Microinfusion of Bupropion Inhibits Putative GABAergic Neuronal Activity of the Ventral Tegmental Area

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A B S T R A C T

Introduction: The most common interpretation for the mechanisms of antidepression is the increase of the brain monoamine levels such as dopamine (DA). The increase of DA can reduce depression but it can also decrease the monoamine release because of autoreceptor inhibition. Although bupropion can decrease the dopamine release, there is evidence about stimulatory effects of chronic application of bupropion on ventral tegmental area (VTA) neurons. In this study, the intra-VTA acute microinfusion of bupropion on putative VTA non-Dopaminergic (VTA-nonDA) neuronal firing rates was evaluated by a single neuron recording technique.

Methods: Animals were divided into 7 groups (sham, and 6 bupropion-microinfused groups with 1, 10⁻¹, 10⁻², 10⁻³, 10⁻⁴, and 10⁻⁵ mol, 1 μl/3 min, intra-VTA). A single neuron recording technique was done according to the stereotaxic coordination. After 10 min baseline recording, ACSF or bupropion was microinfused. The recording continued to recovery period in the treated groups. The prestimulus time (PST) and interspike interval (ISI) histograms were calculated for every single unit. The assessment of the drug effect was carried out by one-way analysis of variance (ANOVA) and Post-hoc test.

Results: 126 non-DA neurons were separated. Bupropion could inhibit 116 neurons and 11 neurons had no significant response. Maximum inhibition was 79.1% of baseline firing rate with 44.3 min duration. The inhibitory effect of bupropion was dose-dependent.

Discussion: The acute inhibitory effects of bupropion on VTA-nonDA neurons can explain the fast inhibitory effects of bupropion and other antidepressants on the VTA. These data can explain some side effects of antidepressants.
Bupropion has introduced as a novel antidepressant (AD) (Soroko et al., 1977) with action on biogenic amine reuptake and acetylcholine receptors (Paterson, 2009). Bupropion inhibits synaptic DA/NE reuptake, as well as antagonizing nicotinic acetylcholine receptors (nAChRs). These dual actions explain their effects as an AD and smoke cessation (Dwoskin et al., 2006). The inhibition of DA reuptake increases the synaptic availability of DA to presynaptic membrane autoreceptors, which is followed by a decline in the release of DA and related to the neuronal firing rate (Ascher et al., 1995).

There is no evidence for the direct effect of bupropion on the VTA-DA or VTA-nonDA neuronal activity. There is a few study that measured the direct effect of bupropion on VTA neurons. Mansvelder et al have studied, the direct effect of bupropion on VTA neurons and determined that the VTA neurons that were stimulated by nicotine in vitro in the brain slices (Mansvelder et al., 2007). The direct effect of the bupropion on the VTA-nonDA neuronal activity has not been studied yet. This study was designed to investigate the direct effect of intra-VTA bupropion on the non-dopaminergic neuronal firing rate.

2. Methods

2.1. Ethical Approval

The local biomedical research ethics committee reviewed and approved the procedures and experiments. All guidelines for the care and use of experimental animals were precisely outlined by the Animal Laboratory Center of Urmia University of Medical Sciences. All experimental procedures and protocols were approved by Urmia Medical Science Research Ethics Committee (UMSREC) and performed in accordance with the national institutes of health (NIH) for the care and use of experimental animals.

2.2. Animals

Healthy male Wistar rats (Pasteur Institute, Tehran, Iran, weight 250-280 gr.) were housed (three in a cage) at a 12h light/dark cycle (7:00am-7:00pm) and controlled temperature (22±2 °C) with the food and water ad libitum. The animals were divided into 7 groups as sham and microinfused with 1, 10⁻¹, 10⁻², 10⁻³, 10⁻⁴, and 10⁻⁵ mol amount of bupropion (intra-VTA, 1μl/3 min, n=6/group). The animals were anesthetized with Urethane (1.2 gr./Kg) which was usually sufficient for the entire recording session, but the booster doses (was about 15-25% of the initial dose) were used if there was any sign of discomfort. The animals’ body temperature was monitored continuously and maintained at ~37 °C during the experiment. The bregma and interaural stereotaxic coordinates were calculated for each rat and the location of electrode insertion for VTA was drilled according to Paxinos and Watson stereotaxic rat brain atlas (Paxinos & Watson, 2007).

