

The Italian Journal of Neurological Sciences

Founded by Renato Boeri

Official Journal of the Italian Neurological Society
and of the Italian Society of Clinical Neurophysiology



SOCIETÀ
ITALIANA
DI
NEUROLOGIA

REPRINT

MASSON

MASSON S.p.A. - Divisione Periodici - Via Statuto 2/4 - 20121 Milano

Circulating levels of anticonvulsant metabolites of progesterone in women with partial epilepsy in the intercritical phase

Galli R.¹, Michelini S.², Bartalena L.³, Massetani R.¹, Pani L.², Grasso L.³, Cassano G.B.², Martino E.³, Purdy R.H.⁴, Murri L.¹

¹ Istituto di Clinica Neurologica, ² Istituto di Clinica Psichiatrica, ³ Istituto di Endocrinologia Università di Pisa,

⁴ Department of Psychiatry, School of Medicine, University of California, San Diego

The 3 α -hydroxy metabolites of progesterone (P), 3 α -hydroxy-5 α -pregnan-20-one (allopregnanolone, A-PREG) and 3 α -hydroxy-5 β -pregnan-20-one (pregnanolone, PREG), have been found to be among the most potent ligands of gamma-aminobutyric (GABA)-A receptors; in experimental animals, they have been found to have anxiolytic, hypnotic and anticonvulsant effects similar to those of the benzodiazepines and barbiturates that interact with GABA-A receptors. The present study was undertaken to determine plasma A-PREG and PREG concentrations in the luteal phase in women with partial epilepsy, in order to determine if an impaired metabolism of P occurs in this convulsive disorder. We measured plasma P, A-PREG and PREG levels in 15 women with partial epilepsy in the intercritical phase, and in 15 age-matched healthy women, during the luteal phase of the ovarian cycle (22nd-24th day). The mean plasma $\pm$ S.E. A-PREG levels (three blood samples) were 0.7 ± 0.6 ng/ml in the epileptic women and 0.5 ± 0.2 ng/ml in controls, with no significant difference between the two groups ($p = \text{NS}$); the PREG levels were also similar (1.4 ± 1 ng/ml and 1 ± 1.1 ng/ml, respectively; $p = \text{NS}$). A significant correlation was found between P levels and both A-PREG and PREG levels ($r = 0.72$, $p < 0.001$ and $r = 0.79$, $p < 0.001$, respectively). There were no significant differences between the two groups in terms of serum adrenocorticotrophic hormone, cortisol, dihydroepiandrosterone-sulfate, follicle stimulating hormone, prolactin, luteinizing hormone or estradiol levels.

Key Words: Progesterone — Allopregnanolone — Pregnanolone — Epilepsy — GABA receptors — Neuroactive steroids — Anxiolytic steroids.

Introduction

The high affinity of naturally occurring progesterone (P) metabolites for GABA-A receptors, and the presence in the brain of both 5 α -steroid reductase and 3 α -hydroxysteroidoxoreductase, have prompted speculations that these natural 3 α -ring-reduced steroids might act as endogenous modulators of GABA-A receptors [13]. It can therefore be postulated that P metabolites similar to the above substances may be involved in the pathogenesis of epilepsy, and that some of these steroids may have potent anticonvulsant properties [9]. This appears to be supported by the observation that ovarian steroids can influence seizure susceptibility. P increases the seizure threshold [8] and its intravenous infusion reduces the entity of EEG discharges in women with partial epilepsy [2]; furthermore, the protective effect of P on seizures has been observed in women treated with medroxyprogesterone acetate, who showed a de-

crease in seizure frequency after this treatment [16]. On the other hand, the facilitating effect of estrogen (E) on seizures has been previously shown by Logothetis et al. [11]; and Logothetis and Harner [12] confirmed the epileptogenic action of E when they applied it either locally on the cortex or parenterally in laboratory animals. Investigation of plasma E and P values throughout the course of the ovarian cycle in epileptic women with catamenial seizure exacerbation has made it possible to relate the frequency of epileptic episodes to an increase in the E/P ratio, with absolute or relative hyperestrogenism [1, 6]. This has led to the suggestion that hormonal imbalance may be one of the mechanisms responsible for the occurrence of seizures during the catamenial period, although it is possible that abnormal epileptic discharges and antiepileptic drugs interfere with pituitary secretion and/or peripheral hormone metabolism [4, 5, 17]: hormonal imbalance is in fact more evident in treated than in drug-free patients with catamenial epilepsy [6]. More recently, it has been reported that one of the structurally-related metabolites of P, 5 α -pregnan-3 α -ol-20-one, is highly effective against bicuculline, metrazol and picrotoxin-induced seizures [3], and that the in-

This work was partially supported by grants from the Ministero della Università e della Ricerca Scientifica e Tecnologica (M.U.R.S.T. 40% and 60%).

