Activation of signal transduction pathways involving trkA, PLCγ-1, PKC isoforms and ERK-1/2 by tetanus toxin

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Abstract Previous reports have demonstrated that tetanus toxin (TeTx) induces activation and down-regulation of protein kinase C (PKC). In the present work the differential activation of PKC isoforms and of signal transduction pathways, including nerve growth factor receptor trkA, phospholipase Cγ-1 (PLCγ-1), and extracellular regulated kinases 1 and 2 (ERK-1/2) by TeTx in a synaptosome-enriched P_2 fraction from rat brain is reported. TeTx induces clear translocation from the soluble (cytosolic) compartment to the particulate (membranous) compartment of PKC-β, -γ and -δ isoforms, whereas PKC-ε showed a slight decrease of its soluble fraction immunoreactivity. On the contrary, the PKC-ζ isoform shows no consistent response, whereas down-regulation of total PKC-α immunoreactivity is shown. Immunoprecipitation assays against phosphotyrosine show an increase of trkA and PLCγ-1 phosphorylation. Moreover, trkA activation is corroborated using phospho-specific antibodies against phosphorylated trkA. On the other hand, TeTx-induced stimulation of mitogen-activated protein (MAP) kinase activity is observed, this event also being detected by Western analysis using phospho-specific antibodies against ERK-1/2.

Key words: Clostridial neurotoxin; Synaptosome; Signal transduction; Rat brain

1. Introduction

Tetanus toxin (TeTx), a holoprotein with Zn^{2+}-dependent proteasic activity produced by some strains of the *Clostridium tetani* bacilli, is one of the most known lethal toxins [1]. TeTx is synthesized as a single polypeptide chain of 150 kDa and is subsequently activated by a bacterial endopeptidase to generate a dipeptide toxin. The main targets of TeTx are both the central and peripheral nervous systems (for review, see [2]), where inhibition of neurotransmitter release occurs, by means of selective cleavage of Synaptobrevin II, a protein involved in synaptic vesicle docking and neurotransmitter release [3,4]. Other molecular mechanisms underlying TeTx toxicity have recently been proposed, such as the activation of Ca^{2+}-dependent, GTP-modulated transglutaminase [5], or those described by our group, i.e. activation and subsequent down-regulation of protein kinase C (PKC) after intracerebral injection of TeTx into adult and neonatal rat brain [6,7]. The increase of inositol phospholipid hydrolysis in rat cerebral cortex preparations treated with TeTx indicates an activation of phospholipases, an effect directly related to PKC activation [8].

Nerve growth factor (NGF) belongs to the neurotrophin family [9] and exerts its effects through the interaction with the trkA membrane receptor (reviewed in [10]), activating the protein kinase intrinsic to the receptor leading to autophosphorylation in tyrosine (Tyr) residues of the receptor. This allows recognition of the receptor by several intracellular signaling proteins that contain src homology (SH2) domains, such as phospholipase Cγ-1, shc proteins and the p85 PI-3 kinase subunit (reviewed in [11]). Such interactions lead to the activation of Ras (a low molecular weight G protein) and of Raf kinase, which in turn phosphorylates and activates MEK (MAP kinase kinase or ERK kinase) [12]. This enzyme then phosphorylates ERK (extracellular regulated kinase), a family of Ser/Thr protein kinases of which the best characterized members are ERK-1 (p44) and ERK-2 (p42), being phosphorylated in Thr and Tyr residues [13].

PKC is a family of protein Ser/Thr kinases, especially abundant in the nervous system, composed of homologous isoforms which can be divided into three subfamilies, based on their requirement for calcium, their susceptibility to phorbol ester-induced down-regulation and their structure [14]. Members of the *classical* group (cPKC), α, βI, βII and γ isoforms, are dependent on calcium and diacylglycerol for activation. On the other hand, the *novel* isoforms (nPKC), δ, ε, θ and η, lack the C2 calcium binding domain common to the cPKC members and are thus calcium-independent. *Atypical* (aPKC) ζ, ι, λ and μ isoforms also lack the C2, as well as one of the repeated Cys-rich zinc binding motifs within the C1 domain [15], being unable to bind phorbol esters, such as 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA). They are, therefore, resistant to phorbol ester-induced down-regulation. TPA, as well as the physiological activators, can cause the translocation of PKC activity from the cytosolic fraction to the membrane [16], initially resulting in activation, but followed, in some cases, by down-regulation, by means of a not yet well-known proteolytic pathway [17]. This effect is not only seen in response to TPA but also to some physiological events, such as dopamine D_1 receptor stimulation in rat hippocampal slices [18].