2.3. VTA Single and Multiple Electrophysiological Recording

Single or multiunit extracellular electrophysiologic activity of the putative VTA-GABAergic neurons was recorded under urethane (1.2 gr./Kg, i.p.) anesthesia as described previously (Mejias-Aponte & Kiyatkin, 2012). Briefly following anesthesia, the animal was mounted in the stereotaxic frame (Steolting, USA). The skull was exposed and the stereotaxic landmarks were determined for opening a burr hole on bregma zero-zero (BZZ) plane. Glass microelectrodes (in vitro impedance 3-6 MΩ) were filled with 2.0% solution of Pontamine Sky Blue in 0.5 % sodium acetate and lowered into the brain in the region of the VTA (Bregma= -6.84, ML= ±0.5, and DV= 8.6 mm from the BZZ plane). The signals were amplified (10,000×) with a high impedance digital amplifier (Electromodule 3111 data acquisition system, ScienceBeam Co, Tehran, Iran) and filtered (300-3000 bandpass, sample rate=50,000) for acquisition by high speed USB port on a PC computer (Windows 7.0 Premium Pro.). Digitized data were displayed on an oscilloscope window of NeuroComet (ver. 2.34, ScienceBeam Co, Tehran, Iran) software. Only spontaneously active right putative VTA-GABAergic neurons were recorded and analyzed. The criteria for isolation of the non-dopaminergic neurons include spontaneous high discharge rate (>15 spike/sec), short-duration spikes (<1.5 ms), and a biphasic (+/-) spike. These criteria were explained in the previous articles (Steffensen et al., 1998; Margolis et al., 2012). The criteria for the dopaminergic neurons include spontaneous low discharge rate (<10 spike/sec), long-duration spikes (>2.5 ms), a triphasic (+/-/+ or biphasic spike with a notch in the positive component. These criteria were explained in the previous articles (White & Wang, 1984; Mansvelder et al., 2002; Marinelli et al., 2003).

Multiunits were observed for 5 min to obtain stable amplitude and firing rate and the units with non-stable firing rate were ignored. The baseline firing rate was recorded for 10 min. In the sham group, the drug vehicle (fresh ACSF) was microinfused in the 11th to 13th min and further 50 min of recording continued for further statistical comparison. Therefore, the neuronal sensitiza-
tion/desensitization can be inhibited due to the volume effect of injected drug vehicle. The groups with bupropion microinfusion received bupropion (intra-VTA, 1 µl/3 min, right VTA in all animals) in the 11th to 13th min. In the treated group, the post-microinfusion recording was continued until the firing rate returned to pre-microinfusion period. The peristimulus time histogram (PSTH) and interspike interval histogram (ISIH) of the units were calculated in a 1 msec bin size. On-line PSTH analysis was used for detection of firing pattern changing. Almost all recordings were multiunit and the PSTH and ISIH were calculated by IGOR pro 6.0 (Wave Metrics, Lake Oswego, OR) software. To process the signals, the principle component analysis (PCA) protocol was used. The duration, amplitude, rising, and falling slope (the accuracy was ±5.0%) were the main parameters used for spike sorting and clustering. All units with stable spontaneous firing were analyzed.

2.4. Bupropion Microinfusion

A 30 gauge stainless steel needle, to the 1 µl Hamilton syringe by PE 10 polyethylene tube (A-M system, USA) was used for bupropion injection. The injection pipette was lowered to the vicinity of recording electrode tip in a proper angle Drug vehicle (fresh artificial cerebrospinal fluid of ACSF) and different concentrations of bupropion were injected for 3 minutes by programmable motorized microsyringe pump (New Era Pump Systems Inc, NY, USA). All injections were 1 µl and the time of microinfusion was 3 min.

2.5. Data Analysis

The Kolmogorov–Smirnov test (K-S test) was used as a goodness of fit test for statistical probability distribution of the data for using parametric or non-parametric statistical tests. As mentioned previously, the single units were isolated using Igor pro 6.0 software by PCA protocol. The PSTH and ISIH were calculated off-line for all recordings. The average of firing rate in pre-microinfusion period (min 1 to 10) was used for each recording as pre-stimulus period and its firing rate was used as a baseline PSTH and ISIH. The data of the period between the 14th min and the minute that firing rate returned to the baseline were calculated for every recording as inhibition or excitation period. The paired t-test was used for analysis of the pre and post-microinfusion statistical analysis for every record. The inhibition or excitation period were calculated for every record by repeated measure one-way ANOVA test. The Igor pro 6.0 software was used for statistical data analysis with p<0.05 as the least level of significance. The information is presented as Mean ± SD.

