Behavioural Brain Research 227 (2012) 109–115

Contents lists available at SciVerse ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

# Effects of apomorphine and β-carbolines on firing rate of neurons in the ventral pallidum in the rats

Abbas Haghparast[a,*], Davood Farzin[b], Mehdi Ordikhani-Seyedlar[a], Shirin Motaman[b], Mojtaba Kermani[a], Pegah Azizi[a]

[a] Neuroscience Research Center, Shahid Beheshti University of Medical Sciences, P.O. Box 19615-1178, Tehran, Iran
[b] Department of Pharmacology, School of Medicine, Mazandaran University of Medical Sciences, Sari 48168, Iran

ARTICLE INFO

*Article history:*
Received 21 September 2011
Received in revised form 23 October 2011
Accepted 25 October 2011
Available online 6 November 2011

*Keywords:*
Ventral pallidum
β-Carboline
Dopamine receptor
Harmane
Harmine
Norharmane
Single unit recording
Rat

ABSTRACT

The ventral pallidum (VP) is a critical element of the mesocorticolimbic system that is inter-connected with motor and limbic structures and may be considered as an interface between motivational and effector neural signals. Dopamine is important in behavioral output of the VP, and dysfunctioning its dopamine quantity leads to various neuropsychiatric disorders. Understanding neural substrate underlying this phenomenon has become an important affair in recent years. In this study, neuronal activities were recorded from the VP in presence or absence of the mixed dopamine D1/D2 receptor agonist, apomorphine, and/or β-carbolines, using an extracellular single-unit recording technique. We reported that subcutaneous administration of apomorphine (0.5 mg/kg) decreased neural activity in the VP. In addition, neither harmine (7.8 mg/kg; i.p.) nor harmane (4 mg/kg; i.p.) and norharmane (2.5 mg/kg; i.p.) had any effect on neural firing in the VP. Finally, pretreatment with β-carbolines prevented the apomorphine-induced inhibition on VP firing rate. Thus, according to the results of aforementioned study and our results in the present study, we can conclude that presumably most responses in the VP are D2 dopamine dependent. Although the β-carbolines were unable to alter neural activity in the VP, interestingly, pretreatment with β-carbolines protect decreasing in firing rate of neurons in the VP followed by apomorphine administration. This protective effect could be explained by interaction between β-carbolines and dopaminergic mechanisms.



## 1. Introduction

The ventral pallidum (VP) is a basal forebrain structure that is interconnected with motor and limbic structures and may be considered as an interface between motivational and effector neural signals. This structure is innervated by dopaminergic, GABAergic and glutamatergic fibers from the ventral tegmental area (VTA) [1,2], the nucleus accumbens (NAc) [3,4] and subthalamic nucleus, amygdala, thalamus and prefrontal cortex [5–9], respectively. Neuronal activity in the VTA dopaminergic neurons has been shown to be under GABAergic control, because dopamine release from these terminals can be increased or decreased after blockade or stimulation of γ-aminobutyric acid type A ($GABA_A$) or type B ($GABA_B$) receptors in the VTA, respectively [8,10,11]. The majority of $GABA_A$ receptors in the VTA are localized to the GABAergic neurons, while $GABA_B$ receptors are primarily localized on the dopaminergic neurons [12,13].

Dopamine in the VP has a significant effect on behavior mediated by the corticolimbic loop of the basal ganglia. The electrophysiological responses of VP neurons are modulated by dopamine D1 and D2 receptors [2,14–16]. Stimulation of the dopamine receptors in the VP through direct and indirect dopamine agonists increases locomotion [1,17–19] and induces conditioned place preference [17]. As regards motor activity, GABA neurotransmission is also involved in the modulation of this process. For example, intra-VTA injection of the $GABA_A$ receptor agonist muscimol increases locomotion [20], produces rewarding effects in the conditioned place preference paradigm [21], and increases dopamine release in the NAc [13]. The inverse agonists at the benzodiazepine binding site of the $GABA_A$ receptor, most notably β-carboline compounds, harmane, harmine and norharmane, also modulate locomotion in rodents [22,23]. β-Carbolines have been found in a number of medicinal plants, tobacco smoke, well-cooked foods [24–26] and endogenously in mammalian tissues [27,28]. These compounds have been of particular interest, because they not only antagonize the actions of benzodiazepines, but also produce intrinsic actions of their own which are opposite to those of the benzodiazepines [27]. Neurochemical and behavioral studies have shown that some β-carbolines facilitate the dopaminergic transmission [29–31] and

* Corresponding author. Tel.: +98 21 2243 1624; fax: +98 21 2243 1624.
*E-mail address:* Haghparast@yahoo.com (A. Haghparast).

