Fibroblast Growth Factor-Induced Decrease in the Phosphorylation of Nsp100 Mediated through a Calcium-Dependent Mechanism and Blocked by Lectins

Seiichi Hashimoto, Akihiko Hagino*, and Yuji Amagai**

Departments of Biochemistry, Biology*, and Physiology**, Ohu University School of Dentistry, Koriyama 963, Japan

Key words: fibroblast growth factor/nerve growth factor/protein phosphorylation/Nsp100/wheat germ agglutinin

ABSTRACT. Separate treatment of PC12h cells with basic fibroblast growth factor (bFGF) and with epidermal growth factor (EGF) induced a selective decrease in the incorporation of radioactive phosphate into a 100,000-dalton soluble protein during phosphorylation with (γ-32P)ATP of soluble extracts from the cells, as was seen previously with nerve growth factor (NGF). This 100,000-dalton soluble protein was designated in earlier studies as nerve growth factor-sensitive protein 100 (Nsp100). The inhibitory effects of bFGF and EGF on Nsp100 phosphorylation were prevented by pretreatment of PC12h cells with the calcium chelator, EGTA. Treatment of PC12h cells with the plant lectin wheat germ agglutinin (WGA), which binds to N-acetylglucosamine and sialic acid residues on glycoconjugates, blocked the inhibitory effects of bFGF, EGF, and NGF on Nsp100 phosphorylation. The blockage by WGA was reversed by the addition of the lectin-specific sugar N-acetylglucosamine to the PC12h cultures. Although pretreatment of PC12h cells with succinylated WGA, which has the ability to bind to N-acetylglucosamine but not to sialic acid residues, failed to block the inhibitory effect of NGF on Nsp100 phosphorylation as described previously, it did prevent the inhibitory effect of bFGF on this phosphorylation. These data suggest that in PC12h cells bFGF and EGF induce a decrease in the phosphorylation of Nsp100 mediated through a Ca2+-dependent mechanism, as in the case of NGF. Furthermore, the blockage of the bFGF-induced inhibition of Nsp100 phosphorylation by WGA and its succinylated form indicates that N-acetylglucosamine residues of bFGF receptor molecules might be involved in the mechanism by which bFGF inhibits the phosphorylation. On the other hand, staurosporine, a protein kinase inhibitor, blocked the NGF-induced decrease in the phosphorylation of Nsp100, but failed to block the bFGF-induced one.

PC12, a clonal line derived from a rat pheochromocytoma, has been widely used as a model for studying neuronal differentiation. Nerve growth factor (NGF) and fibroblast growth factor (FGF) promote neuronal differentiation of PC12 cells (14, 33, 37). On the other hand, although epidermal growth factor (EGF) mimics many of the NGF actions in PC12 cells, in marked contrast to NGF, enhances proliferation of PC12 cells but does not promote neuronal differentiation (24). The actions of these growth factors are certainly initiated by the interaction of the growth factors with their specific receptors on the surface of the target cells. PC12 cells are known to possess two classes of binding sites with their specific receptors on the surface of the target cells. PC12 cells are known to possess two classes of binding sites with different affinity for NGF (26, 34), for EGF (3), and for FGF (31). It has been shown by affinity labeling studies in PC12 cells that apparent Mr = 150,000 and/or Mr = 100,000 [125I]-NGF affinity-labeled species (4, 7, 13, 23, 28), a major Mr = 165,000-170,000 [125I]-EGF affinity-labeled species (4, 8), and a single apparent Mr = 160,000 [125I]-FGF affinity-labeled species (31) are present in these cells. Two distinct classes of NGF receptors have been identified as membrane sialoglycoproteins (23) and possess apparently identical NGF-binding moieties (13). It has been reported that the plant lectin wheat germ agglutinin (WGA), which binds specifically to N-acetyl-D-glucosamine and sialic acid residues of cell membrane glycoproteins, alters the binding of NGF (6, 15, 41) and that of EGF (42) to the factor-specific receptors in PC12 cells. Moreover, inhibition of N-linked glycosylation of NGF receptors by the treatment of PC12 cells with tunicamycin eliminates the rapidly dissociating component of NGF binding and decreases the proportion of PC12 cells capable of elaborating neurites (1). Treatment of human A431 cells with tunicamycin results in an aglyco-receptor species that does not possess the

Abbreviations used: EGF, fibroblast growth factor; EGF, epidermal growth factor; NGF, nerve growth factor; WGA, wheat germ agglutinin; Nsp100, nerve growth factor-sensitive protein 100; dBcAMP, N2',O-dibutyryladenosine 3',5'-cyclic monophosphate.
capacity to bind EGF (35, 36). Recently, it has been reported that in baby hamster kidney cells the receptor for basic FGF (bFGF) contains N-linked glycosidic side chains and that WGA inhibits bFGF binding and the biological activity of bFGF (12). Therefore, it is interesting to investigate whether or not WGA inhibits FGF action in PC12 cells.

