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Acute insulin-induced elevations of circulating leptin and feeding inhibition in lean but not obese rats

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Submitted 1 November 2004; accepted in final form 16 March 2005

Acute insulin-induced elevations of circulating leptin and feeding inhibition in lean but not obese rats. Am J Physiol Regul Integr Comp Physiol 289: R373–R379, 2005. First published March 17, 2005; doi:10.1152/ajpregu.00738.2004.—Insulin has been shown to stimulate leptin mRNA expression acutely in rat adipose tissue, but its short-term effects on circulating leptin levels, and subsequent feeding behavior, have not been well described. We used 11-mo-old female selectively bred obesity-resistant (OR) and obesity-prone (OP) Sprague-Dawley rats maintained on laboratory chow to investigate this question. At testing, body weights and basal leptin levels of the OP rats were significantly elevated compared with the OR rats. In the 3-h fasted state, injection of 2.0 U insulin/kg ip resulted in significant elevations of plasma leptin at 4 h postinjection in both OP and OR groups (hour 4, +2.50 and +5.98 ng/ml, respectively). In separate feeding tests with the same groups, intake of laboratory chow pellets was significantly inhibited during hours 2–4 after 2.0 U/kg of insulin in the OR (−80.1%, P < 0.05), but not in the OP group, compared with intake after saline injections. In feeding tests with palatable moderately high-fat pellets after 2.0 and 3.0 U insulin/kg ip, significant decreases between hours 2 and 4 in intake were seen in the OR group only (−41.0 and −68.3%, respectively). Thus feeding inhibition coincides with insulin-induced elevations of plasma leptin in lean but not obese Sprague-Dawley rats. Our data suggest that elevations of leptin within the physiological range may contribute to short-term inhibition of food intake in rats and that this process may be stimulated by feeding-related insulin release.

hyperphagia; leptin resistance; obese animal model; diet palatability; satiety

LEPTIN IS A PEPTIDE HORMONE encoded by the ob gene that is expressed in and released by the adipose tissue (36). Both peripheral and central administration of leptin have been shown to inhibit feeding, decrease body weight, and alter energy expenditure in rodents (5, 15). These observations suggest that circulating leptin participates in the regulation of food intake and body weight as a negative feedback signal (4, 14). In vivo, leptin expression increases in proportion to the number and size of the body’s adipocytes (13), and circulating leptin levels are highly correlated with body fat mass (7, 12). Thus obese humans and animals express higher plasma leptin levels than normal body weight controls. This paradoxical finding has led to the concept of leptin resistance, since in the obese state, it appears that elevated levels of leptin no longer act to maintain body weight homeostasis (12, 24).

In rodents, insulin is known to stimulate the expression of adipose tissue leptin mRNA within 30 min of its administration (17, 21, 25), and insulin facilitates the release of an intracellular pool of leptin in vitro from rat adipose tissue within 10 min of its application (2). Thus rats show a rapid response to insulin’s stimulatory effect on leptin levels, in contrast to insulin’s effects on leptin secretion in humans, which are considerably slower (13). For example, Saladin et al. (31) demonstrated that a single peripheral insulin injection in fasted rodents increased leptin mRNA within 4 h to levels comparable to those seen in fed animals. Moreover, these investigators showed that leptin mRNA rises continuously during the dark cycle, simultaneous with the onset of spontaneous feeding, and reaches a peak shortly before cessation of feeding. An additional study (27) demonstrated that increases of plasma leptin following a 6-h feeding period in diabetic rats are dependent on the release of endogenous insulin. Finally, Koopmans et al. (19) demonstrated that a 2-h infusion of insulin in rats (18 mU·kg−1·min−1) resulted in a significant increase of circulating leptin levels by 4 h, and they observed a significant reduction in feeding over a 7-day insulin infusion period at an even lower dose of insulin (3 mU·kg−1·min−1). These observations suggest that leptin contributes to the control of feeding in rats on a relatively short-term basis.

