Nerve growth factor and brain-derived neurotrophic factor attenuate angiotensin II-induced facilitation of calcium channels in acutely dissociated nucleus tractus solitarii neurons of the rat

Takayuki Endoh*, Daisuke Sato, Yoshiyuki Wada, Kazuyuki Ishihara, Sadamitsu Hashimoto, Masao Yoshinari, Kenichi Matsuzaka, Masakazu Tazaki and Takashi Inoue

Oral Health Science Center HRC7, Tokyo Dental College, 1-2-2 Masago, Mihama-ku, Chiba 261-8502, Japan

*Corresponding author. E-mail address: tendoh@tdc.ac.jp (T. Endoh)

Keywords: Nerve growth factor, Brain-derived neurotrophic factor, Angiotensin II, Voltage-dependent Ca$^{2+}$ channels current
ABSTRACT

Objective: Neurotrophins, such as Nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), promote neuronal development and neuronal survival. The mechanisms, however, remain controversial. The aim of the present study is to investigate the hypothesis that NGF and BDNF interfere with angiotensin II- and glutamate-induced facilitation of voltage-dependent Ca\(^{2+}\) channels (VDCCs) in the nucleus tractus solitarius (NTS).

Design: The profile of NGF and BDNF actions in acutely dissociated rat NTS has been studied using the whole-cell configuration of the patch clamp technique.

Results: Pretreatment with NGF and BDNF attenuated the Angiotensin II-induced facilitation of VDCCs but not glutamate-induced facilitation of L-type VDCCs current in NTS. NGF-induced attenuations were antagonized by pretreatment with a tyrosine kinase A (TrkA) receptor antagonist K-252a.

Conclusions: NGF attenuated the angiotensin II-induced facilitation of L-type VDCCs mediated by a TrkA receptor in NTS.
1. Introduction

Nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) play critical roles in the development and neuronal survival of the peripheral and central nervous system. NGF is also able to contribute to the neuronal differentiation, neuronal survival, and neuronal long-term maintenance.

Angiotensin II (Ang II), an important mediator of the renin-angiotensin system, does not only exert actions on blood pressure control and body fluid homeostasis but also contributes to cell growth and neuronal differentiation. It has been demonstrated that Ang II induces neurite formation and potentiates the NGF-mediated effect on differentiation.

Voltage-dependent Ca^{2+} channels (VDCCs) serve as crucial mediators of membrane excitability and Ca^{2+}-dependent functions such as neurotransmitter release, enzyme activity, and gene expression. VDCCs also play a variety of roles in many cell processes including neuronal proliferation, neuronal differentiation, and programmed cell death.

The nucleus tractus solitarius (NTS) is known to play a major role in the regulation of cardiovascular, respiratory, gustatory, hepatic, and swallowing functions. In
addition, caudal NTS is believed to belong to the swallowing pattern generators, whereas the rostral NTS is part of the taste pathways. The NTS appears not to be a simple ‘relay’ nucleus, rather it performs complex integration of information from multiple synaptic inputs of both peripheral and central origins.

Previously, we and other groups have demonstrated that Ang-II facilitates VDCCs in NTS. Therefore, this study was designed to test the hypothesis that NGF and BDNF interfere with Ang-II-induced facilitation of VDCCs in NTS.

