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STIMULATION OF NERVE GROWTH FACTOR RECEPTORS IN PC12 BY ACETYL-L-CARNITINE

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Abstract—Acetyl-L-carnitine (ALCAR) prevents some deficits associated with aging in the central nervous system (CNS), such as the aged-related reduction of nerve growth factor (NGF) binding. The aim of this study was to ascertain whether ALCAR could affect the expression of an NGF receptor ($p75^{NGFR}$). Treatment of PC12 cells with ALCAR increased equilibrium binding of ^{125}I-NGF. ALCAR treatment also increased the amount of immunoprecipitable $p75^{NGFR}$ from PC12 cells. Lastly, the level of $p75^{NGFR}$ messenger RNA (mRNA) in PC12 was increased following ALCAR treatment. These results are in agreement with the hypothesis that there is a direct action of ALCAR on $p75^{NGFR}$ expression in aged rodent CNS.

The role played by the nerve growth factor (NGF‖) in the peripheral nervous system (PNS) has been reviewed extensively [1, 2]. During development, tissue levels of NGF are responsible for axonal growth and, once innervation takes place, for a continuous supply of NGF to the innervating neurons by retrograde transport [3, 4]. In the central nervous system (CNS), high concentrations of NGF are found in those areas that receive cholinergic innervation from basal forebrain nuclei, such as the hippocampus and frontal cortex [5]. Although little is known about the physiological role of NGF in the CNS, it has been demonstrated that NGF rescues basal forebrain neurons following fimbria-fornix transection [6, 7] and that it stimulates choline acetyltransferase (ChAT) activity in the hippocampus, neocortex, septum and striatum of neonatal rats [8].

The action of NGF on target tissues requires the binding of the NGF to specific membrane receptors [9–12]. In the nervous system, two distinct NGF binding sites have been described: a high-affinity, low-capacity site with a K_d of about 10^{-11}–10^{-10} M (type I), and a low-affinity, high-capacity site with a K_d of about 10^{-9}–10^{-8} M (type II) [13–17]. Also, there are indirect autoradiographic demonstrations of high-affinity sites for NGF binding in the CNS [18–20], and careful analysis of the published binding data does not rule out the interpretation of the autoradiographic data [15–17]. There are two NGF receptors referred to as $p75^{NGFR}$ and $p140^{prototrk}$ [21–23]. Both $p75^{NGFR}$ and $p140^{prototrk}$ display nanomolar equilibrium binding constants although $p75^{NGFR}$ has fast on and off, association and dissociation, equilibrium rates and $p140^{prototrk}$ has slow on and off, association and dissociation, equilibrium rates [24]. It would appear that both $p75^{NGFR}$ and $p140^{prototrk}$ are each necessary but not sufficient for NGF action in PC12 cells as both receptors are required [21–23]. Here we restricted our studies to the $p75^{NGFR}$ species henceforth called NGFR. The NGFR distribution in the CNS parallels that of cholinergic innervation. NGFR are present in the hippocampus, frontal cortex, basal forebrain and cerebellum [15, 25]. The finding that the highest levels of NGFR messenger RNA in adult rodents are in the basal forebrain nuclei indicates that NGFR in the CNS are predominantly synthesized in the cell bodies of cholinergic neurons and subsequently anterogradely transported to fiber terminals [26]. There, NGFR bind extracellular NGF which is retrogradely transported to basal forebrain nuclei [27, 28]. This continuous flux of NGF from cholinergic terminals to cell bodies most likely provides trophic support for basal forebrain neurons [6, 29].

