ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Aug; 21 (8): 709 - 714
http://www.chinaphar.com

Inhibition of calcium signaling in terminal and soma of carp retinal bipolar cells by GABA

WU Di, ZHU Pei-Hong[1]
(*Shanghai Institute of Physiology, Chinese Academy of Sciences, Shanghai 200031, China*)

KEY WORDS $GABA_A$ receptors; $GABA_C$ receptors; bipolar cells; retina

ABSTRACT

AIM: To investigate the effect of activation of γ-aminobutyric acid (GABA) receptors on high K^+-evoked Ca^{2+} signaling in the terminal and soma of carp retinal ON-type bipolar cells. **METHODS**: Freshly dissociated carp retinal cells were loaded with fluo-3AM and then the fluorescence measurements were performed on a confocal laser-scanning microscope. **RESULTS**: Ca^{2+} signaling evoked by high K^+ 35 mmol/L was completely suppressed in both the terminal and soma of bipolar cells by GABA 100 μmol/L. However, different results were found in the terminal and soma when only one subtype of GABA receptors was activated. While activation of either $GABA_A$ or $GABA_C$ receptors totally suppressed Ca^{2+} signaling in the soma, a gradual elevation of $[Ca^{2+}]_i$ appeared in the terminal. GABA 10 μmol/L could also completely suppress Ca^{2+} signaling in the soma, but could only partially reduce Ca^{2+} signaling in the terminal. **CONCLUSION**: Activation of both $GABA_A$ and $GABA_C$ receptors could completely inhibit high K^+-evoked Ca^{2+} signaling in the terminal and soma of carp retinal ON-type bipolar cells. While activation of either $GABA_A$ or $GABA_C$ receptors alone still totally suppressed Ca^{2+} signaling in the soma, a gradual elevation of $[Ca^{2+}]_i$ appeared in the terminal, which may be due to desensitization of GABA receptors.

INTRODUCTION

γ-Aminobutyric acid (GABA) is the major and the most widely distributed inhibitory neurotransmitter in the vertebrate central nervous system[1]. GABA receptors are classified into $GABA_A$, $GABA_B$, and $GABA_C$ receptors based on their distinct pharmacological characteristics and signal transduction mechanisms. $GABA_A$ and $GABA_C$ receptors are ionotropic receptors and mediate Cl^- currents, while $GABA_B$ receptor is a metabotropic receptor and coupled to potassium and calcium channels via G-proteins[2-4].

$GABA_A$ and $GABA_C$ receptors coexist on retinal bipolar cells in many species, including teleost[5-9]. Up to now, most studies about the inhibitory effect of GABA on bipolar cells have been mainly focused on the terminal[10-13]. It is generally believed that activation of GABA receptors can inhibit Ca^{2+} influx, and then suppress neurotransmitter release from the terminal of bipolar cells[10-13]. However, little is known about that regarding the soma. It has been shown recently that, different from conventional $GABA_C$ receptor[3,4], $GABA_C$ receptor in carp retinal bipolar cells represents striking desensitization in response to maintained GABA application[8]. Therefore, the physiological significance of co-localization of desensitizing $GABA_A$ and $GABA_C$ receptors was also elucidated in the present study.

In the present work, we investigated the inhibitory effect of activating $GABA_A$ and/or $GABA_C$ receptors on high K^+-evoked Ca^{2+} signaling in both the terminal and soma of carp retinal ON-type bipolar cells with a Ca^{2+} imaging system.

MATERIALS AND METHODS

Dissociation of bipolar cells Solitary bipolar cells were acutely dissociated from retina of adult carp (*Carassius auratus*). The dissociation procedure has been described elsewhere[8,14]. In brief, after fish were dark-adapted and anesthetized, eyes were enucleated. Retina was isolated and cut into 8 - 12 pieces, which were incubated for 50 min at 25 ℃ in Hank's solution (see below) containing papain 2×10^4 units/L and *L*-

[1] Correspondence to Prof ZHU Pei-Hong.
Phn 86-21-6437-0080, ext 147. Fax 86-21-6433-2445.
E-mail: phzhu@mail.shcnc.ac.cn
Received 1999-11-17 Accepted 2000-03-01

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Aug; 21 (8)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

cysteine 1 g/L. After the retina pieces were thoroughly rinsed, cells were dispersed mechanically with a glass pipette in Ca^{2+} 0.2 mmol/L Ringer's solution (see below). The cell suspension was plated onto glass coverslip which was attached to the bored bottom of a chamber.

