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Research report

Neurochemical and behavioral effects elicited by bupropion and diethylpropion in rats

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ABSTRACT

This study is an attempt to demonstrate whether bupropion (BP) and diethylpropion (DEP) exert their pharmacological actions by similar neurochemical mechanisms in the dorsal striatum. In this regard, the release of dopamine (DA), glutamate (Glu), and GABA, was determined in the rat dorsal striatum after acute (5 min) and chronic (15 consecutive days) treatments, and subsequently correlated with the locomotor activities produced by these drugs. The results from the acute experiments indicate that BP and DEP (40 mg/kg) increase locomotor activity, whereas chronic DEP treatment decreases locomotor activity by unspecific mechanisms. Acute BP treatment produces significant DA and Glu, but not GABA, releases. A lesser extent of DA release and tissue content of DA and its metabolites, and consequently less locomotor activity, was observed after chronic BP treatment. Acute DEP (5 mg/kg) was only able to slightly increase DA release and to decrease the tissue levels of DA, but no other markers, with practically nil locomotor activity, whereas chronic DEP produced even less neurotransmitter release. The observed difference between BP and DEP might be based on that although both drugs inhibit the DA and norepinephrine transporters, the BP-induced nicotinic receptor inhibition has yet to be demonstrated for DEP.

1. Introduction

Bupropion (BP) has been used for long time as an antidepressant (Wellbutrin®), in the pharmacotherapy for smoking cessation (Zyban®), and more recently as an "off-label" treatment for deficit hyperactivity disorder (reviewed in [1,2]). The most important mechanisms of action include a dual dopamine (DA) and norepinephrine (NE) reuptake inhibition, as well as the noncompetitive inhibition of several nicotinic acetylcholine receptors (AChRs).

Previous microdialysis studies determined the acute effects of BP on the extracellular concentration of DA and its metabolites in freely moving rats [15]. The results showed a peak in the release of DA and its metabolites in the striatum and in the nucleus accumbens that correlated with stereotyped animal behavior. Another experiment using rats chronically administered with BP demonstrated that the DA release is significantly decreased at 2, 6, or 26 h post-insertion of the probe [12]. More recently, it has been demonstrated that BP is responsible for the increased locomotor activity in freely moving rats, and the involved mechanisms include DA reuptake inhibition and blockade of AChRs [24]. However, these studies do not describe the effect of BP on the release of other neurotransmitters including, glutamate (Glu), norepinephrine, and γ-aminobutyric acid (GABA). This is particularly relevant since locomotor activity cannot be conceptualized merely in terms of changes of a single neurotransmitter system. Therefore, new attempts to provide integrative information on different neurotransmitters and their contribution to locomotor alterations are needed.

Diethylpropion (DEP) (Tenuate®) is a mild psychostimulant with structural resemblance to BP, and it is used as an anti-appetite drug and as an "off-label" treatment for migraine. The experimental evidence mounted in the last 30 years suggests that DEP acts in the CNS modulating the catecholaminergic systems (reviewed in [2,26]). Surprisingly, there is limited information regarding the effects of DEP on neurotransmitter release from different neuronal pathways. In this regard, we want to compare the neurochemical effects between BP and DEP to induce the release of different neurotransmitters including, DA, Glu, and GABA, in the rat dorsal striatum, after acute (5 min after injection) and chronic (during 15 consecutive days) treatments. In addition, the tissue content of DA and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), was determined under these treatment protocols. The dorsal striatum (i.e., caudate/putamen) neurons are medium spiny GABAergic projections that receive glutamatergic inputs mainly from cortical areas as well as dopaminergic innervation primarily from the substantia nigra pars compacta and to a lesser degree from the ventral tegmental area ([32] and references therein). We chose the dorsal striatum because this particular

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brain region is probably the main modulatory center for motor activities and it is known to be involved in the integration of sensorimotor, cognitive, and motivational/emotional information for decision-making processes [13,4]. These processes are key for the pharmacological and clinical actions of several drugs including, BP and DEP. In this regard, we want to correlate the neurochemical modulation elicited by BP and DEP with their behavioral effects (i.e., locomotor activity) in a more integrative manner.

