NERVE GROWTH FACTOR INDUCED PHOSPHATIDYLINOSITOL TURNOVER EFFECT OF 6-HYDROXYDOPAMINE TREATMENT

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1. Introduction

There is now abundant evidence that a variety of cell surface stimuli can provoke an increased turnover of phosphorylinositol group of phosphatidylinositol (PI-effect) through specific receptor mechanism(s) [1,2]. Such a phenomenon is reported to occur both in neural and non-neural tissues in response to physiological and pharmacological stimuli [1]. Recently I have shown an increased turnover of phosphatidylinositol in rat superior cervical ganglia (SCG)* and in pineal gland in response to nerve growth factor (NGF) [3,4]. This protein has been implicated in the growth and survival of sympathetic neurones through all ages [5]. Stimulation of PI breakdown in rat SCG and in pineal is reported to be mediated by acetylcholine and noradrenaline respectively, through their specific receptor systems [6–8]. However, the PI-effect elicited by NGF in rat SCG is found to be insensitive to both nicotinic and muscarinic receptor blockers. In pineal glands α-adrenergic receptor blockers did not prevent the NGF specific PI turnover. Moreover, the NGF specific PI effect is observed in bilaterally decentralized superior cervical ganglia as well as in pineals, after bilateral superior cervical ganglionectomy [4]. These studies suggested that the PI-effect observed in response to NGF in both organs may indicate a postsynaptic site of action of NGF. The present study was undertaken with a view to obtaining a better understanding of the site of action of NGF in these organs.

Abbreviations: NGF, nerve growth factor; SCG, superior cervical ganglia; PI, phosphatidylinositol; 6-OHDA, 6-hydroxydopamine; TH, tyrosine hydroxylase

2. Experimental

Nerve growth factor (NGF) 2.5 S was prepared from male mouse submaxillary glands according to the method of Bocchini and Angeletti [9]. 6-Hydroxydopamine (6-OHDA) Hbr. was obtained from Roche Laboratories, Nutley, NJ. Culture medium and antibiotics were obtained from Grand Island Biological Co., NY. All other chemicals were of reagent grade.

A total of 64 newborn rats were used for these studies. Pregnant animals were housed in single cages. As soon as they delivered, each litter was reduced to eight pups and subdivided into two groups. Each group received injection subcutaneously from day 2 through day 9 as follows. The first group received one injection of 100 μl of saline containing 1 mg/ml of ascorbic acid. The second group received 100 μg/g body weight of 6-OHDA freshly prepared in saline containing 1.0 mg/ml of ascorbic acid. The solution was kept under nitrogen atmosphere during the period of injection. The pups were sacrificed 48 h after the last injection. Superior cervical ganglia and pineal glands removed from both groups were organ cultured as described elsewhere [3]. Briefly pairs of ganglia or pineal glands were placed in 250 μl of BGJb medium, Fitton-Jackson modification without phenol red. The medium was supplemented with 0.1% bovine serum albumin. Fraction V and an antibiotic mixture which included penicillin (100 U/ml), and streptomycin (100 μg/ml). Ascorbic acid (0.1 mg/ml) and glutamine (2 mM) were prepared and added just before use. Appropriate concentration of NGF diluted in BGJb medium was added in 5 μl

volume. The incubation started with the addition of labelled myoinositol and the tissues were maintained at 37°C in tissue culture clusters in a humidified atmosphere of 95% oxygen and 5% carbon dioxide for the desired length of time. At the end of incubation, the tissues were removed and rinsed in normal saline containing 10^{-4} M unlabelled myoinositol. Then pairs of ganglia or pineal glands were homogenized in 4 ml of chloroform–methanol mixture (2:1, v/v). The lipid extract was washed four times with 0.2 volume of normal saline containing 10^{-4} M unlabelled myoinositol, transferred to scintillation vials and dried under a stream of nitrogen. They were counted in the presence of scintillation cocktail (5 g PPO and 0.5 g of POPOP for 100 ml toluene).

3. Results

Newborn rats, which had been treated daily with 6-OHDA from day 2 through day 9, did not show any gross change in their external appearance. A low incidence of mortality was noticed. Body weights were slightly reduced compared to saline–ascorbic acid treated rats. A marked decrease in the size of superior cervical ganglia was evident under dissection microscope. The pineal glands of 6-OHDA treated rats appeared to be normal in size. Table 1 shows the effect of NGF on the incorporation of labelled myoinositol into phosphatidylinositol in SCG removed from sham treated and 6-OHDA treated rats. The addition of NGF (1×10^{-8} M) stimulated the incorporation of myoinositol into PI about 57% in SCG removed from sham treated rats suggesting an increased PI turnover in response to NGF. A marked increase of 125% stimulation was observed in SCG removed from 6-OHDA treated rats. However, the SCG of the latter group showed a marked decrease in their basal PI turnover compared to that of sham treated rats. This decrease could be due to the reduction in the number of neuronal cells as a result of 6-OHDA treatment [10]. As shown in table 2 NGF elicited about 62% stimulation of PI turnover in sham treated rats whereas 55% stimulation was observed in pineal glands removed from 6-OHDA treated rats. However, very little difference was noticed in the basal turnover of PI of pineal glands of the two groups.