Given that insulin acutely increases leptin mRNA, as well as plasma leptin, in rodents we hypothesized that physiological doses of insulin should acutely increase plasma leptin and that this increase may promote feeding inhibition in the short term. To test this hypothesis, we administered insulin peripherally to groups of rats fasted for 3 h before injection, and under separate test conditions, we observed changes in both plasma leptin and feeding behavior at intervals following insulin administration. To further probe the ability of endogenous leptin to inhibit feeding, we used two diets for test purposes: standard laboratory chow and a palatable moderately high-fat diet. Finally, to determine whether the obese state interferes with the feeding-inhibitory ability of endogenous leptin, we used groups of selectively bred obesity-prone (OP) and obesity-resistant (OR) Sprague-Dawley rats (22). Differences in response to insulin-induced elevations of endogenous leptin between the OR and OP groups may shed...
further light on the question of whether leptin normally inhibits feeding on a short-term basis in rats.

METHODS

Animals and maintenance. Two groups of female Sprague-Dawley rats, designated as OR and OP were used in this study. These partially inbred substrains were isolated by Levin and colleagues (22) on the basis of body weight and fat changes following 2-3 wk on a high-energy diet, and they were designated DR (diet-resistant) and DIO (diet-induced obesity), respectively. Groups of 3-mo-old OR (n = 7) and OP (n = 8) rats were obtained from a colony maintained at the New York Obesity Research Center. The animals were individually housed at 20–23°C in hanging stainless steel cages and subjected to a 12:12-h light-dark cycle (5 AM:5 PM, respectively). All rats were maintained on a pelleted standard laboratory chow diet (#5001, Purina, St. Louis, MO) and water ad libitum. Food intake and body weights were measured for 24-h periods twice weekly during growth.

For feeding testing, we used pelleted standard chow or a palatable, moderately high-fat diet in pelleted form (19), to which the rats were preexposed before testing (32.5% fat by calories, D12266B, Research Diets, New Brunswick, NJ). All studies were performed in accordance with the guidelines established by the National Institutes of Health Guide for the Care and Use of Laboratory Animals and followed protocols approved by the Institutional Animal Care and Use Committee of the St. Luke’s-Roosevelt Institute for Health Sciences.

Blood sampling. Blood was sampled to determine basal leptin levels at bimonthly intervals throughout the study. For basal samples, blood was gently drawn from the tip of the tail in the nonanesthetized state at midday following a 3-h fast. To determine the effect of injected insulin on endogenous leptin in the absence of feeding, and to ascertain its time course, acute saline and insulin injection tests were conducted starting at 10 mo of age. Blood was sampled from the tail at 2-h intervals during the midday following saline or insulin injections. Specifically, both the OR and OP rats were deprived of food at 8 AM, at which time a basal blood draw was taken. At 11 AM, all rats received an intraperitoneal injection of either saline (control) or regular insulin (Lilly, Indianapolis, IN) at a dose of 2 U/kg body wt. Injection volume was held constant at 1 ml/kg body wt. The groups remained deprived of food for the duration of testing, and blood was sampled again at 1, 3, and 5 PM, (hour 2, 4, and 6, respectively, postinjection). Immediately after blood collection, samples were centrifuged, and plasma was collected and frozen at −20°C. Injection testing was conducted twice a week, with saline injection tests preceding the insulin injection tests. Samples were analyzed for insulin and leptin using rat-specific radioimmunoassays (Linco, St. Louis, MO) and for glucose using a Beckman glucose analyzer.

Feeding testing. Feeding testing was initiated at 11 mo of age, following the conclusion of blood sampling. With our blood sampling method, one cannot both draw blood and monitor feeding in the same animals at the same time without disturbing the validity of both measures. Therefore, to determine the effects of injected insulin on circulating insulin, leptin, and glucose during the 6-h feeding test interval, an initial test was conducted with 2.0 U insulin/kg body wt in OR rats allowed to feed (intake not measured), and blood was sampled. After this initial test, groups were habituated to the feeding test procedure using saline injections and 2-h food intake measurements until intake during each interval of the 6-h test period became stable. Feeding after intraperitoneal injections of regular insulin at doses of 2.0 and 3.0 U/kg body wt was then tested. Feeding testing was conducted once each week with equivolume (1.0 ml/kg body wt) saline control injection tests conducted between insulin injection tests. Feeding after the 2.0-U dose of insulin was tested with both the pelleted laboratory chow diet and the pelleted moderately high-fat diet. Feeding after the 3.0-U dose of insulin was tested with the moderately high-fat diet only. For feeding testing, groups were deprived of food at 8 AM, injected at 11 AM, and refed the test diet immediately after the injection. Subsequent feeding measurements were taken at 1, 3, and 5 PM (hours 2, 4, and 6, postinjection, respectively). Thus the pretest deprivation interval and time of injection was identical in feeding testing and blood sampling, and intake measurements and blood sampling occurred at the same postinjection times over the 6-h test period.

