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Original Article

The Relation between Increasing Anxiety and Prolactin-Releasing Peptide in Rats

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Abstract

PrRP, also known as prolactoliberin, is a bovine hypothalamic extract neurohormone that stimulates prolactin synthesis in a rat pituitary adenoma cell line and lactating rat pituitary cells. PrRP has been shown to control the intake of food and energy expenditure, but it may also have a role in stress sensitivity, reproduction, cardia productivity, secretion of endocrine components, and lately, neuroprotective characteristics, among others. The current study was performed to identify if prolactin-releasing peptide (PrRP) had any effect in increasing anxiety clinical features in rats as an animal model. The study included 114 Wistar handling-acclimated male rats (160 gm, 2 months old); divided randomly into three major groups. The rats were divided randomly into three major groups (38-control animals (38C), and 38-PrRP animals (38P), both were examined using the EPM test to test for stress-related signs, such as fear of height (5 mins duration for each rat). The maze was cleaned with water to eliminate the previous rat odor after the experiment for each rat was completed. The tests were performed between 13:00 to 17:00 of the day. Then, a week later, 38 (19-PrRP animals (19P) and 19-control animals (19C)) were examined using the SP test conducted between 13:00 to 16:00 of the day. Fifteen minutes before EPM, the 38C received intranasal 0.9%-10µl NaCl (per nostril), and 38P received intranasal 10^{-10}mol/l-10 µl PrRP (per nostril), and the anxiety-related signs, such as time spent in open arms (less time means more anxious), during the EPM test were recorded. The 19P and 19C received 10^{-10}mol/l-10µl PrRP and 0.9%-10µl NaCl, respectively, (intranasal, per nostril, and 15 minutes before the SP test, where a stranger rat was placed in a specific cage in front of each of the 19P and 19C animals in a separate cage, in which both cages provided visual and olfactory but no confrontational contact). The results showed that PrRP significantly (P<0.05) decreased the time spent by the treated rats on the open arms. In addition, PrRP revealed significant (P<0.05) decreases in the time spent close to the stranger rat, which means increased anxiety levels. The current findings revealed that prolactin-releasing peptide increases anxiety and decreases sociality in the studied male rats.

Keywords: Anxiety, prolactin-releasing peptide, stress

1. Introduction

PrRP, also known as prolactoliberin, is a hypothalamic neurohormone obtained from a bovine hypothalamic extract and enhances prolactin production in a rat pituitary adenoma cell line and lactating rat pituitary cells. The Prl-RG injection also boosted plasma prolactin levels in male rats and activated concentrations in female rats throughout all stages of the menstrual cycle, including proestrus, estrus, and metestrus (1). The fact that the structural formation of the PrRP is highly consistent across a wide range of animal species suggests that this peptide plays a vital role in body organisms. PrRP has been shown to control the intake of food and energy expenditure, but it may also have a role in stress

sensitivity, reproduction, cardia productivity, secretion of endocrine components, and lately, neuroprotective characteristics, among others. Arrange of inputs, particularly those involving the periphery, control the creation of PrRP in the brain and affect its activity (2-4).

In addition to binding to the G-protein coupled receptor (GPR10) (5), PrRP also shows a lower affinity for the neuropeptide FF (NPFF) receptor type 2 (6). The role of other neuropeptides, including leptin (4), cholecystokinin (7), or neuropeptide Y (8, 9), in the actions of PrRP, is also critical to its success. Novel PrRP analogs with connected fatty acids and modifications in the chain of amino acids were synthesized in structure-activity relationship research to defeat the blood-brain barriers and enhance consistency and bioavailability from the peripheral circulation, thereby displaying attractive therapeutic candidates (10).

Anxiety disorders are the most frequent kind of mental illness and usually begin in a person's adolescence or early adulthood. Anxiety, worry, and perceived risk avoidance are the most common symptoms. Those who suffer from anxiety disorders have problems with brain systems that react to threats. Anxiety disorders are linked to genetic, environmental, and epigenetic variables (11). Anxiety disorders often coexist with other mental and physical problems, including depression and other psychiatric conditions. Generally, comorbidity is associated with more intense symptoms, a higher level of clinical load, and more difficulties in medication. Individuals may reduce the enormous burden of sickness from anxiety disorders by detecting the condition early, accurately administering appropriate therapy, and scaling up treatment as necessary. When treating depression and other mood disorders, patients may benefit from evidence-based psychotherapies like cognitive behavioral therapy and psychoactive drugs like serotonergic substances (12).

The current study was performed to identify if prolactin-releasing peptide (PrRP) increased anxiety clinical features in rats.

