



Studies on Homocysteine and Dehydroepiandrosterone Sulphate Plasma Levels in Alzheimer's Disease Patients and in Parkinson's Disease Patients

S. GENEDANI^a, G. RASIO^b, P. CORTELLI^c, F. ANTONELLI^c, D. GUIDOLINI^d, M. GALANTUCCI^a, K. FUXE^e and L.F. AGNATI^{f,*}

Department of Biomedical Sciences, ^aSection of Pharmacology and ^fSection of Physiology, University of Modena and Reggio Emilia, Italy; ^bLaboratory of Clinical Chemistry, Ospedale "Nuovo Montecchi", Suzzara, Italy; ^cInstitute of Clinical Neurology, University of Modena and Reggio Emilia, Italy; ^dDepartment of Human Anatomy and Physiology, Section of Human Anatomy, University of Padova, Italy; and ^eDepartment of Neuroscience, Karolinska Institutet, Stockholm, Sweden. luigiagnati@tin.it

(Received 28 April 2004; Revised 18 May 2004; In final form 18 May 2004)

Homocysteine (HC) and dehydroepiandrosterone sulphate (DHEAS) plasma levels have been evaluated in groups of male and female patients with Parkinson's Disease (PD) and in a group of female patients with Alzheimer's Disease (AD) and compared with the corresponding plasma levels observed in a group of age-matched subjects. It has been confirmed that HC plasma levels are enhanced in both PD and AD patients.

As far as the DHEAS plasma levels are concerned no changes have been observed in PD patients while a marked decrease has been observed in AD patients. These results support the view that while the pro-oxidant effects of HC and its agonist action at NMDA receptors can play a role in both neurodegenerative diseases, the role of DHEAS is more complex and may be an important factor only in certain neurodegenerative diseases. Thus, according to the present study DHEAS is likely to be involved in AD but not in PD.

Keywords: Homocysteine; Alzheimer's disease; Parkinson's disease; Dehydroepiandrosterone sulphate; Human patients

INTRODUCTION

Homocysteine (HC) metabolism is part of biochemical reactions involved in one-carbon metabolism. In physiological conditions folate is a cofactor in one-carbon metabolism, during which it promotes the remethylation of HC to regenerate methionine. Folate deficiency has been implicated in cardiovascular diseases and, more recently it has been shown to contribute to many

neurological and psychiatric disorders including dementia, Alzheimer's disease (AD), Parkinson's disease (PD), depression and schizophrenia (Ho *et al.*, 2003). Thus, experimental and clinical evidence suggests that folate deficiency may induce neurodegeneration by increasing reactive oxygen species (ROS) production, excitotoxicity and cytosolic calcium accumulation. Folate deficiency causes increases in HC plasma levels (Mattson and Shea, 2003) and the neurotoxic effects may depend on an increase of HC in the brain, since HC is normally present in the brain (up to 10 μ M) (Broch and Ueland, 1984; Hyland and Bottiglieri, 1992) as well as in the cerebrospinal fluid (up to 0.08 μ mol/l) (Fowler, 2001). Recently it has been shown that HC can pass the blood brain barrier and thus HC plasma levels are related to brain levels (Agnati *et al.*, 2004).

It seems clear that deficits in folic acid may lead to hyperhomocysteinemia and this is a risk factor for both cardiovascular and central nervous system diseases. The possibility has been suggested that HC may have toxic effects via its action on *N*-methyl-D-aspartate (NMDA) receptors involving inter alia nitric oxide synthase (NOS) activation and associated free radical formation causing lipid peroxidation and contributing to damage of neuronal DNA, thereby triggering apoptosis (Lipton *et al.*, 1997; Jara-Prado *et al.*, 2003; Mattson and Shea, 2003). It has also been shown that HC via autooxidation generates ROS after intracerebroventricular injections in Wistar rats, and that ROS content can be inhibited by melatonin (Baydas *et al.*, 2003). Thus, it may be stated that HC induced neurodegeneration involves both NMDA receptor stimulation and NMDA receptor independent free radical formation.

