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Neuroactive Steroids in Acute Ischemic Stroke: Association with Cognitive, Functional, and Neurological Outcomes

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Key words
- neuroactive steroids
- acute ischemic stroke
- elderly patients
- cognitive impairment
- functional impairment

Abstract

Despite several scientific and technological advances, there is no single neuroprotective treatment that can reverse the brain damage after acute ischemic stroke (AIS). Neuroactive steroids are cholesterol-derived hormones that have the ability to modulate the normal and pathologic nervous system employing genomic and nongenomic mechanisms. In this work, we first investigated if AIS affects the plasma concentration of 5 neuroactive steroids (cortisol, estradiol, progesterone, testosterone, and 3α-androstenediol glucuronide). Second, we studied if levels of circulating steroids associate with neurological, cognitive, and functional outcome in a cohort of 60- to 90 year-old male and female patients with AIS. For this purpose, we recruited patients who were hospitalized at the Emergency Room of the Central Military Hospital within the first 24 h after stroke onset. We designed 2 experimental groups, each one composed of 30 control subjects and 30 AIS patients, both males and females. The assessment of neurological deficit was performed with the NIHSS and the tests used for the functional and cognitive status were: (1) modified Rankin Scale; (2) Photo test, and (3) abbreviated Pfeiffer's mental status questionnaire. We observed a significant difference in plasma concentration of cortisol and estradiol between both experimental groups. In the AIS group, higher levels of these neuroactive steroids were associated with more pronounced neurological, cognitive and functional deficits in women compared to men. We propose that in elderly patients, high levels of circulating neuroactive steroids like cortisol and estradiol could potentiate AIS-mediated neuropathology in the ischemic and penumbra areas.

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Bibliography
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Introduction

Acute ischemic stroke (AIS) represents a severe challenge to public health and a heavy economic burden to countries with a growing senior population. This illness represented the fourth-leading cause of death in the United States [1]. In Latin America, there are a few epidemiological population-based studies and this information comes from hospital records [2]. In 2011, AIS caused 1 of every 20 deaths in the United States. Stroke incidence rates in low- and middle-income countries now exceed those in high-income countries [3]. It has been shown that the incidence and mortality of AIS is different between sexes [4–6]. Despite advances in the pathophysiology and risk factors of ischemic stroke, there is no effective treatment to cure cerebral ischemic damage. Among the plethora of available drugs employed for CNS diseases, neuroactive steroids are endogenous molecules derived from cholesterol or synthetic compounds that have the ability to cross the blood-brain barrier and modulate brain function in health and disease [7]. The concentrations in plasma and cerebrospinal fluid of these molecules are altered in various neurological diseases [8], although the clinical significance of these alterations remains to be ascertained. In this work, we evaluated whether AIS affects the plasma concentrations of estradiol, progesterone, cortisol, testosterone and 3α-androstenediol glucuronide. As a corollary of these measurements, we also evaluated if changes in circulating steroids bear a relationship with the neurological outcome, cognitive status, and functional dependence of the AIS patients.

Material and Methods

Participants

We recruited patients with AIS from July 2014 to December 2014 who were hospitalized at the Emergency Room of the Central Military Hospital within the first 24 h after stroke onset. Stroke was defined according to the World Health Organization's criteria, and a diagnosis of AIS was confirmed in all patients based on the evidence of neuroimaging including computed tomography and magnetic resonance imaging, following the Recommendations on Stroke Prevention, Diagnosis and Therapy Report [9]. Sixty 90 year-old subjects were randomly selected and distributed in 2 experimental groups: 1) a control group, involving subjects without physical or psychiatric illness and 2) an AIS group, consisting of patients with diagnosis of AIS within the 24 h of their neurovascular event. Subjects were distributed between groups so that each group contained 30 patients (15 women and 15 men). The Ethics Committee of the Cir My 'Dr. Cosme Argerirch' Central Military Hospital approved the study (Act No. 308, February 26, 2014), and the patients or their next-of-kin provided informed consent for participation. **Tables 1S** and **Table 1** shows the criteria employed for inclusion or exclusion of the studied subjects.

