available at www.sciencedirect.com

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Selective septohippocampal – but not forebrain amygdalar – cholinergic dysfunction in diencephalic amnesia

Lisa M. Savage[a,*], *Jessica Roland*[a], *Anna Klintsova*[b]

[a]*Behavioral Neuroscience Program, Department of Psychology, Binghamton University, State University of New York, Binghamton, NY 13902, USA*

[b]*Behavioral Neuroscience Program, Department of Psychology, University of Delaware, Newark, DE 19716, USA*

ARTICLE INFO

Article history:
Accepted 22 December 2006
Available online 8 January 2007

Keywords:
Diencephalon
Hippocampus
Amygdala
Microdialysis
Acetylcholine
Stereology

ABSTRACT

A rodent model of diencephalic amnesia, pyrithiamine-induced thiamine deficiency (PTD), was used to investigate diencephalic–limbic interactions. In-vivo acetylcholine (ACh) efflux, a marker of memory-related activation, was measured in the hippocampus and the amygdala of PTD-treated and pair-fed (PF) control rats while they were tested on a spontaneous alternation task. During behavioral testing, all animals displayed increases in ACh efflux in both the hippocampus and amygdala. However, during spontaneous alternation testing ACh efflux in the hippocampus and the alternation scores were higher in PF rats relative to PTD-treated rats. In contrast, ACh efflux in the amygdala was not suppressed in PTD treated rats, relative to PF rats, prior to or during behavioral testing. In addition, unbiased stereological estimates of the number of choline acetyltransferase (ChAT) immunopositive neurons in the medial septal/diagonal band (MS/DB) and nucleus basalis of Meynert (NBM) also reveal a selective cholinergic dysfunction: In PTD-treated rats a significant loss of ChAT-immunopositive cells was found only in the MS/DB, but not in the NBM. Significantly, these results demonstrate that thiamine deficiency causes selective cholinergic dysfunction in the septo-hippocampal pathway.

Diencephalic lesions are seen in a range of disorders: malnourishment, ischemic infarcts, viral infections, tumors and traumatic damage. Damage to certain nuclei and fiber systems within the diencephalon interrupts the flow of information between key memory structures and thus causes severe and long-lasting amnesia (Langlais and Savage, 1995; Mair, 1994; Reed et al., 2003). Although there is evidence that lesions to particular diencephalic nuclei result in memory impairment in their own right, there is also evidence that damage to diencephalic nuclei can disrupt memory circuits leading to dysfunction in other regions of the brain (Bentivoglio et al., 1997; Mair, 1994; Reed et al., 2003; Savage et al., 2003; Vann and Aggleton, 2003).

In cases of diencephalic amnesia associated with thiamine deficiency (i.e., Wernicke–Korsakoff Syndrome [WKS]) there is neuronal loss and lesions in multiple limbic thalamic nuclei, mammillary bodies (Langlais and Savage, 1995; Langlais et al., 1996; Mair et al., 1988; Mair, 1994), as well as degeneration in key fiber tracts connecting limbic structures (fornix and mammillothalamic tract; Langlais and Zhang, 1993; Langlais and Zhang, 1997). The damage to both key diencephalic nuclei and fiber tracts likely results in a "disconnection syndrome"

* *Corresponding author*. Fax: +1 607 777 4890.
E-mail address: lsavage@binghamton.edu (L.M. Savage).

0006-8993/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2006.12.083

within the limbic system (see Jenkins et al., 2002; Markowitsch and Pritzel, 1985; Warrington and Weiskrantz, 1982).

At one point, WKS patients were labeled as "temporal lobe-related amnesiacs" based on their memory performance (Cohen and Squire, 1980). This clinical population was critical for the development of the memory system classifications (i.e., declarative vs. nondeclarative memory (Squire, 1982)). However, it is clear from neuroimaging studies that the most consistent neuropathology of WKS is damage to the cortex and the diencephalon—particularly the thalamus and mammillary bodies (Jacobson and Lishman, 1997; Kopelman, 1995). One of the best predictors of memory loss in WKS is pathology of the anterior thalamus (Harding et al., 2000). In contrast, the hippocampus is damaged in less than 10% of WKS cases. Although the two types of amnesia differ in structural correlates, the *functional distinction* between temporal lobe amnesia and diencephalic amnesia has been questioned, given the behavioral similarities and the neural connections between the diencephalon and the hippocampus (Aggleton and Brown, 1999; Squire, 1982).