*Corresponding author. Tel.: +39-059-2055345; Fax: +39-059-205363; E-mail: luigiagnati@tin.it

As a matter of fact, HC acts as a partial agonist at the glutamate binding site of the NMDA receptor and as a partial antagonist at the glycine coagonist site (Lipton *et al.*, 1997). Modest plasma levels (10 μ M) are commonly found in adults, and tissue concentrations up to 10 μ M have been measured in brain. However, under pathological conditions in which glycine levels in the nervous system are elevated, such as stroke and head trauma, HC's neurotoxic (agonist) effects (10-100 μ M) outweigh its neuroprotective (antagonist) activity (Lipton *et al.*, 1997). Accordingly, an increased plasma HC level has been shown to be a strong, independent risk factor for the development of dementia and AD (Seshadri *et al.*, 2002).

On the other hand, the neurosteroid dehydroepiandrosterone (DHEA) has antioxidant properties (Aragno *et al.*, 2000). Studies in humans have confirmed a reduction of lipid peroxidation by DHEA. It seems that DHEA acts either by counteracting vitamin E disappearance from low density lipoproteins under oxidation or by scavenging directly the free radicals produced during the oxidative process (Khalil *et al.*, 2000).

Thus, the hypothesis can be put forward that the potentially toxic actions of high brain levels of HC can, *inter alia*, be kept under control by the neurosteroid DHEA sulfate (DHEAS), which becomes gradually reduced in brain during the aging process (Mazat *et al.*, 2001; Schumacher *et al.*, 2003). In agreement with this hypothesis are the data demonstrating that DHEAS protects against beta-amyloid peptide-induced neurotoxicity and amnesia (Maurice *et al.*, 1998; Cardouel *et al.*, 1999; Schumacher *et al.*, 2003). Its local synthesis in glial cells may play a physiological role in antagonizing neurotoxicity. Furthermore, there also exist indications that steroid sulfates can cross the blood-brain barrier to some degree (Wang *et al.*, 1977). In fact, it has recently been shown that DHEAS efflux transport occurs from the brain into the circulation via the organic anion transporting polypeptide 2 in the blood-brain barrier (Asaba *et al.*, 2000). Therefore, a study on the correlation between DHEAS plasma levels seems justified, which also is supported by the suggestions that DHEAS may help slow down the aging process and improve memory in humans (Roberts, 1999). However, we are aware that circulating DHEAS levels may have more of an adrenal than of a brain origin. We have, in the present study, evaluated HC and DHEAS plasma levels in a group of PD patients and in patients affected by cognitive impairments, probably dependent on an AD condition, and compared these levels with the plasma levels in a group of age-matched control patients. Thus, this study may shed new light in

two neurodegenerative diseases on the plasma changes in these two factors, HC potentially favouring neurodegeneration and DHEAS favouring neuroprotection.

METHODS

Subjects

Control patients included 22 females (median age = 81.5 years; interquartile range = 6) and 35 male patients (median age = 73 years; interquartile range = 12) without neurological and metabolic pathologies.

Parkinson (PD, referred both to the Department of Neurology of the University of Modena and to the Psychogeriatric Centre "Villa Azzurra" Mantova, Italy) patients included 29 subjects: 15 female patients (median age = 77 years; interquartile range = 8) and 14 male patients (median age = 67 years; interquartile range = 19). The clinical evaluation of PD patients was carried out according to the Hoehn and Yahr scale (Hoehn and Yahr, 1967). It was assessed that 25% of the patients were in stage I, 29% in stage II, 34% in stage III, 12% in stage IV. Patients were under L-3,4-dihydroxyphenylalanine (L-DOPA) therapy, and the treatment was suspended the day before the blood sampling. We are aware that chronic L-DOPA treatment can produce increases in plasma HC levels in PD patients (Miller *et al.*, 2003).

Patients with dementia of Alzheimer type (AD, referred to the Psychogeriatric Centre "Villa Azzurra" Mantova, Italy for diagnostic examination and treatment) included 22 female patients (median age = 82 years; interquartile range = 11). The assessment of the cognitive state of the patients was carried out according to a standard procedure (Magni *et al.*, 1996). It was assessed that about 90% of the patients obtained a total score < 5/30 while about 10% obtained a score of 13/30.