2.6. Histological Verification

At the end of the experiments, the recording site was marked by passing -20µA current through the recording electrode for 10 min to deposit the Pontamine Sky Blue dye. The animals finally were deeply anesthetized, perfused transcardinally with 10% phosphate buffered formalin solution and then their brains were removed and fixed in the perfused solution. Coronal 40 µm sections were taken on a microtome (SLEE, London) and stained by fast Cresyl violet. The trajectory path and location of tips of infusion cannulae were observed under a light microscopy to verification. The mis-injected rats were excluded from the analysis.

2.7. Drugs and Chemicals

Drugs and chemicals used in the present study included: Bupropion, Formalin, Pontamine Sky Blue, fast Cresyl violet, Urethane (Sigma-Aldrich, USA), Sodium Acetate and Sodium Chloride (Merck, Darmstadt, Germany), Polyethylene microtube (A-M system, USA), Hamilton microsyringes (Hamilton Bonaduz AG, Switzerland).

3. Results

3.1. The Waveform of VTA-nonDA Neurons

In an off-line sorting, based on PCA sorting, the units with spike duration ≤ 1.5 ms were extracted. The PSTH and ISIH of neurons were checked. The PSTH of 10 min of recordings before microinfusion were analyzed as pre-stimulus time histogram. In sham and bupropion microinfused groups, the drug vehicle (fresh ACSF) or drug were microinfused over the 11th to 13th minutes of recordings.

In the present study, the VTA neuronal activities of the 42 healthy male rats were recorded. 127 neurons with spontaneous activity were isolated as putative non-DA neurons (putative VTA-GABAergic neurons). 11 neurons had no response to intra-VTA bupropion microinfusion but the others were inhibited in a dose-dependent manner. The number of neurons in sham group and 1 mol to 10⁻⁵ mol dpse of bupropion was 18, 19, 21, 17, 20, 17, and 15, respectively. The baseline recording and waveform of a typical putative VTA-nonDA neuron is shown in figure 1. Parts A,B and C show the firing of the neurons in different time lines. Part D shows the typical signature of a putative VTA-nonDA neuron. The firing rate of the neuron was 19.5±0.7 spikes/sec and the amplitude of this unit was about 300 µV. The average of amplitude of the extracted spikes was between 285 to 500 µV.
Figure 1. A typical spontaneous firing of a VTA-nonDA (putative GABAergic) neuron. Spontaneous firing rate of the neuron was high and regular (19.5±0.7 spikes/sec) with no bursting or sensitization/desensitization. The time bars were depicted in the parts A, B, and C and the time-amplitude legend was shown in the part D with the shape of the spike.

Figure 2 shows the mean firing rate of the neuron of figure 1. The mean of firing rate in 1 min was calculated and depicted as a PSTH. The fresh ACSF was microinfused over the 11th to 13th minutes of recording. The firing rate of the neuron had no significant changes before and after ACSF microinfusion. The ISIH of the neuron had no significant changes (data not shown here).

3.2. Bupropion Had Inhibitory Effect on the VTA-nonDA Neurons

Figure 3 shows the inhibitory effect of 1 mol dose of bupropion in to the VTA on a typical putative VTA-nonDA neuron is shown in figure 3. Part A shows the firing pattern of the neuron. In the pre-microinfusion period, the firing rate of the neuron was 21.7±1.4 spikes/sec. The bupropion-induced inhibition period in this neuron was 44.3 min. In the inhibition period, the minimum firing rate of the neuron was 4.83±1.2 spikes/sec. The firing rate of the post-microinfusion period was 20.9±1.05 spikes/sec.

Microinfusion of bupropion in the VTA with different doses could inhibit almost all neurons except 11 neurons that showed no significant response to bupropion microinfusion. The inhibition of VTA-nonDA neurons was dose-dependent. The maximum inhibition in 1 mol dose of bupropion lasted about 43±08.9 min. The maximum inhibitory effect decreased the firing rate of the neurons up to 77.8% of its pre-microinfusion period firing rate.
3.3. Bupropion could Decrease the Firing Rate of the VTA-nonDA Neurons Dose-dependently.

