Role of Opioid Tone in the Pathophysiology of Hyperinsulinemia and Insulin Resistance in Polycystic Ovarian Disease

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Hyperinsulinemia secondary to a poorly characterized disorder of insulin action is a feature of polycystic ovarian disease (PCOD). On the other hand, being generally admitted that opioids may play a role in glycoregulation and that opioid tone is altered in PCOD, an involvement of the opioids in determining the hyperinsulinemia of PCOD patients could be suggested. The aim of this study was to evaluate the effect of a chronic opioid blockade on insulin metabolism and peripheral insulin sensitivity in PCOD hyperinsulinemic patients. Twenty-three women with PCOD were studied. An oral glucose tolerance test (OGTT) and a clamp study were performed at baseline (during the follicular phase) and after 6 weeks of naltrexone administration (50 mg/d orally). Based on the insulinemic response to the OGTT, 16 women were classified as hyperinsulinemic and seven as normoinsulinemic. Naltrexone treatment significantly reduced fasting ($P < .05$) and area under the curve (AUC) ($P < .02$) plasma insulin levels only in the hyperinsulinemic group. Moreover, hyperinsulinemic patients showed similar C-peptide incremental areas after naltrexone treatment, whereas in the same patients the fractional hepatic insulin extraction calculated from the incremental areas of insulin and C-peptide was found to be increased after chronic opioid blockade by naltrexone. For peripheral insulin sensitivity, the hyperinsulinemic group showed significantly lower ($P < .01$) total-body glucose utilization (M) compared with the normoinsulinemic group. No change in the M value was found after treatment in both groups. These data suggest that the insulin sensitivity and hyperinsulinemia after an OGTT are two distinct deranged features of the insulin disorder of PCOD patients.

POLYCYSTIC OVARIAN DISEASE (PCOD) is a common syndrome affecting women of reproductive age and is the most frequent cause of infertility.[1] The main features of this syndrome are chronic anovulation, elevated serum androgen concentrations, inappropriate gonadotropin secretion,[2] and the presence of hyperinsulinemia and insulin resistance.[3]

Hyperinsulinemia can be explained by peripheral insulin resistance, which increases the demand to pancreatic β cells for the defect to be overcome and euglycemia to be maintained.[4,5] However, before reaching the periphery, insulin must transverse the liver, where a variable extraction takes place[6]; so it is also possible that abnormal hepatic metabolism of insulin may contribute to the peripheral hyperinsulinemia.

The demonstration of β-endorphins in the human endocrine pancreas[7] and the evidence that they may stimulate insulin and glucagon release in animals and humans[8] suggest that opioids may play a role in glycoregulation. Moreover, elevated plasma immunoreactive β-endorphins have been detected in PCOD women.[9] Therefore, the hypothesis that endogenous opiates are partially responsible for hyperinsulinemia and insulin resistance in PCOD may be considered.

In previous studies, we demonstrated that both acute and chronic inhibition of opioid tone in PCOD patients reduced the exaggerated insulin secretion.[10,11] Moreover, we have suggested that the pharmacological inhibition of opioid tone could improve the plasma insulin concentration by acting chiefly on the liver metabolism of insulin in hyperinsulinemic PCOD patients. The aim of this study was to clarify the role of chronic treatment with the opiate receptor antagonist naltrexone in the pathophysiology of hyperinsulinemia and insulin resistance in PCOD patients.

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SUBJECTS AND METHODS

We studied 23 consecutive women with PCOD aged 17 to 31 years. All of the women were in good health and euthyroid, and had a normal glomerular filtration rate as demonstrated by normal creatinine clearance values. None of the patients had taken any medication known to affect carbohydrate metabolism for at least 3 months before the study. All patients had spontaneous onset of puberty and normal sexual development, and all had been affected by oligomenorrhea with chronic anovulation since puberty. No patient showed evidence of acanthosis nigricans. Obesity was defined as a body mass index (BMI) greater than 25 (normal range, 19 to 25), calculated as body weight in kilograms divided by height in meters squared.