The use of specimens and cognitive data was approved by our Institutional Review Board.

Determination of HC in Plasma

Blood samples were collected into Vacutainers containing EDTA-NaF (17.5 mg; EDTA + NaF; final NaF concentration, 60 mmol/l; Becton Dickinson UK Limited) (Clark *et al.*, 2003) in the morning after an overnight fast. Total plasma HC (tHC) was analyzed by HPLC after the reduction of plasma disulfides with tris(2-carboxyethyl)phosphine, precipitation of proteins with perchloric acid, derivatization with 7-fluoro-2,1,3-benzoxadiazole-4-sulfonate (SBD-F), and fluorescence detection as described previously (Pfeiffer *et al.*, 1999) using *N*-acetyl-cysteine as an internal standard. Performance characteristics of the assay have

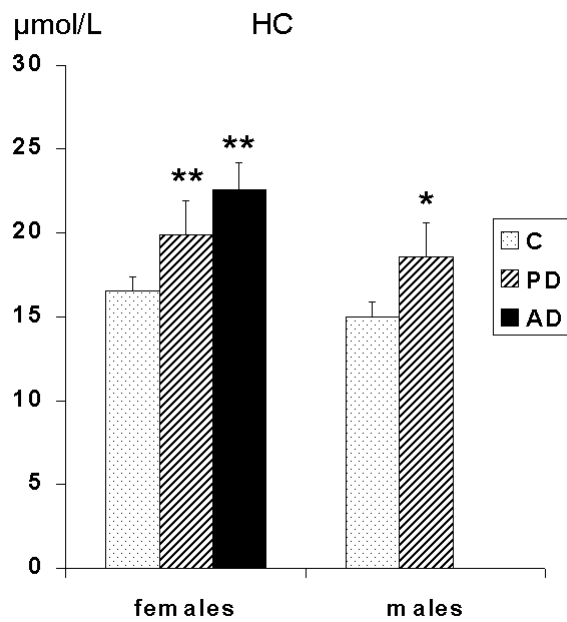


FIGURE 1 Homocysteine (HC) plasma levels (micromole/l) in control subjects (C), Parkinson patients (PD) and Alzheimer patients (AD). Data are shown as means ± S.E.M. ** $p < 0.01$; * $p < 0.05$ vs control group (two-sample t -test for mean comparisons of populations with unequal variances).

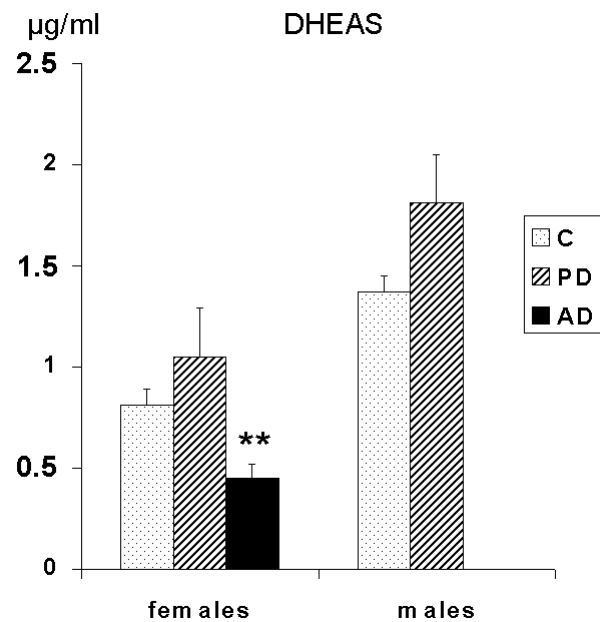


FIGURE 2 Dehydroepiandrosterone sulphate (DHEAS) plasma levels (micrograms/ml) in control subjects (C), Parkinson patients (PD) and Alzheimer patients (AD). Data are shown as means ± s.e.m. ** $p < 0.01$ vs control group (two-sample t -test for mean comparisons of populations with unequal variances).

been described previously (Pfeiffer *et al.*, 1999). HPLC analysis was performed on plasma specimens submitted to the laboratory for tHC determination. HC plasma levels are expressed as micromole/l.