116 out of 127 neurons with spontaneous neuronal activity were inhibited by intra-VTA microinfusion of bupropion. The inhibitory effect was dose-dependent. The firing rate and inhibitory duration of the VTA-nonDA neuronal activity are summarized in Table 1.

Figure 4 shows the mean of firing rate of all groups in the pre-infusion and inhibitory period. The sham group that received fresh ACSF in the VTA had no significant changes in the firing rates. A $10^{-4}$ mol dose of bupropion had no significant effect on the firing rate also. A $10^{-3}$ mol dose of bupropion decreased the firing rate significantly ($p<0.05$) and other doses decreased the firing rate dose-dependently. The maximum effect appeared in the 1 mol dose of bupropion and mean firing rate declined to 19.5% of maximum firing rate.

Figure 5 shows the percentage of neuronal firing rates according to their pre-infusion firing rate as 100% of firing rate.

3.4. Bupropion Could Increase the Inhibition Period of the VTA-nonDA Neurons Dose-dependently.

The inhibition period of the VTA-nonDA neurons in the sham and bupropion groups are shown in Table 1. The longest total inhibition period occurred in 1 mol dose of bupropion. The neurons were inhibited 41.6±6.4 min in 1 mol dose of bupropion with maximum inhibition about 14.5±4.3 min. Figure 6 shows the mean inhibitory duration in all groups. The inhibition period of $10^{-5}$ mol dose of bupropion was very short. Some neurons had no inhibition period in the presence of $10^{-5}$ mol dose of bupropion and few of them showed a non-detectable response.
Figure 4. The Mean±SD of the absolute firing rates of the VTA-nonDA neurons of the sham and groups with bupropion intra-VTA microinfusion (1 µl/3 min, over the 11th to 13th min of the recording). The putative GABAergic neurons were inhibited by the bupropion microinfusion (1 to 10^{-4} mol). The 10^{-5} mol dose of the bupropion and the fresh ACSF had no significant effects. The firing rates of the neurons in the pre and post microinfusion were shown but the data of the recovery periods were not shown here.

(repeated measured one-way ANOVA, Tukey’s post hoc test, * p<0.05, ** p<0.01, and *** p<0.001).

Figure 5. The Mean±SD of the percentage of the firing rates of the VTA-nonDA neurons of the sham and groups with bupropion intra-VTA microinfusion (1 µl/3 min, over the 11th to 13th min of recording). The mean of firing rates in the pre-microinfusion period in every neuronal record was balanced as 100%. The firing rate in the post microinfusion period was compared with its pre-microinfusion period as 100%. The putative GABAergic neurons were inhibited by the bupropion microinfusion (1 to 10^{-4} mol). The 10^{-5} mol dose of the bupropion and the fresh ACSF had no significant effects. The percentage of the firing rates of the neurons in the pre and post microinfusion were shown but the data of the recovery periods were not shown here.

(repeated measured one-way ANOVA, Tukey’s post hoc test, * p<0.05, ** p<0.01, and *** p<0.001).

Figure 6. The Mean±SD of the duration of the inhibition period of the VTA-nonDA neurons (sham and groups with bupropion microinfusion (intra-VTA, 1 µl/3 min, over the 11th to 13th min of the recording). The sham group and group with 10^{-5} mol dose of bupropion had no inhibition period. The inhibition period were increased in a dose dependent manner of bupropion.

(repeated measured one-way ANOVA, Tukey’s post hoc test, * p<0.05, ** p<0.01, and *** p<0.001).
Briefly, the decrease of the neuronal firing rate and inhibitory period of the neurons showed that bupropion can inhibit the VTA-nonDA neurons dose-dependently and it is the possible mechanism for the paradoxical effect of bupropion for the treatment of depression and decreasing the dopamine release in the VTA.

4. Discussion

The results of this research showed that the VTA-nonDA neurons were inhibited by intra-VTA application of bupropion. The inhibitory effects of bupropion on putative VTA-nonDA neuronal activity can explain some paradoxical effects of ADs. The inhibitory effects of bupropion on the VTA-nonDA neuronal activity can be caused by two possible mechanisms, the direct effects and indirect action. The direct action of bupropion on the VTA neurons is the possible mechanism for its antidepressive effects. The antidepressive effect of bupropion proposed that this effect is due to the release of dopamine in the axon terminal of VTA neurons.