2. Material and Methods

2.1. Cell preparation

The study was carried out according to “The guideline for the treatment of experimental animals in Tokyo Dental College”. The animals were individually housed with a 12-h dark/light cycle and free access to food and water. 125 animals were used in all experiment. Young Wistar rats (7-18 days old) were decapitated and their brains were quickly removed and submerged in ice cold artificial cerebrospinal fluid (aCSF) saturated with 95% O₂ and 5% CO₂ of the following composition (in mM): 126 NaCl, 26.2
NaHCO₃, 1 NaH₂PO₄, 3 KCl, 1.5 MgSO₄, 1.5 CaCl₂ and 30 glucose; pH 7.4. The medulla was removed and prepared for in vitro recording as we described previously. The dorsal portion of a block extending from approximately 5 mm caudal to 10 mm rostral to the calamus scriptorius was cut into 400 μm slices with a vibratome (DTK-1000; Dosaka EM Co., Ltd, Kyoto) under oxygenated aCSF. After being sectioned, 3-5 slices obtained from a single brain were transferred to a holding chamber and stored in oxygenated aCSF at room temperature for at least 40 min before use. Slices were then transferred to a conical tube containing gently bubbled aCSF at 36 °C to which 1.8 U/ml dispase (grade II; 0.75 ml/slice) was added. After 60 min incubation, slices were rinsed with enzyme-free aCSF. Under a dissecting microscope, the NTS region was micropunched and placed on a poly-l-lysine-coated coverslip. The cells were then dissociated by trituration using progressively smaller diameter pipettes and allowed to settle on a coverslip for 20 min. After the dissociated neurons had adhered to the bottom of the coverslip (usually within 20 min) the electrophysiological measurements were started. Whole-cell patch clamp recordings were performed 30-50 min after dissociation.

2.2 Whole-cell patch-clamp recordings
Voltage-clamp recordings were conducted using the whole-cell configuration of the patch-clamp technique \(^{24}\). Fabricated recording pipettes (2-3 M \(\Omega\)) were filled with the internal solution of the following composition (in mM): 100 CsCl, 1 MgCl\(_2\), 10 HEPES, 10 BAPTA, 3.6 MgATP, 14 Tris\(_2\)phosphocreatine (CP), 0.1 GTP, and 50 U/ml creatine phosphokinase (CPK). The pH was adjusted to 7.2 with CsOH. After the formation of a giga seal, in order to record VDCCs currents (\(I_{\text{Ca}}\)) carried by Ba\(^{2+}\) (\(I_{\text{Ba}}\)), the external solution was replaced from aCSF solution to a solution containing the following (in mM): 151 tetraethylammonium (TEA) chloride, 5 BaCl\(_2\), 1 MgCl\(_2\), 10 HEPES and 10 glucose. The pH was adjusted to 7.4 with TEA-OH. The \(I_{\text{Ba}}\) were isolated by blocking potassium channels with a CsCl (100  \(\mu\)M) in the internal solution and TEA (151  \(\mu\)M) in the external solution. Using Ba\(^{2+}\) instead of Ca\(^{2+}\) in the external solution in these experiments acted to block Ca\(^{2+}\)-dependent potassium channels. Command voltage protocols were generated with a computer software pCLAMP version 8 (Axon Instruments, Union City, CA, U.S.A.) and transformed to an analogue signal using a DigiData 1200 interface (Axon Instruments, Union City, CA, U.S.A.). The command pulses were applied to cells through an L/M-EPC7 amplifier (HEKA Elektronik, Lambrecht, Germany). The currents were recorded with the amplifier and a computer software pCLAMP 8 acquisition system. Access resistance (< 15 M \(\Omega\)) was determined
by transient responses to voltage commands. Access resistance compensation was not used. To ascertain that no major changes in the access resistance had occurred during the recordings a 5 mV, 10 msec pulses were used before $I_{Ba}$ was evoked. The temperature of the superfusing extracellular solution was maintained at 20-26°C. The neurons were constantly superfused with external solution by gravity flow at a rate of approximately 1 ml/min. All drugs were applied by superfusion; time to reach equilibrium concentrations in the bath was 90-120 s, with 1-2 min exposure times. To avoid desensitization, all drugs were randomly applied to 85 different neurons and non-overlaped drugs were applied to a single neuron.

