Behavioural Brain Research 286 (2015) 318–327

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

The role of catecholamine innervation in the medial prefrontal cortex on the regulation of body weight and food intake

Rita Gálosi[a,*], Andras Hajnal[b], Zoltán Petykó[a,c], Géza Hartmann[a], Zoltán Karádi[a,c], László Lénárd[a,c]

[a] Institute of Physiology, University of Pécs Medical School, Pécs, Hungary
[b] Department of Neural and Behavioral Sciences, Penn State University, College of Medicine, The Milton S. Hershey Medical Center, Hershey, PA, USA
[c] Molecular Neurophysiology Research Group, University of Pécs, Szentágothai Research Center, Pécs, Hungary

HIGHLIGHTS

- 6-OHDA lesion was carried out in the medial prefrontal cortex.
- Catecholamine lesions of the medial prefrontal cortex reduced body weight.
- Catecholamine lesions disrupted habituation in the open field.
- Lesion of dopamine terminals augmented hunger-induced feeding.
- Lesion of dopamine terminals led to enhanced glucose preference.

ARTICLE INFO

Article history:
Received 13 November 2014
Received in revised form 3 March 2015
Accepted 6 March 2015
Available online 14 March 2015

Keywords:
Catecholamine
Medial prefrontal cortex
Body weight
Glucose preference

ABSTRACT

Effects of 6-hydroxydopamine (6-OHDA) lesions in the medial prefrontal cortex with or without protection of norepinephrine (NE) fibers were examined on basic regulatory processes of feeding. Daily body weight, food and water intake were measured. Locomotor activity, ingestion after food or water deprivation, and preference for 5% and 10% glucose solution were examined. Dopamine (DA) and NE content, as well as, tyrosine hydroxylase immunoreactivity were assessed to confirm the neurotoxic effect of treatments. 6-OHDA lesions of the medial prefrontal cortex with or without NE fiber protection resulted in body weight loss. Diminished habituation in open field tests, i.e. a persistently high motor activity, was also observed. Application of 6-OHDA with NE fiber protection led to increased food consumption following food-deprivation and to enhanced glucose preference. Enhanced intake of 10% over 5% glucose solution was also detected. 6-OHDA lesion resulted in a decrease to 20% of NE tissue concentration and only to 75% of DA concentration. In case of lesion with NE protection the NE content decreased to 69% and DA level to 51% with significant loss of tyrosine hydroxylase positive fibers in the deeper layers of the medial prefrontal cortex.

DA depletion in the medial prefrontal cortex resulted in increased behavioral responsiveness to hunger and glucose, as well as, to open field environment. Pronounced lesion of NE terminals caused increased reaction to the environment in open field but not to hunger or glucose solution.

1. Introduction

Large amount of experimental data provide evidences for the involvement of forebrain dopamine (DA) and norepinephrine (NE) systems originating from the ventral tegmental area and locus coeruleus, respectively, in the regulation of hunger motivated behavior [1–4]. In rodents, the medial prefrontal cortex (mPFC), which is defined as the cortical projection area of the mediodorsal thalamic nuclei [5–7], expresses high density of DA containing

Abbreviations: DA, dopamine; NE, norepinephrine; 6-OHDA, 6-hydroxydopamine; DMI, desipramine; PFC, prefrontal cortex; HPLC, high-performance liquid chromatography.

* Corresponding author at: Institute of Physiology, University of Pécs Medical School, Szigeti str. 12, H-7624 Pécs, Hungary. Tel.: +36 72536001x31759; fax: +36 72536244.
E-mail address: rita.galosi@aok.pte.hu (R. Gálosi).

http://dx.doi.org/10.1016/j.bbr.2015.03.017
0166-4328/

terminals from the ventral tegmental area in rats [8–10]. NE fibers originating exclusively from the intermediodorsal nucleus of the locus coeruleus also terminate in the mPFC [11].

The role of DA in the mPFC has been shown in feeding behavior, too. DA activity is enhanced in the mPFC during feeding after food deprivation and stimulation of DA receptors (i.e. D1-type) induces feeding in rats [12–15]. Enhanced DA release occurs to palatable foods in sated rats [16,17], as well as to cues associated with food reward [16,18,19]. The role of DA in enhancement of food reinforcement by hunger has also been demonstrated [20]. These findings together prove that projections of the mesocortical DA system to the mPFC are involved in the regulation of food intake, but until now, the direct effects of DA terminal lesions in the mPFC on feeding behaviors were not demonstrated.

Food intake is dependent on hunger motivation induced by homeostatic need and on the incentive value of food. Glucose solutions are highly preferred by rats based on their sweet taste and the caloric content [21–23]. These qualities of glucose solutions represent a high motivation value and result in enhanced intake. Previous study from our lab showed that mPFC neurons are involved in the mechanisms of sucrose consumption and reward prediction [24]. Furthermore, it has been shown that the intake of sugar solutions is dependent on DA release in the forebrain [25,26], and vice versa, release of DA in the mPFC can be detected when sucrose solution is administrated through an oral cannula [17]. Moreover, DA in the mPFC is involved in the acquisition of conditioned flavor preference induced by post-oral reinforcing properties of glucose solution [27]. These data suggest that DA innervation of the mPFC affects the incentive value of a sugar solution. Therefore, our aim was to determine whether DA depletion in the mPFC alters the intake of a preferred glucose solution.

