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BEHAVIORAL AND IMMUNOHISTOLOGICAL EFFECTS OF CHOLINERGIC DAMAGE IN IMMUNOLESIONED RATS: ALTERATION OF c-Fos AND POLYSIALYLATED NEURAL CELL ADHESION MOLECULE EXPRESSION

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Abstract—The aim of this study was to determine the brain structures as well as the plasticity events associated with the behavioral effects of cholinergic damage. Rats were submitted to injection of 192 IgG-saporin in the medial septum/diagonal band of Broca complex and the nucleus basalis magnocellularis. The immunohistochemical expression of c-Fos protein and PSA-NCAM (polysialylated neural cell adhesion molecule) and the behavioral performances in the non-matching-to-position task were assessed at various post-lesion times. Thus, 3 days after injection of the immunotoxin, increased c-Fos labeling was observed in the areas of infusion, indicating these cells were undergoing some plastic changes and/or apoptotic processes. A drastic increase was observed in the number of PSA-NCAM positive cells and in their dendritic arborization in the dentate gyrus. At 7 days post-lesion, no behavioral deficit was observed in immunolesioned rats despite the drastic loss of cholinergic neurons. These neurons showed decreased c-Fos protein expression in the piriform and entorhinal cortex and in the dentate gyrus. In the latter, PSA-NCAM induction was high, suggesting that remodeling occurred, which in turn might contribute to sustaining some mnemonic function in immunolesioned rats. At 1 month, cholinergic neurons totally disappeared and behavioral deficits were drastic. c-Fos expression showed no change. In contrast, the increased PSA-NCAM-labeling observed at short post-lesion times was maintained but the plastic changes due to this molecule could not compensate the behavioral deficit caused by the immunotoxin. Thus, as the post-lesion time increases, a gradual degeneration process should occur that may contribute to mnemonic impairments. This neuronal loss leads to molecular and cellular alterations, which in turn may aggravate cognitive deficits.

Key words: 192 IgG-saporin, dentate gyrus, T-maze, rat.

The cholinergic basal forebrain in the rat includes cells in the nucleus basalis magnocellularis (NBM), the medial septum (MS), and the diagonal band of Broca (DBB). Cholinergic cells within this system project to the entire rat cortex and hippocampus (Fibiger, 1982). There is a large body of data indicating that loss of cholinergic input to the hippocampus and cortex is one of the major neuropathological components of the cognitive deficits characteristic of Alzheimer's disease (Whitehouse et al., 1982; Gaykema et al., 1992; Rossner et al., 1997; Wrenn and Wiley, 1998). In animals, the involvement of the cholinergic basal forebrain neurons in cognitive functions has been studied using various experimental paradigms, including fimbria-fornix transections and injections of various toxins into the basal forebrain nuclei, their target areas, or the cerebral ventricles (Baxter, 2001; Burk and Sarter, 2001; Waite et al., 1994; Walsh and Opello, 1992; Parent and Baxter, 2004; van der Staay et al., 2006). One of the drawbacks of many of these lesion paradigms is their lack of specificity. The introduction of the immunotoxin 192 IgG-saporin appears to allow for more selective cholinergic lesioning. 192 IgG-saporin is a monoclonal antibody to the rat low-affinity neurotrophin receptor, p75, located on cholinergic nerve terminals in the cortex and hippocampus and on cholinergic cell bodies in the basal forebrain, i.e. the NBM and the MS/DBB (Cuello et al., 1990). When this antibody is coupled with the ribosomal-inactivating protein, saporin, it is able to successfully destroy cholinergic neurons by inhibiting protein synthesis (Wiley, 1992; Wenk et al., 1994). At 3–6 months after intraparenchymal injection of a low dose, 192 IgG-saporin selectively produces lesions of the cholinergic basal forebrain with no specific loss of parvalbumin-, neuropeptide Y-, NADPH-diaphorase, or glutamate decarboxylase–immunoreactive neurons (Baxter et al., 1995; Heckers et al., 1994; Berger-Sweeney et al., 1994; Torres et al., 1994; Wenk et al., 1994; Johnson et al., 2002). No changes in the levels of monoamines and their metabolites in basal forebrain cholinergic projections have been detected after 192 IgG-saporin lesions up to 3 months post-lesion (Walsh et al., 1996; Pizzo et al., 1999). Thus, the immunotoxin is currently considered a valid tool for selectively eliminating cholinergic pathways (McGaughy et al., 2000; Wrenn and Wiley, 1998; Hartonian and de Lacalle, 2005; Pizzo et al., 1999; Perry et al., 2001). Once the cholinergic system is affected, one may assume that as post-lesion time progresses, this cholinergic degeneration induces side effects through trans-synaptic action on other cellular systems. The functional consequences of 192 IgG-saporin-induced lesions have been

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Abbreviations: BDNF, brain-derived nerve factor; ChAT, choline acetyltransferase; DBB, diagonal band of Broca; HBB, horizontal band of Broca; MS, medial septum; NBM, nucleus basalis magnocellularis; NCAM, neural cell adhesion molecule; NGF, nerve growth factor; PBS, phosphate-buffered solution; PSA-NCAM, polysialylated neural cell adhesion molecule; TBS, Tris-buffered saline; VBB, vertical band of Broca.

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widely studied over the last decade and have been the topic of a number of useful reviews (McGaughy et al., 2000; Rossner et al., 1997; Wenk et al., 1997; Wrenn and Wiley, 1998). We have previously shown that the behavioral effects of 192 IgG-saporin-induced cholinergic lesion depended on the time at which testing is conducted after surgery (Paban et al., 2005a). In particular, we demonstrated that no deficit was observed at short post-lesion times whereas rats showed memory impairments at longer post-lesion times.