2. Materials and Methods

2.1. Animals

The study included 114 Wistar handling-acclimated male rats (160gm, 2 months old). The rats were housed under standard conditions with five rats per cage. Before the initiation of the experiment, the rats were exposed to a handling method for 2 weeks every day for 5 mins to avoid responding to the stress of being handled during the experiment. The rats were divided randomly into three major groups (38-control animals (38C), and 38-PrRP animals (38P), both were examined using the EPM test to test for stress-related signs, such as fear of height (5 mins duration for each rat). After the experiment ended for each rat, the maze was wiped with H_2O to get rid of the previous rat smell. The tests were performed between 13:00 to 17:00 of the day. Then, a week later, 38(19-PrRP animals (19P) and 19-control animals (19C)) were examined using the SP test conducted between 13:00 to 16:00 of the day.

2.2. Experiment

Fifteen minutes before EPM, the 38C received intranasal 0.9%-10µl NaCl (per nostril), and 38P received intranasal 10^{-10}mol/l-10µl PrRP (per nostril), and the anxiety-related signs, such as time spent in open arms (less time means more anxious), during the EPM test were recorded. The 19P and 19 Creceived 10^{-10}mol/l-10µlPrRPand0.9%-10µlNaCl, respectively, (intranasal, per nostril, and 15 minutes before the SP test, where a stranger rat was placed in a specific cage in front of each of the 19P and 19C animals in a separate cage, in which both cages provided visual and olfactory but no confrontational contact).

2.3. Statistical test

The data were processed-analyzed using *a* $P<0.05$-level-of-significance-based *t*-test. Mean±standard deviation (M±SD) was used to represent analyzed data.

3. Results and Discussion

The results showed that PrRP significantly ($P<0.05$) decreased the time spent by the treated rats on the open arms with little access to these open arms and fewer hanging times (Table 1). In addition, PrRP revealed

significant (P<0.05) decreases in the time spent close to the stranger rat, which means increased anxiety levels (Table 2).

Table 1. Behaviors of male Wistar rats during elevated plus maze test

Test features	Control NaCl (n=38)	Prolactoliberin 10^{-10} mol/l (n=38)
Seconds spent in open arms	50.7±6.9	31.2±5.1
Overhangs (counts)	5.1±1.1	2.1±0.3
Racks (counts)	7.1±1	5.8±1.1
Motor Activity, cm	300±21.2	320±28.3
Grooming Acts (counts)	3.1±0.5	4.1±0.4

Table 2. Behaviors of male Wistar rats during social preference test

	Seconds spent with "non-stranger"	Seconds spent with "strange" rat	Seconds spent on neutral areas
Control (n=19)	8.1±19.6	150.1±30.1	80.1±12.0
Prolactoliberin 10^{-10} mol/l (n=19)	112±20.7	79.3±31.3	98.6±17

PrRP at low concentrations causes an elevation in anxiety in rats but does not influence their locomotor activity, according to this research. This suggests that PrRP has anxiety-inducing instead of a sedative action. The impact of PrRP on social preference is believed to be directly linked to a shift in anxiety levels. If an animal has a high level of anxiety because of their genetic disposition, they prefer to be with a stranger rather than a known animal (13, 14). Surviving and paying attention to friends and human workers are the criteria for the new environment of the experimental rats that they focus on (15, 16). As a general rule, rats with high baseline anxiety prefer to stay close to their cagemates rather than go out into the surroundings. Because neophobia is less prominent in rats with minimal initial anxiety, they may engage in exploratory behavior toward an unknown animal. In such context, Ben-Ami Bartal, Rodgers (17) detected that rats released stranger rats from a trap similarly to their friendrats.

Many questions remain unsolved about PrRP-psychiatric effects, although it has been demonstrated that it may interact with central nervous system domains that govern emotions and stress response.GPR10isthereceptorthathasastrongaffinityforPrRP.GPR10mRNAisfound in the thalamus, hypothalamus, and postrema region of rat brain. It has been detected in the parabrachial nucleus, the nucleus accumbens, and the hippocampus, which is all engaged in different processes, such as the body's reaction to stress and the nociceptive system (18).

Electric pain stimulation activates the neurons in the median eminence that produce PrRP (19). Knockout mice of the PrRP gene exhibit higher blood glucose and corticosterone levels in response to immobility stress than control animals (20). Stress activates PrRP-nergic neural pathways in the nucleus of the solitary tract (21). McConn, Tachibana (22) reported that PrRP changed the level of food intake in their tested quails, which is linked to alterations in the hypothalamic corticotropin-releasing factor and the gene that encodes the neuropeptide Y receptor gene.

The current findings revealed that prolactin-releasing peptide increases anxiety and decreases sociality in the studied male rats.

Authors' Contribution

Study concept and design: K. G. G.
Acquisition of data: K. G. G.
Analysis and interpretation of data: M. S. K.
Drafting of the manuscript: B. H. A. A.
Critical revision of the manuscript for important intellectual content: K. G. G., M. S. K. and R. A. J.
Statistical analysis: M. S. K.
Administrative, technical, and material support: M. S. K.

