

Inhibition by baclofen of NMDA-activated current in rat dorsal root ganglion neurons¹

SI Jun-Qiang², LI Zhi-Wang³

(Research Center of Experimental Medicine, Tongji Medical University, Wuhan 430030, China)

KEY WORDS baclofen; *N*-methyl-*D*-aspartate receptors; spinal ganglia; saclofen; patch-clamp techniques

ABSTRACT

AIM: To explore the modulatory effect of baclofen on NMDA-activated current in rat dorsal root ganglion (DRG) neurons. **METHODS:** Whole-cell patch-clamp technique was used to record NMDA-activated current in isolated DRG neurons. Drugs were applied by rapid solution exchange. **RESULTS:** Preapplication of baclofen $1 - 100 \mu\text{mol} \cdot \text{L}^{-1}$ induced a concentration-dependent inhibition of the inward NMDA-activated current markedly. NMDA ($100 \mu\text{mol} \cdot \text{L}^{-1}$)-activated current was inhibited by $52 \% \pm 14 \%$ ($n = 11$, $P < 0.01$) by preapplication of baclofen $100 \mu\text{mol} \cdot \text{L}^{-1}$. The inhibitory effect of baclofen was reversible, and was removed by saclofen $100 \mu\text{mol} \cdot \text{L}^{-1}$, which was a selective antagonist of GABA_B receptor. **CONCLUSION:** Preapplication of baclofen exerts an inhibitory effect on NMDA-activated current in the primary sensory neurons.

INTRODUCTION

GABA_A and GABA_B receptors existed separately or coexisted in the membrane of primary sensory neurons^[1-3]. NMDA receptors or glutamate receptors existed in the membrane of dorsal root ganglion (DRG)

neurons^[4-7]. Since these receptors were coexpressed in the same DRG neuron^[8], if they were activated simultaneously, an interaction might occur between the responses mediated by these receptors. It has been shown in our recent investigation that baclofen, a specific agonist of GABA_B receptor, inhibited membrane responses mediated by GABA_A receptor in rat DRG neurons^[9]. GABA_B receptors mediated inhibition of the NMDA component of synaptic transmission in the rat hippocampus^[10]. The present study was to explore the modulatory effect of baclofen on NMDA-activated current in the neurons freshly isolated from rat DRG.

MATERIALS AND METHODS

Cell isolation Young Sprague-Dawley rats (100 - 150 g, Grade II, Certificate No TJLA-Y2) of either sex, were decapitated, the vertebrate column in the thoracic and lumbar segments was dissected out and longitudinally divided into two halves along the median lines on both dorsal and ventral sides. The DRG together with dorsal and ventral roots and spinal nerves attached were taken out from the inner side of each half of dessected vertebra and transferred into Dulbecco's modified Eagle's medium (DMEM, Sigma; DMEM $13.84 \text{ g} \cdot \text{L}^{-1}$, NaCl $2.64 \text{ g} \cdot \text{L}^{-1}$) at pH 7.4, $340 \text{ mOsmol} \cdot \text{kg}^{-1}$. After removal of attached nerves and surrounding connective tissues 8 - 10 DRG were minced with dissecting spring scissors and incubated with trypsin (type III, Sigma) $0.5 \text{ g} \cdot \text{L}^{-1}$, collagenase (type I A, Sigma) $1.0 \text{ g} \cdot \text{L}^{-1}$, and DNase (type IV, Sigma) $0.1 \text{ g} \cdot \text{L}^{-1}$ in DMEM 5 mL at $35 \text{ }^{\circ}\text{C}$ in a shaking bath for 35 - 40 min. Soybean trypsin inhibitor (type II-S, Sigma) $1.25 \text{ g} \cdot \text{L}^{-1}$ was added to stop the enzymatic digestion. The isolated neurons were transferred into a 35-mm culture dish for 30 min. Experiments were performed at $(25 \pm 2) \text{ }^{\circ}\text{C}$.

¹ Project supported by the National Natural Science Foundation of China, No 39370239.