Although the behavioral effects of cholinergic immunolesion are well depicted, data on brain structures involved have not been fully explored, nor have the molecular events underlying cholinergic insult. In particular, it seems of great interest to study the effects associated with the cholinergic insult per se but also those associated with the trans-synaptic degeneration. Hence, we studied the alteration of c-Fos and PSA-NCAM expression at various times following injection of the 192 IgG-saporin. We used the c-Fos protein firstly as a mapping tool and secondly as molecule involved in cellular and synaptic plasticity processes. Indeed, c-Fos can be detected by immunocytochemistry and is widely used as a spatially and temporally specific indicator of neuronal activation (Morgan and Curran, 1989; Curran and Morgan, 1987; Blandini et al., 2006). c-*fos* Is an immediate early gene that is induced transiently in the CNS by a variety of stimuli (Buytaert et al., 2001; Yasoshima et al., 2006; Teather et al., 2005; Aggleton and Brown, 2005; Zimmer et al., 1998; Richardson et al., 1992) and that controls the expression of late response genes such as nerve growth factor (NGF), fibroblast growth factor (FGF), or brain-derived nerve factor (BDNF). It has been suggested that c-*fos* gene and/or c-Fos protein expression may be important in neurodegenerations and cell death, as well as in plasticity and repair (see review Raivich and Behrens, 2006). Interestingly, for the hippocampus of patients with Alzheimer's disease, Zhang et al. (1992) and Marcus et al. (1998) reported an overexpression of c-Fos protein. This overexpression has been associated with stimulation of the genetic program of apoptosis. After cholinergic injury in rodents, expression of c-Fos protein or c-*fos* mRNA is increased upon infusion of toxic and subtoxic doses of excitotoxins (Page et al., 1993) or 192 IgG-saporin immunotoxin (Rossner et al., 1994). In the present study, we conducted a detailed light microscopic expression analysis of the immunochemistry of the c-Fos protein after intraparenchymal injection of 192 IgG-saporin.

To better characterize the cellular and synaptic plasticity events that occurred after cholinergic immunolesioning, we also examined alterations of the expression of the highly polysialylated neural cell adhesion molecule (PSA-NCAM). Neural cell adhesion molecule (NCAM) is a cell surface glycoprotein that is involved in neuronal migration and neurite outgrowth in the CNS (Seki and Arai, 1999). Alpha-2,8-link polysialic acid (PSA) is almost exclusively carried by NCAM (Zuber et al., 1992). PSA-NCAM expression is increased in the dentate gyrus by ischemia (Sato et al., 2001; Macas et al., 2006), brain injury (Zhang et al., 2006; Liu et al., 2006), and enriched environment (Kempermann et al., 2002). Interestingly, Mikkonen et al. (1999) and Jin et al. (2004) found increased PSA-NCAM expression in the hippocampus of Alzheimer's disease patients. PSA-NCAM is expressed in newly generated neurons as well as in neurons undergoing plastic change (Seki and Arai, 1993, 1999; Fox et al., 2001; Seki, 2002; Emery et al., 2005).

In the present study, rats were behaviorally tested in a nonmatching-to-position T-maze task, which depends on the integrity of the hippocampus (Lalonde, 2002), at various post-lesion times. Indeed, it seems of particular interest to compare an experimental condition in which no behavioral effect was observed to one in which a behavioral deficit appeared. This type of study should give more insight into the anatomical and cellular substrates involved in the behavioral deficit induced by cholinergic immunolesioning. Thus, behavioral performances were measured at 7 days and 1 month post-lesion, i.e. at times we previously observed no effect and behavioral deficit respectively (Paban et al., 2005a). At the end of testing, the expression of c-Fos protein was analyzed in different brain regions, i.e. in the immunotoxin injection areas as well as in the main projection regions of the basal forebrain cholinergic system. PSA-NCAM induction was studied in the dentate gyrus. The choice of this brain region was determined by our previous results on the immunohistochemistry of c-Fos protein, by the involvement of this region in hippocampus-dependent tasks, and by the sensitivity of this region to the expression of PSA-NCAM.

EXPERIMENTAL PROCEDURES

Subjects and behavioral testing

Male Wistar rats (Charles River, L'Arbresle, France) were used in the experiments. Animals were housed in standard conditions, i.e. in groups of two to three rats per cage under 12-h light/dark conditions with *ad libitum* access to food and water, except when food-deprived during behavioral training. Every effort was made to minimize animal suffering and to reduce the number of rats used. All experiments were carried out in accordance with the UK Animals (Scientific Procedure) Act 1986 and associated guidelines, the European Communities Council Directive of 24 November 1986 (86/609/EEC) and the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication 8023, revised 1978).

Forty-seven rats were used. Rats were 3 months old at the time of the surgery. They were housed in a standard environment during all the testing period. Six groups of rats were constituted. Two groups of rats were killed 3 days after surgery: groups 3-days. One group was injected with 192 IgG-saporin ($N=4$, 192-IgG SAP) and the other one with phosphate-buffered solution ($N=4$, PBS). They did not perform any behavioral test and were considered the short post-lesion times. Two groups were tested behaviorally 7 days after surgery: groups 7-days ($N=10$, 192-IgG SAP; $N=9$, PBS). Two groups were tested behaviorally 1 month after surgery: groups 1-month ($N=10$, 192-IgG SAP; $N=10$, PBS). At the end of the behavioral test, all rats were killed. Considering the three post-lesion times, this study included the range of times during which sprouting has been observed in the hippocampus.

Cholinergic lesion surgery

Animals were anesthetized with an i.m. injection of a solution of ketamine (Imalgen 500, 62.5 mg/kg), xylazine (Rompun, 3.17 mg/