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Modulatory effect of VIP injected into hippocampal CA1 area on anxiety in olfactory bulbectomized rats

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Vasoactive intestinal peptide (VIP) is a neuropeptide, which is widely distributed in the central nervous system and peripheral tissues, acting both as a neurotransmitter and neuromodulator. Despite its extensive expression in the hippocampus, amygdala and other limbic system structures, the effects of VIP on anxiety and depression have not yet been fully investigated. The aim of the present study was to evaluate the involvement of VIP and VIP receptors in the mechanism of anxiety in rats with a model of depression (bilateral olfactory bulbectomy), using the elevated plus-maze test. VIP and a non-specific antagonist of VIP receptors (VIP_{6-28}) were administered unilaterally into the hippocampal CA1 area of bulbectomized (OBX) rats. VIP (10 ng) showed a tendency for an anxiety-modulatory effect upon right side injection, by reducing significantly the closed arm time and increasing the open arm time. VIP (100 ng) injected unilaterally (left or right) into CA1 area induced an anxiolytic-like effect on the activity of OBX rats (increased the number of open arms entries, open arm time and the ratio open/total number of entries).VIP_{6-28} failed to antagonize the anxiety-related behavior of OBX rats in the plus maze. An unexpected finding in our study was that upon pretreatment with VIP_{6-28}, VIP (10 ng), injected unilaterally (left or right) exerted an anti-anxiety like effect (increased the number of open arm entries, open arm time and the ratio open/total number of entries). Our data point to a possible involvement of hippocampal VIP-ergic neurons in modulating emotional processes or adaptive responses to stressful stimuli in a rat model of depression.

Key words: vasoactive intestinal peptide, olfactory bulbectomy, hippocampus, plus maze, anxiety, rat

INTRODUCTION

The vasoactive intestinal polypeptide (VIP) is a 28-amino acid peptide, which is widely distributed in both the central and peripheral nervous systems (Gozes and Brenneman 1983). VIP is extensively expressed throughout brain regions involved in behavioural reactions, such as the cerebral cortex, amygdala, striatum, hypothalamus and hippocampus (De Souza et al. 1985). Although VIP was initially classified as a gut hormone, over the past two decades experimental studies provide evidence that it also acts as a neurotrasmitter and neuromodulator in both central and peripheral nervous systems.VIP-containing interneurons have been identified in the hippocampus, where the neuropeptide modulates the excitability of CA1 pyramidal neurons (Haas and Gahwiler 1992, Acsady et al. 1996a,b). VIP and pituitary adenylate cyclase-activating polypeptide (PACAP) are two members of a structurally related family of peptides. The physiological actions of VIP and PACAP are produced through activation of three G-protein coupled receptors: VPAC1, VPAC2 and PAC1. VPAC1 and VPAC2 bind VIP and PACAP with similar affinity whereas PAC1 binds PACAP with high affinity and VIP with much lower affinity. All three types of receptors are expressed in different brain areas, including the hippocampus (Harmar et al. 2012).

The neuropeptides have been intensively studied in the past decade for their implication in the pathogenesis of neurological and psychiatric disorders. Recent findings identify neuropeptide systems as potential

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novel therapeutic targets for the treatment of depression and anxiety disorders (Kormos and Gaszner 2013). VIP has been suggested to participate in the pathophysiology of Alzheimer's disease (Jolkkonen et al. 1991), Parkinson's disease (Staines 2007), autism disorder (Nelson et al. 2006), depression (Gjerris et al. 1984) and anxiety (Saiz Ruiz et al. 1992). Contrary to the extensive research done regarding the role of VIP in neurodegenerative neurological disorders, data are scarce about the involvement of VIP in the mechanisms of anxiety and depression.

Olfactory bulbectomy (OBX) is a widely used animal model of depression. Bilateral removal of the olfactory bulbs in rats causes major dysfunction of the cortical-hippocampal-amygdala circuits and produces a complex constellation of behavioral, neurochemical, neuroendocrine, and neuroimmune alterations, many of which reflect symptoms reported in patients with major depression. Behavioral abnormalities of OBX rats include exploratory hyperactivity in response to a novel environmental stress, memory deficits, anxiety symptom-resembling behaviour, etc. (Song and Leonard 2005, Wang et al. 2007).

