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Stimulation of nerve growth factor synthesis/secretion by 1,4-benzoquinone and its derivatives in cultured mouse astroglial cells

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Previously we reported that astroglial cells cultured from mouse brain synthesize and secrete nerve growth factor (NGF) and that, in quiescent cells, catecholamines markedly increase the NGF content in the conditioned medium (CM). We wished to further assess the structural properties required for exhibition of such effect of compounds containing a ring structure analogous to that of catechol on astroglial NGF synthesis. During our study, we found that hydroquinone, which was confirmed not to stimulate NGF synthesis in mouse fibroblast cells in another of our investigations, is a potent stimulator of NGF synthesis in astroglial cells and that 1,4-benzoquinone, an oxidized form of hydroquinone, is a more effective stimulator than hydroquinone itself. In addition, the results of experiments with 1,2-benzoquinone derivatives indicated that the presence of a long aliphatic side chain in the molecule eliminates the stimulatory effect of 1,4-benzoquinone on NGF synthesis in astroglial cells.

Nerve growth factor; 1,4-Benzoquinone; Coenzyme Q_0; Vitamin K_3; (Astroglial cell)

1. INTRODUCTION

NGF is a protein that is required for the development and maintenance of sympathetic and some sensory neurons [1,2]. NGF is thought to be synthesized in innervated end organs and retrogradely transported to the neuronal cell body [3,4]. In fact, mRNA of NGF has been detected in the various end organs which are sympathetically innervated [5,6].

A few years ago, we developed a highly sensitive EIA for mouse β NGF, which is the active subunit of the submaxillary gland NGF known as 7S NGF [7]. By using this EIA system, we demonstrated that fibroblast cells cultured from various organs of mice synthesize and secrete a molecule identical to submaxillary gland β NGF [8,9]. In those studies, we found that catecholamines induce an increase in the NGF content of CM and that this effect is due to the presence of a catechol ring in the molecule [9–11].

NGF has been found in the brain as well, and it has been reported that NGF functions as a neurotrophic molecule for the cholinergic neurons in the basal forebrain nuclei thought to be responsible for learning and memory [12–14]. A recent pharmacological study on NGF has shown that continuous intracerebral infusion of NGF could partly reverse the cholinergic cell body atrophy and improve retention of a spatial memory task in behaviorally impaired aged rats [15]. This effect of exogenously applied NGF suggests that stimulation of synthesis/secretion of endogenous NGF would be a more efficient and rather more practical means for therapy and/or prevention of neuronal disorders in the brain than exogenously applied NGF.

As for cells cultured from mouse brain, we showed that astroglial cells synthesize and secrete NGF, suggesting that glial cells are responsible for regulation of NGF in the brain [16]. Further, we found that addition of catecholamines to quiescent astroglial cells causes acceleration of their NGF synthesis as in the case of mouse fibroblast cells [11,17]. This indicated the possibility to develop new types of drugs that regulate the NGF content in the brain.

To further investigate the structural properties of the compounds exhibiting a stimulatory effect on NGF synthesis in astroglial cells, we examined the effect of compounds that contain a ring structure analogous to that of catechol in their molecules.

2. MATERIALS AND METHODS

2.1. *Materials*

Mouse submaxillary gland β NGF and anti-β NGF antiserum were prepared as described previously [7]. DMEM was obtained from Nissui; FCS, from Bockneck; cell culture vessels, from Falcon; BSA, from Armour; idebenone and hydroquinone-idebenone, from Takeda Chemical Industries, Ltd. All other chemicals were obtained from either Nacalai Tesque Inc. or Woko Chemical Industries.

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Abbreviations: NGF, nerve growth factor; EIA, enzyme immunoassay; BSA, bovine serum albumin; FCS, fetal calf serum; DMEM, Dulbecco's modified Eagle's medium; CM, conditioned medium

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2.2. *Cell culture*

Astroglial cells were cultured from the whole brain of 8-day-old ICR mice and maintained in DMEM containing 10% FCS as described before [16]. The preparation of the quiescent astroglial cells was performed according to the procedures previously reported [17]. Namely, astroglial cells were inoculated into 24-well plates (well surface, 2.1. cm^2) and cultured in FCS-containing DMEM until confluence was reached (about 1.5×10^6 cells/cm^2). Then they were cultured for an additional 2 weeks in FCS-free DMEM containing 0.5% BSA, with a medium change every 3 days. Most of the cells were arrested in the quiescent phase. The cells were cultured for 24 h in 0.5 ml of DMEM containing 0.5% BSA with or without the desired drug, and then the NGF content in the CM of each culture was determined. Since NGF synthesized by astroglial cells is secreted rapidly into the CM, the measurement of NGF content in CM reflects the amount of NGF synthesized [16,17].

