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Evaluation of CART peptide level in rat plasma and CSF: Possible role as a biomarker in opioid addiction

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ABSTRACT

It has been shown previously that cocaine- and amphetamine-regulated transcript (CART) peptide has a modulatory role and homeostatic regulatory effect in motivation to and reward of the drugs of abuse specially psychostimulants. Recent data also showed that in addition to psychostimulants, CART is critically involved in the different stages of opioid addiction. Here we have evaluated the fluctuations in the level of CART peptide in plasma and CSF in different phases of opioid addiction to find out whether CART can serve as a suitable marker in opioid addiction studies. Male rats were randomly distributed in groups of control, acute low-dose (10 mg/kg) morphine, acute high-dose morphine (80 mg/kg), chronic escalating doses of morphine, withdrawal syndrome precipitated by administration of naloxone (1 mg/kg), and abstinent after long-term drug-free maintenance of addicted animals. The level of CART peptide in CSF and plasma samples was measured by enzyme immunoassay. CART peptide concentration in the CSF and plasma was significantly elevated in acute high-dose morphine and withdrawal state animals and down-regulated in addicted rats. In abstinent group, CART peptide level was up-regulated in plasma but not in CSF samples. As the observed results are in agreement with data regarding the CART mRNA and protein expression in the brain reward pathway in opioid addiction phases, it may be suggested that evaluation of CART peptide level in CSF or plasma could be a suitable marker which reflects the rises and falls of the peptide concentration in brain in the development of opioid addiction.

1. Introduction

Substance abuse is characterized by numerous neuroadaptations and neuromodulations in the central nervous system (CNS) which lead to compulsive and uncontrolled drug seeking, tolerance, dependence, withdrawal syndrome and other serious health problems [30,31]. Opiates form a major class of drugs of abuse which act via opioid receptors and mesocorticolimbic regions in the brain [44]. Multiple neurotransmitters such as dopamine, gamma-aminobutyric acid (GABA), glutamate, and etc. are involved in pharmacologic actions of opiate drugs [29,37]. Another neurotransmitter that has prominently attracted attention in recent years is cocaine and amphetamine-regulated transcript (CART) peptide. CART which is involved in various critical processes such as feeding behavior, regulation of hypothalamic–pituitary–adrenal axis, learning and memory, anxiety and stress, depression, and finally rewarding and reinforcing effects of addictive substances, is a neuroendocrine peptide that is variously expressed in different brain regions [33,40] including the reward pathway. It has been shown that CART has complex relation with dopamine as dopamine controls the level of CART and conversely CART modulates the dopamine level in the brain reward system [43].

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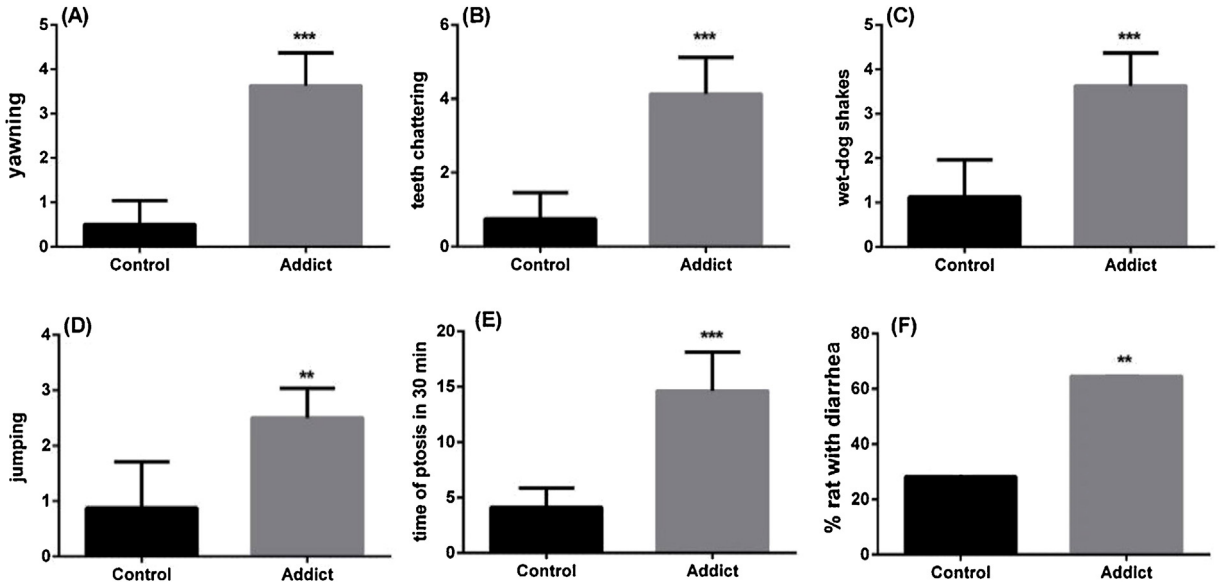

Fig. 1. Morphine withdrawal symptoms during 30 min after injection of naloxone (1 mg/kg) in addicted and control groups. Values are presented as mean ± SEM (n = 6). **P < 0.01, ***P < 0.001 compared to the control group.

