Bisperoxovanadium complex promotes dopamine exocytosis in PC12 cells

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Abstract
The effects of the peroxovanadium complex potassium bisperoxo(1,10-phenanthroline)-oxovanadate (bpV[phen]) have been studied on dopamine (DA) exocytosis in PC12 cells. Bisperoxo(1,10-phenanthroline)-oxovanadate does not elicit dopamine secretion in PC12 cells. However, treatment of PC12 cells with 30 μM bpV[phen] for 20 min significantly enhances the secretion induced by the Ca2+-ionophore A23187. The effects appear to be irreversible, and strikingly different from the transient and suppressing effects of orthovanadate, which, like bpV[phen], is also a protein tyrosine phosphatase inhibitor. Contrastingly, the short-lived peroxovanadates, formed in situ by the addition of hydrogen peroxide and orthovanadate, are relatively ineffective. The Ca2+ chelating agent EGTA abolishes bpV[phen]-enhanced dopamine release. The extracellular-regulated protein kinases (ERK) and synaptophysin, proteins implicated in exocytosis, are both tyrosine-phosphorylated by bpV[phen] in a dose- and time-dependent manner, with a maximal effect at 30 μM. Pre-treatment of cells with PD98059 significantly reduced dopamine release (P < 0.05). These results suggest that this peroxovanadium complex enhances dopamine exocytosis, at least in part, by ERK-mediated signaling pathway and synaptophysin-associated phosphatase(s). © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Exocytosis; Dopamine; PC12 cells; Protein tyrosine phosphatase inhibitors; Vanadate; Synaptophysin; Phosphorylation; Peroxovanadium complexes

1. Introduction
Potassium bisperoxo(1,10-phenanthroline)-oxovanadate (bpV[phen]), a specific inhibitor of protein tyrosine phosphatases (PTPs), belongs to a class of peroxovanadium complexes that are insulinomimetics (Posner et al., 1994; Shaver et al., 1995; Cerovac et al., 1999; Rumora et al., 2001). These complexes are believed to cause irreversible inhibition of PTPs by oxidizing the cysteine residue in the catalytic domain of the phosphatase (Bevan et al., 1995; Huyer et al., 1997; Morinville et al., 2001). The goal of the present study was to determine whether bpV[phen] affects the release of a typical neurotransmitter, dopamine (DA), from PC12 cells, and whether this release process is accompanied by an altered phosphorylation of mitogen-activated protein kinases (MAPK) and synaptophysin.

Protein phosphorylation constitutes an important mechanism in the process of exocytosis. Indeed, several key proteins, such as synapsins and synaptophysin have been shown to undergo phosphorylation during the mobilisation, fusion and recycling of synaptic vesicles (Pang et al., 1988; Abdul-Ghani et al., 1991; Jena et al., 1994; Gomperts et al., 1991; Schweizer et al., 1995; Obendorf et al., 1988; Liu, 1997; Shoji-Kasai et al., 2001). Recently, Becher et al. (1999) demonstrated that synaptophysin–synaptobrevin complex plays an important role in providing a reserve pool of synaptobrevin critical for exocytosis. Members of the synaptophysin family may be involved in fine-tuning of neurotransmitter release (Janz and Sudhof, 1998). Recent studies (Shibaguchi et al., 2000) in Xenopus oocytes show that synaptophysin is implicated in DA release.

The role of tyrosine phosphorylation in the process of neurotransmitter exocytosis is far from clear. It has been reported that the tyrosine kinase pp60c-src could be involved in the regulated secretion of catecholamines from adrenal chromaffin cells (Oddlie et al., 1989; Ely et al., 1994). Synaptophysin has been identified as a potential substrate for pp60c-src (Barneckow et al., 1990). More recently, pp60c-src has been shown, both in vitro and in vivo, to tyrosine phosphorylate the synaptic membrane vesicle transmembrane spanning protein, synaptogyrin and one of its isoforms, cellugyrin (Janz and Sudhof, 1998). In addition, protein tyrosine kinases have been implicated in granular exocytosis from NK and CTL cells (Umehara et al., 1997), in the synthesis and release of PG12 in human umbilical vein endothelial cells (Wheeler-Jones et al., 1996), and...
in lipopolysaccharide (LPS)-stimulated exocytosis in insect hemocytes (Charalambidis et al., 1995). Moreover, a number of other proteins, most notably some MAPK-like proteins and MAPK, have been shown to become tyrosine phosphorylated prior to exocytosis in chromaffin cells (Ely et al., 1990), in neurons (Pang et al., 1988; Baumert et al., 1990) and in a mast cell line, RBL-2H3 (Santini and Beaven, 1993). Evidence for MAPK involvement has also recently been documented in bovine adrenal chromaffin cells (Cox et al., 1996; Cox and Parsons, 1997) and in PC12 cells (Bloch-Shilderman et al., 2001). However, none of these studies have used peroxovanadium complexes as a pharmacological tool to manipulate dopamine release. Taken together, these findings suggest that the role of tyrosine phosphorylation in the exocytotic process merits further investigation.