Fluorescence measurements In Ca^{2+} 0.2 mmol/L Ringer's solution, cells were loaded with fluo-3 AM 10 μmol/L for 20 min in darkness at 20 – 22 ℃ and then continuously superfused with Ringer's solution at a rate of 2 mL·min^{-1}. The volume of the chamber was about 150 μL and the tested agents were applied by superfusion. The bipolar cells used in this study have a bulbous synaptic terminal, which are assumed to be the rod dominant ON-type[15]. Experiments were carried out at room temperature within a few hours after cell dissociation.

Fluorescence measurements were performed on a confocal laser-scanning microscope (MRC1000 UV, Bio-Rad) equipped with a Nikon Diaphot 300 inverted microscope. The fluo-3 loaded cells were excited at a wavelength of 488 nm and the emitted fluorescence was detected at 525 nm. The data of fluorescence intensity was collected at a rate of 0.5 or 1 Hz. The time courses of average fluorescence intensity within rectangular regions of interest could be obtained simultaneously. All of the fluorescence data were stored on a magneto optical disk (Sony) and later processed by Microsoft Excel (Version 7.0). The changes of $[Ca^{2+}]_i$ were expressed with $\triangle F/F_0$, where F_0 was the resting fluorescence and $\triangle F$ Ca^{2+}-dependent increase over F_0.

Solutions and chemicals Hank's solution contained (in mmol/L) NaCl 137, KCl 3, $CaCl_2$ 0.5, $MgSO_4$ 1, Na-pyruvate 1, NaH_2PO_4 1, $NaHCO_3$ 0.5, HEPES 20 and Glucose 16. Ringer's solution consisted of (in mmol/L): NaCl 145, KCl 5, $CaCl_2$ 2, $MgSO_4$ 1, HEPES 10, and Glucose 16. To produce high K^+ solution, Na^+ in Ringer's solution was replaced by an equivalent amount of K^+. The pH of all solutions was adjusted to 7.4 with NaOH. Papain was obtained from Worthington Biochemical Corp; fluo-3 AM was product of Molecular Probes; GABA, bicuculline, and imidazole-4-acetic acid (I4AA) were from Research Biochemicals Inc; all other chemicals were from Sigma.

RESULTS

Inhibitory effect of GABA on Ca^{2+} signaling evoked by high K^+ In response to high K^+ 35 mmol/L-induced depolarization, $[Ca^{2+}]_i$ in both the terminal and soma of ON-type bipolar cells was dramatically elevated (Fig 1, 2). Compared with that in the soma, the increase of $[Ca^{2+}]_i$ in the terminal was evidently larger and faster. As seen previously[16], under resting condition a considerable proportion of ON-type bipolar cells displayed spontaneous $[Ca^{2+}]_i$ oscillations in the terminal but not in the soma (Fig 2).

As shown in Fig 1 and Fig 2, the Ca^{2+} signaling evoked by high K^+ in both the terminal and soma was completely suppressed by GABA 100 – 200 μmol/L (n = 14). Picrotoxin, a chloride channel blocker, could antagonize the inhibitory effect of GABA. In the presence of picrotoxin 200 μmol/L and GABA 100 μmol/L, high K^+-induced $\triangle F/F_0$ in the terminal and soma was 95 % ± 15 % ($P > 0.05$ *vs* control, $n = 6$) and 100 % ± 13 % ($P > 0.05$ *vs* control, $n = 5$) of the control, $\triangle F/F_0$ was induced by high K^+ alone in the terminal and soma (Fig 4). It is indicated that the inhibitory effect of GABA is mediated by $GABA_A$ and/or $GABA_C$ receptors.