2.3. Materials

Ang II, $\beta$-conotoxin GⅡA ($\beta$-CgTx GⅡA) and $\beta$-agatoxin ⅡA ($\beta$-Aga ⅡA) were purchased from Peptide Institute (Osaka, Japan). NGF was purchased from ICN. (RS)-3,5-dihydroxyphenylglycine (DHPG) was purchased from Tocris (Avonmouth, U.K.). BDNF and nifedipine (Nif) were purchased from Sigma (Tokyo, Japan). K-252a was purchased from Calbiochem (La Jolla, CA, U.S.A.).
2.4. Data analysis and statistics

All data analysis was performed using the pCLAMP 8.0 acquisition system. Values in text and figures are expressed as mean ± SEM. Statistical analysis was made by student t-tests for comparisons between pairs of groups and by one-way analysis of variance (ANOVA) followed by Dunnett's test. Probability (p) values of less than 0.05 were considered significant.

3. Results

3.1. Ang-II-induced facilitation of I_{Ba} was attenuated by NGF

Whole-cell patch recordings were obtained from 85 neurons in 420 preparation of 400 μM thick slices of rat brainstem. The average peak I_{Ba} value for neurons recorded was 451.9 ± 156.6 pA (n = 85). The average capacitance for neurons recorded was 8.2 ± 0.4 pF (n = 85). Any drug did not change the capacitance value.

An example of Ang-II-induced facilitation of I_{Ba} is shown in Fig. 1. I_{Ba} was evoked every 20 sec with a 100 msec depolarizing voltage step to 0 mV from a holding potential
of - 80 mV. As shown in Fig. 1A and B, application of 1 µM Ang rapidly and reversibly facilitates $I_{Ba}$. To evaluate the possible contribution of NGF to the Ang-induced facilitation of $I_{Ba}$, the effects of Ang on $I_{Ba}$ in neurons treated with NGF (38 µM) were investigated. In 7 neurons tested, treatment with NGF attenuated the Ang-induced facilitation of $I_{Ba}$ (55.2 ± 4.0 pA/pF for control, 73.1 ± 5.8 pA/pF for Ang only, and 58.4 ± 4.4 pA/pF for Ang in neuron treated with NGF, n = 18, 12 and 7, respectively, P < 0.05).

These results indicated that NGF attenuated the Ang-induced facilitation of $I_{Ba}$ in NTS.

An example of glutamate-induced facilitation of $I_{Ba}$ is shown in Fig. 2. As shown in Fig. 2A and B, application of 100 µM DHPG rapidly and reversibly facilitates $I_{Ba}$. To evaluate the possible contribution of NGF to the glutamate-induced facilitation of $I_{Ba}$, the effects of glutamate on $I_{Ba}$ in neurons treated with NGF (38 µM) were investigated. In 7 neurons tested, treatment with NGF did not attenuate the glutamate-induced facilitation of $I_{Ba}$ (55.2 ± 4.0 pA/pF for control, 78.9 ± 5.3 pA/pF for DHPG only, and 78.4 ± 4.8 pA/pF for DHPG in neuron treated with NGF, n = 18, 7 and 7, respectively).

These results indicated that NGF did not attenuate the glutamate-induced facilitation of $I_{Ba}$ in NTS.
3.2. Ang II-induced facilitation of I_{Ba} was attenuated by BDNF

To evaluate the possible contribution of BDNF to the Ang II-induced facilitation of I_{Ba}, the effects of Ang II on I_{Ba} in neurons treated with BDNF (35 \mu M) were investigated. In 8 neurons tested, treatment with BDNF attenuated the Ang II-induced facilitation of I_{Ba} (55.2 \pm 4.0 \text{ pA/pF} for control, 73.1 \pm 5.8 \text{ pA/pF} for Ang II only, and 56.4 \pm 4.3 \text{ pA/pF} for Ang II in neuron treated with BDNF, n = 18, 12 and 8, respectively, Figs. 3 and 4C, P < 0.05).