Procedures

Patients were diagnosed for AIS by a certified neurologist at the Emergency Room of the Central Military Hospital. Neurological, cognitive, and functional status were determined by NIHSS score, Photo test, Pfeiffer mental status score and by modified Rankin score, respectively. A sample of venous blood was withdrawn in the early morning (07:00 to 09:00 AM) after assessment of neurological and cognitive status. According to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria, stroke subtypes were classified as large-artery atherothrombotic (LAA), cardioembolic (CE), small-artery occlusion (SAO), other causes, and undetermined [10]. Stroke risk factors included a medical history of hypertension, defined as self-reported history of hypertension or using antihypertensive drugs, diabetes mellitus (DM) defined as history of DM or using hypoglycemic medications at discharge, dyslipidemias, defined as self-reported history of all types of dyslipidemia or oral antidyslipidemia drugs or using antidyslipidemia drugs at discharge, atrial fibrillation (AF), defined as history of AF, confirmed by at least one electrocardiogram or the presence of arrhythmia during hospitalization, and modifiable lifestyle factors, including current smoking, alcohol consumption, and obesity (body mass index > 30 kg/m^2).

Table 1 Criteria employed for inclusion or exclusion of the AIS group.

Inclusion	Exclusion
Age between 60 and 90 years	Age < 60 or > 90 years
Agreeing to participate in the study	Hemorrhagic stroke
Acute ischemic stroke of anterior vascular territory and/or posterior vascular territory within 24 h of onset	Transient ischemic attack (TIA)
Nine or more points in the Glasgow Coma Scale	Acute ichemic stroke after 24 h of onset
Female patients in menopause	Hormonal replacement therapy
Patients without cognitive impairment before AIS according to family reference	Immunosuppressive therapy in the last month before AIS (e. g., corticosteroids)
Acceptance of the next of kin proxy in case the participant has sensory impairment	Acute infection (e. g., pneumonia, urinary tract infection)
	Diagnosis of oncologic disease in the last month before AIS
	Diagnosis of endocrinologic disease in the last month before AIS
	Acute or long-term psychiatric illness.
	No agreement to participate in the study
	Eight or less points in the Glasgow Coma Scale
	Female patients with menstrual cycle or in the perimenopause
	Patients with kidney or hepatic illness
	Patients with cognitive impairment before AIS

Measures

Quantitation of neuroactive steroids in plasma

The measurement of estradiol (Estradiol EII) and progesterone (Progesterone II) was performed by electrochemiluminescence immunoassay (ECLIA) employing a Cobas e601 (Roche Diagnostics, Mannheim, Germany). The functional sensitivity of the method of estradiol was 12 pg/ml and the analytical sensitivity was 5.0 pg/ml, while for progesterone the functional sensitivity of the method was 0.15 ng/ml and the analytical sensitivity was 0.03 ng/ml according to the manufacturer. In the case of cortisol and testosterone, they were determined by an immunoassay chemiluminescent microparticle (CMIA) procedure, using a Team Architect i1 000, Abbott Laboratories, Middletown, USA. The cortisol functional sensitivity was 1 µg/dl, and the analytical sensitivity was 0.2 ng/ml, whereas for testosterone the functional sensitivity was 1 µg/dl and the analytical sensitivity was 0.05 ng/ml according to the manufacturer. While 3α-androstenediol glucoronide was determined by radioimmunoassay (RIA) using a DSL 9 200, Beckman Coulter, Texas USA. The functional sensitivity was 0.34 ng/ml according to the manufacturer.

Neurological impairment during AIS

The assessment of neurologic status during the AIS was carried out with the National Institute of health stroke scale at the time of hospitalization (NIHSS, available at (http://www.ninds.nih.gov/doctors/NIH_Stroke_Scale.pdf) [11].

Cognitive testing

At the time of the cognitive assessment, patients with AIS were vigil on 9 or more points on the Glasgow Coma Scale [12]. The cognitive tests used were: (1) test photos [13] and (2) the abbreviated questionnaire of Pfeiffer [14]. These tests were performed within 24 h of the AIS and prior to the extraction of blood for steroid analysis. The reasons for the choice of these tests were: A) Test photos evaluated memory, object recognition and verbal fluency. The level of education of the patient does not influence this test; it is simple and brief in duration (4 min). B) The Pfeiffer Test studied orientation, calculation, recent and remote memory, and information about daily events. It is important that the score of this test depends on the total errors. It is also applicable to people with low educational level, visual or auditory sensory deficit, and advanced age.

Functional dependence for daily activities

The functional status of patients with AIS was measured with the modified Rankin Scale [15] at the time of discharge. Data were collected through an interview designed for the purpose of reducing the variability between evaluators.