PCOD was diagnosed by clinical findings (amenorrhea or oligomenorrhea and hirsutism), plasma androgen levels at the upper limit of the normal range (androstenedione, 1.98 to 5.6 nmol/L; testosterone, 0.59 to 2.01 nmol/L), and bilaterally normal or enlarged ovaries with the presence of at least seven to 10 microcysts (<5 mm diameter) on ultrasonography and/or laparoscopy. A normal luteinizing hormone to follicle-stimulating hormone (LH/FSH) ratio was not considered an exclusion criterion.[12]

Informed consent was obtained from each patient. The study protocol was approved by our ethics committee. All studies were performed in the follicular phase 5 to 8 days after spontaneous or progestin-induced (medroxyprogesterone acetate 10 mg/d orally for 5 days) menses. The patients were hospitalized, and after following a standard carbohydrate diet (300 g/d) for 3 days and fasting overnight for 10 to 12 hours, they underwent an oral glucose tolerance test (OGTT) and basal hormone assay. The following day after another overnight fast, a euglycemic-hyperinsulinemic clamp was performed.

The patients then left the hospital and had 6 to 7 weeks of treatment with 50 mg/d naltrexone, an oral narcotic antagonist (Antaxone; Simes, Vicenza, Italy). In the absence of spontaneous menstrual bleeding, a progestin test was repeated after 5 weeks of naltrexone treatment. Following continuation of the treatment, the OGTT, basal hormone assay, and clamp study were repeated in a second hospitalization at menstrual day 6 to 7.

The OGTTs were performed as follows. At 8 AM, an indwelling

catheter was inserted in the antecubital vein of one arm. Blood samples were collected basally and, after ingestion of 75 g glucose in 150 mL water within 5 minutes, at 30, 60, 90, 120, 180, and 240 minutes.

Samples for glucose were assayed immediately, whereas samples for the other determinations were promptly centrifuged and the plasma was stored at −20°C until assayed. Insulin, glucose, and C-peptide concentrations were measured in all blood samples. Furthermore, LH, FSH, estradiol, 17-hydroxyprogesterone, testosterone, dihydroepiandrosterone sulfate, androstenedione, sex hormone–binding globulin, and cortisol plasma concentrations were also determined in basal conditions and after naltrexone treatment.

The euglycemic-hyperinsulinemic clamp was performed as follows. A retrograde intravenous catheter was inserted into a hand or forearm vein for blood sampling and kept in a warming device at greater than 60°C to arterialize the venous blood samples. Another indwelling catheter was inserted in a contralateral forearm vein for the infusions. Insulin (Actrapid HM; Novo Nordisk, Copenhagen, Denmark) was administered at a dosage of 40 $mU \cdot m^2 \cdot min^{-1}$.[13] After reaching the steady-state velocity for the insulin infusion within 10 minutes in order to achieve steady-state insulin levels of about 717 pmol/L during the clamp (range, 574 to 897), a variable infusion of 20% glucose was begun via a separate infusion pump and the rate was adjusted according to plasma glucose determinations every 5 minutes to maintain plasma glucose between 4.4 and 4.99 mmol/L. The plasma glucose level was determined by the glucose oxidase technique with a glucose analyzer (Beckman Instruments, Palo Alto, CA). Total-body glucose utilization (M) was determined between 90 and 150 minutes of the glucose clamp and expressed as milligrams per kilogram body weight per minute. We prefer this index as the measure of insulin sensitivity because the M/I ratio fails to narrow the range of individual sensitivity values.[14]

All hormone concentrations were determined by commercial radioimmunoassay kits (Radim, Pomezia, Italy). Gonadotropins, insulin, and C-peptide were assayed by a double-antibody technique; all steroids were assayed by a dextran-charcoal technique. Glucose concentrations were determined by the glucose oxidase method. For each determination, all samples from the same patient were assayed simultaneously. Intraassay and interassay coefficients of variation were less than 8% and 15%, respectively, for all determinations.

A normal glycemic response to the OGTT was defined according to the criteria of the National Diabetes Data Group.[15]

All results are expressed as the mean ± SEM. Insulin, glucose, and C-peptide plasma concentrations are also expressed as the area under the curve (AUC) after glucose ingestion, calculated by the trapezoidal rule. The patients were classified as normoinsulinemic and hyperinsulinemic according to the insulin response to the OGTT, with a cut-off value of 107,625 pmol/L · 240 min for the AUC. This cut-off value was calculated using the mean ± 2 SD for about 100 OGTTs performed in control lean subjects and confirmed by a cluster analysis.