2.3. *Two-site EIA*

The contents of NGF in the CM of astroglial cells treated with test samples were determined by our two-site EIA for mouse submaxillary gland β NGF [7]. As hydroquinone and other drugs used in this study, as well as the unconditioned medium, did not affect the EIA system, the CM was directly applied to the wells for the EIA.

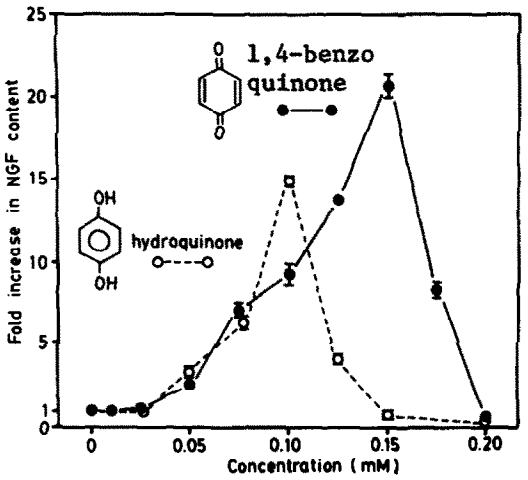

Fig.2. Effect of hydroquinone and 1,4-benzoquinone on the NGF content in medium conditioned by mouse astroglial cells. Same as legend to fig.1 except hydroquinone (○) and 1,4-benzoquinone (●) were tested.

3. RESULTS

The stimulatory effect of catecholamines on NGF synthesis in mouse fibroblast cells was earlier confirmed to be due to the presence of a catechol ring in the molecules [10]. This was based on the result that catechol itself exhibited a weak effect, while its positional isomers, resorcinol and hydroquinone, were ineffective. Since the requirement of a catechol ring for the exhibition of the stimulatory effect in astroglial cells had not been assessed, we examined the effect of those 3 isomers. As shown in fig.1, catechol, possessing its two phenolic hydroxyl groups in the *ortho*-relationship was effective for stimulation of astroglial NGF synthesis in agreement with our previous investigation concerning NGF synthesis in fibroblast cells [10]. Resorcinol, with the groups in the *meta* positions, was ineffective as a stimulator for astroglial cells as well as for fibroblast cells. Hydroquinone, with the two groups in the *para*-configuration and confirmed not to stimulate NGF synthesis in fibroblast cells, surprisingly exhibited a stimulatory effect on NGF synthesis in the astroglial cells. Its dose–response curve was bell-shaped. The NGF content in CM of astroglial cells treated with 0.1 mM hydroquinone corresponded to 15 times that of control cultures, while that of cells treated with 0.15 mM was the same as the control value. This phenomenon was likely caused by the cytotoxicity of hydroquinone, because some dead cells appeared at the concentration of 0.1 mM, and almost all cells died at concentrations over 0.15 mM.

These results present the possibility that drugs other than catechol derivatives may be useful stimulators of NGF synthesis. As the cytotoxicity of derivatives of catechol and its positional isomers can be diminished by modification of the aliphatic side chain or by esterification of the phenolic hydroxyl groups [18], we

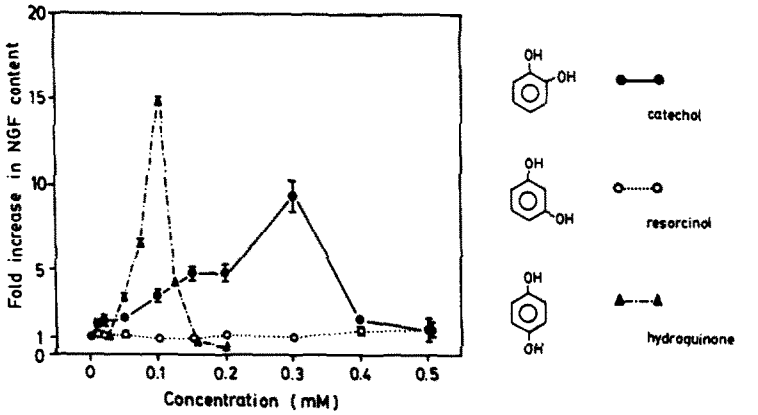

Fig.1. Effects of catechol and its isomers on the NGF content in medium conditioned by mouse astroglial cells. After a 24-hr incubation with various concentrations of catechol (●), resorcinol (○), or hydroquinone (▲), medium was collected. The NGF content in the medium was determined EIA and expressed as fold increase over that in the absence of a given drug. Each point is the mean ± SE of four determinations.

