INVOLVEMENT OF POST-SYNAPTIC CYTOSKELETON IN NERVE GROWTH FACTOR-INDUCED 'PI-EFFECT'

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1. Introduction

One of the selective biochemical responses, subsequent to the interaction of several putative neurotransmitters and hormones with their specific receptors at the cell surface of their target tissue, appears to be increased turnover of phosphorylinositol group of phosphatidylinositol (PI-effect) [1]. Such a phenomenon has been well documented in a wide variety of tissues such as pancreas, pineal gland, sympathetic ganglia, brain, smooth muscle, thyroid, adrenal medulla, adipose tissue, parotid glands and salt glands of birds [1,2]. However to date neither the molecular mechanism(s) nor the functional significance underlying this phenomenon is clear. Recently I have shown an increased phosphatidylinositol (PI) turnover in response to nerve growth factor (NGF) in rat superior cervical ganglia (SCG) and in pineal glands [3,4]. This factor is known to be necessary for the development and survival of sympathetic neurones [5,6].

The two key enzymes, namely diglyceride-kinase and phosphatidylinositol phosphodiesterase, involved in the recycling of membrane phosphoinositides are known to be associated with microtubular subunit protein, tubulin [7,8]. The specific interactions observed between NGF and tubulin led to the suggestion [9] that the tubulin molecules on the cell surface of sympathetic neurones are likely to be the possible NGF receptors in mediating the chain of events inside the target cell. Very recently evidence has been provided for the presence of tubulin on the exterior cell surfaces of the sympathetic neurones [10]. These studies prompted the present investigation on the role of tubulin in NGF-induced stimulation of PI turnover in rat SCG and pineal glands. The results suggest that not only tubulin, the microtubular subunit protein, but also microfilaments are involved in mediating the PI turnover in response to NGF both in target and end organs.

2. Experimental

Nerve growth factor, 2.5 S form was prepared by the method in [11]. [2-^{3}H]Myoinositol (2 Ci/mmol) was obtained from Amersham Searle Corp., Des Plaines, IL. Cytochalasin B was a product of Aldrich Chemical Co. and colchicine was obtained from Sigma. Vinblastine sulphate and diamide were kind gifts of Dr Susil Devare. Culture medium and antibiotics were obtained from Grand Island Biological Co. All other chemicals were of reagent grade.

Superior cervical ganglia excised from day 6–7 rats were decapsulated under a binocular dissection microscope. The pineal glands were removed from adult male rats. The SCG or pineal glands were cultured 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 units/ml) and streptomycin (100 μg/ml). Freshly prepared ascorbic acid (0.1 mg/ml) and glutamine (2 mM) were added just before use. Whenever the effects of vinblastine, cytochalasin B, colchicine and diamide were studied, the SCG and pineal glands were preincubated with the drugs for various times before the addition of labelled myoinositol and

NGF. 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 with 0.9% saline. Pairs of SCG or pineal glands were homogenized in a ground-glass homogenizer with 4 ml chloroform–methanol mixture (2:1, v/v) and transferred to conical centrifuge tubes. The lipid extracts were washed 4 times with 0.2 vol. 0.9% saline containing 10^{-4} M unlabelled myoinositol. They were transferred to scintillation vials and then evaporated to dryness under a stream of nitrogen. A 15 ml scintillation cocktail (5 g PPO and 0.5 g POPOP in 1000 ml toluene) was added before counting. A duplicate sample of tissue-lipid extract was mixed with rat brain synaptosomal lipid fraction (0.5–0.75 μmol lipid P) as carrier and then subjected to two dimensional thin-layer chromatography as in [3]. From 95–98% of the radioactivity was found present in the phosphatidylinositol fraction.

