NERVE GROWTH FACTOR INCREASES GLUTATHIONE S-TRANSFERASE ACTIVITY IN FIMBRIA FORNIX LESIONED RATS

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RESUMEN. Doce ratas Sprague-Dawley fueron sujetas a la lesión de la fimbria fornix (transección de la vía septohipocampal). Seis de estos animales recibieron inyecciones intraventriculares de 3 µg de factor de crecimiento nervioso (FCN) en días alternos y durante dos semanas, mientras que los otros seis recibieron citocromo c en igual esquema y dosis (controles). La actividad específica de la glutatión S-transferasa (GST) se midió en el septum, el hipocampo, el estriado y la corteza frontal. Los animales tratados con FCN mostraron un incremento significativo ($p < 0,002$) de la actividad de la GST en el septum. No hubo diferencias entre los grupos en las otras estructuras estudiadas. Este incremento en la actividad de la GST puede estar vinculado con el efecto protector del FCN sobre la población colinérgica septal.

ABSTRACT. Bilateral fimbria fornix lesion (septohippocampal pathway transection) was performed on 12 male Sprague -Dawley rats. Six animals received intraventricular injections of 3 µgo f nerve growth factor (NGF) on alternate days for two weeks. Six animals received the same amount of cytochrome c (controls). The glutathione S-transferase (GST) specific activity was measured in septum, hippocampus, striatum and frontal cortex. A significant increase of GST activity ($p < 0.002$) was found in the septum of the NGF-treated animals. No differences were found between groups in the other areas. This increase in GST activity might be related to the protecting effect of NGF on the cholinergic septal population.

INTRODUCTION

Glutathione S-transferase (GST) (E.C. 2.5.1.18) includes various isoenzymes which participate in detoxification events of a wide variety of compounds.[1] Multiple isoforms of GST have been reported in almost all the rat tissues investigated so far.[2] Presence of substantial amounts of GST in the brain may indicate a physiological role for this enzyme in neuroprotective mechanisms.[3-5]

Previous work confirmed the presence of GST activity in several areas of normal rat brains. The highest levels were measured in the septum and the hippocampus. A dramatic decrease of GST activity in those areas was detected in aged brains.[6] In young animals after fimbria fornix (FF) lesion (transection of the septohippocampal pathway) the distribution pattern of GST activity showed a reduction of 41 % in the septum.[7] Both, aged and FF-lesioned animals have showed a reduced number of cholinergic septal neurons.[8,9] These evidences suggest that the level of GST activity might be related to the protection of that neuronal population.

Nerve growth factor (NGF) has been shown to promote the survival of cholinergic neurons of septal origin in aged[8] and FF-lesioned animals,[9,10] but the mechanisms of this action are still unknown.

The aim of this study was to determine whether the treatment with NGF to FF-lesioned rats is able to produce changes in the brain GST activity.

MATERIALS AND METHODS

Twelve Sprague-Dawley male rats weighing 270-290 g at the time of surgery were used. Under chloral hydrate narcose (400 mg/kg i.p.) a bilateral aspirative lesion of the FF fiber system was performed. A guide cannula was implanted 1.0mm anterior to bregma, 0.9 mm lateral to the midline and 2.0 mm under the bone surface, in the direction of the right lateral ventricle. Murine NGF (3 µg) dissolved in 10 µL saline were intraventricularly injected through an injection cannula at the time of surgery and from then on, the same amount was injected every second day for two weeks. The injection rate was 1 mL/min; the cannula was left in position for 2 min after finishing the injection. Six animals treated in this way formed the NGF-treated group. A similar group of six rats was lesioned and injected in the same way, but using cytochrome instead of NGF to constitute a control group. Cytochrome c is a protein with a similar molecular weigh but without neurotrophic activity. NGF biological activity was confirmed by the classical assay using sympathetic ganglia from chicken embryos.[11]

The day following the last injection rats were decapitated and their brains extracted. The septum, hippocampus, striatum and frontal cortex were dissected in cold saline, and stored at –70 °C.

