BIBLID: ISSN 0253-9756 中国药理学报 *Acta Pharmacologica Sinica* 1991 Nov; 12 (6) : 494-496

Inhibitory effect of *0,0*-bisacetyldaurisoline on Ca^{2+} influx into synaptosomes[1]

LU You-Ming, LIU Guo-Qing[2]

(*Department of Molecular Pharmacology, Naval Neurobiology Research Centre, Nanjing 210049, [2]China Pharmaceutical University, Nanjing 210009, China*)

ABSTRACT The inhibitory effects of *0,0*-bisacetyldaurisoline (BDSL) on $^{45}Ca^{2+}$ uptake and [^{3}H]GABA release from synaptosomes were investigated. BDSL (1-100 μmol · L^{-1}) produced a concentration-related inhibition of high K^+-stimulated $^{45}Ca^{2+}$ uptake and [^{3}H]GABA release (IC_{50} = 5.6 ± 0.7 and 31.4 ± 2.3 μmol · L^{-1}, respectively) in synaptosomes but verapamil (Ver) was only weakly active. Neither BDSL (100 μmol · L^{-1}) nor Ver (100 μmol · L^{-1}) modified $^{45}Ca^{2+}$ uptake in control medium (K^+ 5 mmol · L^{-1} resting uptake) and [^{3}H]GABA release in Ca^{2+}-free medium (K^+ 45 mmol · L^{-1} basal release). The results suggest that BDSL may be a potent Ca^{2-} channel blocker in neurons.

KEY WORDS *0,0*-bisacetyldaurisoline; verapamil; GABA; calcium channel blockers; synaptosomes

Daurisoline (DSL) selectively blocked calcium channels in neurons and endothelial cells[1,2]. Its derivative *0,0*-bisacetyldaurisoline (BDSL; Fig 1) has not been reported. This paper studied the effects of BDSL on "fast" $^{45}Ca^{2+}$ uptake and [^{3}H]GABA release from synaptosomes.

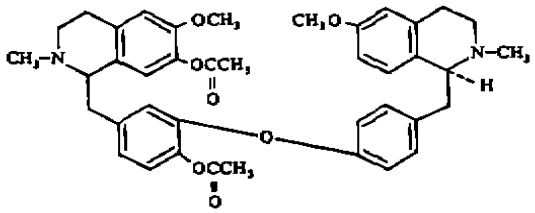

Fig 1. *0,0*-bisacetyldaurisoline (BDSL).

Received 1990 Jun 25 Accepted 1991 Jul 30

[1] Project supported by the National Natural Science Foundation of China, № 38970830

MATERIALS AND METHODS

Chemical reagents $^{45}CaCl_2$ (specific radioactivity = 0.93 MBq · μg^{-1}) and [^{3}H]GABA (specific radioactivity = 1.3 TBq · L^{-1}) were obtained from Beijing Institute of Atomic Energy, Chinese Academy of Sciences; verapamil and norepinephrine were obtained from Tianjing Research Institute of Pharmaceutical Industry; BDSL was synthesized by Dr HUANG Wen-Long in the China Pharmaceutical University. HEPES buffer was obtained from U S Biochemical Co.

Measurement of $^{45}Ca^{2+}$ uptake[1] The net uptake of $^{45}Ca^{2+}$ was calculated as the difference between the influx from standard HEPES buffer and the influx from the stimulating buffer. In each experiment $^{45}Ca^{2+}$ uptake was analysed on 6 replicates.

Measurement of [^{3}H]GABA release[1] Net Ca^{2+}-dependent release of [^{3}H]GABA was calculated by subtracting the basal release values from stimulated release values. All values represent the $\bar{x} \pm s$ of 6 determinations.

RESULTS

Influence of time on $^{45}Ca^{2+}$ uptake in synaptosomes Triggered $^{45}Ca^{2+}$ uptake in high K^+ buffer reached a maximum within 2 min at 37℃ and consisted of a "fast" phase which occurred during the first 20 s of depolarization and a slow phase which appeared 20 s after prolonged stimulation (Fig 2). During the first 20 s of depolarization, $^{45}Ca^{2+}$ uptake was 1.78 ± 0.85 nmol / mg protein which was nearly 3-folds of resting $^{45}Ca^{2+}$ uptake (0.95 ± 0.07 nmol / mg protein).

