

LOCOMOTOR RESPONSES TO VASOACTIVE INTESTINAL PEPTIDE IN BULBECTOMIZED RATS

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ABSTRACT

PURPOSE: The present study aimed at studying the effects of vasoactive intestinal peptide (VIP) microinjected unilaterally into the hippocampal CA1 area on the locomotor activity of rats with olfactory bulbectomy. Hippocampus has been chosen as it is a region with high VIP receptor density as well as a brain structure implicated in many types of disorders, including depression.

MATERIAL AND METHODS: Olfactory bulbectomy (OBX) rat is among the well-validated animal models of depression. The changes in locomotor activity of OBX rats were registered in an Opto Varimex apparatus.

RESULTS: VIP injected into the left CA1 area at doses of 10 ng and 100 ng aggravated OBX rat hyperactivity by increasing the locomotor activity. VIP (in a dose of 100 ng) microinjected into the right CA1 area produced an opposite effect by decreasing the number of both horizontal and vertical movements in OBX rats as compared to saline-treated OBX controls.

CONCLUSION: Our data reveal a pronounced lateralized VIP effect on the locomotor activity of OBX rats and point to a possible VIP involvement in the mechanisms of the olfactory bulbectomy syndrome in rats.

Key words: *vasoactive intestinal polypeptide, olfactory bulbectomy, depression, hippocampus, locomotor activity, rats*

INTRODUCTION

Vasoactive intestinal peptide (VIP) is a neuropeptide which is widely distributed in the central and peripheral nervous systems (24). Studies have provided data that VIP can act as a neurotransmitter, neuromodulator, neurotrophic and neuroprotective factor thus affecting the neural activity and the functioning of different nervous circuits (10,23). VIP

has been highlighted as a key signaling pathway in the suprachiasmatic nuclei of the hypothalamus, which are considered as the master clock controlling daily rhythms in mammals (1).

VIP and its receptors are expressed in many brain regions including cortex, hippocampus, amygdala and hypothalamus (17). VIP has been implicated in a variety of biological activities including hormonal regulation, immunomodulation, regulation of feeding behaviour and modulation of learned behaviours. It has been suggested to participate in the pathophysiology of neurological and psychiatric disorders such as Alzheimer's disease and depression. The publications about VIP lowered levels in the cerebrospinal fluid of patients with depression suggest its possible role in the neurobiological mechanisms of affective disorders (9,20).

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The olfactory bulbectomy (OBX) is an animal model of depression that produces a constellation of behavioural, physiological, neurochemical and neuroendocrine changes of great relevance to clinical depression. The bilateral removal of olfactory bulbs causes a major dysfunction of the cortical-hippocampal-amygdala circuit that underlies the behavioural changes (15,26). OBX rats typically exhibit hyperactivity in an open field test.

There are data about VIP involvement in the locomotor activity of rodents, however, its role in the hyperactivity exhibited by OBX rats has not been examined yet. We have previously demonstrated a modulatory effect of VIP (in a dose of 10 ng) microinjected bilaterally into hippocampal CA1 area on the exploratory behaviour of OBX rats (14).

The objective of the present study was to investigate the effects of VIP microinjected unilaterally into CA1 area on the locomotor activity of OBX rats.

MATERIAL AND METHODS

Experimental model of depression in OBX

Bilateral OBX was performed according to the method described by Kelly *et al.* (15). Male Wistar rats were anaesthetized with Calypsol (in a dose of 50 mg/kg i.p.) and placed in a stereotaxic apparatus (Stoelting Co., USA). Surgical procedure involved drilling two burr holes 2 mm in diameter at the points 8 mm anterior to bregma and 2 mm from the midline on its both sides as coordinates were estimated according to the stereotaxic atlas of Pellegrino and Cushman (19). The bulbs were aspirated with a stainless needle attached to a water pump. After the surgery, the rats were housed in groups of two and were handled and weighed daily during a 15-day period.

Stereotaxic implantation and drug injection into hippocampal CA1 area of OBX rats

After the anaesthesia, OBX rats were placed in the stereotaxic apparatus and guide cannulae (right and left) were implanted into CA1 hippocampal area according to the coordinates of the stereotaxic atlas (10). After surgery, the animals were allowed 7 days to recover prior to the behavioural tests. During the recovery period they were handled daily.

