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Age-Related Alteration of PKC, A Key Enzyme in Memory Processes

Physiological and Pathological Examples

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Abstract

Brain aging is characterized by a progressive decline of the cognitive and memory functions. It is becoming increasingly clear that protein phosphorylation and, in particular, the activity of the calcium-phospholipid-dependent protein kinase C (PKC) may be one of the fundamental cellular changes associated with memory function. PKC is a multigene family of enzymes highly expressed in brain tissues. The activation of kinase C is coupled with its translocation from the cytosol to different intracellular sites and recent studies have demonstrated the key role played by several anchoring proteins in this mechanism. PKC-phosphorylating activity appears to be impaired during senescence at brain level in a strain-dependent fashion in rodents. Whereas the levels of the various isoforms do not show age-related alterations, the enzyme translocation upon phorbol-ester treatment is deficitary among all strains investigated. Anchoring proteins may contribute to this activation deficit. We discuss also modifications of the PKC system in Alzheimer's disease that may be related to pathological alterations in neurotransmission. A better insight of the different factors controlling brain-PKC activation may be important not only for elucidating the molecular basis of neuronal transmission, but also for identifying new approaches for correcting or even preventing age-dependent changes in brain function.

Index Entries: PKC; RACK proteins; brain; aging; Alzheimer's disease.

Introduction

Several cellular processes are regulated via interaction of neurotransmitters/hormones with membrane receptors leading to the activation of the calcium-phospholipid dependent-protein kinase C (PKC) that initiates a cascade of reactions allowing different biological responses. PKC activation is associated with its translocation from the cytosolic pool

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to different intracellular sites, and some investigations have demonstrated the contribution of protein–protein interactions to this process (Mochly-Rosen et al., 1991). PKC plays a key role in modulating neuronal functions such as ion-channel fluxes, receptor desensitization, neurotransmitter release (Tanaka and Nishizuka, 1994). Even though the involvement of a variety of phosphorylating enzymes, e.g., the calcium-calmodulin kinase II (α-CaMKII), the cAMP kinase (PKA), and the nonreceptor tyrosine kinase (Fyn) in learning and memory processes has been widely documented (Silva et al., 1992; Grant et al., 1992; Schwartz, 1994; Izquierdo, 1994; Malenka, 1994), behavioral and pharmacological evidences suggest the relevant contribution of kinase C in memory processes (Wehner et al., 1990; Noguès et al., 1994; Pascale et al., 1994) as well as in long-term changes in synaptic potentiation (LTP) and depression (LTD) (Bliss and Collingridge, 1993; Sacktor et al., 1993; Hrabetova and Sacktor, 1996). Therefore, alterations in neuronal function and in memory mechanisms, observed in physiological or pathological aging, might reflect age-dependent changes in PKC system. The present review will provide a summary of the mechanisms involved in PKC activation and analyze PKC changes in physiological aging and in an age-associated disease characterized by memory disorders (i.e., Alzheimer's disease).

PKC: Structure and Activation

PKC is a family of serine-threonine phosphorylating enzymes distinguished on the basis of calcium-ion requirement into calcium-dependent (conventional PKCs: α, βI, βII, and γ) and calcium-independent (novel PKCs: δ, ε η, θ, and μ; atypical PKCs: ζ, ι, and λ) isoforms (Nishizuka, 1988, 1992; Dekker and Parker, 1994). The primary aminoacidic structure of PKC, deduced from available cDNA sequences, comprises conserved regions (C1–C4) separated by variable domains (V1–V5). Calcium-independent isozymes lack C2 region, although in novel PKCs a C2-like domain has been described (Newton, 1995). The C1 region contains, near the amino terminal, a pseudo-substrate sequence followed by a double cys-rich motif constituting the diacylglycerol/phorbol ester-binding site. The pseudo-substrate sequence is responsible for maintaining the enzyme in the inactive form (folded conformation) in the absence of physiological activators (e.g., calcium, diacylglycerol, phosphatidylserine).