Determination of DHEAS

Plasma levels of DHEAS were determined by an immunoenzymatic method (Adaltis SR1 DHEAS assay for the SR1 analyzer; Adaltis Italia S.p.A, Casalecchio di Reno, Bologna, Italy). Briefly, a rabbit fluoresceine-conjugated anti-DHEAS antibody was used in an enzyme immunoassay system which incorporates mag-

netic solid phase separation (Rosenfeld *et al.*, 1972). DHEAS plasma levels are expressed as micrograms/ml.

RESULTS

The ages and number of the control subjects and of the patients are summarized in Table I. The results obtained in PD and AD patients are summarized in FIGs. 1 and 2.

It was found [two-sample t -test for mean comparisons of populations with unequal variances (Daniel,

Table I

Age and number (N) of the subjects included in the present study

The age (mean ± s.e.m) and number of patients, separated in females and males, are reported for each group. Control patients (C), Parkinson patients (PD), Alzheimer patients (AD).

Subjects	Females		Males	
	Age	N	Age	N
C	81.1 ± 1.3	22	72.9 ± 1.8	35
PD patients	76.7 ± 2.2	15	66.6 ± 3.3	14
AD patients	81.9 ± 1.7	22		

1999)] that in PD patients the HC plasma levels are higher than in the age-matched control subjects both in females ($P < 0.01$) and males ($P < 0.05$) (FIG. 1). In contrast, no difference was observed in the DHEAS plasma levels with respect to the age-matched control subjects (FIG. 2).

The HC plasma levels in AD patients are substantially enhanced ($P < 0.01$) with respect to the age-matched control subjects (two-sample *t*-test for mean comparisons of populations with unequal variances) (FIG. 1). The DHEAS plasma levels were markedly reduced with respect to the age-matched control subjects ($P < 0.01$) (two-sample *t*-test for mean comparisons of populations with unequal variances) (FIG. 2).

GENERAL DISCUSSION

The present results showing a significant increase of HC plasma levels both in PD and in AD patients, support the view that chronic high plasma levels of HC may have deleterious effects on brain function and neuronal survival. In fact, there is increasing evidence that in humans chronic high plasma HC levels play a role in developmental and adult neurological and psychiatric disorders. In fact, it has been shown that individuals with elevated HC plasma levels are at increased risk of these two major neurodegenerative disorders (Mattson and Shea, 2003). Although HC can directly damage neurons, it is also possible that the HC lesioning action on cerebral blood vessels contributes to the neurodegenerative processes observed both in PD and AD patients. In the interpretation of the findings in PD patients it must however be considered that chronic L-DOPA treatment itself may lead to hyperhomocysteinemia, which thus may be pharmacologically induced (Miller *et al.*, 2003).

Very interesting is the observation that while DHEAS plasma levels are not altered in the PD patients, a marked decrease is observed in the female AD patients in view, *inter alia*, of the indications that DHEAS may cross the blood-brain barrier and that plasma DHEAS causes CNS actions (Roberts, 1999; Asaba *et al.*, 2000). Furthermore, there are studies in experimental animals demonstrating that neurosteroids such as DHEA and DHEAS also after peripheral administration may have important modulatory roles on brain function, especially related to memory processes and to neuronal survival (Baulieu *et al.*, 1999; Schumacher *et al.*, 2003). Thus, DHEA and DHEAS improve memory performances in aging mice (Flood and Roberts, 1988) and protect against beta-amyloid peptide-induced neurotoxicity and amnesia (Maurice *et al.*, 1998;

Cardounel *et al.*, 1999), but results from human trials are less clear (Arlt *et al.*, 2000).