Much of our understanding of NGF action and NGFR expression is derived from studies on NGF responsive cell lines such as the rodent adrenal medulla pheochromocytoma cell line PC12 [30, 31]. PC12 cells are similar to undifferentiated sympathetic precursor cells that also do not require NGF for survival when growing in serum containing medium. The PC12 cells display type I and type II NGFR that appear to be identical to the NGFR described for sympathetic and sensory neurons [12]. PC12 cells respond to NGF by extending neurites and differentiating into electrically excitable neuronal cells with some cholinergic properties [32]. Events elicited by NGF in PC12 cells include phosphorylation of several cytoplasmic proteins [33, 34]; stimulation of ornithine decarboxylase messenger RNA tran-

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‖ Abbreviations: NGF, nerve growth factor; PNS, peripheral nervous system; ChAT, choline acetyltransferase; NGFR, p75 nerve growth factor receptor species; ALCAR, acetyl-L-carnitine; PEG, polyethylene glycol; PBS, phosphate-buffered saline; BCA, bicinchoninic acid; SDS, sodium dodecyl sulfate; and MTT, tetrazolium salt.

scription [35]; transcription of some nuclear oncogenes, and stimulation of protein synthesis [36, 37] such as c-*fos* [38, 39]; and protection from peroxidative injury [40]. An obligatory first step to the above is the binding of the NGF to membrane receptors and the subsequent internalization of the NGF–NGFR complex [13]. Thus, changes in NGFR number or NGF affinity for NGFR may be responsible for the fine tuning of NGF action on target tissues.

Acetyl-L-carnitine (ALCAR) is a physiological compound, essential for long chain fatty acid uptake and utilization into mitochondria [41]. Long-term treatment of rodents with ALCAR improves some neurobehavioral impairments associated with aging in the rat [15, 42, 43]. In particular, we have demonstrated that there is a decrease in NGF binding in the CNS of aged rats that can be partially prevented by ALCAR treatment [15]. Decreased levels of NGFR protein and NGFR mRNA in the basal forebrain of aged rats and humans have also been reported [44–47]. These findings would suggest that some degenerative processes of the aged CNS may be associated with reduced activity of central neuronotrophic factors. Thus, the prevention of age-dependent impairments by ALCAR may be due to an enhancement of NGFR expression, and thus NFG action, in the CNS.

The present study was intended to investigate whether there is a direct effect of ALCAR on NGFR expression. We chose the PC12 cell line as a model of NGF responsive neurons.

MATERIALS AND METHODS

Materials. *N'N'*-Methylene-bis-acrylamide, glycine and ammonium persulfate were purchased from Bio-Rad Laboratories; cytochrome *c*, bovine γ-globulins, Nonidet P-40 (NP-40), amberlite GC-400, polyethylene glycol (PEG-8000), lactoperoxidase and H_2O_2 were from the Sigma Chemical Co.; RPMI 1640, fetal bovine serum and horse serum were purchased from Hazelton; ampholines, pH range 3.5 to 10.0, were obtained from LKB Produkter AB, and ultrapure urea was from Mallinckrodt Chemicals; carrier-free Na^{125} I was from Amersham and [α^{32}P]-dCTP from ICN; CM-Sepharose resin was obtained from Pharmacia Fine Chemicals; ALCAR was supplied by the Sigma Tau Co., Italy. The monoclonal antibody 192-IgG was donated by Dr. E. M. Johnson Jr., Washington University, St. Louis, MO; and the cDNA plasmid for the NGFR mRNA was provided by Dr. E. M. Shooter, Stanford University, Stanford, CA. All other chemicals used were either molecular biology or reagent grade.

NGF isolation. The β-NGF subunit was isolated from mouse submaxillary gland according to the method of Mobley *et al.* [48]. After elution from an ion exchange column, the NGF was dialyzed overnight against 0.1% acetic acid. The dialysate was then concentrated by speed vacuum centrifugation to 1 mg/mL, and the final concentration of β-NGF was determined spectrophotometrically. The purity of β-NGF was assayed by isoelectric focusing on polyacrylamide disc gel [49] and specific biological activity was determined by the method developed by Greene [50].

NGF iodination. The iodination of NGF was attained using a modified lactoperoxidase technique, as described elsewhere [13]. Specific activity of the ^{125}I-β-NGF was 2000–3500 cpm/fmol with 85–90cpc of the counts being acid-precipitable. The iodinated NGF was then stored at 4° in phosphate-buffered saline (PBS), pH 7.4, containing 2 mg/mL cytochrome *c* and used within 2 weeks.