To evaluate the possible contribution of BDNF to the glutamate-induced facilitation of I_{Ba}, the effects of glutamate on I_{Ba} in neurons treated with BDNF (35 \mu M) were investigated. In 7 neurons tested, treatment with BDNF did not attenuate the glutamate-induced facilitation of I_{Ba} (55.2 \pm 4.0 \text{ pA/pF} for control, 78.9 \pm 5.3 \text{ pA/pF} for DHPG only, and 76.9 \pm 4.0 \text{ pA/pF} for DHPG in neuron treated with BDNF, n = 18, 7 and 7, respectively).

These results indicated that BDNF also attenuated the Ang II-induced facilitation of I_{Ba} but not glutamate-induced facilitation of I_{Ba} in NTS.

As shown in Figs. 1 and 3, differences of kinetics of I_{Ba} were observed. One possible
explanation is that there is a variation in that VDCCs subtypes distribution in each neuron. L-type VDCCs has long lasting large conductance, whereas N-, P- and Q-types VDCCs show rapid inactivation during voltage step. It can be considered that different distribution has an influence on the differences in the kinetics of the I_{Ba}.

3.3. NGF- and BDNF-induced attenuation of Ang-


NGF binds to two types of receptors: the low-affinity receptor p75 and the high-affinity receptor tyrosine kinase A. The kinase inhibitor K-252a blocks TrkA receptor autophosphorylation and thus prevents NGF signaling through the TrkA receptor. To evaluate the possible contribution of a TrkA receptor to the NGF-induced attenuation of Ang-


In 7 neurons tested, treatment with K-252a antagonized NGF-induced attenuation of the Ang-


Fig. 4, P < 0.05).
To evaluate the possible contribution of a TrkA receptor to the BDNF-induced attenuation of Ang-INDuced facilitation of I\(_{Ba}\), the effects K-252a on BDNF-induced attenuation of Ang-INDuced facilitation of I\(_{Ba}\) were investigated. In 9 neurons tested, treatment with K-252a did not antagonize BDNF-induced attenuation of the Ang-INDuced facilitation of I\(_{Ba}\) (56.4 ± 4.3 pA/pF for Ang in neuron treated with BDNF, 60.2 ± 5.8 pA/pF for Ang in neuron treated with K-252a + BDNF, n= 8 and 9, respectively, Fig. 4).

These results indicated that NGF attenuated the Ang-INDuced facilitation of I\(_{Ba}\) mediated by a TrkA receptor in NTS.

3.4. NGF- and BDNF-induced facilitation of I\(_{Ba}\)

Note that both NGF and BDNF facilitate I\(_{Ba}\) (Fig.1D, 2D, 3B and 3D). The I\(_{Ba}\) current density-voltage relations measured before and during application of NGF (38 nM) and BDNF (38 nM) are shown in Fig. 5A and 5B, respectively. From a holding potential of -80 mV, the I\(_{Ba}\) was activated after -30 mV with a peak current amplitude at 0 mV. As shown in Fig. 5, application of NGF and BDNF resulted in a slight, but non-significant, facilitated peak I\(_{Ba}\) current density. Both NGF (38 nM) and BDNF (35
M) did not shift the $I_{\text{Ba}}$ current density-voltage relations ($n = 5$).

VDCCs were split into two groups on the basis of activation: low voltage activated (LVA)-T-type and high voltage activated (HVA)-L-, N-, P/Q- and R-types. We previously defined pharmacologically the distinct HVA VDCCs in NTS. Mean percentages of L-type $I_{\text{Ba}}$ components ($I_{\text{Ba-L}}$), N-type $I_{\text{Ba}}$ components ($I_{\text{Ba-N}}$), P/Q-type $I_{\text{Ba}}$ components ($I_{\text{Ba-P/Q}}$) and R-type $I_{\text{Ba}}$ components ($I_{\text{Ba-R}}$) of total $I_{\text{Ba}}$ is 42.2 $\pm$ 3.8%, 28.4 $\pm$ 3.4%, 19.3 $\pm$ 3.2% and 10.1 $\pm$ 1.4%, respectively in NTS \textsuperscript{29}. It was then investigated about which types of the VDCCs were facilitated by NGF. When $\alpha$-CgTx G\textsuperscript{A} (N-type VDCCs blocker, 1 $\mu$M) + $\alpha$-Aga \textsuperscript{A} (P/Q-type VDCCs blocker, 1 $\mu$M) were applied first, the resistant $I_{\text{Ba}}$, i.e. L + R components, were facilitated by a subsequent application of NGF. On the other hand, Nif (L-type VDCCs blocker, 10 $\mu$M) + $\alpha$-Aga \textsuperscript{A} (1 $\mu$M) and Nif + $\alpha$-CgTx G\textsuperscript{A} (1 $\mu$M) were applied first, the resistant $I_{\text{Ba}}$, i.e. N + R components and P/Q + R components, respectively, were not affected by a subsequent application of NGF (38 $\mu$M) (Fig.6, Table 1). These results indicated that NGF facilitated L-type $I_{\text{Ba}}$.

