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The effects of intracerebroventrically administered opioid peptide receptor antagonists on exercise performance

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Abstract. Physical exercise increases the level of endogenous opiates that possibly play a major role for integration of hormonal and metabolic responses to exercise. Increased endogenous opioids in response to physical activity may enhance the performance through decreasing pain perception. However, what possible effect the opioid peptides have on physical exercise at the level of central nervous system remains to be elucidated. In this study, naloxone ($N = 6$), and naltrindole ($N = 8$) were administered intracerebroventrically to rats. A physiologic saline group ($N = 10$) and a control group ($N = 8$) were also included in the study. All groups were then subjected to exhaustive exercise on a treadmill. Besides recording the exhaustion time, blood glucose and lactate levels were measured before and after exercise. Treatments with naloxone and naltrindole had no effect on the exhaustion time. In comparison with pre-exercise period, lactate levels increased, while glucose levels decreased significantly in the blood of all animals during post-exercise period. However, decreased level of glucose in the post-exercise period was statistically significant only in those treated with naltrindole, compared with the other groups. Our findings indicate that the central endogenous opioid peptides may have a role in the regulation of glucose metabolism during exhaustive exercise. This effect can be mediated via delta opioid receptors, although they exert no effect on the performance, namely, on the exhaustion time.

Keywords: Exercise, endogenous opioid, delta opioid receptor, rat, naltrindole, blood glucose level

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1. Introduction

Generally, metabolic capacity is considered to be as a limiting factor for the ability of exercise. Several studies associated with the limiting factors for exercise emphasize especially such components of the metabolic capacity as glycogen stores of muscles, maximal oxygen consumption, and aerobic/anaerobic enzyme capacity [1,2].

It has recently been proposed that the sensation of fatigue during physical exercise is regulated by very complex mechanisms between peripheral signals, such as the depleted energy sources and central regulation of homeostasis [1,3].

Beside their analgesic effect, it was reported that the endogenous opioid peptides (EOPs) were involved with the regulation of several physiological processes such as regulation of cardio-respiratory functions [4, 5], locomotor activity [6], and facilitation of muscle glucose uptake [7]. The regulatory effects of EOPs in these instances are exerted through the delta opioid receptors (DORs) [6,8].

It is known that various stress factors induce the activation of EOPs. Studies focusing on the effects of these peptides during the physical exercise appear to be drawing much interest [6]. Based on the published studies, there is evidence showing that the level of opioid peptide increases in response to exercise in humans [6]. It is also known that depending on the strength and type of exercise [9,10], the level of β-endorphin in the plasma varies. Exercise having the intensity threshold somewhere between 60–80% of VO_2 peak results in a signif-

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icant increase in the plasma level of β-endorphin [11, 12].

A previous study indicated that endogenous opioids depress ventilation and may, therefore, play a role in the regulation of respiratory function associated with carbon dioxide, hypoxia and exercise [6]. Moreover, during exercise, the perception of fatigue is also modulated by increased endogenous opioids [6,13]. Sgherza et al. suggested that the decrease in maximal working capacity is associated with the perception of effort rather than physiological fatigue, and this perception can be attenuated by endogenous opioids [14]. To point out the importance of sensation in regard to endurance exercise, Jones and Killian proposed more complex neuronal mechanisms to explain the limitation of exercise endurance instead of limitation-based explanations such as limited capacities of oxygen-delivery mechanisms [15].

A substantial number of studies have encompassed the levels of opioid peptides [7,13] as well as their association with immune system, sympathetic system and stress hormones during the exercise [9,16].

EOPs exert their different effects through μ-,δ-, and κ-receptors. β-Endorphins are mainly found in pituitary gland and released into blood. All the endorphins have an affinity to μ-, δ-, and κ-receptors in variable rates. It is well known that β-endorphins recognize μ-, and δ receptors.

Evidences in the literature about opioidergic system effects on exercise are indirect, such as measurements of β-endorphin levels in plasma and cerebrospinal fluid. However, resulting changes of endorphin levels in the peripheral blood and the cerebrospinal fluids can hardly be related to central opioidergic system. Because once endorphins released, their returning to the brain through blood brain barrier occurs only in marginal circumstances [17,18].

Therefore, the present study was designed to explore the presence of possible relationship between the EOPs and exercise performance at the central nervous system level.

Since the DORs have regulatory effects on most of the exercise related opioid peptide functions, a specific DOR antagonist naltrindole was also used in the experimental protocol in addition to the use of a nonspecific opioid peptid receptor antagonist naloxone.

2. Methods

Thirty two male Wistar albino rats, weighing 212 $\pm$ 21 g were used for the experiments. The rats were maintained under standard animal care conditions (12:12 h light-dark cycle, room temperature 21°C, with standard rat chow and tap water ad libitum). Animal experiments were performed in accordance with the guidelines issued by Ethical Committee of Gazi University Faculty of Medicine. Experimental groups were as follows: control (C, only exhaustive exercise, $N = 8$), saline + exhaustive exercise (S, $N = 10$), naloxone + exhaustive exercise (NX, $N = 6$), naltrindole + exhaustive exercise (NT, $N = 8$).