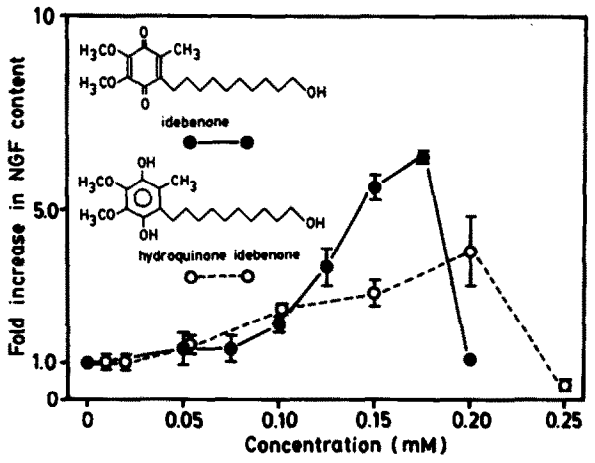

Fig.3. Effects of hydroquinone idebenone and idebenone on the NGF content in medium conditioned by mouse astroglial cells. Same as legend to fig.1 except hydroquinone idebenone (○) and idebenone (●) were tested and three determinations were made.

examined the effect on NGF synthesis of various derivatives of hydroquinone. Fig.2 shows the effect of 1,4-benzoquinone, an oxidized form of hydroquinone. As the phenolic hydroxyl groups of the latter are easily oxidized, the former is more stable. The NGF content in CM from cells treated with 0.15 mM 1,4-benzoquinone was 20 times greater than that of the control. Although the cytotoxicity of 1,4-benzoquinone was also evident, this form was indicated to be more useful than hydroquinone as a stimulator of NGF synthesis.

Fig.3 shows that idebenone (6-(10-hydroxydecyl)-2,3-dimethoxy-5-methyl-1,4-benzoquinone), a 1,4-benzoquinone derivative, had a similar range of effective concentrations as 1,4-benzoquinone had. The increase in NGF content brought about by idebenone at its optimal concentration (0.175 mM) was 7-fold of the control. Fig.3 also shows that idebenone was more effective than its hydroquinone form, 6-(10-hydroxydecyl)-2,3-dimethoxy-5-methylhydroquinone.

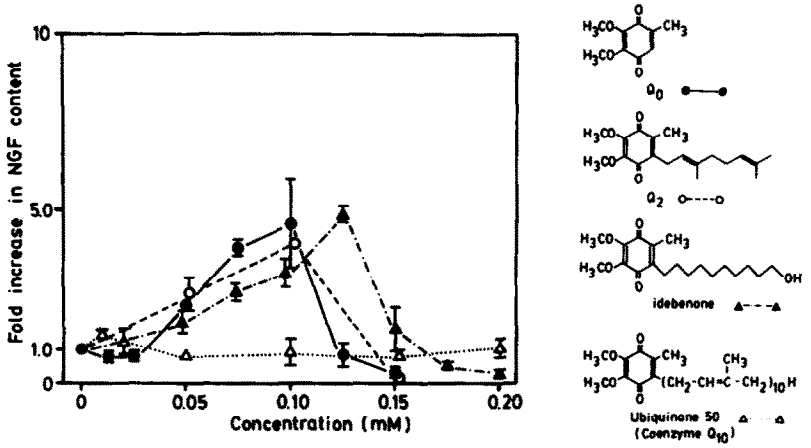

Fig.4. Effects of 2,3-dimethoxy-5-methyl-1,4-benzoquinone derivatives on the NGF content in medium conditioned by mouse astroglial cells. Same as legend to fig.1 except coenzyme Q_0 (●), coenzyme Q_2 (○), idebenone (▲), and ubiquinone 50 or coenzyme Q_{10} (△) were tested and three determinations were made.