A reduction in the CSF levels of DHEAS is present in mild to moderate AD patients (Kim *et al.*, 2003) in spite of increases of DHEA plasma levels in such AD patients (Rasmuson *et al.*, 2002). As a matter of fact, an increase of DHEA levels has also been detected in the cerebrospinal fluid of AD patients, while in the same patients the DHEAS levels were significantly reduced. Thus, it is possible that the DHEA accumulation in the brain and in the plasma results from a decreased production of its biologically active metabolites (Kim *et al.*, 2003). These findings indicate that neuroprotection is mainly exerted by DHEAS while DHEA may exert only minor protective effects. This hypothesis is also supported by the results demonstrating that high levels of key proteins involved in the formation of plaques and neurofibrillary tangles in human brains of AD patients were correlated with decreased levels of DHEAS (Weill-Engerer *et al.*, 2002). However, it should be considered, that DHEA can protect against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induced dopaminergic neurotoxicity (D'Astous *et al.*, 2003).

As to mechanisms of action, it may be speculated that neurosteroids such as DHEA and especially DHEAS may exert at least part of their neuroprotective actions by modulating the plasma membrane receptor function especially in the lipid rafts (LRs), where cholesterol and sphingolipids form assemblies in a fluid bilayer (Simons and Ikonen, 1997). The LRs appear to be ordered membrane platforms for plasma membrane molecular networks built up especially of various types of receptors, and specialized for signal integration and transduction (Razani *et al.*, 2002; Agnati *et al.*, submitted). It seems possible that each neurosteroid can differentially affect the signaling in the LRs via lipid-lipid and lipid-protein interactions, leading to conformational changes in the receptor proteins such as the NMDA and dopamine (DA) receptors with altered transduction and trafficking. Such effects could lead both to neuroprotective and neuromodulatory actions. In fact, early work on estrogens indicated that the density of the D₂ DA receptors in striatal membranes was altered after *in vivo* treatment with estrogens (Fuxe *et al.*, 1979; Di Paolo, 1994), and DHEA and estradiol have been shown to enhance L-DOPA induced locomotor activity in MPTP lesioned monkeys (Belanger *et al.*, 2003). It may be surmised that in PD and AD patients the enhanced HC brain levels exert neurotoxic effects both via overstimulation of NMDA receptors and via oxidative stress of neurons. However, while in PD patients

the neurosteroids and in particular DHEAS can likely still protect some brain areas such as hippocampus, entorhinal cortex and neocortex, a metabolic disruption of DHEAS synthesis in the AD patients may lead to neurodegeneration in such regions which are exquisitely sensitive to metabolic stresses and where the formation of plaques and neurofibrillary tangles especially occur (Brion, 1998).

Acknowledgements

This work has been supported by a grant from the European Commission (QLG3-CT2001-01056) by grants from MIUR, Roma, Italy and from the Swedish Research Council.