² Now in Department of Physiology, Shihezi Medical College, Xinjiang 832002, China.

³ Correspondence to Prof LI Zhi-Wang. Phn 86-27-361-3501. Fax 86-27-585-8920.

Received 1997-10-15

Accepted 1998-10-23

Recording Whole-cell patch-clamp recordings were carried out by a PC-II patch-clamp amplifier (Huazhong Univ of Sci & Tech). The micropipette was filled with internal solution, composed of (mmol·L⁻¹): KCl 140, MgCl₂ 2, HEPES 10, egtazic acid (EGTA) 11, ATP 5. Osmolarity was adjusted to 320 with sucrose and pH was adjusted to 7.4 with KOH. The external solution contained (in mmol·L⁻¹): NaCl 150, KCl 5, CaCl₂ 2.5, MgCl₂ 1, HEPES 10, *d*-glucose 10. Osmolarity was adjusted to 340 with sucrose and pH was adjusted to 7.4 with NaOH. The resistance of recording electrodes was 1–4 MΩ. After a whole cell configuration was established, a series of resistance and capacitance compensations were adjusted before the experiments. Membrane currents were filtered at 10 Hz (–3 dB). Data were analyzed in a super 386 computer with a data acquisitions of software and hardware system (Huazhong Univ of Sci & Tech) or recorded by a pen recorder. Experiments were carried out at a holding potential (HP) of –40 mV.

Drugs *d*, *l*-Baclofen (RBI), *N*-methyl-*D*-aspartic acid (NMDA, Sigma), 2-amino-5-phosphonovalerate (APV, Sigma), saclofen (Sigma), glycine (Gly, Shanghai No 3 Pharmaceutical Factory, China). All drugs were dissolved in external solution and applied by gravity flow from an array of tubules (OD/LD = 500 μm/300 μm), which were connected to a series of independent reservoirs. The distance from the mouth of tubule to the cell was from 90 to 110 μm. This rapid solution exchange system was manipulated by shifting the tubule horizontally with a micromanipulator.

Statistical methods The values of NMDA-activated current were presented as $\bar{x} \pm s$ and compared with *t* test.

RESULTS

The isolated DRG neurons had a round or oval shape with a residue of stem process, which was cut off by enzymatic and mechanical treatment.

NMDA-activated inward currents A large number of cells (68/96, 71 %) were sensitive to NMDA (1–100 μmol·L⁻¹) applied externally. Application of NMDA 1–100 μmol·L⁻¹ induced a concentration-dependent inward current (Fig 1A, *n* = 5 neurons). The concentrations of NMDA that induced

50 % of the maximal response (EC₅₀) and the maximal response (*I*_{max}) were around 12 μmol·L⁻¹ and 287 pA (NMDA 100 μmol·L⁻¹), respectively. The amplitude of NMDA (100 μmol·L⁻¹)-activated current was 194 pA ± 21 pA (*n* = 11), ranging from 129 to 258 pA. A selective NMDA receptor antagonist, 2-amino-5-phosphonovalerate (APV, 100 μmol·L⁻¹), blocked NMDA-activated current (*n* = 6) (Fig 1B).

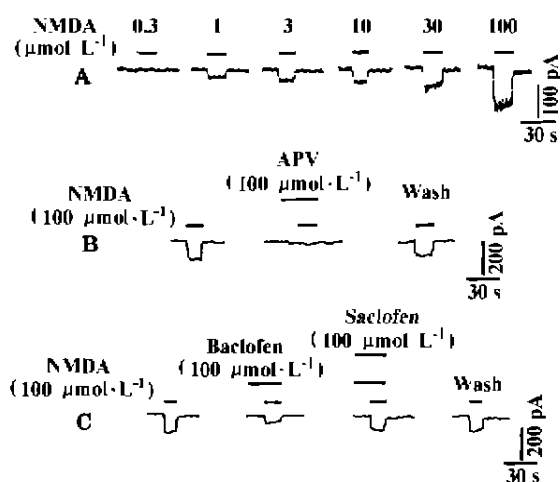


Fig 1. NMDA-activated currents in rat DRG neurons.

