Insulin-Like Growth Factor I Modifies Electrophysiological Properties of Rat Brain Stem Neurons

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INTRODUCTION

Insulin-like growth factor I (IGF-I) is a circulating polypeptide hormone structurally related to insulin that mediates many of the effects of growth hormone in the body (Jones and Cleemans 1995). IGF-I, like other growth factors, also plays an important role in the developing brain (Anlar et al. 1999). In addition, it has an extraordinary variety of effects on the adult brain (Torres-Aleman 1999). Although brain cells synthesize IGF-I, recent evidence indicates that blood-borne IGF-I crosses the blood barrier and modulates brain function in a pleiotropic fashion (Torres-Aleman 1999). IGF-I, like other growth factors, also plays an important role in the developing brain (Anlar et al. 1999). In addition, it has an extraordinary variety of effects on the adult brain (Torres-Aleman 1999).

METHODS

In vitro experiments

Experiments were performed on sagittal brain slices from 14-day-old Wistar rats, following standard procedures described in detail previously (Nuñez and Buno 1999). Briefly, animals were anesthetized with an intraperitoneal injection of pentobarbital (35 mg/kg) and decapitated immediately after disappearance of the pinch reflex. The brain was rapidly removed and submerged in a vial containing cold (4°C) artificial cerebrospinal fluid (ACSF). The ACSF composition was as follows (in mM): 124 NaCl, 2.69 KCl, 1.25 KH2PO4, 2 MgSO4, 26 NaHCO3, 2 CaCl2, and 10 D-glucose. ACSF was main-

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tained at pH 7.4 by bubbling with carbogen (95% O₂–5% CO₂).

Sagittal slices (300–400 μm) were cut with a vibratome and incubated at room temperature. Slices containing the DCN were transferred to a recording chamber placed on an inverted microscope stage and maintained at room temperature.

Cells were impaled with micropipettes filled with potassium acetate (3M; 90–120 MΩ). Signals were amplified through an Axoclamp 2B (Axon Instruments), and the traditional bridge current-clamp method was employed. Synaptic responses were evoked by stimulating dorsal column (DC) fibers with bipolar nichrome electrodes (80 μm). Electrical stimulation was performed by means of a Grass stimulator (0.1–0.3 ms and 0.2–5.0 V). In several experiments, 4-aminoypyridine (4-AP), tetrodotoxin (TTX), and PD 98095 were applied in the bath medium. All chemicals were purchased from Sigma-Aldrich, except IGF-I, which was from GroPep and PD 98095 from Calbiochem. Data were low-pass filtered at 3 kHz, stored with a PCM-videocassette recorder and analyzed in a Pentium-based PC computer through a TL-1 DMA interface (sampling frequency 6–12 kHz).

Patch clamp recordings

Whole cell patch-clamp recordings were obtained from DCN neurons by using patch pipettes pulled from borosilicate glass. Patch electrodes had resistances of 6–10 MΩ when filled with the internal solution that contained (in mM) 100 KMeSO₄, 50 KCl, 10 HEPES, and 4 ATP-Na₄, pH 7.3. Recordings were obtained with an Axoclamp-2A amplifier in either the current-clamp bridge mode or the continuous single-electrode voltage-clamp mode. Signals were fed to a Pentium-based PC computer through a DigiData 1320 interface board (Axon Instruments). Current records were low-pass filtered at 3 kHz. The pClamp 8 software (Axon Instruments) was used for stimulus generation and for data display, acquisition, and storage. To record \( I_M \) cells, signals were voltage-clamped at −90 mV in the presence of TTX (1 μM) to block Na⁺ currents. Capacitance transients and linear leak currents have been subtracted using an on-line subtraction protocol.