To destroy DA terminals, 6-hydroxydopamine (6-OHDA) induced lesion with protection of NE terminals is a widely used method. The neurotoxin 6-OHDA, however, is capable of destroying both DA and NE terminals, thus contribution from a concurrent NE depletion cannot be controlled for. In fact, such an effect has to be considered when investigating the mPFC because of the known role of NE terminals in the elimination of DA in this brain area [28–30]. Moreover, increased NE concentration can be observed during food intake [14,31]. In addition, it is known that 6-OHDA lesion of mPFC causes enhanced locomotor activity, but the results are not consistent in the literature. Enhanced locomotor activity can be found after 6-OHDA lesion with protection of NE terminals in the mPFC [32] or after 6-OHDA lesion without protection of the NE terminals [33,34], while no change in activity was reported after 6-OHDA lesion with [35,36] or without protection of NE terminals [32]. Because enhanced locomotor activity may affect feeding and body weight, it is necessary to examine the effect of 6-OHDA lesion on locomotor activity.

Therefore, in the present experiments, effects of bilateral 6-OHDA injections with enhanced selectivity to DA terminals obtained with a systemic pretreatment with DMI were compared to 6-OHDA lesions without protection of NE terminals in the mPFC. Body weight, food and water intake and locomotor activity were measured. To test the change in homeostatic needs (e.g. hunger and thirst) effects of food and water deprivation on consumptions were examined. In a separate set of rats, preference for 10% or 5% glucose solutions was studied in free choice two-bottle paradigm. Tyrosine hydroxylase immunohistochemical analysis was used to determine the extent of destruction of catecholamine fibers. Effect of 6-OHDA lesions on DA and NE content was determined using high-performance liquid chromatography (HPLC) with electrochemical detection.

2. Materials and methods

2.1. Animals and preoperative procedures

62 young adult (9 weeks old at the beginning of the experiment) male Wistar rats were used in the experiments. 32 animals were used in body weight regulation-related experiments, and the remaining 30 rats were used in the glucose preference tests. Animals were individually caged in a temperature-controlled ($24 \pm 2\,^{\circ}C$) vivarium with 12 h light–dark cycle (light on at 6 a.m.). Rats were cared according to institutional (Medical School of University Pécs) and international standards (NIH Guidelines). Standard laboratory food pellets (11.90 MJ/kg, CRLT/N pellets for rodents, HU 13 1 00039, Hungary) and tap water were available ad libitum except experiments of water deprivation and glucose preference. Following a 1-week habituation period to the vivarium, preoperative measurements commenced for feeding and behavioral tests. Daily food and water consumption and body weight were measured to the nearest gram and milliliter, respectively at 8.00 a.m. Food and water deprivation experiments were carried out on the 14th and 10th days before surgery. Six days prior surgery, animals were habituated to the open field arena, and on the next day locomotor activity of animals was tested.

2.2. Surgery and drugs

On the day of surgery, based on their body weight records, animals were evenly distributed into 3 groups based on the type of neurochemical lesions: lesions with neurotoxin 6-hydroxydopamine [6-OHDA]; 6-OHDA lesions with desmethylimipramine (DMI) premedication [6-OHDA + DMI], and sham-operated controls injected with the vehicle of 6-OHDA solution [CO]. In order to protect NE terminals, NE uptake inhibitor DMI (desipramine hydrochloride, Pertofran, Geigy) was injected in a dose of 20 mg/kg intra-peritoneally 30 min prior 6-OHDA injection [37]. Bilateral microinjections were carried out under ketamine (80 mg/kg i.p., Calypsol, Richter-Gedeon) anesthesia combined with diazepam (2.0 mg/kg i.p., Seduxen, Richter-Gedeon). After exposing the rat's skull, a small hole was drilled. The dura was opened under microscopic control for insertion of the microinjector to the target area by a hydraulic microdrive (Narishige, MO-10, Japan). Stereotaxic coordinates of mPFC were: A:10.0 (from the Bregma:4.2), L:0.8, V:1.8 and 2.8 from the brain surface, according to Pellegrino et al. [38]. Injector was made using fused silica capillary tubing (O.D. 150 μm, I.D. 70 μm, Polymicro Technologies, Phoenix, Arizona, USA) as described in the paper of Parada et al. [39]. To prevent the involuntary side movements of capillary, it was glued in a stainless-steel cannula. The injector was connected by polyethylene tube (PE 20, Beckton Dickinson) to a Hamilton microsyringe mounted on a syringe pump (Cole Parmer EW-74900, VAC 115). Corresponding to the vertical coordinates, given above, two injections (1 μl each) of 7.5 μg/μl 6-OHDA (6-hydroxydopamine hydrobromide, H8523, Sigma–Aldrich) were carried out in the mPFC of both hemispheres. The flow rate was 0.3 μl/min, and after injections the injector remained in place for 5 min. 6-OHDA was dissolved in 0.9% saline (containing 0.1% L-ascorbic acid), was kept at 4 °C and protected from light to prevent oxidation. Control rats received vehicle in an equivalent manner and volume.

2.3. Postoperative behavioral measurements

2.3.1. Body weight, food and water intake measurements

Food pellets and tap water were available ad libitum. In thirty-two animals [6-OHDA, $n = 10$; 6-OHDA + DMI, $n = 12$; CO, $n = 10$] similarly to the preoperative period, daily food and water