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Synthesis and evaluation of a brain-targeted catechol derivative as a potential NGF-inducer

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Abstract

A novel brain-targeted catechol derivative was designed for the potential in vivo induction of nerve growth factor (NGF) biosynthesis within the central nervous system (CNS) after its peripheral administration. The dihydropyridine–pyridinium salt moiety integrated in the structure of the catechol and conferred the brain-selective delivery property to the molecule. After preliminary in vitro evaluation of this brain-targeted derivative and its pyridinium metabolite, in vivo tissue distribution studies demonstrated the selective and sustained delivery of the compound of interest to the brain of a rat. Furthermore, the significant in vitro biological activity of the novel catechol derivative that was demonstrated in the L-M cell line is encouraging for the expected NGF-inducing activity of this novel catechol derivative in vivo.

Keywords: Catechol; Nerve growth factor; Induction; Central nervous system

1. Introduction

One of the best-characterized neurotrophins, nerve growth factor (NGF) is required for the development and survival of sensory and sympathetic neurons in the peripheral nervous system (Barde, 1989) as well as of cholinergic neurons of the basal forebrain in the central nervous system (CNS) (Whittemore and Seiger, 1987; Higgins et al., 1989). Since Alzheimer's disease is characterized by a progressive loss of cholinergic neurons which project from the basal forebrain, NGF has been proposed as a potential treatment for this neurodegenerative disorder (Lapchak, 1993). Indeed many studies (Junard et al., 1990; Fisher et al., 1987) demonstrate the beneficial effect of intracerebroventricular (icv)-administered NGF, such as prevention of the loss of cholinergic neurons in animal models of degeneration, while recently the initial positive results of direct NGF

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infusion into a single Alzheimer's patient were described (Olson et al., 1992; Olson, 1993). However, since the size and the charge properties of the NGF protein prevent it from crossing the blood brain barrier (BBB), alternative approaches have been explored in order to avoid the invasive method of direct icv administration of NGF into the brain. One such approach that has concentrated much interest is the potential use of the pharmacological agents that control endogenous NGF production, enhance NGF biosynthesis and release it in the CNS. Delivery of such neurotrophomodulatory agents to the brain would not only make NGF available to the neurons without brain surgery (and consequently BBB damage), but also would produce NGF in specific CNS cell populations by targeting selected transmitter receptor subtypes, and thus this strategy would have the additional advantage that the needed extra supply of NGF would be produced at physiologically occurring NGF-dependent sites within the brain. Although at present the mechanisms that regulate NGF biosynthesis are not well elucidated, the inducibility of NGF has been extensively demonstrated in a number of in vitro and in vivo systems by a variety of agents (Carswell, 1993; De Bernardi et al., 1991; Thoenen et al., 1991; Mocchetti, 1991).

Among the numerous and diverse compounds known to up-regulate NGF biosynthesis and release, both in vitro and in vivo, are catecholamines (Schwartz, 1988; Follesa and Mocchetti, 1992). Although their activity in the C6-glioma cell line seems to be associated with their β-adrenergic activity, in other cell lines β-receptor stimulation does not seem to be involved in their action and other mechanisms are implicated (Furukawa et al., 1986a,b, 1987, 1989, 1991; Carswell et al., 1992; Kourounakis et al., 1995). A simple catechol derivative such as 4-methylcatechol is one of the most potent stimulators of NGF synthesis and secretion in cultures of fibroblast L-M or L-929 cells (Furukawa et al., 1991; Carswell et al., 1992), as well as in vivo (Kaechi et al., 1993; Hanaoka et al., 1994; Saporito et al., 1993), demonstrating that the only structural requirement for activity lies within the catechol functional moiety of the molecule.

However, the potential direct application, via peripheral administration, of catechols for CNS NGF induction which would be of interest, is of limited use for two major reasons: catechols lack adequate lipophilicity and only minimal amounts are capable of crossing the BBB, and furthermore, they would evoke unwanted side effects due to their demonstrated ability to up-regulate NGF in the periphery.

In an effort to overcome these complications a brain-targeted catechol derivative was designed (Fig. 1) that delivers the catechol functional moiety selectively into the brain. For this purpose, a dihydropyridine–pyridinium salt redox system was incorporated in the structure of 4-ethylcatechol that confers site specificity to the molecule. The chemical delivery system (CDS) approach, the main application of this redox system, has been a useful method for enhancing the selective delivery of drugs to the brain and at the same time of prolonging their presence in the CNS while reducing the peripheral toxic side-effects (Bodor and Brewster, 1983; Bodor and Farag, 1983; Brewster et al., 1989). A brief description of the principle of this targeting system is presented in Fig. 1. In designing the new catechol derivative the following were taken under consideration: the pyridine moiety of the molecule should act as a redox system providing the dual (lipophilic–hydrophilic) character; the 1,4 dihydropyridine-containing form allows BBB penetration, while the in situ-generated hydrophilic pyridinium salt-type form is 'locked' in the brain and readily eliminated in the periphery. The polar functions of the molecule (the phenolic OHs) are also derivatized to impart lipophilic character to the molecule but should be capable of transforming back to the polar species (free phenolic group) in order for the derivative to be pharmacologically active.