Effect of activating one subtype of GABA receptors on Ca^{2+} signaling In order to investigate the role of different subtypes of GABA receptors, I4AA (a potent competitive antagonist for $GABA_C$ receptor) and bicuculline (a specific antagonist for $GABA_A$ receptor) were used to block $GABA_C$ and $GABA_A$ receptors, respectively[8,9]. When GABA 100 μmol/L is applied in the presence of I4AA 200 μmol/L or bicuculline 100 μmol/L, only $GABA_A$ or $GABA_C$ receptors can be activated respectively[8,9]. Ca^{2+} signaling evoked by high K^+ 35 mmol/L in the absence of GABA and any antagonists was taken as the control.

In the presence of GABA 100 μmol/L and I4AA 200 μmol/L, Ca^{2+} signaling in the terminal could be evoked by high K^+ 35 mmol/L exposure. However, compared with a rapid rise of $[Ca^{2+}]_i$ in the control, $[Ca^{2+}]_i$ slowly increased with a latency of a few seconds (Fig 3A). $\triangle F/F_0$ under the condition was 53 % ± 15 % of the control ($n = 6$, Fig 4). Different from that in the terminal, the Ca^{2+} signaling in the soma was still totally suppressed, in spite of the presence of I4AA 200 μmol/L (Fig 3A). Fig 3B shows the effect of activating $GABA_C$ receptors alone on high K^+ response. In this case, $[Ca^{2+}]_i$ in the terminal also slowly elevated with an even longer latency. The peak of Ca^{2+} signaling was

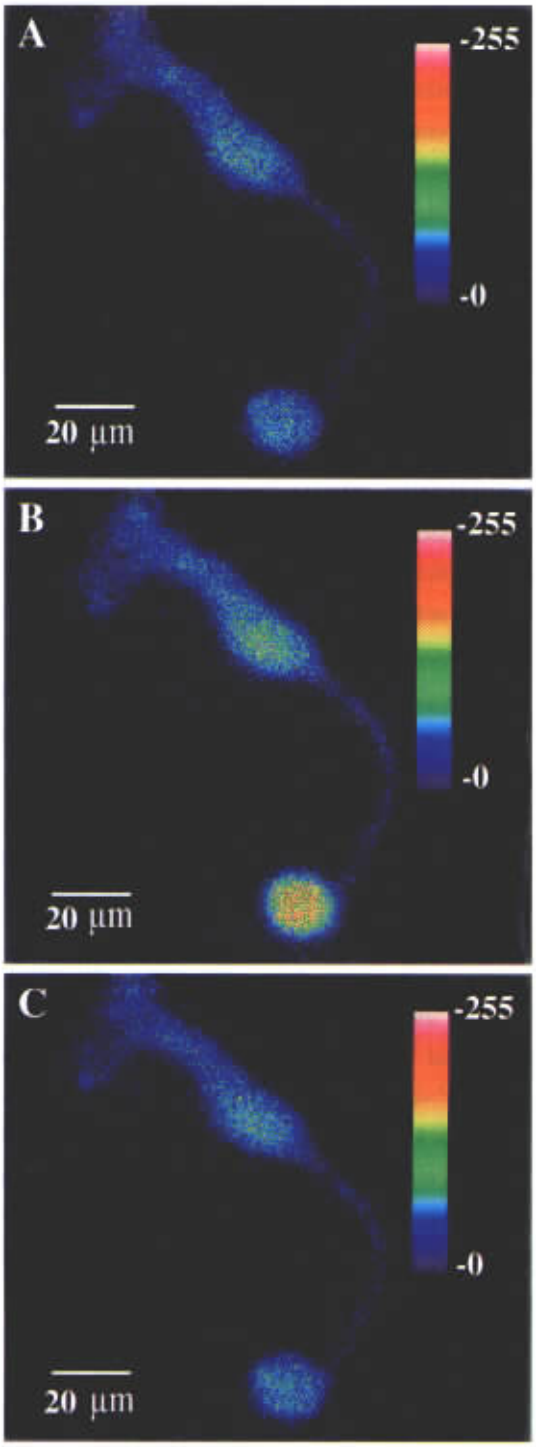

Fig 1. Fluorescence images of a retinal ON-type bipolar cells under resting condition (A), during application of high K^+ 35 mmol/L (B), and during application of high K^+ 35 mmol/L in the presence of GABA 100 μmol/L (C). GABA 100 μmol/L could completely inhibit high K^+ 35 mmol/L-evoked Ca^{2+} signaling in both the terminal and soma of bipolar cells. The vertical bar is an arbitrary 256 point gray scale converted to pseudocolor.

