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THE INTERACTION BETWEEN TROPOMYOSIN-RELATED KINASE B RECEPTORS AND SERINE KINASES MODULATES ACETYLCHOLINE RELEASE IN ADULT NEUROMUSCULAR JUNCTIONS

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Running title: trkB, PKC and PKA in ACh release.

Abbreviations: BDNF, brain-derived neurotrophic factor; CNTF, cilliary neurotrophic factor; CNTF-Rα, cilliary neurotrophic factor receptor; µ-CgTx-GIIIB, µ-conotoxin GIIIB; EPPs, end plate potentials; LAL, *Levator auris longus* muscle; mAChRs, presynaptic muscarinic ACh autoreceptors; MEPPs, miniature endplate potentials; NMJ, neuromuscular junction; NT-4, neurotrophine 4; PKA, protein kinase A; PKC, protein kinase C; trkB, tropomyosin-related tyrosin kinase B receptor; trkC, tropomyosin-related tyrosin kinase C receptor.

Abstract

We conducted an electrophysiological study of the functional link between the tropomyosin-related kinase B (trkB) receptor signaling mechanism and serine-threonine kinases, both protein kinase C (PKC) and protein kinase A (PKA). We describe their coordinated role in transmitter release at the neuromuscular junction (NMJ) of the *Levator auris longus* muscle of the adult mouse. The trkB receptor normally seems to be coupled to stimulate ACh release because inhibiting the trkB receptor with K-252a results in a significant reduction in the size of EPPs. We found that the intracellular PKC pathway can operate as in basal conditions (to potentiate ACh release) without the involvement of the trkB receptor function, although the trkB pathway needs an operative PKC pathway if it is to couple to the release mechanism and potentiate it. To actively stimulate PKA (which also results in ACh release potentiation), the operativity of trkB is a necessary condition, and one effect of trkB may be PKA stimulation.

Key words: protein kinase C; protein kinase A; brain-derived neurotrophic factor.

Introduction

Several signaling mediators and their receptors transmit information between the cells that make up the synapses (pre- and postsynaptic cells and glia). In the neuromuscular synapses on the skeletal muscles, several subtypes of presynaptic muscarinic acetylcholine autoreceptors (mAChRs) [4, 16, 24, 25], adenosine receptors [26] and neurotrophin receptors [2, 17, 18], among others, cooperate to control the functional conditions of transmitter release in response to variable activity demands. These metabotropic receptors are coupled to a limited repertoire of presynaptic effector kinases which finally phosphorylate proteins and regulate neurotransmitter release.

In previous studies, we found that the muscarinic mechanism strongly influences serine-threonine kinase activity, both protein kinase C (PKC) and protein kinase A (PKA) [20, 23]. M1 and M2 subtypes of muscarinic receptors were involved in enhancing and inhibiting ACh release, respectively. Also, PKA (but not PKC) plays a constitutive role in promoting a component of normal release. The selective block of M1 or M2 inverts this kinase function: PKC can then stimulate transmitter release, whereas PKA is uncoupled [21]. Here, we use electrophysiological techniques to investigate the functional interactions between the neurotrophin receptor tropomyosin-related kinase B (trkB) receptor, which binds the target-derived brain-derived neurotrophic factor (BDNF) and neurotrophin-4 (NT-4) [8], and the serine-threonine kinases PKC and PKA and to demonstrate their coordinated role in transmitter release.

It is generally believed that neurotrophin receptors promote local acute effects in nerve endings, and retrograde long-term effects in distant neuronal cell bodies. [6, 7, 9, 11, 13, 14]. Here we analyzed the acute effect on transmitter release resulting from changes in trkB and serine kinases.

Material and Methods

Animals

Experiments were performed on the *Levator auris longus* (LAL) muscle of adult male Swiss mice (30-40 days postnatal; Criffa, Barcelona, Spain). The animals were anesthetized with 2% tribromoethanol (0.15 ml /10 g body weight, I.P.) and killed by exanguination while deeply anesthetized. The total number of mice studied was 95. The animals were cared for in accordance with the guidelines of the European Community's Council Directive of 24 November 1986 (86/609/EEC) for the humane treatment of laboratory animals. This project was approved by the Ethics Committee of "Rovira i Virgili" University.