In vitro stability studies of the dihydropyridine catechol derivative 4, and its quaternary metabolite 3, in various buffer systems and biological media were performed in order to determine the feasibility of delivering the catechol moiety by this method. Subsequently, evaluation of the in vivo (in the rat) tissue distribution of the brain-targeted catechol and its metabolites confirmed the selective and sustained delivery of the com-

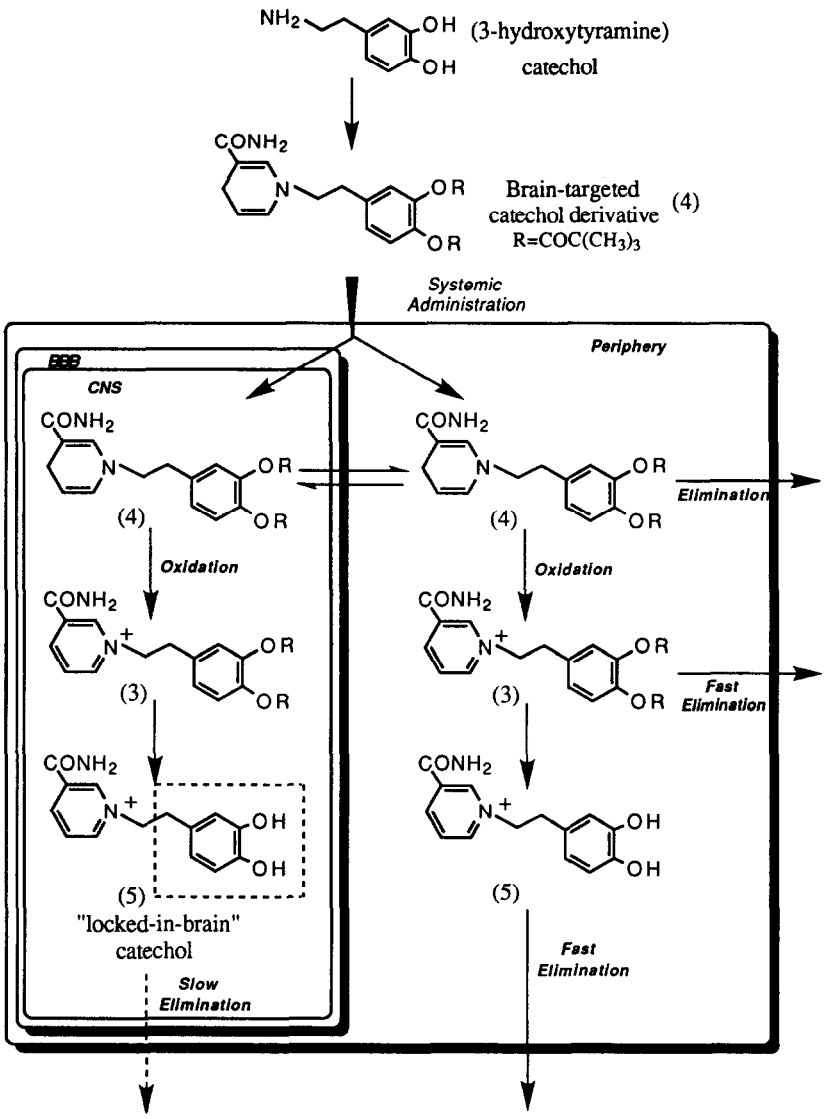


Fig. 1. Design of a brain-targeted catechol derivative 4 that selectively delivers the catechol moiety to the brain and representation of the dihydropyridine–pyridinium salt redox system that promotes CNS retention and accelerated peripheral elimination. The lipoidal dihydropyridine moiety increases the lipophilicity and BBB permeability of the molecule, while upon systemic administration, after extensive distribution, it is oxidized to the charged pyridinium ion, in the brain and in systemic tissues, by the same means as the ubiquitous NADPH–NADP redox system. The charged pyridinium derivative is thus retained, or 'locked', in the brain since the BBB prevents rapid reequilibration of polar species, while in the periphery it is rapidly cleared by renal excretion due to its increased hydrophilicity. The overall result is reduction of peripheral (side) effects or toxicity, and site-specific delivery of the active agent to the CNS.

pounds of interest to the brain. The dihydro moiety was expected to be oxidized (in vivo) after entering the brain and subsequent hydrolysis of the ester moieties would liberate the catechol moiety (Fig. 1). The in vivo studies (in the rat) demonstrated oxidation of the dihydro-catechol derivative, rapid peripheral elimination of the resulting pyridinium salts, and sustained central de-

livery of final compounds (metabolites) of interest. Finally, the pyridinium catechol derivative 5, being the final metabolite of interest, along with its precursors, was evaluated for in vitro biological activity as an NGF stimulator in cell culture, in order to establish that this novel catechol derivative is indeed active, as other catechols, in inducing NGF biosynthesis and secretion.