In the present paper, the existence of a differential activation of PKC isoenzymes by TeTx, as well as the stimulation of PLCγ-1 Tyr phosphorylation has been determined. Since PLCγ-1 activation occurs as a result of its interaction with Tyr kinase membrane receptors, we have addressed trkA activation through its Tyr content. The parallel activation of ERK-1/2 is another finding that points to the triggering of signal transduction pathways by TeTx.

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2. Materials and methods

2.1. Materials

Polyclonal antibodies against PKC isoforms α, γ and ζ, as well as Protein A-agarose beads were purchased from Boehringer Mannheim GmbH (Mannheim, Germany). The specific antibody against phosphotyrosine (Clone PY20) was obtained from Zymed Laboratories Inc. (San Francisco, CA, USA). Monoclonal antibody against phospholipase Cγ-1 and polyclonal anti-trkA were from Upstate Biotechnology (Lake Placid, NY, USA). Phospho-p44/42 MAP kinase (ERK-1/2) E10 monoclonal antibody and polyclonal phospho-specific trkA (Tyr-490) antibody were from New England BioLabs (Beverly, MA, USA). Monoclonal antibody against ERK-1 and anti-mouse and anti-rabbit secondary antibodies conjugated with horseradish peroxidase, as well as monoclonal antibodies against PKC isoforms β, δ and ε were from Transduction Laboratories (Lexington, KY, USA). Despite ERK-1 antibody being described as specific for this kinase, it also detects ERK-2, although with less potency. [γ-^{32}P]ATP was from Amersham International (Buckinghamshire, UK). NGF 2.5S was supplied by Alomone Labs (Jerusalem, Israel) and TeTx by List Biological (Campbell, CA, USA). All other reagents were of the highest grade possible from standard commercial sources.

2.2. Preparation of synaptosomes from rat brain

All experiments were performed with a crude synaptosomal fraction (P_2) prepared from Sprague-Dawley, 4–6 weeks old rat brains according to [19] with slight modifications. The whole brain was homogenized in 40 vol. (wt./vol.) of phosphate buffer at pH 7.4 supplemented with 0.32 M sucrose. Homogenization was performed with 12 strokes (900 rpm) using a Potter homogenizer with a Teflon pestle (0.1–0.15 mm clearance). The homogenate was centrifuged at 4°C for 5 min at $1000\times g$. The resultant supernatant was centrifuged at $12\,000\times g$ for 20 min. The crude synaptosomal pellet obtained from one brain was gently resuspended in 7 ml of Krebs–Ringer bicarbonate buffer containing 125 mM NaCl, 3 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM $CaCl_2$, 22 mM $NaHCO_3$, 1 mM NaH_2PO_4 and 10 mM glucose, and the buffer was gassed before use with a mixture of 95% O_2 and 5% CO_2 for 20 min and adjusted to pH 7.4.

2.3. Subcellular fractionation

When subcellular fractionation was required, synaptosomes were collected by centrifugation after each treatment and resuspended in 0.5 ml of homogenization buffer containing 20 mM Tris–HCl (pH 7.5), 2 mM EDTA, 0.5 mM EGTA, 2 mM dithiothreitol (DTT), 1 mM Na_3VO_4, 50 mM NaF, 2 mM phenylmethyl sulphonyl fluoride (PMSF), 10 μg/ml leupeptin and 25 μg/ml aprotinin and disrupted by sonication in a Dynatech Sonic Dismembrator. The homogenate was centrifuged for 1 h at $100\,000\times g$ to separate the soluble fraction, corresponding to the cytosolic compartment, from the particulate fraction, corresponding to the membranous compartment. The precipitated fraction was further resuspended to the original volume using homogenization buffer supplemented with Triton X-100 (0.3% final concentration) and subsequently sonicated and incubated for 1 h at 4°C. The extract was centrifuged for 1 h at $100\,000\times g$ and the resulting supernatant was considered as the particulate fraction. Protein concentration was determined according to Bradford (1976) using bovine serum albumin (BSA) as the standard.

