L-Arginine: An Ultradian-Regulated Substrate Coupled With Insulin Oscillations in Healthy Volunteers

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OBJECTIVE — Coupled oscillations of 50–110 min in insulin and glucose have been found previously in healthy men under continuous enteral nutrition. Because L-arginine induces insulin release as glucose does, we tested the hypothesis that L-arginine can also display such an ultradian rhythm.

RESEARCH DESIGN AND METHODS — Seven healthy male subjects participated in one experimental night during which blood was sampled every 10 min from 2300 to 0700. Plasma glucose, C-peptide, and L-arginine levels were measured simultaneously. The insulin secretion rate (ISR) was calculated from plasma C-peptide levels by a deconvolution procedure.

RESULTS — Plasma glucose followed the recognizable profiles, with oscillations closely linked to similar changes in the ISR. Pulse analysis of L-arginine profiles revealed significant oscillations linked to glucose and ISR oscillations, with the highest cross-correlation coefficients at time lag 0 ranging from 0.380 to 0.680 for glucose and L-arginine and from 0.444 to 0.726 for ISR and L-arginine ($P < 0.01$). The mean period of L-arginine oscillations was 77.2 ± 6.2 min, and their mean amplitude was 19.9 ± 1.7%, similar to that of glucose (17.0 ± 1.9%), when expressed as the percentage of mean overnight levels.

CONCLUSIONS — This newly discovered ultradian rhythm of L-arginine and its coupling with glucose and ISR oscillations sheds new light on the regulation of L-arginine, the substrate of numerous metabolic pathways, including nitric oxide synthesis. These oscillations may be of significance in conditions of hyperinsulinemia or abnormal glucose tolerance.

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A number of studies have demonstrated that L-arginine triggers insulin release in the presence of D-glucose (1–4). The underlying mechanisms are not fully elucidated. The insulinotropic action of L-arginine has been ascribed to transporter-mediated accumulation of this cationic amino acid inside the β-cells, with resulting depolarization of the plasma membrane (2,3). One other putative mechanism is the formation of nitric oxide (NO) from L-arginine by the action of NO synthase in pancreatic β-cells (4–7). It has been reported that systemic infusion of L-arginine induces vasodilation, inhibits platelet aggregation, and reduces blood viscosity and that these effects are mediated by NO release (8,9). Although there might be a dietary need for L-arginine, it is produced endogenously from the turnover of the urea cycle within the liver and via conversion of citrulline to arginine in the renal proximal tubule (10,11).

In normal humans, the insulin secretion rate (ISR) presents an oscillatory pattern characterized by slow ultradian oscillations with a period of 50–110 min coexisting with rapid small fluctuations of 8–15 min. These ISR oscillations are closely associated with similar oscillations in plasma glucose and are best seen in situations of insulin stimulation, such as during continuous enteral nutrition (12), after meal ingestion (13), and during intravenous glucose infusion (14). Abnormalities in their pattern have been observed in type 2 diabetes (15,16) and in cases of impaired glucose tolerance (17,18). In these situations, the ISR oscillations are less regular. They then have a reduced amplitude, and the tight coupling with glucose oscillations is altered. Because both arginine and glucose stimulate insulin secretion from the pancreatic β-cells, we have tested in healthy volunteers the hypothesis that these substrates can display a common ultradian rhythm.

RESEARCH DESIGN AND METHODS

Subjects

Seven healthy male volunteers aged 20–26 years participated in the experiment after giving their written informed consent. The study was approved by the local ethics committee. All subjects had normal weight, with an average BMI of 22.4 ± 0.7 kg/m^2 and normal routines of work, meals, and sleep. They were selected after medical examination and completing questionnaires on their usual sleep-wake cycle, and work and eating habits. Subjects with a personal history of obesity or sleep disorders, smokers, subjects with underlying signs of disease, and subjects taking medication were excluded from the study.

Procedure

The experiments were performed in soundproof air-conditioned sleep cham-

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Abbreviations: CV, coefficient of variation; ISR, insulin secretion rate; NO, nitric oxide.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

bers, which communicated with an adjoining room where blood samples were collected via a catheter inserted in an antecubital vein and kept patent with heparinized solution. Because plasma glucose and insulin oscillations are amplified during sleep (19), blood was sampled during one experimental night, which was preceded by a habituation night. Subjects were assessed under constant conditions: bed rest and continuous enteral nutrition 4 h before blood sampling (nutrition: Sondalis Iso [Nestlé Clinical Nutrition, Marne-la-Vallée, France]; 50% carbohydrates, 35% fat, and 15% protein; 378 kJ/h).