Cell culture. Rat pheochromocytoma (PC12) cells were provided by Dr. Lloyd Greene, Columbia University, New York, NY. Cells were grown in RPMI 1640, supplemented with 5% heat-inactivated horse serum + 5% heat-inactivated fetal bovine serum, at 37° in a humidified incubator with 5% CO_2 atmosphere and fed on alternate days. At subconfluency, cells were dislodged by vigorous shaking and reseeded at a 1:1 ratio. ALCAR was dissolved in RPMI and added to cells at the final concentrations indicated in the various experiments.

Serum deprivation. PC12 cells were plated out in 24-well plates at a density of 7.5×10^4 cells/mL/well. ALCAR or carnitine was added to the culture medium at a final concentration of either 0.1 or 1 mM. Cells were then cultured for 5 days, and the culture medium was replaced every 24 hr with fresh medium containing either ALCAR or carnitine. On day 5, the medium was replaced with serum-free RPMI 1640 and NGF was added at a final concentration of 1 ng/mL. After 48 hr the cell survival was assayed by a colorimetric assay involving the reduction by viable cells of the tetrazolium salt (MTT) dye according to the method of Hansen *et al.* [51]. The intensity of color development (viable cells) was assessed spectrophotometrically at 570 nm.

Receptor binding assay. After 6 days of ALCAR treatment, PC12 cells were scraped out from culture flasks, centrifuged for 10 min at 800 *g*, and washed twice with PBS. After the second wash, cells were resuspended in PBS containing 0.5% NP-40 and solubilized for 4 hr at 4°. The solubilized cells were then centrifuged for 20 min at 2500 rpm to remove nuclei and cell debris, and the supernatant (soluble receptors) was stored at −80°. An aliquot of each sample was saved for protein determination using the bicinchoninic acid (BCA) assay [52]. The soluble receptor NGF binding assay, was performed as previously described [53]. Briefly, 100-μL aliquots of sample were incubated with increasing concentrations of ^{125}I-NGF for 2 hr at room temperature. Non-specific binding was assessed by carrying out parallel incubations in presence of a 1000-fold excess of unlabeled NGF. At the end of the incubation, samples were cooled in an ice bath for 5 min, and 0.5 mL of ice-cold 21% PEG in PBS, containing 0.3% (w/v) bovine γ-globulins, was added. Tubes were then centrifuged for 20 min at 2000 *g*, the supernatants were aspirated, and the pellets were washed with ice-cold 8% PEG in PBS and centrifuged again. The supernatants were discarded and the pellets counted for radioactivity in a Beckman Gamma-5000. Specific binding was calculated by substracting non-specific binding from total binding and expressed as femtomoles of NGF bound per

Table 1. ALCAR and carnitine contents of PC12 cells treated for 5 days with 1 mM ALCAR

	Cell pellet	Supernatant
Free carnitine	201.7	0.06
Acetyl-L-carnitine (ALCAR)	42.5	0.94
Acyl-carnitine (3<C<10)	228.0	
Acyl-carnitine (C>10)	07.2	

ALCAR and carnitine contents were determined as described elsewhere [56]. Pellets were washed twice with PBS prior to assay. Values in the cell pellet are expressed in nmol/g wet weight and those in the supernatant in mM.

milligram of soluble proteins. All assays were done in triplicate.

Cross-linking of ^{125}I-*NGF and immunoprecipitation.* At confluency cells were harvested and washed twice with PBS. After the second wash, cells were resuspended into 6 mL of PBS and divided into three 2-mL aliquots. ^{125}I-NGF was then added at a concentration of 5 nM and samples were incubated at 37° for 1 hr. To the third aliquot, a 300-fold excess of cold NGF was also added to assess non-specific binding. After incubation the NGF was covalently bound to the receptor by addition of a 20 mM concentration of the cross-linking agent EDAC [1-ethyl-3-(3-dimethyl-aminopropyl)carbodiimide] and incubated at room temperature for 20 min. Cells were then centrifuged, resuspended in PBS containing 0.5% NP-40 and solubilized overnight at 4°. After centrifugation for 1 hr at 100,000 *g*, supernates were transferred to new tubes and 12 μg/mL of the monoclonal antibody 192-IgG, directed against the rat NGFR, was added. The complex was then precipitated by addition of rabbit/anti-mouse IgG antibody coupled to protein A (Pansorbin), loaded onto a 6% sodium dodecyl sulfate (SDS)–polyacrylamide vertical gel and run for 12 hr at 16 mA current. At completion of the electrophoresis, gel was dried and exposed for 48 hr at −80° to the autoradiographic film.