Increasing the catecholamine levels is one of the old theories about the effects of ADs. The primary explanation about this effect of ADs is the direct effect of ADs on the VTA or other brain nuclei (Chenu et al., 2012). The later discoveries about the mechanisms of ADs revealed that many of these drugs inhibit the reuptake of neurotransmitters such as DA, NE, and 5-HT or inhibit the catabolism of neurotransmitters (Randrup & Braestrup, 1977; Sampson et al., 1991). The elevation of DA or NE in the axonal synapses of VTA or LC can explain the antidepressant effects, but in the VTA or LC, the increase of DA or NE can decrease the neuronal activity, respectively. DA or NE reuptake inhibition increases the synaptic availability of DA/NE to presynaptic autoreceptors that followed by the decrease in the release of DA and NE. The increase of NE in the LC nucleus by bupropion can reduce the firing rates of LC-NE neurons dose-dependently (Ascher et al., 1995). The VTA-DA neurons increase the DA secretion due to ADs. Many studies proposed that this increase of DA release is related to the direct effect of ADs on the VTA-DA neurons solely (Mylecharane, 1996; Liu et al., 2006; Schott et al., 2008).

The majority of non-DA neurons of the VTA are GABAergic neurons (Nair-Roberts et al., 2008). The effects of some drugs are mediated by non-DA neurons that are synapses on the DA neurons of the VTA. Opioids excite the VTA-DA neurons by hyperpolarizing the VTA-nonDA neurons (Johnson & North, 1992). Similarly, cocaine, a drug that mainly affects dopamine transporters, decreases GABAergic inhibition and facilitates LTP in

Table 1. The data of the firing rate and duration of inhibition of the VTA-nonDA neurons

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<tr>
<th>Period</th>
<th>Groups</th>
<th>Sham</th>
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<tr>
<td></td>
<td>Bupropion (mol, 1 µl/3 min, intra-VTA)</td>
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<tr>
<td></td>
<td></td>
<td>1</td>
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<tr>
<td>Firing Rate</td>
<td></td>
<td>10.2±4.1</td>
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<tr>
<td>(Spikes/sec)</td>
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<td>18.1±4.3</td>
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<tr>
<td>Inhibitory Period</td>
<td>Recovery</td>
<td>18.3±4.2</td>
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<tr>
<td>(min)</td>
<td>Total Inhibition</td>
<td>0</td>
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<td></td>
<td>Period</td>
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There is evidence about the antagonistic effect of bupropion on α3β2* and α3β4* nAChRs in rat striatum and hippocampus, respectively, across the same concentration range that inhibits DAT and NET function (Miller et al., 2002).
VTA neurons (Liu et al., 2005). The GABA receptors are expressed in DA and GABAergic neurons of the VTA and show the excitatory and inhibitory output to VTA targets. Interestingly, the application of the GABAA receptor agonist muscimol in the VTA can either increase or decrease DA levels in the nucleus accumbens, probably reflecting dose-dependent effects on GABAA receptors expressed by DA cells and GABAergic interneurons (Doherty & Gratton, 2007). Intravenous application of GABA agonist can stimulate firing of A10 dopaminergic neurons (Waszczak & Walters, 1980).

This report is the first report about the direct action of bupropion on the VTA-nonDA neurons in the cellular level in vivo. The paradoxical effect of the bupropion and some other ADs on the VTA neurons revealed that the intra-VTA neuronal circuitry is very complex and make the contribution of the VTA in different aspects of drug-dependence and tolerance to treatment regimen.

Briefly, the bupropion can inhibit the non-DA (putative GABAergic) VTA neurons by the antagonism of nicotine ACh or dopamine autoreceptor inhibition. The involvement of GABA or glutamate neurotransmission for inhibition of VTA-nonDA neurons was also postulated.

Conclusion

In summary, the present study presented new data about the effects of bupropion on the neuronal firing rate of VTA-nonDA (putative GABAergic) neurons. Many previous studies administered the bupropion systemically and explained the general action of bupropion. The present data showed that bupropion can inhibit the VTA-nonDA neurons and produce an intra-VTA dis-inhibition paradigm on the VTA-DA neurons. This is the first study about the cellular effect of bupropion in vivo and can explain some side effects and paradoxical effects of bupropion.

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