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ADAPTIVE CHANGES IN THE EXPRESSION OF CENTRAL OPIOID RECEPTORS IN MICE LACKING THE DOPAMINE D2 RECEPTOR GENE

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Abstract—On the basis of numerous studies that have described interactions between the dopaminergic and opioidergic systems, we have investigated whether genetic deletion of dopamine D2 receptors (D2R) might influence the expression of central opioid receptors. The levels of mu, delta, kappa and nociceptin opioid peptide receptors were determined in the brains and spinal cords of D2R knockout mice using quantitative autoradiography. The significant changes in opioid receptor binding found in the brains of heterozygous and homozygous mice were mainly restricted to the basal ganglia. In homozygous mice, a down-regulation of mu and delta receptors was observed in the striatal and pallidal areas. This alteration may be an adaptive response to the increase in enkephalin levels previously described in the striatum of these mutant mice. On the contrary, an up-regulation of kappa receptors was found in the striatal and nigral regions and might be related to a change in dynorphin levels. Significant increases in nociceptin receptor binding were also observed in homozygous mice in brain areas involved in motor behavior. At the spinal level, only kappa and nociceptin receptor binding showed significant overall differences between genotypes. The functional consequences of these adaptive changes are discussed in relation to the findings of behavioral and neurochemical studies reported to date in D2R knockout mice.

Key words: dopaminergic system, opioidergic system, knockout mice, basal ganglia, autoradiography.

There is considerable evidence showing that the dopaminergic and opioidergic systems interact reciprocally at multiple levels. Thus, in the projection neurons of the dorsal and ventral striatum, dopamine D1- and D2-like receptors regulate the expression of genes coding for the precursors of opioid peptides, dynorphins and enkephalins (Gerfen et al., 1990). More precisely, stimulation of D1 receptors increases the levels of preprodynorphin mRNA in the striatonigral neurons (which constitute the direct striatal pathway) whereas activation of D2 receptors decreases preproenkephalin mRNA expression in the striatopallidal neurons (which correspond to the first segment of the indirect pathway). Conversely, stimulation of mu or delta opioid receptors increases dopamine release in the striatum (preferentially in the nucleus accumbens, the main part of the ventral striatum) whereas activation of kappa opioid receptors decreases dopamine release in this region (Di Chiara and Imperato, 1988; Spanagel et al., 1992). These opposing effects on dopamine transmission are thought to mediate, at least in part, the rewarding and hyperlocomotor properties of mu or delta agonists on the one hand, and the aversive and hypolocomotor properties of kappa agonists on the other (Kalivas et al., 1983; Spanagel and Weiss, 1999; Laviolette et al., 2004). In particular, it has been shown that blockade or genetic disruption of D2 receptors suppressed the rewarding effects of morphine (Maldonado et al., 1997; Manzanedo et al., 2001; Elmer et al., 2002).

Nociceptin, also called orphanin Q (N/OFQ), has been recognized as a member of the opioid peptide family (Meunier et al., 1995; Reinscheid et al., 1995). This neuropeptide, which shares structural homology with dynorphin, has been identified as the endogenous ligand for the nociceptin opioid receptor, cloned soon after that of the traditional opioid receptors. Interestingly, several studies have reported interactions between N/OFQ and dopamine. Thus, N/OFQ decreases extracellular dopamine levels in the nucleus accumbens (Murphy et al., 1996; Koizumi et al., 2004) and suppresses morphine- and cocaine-induced mesolimbic dopamine release and reward (Di Giannuario et al., 1999; Lutfy et al., 2001; Sakoori and Murphy, 2004), similarly to kappa agonists (Funada et al., 1993; Mori et al., 2002).

Another example of an interaction between the dopaminergic and opioidergic systems concerns analgesia. Thus, it has been demonstrated that D2 receptors tonically inhibited opioid analgesia (King et al., 2001). Indeed, genetic deletion of D2 receptors potentiates nociceptin receptor-mediated analgesia at spinal level as well as mu and kappa receptor-mediated analgesia at spinal and supraspinal levels, but does not modify the analgesic response of delta opioid agonists.

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Abbreviations: DAMGO, D-Ala^2-N-methyl-Phe^4-Gly-ol^5 enkephalin; D2R, dopamine D2 receptor; N/OFQ, nociceptin/orphanin Q; [^{3}H]Cl-977, (−) N-methyl-N-[7-(1-pyrrolidinyl)-1-oxaspiro[4,5]dec-8-yl]-4-benzofuranacetamide; [^{3}H]DELT I, [3,5-^{3}H-Tyr]D-Ala-Phe-Asp-Val-Val-Gly-NH_2.

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In the present study, we have used mice lacking the D2 receptor gene in order to further explore the interactions between the dopaminergic and opioidergic systems. The aim of our study was to investigate whether the deletion of D2 receptors might lead to adaptive changes in the expression of central opioid receptors. We have performed a complete quantitative autoradiographic mapping using selective ligands for mu, delta, kappa and nociceptin opioid receptors in the brains and spinal cords of wild-type (dopamine D2 receptor (D2R) +/+), heterozygous (D2R+/−) and homozygous (D2R−/−) mice deficient in D2 receptors.

EXPERIMENTAL PROCEDURES

Animals

Mice lacking the D2 receptor gene were generated as described in details elsewhere (Jung et al., 1999). Briefly, the majority of exon 2 was replaced in mouse embryonic stem cells (129/SvEv). The chimeric male mice obtained were then bred with C57Bl6/J females. Homozygous, heterozygous and wild-type mice were produced by cross-breeding of heterozygous mutants. All experiments were performed in accordance with the guidelines of the Institutional Care and Use Committees of the University of Medicine and Dentistry of New Jersey and the National Institutes of Health and all efforts were made to minimize the number of animals used and their suffering.