NGFR mRNA Northern blot analysis. Total RNA was isolated from PC12 cells according to the method developed by Chomczynski and Sacchi [54]. Briefly, cells were lysed directly in denaturing solution (4 M guanidium thiocyanate; 25 mM sodium citrate; 0.5% *n*-lauryl sarcosine; 100 mM β-mercaptoethanol). After addition of 0.1 vol. of 2 M sodium acetate, 1 vol. acid phenol and 0.2 vol. of chloroform : isoamyl alcohol (49 : 1), the samples were centrifuged at 15,000 *g* for 20 min. The supernates were then collected and the RNA was precipitated twice with isopropanol and ethanol. After the second precipitation, the pellets were resuspended in Tris–EDTA buffer and the RNA was quantified spectrophotometrically using the O.D. absorbance reading at 260 nm. Equal amounts of total RNA from each sample were loaded onto a 1.5% agarose gel containing ethidium bromide and run overnight at 24 V. The quality of the RNA was assessed by the sharpness of the bands obtained after electrophoresis when observed under ultraviolet light. The gel was transferred overnight to a nitrocellulose membrane and hybridized with a [^{32}P]-cDNA probe encoding the NGFR mRNA. The membrane was then exposed for 24 hr to the autoradiographic film.

Statistical analysis. Maximal binding capacity (B_{max}) and dissociation constant (K_d) were calculated from equilibrium binding data according to the method by Scatchard [55]. The B_{max} and K_d values whose confidence limits did not overlap were considered statistically different.

RESULTS

ALCAR concentrations used. The concentrations of ALCAR used resulted in ALCAR concentrations in cell pellets (Table 1) that were identical to those present in the brains of aged rats treated for 6 months with ALCAR at a dose of 100 mg/kg/day in drinking water [56].

Effect of ALCAR on cell survival. ALCAR was compared with carnitine, the deacetylated compound that is not effective in the prevention of age-associated deficits, in the NGF-rescue of serum-deprived PC12 cells at concentrations of 0.1 and 1 mM. The results are shown in Table 2. Neither carnitine nor ALCAR treatment by itself provided any significant protection under these conditions. ALCAR significantly augmented NGF protection at a concentration of 1 mM, whereas carnitine had no significant effect on NGF protection.

NGF equilibrium binding studies. When NGF equilibrium binding assays were carried out using ^{125}I-NGF in solubilized PC12 cells and the results analyzed by Scatchard plot analysis, the resulting regression analysis was curvilinear (Fig. 1). This result has typically been interpreted as demonstrating the presence of two distinct binding sites: a high-affinity, low-capacity site with a K_d in the 10^{-10} M range, and a low-affinity, high-capacity site displaying a K_d in the 10^{-9} M range. After PC12 cells were treated for 6 days with ALCAR (10 mM) and equilibrium binding assays were carried out as before, there was an increase of both type I (high affinity) and type II (low affinity) NGF binding sites, as measured by B_{max} determination (Table 3). The very low levels of type I binding activity measured here made it difficult to accurately assess variations in the levels of high-affinity NGF binding sites

Table 2. Effects of ALCAR on PC12 survival*

Treatment	O.D. (570 nm)
Serum (8)	0.756 ± 0.013
Serum-free (SF) (8)	0.307 ± 0.003
SF + NGF (8)	0.403 ± 0.012
SF + CAR 1 mM (4)	0.317 ± 0.016
SF + ALCAR + mM (4)	0.312 ± 0.014
SF + NGF + CAR 0.1 mM (4)	0.409 ± 0.008 (NS)
SF + NGF + CAR 1 mM (4)	0.411 ± 0.020 (NS)
SF + NGF + ALCAR 0.1 mM (4)	0.396 ± 0.022 (NS)
SF + NGF + ALCAR 1 mM (4)	0.496 ± 0.026†

* Survival was measured by MTT reduction in a serum deprivation assay as described in Materials and Methods. CAR refers to carnitine. NGF was added at 1 ng/mL. Values are means ± SEM; the number of replicates is given in parentheses. NS = not significant.