Data analysis. Basal leptin, body weight, 24-h food intake, hormone, and glucose levels after saline and insulin injections were compared using two-way repeated-measures ANOVA (groups vs. time). Feeding test results were compared using two-way repeated-measures ANOVA (injection condition vs. time) for each group. Comparisons of individual means following significant main effects in ANOVA were made using the Newman-Keuls test. Differences were considered statistically significant when P < 0.05. Data in figures are presented as means ± SE.

RESULTS

Body weight, food intake, and basal leptin. Representative 24-h food intake and basal leptin levels of the groups at three time points during growth are shown in Table 1. By 4 mo of age, the OP group was consuming ~22% more food than the OR group (P < 0.05). Degree of overeating by the OP group was also significantly greater at 8 and 12 mo of age (P < 0.05 and 0.02, respectively). Similarly, basal leptin showed a trend toward an increase in the OP group at 4 mo of age, which became significant at 8 mo (P < 0.05) and remained so thereafter. Figure 1 shows the body weights of the groups over the entire study period. Despite maintenance on the laboratory chow diet, the body weight of the OP group was significantly greater than that of the OR group by 4 mo of age (P < 0.05) and increased at a greater rate than that of the OR group throughout the experiment. At 14 mo of age, the body weight of the OP group was ~67% higher than that of the OR group (P < 0.001).

Leptin response to insulin. In the fasted state, injection of saline to OR and OP groups did not significantly alter leptin levels over the 6-h observation period (Fig. 2). In contrast, injection of 2.0 U/kg body wt insulin resulted in a significant increase of plasma leptin at hour 4 postinjection of 16.4% compared with baseline leptin for the OR group (+2.5 ng/ml, P < 0.05) and in an increase of 21.3% compared with baseline leptin for the OP group (+5.98 ng/ml, P < 0.05). Note that a trend for increasing leptin levels was seen in both groups at

Table 1. Twenty-four-hour food intake and plasma leptin levels during growth

<table>
<thead>
<tr>
<th></th>
<th>4 mo</th>
<th>8 mo</th>
<th>12 mo</th>
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<tbody>
<tr>
<td>Food intake, g</td>
<td>15.1±2.2</td>
<td>17.9±2.2*</td>
<td>20.3±2.2</td>
</tr>
<tr>
<td>Leptin, ng/ml</td>
<td>4.0±3.0</td>
<td>7.3±4.0</td>
<td>15.8±7.6</td>
</tr>
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Values are means ± SD. OR, obesity resistant; OP, obesity prone. *Different from OR, P < 0.05. †Different from OR, P < 0.02. ‡Different from OR, P < 0.01.
hour 2 (not significant). The data in Table 2 demonstrate that this same dose of insulin, when injected into OR rats allowed to feed, did not significantly alter circulating insulin or glucose concentrations in the animals at these time points, compared with injection of saline. Circulating insulin may have been elevated and glucose decreased, however, immediately after injection at 11 AM. In contrast, leptin levels in the OR rats (Table 2) were significantly increased over baseline at all three postinjection time points over saline injection values (P < 0.05), as well as significantly increased at all three postinjection time points over saline injection values (P < 0.02 in all cases). As noted above, feeding was not measured during this test.

Feeding behavior after insulin injections. The effects of injections of 2.0 U/kg insulin/kg body wt on feeding over the 6-h test interval are shown in Figs. 3 and 4. Chow diet results for the groups are shown in Fig. 3, while palatable diet results are presented in Fig. 4. In the OR group, significant decreases of intake were seen during hours 2–4 postinjection for both laboratory chow and the palatable moderately high-fat diet, relative to feeding after saline injections. Thus, during hours 2–4 after the insulin injections, the OR group reduced feeding on the chow diet by 80.1% (Figs. 3–5). These effects were significant (hours 2–4 and 4 postinjection (4.4 and 4.5 ng/ml, respectively, P < 0.05), as well as significantly increased at all three postinjection time points over saline injection values (P < 0.02 in all cases). As noted above, feeding was not measured during this test.

