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Brain Research Reviews 37 (2001) 287–293

BRAIN RESEARCH REVIEWS

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Review

Dehydroepiandrosterone and the relationship with aging and memory: a possible link with protein kinase C functional machinery

Marco Racchi[a,*], Stefano Govoni[a], S. Bruno Solerte[b], Corrado L. Galli[c], Emanuela Corsini[c]

[a]*Department of Experimental and Applied Pharmacology, University of Pavia, Viale Taramelli 14, 27100 Pavia, Italy*
[b]*Department of Internal Medicine, Geriatric and Gerontology Clinic, University of Pavia, Viale Taramelli 14, 27100 Pavia, Italy*
[c]*Department of Pharmacological Sciences, University of Milano, Milan, Italy*

Accepted 30 June 2001

Abstract

A progressive decline of cognitive and memory functions, compared to the average young-life performance, characterizes brain aging. The changes in performance may depend upon altered activity of neurotransmitters acting on attention and memory trace formation (acetylcholine, catecholamines, glutamate, for example) or the failure of the transduction mechanisms linked to receptor activation. One of the fundamental cellular changes associated with brain aging is the alteration of mechanisms involving the activity of the calcium-phospholipid-dependent protein kinase C (PKC). A crucial event for the activation of protein kinase C is 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. The defective activation of PKC-dependent pathways during aging is due to a defective mechanism of translocation of the kinase because of reduced levels of the major anchoring protein RACK-1 (receptor for activated C kinase). Pharmacological strategies aimed at the correction of age-associated memory deficits have been mostly focused on neurotransmitters using direct or indirect agonists. More recently, attention has been paid to the memory enhancing properties of some steroid hormones, namely 'neurosteroids'. Among these the activities of dehydroepiandrosterone (DHEA), pregnenolone (PREG) and their sulfates, have been extensively studied. These neuroactive steroids, can regulate neuronal function through their concurrent influence on transmitter-gated ion channels and gene expression. We addressed the possibility that DHEA, among other neurosteroids, could modulate directly the age-associated impairment of PKC signal transduction and provide experimental evidence that DHEA can revert the alteration of RACK-1 anchoring protein expression.

Theme: Endocrine and Autonomic regulation

Topic: Neuroendocrine regulation: other

Keywords: Aging; Memory; Protein kinase C; Anchoring protein; Dehydroepiandrosterone

Contents

*Corresponding author. Tel.: +39-0382-507-738; fax: +39-0382-507-405.
E-mail address: racchi@unipv.it (M. Racchi).

0165-0173/01/$ – see front matter
PII: S0165-0173(01)00132-1

1. Introduction

The debate whether memory and cognitive function changes during aging are prodromes of an age-associated pathology or expression of a progressive age-related decline has not yet been resolved. Preclinical studies indeed show age-associated changes in the brain, involving impairment of neurotransmission efficiency at the level of signal recognition and transduction in the absence of appreciable neurodegeneration. As an example, laboratory rodents do not present spontaneously amyloid deposition and neurofibrillar degeneration, the neuropathologic markers of human Alzheimer's disease, yet they display age-associated learning and orientation deficits. This observation points to the importance of the age-associated changes in neurotransmission and transduction mechanism which may underlie the age- rather than pathology-associated changes in memory performance. Although most strategies of pharmacologic intervention have focused on boosting the failing neurotransmission, more recently attention has been paid to the memory enhancing properties of neurosteroids.

This review is focused on the possible interplay between these two domains.

2. Protein kinase C: a 'cognitive kinase' needing proper anchoring

In all animals, the processes involved in cognition and memory include the fundamental cellular mechanisms of protein phosphorylation [32]. Among others a major role is played by the calcium-phospholipid-dependent protein kinase C (PKC).

PKC is a multigene family of enzymes with at least 12 different isoforms substantially distinguished in groups on the basis of the requirement of calcium for activation. The groups of isoforms are separated into calcium-dependent (also known as conventional PKCs: α, βI, βII and γ) and calcium-independent, distinguished within the group in novel PKCs: δ, ϵ, η, θ, μ; and atypical PKCs ζ, ι and λ isoforms [13,36]. The primary amino acid structure of PKC, deduced from available cDNA sequences, comprises conserved regions separated by variable domains. One of the conserved regions includes a pseudosubstrate sequence [35], responsible for maintaining the enzyme in the inactive form ('folded conformation') in the absence of physiological activators (e.g. calcium, diacylglycerol, phosphatidylserine). The interaction of PKC with physiological activators allows the opening of the 'folded conformation', in fact, these cofactors are able to reduce the affinity of the pseudo-substrate domain to the catalytic site and induce the activation of the enzyme. This activation mechanism is followed by the translocation of PKC from the cytosol to different intracellular sites [24] and the translocated enzyme has been associated with phosphorylation of specific substrates and with regulation of PKC activation [48]. In the past, lipid–lipid interactions were considered critical for driving PKC translocation, but recent studies have emphasized the role of protein–protein interactions, in particular the involvement of intracellular receptor proteins for activated C kinase (RACKs), in PKC compartmentalization.