Electrophysiological recordings

The LAL muscle with its nerve supply was excised and dissected on a Sylgard-coated Petri dish containing normal Ringer solution (in mM): NaCl, 137; KCl, 5; $CaCl_2$, 2; $MgSO_4$, 1; $NaHCO_3$, 12; Na_2HPO_4, 1 and glucose 11, continuously bubbled with 95% O_2 / 5% CO_2. The preparation was then transferred to a 1.5 ml recording chamber. Experiments were performed at room temperature (22-25 ºC). The bath temperature was monitored during experiments (23.4 ± 1.7 ºC, Digital Thermometer TMP 812, Letica, Barcelona, Spain). Endplate potentials (EPPs) were recorded intracellularly with conventional glass microelectrodes filled with 3 M KCl (resistance: 20-40 MΩ). Recording electrodes were connected to an amplifier (Tecktronics, AMS02), and a distant Ag-AgCl electrode connected to the bath solution via an agar bridge (agar 3.5% in 137 mM NaCl) was used as reference. The signals were digitized (DIGIDATA 1322A Interface, Axon Instruments Inc, CA, USA), stored and computer-analyzed. The

software Axoscope 9.0 (Axon Instruments Inc, CA, USA) was used for data acquisition and analysis.

In previous studies (for PKC drugs, [21]; for PKA drugs, [22]), standard sharp-electrode intracellular recording techniques were used to show that miniature endplate potentials (MEPPs) amplitudes and postsynaptic resting membrane potentials were unaffected and, therefore, that all the compounds used act presynaptically in these conditions. We also analyzed the effect of BDNF and K-252a on MEPPs. In addition, when consecutive incubations were performed with two different substances, we determined whether there was a final change in MEPP amplitude and we found that it did not change in any case. Consecutive incubations with two substances (for instance K-252a and PMA) are used as a pharmacologic tool to investigate the possible occlusive or additive crosstalk effects between them. We recorded control EPPs and measured their size. Then we incubated the muscle for one hour in the first compound. After recording EPPs again, we incubated it for one hour in the second compound (in the presence of the first drug) and then recorded EPPs.

To prevent muscle contraction during EPP recordings, we used μ-conotoxin GIIIB (μ-CgTx-GIIIB, 1-3 μM). After a muscle fiber had been impaled, the nerve was continuously stimulated (70 stimuli at 0.5 Hz) using two platinum electrodes that were coupled to a pulse generator (CIBERTEC CS-20) linked to a stimulus isolation unit. We recorded the last 50 EPPs and used only the results from preparations that had a resting potential of less than –50 mV and which did not deviate by more than 5 mV during the experimental paradigms. The mean amplitude (mV) per fiber was calculated and corrected for non-linear summation (EPPs were usually more than 4 mV) [15] and assuming a membrane potential of – 80 mV. We studied a minimum of 15 fibers per muscle and usually a minimum of five muscles in each type of experiment.

Statistical procedure

Values are expressed as means ± S.E.M. We used a one-way analysis of variance (ANOVA) to evaluate differences between groups and the Bonferroni test for multiple comparisons. When differences were evaluated between only two groups, we used the two-tailed Welch's *t*-test (for unpaired values and not assuming equal variances). Differences were considered significant at $P < 0.05$.

Chemicals

Substances that modulate PKC activity. Phorbol 12-myristate 13-acetate (PMA, Sigma) is made up as a 10 mM stock solution in dimethylsulfoxide (DMSO, TOCRIS). The stock solution of Calphostin C (CaC, Sigma) was made up as a 2.5 mM solution in DMSO. Working solutions were PMA, 10 nM and CaC, 10 µM.

Substances that modulate PKA activity. N-[2-((p-Bromocinnamyl)amino)ethyl]-5-isoquinolinesulfonamide, 2HCl (H-89, Calbiochem) was made up as a 5 mM stock solution in DMSO. The stock solution of Adenosine 3′,5′-cyclic Monophosphorothioate, 8-Bromo-, Rp-Isomer, sodium salt (Sp-8-BrcAMPs, Calbiochem) was made up as a 5 mM solution in deionized water. Working solutions were Sp-8-BrcAMPs 10 µM and H-89 5 µM.

trkB receptor-related agents. K-252a (Calbiochem), 5 mM in DMSO; K-252b (Calbiochem), 5 mM; Anti-trkB (clone 47/trkB, 610102, BD Transduction Laboratories), 250 µg/ml; The trkB ligand N2- (2- {[(2- oxoazepan- 3-yl)amino] carbonyl} phenyl) benzo[b] thiophene-2 carboxamide (ANA-12, Maybridge), was made up as a 10mM in DMSO. Working solutions: K-252a, 200 nM; K-252b, 500 nM; trkB-Fc, 1-5 µg/ml; ANA-12, 10µM.