The amplitude of NMDA (100 μmol·L⁻¹)-activated current was rather small as compared with that of GABA (100 μmol·L⁻¹)-activated current. The recordings of NMDA (100 μmol·L⁻¹) and GABA (100 μmol·L⁻¹)-activated currents showed that the amplitudes for these two kinds of currents estimated in a single DRG cell were 112 pA and 1 nA (voltage clamped at –60 mV), respectively (Fig 2).

Another discrepancy between them was that the desensitization of GABA-activated current was very apparent, *ie*, though GABA is present continually and its concentration remained unchanged, the amplitude of the current decayed exponentially after it reached a peak value (*I*_{peak}) and then maintained at a level of steady state (*I*_{ss}). In contrast to that, NMDA-activated current showed a very slow desensitization (Fig 2).

Inhibitory effect of baclofen on NMDA-activated current No change in membrane current was observed when baclofen (1–100 μmol·L⁻¹) was applied for 30 s prior to application of NMDA, an

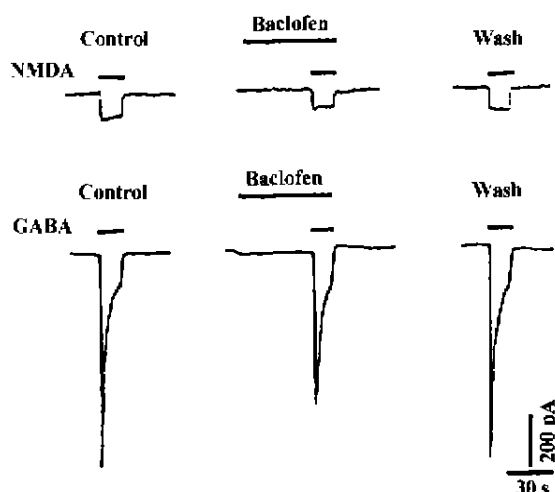


Fig 2. Inhibitory effect of baclofen $100 \mu\text{mol}\cdot\text{L}^{-1}$ on NMDA ($100 \mu\text{mol}\cdot\text{L}^{-1}$)- and GABA ($100 \mu\text{mol}\cdot\text{L}^{-1}$)-activated current in the same DRG neurons ($n=5$).

attenuation of NMDA-activated current occurred. Baclofen reversibly depressed NMDA-activated currents in most of the neurons (49/68, 72%), while in the remaining 19 DRG neurons baclofen did not affect NMDA-activated current (19/68, 28%). The inhibitory effect of baclofen was concentration-dependent and increased gradually with the increase in baclofen concentration (Fig 3).

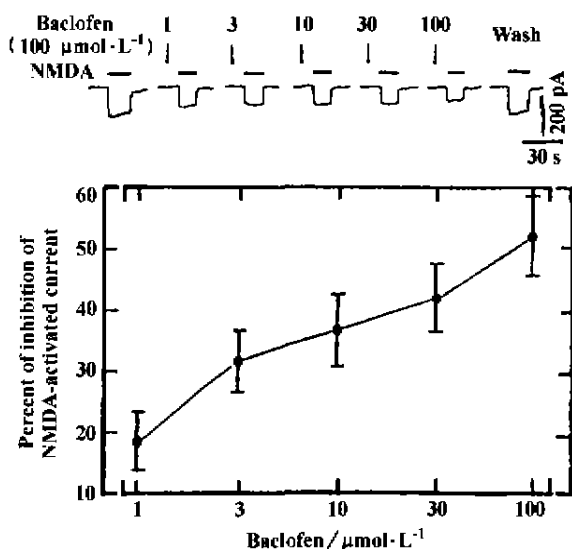


Fig 3. Baclofen inhibition of NMDA ($100 \mu\text{mol}\cdot\text{L}^{-1}$)-activated current. 8–11 DRG cells voltage clamped at -40 mV . $\bar{x} \pm s$.