4. Discussion
This study has shown that NGF and BDNF attenuate the Ang ‡U-induced facilitation of VDCCs mediated in NTS. Especially, NGF attenuated the Ang ‡U-induced facilitation of VDCCs mediated by a TrkA receptor in NTS.

As mentioned above, VDCCs serve as crucial mediators of membrane excitability of neurons and Ca²⁺-dependent functions such as neurotransmitter release, enzyme activity, and gene expression. Additionally, there is some evidence that Ca²⁺ mediates growth factor actions in neurons. In sympathetic neurons, for example, Ca²⁺ is involved in the effect of NGF on neurite outgrowth 30. In addition, the ability of NGF to protect hippocampal neurons against hypoglycemic damage involves modulation of VDCCs 31. Ca²⁺ is also involved in the control of many types of synaptic plasticity 25, and changes in Ca²⁺ conductance can regulate neuronal excitability.

In the present study, we demonstrated that signal transduction of NGF and BDNF attenuate Ang ‡U effects in NTS involving Ca²⁺ current modulation. Interaction between receptor tyrosine kinases (RTK) and G-protein coupled receptors (GPCRs) has recently been recognized as important in the regulation of signaling and the regulation of cellular responses, such as neuronal proliferation and neuronal migration 32. Additionally, it has been demonstrated that activation of GPCRs increases the activation/phosphorylation of a TrkA receptors in the presence of NGF, resulting in the
blockade of apoptosis and enhanced neuronal differentiation and growth. It is known that survival signals produced by receptor activation travel retrogradely along to cell bodies. Several studies focused on the retrograde transport of NGF and a TrkA receptor which is also retrogradely transported. In addition, both NGF and phosphorylated TrkA are found in endosomes. It has also been reported that K252a blocks retrograde NGF signaling and neuronal survival in sympathetic neurons. BDNF is also an important signaling molecule that regulates neuroplasticity in multiple brain regions.

The mechanism of Ang II and NGF interaction is not known, but it might involve mitogen activated protein kinases (MAPK). It has been shown that Ang II stimulation inhibits NGF-stimulated p42/p44 MAPK phosphorylation. Previously, we and Wang et al. have demonstrated that Ang II facilitates L-type VDCCs via Src tyrosine kinase, p38 MAPK kinase and Nox2-dependent reactive oxygen species (ROS) production mediated by AT1 receptors. p38 MAPK has multiple actions in neuronal and other tissues. Its initial characterization was as a signaling pathway activated by pathological conditions such as osmotic stress or inflammation mediated by interleukin-1 and tumor necrosis factor. For this reason, p38 MAPK is an active focus of study in chronic disease as rheumatoid arthritis and neurogenic pain syndromes. Physiological roles of p38 MAPK in neurons have begun to follow that of its better characterized MAPK
family, ERK1/2, which in addition to its initial discovery as a mediator of mitogenic stimulation has also been found to modulate a number of ion channels and neuronal plasticity processes \(^{44,45}\). Additionally, in this study we also observed that NGF did not attenuate the glutamate-induced facilitation of VDCCs in NTS. We have previously reported that glutamate facilitates L-type VDCCs involving protein kinase C (PKC) including inositol-1,4,5-trisphosphate (IP\(_3\)) formation \(^{46}\). This data supports the notion that NGF and BDNF modulate Ang \(\V^
abla\)-induced facilitation of VDCCs via MAPK and/or tyrosine kinase pathways, but not protein kinase pathway.