Ethics

The study design was approved by the ethics committee of the University of AL-Qadisiyah, Al-Qadisiyah, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Dodd GT, Luckman SM. Physiological Roles of GPR10 and PrRP Signaling. Front Endocrinol (Lausanne). 2013;4:20.
2. Lin SH. Prolactin-releasing peptide. Results Probl Cell Differ. 2008;46:57-88.
3. Prazienkova V, Popelova A, Kunes J, Maletinska L. Prolactin-Releasing Peptide: Physiological and Pharmacological Properties. Int J Mol Sci. 2019;20(21).
4. Tachibana T, Sakamoto T. Functions of two distinct "prolactin-releasing peptides" evolved from a common ancestral gene. Front Endocrinol (Lausanne). 2014;5:170.
5. Davenport AP. Peptide and trace amine orphan receptors: prospects for new therapeutic targets. Curr Opin Pharmacol. 2003;3(2):127-34.
6. Buffel I, Meurs A, Portelli J, Raedt R, De Herdt V, Sioncke L, et al. Neuropeptide FF and prolactin-releasing peptide decrease cortical excitability through activation of NPFF receptors. Epilepsia. 2015;56(3):489-98.
7. Pirnik Z, Korinkova L, Osacka J, Zelezna B, Kunes J, Maletinska L. Cholecystokinin system is involved in the anorexigenic effect of peripherally applied palmitoylated prolactin-releasing peptide in fasted mice. Physiol Res. 2021;70(4):579-90.
8. Rettori V, Milenkovic L, Riedel M, McCann SM. The role of neuropeptide Y (NPY) in control of gonadotropin and prolactin release in the rat. Gynecol Endocrinol. 1990;4(3):169-79.
9. Wang G, Tachibana T, Gilbert ER, Cline MA. Exogenous prolactin-releasing peptide's orexigenic effect is associated with hypothalamic neuropeptide Y in chicks. Neuropeptides. 2015;54:79-83.
10. Karnosova A, Strnadova V, Hola L, Zelezna B, Kunes J, Maletinska L. Palmitoylation of Prolactin-Releasing Peptide Increased Affinity for and Activation of the GPR10, NPFF-R2 and NPFF-R1 Receptors: In Vitro Study. Int J Mol Sci. 2021;22(16).
11. Penninx BWJH, Pine DS, Holmes EA, Reif A. Anxiety disorders. Lancet. 2021;397(10277):914-27.
12. Vos T, Abajobir AA, Abate KH, Abbafati C, Abbas KM, Abd-Allah F, et al. Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet. 2017;390(10100):1211-59.
13. Robinson GE, Fernald RD, Clayton DF. Genes and social behavior. Science. 2008;322(5903):896-900.
14. Tan T, Wang W, Williams J, Ma K, Cao Q, Yan Z. Stress Exposure in Dopamine D4 Receptor Knockout Mice Induces Schizophrenia-Like Behaviors via Disruption of GABAergic Transmission. Schizophr Bull. 2019;45(5):1012-23.
15. Takano Y, Ukezono M. An experimental task to examine the mirror system in rats. Sci Rep. 2014;4:6652.
16. Turner KM, Peak J, Burne TH. Measuring Attention in Rodents: Comparison of a Modified Signal Detection Task and the 5-Choice Serial Reaction Time Task. Front Behav Neurosci. 2015;9:370.
17. Ben-Ami Bartal I, Rodgers DA, Bernardez Sarria MS, Decety J, Mason P. Pro-social behavior in rats is modulated by social experience. eLife. 2014;3:01385.
18. Davis XS, Grill HJ. The hindbrain is a site of energy balance action for prolactin-releasing peptide: feeding and thermic effects from GPR10 stimulation of the nucleus tractus solitarius/area postrema. Psychopharmacology (Berl). 2018;235(8):2287-301.
19. Morales T, Sawchenko PE. Brainstem prolactin-releasing peptide neurons are sensitive to stress and lactation. Neuroscience. 2003;121(3):771-8.
20. Mochiduki A, Takeda T, Kaga S, Inoue K. Stress response of prolactin-releasing peptide knockout mice as to glucocorticoid secretion. J Neuroendocrinol. 2010;22(6):576-84.
21. Card JP, Johnson AL, Llewellyn-Smith IJ, Zheng H, Anand R, Brierley DI, et al. GLP-1 neurons form a local synaptic circuit within the rodent nucleus of the solitary tract. J Comp Neurol. 2018;526(14):2149-64.
22. McConn BR, Tachibana T, Gilbert ER, Cline MA. Prolactin-releasing peptide increases food intake and affects hypothalamic physiology in Japanese quail (Coturnix japonica). Domest Anim Endocrinol. 2020;72:106464.