Statistical analysis

Statistical analysis was carried out using GraphPad Prism 5.03. Data were subjected to the Shapiro Wilks test to ascertain a normal distribution. The statistical tests used were column statistics (average), 2-tailed Student' *t*-test when only 2 groups were compared, and one-way ANOVA followed by the post-hoc Newman-Keuls multiple comparison test when several groups were analyzed. Results were expressed as the mean ± SEM) of 15 patients per experimental group. A p-value < 0.05 was considered statistically significant.

Ethical aspects and institutional approval

The used Protocol was approved by the institutional review board of clinical trials (C.I.R.E.C.) of the Cir My 'Dr. Cosme Argerich' Central Military Hospital according to the Act No. 308, February 26, 2014.

Results

Sociodemographic variables and clinical measurements

The age of presentation of AIS in women was significantly higher than men ($p < 0.05$). There were no differences in the body mass index (BMI). Both male and female AIS patients received at the Emergency Room showed more than 140/90 mmHg blood pressure values, blood glucose levels greater than 140 mg/dl, HbA1c values lower than 52 mmol/mol in nondiabetic patients, more than 53 mmol/mol up to 69 mmol/mol in diabetic patients and were normothermic. The main risk factor of AIS in both sexes was high blood pressure while the main cause of the AIS in women was cardioembolic (47%) and in men was atherothrombotic (38%). Females presented a greater total involvement of the anterior vascular circuit with respect to men (53 vs. 31%), whereas in males we observed a greater partial involvement of the anterior circulation (44 vs. 35%), following the nomenclature of the Classification of the Oxfordshire Community Stroke. From the anatomical point of view, AIS more frequently affected the left cerebral hemisphere than the right hemisphere in both sexes (data shown in **Table 2**).

Neurological impairment during AIS

The neurological deficit quantified by the NIHSS score was significantly higher in women than men ($p < 0.05$) in the first 24 h of hospitalization from the beginning of AIS (**Fig. 1**).

Cognitive status

Photo test

The cognitive status quantified by the Photo test was significantly lower in women within the AIS group compared to the control group and men from both the control and AIS groups ($p < 0.001$) (**Fig. 2a**). No statistical differences in the Pfeiffer mental status score were observed but there was a tendency of female AIS to perform worse compared to the other experimental groups (**Fig. 2b**).

Pfeiffer mental status score

No statistical differences in the Pfeiffer mental status score was observed, although a tendency existed for women with AIS to perform worse compared to the other experimental groups (**Fig. 2b**).

Plasma concentration of neuroactive steroids

Cortisol

Whereas AIS may be a reaction to an acute stressful event, it constitutes a stressful condition with activation of cortisol secretion. Thus, it came to no surprise that AIS was followed by a significant increase in serum cortisol concentrations both in women and men compared to their gender control subjects ($p < 0.001$ and $p < 0.05$ respectively) (**Fig. 3a**).

Estradiol

Table 2 shows that both control women and women with AIS passed their menopause age by several years. In spite of their hypoestrogenic stage, women with AIS showed an increase in plasma estradiol values compared to their control group ($p < 0.01$). We found no differences in men between those with AIS and control subjects. Our analysis also showed that AIS increased plasma estradiol in men compared to women in the control group ($p < 0.001$) (**Fig. 3b**).

Progesterone, testosterone, and 3α-androstenediol glucuronide

In contrast to changes of cortisol and estradiol, we found no significant differences in plasma concentrations of the 3 neuroactive steroids between sexes or between AIS and control groups.

Degree of functional dependence

At the time of discharge, the degree of functional dependence quantified by the modified Rankin scale was higher in AIS women compared to AIS men ($p < 0.05$) (**Fig. 4**).

Discussion

The present investigation demonstrated that the age of presentation of AIS in women was significantly higher compared to men. There are already reports showing differences of AIS incidence between women and men, with men showing a greater vulnerability at early age periods in comparison to women. However, this relationship is inverted in older women who are more prone to suffer an ischemic stroke than men [5, 16]. Our behavioral data also determined that women with AIS showed greater cognitive and memory impairment and neurological disability at discharge compared with men with AIS.

