PERIPHERAL INJECTION OF DEXAMETHASONE MODULATES ANXIETY RELATED BEHAVIORS IN MICE: AN INTERACTION WITH OPIOIDERGIC NEURONS

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ABSTRACT
Stress and anxiety initiates a cascade of biochemical and endocrine event which results in behavioral and electrophysiological effects in both animals and humans. In this study, we investigated the effects of dexamethasone (DEX), as a synthetic glucocorticoid, and its interaction with opioidergic system on anxiety related behavior in mice. Young adult male mice were used in this study. A standard elevated plus-maze was used to determine anxiety levels in animal. Different doses of DEX (0.1, 0.5, 1, 2 and 10 mg/kg, SC) or vehicle was injected 30 min before of evaluation. Naloxone (1 and 2 mg/kg, IP) was injected 5 min before the DEX (0.5 and 1 mg/kg) administration. Results indicated that DEX at doses of 0.5 and 1 reduced and in dose of 10 mg/kg increased anxiety related behaviors significantly ($P<0.05$ in all cases). Also pretreatment of naloxone at doses of 1 and 2 mg/kg attenuated the effects of lower doses of DEX on anxiety related behaviors. Finding above indicated that peripheral administration of glucocorticoids induces biphasic effects on anxiety related behaviors: anxiolytic effects in lower doses and anxiogenic effects in a high dose. Data also revealed an involvement of opioidergic system in anxiolytic effects of glucocorticoids.

Keywords: Glucocorticoids, anxiety, dexamethasone, opioid system, elevated plus maze, mice.

INTRODUCTION

Anxiety disorders are the most common type of psychiatric disorders, with an incidence of 18.1% and a lifetime prevalence of 28.8% (Kessler *et al.,* 2005). The term of anxiety is characterized by somatic, cognitive, behavioral and perceptual symptoms. Also many endocrine, autoimmune, metabolic and toxic disorders as well as medi-cation adverse effects are known to generate anxiety (Kaplan *et al.,* 2004). In addition, anxiety is experienced in situations where an individual has a need that they see no means of satisfying, or is faced with the threat of punishment that they see no means of avoiding (Korneyev, 1997). Previous evidences indicate that pretreatment with anti-anxiety agents attenuates various behavioral consequences of stress and anxiety (Eisenberg, 1993). Many evidences indicate that stress and anxiety initiates a cascade of biochemical and endocrine event which results in behavioral and electrophysiological effects in both animals and humans (Korneyev, 1997). According to gray anti-anxiety affects are achieved through the inhibition of noradrenergic, adrenergic and serotonnergic neurons located in the locus ceruleus and raphe nuclei. These neurons initiate a stress and anxiety reaction through the stimulation of CRH release from the paraventricular nucleus (Kaplan *et al.,* 2004).

It seems that the hypothalamic-pituitary-adrenal (HPA) axis plays a major role in the stress and anxiety reaction. Cortisol is a steroid hormone which affects all cells and maintains physiologic integrity within the body. Studies over decades have consistently shown a positive correlation between the severity of a stressor and the level of cortisol in the bloodstream a (Fernandes *et al.,* 1997). There is now considerable evidence that dysregulation of the HPA axis is implicated in the pathophysiological of affective and anxiety disorders (Boyle *et al.,* 2006; Velisek, 2006).

Glucocorticoid hormones readily enter the brain and activate adrenal steroid receptors. They modulate a variety of behavioral responses through an interaction with several neurotransmitter systems (Soravia *et al.,* 2006). The aim of this study was to determine the effects of dexamethasone, as a synthetic glucocorticoid, and its interaction with opioidergic system on anxiety related behaviors in mice.

MATERIAL AND METHODS

Subjects

Young adult male albino mice (n=120), weighting between 25-30 grams, were used in this experimental study. They were housed in groups of ten in plastic cages in a room with constant temperature (22°-24°) and natural lighting (12h light-12h dark) conditions. Water and food were freely available. The treatment of the animals was approved by the Institute's Ethical Committee, and conformed to NIH guidelines and to the Animal Protection Law of the Iran.