References

- Agnati LF, S Genedani, G Rasio, M Galantucci, S Saltini, M Filaferro, R Franco, F Mora, S Ferrè and K Fuxe (2004, 12 May online) Studies on homocysteine plasma levels in Alzheimer's patients, relevance for neurodegeneration. *J. Neural Transm.*
- Agnati LF, D Guidolin, S Genedani, S Ferrè, A Bigiani, A Woods and K Fuxe (submitted) How proteins come together in the plasma membrane and function in macromolecular assemblies: focus on receptor mosaics. *J. Mol. Neurosci.*
- Aragno M, S Parola, E Tamagno, E Brignardello, R Manti, O Danni and G Boccuzzi (2000) Oxidative derangement in rat synaptosomes induced by hyperglycaemia: restorative effect of dehydroepiandrosterone treatment. *Biochem. Pharmacol.* **60**, 389-395.
- Arlt W, F Callies and B Allolio (2000) DHEA replacement in women with adrenal insufficiency: pharmacokinetics, bioconversion and clinical effects on well-being, sexuality and cognition. *Endocr. Res.* **26**, 505-511.
- Asaba H, K Hosoya, H Takanaga, S Ohtsuki, E Tamura, T Takizawa and T Terasaki (2000) Blood-brain barrier is involved in the efflux transport of a neuroactive steroid, dehydroepiandrosterone sulfate, via organic anion transporting polypeptide 2. *J. Neurochem.* **75**, 1907-1916.
- Baulieu EE, P Robel and M Schumacher (1999) *Neurosteroids, A New Regulatory Function in the Nervous System* (Humana Press: Totowa, NJ, USA).
- Baydas G, S Kutlu, M Naziroglu, S Canpolat, S Sandal, M Ozcan and H Kelestimur (2003) Inhibitory effects of melatonin on neural lipid peroxidation induced by intracerebroventricularly administered homocysteine. *J. Pineal Res.* **34**, 36-39.
- Bélanger N, L Grégoire, PJ Bédard and T Di Paolo (2003) Estradiol and dehydroepiandrosterone potentiate levodopa-induced locomotor activity in 1-methyl-4-phenyl-,2,3,6-tetrahydropyridine monkeys. *Endocrine* **21**, 97-101.
- Brion JP (1998) Neurofibrillary tangles and Alzheimer's disease. *Eur. Neurol.* **40**, 130-140.
- Broch OJ and PM Ueland (1984) Regional distribution of homocysteine in the mammalian brain. *J. Neurochem.* **43**, 1755-1757.
- Cardounel A, W Regelson and M Kalimi (1999) Dehydroepiandrosterone protects hippocampal neurons against neurotoxin-induced cell death: mechanism of action. *Proc. Soc. Exp. Biol. Med.* **222**, 145-149.
- Clark S, LD Youngman, J Sullivan, R Peto and R Collins (2003) Stabilization of homocysteine in unseparated blood over several days: a solution for epidemiological studies. *Clin. Chem.* **49**, 518-520.
- Daniel WW (1999) *Biostatistics: A Foundation for Analysis in the Health Sciences* (John Wiley: New York NY, USA).
- D'Astous M, M Morissette, B Tanguay, S Callier and T Di Paolo (2003) Dehydroepiandrosterone (DHEA) such as 17beta-estradiol prevents MPTP-induced dopamine depletion in mice. *Synapse* **47**, 10-14.
- Di Paolo T (1994) Modulation of brain dopamine transmission by sex steroids. *Rev. Neurosci.* **5**, 27-41.
- Flood JF and E Roberts (1988) Dehydroepiandrosterone sulphate improves memory in aging mice. *Brain Res.* **448**, 178-181.
- Fowler B (2001) Transport and tissue distribution of homocysteine and related S-adenosyl compounds, In *Homocysteine in Health and Disease* (Carmel R and DW Jacobsen, Eds.) Cambridge University Press: Cambridge, pp 163-175.
- Fuxe K, K Andersson, R Schwarcz, LF Agnati, M Pérez de la Mora, T Hökfelt, M Goldstein, L Ferland, L Possani and R Tapia (1979) Studies on different types of dopamine nerve terminals in the forebrain and their possible interactions with hormones and with neurons containing GABA, glutamate and opioid peptides. *Adv. Neurol.* **24**, 199-215.
- Ho PI, D Ashline, S Dhitavat, D Ortiz, SC Collins, TB Shea and E Rogers (2003) Folate deprivation induces neurodegeneration: roles of oxidative stress and increased homocysteine. *Neurobiol. Dis.* **14**, 32-42.
- Hoehn MM and MD Yahr (1967) Parkinsonism: onset, progression and mortality. *Neurology* **17**, 427-442.
- Hyland K and T Bottiglieri (1992) Measurement of total plasma and cerebrospinal fluid homocysteine by fluorescence following high-performance liquid chromatography and precolumn derivatization with o-phthalaldehyde. *J. Chromatogr.* **579**, 55-62.
- Jara-Prado A, A Ortega-Vazquez, L Martinez-Ruano, C Riosand and A Santamaria (2003) Homocysteine-induced brain lipid peroxidation: effects of NMDA receptor blockade, antioxidant treatment, and nitric oxide synthase inhibition. *Neurotox. Res.* **5**, 237-243.
- Khalil A, JP Fortin, JG LeHoux and T Fulop (2000) Age-related decrease of dehydroepiandrosterone concentrations in low density lipoproteins and its role in the susceptibility of low density lipoproteins to lipid peroxidation. *J. Lipid Res.* **41**, 1552-1561.
- Kim SB, M Hill, YT Kwak, R Hampl, DH Jo and R Morfin (2003) Neurosteroids: cerebrospinal fluid levels for Alzheimer's disease and vascular dementia diagnostics. *J. Clin. Endocrinol. Metab.* **88**, 5199-5206.
- Lipton SA, WK Kim, YB Choi, S Kumar, DM D'Emilia, PV Rayudu, DR Arnelle and JS Stamler (1997) Neurotoxicity associated with dual actions of homocysteine at the N-methyl-D-aspartate receptor. *Proc. Natl. Acad. Sci. USA* **94**, 5923-5928.
- Magni E, G Binetti, A Bianchetti, R Rozzini and M Trabucchi (1996) Mini-mental state examination: a normative study in Italian elderly population. *Eur. J. Neurol.* **3**, 1-5.
- Mattson MP and TB Shea (2003) Folate and homocysteine metabolism in neural plasticity and neurodegenerative disorders. *Trends Neurosci.* **26**, 137-146.
- Maurice T, JL Junien and A Privat (1998) Sigma1 receptor agonists and neurosteroids attenuate B25-35-amyloid peptide-induced amnesia in mice through a common mechanism. *Neuroscience* **83**, 413-428.
- Mazat L, S Lafon, B Debuire, JF Tessier, JF Dartigues and EE Baulieu (2001) Prospective measurements of dehydroepiandrosterone sulfate in a cohort of elderly subjects: relationship, to gender, subjective health, smoking habits, and 10-year mortality.