RACK proteins are 30–36 kDa proteins located in cytoskeletal compartments initially described in rat heart by Mochly-Rosen et al. (reviewed in Ref. [33]). PKC binding to RACKs is dependent on the presence of PKC activators [33,42], suggesting the pivotal role of RACK proteins in driving PKC translocation following its activation. Similar to what occurs for the interaction of the pseudosubstrate domain of PKC, the presence of a pseudo-RACK sequence associated with the RACK-binding site when PKC is inactive has been suggested [34].

RACK-1, cloned from a rat brain cDNA library, is the best characterized member of this family of proteins. RACK-1 seems to preferentially interact with specific PKC isoforms and in particular PKCβ, most likely in its PKCβII form [44].

Several studies have demonstrated the involvement of PKC in the long-term changes that reflect persistent biochemical and morphological alterations characterizing learning and memory mechanisms. In particular, PKC has been involved both in the induction and in the maintenance phases of Long Term Potentiation (LTP) [47]. Such electrophysiological phenomenon, first described in 1973 in the hippocampus by Bliss and Lømo, reproduces a synaptic model of memory where brief trains of high-frequency stimulation to monosynaptic excitatory pathways produce a sustained increase in the efficiency of synaptic transmission (for a review, see Refs. [10,22]). Various in vivo and ex vivo models have been used to demonstrate the involvement of PKC and its isoforms in LTP (reviewed in Ref. [39]). In addition, behavioral studies indicate that the response to a large variety of memory tasks is related to the PKC functional machinery because hippocampal PKC in mammals is activated by learning [37,49], although, the kind of information processed, the type of task, and the dynamics of learning processes all induce differential changes in the mode of PKC activation and in its anatomy [38]. As an example, adult and aged mice were submitted to a discrimination task in a radial maze (regular trials — two arms), and then to probe trials (three arms) requiring them to construct relationships with the information learned in the first phase of the trials, a process indicated as relational representation. It was shown that the performance of aged animals was impaired on probe but not on regular trials in correlation with lower hippocampal cytosolic calcium-dependent and -independent PKC activities compared to adults. The analysis of the results suggested a specific relationship between the ability to form relational representations and hippocampal PKC activity [41]. Thus, it is

established that both in vertebrates as in invertebrates, such as the marine snail Hermissenda (reviewed in Refs. [38,39]), PKC has a key role in learning and memory, probably given its involvement in synaptic plasticity.

3. Failure of PKC and its anchoring system in brain aging

Brain aging is characterized by a progressive impairment of multiple neurotransmitters systems, most of them acting through a signalling cascade involving activation of PKC. These alterations may lead to modified interneuronal communications representing possibly the first step towards cognitive deficits. Because protein kinases play a critical role in converting extracellular signals to biological responses, alterations in kinase function might directly contribute to neuronal dysfunction.

PKC-phosphorylating activity appears to be impaired during senescence at brain level in a strain-dependent fashion in rodents. The calcium-dependent activity (classical PKCs) has been found reduced in brain cortex of Fisher 344 and Sprague–Dawley strains of rats [1,18,30] both in the cytosolic and membrane subcellular fractions, while these levels were found unmodified in Wistar animals [2], suggesting the presence of strain-related differences.

Technical caveats may apply to these studies because PKC activity measurements were assessed in vitro under optimal conditions of activators and substrates, however these methods may diverge from the physiological milieu during senescence. The subsequent analysis of the functional response in terms of enzyme translocation from soluble to membrane compartments has given more indicative results.

Calcium-dependent kinase C translocation upon phorbol ester treatment of tissues slices is impaired in rat physiological aging [2,3,18] and no specific differences were found among all strains investigated. The translocation mechanism has been recently observed to be defective also in terms of calcium-independent activity in cortical tissues of aged Wistar rats [24]. Challenge with phorbol esters (molecules that directly activate the kinase) is a method that bypasses the mechanism of receptor activation and indicates that the deficit of translocation is independent of neurotransmitter levels, receptor availability and second messenger production. Of pivotal importance is the fact that in spite of strain-related differences in brain basal PKC activity, the translocation process appears to be the common component of a defective functional machinery in the aging brain.