3. Results

Figures 1a and 1b indicate that NGF stimulates PI turnover in SCG and pineal, respectively. Colchicine (1×10^{-6} M), a potent drug that binds to dimeric subunit of microtubule tubulin, leading to inhibition of microtubule assembly [12], did not prevent the NGF-specific PI turnover. NGF-mediated PI turnover was indeed stimulated by colchicine in rat SCG and pineal glands. Preincubation of these organs with vinblastine (1×10^{-6} M), which is known to induce depolymerization of microtubules [13], resulted in decreased entry of labelled myoinositol into PI, indicating a decrease in the NGF-induced turnover of PI. However the contradictory data obtained with vinblastine and colchicine can be explained on the basis that these drugs bind to tubulin at distinct sites [14]. In order to confirm further the involvement of microtubules in NGF-mediated PI turnover diamide, a drug recently shown to inhibit the polymerization of tubulin as well as to cause the disassembly of preformed microtubules [15,16] was used. It is clear from table 1 that the preincubation of SCG and pineal glands with diamide (5×10^{-3} M) resulted in a decrease in the NGF-induced PI turnover. Since microfilaments have been suggested [17] to link microtubules and receptors, the involvement of microfilaments in NGF-mediated PI effect was tested using cytochalasin B as a probe. The addition of cytochalasin B (0.75 μg/ml) also decreased the NGF-specific PI turnover (table 2) thereby suggesting the involvement of microfilaments

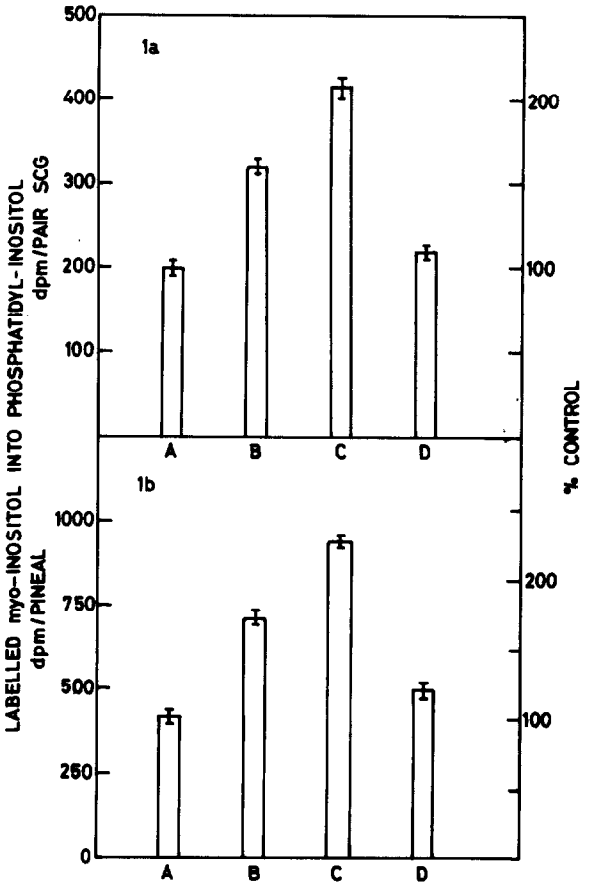

Fig.1. Effect of colchicine and vinblastine on NGF-induced PI turnover in rat SCG and pineal glands. SCG (3 pairs/well) or pineal glands (2 pairs/well) were preincubated with or without colchicine (final conc. 1×10^{-6} M) for 6 h. In the case of vinblastine (final conc. 1×10^{-6} M) the tissues were preincubated with or without the alkaloid for 30 min, at 37°C. At the end of preincubation, 12.5 μCi of [2-^{3}H]-myoinositol (2 Ci/mmol) and NGF (final conc. 1×10^{-8} M) or buffer were added in 5 μl aliquots. The incubation was continued further 12 h. The tissues were removed, rinsed, processed for lipid extraction and then counted as in section 2. Radioactivity was expressed as dpm/pair SCG or dpm/pineal gland. Each bar represents the mean ± SEM of at least 12 pairs of SCG or 8 pineal glands. (A) Control; (B) NGF, (C) colchicine plus NGF, (D) vinblastine plus NGF.