In vivo procedures

Data were obtained from 25 urethan-anesthetized (1.6 g/kg ip) and 12 ketamine hydrochloride/xylazine-anesthetized (100 mg and 20 mg/kg ip, respectively) young adult Wistar rats of either sex, weighing 180–250 g. Animals were placed in a stereotactic device, and the body temperature and end-tidal CO₂ concentration were controlled. To record the electroencephalogram (EEG), a macroelectrode (nichrome electrode; 120 μm diam) was lowered 1.5 mm from the cortical surface into the frontal lobe. The EEG was filtered between 0.3 and 30 Hz and was continuously monitored in the oscilloscope. Supplemental doses of the anesthetic were given when a decrease in the amplitude of the EEG slow waves was observed. To obtain single-unit recordings, the cisterna magna was opened to introduce tungsten microelectrodes (0.3–3 kHz), and the cisterna magna was opened to introduce tungsten microelectrodes at the depth of 100° angle over the surface of the nucleus (Panetsos et al. 1997; Paxinos and Watson 1998). Extracellular recordings were filtered (0.3–3 kHz), amplified and fed to a Macintosh computer (10-kHz sample frequency) for off-line analysis. The unit activity was extracted from the extracellular records using a window discriminator. Once single neurons were isolated, their cutaneous receptive fields (RFs) were mapped by a small hand-held brush while listening to their amplified neuronal discharges over an acoustic speaker. RFs were defined by the limits at which these stimuli elicited changes in the unit discharge pattern. Precise cutaneous stimulation was performed by an electronically controlled solenoid with a polyethylene probe 1 mm in diameter that induced <0.5 mm skin deflection. Stimulation consisted of 20-ms stimulation delivered at 0.5 Hz and was directed to a small area of the fore- or hindlimb. Two equal cutaneous stimulations (conditioning and test stimuli) were delivered on the RF with two different delays (50 and 100 ms) between them. As a measure of the inhibition, the decrease of the response to the test stimulus was calculated for each cell as the number of spikes elicited by 20 test stimuli divided by the number of spikes elicited by the previous 20 conditioning-stimuli delivered in the same place. Intracarotid injection of IGF-I (10 μg/rat in 100 μl saline) was performed through a cannula placed in the common carotid artery. PD 98095 was injected into the DCN through a microsyringe (500 ng/μl; 5 μl). Control animals received saline infusions. Summed peristimulus time histograms (PSTHs) were calculated off-line with Spike 2 software (Cambridge Electronic Design) running on a Macintosh computer, using 2 ms bins.

A paired t-test was applied in vivo and in vitro experiments and differences were considered statistically significant when \( P < 0.01 \). All data are shown as means ± SE. All experiments were carried out in accordance with the European Communities Council Directive (86/609/EEC).

RESULTS

IGF-I modifies the electrophysiological properties of DCN cells

Intracellular recordings with stable membrane potentials \( (V_m) \) and overshooting action potentials were obtained in brain stem slices from 32 DCN neurons. In a previous report using similar techniques, two types of dorsal column nuclei cells were defined based on their membrane properties and morphological characteristics (Núñez and Buñó 1999). Because IGF-I effects were similar in both types of neurons, all of the DCN neurons were grouped together in this analysis. Cells showed a stable resting \( V_m \) of −70.2 ± 1.1 mV and an input resistance of 42.3 ± 3.5 MΩ and fired spikes with mean amplitude of 73.5 ± 1.8 mV when depolarized above their threshold. Addition of IGF-I (10 μM; 1 μM; final concentration: 10 mM) to the slice bath induced a sustained depolarization of 2–5 mV and spike firing, with a latency of 30–60 s and duration of 30 to 50 min in 22 of 27 neurons (81%; Fig. 1, A and B). The peak amplitude of the depolarization had a mean value of 3.5 ± 0.9 mV measured at the resting \( V_m \) (n = 22). No differences were found in the amplitude of the IGF-I evoked depolarization at \( V_m \) between −90 and −50 mV (Fig. 1C). Intracarotid injection of short-lasting hyperpolarizing pulses was used to monitor the IGF-I effect on input resistance (Fig. 1, A and B). The evoked depolarization was accompanied with a statically significant increase in the input resistance (48.8 ± 3.9 MΩ; \( P = 0.005; n = 22 \) ), as is observed in the average of hyperpolarizing pulses (n = 6) delivered at the same \( V_m \) (Fig. 1D, left). Increased input resistance was also evidenced by an increase in hyperpolarizing pulse rebounds that evoked action potentials (Fig. 1D, right). The IGF-I actions reported here were insensitive to TTX (1 μM), which, however, blocked spike firing and therefore synaptic transmission. In the presence of TTX in the recorded bath, the mean peak amplitude of the depolarization induced by IGF-I was 3.1 ± 1.3 mV measured at the resting \( V_m \) (n = 4). Consequently, IGF-I acted at postsynaptic membrane sites.