Experiments in the presence of K-252a were conducted with no direct illumination. The final DMSO concentration in control and drug-treated preparations was 0.1% (v/v). In control experiments this concentration of DMSO did not affect any of the parameters studied (data not shown).

Results and Discussion

In a previous study [8], we found that exogenously added BDNF (10 nM) potentiates evoked ACh release in a trkB and $p75^{NTR}$ receptor dependent manner because potentiation is prevented by the pharmacological block of trkB (K-252a, 200 nM, 1 hour) or $p75^{NTR}$ (Pep5, 1 µM, 1 hour). The inhibition of the trkB receptor with K-252a does not merely prevent the effect of exogenous BDNF, but also gives rise by itself to a significant reduction in the size of EPPs (~50%) after one hour of incubation (Figures 1 and 2, the first white column). However, MEPP amplitude does not change (data not shown), indicating that AChR are unaffected and ACh release is truly inhibited. Thus, the trkB receptor can modulate transmitter release without stimulation by added BDNF. This indicates that the trkB receptor is coupled to tonically potentiate ACh release in basal conditions. K-252a is rather a broad-spectrum kinase inhibitor [10, 12] and therefore, we performed several control experiments. The specificity of the K-252a blocking effect on trkB was confirmed with the anti-trkB antibody 47/trkB (% of inhibition: 34.74 ± 6.57; n=5 muscles, mínimum 15 fibres per muscle, $p< 0.05$) that reduces evoked ACh release as K-252a does, whereas the non-permeant tyrosine kinase blocker K-252b does not change release (% of variation 1.25 ± 7.42; n=5 muscles, mínimum 15 fibres per muscle, $p>0.05$) and the NT-3-trkC pathway does not seem to be coupled to release in our conditions [8]. In addition, we repeated several key experiments by using ANA-12 instead of K-252a. ANA-12 showed direct and selective binding to trkB and inhibited processes downstream of trkB without altering trkA and trkC functions [5]. ANA-12 fully inhibits BDNF-induced trkB phosphorylation and therefore prevents receptor activation. In our experiments with ANA-12 the compound blocks many downstream processes beyond trkB, but some trkB functions are preserved. For instance, the compound blocks the ACh release potentiating effect of BDNF (as K-

252a; 3.96 ± 6.58) but was unable to suppress the constitutive tonic contribution of trkB receptors to release (8). In the present study we used ANA-12 to complement K-252a.

We investigated how blocking trkB signaling affects coupling to the release of PKC and reciprocally (Figure 1). Sequential incubations with two substances (for instance K-252a and PMA) are used as a pharmacologic tool to see occlusive or additive effects between them. The numbers (1: or 2:) in front of the chemical drugs indicate the order of their serial application. We found that PKC can be stimulated by the phorbol ester PMA, resulting in ACh release potentiation (raw data in figure 1). However, in the resting NMJs, PKC does no play a constitutive role in promoting a component of normal release because PKC inhibition with calphostin C (CaC) does not reduce ACh release (white columns in figure 1 for the PMA and CaC effects; see also [20]. However, PKC stimulates release (and therefore CaC reduces it) in certain circumstances, such as when there is continuous synaptic activity and when the release modulatory mechanism of the mAChRs is perturbed [19, 22, 23]. The figure 1 shows that after K-252a preincubation, the PKC stimulator (PMA) can normally affect ACh release potentiation and the PKC inhibitor (CaC) has no effect, as in previously untreated muscles (black columns in figure 1). Thus, the intracellular PKC pathway can operate in relation with ACh release without the involvement of the trkB receptor function. Alternatively, the same experiments conducted in a reciprocal manner (a first one-hour incubation with a PKC modulator followed by a second incubation with K-252a to block trkB) show that K-252a normally reduces release after PKC stimulation but does not affect release when PKC is previously blocked (gray columns in figure 1). This indicates that the trkB pathway needs an operative PKC pathway if it is to couple to the release mechanism. We repeated experiments by using ANA-12 instead of K-252a (Figure 1). Although this compound was unable to suppress the constitutive

