Neuronal dysfunction in Down syndrome: Contribution of neuronal models in cell culture

Katherine Saud [a], Christian Arriagada [b], Ana María Cárdenas [c], Takeshi Shimahara [d], David D. Allen [e], Raúl Caviedes [a], Pablo Caviedes [a,*]

[a] *Program of Molecular and Clinical Pharmacology, ICBM, Faculty of Medicine, University of Chile, Independencia 1027, Clasificador No. 7, Independencia, Santiago, Chile*
[b] *Program of Anatomy and Developmental Biology, ICBM, Faculty of Medicine, University of Chile, Santiago, Chile*
[c] *CNV, University of Valparaíso, Chile*
[d] *NBCM, CNRS, Gif sur Yvette, France*
[e] *Department of Pharmaceutical Sciences, Texas Tech University HSC School of Pharmacy, Amarillo, TX, 79106-1712, USA*

Abstract

Down syndrome (DS) in humans, or trisomy of autosome 21, represents the hyperdiploidy that most frequently survives gestation, reaching an incidence of 1 in 700 live births. The condition is associated with multisystemic anomalies, including those affecting the central nervous system (CNS), determining a characteristic mental retardation. At a neuronal level, our group and others have shown that the condition determines marked alterations of action potential and ionic current kinetics, which may underlie abnormal processing of information by the CNS.

Since the use of human tissue presents both practical and ethical problems, animal models of the human condition have been sought. Murine trisomy 16 (Ts16) is a model of the human condition, due to the great homology between human autosome 21 and murine 16. Both conditions share the same alterations of electrical membrane properties. However, the murine Ts16 condition is unviable (animals die in utero), thus limiting the quantity of tissue procurable. To overcome this obstacle, we have established immortal cell lines from normal and Ts16 mice with a method developed by our group that allows the stable in vitro immortalization of mammalian tissue, yielding cell lines which retain the characteristics of the originating cells. Cell lines derived from cerebral cortex, hippocampus, spinal cord and dorsal root ganglion of Ts16 animals show alterations of intracellular Ca^{2+} signals in response to several neurotransmitters (glutamate, acetylcholine, and GABA). Gene overdose most likely underlies these alterations in cell function, and the identification of the relative contribution of DS associated genes on such specific neuronal dysfunction should be investigated. This could enlighten our understanding on the contribution of these genes in DS, and identify new therapeutic targets.

Keywords: Down syndrome; Cell lines; Gene dosage; Neuron

1. Introduction

Aneuploidy is an adverse condition for development, and generally results in death in utero (Epstein, 1986a). In the case of trisomies, the disruption of homeostasis is determined by an increased genetic material and the conse-quent effects of gene dosage excess (Epstein, 1986b). The development of therapies has diminished the incidence of mental retardation by causes such as trauma or connatal infection, and has enhanced the importance of genetic alterations in the genesis of this type of pathologies (Oster-Granite, 1986). In humans, the best known cause of mental retardation that most frequently survives gestation is the trisomy of chromosome 21 or Down syndrome (DS) which occurs in 1 of every 700 live births (Reeves et al., 1986), representing 30% of all cases of mild to severe

* Corresponding author. Tel.: +562 678 6559; fax: +562 737 2783.
 E-mail address: pcaviede@med.uchile.cl (P. Caviedes).

mental retardation (Brousseau and Brainerd, 1928; Pulsifer, 1996). The condition also determines muscle hypotonia, increased frequency of cardiovascular malformations, immunological defects, a greater incidence of leukemia, and a relation with Alzheimer's disease represented by the early onset of similar and typical neuropathology by the fourth decade of life: presence of senile plaques, amyloid deposits, neurofibrillary tangles and gliosis (Ault et al., 1989a; Cárdenas et al., 1999; Epstein, 1986a,b; Reeves et al., 1986).

Human chromosome 21 has been mapped (Hattori et al., 2000), and 127 genes and 98 possible new genes have been identified, all of which can potentially be overexpressed (Epstein, 1986a). It is currently known that for the development of DS requires at least the presence of a definite portion of the chromosome 21 (bands 21q22.1 to qter), termed the DS region. The syndrome is clearly not based on mutations, deletions or connatal pathology, but in the excess of gene normal products. Nevertheless, the specific implications to function at the cell level by this generalized overexpression remain obscure.