The tissue samples of each cerebral area were individually homogenized using a glass-teflon homogenizer and centrifuged at 16 000 g for 30 min at 4 °C . The supernatant was assayed for soluble proteins according to the method of Bradford.[12] GST activity was determined by coupling chloro-2,4-dinitrobenzene (CDNB) to reduced glutathione (GSH). Reaction progress was followed by recording the absorbance increase at 340n m . One unit of GST activity was the quantity of enzyme required to catalyze the conjugation of 1 mol/min of CDNB to GSH usinge=96 00 $L \cdot mol^{-1} \cdot cm^{-1}$ and the conditions describedby Habig.[13] Assays were carried out in triplicate.

All values were expressed as the mean ± S.E.M. Differences between means were analyzed by the Mann-Whitney's test.

RESULTS AND DISCUSSION

The GST activity showed a difference between the animal groups studied. The GST activity in septum increased significantly ($p < 0.002$) in those animals treated with NGF after FF lesion when compared with cytochrome c-treated ani-

mals.The differences between groups were not significant for striatum, hippocampus and frontal cortex (Table I).

TABLE I
Glutathione S-transferase specific activity in the septum, hippocampus, striatum and frontal cortex from fimbria fornix lesioned rats treated with cytochrome c

Brain area	Cytochrome c	NGF
Septum	78.60 ± 3.25	99.23 ± 2.66*
Hippocampu	128.99 ± 11.24	109.40 ± 10.45
Striatum	112.35 ± 4.38	113.83 ± 5.91
Frontal Cortex	86.99 ± 4.30	94.16 ± 7.31

* Significantly different from cytochrome c-treated group ($p < 0.002$). ($n = 6$) or NGF ($n = 6$). NGF Nerve growth factor.
Values are expressed in nmol · min^{-1} · mg of $protein^{-1}$ as mean S.E.M.

The fact that only the GST in the septum showed an effect after NGF treatment should not be ascribed to the vicinity of this area to the ventricular system. Other structures investigated in this report as the hippocampus or the striatum are also near the ventricles and no such differences were noticed. Using radiolabeled NGF, Yan *et al.*[14] have shown that the intraventricularly injected neurotrophic substances are readily distributed across the brain tissue, though it finally accumulates in the septum due to the high concentration of NGF-receptors in this area. Therefore, the effect reported in this paper seems to be selective for this brain area.

It has been suggested that NGF increases cell survival by enhancing cellular defense systems, which would include antioxidant enzymes and low molecular weight scavengers. For example, NGF has been reported to rescue PC 12 cells from hydrogen peroxide attack[15] and to stimulate glutahione peroxidase, glucose-6-phosphate-dehydrogenase and gamma-glutamylcysteine synthetase activities in this cellular line.[16] Besides, this neurotrophin restores decreases in catalase activity and stimulates glutathione peroxidase activity in the aged rodent brain, [17] while induces catalase expression in young rat striatum.[18] An effect of NGF on GST activity has not been previously reported.

An early common event in many growth factor ligand-receptor interactions, including NGF ones,[19] is the induction of c-*fos,* a member of the heterodimeric protein complex AP-1. A regulatory AP-1-like binding site located upstream a GST gene has been demonstrated to be involved in the induction mechanisms of the enzyme.[20] These facts suggest that the stimulation of GST activity by NGF could be mediated by the induction of the transcription factor AP-1.

As previously mentioned, NGF shows a neuroprotective action on the septal cholinergic neurons, which abundantly express NGF receptors.[9,10,14] The specific and highly significant effect of NGF on the GST activity in septum should not be a consequence of an increased survival of the septal cholinergic population, since GST is mainly expressed by glial cells.[5] On the contrary, an increase in GST activity by NGF could be important for the protection of those neurons. A neuron-glia-neuron interaction might be considered.

A neuroprotective action by NGF has also been reported in aged, memory deficient animals,[9] but there are no indications of what the mechanisms of this action might be. In a previous report a significant decrease in GST activity in septa obtained from aged rats was described.[6] It is tempting to speculate that the loss of cholinergic septal neurons might be influenced by the reduced GST activity. Future studies on the effect of NGF on GST activity in naive animals will further confirmed this hypothesis.

CONCLUSION

The effect of NGF increasing the GSTacitivity in septum seems to be plausibly linked to its neuroprotective action on the same brain area.

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