4. Discussion

Increased turnover of phosphatidylinositol in response to hormones and putative neurotransmitters is now widely documented. This phenomenon is probably mediated at the cell surface through specific receptor mechanism(s) [1]. In rat SCG, NGF induced 'PI-effect' appears to be a receptor specific event [3]. No other hormone could substitute for NGF. The PI-effect is demonstrable in physiological range of con-

Table 1
Effect of 6-OHDA treatment on nerve growth factor induced PI turnover in rat SCG

Additions to culture	Sham treated SCG		6-OHDA-treated SCG	
	[^{3}H]PI dpm/pair SCG	Stimulation	[^{3}H]PI dpm/pair SCG	Stimulation
None	270 ± 24 [1180 ± 105]	–	100 ± 12 [625 ± 75]	–
NGF (1×10^{-8} M)	425 ± 30[a] [1860 ± 130]	57%	225 ± 20[a] [1406 ± 125]	125%

[a] $P<0.01$ compared to control. The value in parentheses represents dpm/mg protein

Pairs of superior cervical ganglia of immature rats were organ cultured for 20 h in the presence of 12.5 μCi [2-^{3}H]myoinositol (spec. activity 2 Ci/mmol) as described in text. Each value represents the mean ± SE from 12 pairs of SCG

Table 2
Effect of 6-OHDA treatment on nerve growth factor induced PI turnover in rat pineal

Additions to culture	Sham treated pineal		6-OHDA-treated pineal	
	[^{3}H]PI (dpm/pineal)	Stimulation	[^{3}H]PI (dpm/pineal)	Stimulation
None	220 ± 22 [1403 ± 140]	–	200 ± 18 [1220 ± 110]	–
NGF (1×10^{-8} M)	356 ± 30[a] [2271 ± 190]	62%	310 ± 24[a] [1892 ± 146]	55%

[a] $P<0.01$ compared to control. The value in parentheses represents dpm/mg protein

Pairs of pineal glands of immature rats were organ cultured for 20 h in the presence of 12.5 μCi of [2-^{3}H]myoinositol (spec. activity 2 Ci/mmol) as described in text. Each value represents the mean ± SE of at least twelve pineal glands

centration of NGF. Also NGF specific 'PI-effect' is sensitive to NGF antibody. We have also presented evidence elsewhere for the probable site of action of NGF [4]. NGF specific PI turnover was found to be insensitive to various neurotransmitter receptor specific blockers. Surgical denervation did not abolish the PI-effect in rat SCG and in pineal glands. These results indicated that the PI-effect in response to NGF might occur on the post-synaptic structures. In the present paper, we have made use of the property of 6-OHDA to extend and confirm our earlier findings.

6-OHDA treatment in newborn rats not only destroys adrenergic nerve terminals but also produces widespread lesions in the cell body of these neurones [11]. In rat SCG, 6-OHDA treatment is also known to decrease neuronal cell population without affecting the supporting cells [10]. The addition of NGF did elicit a 'PI-effect' in SCG and in pineal of 6-OHDA treated rats. Thus it is conceivable from the results that the NGF-specific PI turnover is on the post-synaptic structures both in pineal and SCG, and in the case of ganglia, the site may be localized on the neuronal perikaryon. Another aspect of the results presented in this paper deserves comment, namely, the enhanced stimulation (125%) of NGF specific 'PI-effect' in SCG of 6-OHDA treated rats compared to the stimulation (57%) observed in SCG of sham treated rats. It appears that the 6-OHDA treatment does potentiate the 'NGF receptor' specific PI turnover. This is interesting in the light of the report by Levi-Montalcini et al. [12] that the combined treatment of NGF and 6-OHDA, versus NGF alone, resulted in a paradoxical volume increase of SCG and a much larger increase of total as well as the specific activities of TH in the ganglia. During a short time in vivo uptake studies, Levi-Montalcini et al. [13] also noticed a greater accumulation of ^{125}I-NGF in the cell bodies of sympathetic neurones of rats subjected for two weeks period to dual NGF and 6-OHDA treatment, in contrast to NGF or saline treatment.

Thus the results presented in this paper, taken together with the findings of Levi-Montalcini et al. [13], led to suggest that 6-OHDA treatment to newborn rats results in an increased NGF-receptor density on the cell body of adrenergic neurones. Such an alteration in the NGF receptor density could be interpreted as a fine control mechanism for the survival of neurones against the cytotoxic action of 6-OHDA. Alternatively the lack of evidence for the increased receptor density in pineal after 6-OHDA treatment, means that this organ does not need NGF for its ultimate survival.

Further work in progress includes (a) elucidation of the functional significance of PI turnover, (b) whether the increased NGF-receptor density after 6-OHDA treatment is a permanent or a transient feature of adrenergic neurones, and (c) how this

property could be used as a tool to isolate and identify the NGF receptor molecules.

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