The results from the present studies show that bpV[phen] significantly enhances DA release in a dose- and time-dependent manner in PC12 cells. Bisperoxo(1,10-phenanthroline)-oxovanadate produces a long lasting stimulatory effect as opposed to the transient and inhibitory effect induced by vanadate. In addition, bpV[phen] alters the phosphorylation state of MAPK and synaptophysin, proteins implicated in exocytosis (Cox and Parsons, 1997; Cox et al., 1996; Thoudis et al., 1998; Volkman, 1995; Navone et al., 1986; Rubinstein et al., 1993; Bloch-Shilderman et al., 2001).

2. Materials and methods

2.1. Cell culture

The PC12 cells used in this study, clone number 13088, were recently obtained from the American Type Culture Collection (ATCC) and were used for no more than 10–12 passages. Growth medium consisted of RPMI (Sigma) supplemented with 10% horse serum (Gibco) and 5% fetal bovine serum (Gibco), as well as antibiotics (penicillin and streptomycin; Gibco). One day prior to an experiment, a flask of confluent cells was passaged and plated onto a poly-l-lysine-coated 24-well tissue culture plate at a density of 50,000 cells per well.

2.2. Cell treatments

After 24–30h, the culture medium was removed and replaced with pre-warmed stimulation buffer (10 mM HEPES, pH 7.2, 150 mM NaCl, 3 mM CaCl2, 2 mM KCl, 1 mM sodium orthovanadate, and “Complete protease inhibitor” (Boehringer Mannheim, Indianapolis, IN) as per the manufacturer’s instructions). The lysates were subsequently centrifuged at 14,000 rpm for 20 min at 4 °C and the protein content was determined using Bio-Rad protein assay dye reagent by the method of Bradford (1976) with bovine albumin as the protein standard. Samples were denatured by boiling for 3 min with either a 1:1 dilution with 2× Laemmli sample buffer (0.125 M Tris-HCl pH 6.8, 4% w/v SDS, 1% 2-mercaptoethanol, 0.2% w/v bromophenol blue, 4% β-mercaptoethanol in double distilled water) or 4:1 with 6× Laemmli sample buffer (0.375 M Tris-HCl pH 6.8, 12% w/v SDS, 3% w/v glycerol, 0.2% w/v bromophenol blue, 12% β-mercaptoethanol in doubly distilled water). Samples were frozen at −20 °C until use. An equal amount of protein was added to each well in a 1:1 dilution with 2× Laemmli sample buffer.
loaded for each sample onto a 12% polyacrylamide gel and transferred onto nitrocellulose film. An additional 20 min was required for electrophoresis, followed by 20 min in blocking buffer (2% milk, Bio-Rad). All antibody dilutions were performed in PBS (25 mM Tris, 150 mM NaCl, 0.05% Tween, 2% bovine albumin) in washing buffer (25 mM Tris, 150 mM NaCl, 0.05% Tween 20 in doubly distilled water). Primary antibodies were diluted in blocking buffer, at a dilution of 1:1000 (Phosphotyrosine (Upstate Biotechnology, Lake Placid, NY) ERK1 (Santa Cruz Biotechnology, Santa Cruz, CA)), or 1:20000 (Anti-active MAPK, Promega (Madison, WI)). Anti-active-MAPK recognizes only phosphorylated MAPK. The anti-ERK1 antibody used in this study is known to cross-react with anti-ERK2 and detects both phosphorylated and non-phosphorylated MAPK. Anti-mouse horseradish peroxidase-linked anti-rabbit horseradish peroxidase-linked antibodies (dilution of 1:4000 in washing buffer with 5% milk) were obtained from Amersham Life Sciences, Inc. (Little Chalfont, Buckinghamshire, England). The chemiluminescence reagents and film were purchased either from Amersham LifeSciences, Inc. (ECL and hyperfilm, respectively) or Dupont (Boston, MA, NEN Renaissance reagents and autoradiography film, respectively) or Kodak (X-OMAT Blue film). Membranes were stripped by incubating at 65 °C for 30 min in stripping buffer (100 mM β-mercaptoethanol, 2% w/v SDS and 62.5 mM Tris–HCl pH 6.7). Immunoblots were scanned using a Vista scan V2.37 scanner and the Presto PageManager V1.22 Rev03 software (NewSoft America Inc., Fremont, CA). The integral density for each band was determined using software from Scionimage (Scion Corporation, Frederick, MD).