The rats were microinjected into left or right hippocampal CA1 areas with VIP (in a dose of 10

ng and 100 ng) or with saline (1 μ L). VIP (Sigma) was dissolved *ex tempore* in saline and 1 μ L of VIP solution (pH 7.4) was infused 5 min before the test for locomotor activity. Immediately prior to sacrifice, the rats were injected with 1 μ L of 2% fast green dye through the injection cannula. Brains were removed, and bulbectomy was verified macroscopically. Injection sites were verified anatomically in 25 mm coronal brain sections cut through the hippocampus.

Test for locomotor activity

Locomotor activity was recorded in an Opto Varimex apparatus (Columbus Instruments, USA). The apparatus recorded the number of photo beam interruptions during animal movements in the experimental chamber. It provided selective counting of the number of horizontal and vertical movements in arbitrary units (AU). The rats were placed in the central quadrant of the activity monitor 5 min after VIP microinjection. The information was recorded automatically for a 30-min period of observation.

Statistical analysis

Data were processed by analysis of variance (ANOVA). Two-way ANOVA was used to process the data obtained for the total number of movements of VIP-treated OBX rats for a 30-min period. Where appropriate, ANOVA data were further analyzed by the *t*-test for post hoc group comparisons.

RESULTS

Repeated two-way ANOVA analysis on the effect of unilateral VIP microinjection on the number of horizontal movements in OBX rats for a 30-min period of observation showed a significant effect of the factor 'side' ($F_{1,47}=87,058$; $p\leq 0,001$) and a significant interaction between the factors 'drug' x 'side' ($F_{2,47}=40,667$; $p\leq 0,001$).

VIP administered into the left CA1 area of OBX rats increased the total number of horizontal movements in both doses: for VIP (10 ng) ($t=2,80$; $p\leq 0,05$) and for VIP (100 ng) ($t=3,56$; $p\leq 0,1$), respectively, while VIP microinjection (of 100 ng) into the right CA1 area significantly decreased the total number of horizontal movements ($t=2,95$; $p\leq 0,05$) as compared to the saline-treated OBX controls (Fig. 1). VIP injected into the left CA1 area produced a greater total number of horizontal movements in both doses: for VIP (10 ng) ($t=7,89$; $p\leq 0,001$) and for VIP (100

ng) ($t=10,74$; $p\leq 0,001$) as compared to the right-side microinjections (Fig. 1).

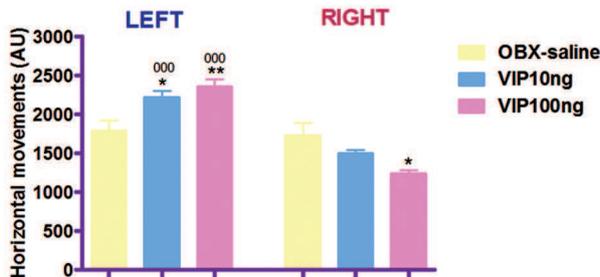


Fig. 1. VIP effects (10 ng and 100 ng) after unilateral injection into the hippocampal CA1 area of OBX rats on the total number of horizontal movements for a 30-min period of observation. AU - arbitrary units, * $p\leq 0,05$; ** $p\leq 0,01$ - comparison to the respective saline-injected OBX controls; *** $p\leq 0,001$ - comparison between right-side and left-side treated animals. $n=8$. Means (\pm S.E.M.) are presented

Two-way ANOVA analysis of VIP effect (in a dose of 10 and 100 ng) on the number of vertical movements of OBX rats demonstrated a significant effect of the factor 'drug' ($F_{2,47}=12,161$; $p\leq 0,001$), the factor 'side' ($F_{1,47}=159,614$; $p\leq 0,001$) and an interaction between both factors 'side' x 'drug' ($F_{2,47}=32,538$; $p\leq 0,001$).

The number of vertical movements was significantly increased following VIP injection into the left CA1 area: for VIP (10 ng) ($t=8,80$; $p\leq 0,001$) and for VIP (100 ng) ($t=6,14$; $p\leq 0,001$), respectively, while VIP

