

# Involvement of High-Conductance Calcium-Dependent Potassium Channels in Short-Term Presynaptic Plasticity in the Rat Dentate Gyrus

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We examined the involvement of high-conductance calcium-dependent potassium (BK) channels in short-term presynaptic plasticity in the rat dentate gyrus; a paired-pulse stimulation protocol was used for evaluation of this phenomenon. Paired-pulse responses were recorded from the dentate gyrus of rats while stimulating the medial part of the perforant path with different interpulse intervals (IPIs). Iberiotoxin (IbTX), a selective blocker of BK channels, at doses of 50 and 100 µg/kg was i.p. administered 30 min presurgery. The population spike (PS) amplitude ratio and field EPSP (fEPSP) amplitude and slope ratios were measured at IPIs 10, 20, 30, and 50 msec as indices of synaptic facilitation and/or depression. At IPIs of 10 and 20 msec, there was no significant increase in the PS amplitude ratio after IbTX. However, at longer IPIs (30 and 50 msec), there was a significant dose-dependent increase in this ratio vs the vehicle group ( $P < 0.05$  and  $P < 0.01$ , respectively). There were a slight decreases in the fEPSP amplitude ratio at short IPIs (10, 20, and 30 msec) in rats pretreated with IbTX, while mild increases in the fEPSP amplitude ratio were observed at longer IPIs (50 msec). With respect to the fEPSP slope ratio, IbTX dose-dependently and insignificantly increased it. In addition, longer IPIs did not provide significant changes in the fEPSP slope ratio. Thus, high-conductance calcium-dependent potassium channels in the rat dentate gyrus have a modulatory (inhibitory) and (apparently) regulatory role in short-term presynaptic plasticity at relatively long ISIs, and blocking of these channels leads to paired-pulse facilitation.

**Keywords:** high-conductance calcium-dependent potassium channels, iberiotoxin, short-term plasticity, paired-pulse stimulation protocol, hippocampus.

## INTRODUCTION

Potassium channels are known as the most diverse ion channels involved in the regulation of neuronal excitability [1]. These channels pass outward potassium currents, which leads to hyperpolarization of the cell membrane and in this way attenuates the effects of excitatory influences. Since potassium channels reduce the neuronal excitability, they are regarded as inhibitory [1]. Calcium-activated potassium channels form a large family of potassium channels that are found throughout the CNS and are activated following elevation of the level of cytosolic calcium, largely in response to calcium influx through voltage-operated calcium channels open during action potentials (APs) [2]. The involvement of these channels has been reported in

the pathogenesis of some neurological and mental disorders [2]. One kind of these channels known as large (high-)conductance calcium- and voltage-dependent potassium channels (also called BKCa, MaxiK, or BK channels) is widely expressed throughout the nervous system of vertebrates [1]. These channels are activated in response to calcium influx during APs and are responsible for spike repolarization and fast after-hyperpolarization [3]. Due to this, they can regulate the cell excitability and contribute to AP repolarization and spiking frequency adaptation [4]. Immunohistochemical and radioligand binding studies have revealed the presence of BK channels in the membranes of neuronal somata, processes, and axon terminals in several brain structures, including the hippocampus where they are particularly abundant [2]. The hippocampus is a key structure for certain kinds of learning and memory phenomena [5, 6]. Synaptic plasticity in the hippocampus is known to be the neural substrate of the mnemonic processes [5, 6].

As is known, there are several forms of synaptic

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plasticity, including short- and long-term types. Paired-pulse stimulation is a standard technique for evaluation of short-term synaptic plasticity, and its effects in the hippocampus and the mechanisms underlying these effects have been intensely investigated, in particular in rodents [7, 8]. Paired-pulse facilitation (PPF) and paired-pulse depression (PPD) appear in the dentate gyrus of rats in a manner dependent on the stimulation site, stimulus intensity, and interpulse intervals (IPIs) [9]. Although paired-pulse responses in the hippocampus have been well characterized in rodents as an index of short-term plasticity [9], and the role of voltage-gated calcium channels have been reported in this respect [10], there is no information on the involvement of BK channels in modulation of this kind of plasticity in the dentate gyrus. Therefore, our study was designed to evaluate whether iberiotoxin (IbTX, a highly specific inhibitor of these channels) can modulate short-term presynaptic plasticity in the rat dentate gyrus following stimulation of the medial perforant path with different IPIs.