2.5. Immunoprecipitation

Anti-synaptophysin (MAB 368, Canadian Chemicon, Bio/Can Scientific) was added (2 µl/100 µl) to cell lysates and incubated at 4 °C overnight. Precipitated synaptophysin was separated by adding protein A agarose beads (30 µl per sample, Bio-Rad), incubated for an additional hour and beads were separated by centrifugation (2 min, 12 000 rpm). Beads were washed 3× with lysis buffer, centrifuged and the pellet was boiled (5 min) with 2× Laemmli sample buffer (30 µl), cooled on ice and stored at −20 °C. Proteins were separated using pre-made gels (12%, Novex) and transferred onto nitrocellulose membrane (0.2 µm, Bio-Rad). All subsequent steps were as described for antiphosphotyrosine Western blotting.

3. Results

3.1. Potentiation of DA release by bpV[phen]

is dose-dependent

PC12 cells were first treated with bpV[phen] for 30 min in the absence of secretagogues in order to determine whether bpV[phen] itself could alter basal DA secretion. None of the concentrations tested (between 0 and 300 µM) could alter low basal DA release (20–80 pg/ml, Fig. 1).

We next investigated the ability of bpV[phen] to alter DA release induced by several different secretory stimuli. The Ca2+ ionophore A23187, at a concentration of 1–5 µM, induced a robust DA secretion from PC12 cells into the stimulation medium (Fig. 1). When the cells were pre-treated for 20 min with various concentrations of bpV[phen] and then stimulated with A23187 in the continued presence of bpV[phen], a characteristic dose–response curve was obtained (Fig. 1). Secretion of DA was maximal at 30 µM bpV[phen], reaching 150–200% of the levels observed in cultures not pre-treated with bpV[phen]. While the absolute amounts of DA exocytosed varied from one experiment to another, the shape of the dose–response curves was consistent in five independent experiments, peaking at 30 µM. However, triplicate samples analysed from each experiment were very reproducible and within 5% experimental error. At concentrations of 100 μM or more, bpV[phen] still enhanced DA release, but to a lesser extent than at 30 µM (Fig. 1). DA release induced by depolarisation of cells with high extracellular K+ (55 mM KCl added to the stimulation buffer) was less affected by bpV[phen] (Fig. 2). Although the dose–response curve again showed the peak at 30 µM, the maximal increase in DA release was consistently lower than that observed in experiments with A23187. Other secretagogues tested, notably histamine (not shown) and nicotine (Fig. 2), failed to elicit a high enough level of DA release to merit further evaluation. There was no evidence of toxicity to the cells exposed to any of the concentrations of bpV[phen] used during the time periods employed in the described experiments.

3.2. Bisperoxo(1,10-phenanthroline)-oxovanadate effects on DA release are time-dependent

The experiments shown in Fig. 1 revealed the cumulative effects of bpV[phen] during a 40 min exposure period (20 min pre-incubation and 20 min stimulation). To inves-

Fig. 2. The effect of bpV[phen] pre-treatment over time on dopamine release induced by secretagogues. PC12 cells were treated with 30 μM bpV[phen] for 20 min and were subsequently incubated with either 100 μM nicotine, KCl 55 mM or A23187 5 μM. Dopamine concentrations were determined by HPLC as described in Section 2. Basal dopamine release (i.e. in the absence of secretagogues) was between 31 ± 8 pg/ml. Values represent the means of three independent determinations.