Table 1
Effect of diamide on NGF-induced PI turnover in rat SCG and pineal glands

Tissue	Addition to culture	Radioactivity in phosphatidylinositol
Superior cervical ganglia	Control	220 ± 25 (100%)
	NGF	378 ± 30 (171%)
	Diamide + NGF	182 ± 26 (82%)
Pineal glands	Control	390 ± 36 (100%)
	NGF	605 ± 25 (155%)
	Diamide + NGF	273 ± 26 (70%)

SCG (3 pairs/well) or pineal glands (2 pairs/well) were preincubated with diamide (final conc. 5×10^{-3} M) for 15 min before adding [2-^{3}H]myoinositol (12.5 μCi) and NGF (final conc. 1×10^{-8} M). The other experimental details are as in the text and fig.1

in NGF-mediated PI turnover. Preincubation of tissues with diamide and cytochalasin B resulted in a decrease of NGF-induced turnover of phosphatidylinositol which is lower than the basal PI turnover. This suggests that the basal PI turnover may be related to integrity of microtubule and microfilaments of the membrane.

4. Discussion

Colchicine and vinblastine are known to inhibit selectively microtubule assembly by virtue of their specific interactions with tubulin, in an unusual substoichiometric fashion [14,20]. However the mechanism by which diamide inhibits microtubule assembly appears to be different from that of colchicine and vinblastine [15]. Cytochalasin B is thought to disrupt the microfilament system containing actomyosin located at the cell surface [18,19]. Moreover recent investigations have provided both biochemical and morphological evidence towards the specific interactions between NGF and tubulin dimers, NGF and preformed microtubules and NGF and actin [21–23]. For these reasons as well as those mentioned in the introduction, the present investigation has made use of the above-mentioned drugs to probe the involvement of microtubule subunit protein in NGF-specific stimulation of PI turnover. The results provide evidence for the participation of microtubules and microfilaments in NGF-stimulated PI turnover. The primary locus of NGF-stimulated PI turnover as the post-synaptic structures in the target as well as end organs was revealed in [4] since surgical denervation did not abolish NGF-induced PI turnover. Taken together, these observations indicate that the microtubules and microfilaments (cytoskeleton) of the post-synaptic structures are primarily involved in mediating the NGF-specific PI turnover. Although the functional significance(s) for the presence of microtubules and microfilaments in relation to synaptic function is largely unknown, their presence as post-synaptic structural matrix at least in cerebral

Table 2
Effect of cytochalasine B on NGF-induced PI turnover in rat SCG and pineal glands

Tissue	Addition to culture	Radioactivity in phosphatidylinositol
Superior cervical ganglia	Control	200 ± 20 (100%)
	NGF	340 ± 28 (170%)
	Cytochalasin B + NGF	180 ± 24 (90%)
Pineal glands	Control	430 ± 25 (100%)
	NGF	690 ± 44 (160%)
	Cytochalasin B + NGF	390 ± 32 (90%)

SCG (3 pairs/well) or pineal glands (2 pairs/well) were preincubated with or without cytochalasin B (final conc. 0.75 μg/ml) for 30 min at 37°C before the addition of [2-^{3}H]myoinositol (12.5 μCi) and NGF (final conc. 1×10^{-8} M). The other experimental details are in the text and fig.1

cortex in situ, has been repeatedly confirmed by biochemical, immunochemical and morphological techniques [24–27].

Hypotheses proposed to explain the physiological significance of PI turnover elicited by a variety of stimuli at the cell surface include:

(i) Permeability changes accompanying selective ion transport during synaptic transmission.
(ii) Packaging and translocation of macromolecules.
(iii) An intrinsic function probably mediated by the receptor sites at the cell surface [1].

However none of these hypotheses have any sound experimental basis. The identification of the involvement of microtubules and microfilaments in NGF-mediated PI turnover provide some new perspectives for further investigations as to which of these hypotheses is relevant to NGF action.

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