Acute Cold / Restraint Stress in Castrated Rats

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Received January 2008; Revised and accepted July 2008

Abstract

Objective: The present study aimed to determine whether castration altered osmotically stimulated vasopressin (VP) release and urinary volume and what is the role of endocrine-stress axis in this process.

Materials and methods: Totally 108 mice were studied in two main groups of castrated (n=78) and control (n=30). Each group was extracted by acute cold stress (4◦C for 2h/day), restraint stress (by syringes 60cc 2h/day) and cold/restraint stress. The castrated group was treated in sub groups of testosterone, control (sesame oil as vehicle of testosterone). Propranolol as blocker of sympathetic nervous system was given to both groups of castrated mice and main control.

Results: Our results showed that, there is interactions between testosterone and sympathetic nervous system on vasopressin, because urine volume was decreased only in testoctomized mice with cold/restraint and cold stress ($P<0.001$); propranolol as the antagonist of sympathetic nervous system could block and increase urine volume in castrated mice. This increased volume of urine was due to acute cold stress, not restraint stress ($p<0.001$). The role of testosterone, noradrenalin (NA) and Vasopressin (VP) in the acute cold stress is confirmed, because testosterone could return the effect of decreased urine volume in control group ($P<0.001$).

Conclusion: Considering the effect of cold/restraint stress on urinary volume in castrated mice shows that there is interaction between sex hormone (testosterone), vasopressin and adrenergic systems.

Keywords: Cold/restraint stress, Castrated mice, Urinary volume, Testosterone, Propranolol

Introduction

Stress is a normal part of the today life. The stress response is a necessary mechanism but disrupts homeostatic process. Stress has been defined in many ways. To the physicist, the term refers to a force, strain or pressure applied to a system. The events or environmental agents responsible for initiating the stress response are called stressors. According to Hans Sely, stressors include mental, psychological or sociological ones that all disturb the stable internal environment, which may contribute directly to the production of disease or to the development of a behavior, which increases the risk of disease. Most of the stressors produce specific and nonspecific responses. The individual specific responses alter in the presence of the stressors, which involves neuroendocrine responses such as increased autonomic nervous system activity (1, 2). The physiologic responses to stress are initiated by the activation of the sympathetic–adrenomedullary system (SAS) and the hypothalamic–pituitary–adrenal (HPA) axis, resulting in the release of catecholamines

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Table 1: Comparison of urine volume between castrated and normal mice at different conditions

Stresses		Drugs		Urine volume (ml)		P-Value
Cold	Restrained	Testosterone (Sesame oil)	Propranolol	Castrated	Normal	
-	-	-	-	0.25 ± 0.19	0.06 ± 0.10	0.229
✓	-	-	-	0.30 ± 0.83	1.05 ± 0.27	**0.001**
-	✓	-	-	1.06 ± 0.87	1.27 ± 0.23	0.132
✓	✓	-	-	0.11 ± 0.44	0.86 ± 0.71	**0.003**
-	-	✓	-	0.15 ± 0.74	0.08 ± 0.14	0.801
✓	-	✓	-	0.44 ± 0.84	0.45 ± 0.44	0.894
✓	✓	✓	-	0.81 ± 0.65	0.15 ± 0.86	**0.021**
-	-	-	✓	0.03 ± 0.32	0.05 ± 0.30	0.578
✓	-	-	✓	0.39 ± 0.54	0.02 ± 0.51	0.067
-	-	✓	✓	0.08 ± 0.69	1.03 ± 0.69	< **0.001**