The time course of bpV[phen] action, PC12 cells were pre-incubated in the stimulation buffer in the absence of bpV[phen] and then stimulated in the presence of 30 μM bpV[phen], the maximally effective concentration. DA release was unaffected by bpV[phen] until about 10 min, after which time, increased release was observed. This latency of activation was observed with both A23187- and KCl-induced release (the results with 5 μM A23187 are shown in Fig. 2). When PC12 cells were pre-treated with bpV[phen], for 30 min prior to the addition of secretagogue, significantly increased DA release was apparent after 10 min of stimulation (Fig. 3).

3.3. Bisperoxo(1,10-phenanthroline)-oxovanadate reversibility

To determine whether the effect of bpV[phen] is reversible, we pre-treated PC12 cells for 20 min with bpV[phen], then washed the cells twice for 10 min with stimulation buffer and stimulated them with A23187 in the absence of bpV[phen] (WASHOUT, Fig. 4). DA release from these cultures was compared with parallel cultures pre-treated with bpV[phen] for 20 min and subsequently stimulated with A23187 in the presence of bpV[phen] (CONTINUOUS, Fig. 4). The results show that the effects of bpV[phen] could not be completely reversed, possibly because of an inability of the washing procedure to totally eliminate the compound. Dopamine release from pre-treated and washed cells remained elevated relative to untreated controls, but it was lower than DA release from cells continuously exposed to bpV[phen]. These results suggest that the effects produced by bpV[phen] on DA exocytosis are only partially reversible.

3.4. Calcium dependence

We next sought to determine whether the bpV[phen]-induced dopamine release is dependent on calcium. Incubation in the presence of the Ca2+-chelating agent EGTA did not change A23187-induced release of dopamine (Fig. 5, columns 1 and 3). However, incubation of bpV[phen] with then washed the cells twice for 10 min with stimulation buffer and stimulated them with A23187 in the absence of bpV[phen] (WASHOUT, Fig. 4).

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Fig. 3. Time course of dopamine release from PC12 cells stimulated with 3 μM A23187 in the presence of 30 μM bpV[phen]. Cells were either exposed to 30 μM bpV[phen] for 20 min (PRE), or not, prior to stimulation with 3 μM A23187 in the presence of 30 μM bpV[phen]. Dopamine concentrations were determined as described in Section 2. Basal dopamine release (i.e. in the absence of secretagogues) was between 35 and 52 pg/ml. Each point represents the mean of five independent determinations. Variability between the individual values for dopamine determination under each condition was less than 5%.

Fig. 4. Partial irreversibility of the effects of bpV[phen] on dopamine release. PC12 cells were pre-treated with 30 μM bpV[phen] for 20 min, and the cells were either subsequently washed with buffer prior to exposure to 5 μM A23187 (WASHOUT) or were continuously exposed to bpV[phen] during stimulation with 5 μM A23187 (CONTINUOUS). Dopamine was determined as described in Section 2. Basal values of dopamine release were between 28 and 37 pg/ml.

Fig. 5. Partial irreversibility of the effects of bpV[phen] on dopamine release. PC12 cells were pre-treated with 30 μM bpV[phen] for 20 min, and the cells were either subsequently washed with buffer prior to exposure to 5 μM A23187 (WASHOUT) or were continuously exposed to bpV[phen] during stimulation with 5 μM A23187 (CONTINUOUS). Dopamine was determined as described in Section 2. Basal values of dopamine release were between 28 and 37 pg/ml.

Fig. 5. Potentiation of dopamine release by bpV[phen] complex depends on extracellular calcium. PC12 cells were untreated, treated with EGTA, or pre-treated with bpV[phen] (30 μM) in the presence and absence of EGTA (1 mM), and subsequently stimulated with A23187 to induce dopamine release (determined as described previously). Bars represent the mean ± S.E.M. (*p < 0.001).
Fig. 6. Comparison between the effects of bpV[phen] with orthovanadate and H$_2$O$_2$. (A) Orthovanadate and bpV[phen] at the same concentrations exert different effects on A23187-induced dopamine release (as described in Section 2). (B) Comparison between drugs applied in equimolar concentrations (30 μM) of bpV[phen], orthovanadate, H$_2$O$_2$, and pervanadate (formed by the addition of H$_2$O$_2$ and orthovanadate) for 20 min in the presence of A23187 (3 μM). Dopamine concentrations were determined by HPLC as described in Section 2. Each point represents the mean from three to five independent determinations. Asterisk denotes statistical significance between bpV[phen] and other treatments or untreated cells (p < 0.001).