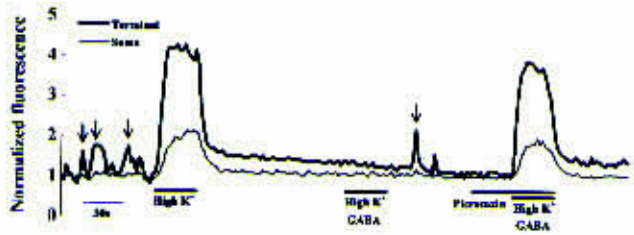

Fig 2. Effect of GABA on high K^+-evoked Ca^{2+} signaling in the terminal and soma of a carp retinal ON-type bipolar cell. Ca^{2+} signaling in both the terminal and soma was evoked by high K^+ 35 mmol/L exposure. When GABA 100 μmol/L was applied with high K^+, no detectable change in $[Ca^{2+}]_i$ was seen. Picrotoxin 200 μmol/L could block the inhibitory effect of GABA 100 μmol/L. ↓: spontaneous Ca^{2+} oscillation. The vertical axis is the fluorescence normalized to that of the resting level.

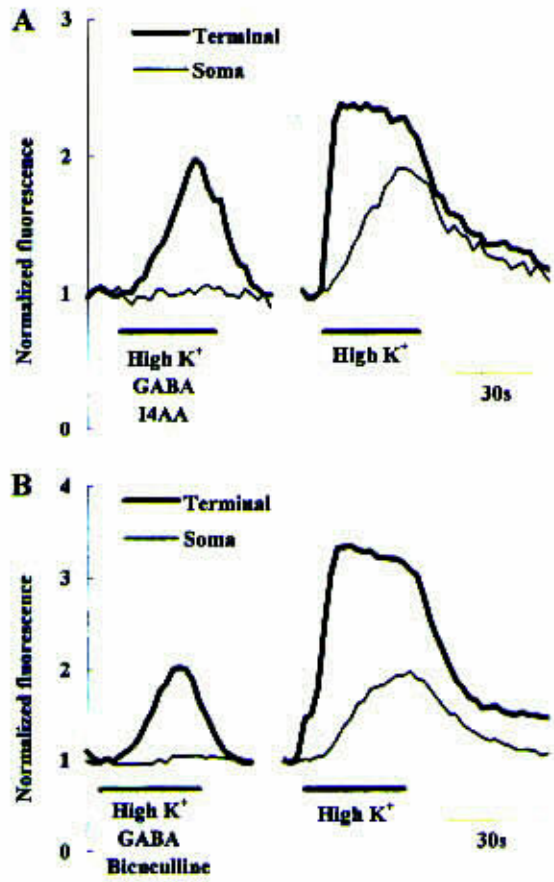

Fig 3. Effect of activating different subtypes of GABA receptors on Ca^{2+} signaling in the terminal and soma of ON-type bipolar cells. In the presence of I4AA 200 μmol/L (Fig 2A) or bicuculline 100 μmol/L (Fig 2B), only $GABA_A$ or $GABA_C$ receptors could be activated by GABA 100 μmol/L, respectively. About 5 min were allowed between two high K^+ 35 mmol/L exposures.

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Aug; 21 (8)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