and stress hormones, such as glucocorticoids from the adrenal glands (3). Early life events influence life-long patterns of emotionality and stress responsiveness and alter the rate of brain and body aging. The hippocampus, amygdale, and prefrontal cortex undergo stress-induced structural remodeling, which alters behavioral and physiological responses. As an adjunct to pharmaceutical therapy, social and behavioral interventions such as regular physical activity and social support reduce the chronic stress burden and benefit brain and body health and resilience (4). The orchestrated interplay of several neurotransmitter systems in the brain underlies the characteristic phenomenology of behavioral, endocrine, autonomic and immune responses to stress (5). These transmitters include corticotrophin releasing hormone (CRH), arginine vasopressin (AVP), opioid peptides, dopamine and nor epinephrine. Shortly after its isolation, it became apparent that CRH was implicated in other components of the stress response, such as arousal and autonomic activity. Supportive evidence was derived from intracerebroventricular or selective brain administration of CRH in rodents and nonhuman primates, which precipitated several coordinated responses characteristic of stress (6). Elevation of glucocorticoid level provokes both short- and long-term effects in the brain. These changes indicate that stress can affect hippocampus structure and function. On the other hand, the hippocampus can also suppress stress reaction through the feedback regulation of the HPA axis (7). Although all stressors activate HPA and SAS, the degree of their activation depends on stress duration, type and intensity. Social interaction is an important source of stress. Originally, stress was considered to be a non-specific phenomenon, but it is now clear that different types of stressors elicit specific responses. For example, immobilization stress elicits a robust increase in the plasma concentrations of both adrenaline and nor adrenaline (NA). By contrast, cold stress triggers an equally robust increase in plasma NA, but only small rise in adrenaline, and insulin-induced hypoglycemia raises plasma adrenaline, but is less effective in rising NA (8).This process is mediated by transcriptional mechanisms in the adrenal medulla and the locus coeruleus. The persistence of transcriptional activation depends on the duration and repetition of the stress (9). The results of immobilization (IMMO) in rats indicate that acute IMMO increases synthesis, release, and metabolism of NA in the central nucleus of the amygdale and that repetition of IMMO decreases basal catecholamine synthesis and noradrenergic turnover in this brain region, without inhibiting acute noradrenergic responses (10). The neurohypophysial hormone vasopressin (Avp) is the hormonal regulator of water homeostasis and has major effects on behavior and vascular tone (11). It exerts major physiological actions through three distinct receptor isoforms designated as V1a, V1b, and V2. Among these three subtypes, the vasopressin V1b receptor is specifically expressed in pituitary corticotrophs and mediates the stimulatory effect of vasopressin on adrenocorticotropin releasing hormone (ACTH) release and it is clearly demonstrated that the V1b receptor plays a crucial role in regulating hypothalamic-pituitary-adrenal axis activity. It does this by maintaining ACTH and corticosterone levels, not only under stress but also under basal conditions (12). In addition to this, it is a key regulator of the hypothalamic-pituitary-adrenal (HPA) axis (12). These actions are mediated through a family of G protein - coupled receptors; the Avp 1a receptor (Avpr1a) which regulates vascular tone and has many

putative roles in the central nervous system, the Avp 2 receptor (Avpr2) which controls renal collecting duct water permeability, and the Avp 1b receptor (Avpr1b or V3R) which is predominantly found in the corticotrophs of the anterior pituitary, where it is involved in the regulation of ACTH release (13). Diverse homeostatic challenges including cognitive (e.g. restraint) and noncognitive (e.g. infection) stressors activate the HPA axis and sympathoadrenal systems (14-15). The present study aimed to determine whether castration altered osmotically stimulated vasopressin (VP) release and urinary volume and what is the role of endocrine-stress axis in this process.