Anchoring proteins may contribute to this activation deficit. The results obtained from the investigation of this hypothesis have shown that cortical slices from aged rats express RACK-1 levels that are 50% of the values obtained in young adult and middle-aged animals [40]. Consistent with these results it has been shown that the PKC isoenzymes known to interact with it do not translocate to membrane compartments upon stimulation. As mentioned, RACK1 preferentially interacts with PKC β, δ and ϵ [33,43,44] and the translocation of these isoforms has been showed defective in the brain of senescent rats [3,40], suggesting that in the absence of adequate levels of RACK-1, the corresponding target kinase C isoforms may not find the appropriate milieu for interaction with the membrane compartment. Finally, it has been shown that in Alzheimer's disease, a neurodegenerative condition significantly affecting cognition and memory, a reduced level of RACK-1 protein can be observed in the brain of affected patients [4], a finding that interestingly link all previous observations concerning a defective PKC machinery in Alzheimer's brain and peripheral tissues [5,19].

From this information we are gaining a better insight on the different factors controlling brain PKC activation. As a consequence, we may be able to identify new approaches for correcting or even preventing age-related changes in brain function.

4. DHEA, a neurosteroid acting on memory in search for a mechanism

The previous considerations, upon the involvement of specific signal transduction deficits in age-related impairment in cognitive functions, will be now integrated with a brief review of the extensive discussion currently centered upon the physiological role of neurosteroids in the modulation of age-related dysfunctions of the central nervous system. The presence of high concentrations of certain steroids in the central nervous system has prompted approximately 20 years ago, the definition of the term 'neurosteroid' [8]. These molecules accumulate in the nervous system partially independently of supply by peripheral endocrine glands. They are synthesized in the central and peripheral nervous system, particularly but not exclusively, by myelinating glial cells, from cholesterol or steroidal precursors imported from peripheral sources and include, among others, compounds such as pregnenolone (PREG) and dehydroepiandrosterone (DHEA), their sulfates, and reduced metabolites.

Normal aging is associated with several alterations in hormone production and secretion. In humans, DHEA and DHEA sulfate (DHEAS) represent the major androgens secreted by the adrenal gland and their levels are known to increase throughout childhood and puberty and then to decrease in old age. Various functions including metabolic, immune, and cognitive effects have been attributed to these steroids and since the levels of DHEA correlate with general good health, a growing interest in replacement of DHEA in elderly people has developed [23]. Although the preclinical data are intriguing there is a general lack of solid scientific evidence on the effectiveness of these compounds [50]. A recent systematic review of the litera-

ture has been conducted [20], particularly over the concern upon the fact that DHEA is currently being sold in large quantities in health food stores, particularly in the USA. Particular attention was given to the effect of DHEA or DHEAS in the improvement of psychological well-being and/or reduction of the rate of decline of cognitive function in older adults or in individuals with dementia. The results of the study offer limited support for improvement in a sense of well-being following DHEA treatment, with non significant results concerning improvement in memory or other aspects of cognitive function, although cognitive function was only measured in trials of relatively short duration. Another study, recently published, indicated that long-term (1 year) treatment of elderly men and women with 50 mg DHEA daily, normalized some parameters of well being affected by aging, particularly in women, without apparent side effects [7]. This study, however, did not provide evidence concerning the cognitive effect of the treatment [7].

The cognitive effect of DHEA and other neurosteroids has been extensively studied in rodents. Several studies have reported positive effects of the acute administration of DHEA, pregnenolone or their sulfates on memory performances of rats or mice, in particular on paradigm of passive avoidance [21]. Flood et al. [14,15] showed that the intracerebroventricular administration to male mice of various neurosteroids could improve retention of previously performed footshock active avoidance training. The same authors demonstrated that DHEAS was able [16] to improve retention of footshock active avoidance training both in middle-aged and old mice to the same levels observed in young mice. In addition, the infusion of pregnenolone sulfate into the nucleus basalis magnocellularis of the rat was shown to enhance memory performance when injected after an acquisition trial [29].