2. Mental retardation: A problem of development and structure vs function

The basis of mental retardation in DS remains unknown (Epstein, 1986b; Haxby, 1989; Pulsifer, 1996; Schapiro et al., 1989). Post-mortem studies suggest the existence of cerebral atrophy (Marin-Padilla, 1972, 1976), but axial computerized tomography studies in young DS patients do not show atrophy or developmental alterations (Schapiro et al., 1987). Further, the reduction of the total volume of the brain observed in these subjects is matched by their characteristic low height, giving an equivalent cerebral/height relation to that of normal subjects (Dekaban, 1978; Schapiro et al., 1987). On the other hand, more recent studies with magnetic nuclear resonance (NMR) have shown reduction in the volume of cerebral and cerebellar hemispheres (Raz et al., 1995), pons, mamillary bodies and hippocampus (Aylward et al., 1999), after correction by size. The same study also revealed an enlarged parahippocampal gyrus. Functional studies, using positron emission tomography (PET), have reported normal regional cerebral glucose utilization levels in young DS subjects (Horwitz et al., 1990). Nevertheless, the correlation among regional levels of metabolism is reduced, suggesting a functional decrease of interactions among different regions of the brain and alterations in the neuronal circuits (Horwitz et al., 1990). The latter appears particularly clear in language areas, a function deeply affected in DS (Azari et al., 1994).

Morphological studies have yielded contradictory evidence of alterations in DS brains. Studies in fetal brains reveal neuronal morphology and dendritic spines numbers that are comparable to those of age-matched normal fetuses. However, young and adult patients present shorter dendrites, reduction in number and altered shape of dendritic spines, and alterations in cortical layers (Schmidt-Sidor et al., 1990; Takashima et al., 1981). At the same time, other studies show delayed myelinization (Mito et al., 1991; Schmidt-Sidor et al., 1990) and dendritic alterations in post-mortem brains of both newborn and young adults DS patients (Marin-Padilla, 1972, 1976; Wisniewski et al., 1985). However, the latter findings also exist in mentally retarded subjects which lack chromosomal anomalies (Purpura, 1974), suggesting that these alterations are non-specific. All this contradictory morphological evidence reported for brains of fetuses, young and adult DS subjects, has led to believe that CNS dysfunction in DS is a reflection of functional alteration rather than a structural one (Galdzicki et al., 2001), particularly in early phases of the development. The alteration(s) can exist at multiple levels, and can certainly affect development subsequently.

Some of these anomalies can be encountered at the following levels:

1. Electrical membrane properties of individual neurons and their interaction with other cells.
2. Synaptic plasticity, also including coupling to signal transduction mechanisms and gene transcription regulation.
3. Formation and destruction of synapses.
4. Altered intracellular signaling mechanisms.
5. Altered development and integrity of neuronal circuits (Koch, 1997; Llinas, 1988).

Regarding neuronal function, several alterations in neurotransmitter systems exist in DS, all of which can contribute to the multiple neurological and neuromuscular alterations (hypotonia) (Cárdenas et al., 2002a; Collingridge et al., 1983) that characterize the syndrome. Indeed, these patients present electroencephalographical alterations related to age (Gibbs and Gibbs, 1964), and deficits in visual and auditory sequential memory (Luria, 1963; Marcell and Armstrong, 1982). Also, platelets and post-mortem brains show decreased serotonin content (Boullin and O'Brien, 1971; McCoy and England, 1968; Tu and Zellweger, 1965). Further, there are altered noradrenalin contents in the hypothalamus and decreased choline acetyl transferase activity in the brain (Yates et al., 1983). Regarding electrical membrane properties, studies in normal and trisomic human dorsal root ganglia neurons in primary culture have demonstrated specific alterations in DS cells, namely at the action potential and ionic current level (Ault et al., 1989b; Caviedes et al., 1990; Nieminen et al., 1988). Briefly, trisomic neurons exhibit accelerated rates of depolarization and repolarization of the action potential, yielding a shortened duration of the spike. The accelerated depolarization could be explained by in a differential sensitivity to potential of the inactivation gate of two populations of Na^+ channels, one TTX-sensitive and another TTX-resistant. This displacement was estimated in 10 mV towards more depolarized potentials in