Hepatic insulin extraction was estimated from (1) the C-peptide to insulin molar ratio in the fasting state and after glucose loading; and (2) the difference between the incremental areas of C-peptide and insulin divided by the incremental area of C-peptide, as proposed by other groups.[16,17]

The incremental area was calculated by the difference between the AUC and basal AUC (basal AUC = area of the curve due to basal unstimulated secretion and calculated assuming a constant value during a 4-hour period).

Statistical analysis was performed using the nonparametric Mann-Whitney test for two-group comparisons; within-group comparisons were performed by the Wilcoxon matched-pairs, signed-rank test. Linear regression analysis was used to analyze possible correlations between findings, after logarithmic transformation when necessary. Differences were considered significant at P less than .05.

RESULTS

Based on the insulinemic response to the OGTT, patients were classified as hyperinsulinemic (n = 16, 69%) or normoinsulinemic (n = 7, 31%). Table 1 lists endocrine and metabolic parameters for the two groups at baseline and after 6 weeks of naltrexone therapy. Similar BMI values were found in the two groups: obese subjects comprised 69% and 43% of the hyperinsulinemic and normoinsulinemic groups, respectively. No endocrine parameters changed after long-term treatment, and the BMI did not change during treatment. For the glycemic response to the OGTT, two patients (both hyperinsulinemic) showed impaired glucose tolerance. No patient had a change in glucose tolerance after therapy.

Figure 1 shows insulin and C-peptide plasma levels after the glucose load and their incremental areas in the two groups before and after naltrexone administration. The curves of the group data showed a similar course, with a peak reached within 60 to 90 minutes and then a descending slope. In basal conditions, hyperinsulinemic patients were characterized by significantly greater C-peptide and insulin incremental areas in

Table 1. Metabolic and Endocrine Features of the PCOD Patients Before and After Naltrexone Treatment

Feature	Normoinsulinemic (n = 7)		Hyperinsulinemic (n = 16)	
	Before Treatment	After Treatment	Before Treatment	After Treatment
BMI (kg/m^2)	25.36 ± 0.96	24.9 ± 0.9	28.13 ± 1.26	27.63 ± 1.17
Glucose AUC (mmol/L · 240 min)	1,382.9 ± 86.7	1,421.7 ± 86.2	1,532.4 ± 46.57	1,529.1 ± 58
Insulin AUC (pmol/L · 240 min)	91,280 ± 14,547*	99,168 ± 15,115	197,008 ± 23,219	131,073 ± 21,349†
Sex hormone–binding globulin (nmol/L)	36.55 ± 8.53	35.93 ± 5.11	22.36 ± 4.09	23.79 ± 3.46
FSH (IU/L)	5.9 ± 0.67	6.3 ± 0.8	6.72 ± 0.44	5.23 ± 0.35
LH (IU/L)	10.37 ± 2.03	17.73 ± 5.85	10.27 ± 1.15	6.24 ± 0.76
Estradiol (pmol/L)	108.9 ± 24.8	81.1 ± 13.87	130.5 ± 16.22	114.9 ± 14.7
Cortisol (nmol/L)	348.5 ± 35.9	367.2 ± 31.1	357 ± 40.17	300.3 ± 23.56
Testosterone (nmol/L)	2.08 ± 0.24	2.35 ± 0.14	1.8 ± 0.09	1.94 ± 0.21
Androstenedione (nmol/L)	5.41 ± 0.35	6.42 ± 1.46	5.58 ± 0.45	5.51 ± 0.45
17-Hydroxyprogesterone (nmol/L)	1.59 ± 0.05	2.76 ± 0.57	1.94 ± 0.16	1.81 ± 0.19
DHEAS (μmol/L)	5.28 ± 0.44	5.88 ± 0.51	6 ± 0.47	5.18 ± 0.39

NOTE. Data are the mean ± SEM.

Abbreviation: DHEAS, dehydroepiandrosterone sulfate.

*$P < .01$, normoinsulinemic *v* hyperinsulinemic.

†$P < .01$, within hyperinsulinemic group.