Administration of neurosteroids also reduces the effect of amnestic treatments. The use of the NMDA antagonist dizocilpine in rodents induces an amnestic cognition deficit which is antagonized by pregnenolone and its metabolic derivatives [11,28]. In addition, while the subcutaneous administration of scopolamine dose-dependently impairs learning during an appetitively reinforced visual discrimination task, the intracerebroventricular administration of pregnenolone sulfate, dose-dependently blocks learning deficits induced by scopolamine with an additive memory-enhancing effect, when administered alone after the first training session. The rapid effect on memory processes has been attributed to interaction with NMDA and/or GABA-A receptors [31]. Finally memory enhancement in rodents has been more recently demonstrated by administration of the steroid sulfatase inhibitor *p*-*O*-(sulfamoyl)-*N*-tetradecanoyl tyramine (DU-14) alone or in combination with DHEAS to rats which were then tested for the reversal of scopolamine-induced amnesia. A single dose of DU-14 produced inhibition of steroid sulfatase activity and enhanced the reversal of amnesia by DHEAS [27]. Another steroid sulfatase inhibitor, estrone-3-*O*-sulfamate (EMATE), potentiated the effect of DHEAS on memory retention such that lower doses of DHEAS were necessary to obtain significant effects. EMATE, administered peripherally, potentiated the effect of centrally administered DHEAS on memory retention, however it did not potentiate the effect of centrally administered pregnenolone sulfate [17].

With an original approach, the long-term administration of pregnenolone sulfate in adult male rats was obtained by intracerebroventricular infusion with the aid of an osmotic minipump, and the effect of steroid administration was studied on memory performances in a Y-maze arm discrimination test. Since a 'basal' level of steroids is continuously present in the nervous system, this approach was expected to more closely approximate the physiological conditions. The results demonstrated that the infusion of pregnenolone sulfate induces memory enhancing effects in mice, using a non-stressful spatial memory task [26].

Summarizing the studies mentioned above, there is substantial evidence in the literature supporting a role of neurosteroids in maintaining cognitive functions and possibly restoring these functions in aged animals.

Many hypotheses concerning the mechanism of action of neurosteroids have been explored. The classical mechanism of steroids involves the binding to intracellular receptors that can act as transcription factors and regulate gene expression. In addition, some neurosteroids such as pregnenolone, DHEA and their sulfates are potent modulators of an array of ligand-gated ion channels and of distinct G-protein coupled receptors via nongenomic mechanisms (reviewed in Ref. [45]), in particular acting as allosteric modulators of neurotransmitter receptors, such as GABA-A, NMDA and sigma receptors [6]. The observation that DHEA can have memory enhancing effects even when given at 1 h after training [14], suggested that the effects of the active steroids may imply the facilitation of transcription of immediate-early genes. It has been demonstrated that neuroactive steroids can regulate gene expression via the progesterone receptor after intracellular oxidation [46]. Those molecules for which receptors have not yet been demonstrated, may exert their effects by serving as precursors for the formation of a panoply of different steroids eventually modulating the plastic changes of memory processes.

5. Experimental evidence of the role of DHEA in reverting age-associated impairment in PKC machinery

In light of the observations outlined above concerning the age-related changes in signal transduction that parallel cognitive dysfunctions, as well as the potential cognitive enhancing effect of neurosteroids, we have speculated that DHEA among other neurosteroids might influence brain

functionality, possibly interacting with the PKC signal transduction system.

To test this hypothesis we have investigated the effects of DHEA on brain RACK-1 expression in two different animal models.

For the first model, we have used castrated young male Sprague–Dawley rats. Animals were orchiectomized at 8 weeks of age and compared with control (sham operated rats) 8 weeks after castration. Castration of males reproduces a hormonal situation similar to that observed in old rats, as it has been reported that castration is associated with the loss of both testosterone and DHEA [9]. As shown in Fig. 1 lane 3, male castration was associated with a 54% decrease in the expression of RACK-1 compared to sham-operated rats, whereas no differences were detected in the expression of total PKCβII. We sought to determine whether DHEA was able to restore RACK-1 levels to normal and, to achieve our goal we subcutaneously implanted animals with ALZET™ 2ML2 osmotic pumps. The use of osmotic pumps was chosen to minimize animal handling and stress. The subcutaneous route was chosen since it has been reported to allow a 100% bioavailability [25]. DHEA 2.5 mg/kg was delivered at a rate of 0.75 mg/day over a 2-week period. The control groups received only propylene glycol (vehicle). Animals were sacrificed 2 weeks after implantation and the hippocampus was isolated, homogenized and proteins analysed by Western blot to determine RACK-1 and PKCβII immunoreactivity. As shown in Fig. 1 lane 4, DHEA administration completely restored the castration-associated decrease in RACK-1 expression, while no changes were observed in sham-operated rats treated with DHEA. Again no changes were observed in the immunoreactivity of PKCβII.

The ability of DHEA to restore the castration-associated decrease in RACK-1 expression led us to investigate the effect of DHEA treatment on RACK-1 expression in old rats.