Materials

[^{3}H] DAMGO (D-Ala^2-N-methyl-Phe^4-Gly-ol^5 enkephalin, 2.59 TBq/mM), [^{3}H]CI-977 ((−) N-methyl-N-[7-(1-pyrrolidinyl)-1-oxaspiro[4,5]dec-8-yl]-4-benzofuranacetamide, 1.52 TBq/mM) and [^{3}H] nociceptin (6.18 TBq/mM) were purchased from Amersham Life Sciences (Buckingham, UK). [^{3}H]DELT I ([3,5-^{3}H-Tyr]D-Ala-Phe-Asp-Val-Val-Gly-NH_2; 1.85 TBq/mM) was custom synthesized by Zeneca (Cheshire, UK). [^{3}H] raclopride (78.4 Ci/mmol) was obtained from New England Nuclear (Boston, MA, USA). Naloxone and sulpiride were purchased from Sigma (Dorset, UK). Unlabeled nociceptin was obtained from Bachem (St. Helens, UK).

Autoradiographic procedures

Adult male mice (25–30 g) were killed by cervical dislocation, and their brains and spinal cords were rapidly removed, immediately frozen in isopentane and stored at −80 °C until sectioning in a cryostat (Zeiss Microm 505E) at −20 °C. Sequential coronal sections of 20 μm thickness were thaw-mounted onto gelatin-coated slides and stored at −20 °C before use. Determination of total binding for mu, delta, kappa and nociceptin opioid receptors was performed using 4 nM [^{3}H]DAMGO, 7 nM [^{3}H]DELT I, 2.5 nM [^{3}H]CI-977 or 0.4 nM [^{3}H] nociceptin, respectively. Non-specific binding was defined in adjacent sections in presence of naloxone (1 μM for mu and kappa binding or 10 μM for delta binding) or unlabeled nociceptin (1 μM) for nociceptin binding. The binding experiments were conducted as already described in detail previously (Kitchen et al., 1997; Slowe et al., 2001). Labeling of D2-like receptors was also performed using [^{3}H] raclopride (Léna et al., 2004). Sections were first prewashed for 20 min in 50 mM Tris buffer, pH 7.4, containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$ and 1 mM $MgCl_2$ at room temperature. Slides were then incubated for 60 min in the same buffer with 4 nM [^{3}H] raclopride. Non-specific binding was determined in adjacent sections in the presence of 10 μM sulpiride. After incubation, slides were washed (6×1 min) in ice-cold 50 mM Tris buffer (pH 7.4), then dipped into ice-cold distilled water.

Brain sections were then apposed to tritium sensitive film (Hyperfilm, Amersham, UK) for a period of 3 (mu, delta and nociceptin receptors), 6 (kappa receptors) or 10 (D2 receptors) weeks. Spinal cord sections were apposed for a period of 8 (nociceptin receptors), 10 (D2 receptors), 11 (mu receptors), 14 (delta receptors) or 18 (kappa receptors) weeks. Films were developed in 50% Kodak D19 developer. Quantitative analysis of autoradiograms was carried out by video-computerized densitometry using an MCID image analyzer (Imaging Research, Canada) as previously described (Kitchen et al., 1997) and brain structures were identified using the mouse brain atlas of Franklin and Paxinos (1997). Brain or spinal cord sections from D2R+/+, +/− and −/− animals were processed simultaneously in a completely paired protocol such that identical radioligand solutions were used between genotypes and that apposition to the same tritium-sensitive film and parallel image analysis could be performed.

Statistical analysis

Data were analyzed using two-way ANOVA with genotype and regions as independent factors. Except for D2 receptors, a separate two-way ANOVA analysis of cortical and non-cortical regions was performed. Comparisons of specific binding in each region between the three genotypes were carried out using Fisher's post hoc test.

RESULTS

D2 receptors

The quantitative distribution of D2-like receptors in the brains and spinal cords of D2R+/+, +/− and −/− mice is shown in Table 1. In D2R+/+ brains, the highest levels of [^{3}H] raclopride binding were seen in the caudate-putamen, olfactory tubercles, nucleus accumbens and glomerular layer of the olfactory bulb. Moderate to low binding was observed in the lateral septum, ventral tegmental area, ventral pallidum, globus pallidus and inferior colliculus. Very low binding was found in the other brain structures examined and those in which specific binding was inferior to 7 fmol/mg tissue were omitted in Table 1. Two-way ANOVA analysis of these data indicated significant effects of region and genotype ($P<0.0001$ for both). As expected, there was an overall 46% decrease in D2-like binding sites in D2R+/− brains compared with wild-type mice. In D2R−/− brains, [^{3}H] raclopride binding was not detectable in most of the structures studied except, at a very low level, in the nucleus accumbens, olfactory tubercles and substantia nigra (cf. Table 1), as well as, at a higher level, in the minor (48.1±3.4 fmol/mg tissue) and major (25.0±0.9 fmol/mg tissue) Islands of Calleja, regions known to contain high density of D3 receptors (Levesque et al., 1992) (Fig. 1).

In the spinal cords of wild-type mice, the densest labeling of D2-like receptors was localized in the superficial layers of the dorsal horn at cervical, thoracic and lumbar levels (Table 1) as described in previous autoradiographic studies (Bouthenet et al., 1987; Levant and McCarson, 2001). In the other layers, [^{3}H] raclopride binding was very low (between 1.9 and 3.9 fmol/mg tissue). Similarly to the brains, D2-like binding sites decreased significantly by about twofold in the spinal cords of heterozygous mice