In the presence of IGF-I, depolarizing current pulses evoked...
larger voltage deflections and increased the spike-firing rate as compared with control conditions (Fig. 2A). This increment of excitability was due to a decrease in the delay to the first action potential and an increase in the repolarizing slope that followed the postspike afterhyperpolarization (Fig. 2B). No statistically significant differences were found in the spike width (0.7 ± 0.05 vs. 0.7 ± 0.08 ms) or threshold (−53 ± 0.19 vs. −54 ± 0.11 mV) after addition of IGF-I. Some cells displayed a repolarizing sag during hyperpolarizing current injection, as the neuron shows in Fig. 2A. This repolarizing sag is due to the activation of the Na⁺- and K⁺-mediated Q current (Núñez and Buño 1999). Addition of IGF-I did not modify the amplitude of repolarizing sag. A representative sample of 10 DCN neurons displayed an average V-I relationship, which illustrated the steady-state voltage responses as a function of injected current intensity, as is shown in Fig. 2C. IGF-I increased the input resistance of this DCN cell population. Under IGF-I, the mean firing rate measured during the injection of 200-ms depolarizing pulses increased and was statistically significant at intensities of 0.3 and 0.6 nA (n = 22; P < 0.005; Fig. 3A). The instantaneous frequency was calculated to study the spike firing adaptation during the injection of those depolarizing current pulses. The spike firing adaptation was not modified under IGF-I (Fig. 3B). Interestingly, IGF-I induced an increase
of the instantaneous frequency of the first spikes; however, after spike adaptation the instantaneous frequency was not modified by IGF-I.

Depolarizing pulses, applied at different hyperpolarization levels, displayed a slow $V_m$ increment that delayed action potential firing. This effect was voltage dependent and increased with the level of hyperpolarization (Fig. 4A, control). Application of 4-AP (200 µM, $n = 4$) to the bath blocked both the slow return to the resting $V_m$ and the delayed action potential firing (Fig. 4A, 4-AP). This indicates the existence of a transient K$^+$-mediated A current ($I_A$), as previously described (Núñez and Buño 1999). Application of IGF-I produced a similar, but smaller, effect to 4-AP application in 10 of 12 neurons (Fig. 4B). Moreover, in presence of 4-AP (200–500 µM, $n = 5$) in the bath, IGF-I evoked a sustained depolarization of smaller amplitude than the depolarization evoked by IGF-I in absence of 4-AP (peak amplitude of 2.3 ± 0.6 mV; $n = 4$); however, differences were not statistically significant and the firing rate during the injection of depolarizing current pulses was not increased (data not shown). This result suggests that IGF-I could have increased the neuronal excitability of DCN cells by reducing the $I_A$.

The effect of IGF-I on the $I_A$ was examined using the patch- and voltage-clamp techniques. Recordings ($n = 6$) were performed after the addition to the recording bath of 1 µM of TTX to block sodium channels. From a holding −90 mV to remove the current inactivation, the $V_m$ was stepped to a test potential of +20 mV preceded by a variable 50-ms depolarizing prepulse (from −90 to +20 mV). In this condition, a fast-transient outward current was elicited, which completely decayed within the first 20–40 ms (Fig. 5A). Peak amplitude of the outward current was measured, and the average of the six cells was plotted versus prepulse potential after they were normalized (inactivation curve). To determine the activation curve, the $V_m$ was stepped to a test potential (-90 to +20 mV) by a 50-ms depolarizing pulse from a holding potential of −90 mV, and the average of the six cells was plotted versus the holding potential after they were normalized. Both activation and inactivation curves of the outward current (Fig. 5B) together with the fact that it was blocked by the application of 200 µM 4-AP to the recording bath (Fig. 5C) strongly suggest that it was an $I_A$ current. Averages of five voltage steps displayed that $I_A$ was decreased under IGF-I (Fig. 5C). Measure of the normalized peak amplitude of the $I_A$ elicited by a voltage step from −90 to +20 mV displayed a significantly decreased the $I_A$, which was completely blocked by 4-AP. A slow, 4-AP-resistant current was also observed that resembled the delayed rectifier potassium conductance. It was also distinguished from the $I_A$ because it displayed a slower activation. The delayed rectifier potassium conductance was not significantly modified by IGF-I (data not shown).