2.4. Immunoprecipitation and immunoblotting analysis

The synaptosome suspension was diluted to a final protein concentration of 1 mg/ml and split into the necessary aliquots. After treatment, synaptosomes were collected by centrifugation and the reaction medium was eliminated. Next, 1 ml of homogenization buffer supplemented with 0.3% Triton X-100 was added and synaptosomes were disrupted by sonication (3×10 s). For immunoprecipitation, 1 mg of protein was incubated by gently rocking at 4°C overnight in the presence of 4 μg of antibody. The immunocomplex was then captured by adding 50 μl of washed Protein A-agarose bead slurry (25 μl of packed beads) and gently rocked at room temperature for 2 h. The agarose beads were collected by pulsing in a microcentrifuge and the supernatant was then drained off. The beads were washed three times with ice-cold PBS and resuspended in 100 μl $2\times$ reducing sample buffer and boiled for 2 min. Next, the agarose beads were separated by pulsing, and 15 μl of each sample were analyzed in polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS–PAGE), according to Laemmli (1970). All of the electrophoresis reagents were of analytical grade (Pharmacia Biotech, Upsala, Sweden). The separated proteins were transferred to a polyvinylidene fluoride (PVDF) membrane (from Millipore, Bedford, MA, USA), using a Mini TransBlot Cell II (Bio-Rad, CA, USA) at 100 V for 1 h. The blotting buffer used contained 48 mM Tris, 39 mM glycine, 1.3 mM SDS and 20% methanol (pH 8.3). The membrane filters were blocked for 1 h with PBS supplemented with 0.1% Tween 20 and 5% defatted

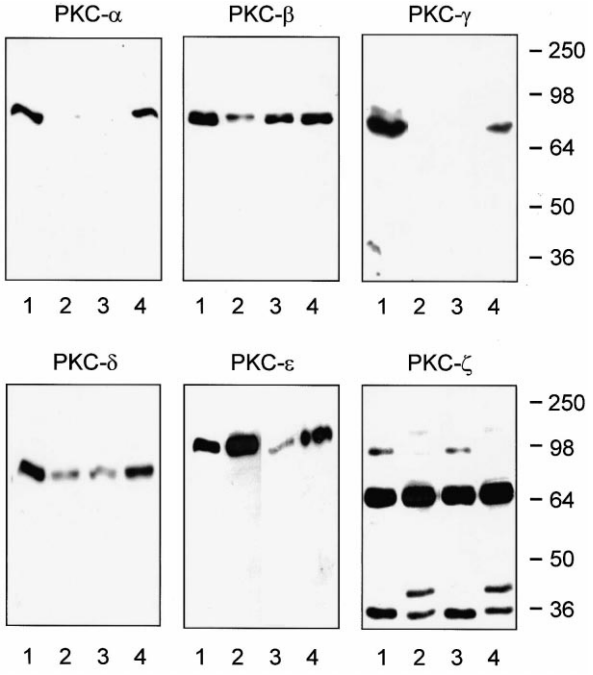

Fig. 1. PKC isoform subcellular distribution in rat brain synaptosomes and their response to TPA. Synaptosomes were exposed to 100 nM TPA for 5 min, and denatured soluble and particulate fractions were prepared as described in Section 2. For SDS–PAGE, protein loading was 20 μg/ml and then transfer of protein to PVDF membrane was carried out with ECL detection. Experiments were carried out on eight separate occasions with similar results. Western blots show representative immunodetections of PKC-α, -β, -γ, -δ, -ε and -ζ isoforms in soluble (lines 1 and 3) or in particulate fraction (lines 2 and 4), lines 1 and 2 being from vehicle-treated synaptosomes and lines 3 and 4 from TPA-treated synaptosomes. The standard molecular weight is shown on the right in kDa.