† $P < 0.05$ vs SF + NGF group (ANOVA).

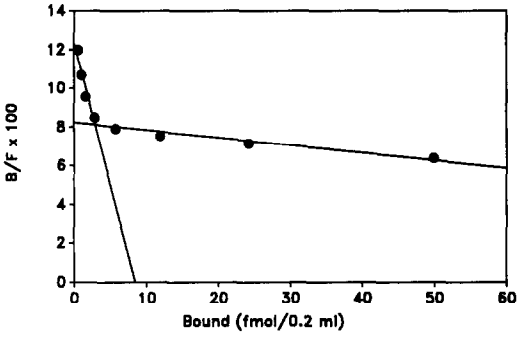

Fig. 1. ^{125}I-β-NGF binding to soluble receptors from PC12 cells. A high-affinity, low-capacity site with a K_d of about 10^{-10} M and a low-affinity, high-capacity site with a K_d of about 10^{-9} M are shown.

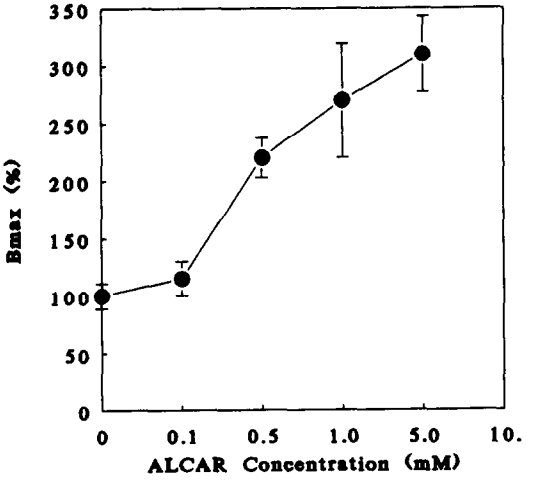

Fig. 2. Percent B_{max} values for NGF binding in PC12 cells treated for 6 days with ALCAR at various concentrations. See Materials and Methods for details.

following treatment with ALCAR. Thus, we restricted our measurements to the determination of total B_{max} in all subsequent experiments. This approach is in part justified since type I and type II NGFR are functionally and quantitatively interrelated [24, 57].

As shown in Fig. 2, ALCAR increase of NGF specific binding activity was evident at low concentrations (0.5 to 5.0 mM). After PC12 cells were treated with 0.5 mM ALCAR for 6 days, the calculated B_{max} (fmol/mg protein) for NGF binding was more than twice that of the untreated cells. There was no enhancement of NGF binding to PC12 cells treated with ALCAR at 0.1 mM, in contrast to the effect at a concentration of 1.0 mM ALCAR.

Table 3. ^{125}I-NGF binding in PC12 cells treated for 6 days with 10 mM ALCAR

	B_{max1} (fmol/mg protein)	K_{d1} (nM)	r^1	B_{max2} (fmol/mg protein)	K_{d2} (nM)	r^2
Control	80	0.33	−0.983	225	1.96	−0.999
ALCAR	113	0.53	−0.886	356	2.32	−0.992

The headings r^1 and r^2 are for the correlation coefficient for type I and type II equilibrium binding constants, respectively.

Table 4. K_d values obtained by NGF equilibrium binding of ^{125}I-NGF

	K_d (conf. limits) (nM)
Experiment 1	
Control	0.69 (0.38–0.99)
ALCAR 0.1 mM	0.47 (0.27–1.53)
ALCAR 1 mM	1.68 (0.65–2.70)
ALCAR 10 nM	1.49 (0.43–2.56)
Experiment 2	
Control	3.1 (2.1–4.2)
ALCAR 0.5 mM	3.9 (2.9–4.9)
ALCAR 1 mM	2.9 (1.3–4.5)
ALCAR 5 mM	2.2 (1.4–3.1)

Thus, we observed a concentration-curve of changes in NGF binding in response to various ALCAR concentrations (Fig. 2). This stimulation was half-maximal between 0.1 and 0.5 mM ALCAR. No K_d values calculated from equilibrium binding data were changed significantly after ALCAR treatment in any of the binding experiments (Table 4).