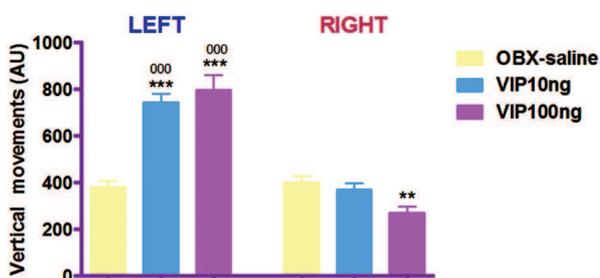


Fig. 2. VIP effects (100 ng) after unilateral injection into the hippocampal CA1 area of OBX rats on the total number of vertical movements for a 30-min period of observation. AU - arbitrary units, ** $p\leq 0,01$; *** $p\leq 0,001$ - comparison to the respective saline-injected OBX controls. *** $p\leq 0,001$ - comparison between right-side and left-side treated animals. $n=8$. Means (\pm S.E.M.) are presented

injection at a dose of 100 ng significantly decreased the number of vertical movements ($t=3,61$; $p\leq 0,01$) as compared to the saline-treated OBX rats. Both doses of VIP increased the number of vertical movements in OBX rats following microinjection into the left CA1 area as compared to the right CA1: for VIP (10 ng) ($t=8,70$; $p\leq 0,001$) and for VIP (of 100 ng) ($t=7,70$; $p\leq 0,001$) (Fig. 2).

DISCUSSION

The present study showed a pronounced lateralized effect of VIP on the locomotor activity of rats with a model of depression. Unilateral VIP microinjection (in a dose of 10 ng and 100 ng) into the left hippocampal CA1 area enhanced OBX rat locomotor activity expressed as an increased number of both horizontal and vertical movements, while following injection into the right CA1 area, the higher VIP dose (100 ng) exerted an opposite, inhibitory effect expressed as a decreased number of movements when compared to the saline-treated OBX controls.

The hyperactivity in an open field is a typical behavioural phenomenon in OBX rats. It is considered an index of depressive-like behaviour in this animal model (15). In this respect, VIP locomotor stimulatory effect after administration into the left CA1 area could be considered a sign of aggravated depression-like state.

Although behavioural VIP effects have been investigated, its involvement in the mechanisms of the depressive disorders has not been studied yet. Recent data about the behavioural responses to VIP after administration in the brain show different, sometimes opposite effects, which may be also dependent on the initial state of the experimental animal. Stimulatory VIP effects on rodent locomotor activity after i. c. v. administration were reported (12,16) while VIP injection after brain injury (lobectomy) showed a regulatory influence and restored the impaired locomotion (21). An i.c.v. VIP injection reduced food intake and increased both activity and energy expenditure in rats. Thus its endogenous role in the hypothalamic control of energy homeostasis was suggested (8).

A correlation between locomotion and hippocampal VIP was reported, where rat locomotor activity was associated with an increased VIP

expression in hippocampal rat neurons along with an increased VIP mRNA in the hippocampus and motor cortex (7).

We have previously demonstrated a lateralized VIP effect on the exploratory activity of rats (number of movements in a 5-min period of observation) after administration into hippocampal CA1 area. VIP injection (50 ng and 100 ng) into the right CA1 area stimulated the activity, while that of 100 ng into the left CA1 decreased the number of movements (13). Interestingly, in the present study, the locomotor stimulatory effect was exhibited after VIP injection into the left CA1 area in OBX rats. It is difficult to explain both mechanisms and the change of the lateralized locomotor effect in the OBX rats. It could be speculated that VIP modulatory effects on the neuronal excitability and on the release of neurotransmitters in the hippocampus (3,4,18) might be involved in the behavioural effect observed in the present investigation.

Left-right brain asymmetry (laterality), once believed to be a human characteristics, has now been found to be widespread among vertebrates (11). There is a lot of data concerning the functional, anatomical and neurochemical asymmetries in the brain of non-human primates and other animals including rats (5,6,22). There is evidence that rat asymmetric behavioural responses are, probably, due to asymmetry in some pathways or neurotransmitter systems. Recently, a morphological left-right asymmetry at the synaptic level in the hippocampus has been reported (25).

Changes in the expression of neuropeptides in some brain areas have been established following bulbectomy (26). It could be hypothesized that an altered hemispheric asymmetry in the number of VIP interneurons, the density of VIP receptors in the hippocampus, or asymmetry in the neurotransmitter systems with which VIP interacts might account for the lateralized behavioural responses of OBX rats. The neurodegenerative changes in the hippocampus after bulbectomy (26) and the compensatory reorganization of neuronal circuits could contribute to the lateralized VIP effects on locomotion of OBX rats, too. In this respect, our results are in accordance with the interhemispheric differences in OBX rats reported elsewhere (2).

CONCLUSION

The present study reveals a pronounced lateralized VIP effect following unilateral microinjection into the hippocampal CA1 area of rats with OBX model of depression. The asymmetry in the behavioural responses to VIP suggests a possible involvement of VIP in the mechanisms of the olfactory bulbectomy syndrome in rats.

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