contribution of trkB receptors to release, the result of an initial incubation with ANA-12 instead of K-252a (before the second incubation with the PKC stimulator PMA) is no different from an initial incubation with K-252a. Figure 1 shows some representative raw data. The first entry (on the left) shows that the phorbol ester PMA (10 nM) can double the amplitude of end plate potential (EPP) from a single fiber whereas the second and third records show how this potentiating action persists even though the sample was previously incubated with the trkB blockers K-252a (200nM) or ANA-12 (10 μM). We conclude that PKC operates independently of the trkB. In the present experiments we used PMA as a tool for PKC stimulation. The PKC stimulatory phorbol esters such as PMA can also bind and activate Munc13, a presynaptic high-affinity phorbol ester receptor, and diacylglycerol receptor with ligand affinities similar to those of PKC that acts in parallel with this kinase to regulate neurotransmitter secretion [1, 3]. Specifically, the docking of vesicles at the transmitter release sites is a process that involves the scaffold protein RIM (Rab3A interacting molecule) and its binding partner Munc-13. If a component of the PMA action were Munc-13 stimulation, the observation made here that, after K-252a incubation, PMA can normally induce ACh release potentiation and, therefore, that the intracellular PKC pathway can operate without the involvement of the trkB receptor function should be handled with caution.

The interaction between the PKA and trkB pathways is different (Figure 2). PKA can be stimulated by Sp-8-BrcAMPs resulting in ACh release potentiation (the raw data on the left in figure 2) and this kinase played a constitutive role in promoting a component of normal release because PKA inhibition with H-89 reduces release {white columns in figure 2; see also Santafe and coworkers (2009), [20]}. We observed that PKA inhibition with H-89 incubation reduces release as it normally does in the presence of K-252a (figure 2, black down column) as well as reciprocally (figure 2, the last gray

down column), suggesting that the coupling of these two molecules to ACh release may be unrelated. However, PKA activation with Sp8Br cannot stimulate release in the presence of K-252a (figure 2, black column going up and associated raw data) and K-252a cannot significantly inhibit release in the presence of Sp8Br (figure 2, first gray column). Thus, to stimulate PKA (with Sp8Br) above a certain normal level, the normal operation of the trkB receptor may be a permissive condition. In addition, activated PKA by Sp8Br induces some change in the trkB receptor that makes it resistant to the blocking action of the K-252a inhibitor. We repeated experiments using ANA-12 instead of K-252a and observed that the result of an initial incubation with ANA-12 (before the second incubation with Sp8Br) is no different than the result when K-252a is used (we include raw data).

In conclusion, we previously described that the muscarinic mechanism strongly influences how the serine-threonine kinases (both PKC and PKA) couple to ACh release [22]. Here we expanded the knowledge of the functional links between these molecules by including some molecular interactions between PKC, PKA and the trkB receptor in modulating ACh release in the NMJ. We found that the intracellular PKC pathway can operate as in basal conditions (to potentiate ACh release) without the involvement of the trkB receptor function, although the trkB pathway needs an operative PKC pathway if it is to couple to the release mechanism and potentiate it. To actively stimulate PKA (which also results in ACh release potentiation), the operativity of trkB is a necessary condition, and one effect of trkB may be PKA stimulation.

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References

[1] A. Betz, U. Ashery, M. Rickmann, I. Augustin, E. Neher, T.C. Sudhof, J. Rettig, N. Brose, Munc13-1 is a presynaptic phorbol ester receptor that enhances neurotransmitter release, Neuron 21 (1998) 123-136.

[2] M. Bibel, Y.A. Barde, Neurotrophins: key regulators of cell fate and cell shape in the vertebrate nervous system, Genes Dev 14 (2000) 2919-2937.

[3] N. Brose, C. Rosenmund, Move over protein kinase C, you've got company: alternative cellular effectors of diacylglycerol and phorbol esters, J Cell Sci 115 (2002) 4399-4411.

[4] M.P. Caulfield, Muscarinic receptors--characterization, coupling and function, Pharmacol Ther 58 (1993) 319-379.