As a component of the limbic system the hippocampus is involved in the processes of learning and memory, in the control of emotions and in the response to stress (Eichenbaum 2004, Engin and Treit 2007). Accumulating evidence indicates that neuropeptides such as VIP, neuropeptide Y (NPY) and galanin play important role in the control of hippocampal neurogenesis and may be involved in the modulation of hippocampal-dependent memory and anxiety related behavior (Zaben and Gray 2013). The accumulation of numerous data supporting the neuromodulatory role of VIP in different brain structures necessitates additional research to explore the mechanism underlying the behavioral effects of VIP.

Previously, our laboratory has investigated the anxiety-modulating effects of neuropeptides in the hippocampal CA1 area. Uni- or bilateral administration of VIP induced an anxiogenic-like effect in rats tested on EPM test, while the angiotensin II injected bilaterally showed an anxiolytic-like effect (Belcheva et al. 1997, Ivanova et al. 2010). Using the OBX model of depression, a different behavioral effects of VIP microinjected into the CA1 area have been reported. VIP injected into the left CA1 produced an anti-nociceptive effect; in the right CA1 VIP showed a tendency to improve the memory deficits, induced by the bulbectomy; while the bilateral injection showed anxiolytic-like effect (Belcheva et al. 2009, Ivanova et al. 2011, 2012). Based on the above mentioned findings, the aim of our study is to evaluate the involvement of VIP, and VIP receptors in the mechanism of anxiety in rats with OBX model of depression.

METHODS

Animals

Adult male Wistar rats (2 month old at the time of surgery) were used for the experiments. The animals were housed individually in polypropylene boxes with free access to food and water. The animals were maintained in a constant temperature environment (22±2°C) on a 12 h light/dark cycle (lights on at 06:00 AM). The behavior experiments were performed between 10:00 AM and 01:00 PM. The experiments were carried out according to the "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985), and the rules of the Ethics Committee of the Institute of Neurobiology, Bulgarian Academy of Sciences (registration FWA 00003059 by the US Department of Health and Human Services).

Surgical procedures

Experimental model of depression – bilateral olfactory bulbectomy (OBX)

Bilateral OBX was performed according to the method described by Kelly and coauthors (1997). Animals were anesthetized with Calypsol (50 mg/kg ip). The top of the skull was shaved and swabbed with an antiseptic, after which the animals were placed in a stereotaxic apparatus Stoelting, USA) and a midline frontal incision was made in the scalp, with the skin being retracted bilaterally. The 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 it's both sides (coordinates of olfactory bulbs were detected according to the stereotaxic atlas of Pellegrino and Cushman (1967). The bulbs were aspirated with a stainless needle attached to a water pump. The cavity was packed with Gelaspon used as a haemostatic. The area was treated with Nemibacin and the skin was sewed with stitches. After the surgery the rats were

housed in groups of two and were handled and weighed daily during a 15 day period. The sham operation was performed in the same way as in the case of olfactory bulbectomy without the removal of the olfactory bulbs.

Stereotaxic implantation and drug microinjection into the hippocampal CA1 area

Seven days post bilateral olfactory bulbectomy a surgical implantation of cannulae was conducted. After anesthesia (Calypsol 50 mg/kg ip) the OBX rats were placed in a stereotaxic apparatus (Stoelting, USA) and guide cannulae (right and left) were implanted into left and right CA1 hippocampal areas according to stereotaxic coordinates from Pellegrino and Cushman atlas (Pellegrino and Cushman 1967) (P=4.3 mm; L=±2.0 mm; h=−3.0 mm). The cannulas were fixed on the surface of the skull with dental cement. After surgery the animals were allowed 7 days to recover before the behavioral studies. During the recovery period the rats were handled daily.

VIP (Sigma) or VIP_{6-28} (Tocris) were dissolved *ex tempore* in saline. One µl of VIP (pH 7.4) or 1 µl VIP_{6-28} solution (pH 7.4) were infused unilaterally through an injection cannula connected by polyethylene tubing with a constant rate microsyringe (Hamilton, Reno, NV, USA) over a period of 1 min into the hippocampal CA1 area,15 min before the plus maze test. The injection cannula was left in place for additional 30 s. The combination (VIP_{6-28}+VIP) was applied by separate microinjections with a 10-minute lag (i.e., VIP was microinjected into the same side ten minutes after the VIP_{6-28} injection). Five minutes later the rats were placed into the plus maze.