- Proc. Natl. Acad. Sci. USA* **98**, 8145-8150.
- Miller JW, J Selhub, MR Nadeau, CA Thomas, RG Feldman and PA Wolf (2003) Effect of l-dopa on plasma homocysteine in PD patients: relationship to B-vitamin status. *Neurology* **60**, 1125-1129.
- Pfeiffer CM, DL Huff and EW Gunter (1999) Rapid and accurate HPLC assay for plasma total homocysteine and cysteine in a clinical laboratory setting. *Clin. Chem.* **45**, 290-292.
- Rasmuson S, B Bnasman, K Carlstrom and T Olsson (2002) Increased levels of adrenocortical and gonadal hormones in mild to moderate Alzheimer's disease. *Dement. Geriatr. Cogn. Disord.* **13**, 74-79.
- Razani B, SE Woodman and MP Lisanti (2002) Caveolae: from cell biology to animal physiology. *Pharmacol. Rev.* **54**, 431-467.
- Roberts E (1999) The importance of being dehydroepiandrosterone sulfate (in the blood of primates). A longer and healthier life? *Biochem. Pharmacol.* **57**, 329-346.
- Rosenfeld RS, L Hellman and T Gallagher (1972) Metabolism and interconversion of DHEA and DHEA-S. *J. Clin. End. Metab.* **35**, 187-193.
- Schumacher M, S Weill-Engerer, P Liere, F Robert, RJM Franklin, LM Garcia-Segura, JJ Lambert, W Mayo, RC Melcangi, A Parducz, U Suter, C Carelli, EE Baulieu and Y Akwa (2003) Steroid hormones and neurosteroids in normal and pathological aging of the nervous system. *Prog. Neurobiol.* **71**, 3-29.
- Seshadri S, A Beiser, J Selhub, PF Jacques, IH Rosenberg, RB D'Agostino, PW Wilson and PA Wolf (2002) Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. *New Engl. J. Med.* **346**, 476-483.
- Simons K and E Ikonen (1997) Functional rafts in cell membranes. *Nature* **387**, 569-572.
- Wang M, G Wahlström and T Bäckström (1977) The regional brain distribution of the neurosteroids pregnenolone and pregnenolone sulfate following intravenous infusion. *J. Steroid. Mol. Biol.* **62**, 299-306.
- Weill-Engerer S, JP David, V Sazdovitch, P Liere, B Eychenne, A Pianos, M Shumacher, A Delacourte, EE Baulieu and Y Akwa (2002) Neurosteroids quantification in human brain regions: comparison between Alzheimer's and nondemented patients. *J. Clin. Endocrinol. Metab.* **87**, 5138-5143.