**Effect of the IGF-I on the synaptic responses of DCN cells**

In addition, we analyzed the effects of IGF-I on the synaptic responses of DCN cells. The characteristics of the excitatory postsynaptic potential (EPSP) evoked by dorsal column (DC) stimulation (DC-EPSP) are mediated by the activation of glutamatergic, non-N-methyl-D-aspartate (NMDA) receptors, as described in detail previously (Núñez and Buño 1999, 2001). Under control conditions, the EPSPs elicited by DC stimulation had a mean latency of 2.3 ± 0.2 ms and a mean duration of 10.6 ± 2.3 ms when the intensity of the stimulus was twice the threshold to evoking the EPSP (Fig. 6A, control). Addition of IGF-I to the recording bath (10 µl, 1 µM) induced, in 78% of the cells tested (14 of 18 neurons), an increase in DC-EPSP peak amplitude (from $6.7 ± 0.8$ mV in control conditions to $8.4 ± 1.0$ mV after IGF-I; $P = 0.008; n = 14$) and in the rising
slope of the DC-EPSP, measured at the resting \( V_m \) (from 2.8 ± 0.27 V/s in controls to 3.7 ± 0.29 V/s after IGF-I; \( P = 0.003; n = 14 \); Fig. 6A). This increase in DC-EPSP enhanced the probability of eliciting spike firing. However, no differences were observed in the mean latency and duration of the DC-EPSP after IGF-I application (2.5 ± 0.4 and 12.8 ± 3.1 ms, respectively).

To test if IGF-I has a presynaptic effect, a paired-pulse protocol was used. We had previously observed that the depression of the second DC-EPSP after paired-pulse stimulation of DC fibers at delays shorter than 50 ms was probably mediated by a presynaptic mechanism (Nunéz and Buño 2001). A 10–25% depression of the second DC-EPSP was found in all cells tested under basal conditions (\( n = 10; \) Fig. 6B, thin traces). Under IGF-I, the depression of the conditioned DC-EPSP decreased (8 out of 10 neurons; Fig. 6B, thick traces).

Plotting the mean area of the test DC-EPSP relative to the conditioned DC-EPSP area, as a function of the paired-pulse interval, showed that at 20 ms delays IGF-I significantly reduced the depression of the test DC-EPSP (Fig. 6B, plot; \( P = 0.008; n = 8 \)), suggesting that IGF-I has a presynaptic effect on DCN neurons.

Because a major intracellular pathway activated by IGF-I involves MAPK, a Ser-kinase that is also implicated in modulation of \( K^+ \) currents (Aimond et al. 2000), we analyzed whether p38 mitogen-activated protein kinase (MAPK) activation mediated the effects of IGF-I on DCN cells. Indeed, MAPK inhibition with the MAPK kinase (MEK) inhibitor PD 98059 blocked the effects of IGF-I. Adding of PD 98059 (10 \( \mu \)l; 50 \( \mu \)M; final concentration 0.5 \( \mu \)M) to the recording bath blocked the depolarization and decreased the enhanced excitability of DCN neurons as well as inhibited the reduction in the \( I_A \) current, all of which were evoked by IGF-I (\( n = 6; \) Fig. 7, A and B). Moreover, it also reduced the amplitude of the EPSP by 5–10% in DCN neurons previously activated by IGF-I (\( n = 5 \); Fig. 7, B and C) and abolished the effects of subsequent applications of IGF-I on DCN neurons.