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Drug and Injection Procedure

Dexamethasone (Synopharm, Italy) as a specific GR agonist (0.1, 0.5, 1, 2 and 10 mg/kg) or vehicle (VEL) were systemic injected subcutaneously. DEX was dissolved initially in 100% ethanol and diluted to a final concentration of 2% ethanol in 0.9% saline. A 2% ethanol solution in saline was used for vehicle control injection. Also naloxone (Sigma Co.) as an opioidergic antagonist (1 and 2 mg/kg IP) or saline (SAL) were systemic injected IP. NAL was dissolved initially in SAL. All drugs and solutions were freshly prepared before each experiment. Doses of drug determined with our pilot study and also a survey of other studies using these drugs (Boyle *et al.,* 2006; Korneyev, 1997, Soravia *et al.,* 2006).

Behavioral test and apparatus

Elevation plus maze (EPM) task

Apparatus

The elevated plus maze test was made of wood with two open arms (50 x 5 cm) and opposite closed arms of the same size but with 40-cm high walls. The arms were connected by a central square and thus formed a plus sign. The apparatus was elevated 50 cm above the floor. Each of mice was placed in the central square of the plus maze facing an enclosed arm. The time spent in enclosed and open arms was scored for 5 min. An arm entry was defined as an animal entering the arm with all four feet and the number of entries into open and enclosed arms was scored (Zarrindast *et al.,* 2001). This instrument is commonly used to assess anxiety-like behavior in laboratory animals. The open arms are perpendicular to the closed arms, with the four arms intersecting to form the shape of a plus sign. Security is provided by the closed arms while the open arms over exploratory value. Therefore, one might expect anxious rats to spend less time in the open arms than those that are less fearful. When placed in an elevated plus-maze for the first time, a mice's behavior is largely based on its anxiety level. Normal mice's that have not received any anti-anxiety drugs will become moderately anxious in this new environment. Thus, they tend to prefer the closed arms over the less secure open arms (Dawson and Tricklebank, 1995).

Meanwhile, mice's treated with anti-anxiety drugs (diazepam) tend to be less anxious, so they spend more time in the open arms compared to normal mice's, and they are generally less active (Fernandes *et al.,* 1997). Two behavioral measures were used: the percentage of time spent in the open arms and the ratio of open arm entries to total entries during 5 min. More entries into the open arms and more time spent in the open arms were interpreted as indicating lower levels of anxiety.

Statistics

Data were analyzed by one-way and two-way analysis of variance (ANOVA), followed by Tukey's test for multiple comparisons. Values of $P<0.05$ were considered significant.

EXPERIMENTAL PROTOCOL

Experiment 1

The aim of Expt.1 was to determine the effect of systemic injection of DEX on anxiety related behavior in EPM task. Sixty mice were divided into six groups, SAL+VEL (n =10), SAL+DEX (n=50 in five groups), and EPM evaluation occurred in a single 5 min session according to procedure described in Section 2. 30 min before evaluation, control and treatments groups received systemic injections of SAL (1 ml/kg IP) and VEL (1ml/kg SC) or SAL (1 ml/kg IP) and DEX (0.1, 0.5, 1, 2 and 10mg/kg SC), respectively. EPM evaluation was assessed during a 5 min.

Experiment 2

The aim of Expt.2 was to determine the role of the opioid system on angiolytic effects of DEX. Sixty mice were divided into six groups: NAL+VEL (n =20 in two groups), and NAL+DEX (n=40 in four groups). EPM evaluation occurred in a single 5 min session according to procedure described in Section 2. Control and treatments groups received systemic injections of NAL (1 and 2 mg/kg, IP) plus VEH (1 ml/kg SC). Treatments groups received NAL (1 or 2 mg/kg IP) plus DEX (0.5 or 1 mg/kg SC). EPM evaluation was assessed during a 5 min.

RESULTS

Experiment 1

Analysis of data indicated that DEX–treated mice showed anxiolytic responses in doses of 0.5 and 1 mg/kg. These mice spent more percent time in open arms ($P<0.02$) and higher number of entrances to open arm ($P<0.01$) than to control groups. DEX at a higher dose (10 mg/kg) induced an anxiogenic response in mice (fig.1 A and B). Thus, DEX induces a biphasic effects on anxiety related behaviors.