## METHODS

Thirty male Wistar rats (220-250 g) were used. The animals were housed in Plexiglas cages (3-4 rats in each cage) and kept at a temperature of  $22 \pm 2^\circ\text{C}$  with a 12/12 light/dark cycle. Food and water were provided *ad libitum*. All experimental procedures were conducted according to the Guide for the Care and Use of Laboratory Animals of NIH and those of the Tehran University of Medical Sciences (Tehran, Iran).

Rats were i.p. pretreated with iberiotoxin, IbTX (Sigma, USA, 50 or 100  $\mu\text{g}/\text{kg}$ ), 30 min before surgery. Iberiotoxin was dissolved in normal saline. Then, the animals that have received IbTX or vehicle were prepared for electrophysiological experiments. They were anesthetized with 1.5 g/kg of urethane (i.p.) and fixed in a stereotaxic device. Body temperature of the animals was maintained at  $37^\circ\text{C}$  using a homeothermic blanket system. Small holes were drilled in the skull at the positions of the stimulating and recording electrodes. A recording electrode was positioned in the granular cell layer of the dentate gyrus (AP =  $-3.8$ , L = 2.5, V = 2.8 to 3.2 mm from the skull surface with respect to the bregma) according to the coordinates by Paxinos and Watson. A stimulating electrode was positioned at the angular bundle of the medial perforant

path (4.2-4.3 mm lateral to the lambda, depth  $\approx 2.8$  mm). The electrodes were lowered very slowly in order to minimize tissue damage. Final positions of the electrodes were estimated by observing evoked responses. Correct implantation of the electrodes was also confirmed by histological assessment. Field-potential recordings were obtained from the dentate gyrus following stimulation of the medial perforant path. Bipolar stimulating electrodes were made from Teflon-coated stainless steel (AM Systems, USA) with bare tips (diameter 0.125 mm). Stimuli were generated using a constant-current isolated stimulator unit (NPI, Germany). Glass capillary recording electrodes (WPI Instruments, USA) had the resistance of 6-10 M $\Omega$ . Extracellular evoked potentials were preamplified (using an appropriate headstage from NPI, Germany), amplified ( $\times 1000$ ), filtered (100 Hz to 5 kHz bandpass), digitized at  $10^4 \text{ sec}^{-1}$ , and recorded with a differential extracellular amplifier (NPI, Germany). Data were analyzed using customized software.

**Current-Response Relationship.** Single stimuli (100-800  $\mu\text{A}$ , pulse width 50  $\mu\text{sec}$ ) were applied to the medial perforant path. Each current intensity was tested in a randomized order at least three times with 10-sec interstimulation intervals. Evoked responses were recorded and analyzed online. The characteristic field response in the dentate granule cells to perforant path stimulation consisted of a positive fEPSP with a superimposed negative-going population spike (PS). The fEPSP slope (25 to 75% of the initial rising phase) and PS amplitude were measured for each response. In this respect, the PS amplitude was measured as the voltage difference between the peak of the first positive wave and the peak of the first negative deflection.

**Paired-Pulse Protocol.** Pairs of identical stimuli were delivered to the medial perforant path and initiated four consecutive evoked responses to paired pulses at 10-, 20-, 30-, and 50-msec-long interstimulus intervals. The stimulus intensity (typically 800  $\mu\text{A}$ , 50  $\mu\text{sec}$ ) was chosen such that the first evoked PS was maximal. The PS amplitude ratio (second PS amplitude/first PS amplitude qualified as paired-pulse index, PPI) was measured at different IPIs. Values below 100% indicated paired-pulse inhibition (PPD), whereas values above 100% were indicative of PPF. The same measurement was also made for fEPSPs. In addition, the fEPSP slope ratio (second fEPSP slope/first fEPSP slope) was also measured.