2. Experimental section

2.1. Synthesis

2.1.1. Synthesis of 3-carbamoyl-1-(2,4-dinitro)phenylpyridinium chloride ('Zincke-type reagent') (1)

Nicotinamide (0.065 mol) and 2,4-dinitrochloro-benzene (0.098 mol) were mixed and heated at 90 ± 2°C in an oil bath for 75 min. The melt was dissolved in MeOH and ether was added to precipitate a yellow solid. Using the same system the product was re-precipitated three times and then dissolved in water and treated with charcoal to afford after freeze-drying 11.76 g (56%) of compound 1: pale yellow-white solid, m.p. 141–142°C. ^{1}H-NMR (dimethyl sulfoxide (DMSO)-d_6) δ (ppm): 9.8 (bs, 1H); 9.4 (d, 1H); 9.1 (d, 1H); 8.9–8.7 (m, 3H); 8.4–8.2 (m, 2H); 8.1 (bs, 1H).

2.1.2. Synthesis of 3',4'-dipivaloylphenethylamine (trifluoroacetate) (2)

In a suspension of 3-hydroxytyramine hydrochloride (3HT·HCl) (4 g) in trifluoroacetic acid (TFA) (20 ml), pivaloyl chloride (10 ml) was added dropwise. After stirring for 45 h, the TFA was removed under vacuum and the product was chromatographed (silica gel, 10% methanol in chloroform) to afford 8.1 g (92%) of compound 2: pale white solid, m.p. 108–109°C. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.7 (br, 2H); 7 (3H); 3 (m, 4H); 1.3 (s, 18H). Elemental analysis for $C_{20}H_{28}F_3NO_6$. Theory: C, 55.17; H, 6.40; N, 3.20. Found: C, 54.85; H, 6.50; N, 3.12.

2.1.3. Synthesis of 1-(3',4'-dipivaloylphenethyl)-3-carbamoylpyridinium trifluoroacetate (3)

To a solution of compound 1 (4.62 mmol) and compound 2 (9.25 mmol) in anhydrous MeOH (10 ml), triethylamine (TEA) (1.28 ml in 6 ml MeOH) was added dropwise. The mixture was stirred at room temperature for 30 min and then refluxed for 30 min, after which, upon cooling, a yellow solid precipitated (by-product) and was removed. The filtrate was evaporated and dissolved in a small amount of MeOH and ether was added to precipitate a pale solid that was recrystallized three times from methanol/ether to afford 1.2 g (52%) of compound 3: pale-white solid, m.p. 194–198°C (uncov.). ^{1}H-NMR (DMSO-d_6) δ (ppm): 9.61 (s, 1H); 9.15 (d, 1H); 8.96 (d, 1H); 8.63 (s, 1H, for 1H of $CONH_2$); 8.25 (t, 1H); 8.16 (s, 1H, for 1H of $CONH_2$); 7.2 (m, 3H); 4.9 (t, 2H); 3.15 (m, for H_2O and CH_2–Ph); 1.3 (d, 18H). (MW = 540) EI MS 427 ($M-CF_3COO^-$, 100). Elemental analysis for $C_{26}H_{31}F_3N_2O_7$. Theory: C, 57.70; H, 5.74; N, 5.18. Found: C, 57.70; H, 5.73; N, 5.21.

2.1.4. Synthesis of 1-(3',4'-dipivaloylphenethyl)-3-carbamoyl-1,4 dihydropyridine (4)

Compound 3 (1.076 mmol) was dissolved in deaerated water, peroxide-free ether was added and the mixture cooled. $NaHCO_3$ solution (5 mmol) was added slowly followed by $Na_2S_2O_4$ (5 mmol) and the reaction mixture was stirred in an ice-bath (1–2°C), under N_2, for 3 h. The two layers were subsequently separated, washed three times with distilled water, and the combined ether solutions dried and evaporated to afford 0.21 g (45.5%) of compound 4: yellow solid, m.p. 62–66°C, reduces instantly methanolic silver nitrate. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.06 (s, 2H); 6.96 (s, 1H); 6.91 (s, 1H for ArH and dihydropyridine H-2); 5.65 (d, 1H for dihydropyridine H-6); 5.25 (bs, 2H for $CONH_2$); 4.71 (m, 1H for dihydropyridine H-5); 3.32 (t, 2H); 3.13 (s, 2H for dihydropyridine 2 H-4); 2.81 (t, 2H); 1.35 (d, 18H). (MW = 428) EI MS 451 ($M + Na^+$, 100). UV (in MeOH) λ_{max} 270, 345 nm. Elemental analysis for

$C_{24}H_{32}N_2O_5$. Theory: C, 67.29; H, 7.48; N, 6.54. Found: C, 67.58; H, 7.65; N, 6.28.