Experiment 2

Analysis of data (fig. 2A and B) indicated that pretreatment of NAL at both doses attenuated the effects of DEX on anxiety related behaviors. NAL alone did not change the behavioral measures (time spent in open arm and number of entrances to open arm) of anxiety related behaviors in comparison with control groups, but significantly reduced both measures in DEX-treated mice ($P<0.05$). Thus, NAL attenuates anxiolytic effects of DEX.

DISCUSSION

The major finding of the present experiment is that systemic injection of the dexamethasone has a biphasic

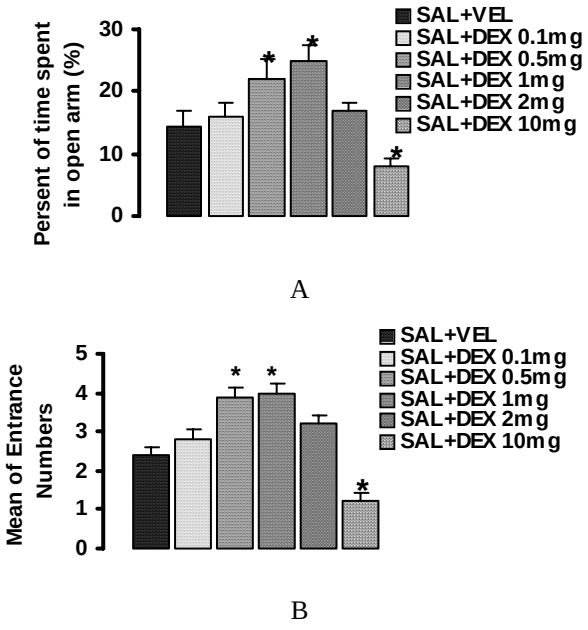

Fig. 1: The effect of dexamethasone (0.1, 0.5, 1, 2 and 10 mg/kg, SC) on anxiety related behaviors in mice in Expt.1. Mean ± SEM of percent of time spent in open arm (A) and number of entrances (B) during a 5 min test. *P < 0.02 as compared with control group.

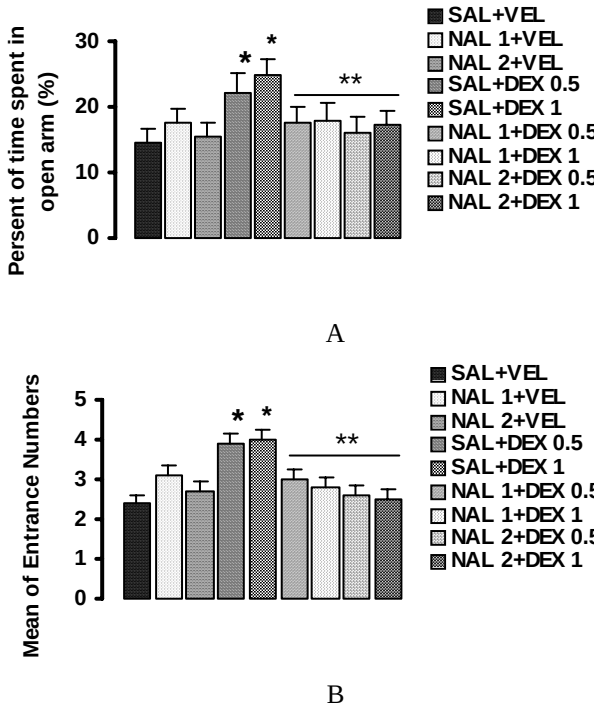

Fig. 2: The effect of naloxone on anxiolytic effects of dexamethasone in Expt. 2. Mean ± SEM of percent of time spent in open arm (A) and number of entrances (B) during a 5 min test.* P<0.01 as compared with saline + VEH group. ** P<0.01 as compared with saline + DEX groups. SAL: saline; NAL: Naloxone; DEX: dexamethasone. N= 10 for each group.

effects on anxiety related behaviors in mice: in lower doses induces an anxiolytic while in a higher dose induces an anxiogenic effects. Data also revealed an involvement of opioidergic system in anxiolytic effects of glucocorticoids.