the trisomic condition, determining that, at resting potential levels, there is a greater quantity of Na^+ channels available for activation (non-inactivated) (Caviedes et al., 1990). At the same time, the TTX-resistant Na^+ current presents prolonged time-dependent kinetics in the trisomic condition, resulting in a persistent Na^+ current after the action potential that reduces the refractory period. The latter could be related to the greater excitability observed in ganglionic and spinal trisomic neurons (Orozco et al., 1987; Scott et al., 1981). Finally, at the same time, an accelerated temporal kinetics in the activation of K^+ currents in DS neurons could explain the greater acceleration in the rate of repolarization (Nieminen et al., 1988). These alterations can alter the critical sequence of events in the processing of information in CNS neuronal networks, determining a generalized dysfunction (Caviedes et al., 1990) and limitations in the cell phenomena that are dependent of electrical membrane stimulation (i.e.: Reduction of intracellular Ca^{2+} and neurotransmitter release) (Ault et al., 1989a; Caviedes et al., 1990; Nieminen et al., 1988).

3. Murine trisomy 16 and others, cell lines

Ethical and practical problems are clear issues in the procurement and use of human tissues to carry out cellular pathophysiological studies in any given genetic alteration (Fig. 1). Fortunately, an animal model of human trisomy 21 does exist, which is the trisomy 16 in the mouse, whose triplicated chromosome presents great genotypical homology with the human condition (Epstein et al., 1985).

More recently, partial trisomies have been developed (Davisson et al., 1990; Davisson et al., 1993; Sago et al., 1998), that only have an extra copy of segments of the DS region in chromosome 16. Using cultured neurons of the complete trisomy, our group and others have described essentially the same electrophysiological alterations found in the human condition (Caviedes et al., 1990; Nieminen et al., 1988), along with altered Ca^{2+} currents and central cholinergic function (Fiedler et al., 1994). These results have essentially validated the animal model for the study of DS pathophysiology. Indeed, the trisomic condition in both man and mouse determines comparable alterations in mechanisms intimately related to the cell membrane: (a) Electrical membrane properties (increased rates of depolarization, repolarization and reduced spike duration; determined by altered density and kinetics of Na^+, K^+ and Ca^{2+} channels) (Caviedes et al., 1990, 1995; Nieminen et al., 1988), (b) Neurotransmitter function (altered amplitude and kinetics of intracellular Ca^{2+} responses to agonists) (Allen et al., 2002; Cárdenas et al., 1999, 2002a,b; Schuchmann et al., 1998), and (c) Cholinergic dysfunction (reduced choline uptake, reduced expression and activity of choline acetyltransferase—ChAT, and alterations of fractional choline release after external stimulation) (Allen et al., 2000, 2002; Cárdenas et al., 1999, 2002a,b; Fiedler et al., 1994). However, these alterations differ quantita-

tively and qualitatively in different regions from the nervous system. In the septum, spinal cord and ganglion neurons, there is increase in the rate of depolarization and repolarization, with shortened spike duration (Acevedo et al., 1995; Ault et al., 1989a,b). Nevertheless, in cultured hippocampal neurons the situation is inversed, and trisomic neurons exhibit slower depolarization of the action potential and increased duration, possibly due to decreased Na^+ current density (Galdzicki et al., 1993). Conversely, ganglionic neurons exhibit an increased Na^+ current density, which agrees with the enhanced depolarization phase previously noted (Orozco et al., 1988). Further, Galdzicki et al. (1998) showed an increase of type L Ca^{2+} current density in hippocampal Ts16, while our group showed reduction in such currents in ganglionic neurons. In this regard, Schuchmann et al. (1998) reported that cultured hippocampal neurons of Ts16 mice exhibit slower inactivation kinetics in response to glutamatergic stimulation. Finally, our studies of cholinergic function in cultured brain a spinal cord neurons indicated that Ts16 cells have reduced high affinity choline uptake (Na^+ dependent, sensitive to hemicolinium-3) in both territories compared to control cultures. Fractional choline release was diminished in trisomic brain cultures, whereas spinal cord cells exhibited no differences between two conditions (Fiedler et al., 1994). Also, abnormalities have been described in pathways involving protein kinase A (PKA) and C (PKC) (Rapoport and Galdzicki, 1994), which could be related to elevated myo-inositol levels in trisomic brains (Huang et al., 2000; Shetty et al., 1996). The findings detailed above reinforce the notion that trisomy in both humans and mice determine different membrane-related effects in diverse territories of the central nervous system (CNS). It is therefore necessary to carry out functional studies in nerve cells of different territories along with DS-related gene expression, in order to relate overexpression with specific regional dysfunctions.