Immunoprecipitation of the NGFR. Immunoprecipitation of NGFR cross-linked to ^{125}I-NGF using the monoclonal antibody 192-IgG was performed on PC12 cells that had been treated for 6 days with ALCAR (10 mM). The experiment was done to test the hypothesis that the increase in B_{max} obtained in the equilibrium NGF binding assays in response to ALCAR treatment in PC12 cells was due to increased levels of NGFR protein. As shown in Fig. 3, electrophoresis of the immunoprecipitated NGF–NGFR complex on SDS–polyacrylamide gels revealed a 49% increase in intensity of the radioactive band associated with a 92.2 kDa NGF–NGFR complex and a 91% intensity increase of the band associated with a 170.9 kDa NGF–NGFR complex, as measured by computerized image analysis of the autoradiographic film. The low molecular weight protein band corresponds to the low-affinity NGFR while the high molecular weight band has been reported to be associated with the high-affinity NGFR, probably being derived from the association of two low-affinity NGFR molecules or of a 60 kDa receptor-associated protein with the low-affinity NGF binding site [49, 53]. Since the molecular weights reported here are for the NGF–NGFR complex, if we subtract the molecular weight of the NGF covalently bound to the receptor, we would report NGFR molecular species of about 79 and 158 kDa, respectively. The presence of a 300-fold excess of unlabeled NGF in the cross-linking mixture abolished most of the radioactivity associated with the 92.2 and 170.9 kDa bands, demonstrating the

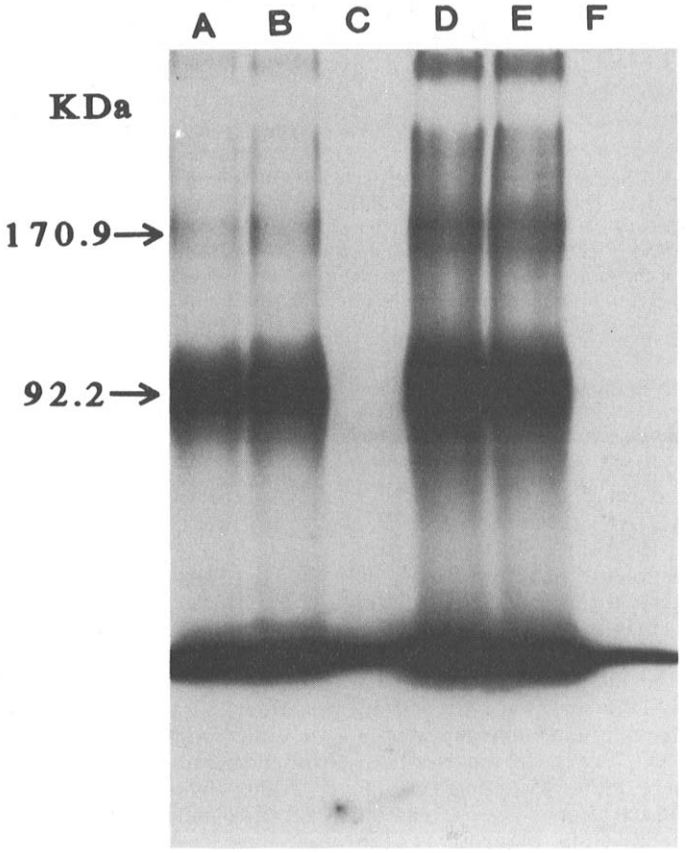

Fig. 3. Autoradiograph of ^{125}I-NGF cross-linked NGFR immunoprecipitated with the Mab 192-IgG from PC12 cells treated for 6 days with ALCAR (10 mM). Lanes A and B: control cells; lane C: control cells + 300-fold excess of unlabeled NFG; lanes D and E: ALCAR-treated cells; lane F: ALCAR-treated cells + 300-fold excess of unlabeled NGF.