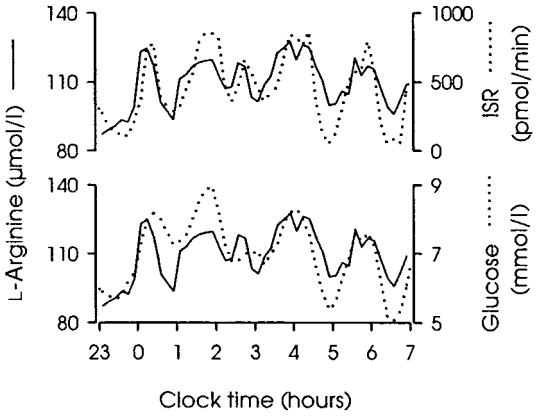

Figure 1—*Ultradian oscillations in ISR, plasma glucose, and L-arginine in one representative subject studied overnight during continuous enteral nutrition with a 10-min blood sampling interval.*

Blood sampling and assays

Blood was sampled every 10 min from 2300 to 0700 and placed in ethylenediamine tetraacetate-K_2–treated tubes (1 mg/ml) using a peristaltic pump. Samples were immediately centrifuged at 4°C, and plasma was stored at −25°C until assay. Plasma glucose levels were measured using a glucose oxidase method (BioMérieux, Marcy-l'Etoile, France), with an intra-assay coefficient of variation (CV) of <1.3%. Plasma C-peptide was measured by radioimmunoassay (Diagnostic Systems Laboratories, Webster, TX) with a detection limit of 0.01 ng/ml. The mean intra-assay CV was 4.3% for concentrations between 0.01 and 4.4 ng/ml and 7.9% for concentrations >4.4 ng/ml. L-Arginine was measured by high-performance liquid chromatography. The intra-assay CV was 5.0% for values between 90 and 140 μmol/l. All samples from one individual were analyzed in a single series.

Data analysis

Determination of the ISR. For each subject, the ISR during each 10-min interval was mathematically derived from plasma C-peptide levels using a two-compartment model. This deconvolution method is based on the fact that insulin and C-peptide are cosecreted in equimolar amounts and that C-peptide, unlike insulin, is not significantly extracted by the liver and has a constant metabolism throughout the day. The kinetic parameters for C-peptide distribution and metabolism were obtained from published data adjusted for sex, age, and body surface area (20). No assumption was made for the shape of the secretory pulses. Statistical error propagation of the uncertainty in the C-peptide measurements was taken into account in the determination of the secretory profiles, and the SD associated with each estimated secretory rate was calculated.

Ultradian rhythm analysis.
The individual nocturnal plasma glucose, ISR, and L-arginine profiles were analyzed for pulse identification using the computer program ULTRA (21). The threshold for pulse detection was set at twice the intra-assay CV in the relevant range of concentration for glucose and L-arginine and at thrice the SD associated with the estimated ISR. For each significant pulse, the time of occurrence, the increment and decrement, and the total duration were determined.

The temporal link between the overnight profiles of L-arginine, glucose, and ISR was quantified using cross-correlation analysis. The association between individual oscillations of L-arginine and glucose and ISR oscillations was tested by lagged coincidence analysis, based on a model of conditional probability derived from two binomial distributions leading to a hypergeometric probability density function, as proposed by Veldhuis et al. (22). Two pulses were considered to be concomitant if their peaks occurred within ±10 min of each other.

Mean ISR oscillations were calculated by aligning significant individual pulses by their maximum and averaging them for each subject. A mean pulse was then obtained for the group of seven subjects.

RESULTS

— Figure 1 illustrates the overnight profiles of ISR, glucose, and L-arginine in one representative subject. Figure 2 gives the mean oscillations aligned by the maximum of ISR. ISR and glucose followed the recognizable profiles (19), with oscillations of ISR having a period of 75.2 ± 6.7 min. Their mean number was 5.6 ± 0.4 and their mean amplitude was 123.9 ± 12.4%, expressed as a percentage of the mean overnight levels. Plasma glucose showed similar oscillations with a mean period of 75.0 ± 6.6 min. An average of 5.7 ± 0.5 glucose oscillations was detected throughout the night, with a mean amplitude of 17.0 ± 1.9%. ISR and glucose oscillations were closely linked, with the highest cross-correlation coefficients at time lag 0 ranging from 0.767 to 0.918 ($P < 0.001$).