In this study, treatment with K-252a, TrkA receptor inhibitor, antagonized NGF-induced attenuation, but not BDNF-, of the Ang \(\V^
abla\)-induced facilitation of VDCCs. In fact, NGF preferentially activates a TrkA receptors and BDNF is selective for a TrkB receptors \(^{47,48}\). In the basal forebrain, neurotransmitter synthesis as well as neuronal survival after injury are regulated by NGF \(^{49,50}\), while BDNF influences neuronal survival \(^{51}\). BDNF activate intracellular signaling pathways, which include PLC-\(\V\), ERK1/2 and phosphatidylinositol-3 (PI3)-kinase signaling pathway \(^{52}\). Interestingly, it has been reported that TrkA receptor induces neuroprotection involving PI3-kinase/Akt pathways, whereas MAPK and Erk1/2 are not involved \(^{53}\).

Several studies have demonstrated that neurotrophins can alter the expression of
specific ion channel proteins associated with action potential characteristics but relatively few studies have reported immediate effects of neurotrophins on electrophysiological properties. For example, NGF prolongs the action potential duration of sensory neurons from mature mice and BDNF rapidly depolarizes neonatal CNS neurons. Levine et al. demonstrated that NGF facilitates $I_{Ca}$ in cultured basal forebrain neurons. In contrast, BDNF did not modulate $I_{Ca}$ in this neuron. As shown in the present study, both NGF and BDNF facilitate $I_{Ca}$ in NTS.

During differentiation, L-type VDCCs-mediated Ca$^{2+}$ influx is essential for neurite outgrowth, synapse formation, neuronal survival, and the shift to the mature action potential profile. In addition, L-type VDCCs also have been implicated in synaptic plasticity and regulation of gene expression.

In conclusion, the findings present new evidence suggesting that NGF and BDNF can not only promote VDCCs modulation, but also can further long-lasting neuronal changes including cell growth, neuronal differentiation, neuronal survival and long-term maintenance. It can be considered that NGF- and BDNF-induced attenuation of Ang-2-induced facilitation of VDCCs is important role in these events in NTS.

NTS are divided into subtypes: rostral, medial and caudal NTS. Comparison between rostral and caudal NTS neurons indicate that they have comparable intrinsic properties,
which suggests some similarities in the processing of sensory information. However, caudal NTS are believed to belong to the respiratory or swallowing central pattern generators, whereas the rostral NTS are part of the taste and somatosensory pathways. However, it is not possible to identify distinct NTS subtypes in this study. Therefore the NTS subtypes mechanisms discrepancy must be investigated in a further study.

Acknowledgements

This research was supported by Oral Health Science Center Grant HRC7 from the Tokyo Dental College by a “High-Tech Research Center” Project for Private Universities and matching fund subsidy from MEXT (Ministry of Education, Culture, Sports, Science and Technology) of Japan and 2007-2010 (No.19592414). The authors would like to thank Mr. Bryan Loudon for his assistance with the writing of the English manuscript.
REFERENCES


5. Tsuzuki S, Eguchi S, Inagami T. Inhibition of cell proliferation and activation of protein tyrosine tyrosine phosphatase mediated by angiotensin II type 2 (AT2) receptor in R3T3 cells. Biochem Biophys Res Commun 1996;228:825-830.


Nox2, Ca\(^{2+}\), and protein kinase C play a role in angiotensin \(\beta\)-induced free radical production in nucleus tractus solitarius. Hypertension 2006;48:482-489.