TABLE I. Clinical features of epileptic patients.

Patients (No.)	Age (yrs)	Onset of disease (yrs)	Type of seizures	Frequency of seizures More than 4 seizures per month: > Less than 4 seizures per month: <	Catamenial exacerbation	EEG focus	Neuroimaging	Therapy (mg/die)
1	40	10	CP	<	No	rTL	Previous Cerebral Neoplasia	PB: 150; PHT: 300; VGB: 1000
2	35	34	SP	>	Yes	ITL	Previous Cerebral Neoplasia	CBZ: 600; VGB: 1500
3	50	47	SP	>	No	ITL	Previous Cerebral Neoplasia	PHT: 250; CBZ: 1200; VGB: 1000
4	27	17	CP	<	No	ITL	No Lesion	PB: 50; CBZ: 800; VGB: 2000
5	29	23	CP	<	Yes	ITL	No Lesion	PHT: 300; VGB: 1000
6	33	10	CP	<	No	ITL	Left Temporal Cortical Atrophy	CBZ: 800; PB: 150
7	34	25	CP	<	Yes	Tb	Bilateral Parietal- Occipital Cortical Atrophy	CBZ: 800
8	24	18	CP	>	No	ITL	No Lesion	PB: 100; CBZ: 1600; VGB: 2500
9	25	15	CP	>	Yes	Tb	No Lesion	CBZ: 1000; VPA: 1000
10	38	6	CP	<	Yes	ITL	No Lesion	CBZ: 1600; VGB: 3000
11	42	25	CP	<	No	Tb	Subcortical Atrophy	CBZ: 1000; VPA: 1500
12	43	1	CP	>	Yes	ITL	Bilateral Temporal Cortical Atrophy	PB: 100; CBZ: 600; PHT: 300; VGB: 2000
13	30	25	CP	<	No	rTL	Previous Cerebral Neoplasia	PB: 100; CBZ: 1000
14	33	24	SP	<	No	Tb	No Lesion	CBZ: 800; VGB: 1500
15	18	10	CP	>	No	Tb	Bilateral Temporal Cortical Atrophy	PB: 100; VPA: 2500; VGB: 3000

CP = Complex Partial; SP = Simple Partial; rTL = right Temporal Lobe; ITL = left Temporal Lobe; Tb = Temporal bilateral; CBZ = carbamazepine; PHT = phenytoin; VGB = γ -vinyl-GABA; PB = phenobarbital; VPA = sodium valproate.

traperitoneal administration of 5 β -pregnan-3 α -ol-20-one, 5 α -pregnan-3 α -ol-20-one and 5 α -pregnan-3 α , 21-diol-20-one actively protects against kainic acid-induced limbic status epilepticus in mice [10]. For these reasons, this study was designed to measure plasma A-PREG and PREG concentrations in patients with temporal lobe partial epilepsy, in whom the epileptogenic focus is localized in the limbic structures, and anatomically and physiologically related to the central neuroendocrine structures of the hypothalamus and pituitary.

Methods

Patient population

Fifteen women (aged 18-50 years; mean 33.7) with temporal lobe partial epilepsy diagnosed on the basis of the Classification of Epilepsies and Epileptic Syndromes of

the International League Against Epilepsy (1989) [7] were included in this study after having given their informed consent. Table I shows the clinical features of these patients. In particular, six patients had more than four seizures per month and nine more than one but less than four seizures per month. Catamenial exacerbation was present in six patients. EEG revealed a unilateral temporal focus in ten patients (two in the right and eight in the left hemisphere), and bilateral temporal abnormalities in five. Neuroimaging (CT scan and/or NMR) detected cortical or subcortical atrophy in five cases, sequelae of previous surgery for cerebral neoplasia in four cases, and no lesion in six cases. All of the patients were treated with more than one drug and had serum drug levels within the therapeutic range.