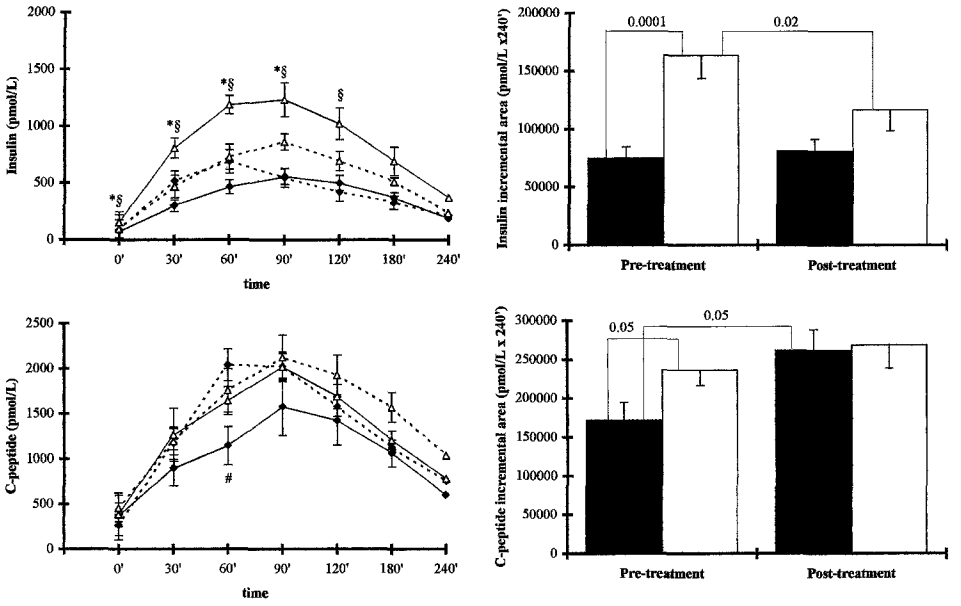

Fig 1. Stimulated insulin and C-peptide plasma levels in hyperinsulinemic (△) and normoinsulinemic (◆) patients before (—) and after (--) naltrexone administration, and insulin and C-peptide incremental areas after glucose loading at baseline and after naltrexone in hyperinsulinemic (□) and normoinsulinemic (■) patients. *$P < .05$ within hyperinsulinemic group; §$P < .01$, hyperinsulinemic *v* normoinsulinemic; #$P < .02$ within normoinsulinemic group. Data are the mean ± SEM.

comparison to the normoinsulinemic group; however, in the curve analysis, the difference reached statistical significance only for the insulin response to the OGTT. As expected, naltrexone treatment reduced fasting and stimulated plasma insulin levels only in the hyperinsulinemic group, abolishing the difference between normoinsulinemic and hyperinsulinemic patients. Moreover, despite the significant reduction in the insulin incremental area shown by this last group after naltrexone treatment, no significant variation in the C-peptide level after the glucose load, as well as its incremental area, was observed. Normoinsulinemic patients showed significantly higher C-peptide incremental areas after naltrexone treatment, whereas curve analysis showed a significant difference only at 60 minutes.

The fractional hepatic insulin extraction calculated by the formula, (C-peptide- insulin)/C-peptide incremental areas, is shown in Fig 2. In the hyperinsulinemic group, we found significantly lower values for this parameter; moreover, these patients showed a significant increase in liver insulin metabolism after treatment. In the normoinsulinemic group, the increase in hepatic insulin uptake did not reach statistical significance even if the C-peptide to insulin molar ratio incremental area was significantly higher after naltrexone administration (data not shown).

Total-body glucose utilization (M) in the two groups is shown in Fig 3. Hyperinsulinemic patients had lower M values ($P < .01$) compared with normoinsulinemic patients. Total-body glucose utilization was unchanged after naltrexone treatment in both groups.

Moreover, we found basally a positive linear relation between insulin resistance and insulinemic status ($P < .003$, $r = .65$) or BMI ($P < .0001$, $r = .77$).

