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Presence of Depolarization-Induced Suppression of Inhibition in a Fraction of GABAergic Synaptic Connections in Rat Neocortical Cultures

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Brief depolarization of postsynaptic neurons in hippocampus and cerebellum results in a transient depression of GABAergic inhibitory input, called "depolarization-induced suppression of inhibition" (DSI). We studied whether a similar phenomenon occurs in the rat neocortical neurons. Using patch-clamp technique in neocortical cell cultures, we examined the effects of a 5-second depolarization of postsynaptic neurons on evoked GABAergic inhibitory post-synaptic currents (IPSCs). We found that the depolarization evoked a suppression of IPSC amplitude in 6 out of 26 neuronal pairs tested. The suppression of IPSC amplitude lasted for ~70 seconds and was accompanied by changes of paired-pulse ratio and IPSC coefficient of variation (CV), which is suggestive of a presynaptic mechanism. These results are in agreement with previous observations in hippocampal cell cultures and suggest that neocortical neurons express DSI.

KEY WORDS: GABA, synaptic plasticity, depolarization-induced suppression of inhibition, retrograde signaling, endocannabinoids.

GABA is the major inhibitory neurotransmitter in the adult central nervous system (CNS) and plays important roles in several physiological processes including network synchronization and generation of theta and gamma rhythms [2]. Several forms of use-dependent plasticity of GABAergic transmission have been documented. In particular it has been shown that, depending on the pattern of activity of a presynaptic neu ron, a decrease or an increase of the efficiency of GABAergic transmission may occur [8, 17, 22]. On the other hand, activity of the postsynaptic neuron also plays an important role in the short-term plasticity of GABAergic transmission. Postsynaptic spike firing or brief depolarization of the membrane of postsynaptic neuron results in a transient suppression of GABAergic synaptic transmission. This phenomenon termed "depolarization-induced suppression of inhibition" (DSI), was observed in hippocampal CA1 pyramidal cells [15, 16] as well as in Purkinje neurons in cerebellum [11]. Interestingly, DSI in hippocampal cell cultures was observed not only in glutamatergic, but also in GABAergic neurons [14]. Although DSI is triggered by a postsynaptic increase of calcium ion levels, its expression is presynaptic ([1] see also [24] for a review). Recent studies have revealed that endocannabinoid substances play a key role in DSI. These lipid mediators may be released from postsynaptic neurons and, travelling backward across synapses, activate CB_1 receptors on axon terminals and suppress GABA release [6, 13, 23, 24]. Indeed, activity-dependent synthesis and release of endocannabinoids [3, 4] and regulation of GABA release mediated by CB_1 receptors [9, 10] have been well documented. Presence of CB_1 receptors in the neocortex [20] and the presence of endocannabinoids in neocortical neurons [3, 4] suggest that DSI may be also present in neocortex. Presence of DSI has been recently reported in mice neocortical slices [18, 19], It is not known, however, whether rat neocortex is similar in this respect. Additionally, while evidence has been provided that DSI in (mice) neocortex is selectively expressed in a