Fifteen age-matched healthy female volunteers (aged 25-46 years; mean 33) with no present or past history of psychiatric, neurological or endocrine disorders served as controls. A full medical history was taken and a complete physical examination carried out for each patient. All of

TABLE II. Endocrine profile of epileptic patients and controls.

Hormones		Patients (Mean±SE)	Controls (Mean±SE)
ACTH	(pg/ml)	10.43±0.75	11.71±0.74
CORTISOL	(ng/ml)	184.27±19.27	172.09±15
DHEA-S	(ng/ml)	2223±304.07	2031.80±4
FSH	(mIU/ml)	11.95±2.18	8.61±1.85
PRL	(ng/ml)	13.92±1.91	10.5±1.5
LH	(mIU/ml)	18.91±5.01	19.5±6.81
E ₂	(pg/ml)	114.4±18.6	137.6±25.2

the endocrine evaluations, which included the measurement of the plasma concentrations of P, A-PREG, PREG, ACTH, cortisol, DHEA-S, FSH, PRL, LH and E₂, were made during the advanced luteal phase of the menstrual cycle (22nd-24th day); the patients had been seizure-free for at least two days.

Hormonal Assays

Commercial assays were used to determine the levels of the following hormones: P (PROG-CTK-2, Sorin Biomedica Diagnostic Division, Saluggia, Italy); ACTH (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA); cortisol and DHEA-S (Radim S.p.A., Pomezia-Rome, Italy); FSH (FSH-CTK-IRMA, Sorin Biomedica Diagnostic Division, Saluggia, Italy); PRL (Hybritech Inc., San Diego, CA, USA); LH (ELSA-2 LH, Sorin Biomedica Diagnostic Division, Saluggia, Italy); E₂ (Diagnostic Products Co., Los Angeles, CA, USA).

A-PREG and PREG Assays

A) Extraction. The plasma samples were extracted as described by Purdy et al. [22] with minor modifications. Briefly, 300 µl plasma, 50 µl 0.3 NaOH and trace amounts of 3H-labeled A-PREG and PREG to evaluate recovery (100 µl, 3,000 dpm), were incubated at 24 °C for 15 min. After the addition of 0.6 ml of water, hexane (3 ml) was used for extraction: after 15-min rotation, the upper phase was recovered and the lower phase was reextracted as above to maximize sensitivity. The extracts were then combined and dried using a Speed-Vacuum apparatus. B) Purification. The plasma extracts were purified by means of high-pressure liquid chromatography (HPLC) using a Beckman 25 cm×4.6 mm-5 µm ultrasphere silica column and a Beckman guard column. To calibrate the HPLC system, aliquots of 3H-labeled A-PREG and PREG were applied using 0.2% 95%-ethanol in dichloromethane (v/v) at a flow rate of 2 ml/min as described by Purdy et al. [22], with minor modification. Fractions were collected at 0.2-min intervals and their radioactivity counted in order to define the retention time of the A-PREG and PREG standards. The calibration procedure showed that A-PREG was eluted after 25-26 min, and PREG after 37-38 min; under these conditions, P eluted after 41-42 min, thus allowing a good separation of the steroids. The plasma extract was dissolved in dichloromethane (50 µl) and applied to the column. The eluates in the area of A-PREG and PREG were collected

and dried under nitrogen steam. The pellet was suspended in 300 µl of assay buffer [22]; a 50 µl aliquot was used to determine recovery after extraction and HPLC separation. C) Assay procedure. Standard solutions were freshly prepared. The solutions of 3H-A-PREG and 3H-PREG were prepared in the assay buffer at a concentration of 200 pg/ml. Previously characterized [22] antisera to both of these P metabolites were diluted in phosphate-buffered saline (PBS) containing 0.1% bovine serum albumin (PBS-BSA), and used at a working dilution of 1:3,000. The standard curves were constructed using serial concentrations of unlabeled A-PREG or PREG of from 25 to 1000 pg/assay tube in 250 µl of PBS-BSA. Aliquots (100 µl) of the resuspended pellets (see above) were diluted in PBS-BSA to 150 µl. The antiserum (250 µl) and labeled steroids (250 µl, 50 pg) were added to standard and sample tubes, mixed and incubated overnight at 4 °C. After the addition of 200 µl dextran-coated charcoal (1 g charcoal, 0.1 g dextran and 0.2 g human gamma-globulin in 200 ml deionized water), each tube was again mixed and cooled at 4 °C for 20 min. Following centrifugation at 1000×g for 6 min, 500 µl of each supernatant were aliquoted into scintillation fluid, and counted to a relative error of less than 2% in an LS 7500 Beckman beta-scintillation counter. Both the A-PREG and PREG assays had a sensitivity of 0.08 nmol/L.