EGTA abolished the potentiation by 30 μM bpV[phen] of DA release induced by A23187 (Fig. 5, columns 2 and 4). These results suggest that bpV[phen] potentiates dopamine release through the mobilisation of Ca$^{2+}$.

3.5. The effect of bpV[phen] differs from orthovanadate

As orthovanadate and peroxovanadate are both inhibitors of PTPs, it was hypothesised that their effects would be similar. Accordingly, analogous experiments were conducted in PC12 cells employing similar experimental conditions for orthovanadate as for bpV[phen]. Orthovanadate could completely suppress DA release induced by either KCl (not shown) or A23187 (Fig. 6A) whereas basal release appeared to be unaffected (it stayed within 25–40 pg/ml). Washout experiments with orthovanadate suggested that effects of orthovanadate, unlike the case with bpV[phen], are reversible (not shown).

3.6. Orthovanadate and H$_2$O$_2$ in combination do not reproduce the effects of bpV[phen] on DA release

The observed differences between bpV[phen] and orthovanadate most plausibly arise from the two peroxo groups present in the bpV[phen], which are absent for orthovanadate. Thus, a mixture of orthovanadate and H$_2$O$_2$ (referred to as “pervanadate”) could be expected to have effects analogous to those of bpV[phen] on DA release. A number of stimulation experiments were conducted with orthovanadate and H$_2$O$_2$ either in 1:1 or 1:2 molar ratios, respectively, in order to determine whether this is indeed the case. Hydrogen peroxide itself could enhance DA release only at concentrations greater than 100 μM (not shown). However, no synergistic effects of H$_2$O$_2$ and orthovanadate were observed. Addition of H$_2$O$_2$ merely blocked the inhibitory effects of orthovanadate without significantly enhancing DA release (Fig. 6B).

3.7. Bisperoxo(1,10-phenanthroline)-oxovanadate produces a dose- and time-dependent increase in protein tyrosine phosphorylation

Lysates of PC12 cells treated with orthovanadate or bpV[phen] were subjected to gel electrophoresis and probed with anti-phosphotyrosine antibody to discover any overt differences. Predictably, a large number of proteins were tyrosine-phosphorylated following treatment with bpV[phen] (Fig. 7). Proteins become weakly tyrosine-phosphorylated after 5 min and are maximally phosphorylated in the presence of 30 μM bpV[phen] by 20 min (Fig. 7). On the other hand, treatment of PC12 cells with orthovanadate for similar times and concentrations as bpV[phen] produced very low tyrosine phosphorylation levels, which were similar to those observed in untreated cells (results not shown).

3.8. Bisperoxo(1,10-phenanthroline)-oxovanadate produces an increase in synaptophysin and MAPK phosphorylation

Among the proteins that underwent tyrosine phosphorylation in response to bpV[phen] is synaptophysin (Fig. 8). As can be seen, tyrosine-phosphorylated synaptophysin can first be detected with 10 μM bpV[phen] and appears to...
reach a maximum with 30 μM (Fig. 8). This dose–response profile parallels the enhanced dopamine release achieved with 30–100 μM bpV[phen], suggesting the association of synaptophysin phosphorylation with dopamine release engendered by bpV[phen].

Enhanced phosphorylation of ERK in the presence of bpV[phen] has been previously demonstrated (Cerovac et al., 1999; Band and Posner, 1997). To determine if this increased ERK phosphorylation might play a role in enhanced dopamine exocytosis in the presence of bpV[phen], the MAP kinase (MEK) inhibitor PD98059 was employed. Very low levels of active MAPK were observed in untreated PC12 cells or cells treated with PD98059 (Fig. 9A, lanes 1 and 3). In contrast, strong phosphorylation of ERK could be observed in PC12 cells treated with bpV[phen] (Fig. 9A, lane 2); treatment with PD98059 prior to the addition of bpV[phen] reduced the level of MAPK phosphorylation (Fig. 9A, lane 2); treatment with PD98059 prior to the addition of bpV[phen] reduced the level of MAPK phosphorylation (Fig. 9A, lane 2). Treatment with PD98059 significantly reduced dopamine release engendered by bpV[phen] (Fig. 9B, columns 2 and 4; P < 0.05).