The animals were divided in three independent groups: 1st group rats with OBX (n=10) and sham operated rats (n=10). Other OBX rats with bilaterally implanted cannulas were divided in two groups: 2nd group (n=8) microinjected unilaterally (left or right) into hippocampal CA1 areas every third day with randomly selected VIP (10 and 100 ng) or saline. In this manner each rat received 6 injections. Drug doses were individually randomized for each rat; 3rd group (n=8) microinjected into the left or right CA1 areas every third day with randomly selected VIP_{6-28} (10 ng), combination (VIP_{6-28} +VIP) or saline. Thus, each rat received 6 injections. All animals were placed in individual holding cages until testing.

Immediately prior to scarification the animals were injected with 1 µl 2% fast green dye through the injection cannula. Brains were removed, and successful bulbectomy was verified macroscopically by comparison with the bulbs of an intact rat brain. Animals in which <80% of the bulbs had been removed were omitted from the analysis. Injection sites were then verified histologically *post-mortem* in 25 µm coronal brain sections cut through the hippocampus. Animals with cannulae placement outside the CA1 area or with cannulae placement that was not symmetrical were excluded from the statistical analysis. Thus, the histological verification of the surgical procedures resulted in exclusion of 6 out of 42 rats.

Elevated plus-maze test

Elevated plus-maze (EPM) test was performed according to the method described by Pellow and colleagues (1985). The apparatus was made of wood and consisted of four arms, painted in black – two open arms (50×10 cm) and two closed arms (50×10×40 cm), with an open roof. The apparatus was elevated 50 cm above the floor. Two open arms were opposite to each other and were illuminated by a 40 W bulb positioned 50 cm above the apparatus. Experiments were performed between 10:00 AM and 01:00 PM. Each session lasted 5 min. Behavioral analysis was performed by a trained experimenter who was blind to treatment condition. Each rat was placed in the centre of the plus-maze facing one of the open arms. After each trial, the maze was thoroughly cleaned. A number of parameters were collected: (1) Number of open arm entries (the number of rat entries with all four paws into the open, unprotected arms); (2) Open arm time – the total amount of time the rat spent in the open arms; (3) Number of closed arm entries; (4) Closed arm time; (5) Ratio of open to total arm entries. The number of open arm entries and the open/total number ratio have been suggested as most valid measures of anxiety-like behavior in the EPM test (Pellow et al. 1985). Effects of exploration and locomotion were loaded on the number of closed arm entries. Accordingly, a sedative effect and reduced locomotion should be suspected by a reduction in the number of closed arm entries, whereas an increased number of closed arm entries reflect an increased locomotor activity.

Statistical analysis

Data were processed by analysis of variance (ANOVA). One-way ANOVA was used to analyze the data obtained for olfactory bulbectomy. Separate two-factor ANOVA analysis was used for evaluation of data about unilateral injections of the drugs. For VIP factors were "side" of injection (right or left) and "dose" (3 levels: VIP 10 ng, VIP 100 ng and saline). For VIP_{6-28} (10 ng) and the combination VIP_{6-28}+VIP the factors were "side" of injection and "drug" (3 levels: VIP_{6-28}; combination VIP_{6-28}+VIP and saline). ANOVA data were further analyzed by *post-hoc* Student-Newman-Keuls (SNK) test, where appropriate.

RESULTS

One-way ANOVA analysis of the plus-maze behavior of OBX rats (without implanted cannulas) demonstrated a significant effect for the factor "bulbectomy" for the open arm entries ($F_{1,19}$=13.186; $P\leq0.01$), the time spent in the open arms ($F_{1,19}$=62.031; $P\leq0.001$);the ratio open/total entries($F_{1,19}$=29.376; $P\leq0.001$)**;** the closed arm entries ($F_{1,19}$=26.973; $P\leq0.001$), and the time spent in the closed arms ($F_{1,19}$=62.031; $P\leq0.001$); *Post-hoc* SNK comparisons demonstrated a decrease of the open arm entries ($P\leq0.01$), time spent there ($P\leq0.001$), ratio open/total entries ($P\leq0.001$) and an increase of closed arm entries ($P\leq0.01$) and closed arm time ($P\leq0.001$) as compared to the sham operated controls (Figs 1, 2, Table I).