It is clear that NGF influences the phosphorylation of a number of specific proteins in target cells. Among the alterations in protein phosphorylation mediated by NGF, the action of NGF on a 100,000-dalton soluble protein, designated as Nsp100 in early studies (10, 11), has been looked at in some detail. The treatment of PC12 cells with NGF causes a decreased phosphorylation of Nsp100 in intact cells labeled with 32P-orthophosphoric acid (10). The NGF-induced decrease in Nsp100 phosphorylation is also observed when cell-free preparations from treated cells are incubated with (γ-32P)ATP (11). A similar alteration in the phosphorylation of this protein is also observed with FGF in an intact-cell phosphorylation system (38) and with EGF in a cell-free phosphorylation system (11). We have shown that in PC12h cells the NGF-induced decrease in the cell-free phosphorylation of Nsp100 is blocked by treatment of the cells with WGA (20) and is certainly mediated through a Ca2+-dependent mechanism, and that, moreover, not only calcium-mobilizing stimuli such as calcium ionophores and depolarizing agents, but also agents that raise the cAMP level in the cells, specifically dBcAMP and cholera toxin, also act on Nsp100 through a Ca2+-dependent mechanism and result in the decreased phosphorylation of Nsp100 (21). Nsp100 kinase, which phosphorylates Nsp100, has been partially purified and characterized (39). It appears that Nsp100 and Nsp100 kinase are identical with elongation factor 2, which participates in protein synthesis, and Ca2+/calmodulin kinase III, respectively (30). Phosphorylated elongation factor 2 is completely inactive in translation and, moreover, inhibits the activity of non-phosphorylated elongation factor 2 (32).

In this paper, we show that the FGF-induced decrease in the phosphorylation of Nsp100 is also mediated through a Ca2+-dependent mechanism and that both native and succinylated WGA prevent the inhibitory effect of FGF on Nsp100 phosphorylation.

MATERIALS AND METHODS

Materials. NGF (2.5S) was prepared by the procedure of Bocchini and Angeletti (2). EGF and pituitary basic FGF were purchased from Biomedical Technologies, Inc. Dibutyryl cyclic AMP (dBcAMP), A23187, and cholera toxin were purchased from Sigma. WGA and succinylated WGA were obtained from Vector Laboratories, Inc. (γ-32P)ATP was obtained from New England Nuclear. Staurosporine was generously supplied by Y. Matsuda (Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) molecular mass standards were from Bio-Rad Laboratories.

Cell culture. PC12 subclone h cells (22) were grown in DME supplemented with 1% FBS and 1% horse serum. They were kept in a humidified atmosphere containing 95% air and 5% CO2 in 75 cm2 tissue culture flasks at 37°C. The cells were subcultured once a week at a 1:6 split ratio, and the culture
medium was changed once between splits.

**Protein phosphorylation.** After treatment of PC12h cells with various agents, the medium was removed, and the cells were collected and washed with buffer A (composed of 0.32 M sucrose containing 3 mM CaCl₂, 1 mM MgCl₂, and 1 mM sodium phosphate [pH 6.5]). The cell pellet was suspended in 700 µl or 1 ml of sonication buffer consisting of 100 mM piperazine-N,N'-bis(2-ethanesulfonic acid), 1 mM MgSO₄, 0.1 mM Na₂MoO₄, 10 mM EGTA, and 1 mM PMSF. The cell suspension was sonicated for 30s (Sonifier model no. 200) and centrifuged at 100,000×g for 60 min. The supernatant was diluted to an equal concentration of protein and the phosphorylation was carried out as described previously (11). Protein determination was done by the method of Bradford (5). The incubation mixture contained 50 mM 2-(N-Morpholino)ethanesulfonic acid (pH 6.2), 10 mM MgSO₄, 0.1 mM Na₂MoO₄, and 4 µCi of (γ-³²P)ATP in a final volume of 200 µl. Incubation was carried out at 37°C for 10 min and terminated by the addition of 200 µl of SDS sample buffer. After boiling, the samples were subjected to SDS-PAGE.