2.1.5. Synthesis of 1-(3',4'-dihydroxyphenethyl)-3-carbamoylpyridinium chloride (5)

To a solution of 3-hydroxytyramine hydrochloride (3HT·HCl) (4.6 mmol), in methanol (10 ml), TEA (4.6 mmol) was added followed by compound 1 (2.3 mmol) in methanol (3 ml). The mixture was refluxed for 1 h and upon cooling the yellow precipitate was removed. Addition of ether to the filtrate resulted in a precipitate that was collected and dried to afford 0.2 g (30%) of compound 1: yellow solid, m.p. 234–235°C (dec). ^{1}H-NMR (DMSO-d_6) δ (ppm): 9.3 (s, 1H); 9.05–8.8 (m, 5H); 8.2 (m, 2H); 6.6 (m, 2H); 6.4 (d, 1H); 4.8 (t, 2H for N–CH_2); 3.1 (t, 2H for Ar–CH_2). (MW = 294) EI MS 259 (M – Cl^-, 100). Elemental analysis for $C_{14}H_{15}ClN_2O_3$. Theory: C, 57.04; H, 5.09; N, 9.51; Cl, 12.05. Found: C, 57.40; H, 5.18; N, 9.19; Cl, 11.59.

2.2. In vitro stability studies

2.2.1. Analytical method

A high performance liquid chromatographic (HPLC) method was developed to assay the CDSs and their metabolites. The HPLC system consisted of an Autochrom M500 pump, Rheodyne injector, Waters RCM C-18 column, Spectroflow 757 absorbance detector and a Fisher Recordall series 5000. The mobile phase consisted of aqueous phosphate buffer solution (0.037 M, pH 6.5) and acetonitrile in different proportions. HPLC peak heights were used as a measure of the concentration of the compounds (assay detection limit 1 mg/ml) and were plotted against time to evaluate the disappearance rates of the compounds. The stabilities were determined by measuring the pseudo-first-order rate constant (k_{obs}, min^{-1}) or the half-life ($t_{1/2}$, min) of disappearance of the compound in the solution. k_{obs} is determined from the slope of the log of the disappearance curve (k_{obs} = slope × 2.303) and the $t_{1/2}$ of the compounds is calculated from the relation $t_{1/2} = 0.693/k_{obs}$.

2.2.2. Stability in buffers

USP standard phosphate buffer solutions (0.2M) (USP XXI, 1985) in the pH range 4.5–9.5 were used in this study. Solutions of compounds 4 and 3 in buffers were made at a concentration of 1 mg/ml. The solutions were kept at 37°C and aliquots were taken at frequent time intervals and injected in the HPLC. The study was repeated three times, and the half-life at each pH was calculated from the averages of the values obtained.

2.2.3. Stability in biological media

A 0.3-ml aliquot of stock solution (3 mg/ml DMSO) of compound 4 or its quaternary metabolite 3 was added respectively to 3 ml of biological medium (whole heparinized rat blood, 20% rat brain homogenate, or 20% rat liver homogenate, in isotonic phosphate buffer of pH 7.4), which was kept in a 37°C water bath, to obtain a final concentration of 0.33 mg/ml biological media. Samples of 0.1 ml were taken at appropriate time intervals and were mixed with 0.2 ml acetonitrile containing 5% DMSO. The mixtures were centrifuged and the supernatants injected in the HPLC. The experiment was repeated again and the average half-lives were calculated.

2.3. In vivo distribution study

2.3.1. Analytical method

The HPLC system consisted of an SP 8810 precision isocratic pump, SP4290 injector, Waters RCM C-18 column, SP 8450 UV/visible detector and SP4290 integrator. The mobile phase consisted of aqueous phosphate buffer solution (0.05 M, pH 6.5) and acetonitrile in different proportions for the detection of the dihydro compounds, quaternary metabolites and final metabolites. The area under the peak was used as a measure of the concentration of the compound. Detection was made at 345 nm for the dihydro compounds and at 254 nm for metabolites.

2.3.2. Experimental procedure

Male Sprague Dawley rats (body weight 200–220 g) were injected intravenously (tail vein) with compound 4 (50 mg/kg body weight) in 1:1

DMSO and 50% 2-hydroxypropyl-β-cyclodextrin in water. Animals were sacrificed at the appropriate time points and trunk blood, whole brain and liver tissue collected. Samples were mixed or homogenized with 2 vol. of acetonitrile containing 5% DMSO, centrifuged, and supernatants analyzed by HPLC, as previously described in Section 2.2.

Calibration curves were used to determine the concentrations of the compounds under investigation in blood, brain or liver, respectively.

2.4. In vitro NGF-stimulatory activity

2.4.1. Cell cultures

Mouse L-M cells were obtained from the American Type Culture Collection (ATCC) (Rockville, MD) and maintained as monolayer cultures in medium 199 (Sigma, St. Louis, MO) supplemented with 0.5% bactopeptone (Difco Laboratories, Detroit, MI), antibiotic-free. Stock cultures were grown in 25-cm^2 flasks and subcultured once a week. To study the effect of the compounds under investigation, cells were inoculated in 24-well plates (well surface 2.1 cm^2) at a cell density of $4-8 \times 10^4$ cells/well and cultured for 3 days in peptone-containing medium, after which the medium was changed to medium 199 containing 0.5% bovine serum albumin (BSA) with or without any compounds, and the cells were cultured for 24 h.

