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Short communication

Excitotoxic lesions of the prefrontal cortex reduce dopamine D_1-like receptors in the ventral tegmental area

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Abstract

Ventral mesencephalic dopamine D_1-like receptors were quantified in brains of male rats ten days after unilateral microinjections of ibotenic acid (2 or 10 μg/μl) or its vehicle into the medial prefrontal cortex. The density of dopamine D_1-like receptors was reduced by more than 40% in the ipsilateral ventral tegmental area (both doses) and by 15% (low dose) and 44% (high dose) in the contralateral side; no significant reduction was observed in the substantia nigra. These results suggest that a significant number of ventral tegmental D_1-like receptors are localized on afferent terminals from the medial prefrontal cortex. © 1997 Elsevier Science B.V.

Keywords: Dopamine D_1 receptor; Medial prefrontal cortex; Substantia nigra; Ventral tegmental area

1. Introduction

Repeated exposure to amphetamine results in a progressive increase in its locomotor activating effect, a phenomenon known as behavioral sensitization (Kalivas and Stewart, 1991). Although the exact neural mechanism(s) involved in the development of amphetamine sensitization remain(s) to be determined, several studies suggest that activation of ventral mesencephalic dopamine D_1-like receptors is important (see Vézina, 1996). The failure to observe dopamine D_1 receptor mRNA expression in the ventral mesencephalon (Mengod et al., 1992), suggests that dopamine D_1 receptors are located on afferent terminals to this region, possibly originating from the medial prefrontal cortex. This hypothesis is supported by previous studies showing that some medial prefrontal cortex neurons project to the ventral tegmental area (Sesack and Pickel, 1992) and that a significant number of prefrontal cortex neurons contain dopamine D_1 receptor mRNA (Gaspar et al., 1995; Lu et al., 1997). The presence of dopamine D_1-like receptors on medial prefrontal cortex afferents in the ventral tegmental area would be consistent with the demonstration that destruction of neuronal cell bodies in the prefrontal cortex attenuates the development of amphetamine sensitization (Wolf et al., 1995). Consequently, this study was aimed at determining the effect of excitotoxic lesions of the medial prefrontal cortex on dopamine D_1-like receptors in the ventral tegmental area and in the substantia nigra.

2. Materials and methods

2.1. Animals

Male Wistar rats (Charles River, St. Constant, Québec, Canada) weighing 300–350 g were used. Animals were housed two per cage with free access to food and water in a temperature- and humidity-controlled room with a 12 h light/dark cycle (lights on at 06.30 h).

2.2. Surgery

Animals were injected with atropine methylnitrate (0.4 mg/kg, i.p.), anesthetized 20 min later with sodium pentobarbital (65 mg/kg, i.p.) and then placed in a stereotaxic frame. The surface of the cranium was exposed and the bone and dura above the left medial prefrontal cortex were removed. 1 μl of 0.9% saline containing 2 or 10 μg of ibotenic acid (Research Biochemicals International, Natick, MA, USA) or the vehicle alone was unilaterally

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injected at three different anterior–posterior locations (spaced 0.75 or 0.5 mm apart) between 3.25 and 4.5 mm anterior to bregma, 0.5 mm lateral to midline and 4.5 mm ventral to the surface of the cranium (Paxinos and Watson, 1986). In a first group of animals, 1 μl of 10 μg ibotenic acid was injected using a 5 μl Hamilton syringe. This procedure resulted in large non-specific damage to the neural tissue that often spread to the contralateral side. In the following experiments, 1 μl of 2 μg ibotenic acid (or its vehicle) was injected using a glass micropipette with the tip broken down to 35–45 μm. The solution was injected over a period of 6 min using a microinfusion pump to activate the Hamilton microsyringe connected to the micropipette with a polyethylene tubing; the pipette was moved up by 0.5 mm after injection of 0.5 μl and was left in place for an additional minute after the injection. Ten days after injection, animals were killed by decapitation, the brains removed and blocked into the forebrain and the midbrain, which were stored in 10% formalin and at −80°C, respectively. Coronal sections (40 μm) of the medial prefrontal cortex were cut on a cryostat and subsequently stained with a formal–thionin solution and the extent of the lesions was determined under light microscopic examination. 20 μm cryostat sections containing the substantia nigra and ventral tegmental area were taken from the midbrain block, thaw mounted onto gelatin coated slides and kept at −80°C until processed for quantitative ligand binding autoradiography.

