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AGE-RELATED CHANGES IN THE CONTENT OF INSULIN - LIKE GROWTH FACTOR-I IN RAT BRAIN

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Although there has been extensive research on the effect of IGF-I on muscles and bone tissue, the effect on brain aging has received little attention. We investigated the IGF-I content in brains of differently aged rats. The IGF-I contents in cerebellar and cerebral cortex were found to be higher in immature rats (4-5 days old) compared to young adult (2.5 months old) and middle-aged (7.5-9 months old) rats. However, the decrease of mean IGF-I in middle-aged rats compared to immature animals was statistically significant only in the cerebellar cortex. Our results indicate that IGF-I content decreases through the lifespan and maybe selectively in some brain regions.

Key words: IGF-I, brain, cerebellar cortex, ageing, rat

INTRODUCTION

The insulin-like growth factor (IGF) system, which includes: two peptides (IGF-I and -II) with structural homology to insulin, two membrane receptors (type I and II) and IGF binding proteins (at least 9), is present in the brain and its role is becoming apparent (Jones and Clemmons, 1995; D'Ercole *et al.*, 1996). All types of brain cells (neurons and glia) may express components of the IGF system and/or constitute targets of IGFs at some time during the life of an organism (Torres-Aleman, 1999). In particular, IGF-I has been found to be involved in neuronal growth, differentiation, brain metabolism and neural transmission. Serum and brain IGF-I levels appear to change with age and this decrease may contribute to neurodegenerative diseases (D'Costa *et al,* 1995; Sonntag *et al,* 1997, 2000 a). The aim of this study was to provide more quantitative data about the IGF-I content in cerebellar and cerebral cortex of young and adult rats.

MATERIAL AND METHODS

The experiments were performed on three groups of male Wistar rats kept under optimum temperature and 12:12 hours light:dark conditions. They were fed ad libitum. All efforts were made to minimize animal suffering and reduce the number of animals used. There were three age groups: pups aged 4-5 days (N=12, immature group), 2.5 - 3.5 month-old rats (N=11, young adult group) and

7.5-9 month-old rats (N=10, middle-aged group). The young adult and middle-aged animals were decapitated in deep Nembutal anesthesia (Nembutal, Serva, Germany, 50mg/kg i.p), while the pups were killed in hibernation. There after the brains were quickly removed and samples of parietal cerebral and cerebellar cortices (of both hemispheres), each about 0.2 g in weight, were placed in 10 volumes of 0.5 M acetic acid and extracted for 10 minutes in a water bath at 100°C. After cooling the extracts were stored at -20 °C until analysis. Aliquots of the supernatant were included in a competitive binding assay for IGF-I using recombinant human ^{125}IGF-I (ICN, USA) as the labeled ligand, as described earlier (Nikolić *et al,* 1998). Analysis of variance (ANOVA) and Student's t-test (Njegić *et al.* 1989) were used to determine the significance of differences between mean IGF-I contents in cerebral and cerebellar cortices of the three animal age groups.

RESULTS

IGF-I was determined in all extracts but was below the level of detection (~0.2 μg/kg) in several cerebral extracts from young adult animals and four cerebellar extracts from middle-aged rats. The mean IGF-I concentrations found in all (66) brain samples are shown in Fig. 1. The IGF-I contents in cerebellar and cerebral cortices were highest in immature rats (4-5 days old), while there was a ten-