Our study also disclosed that AIS induces in women and men changes of neuroactive steroids in plasma. Notably, plasma cortisol was increased in both sexes, as expected from patients undergoing a very stressful situation. In this sense, ischemic stroke induces a strong activation of the hypothalamic-pituitary-adrenal (HPA) axis, with increases in adrenal cortisol secretion [17]. This neuroactive steroid plays an important role in brain ischemia [18]. A brain region showing high vulnerability to excess glucocorticoid exposure is the hippocampus [19–21], due to its high expression of mineralocorticoid and glucocorticoid receptors [22]. In the hippocampus, besides other regions, glucocorticoids exert a negative feedback to inactivate the HPA overdrive occurring during stress [21]. However, a faulty hip-

Table 2 A comparison of sociodemographic variables and measurements in control and AIS patients.

Variables	Women		Men	
Age (years, mean ± SD)	Control: 79 ± 3; AIS: 82 ± 9 (ns)		Control: 65 ± 9; AIS: 70 ± 13 (ns)	
Body mass index (kg/m^2)	Control: 27.8; AIS: 27.3 (ns)		Control: 27.7; AIS: 28.1 (ns)	
Education (years)	Control: 13.8; AIS: 12 ($p<0.05$)		Control: 16; AIS: 16.01 (ns)	
Modifiable risk factors				
Hypertension	100 % (15)		81 % (12)	
Atrial fibrillation	18 % (3)		6 % (1)	
Diabetes	12 % (2)		31 % (5)	
Dyslipidemic	18 % (3)		44 % (7)	
Current smoking	6 % (1)		31 % (5)	
Obesity	0 % (0)		0 % (0)	
Alcohol consumption	0 % (0)		0 % (0)	
TOAST classification				
LAA	23 % (3)		38 % (6)	
CE	47 % (7)		31 % (5)	
SAO	12 % (2)		31 % (5)	
Undetermined etiology	12 % (2)		0 %	
Other determined etiology	6 % (1)		0 %	
Oxford Community Stroke Project classification				
Total anterior circulation stroke	53 % (8)		31 % (5)	
Partial anterior circulation stroke	35 % (5)		44 % (7)	
Lacunar stroke	6 % (1)		25 % (3)	
Posterior circulation stroke	6 % (1)		0 %	
Brain hemisphere				
Left	53 % (8)		63 % (9)	
Right	47 % (7)		37 % (6)	
Neuroactive steroids	Control	AIS	Control	AIS
Cortisol (µg/dl)	7.04	20.18 ($p<0.001$)	6.5	15.34 ($p<0.05$)
Estradiol (pg/ml)	13.83	28.22 ($p<0.01$)	24.15	33.14 (ns)
Progesterone (ng/ml)	0.4	0.49 (ns)	0.41	0.55 (ns)
Testosterone (ng/ml)	0.37	0.38 (ns)	4.31	4.1 (ns)
3α-androstenediol glucuronide (ng/ml)	2.56	2.76 (ns)	5.01	3.77 (ns)

CE: Cardioembolic; LAA: Large-artery atherotrhombotic; SAO: Small artery occlusion; TOAST: Trial Org 10172; ns: Not significant

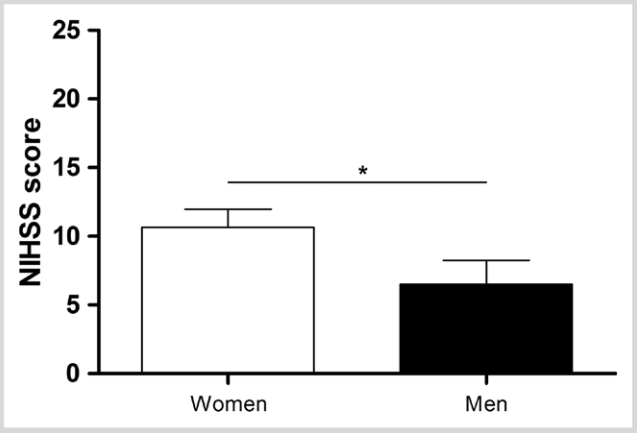

Fig. 1 NIHSS score of patients who were hospitalized at the Emergency Room of the Central Military Hospital within the 24 h after onset of ischemic stroke. The NIHSS score evaluates neurological, cognitive and functional status. Statistical analysis by Student's *t*-test showed $p<0.05$ between scores of women and men (n = 15 in each group).

pocampus would be resistant to the negative glucocorticoid feedback, with unopposed activation of the HPA axis. Therefore, it is possible that excess levels of cortisol in AIS negatively affect the metabolic, neuroendocrine, and behavioral functions of the hippocampus, and contribute to the cognitive and memory impairment of the stroke patients. However, this mechanism would equally apply to women and men, since both genders showed comparable increases of plasma cortisol. Therefore, in order to account for their greater neurological disability and cognitive impairment, other factors could play a role in old women with AIS. In our studies, the enhanced vulnerability in women with AIS was revealed by the Photo test, which combines changes in object recognition, free memory, facilitated memory, and fluency.

