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Postsynaptic Response to Stimulation of the Schaffer Collaterals with Properties Similar to Those of Synaptosomal Aspartate Release

Xuying Zhang and J. Victor Nadler*

Department of Pharmacology and Cancer Biology and Department of Neurobiology, Duke University Medical Center, Durham, NC 27710, USA

Abstract

Aspartate satisfies all the criteria normally required for identification of a CNS neurotransmitter. Nevertheless, little electrophysiological evidence supports the existence of aspartate transmission. In studies with rat hippocampal synaptosomes, chemically-evoked aspartate release differed from glutamate release in its relative sensitivity to increased Ca^{2+} concentration outside the presynaptic active zones, inefficient coupling to P/Q-type Ca^{2+} channels, sensitivity to KB-R7943, and resistance to native Clostridial toxins. We took advantage of these differences to search for a potential aspartate-mediated response at Schaffer collateral synapses in organotypic hippocampal slice cultures. The slice cultures were pretreated with botulinum neurotoxin C (BoNT/C) to eliminate most of the glutamate release so that an expectedly smaller aspartate-like component of the compound EPSC could be detected by whole cell patch clamp recording. In control cultures, NMDA receptor activation accounted for only 18% of the evoked EPSC and an NR2B-selective antagonist reduced the NMDA receptor-mediated component by only 20%. Block of P/Q-type Ca^{2+} channels essentially eliminated the response and 0.1 μM KB-R7943 had no significant effect. In BoNT/C-pretreated cultures, however, NMDA receptor activation accounted for 77% of the evoked EPSC and an NR2B-selective antagonist reduced the NMDA receptor-mediated component by 57%. Block of P/Q-type Ca^{2+} channels reduced the response by only 28%, but 0.1 μM KB-R7943 reduced it by 45%. These results suggest that part of the Schaffer collateral synaptic response has pharmacological properties similar to those of synaptosomal aspartate release and may therefore be mediated at least partly by released aspartate.

Keywords

Aspartate; Hippocampus; Schaffer collaterals; KB-R7943; NMDA receptor

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*Corresponding author. Department of Pharmacology and Cancer Biology, Box 3813, Duke University Medical Center, Durham, NC 27710, USA. Fax: +1 919 681-8609. nadle002@acpub.duke.edu (J.V. Nadler)

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Section: Neurophysiology, Neuropharmacology and other forms of Intercellular Communication

1. Introduction

Aspartate satisfies all the criteria normally required for identification of a neurotransmitter. It is accumulated (Fleck et al., 2001b; Miyaji et al., 2008) and stored (Gundersen et al., 1998, 2001, 2004) in synaptic vesicles, released in a Ca^{2+} -dependent manner by exocytosis (Wang and Nadler, 2007; Holten et al., 2008; Cavallero et al., 2009), and activates postsynaptic NMDA receptors selectively (Patneau and Mayer, 1990; Curras et al., 1992). There is no evidence to suggest that aspartate serves as the principal transmitter of any pathway in the brain. Rather, aspartate may be employed as a co-transmitter by pathways known to release either glutamate (Nadler et al., 1976; Burke and Nadler, 1988, 1989; Gundersen et al., 1998, 2001) or GABA (Gundersen et al., 2004) as their primary transmitter. There is little electrophysiological support for this hypothesis, however.