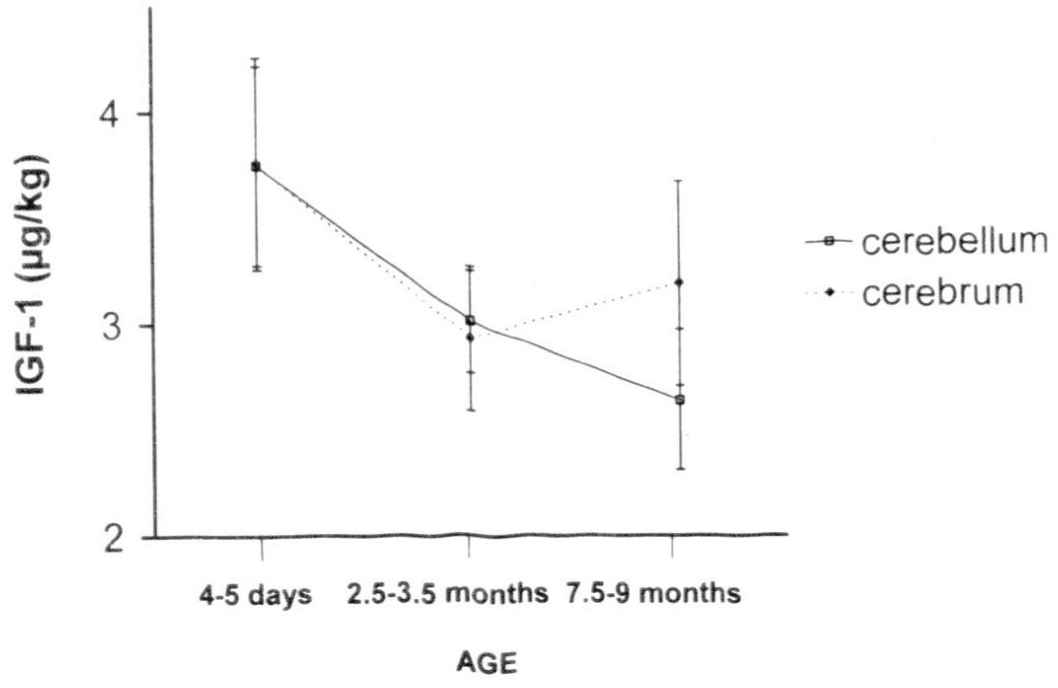

Figure 1. Mean values (± SE) of IGF-1 concentrations in cerebral cortex and cerebellar cortex of immature, young adult and middle-aged rats.

dency for decreasing IGF-I levels to occur with aging. A statistically significant decrease of IGF-I (Student's t-test, $p<0.05$) was observed only in the cerebellar cortex in middle-aged rats (7.5-9 months old) compared to the immature animals. However, the two-way ANOVA method was used to test the significance of the effects of two factors: animal age (A) and brain structure (S), on the detected levels of IGF-I concentrations. Results of the ANOVA test (Table 1) showed that animal age but not the type of structure had a significant influence on the dependent variable values. In addition, interaction between the two observed factors (A and S) was not statistically significant.

Table 1.Two-way ANOVA of IGF-I concentrations in cerebrum and cerebellum of differently aged rats

Source	SumSquSd of IGF conc	Degrees of freedom	IGF conc Variance	F	Probability
Age (A)	14.0143	2	7.007167	3.874214	0.02
Structure (S)	0.0400	1	0.040042	0.022139	0.88
Interact. A-S	0.0001	2	0.000050	0.000028	>0.9
Error	97.6681	54	1.808668		
Total	111.7225	59			
No Observ.	60				

DISCUSSION

Expression patterns of the IGF system proteins suggested highly regulated and developmentally timed IGF actions on specific cell populations in various brain regions (D'Ercole *et al.*, 1996).

The IGF-I system of brain regions is particularly well represented in young and adult cerebellum (Andersson *et al,* 1988; Aguado *et al,* 1992). However, the data on IGF-I contents in brain throughout the lifespan are ambiguous, partly because most studies have been qualitative investigations of the distribution of IGF-I immunoreactivity in different brain regions. An age-related decline has been found in plasma IGF-I as well as in cerebral cortex IGF-I protein levels (by 36.5% in 32 month-old rats compared with 11 month-old rats), despite the absence of altered gene expression (Sonntag *et al.*, 1999). This was related to a decrease in cerebral microvasculature, even though IGFs in the peripheral circulation are not considered to be a major source of IGFs for the central nervous system (Sonntag *et al.*, 1997). Our results indicate that the IGF-I levels in the cerebellar cortex started to decline in middle adulthood (at 7.5 to 9 months old), while similar changes did not occur in the cerebral cortex. It will be interesting if our histochemical study in progress will confirm the radioimmunoassay results for differences in IGF-I contents in adult rat cerebrum and cerebellum. Our previous pilot

study on IGF-I concentrations in a model of epilepsy did not reveal any particular differences in the IGF-I brain concentrations of penicillin treated rats compared to untreated rats; in all animals the serum contents were about 100 fold greater than the cerebral levels (Ćulić *et al.*, 1998). IGF-I-like immunoreactivity was found to be selectively distributed in the frontal cortical and hippocampal glial cells and in cerebellar Purkinje neurons of age-matched control humans, as well as in Alzheimer disease (AD) brains (Jafferali *et al.*, 2000). The same authors suggested a role for IGF-I in compensatory plasticity and/or survival of the susceptible neurons in AD brains, because the amyloid peptide containing neuritic plaques, apart from astrocytes, exhibited IGF-I immunoreactivity. Although research on the IGF system and brain ageing is still evolving, it suggests that the decreases in growth hormone and IGF-I with age have both beneficial and deleterious effects (Sonntag *et al.*, 2000 b).

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STAROSNE PROMENE U SADRŽAJU INSULIN - SLIČNOM FAKTORA RASTA I U MOZGU PACOVA

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SADRŽAJ

Vršena su istraživanja insulinu sličnog faktora rasta (IGF-I) na mišićno i koštano tkivo, ali je posvećena mala pažnja efektu na mozak u toku starenja. Mi smo ispitivali sadržaj IGF-I u moždanom tkivu pacova različite starosti. Nađeno je da su IGF-I koncentracije u kori malog mozga kao i velikog mozga mladih pacova (4-5 dana starih) više u poređenju sa sadržajima grupe tek-odraslih pacova starosti 2,5 meseca i grupe nešto starijih odraslih pacova (7,5-9 meseci starih). Međutim, smanjenje koncentracije IGF-I sadržaja samo u kori malog mozga nešto starijih pacova (7,5-9 meseci) bilo je značajno u odnosu na vrednosti u novorođenih (4-5 dana starih pacova). Naši rezultati ukazuju da IGF-I opada tokom života i moguće - selektivno u određenim moždanim regionima.