Our cohort also showed changes of estradiol levels, which were elevated in women with AIS compared to their control group. In men with AIS, estradiol levels were higher than in control women, but did not increase further after stroke. There is now abundant evidence for estrogen neuroprotection in several stroke models [23], which include transient and permanent middle cerebral artery occlusion, global forebrain ischemia, and glutamate-induced focal cerebral ischemia [24–27]. However, estrogen effects change drastically from neuroprotective to neurotoxic and neurodegenerative in reproductively senescent animals [28,29]. This evidence supports the "window of opportunity" hypothesis for estrogen replacement therapy for perimenopausal women [30]. Instead, in old women the window is missing and together with high cortisol, may contribute to the damaging effect of stroke.

It is important at this point to discuss the origin of plasma estradiol in AIS patients. In postmenopausal women and elderly men, estrogen synthesis is predominantly extragonadal, due to the aromatase expressed by white adipose tissue and other periph-

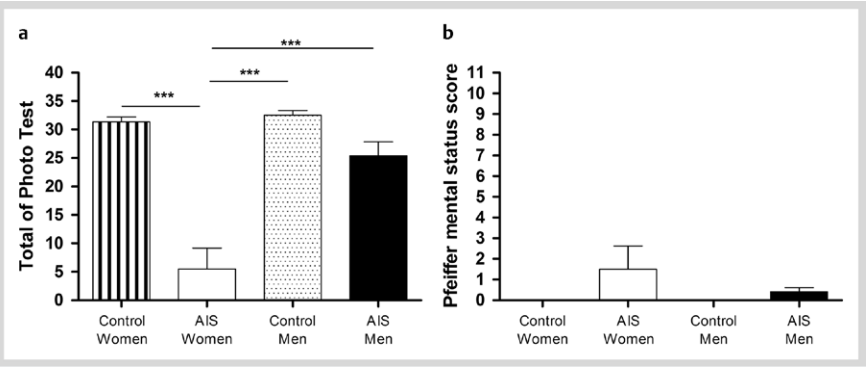

Fig. 2 Control and AIS groups evaluated by Photo Test **a** and by Pfeiffer mental status score **b** within the 24 h from onset of ischemic stroke. The total of Photo test was the sum of score of object recognition, free memory, facilitated memory, and verbal fluency. Statistical analysis carried out using ANOVA followed by the Newman-Keuls post-test demonstrated significant differences between control and AIS women (* * * $p<0.001$), control and AIS men (* * * $p<0.001$) and AIS women and control men (* * * $p<0.001$) in Photo Test. No significant differences were found for gender in either the control subjects or the AIS group in Pfeiffer mental status score (n = 15 control subjects or patients in each group).

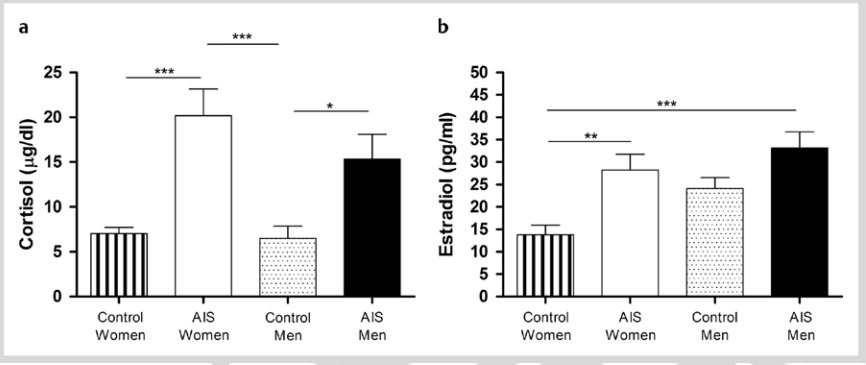

Fig. 3 Levels of plasma cortisol (μg/dl) **a** and estradiol (pg/ml) **b** within the 24 h from onset of ischemic stroke. Statistical analysis by ANOVA followed by the Newman-Keuls post-hoc test demonstrated increased plasma cortisol in men with AIS (* $p<0.05$ vs. control men), which was of higher statistical magnitude in women with AIS (* * * $p<0.001$ vs. control women). On the other hand, the statistical analysis demonstrated that AIS increased plasma estradiol in women ($p<0.01$) but not in men (n = 15 control subjects or patients in each group).