As a second animal model, we used 21-month-old male Sprague–Dawley rats that were subcutaneously implanted with ALZET™ 2ML2 osmotic pumps. DHEA 2.5 mg/kg was delivered at a rate of 2 mg/day over a 2-week period, control group (aged animals) received propylene glycol as vehicle control. Animals were sacrificed 2 weeks after implantation and the hippocampus was collected. The samples of each animal ($n=5$) were split in two; one sample was processed for Western blot analysis and the second served for the extraction of RNA for semiquantitative RT-PCR analysis. As shown in Table 1, the treatment with DHEA was able to restore, as shown previously in castrated young animals, the levels of expression of

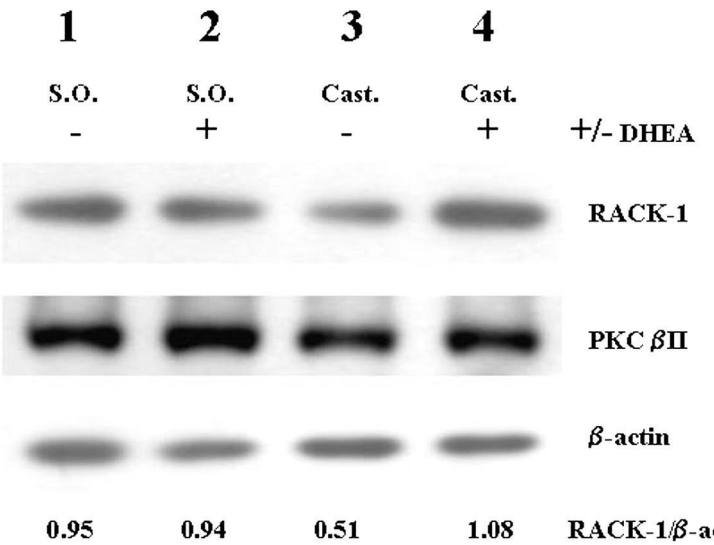

Fig. 1. Effect of DHEA on RACK-1 expression in castrated male rats. Young male Sprague–Dawley rats ($n=4$) were orchiectomized at 8 weeks of age and compared with control (sham-operated rats) 8 weeks after castration. Male castration was associated with a 54% decrease in the expression of RACK-1 (lane 3) in hippocampus, compared to sham-operated rats, whereas no differences were detected in the expression of total PKCβII. DHEA administration (0.75 mg/day) with ALZET™ osmotic pumps, over a period of 2 weeks, completely restored castration-associated decrease in RACK-1 expression (lane 4), while no changes were observed in sham-operated rats treated with DHEA. Again no changes were observed in the immunoreactivity of PKCβII. The Western blot analysis was performed on homogenates of rat brain areas (hippocampus) obtained in a buffer composed of 50 mM Tris, 150 mM NaCl, 5 mM EDTA pH 7.5, 0.5% Triton X-100, 50 μM PMSF, 2 μg/ml aprotinin, 1 μg/ml pepstatin and 1 μg/ml leupeptin. The protein content of the cell lysate was measured using a commercial kit (Bio-Rad). The proteins (5 μg per lane) were then electrophoresed on to a 12% SDS–polyacrylamide gel and transferred to PVDF membrane in 20 mM Tris, 150 mM glycine, 20% methanol, 0.03% SDS. The different proteins were visualized using a RACK-1 monoclonal antibody at 1:2500 (Transduction Laboratories) a PCKβII monoclonal antibody 1:5000 (Santa Cruz) and a β-actin monoclonal antibody (Sigma) 1:5000 as the primary antibodies and developed using enhanced chemiluminescence according to the manufacter's instruction (ECL, Amersham). Semiquantitative analysis of Western blots was performed by calculating the relative intensity of the immunoreactive bands after acquisition of the blot image trough a Nikon CCD videocamera module and analysis by means of the Image 1.47 program (Wayne Rasband, NIH, Research Service Branch, Nimh, Bethesda, MD, USA).

Table 1
In vivo effects of DHEA on RACK-1 protein and mRNA expression in rat hippocampus

Group	Western blot analysis RACK-1/β-actin	RT-PCR analysis RACK-1/β-actin
YOUNG (3 months)	1.02±0.06	1.10±0.05
OLD (21 months)	0.21±0.02*	0.14±0.01*
OLD+DHEA 2.5 mg/kg	1.09±0.08§	1.20±0.05§