Pulse analysis of L-arginine profiles

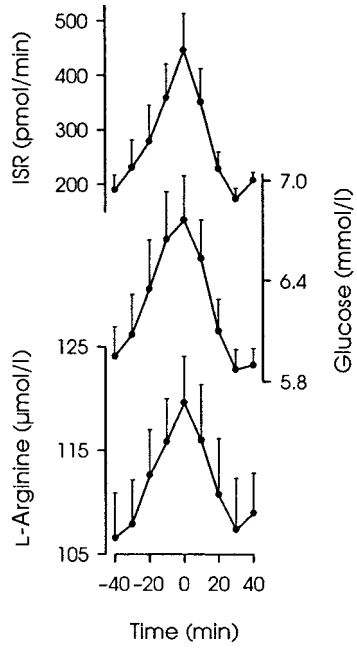

Figure 2—*Mean (±SE) oscillations in ISR, plasma glucose, and L-arginine in seven subjects. L-Arginine and glucose were aligned by the maximum of ISR oscillations.*

revealed an average of 5.1 ± 0.4 significant oscillations during the night. Their mean period was 77.2 ± 6.2 min, and their mean amplitude was 19.9 ± 1.7%, similar to that of glucose. These oscillations were in phase with ISR and glucose. The highest cross-correlation coefficients were at time lag 0 and ranged from 0.380 to 0.680 for glucose and L-arginine and from 0.444 to 0.726 for ISR and L-arginine. Coincidence analysis revealed that, on average, 85.7% of ISR and 78.4% of the glucose oscillations were associated with L-arginine oscillations within a time lag of 0 ± 10 min ($P < 0.001$).

CONCLUSIONS — This newly discovered ultradian rhythm of L-arginine and its coupling with ISR and glucose oscillations lead to an unconventional mode of thinking on the regulation of L-arginine, a nonessential amino acid for healthy subjects. In our experimental conditions, confusing or masking effects of repeated food ingestion were avoided by enteral nutrition, which provides a constant L-arginine intake. Any influence of external factors was eliminated because the subjects were asleep, which amplified glucose and insulin oscillations (19). An habituation session minimized the stress effect due to laboratory procedures. Under these constant conditions, the ultradian rhythm of L-arginine was clearly apparent.

The concomitant rhythmic increases of L-arginine, glucose, and ISR may reflect the stimulatory action of both substrates on insulin secretion. Over the past years, there has been considerable interest in the involvement of L-arginine in the release of insulin through NO pathways (4–7,23), which may explain the hemodynamic and vascular effects of insulin (8,9). The coupling of L-arginine, insulin, and glucose in a rhythmic way sheds new light on this complex interplay that might be significant in pathological states. In type 2 diabetic patients, in whom an altered ultradian insulin rhythm has been reported (15,16) together with an impaired L-arginine uptake in platelets (24), a disordered ultradian organization of L-arginine might occur, and such an imbalance could be implicated in pancreatic β-cell dysregulation. Also, one can inquire whether such coupled ultradian rhythms persist in heart failure patients in whom insulin resistance and impaired NO synthesis have been described (25,26).

The numerous therapeutic properties of L-arginine, namely its anti-asthenic and detoxifying activities, have led to the use of this amino acid as a dietary supplement consumed by millions of people. L-Arginine also exerts favorable effects in the prevention and treatment of endothelial damage and the restoration of endothelial function in patients with cardiovascular risk factors or severe chronic cardiovascular disorders. L-Arginine administration is likely to represent a potentially novel therapeutic strategy (27,28). The possibility that L-arginine acts synergistically in mediating insulin release has been poorly investigated.

In conclusion, the results presented here suggest that L-arginine and glucose share not only their insulinotropic action but also their ultradian regulatory mechanisms, which raises the question of whether there is common insulin control of both substrates. It may be conceivable that L-arginine, a substrate of numerous pathways, protein synthesis, ureagenesis, and NO release, is submitted to mechanisms controlling its cellular availability, which have already been described for glucose. Therefore, the present findings of coupled oscillations of arginine and glucose suggest that further research on common regulatory pathways for these substrates is warranted.