Reports by ourselves (Peterson et al., 1995; Zhou et al., 1995; Bradford and Nadler, 2004) and others (Herrero et al., 1998; Fleck et al., 2001a; Cavallero et al., 2009) favored independent mechanisms of aspartate and glutamate release. In studies with rat hippocampal synaptosomes, for example, aspartate release was relatively sensitive to increases in intracellular $[\text{Ca}^{2+}]$ outside the active zones, inefficiently coupled to P/Q-type voltage-activated Ca^{2+} channels, reduced by KB-R7943 (an inhibitor of reversed $\text{Na}^+/\text{Ca}^{2+}$ exchange (Iwamoto et al., 1996)) and resistant to both bafilomycin A_1 and native Clostridial toxins (Bradford and Nadler, 2004). In contrast, glutamate release measured simultaneously from the same preparations was less sensitive to increases in intracellular Ca^{2+} outside the active zones, driven mainly by Ca^{2+} influx through P/Q-type channels, insensitive to KB-R7943, and reduced strongly by both bafilomycin A_1 and native Clostridial toxins. These results suggested that, whereas glutamate is released mainly at presynaptic active zones, aspartate is released at some distance from the active zones by a somewhat different mechanism.

We took advantage of some of the unique features of synaptosomal aspartate release to search for a potential aspartate-mediated response at Schaffer collateral synapses in organotypic hippocampal slice cultures. Schaffer collateral terminals contain both the vesicular glutamate transporter VGLUT1 (Kaneko et al., 2002) and sialin (Yarovaya et al., 2005). Sialin, a lysosomal H^+ /sialic acid cotransporter, accumulates aspartate and glutamate in synaptic vesicles by a proton- and voltage-dependent mechanism independent of sialic acid transport (Miyaji et al., 2008). Accordingly, synaptic vesicles in Schaffer collateral terminals exhibit intense immunoreactivity for both aspartate and glutamate (Gundersen et al., 1998, 2001; Holten et al., 2008). These terminals also release aspartate, along with glutamate, in a Ca^{2+} -dependent manner (Nadler et al., 1976; Burke and Nadler, 1988, 1989; Zhou et al., 1995; Gundersen et al., 1998). Hippocampal slice cultures were used for these studies in preference to acutely-prepared slices, because slice cultures retain the anatomical structure of the hippocampus while permitting the use of Clostridial toxins (Capogna et al., 1997). It is very difficult to use Clostridial toxins in studies with acutely-prepared brain slices, because these proteins do not penetrate into the tissue well. The only known non-metabolic action of aspartate in mammalian brain is the selective activation of NMDA receptors. Therefore the NMDA receptor-mediated component of the compound Schaffer collateral EPSC was recorded under conditions expected to suppress the release of glutamate with little reduction of aspartate release. If aspartate is indeed released largely outside the presynaptic active zones, we expected it would activate mainly extrasynaptic NMDA receptors. These receptors are more likely than synaptic receptors to contain the NR2B subunit (Kirson and Yaari, 1996; Stocca and Vicini, 1998; Tovar and Westbrook, 1999; Lozovaya et al., 2004).

2. Results

The design of these studies was guided by the properties of aspartate release revealed in previous neurochemical studies. Release studies indicated that aspartate release is favored relative to glutamate release by prolonged high-frequency stimulation (Szerb, 1988), a low extracellular glucose concentration (Fonnum et al., 1986; Szerb and O'Regan, 1987; Szerb, 1988; Burke and Nadler, 1989; Fleck et al., 1993), and pretreatment with Clostridial toxin (Bradford and Nadler, 2004; Cavallero et al., 2009). Accordingly, recordings were made during stimulation of Schaffer collateral fibers with a train of 10 pulses delivered at a frequency of 30 Hz in the presence of 0.1 mM glucose. No postsynaptic effects of low glucose media (as low as 0.1–0.2 mM) were detected in previous studies of this type (Fleck et al., 1993). Preliminary experiments also detected no effect of 0.1 mM, compared to 10 mM, glucose on resting V_m or R_N , and a previous study found that any deficit in AMPA/kainate receptor-mediated transmission reverses rapidly and completely upon restoration of 10 mM glucose (Burke and Nadler, 1989). Responses of CA1 pyramidal cells to stimulation of the Schaffer collaterals were recorded at a holding potential of +40 mV in order to maximize the contribution of NMDA receptors. Results obtained from BoNT/C-pretreated cultures were compared to those obtained from control cultures.