**PAGE.** SDS-PAGE was performed at 150 V for 5-6 h using 7.5% acrylamide slab gels containing 0.1% SDS (25). Gels were stained overnight with Coomassie Blue staining solution (0.25% Coomassie Blue in 9% acetic acid and 45% ethanol) and destained initially with 42% ethanol and 7% acetic acid, and finally with 7% acetic acid.

**Autoradiography.** The slab gels were dried in vacuo and exposed to XAR-5 film at room temperature. After an appropriate period of time, the film was processed using standard procedures. Autoradiograms were scanned using a Shimadzu CS-900, and the areas under the relevant peaks were measured.

**RESULTS**

Our previous work (21) showed that treatment of PC12h cells with NGF causes a decrease in the phosphorylation of a specific protein having an apparent molecular mass of 100,000 D during incubation of the cell extracts with (γ-³²P)ATP and that the inhibitory effect of NGF on this protein phosphorylation is mediated through a Ca²⁺-dependent mechanism. In earlier studies using conventional PC12 cells, this 100,000-D NGF-sensitive protein was designated as Nsp100 (10, 11). Treatment of PC12h cells with bFGF (50 ng/ml) or EGF (50 ng/ml) also caused a marked and selective decrease in the phosphorylation of Nsp100 without any marked phosphorylative alterations of other phosphoproteins, as measured by the subsequent incubation of cell extracts from these treated cells with (γ-³²P)ATP (Fig. 1). Moreover, pre-addition of an excess amount of the calcium chelator EGTA to the cultures blocked the inhibitory effect of bFGF and of EGF on Nsp100 phosphorylation (Fig. 2), suggesting that both bFGF and EGF act on Nsp100 through a Ca²⁺-dependent mechanism as does NGF.

Previously, we showed that treatment of PC12h cells with WGA prevents NGF-induced changes in the phosphorylation of specific proteins including Nsp100 (20). When PC12h cells were pretreated with WGA (100 µg/ml), the inhibitory effect of bFGF and of EGF as well as that of NGF on Nsp100 phosphorylation was also prevented (Fig. 3, A, B, and C). The blockage by WGA of the inhibitory effects on Nsp100 phosphorylation elicited by these three growth factors was reversed by the addition of the lectin-specific sugar N-acetylglucosamine to the culture (Fig. 3, A, B, and C). Pretreatment of the cells with succinylated WGA (100 µg/ml) did not block the inhibitory effect of NGF on Nsp100 phosphorylation, as described previously (19); in contrast, the inhibitory effect of bFGF was blocked...
by such pretreatment (Fig. 4).

As mentioned earlier, dBcAMP and cholera toxin mimic the NGF-induced decrease in the phosphorylation of Nsp100; so we examined the effect of WGA on their action. Incubation of cells with dBcAMP at a concentration of 1 mM for 3 hours induced a large increase in the phosphorylation of tyrosine hydroxylase and a marked decrease in the phosphorylation of Nsp100 in cell-free preparations (Fig. 5). Either pretreatment of PC12h cells with WGA (100 μg/ml) 60 min before or post-treatment with it 30 min after dBcAMP addition resulted in no apparent inhibition of the phosphorylation of tyrosine hydroxylase (Fig. 5). These pre- and post-treatments with WGA, on the other hand, induced
Inhibition of FGF Action by Lectins

some change in the phosphorylation levels of Nsp100; however, the phosphorylation level of Nsp100 after treatment of the cells with dBcAMP was substantially lower from that seen with WGA alone (Fig. 5). The data indicate that WGA does not block the effect of dBcAMP on the cell-free phosphorylation of these proteins. Treatment of cells with cholera toxin, which induces an increased level of intracellular cyclic AMP, also induced a decrease in the phosphorylation of Nsp100 and an increase in that of tyrosine hydroxylase in the subsequent cell-free phosphorylation system. When the cells were pre-treated with WGA (100 μg/ml) 60 min before cholera toxin addition (50 ng/ml), results similar to those seen with dBcAMP were obtained. That is, WGA did not block the inhibitory and stimulatory effects of cholera toxin on the phosphorylations of Nsp100 and tyrosine hydroxylase, respectively, in PC12h cells (Fig. 6). As our previous work showed that calcium-mobilizing stimuli, for example, A23187 (calcium ionophore) and high K+ depolarization, also induce a decrease in the phosphorylation of Nsp100 (21), we therefore investigated whether or not WGA prevents the inhibitory effects of these stimuli on Nsp100 phosphorylation. The autoradiograms demonstrated that both A23187 (20 μM) and high K+ (64.4 mM) caused a decreased phosphorylation of Nsp100 and an increased