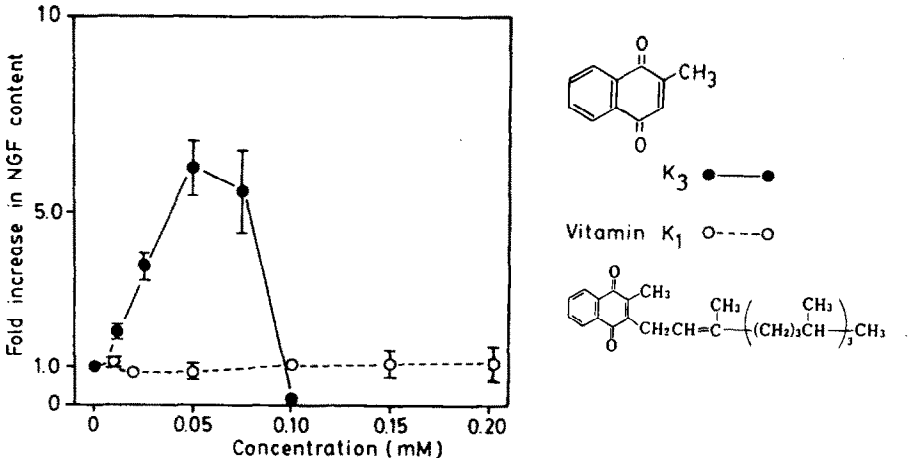

Fig.5. Effects of vitamins K_1 and K_3 on the NGF content in medium conditioned by mouse astroglial cells. Same as legend to fig.1 except vitamin K_1 (○) and K_3 (●) and three determinations were made.

Fig.4 reveals that the effects of 2,3-dimethoxy-5-methyl-1,4-benzoquinone (coenzyme Q_0) and coenzyme Q_2 on NGF synthesis were almost the same as the effect of idebenone. On the other hand, ubiquinone 50 (coenzyme Q_{10}), which has a much longer aliphatic chain, was ineffective for stimulation of NGF synthesis. Fig.5 demonstrates that vitamin K_3 (2-methyl-1,4-naphthoquinone) was effective for stimulation on NGF synthesis, while vitamin K_1, possessing an aliphatic side chain consisting of 20 carbons, was ineffective. These results indicate that the presence of a long aliphatic side chain hinders the stimulatory effect of 1,4-benzoquinone and that the presence of less than 10 carbons is likely to be permissive for the effect. The decreases observed in their effects at high concentrations was caused by their cytotoxicity.

4. DISCUSSION

The present results indicate that a catechol ring is not always necessary for exhibition of a stimulatory effect on NGF synthesis in mouse astroglial cells. We found that hydroquinone and its oxidized form (1,4-benzoquinone) also have a stimulatory effect on NGF synthesis. As they were cytotoxic at their high concentration, we focused our attention on examining the effect of their derivatives, that is, idebenone, coenzyme Q, and vitamin K. We have not yet succeeded to find any drugs without cytotoxicity. In the future we will synthesize various drugs having the 1,4-benzoquinone ring and examine their effect on NGF synthesis and cytotoxicity.

Although we have not yet examined how 1,4-benzoquinone derivatives affect the NGF content, we predict that they stimulate the de novo synthesis of NGF protein, as catechol is known to do [11,17]. In preliminary experiments, we observed that these drugs did not affect [^{3}H]thymidine incorporation, suggesting their independence of cell growth. 1,4-Benzoquinone might specifically affect the NGF mRNA level.

Several recent findings have indicated that intraventricular or intracerebral injection of NGF prevents neuronal death in rat brain lesioned mechanically or chemically [13–15], which opens up the interesting possibility that NGF may be useful as a therapeutic agent for diseases of the central nervous system such as Alzheimer's disease. As NGF itself does not cross the blood–brain barrier, and human NGF is not available, it would appear worthwhile to attempt to develop synthetic compounds having the ability to stimulate the synthesis of endogenous NGF. Although benzoquinone may be useful, it seems to be easily metabolized and does not cross the blood–brain barrier. On the other hand, benzoquinone derivatives with short aliphatic side chains in the benzene ring are able to pass the blood–brain barrier, as idebenone has proven to be a drug that improves blood flow in the brain and has been already put to use. We thus plan to develop compounds employing the 1,4-benzoquinone ring as the basal skeletal structure as potential candidates of drugs that regulate the NGF content in the brain. Derivatives of coenzyme Q or vitamin K might prove to be useful drugs.

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