

## Amyloid beta protein affects poly(ADP-ribose) polymerase activity in PC-12 cells in culture

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**INTRODUCTION AND METHODS.** Poly(ADP-ribose) polymerase (PARP; EC 2.4.2.30) is responsible for a post-translational modification of proteins, including PARP itself called poly(ADP-ribosyl)ation. It is a nuclear, DNA dependent enzyme that consumes cellular NAD<sup>+</sup> to produce chains of ADP-ribose. Its biological role(s) is not yet fully understood, but numerous studies imply its role in DNA repair and other cellular responses to DNA damage (1). The mechanisms by which amyloid beta protein (A $\beta$ ) mediates cell death, or the question whether A $\beta$  is cytotoxic *in vivo* in Alzheimer's disease are issues which are not yet solved. The aim of this study was to investigate the effects of amyloid beta peptides on PARP activity and cell death in pheochromocytoma cells (PC-12) in culture. The PC-12 cells nuclei were visualized using Hoechst 33258 staining and their viability was evaluated under a fluorescent microscope. DNA samples were electrophoresed through 1,1% agarose gel and DNA bands were visualized by staining with ethidium bromide. PARP assay was carried out as described previously by Ueda et al. (2) with authors modifications. A $\beta$  fragment 25-35 after aggregation (1h incubation at 37°C) was added to the cell culture at a final concentration from 1 to 100  $\mu$ M. The cells were exposed for 2, 6, 48 h to the action of amyloid. As a control reverse sequence 35-25 of A $\beta$  was used.

**RESULTS AND DISCUSSION.** A significant effect of amyloid beta action was observed after 48 h (Fig.1). After that time even 1  $\mu$ M A $\beta$  (25-35) caused activation of PARP but significant stimulation of PARP activity was observed at 10  $\mu$ M A $\beta$  as compared with control samples (Fig.1). Interestingly at 20, 30, and 100  $\mu$ M of A $\beta$  (25-35), PARP activity was inhibited. The above effects of A $\beta$  (25-35) were pH dependent (Fig.1). PARP activity in untreated PC-12 cells was also pH dependent, meaning it was about 50 % higher at pH 9.0 than at pH 8.0. We also noted that under the same conditions, endogenous activity(ies) of phosphodiesterase(s), an enzyme which takes part in degradation of ADP-ribose chains, was more than two times lower at pH 9.0 than at pH 8.0. In addition, the Hoechst staining revealed that only a few (<5%) apoptotic cells were observed after treatment with A $\beta$  (25-35) (48 h, 10  $\mu$ M) in contrast to staurosporine treatment (48 h, 0.1 $\mu$ M), which promoted many cells to undergo apoptotic changes. Furthermore, an analysis of DNA integrity using agarose gel electrophoresis revealed, that A $\beta$  (25-35) treatment of PC-12 cells did not result in DNA laddering which is one of the apoptosis markers. Our study indicates a possible link between PARP and amyloid beta action *in vivo*, but the underlying mechanism(s) and its biological role remains unclear and needs further investigation.

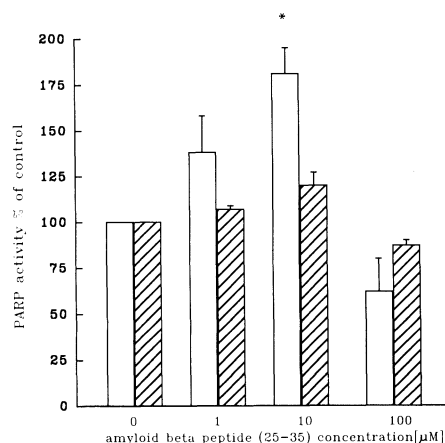


Fig. 1. Effect of A $\beta$  (25-35) on PARP activity in PC-12 cells. PARP activity was determined at pH 8.0 (open bars) and at pH 9.0 (hatched bars). The control activity of enzyme was at pH 8.0 *ca.* 3.57 nmol/mg protein/min and at pH 9.0 *ca.* 5.20 nmol/mg protein/min. Incubation time with A $\beta$ (25-35) was 48 h. The data are mean  $\pm$  SD from 3-4 experiments carried out in triplicate. Statistical significance was evaluated by Student *t*-test, \**P*<0.05.

1. Szabo C. (1996) Free Rad. Biol. Medicine 21: 855-69.

2. Ueda K., Kawaichi M., Hayaishi, O. (1982) In: ADP-Ribosylation reactions: biology and medicine (Eds. O. Hayaishi and K. Ueda). Academic Press, New York, p. 117-155.