In control cultures, NMDA receptor activation accounted for <20% of the charge transferred by the evoked compound EPSC (Fig. 1). In cultures pretreated with BoNT/C, however, 77% of the charge, on average, was transferred through NMDA channels. The decay time constant of the NMDA receptor-mediated response was reduced significantly [control ($n = 6$): 377 ± 17 ms; BoNT/C ($n = 9$): 260 ± 22 ms; $P < 0.001$ by Student's *t*-test].

The contribution of NR2B subunits to the NMDA receptor-mediated EPSC was assessed with use of the selective antagonist RO 25-6981 (1 μ M) (Fischer et al., 1997). NR2B-containing receptors contributed relatively little (20%) to this response when evoked in control cultures (Fig. 1). In contrast, the NMDA receptor-mediated EPSC in BoNT/C-pretreated cultures depended largely on the activation of NR2B-containing receptors (57%).

In control cultures, the NMDA receptor-mediated EPSC was completely insensitive to a low concentration of KB-R7943 (0.1 μ M) (Fig. 2). However, the same concentration of KB-R7943 reduced the charge transferred by an average of 45% after cultures were preincubated with BoNT/C. Pretreatment with BoNT/C also changed the response to aga IVA (1 μ M), a selective blocker of P/Q-type voltage-activated Ca^{2+} channels (Mintz et al., 1992). In control cultures, aga IVA essentially abolished the Schaffer collateral synaptic response (Fig. 3), as reported previously in acutely-prepared hippocampal slices (Wheeler et al., 1994). However, the response was much less affected by aga IVA when the slice culture was preincubated with BoNT/C. The percentage depression of Schaffer collateral transmission by aga IVA was about the same as that produced by blocking AMPA/kainate receptors with NBQX.

Preincubation with BoNT/C did not significantly affect the resting membrane potential or input resistance of CA1 pyramidal cells (Table 1). In addition, control and BoNT/C-pretreated cultures were studied, on average, about the same time after they were prepared and about the same time after the birth of the rat. There were no noticeable differences between results obtained from cultures maintained for 2 or 4 weeks in vitro.

3. Discussion

These studies identified in BoNT/C-pretreated organotypic hippocampal slice cultures an NMDA receptor-mediated response to stimulation of Schaffer collaterals with properties similar to those of synaptosomal aspartate release. The response was relatively resistant to BoNT/C and block of P/Q-type Ca^{2+} channels with aga IVA, but was depressed by KB-R7943.

In contrast, responses recorded from control cultures were strongly depressed by aga IVA and unaffected by KB-R7943. The response also was mediated to a considerably greater degree by NR2B-containing receptors. Although the normal NMDA receptor-mediated EPSC is produced mainly by glutamate released through the VGLUT1 mechanism, our results are consistent with production of a small component by aspartate and some glutamate released at a distance from the active zones through the sialin mechanism.

Hippocampal slice cultures were exposed to BoNT/C with the intent of suppressing most of the glutamate release evoked by Schaffer collateral stimulation without much affecting aspartate release. The pronounced shift of the compound EPSC in favor of mediation by NMDA receptors is consistent with this objective having been accomplished. We cannot rule out a postsynaptic action of BoNT/C, however. Exposure to BoNT/C can initiate neurite degeneration (Berliocchi et al., 2005) and Clostridial toxins can block the delivery of AMPA receptor subunits to the plasma membrane (Lledo et al., 1998). Neither of these actions appears to explain our results. Neurodegenerative effects of BoNT/C have not been observed after so short an exposure time as 3 h. A 3 h exposure to Clostridial toxin can block the insertion of AMPA receptors into the plasma membrane and thus the expression of long-term potentiation. However, the baseline AMPA receptor-mediated response to synaptic activation or application of exogenous agonist is unaffected. Whether BoNT/C possesses additional postsynaptic actions that could have produced our findings requires further investigation. Definitive studies of this issue would require experimental approaches quite different from that employed in the present study.