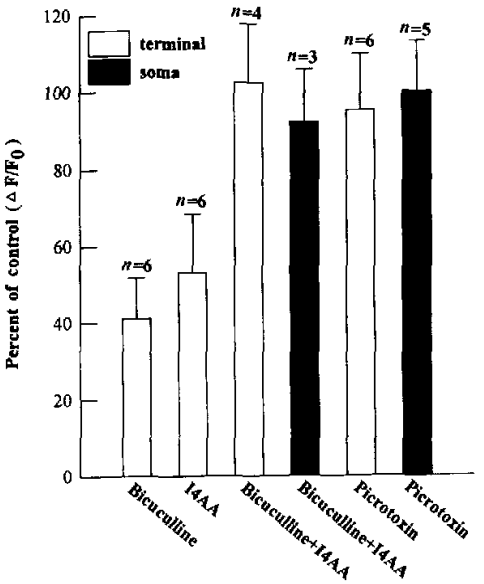

Fig 4. In the presence of GABA 100 μmol/L, effects of bicuculline 100 μmol/L , I4AA 200 μmol/L and picrotoxin 200 μmol/L on the peak of high K^+ 35 mmol/L-evoked Ca^{2+} signaling in the terminal and soma of ON-type bipolar cells. Control: The peak of Ca^{2+} signaling (expressed by $\triangle F/F_0$) evoked by high K^+ 35 mmol/L. *n* = the number of examined cells. $x \pm s$.

41 % ± 11 % of the control ($n = 6$, Fig 4). It is obvious that the activation of $GABA_C$ receptors alone is also enough to completely suppress Ca^{2+} signaling in the soma (Fig 3B). If all of $GABA_A$ and $GABA_C$ receptors were blocked by bicuculline and I4AA, Ca^{2+} signaling evoked by high K^+ 35 mmol/L in the terminal and soma was no longer affected by GABA 100 μmol/L (Fig 4).

EC_{50} of GABA for activating $GABA_C$ receptors in carp retinal bipolar cells is 5.5 μmol/L[8]. $GABA_A$ receptors usually are in an order of magnitude less sensitive than $GABA_C$ receptors[17]. Thus, GABA 10 μmol/L can activate most $GABA_C$ receptors, while its effect on $GABA_A$ receptors may be negligible. In consistence with the result represented in Fig 3, it was found that GABA 10 μmol/L could completely suppress Ca^{2+} signaling evoked by high K^+ 35 mmol/L in the soma, but only partially reduce Ca^{2+} signaling in the terminal ($\triangle F/F_0$ is 38.3 % ± 9.8 % of control, $n = 5$).

DISCUSSION

Illumination of the receptive field center of ON-type bipolar cells causes graded depolarization[15] and opening of voltage-gated *L*-type Ca^{2+} channels[18]. It have been shown that, bipolar cells receive negative feedback inputs from amacrine cells in the inner plexiform layer and feed-forward inputs from horizontal cells in the outer plexiform layer, and GABA is involved in regulating the activities at these synapses[17]. The results obtained in this study, ie high K^+-evoked Ca^{2+} signaling in both the terminal and soma of carp retinal ON-type bipolar cells totally suppressed by GABA, may be relevant to the *in vivo* physiological processes. Since the effect of GABA could be blocked by picrotoxin, it is indicated that GABA exerts its effect by activating Cl^- conductance via $GABA_A$ and/or $GABA_C$ receptors.

Since high K^+-induced Ca^{2+} signaling disappeared *in the presence of nifedipine 100 μmol/L, it is confirmed* that Ca^{2+} influx through *L*-type Ca^{2+} channels of the terminal and soma is essential for the Ca^{2+} signaling[16]. It has been shown that voltage-gated *L*-type Ca^{2+} channels predominantly locate in the terminal of ON-type bipolar cells[18,19]. *This localization has been confirmed by this* study. Compared with that in the soma, the amplitude and speed of the increase of $[Ca^{2+}]_i$ evoked by high K^+ in the terminal was obviously greater. Since the mechanism of Ca^{2+}-induced Ca^{2+} release (CICR) from caffeine-sensitive Ca^{2+} stores is present in the soma of ON-type bipolar cells, the high K^+-induced Ca^{2+} signaling in the soma comprises of Ca^{2+} influx through Ca^{2+} channels and the Ca^{2+}-induced Ca^{2+} release from intracellular stores[16].