Old male Sprague–Dawley rats (n=5) were subcutaneously implanted with ALZET™ 2ML2 osmotic pumps. DHEA 2.5 mg/kg was delivered at a rate of 2 mg/day over a 2-week period, control groups received propylene glycol as vehicle control. Animals were terminated 2 weeks after implantation and the hippocampus was removed and split in two for Western blot and RT-PCR analysis. For Western blots, 5 μg of tissue homogenate was loaded on 12% SDS–PAGE and the rest of the procedure was as indicated in the legend of Fig. 1. For the determination of RACK-1 mRNA levels, total RNA was isolated from tissues by guanidinium-thiocyanate phenol–chloroform extraction followed by reverse transcription as previously described [12]. For RT-PCR, 0.5 and 100 ng of reverse-transcribed total RNA was amplified for RACK-1 and β-actin, respectively, using the following pairs of primers:
rat RACK-1: forward, 5′-GGT CAC TCC CAC TTT GTT AG-3′; reverse, 5′-AGA AGC GGA CAC AAG ACA-3′;
mouse β-actin: forward, 5′-GTG GGC CGC TCT AGG CAC CAA-3′; reverse, 5′-CTC TTT GAT GTC ACG CAC GAT TTC-3′.
The amplified PCR products are 288 bp for RACK-1 and 540 bp for β-actin. In preliminary experiments, RNA concentrations and PCR cycles were titrated to establish standard curves to document linearity and to permit semi-quantitative analysis of signal strength. Gels were photographed with type 55 film (Polaroid; Cambridge, MA) and images acquired and processed as indicated in the legend to Fig. 1.

RACK-1 to the levels found in young rats, both at the protein and mRNA level. The expression of RACK-1 in old animals was found to be reduced to the same extent as previously found in Wistar rats [40], and the fact that DHEA can restore its levels to those expressed by young animals suggests that DHEA can also improve the translocation deficit observed in aging animals.

Altogether these data support further investigation into the mechanism of DHEA and possibly other neurosteroids on the molecular mechanisms supporting brain cognitive functions. Previous considerations on the role of PKC in memory processes suggested that signal transduction mechanisms are primarily involved in age-associated cognitive deficiencies. The results presented here are the first indication that the activity of DHEA, and possibly of other neurosteroids, on the mechanisms of cognition and memory in aging is also exerted through the restoration of defective PKC signal transduction machinery.

Acknowledgements

We acknowledge the financial contribution of the University of Pavia (Progetto Ateneo). We wish to thank Dr Alessia Pascale, Dr Marisa Fioravanti and Laura Lucchi for helpful discussion and technical assistance.