The C2 domain represents the recognition site for calcium ion and phosphatidylserine, although some studies have additionally indicated the importance of V1 and C1 regions in mediating this interaction (Moisor and McLaughlin, 1991; Luo et al., 1993). Finally, C3 and C4 contain the ATP and the substrate-binding sites, respectively. The regulatory (N-terminal) and the catalytic (C-terminal) subunits are separated by an hinge region (V3) that is sensitive to proteolytic cleavage leading to the production of a constitutively active kinase (PKM) (for a review, *see* Stabel and Parker, 1991; Hug and Sarre, 1993; Newton, 1995; Nishizuka, 1995). The interaction of PKC with cofactors allows the activation of the enzyme by means of the opening of the folded conformation, reducing the affinity of the pseudo-substrate domain for the catalytic site that can therefore exert its phosphorylating activity (Fig. 1). This activation mechanism is related to translocation of PKC from the cytosol to different intracellular sites (Kraft and Anderson, 1983) and the translocated enzyme has been associated with phosphorylation of specific substrates and with regulation of PKC activation (Stabel and Parker, 1991; Tanaka et al., 1994). In the past, lipid–lipid interactions were considered the only determinant for driving PKC translocation, but studies on trypsin sensitivity of PKC binding to plasma membrane (Mochly-Rosen et al., 1991) have emphasized the additional role of protein–protein interactions in the mechanism of PKC activation. Recent studies have characterized a number of PKC-binding proteins that are present in different subcellular structures. Among these proteins, PSPB (PKC substrate-binding pro-