It is known that $GABA_A$ and $GABA_C$ receptors coexist in the terminal and soma/dentrites of carp retinal ON-type bipolar cells. In addition to that, there is no difference in the Cl^- current mediated by $GABA_A$ receptors in either the terminal or soma/dentrites. The same is for the Cl^- current mediated by $GABA_C$ receptors (personal communication). Although both $GABA_A$ and $GABA_C$ receptors were involved in the suppression of Ca^{2+} signaling, this study found that activation of one subtype of GABA receptors displayed different effects in the terminal and soma. Activation of either $GABA_A$ or $GABA_C$ receptors alone was enough to totally suppress Ca^{2+} signal-

ing evoked by high K^+ in the soma, while a gradual elevation of $[Ca^{2+}]_i$ still appeared in the terminal. This difference may be well explained by the presence of CICR in the soma, but not in the terminal of bipolar cells[16]. When one subtype of GABA receptors is activated, high K^+-induced depolarization is antagonized to some extent. Consequently, Ca^{2+} influx and in turn Ca^{2+} signaling in the terminal are partially decreased. However, in the soma possessing only a small amount of voltage-gated Ca^{2+} channels, as a result of the decrease of Ca^{2+} influx, $[Ca^{2+}]_i$ can not be raised to a level necessary for inducing CICR. Therefore, Ca^{2+} signaling in the soma under these conditions may be completely suppressed.

Another interesting finding is that, in response to high K^+ exposure, $[Ca^{2+}]_i$ of the terminal slowly increased when only one subtype of GABA receptors was activated. It has been shown previously that high K^+ can cause a slow increase of $[Ca^{2+}]_i$ in the terminal of rat retinal bipolar cells when only $GABA_A$ receptors are activated[12]. However, different from the results of this study, when only $GABA_C$ receptors of rat retinal bipolar cells were activated, the inhibitory effect appeared slowly in the terminal and at last high K^+-induced response was totally suppressed[12]. This difference may be interpreted as that the $GABA_C$ receptors of bipolar cells in carp retina but not in rat retina can be desensitized by sustained GABA application[8]. But the desensitizing rate of $GABA_C$ receptors was significantly slower than that of $GABA_A$ receptors[9]. In accordance with slower desensitization of $GABA_C$ receptors, the latency of Ca^{2+} signaling seen with activation of $GABA_C$ receptors seems longer than that with activation of $GABA_A$ receptors as shown in Fig 3. Because of low sampling rate and low signal/noise ratio of Ca^{2+} signaling, the latency of Ca^{2+} signaling represented in Fig 3 could not be measured quantitatively.

Compared with $GABA_A$ receptor, $GABA_C$ receptor is more sensitive to GABA. According to the present results, low concentration of GABA could partially inhibit Ca^{2+} signaling in the terminal through selectively activating $GABA_C$ receptors and reducing neurotransmitter release from the terminal, while high concentration of GABA displays complete inhibitory effect by activating both $GABA_A$ and $GABA_C$ receptors. Therefore, GABAergic neurons in retina could regulate Ca^{2+} signaling of bipolar cells through releasing different amounts of GABA. The physiological significance of activating one subtype of GABA receptors enough to totally suppress Ca^{2+} signaling evoked by high K^+ in the soma is not clear. As a possibility, it many be that frequent increase of $[Ca^{2+}]_i$ accompanying the activation of ON-type bipolar cells is not useful or even harmful for the soma.