Fig. 5. Effect of pre- and post-treatment of PC12h cells with wheat germ agglutinin on the subsequent cell-free phosphorylation of soluble proteins from PC12h cells treated with NGF or dibutyryl cyclic AMP. Cells were treated with control culture medium (C); NGF at 50 ng/ml for 3 h (N); WGA at 100 μg/ml for 4 h (W); W+N (WGA at 100 μg/ml for 4 h and NGF at 50 ng/ml for the last 3 of the 4 h); N+W (NGF at 50 ng/ml for 3 h and WGA at 100 μg/ml for the last 2.5 of the 3 h); 1 mM dibutyryl cyclic AMP for 3 h (dBc); WGA+dBc (WGA at 100 μg/ml for 4 h and 1 mM dibutyryl cyclic AMP for the last 3 of the 4 h); or with dBc+W (1 mM dibutyryl cyclic AMP for 3 and WGA at 100 μg/ml for the last 2.5 of the 3 h). The extracts were prepared, phosphorylated, and analyzed as described in Fig. 1. The upper and lower arrows in the autoradiograms indicate the position of Nsp100 and tyrosine hydroxylase, respectively. The relative densities of Nsp100 are as follows: C, 100; W, 124; N, 65; W+N, 129; N+W, 134; dBc, 43; W+dBc, 82; dBc+W, 67; and those of tyrosine hydroxylase: C, 100; W, 131; N, 136; W+N, 113; N+W, 102; dBc, 1418; W+dBc, 1599; dBc+W, 1522.

Fig. 6. Effect of pretreatment with wheat germ agglutinin on the subsequent cell-free phosphorylation of soluble proteins from PC12h cells treated with cholera toxin. Cells were treated with control culture medium (C); cholera toxin at 50 ng/ml for 3 h (CT); WGA at 100 μg/ml for 4 h (W); W+CT (WGA at 100 μg/ml for 4 h and cholera toxin at 50 ng/ml for the last 3 of the 4 h); or with W+NGc+CT (WGA at 100 μg/ml for 4 h; 100 mM N-acetylglucosamine for the final 3.5 h; and cholera toxin at 50 ng/ml for the final 3 h of the 4 h). The extracts were prepared, phosphorylated, and analyzed as described in Fig. 1. The upper and lower arrows beside the autoradiograms indicate the position of Nsp100 and tyrosine hydroxylase, respectively. The relative densities of the Nsp100 peak are as follows: C, 100; CT, 38; W, 96; W+CT, 43; W+NGc+CT, 38. The relative densities of tyrosine hydroxylase peak are: C, 100; CT, 381; W, 72; W+CT, 394; W+NGc+CT, 337. Bars on the right indicate the positions of molecular mass standards (from the top, 200 kDa, 116 kDa, 92.5 kDa, 66 kDa, and 45 kDa).
Fig. 7. Effect of pretreatment with wheat germ agglutinin on the subsequent cell-free phosphorylation of soluble proteins from PC12h cells treated with calcium-mobilizing stimuli. Cells were treated with control culture medium (C); $2 \times 10^{-5}$ M A23187 (A) or 64.4 mM K$^+$ (K$^+$) for 3 h; WGA at 100 $\mu$g/ml for 4 h and $2 \times 10^{-3}$ M A23187 or 64.4 mM K$^+$ for the last 3 of the 4 h; or with W+NGc+A or W+NGc+K (WGA at 100 $\mu$g/ml for 4 h; 100 mM N-acetylglucosamine for the final 3.5 h; and $2 \times 10^{-3}$ M A23187 or 64.4 mM K$^+$ for the final 3 h of the 4 h). The extracts were prepared, phosphorylated, and analyzed as described in Fig. 1. The upper and lower arrows in the autoradiograms indicate the position of Nspl00 and tyrosine hydroxylase, respectively. In A, the relative densities of the Nspl00 peak are as follows: C, 100; A, 63; W, 110; W+A, 58; W+NGc+A, 52; and those of tyrosine hydroxylase peak: C, 100; A, 190; W, 121; W+A, 228; W+NGc+A, 191. In B, the relative densities of the Nspl00 peak are as follows: C, 100; K$^+$, 59; W, 119; W+K$^+$, 78; W+NGc+K$^+$, 52; and those of the tyrosine hydroxylase peak: C, 100; K$^+$, 186; W, 100; W+K$^+$, 153; W+NGc+K$^+$, 159. In B, bars on the right indicate the positions of molecular mass standards (from the top, 200 kDa, 116 kDa, 92.5 kDa, 66 kDa, and 45 kDa).