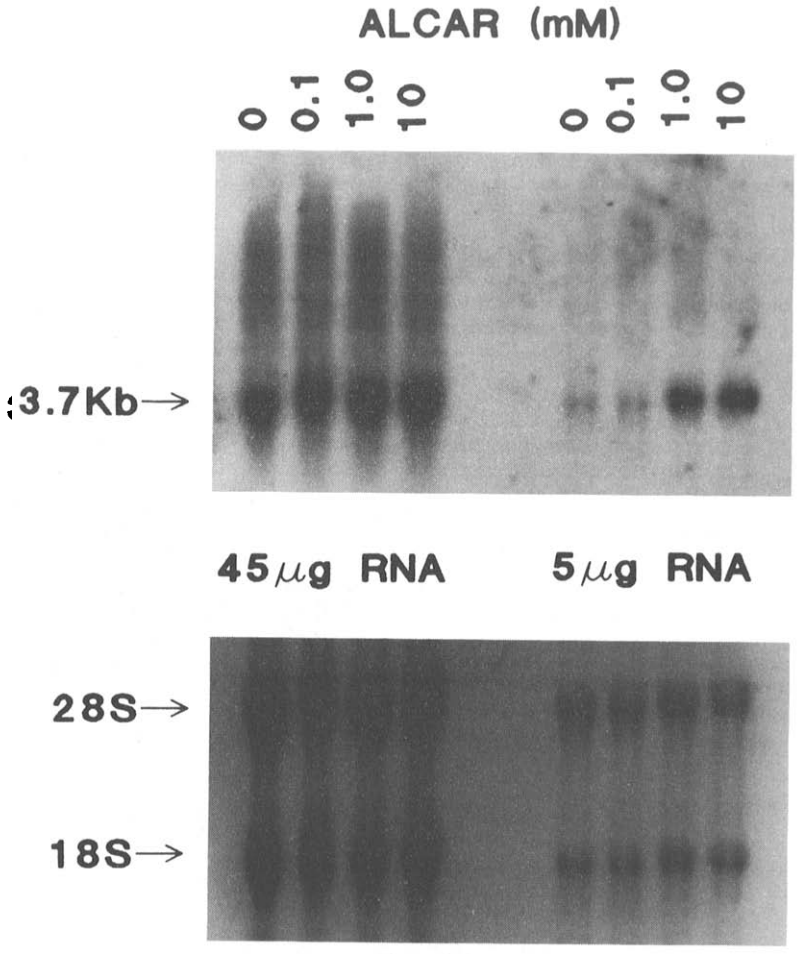

Fig. 4. Northern blot analysis of total RNA isolated from PC12 cells treated with different concentrations of ALCAR for 6 days. Forty-five (left side) and five (right side) micrograms of total RNA from each sample were added to lanes as shown. Upper panel: autoradiograph of the nitrocellulose membrane after blotting with a [^{32}P]cDNA probe encoding for the NGFR mRNA. Lower panel: photograph under ultraviolet light of the same gel prior to transferring to the nitrocellulose membrane. The same amount of 18S and 28S RNA in each sample is shown.

specificity of ^{125}I-NGF binding to both NGFR species (Fig. 3 lanes C and F).

NGFR mRNA Northern blot analysis. Northern blot assay of NGFR mRNA extracted from PC12 cells that were treated for 6 days with different concentrations of ALCAR revealed a 56–71% increase in NGFR mRNA in cells treated with ALCAR at concentrations of 0.5 to 1.0 mM, as measured by computerized image analysis of the autoradiogram (Fig. 4). It is interesting to notice that the concentrations of ALCAR that induced NGFR mRNA are in the same range as the concentrations of ALCAR that were needed to increase the ^{125}I-NGF equilibrium binding to soluble NGFR from treated PC12 cells. In the Northern blot analysis shown here, either 45 or 5 μg of total RNA was added from each sample. The increase in NGFR mRNA was more easily observed for the lanes containing 5 μg of RNA (right side) as compared to the ones containing 45 μg of RNA (left side) because in the latter the autoradiographic film was overexposed, due to the amount of hybridization with the labeled probe. The bottom panel of Fig. 4 is a photograph of the agarose gel, taken under ultraviolet light before transfer to the nitrocellulose membrane, to show that equal amounts of total RNA were actually applied to the gel.