A 10-min interval was interposed between each baclofen preapplication and recovery responses of NMDA-activated currents. On average, the current activated by NMDA $100 \mu\text{mol}\cdot\text{L}^{-1}$ was suppressed by $19\% \pm 5\%$, $32\% \pm 5\%$, $37\% \pm 6\%$, $42\% \pm 6\%$, $52\% \pm 6\%$ by baclofen 1, 3, 10, 30, and $100 \mu\text{mol}\cdot\text{L}^{-1}$, respectively. The IC_{50} (95% confidence limits) were $18 (15.75 - 20.25) \mu\text{mol}\cdot\text{L}^{-1}$.

Selective GABA_B receptor antagonist saclofen ($100 \mu\text{mol}\cdot\text{L}^{-1}$) prevented the suppression of NMDA-activated current by baclofen $100 \mu\text{mol}\cdot\text{L}^{-1}$ ($1 - 100 \mu\text{mol}\cdot\text{L}^{-1}$) ($n=7$, Fig 1C).

Not only NMDA-activated current, but also GABA-activated current were inhibited by baclofen ($100 \mu\text{mol}\cdot\text{L}^{-1}$) in the same DRG neuron ($n=5$, Fig 2).

Effect of voltage on the inhibition by baclofen of NMDA-activated current It was desirable to hold neurons at depolarized potentials for long periods. The pipette solution contained ($\text{mmol}\cdot\text{L}^{-1}$): CsCl 140, MgCl_2 2, EGTA 11, HEPES 10. pH was adjusted to 7.4 with CsOH. Baclofen suppressed NMDA-activated current at all holding potentials between -180 mV and $+40 \text{ mV}$. The reversal potential for NMDA-activated current was around $+10 \text{ mV}$ ($n=5$). Strong outward rectification of NMDA-induced current at membrane potentials more hyperpolarized than -40 mV . The suppression by baclofen of NMDA-activated current was in a voltage-independent manner and the reversal potential of NMDA-activated current remained unchanged in the presence of baclofen ($100 \mu\text{mol}\cdot\text{L}^{-1}$) ($n=5$, Fig 4).

Inhibition of NMDA-activated current by baclofen The inhibition of NMDA-activated current appeared at 30 s after preapplication of baclofen ($100 \mu\text{mol}\cdot\text{L}^{-1}$, $n=7$) and reached its peak at about 2–4 min. It took about 8–10 min to get a full recovery from baclofen inhibition. If the interval between pretreatment with baclofen and application of NMDA was less than 30 s, no effect was observed (Fig 5).

DISCUSSION

In the present study, the NMDA-activated inward current was identified to be the current mediated by NMDA receptors, since it could be potentiated and attenuated by coapplication of Gly and Mg^{2+}

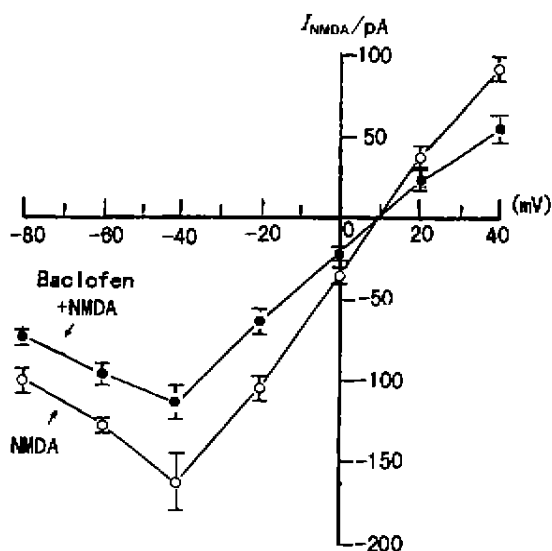


Fig 4. I-V relation for NMDA ($100 \mu\text{mol} \cdot \text{L}^{-1}$)-activated currents with and without pretreatment of baclofen ($100 \mu\text{mol} \cdot \text{L}^{-1}$) ($n = 5$ neurons).