ANOVA analysis of the elevated plus-maze data revealed a significant effect of VIP dose (10 and 100 ng) for the number of open arm entries ($F_{2,47}$=11.975; $P\leq0.001$), open arm time ($F_{2,47}$=16.9; $P\leq0.001$), the ratio open/total entries ($F_{2,47}$=7.387; $P\leq0.01$), the number of closed arm entries ($F_{2,47}$=11.961; $P\leq0.001$) and closed arm time ($F_{2,47}$=16.9; $P\leq0.001$). *Post-hoc* comparisons showed that the microinjection of VIP (10 ng) into the right CA1 area increased significantly the time spent in the open arms ($P\leq0.05$) and decreased the time spent in the closed arms ($P\leq0.05$) (Table I). The microinjection of VIP (100 ng) into the left CA1 area increased significantly the number of open arm entries ($P\leq0.001$), open arm time ($P\leq0.001$) the ratio open/total entries ($P\leq0.01$) and decreased closed arm time ($P\leq0.001$) as compared to the respective saline-injected OBX controls (Figs 1, 2, Table I). VIP (100 ng) injected into the right CA1 area increased the number of open arm entries ($P\leq0.05$), open arm time ($P\leq0.01$), the ratio open/total entries ($P\leq0.05$) and decreased closed arm time ($P\leq0.01$) as compared to the controls. There was no significant change in the number of closed arm entries upon inhjection of VIP (100 ng) (Figs 1, 2, Table I).

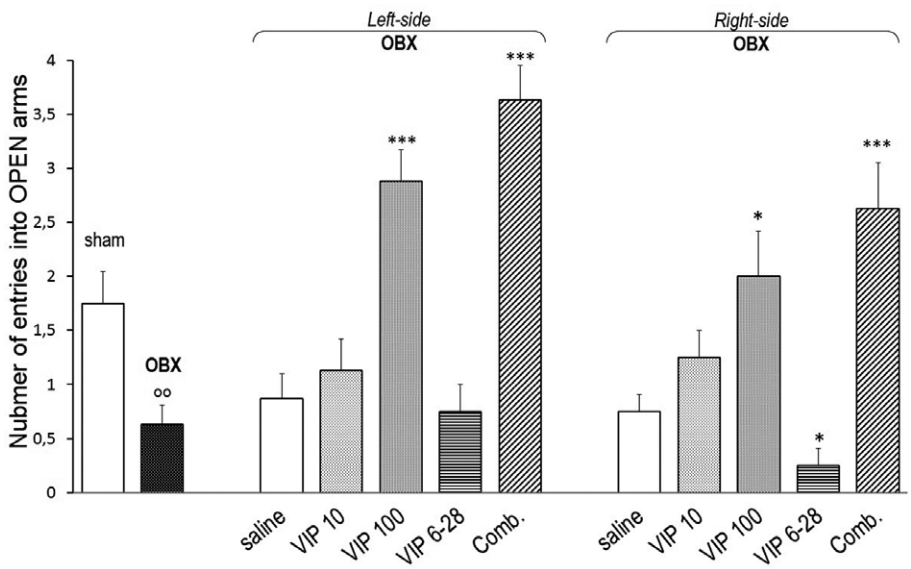

Fig. 1. Effects of VIP (10 ng and 100 ng), VIP_{6-28} (10 ng) and combination /VIP_{6-28} (10 ng) + VIP (10 ng)/ microinjected unilaterally (left side or right side) into hippocampal CA1 area on the number of open arm entries of OBX rats in a plus maze test. °°$P\leq0.01$; °°°$P\leq0.001$(circles depict OBX vs. sham-operated controls, n=10). *$P\leq0.05$; **$P\leq0.01$; ***$P\leq0.001$ (asterisks depict VIP, VIP_{6-28} or combination vs. saline-treated controls (n=8). Mean ± SEM are presented.

ANOVA analysis of the data after microinjection ofVIP_{6-28}or the combination (VIP_{6-28}+VIP) showed a significant effect for "drug" factor for the number of open arm entries ($F_{2,47}$=54.448; $P\leq0.001$), the time spent in the open arms ($F_{2,47}$=20.106; $P\leq0.001$), the ratio open/total entries ($F_{2,47}$=41.616; $P\leq0.001$), and for the number of closed arm entries ($F_{2,47}$=18.568; $P\leq0.001$). A significant effect for the "side" factor was found regarding the number of open arms entries ($F_{1,47}$=5.945; $P\leq0.05$) and the number of closed arms entries ($F_{1,47}$=6.018; $P\leq0.05$).