0166-4328/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbr.2011.10.041

interact with D1 and D2 dopaminergic receptors [32,33]. We consider that these compounds might be useful in further clarifying the role of benzodiazepine recognition sites and its interaction with other neurotransmitter system, particularly dopamine, in the regulation of neural activity in the VP.

Therefore, we studied the effects of β-carbolines harmane, harmine and norharmane on the firing rate of VP neurons in the presence or absence of the mixed dopamine D1/D2 receptor agonist, apomorphine by single unit recording technique in anaesthetized rats.

## 2. Materials and methods

### 2.1. Subjects

Adult male Wistar albino rats (250–350 g) were purchased from Pasteur institute, Tehran, Iran. Animals were housed in groups of three per cage at a room controlled temperature (22 ± 2 °C) and were maintained on a 12-h light/dark cycle (light on 07:00 h) with free access to the standard rat breeding diet and tap water. All experiments were conducted during the light period of a day–night cycle. Animals were handled daily for at least three days before electrophysiological experiments. All experiments were done in accordance with the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health (NIH Publication No. 80-23, revised 1996) and was also approved by the Research and Ethics Committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran. Each animal was used only once.

### 2.2. Drugs

The following drugs were used: R(−)-apomorphine HCl (Research Biochemicals, USA), Harmane HCl (Sigma, USA), Harmine HCl (Sigma, USA), Norharmane HCl (Sigma, USA). The drugs were prepared immediately before injection and administered in a volume of 1 ml/kg. The doses of drugs were usually those used previously and shown to be pharmacologically active [34].

### 2.3. Experimental protocol

Testing sessions were carried out in a quiet room, with the room temperature kept at 25 ± 1 °C. A total of 76 male Wistar albino rats (260–350 g) were tested in these experiments. There were eight groups as follow: (1) saline + saline, control group ($n$ = 12), which was tested merely for determining spontaneous firing rate of VP neurons for a 95-min period; (2) saline + apomorphine group ($n$ = 12) which received saline (1 ml/kg; intraperitoneal (i.p.)) after baseline recording (15 min) and 20 min prior to apomorphine injection (0.5 mg/kg; s.c.); (3–5) β-carbolines + saline groups which received β-carbolines ($ED_{50\%}$) 15 min following baseline recording and then saline (1 ml/kg; s.c.) injected 20 min after i.p. administration of β-carbolines. These β-carbolines were *Harmine* (7.8 mg/kg; $n$ = 8), *Harmane* (4 mg/kg; $n$ = 8) and *Norharmane* (2.5 mg/kg; $n$ = 8) in this study; (6–8) β-carbolines + apomorphine groups which received β-carbolines ($ED_{50\%}$) at the same doses, 15 min after baseline recording and then apomorphine (0.5 mg/kg; s.c.) injected 20 min following i.p. administration of β-carbolines. In all aforementioned experimental groups, after the second injection, single unit recording continued for 60 min. In this study, a total of 90 single VP neurons were analyzed. Only one cell was recorded from each animal except control group (26 cells/12 rats) for determining the baseline firing rate of VP neurons.

### 2.4. Electrical recording and data acquisition

Animals were initially anesthetized with urethane intraperitoneally (1.2 g/kg) with additional doses (0.1 g/kg) were administered every 1 h as needed for maintaining a deep and constant level of anesthesia as determined by lack of movement in response to a strong tail pinch. Then, animals were placed in a stereotaxic instrument (Stoelting; USA). Body temperature was maintained at 36–37 °C with a thermistor-controlled heating pad. A 2-mm diameter hole was drilled in the skull above the VP (0.2–0.5 mm caudal to bregma, and 2.2–2.6 mm lateral to the sagittal suture according to the rat brain atlas [35], and the dura was removed. Extracellular recording from individual neurons was obtained with tungsten microelectrode (Harvard Apparatus, USA; Parylene Coated; 127-μm diameter shaft with extra fine tip; 5 MΩ impedance tip). Microelectrode was stereotaxically advanced into the VP (approximately 7.5–8.3 mm ventral to skull surface). Spike signals were amplified by a differential amplifier (DAM-80, WPI, USA; ×1000 gain; 300 Hz and 10 kHz for low and high filters, respectively) and were displayed continuously on a storage oscilloscope (OS-3060D; EZ Digital Co, Korea) as signals, and were also monitored with an audio monitor. Only single cells having a consistent spike amplitude and waveform during the experimental procedure were studied. The action potentials were categorized as biphasic or triphasic and by the initial direction of the voltage deflection (positive or negative); amplitude (peak-to-peak) and duration were also determined. Action potentials were isolated from background activity with a window discriminator (W3205; WSI; Iran) which generated output pulses for signal units based on spike height, and which counted the number of spikes per unit time (bin widths were 1000 ms). Sampling of extracellular recordings was done using an electrophysiological data acquisition (D3109; WSI; Iran) on an IBM Pentium computer for on-line data collection. In this manner, computer saves number of output signals as spikes in time unit that is set manually (0.1–12,000 s). In these experiments, time setting for data collection was 5700 s with 1000 ms bin size as a file which was saved continuously during experiment in hard disk, and unit activity was calculated by computer as an average frequency (spikes $s^{-1}$). In the present study, the signal to noise ratio was considered at least 4 to 1. For data presentation, unit activity is shown at 1- and/or 5-min intervals.