Unfortunately, the murine model has certain drawbacks. An important limitation is the impossibility to carry out cognitive or behavioral studies, because the hyperdiploid condition in the mouse is inviable, with death occurring in utero around 14–16 days of gestation. Strategies used to circumvent this obstacle have been of an additive nature, starting with transgenic mouse models for individual DS-related genes, and later with the use of partial Ts16 animals (Ts65Dn, Ts1Cje) (Davisson et al., 1990, 1993; Sago et al., 1998). Although these models survive gestation and develop into adulthood, they necessarily renounce to genes of the definite portion of murine chromosome 16 that bears homology with the critical distal segment of the long arm of human autosome 21. This can compromise the interactions of all gene products involved in the full trisomy, and thus compromise conclusions related to the pathology.

Why establish cell lines? Being the Ts16 condition inviable in the mouse, and considering the limitations of primary culture techniques (variability, limitations in
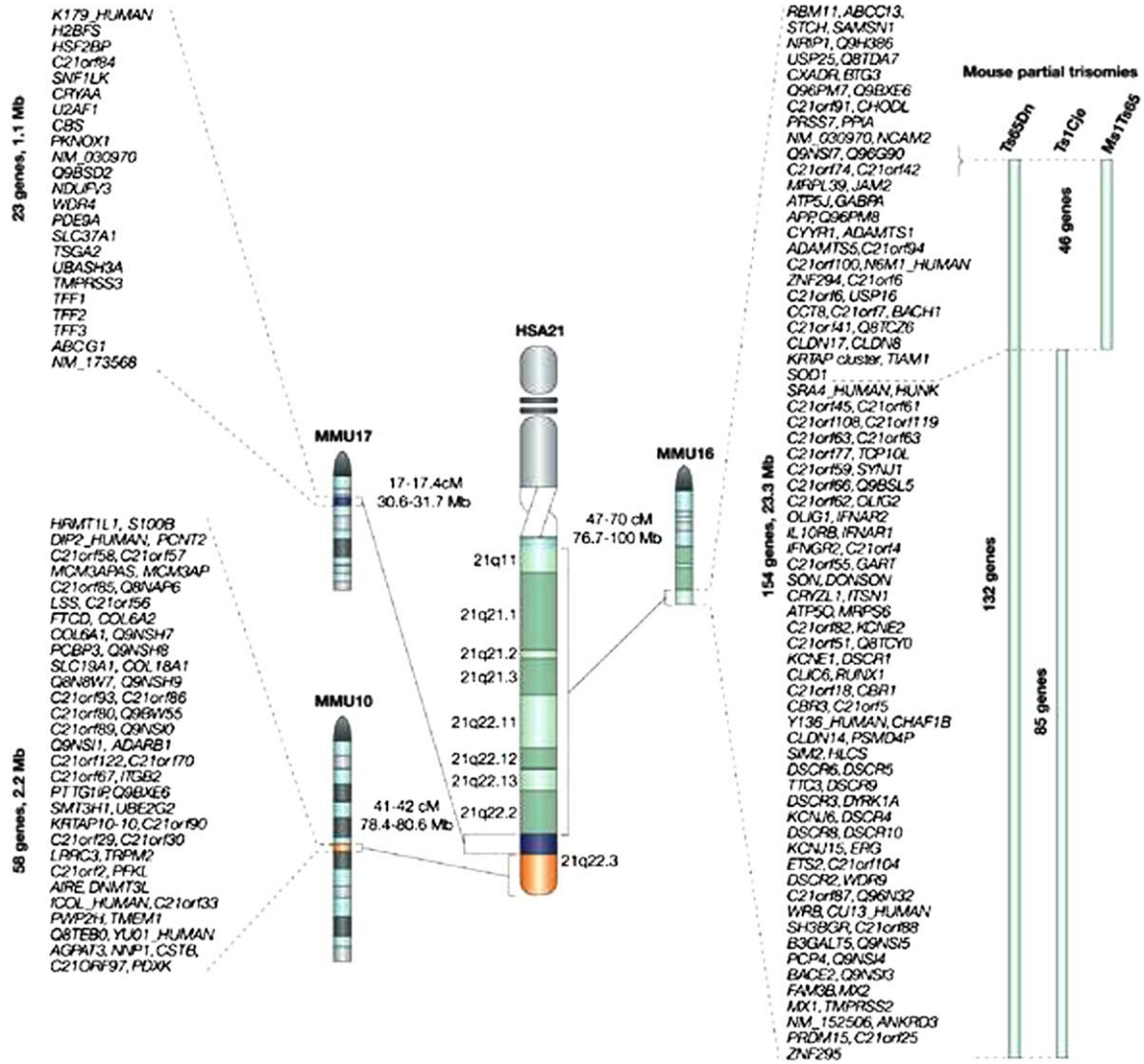
Fig. 1. Synteny between human chromosome 21 (HSA21) and mouse chromosomes (MMUs) 16, 17 and 10. Three partial trisomy mouse models of human trisomy 21 are shown, all trisomic for a portion of MMU16. The gene content of these partial trisomies is shown on the right. Reproduced by permission from (Antonarakis et al., 2004).