phosphorylation of tyrosine hydroxylase (Fig. 7, A and B). The inhibitory and stimulatory effects of A23187 and high K$^+$ depolarization on the phosphorylation of Nspl00 and tyrosine hydroxylase, respectively, in PC12h cells were not blocked by the pretreatment of the cells with WGA (Fig. 7, A and B). Thus, WGA specifically blocked the action of NGF, bFGF, and EGF, but did not block the action of other stimuli such as dBcAMP, cholera toxin, A23187, and high K$^+$ depolarization. In our previous work (18), we showed that pretreatment of PC12h cells with a protein kinase inhibitor staurosporine (30-100 nM) was required to block the effect of NGF. Therefore, we employed 100 nM staurosporine to investigate whether the inhibitor blocks the effect of bFGF on Nspl00 phosphorylation. Fig. 8 shows that 100 nM staurosporine blocked the effect of NGF, but failed to block that of bFGF on Nspl00 phosphorylation.

DISCUSSION

The initial step in the activation of target cells by peptide growth factor is the binding of the growth factor to its specific receptor. The interaction of peptide growth factor with its cell-surface membrane receptor appears to be the trigger for all the subsequent and various reactions induced by the factor. It is known that in general receptors for peptide growth factors are membrane...
Inhibition of FGF Action by Lectins

Fig. 8. Effect of pretreatment with staurosporine on the subsequent cell-free phosphorylation of soluble proteins from PC12h cells treated with NGF or bFGF. Cells were cultured in control culture medium (Normal Control); NGF at 50 ng/ml for 3 h (Normal NGF); bFGF at 20 ng/ml for 3 h (Normal FGF); staurosporine at 100 nM for 4 h (SS 100 nM Control); staurosporine at 100 nM for 4 h and NGF at 50 ng/ml for the last 3 of the 4 h (SS 100 nM NGF); staurosporine at 100 nM for 4 h and bFGF at 20 ng/ml for the last 3 of the 4 h (SS 100 nM FGF). The extracts were prepared, phosphorylated, and analyzed as described in Fig. 1. The arrows in the densitometer tracings of the autoradiogram indicate the position of Nsp100. The relative densities of the Nsp100 peak for the normal cells are as follows: Control, 100; NGF, 61; FGF, 74. The relative densities of the Nsp100 peak for the staurosporine-pretreated cells are as follows: Control, 100; NGF, 96; FGF, 64.

glycoproteins; and NGF, EGF, insulin, and platelet-derived growth factor are examples of such receptors.

Lectins are known to interact with cell surface glycoproteins and to influence the binding of a variety of peptide growth factor receptors to their specific factor. For example, Costin and Kogan (9) showed that plant lectins Con A and WGA inhibit NGF binding to its receptor in rabbit superior cervical ganglia. Vale and Shooter (41) subsequently reported that WGA inhibits NGF binding to its rapidly dissociating (low affinity) receptor, and, moreover, converts the receptor rapidly to its slowly dissociating (high affinity) state and that Con A also produces similar but weaker modification of the binding properties of the receptor in PC12 cells. WGA-mediated receptor conversion from rapidly to slowly dissociating receptor state has also been shown by Grob and Bothwell (15) and by Buxser et al. (6). The cleavage of sialic acid residues from NGF receptors with neuraminidase decreases WGA-promoting receptor conversion and apparent molecular weights of both rapidly and slowly dissociating receptors by 10,000 daltons (40); and succinylated WGA, which binds N-acetylglucosamine but not sialic acid residues, does not affect the binding of NGF to its receptors (40). These findings suggest that WGA interacts with sialic acid residues of NGF receptors, resulting in a change in the binding properties of the receptor. Our previous studies showed that treatment of PC12h cells with WGA strongly inhibited the action of NGF (19, 20); moreover, Con A and LCA, both of which recognize the same specific sugars (mannose, glucose), display only a moderate inhibition of NGF action (19). In contrast, succinylated WGA and other lectins such as peanut lectin, soybean lectin, pokeweed mitogen, and mushroom lectin do not inhibit the action of NGF on Nsp100 (19). The blockage of the biological action of NGF by WGA was also reported by Landreth et al. (27). These observations again suggest that WGA acts on sialic acid residues of the NGF receptor molecule and results in the inhibition of biological actions of NGF. Lectin-induced modification of properties of EGF receptors in PC12 cells has also been revealed. Vale and Shooter demonstrated that WGA and Con A caused a decrease in binding affinity and in the apparent number of binding sites for EGF in PC12 cells (42). On the other hand, Boonstra et al. reported that Con A primarily decreases the affinity of the high-affinity class of binding sites without affecting the affinity of the low-affinity ones, but that the apparent number of binding sites of the low-affinity class receptor is significantly decreased (3). Thus, lectins such as WGA and Con A inhibit the binding of NGF and EGF to their specific receptors in PC12 cells.

