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Effects of D-galactose on the expression of hippocampal peripheral-type benzodiazepine receptor and spatial memory performances in rats

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Summary The changes in spatial memory performances and the binding of hippocampal peripheral-type benzodiazepine receptor (PBR) induced by D-galactose (D-gal) were investigated in rats. The animals were randomly divided into two groups: saline-treated group and D-gal-induced aging group. All rats received 56 days of injection followed by 5 days of behavioral tests. The D-gal-induced aging rats presented significant impairment in water maze performance, compared with that in the saline-treated rats. A significant decrease in [3H]PK11195 binding in the synaptosomes from hippocampus in the D-gal-induced aging rats was observed, compared to that in the saline-treated rats. Meanwhile, the Scatchard analysis revealed that there was a decrease in B_{max}, with no significant change in K_D. Further analysis demonstrated that water maze performance was closely related to the PK11195 binding in all rats. These results suggest that D-gal decreased the density of PBR in hippocampal synaptosomes, which may be attributable to the progressive pathogenesis of aging in rats.

1. Introduction

The peripheral-type benzodiazepine receptor (PBR) is distinct from the central-type benzodiazepine receptor (CBR) structurally, functionally and pharmacologically. PBR exists as a hetero-oligomeric complex of at least three proteins viz, of which the 18 kDa protein carries both antagonist (binds to the isoquinoline carboxamide PK11195) and agonist (binds to the benzodiazepine Ro5-4864) binding domains (Joseph-Liauzun et al., 1997). In mammalian central nervous system, PBR is mainly localized at the outer mitochondrial membrane. The PBR related mechanism in neurodegenerative diseases is considered to contribute to the dysfunction of the mitochondria, which is one of the key factors in apoptosis. Previous binding studies on the changes of PBR during aging were mostly performed on

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crude membranes of brain tissues. A numbers of investigators reported the changes of PBR in different brain areas. However, the findings on the changes of [^{3}H]PK11195 binding as the effect of aging appeared to be rather inconsistent. During aging, the binding to PBR has been reported to be decreased in cerebral cortex, striatum and midbrain (Dalezios and Matsokis, 1998) or to remain unchanged in cerebral cortex and hippocampus (Komiskey and MacFarlan, 1983; Anholt et al., 1985; Fares et al., 1987) and in cerebellum (Dalezios and Matsokis, 1998) or to be increased in cerebral cortex and cerebellum (Daval et al., 1988). The densities of omega 3 sites have also been studied by autoradiography using [^{3}H]PK11195 in four groups of Fischer 344 rats aged 3, 12, 22, and 30 months, no age-related changes were noted except in the 30-month-old group in which discrete and focal increases (reflecting tumoral processes) were observed in various brain regions (Benavides et al., 1990). One reason for the discrepancy observed in the development of the brain PBR binding sites between the different time points may result from the different sampling time or the different radioligands used in various studies for labeling PBR sites since subtypes of PBR may exist in rat brain (Itzhak et al., 1995). Another reason may arise from the variability of the data, i.e. the differences in species and the brain regions examined. Majority of previous reports based on the single-point binding experiments may be another reason. These reports did not provide substantial information on the type of alterations, whether the change was quantitative (density of receptors) or qualitative (affinity), or even both. It should be noted that the above studies were designed to analyze the PBR binding sites in those identically prepared crude membrane, in which internal contamination of brain extract fractions may exist. In addition, previous studies in PBR levels during aging did not provide the results of kinetics of radioactive ligand binding to hippocampus or only present single determination (Weissman et al., 1984) due to less amount of hippocampus matrix. Furthermore, the implication of PBR activity in cognitive behavior in rodents has not been documented.

It was reported that a low dose of D-galactose (D-gal) caused mental retardation and cognitive dysfunction as measured by open field, avoidance/escape, T-maze, Y-maze and Morris Maze in mice (Shen et al., 2002; Xu and Zhang, 2002; Ho et al., 2003). In the past decade, this model was widely used for aging. However, the mechanism of D-gal-induced neurodegeneration remains unclear. Different mechanisms have been proposed. The most attractive assumption is that D-gal causes oxidative stress (Cui et al., 2004). According the free radical theory of aging, when the production of reactive oxygen species (ROS) overwhelms a cell's antioxidant capacity, oxidative damages to macromolecules such as membrane receptors may occur, hence, result in degenerative process (Ames et al., 1993). Higher eukaryotes have developed elaborated strategies to deal with reactive byproducts of their own metabolism, including enzymatic systems, such as superoxide dismutase, catalase, and glutathione peroxidase (Cui et al., 2004; Nakamura et al., 2002). Also, ROS have been increasingly implicated in AD, Parkinson's disease, and the ageing process caused by D-gal. Initial studies indicated that PBR could mediate protective effects against ROS damage (Carayon et al., 1996).

In this study, our aims are to observe the effects of D-gal on the learning-memory behavior and the expression of PBR in hippochampal synaptosomes in Sprague–Dawley rats.

2. Materials and methods

2.1. Animals

Male and female Sprague–Dawley rats (12-month-old) weighting between 300 and 400 g ($n=24$) were purchased from Experimental Animal Center (Beijing, China). Rats were housed in polycarbonate cages in a temperature-controlled room with a 12:12-h light/dark cycle. All rats were housed individually, and fed with an equilibrated standard sodium diet and water ad libitum. The animals were randomly divided into the following two groups ($n=12$ in each group, six male and six female): (1) saline-treated controls (0.9% saline, 1 ml/kg body weight, s.c., once a day for 56 days); (2) D-gal-induced aging group (D-gal, 100 mg/kg s.c., dissolved in saline solution, in a total volume of 1 ml/kg, every day for 56 days).

2.2. Chemicals

[^{3}H]PKlll95 (specific activity, 83.5 Ci/mmol; radiochemical purity, 97%) was purchased from Perkin–Elmer Life Sciences (Boston, MA). Sucrose, POPOP and unlabelled PKlll95 were purchased from Sigma Chemical Co. (St Louis, MO). PPO and Coomassie brilliant blue G-250 were purchased from Fluka Ltd (Buchs, Switzerland). Triton X-100 was purchased from Amersham Pharmacia Biotech, Inc. (Uppsala, Sweden). D-gal was purchased from Amresco (Solon,