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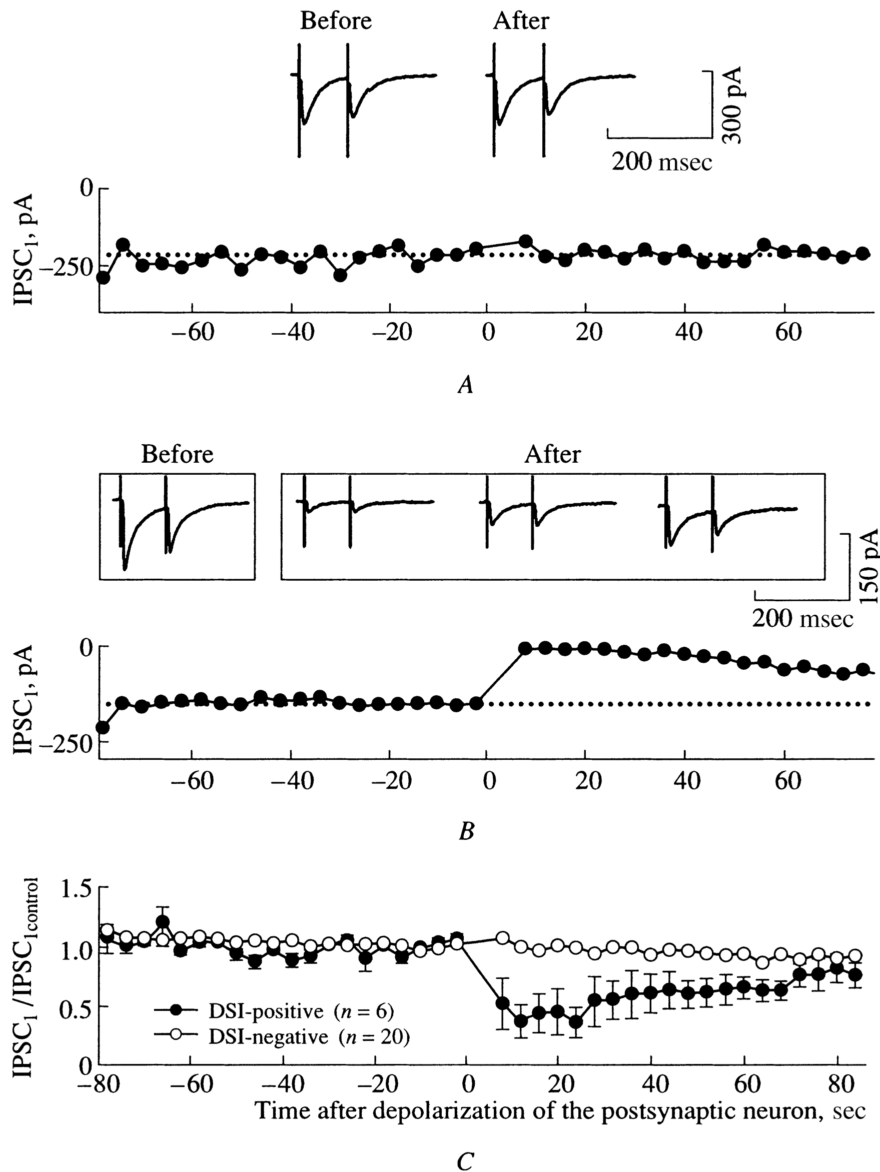

Fig. 1. A fraction of GABAergic connections in neocortical neurons is susceptible to DSI: *A*) an example of a synaptic connection without DSI ("DSI"-negative connection). Upper panel – averages of five sequential traces before and after the depolarization of the postsynaptic neuron. Lower panels – amplitudes of (first in a pair) IPSC plotted as a function of time. Depolarization of the postsynaptic neuron (5 sec) was started at time 0; *B*) an example of DSI in neocortial culture. Original traces represent averages of 5 sequential traces before the depolarization (for 5 sec) of the postsynaptic neuron and averages for time windows 28–44 sec; 48–64 sec and 68–84 sec after the depolarization respectively; *C*) average time courses of IPSC amplitudes in DSI-positive ($n = 6$) and DSI-negative pairs ($n = 20$). IPSC amplitudes in each experiment were normalized to the mean amplitude of 5 IPSC preceding the depolarization of the postsynaptic neuron before pooling.

subpopulation of GABAergic connections [18], this has not been directly demonstrated. The aim of this study is to check whether DSI is present in rat neocortical neurons and to examine directly whether DSI is selectively expressed in a fraction of GABAergic connections. To address these questions, in the present study we used patch-clamp technique to examine the effects of postsynaptic depolarization on efficacy of GABAergic transmission in rat neocortical neurons in culture.

Differences in synaptic properties can be masked when terminals originating from multiple cell types are stimulated simultaneously. Since only a fraction of GABAergic connections is susceptible to DSI in hippocampus (see [24] for a review), it is crucial to study responses evoked by the