DISCUSSION

The values of ALCAR present in cell pellets reflect intracellular values and are similar to those detected in rodent brain after exogenous ALCAR treatment in drinking water. Whether intracellular distribution is the same in these two instances cannot be directly determined by these techniques and is not addressed here.

Although neither carnitine nor ALCAR treatment had any significant effect on PC12 cell survival under serumless conditions, there was a dramatic effect on PC12 cell survival in the presence of suboptimal concentrations of NGF (1 ng/mL). From a strictly mass law action rationale, one can explain this potentiation of the effect of NGF on survival if

ALCAR increases NGF or NGFR levels. Since NGF levels here were controlled exogenously, one partial explanation for the results observed would be an increase in NGFR expression.

The increase in NGF equilibrium binding values (B_{max}) displayed by ALCAR-treated PC12 cells, without any detectable changes in K_d, is consistent with there being an increase in the apparent number of NGFR sites. The SDS–polyacrylamide gel analysis of immunoprecipitated ^{125}I-NGF cross-linked to NGFR clearly showed that there was an increase in NGFR proteins following ALCAR treatment in agreement with the results obtained by equilibrium binding of ^{125}I-NGF. These binding assays also showed that there was an increase of the type I (high-affinity) NGFR, although the measurements of type I binding were seldom consistent. In the immunoprecipitation experiments reported here, we also observed that after ALCAR treatment there was an increase in a radioactive band of about 170 kDa that is likely to be associated with high-affinity binding of NGF [49, 58].

There is evidence that ALCAR treatment reduces the membrane cholesterol content in PC12 cells.* Because decreased cholesterol levels in the cell membrane are associated with increased membrane fluidity [59], decreased levels of cholesterol after ALCAR treatment may facilitate NGFR entrance into the membrane and its displacement there. This phenomenon would result in an increased apparent NGFR number on the cell surface without concomitant increase in NGFR protein or mRNA. However, the observed increase in NGFR protein and mRNA for PC12 cells that have been treated with ALCAR would suggest that ALCAR acts at a site other than the plasma membrane. The increase in NGFR number at the plasma membrane is likely to be due to stimulation of NGFR mRNA levels as opposed to specific modifications in the physico-chemical state of the cell membrane. However, the two phenomena are not mutually exclusive. There is support for this hypothesis in that the same minimal concentrations of ALCAR that are needed to significantly increase NGF binding and NGFR mRNA levels are those required for effects of ALCAR on NGF rescue from "serumless death". Presently we have no evidence for a direct action of ALCAR on the PC12 genome. ALCAR treatment may stabilize NGFR mRNA molecules, thus increasing levels of NGFR mRNAs and proteins.

In vivo, long-term ALCAR treatment has been shown to prevent several behavioral impairments in the aged rat [43]. In the hippocampus of aged rats, ALCAR rescues pyramidal neurons of Ammon's horn and granular cells in the dentate gyrus, where it abolishes the aged-associated loss of glucocorticoid receptors [60]. Similarly, ALCAR retards aged-associated losses of NGFR in the hippocampus and basal forebrain [15]. Since NGF provides trophic support to basal forebrain cholinergic neurons by the continuous influx of NGF–NGFR complexes from hippocampus and NGF infusion rescues septal cholinergic neurons of aged rats and improves their behavioral performances in an spatial orientation task [61, 62], the enhanced NGFR expression and possible improved retrograde transport of NGF after ALCAR treatment may explain some of the reported effects of ALCAR on the aged rodent CNS. Since one component measured here, p75^{NGFR}, is a common receptor to all neurotrophins, these results may be applicable to other members of the neurotrophin family such as BDNF and NT-3 and hence may bear relevance to a broader category of degenerative events in the CNS. Taken together, these results would suggest that some degenerative processes during CNS aging may be a consequence of a reduced expression of neurotrophin activity.

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* Werrbach-Perez *et al.*, manuscript in preparation.

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