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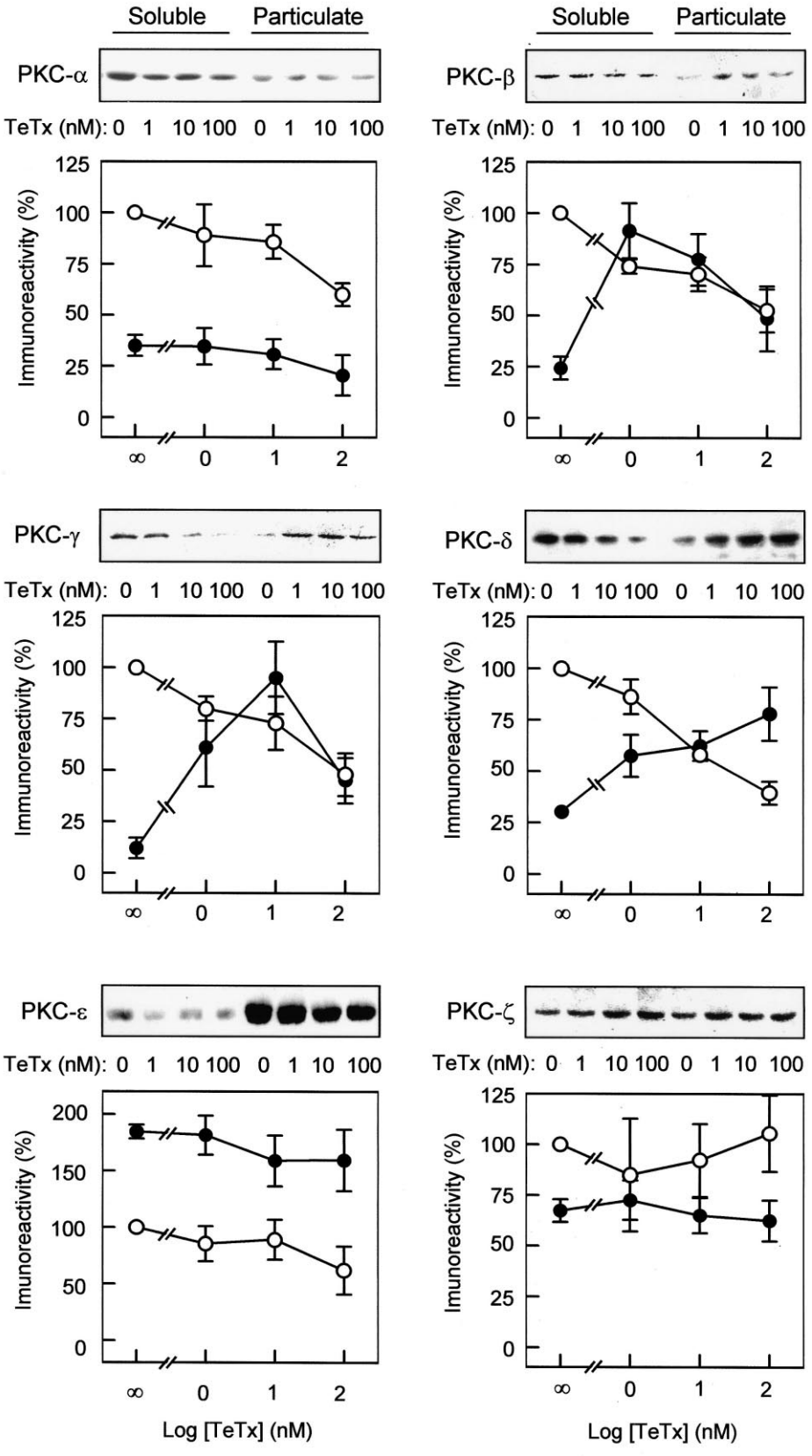
Fig. 2. TeTx-induced PKC isoform translocation. Synaptosomes were exposed to 1, 10 and 100 nM TeTx for 15 min, and then soluble and particulate fractions were prepared. For SDS–PAGE, protein loading was 20 μg/lane for both fractions. Following SDS–PAGE and transfer of protein to PVDF membrane, immunoblotting was carried out as described in Section 2 with ECL detection. The upper panels show representative blottings of the results obtained with each isoform (PKC-α, -β, -γ, -δ, -ε and -ζ), as is indicated on the left of each blot. The lower panels show the quantitation by laser scanning densitometry of the immunoreactivity variations in the soluble (○) and particulate (●) fractions. Data were normalized, taking the control soluble arbitrary absorbance units value as 100%. The amounts of immunoreactive PKC isoform in the soluble or particulate fraction (expressed as %, with respect to control soluble) were plotted against log TeTx concentration. Data from three separate experiments were expressed as means ± S.D.