Post-hoc comparisons revealed that microinjection of VIP_{6-28} into the left CA1 significantly reduced the number of closed arm entries only ($P\leq0.01$), while the administration into the right CA1 area significantly decreased both the number of open armentries ($P\leq0.05$) and closed arm entries ($P\leq0.001$) as compared to the saline-treated OBX controls. All the other tested parameters did not show a significant change (Fig. 1, Table I).

The combination (VIP_{6-28}+VIP), administered into the left CA1 area increased significantly the number of open arm entries ($P\leq0.001$), the time spent in the open arms ($P\leq0.01$), the ratio open/total entries ($P\leq0.001$) and decreased closed arm time ($P\leq0.01$) as compared to the respective controls (Figs 1, 2, Table I). The right-side microinjected OBX rats displayed a significant increase in the open arm entries ($P\leq0.001$), time spent in the open arms ($P\leq0.05$), the ratio open/total entries ($P\leq0.001$), together with a significant reductions in the closed arm entries ($P\leq0.01$) and closed arm time ($P\leq0.05$) as compared to the controls (Figs 1, 2, Table I).

The comparison between left-side and right-side effects of the treatment with VIP, VIP_{6-28} or the combination (VIP_{6-28}+VIP) did not show any significant difference in all tested parameters with an exception of the number of closed arm entries, which were significantly decreased upon right side injection of the combination VIP_{6-28} +VIP ($P\leq0.05$).

DISCUSSION

The hippocampus is an important site in modulation of anxiety- and mood-related behaviors. It is also a brain structure with high concentration of VIP and VIP receptors (see Introduction). We have investigated the effects of VIP and VPAC receptor antagonist VIP_{6-28} microinjected into hippocampal CA1 area on the performance of bulbectomized rats in the EPM test. OBX rats made fewer open arm entries and spent less time in the open arms as compared to the sham operated controls. Both of these measures are indications of elevated anxiety in this animal model. Our results are consistent with the reported by Saitoh and others (2007, 2008) hyper emotionality and anxiety-like behavior of OBX rats.

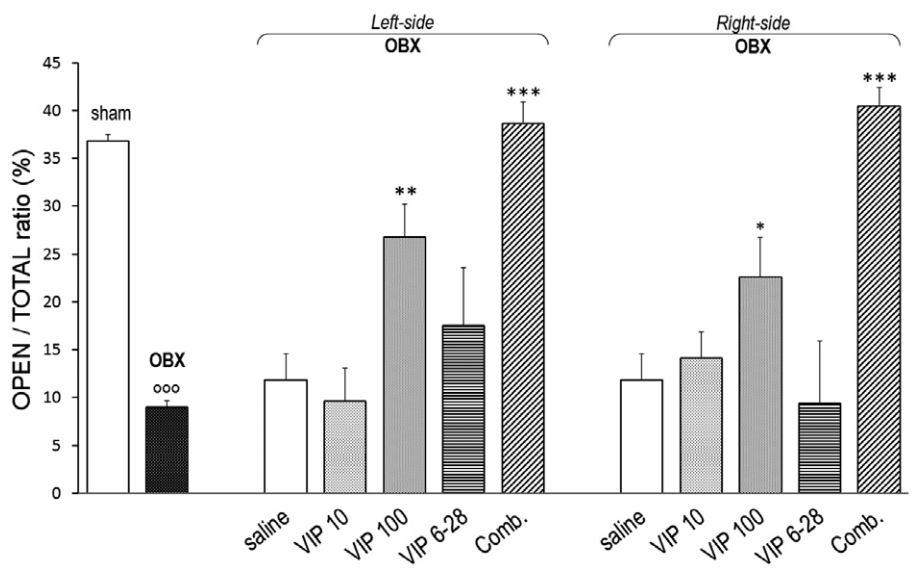

Fig. 2. Effects of VIP (10 ng and 100 ng), VIP_{6-28} (10 ng) and combination /VIP_{6-28} (10 ng) + VIP (10 ng)/ microinjected unilaterally (left side or right side) into hippocampal CA1 area on the ratio open/total arm entries of OBX rats in a plus maze test. °°$P\leq0.01$; °°°$P\leq0.001$(circles depict OBX vs. sham-operated controls, n=10). *$P\leq0.05$; **$P\leq0.01$; ***$P\leq0.001$ (asterisks depict VIP, VIP_{6-28} or combination vs. saline-treated controls (n=8). Mean ± SEM are presented.