2. Material and methods

2.1. Materials

Bupropion hydrochloride (BP), DA, DOPAC, HVA, o-phtaldialdehyde (OPA), and a kit of amino acids were purchased from Sigma–Aldrich Co. Ltd. (St. Louis, MO, USA). DEP was obtained from Neobes Medix (Mexico City, Mexico). Sodium pentobarbital was obtained from Pet's Pharma (Mexico City, Mexico). All other reagents were of the highest purity available.

2.2. Animals

Sixty-three adult male Wistar bred-in-house rats (270–320 g) were used throughout the study. For all experimental purposes, animals were housed five per cage in polycarbonate box cages and provided with a standard commercial diet (Laboratory rodent diet 5001, PMI Feeds Inc., Richmond, IN, USA) and water *ad libitum*. Housing room was maintained under constant conditions of temperature ($25 \pm 3\,^{\circ}C$), humidity ($50 \pm 10\%$), and lighting (12 h light/dark cycles, light on at 7:00 am). All procedures with animals were strictly carried out according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the local guidelines on the ethical use of animals from the Health Ministry of Mexico. During the experiments, all efforts were made to minimize animal suffering.

2.3. Experimental design

Rats were randomly assigned to the different treatment groups: systemic saline, systemic BP, or systemic DEP. For all purposes, each group consisted of a minimum of six rats. For locomotor activity and microdialysis protocols, acute and chronic schemes of drug administration were evaluated. For the acute protocol, four basal samples were collected and then rats were given with a systemic infusion (i.p.) of saline solution (0.9% NaCl), BP (40 mg/kg), or DEP (5 mg/kg). Dosages for both BP and DEP were decided on the basis of previous studies demonstrating maximal neurochemical effects of these drugs in rats [8,24]. For comparative purposes, the effect of 5 mg/kg BP and 40 mg/kg DEP were tested by additional locomotor activity experiments. BP and DEP were dissolved in saline solution and administered in a volume of 1 mL/kg of weight. The solution's pH was adjusted to 7.4 before systemic administration. For the chronic scheme, similar doses of the drugs were administered for 15 consecutive days. In this case, four basal samples were also collected, but this time sample collection started just before the last drug administration. In both cases (acute and chronic schemes), dialysates were sampled every 20 min for a total span time of 360 min, in addition to the first four samples.

2.4. Assessment of locomotor activity patterns in rats

Independent groups of rats ($n = 6$–7 rats per group) from all treatment groups were evaluated in regard to their locomotor activity patterns, according to methods recently described by our laboratory [17,25]. Rats were first habituated to motor activity chambers for 1 h before any drug treatment. Then, 5 and 40 (see [24]) mg/kg BP or 5 (see [8]) and 40 mg/kg DEP, respectively, were injected (i.p.) and the animal motor/kinetic activity was recorded in a Versamax Animal Activity Monitor and Analyzer (AccuScan Instruments, Inc., Columbus, Ohio, USA) for 60 min. Two treatment protocols were used: acute (5 min after injection) and chronic (daily injection for 15 consecutive days) drug administration. In both cases, locomotor activity patterns were recorded 5 min after the single injection (acute protocol) or 5 min after the injection on the last (15th) day of the chronic treatment. The locomotor effect elicited by the drug was compared to that for saline treatment (0.9% NaCl, w/v; Control). The device detects movements, recorded as counts in different directions based on the ability of animals to cross virtual lines projected by infrared light. The device also follows the precise trajectories and distances traveled by the animals. The criteria collected in this device included total distance traveled as well as total horizontal and vertical activities. External light and sound conditions were minimized by performing the experiments in an isolated room. The results were expressed either as total distance traveled (cm) in 60 min, or as total counts per 60 min, respectively. In order to reduce false recordings and bias, all animals were habituated in the device in one session of 10 min, practiced 24 h prior the activity tests. Body weight of animals was registered every day for the 15 days that the drug administration scheme lasted.