Statistical analysis

The results were expressed as mean values ± SE and analyzed by means of Student's t test for unpaired data and linear regression, as appropriate.

Results

As shown in Table II, there were no significant differences in serum ACTH, cortisol, DHEA-S, FSH, PRL, LH and E₂ levels between the epileptic patients and the control subjects.

The data for P and its metabolites are summarized in Table III. The mean P concentration (three blood samples) during the luteal phase (22nd-24th day) was 6.7±4.8 ng/ml in the epileptic patients and 9.1±4.4 ng/ml in the control group (p=NS). The mean A-PREG and PREG values in the epileptic patients were 0.7±0.6 ng/ml (range <0.12 - 2.1 ng/ml) and 1.4±1 ng/ml (range <0.12-3.18 ng/ml), respectively; these values did not significantly differ from those found in the controls (A-PREG, 0.5±0.2 ng/ml, range <0.12-0.9 ng/ml; PREG, 1±1.1 ng/ml, range <0.12-4.3 ng/ml). Likewise, no differences were found in the A-PREG/P, PREG/P or A-PREG + PREG/P ratios between patients and controls, excluding a relative deficiency in 5α-reductase and/or 5β-reductase activity. When the patient and control values were considered together, a significant correlation was found between P and A-PREG (r=0.72, p<0.001) and PREG levels (r=0.79, p<0.001).

Discussion

It is widely recognised that GABA-A receptors mediate

TABLE III. Progestinic profile in epileptic patients and in control subjects.

(No.)	P (ng/ml)		A-PREG (ng/ml)		PREG (ng/ml)	
	Controls	Patients	Controls	Patients	Controls	Patients
1	15.8	6.7	0.9	0.34	4.3	1.84
2	3.7	2	0.45	0.12	0.12	0.7
3	2.1	2.6	0.12	0.12	0.12	1.4
4	7.4	7.9	0.12	0.47	0.12	0.18
5	13.2	1	0.53	0.12	0.96	2.3
6	7.3	12.5	0.53	0.3	0.12	2.5
7	7.8	10	0.62	0.12	0.36	2.8
8	18.1	7	0.81	0.73	2.1	1.9
9	13.2	1.2	0.8	1.35	1.8	0.12
10	6	2.6	0.32	0.85	1.2	0.12
11	8.2	12.7	0.65	2.1	0.8	2.6
12	9.1	9	0.72	0.61	1.1	0.7
13	6.5	8.6	0.44	1.43	0.4	1.5
14	7.3	1	0.7	1.45	0.7	0.11
15	12	15.8	0.9	1.59	1.3	3.18
Mean	9.1	6.7	0.5	0.7	1	1.4
SE	4.4	4.8	0.2	0.6	1.1	1

the anxiolytic, anesthetic, sedative and anticonvulsant effects of benzodiazepines and barbiturates [18]. The 3 α -hydroxysteroid metabolites of P, A-PREG and PREG, have been shown to be among the most potent ligands of GABA-A receptors, with an affinity equal to or greater than that of benzodiazepines and barbiturates (for review see 19); in keeping with this, the administration of neuroactive steroids has been associated with anxiolytic, hypnotic and anticonvulsant effects in laboratory animals (for review see 14, 15, 19). Most of the A-PREG and PREG in human plasma are of ovarian origin, but they may also be synthesized in the brain [20, 22]. Within the central nervous system (CNS), they may act as locally-produced endogenous modulators of GABA-A receptors [13], and the impact of such an action could be relevant because, as these modulators are devoid of any endocrine properties, their CNS effects could be exploited for the development of new anxiolytic, hypnotic and anticonvulsant drugs.

