological study.
Biochim Biophys Acta 1989; 979: 305 – 10.

13 Freedman SB, Dawson G, Villereal ML, Miller RJ. Identification and characterization of voltage-sensitive calcium channels in neuronal clonal cell lines. J Neurosci 1984; 4: 1453 – 67.

14 Noronha-Blob L, Richard C, U'Prichard DC.
Voltage-sensitive calcium channels in differentiated neuroblastoma × glioma hybrid (NG108-15) cells: characterization by quin 2 fluorescence.
J Neurochem 1988; 50: 1381 – 90.

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尼卡地平抑制 dbcAMP 分化的神经母细胞瘤×神经胶质瘤杂种细胞(NG 108 – 15)内 N 型钙通道

Brao., M

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dbcAMP

关键词 二氢吡啶; 尼卡地平; 硝苯地平; 钙通道; Fura-2; 环腺苷一磷酸; NG 108-15 细胞

神经母细胞瘤

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