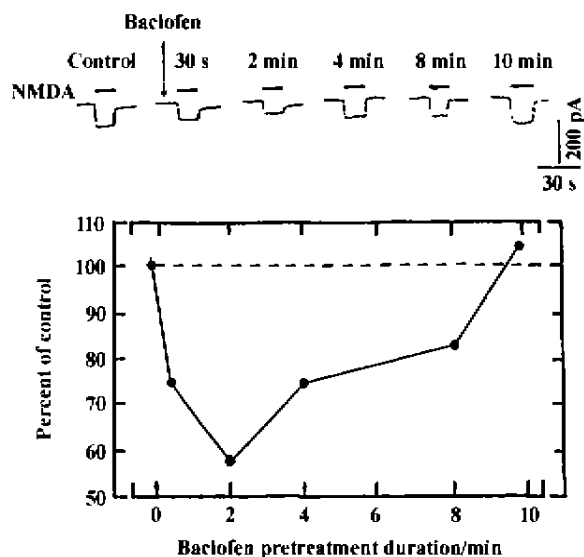


Fig 5. Baclofen ($100 \mu\text{mol} \cdot \text{L}^{-1}$) inhibition of NMDA ($100 \mu\text{mol} \cdot \text{L}^{-1}$)-activated current ($n = 7$ neurons).

respectively and blocked by NMDA receptor antagonist APV completely. The amplitude of NMDA-activated current recorded was too small to detect in the normal external solution at holding potential of -60 mV , so in this experiment the holding potential was always clamped at the level of -40 mV for favouring to get

more obvious recordings.

The result that preapplication of baclofen produced an inhibitory effect on the NMDA-activated current was convincing, because the GABA_B receptor antagonist saclofen could almost reverse the inhibitory effect of baclofen completely (Fig 1C), in addition, the baclofen inhibition revealed an obvious concentration-dependent relation (Fig 3). It is evident that the attenuation of NMDA-activated current by baclofen is more profound than the blockade by high Mg^{2+} of NMDA-activated current.

What is the physiological significance for the inhibition by baclofen of NMDA-activated current? It is not clear now. As we know gamma-aminobutyric acid (GABA) is the major neurotransmitter related to the generation of presynaptic inhibition of primary afferent terminals^[11]. GABA as an inhibitory neurotransmitter acts not only on GABA_A receptor, opening the Cl^- channel and involving in formation of primary afferent depolarization (PAD), but also on GABA_B receptor, mediating inhibition of Ca^{2+} influx and shortening APD during action potential^[12,13]. These two actions of GABA result in the decrease of release of neurotransmitter including substance P (SP) and glutamate (Glu) from primary afferent terminals. According to the reports published recently^[5-7], there exists NMDA/Glu autoreceptor in the presynaptic sensory terminals, which enhances the further release of Glu via positive feedback mechanism. Taking these findings with the present study together, it suggests that the activation of GABA_B receptor in the primary afferent terminals may produce presynaptic inhibition of Glu release both by suppressing of Ca^{2+} influx during AP directly and by breaking down the positive feedback linking of activation of NMDA autoreceptor immediately.

There are several possible routes of intramembranous and intracellular transduction. It is believed reasonably that the activation of PKC gives rise to phosphorylation at the site for cAMP-dependent phosphorylation in the cytoplasmic loop of NMDA receptor and results in closure of NMDA receptor channel and thus decreases in Ca^{2+} influx in DRG neurons^[14].

REFERENCES

- Desarmenien M, Feltz P, Occhipinti G, Santangelo F.

Schlichter R. Coexistence of GABA_A and GABA_B receptors on A_α and C primary afferents. *Br J Pharmacol* 1984; 81: 327-33.

2 Lin ZW, Li ZW. Responses mediated by GABA_A and GABA_B receptors on the somatic membrane of toad dorsal root ganglion neurons. *Acta Physiol Sin* 1993; 45: 117-23.