quantity of procurable tissue, in vitro senescence), the establishment of immortalized cell lines appears as an excellent alternative to preserve the trisomic condition indefinitely in vitro, and thus generate immortal models that are clonable, easy to access and manipulate, and that provide unlimited amounts of cells. In this regard, attempts to immortalize Ts16 mouse tissue using oncogene transfection methods have not been successful (Frederiksen et al., 1996; Kim and Hammond, 1995), fundamentally due to viability problems or poor stability of neuronal traits after transformation. As previously mentioned, our laboratory, using an original procedure (Caviedes et al., 1993), has managed to successfully establish neuronal cell lines in permanent culture from diverse origins of the CNS of normal and trisomic mice, which after six years retain neuronal stably (Caviedes et al., 1993; Liberona et al., 1997). Briefly, the immortalization procedure consists in maintaining neu-

ronal primary cultures in the presence of media conditioned by the rat thyroid cell line UCHT1. This treatment induces tissue immortalization in variable periods of time (Caviedes et al., 1993). With this procedure, we have been the first group to establish continuously growing cell lines that express stable neuronal phenotypes and lack glial characteristics. Our experience with these and other lines so established indicates us that with this procedure the lines generated retain differentiated traits, some for decades (Allen et al., 2000; Cárdenas et al., 1999; Caviedes et al., 1995; Liberona et al., 1997). In this fashion, mouse Ts16 cell lines were established from the cerebral cortex (named CTb), hippocampus (HTk), spinal cord (MTh) and dorsal root ganglion (GTl). Simultaneously, cell lines from the same territories were established from normal, age-matched littermates, that have served as controls. All these lines retain neuronal markers (Neural

specific enolase—NSE, Choline acetyl transferase—ChAT, synaptophysin, microtubule associated protein 2—MAP-2) and lack glial traits (GFAP, galactocerebroside, S-100). Our prior studies have also shown that these cells express functional neurotransmitter receptors, as shown by intracellular Ca^{2+} responses to neurotransmitter agonists (Cárdenas et al., 1999), and also cholinergic function (Allen et al., 2000, 2002; Cárdenas et al., 2002a,b), and present alterations of the aforementioned functions in a manner similar to that observed in similar tissues kept in primary culture conditions.