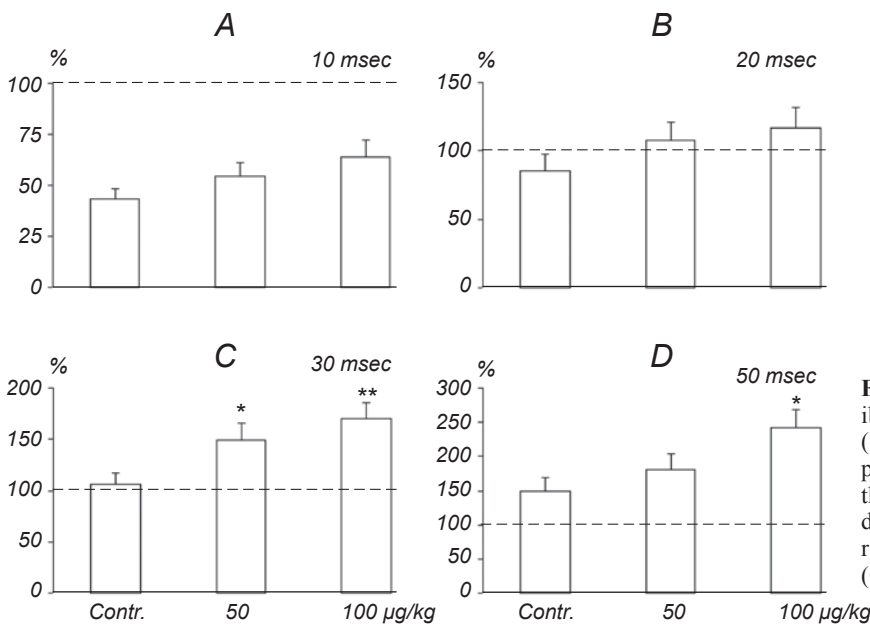
An average value was calculated from three

successive trials with 15-sec-long intervals between consequent trials. The interval at which 50% inhibition of the second PS would occur was calculated for each individual paired-pulse profile for determination of any synaptic changes of neurons of the dentate gyrus and its inhibitory interneurons and to evaluate short-term plasticity.

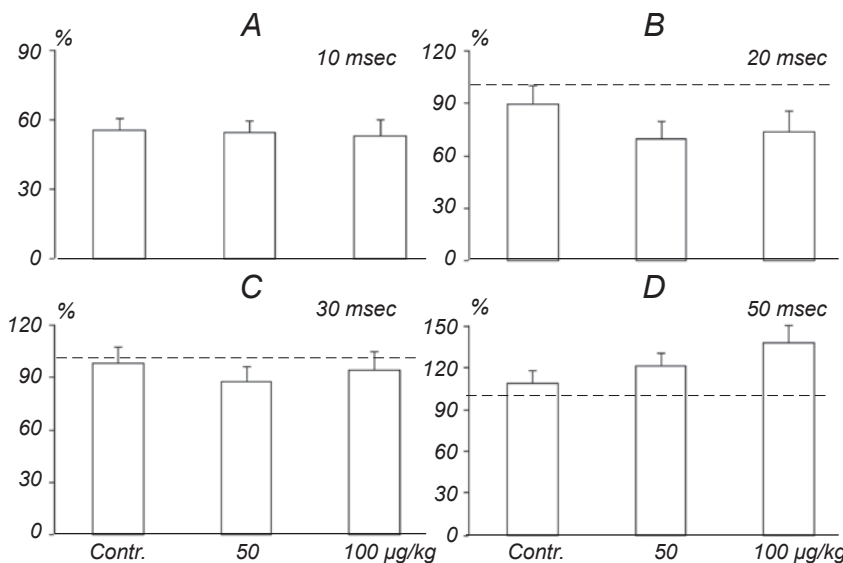
**Statistical Analysis.** All data were expressed as means  $\pm$  s.e.m. and analyzed using one-way ANOVA and the Tukey post-test. *P* values less than 0.05 were considered significant.