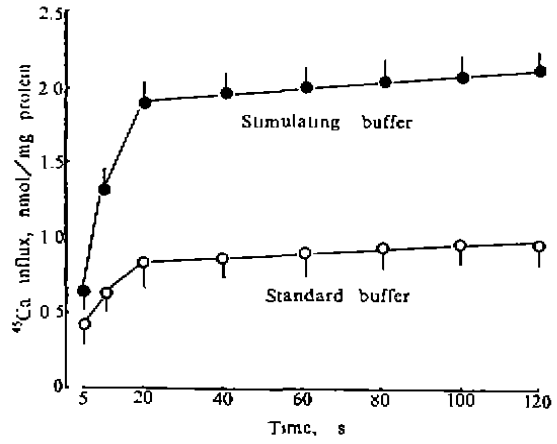

Fig 2. $^{45}Ca^{2+}$ uptake during incubation with standard buffer or stimulating buffer. $n=7$, $\bar{x}\pm s$.

Effects of BDSL and Ver on $^{45}Ca^{2+}$ uptake in synaptosomes At the concentration of 100 μmol · L^{-1}, BDSL and Ver had little or no effect on resting $^{45}Ca^{2+}$ uptake. The most prominent action produced by BDSL was a concentration-dependent inhibition on stimulated "fast" $^{45}Ca^{2+}$ uptake (IC_{50}= 5.6 ± 0.7 μmol · L^{-1}, $n=7$). Meanwhile, Ver (100 μmol · L^{-1}) only blocked 40% of the maximal $^{45}Ca^{2+}$ uptake (Fig 3).

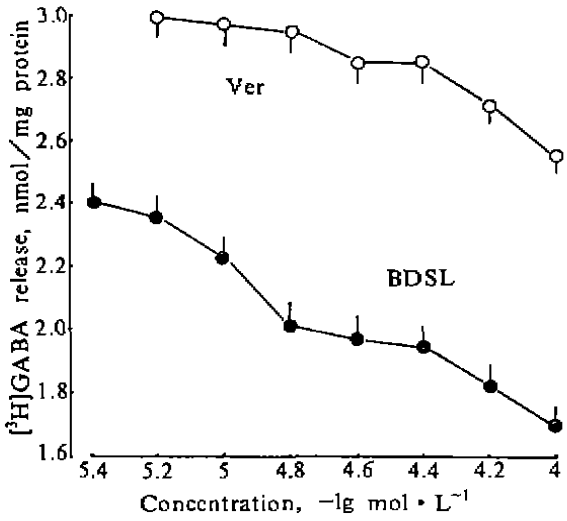

Fig 3. Effects of verapamil and BDSL on $^{45}Ca^{2+}$ uptake in synaptosomes induced by K^+ (75 mmol · L^{-1}). $n=6$, $\bar{x}\pm s$.

Effect of BDSL on [^{3}H]GABA release Addition of $CaCl_2$ (0.2–1.2 mmol · L^{-1}) produced a concentration-related increase in [^{3}H]GABA release. The maximal release was 2.98 ± 0.33 nmol / mg protein. The IC_{50} of BDSL (1–100 μmol · L^{-1}) on K^+-stimulated [^{3}H]GABA release was 31.4 ± 2.3 μmol · L^{-1} (Fig 4). Ver (50 μmol · L^{-1}) only slightly inhibited (11.1 ± 0.9%) [^{3}H]GABA release. Neither BDSL (100 μmol · L^{-1}) nor Ver (100 μmol · L^{-1}) decreased the basal release in Ca^{2+}-free buffer (0.99 ± 0.10 nmol / mg protein).

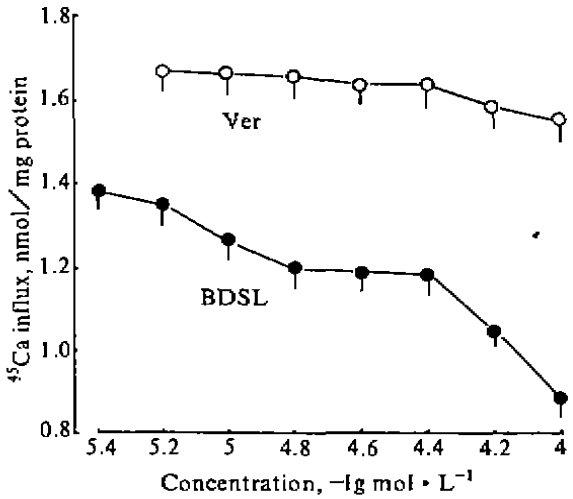

Fig 4. Effects of verapamil and BDSL on [^{3}H]GABA release from synaptosomes. $n=6$, $\bar{x}\pm s$.