The aim of this study was to determine whether epileptic patients in intercritical phase may have a relative deficiency in A-PREG and/or PREG formation, leading to reduced GABA activity in the CNS. To test this hypothesis, we measured plasma A-PREG and PREG levels in epileptic women during the luteal phase of the ovarian cycle, at least two days after their last seizure, when the peaks of P and its metabolites are easily detectable. We did not find any significant difference in plasma A-PREG and PREG concentrations between the patients and normal controls, or any evidence of the activation of the pituitary-adrenal axis (as is demonstrated by the similar levels of adrenal steroids). Plasma A-PREG and PREG levels correlated with those of P. These results in epileptic patients are in agreement with the data recently reported by Schmidt et al. [23] in women with

premenstrual syndrome (PMS), who were found to have normal A-PREG and PREG concentrations. The normal levels of P metabolites in PMS and in epileptic women may be indicative of the lack of involvement of neuroactive steroids in both disorders, which would therefore imply a different pathogenetic mechanism. However, it can be argued that the measurement of the plasma levels of these compounds does not necessarily reflect what is going on in the CNS, especially if the putative deficiency is related to locally-synthesized steroids. Furthermore, it should also be pointed out that (at least in stress-exposed animals) variations in both plasma and CNS neuroactive steroid levels occur very rapidly and are transient [22].

It is thus conceivable that, if variations in the plasma concentrations of P metabolites occur in epileptic patients, they should be looked for and detected during or shortly after an attack. Pritchard et al. [20] found transient hyperprolactinemia only immediately after complex partial seizures, whereas interictal serum PRL levels were invariably normal.

Finally, since our study did not find any significant differences in the plasma concentrations of P and its metabolites between the patients and control subjects, we feel that the role played by enzymatic induction secondary to treatment with antiepileptic drugs is insignificant.

In conclusion, no differences in the plasma levels of A-PREG and PREG were found between interictal epileptic patients and normal controls during the luteal phase of the ovarian cycle. It cannot be excluded that variations in these neuroactive steroids may occur within the CNS but are not reflected by their plasma levels or, alternatively, that relative changes in plasma concentrations in epileptic patients only occur during or immediately after seizures.

Sommario

I 3alfa-idrossimetaboliti del P, 3alfa-idrossi-5alfa-pregnan-20-one (allopregnanolone, A-PREG) e 3alfa-idrossi-5beta-pregnan-20-one (pregnanolone, PREG) sono risultati tra i più potenti ligandi dei GABA-A recettori; nell'animale da esperimento essi hanno mostrato infatti di possedere proprietà ansiolitica, ipnotica ed anticonvulsivante sovrapponibile alle benzodiazepine ed ai barbiturici. Questo studio è stato condotto dosando la concentrazione plasmatica di A-PREG e PREG nella fase luteale del ciclo mestruale in 15 pazienti affette da epilessia parziale del lobo temporale in fase intercritica ed in 15 soggetti omogenei per sesso ed età, al fine di evidenziare un eventuale alterato metabolismo del P e dei suoi metaboliti in soggetti con sindrome comiziale. La concentrazione plasmatica media (sono stati effettuati 3 prelievi ematici) di A-PREG era di 0.7 ± 0.6 ng/ml nelle pazienti e di 0.5 ± 0.2 ng/ml nei controlli, mentre quella di PREG risultava essere rispettivamente 1.4 ± 1 ng/ml e 1 ± 1.1 ng/ml, senza che per nessuno dei due ormoni fosse possibile evidenziare una differenza statisticamente significativa tra pazienti e controlli ($p = NS$).