46. Endoh T. Characterization of modulatory effects of postsynaptic metabotropic


54. Shen KF, Crain SM. Nerve growth factor rapidly prolongs the action potential of mature sensory ganglion neurons in culture, and this effect requires activation of Gs-coupled excitatory kappa-opioid receptors on these cells. J Neurosci 1994;14:5570-5579.


57. Spitzer NC. Spontaneous Ca\textsuperscript{2+} spikes and waves in embryonic neurons: signaling systems for differentiation. Trend Neurosci 1994;17:115-118.

58. Kullman DM, Perkel DJ, Manabe T, Nicoll RA. Ca\textsuperscript{2+} entry via postsynaptic voltage-sensitive Ca\textsuperscript{2+} channels can transiently potentiate excitatory synaptic transmission in the hippocampus. Neuron 1992;9:1175-1183.

Fig. 1. NGF attenuated Ang ‡-induced facilitation of I_{Ba}. (A) Typical superimposed I_{Ba} traces at the times indicated in the time course graph B. I_{Ba} were evoked from a holding potential of -80 mV by a 100 msec voltage step to 0 mV at 20 sec intervals. (B) Typical time course of Ang ‡-induced facilitation of I_{Ba} current density (pA/pF). The peak I_{Ba} was divided by cell capacitance to obtain the current density. Ang ‡ (1 μM) was bath-applied during the time indicated by the filled bar. (C) Typical superimposed I_{Ba} traces at the times indicated in the time course graph D. (D) Typical time course of NGF-induced attenuation of Ang ‡-induced facilitation of I_{Ba} current density. NGF (38 μM) and Ang ‡ (1 μM) were bath-applied during the time indicated by the open and filled bar, respectively.
Fig. 2. NGF did not attenuate glutamate-induced facilitation of $I_{Ba}$. (A) Typical superimposed $I_{Ba}$ traces at the times indicated in the time course graph B. (B) Typical time course of DHPG-induced facilitation of $I_{Ba}$ current density. DHPG (100 µM) was bath-applied during the time indicated by the filled bar. (C) Typical superimposed $I_{Ba}$ traces at the times indicated in the time course graph D. (D) Typical time course of the effect of NGF on DHPG-induced facilitation of $I_{Ba}$ current density. NGF (38 µM) and DHPG (100 µM) were bath-applied during the time indicated by the open and filled bar, respectively.
Fig. 3. BDNF attenuated Ang ‡-induced facilitation of $I_{Ba}$. (A) Typical superimposed $I_{Ba}$ traces at the times indicated in the time course graph B. (B) Typical time course of the effect of BDNF on Ang ‡-induced facilitation of $I_{Ba}$ current density. BDNF (35 μM) and Ang ‡ (1 μM) were bath-applied during the time indicated by the open and filled bar, respectively. (C) Typical superimposed $I_{Ba}$ traces at the times indicated in the time course graph D. (D) Typical time course of the effect of BDNF on DHPG-induced facilitation of $I_{Ba}$ current density. BDNF (35 μM) and DHPG (100 μM) were bath-applied during the time indicated by the open and filled bar, respectively.
Fig. 4. K-252a blocked NGF-induced attenuation of the Ang ‡-induced facilitation of
$I_{Ba}$. (A) Typical superimposed $I_{Ba}$ traces at the times indicated in the time course graph
B. (B) Typical time course of the effect of K-252a and NGF on Ang ‡-induced
facilitation of $I_{Ba}$ current density. K-252a (10 $\mu$M), NGF (38 $\mu$M) and Ang ‡ (1 $\mu$M)
were bath-applied during the time indicated by the stripe, open and filled bar,
respectively. (C) Histogram demonstrating the degree of $I_{Ba}$ current density, control
(untreated neurons), after Ang ‡ (1 $\mu$M), after NGF (38 $\mu$M) + Ang ‡, after
BDNF (35 $\mu$M) + Ang ‡, after K-252a (10 $\mu$M) + NGF + Ang ‡, and after
K-252a + BDNF + Ang ‡. Numbers in parentheses indicate the number of neurons
tested. * < 0.05 compared with control, ANOVA.
Fig. 5. NGF and BDNF facilitated $I_{Ba}$ density. (A) $I_{Ba}$ current density-voltage relations evoked by a series of voltage steps from a holding potential of $-80$ mV to test potentials between $-80$ and $+40$ mV in $+10$ mV increments in the absence (opened points) and presence (filled points) of NGF (38 µM). (B) $I_{Ba}$ current density-voltage relations evoked by a series of voltage steps from a holding potential of $-80$ mV to test potentials between $-80$ and $+40$ mV in $+10$ mV increments in the absence (opened points) and presence (filled points) of BDNF (35 µM). Values of $I_{Ba}$ are the averages of five neurons.
Fig. 6. NGF and BDNF facilitated L-type $I_{\text{Ba}}$ density. Histogram demonstrating the degree of $I_{\text{Ba}}$ current density by NGF (38 nM) and BDNF (35 nM) on L + R-types (after treatments with $\alpha$-CgTx G A + $\alpha$-Aga A A), N + R-types (after treatments with Nif + $\alpha$-Aga A A) and P/Q + R-types (after treatments with Nif + $\alpha$-CgTx G A) VDCCs. Numbers in parentheses indicate the number of neurons tested. *< 0.05 compared with L + R-types, ANOVA.
Table 1. Comparison of I_{Ba} density in NTS. Values are mean ± SEM.
Fig. 1