The C6 glioma cell line was also obtained from the ATCC and was grown as monolayer cultures in RPMI-1640 (Sigma) serum-free (SF) medium supplemented with 1% L-glutamine and 0.5% penicillin/streptomycin in a humidified chamber with 95% air and 5% CO_2 at 37°C. Incubation with compounds was performed in 25-cm^2 flasks ($1-1.5 \times 10^6$ cells/flask) in the same SF medium at 37 ± 0.5°C for 17 h.

In all cases, the concentration of compounds used did not affect cell viability (viability was $> 90\%$ compared to control) as determined by the Trypan Blue dye exclusion assay.

2.4.2. Determination of NGF levels

L-M cells: the conditioned medium was applied directly to the double antibody enzyme-linked immunosorbent assay (ELISA). C6 glioma cells: the NGF in the cell culture medium was quantitated using the double antibody ELISA. Also, the cell monolayers were washed with 2 ml of EDTA 0.02% solution (Sigma) (37°C) that was also immediately collected for NGF-ELISA analysis. Since neither the compounds used in this study nor the medium or EDTA solution affected the ELISA, they were directly applied to the ELISA. NGF-ELISA: the amount of NGF protein that is produced and secreted by the cells in the cell culture medium is measured with the double antibody ELISA. The principle involves sandwiching the NGF protein with two antibodies. The two monoclonal antibodies against NGF were obtained from Boehringer Mannheim, Germany. In brief, immunoplates were coated with purified anti-NGF monoclonal antibody. Following incubation with 1% BSA, culture medium or NGF standards (6.25–800 pg/ml) were added in triplicate to the plates. After overnight incubation, the immunoplate wells were incubated with anti-NGF monoclonal antibody conjugated with β-galactosidase. By adding the enzyme substrate chlorophenol red-β-galactopyranoside, the intensity of the color produced is measured photometrically at a wavelength of 574 nm. Statistical analysis of the data was performed by using analysis of variance (ANOVA) followed by the multiple comparison Fisher PLSD.

3. Results and discussion

3.1. Synthesis

The synthetic scheme used for the synthesis of the catechol derivatives is represented in Fig. 2. The reaction used for the synthesis of the pyridinium derivatives (esterified or free catechol functions), compounds 3 and 5, involves nucleophilic attack of the amine group of the (protected or not) 3-hydroxytyramine at the pyridinium ring of the 'Zincke reagent' (Lettre et al., 1953; Zincke, 1903). The strong electron-withdrawing N-substituent (2,4 dinitrophenyl) of the pyridinium ring of the 'Zincke reagent' facilitates ring opening upon reaction with the amine group