Feeding behavior after insulin injections. The effects of injections of 2.0 U/kg insulin/kg body wt on feeding over the 6-h test interval are shown in Figs. 3 and 4. Chow diet results for the groups are shown in Fig. 3, while palatable diet results are presented in Fig. 4. In the OR group, significant decreases of intake were seen during hours 2–4 postinjection for both laboratory chow and the palatable moderately high-fat diet, relative to feeding after saline injections. Thus, during hours 2–4 after the insulin injections, the OR group reduced feeding on the chow diet by 80.1% (−1.0 g, P < 0.01), and reduced feeding on the palatable diet by 41.0% (−1.1 g, P < 0.05), compared with feeding during the same interval after saline injection. In contrast, in the OP group, administration of 2.0 U/kg insulin/kg body wt failed to result in significant feeding inhibition during any postinjection interval on either laboratory chow or the palatable diet. A tendency toward a reduction of feeding on the chow diet was seen in the OP group between hours 2 and 4, but it was not statistically significant. Indeed, when offered the palatable diet, the OP group actually consumed 18.7% more food between hours 2 and 4 after insulin injection than after saline. To test whether a higher dose of insulin would stimulate feeding inhibition in the OP rats, the groups were injected with 3.0 U insulin/kg body wt and tested with the palatable diet. Feeding of the groups after administration of 3.0 U insulin is shown in Fig. 5. The OR group again exhibited a significant decrease of intake of −68.3% between hours 2 and 4 after insulin injection (−1.6 g, P < 0.05). Although a similar trend was observed in feeding by the OP group between hours 2 and 4, it was found to be insignificant. In the case of each significant feeding reduction in the OR group between hours 2 and 4, what appears to be compensatory feeding occurred between hours 4 and 6 (see Figs. 3–5). These trends approached, but did not achieve, statistical significance. Total (6-h) intake of each group did not differ between saline vs. insulin test conditions over the 6-h test period for either diet (data not shown). The groups were not tested with the 3.0-U dose of insulin on the laboratory chow diet. Note that intake of the moderately high-fat diet in each test by both OR and OP groups between hours 0 and 2 was more than twice as great as that of chow (Figs. 3–5). These effects were significant (hours 2–4 and 4 postinjection (2.7–2.9 g, 79.5–87.0, and 12.4–13.9, respectively, P < 0.05).
intake of moderately high-fat vs. chow diet for saline-injected OR and OP groups both $P < 0.01$ and demonstrate the increased palatability of this diet in relation to laboratory chow.

**DISCUSSION**

The results of the present study demonstrate a clear temporal correlation between insulin-induced elevations of endogenous leptin and feeding inhibition in lean OR rats but not in OP rats. Significant feeding inhibition in the OR group was observed between hours 2 and 4 postinsulin injection on both laboratory chow and a palatable, moderately high-fat diet, following doses of both 2.0 and 3.0 U/kg insulin. Feeding inhibition after insulin administration was not preceded by a period of hyperphagia in the OR group, rendering the effect unlikely to be the result of caloric compensation. In contrast, a trend for compensatory feeding between hours 4 and 6 followed each case of significant intake inhibition in the OR group, suggesting that a caloric deficit had taken place. Saladin et al. (31) showed that onset of dark-cycle feeding stimulates leptin mRNA expression within 4 h in normal rats and that leptin mRNA expression peaks shortly before cessation of feeding at the end of the dark cycle. Koopmans et al. (19) showed that infusion of physiological levels of insulin ($3 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) to normal rats stimulated an increase in circulating leptin levels within 4 h, accompanied by a significant decrease in feeding of 2.5 g/day when the infusion was maintained over a 7-day period. The mean increase of plasma leptin concentrations observed in the infused animals over this period was 1.0 ng/ml. In addition, Haltiner et al. (16) showed that a 13-day period of exogenous leptin infusion in low-fat-fed and high-fat-fed mice significantly increased circulating leptin in the groups by 1.1 and 2.3 ng/ml, respectively, and was accompanied by significantly decreased food intake in both groups. In the present study, we have shown that insulin elevates endogenous leptin acutely in both lean and obese rats within 4 h after its administration. The increase of endogenous leptin, which was correlated with reduced feeding at hour 4 by the OR rats in this study, was 2.5 ng/ml. Thus our data are consistent with the magnitude of leptin increases associated with a feeding-inhibitory effect and suggest that elevations of endogenous leptin stimulated by insulin may underlie feeding inhibition observed in the OR rats in this study.