Table I

Effect of VIP (10 and 100 ng), VIP_{6-28} (10 ng) and combination /VIP_{6-28} (10 ng) + VIP (10 ng) microinjected unilaterally into the left and right hippocampal CA1 area on the behavior of plus maze tested rats

SITE	Group	ENTRIES INTO CLOSED ARMS (± SEM)		ENTRIES INTO OPEN ARMS (± SEM)
		Number	Time	Time
	Sham Operated	2.75±1.16	285.0±3.12	15.0±3.12
	OBX	7.0±2.0^{oo}	297.0±2.98^{ooo}	3.0±2.98^{ooo}
LEFT	OBX-saline	6.5±0.68	296.87±1.11	3.13±1.11
	VIP (10 ng)	7.37±0.57	293.13±1.79	6.87±1.79
	VIP (100 ng)	8.25±0.7	286.25±1.24***	13.75±1.23***
	VIP_{6-28}	3.5±0.32**	295.25±1.72	4.75±1.72
	VIP_{6-28} +VIP	5.88±0.55	278.37±5.09**	21.63±5.09**
RIGHT	OBX-saline	6.37±0.59	297.25±0.77	2.75±0.77
	VIP (10 ng)	7.25±0.62	294.13±10.79*	5.88±1.07*
	VIP (100 ng)	6.87±0.74	290.13±1.76**	9.87±1.76**
	VIP_{6-28}	2.63±0.32***	298.0±0.5	2.0±1.36
	VIP_{6-28} +VIP	3.88±0.58**	281.63±5.84*	18.37±5.84*

$^{oo}P\leq0.01$; $^{ooo}P\leq0.001$ (sham operated vs. OBX rats, n=10). * $P\leq0.05$; ** $P\leq0.01$; *** $P\leq0.001$ (drug treated vs. respective saline-treated OBX controls, n=8).

VIP (100 ng) microinjected unilaterally into the left or right hippocampal CA1 area of OBX rats increased significantly the number and time of open arm entries, the ratio open/total entries and reduced the time spent in the closed arms in comparison to the saline-treated OBX controls.This effect of VIP to selectively increase the percentage of entries into the open arms without affecting closed arm entries is indicative of anxiolytic-like activity. Furthermore, VIP not only attenuated the anxiogenic-like behavior of OBX rats, but reversed the behavioral scores to the values of the sham operated controls. The lower dose of VIP (10 ng) showed a tendency for an anxiety-modulatory effect upon right side administration only, by reducing significantly the closed arm time and increasing the open arm time.

Diverse mechanisms appear to be involved in the regulation of anxious states. Dysfunction of γ-aminobutyric acid (GABA) and serotonin systems has been generally related to anxiety disorders (Sandford et al. 2000). In addition to the GABAergic neurotransmission (a key modifier of anxiety), and conventional neurotransmitters such as monoamines, glutamate and acetylcholine, many other modulators including neuropeptides have been implicated in the control of anxious states. Intrahippocampal administration of drugs modulating serotoninergic, glutamatergic and cholinergic systems has been shown to affect the anxiety triggered by the EPM test (Padovan et al. 2000, Degroot et al. 2001, Alves et al. 2004).

VIP regulates neuronal excitability and synaptic efficiency in the hippocampus (Haas and Gahwiler 1992, Ciranna and Cavallaro 2003) where it exerts a modulatory effect on neurotransmitter systems such as GABA, 5-HT, acetylcholine and glutamate (Rostene et al. 1983, Masuo et al. 1993, Cunha-Reis et al. 2004). VIP modulates GABAergic transmission to hippocampal interneurons, thus increasing synaptic transmission to CA1 pyramidal cells (Cunha-Reis et al. 2004). In addition, VIP has been shown to regulate NMDA receptors on CA1 pyramidal neurons (Yang et al. 2009).