References

[1] F. Battaini, R. Del Vesco, S. Govoni, M. Trabucchi, Regulation of phorbol ester binding and protein kinase C activity in aged rat brain, Neurobiol. Aging 11 (1990) 563–566.
[2] F. Battaini, S. Govoni, L. Lucchi, V. Ladisa, S. Bergamaschi, M. Trabucchi, Age-related changes in brain protein kinase C expression, activity, and translocation, Drugs Dev. 2 (1993) 275–282.
[3] F. Battaini, S. Elkabes, S. Bergamaschi, V. Ladisa, L. Lucchi, P.N.E. De Graan, T. Schuurman, W.C. Wetsel, M. Trabucchi, S. Govoni, Protein kinase C activity, translocation and conventional isoforms in aging rat brain, Neurobiol. Aging 16 (1995) 137–148.
[4] F. Battaini, A. Pascale, L. Lucchi, G.M. Pasinetti, S. Govoni, Protein kinase C anchoring deficit in postmortem brains of Alzheimer's disease patients, Exp. Neurol. 159 (1999) 559–564.
[5] F. Battaini, A. Pascale, R. Paoletti, S. Govoni, The role of anchoring protein RACK1 in PKC activation in the ageing rat brain, Trends Neurosci. 20 (1997) 410–415.
[6] E.E. Baulieu, Neurosteroids: a novel function of the brain, Psychoneuroendocrinology 23 (1998) 963–987.
[7] E.E. Baulieu, G. Thomas, S. Legrain, N. Lahlou, M. Roger, B. Debuire, V. Faucounau, L. Girard, M.P. Hervy, F. Latour, M.C. Leaud, A. Mokrane, H. Pitti-Ferrandi, C. Trivalle, O. de Lacharriere, S. Nouveau, B. Rakoto-Arison, J.C. Souberbielle, J. Raison, Y. Le Bouc, A. Raynaud, X. Girerd, F. Forette, Dehydroepiandrosterone (DHEA), DHEA sulfate, and aging: contribution of the DHEAge Study to a sociobiomedical issue, Proc. Natl. Acad. Sci. USA 97 (2000) 4279–4284.
[8] E.E. Baulieu, Steroid hormones in the brain: several mechanisms, in: K. Fuxe, J.A. Gustafsson, L. Wetterberg (Eds.), Steroid Hormones Regulation of the Brain, Pergamon, Oxford, 1981, pp. 3–14.
[9] B. Belanger, A. Belanger, F. Labrie, A. Dupont, L. Cusan, G. Monfetta, Comparison of residual C-19 steroids in plasma and prostatic tissue of human, rat and guinea pig after castration: unique importance of extratesticular androgens in men, J. Steroid Biochem. 32 (1989) 695–698.
[10] T.V.P. Bliss, G.L. Collingridge, A synaptic model of memory: long-term potentiation in the hippocampus, Nature 361 (1993) 31–39.
[11] D.L. Cheney, D. Uzunov, A. Guidotti, Pregnenolone sulfate antagonizes dizocilpine amnesia: role for allopregnanolone, Neuroreport 6 (1995) 1697–1700.
[12] E. Corsini, F. Battaini, L. Lucchi, M. Marinovich, M. Racchi, S. Govoni, C.L. Galli, A defective protein kinase C anchoring system underlying age-associated impairment in TNF-α production in rat macrophages, J. Immunol. 163 (1999) 3468–3473.
[13] L.V. Dekker, P.J. Parker, Protein kinase C — a question of specificity, Trends Biochem. Sci. 19 (1994) 73–77.
[14] J.F. Flood, G.E. Smith, E. Roberts, Dehydroepiandrosterone and its sulfate enhance memory retention in mice, Brain Res. 447 (1998) 269–278.
[15] J.F. Flood, J.E. Morley, E. Roberts, Memory-enhancing effects in male mice of pregnenolone and steroids metabolically derived from it, Proc. Natl. Acad. Sci. USA 89 (1992) 1567–1571.
[16] J.F. Flood, E. Roberts, Dehydroepiandrosterone sulfate improves memory in aging mice, Brain Res. 448 (1988) 178–181.
[17] J.F. Flood, S.A. Farr, D.A. Johnson, P.K. Li, J.E. Morley, Peripheral steroid sulfatase inhibition potentiates improvement of memory retention for hippocampally administered dehydroepiandrosterone sulfate but not pregnenolone sulfate, Psychoneuroendocrinology 24 (1999) 799–811.
[18] E. Friedman, H-Y. Wang, Effect of age on brain cortical protein kinase C and its mediation of 5-hydroxythryptamine release, J. Neurochem. 52 (1989) 187–192.
[19] L. Gasparini, M. Racchi, G. Binetti, M. Trabucchi, S.B. Solerte, D. Alkon, R. Etcheberrigaray, G. Gibson, J. Blass, R. Paoletti, S.