[5] M. Cazorla, J. Premont, A. Mann, N. Girard, C. Kellendonk, D. Rognan, Identification of a low-molecular weight TrkB antagonist with anxiolytic and antidepressant activity in mice, J Clin Invest 121 (2011) 1846-1857.

[6] B. Friedman, D. Kleinfeld, N.Y. Ip, V.M. Verge, R. Moulton, P. Boland, E. Zlotchenko, R.M. Lindsay, L. Liu, BDNF and NT-4/5 exert neurotrophic influences on injured adult spinal motor neurons, J Neurosci 15 (1995) 1044-1056.

[7] H. Funakoshi, N. Belluardo, E. Arenas, Y. Yamamoto, A. Casabona, H. Persson, C.F. Ibanez, Muscle-derived neurotrophin-4 as an activity-dependent trophic signal for adult motor neurons, Science 268 (1995) 1495-1499.

[8] N. Garcia, M. Tomas, M.M. Santafe, N. Besalduch, M.A. Lanuza, J. Tomas, The Interaction between Tropomyosin-Related Kinase B Receptors and Presynaptic Muscarinic Receptors Modulates Transmitter Release in Adult Rodent Motor Nerve Terminals, J Neurosci 30 (2010) 16514-16522.

[9] M. Gonzalez, F.P. Ruggiero, Q. Chang, Y.J. Shi, M.M. Rich, S. Kraner, R.J. Balice-Gordon, Disruption of Trkb-mediated signaling induces disassembly of postsynaptic receptor clusters at neuromuscular junctions, Neuron 24 (1999) 567-583.

[10] Y. Hashimoto, T. Nakayama, T. Teramoto, H. Kato, T. Watanabe, M. Kinoshita, K. Tsukamoto, K. Tokunaga, K. Kurokawa, S. Nakanishi, et al., Potent and preferential inhibition of Ca2+/calmodulin-dependent protein kinase II by K-252a and its derivative, KT5926, Biochem Biophys Res Commun 181 (1991) 423-429.

[11] C.E. Henderson, W. Camu, C. Mettling, A. Gouin, K. Poulsen, M. Karihaloo, J. Rullamas, T. Evans, S.B. McMahon, M.P. Armanini, et al., Neurotrophins promote motor neuron survival and are present in embryonic limb bud, Nature 363 (1993) 266-270.

[12] H. Hidaka, R. Kobayashi, Pharmacology of protein kinase inhibitors, Annu Rev Pharmacol Toxicol 32 (1992) 377-397.

[13] V.E. Koliatsos, M.H. Cayouette, L.R. Berkemeier, R.E. Clatterbuck, D.L. Price, A. Rosenthal, Neurotrophin 4/5 is a trophic factor for mammalian facial motor neurons, Proc Natl Acad Sci U S A 91 (1994) 3304-3308.

[14] V.E. Koliatsos, R.E. Clatterbuck, J.W. Winslow, M.H. Cayouette, D.L. Price, Evidence that brain-derived neurotrophic factor is a trophic factor for motor neurons in vivo, Neuron 10 (1993) 359-367.

[15] E.M. McLachlan, A.R. Martin, Non-linear summation of end-plate potentials in the frog and mouse, J Physiol 311 (1981) 307-324.

[16] J. Minic, J. Molgo, E. Karlsson, E. Krejci, Regulation of acetylcholine release by muscarinic receptors at the mouse neuromuscular junction depends on the activity of acetylcholinesterase, Eur J Neurosci 15 (2002) 439-448.

[17] E.V. Pitts, S. Potluri, D.M. Hess, R.J. Balice-Gordon, Neurotrophin and Trk-mediated signaling in the neuromuscular system, Int Anesthesiol Clin 44 (2006) 21-76.

[18] P.P. Roux, P.A. Barker, Neurotrophin signaling through the p75 neurotrophin receptor, Prog Neurobiol 67 (2002) 203-233.

[19] M.M. Santafe, N. Garcia, M.A. Lanuza, J. Tomas, Protein kinase C activity affects neurotransmitter release at polyinnervated neuromuscular synapses, J Neurosci Res 85 (2007) 1449-1457.

[20] M.M. Santafe, N. Garcia, M.A. Lanuza, M. Tomas, J. Tomas, Interaction between protein kinase C and protein kinase A can modulate transmitter release at the rat neuromuscular synapse, J Neurosci Res 87 (2009) 683-690.