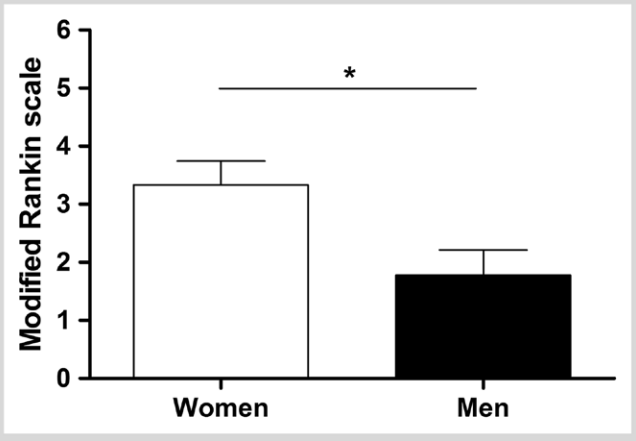

Fig. 4 Degree of disability or dependence in the daily activities by modified Rankin scale at the time of patient discharge. AIS produced greater disability in women than men (Student's *t*-test, $p<0.05$).

eral tissues. One of the stimulatory factors for estrogen synthesis in the white adipose tissue is cortisol [31–33]. Therefore, we hypothesized that cortisol-stimulated extragonadal aromatase accounts for the high plasma estradiol levels in patients with AIS. Again, this mechanism could occur in both sexes, unless ischemia more exclusively sensitizes the brain of elderly women to the damaging effects of estradiol and cortisol.

Finally, although these results reflect the peripheral endocrine effect of AIS, we could consider that the brain itself could be an additional site of synthesis of estrogen-induced injury through rapid transcription and translation of aromatase agree on some observations in the literature. This induction seems to occur regardless of location of primary brain damage [34]. The induced expression of aromatase could elevate local estrogen levels enough to exacerbate the damage induced by AIS.

Conclusions

Our results showed that some, but not all neuroactive steroids change their concentrations under life-threatening conditions. In addition, these hormones modulate the functionality of the nervous system in a sex and age dependent manner. The age of AIS in our population distribution was similar to that reported in other studies. Additionally, we found that the neuroendocrine status of the patients may play a role in the cognitive status and neurological outcome of stroke. In principle, the female brain may be better protected against cerebral ischemic damage than men during adulthood. However, the decline of sex steroids after menopause together with increased HPA activation change the role of sex steroids from neuroprotective to neurotoxic according to recent theories. It is at this moment when women equal and surpass men in increased susceptibility to ischemic vascular event. In the future, these molecules (cortisol and estradiol) could be variables to consider during the acute treatment of ischemic stroke for the purposes of improving the neurological, cognitive, and functional status of patients.

Contributors

Sebastian Casas designed the original study and wrote the protocol. Sebastian Casas and Alejandro De Nicola wrote the manuscript. Alejandro De Nicola, Maria Gonzalez Deniselle, and Gisella Gargiulo-Monachelli made contributions to the conceptualization of the study, conducted the literature review, and statistical analyses. Andres Perez, Martin Tourreilles, and Marcelo Mattiazzi contributed to recruit patients. Cristian Ojeda and Daniel Lotero Polesel quantified neuroactive steroids in plasma. All of the authors assisted in and reviewed the analyses. All authors contributed to and approved of the final manuscript.

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Conflict of Interest

The authors have no conflicts of interest to disclose.