2.3. Receptor autoradiography

Dopamine D_1-like receptors were visualized using the selective dopamine D_1 receptor ligand R(+)-8 [^{125}I]iodo-7-hydroxy-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepine ([^{125}I]SCH23982, 2200 Ci/mmol, DuPont, Boston, MA, USA). Sections were incubated for 30 min at 25°C in 50 mM Tris–HCl buffer containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$ and 1 mM $MgCl_2$. They were then incubated at 25°C for 60 min in the same buffer in the presence of 0.1 nM [^{125}I]SCH23982 and 100 nM mianserin (Research Biochemicals International) to block binding to 5-HT2 receptors. Non-specific binding was determined in adjacent sections incubated with the radioligand but in the presence of 30 μm (±)-1-phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8-diol hydrochloride ((±)-SKF38393, Research Biochemicals International). Following incubation, sections were washed with ice-cold buffer (3 × 10 min), air dried, then apposed to ^{3}H-sensitive

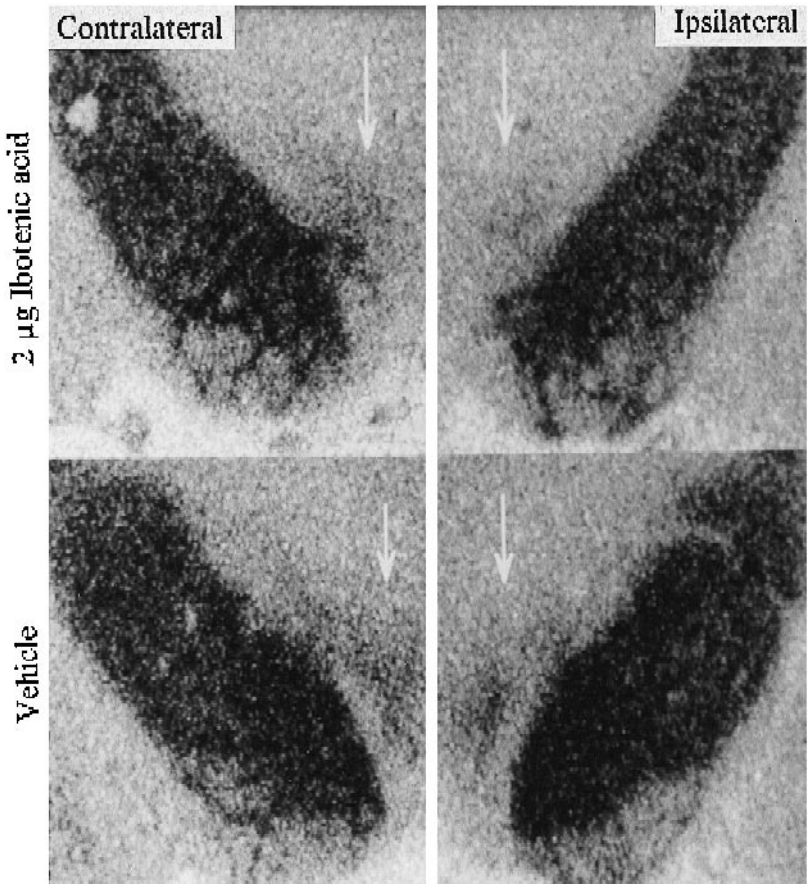

Fig. 1. Computer-generated autoradiogram illustrating D_1-like receptor binding in the ventral midbrain of one lesioned (2 μg ibotenic acid, top panel) and one vehicle treated (bottom panel) animal. The side ipsilateral to the injection is shown on the right of each panel and the arrows point to the ventral tegmental area in which a clear decrease in binding can be observed.

film (Amersham, Amersham, UK) together with iodinated standards (Microscales, Amersham). Autoradiographic exposures lasted 3–5 days and the films were developed in D-19 (Kodak, Rochester, NY, USA) for 4 min at 19°C. [^{125}I]SCH23982 binding was quantified in the ventral tegmental area and the substantia nigra (boundaries as defined by Paxinos and Watson, 1986) of 6 control and 5 (high dose) and 8 (low dose) lesioned animals. Quantitative measurements were obtained with a microcomputer-based image analysis system (MCID, Imaging Research). Standard curves were generated from [^{125}I]microscales and were used to convert density values into fmol/mg of protein assuming a 30% protein content in the tissue. Multiple readings were made for each region (5–6 sections for each brain region per animal) and non-specific binding was determined in adjacent sections.

3. Results

Histological analysis revealed that the low neurotoxin dose produced selective decreases in cell bodies over a region extending from the anterior portion of the medial prefrontal cortex to the rostral pole of the cingulate cortex, being mainly restricted to the cingulate and infralimbic cortices; no apparent damage was found in the cortical tissue contralateral to the injection site. The higher dose produced non-specific destruction of the tissue within a similar anterior–posterior axis as the low dose, with damage, however, often spreading to the most medial portion of the contralateral hemisphere.