[21] M.M. Santafe, M.A. Lanuza, N. Garcia, J. Tomas, Calcium inflow-dependent protein kinase C activity is involved in the modulation of transmitter release in the neuromuscular junction of the adult rat, Synapse 57 (2005) 76-84.

[22] M.M. Santafe, M.A. Lanuza, N. Garcia, J. Tomas, Muscarinic autoreceptors modulate transmitter release through protein kinase C and protein kinase A in the rat motor nerve terminal, Eur J Neurosci 23 (2006) 2048-2056.

[23] M.M. Santafe, M.A. Lanuza, N. Garcia, M. Tomas, J. Tomas, Coupling of presynaptic muscarinic autoreceptors to serine kinases in low and high release conditions on the rat motor nerve terminal, Neuroscience 148 (2007) 432-440.

[24] M.M. Santafe, I. Salon, N. Garcia, M.A. Lanuza, O.D. Uchitel, J. Tomas, Modulation of ACh release by presynaptic muscarinic autoreceptors in the

neuromuscular junction of the newborn and adult rat, Eur J Neurosci 17 (2003) 119-127.

[25] I. Slutsky, H. Parnas, I. Parnas, Presynaptic effects of muscarine on ACh release at the frog neuromuscular junction, J Physiol 514 (Pt 3) (1999) 769-782.

[26] W.J. Song, T. Tkatch, D.J. Surmeier, Adenosine receptor expression and modulation of Ca(2+) channels in rat striatal cholinergic interneurons, J Neurophysiol 83 (2000) 322-332.

Legends to the figures

Figure 1. trkB and PKC coupling to ACh release.

The figure shows that blocking neurotrophin-trkB signaling affects PKC coupling to ACh release. The white columns show the action of several drugs in **basal conditions**. PMA (10nM) used as a PKC agonist can enhance ACh release. PKC inhibition with CaC (10 µM) does not. The black columns show the effect of PKC agents in the presence of K-252a (200 nM). In this situation, the PKC stimulator PMA and inhibitor CaC have their normal pharmacological effect on ACh release. The gray columns indicate the results of the same experiments conducted in a reciprocal manner: the K-252a effect on the presence of PKC agents. In this set of experiments, the K-252a normally reduces release after PKC stimulation with PMA but has no effect when PKC is previously blocked with CaC. The numbers (1: or 2:) in front of the chemical drugs indicate the order of their serial application. For each column: 5 muscles, minimum 15 fibers per muscle. Values are expressed as mean ± SEM. * $P<0.05$ with respect to initial values. The figure contains representative raw data of the interaction of PKC with trkB receptors in the release of ACh. The first entry (on the left) shows that the nonspecific agonist of all PKC isoforms PMA (10 nM) can double the amplitude of the EPPs from a single fiber. The second and third entries show that this potentiating effect can be reproduced even though the sample was previously incubated with the trkB blockers K-252a (200nM) or ANA-12 (10 µM). Scale bar: horizontal, 5ms; Vertical value is shown in each recording.

Figure 2. trkB and PKA coupling to ACh release .

Blocking neurotrophin-trkB signaling affects PKA coupling to ACh release. As in the previous fugure, the white columns show the action of several drugs in basal conditions. The PKA agonist Sp-8-BrcAMPs (10 µM) can enhance ACh release, whereas PKA inhibition with H-89 (5 µM) reduces it. The black columns show the effect of the PKA agents in the presence of K-252a (200 nM). In this situation, whereas H-89 reduces release as normal in the presence of K-252a, Sp8Br cannot stimulate release in the presence of K-252a. The gray columns indicate the results of the same experiments conducted in a reciprocal manner: the K-252a effect on the presence of PKA agents. In this set of experiments, whereas K-252a cannot inhibit release in the presence of Sp8Br, in the presence of H-89 it can. The numbers (1: or 2:) in front of the chemical drugs indicate the order of their serial application. For each column: 5 muscles, minimum 15 fibers per muscle. Values are expressed as mean ± SEM. * $P<0.05$ with respect to initial values. The figure contains representative raw data of the interaction of PKA with trkB receptors in the release of ACh. The first entry on the left shows the potentiating action of the PKA agonist Sp-8-BrcAMPs (10 µM) on EPP from a single fiber. The second and third entries show that Sp-8-Br cannot enhance evoked neurotransmitter release when the trkB is blocked by K-252a or ANA-12. Scale bar: horizontal, 5ms; Vertical value is shown in each recording.