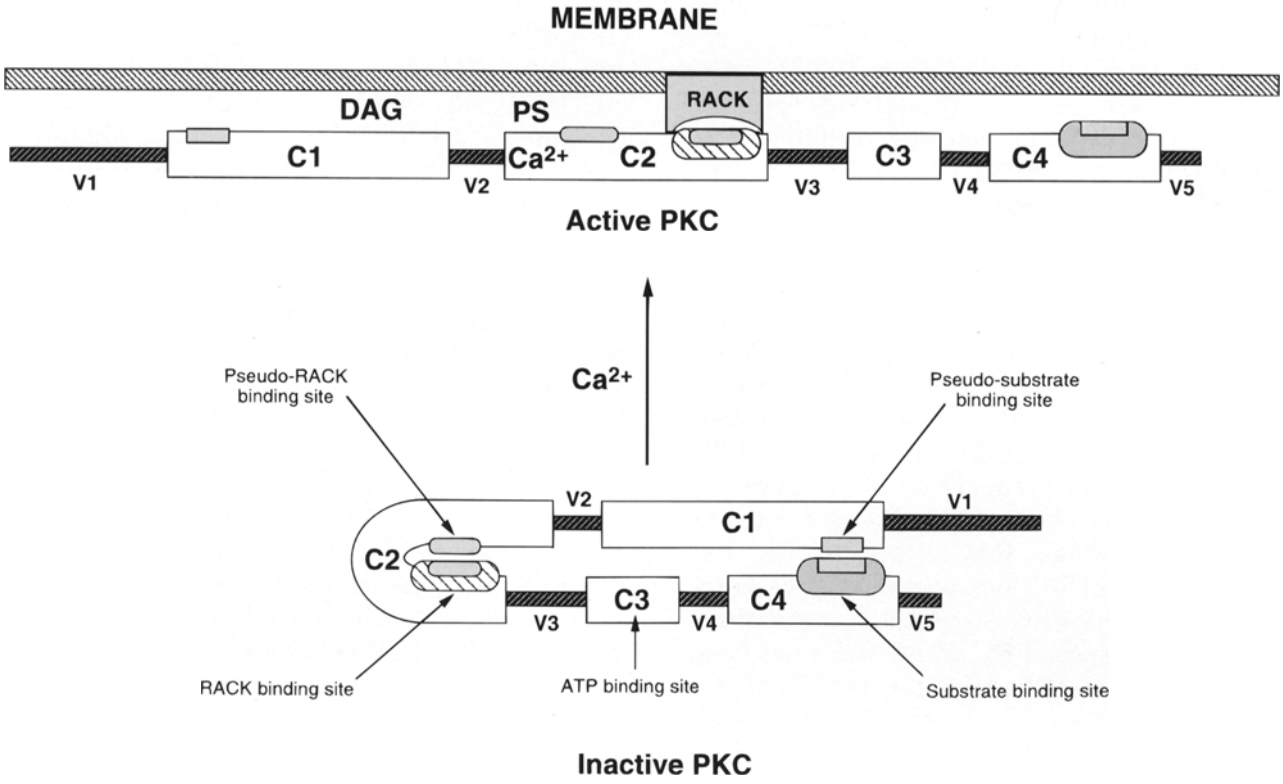

Fig. 1. Schematic picture of the PKC activation process: The figure represents the differents domains characterizing PKC structure. The interaction of kinase C with the physiological activators (Ca^{2+}, diacylglycerol [DAG] and phosphatidylserine [PS]) opens the "folded conformation" through inhibition of interaction of the pseudo-substrate region with the substrate binding site. The figure also depicts the additional role played by RACK proteins in driving the translocation process of PKC from the cytosol to the membrane compartments (*see text*).

teins) interact with PKC through a PS bridge in the regulatory region, whereas the perinuclear PICKs (proteins interacting with C kinase) bind the catalytic region of kinase C. A binding site for PKC has been described also in the case of AKAP79 (A kinase anchoring protein 79) (Faux and Scott, 1996). Finally, a class of proteins generally referred to as receptors for activated C kinase (RACKs) has been characterized and, among all PKC-binding proteins are the only members demonstrated important for PKC-mediated functions (Mochly-Rosen, 1995).

RACK Proteins

RACK are 30- and 36-kDa proteins located in cytoskeletal compartments firstly described in rat heart by Mochly-Rosen et al. (1991). PKC binding to RACKs is dependent on the presence of kinase C activators in a specific and saturable manner (Mochly-Rosen, 1991; Robles-Flores and Garcia-Sainz, 1993) and in vivo data suggest the role of RACK proteins in driving PKC translocation following its activation (Ron et al., 1995). In vitro PKC does not interact with RACKs via the substrate binding site in the catalytic domain, since an excess of substrate peptide does not inhibit this interaction: On the contrary, as suggested by Mochly-Rosen's group, at least part of the C2 region could be involved in binding RACK proteins. In analogy with the pseudo-substrate domain, it has been demonstrated that the presence in the PKC structure of a pseudo-RACK sequence associated in an intramolecular interaction with the

RACK-binding site when PKC is inactive (Mochly-Rosen et al., 1995).

The in vivo role of RACKs in PKC function has been emphasized by studies utilizing *Xenopus* oocyte. Insulin induces oocyte maturation through the translocation of PKC from the cytosol to the particulate fraction. Microinjection of purified rat-brain RACKs both inhibits PKC translocation and delays oocyte maturation, suggesting that exogenous RACKs compete with the endogenous receptors, preventing PKC translocation (Smith and Mochly-Rosen, 1992).

RACK1 is the best characterized member of this family of proteins, but in spite of its cloning from a rat-brain cDNA library, most of the studies have been performed up to now in nonneuronal tissues. RACK1 seems to preferentially interact in vivo with PKCβ as compared with other PKC isozymes. A more direct correlation between PKC and RACK1 has been recently demonstrated in cardiac myocytes where phorbol ester-activated PKCβ colocalizes with RACK1 (Ron et al., 1995), strenghtening the key involvment of RACKs in PKC-mediated functions. Recent investigations also indicate that PKCε may be anchored in cardiac tissues by another component of this family referred to as RACK2 (Mochly-Rosen, personal communication). The site of interaction with PKC has been mapped on the V1 region because a peptide derived from this region inhibits specifically the translocation of PKCε in both cardiac (Johnson et al., 1996) and pancreatic islet cells (Yedovitzky et al., 1997). PKC isoform-specific binding proteins seem therefore to localize kinase C to subcellular sites.

PKC Involvement in Memory Processes

Memory processes allow organisms to keep experiences. Long-term changes in the efficacy of cell-to-cell communication (long-term potentiation and depression) are assumed to reflect persistent biochemical and morphological alterations characterizing encoding mechanisms that are believed to underlie learning and memory. The role of long-term potentiation (LTP), firstly described in 1973 in the hippocampus by Bliss and Lφmo, in-memory storage has been widely explored. In this synaptic model of memory, brief trains of high-frequency stimulation to monosynaptic excitatory pathways in tissue slices produce a sustained increase in the efficiency of synaptic transmission (for reviews, *see* Bliss and Collingridge, 1993; Izquierdo, 1994).