Although CART is found at high levels in the CNS, many studies have shown that it is also expressed in areas outside the brain such as adrenal medulla, gut, sympathetic ganglion neurons, islets of the pancreas, and etc. [14,25]. It is suggested that CART peptide may have potential endocrine and/or paracrine functions in the periphery in addition to its activity in the CNS [24]. CART is also found in blood [24] and is able to pass the blood-brain-barrier (BBB) and reach the brain and cerebrospinal fluid (CSF) [26]. However, our knowledge about the source, destiny, and mechanisms involved in the control of circulating CART are very limited.

Evaluation of the diurnal variations of CART peptide in rat blood samples has shown that CART 55–102 which is the major immunoreactive fragment of the peptide in the circulation, exhibits a daily rhythm. Under the normal 12 h light/dark cycle, the peak plasma level of the peptide is at 5:00 P.M. and the minimum level is at 5:00 A.M. [46]. As adrenalectomy can diminish the circulating level of CART by 70% and affect its daily variations, it was suggested that circulating glucocorticoids might be involved in this diurnal pattern of CART blood level [15,32]. Furthermore, it was reasonably proposed that corticosterone impacts blood level rhythm of CART through hypothalamic-pituitary paths where CART is plentifully expressed [45]. Similar studies have been performed to discover whether centrally expressed CART also exhibits diurnal regularity. The morning lower level of the peptide compared to the evening level was also observed in the hypothalamus, nucleus accumbens and amygdala [46,48]. It is proposed that alterations of CART blood level reflect its mRNA expression level in anterior pituitary gland [38]. Furthermore, It has been claimed that gut, pancreas and adrenal gland release CART peptide in blood in addition to other possible sources and around 30% of CART in plasma has non-adrenal tissue origin [46]. More studies demonstrated that diurnal mRNA expression of central CART in hypothalamus and mesolimbic regions as well peripheral plasma fluctuations of the peptide level depend on food intake, reward and motivation [39,41,47]. According to CART ability to cross the BBB, Vicentic and colleagues suggested that those parts of the brain which considerably express CART peptide may be the sources for appearance of this peptide in blood [45]. Rhythmic expression of CART in mesolimbic regions and hypothalamus can be a significant signal for integration of apparently different periodic activities like food intake, energy expenditure, wakefulness, reward and motivation [1,19,45]. Studies have shown that food availability affect expression of CART gene and peptide either directly or indirectly by altering blood hormones such as glucocorticoids. More studies have also demonstrated that circadian gene transcription network may tightly regulate the daily variations of CART peptide [10,16,42].

Previous studies have found a vast body of evidence for the interaction between CART, dopamine, and addictive drugs. Our previous study strongly demonstrated that the level of CART mRNA and peptide changes in some critical parts of the brain reward system when acute or chronic morphine is administered to male rats. Furthermore, it was found that acute morphine withdrawal and long-term abstinence also change the level of CART peptide in the reward pathway [2]. After observing these results, we were encouraged to evaluate whether such a relation also exist between CSF and plasma CART level and different stages of opioid addiction such as acute and chronic abuse or acute and spontaneous withdrawal state. The answer of this question could help us to find out whether CART level in CSF or blood plasma could serve as a peripheral marker to evaluate different stages of opiate addiction.

2. Materials and methods

2.1. Animals

Forty-two male Wistar rats weighing between 250 and 290 g and bought from Iran University of Medical Sciences (Tehran, Iran) were used in the study. Animals were kept four per Plexiglas cages (40 * 30 * 25 cm) with food and water freely available ad-libitum, under 12:12-h light/dark cycle (light on at 07:00 A.M.) and temperature maintained at 22 ± 2 °C. Before the start of any experiment, rats were first allowed to adapt to the laboratory conditions for a minimum of one week. Maintenance of all the animals and performance of all the procedures were in accordance with institutional guidelines for animal care and use. Experimental protocols were approved by the Research and Ethics Committee of Tehran University of Medical Sciences.

2.2. Drugs

Morphine sulfate (Temad, Tehran, Iran) and naloxone hydrochloride (Tolid-Daru, Tehran, Iran) were used in the