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References

1. Blachier F, Leclercq-Meyer V, Marchand J, Woussen-Colle MC, Mathias PC, Sener A, Malaisse WJ: Stimulus-secretion coupling of arginine-induced insuline release: functional response of islets to L-arginine and L-ornithine. *Biochim Biophys Acta* 1013:144–151, 1989
2. Thams P, Capito K: L-arginine stimulation of glucose-induced insulin secretion through membrane depolarization and independent of nitric oxide. *Eur J Endocrinol* 140:87–93, 1999
3. Sener A, Best LC, Yates AP, Kadiata MM, Olivares E, Louchami K, Jijakli H, Ladrière L, Malaisse WJ: Stimulus secretion coupling of arginine-induced insulin release. *Endocrine* 13:329–340, 2000
4. Vincent SR: Nitric oxide and arginine-evoked insulin secretion. *Science* 258: 1376–1378, 1992
5. Spinas GA, Laffranchi R, Francoys I, David I, Richer C, Reinecke M: The early phase of glucose-stimulated insulin secretion requires nitric oxide. *Diabetologia* 4:292–299, 1998
6. Spinas GA: The dual role of nitric oxide in islet β-cells. *N Physiol Sci* 14:49–54, 1999
7. Kahn NN, Acharya K, Bhattacharya S, Acharya R, Mazumder S, Bauman WA, Sinha AK: Nitric oxide: the "second messenger" of insulin. *Life* 49:441–450, 2000
8. Baron AD: Hemodynamic actions of insulin. *Am J Physiol* 267:E187–E202, 1994
9. Giugliano D, Marfella R, Verrazzo G, Acampora R, Coppola L, Cozzolino D, d'Onofrio F: The vascular effects of L-arginine in humans. *J Clin Invest* 99:433–438, 1997
10. Reys AA, Karl IE, Klahr S: Role of arginine in health and renal disease. *Am J Physiol* 267:F331–F346, 1994
11. Yu YM, Burke JF, Tompkins RG, Martin R, Young VR: Quantitative aspects of interorgan relationships among arginine and citrulline metabolism. *Am J Physiol* 271:E1098–E1109, 1996
12. Simon C, Brandenberger G, Follenius M: Ultradian oscillations of plasma glucose, insulin and C-peptide in man during continuous enteral nutrition. *J Clin Endocrinol Metab* 64:669–674, 1987
13. Simon C, Follenius M, Brandenberger G: Postprandial oscillations of plasma glucose, insulin and C-peptide in man. *Diabetologia* 30:769–773, 1987
14. Shapiro ET, Tillil H, Polonsky KS, Fang US, Rubenstein AM, Van Cauter E: Oscillations in insulin secretion during constant glucose infusion in normal man: relationship to changes in plasma glucose. *J Clin Endocrinol Metab* 67:307–314, 1988
15. Simon C, Brandenberger G, Follenius M, Schlienger JL: Alteration in the temporal organisation of insulin secretion in type 2 (non-insulin-dependent) diabetic patients under continuous enteral nutrition. *Diabetologia* 34:435–440, 1991
16. Polonsky KS, Given BD, Hirsch LJ, Tillil H, Shapiro ET, Beebe C, Frank BH, Galloway JA, Van Cauter E: Abnormal patterns of insulin secretion in non-insulin-dependent diabetes mellitus. *N Engl J Med* 318:1231–1239, 1988
17. O'Meara NM, Sturis J, Van Cauter E, Polonsky KS: Lack of control by glucose of ultradian secretory oscillations in impaired glucose tolerance and in non-insulin-dependent diabetes mellitus. *J Clin Invest* 92:262–271, 1993
18. Scheen AJ, Sturis J, Polonsky KS, Van Cauter E: Alterations in the ultradian oscillations of insulin secretion and plasma glucose in aging. *Diabetologia* 39:564–572, 1996
19. Simon C, Brandenberger G, Saini J,

Ehrhart J, Follenius M: Slow oscillations of plasma glucose and insulin secretion rate are amplified during sleep in humans under continuous enteral nutrition. *Sleep* 17:333–338, 1994
20. Van Cauter E, Mestrez F, Sturis J, Polonsky KS: Estimation of insulin secretion rates from C-peptide levels: comparison of individual and standard kinetic parameters for C-peptide clearance. *Diabetes* 18: 716–738, 1992
21. Van Cauter E: Quantitative methods for the analysis of circadian and episodic hormone fluctuations. In *Human Pituitary Hormones: Circadian and Episodic Variations*. Van Cauter E, Copinschi G, Eds. The Hague, the Netherlands, Martinus Nighof, 1981, p. 1–25
22. Veldhuis JD, Johnson ML, Seneta E: Analysis of the co-pulsatility of anterior pituitary hormones. *J Clin Endocrinol Metab* 73:569–576, 1991
23. Kerwin JF, Heller M: The arginine-nitric oxide pathway: a target for new drugs. *Med Res Rev* 14:23–74, 1994
24. Signorello MG, Giovine M, Pascale R, Bordone C, Benatti U, Leoncini G: Impaired L-arginine uptake in platelets from type-2 diabetic patients. *Biotechnol Appl Biochem* 34:19–23, 2001
25. Anker SD, Rauchhaus M: Heart failure as a metabolic problem. *Eur J Heart Failure* 1:127–131, 1999
26. Goumas G, Tentolouris C, Tousoulis D, Stefanadis C, Toutouzas P: Therapeutic modification of the L-arginine-eNOS pathway in cardiovascular disease. *Atherosclerosis* 154:255–267, 2001
27. Tenenbaum A, Fisman EZ, Motro M: L-arginine: rediscovery in progress. *Cardiology* 90:153–159, 1998
28. Tentolouris C, Tousoulis D, Goumas G, Stefanadis C, Davies G, Toutouzas P: L-arginine in coronary atherosclerosis. *Int J Cardiol* 75:123–128, 2000