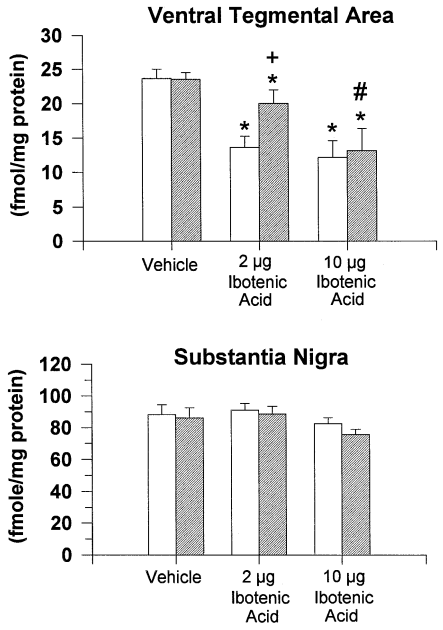

Fig. 2. Mean [^{125}I]SCH23982 binding measured in the ipsi- (white bars) and contralateral (striped bars) ventral tegmental area (top panel) and substantia nigra (bottom panel) in control ($n = 6$); 2 µg ibotenic acid ($n = 8$) and 10 µg ibotenic acid-lesioned ($n = 5$) animals. For the ventral tegmental area, a one way analysis of variance yielded a significant effect of treatments ($F_{(2,16)} = 7.6$, $P < 0.01$) and a Newman–Keuls post-hoc test revealed a significant difference ($P < 0.01$) between vehicle and lesioned animals (*), between the ipsi- and contralateral side of lesioned animals ($+$) and between small and large lesion groups but only on the contralateral side (#). For the substantia nigra, the analysis of variance yielded no effect of treatment ($F_{(2,16)} = 1.2$, $P = 0.32$) or of treatment versus side interaction ($F_{(2,16)} = 2.1$, $P = 0.14$).

Computer-generated autoradiograms illustrating dopamine D_1-like receptor binding in the ventral tegmental area of one lesioned (2 µg ibotenic acid, top panel) and one vehicle injected (bottom panel) animal, are shown in Fig. 1. A reduced density of receptor binding can be observed in the lesioned side (top right panel) compared to the vehicle injected control (bottom right panel); binding was also slightly decreased in the contralateral side in the lesion animal (compare top left with bottom left panel).

The mean density of dopamine D_1-like receptor binding measured in the three groups of animals is shown in Fig. 2. The low dose lesions produced a 43% and 15% reduction in receptor binding in the ipsi- and contralateral sides, respectively (top panel), that was statistically significant ($P < 0.05$) when compared to the control group. The large lesions resulted in a reduction of more than 40% in the number of receptors on both sides. It is noteworthy that the extent of reduction in binding in the ipsilateral ventral tegmental area was similar with the two types of lesions. In contrast, no significant change in the density of binding sites was found in the substantia nigra (Fig. 2, bottom panel).

4. Discussion

The present results show that destruction of neuronal cell bodies in regions of the medial prefrontal cortex known to contain dopamine D_1 receptor mRNA produces a significant reduction in dopamine D_1-like binding sites in the ipsilateral ventral tegmental area but not into the substantia nigra. Moreover, a small but statistically significant reduction was observed in the ventral tegmental area on the side contralateral to the lesion. The most obvious explanation of the present findings is that a certain population of dopamine D_1-like receptors in the ventral tegmental area are located on afferent terminals from the medial prefrontal cortex. This hypothesis is consistent with the presence of a significant efferent projection from the medial prefrontal cortex to the ventral mesencephalon (Sesack et al., 1989) and that 37% of prefrontal cortical neurons that project to the ventral tegmental area express dopamine D_1 receptor mRNA (Lu et al., 1997). Since dopamine D_1 receptor mRNA expression has not been found in the ventral tegmental area (Mengod et al., 1992) dopamine D_1 receptors appear to be located only on afferent nerve terminals in this region. Although the results obtained with the high dose injection may be less reliable due to its highly unspecific nature, they provide additional interesting insights. The magnitude of the decrease in dopamine

D_1-like receptors observed in the ipsilateral ventral tegmental area in these animals was very similar to that found in animals with smaller lesions suggesting a floor effect and that around 40% of dopamine D_1-like receptors in the ventral tegmental area are localized on afferents from medial prefrontal cortex neurons. This reinforces the hypothesis that the reduction in dopamine D_1-like receptors is specifically due to a destruction of the medial prefrontal cortical efferents and not to its afferents. The larger decrease in dopamine D_1-like receptor binding observed in the contralateral ventral tegmental area with the high dose lesion is likely explained by spreading of the lesion to the contralateral hemisphere. The present results also show that very few dopamine D_1-like receptors in the substantia nigra are located on prefrontal cortex afferent terminals, a finding consistent with the virtual absence of medial prefrontal afferents to this region (Sesack et al., 1989).

On the basis of recent findings, it is tempting to propose that a significant amount of dopamine D_1-like receptors in the ventral tegmental area are located on prefrontal cortex afferent containing glutamate and/or aspartate. Indeed, there is evidence that the ventral tegmental area receives excitatory amino acid innervation that is regulated by dopamine D_1-like receptors located on their terminals since the activation of dopamine D_1-like receptors in this region increases extracelluar levels of glutamate (see Kalivas and Duffy, 1995). Furthermore, the location of dopamine D_1-like receptors on prefrontal cortex afferents could explain why the destruction of prefrontal cortex neurons attenuates amphetamine sensitization (Wolf et al., 1995) and why NMDA antagonists block the development of this sensitization (Karler et al., 1989).

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