### 2.5. Data analysis

In this study, discharges of each neuron were counted in 60-s bins using a data acquisition interface program to construct peri-stimulus time histograms (PSTHs), with a time range from 15 min before (baseline recording), to 80 min after the stimulation (first and second injections of saline, β-carbolines and/or apomorphine). The data were later analyzed off-line with the homemade analysis software for windows. In order to detect the neuronal response patterns to saline, β-carbolines and/or apomorphine administration over time, the whole period of observation was sectioned into 1-min time bins. An increase or decrease of firing rates over two-fold of the standard deviation of the baseline for at least 3 consecutive bins (i.e., 3 min) was considered as an excitatory or inhibitory response, respectively. A clustering analysis (K-means, SPSS) was performed to classify neuronal responses depending on the similarities in patterns of excitation or inhibition (both latency and duration of response) induced by injection of drugs. Numbers of neurons exhibiting excitatory or inhibitory response to β-carbolines and apomorphine, alone or concurrent injections at each time bin were counted.

To calculate the significance, the mean and SEM values of the 1- and/or 5-min time blocks, representing the vehicle (saline), β-carbolines and/or apomorphine responses of each of the groups were compared to each other. Statistical analysis of the data was done using one-way ANOVA and Student-Newman-Keuls test for multiple comparisons. In addition, we did a chi-square analysis to compare the distribution of neurons among different clusters in experimental groups with or without administration of β-carbolines and/or apomorphine. A difference in responses between groups was considered significant with a $P$-value ≤0.05.

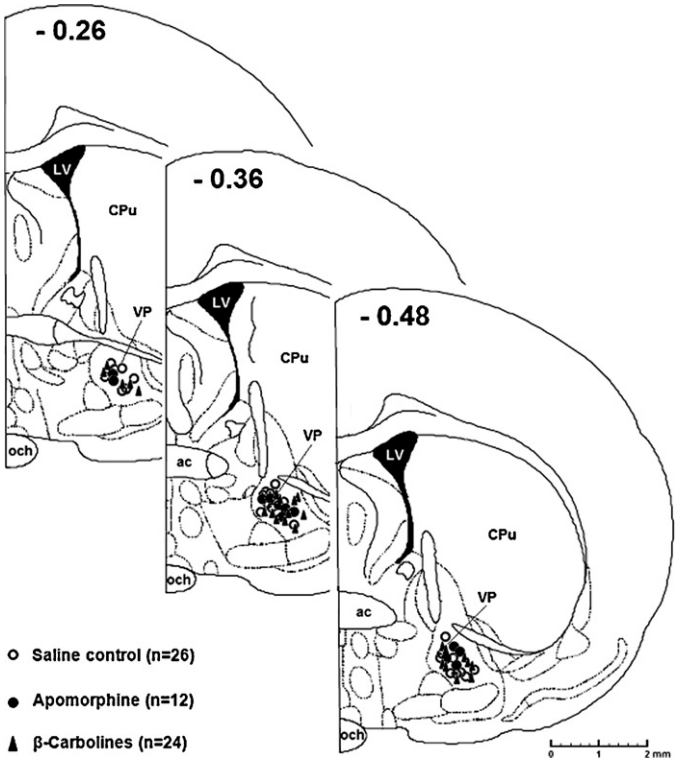


**Fig. 1.** Schematic diagram of coronal sections depicting location of recording sites in the ventral pallidum in control and experimental groups. Sections correspond to 0.2–0.5 mm caudal from bregma in the rat brain atlas. ac, anterior commissure; CPu, caudate putamen; LV, lateral ventricle, VP, ventral pallidum.