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References

1 *Heron M*. Deaths: leading causes for 2007. Natl Vital Stat Rep 2011; 59: 1–95
2 *Feigin V*. Stroke epidemiology in the developing world. Lancet 2005; 365: 2160–2161
3 *Feigin VL*, *Lawes CM*, *Bennett DA*, *Barker-Collo SL*, *Parag V*. Worldwide stroke incidence and early case fatality reported in 56 population-based studies: a systematic review. Lancet Neurol 2009; 8: 355–369
4 *Kelly-Hayes M*, *Beiser A*, *Kase CS*, *Scaramucci A*, *D'Agostino RB*, *Wolf PA*. The influence of gender and age on disability following ischemic stroke: the Framingham study. J Stroke Cerebrovasc Dis 2003; 12: 119–126
5 *Kapral MK*, *Fang J*, *Hill MD*, *Silver F*, *Richards J*, *Jaigobin C*, *Cheung AM*. Sex differences in stroke care and outcomes: results from the registry of the Canadian stroke network. Stroke 2005; 36: 809–814
6 *Reeves MJ*, *Bushnell CD*, *Howard G*, *Gargano JW*, *Duncan PW*, *Lynch G*, *Khatiwoda A*, *Lisabeth L*. Sex differences in stroke: epidemiology, clinical presentation, medical care, and outcomes. Lancet Neurol 2008; 7: 915–926
7 *Morrow AL*, *Porcu P*. In: Neuroactive Steroids. Price LH, Stolerman I. (eds.). Encyclopedia of Psychopharmacology. Berlin: Springer; 2014
8 *Porcu P*, *Barron AM*, *Frye CA*, *Walf AA*, *Yang SY*, *He XY*, *Morrow AL*, *Panzica GC*, *Melcangi RC*. Neurosteroidogenesis today: Novel targets for neuroactive steroid synthesis and action and their relevance for translational research. J Neuroendocrinol 2016; 28: 12351
9 Recommendations on stroke prevention, diagnosis, and therapy. Report of the WHO task force on stroke and other cerebrovascular disorders. Stroke 1989; 20: 1407–1431
10 *Adams HP*, *Bendixen BH*, *Kappelle LJ*, *Biller J*, *Love BB*, *Gordon DL*, *Marsh EE*. Classification of subtype of acute ischemic stroke: definitions for use in a multicenter clinical trial: TOAST: Trial of Org 10172 in Acute Stroke Treatment. Stroke 1993; 24: 35–41
11 *Brott T*, *Adams HP*, *Olinger CP*, *Marler JR*, *Barsan WG*, *Biller J*, *Spilker J*, *Holleran R*, *Eberle R*, *Hertzberg V*, *Rorick M*, *Moomaw CJ*, *Walker M*. Measurements of Acute Cerebral Infarction: A Clinical Examination Scale. Stroke 1989; 20: 864–870
12 *Fischer M*, *Rüegg S*, *Czaplinski A*, *Strohmeier M*, *Lehmann A*, *Tschan F*, *Hunziker P*, *Marsch S*. Research Inter-rater reliability of the Full Outline of UnResponsiveness score and the Glasgow Coma Scale in critically ill patients: a prospective observational study. Critical Care 2010; 14: R64
13 *Radak D*, *Resanovic I*, *Isenovic ER*. Changes in hypothalamus-pituitary-adrenal axis following transient ischemic attack. Angiology 2014; 65: 723–732
14 *Pfeiffer E*. A short portable mental status questionnaire for the assessment of organic brain deficit in elderly patients. J Am Geriatr 1975; 10: 433–441
15 *Wilson JT*, *Hareendran A*, *Grant M*, *Baird T*, *Schulz UG*, *Muir KW*, *Bone I*. Improving the Assessment of Outcomes in Stroke Use of a Structured Interview to Assign Grades on the Modified Rankin Scale. Stroke 2002; 33: 2243–2246
16 *Yu C*, *An Z*, *Zhao W*, *Wang W*, *Gao C*, *Liu S*, *Wang J*, *Wu J*. Sex Differences in Stroke Subtypes, Severity, Risk Factors, and Outcomes among Elderly Patients with Acute Ischemic Stroke. Front Aging Neurosci 2015; 7: 174
17 *Barugh AJ*, *Gray P*, *Shenkin SD*, *MacLullich AM*, *Mead GE*. Cortisol levels and the severity and outcomes of acute stroke: a systematic review. J Neurol 2014; 261: 533–545
18 *Baird TA*, *Parsons MW*, *Planh T*, *Butcher KS*, *Desmond PM*, *Tress BM*, *Colman PG*, *Chambers BR*, *Davis SM*. Persistent poststroke hyperglycemia is independently associated with infarct expansion and worse clinical outcome. Stroke 2003; 34: 2208–2214
