A new treatment regime for sodium azide to evoke experimental Alzheimer's disease for pharmacological screening

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Alzheimer's disease (AD) is characterized by a progressive decline of cognitive functions. Comparative studies identified that there are no detectable signs of drug-induced changes in cognitive functions in healthy animals brain versus diseased brain, therefore, to see decreased behavioral manner of AD suffered animals needs to mimic all specific features of the disease [1]. More evidence suggests the early role of reactive oxygene species in AD major source of which is the mitochondria [2]. The mitochondrial poisoning hypothesis of AD underlying is based on the fact that in human post-mortem AD brains the complex IV activity declines [3]. Inhibition of this complex could be evoked by chronic subcutaneous sodium azide (NaN_3) treatment via implanted osmotic minipumps in animals [4]. For screening, however, minipumps are not ideal tools due to high cost, one-time usability and long treatment time. We developed a new method to produce AD–like dementia by selective inhibition of cytochrome C complex using intraperitoneally (ip.) injected NaN_3 in various doses (10–15 mg/kg/day) in rats. We explored the dose-effect relationships of the ip. applied mitochondrial poison and established a complex test system to study alterations of the cognitive functions caused by NaN_3 treatment. Furthermore, we found the optimal dose and treatment regime of NaN_3 to evoke histopathological changes in treated animals.

The mechanism of spatial learning behavior in mammals is critically dependent on the proper function of the hippocampus. Based on these data we concluded that the hippocampal CA1– and CA3–regions are crucial for acquisition and memory consolidation therefore, the Morris water maze test was selected to examine the changes in hippocampal-dependent cognitive functions [5]. We measured learning function after 5 days ip. treatment using the TSE VideoMot2 system. This is a system for the camera-monitored, computer-supported, automated observation and analysis of activity and locomotion of animals. It captures the movement of animals in the pool, collects, analyzes the data and draws the swimming path.

Methods

We used male Sprague-Dawley rats (400–430 g) from our own breeding stock. Animals received extruded complete diet and water, ad libitum. Our Local Committee based on the 86/609/EEC directive approved the protocol. During acquisition trials animals placed into the water were only able to escape by finding a platform within 120 s, which was hidden 1 cm under the water in center of one of the quadrants of the maze (target). Each rat received 4 trials/day for 3 days. The trial ended automatically when the target was reached and the elapsed time was called "escape latency". After 14 days NaN_3 treatment we studied the memory functions. We removed the platform from the pool and animals had only one trial (120 s). We registered three parameters: the time spent in the original target quadrant of the pool (1^{st}), the number of crossings through the original position of the platform (2^{nd}), and the latency of the first crossing of the platform original location (3^{rd}). When 12.5 mg/kg/day NaN_3 dose was reduced after 5 days to 10 mg/kg/day, considerable loss of memory functions was revealed on this day, specifically a significant decrease in the 1^{st} and 2^{nd} and increase in the 3^{rd} measured memory parameter. One hour later we tested the re–learning capability of the animals by putting back the platform into a new quadrant of the pool. Animals had re–learning trials from three directions. In this experiment only animals treated with 12.5 mg/kg/day NaN_3 than reduced to 10 mg/kg/day had significantly longer escape latency. To investigate NaN_3-induced interactions we measured the animals' spontaneous locomotor activity (SMA), and the weight of their body and adrenal glands.

Results and Discuission

According to swimming velocity results the observed effects developed without the loss of movement ability. 24 hours after the last NaN_3 treatment SMA, body and adrenal glands weights of animals in any of the NaN_3-treated group did not differ from the control, indicating the lack of any non-specific effect (e.g. stress). Measurement of corticosteron-regulated immunological markers (IL-8, IL-10) is in progress. Detailed histopathology of the different regions of brain was performed at the termination of the study. Neuronal degeneration and necrosis were seen in the cortical and hippocampal areas in the treated rats. Pathological changes in the ultrastructure of mitochondria and glial cells were detected in NaN_3–treated animals suggesting some degree of disruption of blood–brain barrier. Significant loss of neurons was detected in the hippocampal CA–1 and CA–3 regions. Ki67 immunochemistry to evaluate neurogenesis demonstrated decreased rate of cellular proliferation in the dentate gyrus of the treated rats.

These results show that treatment regime of 12.5 mg/kg/day ip. NaN_3 for 5 days with a subsequent reduction to 10 mg/kg/day for the following 9 days is a suitable method to produce dementia in rats.

We confirmed that with the ip. injection method of NaN_3 for 15 days produces comparable level of dementia caused by 31 days infusion of NaN_3 using implanted osmotic minipumps in rats. In summary, our developed new treatment regime seems to be an improved method for pharmacological screening of neuroprotective compounds, where low cost, reproducibility and short experiment time are required.

References.

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