**RESULTS**

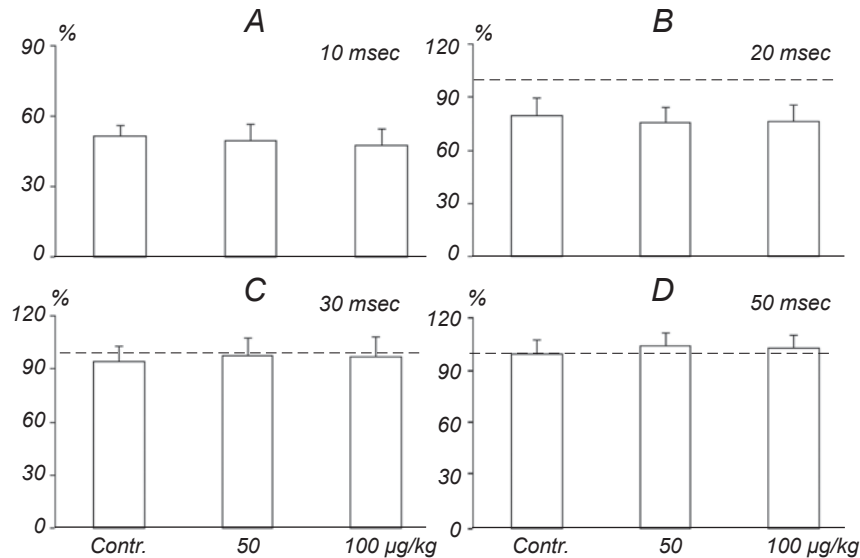
In our study, the effect of IbTX as a blocker of BK channels on short-term synaptic plasticity in the hippocampal dentate gyrus was evaluated. For this purpose, the PPI as an index of presynaptic facilitation or inhibition was determined at different IPIs intervals (10, 20, 30, and 50 msec). In this respect, as is shown in Fig. 1, at IPIs of 10 and 20 msec there were some increased in the above index for PSs (their amplitude ratio; the



**Fig. 1.** Effects of the blocker of BK channels iberiotoxin at doses of 50 and 100 µg/kg (50 and 100, respectively) on the ratio of population spike (PS) amplitudes (%) in the dentate gyrus of the rat hippocampus at different interpulse intervals as shown at the right (A-D). \**P* < 0.05, \*\**P* < 0.01 vs sham (Contr.).



**Fig. 2.** Effects of iberiotoxin (IbTX) at doses of 50 and 100 µg/kg on the ratio of fEPSP amplitudes (%) in the dentate gyrus of the rat hippocampus at different interpulse intervals as shown at the right (A-D). Other designations are the same as in Fig. 1.



**Fig. 3.** Effects of iberiotoxin (IbTX) at doses of 50 and 100 µg/kg on the fEPSP slope ratio (%) in the dentate gyrus of the rat hippocampus at different interpulse intervals as shown at the right (A-D). Other designations are the same as in Fig. 1.

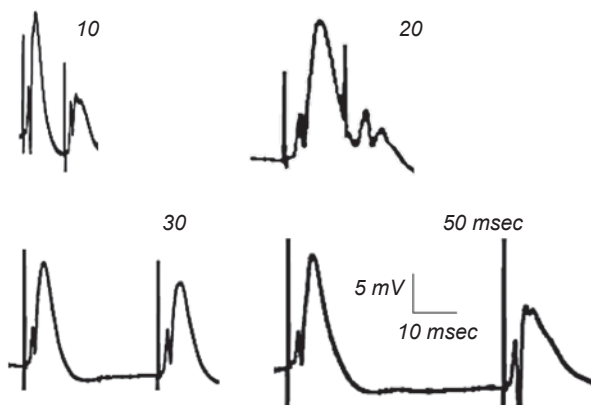
difference, however, did not reach the significance level). However, at longer IPIs (30 and 50 msec), the PPI for PSs was significantly greater after IbTX pretreatment ( $P < 0.05$  and  $P < 0.01$ ), and the observed responses followed a dose-response pattern. In other words, PPF was observed at IPIs of 30 and 50 msec in rats pretreated with IbTX (at doses of 50 and 100 µg/kg), as compared to the vehicle group.