The present study aims at examining the role of hippocampal VIP in the modulation of anxiety-related behavior of bulbectomized rats. The bilateral olfactory bulbectomy model is a well validated animal model of depression. Abnormalities in many brain neurotransmitter systems have been reported post bulbectomy such as reduction of the catecholamine, acetylcholine and glutamate levels in the brain, reduced serotonin synthesis, reduced density of the N-methyl-d-aspartate (NMDA) sites, etc. (Song and Leonard 2005). Taking into consideration the above mentioned data, it is likely that the interaction of VIP with different neurotransmitter systems in the hippocampus could be a factor, contributing to the restoration of the neurotransmitter balance, thus modulating the anxiety-like behavior of OBX rats in the EPM test.

Data are scarce when it comes to evaluating the effects of centrally applied VIP in the modulation of anxiety-related behavior and stress-induced reactions in rodents. Cottrell and coworkers (1984) observed that intracerebroventricular (icv) administration of VIP suppressed the "fear-motivated behaviour", i.e. exerted an anxiolytic-like effect. Intracerebroventricular infusions of VPAC receptor antagonist (neurotensin6-11 mouse $VIP_{7–28}$) in gregarious finches reduced social contact when animals were tested in a novel environment but did not modulate general anxiety-like behavior (Kingsbury et al. 2013).

The present study revealed an anxiolytic-like effect of VIP, which attenuated the anxiogenic-like behavior of bulbectomized rats. Our laboratory has previously reported that unilateral administration of VIP (10, 50, 100 ng) into the hippocampal CA1 area induced an anxiogenic-like effect in rats submitted to the EPM test (Ivanova et al. 2010). Studies have shown that direct infusions of the structurally related to VIP neuropeptide PACAP into the central nucleus of the amygdala produced an anxiogenic effect (Legradi et al. 2007) while icv injection of PACAP induced acute stress-related behavior in rats (Agarwal et al. 2005). Despite the extensive research, the role of PACAP in behavioral stress responding is not yet clear. Genetically modified mice lacking either PACAP or PAC1receptor exhibited reduced anxiety levels (Hashimoto et al. 2001, Otto et al. 2001). Unlike PAC1, the contribution of VIP and VPAC receptor subtypes in the emotional processes is still mostly unknown. Our experiments are the first to provide data about the effects of VIP injected into hippocampal CA1 area on the anxiety-like behavior of OBX rats.

While activation of PAC1 receptor tends to increase the anxiety levels in rats, data are lacking about such effects in experimental models of depression. The hippocampus is a brain structure where neurodegenerative changes after olfactory bulbectomy have been reported (Carlsen et al. 1982). While much focus has been put on the changes in the classical neurotransmitter systems, data is scarce about alteration in the expression of neuropeptides or their receptors following bulbectomy. For example, increased prepro-enkephalin mRNA and substance P levels have been detected in different brain structures in rodent OBX model (Holmes et al. 1998, Holmes 1999). There are numerous reports about an elevated expression of endogenous PACAP and its specific PAC1 receptor in different parts of the central and peripheral nervous systems following traumatic injuries (Skoglosa et al. 1999, Van Landeghem et al. 2007). Significantly low VIP levels have been detected in contralateral cortical areas after unilateral ablation of the sensorimotor cortex in rats (Culić et al. 1995). Although data are lacking about VIP levels in the brain of OBX rat, it is likely that that the anxiolytic-like effect of VIP in OBX rats might beassociated with neurodegenerative, structural and neurochemical changes in the limbic system which occur after bulbectomy, including alteration in the VIP or PACAP systems.

VIP and PACAP exert their actions through three G-protein coupled receptors (GPCRs) – VPAC1, VPAC2 and PAC1.VIP interacts with high affinity with VPAC1 and VPAC2 receptors and with low affinity with PAC1 receptor. PAC1 is a PACAP specific receptor which also can interact with VIP at high VIP concentration (Harmar et al. 2012). All three types of VIP receptors are expressed in high concentration in the hippocampal CA1–CA3 areas and dentate gyrus (Vaudry et al. 2000).