Materials and methods

Male Naval Medical Research Institute (NMRI) mice (20-25g) were obtained from animal house center of Ahwaz University of Medical Sciences. All mice were maintained on a 12-h light/ dark cycle and animals were allowed at least 1 week of habituation in the animal room before the experiment. All of the animal studies were also approved by the Ethics Committee of Tehran University of Medical Sciences. Experiments were performed in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (publication No. 85-23, revised 1985). Mice were divided in two main groups of control and castrated which were studied in 18 sub groups each containing 6 animals. In the first experiment (castration experiment), gonadectomy or Sham operation was performed and an interval of 2 weeks was allowed to reach the hormonal equilibrium. The castrated (cast) and control (normal) mice were divided in three main sub groups: cold stress, restraint stress (rest) and cast-cold/restrained stress. Mice used for the acute cold stress procedure were transported, in their cages, to a 4°C cold room and remained there for 1h. Acute restraint stress mice were individually restrained in well- ventilated plastic 60-ml syringes in a way that, mice can move forward and backward in the syringe but can not turn head to tail. Mice used for the acute cold/restraint (ACRS) in order to induce cold stress and restraint were in syringes and then all animals were placed in 4°C cold room. The ACRS was always performed between 10 A.M. and noon (16). ACRS is considered a physical and psychological stressor. Control mice were left in their original cages undisturbed during the same time period. In Castrated-testosterone group, testosterone propionate was used (Abooraihan Drug Company 1 ng IP) and for control group sesame oil as vehicle of testosterone was used. Propranolol hydrochloride (Kenoll Drug Company) as blocker of sympathetic nervous system had been given to both groups of castrated and control mice (2.5 mg/kg IP). Study groups are presented in table 1. Urine volume of castrated and normal groups is presented as mean ± standard deviation and comparison performed with independent t test in Excel worksheet (Microsoft Co., USA). The p-value less than 0.05 was considered for statistical significant.

Results

Urinary volume in castrated and normal mice without any stress and after restrained stress had not any significant difference (respectively, p=0.229, p=0.132). After cold stress urinary volume in castrated mice was 0.3±0.83 which was statistically lower than normal mice 1.05±0.27 (p=0.001). Also after cold plus restrained stress urinary volume in castrated mice was 0.11±0.44 which was statistically lower than normal mice 0.86±0.71 (p=0.003). Comparison of urinary volume in testectomized mice with and without cold stress did not show any significant difference (respectively, p=0.801, p=0.894). Cold plus restrained stress made urinary volume in castrated mice higher than testectomized normal mice (p=0.021). Castrated mice had lower urinary volume than normal mice when receiving propanalol and testosterone ($p < 0.001$).

Discussion

Cold stress was shown to have a critical role in this study and restrained group did not have signifycant response. Originally, stress was considered to be a non-specific phenomenon, but it is now clear that different types of stressors elicit specific responses. For example, Kvetnansky and et al. and Fiedler showed that, immobilization stress elicits a robust increase in the plasma concentrations of both adrenaline and NA. By contrast, cold stress triggers an equally robust increase in plasma NA, but only small rise in adrenaline and insulin-induced hypoglycemia raises plasma adrenaline, but is less effective in raising NA (16, 17). This process is mediated by transcriptional mechanisms in the adrenal medulla and the locus coeruleus. The persistence of transcriptional activation depends on the duration and repetition of the stress (18) and recent studies suggest thyrotrophin-releasing hormone (TRH) plays an important part in mediating the body response to cold by serving both as a neurohormone

(19). The origin of neurohormonal TRH has been localized to the medial parvocellular and periventricular subdevisions of the middle zone of the paraventricular nucleus (PVN) (20, 21). Cells linking the PVN to sympathetic structures have been considered classically to be located in the parvocellular region of the mid-PVN. However, there is additional evidence suggesting that the magnocellular region of the mid-PVN is also involved in sympathetic control (22). As a result of extensive catecholamine release, stress reduces a cellular catecholamine level, which is subsequently compensated for by increased biosynthesis. For example, even a single episode of immobilization stress can lead to a ~ 30% decrease in levels of rat adrenaline, this remains low, but returns to basal levels after three weeks of repeated (daily) immobilization stress (23, 24). In the brain, acute stress causes NA to be released in the terminal fields of neurons that are localized in the locus coeruleus (LC), leading to a depletion of NA and increase in the concentrations of NA metabolites (11, 12). Our study on castrated mice showed that, there is an interaction between testosterone and adrenaline in, because urine volume was decreased only in testoctomized mice with cold/restraint and cold stress ($P<0.001$) and in restraint only mice the result was not significant. So it is shown that restraint stress alone can not affect; but together with cold stress can affect urinary volume. The role of testosterone, NA and AVP in the acute cold stress is confirmed, because testosterone could return the effect of decreased urine volume in normal case and propranolol as sympathetic antagonist in testoctomized mice with only cold stress blocked it ($P< 0.001$).

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