**IGF-I increases the receptive field of somatosensory neurons in the DCN**

To determine if the increased excitability evoked by IGF-I in in vitro preparations has functional implications in the response of DCN neurons to natural stimuli, we performed extracellular in vivo recordings in 24 units (18 from the gracilis and 6 from the cuneatus nucleus) of rats anesthetized with either urethan or ketamine-xylazine. As expected from previous observations (Carro et al. 2000), intracarotid injection of IGF-I (10 \( \mu \)g per rat of IGF-I) increased both the spontaneous activity as well as the spikes evoked by cutaneous stimulation in 83% of the cells tested (20 of 24 cells; compare upper PSTHs in Fig. 8A). In these 20 neurons affected by systemic injection of IGF-I, spontaneous activity significantly increased from 1.3 ± 0.38 to 2.8 ± 0.57 spikes/s (\( P = 0.008 \)). Moreover, cutaneous stimulation induced a mean of 1.02 ± 0.15 spikes per stimulus, and this increased to 2.2 ± 0.31 spikes per stimulus 15 min after of IGF-I injection (\( P = 0.005; n = 20 \)). This stimulatory effect of IGF-I was accompanied by an increase in the extension of the RF of DCN neurons because a wider contiguous area in the skin elicited spike responses (Fig. 8A; compare lower post-
stimulus histograms and see also the increase of the RF area shown in the inset). Both the increase in evoked spikes and the RF expansion were evident 5 min after the injection of IGF-I and lasted for 30–40 min. After that time, the spontaneous activity and the spikes evoked by cutaneous stimulation, as well as the RFs, returned to the control values (1.5 ± 0.47 and 1.18 ± 0.18 spikes per stimulus, respectively).

We tested whether the reorganization evoked by IGF-I involved changes in inhibitory processes within the DCN using a two-stimulus conditioning-test paradigm consisting of two identical stimuli (conditioning and test stimuli) delivered to the same location in the RF with two different delays (50 and 100 ms). After calculating the pair-pulse inhibition, no statistically significant effects were seen with IGF-I, either with 50- or 100-ms delays (Fig. 8B; n = 12).

Because it has been demonstrated in vitro that inhibition of MAPK with the MEK inhibitor PD98059 diminished or abolished the effects of IGF-I, we also studied the effect of the injection of PD98059 into the DCN. This drug was injected into the DCN (500 ng/μl; 5 μl) 20 min before intracarotid injection of IGF-I (10 μg/rat). Unit recordings were performed in an area 500 μm away from the injection site, and cutaneous stimuli were delivered at their RF. Under PD98059, DCN neurons responded to sensory stimulation, as can be observed in the PSTH shown in Fig. 9 (left). In this situation, intracarotid injection of IGF-I (10 μg/rat) did not increase the RF (n = 5). Moreover, neither spontaneous activity (1.1 ± 0.38 spikes/s under PD98059, to 0.9 ± 0.57 spikes/s after IGF-I) nor the mean spikes evoked by the cutaneous stimulation (1.1 ± 0.38 spikes per stimulus under PD98059, to 1.3 ± 0.27 spikes per stimulus after IGF-I; Fig. 9, middle) were significantly affected. PD98059 effect lasted for ~90 min, and after that time, a new intracarotid injection of IGF-I increased the spontaneous activity (3.1 ± 0.43 spikes/s) and the sensory responses (2.1 ± 0.21 spikes/stimulus) in DCN neurons (n = 6; Fig. 9, right). Saline injection into the DCN (5 μl) did not modify spontaneous activity (1.3 ± 0.69 spikes/s), sensory responses (1.12 ± 0.11 spikes per stimulus), or the facilitation evoked by IGF-I (n = 5).

**DISCUSSION**

Neurotrophic factors, particularly the neurotrophins, have recently been shown to act as rapid and potent modulators of neuronal activity in the adult brain (Thoenen 2000). Our results indicate that yet another neurotrophic factor, IGF-I, also mod-
ulates the excitability of central neurons. Analysis of the cellular mechanisms that underlying the stimulatory effects of IGF-I on DCN neurons indicates that this trophic factor modifies their intrinsic electrophysiological properties and facilitates their synaptic responses. Furthermore, in vivo analysis revealed that on systemic administration to adult rats, IGF-I

![Image](https://example.com/image1.png)

**FIG. 6.** IGF-I increases the amplitude and the efficiency of the dorsal column (DC)-excitatory postsynaptic potential (EPSP). A: electrical stimulation of DC fibers evoked EPSPs in DCN neurons (control). With IGF-I in the recording bath, the DC-EPSP amplitude increased and favored the generation of action potentials. B: paired DC-EPSP averages ($n = 4$) showed a depression of the 2nd DC-EPSP at stimulation intervals of 20 and 50 ms (top and bottom, respectively) under control conditions (thin traces). After IGF-I bath application, the DC-EPSP amplitude increased and the depression of the 2nd EPSP diminished (thick traces). Plot of the ratio between the area of test vs. conditioned EPSPs revealed a smaller depression of the test stimuli under IGF-I at 20-ms delay that was not observed at 50-ms delay (C in the plot indicates control values; *, $P = 0.008$).