DISCUSSION

The blockage of "fast" $^{45}Ca^{2+}$ uptake and evoked [^{3}H]GABA release by BDSL were found. The results also showed that the resting $^{45}Ca^{2+}$ uptake and basal [^{3}H]GABA release were not altered by BDSL. Furthermore, the previous results have indicated that the "fast" $^{45}Ca^{2+}$ uptake was mediated by voltage-dependent Ca^{2+} channel and associated with release of the transmitter from synaptosomes[4]. We conclude that only depolarization-induced transmembrane Ca^{2+}

movements are involved in synaptic effects of BDSL.

REFERENCES

1 Lu YM, Liu GQ. The effects of (−)-daurisoline on Ca^{2+} influx in presynaptic nerve terminals. *Br J Pharmacol* 1990; **101**: 45

2 Lu YM, Liu GQ. (−)-Daurisoline inhibits the endothelium-dependent relaxation and cycle GMP formation in rat aorta. *Asia Pac J Pharmacol* 1990; **5**: 207

3 Nachshen DA, Blaustein MP. The effects of some organic "calcium antagonists" on calcium influx in presynaptic nerve terminals. *Mol Pharmacol* 1979; **16**: 579

4 Suszckiw JB, O'leary ME, Rurawsky MM, Wany T. Presynaptic calcium channel in rat cortical synaptosomes: fast kinetics of phasic calcium influx, channel inactivation and relationship to nitrendipine receptor. *J Neurosci* 1986; **6**: 1340

5 Nachshen DA. The early time-course of potassium stimulated calcium uptake in presynaptic nerve terminals isolated from rat brain. *J Physiol* 1985; **361**: 251

6 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; **193**: 265

0,0-二乙酰蝙蝠葛苏林碱对突触体钙内流的抑制作用[1]

鲁友明、刘国卿[2]

(海军神经生物研究中心分子药理研究室，南京 210049；[2] 中国药科大学，南京 210009，中国)

提要 观察 *0,0*-二乙酰蝙蝠葛苏林碱(BDSL)对突触体$^{45}Ca^{2+}$摄取和钙依赖[^{3}H]-γ-氨基丁酸([^{3}H]GABA)释放的作用. BDSL (1−100 μmol · L^{-1})抑制突触体高钾除极快相$^{45}Ca^{2+}$摄取和[^{3}H]GABA 释放，IC_{50} 分别为 5.6 ± 0.7 和 31.4 ± 2.3 μmol · L^{-1}. 维拉帕米(Ver)作用极弱. BDSL (100 μmol · L^{-1})和 Ver (100 μmol · L^{-1})均不影响静息$^{45}Ca^{2+}$摄取和基础[^{3}H]GABA 释放. 表明 BDSL 可能是一个强效神经元钙通道阻滞剂.

关键词 *0,0*-二乙酰基蝙蝠葛苏林碱；维拉帕米；γ-氨基丁酸；钙通道阻滞剂；突触体

《中国药学杂志》1992 年征订

《中国药学杂志》(原名《药学通报》)为中国药学会主办的、国内外公开发行的综合性药学学术刊物，是一本反映我国药学各科进展和动态的高、中级专业性学术期刊，以高、中级药学工作者及其他医药卫生人员为读者对象.

本刊内容包括药剂学，临床药学、药理学、药品检验学，药物化学、药品生物化学、中药及天然药物学等. 辟有专题笔谈、药品生产、中药及天然药物、药理，药剂、药品质量与检验、药物与临床，药物不良反应、新药评价，综述，药学史，知识介绍、科学管理、问题讲座，科研简讯、文摘与资料、药学信息等 20 多个栏目，欢迎订阅. 现已开始 1992 年征订，请及早到当地邮局订阅.

本刊国内统一刊号 CN11-21621R，邮发代号 2-232，为 16 开本 64 页，每期定价 2.00 元.