Feeding inhibition following insulin administration at doses of 2.0 and 3.0 U/kg was not observed in this study in similarly tested OP rats that had become obese. This was true, although the OP group showed an even greater absolute ($+6.0 \text{ ng/ml}$) and percent ($+21.3\%$) elevation of plasma leptin than the OR group after 2.00 U/kg insulin administration. It is well documented that outbred rodent strains rendered obese and hyperleptinemic by high-energy diets are resistant to the feeding inhibitory effects of leptin, whether administered centrally or peripherally (15, 34). Thus it is interesting to note that in this study, a partially inbred strain of rats, which became obese and hyperleptinemic while consuming laboratory chow, also appeared resistant to the feeding inhibitory effects of elevated circulating leptin, in this case, induced by exogenous insulin administration. We have previously observed that maintenance of obese Sprague-Dawley rats on a high-energy diet is not a requirement for the expression of leptin resistance (unpublished observations). The nature of the potential hormonal or metabolic alterations underlying the propensity to obesity in
OP rats has been investigated by Ricci and Levin (29), who, as noted above, designated this strain as DIO. These investigators observed reduced sensitivity at 7 wk of age in DIO rats to the potential feeding-inhibitory effects of large increases of endogenous leptin in response to consumption of a high-energy diet. Our results showing the absence of feeding inhibition in this strain to acute elevations of circulating leptin are therefore consistent with the findings of Ricci and Levin. Apparently, increased adiposity may not be required for this loss of sensitivity to the feeding inhibitory effects of leptin in OP rats.

It is important to note that when the OR group injected with 2.0 U insulin was challenged with a palatable moderately high-fat diet, the feeding inhibitory effect of elevated endogenous leptin remained intact, but was smaller in magnitude, compared with feeding inhibition on the Chow diet. The percentage by which the OR rats inhibited their feeding in response to the palatable moderately high-fat diet after administration of 2.0 U insulin was reduced to approximately half of that seen under the same conditions with the standard Chow diet (41.0 vs. 81.1% reductions, respectively). Thus, in lean rats, the inhibitory effects of leptin were modified by diet type. Although macronutrient effects on leptin expression and levels in rodents and humans have been observed (17, 28), and high-fat diets have been reported to rapidly induce leptin resistance (35), it is likely that the diet effects observed in this case were dependent primarily on sensory quality. Thus, because the OR group was not maintained on the moderately high-fat test diet for any length of time, and exposure to the diet for 6 h during testing is unlikely to have metabolic effects that would alter leptin sensitivity. Thus our data suggest that the OR rats are not unresponsive to the sensory effects of the moderately high-fat diet. Previous observations indicate, however, that the OR strain would react to long-term maintenance on such a diet with appropriate weight regulatory responses (29).