4. Discussion

The results of the present study show that in a concentration range between 10 and 100 μM, bpV[phen] enhances dopamine release in response to two secretory stimuli associated with entirely different mechanisms of action: KCl-induced influx of Ca2+ via voltage-gated calcium channels and the A23187-induced increase in intracellular calcium which occurs independent of these channels. It seems that bpV[phen] acts at a common point downstream from these channels where the signaling pathways triggered by depolarisation and A23187 converge. Studies employing caged-Ca2+ compounds have provided new evidence for intermediate steps in the series of subcellular events leading to exocytosis (Kasai, 1999).

The effects of bpV[phen] in potentiating the dopamine release induced by A23187 are time-dependent, being maximal after about 20 min of incubation periods. This result suggests that it requires approximately 20 min for the unopposed action of the kinases to become apparent on exocytotic processes.

The actions of bpV[phen] in enhancing dopamine exocytosis in the presence of A23187 appear to be partially irreversible. Indeed, peroxovanadium complexes are commonly believed to be irreversible PTP inhibitors (Huyer et al., 1997; Bevan et al., 1995; Posner et al., 1994; reviewed in Morinville et al., 1998). The catalytic domain of PTPs is well conserved and contains a critical cysteine residue that is oxidised by peroxovanadium complexes (Huyer et al., 1997; Bevan et al., 1995). It is conceivable that in the time period following the end of incubation to the start of stim-
calculation (10 min), enough PTPs were inhibited to allow the unopposed action of the kinase to be observed. The actions of bpV[phen] on potentiating DA release induced by ionophore A23187 appear to depend on extracellular Ca\(^{2+}\), as was demonstrated in the experiments in the presence of EGTA. This peroxovanadium complex may induce the mobilisation of Ca\(^{2+}\) in order to affect the exocytosis of DA. Indeed, calcium has previously been implicated in the exocytosis of neurotransmitters and most recently in the release of \(\alpha\)-aspartate stored in secretory vesicles in PC12 cells (Nakatsuka et al., 2001). This mobilisation could be a result of phosphatase inhibition, which would allow the unopposed action of kinases to lead to calcium entry from the extracellular milieu or intracellular stores.

A striking result of these studies is the difference between the effects of bpV[phen] (a typical representative of the peroxovanadium complexes) and of orthovanadate on DA release in PC12 cells. This difference can best be explained by the different specificities of the two compounds. Although both are PTP inhibitors, the peroxovanadium complex used in this study is far more potent (about 100 times) and more selective for PTPs (Posterus et al., 1994), than orthovanadate (Hayer et al., 1997). Orthovanadate also inhibits protein serine/threonine phosphatases, which peroxovanadium complexes are ineffective at inhibiting (reviewed in Morinville et al., 1998). Thus, it is likely that bpV[phen] and orthovanadate each affect a different set of phosphatases and thereby interfere with different parts of the exocytotic process. The mixture of orthovanadate and \(\text{H}_2\text{O}_2\), meant to simulate bpV[phen], proved not to be effective in stimulating exocytosis, a result probably explained by the instability of aqueous solutions of pervanadate, which may induce the mobilisation of Ca\(^{2+}\) in order to affect the exocytosis of DA. Indeed, calcium has previously been implicated in the exocytosis of neurotransmitters and most recently in the release of \(\alpha\)-aspartate stored in secretory vesicles in PC12 cells (Nakatsuka et al., 2001). This mobilisation could be a result of phosphatase inhibition, which would allow the unopposed action of kinases to lead to calcium entry from the extracellular milieu or intracellular stores.

The observed enhancement of ERK phosphorylation by bpV[phen] suggest that it could be involved in the potentiation of dopamine release. Peroxovanadium complexes exert their effects through inhibition of PTPs, rather than direct activation of kinases, eventually resulting in the accumulation of phosphorylated proteins. Inhibition of MEK with PD98059 reduced ERK phosphorylation levels, indicating that this enhanced ERK phosphorylation proceeds partly through the MEK pathway. Similarly, incubation with PD98059 significantly reduced dopamine release. These results implicate phosphorylation of ERKs in potentiation of dopamine release induced by A23187. Phosphorylation of ERKs has previously been implicated in the release of neurotransmitter from bovine chromaffin cells, which are closely related to the PC12 cells used in our studies (Cox et al., 1996; Cox and Parsons, 1997). More recently, ERK involvement in dopamine release was demonstrated in PC12 cells treated with parafinix (Bloch-Shilderman et al., 2001).

In conclusion, the present findings implicate ERK signalling and synaptophysin phosphorylation in ionophore-induced bpV[phen]-enhanced dopamine release.

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References