For familiarization to motor driven leveled custom made rat treadmill (Department of Electrical Engineering and Electronics, Ankara University), rats were allowed to run on the treadmill for 10 minutes by a mild electrical stimulus to the tails of the rats that caused no injury. Familiarization period was 5 consecutive days. The angle of treadmill was 15° grade and speed was progressively increased from 7 m/min to 20 m/min by the end of familiarization period. For intracerebroventricular (i.c.v) drug applications, rats were anesthetized with ketamine (90 mg/kg) and xylazine (5 mg/kg) and then placed in a stereotaxic instrument (*Stoelting Co., Wood Dale, IL*). A 22-gauge stainless steel guide cannula was inserted into lateral cerebral ventricle (AP: +0.8 mm, L: −1.5 mm) [19]. Injection route was confirmed by the staining of methylen blue. Animals were allowed to recover for one week before conducting experiments.

On the day of the experiment, each animal was sedated by isofluran followed by drug administration (50 μg/10 μl naloxone, 50 μg/10 μl naltrindole or vehicle 10 μl physiologic saline) via i.c.v canulla. At concentrations ranging from micromolar to nanomolar levels, both naloxone and naltrindole have inhibitory effects in different experimental models of opiodergic system studies [20,21]. Based on this published fact, the pilot-experiments have been conducted as to finding out the most appropriate dose.

Using doses over 50 μg of both antagonists caused the animals to become oversensitive to any kind of stimulus. Therefore, fifty microgram was chosen for both antagonists. Under the anesthesia, blood samples were collected from tail veins for measuring lactate and glucose levels. The measurements were carried on by using glucometer (Accu-check Glucose, Roche) and lactometer (Accutrend Lactate, Roche). Afterwards, the opioid antagonists were injected into the lateral cerebral ventricule by a 29 Gauge Hamilton micro syringe. Approximately 90 minutes following the administration of either drug or vehicle, all animals were subjected to the exhaustive exercise on a treadmill (20 m/min,

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Table 1
Exhaustion times of the exercised rats (min ± SD)

Groups	Exhaustion time
C ($n = 8$)	67.3 ± 36.6
S ($n = 10$)	70.3 ± 19.2
NX ($n = 6$)	59.8 ± 30.4
NT ($n = 8$)	86.6 ± 34.6

Table 2
Blood glucose levels before and after exhaustive exercise (mg/dl ± SD)

Groups	BE	AE	p
C ($n = 8$)	133 ± 24	65.4 ± 34.8*	0.012
S ($n = 10$)	125.1 ± 15.3	54.9 ± 17.3†	0.005
NX ($n = 6$)	125 ± 17.6	56.3 ± 26,1‡	0.028
NT ($n = 8$)	114 ± 22.7	37.8 ± 7.5*†‡	0.012

Blood glucose levels. Naltrindole (NT) decreased post-exercise glucose levels more significantly compared to other groups. *NT versus Control (C) $p = 0.013$; †: NT versus Physiologic Saline (S) $p = 0.009$; ‡: NT versus Naloxone (NX) $p = 0.038$. BE: Before exhaustive exercise; AE: after exhaustive exercise; n: number of the subjects.

inclination: 15%). The time lag between the beginning of the exercise and the exhaustion was recorded and called as exhaustion time. Upon exhausted, the animals were immediately sedated for collecting blood samples to measure post-exercise lactate and glucose levels. Then, they were sacrificed.

Blood levels of glucose and lactate in pre- and post-exercise periods were evaluated statistically by Wilcoxon Test. Kruskal Wallis Test was used for the evaluation of the exhaustion time and levels of glucose and lactate. The significancy levels of the differences were analyzed by Mann Whitney U Test. Values are expressed as mean ± S.D. $p < 0.05$ was considered significant.

3. Results

Naloxone and naltrindole treatments had no effects on the exhaustion time of exercised rats ($p > 0.05$) (Table 1). However, the decreased glucose level was statistically more significant in naltrindole-treated group ($p < 0.05$) compared to the other groups (Table 2). Moreover exhaustive exercise caused increased blood lactate levels ($p < 0.05$) (Table 3) in the post-exercise period of all groups.

4. Discussion

The main finding of this study was that i.c.v. administration of naltrindole decreased the blood level of glucose significantly after the exhaustive exercise.

Table 3
Blood lactate levels before and after exhaustive exercise (mmol/L ± SD)

Groups	BE	AE	p
C ($n = 6$)	2.7 ± 0.2	8.9 ± 6*	0.028
S ($n = 8$)	3.1 ± 0.3	6.1 ± 2.7*	0.012
NX ($n = 6$)	2.7 ± 1.2	7.6 ± 2.7*	0.028
NT ($n = 7$)	2.7 ± 0.6	5.1 ± 2*	0.028

*After the exhaustive exercise in all groups, blood lactate levels increased ($p < 0.05$). BE: Before exhaustive exercise; AE: after exhaustive exercise; C: Control, S: Physiologic Saline, NX: Naloxone, NT: Naltrindole.