In addition, as is shown in Fig. 2, the fEPSP amplitude ratio was also calculated as another index for presynaptic facilitation or depression. At relatively short IPIs (10, 20, and 30 msec), slightly smaller fEPSP amplitude ratios were found in rats pretreated with IbTX. Thus, mild (insignificant) presynaptic depression was observed under the action of IbTX. In addition, at longer IPIs (50 msec), there were clearly but insignificantly greater fEPSP amplitude ratios, indicating slight presynaptic facilitation.

Regarding the fEPSP slope ratio (Fig. 3), IbTX pretreatment somewhat increased it in a dose-dependent manner at long ISIs, but intergroup differences did not reach the significance level. In addition, an increase in the duration of IPIs did not cause marked changes in the fEPSP slope ratio. Some representative traces of paired-pulse evoked responses after IbTX administration at a dose of 100 µg/kg are demonstrated in Fig. 4.

## DISCUSSION

Following IbTX, there was no significant increase in the PS amplitude ratio at IPIs equal to 10 or 20 msec. However, at longer IPIs (30 and 50 msec), we observed significantly greater PS amplitude ratios vs the vehicle group, and this increase was dose-dependent. Regarding the fEPSP amplitude ratio, there were slight decreases in the fEPSP



**Fig. 4.** Representative traces of paired-pulse evoked responses in the dentate gyrus of the rat hippocampus following stimulation of the medial perforant path at different interpulse intervals after iberiotoxin administration at a dose of 100 µg/kg.

amplitude ratio in rats pretreated with IbTX at short IPIs (10, 20, and 30 msec), while at longer IPIs (50 msec), there was an insignificant increase in this index. With respect to the fEPSP slope ratio, IbTX dose-dependently but insignificantly increased it. In addition, increasing durations of IPIs did not cause significant changes in the fEPSP slope ratio.

The dentate gyrus receives its major input from the entorhinal cortex via the so-called perforant pathway. The respective contacts are localized primarily on the dendrite spines of granule cells, although a small number of perforant path fibers also form asymmetric synapses on the shafts of GABA-positive interneurons [11]. It seems that IbTX inhibits BK channels on glutamatergic terminals of this pathway at longer IPIs and, therefore, provides augmented glutamate release leading to short-term facilitation. In addition, it appears that GABA-ergic neurons have a relatively low density of such channels and, because of this, synaptic facilitation was observed in our study following IbTX treatment. In support of this hypothesis and according to the existing literature, BK channels can preferentially control glutamate release, whereas they appear to exert only a minor influence over GABA release [12]. Although the excitability of granular and pyramidal cells in the hippocampus is believed to be regulated by both feedback and feedforward GABA-mediated mechanisms [10], our results clearly suggest that the blockade of BK potassium channels may increase the total calcium signal in the terminals provoking the enhancement of glutamate release from medial perforant path terminals on cells of the dentate gyrus and, to a lesser degree, intensifying the release of GABA from terminals of GABA-ergic interneurons. Thus, the net effect would be PPF at longer IPIs, which was precisely observed in our study. In addition, according to previous reports, PPF is prominent in synapses with a low initial probability of transmitter release and is characterized by increase in the amount of a neurotransmitter released in response to the second stimulus [10]. Since the fEPSP2/fEPSP1 amplitudes and slope ratios did not significantly change in IbTX-pretreated rats, it is possible that projections of inhibitory interneurons terminate on the somata of cells of the dentate gyrus and, therefore, determine the input/output of the target cells. To conclude, our study showed that BK channels in the rat dentate gyrus have a modulatory (inhibitory) and an apparently regulatory role in short-term presynaptic

plasticity at relatively long interstimulus intervals, and their blockade leads to PPF.

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