4. Gene overdose in the cell membrane

The evaluation of any therapy in the DS requires the precise definition of the multisystemic alterations and their specific relation with the trisomy itself. Therefore, the knowledge of the action of genes specifically located between bands 21q22.1 to qter of chromosome 21 is of capital importance. The alterations in DS probably result from the overexpression of a group of genes that alter the integration of gene expression (Rapoport, 1988). Among the genes that are likely to affect CNS function if overexpressed we can mention: Cu/Zn superoxide dismutase (Sod1), amyloid precursor protein (App); kainate receptor subunit (Grik1); cytokine receptor, family 2 (Crfb4), α and β interferon receptor (Ifnar1), Drosophila single-minded homologue (Sim2); Drosophila minibrain homologue (Mnb); inward rectifying K^+ channels (Kcnj6/Girk2 and Kcnj15/IRKK); Na^+/myo-inositol cotransporter (Slc5a3), and synaptojanin-1 (SYNJ1). In spite of the critical contribution of the mapping of human chromosome 21 and murine 16, the specific cellular and physiological implications of the excess gene products in the overall syndrome remains obscure. Our group has made fundamental contributions to clarify cell membrane-related mechanisms in both human and murine trisomic neurons, which currently apply to processes related to mental retardation and muscle hypotonia (Ault et al., 1989a,b; Caviedes et al., 1990, 1991, 1995; Fiedler et al., 1994). At present, there is documented evidence that relates the overexpression of certain DS-related genes to these and other membrane-related mechanisms. Among these, Sod1, App and Scl5a3 relate to general cellular mechanisms that can affect diverse membrane function, whereas Grik1 would primarily alter glutamatergic transmission. A detailed discussion is presented below.

(A) *Sod1:* It is certainly one of the most thoroughly studied. This gene encodes for the enzyme Cu/Zn superoxide dismutase (Sod1) (Epstein et al., 1985; Heizmann and Braun, 1992), which catalyzes the conversion (dismutation) of superoxide radicals to H_2O_2 and O_2. This gene is reportedly overexpressed at the mRNA level by 1.4 fold in Ts16 (Stoll and Galdzicki, 1996) and in the murine partial trisomy, Ts65Dn (Holtzman et al., 1996). This agrees with our previous results in the cortical trisomic line, CTb (Arriagada et al., 2000; Mendoza et al., 2002) and trisomic

spinal cord, MTh (Cárdenas et al., 2002a), who overexpress Sod1 mRNA by similar proportion, compared to normal lines. An overexpression of Sod1 could determine greater production of H_2O_2, which in turn is a precursor of hydroxyl radicals (Brooksbank and Balazs, 1984; Brooksbank et al., 1988). Such radicals are capable of altering the function of a vast number of biomolecules (Double et al., 2000; Matarredona et al., 1997; Mohanakumar et al., 1998). At the cell membrane, hydroxyl radicals induce lipoperoxidation in the cell membrane, altering the function of membrane molecules (i.e. ionic channel kinetics, neurotransmitter receptor function) (Cox et al., 1980; Yoshikawa and Kuriyama, 1980), and directly activate guanylate cyclase (Epstein et al., 1985; Yoshikawa and Kuriyama, 1980). The latter could affect α-adrenergic, cholinergic, histaminic (Snider et al., 1984) and glutamatergic metabotropic membrane receptor function (Siarey et al., 1997). Nevertheless, it should be noted that our studies in a genetically modified mouse model, which overexpresses this gene by 50%, revealed normal action potentials of action in neurons cultured from these animals (Ault et al., 1989b). Again, this reaffirms the notion that the overexpression of a single gene in a normosomic subject is not sufficient to produce the alterations described, and that the effect of this and other genes must be evaluated in a new "steady-state", where potentially all genes of the DS region are in triplicate and can exercise mutual interactions that can trigger the syndrome.

(B) *App:* This gene encodes for the amyloid precursor protein, App, a protein that is linked to Alzheimer's disease neurodegenerative mechanisms, and whose gene is present in human autosome 21, and which is reportedly overexpressed in DS (Busciglio et al., 2002; Hartmann et al., 1997; Hosoda et al., 1998). The progressive cerebral dysfunction observed in DS and Alzheimer's disease is manifested by (i) the formation of extracellular amyloid deposits in the form of senile plaques in brain regions with greater neurodegeneration (Finch and Cohen, 1997), (ii) diffuse aggregates in regions with little or no degeneration and (iii) microvascular amyloid deposits (Finch and Cohen, 1997; Haass et al., 1992a,b). The amyloid fibrils are composed of a protein of 39–43 residues called β amyloid (Aβ), a fragment derived from App processing (Selkoe et al., 1996). App is a type I integral membrane protein that possesses a short intracellular carboxyl intracellular fragment (Figueiredo-Pereira et al., 1999) and a large extracellular fragment (Sisodia and Price, 1995). In the region spanning the transmembrane segment and the first 17–18 extracellular aminoacid residues is the so called Aβ segment, which undergoes β-pleated sheet conformation when completely released by enzimatic cleavage. The processing of App is made by enzymes called secretases that are ubiquitously situated in different subcellular compartments. These enzymes produce a secretable peptide (sAppα or sAppβ) which varies in length depending of the secretase involved in the intracellular processing. These enzymes cleave App in different portions, in relation to the App