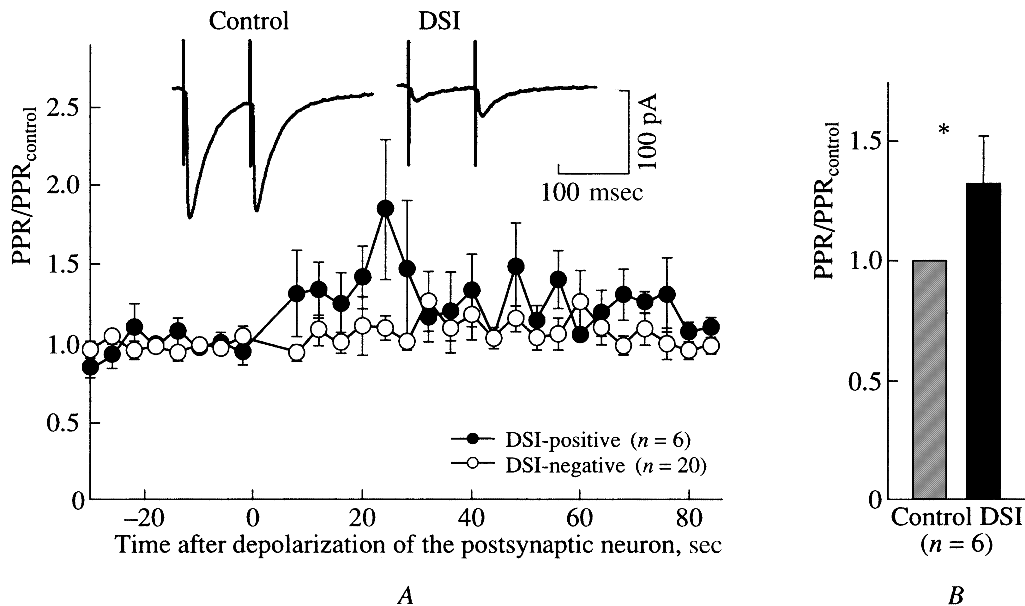

Fig. 2. DSI in neocortical cultures is accompanied by changes of paired-pulse ratio: *A*) upper panel – example of original traces representing averages of 5 sequential traces before and after the depolarization of the postsynaptic neuron for 5 sec; lower panel – average time courses of paired-pulse ratios ($IPSC_2/IPSC_1$) in DSI-positive ($n = 6$) and DSI-negative pairs ($n = 20$). PPRs in each experiment were normalized to the mean value of this parameter for 5 traces preceding the depolarization of the postsynaptic neuron; *B*) graph summarizing changes of paired-pulse ratios observed in the above-mentioned experiments. Asterisk indicates statistically significant difference ($^*p < 0.05$) as compared to respective control.

stimulation of a single neuron. We therefore used cultures of neocortical neurons, a preparation which allowed us to study relatively easily responses evoked by the stimulation of a single presynaptic neuron.

METHODS

Cell cultures were prepared as described previously [17] except neocortex was used for preparation. All cultures were kept at 36°C in humidified air with 5% CO_2 and were used for the experiments 14–22 days after plating. Unless otherwise noted, relatively low-density areas of coverslips with cultured cells (2–5 neurons in 400 μm diameter view-field) were selected for the experiments. Synaptic responses were evoked by applying voltage pulses (0.2–1 msec, 20–100 V) to an extracellular electrode (a patch electrode filled with the extracellular solution) positioned in the vicinity of the presynaptic neuron soma or neurite. Such an approach allows local ("down to" a single synaptic bouton) extracellular stimulation [5]. Whole-cell patch-clamp technique was employed to record responses (IPSCs) from postsynaptic neurons. Slow (as compared to glutamatergic) evoked responses were assumed to be mediated by $GABA_A$ receptors since they reversed near the chloride equilibrium potential (this was checked at the beginning of each experiment) and were blocked by bicuculine (10 μM). Experiments were done at room temperature (20–22°C). The intracellular solution contained (in mM): Cs gluconate 100, CsCl 30, $MgCl_2$ 4, Na_2ATP 4, EGTA 5, N[2-hydroxyethyl]piperazine-N′-[2-ethane-sulfonic acid] (HEPES) 10. The extracellular solution contained (in mM): NaCl 140, KCl 4, $CaCl_2$ 2, $MgCl_2$ 1, HEPES 10, glucose 10; pH of all solutions was 7.4. 10 μM of 6-cyano-7-nitroquinoxaline-2.3-dione (CNQX) and 50 μM of DL-2-amino-5-phosphonovaleric acid (APV) were added to extracellular solution to block ionotropic glutamate receptors to study pharmacologically isolated GABAergic responses. All chemicals were obtained from Sigma (USA). All potentials indicated were corrected for liquid junction potentials as suggested earlier [12].