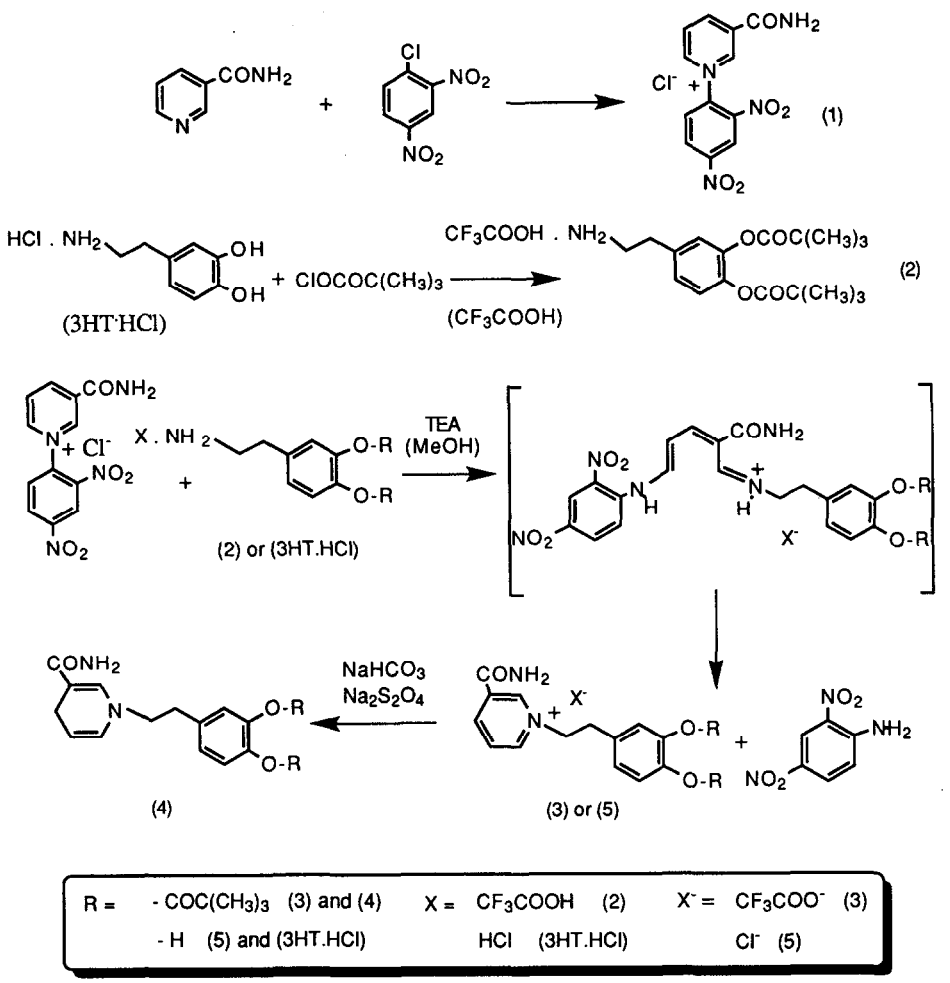


Fig. 2. Synthesis of the brain targeted catechol derivative 4 and the pyridinium catechol derivative 5.

of 3-hydroxytyramine to produce the intermediate dianil (Fig. 2). Subsequent recyclization renders the desired pyridinium products.

3.2. *In vitro stability studies*

3.2.1. *Stability in buffers*

Theoretically, compounds with potential biological activity, apart from potency, should exhibit some stability in aqueous solutions, in order to be useful for therapeutic purposes, for example in order to make stable pharmaceutical formulations. The in vitro stability study conducted herein constituted an approach to investigate these properties.

The pH stability profiles (in USP standard and phosphate buffers) of the dihydro compound 4 and the corresponding quaternary salt 3 are represented in Fig. 3. As expected, the dihydro compound 4 exhibited sufficient stability in alkaline conditions ($t_{1/2} = 2$ h in pH = 8), while its instability in acidic environment (below pH 5.5) is due to hydration (water addition) at the dihydro ring. In the case of the quaternary salt 3, hydrolysis of the pivaloyl esters is the only major expected transformation which occurs in alkaline conditions ($t_{1/2} =$ 48 h in pH = 8), while it is very stable in acidic environment.

3.2.2. Stability in biological media

In vitro stability studies in biological matrices give a relative picture of the stability profile of the compounds under investigation and indications of the possible metabolic profile in in vivo conditions. Stability in the rat brain homogenate: the dihydro compound 4 exhibited a half-life of 12 min in the rat brain homogenate. Degradation pathways for the dihydro consisted mainly in oxidation to the quaternary and sequential hydrolysis of the two ester bonds. On the other hand, the quaternary derivative showed a much longer half-life than the dihydro, demonstrating that the charged side chain stabilizes the molecule relatively to the dihydro which is more vulnerable to degradation. Stability in whole rat blood: shorter half-lives, as expected, due to the abundance of esterases in the blood compared to the brain, were obtained in whole rat blood for both the dihydro compound and its quaternary metabolite. Stability in rat liver homogenate: half-life values of compounds 4 and 3 in the rat liver homogenate ranged between the values observed in the blood and the brain homogenate (Fig. 4), with the dihydro retaining its faster degradation compared with the quaternary. Although metabolic enzymes are primarily found in the liver homogenate, degradation of compounds 4 and 3 by ester hydrolyses requires mostly unspecific esterases found in abundance in the blood.

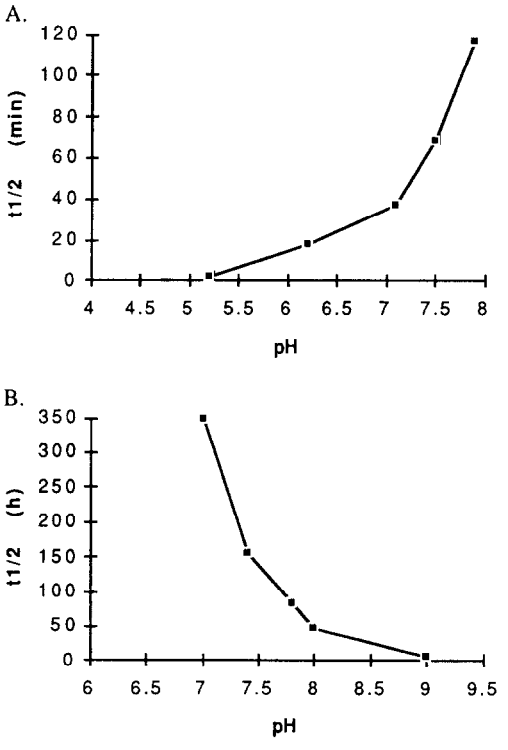


Fig. 3. pH profiles of A) dihydro compound 4 and B) its quaternary metabolite 3. Each value is the mean of three independent determinations.