powdered milk. Next, the membranes were incubated overnight with the corresponding antibody diluted in blocking buffer. Then, the membrane filters were incubated for 1 h with a secondary antibody conjugated with horseradish peroxidase diluted in blocking buffer. Several washes with PBS/0.1% Tween 20 were performed between each one of the steps. The Western blots were developed using Super-Signal West Pico Chemiluminescent Substrate from Pierce (Rockford, IL, USA) and exposed to Amersham ECL films. The computer-assisted analysis of the bands was performed with a Bio-Rad GS700 system (Bio-Rad, CA, USA), and data were processed with a Bio-Rad Molecular Analyst image program using a DELL work station. Repeated scans were taken for film non-linearity corrections.



2.5. MAP kinase assay

MAP kinase assays were performed as described [20], with modifications. Briefly, 15 μl of soluble fraction (at a protein concentration of 40 μg/ml) was added to 35 μl of a solution containing MBP (0.3 mg/ml), 20 mM Tris–HCl pH 7.5, 200 μM ATP (with [γ^{32}]ATP at a specific radioactivity of 10 000 cpm/pmol), 25 mM magnesium acetate, 2 μM PKi, 200 μM Na_3VO_4 and 200 μM EGTA. The phosphorylation reaction was conducted at 37°C for 10 min. In order to terminate the reaction, 40 μl of the reaction solution were absorbed in phosphocellulose P81 Whatman paper (2 cm×2 cm), rinsed in 0.5% phosphoric acid and washed five times (1 min each). After that, the radioactivity incorporated was determined by liquid scintillation counting.

3. Results

3.1. Redistribution of PKC isoforms by TPA in the rat brain synaptosomal fraction

The *classical* PKC isoforms (PKC-α, -β and -γ), as well as PKC-δ, were detected in rat brain synaptosomes, appearing in SDS–PAGE at an apparent molecular weight of 80 kDa, whereas *novel* PKC-ε showed an apparent molecular mass of 110 kDa, and *atypical* PKC-ζ a molecular mass of 68 kDa. The specificity of the antibody against the PKC-ζ isoform has been tested through blocking the antibody with the corresponding antigenic peptide, amino acids 577–592 of PKC-ζ, since it is the only antibody that recognizes more than one band (Fig. 1). The incubation of 100 nM TPA for 5 min induces translocation from the soluble fraction to the particulate fraction of the *classical* isoforms (PKC-α, -β, -γ), as well as the translocation of the *novel* PKC-δ, indicating activation of these isoforms by TPA. A large amount of PKC-ε was found associated to the particulate fraction under basal conditions in synaptosomes, and no translocation of this isoform was detected, although a visible diminution in the soluble fraction was seen. In contrast, no effect was observed in the case of the PKC-ζ, as has been extensively described (Fig. 1).