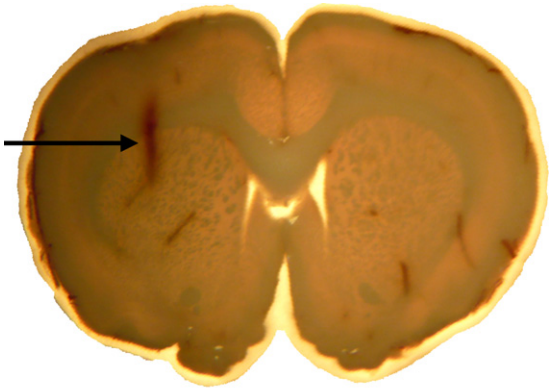

Fig. 1. Verification of the placement of the microdialysis probe. The photograph shows a representative coronal section of the brain from a rat previously implanted with a guide probe at the level of the dorsal striatum. The arrow indicates the cannulae placement.

2.5. Microdialysis in freely moving rats

The brain content of the neurotransmitters DA and its metabolites, Glu, and GABA were measured in awake animals. The methodological procedures to collect microdialysates were based on a previous report from our group [23]. Rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and transferred to a Stoelting stereotaxic frame (Harvard Bioscience, Holliston, Mass, USA). Body temperature in animals was always maintained at 37 °C using a homeothermic blanket. A brain microdialysis probe (2 mm membrane, BAS, West Lafayette, IN, USA) was implanted into the right dorsal striatum at the following stereotaxic coordinates: anterior +0.5 mm, lateral +2.6 mm, ventral −5.4 mm from Bregma [16]. The corresponding guide cannula and probe were secured with dental cement, and the wound sealed. For the acute drug-exposure protocol, all cannula and probe implantations were practiced at least 72 h before the microdialysate collection, to give the animals a reasonable period of post-surgery recovery. For the chronic drug-exposure protocol, animals were implanted with the guide cannula 72 h prior to the first drug injection, whereas the probe was inserted 4 h before the last drug injection on the 15th day. In both cases, following surgery and treatments, rats were placed in the circular chamber of the Raturn microdialysis system (BAS, West Lafayette, IN, USA) with the microdialysis probe connected to a liquid swivel and a counter-balanced arm to allow unrestricted movement. Rats received food and water *ad libitum*. Placement of microdialysis probes was verified in a small group of rats at the end of the experiments (see Fig. 1). Probes were continuously perfused at a flow rate of 2.0 μL/min with a physiological buffer mimicking the cerebrospinal fluid (125 mM NaCl, 2.5 mM KCl, 1.18 mM $MgCl_2$, 1.26 $CaCl_2$, 100 mM Na_2HPO_4, adjusted to pH 7.4 with H_3PO_4). Samples were collected at 20 min intervals by a sample collector (BAS, West Lafayette, IN, USA) into glass sample vials containing 10 μL of perchloric acid (0.1 M) (only for DA detection). For microdialysates collection, the first five samples (equivalent to 100 min) perfused with buffer solution were discarded in order to establish an accurate baseline. Neurotransmitters from both dialysates and tissue samples were measured by HPLC immediately after collected in order to avoid any possible loss over time. However, a second chromatographic analysis was performed to each stored sample 24 h later. No significant differences in the levels of neurotransmitters per sample were detected between "fresh" and stored samples.

2.6. Dopamine and metabolite analyses

The extracellular content of DA in striatal microdialysates, as well as the striatal tissue content of DA and its metabolites, was analyzed by HPLC with electrochemical detection, as previously described [9]. Briefly, fractions of the microdialysates (~40 μL) were collected in an automated fraction collector with refrigeration (5 °C) coupled to the microdialysis system. When applicable, tissue samples were obtained after the injection on the last (15th) day of the chronic treatment from different groups by dissection and immediately sonicated on ice in 300 μL of perchloric acid 0.4 N and 0.1% (w/v) sodium metabisulfite, followed by a 10 min-centrifugation step at $4000 \times g$ at 4 °C. Tissue samples belonged to the right striata from those animals previously challenged for microdialysis, and obtained immediately after the microdialysis assessments were completed. Supernatants and microdialysates were analyzed immediately after collected, or kept frozen at −70 °C for a second confirmatory chromatographic analysis.

For chromatographic analysis, a PerkinElmer LC-4C liquid chromatograph with a BASS CC-5 electrochemical detector was used. Detection limit of DA was 5 fmol. Peaks were integrated with a PerkinElmer Turbochrom Navigator 4.1 data station. An Alltech Adsorbosphere Catecholamine (100 mm × 4.6 mm) column with 3 μm of particle size was used. Mobile phase consisted of an aqueous phosphate buffer