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# *In Vivo* Modulation of Catecholamines Release from Cortex and Hippocampus by Vasoactive Intestinal Peptide

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The aim of this work was to collect data regarding *in vivo* effects of vasoactive intestinal peptide (VIP), administered locally through the microdialysis probe, on catecholamines release from the cortex and hippocampus. Studies were performed in awake, male Wistar rats by means of transversal microdialysis technique and high-performance liquid chromatography (HPLC). VIP (0.1 to 10 nM) decreased in a dose-dependent manner the release of endogenous dopamine in cortex, while in hippocampus its effect on catecholamines release was stimulatory. Our results suggest that VIP affects dopaminergic neurotransmission both in hippocampus and cortex, probably via two different mechanisms.

*Key words*: VIP , dopamine, cortex, hippocampus, microdialysis.

## Introduction

Vasoactive intestinal peptide (VIP) is a 28-amino acid peptide. It is widely distributed in the central and peripheral nervous system, showing rich profile of biological activities - from neurotransmission to neuromodulation and neurotrophic properties. The cerebral cortex and hippocampus have the highest ratio of VIP immunoreactive neurons and VIP receptors. VIP has potential to be used in therapy of Parkinson's disease (PD) because of its potent antioxidant, antiinflammatory and neuroprotective activity [4, 2]. However, there are not enough data regarding effects of VIP on dopaminergic neurotransmission in normal and PD animal models. In order to study the action of VIP in Parkinson's disease model, it is important to know its effect under normal conditions. The aim of this study, therefore, was to investigate the effect of VIP *in vivo* on catecholamines (dopamine, norepinephrine and epinephrine) release in hippocampus of freely moving rats. In additional experiments effect of VIP on dopamine release in rat cortex has been investigated.

## Materials and Methods

Adult male Wistar rats (250-300 g) were housed on a 12 h/12 h light/dark cycle at a constant temperature and free access to food and water. The rats were anaesthetized

with chloral hydrate (400 mg/kg i.p.) and placed in stereotaxic apparatus. Microdialysis tubes (molecular weight cut-off 15 000 Da) were inserted transversally into hippocampus (AP — 3.3 mm posterior from the bregma and H +3.3 mm) or cortex (AP — 1.0 mm posterior from the bregma and H +2.0 mm). One day after surgery, the inlet of the microdialysis probe was connected to a microperfusion pump and perfused with Ringer solution at a constant rate of 2 μl/min. After 1 h stabilization period, during which the animals were perfused without collecting the dialysate, samples were collected at 40-min intervals. After collecting the first four samples to measure the basal outflow, drugs (dissolved in Ringer solution) were administered locally through the dialysis membrane for 80 min. Dialysates, derived from hippocampus, were analyzed for dopamine, norepinephrine and epinephrine using high-performance liquid chromatography apparatus with fluorescent detector. Separation was achieved on a column Hypersil C18 bonded phase, 5 μM particle size (Hewlett-Packard). Data acquisition and processing were carried out with program DataApex (Czech Republic). The peak area of the external standard was used to quantify the sample peak. At the end of experiment the rats were anaesthetized with chloral hydrate (800 mg/kg, i.p.) and sacrificed by decapitation. The brain was rapidly removed and placed in a vial containing 10 ml of 9 % formaldehyde solution in phosphate buffer. Three days later thin hippocampal or cortical slices were cut to verify the position of the dialysis membrane. Data obtained from rats in which the dialysis membrane was positioned outside of the cortical or hippocampal brain structure were discarded (< 5 %).

## Results and Discussion

After 60 min recovery period basal extracellular levels of dopamine, norepinephrine and epinephrine in microdialysis perfusate from hippocampus were 6.68 pmol/40 min, 5.90 pmol/40 min and 15.84 pmol/40 min, respectively. Basal level of dopamine in cortex was 2.98 pmol/40 min. Catecholamine levels remained relatively constant from one collection period to the next through each experiment (up to 6th hour for hippocampus, and up to 8th hour for cortex). In cortex VIP (0.1, 1 and 10 nM) was applied for 80 min locally through microdialysis probe. The neuropeptide induced dose-dependent decrease in extracellular dopamine levels, the most effective concentration being 0.1 nM VIP (Fig. 1). The maximum decrease by 65.59 % was observed about 80 min after VIP administration (Fig. 1A). On the contrary, applied locally through microdialysis membrane in hippocampus, VIP (0.1, 1 and 100 nM) induced an increase of extracellular dopamine, norepinephrine and epinephrine levels (Fig. 2). The maximum increase of 59.61 % and 38.19 % for dopamine and epinephrine, respectively, was observed at concentration of 1 nM VIP (Figs. 2A, 2B). VIP (100 nM) enhanced the norepinephrine release by 108.04 %, while after 1 nM VIP the increase was only 26.11 % as compared to the controls (Fig. 2C). There are not enough data in the literature regarding interaction between dopaminergic and VIPergic neurotransmitter systems, but a suggestion that VIP facilitates the dopamine metabolism in the brain has been made [3]. Our results suggest that VIP can modulate dopamine neurotransmitter system in hippocampus and cortex. We observed different effects of VIP on dopamine release in those structures: in hippocampus VIP provoked an increase in extracellular catecholamine levels, including dopamine, while in cortex the peptide decreased the release of dopamine. In the cortex VIP exhibits over 70 % co-localization with acetylcholine and 30 % co-localization with γ-aminobutyric acid (GABA) [1], while in hippocampus one third to one