In this paper, we made the following observations in PC12h cells: (1) bFGF and EGF induced a decrease in the phosphorylation of Nsp100, as seen previously with NGF; (2) the inhibitory effect on Nsp100 phosphorylation elicited by bFGF and by EGF was prevented by pretreatment of the cells with the calcium chelator EGTA; (3) WGA selectively blocked the inhibitory effect of bFGF and EGF as well as that of NGF, but did not block that elicited by the calcium ionophore A23187 or high K+ depolarization, both of which raise the level of calcium in the cells; or that by dBcAMP or cholera toxin, both of which raise the level of cyclic AMP in the cells; (4) the blockage by WGA of the inhibitory effect of these three growth factors on Nsp100 phosphorylation was reversed by the addition of the lectin-specific
sugar N-acetylgalcosamine to the PC12 cultures; (5) succinylated WGA prevented the inhibitory effect of bFGF, but failed to block that of NGF; (6) staurosporine, a protein kinase inhibitor, blocked the inhibitory effect of NGF, but failed to block that of bFGF.

GM1 gangliosides on the cell surface are known to be binding sites for cholera toxin. It has been reported that WGA at concentrations up to 200 μg/ml does not aggregate vesicles containing GM1 (29). Therefore, it is reasonable that WGA did not block the action of cholera toxin.

The three observations, from (3) to (5), listed above suggest that WGA acts on glycosyl residues of the specific receptors for bFGF, EGF and NGF. In particular, the blockage of bFGF-induced inhibition of Nsp100 phosphorylation by succinylated WGA suggests involvement of N-acetylgalcosamine residues of the bFGF receptor molecule in the mechanism by which the native and modified lectins inhibit the bFGF action. However, at this point, it is not clear whether or not the binding of WGA to sialic acid residues of the bFGF receptor molecule is also involved in preventing the action of bFGF.

Involvement of protein kinase C in the inhibition of Nsp100 kinase by NGF has been suggested by Hama et al. (16). That is, they proposed that NGF activates protein kinase C, which, when activated, phosphorylates and inactivates Nsp100 kinase, resulting in a decrease in the phosphorylation of Nsp100. In contrast, Nairn et al. made the following observations: treatment of PC12 cells with phorbol ester, under conditions where protein kinase C would be activated, had little effect on Ca2+/calmodulin kinase III activity (30). These findings indicate that protein kinase C is not involved in the mechanism of NGF-induced decrease in the activity of Ca2+/calmodulin kinase III (Nsp100 kinase). Consistent with the work of Nairn and coworkers, chronic treatment of PC12h cells with phorbol ester did not block the NGF-induced decrease in the phosphorylation of Nsp100 in our experiments (unpublished data). Therefore, the molecular mechanism of NGF-induced decrease in the phosphorylation of Nsp100 is still not clear.

Recently we reported that pretreatment of the cells with staurosporine at a concentration of 100 nM blocked the NGF-induced decrease in Nsp100 phosphorylation, but did not prevent the bFGF-induced one (Fig. 8). Therefore, it appears that the mechanism by which bFGF induces a decrease in the phosphorylation of Nsp100 is different, at least in part, from that underlying NGF action.

REFERENCES

Inhibition of FGF Action by Lectins


(Received for publication, March 3, 1990 and in revised form, May 29, 1990)