segment involved in the generation of the Aβ peptide. In this way, α-secretase cleaves APP between the residues 17–18 of the βA4 sequence (Esch et al., 1990), β-secretase in the amino terminal end of the βA4 sequence and the γ-secretase in the transmembrane region comprising the carboxyl terminal end of the βA4 sequence (Fu et al., 1998; Kowall et al., 1992; Fukuchi et al., 1992; Louzada et al., 2001; Pike et al., 1991a,b; Price et al., 1992; Shoji et al., 1992; Yankner et al., 1990). These enzymes have discrete intracellular localizations: α-secretase is mainly located in cell surface while β-secretase have been located in compartments corresponding to the endosomal–lysosomal pathway, trans-Golgi Network etc. The amyloidogenic processing refers to the generation of Aβ peptide and secreted APP (sAppβ) by the action of the β- and γ-secretase, whereas the non-amyloidogenic pathway is referred to the action of α-secretase and the generation of secreted, soluble Apps (sAppα) and carboxyl terminal fragments associated to the plasma membrane. When App is overexpressed, a potential overload of the endosomal–lysosomal pathway, where enzymes responsible of the amyloidogenic pathway are referentially located, could result in excess Aβ production and toxicity. In the mouse, previous studies have shown 2–2.5 times App mRNA overexpression in both Ts16 and Ts65Dn models (Holtzman et al., 1996) compared to normal controls. Also, Ts16 exhibits overexpression of App (Fisher and Oster-Granite, 1990). Again, this agrees with our previous results in the CTb line (Arriagada et al., 2000; Mendoza et al., 2002), which overexpresses this protein and accumulates it in intracellular, vacuole-like compartments, where important metabolic pathways have been described (Cole et al., 1989; Cook et al., 1997; Coughlan and Breen, 2000; Hartmann et al., 1997; Hartmann, 1999; Koo et al., 1996; Scott Turner et al., 1996; Skovronsky et al., 1998; Tomita et al., 1998). Relating its effects on membrane function, increased APP in DS neurons has been also linked to lipoperoxidation (Begnia et al., 2003). Pollard et al. (1995) described that the Aβ peptide forms Ca^{2+} channels in artificial bilayers, with conductances in the order of 400–4000 pS (Arispe et al., 1993; Arispe et al., 1994; Kawahara et al., 2000). A channel of such conductance would be capable of dissipating the Ca^{2+} gradient quickly, altering the intracellular homeostasis of the cation, which can deeply impair cell function. Interestingly, our cortical trisomic line CTb presents high intracellular basal Ca^{2+} levels compared to normal controls (Cárdenas et al., 1999). It is then tempting to speculate that this mechanism could be operating in CTb cells, a hypothesis which deserves attention. Also, altered APP metabolism in DS impairs mitochondrial function, which could render cell more susceptible to oxidative stress and alter Ca^{2+} homeostasis (Busciglio et al., 2002). Further, the protein reportedly has a role in cholinergic dysfunctions (Giovannini et al., 2002; Hunter et al., 2003; Kar et al., 1996, 1998), which appear similar to those documented in Ts16 central neurons in primary culture (Fiedler et al., 1994) and in our CTb and HTk cell lines

(Allen et al., 2000; Cárdenas et al., 2002b). This postulates App overexpression as another potential target to study in our CNS lines. Finally, recent results indicate that the use of phenol-derived compounds diminish the accumulation of extracellular amyloid (De Felice et al., 2001), and also in the vacuoles of CTb cells (Paula Lima et al., 2002). This presents these cells as a possible model to evaluate therapeutic agents that reduce the amyloid content in the cell.