3.3. In vivo distribution studies

The dihydro catechol derivative 4 readily crossed the BBB after i.v. administration, and was converted rapidly in the rat brain, even at an early stage, to the quaternary metabolite 3 ('lock-in' form), concentrations of which were sustained for long periods. The quaternary metabolite 3 released the final metabolite 5 that was maintained at higher levels in the brain than the quaternary 3 for at least 2 h (Fig. 5(A)).

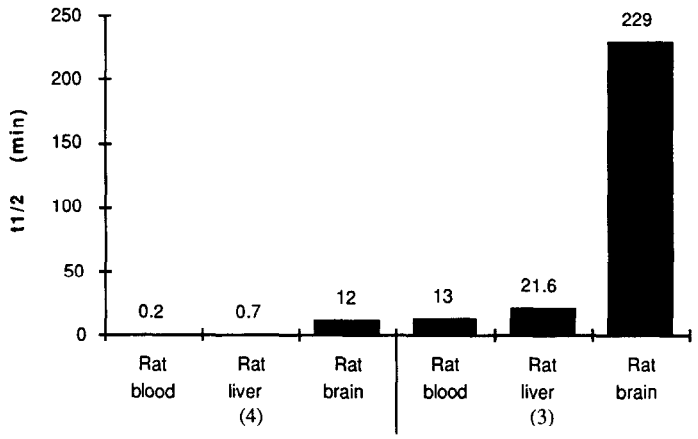
Blood levels of the CDS 4 were undetectable even at early times and only the quaternary metabolites (3 and 5), which were in turn rapidly eliminated, were observed, as shown in Fig. 6. While these metabolites rapidly disappeared from the periphery, they were sustained in much higher levels and for longer in the brain (Fig. 5).

The presence and metabolism of compound 4 and its metabolites was also investigated in the liver tissue. As expected, the dihydro was rapidly converted to the quaternary 3 and that in turn to the pyridinium catechol derivative 5 (Fig. 5(B)).

3.4. In vitro NGF-stimulatory activity

The in vitro activity of the pyridinium catechol derivative 5 in the C6-glioma cells and fibroblastic L-M cells and of its precursors (4 and 3) in L-M cells is shown in Figs. 7–9.

Although catecholamines are good NGF-inducers in C6-glioma cells, the catechol derivative 5 showed no significant activity in this cell line. The bulky and charged side chain of this derivative is probably interfering/preventing with β-adrenergic receptor interaction, which is the proposed mech-



	Blood (4)	Liver (4)	Brain (4)	Blood (3)	Liver (3)	Brain (3)
r	0.986	0.99	0.991	0.995	0.989	0.612
k	3.236	1.0187	0.0574	0.053	0.032	0.003
t1/2 (min)	0.2	0.7	12	13	21.6	229

Fig. 4. In vitro stability of compounds 4 and 3 in various biological media. The first three columns refer to the dihydro 4, while the last three columns refer to the quaternary 3. Each value is the mean of two independent determinations. r is the regression coefficient, k is the rate of disappearance and $t_{1/2}$ is the half life in minutes.

anism of action of catecholamines in this cell line. However, the different mechanism of action of catechols in L-M cells is also reflected by the activity exhibited by compound 5 (NGF content increased more than 2-fold over the control) in this cell line. Although the mechanism is not yet well elucidated, results seem to confirm the already proposed requirement (Furukawa et al., 1986a,b, 1987, 1989, 1991; Kourounakis et al., 1995) and necessity of the catechol moiety for activity. The lower activity of compound 5 compared to other active catechol derivatives can be attributed to its charged pyridinium side chain that could interfere with cellular uptake. Accordingly, compounds 3 and 4, with protected catechol functions, show minimal activity since the catechol moiety needs to be liberated first (hydrolysis of the esters), which does not readily proceed in the culture medium of these cells (presence of esterases or alkaline pHs facilitate this conversion).

4. Conclusion

The brain-targeted catechol derivative successfully demonstrated selective and sustained delivery of the catechol moiety to the brain. The relative rapid disappearance of the metabolites of interest from the periphery, in combination with its long-lasting presence within the CNS, provides support for the expected reduction in peripheral side effects along with longer CNS duration of action of this potential NGF-inducer after peripheral administration.

Furthermore, the in vitro activity in L-M cells exhibited by the novel catechol derivative 5 is encouraging for its potential effectiveness in stimulating NGF biosynthesis in vivo within the brain.