3.2. Effect of TeTx treatment on specific PKC isoform redistribution

Clear translocation of PKC-β, -γ and -δ isoforms was observed when synaptosomes were incubated with increasing concentrations of TeTx, during 15 min. PKC-β is the more sensitive isoform, being translocated at 1–10 nM TeTx, whereas at 100 nM the total immunoreactivity decreases, revealing the appearance of down-regulation. The response of PKC-γ isoform was similar, although down-regulation appears at a higher TeTx concentration (100 nM) (Fig. 2). On the other hand, PKC-δ showed a sustained translocation, without down-regulation at any concentration tested. In the case of the PKC-α isoform, a diminution in its total immunoreactivity (40% of diminution in the soluble fraction and 41% in the particulate fraction) was detected at the highest concentration tested (100 nM). No consistent translocation of PKC-ε and PKC-ζ following exposure of synaptosomes to TeTx was found. Despite this, a slight diminution in the immunoreactivity corresponding to the soluble fraction of PKC-ε was observed (38% diminution), whereas no increase in the particulate fraction occurs. This absence of translocation cannot be attributed to either a saturation of the immunoblot technique or an overexposure of the film during ECL detection. In order to obtain internal control in PKC translocation experiments TPA was included separately in each experiment (not shown).

3.3. TeTx stimulates tyrosine phosphorylation of trkA and of PLCγ-1

To determine the possible effect of TeTx on cellular signal transduction pathways that involve Tyr phosphorylation, immunoprecipitation experiments using anti-phosphotyrosine antibodies (clone PY20) were performed. In each experiment whole synaptosomal homogenates were immunoprecipitated using PY20 antibodies, and the contents of two proteins which are phosphorylated in Tyr, i.e. trkA and PLCγ-1, were determined, using specific antibodies, by Western blot. NGF was used as control of a typical signal transduction activator (Fig. 3A). A single band corresponding to the trkA receptor appeared at, approximately, 140 kDa of molecular weight and a single band corresponding to PLCγ-1 at 150 kDa, both in accordance with the literature. This effect was not unspecific, since the amount of signal due to FGFR-1 (fibroblast growth factor receptor-1) Tyr phosphorylation was not altered (results not shown). The amount of Tyr phosphorylation which appeared in trkA and in PLCγ-1 is time-dependent, the onset of the signal being rapid and detectable after 1 min of treatment (Fig. 3A). In the two phosphoproteins tested, the maximal amounts of signal were seen in 10 min, whereas after 30 min the phosphorylation level had decreased. The PLCγ-1 phosphorylation was further supported by our finding that TeTx induces a modest but significant polyphosphoinositide hydrolysis increase in rat brain synaptosomes, which is in agreement with PLC phosphorylation and activation (manuscript submitted). Moreover, Western analysis using a phospho-specific antibody recognizing trkA

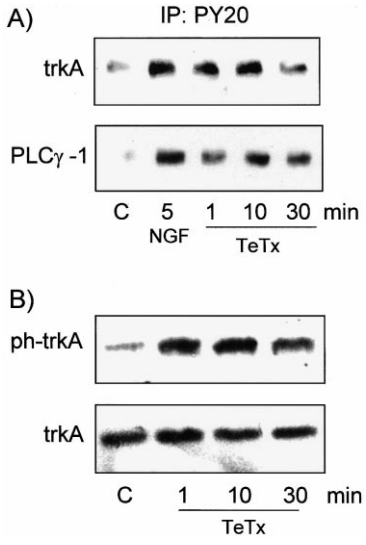


Fig. 3. TeTx-induced Tyr phosphorylation of trkA and PLCγ-1. A: Synaptosomes in suspension in Krebs–Ringer buffer supplemented with 10 mM glucose were incubated at 30°C with NGF (50 nM) as positive control for 5 min or with 100 nM TeTx at different times. Equal amounts of lysate from each time point, obtained by sonication and with a protein concentration of 1 mg/ml, were immunoprecipitated, as described in Section 2, with 4 μg of antibody PY20 directed against phosphotyrosine residues, and blots were probed with anti-trkA or anti-PLCγ-1. Each experiment was repeated three times, and similar results were obtained. B: Time-course of the TeTx-stimulated phosphorylation of trkA in Tyr-490. The upper blot was incubated with phospho-specific antibody for phosphorylated trkA (Tyr-490). The lower blot shows immunoreactivity obtained with antibody specific for trkA, in a manner independent of its phosphorylation state. Blots are representative of three separate experiments.