In an attempt to explore the mechanism underlying the effect of VIP on the plus maze performance of OBX rats we used a VPAC receptor antagonist VIP_{6-28}. The right side injection of $VIP_{6–28}$ decreased the number of both closed arm and open arm entries, while a reduction of closed arm entries only was observed upon left-side administration as compared to the saline-treated OBX controls. These findings can be interpreted as a sign of suppressed locomotor activity as well as a failure of $VIP_{6–28}$ to antagonize the effects of VIP on the behavior of OBX rats submitted to the EPM test.Thus, our results point to an involvement of hippocampal PAC1 receptors in the behavioral effects of VIP.

Regarding the inability of VIP_{6-28} to reverse the anxiolytic-like effect of VIP in OBX rats it is worth mentioning that while VIP_{6-28} is frequently used as a VIP antagonist, it is moderately effective even in large concentrations, it may not be a competitive antagonist and it may interact with other receptors (Markos et al. 2002). In addition, it should be taken into consideration that the effectiveness of VIP receptor antagonist in the brain seems to be dependent on the concentration of the injected drug. For example, the effect of centrally administered VIP (1 μg), on median eminence 3,4-dihydroxyphenylalanine was blocked by coadministration of VIP_{6-28} in 10- and 30-μg, but not in 1- or 0.1-μg doses (Huang and Pan 1996).

The most striking and unexpected observation of our study was that upon pretreatment with VIP_{6-28}, VIP injected unilaterally into the right or left CA1 area stimulated the exploration of the open arms (increased the number of open arm entries, the time spent there, the ratio open/total entries and decreased closed arm time), thus suggesting an anxiolytic-like behavior of OBX rats. A reduction in closed arm entries was significant for the combination VIP_{6-28}+VIP administered into the right CA1 area. If we consider the observed tendency of VIP_{6-28} to inhibit the locomotor activity upon separate, right-side injection, the effect of the combination in the right side could be attributed to that sedative effect of VIP_{6-28}. The pretreatment with VIP_{6-28} enhanced the effects of VIP in the left CA1 area without affecting the locomotor activity and elicited a pronounced anxiolytic-like response in the EPM test which completely abolished the bulbectomy-induced anxiety.

In an attempt to explain the mechanism of the antagonist-agonist interaction we can only make speculative assumptions.VIP and PACAP are known to activate G-protein coupled receptors that stimulate a number of second messenger systems which regulate neuronal excitability. All currently available VIP receptor antagonists are peptide derivatives that include large sections of the original VIP. In VIP_{6-28}, the first five amino acids have been cleaved, leaving a shortened VIP molecule that is thought to bind to the VIP receptors without eliciting an effect (Fishbein et al. 1994). Since VIP_{6-28} includes large sections of the original VIP molecule, it ispossible that the antagonist might have triggered some intracellular events that could have resulted in an increased neuronal excitability**.** Another factor that must be considered regarding VIP receptor antagonists is that they do induce some partial agonism. Markos and Snow (2001) have observed an increase of heart rate following administration of VIP, VIP antagonist, noradrenaline and stimulation of the sympathetic nerves to the heart. A modulatory effect of VIP_{6-28} on the locomotor activity can not be excluded as well, although there were no significant changes observed upon left-side administration.

The unexpected anxiolytic-like effect of the combination (VIP_{6-28}+VIP) in OBX rats is difficult to explain. However, similar effects involving opioid antagonists have been reported. Laboratory research has demonstrated paradoxical effects of opioid antagonists for enhancing rather than attenuating analgesic effects of opioids. The administration of low doses intrathecal or systemic opioid antagonist naloxone (which selectively inhibits the excitatory effects of morphine), augmented the antinociception produced by an acute dose of morphine in the tail-flick test (Powell et al. 2002). Further studies are needed to explain the interaction between VIP and VIP receptor antagonist VIP_{6-28} in CA1 area of OBX rats.

CONCLUSIONS

Our results suggest an involvement of hippocampal VIPergic system in the mediation of anxiety-related behavior in rat OBX model. VIP injected unilaterally into the CA1 hippocampal area produced an anxiolytic-like effect on the plus maze activity of OBX rats, while VPAC receptor antagonist VIP_{6-28} failed to antagonize the behavioral effect of VIP. The pretreatment with VIP_{6-28} potentiated the effects of VIP especially in the left CA1 area. We are inclined to assume that most likely PAC1 receptors are involved in the modulation of anxiogenic profile of OBX rats. Future research using a selective PAC1 receptor antagonist will provide additional data for the mechanism of the behavioral effects of VIP.

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