A

B

Ang II (1 μM), bath solution

C

D

NGF (38 μM), bath solution
Fig. 3

A

---

B

Ang II (1 μM), bath solution

BDNF (35 μM), bath solution

C

---

D

DHPG (100 μM), bath solution

BDNF (35 μM), bath solution

---
Fig. 4

A

B

C

Ang II (1 \mu M), bath solution

NGF (38 \mu M), bath solution

K-252a (10 \mu M), bath solution

I_{Ba} density (pA/pF)

Time (min)

Cont

Ang II

NGF + Ang II

BDNF + Ang II

K-252a + NGF + Ang II

K-252a + BDNF + Ang II

* *
Fig. 5

A

Test potential (mV)

Peak \( I_{\Delta A} \) density (pA/pF)

Before application

NGF (38 \( \mu \text{M} \)), bath solution

B

Test potential (mV)

Peak \( I_{\Delta A} \) density (pA/pF)

Before application

BDNF (35 \( \mu \text{M} \)), bath solution
<table>
<thead>
<tr>
<th></th>
<th>$I_{\text{Ba}}$ density (pA/pF)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td>55.2 ± 4.0</td>
<td>18</td>
</tr>
<tr>
<td><strong>Ang</strong></td>
<td>73.1 ± 5.8</td>
<td>12</td>
</tr>
<tr>
<td><strong>DHPG</strong></td>
<td>78.9 ± 5.3</td>
<td>7</td>
</tr>
<tr>
<td><strong>No Response</strong></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td><strong>NGF + Ang</strong></td>
<td>58.4 ± 4.4</td>
<td>7</td>
</tr>
<tr>
<td><strong>NGF + DHPG</strong></td>
<td>78.4 ± 4.8</td>
<td>7</td>
</tr>
<tr>
<td><strong>BDNF + Ang</strong></td>
<td>56.4 ± 4.3</td>
<td>8</td>
</tr>
<tr>
<td><strong>BDNF + DHPG</strong></td>
<td>76.9 ± 4.0</td>
<td>7</td>
</tr>
<tr>
<td><strong>K-252a + NGF + Ang</strong></td>
<td>74.4 ± 6.0</td>
<td>7</td>
</tr>
<tr>
<td><strong>K-252a + BDNF + Ang</strong></td>
<td>60.2 ± 5.8</td>
<td>9</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>85</td>
</tr>
</tbody>
</table>