Govoni, Peripheral markers in testing pathophysiological hypotheses and diagnosing Alzheimer's disease, FASEB J. 12 (1998) 17–34.
[20] F.A. Huppert, J.K. Van Niekerk, J. Herbert, Dehydroepiandrosterone (DHEA) supplementation for cognition and well-being (Cochrane Review), in: The Cochrane Library, Issue 1, Update Software, Oxford, 2001.
[21] R.L. Isaacson, J.A. Varner, J.M. Baars, D. De Wied, The effects of pregnenolone sulfate and ethylestrenol on retention of a passive avoidance task, Brain Res. 689 (1995) 79–84.
[22] I. Izquierdo, Pharmacological evidence for a role of long-term potentiation in memory, FASEB J. 8 (1994) 1139–1145.
[23] O. Khorram, DHEA: a hormone with multiple effects, Curr. Opin. Obstet. Gynecol. 8 (1996) 351–354.
[24] A.S. Kraft, W.B. Anderson, Phorbol esters increase the amount of calcium, phospholipid-dependent protein kinase associated with plasma membrane, Nature 301 (1983) 621–623.
[25] C. Labrie, M. Flamand, A. Belanger, F. Labrie, High bioavailability of dehydroepiandrosterone administered percutaneously in the rat, J. Endocrinol. 150 (1996) S107–S118.
[26] N. Ladurelle, B. Eychenne, D. Denton, J. Blair-West, M. Schumacher, P. Robel, E.E. Baulieu, Prolonged intracerebroventricular infusion of neurosteroids affects cognitive performances in the mouse, Brain Res. 858 (2000) 371–379.
[27] P.K. Li, M.E. Rhodes, A.M. Burke, D.A. Johnson, Memory enhancement mediated by the steroid sulfatase inhibitor (p-O-sulfamoyl)-N-tetradecanoyl tyramine, Life Sci. 60 (1997) 45–51.
[28] C. Mathis, S.M. Paul, J.N. Crawley, The neurosteroid pregnenolone sulfate blocks NMDA antagonist-induced deficits in a passive avoidance memory task, Psychopharmacology 116 (1994) 201–206.
[29] W. Mayo, F. Dellu, P. Robel, J. Cherkaoui, M. Le Moal, E.E. Baulieu, H. Simon, Infusion of neurosteroids into the nucleus basalis magnocellularis affects cognitive processes in the rat, Brain Res. 607 (1993) 324–328.
[30] M. Meyer, J.H. Judkins, A.E. Momol, E.O. Hardwik, Effects of peroxidation and aging on rat neocortical Ach release and protein kinase C, Neurobiol. Aging 15 (1994) 63–67.
[31] H. Meziane, C. Mathis, S.M. Paul, A. Ungerer, The neurosteroid pregnenolone sulfate reduces learning deficits induced by scopolamine and has promnestic effects in mice performing an appetitive learning task, Psychopharmacology 126 (1996) 323–330.
[32] J. Micheau, G. Riedel, Protein kinases: which one is the memory molecule?, Cell. Mol. Life Sci. 55 (1999) 534–548.
[33] D. Mochly-Rosen, Localization of protein kinases by anchoring proteins: a theme in signal transduction, Science 268 (1995) 247–251.
[34] D. Mochly-Rosen, B.L. Smith, C.H. Chen, M.H. Disatnik, D. Ron, Interaction of protein kinase C with RACK1, a receptor for activated C kinase: a role in β protein kinase C mediated signal transduction, Biochem. Soc. Trans. 23 (1995) 596–600.
[35] A.C. Newton, Protein kinase C: structure, function, and regulation, J. Biol. Chem. 270 (1995) 28495–28498.
[36] Y. Nishizuka, Intracellular signaling by hydrolysis of phospholipids and activation of protein kinase C, Science 258 (1992) 607–614.
[37] X. Noguès, J. Micheau, R. Jaffard, Protein kinase C activity in the hippocampus following spatial learning tasks in mice, Hippocampus 4 (1994) 71–78.
[38] X. Nogues, Protein kinase C, learning and memory: a circular determinism between physiology and behaviour, Prog. Neuropsychopharmacol. Biol. Psychiatry 21 (1997) 507–529.
[39] A. Pascale, S. Govoni, F. Battaini, Age-related alteration of PKC, a key enzyme in memory processes, Mol. Neurobiol. 16 (1998) 49–62.
[40] A. Pascale, I. Fortino, S. Govoni, M. Trabucchi, W.C. Wetsel, F. Battaini, Functional impairment in protein kinase C by RACK1 (receptor for activated C kinase 1) deficiency in aged rat brain cortex, J. Neurochem. 67 (1996) 2471–2477.
[41] A. Pascale, X. Nogues, A. Marighetto, J. Micheau, F. Battaini, S. Govoni, R. Jaffard, Cytosolic hippocampal PKC and aging: correlation with discrimination performance, Neuroreport 9 (1998) 725–729.
[42] M. Robles-Flores, J.A. Garcia-Sainz, Activated protein kinase C binds to intracellular receptors in rat hepatocytes, Biochem. J. 296 (1993) 467–472.
[43] D. Ron, C.H. Chen, J. Caldwell, L. Jamieson, E. Orr, D. Mochly-Rosen, Cloning of an intracellular receptor for protein kinase C: a homolog of β subunit of G proteins, Proc. Natl. Acad. Sci. USA 91 (1994) 839–843.
[44] D. Ron, Z. Jiang, L. Yao, A. Vagts, I. Diamond, A. Gordon, Coordinated movement of RACK1 with activated betaII PKC, J. Biol. Chem. 274 (1999) 27039–27046.
[45] R. Rupprecht, F. Holsboer, Neuroactive steroids: mechanisms of action and neuropsychopharmacological perspectives, Trends Neurosci. 22 (1999) 410–416.
[46] R. Rupprecht, The neuropsychopharmacological potential of neuroactive steroids, J. Psychiatr. Res. 31 (1997) 297–314.
[47] T.C. Sacktor, P. Osten, H. Valsamis, X. Jiang, M.U. Naik, E. Sublette, Persistent activation of the ζ isoform of protein kinase C in the maintenance of long-term potentiation, Proc. Natl. Acad. Sci. USA 90 (1993) 8342–8346.
[48] C. Tanaka, Y. Nishizuka, The protein kinase C family for neuronal signaling, Annu. Rev. Neurosci. 17 (1994) 551–567.
[49] J.M. Wehner, S. Sleight, M. Upchurch, Hippocampal protein kinase C activity is reduced in poor spatial learners, Brain Res. 523 (1990) 181–187.
[50] S.S. Yen, G.A. Laughlin, Aging and the adrenal cortex, Exp. Gerontol. 33 (1998) 897–910.