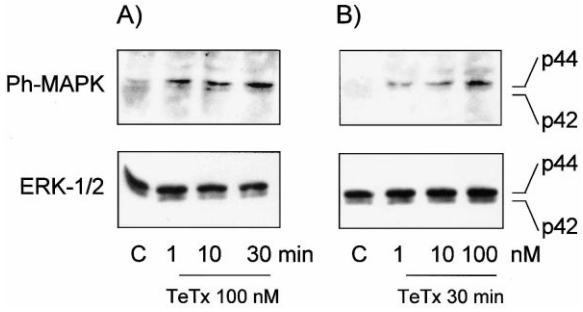


Fig. 4. Activation of ERK-1/2 by TeTx. Synaptosomes were incubated at 30°C with TeTx (100 nM) for different times in A or were incubated at increasing TeTx concentration for 30 min in B. For immunoblot 20 μg/lane of total synaptosomal lysates were loaded in each SDS–PAGE. Upper blots were analyzed using antibody specific for fully Thr- and Tyr-phosphorylated ERK-1 (p44) and ERK-2 (p42) (phospho-p44/42 MAP kinase E10 monoclonal antibody). Lower blots show the immunoreactivity obtained for samples as in upper blots with antibodies specific for ERK-1 and -2, independent of their phosphorylation state. All blots are representative of three separate experiments.

phosphorylated in Tyr-490 reveals a rapid increase of TeTx-induced trkA phosphorylation, while signal decreasing is detected at 30 min after treatment (Fig. 3B). Equal loading of protein between samples was confirmed by detection with phosphorylation state-independent anti-trkA. This result is in agreement with the signal which appeared in immunoprecipitation experiments.

3.4. Enhancement of ERK-1/2 phosphorylation status and of MAP kinase activity

A series of Western analysis using an antibody specific for dually Thr- and Tyr-phosphorylated ERK-1 (p44) and ERK-2 (p42) was performed (Fig. 4). In time-course experiments, where synaptosomes were treated with TeTx (100 nM), an induction of ERK phosphorylation was seen. The strongest signal appeared after 10 min of treatment, being maintained in the 30 min treatment. Equal loading of protein between samples was confirmed by detection with phosphorylation state-independent anti-ERK-1, although this antibody recognizes both p44 and p42 in the assayed conditions. The determination of MAPK activity present in the synaptosomal soluble fraction, using myelin basic protein (MBP) as substrate, shows an increase in MBP phosphorylation induced by TeTx, this being slight at 10 nM (154%) and more marked at 100 nM (245%) (Fig. 5).

4. Discussion

The influence of TeTx over some members of signal transduction pathways has been described in several works. The first work on this topic described the decrease of PKC activity, in soluble fraction as well as in particulate fraction, in TeTx-treated macrophages and in the spinal cord of mice with generalized tetanus [21]. Our group described TeTx-induced PKC activation/translocation and down-regulation after intraventricular toxin administration into adult rats [6]. In perinatal rat brain, TeTx causes PKC activation parallel with an increase of serotonin turnover [22,7]. Later, treatment of rat brain primary neuronal cultures with the neurotoxin revealed PKC activity translocation and increases in polyphosphoinositide hydrolysis [8]. In the present work, a detailed investigation focusing on which PKC isoforms are affected by TeTx is performed, observing a puzzling diminution in total PKC-α immunoreactivity, which could be related to a high degree of stimulus-triggered PKC hydrolysis detected in rat brain synaptosomes [23]. PKC translocation observed in some isoforms in this work is directly related to the activation of PLCγ-1, since the phospholipase products (diacylglycerol and inositol 1,4,5-triphosphate, as a calcium mobilizer) are activators of PKC and induce its translocation. Moreover, PLCγ-1 phosphorylation is in consonance with polyphosphoinositide hydrolysis enhancement by TeTx observed in primary neuron cultures from fetal rat brain and in slices from adult rat cerebral cortex [8]. This phospholipase C activity increase, which is modest but significant, is very similar to that exerted by NGF in rat brain synaptosomes [23]. In the same work, an NGF-activated PKC-γ and -δ translocation was described, although PKC-β translocation was slight and difficult to detect. TeTx-stimulated inositol phospholipid hydrolysis has also been detected in rat brain synaptosomes, in a comparable level to that exerted by NGF (results not shown). NGF is a signaling molecule that acts through PLCγ activation [24], this specific activation being related to the modest increase of polyphosphoinositide hydrolysis. Additionally, PKC activation in synaptosomes could be related to serotonin transport inhibition caused by TeTx or by its H_C fragment [25], since serotonin transporter phosphorylation by PKC causes a diminution in its transport capacity [26]. Studies focusing on H_C-TeTx-induced redistribution of PKC isoenzymes are now in progress in our group. On the other hand, TeTx-induced phosphorylation of trkA in Tyr-490 points to a parallel association of shc proteins with the trkA, an important event implicated in neuronal differentiation [27]. This putative association is strongly supported by ERK-1/2 phosphorylation observed in this work, since the shc adapter proteins are the first link of a pathway that results in ERK-1/2 activation [12]. In addition, the activation of MAPK pathway could explain the changes in the C-*fos* and Fos-like immunoreactivity observed in the cortex of rats with tetanus toxin-induced epilepsy [28], since growth factor-induced c-fos expression is activated through transcription factors, such as Elk-1 or SAP-1, that are targets of the ras/MAPK [29] or PKC mediated phosphorylation [30].