Digitized currents were analyzed using ANDATRA software kindly provided by Yaroslav Boychuk (A. A. Bogomoletz Institute of Physiology, Kiev, Ukraine). The data are presented as a mean ± *S.E.M.*

RESULTS AND DISCUSSION

Briefly, we stimulated presynaptic neuron extracellularly with a pair of pulses (interstimulus interval – 100 msec) every 4 sec. Recordings were made from postsynaptic neurons voltage-clamped at membrane potentials –75 to –70 mV. After at least 20 control sweeps, the postsynaptic

neuron was depolarized to –10 mV for 5 sec and 20–40 more sweeps were collected. To quantify the suppression of synaptic currents evoked by the depolarization of the postsynaptic neuron, the mean amplitude of five consecutive IPSCs following the depolarization was expressed as a fraction of five IPSCs before the depolarization. As suggested previously [14], connections were considered as "DSI" positive when more than 15% suppression was observed. Using this experimental protocol, we studied 26 neuronal pairs. No IPSC decrease following the depolarization of the postsynaptic neuron was observed in 20 pairs (e.g., Fig. 1, *A*); DSI was observed in 6 (Fig. 1, *B*). In 2 out of 6 pairs with DSI, duration of IPSC amplitude decrease was greater than 80 sec (Fig. 1, *B*); full restoration of the IPSC amplitude was observed in 4 (not illustrated). On average, duration of DSI in our experiments also appeared to be greater than 80 sec (Fig. 1, *C)*. However, a comparison of DSI time course with time-dependence of IPSC amplitude in DSI-negative pairs (Fig. 1, *C*) suggests that actually DSI duration is shorter: about 70 sec. Therefore, the duration of DSI in our experiments is comparable to that of DSI in hippocampal cell cultures [14]. Usually, but not always, pronounced changes of pair–pulse ratio (PPR) were observed during DSI (Fig. 2, *A*). On average PPR during DSI increased by 32 ± 20% ($n = 6$) as compared with PPR before DSI induction (Fig. 2, *B*). The increase of PPR was statistically significant ($p < 0.05$; Wilcoxon signed rank test). Thus, our results are in agreement with previously observed changes of PPR during DSI in hippocampal cell cultures [14]. In hippocampal cultures, changes of the quantal content affected PPR [14, 22], while changes of membrane potential in the postsynaptic neuron or partial block of $GABA_A$ receptor did not [13, 14, 22]. Assuming that the origin of PPR changes in neocortical and hippocampal GABAergic synapses is similar (i.e., presynaptic), our results support a presynaptic origin of DSI in neocortex.

To further confirm involvement of presynaptic mechanisms in DSI in neocortical neurons, we also calculated and compared coefficient of variations (CV) of IPSC amplitude before and during depolarization-induced suppression of inhibition. The IPSC amplitude during DSI is not stationary and this may result in an overestimate of IPSC CV. To minimize possible error due to the non-stationarity, the following approach was used. In each experiment, DSI period was fitted by a linear function using 'least square' method. Then, variance of 'fitted IPSCs' was subtracted from the variance of the measured ones:

$$\text{VarianceIPSC}_{\text{corrected}} = \text{VarianceIPSC}_{\text{measured}} - \text{VarianceIPSC}_{\text{fitted}}.$$

After this, $CV_{\text{corrected}}$ was calculated as

$$SD_{\text{corrected}}/IPSC_{\text{mean}}.$$

Then results for 6 pairs with DSI were normalized and pooled. We found that during DSI IPSC CV is increased to 344 ± 122% ($n = 6$) as compared with IPSC CV before DSI induction. The increase of IPSC CV was statistically significant ($p < 0.05$; Wilcoxon signed rank test).

CONCLUSION

We found that the depolarization of the postsynaptic neuron evokes transient decrease of IPSC amplitude in a fraction (~23%) of rat neocortical connections. Considering similarities of DSI in rat neocortical and hippocampal cell cultures, and presence of CB_1 receptors [20] and endocannabinoids in neocortex [3, 4], we hypothesize that DSI in rat neocortex is also due to endocan-nabinoid-mediated retrograde signaling. This hypothesis is strongly supported by recent observations that endocannabinoids are involved in DSI in *mice* neocortical slices [18, 19]. Nevertheless, considering that involvement of other retrograde messengers in synpatic plasticity in neocortex has also been reported [21, 7], further experiments are required to test this hypothesis directly in *rat* neocortical neurons.

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