DISCUSSION

Hyperinsulinemia secondary to a poorly characterized disorder of insulin action is a feature of PCOD. Moreover, PCOD and obesity have a synergistic deleterious effect on glucose homeostasis.[4] About 20% of obese women with PCOD already have impaired glucose tolerance or frank diabetes mellitus by

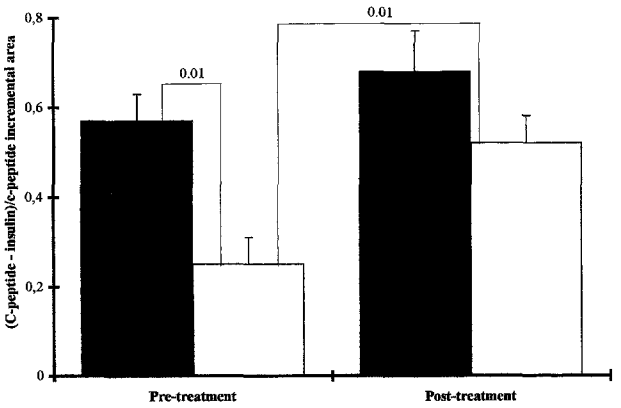

Fig 2. Hepatic extraction as evaluated by the formula, (C-peptide insulin)/C-peptide incremental area, after glucose loading at baseline and after naltrexone treatment in hyperinsulinemic (□) and normoinsulinemic (■) patients. Data are the mean ± SEM.

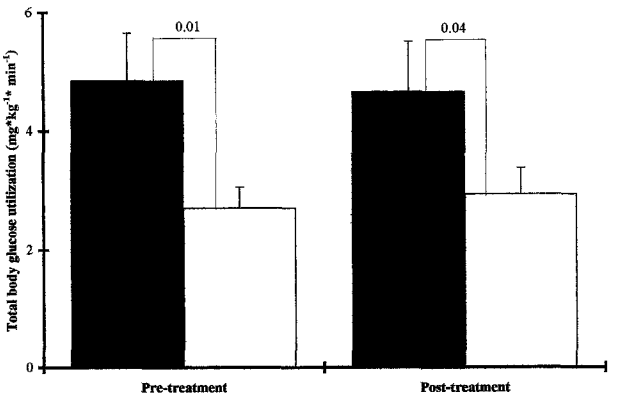

Fig 3. Total-body glucose utilization (M) at baseline and after naltrexone treatment in normoinsulinemic (■) and hyperinsulinemic (□) patients. Data are the mean ± SEM.

their third decade, indicating that PCOD is a previously unappreciated risk factor for non–insulin-dependent diabetes mellitus.[4,18]

On the other hand, being generally admitted that opioids may play a role in glycoregulation[8] and that opioid tone is altered in PCOD,[9] an involvement of the opioids in determining the hyperinsulinemia of PCOD patients could be suggested.

In previous studies, we demonstrated that both acute and chronic inhibition of opioid tone in PCOD patients reduced the exaggerated insulin secretion by about 30% without affecting the glycemic levels.[10,11] Moreover, we have shown that a similar treatment failed to cause a change in the insulinemic status in normoinsulinemic PCOD patients and control subjects.[10,12]

In this study, we have focused on the possible mechanisms by which opiates could lead to elevated insulin levels in PCOD. We have shown that a long-term naltrexone treatment could reduce the hyperinsulinemia of PCOD patients without affecting β-cell secretion, since C-peptide levels are similar before and after treatment. Furthermore, insulin sensitivity also was not improved by the treatment, since no patient showed improvement in total-body glucose utilization.

On the contrary, the specific parameters of liver insulin metabolism were significantly improved with opioid antagonist administration. For estimation of hepatic insulin extraction, we used the formula reported by Faber et al[16] and Bonora et al,[17] which may raise some criticism in terms of mathematical calculations[6] but could be reasonably considered a biological index to compare the same patients before and after a specific treatment. On the other hand, the C-peptide curves of the group data showed a similar course before and after therapy, so our results do not seem to be dependent on an artifact of the protocol design, due to a "truncated" C-peptide response to the glucose load. Furthermore, O'Meara et al,[19] using more specific methods, also showed that the increased insulin concentration in PCOD reflected both a reduced clearance and an increased secretion of insulin.

Even if it is generally admitted that altered hepatic insulin removal is related to obesity,[16,17] data from the present study could suggest that in PCOD patients hyperinsulinism can influence the hepatic removal more than obesity, confirming our previous data[20]; in fact, normoinsulinemic and hyperinsulinemic patients had similar BMI values and did not record any body weight modification after naltrexone therapy. These data are partially in agreement with those reported by Buffington and Kitabchi,[21] who showed impaired insulin clearance in obese PCOD patients compared with obese or lean control subjects. On the other hand, it has to be considered that in their study only obese PCOD patients were evaluated, without normoinsulinemic or hyperinsulinemic subjects. Moreover, they based their conclusions on the data for similar hepatic insulin clearance between lean and obese control subjects, in contrast to data from the literature.