![Image](https://example.com/image2.png)

**FIG. 7.** The MEK inhibitor PD98059 partially blocks the effects of IGF-I on DCN cells. A: in presence of PD98059 in the recording bath (10 μl; 50 μM), application of IGF-I (10 μl; 1 μM, vertical arrow) did not modify $V_m$. B: addition of PD98059 to the bath decreased the enhanced excitability of DCN neurons evoked by IGF-I. C: PD98059 reduced the amplitude of the DC-EPSP in DCN neurons previously activated by IGF-I.
increased the excitability and RF of these brain stem neurons probably by means of the mechanisms demonstrated in vitro. These observations confirm and extend previous findings indicating that IGF-I increases the spontaneous firing rate as well as the response to afferent stimulation in target neurons, including DCN cells (Carro et al. 2000). Together with the anti-apoptotic and the antiinflammatory effects of IGF-I on nerve tissue, enhancement of neuronal excitability evoked by IGF-I may contribute to the known neuroreparative effects of IGF-I (Torres-Aleman 2000). Indeed, recent evidence indicates that increased neuronal activity after neuronal damage contributes to functional recovery (Al Majed et al. 2000).

Our results suggest that increased excitability of DCN neurons after IGF-I is, at least, partially mediated through blockade of a K+mediated $I_A$ current, as has been demonstrated here by voltage-current studies using patch-clamp recordings. Although only 14% of the $I_A$ is activated at rest, the blockade of $I_A$ would induce a modest depolarization, as is induced by IGF-I. However, we cannot exclude that other mechanisms may be involved in the increased excitability of DCN neurons evoked by IGF-I. In this regard it has recently been shown that increased excitability of midbrain neurons after exposure to another growth factor, glial derived growth factor, also involves inhibition of the $I_A$ current (Yang et al. 2001). Although IGF-I induced a long-lasting depolarization of the $V_m$ of DCN cells together with a moderate increase in their firing rate (see FIG. 8).

**FIG. 8.** Systemic IGF-I increases sensory responses and the cutaneous receptive field (RF) of DCN neurons recorded in vivo. A: poststimulus time histograms (PSTHs) of cutaneous stimulation responses (20 stimuli of 20-ms duration applied on the RF) in control and after IGF-I intracarotid injection are shown. Responses to cutaneous stimulation applied at site 1 of the RF (see inset) increased after IGF-I and unmasked a new RF (site 2, hatched area in the cutaneous RF representation). Zero references in PSTHs indicate sensory stimulation onset (vertical arrows). Insets: raw data of neuronal responses in control and after IGF-I intracarotid injection (10 μg/rat) and a diagram of the RF in the hindpaw under control condition (solid area), and after intracarotid injection of IGF-I (hatched area) the RF was enlarged and the magnitude of the neuronal response increased. B: PSTHs of paired-pulse cutaneous stimulation (20 stimuli of 20-ms duration applied on the RF; 2-ms bin size) at 50- and 100-ms delays (top and bottom, respectively) and plot of the response ratio between the test vs. conditioned stimuli when paired pulses were delivered at 50- and 100-ms delays. No differences were observed between control and IGF-I injected rats. Control values (C, open bars) and at 10, 20, and 30 min after IGF-I injection are shown.

**FIG. 9.** PD98059 blocks the sensory response facilitation evoked by IGF-I in vivo. PSTHs (2-ms bin size) obtained after PD98059 injection in the gracile nucleus (PD98059), after intracarotid injection of IGF-I, 20 min after PD98059 application (PD98059 + IGF-I, after 20 min), and after a new application of IGF-I, 90 min following PD98059 application (PD98059 + IGF-I, after 90 min). PD98059 blocked the facilitatory effect described for IGF-I. However, the inhibition of the IGF-I effects by PD98059 was transient; 90 min after the initial application of PD98059 the stimulatory effects of IGF-I were recovered. PSTHs show sensory responses (20 stimuli of 20-ms duration applied on the RF) from the same neuron. Insets: raw data.
been shown to inhibit a subtype of kainate receptors have been demonstrated in DCN cells responses. Indeed, a kainate component for EPSP in DCN neuronal excitability that could involve other K⁺-mediated currents. The I₄ current has been described in dendrites of hippocampal pyramidal cells, where it controlled the generation of dendritic action potentials and altered the shape of the EPSPs (Hoffman et al. 1997). Thus IGF-I will facilitate the possibility of both remote dendritic EPSPs reaching the soma and inducing spikes.