Aspartate release is not completely resistant to Clostridial toxins. It can be reduced by applying a sufficiently high toxin concentration (Gundersen et al., 1998, 2001), by prolonged exposure to a lower concentration (Cavallero et al., 2009), or by incorporating toxin light chain into synaptosomes by electroporation (Wang and Nadler, 2007). The light chain of BoNT/C inhibits exocytosis in an all-or-none fashion by cleaving and inactivating the SNARE proteins syntaxin 1 and SNAP-25 (Simpson, 2004; Sakaba et al., 2005; Rossetto et al., 2006). Progressive inhibition of aspartate release due to accumulation of BoNT/C light chain in synaptic terminals may have contributed to loss of the NMDA receptor-mediated EPSC during incubations longer than 3 h.

KB-R7943 is the only compound tested to date that has inhibited aspartate release without affecting significantly the quantity of glutamate released simultaneously from the same preparation. Therefore its marked reduction of the NMDA receptor-mediated EPSC after, but not without, pretreatment of the slice culture with BoNT/C is consistent with aspartate transmission. KB-R7943 has been used extensively to block Ca^{2+} influx through $\text{Na}^+/\text{Ca}^{2+}$ exchangers. It is possible that aspartate release, but not glutamate release, depends, in part, on Ca^{2+} influx by this mechanism. However, KB-R7943 probably did not function as an inhibitor of reversed $\text{Na}^+/\text{Ca}^{2+}$ exchange in this instance. The concentration used was an order of magnitude below the IC_{50} value for inhibiting reversed $\text{Na}^+/\text{Ca}^{2+}$ exchange (Iwamoto et al., 1996), our study of hippocampal synaptosomes indicated no clear correlation between this action and its inhibition of aspartate release (Bradford and Nadler, 2004), and KB-R7943 has many other actions at concentrations similar to those needed to inhibit exchanger-mediated Ca^{2+} influx (Sobolevsky and Khodorov, 1999; Arakawa et al., 2000; Pintado et al., 2000; Kraft, 2007; Liang et al., 2008). A possible candidate mechanism would be block of cation current through TRPC channels. TRP channels are non-selective cation channels permeable to Ca^{2+} , and they contribute to cellular Ca^{2+} influx. TRPC channels are expressed densely by hippocampal pyramidal cells (Chung et al., 2006), about 30% of these channels are expressed on presynaptic membranes (Zhou et al., 2008), and KB-R7943 blocks them at concentrations several-fold lower than those needed to inhibit reversed $\text{Na}^+/\text{Ca}^{2+}$ exchange (Kraft, 2007). This hypothesis merits evaluation.

Hippocampal NMDA receptors are composed of two NR1 subunits and two NR2 subunits, which may be either NR2A or NR2B. NR2B-containing receptors contributed relatively little to the NMDA receptor-mediated EPSC in control slice cultures. In contrast, the response depended largely on NR2B-containing receptors after pretreatment of the slice culture with BoNT/C. The EC₅₀ for aspartate is 3.3-fold lower for recombinant NR1/NR2B receptors (14.3 μ M) than for NR1/NR2A receptors (47.8 μ M) (Erreger et al., 2007), and these receptors were unlikely to have been saturated by the probable extracellular concentrations of aspartate outside the synaptic cleft. Glutamate released into the synaptic cleft should have saturated both types of NMDA receptor (EC₅₀ values of 2.9 μ M for recombinant NR1/NR2B and 3.3 μ M for NR1/NR2A (Erreger et al., 2007)). NR2B subunits appear to be localized mainly outside synapses and NR2A subunits mainly in the postsynaptic membrane, although both subunits are expressed in both locations (Thomas et al., 2006). Thus NR2B-containing NMDA receptors may be activated more readily than NR2A-containing receptors by released aspartate, due both to their higher affinity for aspartate and their largely extrasynaptic localization. The greater contribution of NR2B-containing receptors to the NMDA receptor-mediated EPSC after exposure to BoNT/C might thus be explained by an increase in the proportion of transmitter release contributed by aspartate. Enhanced spillover of glutamate from synapses could have produced a similar result. However, NMDA receptor-mediated EPSCs evoked in BoNT/C-pretreated slice cultures were smaller on average and decayed more rapidly than those evoked in untreated cultures. The decay rate of the NMDA receptor-mediated EPSC increased despite the greater involvement of NR2B-containing NMDA receptors. Responses mediated by NR1/NR2B receptors decay more slowly than those mediated by NR1/NR2A receptors (Flint et al., 1997; Vicini et al., 1998; Lozovaya et al., 2004). Thus enhancement of glutamate spillover would be expected to prolong, rather than to accelerate, the decay of NMDA receptor-mediated responses. Faster decay is, however, consistent with activation of the receptor by an agonist with lower affinity than glutamate, such as aspartate (Lester and Jahr, 1992). Thus aspartate release outside the presynaptic active zones offers a more plausible explanation for the greater contribution of NR2B-containing NMDA receptors than increased glutamate spillover.