Studies searching the possible effects of endogenous opioid peptides on exercise performance are very limited. Moreover, the outcomes of these studies are contradictory. For instance, some reports suggest that the opioid peptides contribute remarkably to exercise performance by maintaining muscle contractility, facilitating glucose uptake to the muscle, and attenuating the pain perception during the exercise [6,13], while other propose that opioid peptides are not involved with the exercise performance [24,25]. Potter et al. showed that the non-specific opioid receptor antagonist naltrexone decreases the total running activity and speed, suggesting that the activation of endogenous opioid receptors facilitates the voluntary exercise. On the other hand, these receptors are not involved in the initiation of exercise in female hamsters [26]. Sgherza et al. showed that naloxone causes a significant decrease in VO_2 max and exercise in human subjects [14]. The findings of the present study were not consistent with those of the aforementioned reports, several factors of which can be accounted for. Employing different species and/or experimental methodology, and a relatively small number of subjects with high standard deviations could be the determining primary factors. The subjects of the published two reports mentioned above were human beings and hamsters. Furthermore, exercise in these studies was ended by subjects. On the other hand, the rats in the present study underwent exhaustive exercise. Variations in the dose and route of the drug administration can also account for the variations in the outcomes; the opioid antagonists used in the other studies were given either intraperitoneally or intravascularly.

Nevertheless, our findings are consistent with a number of reports suggesting that opioid peptides do not contribute to exercise performance. McMurray et al. showed that naloxone administration had no effects on exercise performance while altering the cardiorespiratory responses in human subjects experiencing exhaustive exercise [24]. Gullestad et al. also demonstrated that naloxone administration was not involved with the perception of fatigue [25].

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During the prolonged exercise, glucose output of liver does not meet the muscles glucose demand and plasma glucose concentration decreases. The level of the circulating blood glucose content may also fall in this situation. Indeed in all groups the exhaustive exercise decreased the blood glucose levels ($p < 0.05$) during the post-exercise period (Table 2). In the present study, a specific δ-opioid receptor antagonist, naltrindole, decreased significantly the level of blood glucose following the exhaustive exercise. However, it did not alter the level of lactate in blood. Even though it is well known that there is a close relationship between blood lactate concentration and fatigue during exercise, no correlation between opioid peptides and increased level of lactate has been reported in severe endurance exercise [22,23]. Sgherza et al. [14] reported that there was no significant difference between control and naloxone groups in terms of lactate levels in human subjects performing maximally on a cycle ergometer. Furthermore, there was no relationship between the increase in β-endorphin level and high lactate level in long term severe endurance exercise [17,27].

It is well established that EOPs have a widespread tissue distribution. For instance they participate in the regulation of glucose homeostasis, with their presence in pancreas and pituitary gland [28]. Intracerebroventricular infusion of β-endorphin or morphine increases the levels of glucose and catecholamines in dogs [29], while peripheral infusion of β-endorphin has no effect on the level of catecholamine [30]. Marczak et al. also showed that a μ-/δ-opioid receptor antagonist MZ-2 decreases serum glucose and insulin levels in voluntarily exercising animals [31]. Therefore, it is believed that EOPs have a role in the regulation of glucose metabolism during exercise [32]. Among the EOPs, β-endorphins facilitate especially glucose uptake into the muscle during exercise [32]. Evans et al. suggested that β-endorphin facilitated glucose uptake to the contracting diaphragm muscle in mice [33]. They have also demonstrated that the δ-opioid receptor was involved with the glucose uptake to the muscle [7,8].

In the present study, glucose levels decreased more significantly in naltrindole-treated groups than in the other groups in post-exercise period. Even though this finding appears to be inconsistent with those of previous reports, similar observation was also made by Angelopoulos et al. [32,34]. These reports suggest that the opioid antagonism induced by intravenous naloxone administration increases plasma β-endorphin level during exercise. When it binds to opioid receptors, naloxone leads to an increased release of β-endorphin by the pituitary gland via a positive feedback mechanism. In spite of the fact that naloxone is a well known non-specific opioid receptor antagonist, the mechanism(s) underlying the action of naloxone deserve further investigation.

It should be borne in mind that naloxone is a primary antagonist at μ- and κ-opioid receptors, and to a lesser extent at δ-opioid receptors, whereas naltrindole is a specific δ-opioid receptor antagonist. In the present study, i.c.v. administration of naltrindole, could have triggered a positive feedback mechanism mentioned above, which might have been involved with delta opioid receptors. Taken together, our findings suggest that central opioid antagonism results in enhanced glucose uptake into the muscles during the exercise. In the light of findings of the present study, further studies are needed to explore how central opioid peptides regulate the glucose mechanism during the exhaustive exercise and what are the mechanism(s) by which regulation takes place.

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