3 Guan BC, Li ZW, Zhou XP. Modulatory effect of substance P on the membrane responses mediated by GABA_A and GABA_B receptors in DRG neurons. *Acta Physiol Sin* 1994; 46: 441-50.

4 Lovinger DM, Weight FF. Glutamate induces a depolarization of adult rat dorsal root ganglion neurons that is mediated predominantly by NMDA receptors. *Neurosci Lett* 1988; 94: 314-20.

5 Sato K, Kiyama H, Park HT, Tohyama M. AMPA, KA, and NMDA receptors are expressed in the rat DRG neurons. *Neuroreport* 1993; 4: 1263-5.

6 Liu H, Wang H, Sheng M, Jan YN, Basbaum AI. Evidence for presynaptic *N*-methyl-*D*-aspartate autoreceptors in the spinal cord dorsal horn. *Proc Natl Acad Sci USA* 1994; 91: 8383-7.

7 Si JQ, Hu HZ, Wu XP, Fan YZ, Liu CJ, Li ZW. A new method combining electrophysiological identification of membrane receptors with immunohistochemical detection of intracellular neurotransmitters in the same isolated rat DRG neuron. *Chin J Appl Physiol* 1996; 12: 77-81.

8 Si JQ, Li ZW, Fan YZ. Modulatory effect of SP on GABA_A and NMDA-activated currents in neurons freshly isolated from rat DRG. *Chin J Neurosci* 1996; 3: 114-9.

9 Si JQ, Li ZW, Hu HZ, Zhou XP, Guan BC. Inhibitory effect of baclofen on GABA-induced depolarization and GABA-activated current in primary sensory neurons. *Neuroscience* 1997; 81: 821-7.

10 Morrisett RA, Mott DD, Lewis DV, Swartzwelder HS, Wilson WA. GABA-receptor-mediated inhibition of *N*-methyl-*D*-aspartate component of synaptic transmission in the rat hippocampus. *J Neurosci* 1991; 11: 203-9.

11 Barber RP, Vaughn JF, Saito K, Melaughlin BJ. GABAergic terminals are pre-synaptic to primary afferent terminals in the substantia gelatinosa of the rat spinal cord. *Brain Res* 1978; 141: 35-55.

12 Dolphin AC, Scott RH. Calcium channel currents and their inhibition by (-)-baclofen rat sensory neurones: modulation by guanine nucleotides. *J Physiol (Lond)* 1987; 386: 1-17.

13 Campbell V, Berrow N, Dolphin AC. GABA_B receptor modulation of Ca²⁺ currents in rat sensory neurones by the G protein G_o: antisense oligonucleotide studies. *J Physiol (Lond)* 1993; 470: 1-11.

14 Mayer ML, Westbrook GL. The physiology of excitatory amino acids in the vertebrate central nervous system. *Prog Neurobiol* 1987; 28: 197-276.

324-328

巴氯芬对大鼠背根神经节神经元 NMDA 激活电流的抑制作用

R338-21

R96p

司军强, 李之望 (同济医科大学
实验医学研究中心, 武汉 430030, 中国)

关键词 巴氯芬; *N*-甲基-*D*-精氨酸受体;
脊神经节; 沙氯芬; 膜片箝技术

目的: 探索巴氯芬对大鼠初级感觉神经元膜 NMDA 激活电流的调制作用. 方法: 全细胞膜片箝技术在新鲜分离的背根神经节(DRG)细胞上进行实验. 结果: DRG 细胞外加巴氯芬(1-100 μmol·L⁻¹)未记录到可检测的膜电流改变, 但预加巴氯芬对 NMDA 激活电流则有明显的抑制作用. 巴氯芬 100 μmol·L⁻¹对 NMDA (100 μmol·L⁻¹)激活电流的抑制可达 52% ± 14% (n = 11, P < 0.01). 此抑制作用被 GABA_B 受体的拮抗剂沙氯芬(100 μmol·L⁻¹)所取消. 结论: 初级感觉神经元膜上 GABA_B 受体的激活对 NMDA 受体介导的膜电流具有抑制作用.

(责任编辑 李颖)