The small effect of aga IVA on Schaffer collateral synaptic transmission in BoNT/C-pretreated slice cultures implies that the opening of P/Q-type channels accounts for little of the relevant action potential-evoked presynaptic Ca²⁺ influx under these conditions. Similarly, aspartate release from hippocampal synaptosomes appeared poorly coupled to these channels (Bradford and Nadler, 2004). In contrast, transmitter release at these synapses normally depends strongly on Ca²⁺ influx through P/Q-type channels, as does glutamate release from hippocampal synaptosomes. Studies of the calyx of Held revealed that P/Q-type channels are localized predominantly at the presynaptic active zones (Wu et al., 1999). If this localization is also true for Schaffer collateral synaptic terminals, then our results with aga IVA suggest that, in BoNT/C-pretreated slice cultures, transmitter is released mainly at a distance from the active zones as proposed for aspartate.

In summary, these electrophysiological tests, taken together, suggest that aspartate release from Schaffer collateral terminals may contribute to synaptic transmission in CA1 pyramidal cells. The conditions we used were designed to maximize aspartate release and minimize glutamate release. It remains to be determined how much aspartate release might contribute to synaptic transmission under more physiological conditions. Major difficulties in extending these studies include our inadequate knowledge of aspartate mechanisms and the lack of reagents to distinguish postsynaptic responses evoked by aspartate and glutamate. The actions of KB-R7943 and the recent discovery of aspartate transport by sialin-expressing synaptic vesicles may lead to advances in this area. It will be crucial to establish the mechanism by which KB-R7943 inhibits aspartate release and reduces the size of the putative aspartate-mediated EPSC and to determine the role of sialin-expressing vesicles in synaptic function.

4. Materials and Methods

Transverse hippocampal slices were prepared from 6 or 7 day old rat pups and cultured on semipermeable membranes (Stoppini et al., 1991; Bausch et al., 2000). After 2-4 weeks in vitro, half the slice cultures were exposed to 50 nM BoNT/C for 3 h at 37°C. They were then transferred back to toxin-free culture medium prior to recording. The goal of BoNT/C pretreatment was to eliminate most of the glutamate release so that an expectedly smaller aspartate-like component of the compound EPSC could be detected. Because aspartate activates only NMDA receptors, the ratio of the AMPA/kainate receptor-mediated Schaffer collateral EPSC to the NMDA receptor-mediated EPSC was used to assess the efficacy of BoNT/C. The goal was to reduce this ratio as much as possible while retaining a large NMDA component. By varying the concentration and exposure time in preliminary studies, we determined that preincubation with 50 nM BoNT/C for 3 h yielded optimal results. Shorter preincubations and lower concentrations resulted in higher AMPA/kainate to NMDA ratios, whereas longer preincubations and higher BoNT/C concentrations usually abolished all evoked synaptic response.