References

- [1] BACKSTROM T.: *Epileptic seizures in women related to plasma estrogens and progesterone during the menstrual cycle*. Acta Neurol. Scand., 54: 34, 1976.
- [2] BACKSTROM T., ZETTERLUND B., BLUM S., ROMANO M.: *Effects of i.v. progesterone infusions on epileptic discharge frequency in women with partial epilepsy*. Acta Neurol. Scand., 69: 240-248, 1984.
- [3] BELELLI D., BOLGER M.B., GEE K.W.: *Anticonvulsant profile of the progesterone metabolite 3alpha-pregnan-3alpha-ol-20-one*. J. Pharmacol., 166: 325-329, 1989.
- [4] BONUCCELLI U., MURIALDO G., MARTINO E. et al.: *Effects of carbamazepine on secretion in normal and in epileptic subjects*. Clin. Neuropharmacol., 8: 165-174, 1985.
- [5] BONUCCELLI U., MURIALDO G., ROSSI G. et al.: *Prolactin secretion in epileptic subjects treated with phenobarbital: sex differences and circadian periodicity*. Epilepsia, 27: 142-148, 1986.
- [6] BONUCCELLI U., MELIS G.B., PAOLETTI A.M. et al.: *Unbalanced progesterone and estradiol secretion in catamenial epilepsy*. Epilepsy Res., 3: 100-106, 1989.
- [7] Commission on Classification and Terminology of the International League Against Epilepsy: *Proposal for revised classification of epilepsies and epileptic syndromes*. Epilepsia, 30: 389-399, 1989.
- [8] HOLMES G.L., KLOCZKO N., WEBER D.A., ZIMMERMANN A.W.: *Anticonvulsant effect of hormones on seizures in animals*. In: Porter R.J., Mattson R.H., Ward A.A.Jr., Dam M. (Eds.), *Advances in epileptology: XVth Epilepsy International Symposium*. New York, Raven Press, pp. 265-268, 1984.
- [9] GEE K.W., BRINTON R.E., CHANG W.C., MC EWEN B.S.: *Gamma-aminobutyric acid-dependent modulation of the chloride ionophore by steroids in rat brain*. European J. Pharmacol., 136: 419, 1987.
- [10] KOKATE T.G., ROGAWSKI M.A.: *Neurosteroid effects on experimental limbic status epilepticus induced by kainic acid in mice*. Epilepsia, 35 (Suppl. 8): S68, 1994.
- [11] LOGOTHETIS J., HARNER R., MORREL F., TORRES F.: *The role of estrogens in catamenial exacerbation of epilepsy*. Neurology, 9: 352-360, 1958.
- [12] LOGOTHETIS J., HARNER R.: *Electrocortical activation by estrogens*. Arch. Neurol., 3: 290-297, 1960.
- [13] MAJEWSKA M.D., HARRISON N.L., SCHWARTZ R.D. et al.: *Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor*. Science, 232: 1004, 1986.
- [14] MAJEWSKA M.D.: *Steroids and brain activity: essential dialogue between body and mind*. Biochem. Pharmacol., 36: 3781-3787, 1987.
- [15] MAJEWSKA M.D.: *Neurosteroids: endogenous bimodal modulators of the GABA-A receptors. Mechanism of action and physiological significance*. Progress in Neurobiology, 38: 379-395, 1992.
- [16] MATTSON R.H., CRAMER J.A., CALDWELL B.V., SICONOLFI B.C.: *Treatment of seizures with medroxyprogesterone acetate: preliminary report*. Neurology, 34: 1255-1258, 1984.
- [17] MATTSON R.H., CRAMER J.A.: *Epilepsy, sex hormones and antiepileptic drugs*. Epilepsia, 26: 40-51, 1985.
- [18] MORROW A.L., PAUL S.M.: *Benzodiazepine enhancement of gamma-aminobutyric acid-mediated chloride ion flux in rat brain synaptoneurosomes*. J. Neurochem., 50: 302-306, 1988.
- [19] PAUL S.M., PURDY R.H.: *Neuroactive steroids*. FASEB J., 6: 2311-2322, 1992.
- [20] PRITCHARD P.B.III., WANNAMAKER B.B., SAGEL J. et al.: *Endocrine function following complex partial seizures*. Ann. Neurol., 14: 27-32, 1983.
- [21] PURDY R.H., MOORE P.H.Jr., RAO P.N. et al.: *Radioimmunoassay of 3alpha-hydroxy-5alpha-pregnan-20-one in rat and human plasma*. Steroids, 50: 290-296, 1990.
- [22] PURDY R.H., MORROW A.L., MOORE P.H.Jr., PAUL S.M.: *Stress-induced elevations of gamma-aminobutyric acid type A receptor-active steroids in the rat brain*. Proc. Natl. Acad. Sci., 88: 4553-4557, 1991.
- [23] SCHMIDT P.J., PURDY R.H., MOORE P.H.Jr. et al.: *Circulating levels of anxiolytic steroids in the luteal phase in women with premenstrual syndrome and in control subjects*. J. Clin. Endocrinol. Metabolism, 79: 1256-1260, 1995.

Address reprint requests to: Prof. Luigi Murri, Istituto di Clinica Neurologica, Università di Pisa, Via Roma 67, 56100 Pisa, Italy.