In addition to the potential therapeutic implications, use of such or similar derivatives for CNS NGF induction may further serve as a better means for studying the as yet not fully elucidated

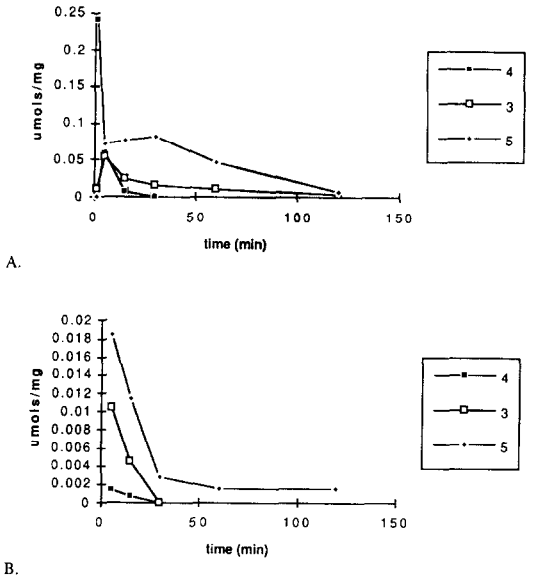


Fig. 5. In vivo concentrations of compound 4, its metabolite 3 and final metabolite 5, in A) rat brain and B) rat liver. Each value is the mean of two independent determinations.

in vitro and/or in vivo mechanisms involved in regulating NGF biosynthesis.

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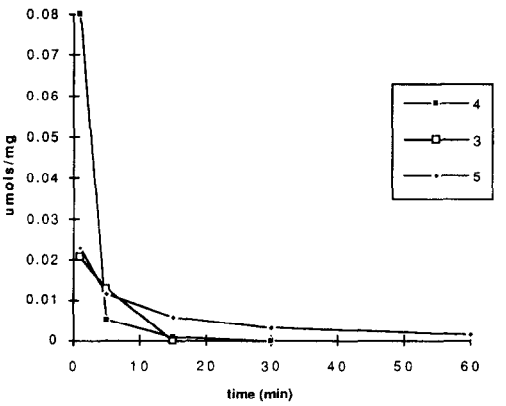

Fig. 6. In vivo concentrations of compound 4, its metabolite 3 and final metabolite 5, in rat blood. Each value is the mean of two independent determinations.

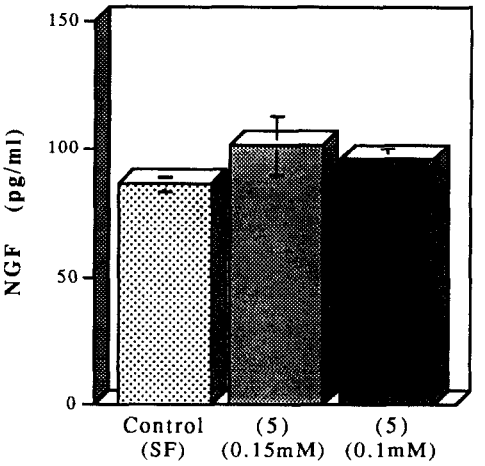

Fig. 7. Effect of catechol derivative 5 on NGF content in the culture medium of the C6 glioma cells. Each value is the mean ± S.E. of five independent determinations, each assayed in duplicate. * $P < 0.05$ relative to control according to Fisher PLSD.

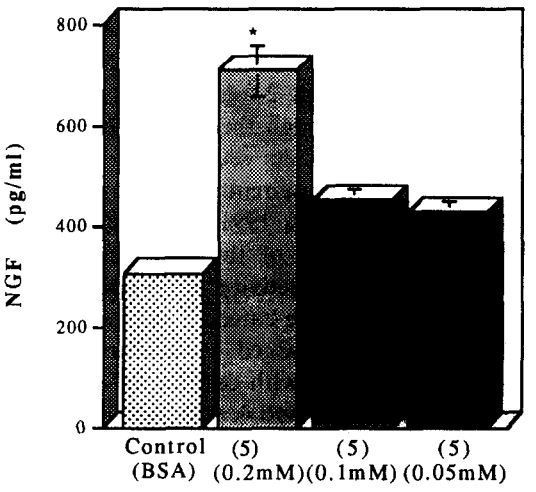

Fig. 8. Effect of catechol derivative 5 on NGF content in the culture medium of the L-M cells. Each value is the mean ± S.E. of five independent determinations, each assayed in duplicate. * $P < 0.05$ relative to control according to Fisher PLSD.

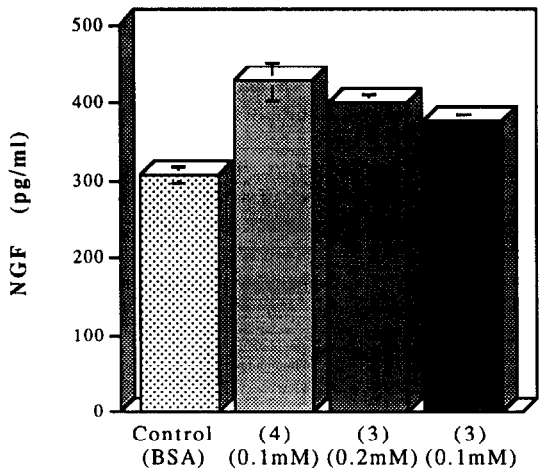

Fig. 9. Effect of the dihydro catechol derivative 4 and its quaternary metabolite 3 on NGF content in the culture medium of the L-M cells. Each value is the mean ± S.E. of five independent determinations, each assayed in duplicate.

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