(C) *Slc5a3:* Encodes for the Na^+/myo-inositol cotransporter. Its overexpression determines increased myo-inositol content in the cell, which has been described in brains of Ts16 and Ts65Dn mice, and in human DS. The latter also has increased myo-inositol content in the cerebrospinal fluid (Huang et al., 2000; Shetty et al., 1996). Myo-inositol is a precursor of the phosphatidylinositol (IP) and 4,5-phosphatidylinositol diphosphate (PIP), which participates in the phosphoinositide cycle. PIP is hydrolyzed by phospholipase C to produce diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP3). DAG is activated by the protein kinase C (PKC) and is a substrate of the DAG lipase that generates araquidonic acid. On the other hand, IP3 induces Ca^{2+} release from intracellular stores, which in turn regulates various kinases and phosphatases. Therefore, the presence of high levels of myo-inositol in the nervous system can modify the phosphoinositide cycle and stimulate PKC and Ca^{2+}/calmoduline kinase II by excess DAG and IP3, respectively. This could deeply alter the kinetic properties of ionic channels such as Na^+ and Ca^{2+}, which are subject to PKC regulatory mechanisms (Costa and Catterall, 1984; Rossie et al., 1987). Other cell processes linked to metabotropic glutamate, glutamate triggered intracellular Ca^{2+} release) (Kirischuk et al., 1999) and cholinergic function (Harvey et al., 2002; Yao et al., 2000) may also be affected by Slc5a3 overexpression. In this regard, preliminary results in our laboratory indicate that the lines CTb and HTk possess an increase of PKC phosphorylation. Considering the deregulation of basal Ca^{2+} levels present in the CTb line, the contribution of this transporter in DS related neuronal dysfunction certainly deserves study.

(D) *Grik1:* In human chromosome 21 and murine 16, there is one gene that encodes for a glutamatergic receptor subunit, which is reportedly overexpressed. This gene, named Grik1, encodes for subunit 5 of the kainate receptor (Bettler et al., 1990; Gregor et al., 1993), which is important in the activation of NMDA receptors. Glutamate is the most widely distributed excitatory neurotransmitter in the CNS, and acts on ionotropic and metabotropic receptors. In the hippocampus of adult DS subjects, a significant deficit of glutamate has been described (Reynolds and Warner, 1988), and receptors are overexpressed in various territories of the brain (Arai et al., 1996; Oka and Takashima, 1999). Among them, the *N*-methyl-D-aspartate ionotropic receptor (NMDA) is important in mechanisms related to synaptic plasticity and long term potentiation (LTP) (Collingridge et al., 1983), two cell mechanisms that are key in learning and memory. Grik1 has a role in noci-

ception (Simmons et al., 1998), which is reportedly altered in the Ts65Dn partial trisomy mouse model (Martínez-Cué et al., 1999). An overexpression of this protein can alter the response of these receptors to their specific agonists, and also affect the expression of Na^+ and K^+ channels, which would alter the action potential (Ishikawa et al., 1999). In this regard, our cell lines derived from trisomic animals manifest responses to glutamatergic stimuli that differ from those derived from normal animals (Allen et al., 2002; Cárdenas et al., 1999, 2002a,b). Briefly, our CTb line, presented with stimulation by glutamate, shows a greater amplitude and slower decay in intracellular Ca^{2+} increases in comparison to normal CNh cells (Cárdenas et al., 1999). At the same time, our line derived from trisomic hippocampus HTk line, also exhibits slower decay kinetics in response to glutamate when compared with the H1b normal control line (Cárdenas et al., 2002b), in a manner similar to that observed in Ts16 hippocampal neurons kept in primary culture conditions (Schuchmann et al., 1998). This suggests that the pathophysiological mechanism that alters the function of these receptors is reproduced in our trisomic lines. Grik1 overexpression could alter membrane excitability by increased sensitivity to glutamate in brain and spinal cord (Martínez-Cué et al., 1999), which would contribute to the alterations described.

5. Conclusion

Due to the above, we believe that the study of the relationship of these genes and others with glutamatergic and cholinergic dysfunction is of great relevance to the neuronal pathophysiology in DS. Further, with the use of antisense or siRNA techniques, we have a unique opportunity to verify the specific contribution of individual genes in these models in a context of a generalized overexpression, which could provide very pertinent information at the moment of proposing therapies and identifying potential drug targets. Each of the aforementioned models offers significant potential for evaluation of therapeutic candidates.

Acknowledgements

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