Early studies show that TeTx binds to gangliosides contain-

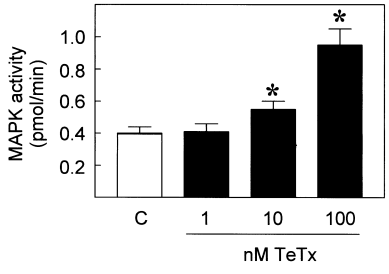


Fig. 5. MAPK activity induction by TeTx, at 1, 10 and 100 nM concentration. The MAPK activity present in the soluble fraction was assessed at a final protein concentration of 10 μg/ml using [γ-^{32}P]ATP and MBP as substrate, as described in Section 2. The results are expressed as means ± S.E.M. of the percentage of three separate experiments. Differences between groups were assessed using the one-way ANOVA test ($P < 0.001$) and one-way Dunnett's test for multiple comparisons. '*' represents significantly different from the control group ($P < 0.05$).

ing '1b' structure, such as GT_{1b} or GD_{1b} with a high affinity [31]. Ganglioside binding has also been demonstrated for Type A and for Type E *Clostridium botulinum* neurotoxins [32]. On the other hand, the involvement of a protein component has been postulated, since TeTx binding to cells and neuronal membranes is sensitive to proteases [33]. According to this, a two receptor model has been postulated in which the initial toxin binding to gangliosides is followed by lateral movement in the membrane until the toxin reaches a protein receptor, then given internalization [34]. Despite efforts made, the nature of the neurospecific protein receptor of TeTx is still unknown, although cross-linking experiments in NGF-differentiated PC12 cells suggest that an *N*-glycosylated protein with an apparent molecular weight of 15–20 kDa is involved in the neurospecific binding of TeTx [35,36]. Recently, the binding of the H_C fragment from BoNT/A, which shares the feature with TeTx and with trk receptors of being retroaxonally transported [37], to three proteins of approximately 150, 120 and 75 kDa of molecular mass in rat synaptosomes has been reported [38].

In summary, this work determines differential translocation of synaptosomal PKC isoforms in response to TeTx. Likewise, this PKC activation is directly related to PLCγ-1 phosphorylation and to trkA activation, assessed by means of its phosphotyrosine content. Furthermore, the downstream activation of MAP kinases ERK-1 and -2 also exists, displaying part of a signal transduction pathway, whose complexity must be assessed, triggered by TeTx. On the other hand, the interaction of TeTx with p140 trkA represents a mechanism by which the toxin could take advantage of trkA mobility in its tropism to the central nervous system. Further work, which is in progress in our group, will shed light on the specific relations between activation of signal proteins by TeTx and the manner of action of this lethal toxin on the nervous system.

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