Several studies have demonstrated the involvement of PKC both in the induction and in the maintenance phases of LTP (Bliss and Collingridge, 1993; Colley and Routtenberg, 1993; Sacktor et al., 1993). In particular, the role of PKCγ, a brain-specific isoform, in LTP has been analyzed. Messenger RNA of PKCγ increases after LTP (Thomas et al., 1994) and mice deficient in this isozyme present an aberrant LTP, although they show only very mild disturbances in spatial and contextual learning (Abeliovich et al., 1993), which questions the importance of this isoform in these particular learning paradigms. In addition, following LTP there is an increased phosphorylation of B-50/GAP-43 (De Graan et al., 1988; Gianotti et al. 1992; Leahy et al., 1993; Ramakers et al. 1995), one of the major substrates of PKC in the nervous system (Gispen et al., 1991), that has been correlated to the modulation of neurotransmitter release that plays a prominent role in LTP mechanism (Dekker et al., 1989; De Graan et al., 1991). Recent observations indicate that kinase C is also involved in LTD phenomena. It has been suggested that PKC content may decrease in LTD and increase in LTP, hypothesizing a bidirectional control mechanism in synaptic plasticity particularly evident for PKMζ (Hrabetova and Sacktor, 1997).

The processes of memorization in the intact animal have confirmed and strenghtened the concept of PKC as being involved in memory functions. Investigations on the marine snail *Hermissenda crassicornis* first demonstrated the critical role of PKC in neuronal changes in excitability correlated to associative learning

(Alkon, 1989). Pavlovian conditioning also induces PKC translocation in rabbit hippocampus (Olds et al., 1989). Further behavioral studies indicate that spatial memory is also related to the PKC system (Wehner et al., 1990; Van der Zee et al., 1992; Noguès et al., 1994). In accord with this concept, intraventricular injection of phorbol esters (PKC activators) improves spatial learning performance in rodents (Paylor et al., 1991), whereas injections of kinase C inhibitors impairs this behavior (Mathis, 1992). Moreover, PKC activity is reduced in hippocampus of poor spatial-learner mice (Wehener et al., 1990) and this is caused by decreased levels of PKCγ (Bowers et al., 1995). In line with these studies, pharmacological approaches demonstrated that in-brain tissues transient PKC activation is observed with nootropic drugs (Lucchi et al., 1993; Pascale et al., 1994) at doses facilitating cognitive processes (Spignoli and Pepeu, 1986; Lopez et al., 1991), further supporting the importance of kinase C in memory and suggesting its possible pharmacological modulation in altered mnemonic states.

Aging

Morphological analysis reported in older studies indicate that senescence is associated with neuronal loss in cortical structures: remaining neurons compensate these alterations by a wide dendritic arborization until the degeneration exceedes a threshold, thus manifesting the damage. Also the hippocampus appears to be a cerebral area particularly sensitive to physiological decline both morphologically and functionally, in fact neurobiological measures significantly correlate with the severity of spatial learning impairment in rats (Ingram et al., 1981; Gallagher et al., 1988). In agreement with these data, a significant correlation was found between the duration of LTP and a rat's performance. In fact, old animals showing the poorest behavior on spatial tasks also showed the fasted decay in synaptic enhancement (Barnes, 1979); moreover differences in decay rate of LTP in aged and adult rats are similar to differences in the forgetting rate on a spatial memory protocol (Barnes and McNaughton, 1985). The rapid decay in LTP in aged animals could be caused by either a loss in the capacity to actively maintain synaptic enhancement or, alternatively, to increased mechanisms for actively depressing potentiated synapses (Norris et al., 1996). However, recent advances in total neuron number evaluation with stereological techniques applied to aging studies have disputed previous data. Research on physiological aging in human tissues have suggested that, at least for the cerebral cortex, neuronal loss may be less than previously hypothesized. In fact, in normal brains no age-related differences in cell numbers between ages 60 and 90 have been observed in the enthorinal cortex nor in the superior temporal sulcus, an area belonging to the neocortex structure (for a discussion, *see* Wickelgren, 1996). Also, no neuronal decline is present at hippocampal level of aged rats showing age-dependent deficits in water-maze task, providing evidence that neuronal degeneration in the hippocampus is not correlated to behavioral impairments observed during senescence (Rapp and Gallagher, 1996).