REFERENCES

1 Sivilotti L, Nistri A. GABA receptor mechanisms in the central nervous system. Prog Neurobiol 1991; 36: 35 – 92.
2 Bormann J. Electrophysiology of $GABA_A$ and $GABA_B$ receptor subtypes. Trends Neurosci 1988; 11: 112 – 6.
3 Bormann J, Feigenspan A. $GABA_C$ receptors. Trends Neurosci 1995; 18: 515 – 9.
4 Cherubini E, Strata F. $GABA_C$ receptors: A novel receptor family with unusual pharmacology. News Physiol Sci 1997; 12: 136 – 41.
5 Feigenspan A, Wässle H, Bormann J. Pharmacology of GABA receptor Cl^- channels in rat retinal bipolar cells. Nature 1993; 361: 159 – 62.
6 Lukasiewicz PD, Maple BR, Werblin FS. A novel GABA receptor on bipolar cell terminals in the tiger salamander retina. J Neurosci 1994; 14: 1202 – 12.
7 Qian H, Dowling JE. $GABA_A$ and $GABA_C$ receptors on hybrid bass retinal bipolar cells. J Neurophysiol 1995; 74: 1920 – 8.
8 Han MH, Li Y, Yang XL. Desensitizing $GABA_C$ receptors on carp retinal bipolar cells. Neuroreport 1997; 8: 1331 – 5.
9 Han MH, Yang XL. Zn^{2+} differentially modulates kinetics of $GABA_C$ *vs* $GABA_A$ receptors in carp retinal bipolar cells. Neuroreport 1999; 10: 2593 – 7.
10 Heidelberger R, Matthews G. Inhibition of calcium influx and calcium current by γ-aminobutyric acid in single synaptic terminals. Proc Natl Acad Sci USA 1991; 88: 7135 – 9.
11 Matthews G, Ayoub GS, Heidelberger R. Presynaptic inhibition by GABA is mediated via two distinct GABA receptors with novel pharmacology. J Neurosci 1994; 14: 1079 – 90.
12 Pan ZH, Lipton SA. Multiple GABA receptor subtypes mediate inhibition of calcium influx at rat retinal bipolar cell terminals. J Neurosci 1995; 15: 2668 – 79.
13 Wellis DP, Werblin FS. Dopamine modulates $GABA_C$ receptors mediating inhibition of calcium entry into and transmitter release from bipolar cell terminals in tiger salamander retina. J Neurosci 1995; 15: 4748 – 61.
14 Shen Y, Lu T, Yang XL. Modulation of desensitization at glutamate receptors in isolated crucian carp horizontal cells by concanavalin A, cyclothiazide, aniracetam and PEPA. Neuroscience 1999; 89: 979 – 90.
15 Tachibana M, Kaneko A. γ-Aminobutyric acid exerts a local inhibitory action on the axon terminal of bipolar cells: Evidence for negative feedback from amacrine cells. Proc Natl Acad Sci USA 1987; 84: 3501 – 5.
16 Wu D, Zhu PH. Caffeine-sensitive Ca^{2+} stores in carp retinal

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Aug; 21 (8)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

bipolar cells. Neuroreport 1999; 10: 3897 - 901.
17 Djamgoz MBA. Diversity of GABA receptors in the vertebrate outer retina. Trends Neurosci 1995; 18: 118 - 20.
18 Heidelberger R, Matthews G. Calcium influx and calcium current in single synaptic terminals of goldfish retinal bipolar cells. J Physiol 1992; 447: 235 - 56.
19 Tachibana M, Okada T, Arimura T, Kabayashi K, Piccolino M. Dihydropyridine-sensitive calcium current mediates neurotransmitter release from bipolar cells of the goldfish retina. J Neurosci 1993; 13: 2898 - 909.

GABA 抑制鲫鱼视网膜双极细胞的轴突末梢和胞体内的钙信号

吴 迪，朱培闳[1]
（中国科学院上海生理研究所，上海 200031，中国）

关键词 $GABA_A$ 受体；$GABA_C$ 受体；双极细胞；视网膜

目的：研究 GABA 受体的激活对于鲫鱼视网膜 ON-型双极细胞的胞体和轴突末梢内高钾引起的钙信号的影响. **方法**：急性分离的鲫鱼视网膜细胞经 fluo-3AM 孵育后，用激光共聚焦显微镜检测荧光变化. **结果**：GABA 100 μmol/L 可以完全抑制双极细胞胞体和轴突末梢内高钾 35 mmol/L 引起的钙信号. 仅激活 GABA 受体的一种亚型时，轴突末梢和胞体内出现了不同的结果. 当 $GABA_A$ 受体或 $GABA_C$ 受体被单独激活时，尽管胞体内的钙信号仍旧被完全抑制，但轴突末梢的 $[Ca^{2+}]_i$ 逐渐升高. GABA 10 μmol/L 仍旧可以完全抑制胞体内的钙信号且部分抑制轴突末梢内的钙信号. **结论**：激活 $GABA_A$ 和 $GABA_C$ 受体能够完全抑制鲫鱼视网膜 ON-型双极细胞内高钾引起的钙信号. 仅激活 $GABA_A$ 或 $GABA_C$ 受体时可以完全抑制胞体内的钙信号，而轴突末梢的 $[Ca^{2+}]_i$ 逐渐升高，这可能与 $GABA_A$ 或 $GABA_C$ 受体的脱敏有关.

（责任编辑 刘俊娥）