19 *Sapolsky RM*. Glucocorticoid Toxicity in the Hippocampus: Reversal by Supplementation with Brain Fuels. The Journal of Neuroscience 1986; 6: 2240–2244
20 *Herman JP*, *Schäfer MK*, *Young EA*, *Thompson R*, *Douglass J*, *Akil H*, *Watson SJ*. Evidence for hippocampal regulation of neuroendocrine neurons of the hypothalamo-pituitary-adrenocortical axis. J Neurosci 1989; 9: 3072–3082
21 *Kim JJ*, *Diamond DM*. The stressed hippocampus, synaptic plasticity and lost memories. Nat Rev Neurosci 2002; 3: 453–462
22 *De Kloet ER*, *Karst H*, *Joëls M*. Corticosteroid hormones in the central stress response: quick-and-slow. Front Neuroendocrinol 2008; 29: 268–272
23 *McCullough LD*, *Hurn PD*. Estrogen and ischemic neuroprotection: an integrated view. Trends Endocrinol Metab 2003; 14: 228–235
24 *Yang SH*, *Liu R*, *Perez EJ*, *Wang X*, *Simpkins JW*. Estrogens as protectants of the neurovascular unit against ischemic stroke. Curr Drug Targets CNS Neurol Disord 2005; 4: 169–177
25 *Sudo S*, *Wen TC*, *Desaki J*, *Matsuda S*, *Tanaka J*, *Arai T*, *Maeda N*, *Sakanaka M*. Beta-estradiol protects hippocampal CA1 neurons against transient forebrain ischemia in gerbil. Neurosci Res 1997; 29: 345–354
26 *Fukuda K*, *Yao H*, *Ibayashi S*, *Nakahara T*, *Uchimura H*, *Fujishima M*, *Hall ED*. Ovariectomy exacerbates and estrogen replacement attenuates photothrombotic focal ischemic brain injury in rats. Stroke 2000; 31: 155–160
27 *Mendelowitsch A*, *Ritz MF*, *Ros J*, *Langemann H*, *Gratzl O*. 17beta-Estradiol reduces cortical lesion size in the glutamate excitotoxicity model by enhancing extracellular lactate: a new neuroprotective pathway. Brain Res 2001; 901: 230–236
28 *Azcoitia I*, *Arevalo MA*, *De Nicola AF*, *Garcia-Segura LM*. Neuroprotective actions of estradiol revisited. Trends Endocrinol Metab 2011; 22: 467–473
29 *Velarde MC*. Pleiotropic actions of estrogen: a mitochondrial matter. Physiol Genomics 2013; 45: 106–109
30 *Brinton RD*, *Yao J*, *Yin F*, *Mack WJ*, *Cadenas E*. Perimenopause as a neurological transition state. Nat Rev Endocrinol 2015; 11: 393–405
31 *DiSilvestro D*, *Petrosino J*, *Aldoori A*, *Melgar-Bermudez E*, *Wells A*, *Ziouzenkova O*. Enzymatic intracrine regulation of white adipose tissue. Horm Mol Biol Clin Investig 2014; 19: 39–55
32 *Simpson E*, *Ackerman G*, *Smith M*, *Mendelson C*. Estrogen formation in stromal cells of adipose tissue of women: Induction by glucocorticosteroids. Proc Nati Acad Sci USA 1981; 78: 5690–5694
33 *Nelson LR*, *Bulun SE*. Estrogen production and action. J Am Acad Dermatol 2001; 45: 116–124
34 *Saldanha CJ*, *Duncan KA*, *Walters BJ*. Neuroprotective Actions of Brain Aromatase. Front Neuroendocrinol 2009; 30: 106–118

Supporting Information

Neuroactive Steroids in Acute Ischemic Stroke: Association with Cognitive, Functional, and Neurological Outcomes

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Table 1S This table shows the criteria employed for inclusion or exclusion of the control subjects.

INCLUSION	EXCLUSION
Age between 60 and 90 years.	Age <60 or >90 years.
Agreeing to participate in the study.	Hormonal replacement therapy.
Fourteen or more points in the Glasgow Coma Scale.	Immunossupresive therapy in the last month (Example corticosteroids).
Female control in menopause.	Acute infection (Example, pneumonia, urinary tract infection).
Control subjects without cognitive impairment according to certified neurologist.	Diagnosis of oncologic disease in the last month.
	Diagnosis of endocrinologic disease in the last month.
	Acute or long-term psychiatric illness.
	No agreement to participate in the study
	Thirteen or less points in the Glasgow Coma Scale.
	Female patients with menstrual cycle or in the perimenopause.
	Patients with kidney or hepatic illness.
	Patients with cognitive impairment.