Numerous studies indicate that IGF-I modulates membrane other ion channels, for instance Ca²⁺ and K⁺ channels (Blair and Marshall 1997; Fadool et al. 2000; Kanzaki et al. 1999; Sakagami et al. 1999) or ionotropic receptors such as those of glutamate, GABA, or cyclic nucleotides (Gonzalez de la Vega et al. 2001; Man et al. 2000; Savchenko et al. 2001; Wan et al. 1997). It is thus possible that in DCN cells I₄ is modulated by IGF-I, perhaps through a phosphorylation-dependent pathway. In this sense, the MAPK pathway appears to be a likely candidate because inhibition of this pathway blocks the stimulatory effects of IGF-I on DCN cells both in vitro and in vivo. Accordingly, MAPK activation by IGF-I on target cells is a very common step in IGF-I signaling (LeRoith 2000), and MAPK is also known to modulate other K⁺-mediated currents (Aimond et al. 2000).

Previous studies have shown that DC-EPSP is caused by activation of non-NMDA glutamatergic receptors and that it lacks an NMDA component (Nuñez and Buño 1999, 2001). Recently, it was shown that IGF-I decreases AMPA-mediated currents in cerebellar Purkinje neurons without affecting NMDA-mediated currents (Wang and Linden 2000; see also Man et al. 2000). However, IGF-I potentiates kainate-mediated responses in the same cell type (Gonzalez de la Vega et al. 2001). Consequently, the IGF-I effect on the DC-EPSP amplitude could be due to potentiation of kainate-mediated responses. Indeed, a kainate component for EPSP in DCN neurons has not been ruled out (Nuñez and Buño 2001) because kainate receptors have been demonstrated in DCN cells (Hwang et al. 2001) and, more recently, kainate receptors have been shown to inhibit a subtype of K⁺ current (Melyan et al. 2002).

The increase of input resistance cannot account for the increase of the EPSP rising slope evoked by IGF-I, suggesting that IGF-I enhances synaptic potentials through a preand/or postsynaptic facilitatory effect. Paired-pulse depression of DC-EPSP has been previously described in DCN neurons (Nuñez and Buño 1999 2001). It is usually assumed to be due to presynaptic processes (He et al. 2002; Zucker 1999) or caused by loss of action potential conduction in the axons of the dorsal column (Newberry and Simmonds 1984). IGF-I decreased the depression of the conditioned DC-EPSP in the paired-pulse protocol performed in vitro preparations. Therefore it is likely that IGF-I has a presynaptic facilitatory effect in addition to its probable postsynaptic facilitation of kainate-mediated responses.

Transient deafferentation of DCN neurons by subcutaneous injection of the local anesthetic lidocaine induces an expansion of the RF that is probably due to desinhibition of small or remote EPSPs evoked by sensory stimulation of the DCN cells (e.g., Panetsos et al. 1997). Because the inhibitory synaptic network properties of the DCN were unaffected by IGF-I (this was determined by a paired-pulse protocol performed in vivo because inhibitory postsynaptic potentials (IPSPs) were not study in vitro because they were very infrequent), the expansion of the DCN cell RFs may be due to the increment of the DCN cellular excitability as has been indicate in the preceding text. The rapid expansion of the RF of DCN cells after intracarotid injection of IGF-I probably involves a central effect of this trophic factor on DCN neurons rather than a peripheral effect of IGF-I on nerve endings. Central neurons, including DCN cells, show marked IGF-I staining within 5 min of systemic administration of IGF-I (Carro et al. 2000). Thus systemic IGF-I has the potential of affect central neurons very rapidly.

Because levels of circulating IGF-I vary not only with physiological stimuli but also in many pathological situations (Torres-Aleman 2000) and because circulating IGF-I can reach the brain (Carro et al. 2000), it is possible that increased availability of IGF-I in injured areas of the brain could contribute to rapid reorganization of the RFs in deafferentated neurons (Busiguina et al. 2000). It is also possible that plastic changes involved in functional reorganization of the brain under normal circumstances also involve IGF-I modulation of neuronal excitability (Castro-Alamancos et al. 1994).

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