It therefore appears that although cortical and hippocampal cells may not degenerate during aging, other modifications such as those implying changes in signal transduction might affect cerebral functions. Accordingly, increasing evidence suggest that alterations associated with the senescent brain involve a number of neurotransmitter systems leading to a modified interneuronal communication that may therefore represent rather than morphological changes the primum movens towards cognitive deficits. The cholinergic system has been extensively investigated and initial clinical and pharmacological research led Bartus to formulate the cholinergic hypothesis of geriatric memory dysfunction (Bartus et al., 1982). However several pathways have been demonstrated to be deficitary during senescence (Agnati et al., 1990; Fulop and Seres, 1994) and the results of such studies indicate that signal-transduction changes occur with aging. Because protein ki-

nases play a critical role in converting extracellular signals to biological responses, it is likely that alterations in kinase function might directly contribute to neuronal dysfunction in aging.

Age-Related Alterations of PKC Signal Transduction

The observation that PKC activity is closely associated with the cell-membrane lipid activators and with the availability of free intracellular calcium levels suggests that age-related alterations in membrane constituents and properties, in calcium and lipid metabolism may affect the action and the cellular functions of this enzymatic family (Oestreicher et al., 1986; Gibson and Peterson, 1987; Undie et al., 1995).

In keeping with this notion in cortical structures of aged (24–28 mo old) Fisher 344 (Friedman and Wang, 1989; Meyer et al., 1994) and Sprague-Dawley (Battaini et al., 1990) rats, the calcium-dependent PKC activity is reduced both in soluble and in particulate fractions when compared with middle-aged (12–15 mo old), adult (5–8 mo old), and young (3–4 mo old) animals. On the other hand, unmodified PKC activity values in aged Wistar rats were observed (Battaini et al., 1993), suggesting the presence of strain-related changes. In cortex of Wistar strain, no variations were observed both in activity and in protein levels of calcium-independent isoforms (Fig. 2) whose differential temporal sensitivity to activation (Pascale et al., 1996a) and importance in brain function and in memory phenomena has been recently emphasized (Sacktor et al., 1993; Tanaka and Nishizuka, 1994).

In the described studies, PKC activity measurements were assessed in vitro under optimal conditions of activators and substrates, but, as pointed out, this may not be the physiological milieu during senescence. Within this context, the analysis of the functional response of PKC system upon activation in terms of enzyme translocation from soluble to membrane compartments might be more physiologically relevant.

Translocation of calcium-dependent kinase C activity upon phorbol-ester treatment of tissues slices has been explored in physiological rat brain aging and reported to be impaired (Fig. 3A) among all strains investigated (Friedman and Wang, 1989; Battaini et al., 1993, 1995). The translocation mechanism has been recently observed to be deficitary also concerning calcium-independent activity in cortical tissues of aged Wistar rats (Fig. 3B; *see also* Pascale et al., 1996b). The phorbol esters challenge bypass-receptor activation and the following diacylglycerol production, suggesting that the translocation deficit is independent of neurotransmitters levels, receptor availability, and second-messenger production that may or may not be modified as a consequence of senescence. Therefore in spite of strain-related changes in basal PKC activity, the translocation process appears to be the common component of the kinase C system sensitive to aging.