In assessing the significance of the effects demonstrated here, it is important to distinguish feeding inhibition induced by insulin-stimulated leptin release from the phenomenon of low-dose insulin-induced satiety per se. Several investigators have demonstrated the short-term feeding inhibitory effects of acute exogenously administered insulin (1, 23) or endogenously stimulated insulin release (32), which raise insulin concentrations within the physiological range. These effects occur rapidly (30–45 min) and are believed based on nutrient utilization (10). Although a nutrient-based insulin-induced satiety mechanism may well underlie rapid short-term feeding inhibition, this does not appear to be the phenomenon we have identified here. Significant feeding inhibition was not observed in our experiment until an interval 2–4 h after insulin administration, and on the basis of data obtained in insulin-injected OR rats permitted to feed (Table 2), the effect does not appear to be based on glucose clearance from the circulation during this interval. Thus we appear to be dealing with another distinct feeding-inhibitory effect induced by insulin, of somewhat longer onset and duration. Indeed, our results are consistent with the more long-term process of leptin expression and release from the adipose tissue (25). The two hypothesized satiety effects of insulin discussed above may actually participate in the control of feeding simultaneously, as a study by Vanderweele et al. (33) using chronic insulin infusions suggests. Minipump infusions of insulin, which elevated circulating insulin levels within the physiological range for a 7-day period in normal rats, inhibited food intake by reducing meal size and also limited weight gain. Results obtained by Vanderweele et al. with chronic insulin infusion are, in fact, consistent with the effects of chronically infused leptin in normal rats, which also include specific reductions of meal size and inhibition of weight gain (18). Thus insulin-mediated nutrient utilization after a meal, and subsequent elevations of endogenous leptin, may both be insulin-stimulated feeding-inhibitory factors involved in the short-term control of food intake in rodents. Their effects are most likely expressed through reductions of meal size. These relatively short-term feeding effects of insulin should be distinguished from more long-term weight regulatory effects also proposed for the hormone (26).

Our data, and those of several other investigators (4, 19, 27, 31), suggest that small but significant elevations of circulating leptin within the physiological range may contribute to the control of food intake and maintenance of body weight on a daily basis in rats. This notion differs from suggestions advanced by others (11, 30) that leptin’s primary role is to serve a protective function during states of starvation or caloric restriction, via decreases in its levels, which trigger homeostatic behavioral and/or metabolic mechanisms. Although the latter concept is strongly supported by numerous data and generally well accepted, it does not preclude a more subtle role for leptin in the day-to-day maintenance of energy balance, via both alterations of feeding and energy expenditure (14). Although such a maintenance role for leptin in body weight regulation appears plausible for animal species such as rodents, which have relatively rapid leptin release in response to nutrient stimulation, the notion is more difficult to support in the

![Fig. 5. Feeding of palatable moderately high-fat diet by OR (left) and OP (right) groups after injection of saline or 3.0 U regular insulin/kg ip. Groups were injected at 11 AM after a 3-h fast. *Different from saline condition, same interval, P < 0.05.](http://ajpregu.physiology.org/10220/33.5)
case of human weight regulation (11, 30). Nevertheless, there is evidence that leptin is one of the factors that participates in perceptions of hunger and satiety in humans (3), and a recent human study demonstrated alterations of leptin level in response to short-term (24-h) energy imbalances, which are predictive of subsequent meal intake (6). The issue remains unresolved, but the authors hope additional studies might focus on the more subtle effects of leptin as it is involved in the maintenance of energy balance.

In conclusion, we have demonstrated that administration of insulin at physiological doses is accompanied by a significant feeding-inhibitory effect in OR rats between 2 and 4 h after injection. This effect appears to be distinct from the rapid satiety effects of injected insulin associated with nutrient absorption. Less feeding inhibition was observed in the OR rats in response to a palatable moderately high-fat diet, indicating that feeding inhibition in response to elevated endogenous leptin is influenced by the sensory quality of the diet. In contrast, OP rats did not exhibit feeding inhibition at 4 h after insulin injection under the same test conditions, demonstrating what appeared to be leptin insensitivity. Both groups of rats, however, did respond to insulin injections with significant elevations of plasma leptin 4 h after insulin injection. Thus in OR rats a close temporal correlation between feeding inhibition and elevations of endogenous leptin was observed. Our data suggest that meal-related insulin-induced elevations of leptin may play a role in short-term adjustments of energy intake in rodents, although a direct test of this possibility awaits further study.

ACKNOWLEDGMENTS

The authors thank Dr. B. Levin for generously providing breeding pairs of the DR and DIO substrains to the New York Obesity Center. We thank C. A. Maggio for her helpful comments on the manuscript.

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GRANTS

This work was supported by Weight Risk Investigators Study Council Award 399-24 to J. R. Vessalli, a Sigma Xi Undergraduate Grant-in-Aid to K. A. Singh, and National Institutes of Diabetes and Digestive and Kidney Diseases Award P-30-DK-26687 to the Obesity Research Center, St. Luke’s-Roosevelt Hospital, F. X. Pi-Sunyer.

REFERENCES