Toxin-treated and untreated slice cultures were studied in a recording chamber mounted on a microscope stage and superfused with ACSF at 3 ml/min and 32°C. The ACSF contained 122 mM NaCl, 25 mM NaHCO₃, 3.1 mM KCl, 1.8 mM CaCl₂, 1.2 mM MgSO₄, 0.4 mM KH₂PO₄, 0.1 mM D-glucose, pH 7.4 by gassing with 95% O₂/5% CO₂ and adjusted to 330-340 mosm by adding sucrose. A knife cut was placed between areas CA3 and CA1 to block the spread of epileptiform activity.

CA1 hippocampal pyramidal cells were identified with use of a Nikon E600FN microscope equipped with IR-DIC optics. Whole cell patch clamp recordings were made beginning 10 min after breakin. Patch electrodes were pulled from borosilicate glass to a tip resistance of 3-5 MΩ. The tip was filled with a solution that contained 140 mM cesium gluconate, 15 mM HEPES, 3.1 mM MgCl₂, 1 mM CaCl₂, 11 mM EGTA, pH 7.2 and 310 mosm. The electrode was then backfilled with an internal solution that consisted of 120 mM cesium gluconate, 10 mM HEPES, 2 mM MgATP, 20 units/ml creatine phosphokinase, 5 mM disodium creatine phosphate, 20 mM QX-314, 0.3 mM NaGTP, pH 7.2 and 310 mosm. Whole cell access was achieved in current clamp mode and resting V_m was determined immediately. A liquid junction potential of 10 mV was subtracted from all membrane potentials. Only cells with $V_m > -55$ mV upon breakin were accepted for study. Series resistances of 8-15 MΩ remained stable during the experiment ($\pm 20\%$) and were compensated $\geq 75\%$. R_N was calculated from the current deflection produced by a 200 ms, 5 mV hyperpolarization from a holding potential of +40 mV. Signals were filtered below 2 kHz and stored to disk with use of pClamp8. A 25 μ m-diameter nichrome wire insulated to the tip with Formvar was used as a monopolar stimulating electrode. To stimulate Schaffer collaterals, the electrode was placed in stratum radiatum of area CA1 50-100 μ m from the pyramidal cell body layer. Ten stimuli of 100 μ s duration were presented at a frequency of 30 Hz. Ten such trains were presented under each experimental condition and the effects of test compounds were computed from the average of the 10 evoked responses. Total charge transferred during the compound EPSC was compared before and at the end of a 10 min exposure to the test compound. Stimulus currents ranged from 6 to 20 μ A for control cultures and from 17 to 350 μ A for BoNT/C-treated cultures.

Synaptic currents were recorded at a holding potential of +40 mV. The NMDA receptor-mediated component was the difference between the current recorded in the presence of 10 μ M NBQX and 30 μ M bicuculline and the current recorded in the presence of 10 μ M NBQX, 30 μ M bicuculline and 50 μ M D-AP5. The AMPA/kainate receptor-mediated component was the difference between the current recorded with bicuculline-containing medium in the presence and absence of 10 μ M NBQX.

BoNT/C was purchased from Wako Chemicals USA (Richmond, VA, USA), aga IVA from Peptides International (Louisville, KY, USA), NBQX, D-AP5 and RO 25-6981 from Tocris Cookson (Baldwin Park, MO), bicuculline methiodide from RBI (Natick, MA, USA), QX-314 from Alomone Laboratories (Jerusalem, Israel) and D-gluconic acid lactone, cesium hydroxide (99.9%; 50% by weight), HEPES, EGTA, NaGTP, MgATP, disodium creatine phosphate and creatine phosphokinase from Sigma Chemical Co. (St. Louis, MO, USA). KB-R7943 was a gift from Nippon Organon KK (Osaka, Japan).