Since several investigations, mostly performed in nonneuronal tissues and cells, suggest that RACK proteins play a key role in PKC activation and in membrane anchoring, subsequent investigations have been focused on the analysis of RACK1 protein content at brain level by immunoblot studies in animals of different ages. We have shown that cortical slices from aged rats (24–28 mo old) have reduced levels of RACK1 protein (approx 50%) in comparison with those observed in adult (5–8 mo old) and middle-aged (12–15 mo old) animals (Pascale et al., 1996b). In vitro RACK1 is reported to interact with PKC β, δ, and ε (Ron et al., 1994) and therefore we have investigated the translocation of these isoforms in aged cerebral tissues. The translocation of these isozymes, evaluated both as immunoreactivity and activity, has been shown to be deficient in senescent rats (Battaini et al., 1995; Pascale et al., 1996b). These observations further support the involvement of RACK proteins in the PKC-translocation process, suggesting that in aged animals, PKC may not find the appropriate milieu for interaction and anchoring with the membrane and therefore is not able to bind to membrane upon activation. Moreover, the

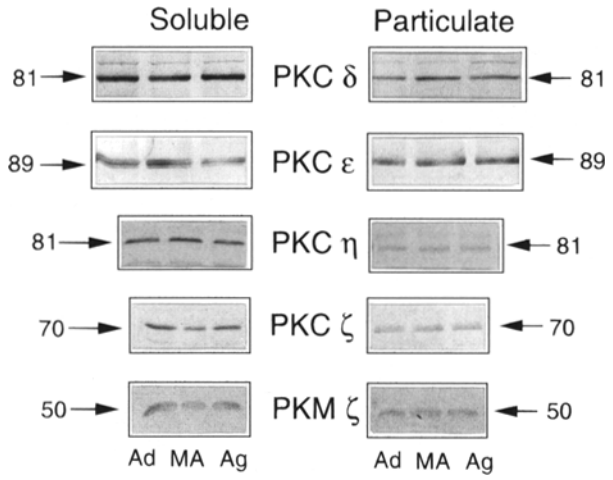

Fig. 2. Representative Western analysis of calcium-independent isoforms during aging: Aliquots of cortical tissues from at least four rats dissected from adult (Ad), middle-aged (MA), and aged (Ag) animals were pooled. Ten μg of proteins from either soluble and particulate fractions were electrophoresed, electroblotted to nitrocellulose, and incubated with specific antisera as detailed previously (Battaini et al., 1994). The arrows refer to the specific immunoreactive band for each of the different PKCs and the molecular weights are reported in kDa.

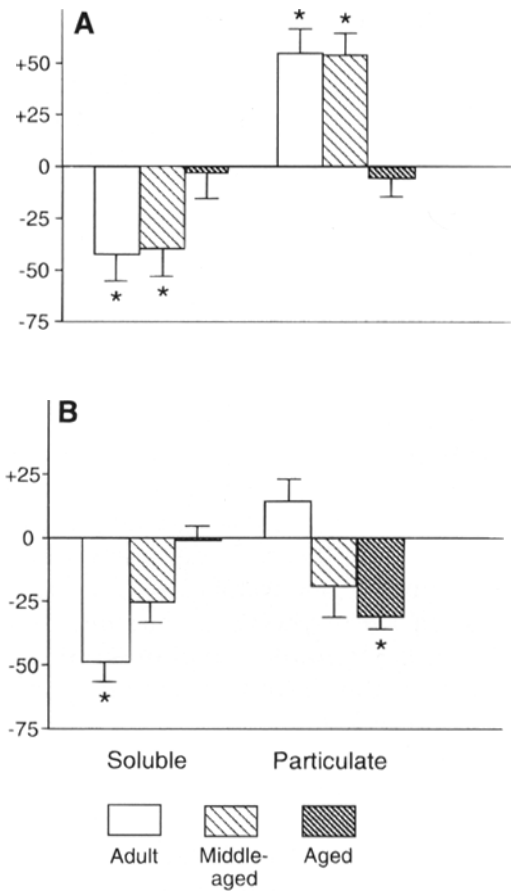

Fig. 3. Calcium-dependent and calcium-independent PKC translocation during aging in rat cortical tissues. Calcium-dependent **(A)** and calcium-independent **(B)** PKC activity translocation in response to in vitro phorbol esters (PMA) in rats of different ages. Data are mean ± SEM (bar) values from 7–9 individual measurements. Control PKC phosphorylating activities in soluble and particulate fractions of adults were 2.3 ± 0.13 and 2.4 ± 0.25 **(A)** and 2.3 ± 0.55 and 3.01 ± 0.66 **(B)** nmol/min/mg protein, respectively. These values did not significantly change as a function of age. All values are expressed as a percent response to PMA with respect to the relative control value (0%). Calcium-dependent and calcium-independent PKC activities were assayed utilizing histone H1 and peptide (Ser ^{25}) PKC_{19-36} as substrates, respectively. *$p < 0.01$ PMA vs vehicle group.