Concerning the peripheral insulin resistance, we have found that opioids are not responsible for the decreasing insulin sensitivity of PCOD; in fact, no patient showed improvement of the M value after opioid antagonist administration. Thus, it could be suggested that insulin resistance and hyperinsulinemia after a glucose load are two distinct deranged features of the insulin disorder of PCOD patients, and opioids could be involved only in the latter. This hypothesis is in agreement with data from Holte et al,[22] who have demonstrated that it is possible to improve insulin sensitivity in severely insulin-resistant women with PCOD by weight reduction, to a level that does not differ significantly from that of BMI-matched women with normal ovaries, while the enhanced early insulin response to intravenous glucose remains unchanged.

Since no improvement occurred in peripheral insulin resistance after naltrexone treatment, an open problem is that our hyperinsulinemic PCOD patients showed a reduction of the insulin response to a glucose load without any variation in glycemic levels. A possible explanation is that PCOD could have increased the β-cell mass or enhanced the sensitivity to glucose. This is suggested by the finding of decreased glycated hemoglobin in PCOD.[23,24] Furthermore, lower fasting plasma glucose[25] and 24-hour plasma glucose[26] have been observed in lean women with PCOD compared with control subjects; in addition, both obese and lean women with PCOD were found to have a low postprandial glucose increase.[26] Another explanation could be that the effects of the opioid antagonist on insulin levels in PCOD are indirectly mediated by an action on the hormones counteracting insulin. A recent report showed that β-endorphin binding, even if also occurring in insulin-containing β cells, was primarily concentrated in the glucagon α cells and somatostatin-containing δ cells.[27]

On the basis of the bulk of our data, it could be suggested that opioid tone plays a role in the insulin alterations of PCOD by acting chiefly on hepatic insulin clearance. These data are in disagreement with data from the literature, which clearly showed an opioid action on insulin release by β cells.[7,8] Nevertheless, it has to be considered that the studies reported an association between β-endorphins and β cells, but in light of a recent report,[27] we could also hypothesize that this association is also indirect, probably mediated by glucagon release by α cells.

On the other hand, a basic difference between our data and data from the literature is that we evaluated a long-term opioid blockade: it is possible that while the acute response to naloxone could influence insulin release, chronic administration of naltrexone could lead to improvement of liver metabolism of insulin without affecting pancreatic secretion of the hormone, or via a resetting of pancreatic secretion due to chronic treatment. We previously observed in a group of PCOD patients a lack of linear correlation between insulin and C-peptide incremental areas, whereas after naltrexone therapy, even without any variation in C-peptide levels, we recorded a significant relation between these parameters, thus suggesting that chronic opioid blockade could affect the insulin metabolism but not insulin release by β cells.

In terms of opioid action on the liver, we are not able to specify whether it is direct or indirect, probably mediated by another unknown factor. Moreover, we are not able to clarify if the action is at the level of insulin receptor binding or the enzymatic processes of metabolism. Recent reports showed the presence of μ and δ opioid receptors in the rat liver[28] and the presence of elevated met-enkephalin and β-endorphins in patients with liver failure.[29] On the other hand, we have shown that insulin sensitivity was not modified from chronic opioid blockade. Thus, it could be suggested that there is a different role of opioids on different aspects of insulin metabolism.

Finally, other effects of naltrexone could be hypothesized. For example, the presence of opioid receptors has been reported also in the gastrointestinal tract.[30] Therefore, it is possible to hypothesize an indirect action of naltrexone on insulin metabolism, mediated by enterohormones.

In conclusion, considering our data and those from the literature, we can hypothesize that the insulin sensitivity and hyperinsulinemia in response to a glucose load are two distinct deranged features of the insulin disorder of PCOD patients. Chronic pharmacological inhibition of opioid tone could improve the insulin concentration in the plasma by acting chiefly on insulin metabolism in the liver of hyperinsulinemic PCOD subjects. Further studies are needed to clarify the improved liver function occurring after the depressed endorphin levels.

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