Figure
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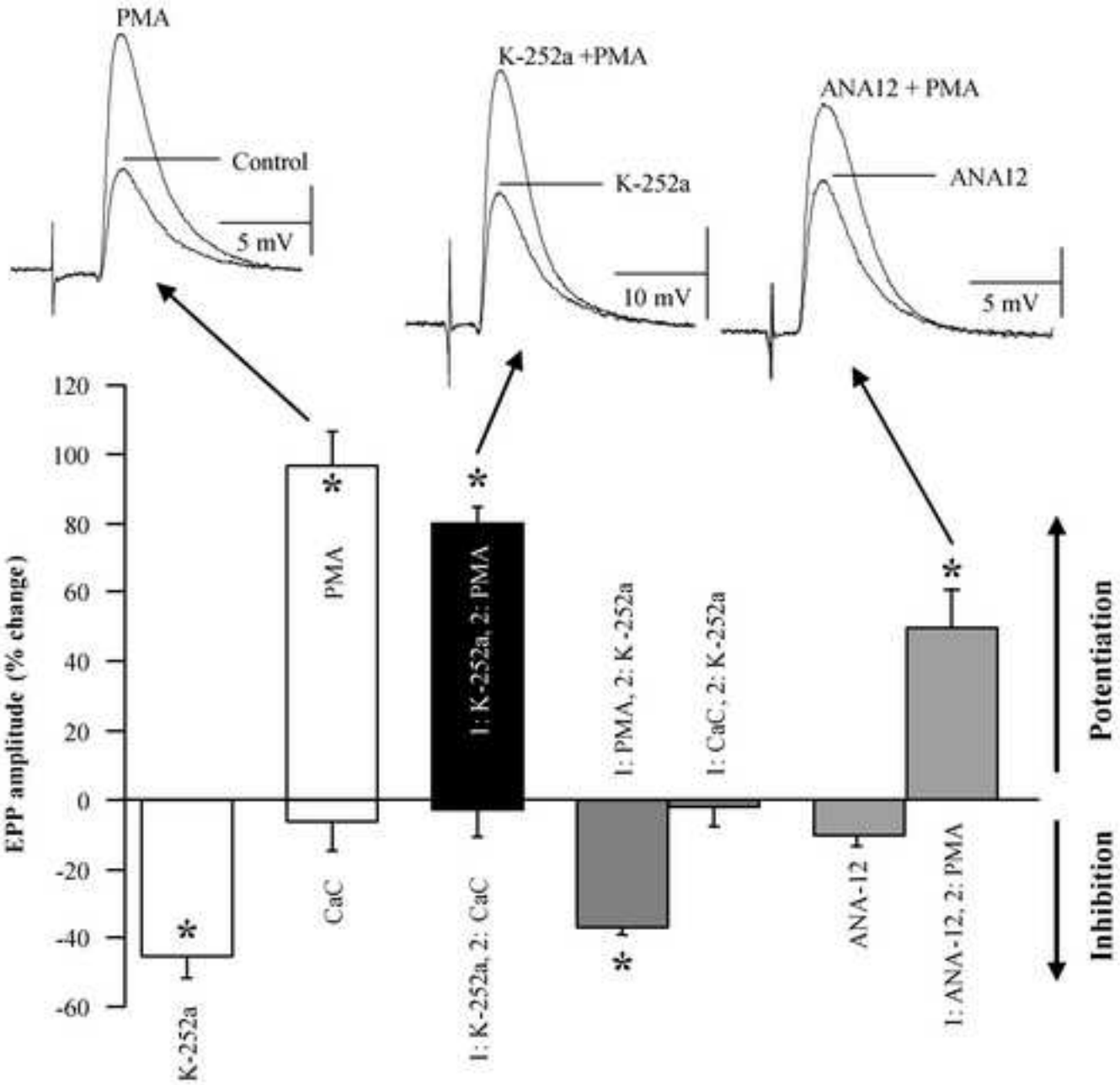
Figure 1



Figure
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Figure 2