Factors affecting the β-amyloid precursor in PC12 cells.

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β-amyloid precursor protein (APP) mismetabolism probably contributes to formation of neuroatheriological changes, including deposits (e.g., senile plaques) in non-familial Alzheimer’s disease, the common form of the disease. Causative processes are unknown but abnormal energy metabolism (“oxidative stress”) is implicated by some studies [1]. APP is normally processed by chloroquine-sensitive lysosomal and secretory pathways. At least one secretory pathway is stimulated by activation of phosphoinositide (PI) linked receptors, probably by altering protein kinase C-mediated phosphorylation events [2]. Here PC12 cells [3] have been used to study the effect of oxidative stress, in combination with kinase stimulation and chloroquine, on amounts of APP.

Cells (ATCC, CRL 1712, Poron Down) were grown on 35 mm collagen-coated plates (15°C, 5% CO2, 95% air) in medium (serum free with Na selenite, insulin, transferrin, Boehringer Mannheim); seeding density, 7 x 103 cells/ml. For neuronal differentiation, nerve growth factor (NGF, 400ng/µl, 7S, murine, Promega) was added 24 h after seeding (Fig 1B, less NGF gave less APP, not shown, band identity in [2]) and continued daily for 3 days. For oxidative stress, cells were incubated (3h) in medium (no glucose or pyruvate), with 5mM deoxyglucose and 0.2mM oligomycin [3] and where appropriate NGF, Chloroquine, phorbol-12-13-dibutyrate and bradykinin, respectively. Cells, separated from medium (3500g, 7 min), were resuspended (cold PBS, with 0.5mM deoxyglucose and 0.2mM oligomycin) and 10% glycerol (A, B, C-D; incubation for 10 days) or centrifuged and pellet sonicated with 100mM phenylmethylsulphonyl fluoride (PMSF) for determination of cell viability (trypan blue exclusion), ATP [34] and APP. For latter (from 1.35 ml medium), suspension was centrifuged and pellet sonicated with 285µl, 50mM Tris-HCl, pH 6.8, 4% SDS, 10mM EGTA, 2mM PMSF and 10% glycerol (A) and centrifuged (18000g, 15 min). 70µl supernatant was removed (for total protein) and diithiothreitol and bromophenol blue added (40µl, final concentrations 100mM and 0.1% respectively, B) Medium (6x, Amicon Centricon 10 tubes) was mixed with sample buffer (A plus B). After 100°C (4 min) samples (cell lysates, 100 µg protein, 300µl medium, original volume) were electrophoresed in triplicate in 7.5% (10% medium) polyacrylamide-SDS gels with transfer to nitrocellulose. Density of a band (antibody 22CI1, Boehringer Mannheim) was normalised to density of same band under control conditions ([5], see Stratmann this meeting).

Incubation (3h) of non-NGF treated cells with chloroquine significantly increased (t-statistic) amount of intracellular APPm5 (125 ± 3% of value for incubations without drug, mean ± SEM, n=3) and APPm6 (10.6 ± 0.4%, as described [2]. Oxidative stress did not effect cell viability (69 ± 15 and 64 ± 18% of cells were viable, control and stressed cells respectively, n=7, not significantly different, Student’s t-test). The stress decreased amount of ATP and intracellular (Table 1, Fig 1; G, H, J, K) as well as secreted (Table 2) APPs. This was not reversed by chloroquine except for intracellular APPm6 in NGF treated cells (Table 1, Fig 1 H, J), although when NGF was absent mean values were higher (70 and 68% compared with 48 and 58% Table 1 Fig 1 J, L). Incubation (3h) of NGF treated cells, under oxidative stress with chloroquine did not affect (t statistic) the amount of APP in medium (66 ± 2 and 67 ± 11% compared with control, 68 and 65%, Table 2). Bradykinin and phorbol ester increased the amount of APP in medium from normal cells, as described [2,6] and was most obvious with APPm5. For incubations with stressed cells both compounds restored values to those for normal cells (Table 2, compare 89 - 101% with 100% Table 2).

These data suggest that APPm5 and APPm6 are not subject to the same processes of regulation, as in stressed cells only APPm5 showed clear evidence of sensitivity to chloroquine. This result may be taken as evidence that the depletion of APPm5 was a consequence of increased degradation. Further work is needed to establish whether synthesis of APPm5 by cells is selectively affected by oxidative stress, an important issue as this species is particularly obvious in human but not rat brain.

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Fig. 1. Illustrates immunoreactivity in medium (A-F) and cell lysate (G-I), with NGF (B-C-D), incubation for 10 days (A,B), 1h (C-D), 3h (E-F), with bradykinin (D), oxidative stress (F,H,K) plus chloroquine (LL).