complete loss of PKC translocation (both calcium-dependent and calcium-independent) in aged animals in spite of a 50% decrease in RACK1 levels indicates that other anchoring proteins may be affected during senescence. The levels of other RACKs, such as the previously mentioned RACK2, that is specific for PKCε, or of other anchoring proteins for PKC that have not been investigated as yet during aging could further clarify selectivity of anchoring proteins for PKC isoforms. The in vivo specificity of interaction of RACK1 with PKCβ, demonstrated in cardiac tissues, is not known in neuronal tissues and cannot be verified by our observations. In addition to anchoring proteins, other factors may contribute to the deficit in PKC translocation: Free radicals may be contributors to this deficit. In fact, in vitro peroxidation impairs PKC translocation in young animals (Meyer et al., 1994) and the chronic exposure to spin-trapping compounds can restore the blunted phorbol ester-dependent phosphorylation of synapsin I in aged brain tissues (Eckles et al., 1995).

Alzheimer's Disease

Alzheimer's disease (AD), the major form of senile dementia, is characterized by gradual deterioration of cognitive function and memory coupled with widespread neuronal death. The basal forebrain cholinergic system, brain stem monoaminergic systems, anterior thalamic limbic circuit, amygdala, hippocampus, and neocortex appear mostly sensitive to neurodegeneration (reviewed in Price and Sisodia, 1994).

Microscopic hallmarks of AD are neurofibrillary tangles and neuritic plaques, the latter mainly constituted by β-amyloid (βA). Neurofibrillary lesions contain paired helical filaments (PHFs) that are derivatized forms of abnormally phosphorylated central nervous system tau proteins. It has been suggested that this aberrant phosphorylation might depend on the failure of protein phosphatases (Trojanowski and Lee, 1995).

βA deposits are generally assumed to contribute to progressive neurodegeneration in the disease. βA is a 39–43 amino acid protein with a molecular weight of 4 kDa, derived by an aberrant proteolytic cleavage of a much larger precursor referred to as APP (amyloid protein precursor; *see* Bush et al., 1992; Cordell, 1994). The metabolism of the precursor can occur through two principal pathways: not amyloidogenic or amyloidogenic. The former leads to the secretion of the soluble fragment of APP (APPs) by means of the α-secretase enzyme that cuts APP within the βA sequence, the latter generates intact βA fragment through the intervention of β-secretase and γ-secretase enzymes. The APP gene is expressed at high levels in brain, but it is also present in many peripheral tissues. APP is an integral transmembrane protein whose biological function is still unclear and it is possible that individual proteolytic derivatives of APP are involved in distinct pathways. Evidences indicate that APP promotes cell–cell and cell–surface adhesion (Breen et al., 1991) as well as neurite outgrowth (Milward et al., 1992).

Synthetic fragments of the βA sequence are reported to have neurotoxic properties in neuronal cell culture (Yankner et al., 1990). In addition, the application of βA aggregate peptides induces a dysregulation of cellular calcium homeostasis resulting in elevation of intracellular calcium levels (Mattson et al., 1992). Recently, it has been hypothesized that free radicals may contribute to the increase of calcium influx observed following βA exposure (Mark et al., 1995; Ueda et al., 1997). Peroxide accumulation and lipid peroxidation may in fact lead to an impairment of plasma-membrane proteins involved in calcium regulation, thus producing a loss of calcium homeostasis and cellular death (Mark et al., 1995, 1997).

Additionally, βA inhibits a specific potassium channel whose function has been observed to be impaired also in fibroblasts from AD patients (Etcheberrigaray et al., 1993).

βA deposition appears also associated with the overexpression of APP in Down's subjects (Armstrong, 1994; Govoni et al., 1996a) that develop histopathology, and often neurological symptoms, that are indistinguishable from AD patients, further supporting the amyloid-cascade hypothesis of AD.