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Abbreviations

ACSF, artificial cerebrospinal fluid
 aga IVA, Ω -agatoxin IVA
 D-AP5, D-2-amino-5-phosphonopentanoate
 BoNT/C, botulinum neurotoxin C
 EPSC, excitatory postsynaptic current
 KB-R7943, 2-[2-[(4-nitrobenzyloxy)phenyl]ethyl]isothiourea methanesulfonate
 NBQX, 2,3-dihydroxy-6-nitro-7-sulfamyl-benzo[F]quinoxaline
 QX-314, N-ethyl lidocaine
 R_N , input resistance
 RO 25-6981, (α R, β S)- α -(4-hydroxyphenyl)- β -methyl-4-(phenylmethyl)-1-piperidinepropanol maleate
 V_m , membrane potential
 VGLUT1, vesicular glutamate transporter 1

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Fig. 1.

Pretreatment of hippocampal slice cultures with BoNT/C increased the fraction of the Schaffer collateral compound EPSC mediated by NMDA receptors and the fraction of the NMDA component mediated by NR2B-containing receptors. Top: representative recordings. Slice cultures were exposed to 50 nM BoNT/C for 3 h at 37°C before recordings began. Recordings were made from CA1 pyramidal cells at 32°C and a holding potential of +40 mV. All solutions contained 30 μ M bicuculline. Ten stimuli were applied to the Schaffer collaterals at a frequency of 30 Hz. Traces shown are the averaged responses to 10 such trains. Vertical deflections are stimulus artifacts. The NMDA component of the EPSC was isolated pharmacologically by adding 10 μ M NBQX to the superfusion medium. The difference between recordings made in the presence and absence of 1 μ M RO 25-6981 represents the portion of the NMDA component mediated by NR2B-containing receptors. The RO 25-6981-resistant response (equivalent to the NR2A-mediated EPSC) was abolished by 50 μ M D-AP5. Bottom: grouped data from 6 control cultures and 9 cultures pretreated with BoNT/C. Values are means $\pm$ SEM.

*Significantly different from control at $P < 0.001$ (Student's t -test).

Fig. 2. KB-R7943 (0.1 μ M) reduced the size of the synaptic NMDA current only after pretreatment of the hippocampal slice culture with BoNT/C. Top: representative recordings. Recordings were made as described in figure 1 with a superfusion medium that contained 30 μ M bicuculline and 10 μ M NBQX. Bottom: grouped data from 7 control cultures and 8 cultures pretreated with BoNT/C. Values are means $\pm$ SEM. *Significantly different from control at $P = 0.004$.

Fig. 3.

Aga IVA (1 μ M) inhibited synaptic transmission less effectively when the hippocampal slice culture was pretreated with BoNT/C. Top: representative recordings. In control cultures, aga IVA essentially abolished Schaffer collateral transmission, both the AMPA/kainate and NMDA components. However, the NMDA receptor-mediated EPSC was reduced by only 28% when the culture was pretreated with BoNT/C. See figure 1 for other details. Bottom: grouped data from 5 control cultures and 5 cultures pretreated with BoNT/C. Values are means $\pm$ SEM. *Significantly different from control at $P < 0.001$ (Student's t -test).

Table 1

Resting membrane properties and ages of CA1 pyramidal cells studied

	Control	BoNT/C
V_m (mV)	-66 ± 1 (18)	-64 ± 1 (22)
RN ($M\Omega$)	123 ± 10 (17)	161 ± 18 (20)
Days in culture	19 ± 1 (18)	21 ± 1 (22)
Days since birth	26 ± 1 (18)	28 ± 2 (22)

Hippocampal slice cultures were exposed to 50 nM BoNT/C for 3 h at 37°C before recordings began. Values are means $\pm$ SEM for the number of cells in parentheses. Student's *t*-test revealed no significant between-group differences ($P > 0.05$).