Previous study indicated that stress and anxiety activates the hypothalamus-pituitary-adrenal axis and causes release of glucocorticoids (Fernandes *et al.,* 1997). It seems that this sequence of events contributes to anxiety induced behavioral responses (Korneyev, 1997). The finding of the present experiments supports the hypothesis that glucocorticoid receptors (GR) affect anxiety related behaviors. We have found that systemic administration of DEX modulates anxiety related behaviors, suggesting an involvement of GR in anxiety behaviors. However, with a systemic administration it is not possible to determine that anatomical locations of GR that involved in anxiety. Our finding consistent with previous studies showing that the HPA axis plays a major role in the stress and anxiety reaction. There is now considerable evidence that dysregulation of HPA axis is implicated in the pathophysiology of affective and anxiety disorders (Heim *et al.,* 2000, Holsboer, 2000).

Previous study indicated that many of neurotransmitter systems have been implicated in the neurobiology of anxiety disorders. There is growing evidence that alterations in several neurotransmitter systems (such as GABA, serotonin, adrenergic, glutamate, opioids and etc) may be involved in general anxiety disorders (Nutt, 2001). Also many evidence indicates that these systems and HPA may interact in influencing have behaviors (Soravia *et al.,* 2006). For example, a previous study indicated that high levels of corticosteroids can also down-regulate serotonin 5-HT_{1A} receptor messenger ribonucleic acid (mRNA) and 5-hydroxytryptamine (5-HT) binding in the hippocampus (Lopez *et al* 1999), suggesting an important and complex triangular relationship among the HPA axis, glutamate, and serotonin in the neural circuits implicated in stress and anxiety responses. Also extensive preclinical evidence suggests that glutamate serves an important role in modulating actions of CRF and glucocorticoids, particularly in brain regions important in anxiety and mood regulation such as the amygdala, hypothalamus, mono-aminergic brainstem nuclei, and hippocampus (Mathew *et al.,* 2001).

Our finding also indicated that naloxone did not affect anxiety related behavior alone, but rather blocked the angiolytic effects of dexamethasone. This finding indicates involvement of naloxone sensitive pathway in mediating the influences of glucocorticoids on anxiety. There are two possible explanations for an interaction between dexamethasone and naloxone on anxiety. First, it is likely that dexamethasone activates the endogenous opiate system and then that mediates their influences on anxiety. Although, there are some evidences indicating that the effects of DEX on some behaviors such as analgesia and emotional memory mediated, at least in part, by the endogenous opiate system (Rashidy-Pour *et al* 2004, Stevens and Pezulla 1989), but to our knowledge there are no reports in literature concerning with such mediation on anxiety. Another explanation for these results might be that naloxone interacts with stress and anxiety induced glucocorticoids or dexamethasone in plasma membrane of target neurons in brain. As noted above, anxiety modulate was found as soon as 30 min after dexamethasone injection. This time is too fast to explain genomic action of glucocorticoids via intracellular receptors and it may reflect an involvement of membrane receptors. In light of this idea, recently a membrane receptor for glucocorticoids in brain of the roughskin newt (*Taricha granulosa*) as an amphibian model has been identified (Evans *et al.*, 2000a), and this receptor appears to be a G-protein coupled receptor super-family (Orchinik *et al.*, 1992). This receptor mediates the rapid effects of stress or corticosterone on reproductive behaviors and neural activity of the animal (Hua and Chen 1989, Joles and de Kloet, 1989). Ligand binding assays with radiolabel corticosterone revealed that this receptor not only is the highly selective for corticosterone and Cortisol, but also recognizes specific opiate ligands such as dynorphine and naloxone. These opiate ligands interact directly by competing for the same binding site (Evans *et al.*, 2000b). Therefore, based on these data, opiate antagonists that bind to membrane receptor for glucocorticoids should block the behavioral effects of glucocorticoids. This idea supported by the present findings, which indicate naloxone blocks, the deteriorating effects of dexamethasone on anxiety related behavior.

CONCLUSION

In conclusion, the presents results provide evidence for this hypothesis that peripheral administration of glucocorticoids produce a biphasic effects on anxiety related behavior in mice: in lower doses induce an anxiolytic while in a higher dose induces an anxiogenic effects. Further, the opiate system may mediate the anxiolytic effects of glucocorticoids. Further studies are required to determine the underlying mechanisms.

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