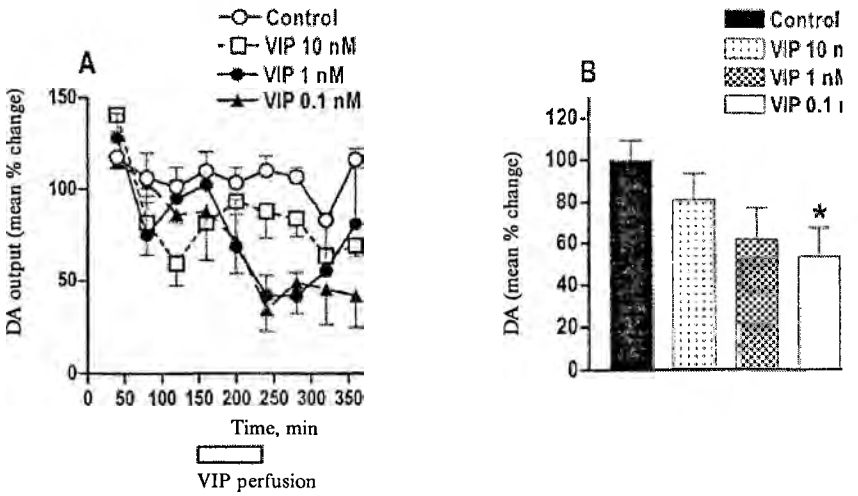


Fig. 1. Effect of vasoactive intestinal peptide (VIP) on dopamine (DA) release from cortex of freely moving rats. Dialysate samples were collected every 40 min. The release of DA was expressed as per cent change over the mean of the first three basal samples. VIP (0.1, 1 and 10 nM) was administered for a period of 120 min after collection of the first three basal samples (A); Bars in Fig. 1B represent mean percent change of area under the curve in Fig. 1A, calculated from time 160 min to time 360 min. Results are expressed as mean ± SEM of at least four experiments in each group. Differences among groups were evaluated by Student's t-test, $*P < 0.05$ versus control

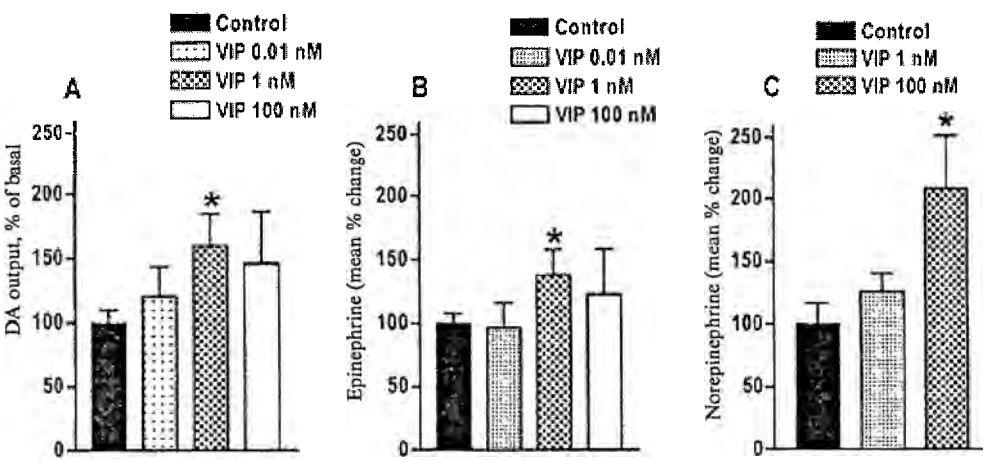


Fig. 2. Effect of vasoactive intestinal peptide (VIP) on dopamine (A), epinephrine (B) and norepinephrine (C) release from hippocampus of freely moving rats. Dialysate samples were collected every 20 min. The release of catecholamines was expressed as per cent change over the mean of the first four basal samples. VIP (1 and 100 nM) was administered for a period of 40 min after collection of the first four basal samples. Results are expressed as mean ± SEM of at least four experiments in each group. Differences among groups were evaluated by Student's t-test, $*P < 0.05$ versus control

half of the VIP-ergic neurons contain only GABA and/or cholecystokinin [5]. Taken together, the above-mentioned data and our results suggest that probably two different mechanisms underlie the effect of VIP on dopamine release in rat hippocampus and cortex.

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