Using a rodent model of WKS, pyrithiamine-induced thiamine deficiency (PTD), we have demonstrated that the hippocampus is *functionally impaired*: When PTD-rats are performing a spontaneous alternation task (Savage et al., 2003) or learning a nonmatching-to-position task (Roland and Savage, 2007) the rise in hippocampal acetylcholine (ACh) efflux is blunted relative to control rats. This impairment in hippocampal ACh efflux is only evident during behavioral testing as baseline levels of hippocampal ACh are normal in the PTD model. Given the known importance of ascending cholinergic systems in a range of behaviors and neural physiology within the limbic system, understanding the role of cholinergic dysfunction in diencephalic amnesia will both further our understanding of the complex role of ACh in learning and memory and aid in the development of pharmacotherapies.

The impairment of functional ACh hippocampal release during behavioral testing in the PTD model could be driven by a number of neuroanatomical changes associated with thiamine deficiency and diencephalic amnesia. We have previously documented a loss of choline acetyltransferase (ChAT) immunopositive neurons in the medial septal/diagonal band (MS/DB) in the PTD model using a profile counting method (Pitkin and Savage, 2001; Pitkin and Savage, 2004). Furthermore, Nakagawasai and colleagues (Nakagawasai et al., 2000; Nakagawasai et al., 2004; Nakagawasai, 2005) have demonstrated a decrease in the intensity of ChAT positive fibers in the hippocampus, cortex and thalamus after thiamine deficiency. However, this decrease in ChAT immunofluorescence is not observed in the amygdala. Pires and colleagues have also demonstrated that after mild thiamine deficiency (Pires et al., 2001) or PTD treatment (Pires et al., 2005) stimulated ACh release is decreased in the cortex. Furthermore, these authors found that acetylcholinesterase (AChE) activity was decreased in both the hippocampus and cortex after PTD treatment (Pires et al., 2005). In addition, compounds that enhance ACh levels decrease mortality and sickness behaviors (attacking, antinociception, altered startle response) as well as improve learning in thiamine-deficient mice (Nakagawasai et al., 2000) and spontaneous alternation in PTD-treated rats [unpublished data].

Reviews of the pathology produced by the PTD treatment have consistently demonstrated that although mild to moderate cell loss occurs outside the diencephalon, the anterior and midline thalamic damage are critical and responsible for the majority of the loss of learning and memory function that occurs after thiamine deficiency (Langlais et al., 1996; Mair, 1994). These changes, along with the white matter loss that occurs in key fiber tracts from the diencephalon after PTD treatment (Langlais and Zhang, 1997), suggest that thiamine deficiency likely causes system level dysfunction. There is neurobiological evidence that even discrete diencephalic damage alters the activation of other limbic regions—in particular the hippocampus (see Jenkins et al., 2002; Reed et al., 2003; Savage et al., 2003). Thus, damage to the diencephalon produced by thiamine deficiency could produce dysfunction in other limbic regions.

In the current experiment we examined whether thiamine deficiency, which causes diencephalic pathology, alters both hippocampal and amygdalar ACh efflux when rats are actively exploring a maze. Measurement of ACh efflux in the brains of rats during learning appears to be a useful marker of activation of a given neural system—particularly the hippocampus and amygdala (Chang and Gold, 2004; Gold, 2003; McIntyre et al., 2002; McIntyre et al., 2003a; McIntyre et al., 2003b). We chose a task, spontaneous alternation, which is sensitive to diencephalic damage (Langlais and Savage, 1995) and to changes in release of ACh in both the hippocampus and amygdala (Chang and Gold, 2004; McIntyre et al., 2002; McIntyre et al., 2003a). In addition, to understand the neuroanatomical correlates of this dysfunction we used unbiased stereological techniques to estimate ChAT cell populations in the MS/DB (that projects to the hippocampus) as wells as the nucleus basalis of Meynert (NBM; that projects to the amygdala). Given our behavioral data (Savage and Langlais, 1995), that suggests intact amygdala functioning in the PTD model, we expect that during behavioral testing there will be a blunted rise in hippocampal – but not amygdalar – ACh efflux in the PTD treated rats. Furthermore, we expect PTD-treated rats to display a loss of ChAT immunopositive cells in the MS/DB (Pitkin and Savage, 2001; Pitkin and Savage, 2004). However, no one has examined cell loss in the NBM of this model. Our goal was to determine the selectivity of cholinergic dysfunction by measuring both ACh efflux during behavioral testing in two structures and ChAT immunopositive cell loss in the regions that project to these structures. The data from this study which combines behavioral, neurochemical and histological measures will give a more complete picture of the role of cholinergic abnormalities to the learning and memory problems associated with the thiamine deficiency form of diencephalic amnesia.

1. Results

1.1. *Statistical analyses*

The mean percent alternation and total number of arm entries during spontaneous alternation, as well as ChAT immunopositive cell counts (MS/DB, NBM) were analyzed with a 1-factor (Group = PF vs. PTD) ANOVA. The neurochemical microdialysis/HPLC data were analyzed with a one-between (Group), two-