PKC Involvement in AD

Several in vitro studies demonstrate a direct role of PKC in the regulation of APP metabolism. In fact, phorbol esters increase the secretion of soluble APP (Gillespie et al., 1992) and reduce the release of βA (Buxbaum et al., 1993), suggesting a PKC involvement in nonamyloidogenic pathway.

AD brain presents both reduced kinase C activity and a deficitary PKC translocation in cortical and hippocampal structures (Wang et al., 1994). PKC levels are extremely low in AD brain (Cole et al., 1988) as measured by radioactive-phorbol-ester binding as well as by immunochemical analyses (Masliah et al., 1990). In particular, PKCβ (Shimohama et al., 1993) and PKCε (Matsushima et al., 1996) have been observed to be decreased in AD temporal cortex. The importance of PKCβ (βII) in the pathogenesis of AD is further pointed out by its colocalization

with diffuse plaques that are regarded as an early AD pathological change (Masliah et al., 1991).

Several biochemical abnormalities associated with the disease are not exclusively observed at brain level, but also in peripheral tissues from AD patients. Within this context, various groups, including ours, have reported a decreased PKC activity in cultured fibroblasts from AD subjects (Van Huynh et al., 1989; Govoni et al., 1993). This deficit is related to a reduced basal secretion of APP as well as to a reduced response at low concentrations (in the range of Kd values for PKC) of phorbol esters (Bergamaschi et al., 1995). PKCα, the only calcium-dependent isoform detected in human fibroblasts (Racchi et al., 1994), is significantly decreased in fibroblast cell lines prepared from AD patients (Govoni et al., 1996b). Skin fibroblasts from AD patients also present decreased levels of cp20 protein that is a PKC substrate involved in associative learning (Nelson and Alkon, 1995). In fact, the incubation of control fibroblasts with low concentration of βA reproduced the AD phenotypes for cp20 (Kim et al., 1995), suggesting that substrate alterations may contribute to the deficitary function of PKC system. It might be hypothesized that in AD patients, βA exposure could alter the kinase C system. In agreement with this concept, it has been reported in rat brain that low micromolar concentrations of βA stimulate and high micromolar βA concentrations inhibit PKC activity (Chauhan et al., 1991). Some researchers have speculated that the reduced levels or activity of kinase C, observed in AD subjects, might also reflect a downregulation mechanism following an initial enzyme overactivation (*see* Jin and Saitoh, 1995). In fact, it has been observed that PKC activators, phorbol esters, induce neurodegeneration in human cerebral cortical cells and that this activation of PKC is also related to increased immunoreactivity toward antibodies that recognize the microtubule-associated protein, tau, in Alzheimer neurofibrillary tangles. Therefore PKC might directly lead to degenerative changes in neurons by means of an excessive phosphorylation of regulatory proteins disrupting their normal functioning (Mattson, 1991).

In line with this hypothesis, PKC inhibitors may prevent neurotoxicity mediated by PKC overactivation (Manev et al., 1990).

The qualitatively similar changes of PKC in fibroblasts and in brain of AD patients support the concept that the analysis of this family of enzymes may represent a useful approach to explore the cellular pathophysiology of AD, further suggesting skin fibroblasts as an interesting model to study this pathology.

Conclusions

The major hallmark of brain PKC function in physiological aging seems to be a deficit in enzyme translocation that is related, in cerebral cortex of Wistar rats, to decreased levels of RACK1 protein. This observation underscores the key emerging role of protein–protein interactions in the activation mechanism of kinase C (for a review, *see* Battaini et al., 1997), suggesting that RACK proteins might represent a new pharmacological target for modulation of PKC function. Within this context, PKC translocation activators/inhibitors have been synthesized on the basis of sequence homologies with the PKC-RACK interaction site (Ron and Mochly-Rosen, 1994).

PKC translocation has been reported impaired in Alzheimer's disease (Wang et al., 1994) and this could be correlated with alterations in the nonamyloidogenic cascade of APP.

A better insight of mechanisms involved in PKC activation may provide a more complete picture of neuronal function and could represent a starting point for new strategies aimed to correct or prevent age-dependent alterations in signal transduction.

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