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Research report

Rats bred for enhanced apomorphine susceptibility have elevated tyrosine hydroxylase mRNA and dopamine D₂-receptor binding sites in nigrostriatal and tuberoinfundibular dopamine systems

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Abstract

From a Wistar population two rat lines were generated using as criterion the behavioral response to the dopamine agonist apomorphine. Rats of the apomorphine-susceptible (apo-sus) line revealed a vigorous gnawing response to apomorphine administration while the other rat line, the apomorphine-unsusceptible (apo-unsus) line, was selected for lack of response to the drug. In the present study using the 12th and 13th generation of these genetically selected lines, we have investigated whether this difference in apomorphine responsiveness was correlated with changes in dopamine neurochemistry. Therefore, we measured tyrosine hydroxylase (TH), the rate limiting enzyme in dopamine synthesis, as well as dopamine D₁ and D₂ receptor mRNA levels in discrete brain regions by *in situ* hybridization. Dopamine (D₂/D₃) receptor binding was assessed with [¹²⁵I]iodosulpride in a membrane binding assay and by quantitative autoradiography on tissue sections. [³H]SCH 23390 was used to analyze D₁ receptor binding. Apo-sus rats displayed significantly higher TH mRNA levels in the A₉ cell group of the substantia nigra pars compacta and in the A₁₂ cell group of the arcuate nucleus. No difference was found in the A₁₀ cell group of the VTA and the A₆ cell group of the locus coeruleus. The density of D_{2/3} binding sites as well as D₁ receptor mRNA levels in the striatal projection area of the A₉ substantia nigra neurons, were significantly elevated in apo-sus rats. Dopamine D₂ receptor mRNA and D₁ receptor binding levels in caudate putamen and nucleus accumbens, however, were similar in rats of both lines. In conclusion, high apomorphine susceptibility is related to a potentially enhanced dopamine responsiveness selective for the nigrostriatal and tuberoinfundibular pathways.

Keywords: Stress; HPA axis; Dopamine system; Selection line; Corticosteroid receptor; Free corticosterone

1. Introduction

Rats of a normal population show remarkable individual differences in physiological and behavioral responses to environmental stimuli. During the past years numerous attempts were made to explore possible mechanisms underlying these individual differences. Some groups compared different strains, Lewis and Fisher rats [27,28], while others used the psychogenetically selected Roman High Avoidance (RHA) and Roman Low Avoidance (RLA) rats

[2–4]. For our study, rats of a normal outbred Wistar population were selected on the basis of high or low behavioral responsiveness to the dopamine agonist apomorphine which was injected subcutaneously at a dose of 1.5 mg/kg BW [6]. Rats which showed a high stereotypic gnawing score in response to apomorphine were thus referred to as apomorphine-susceptible (apo-sus) rats as opposed to rats that showed a minimal gnawing response, the apomorphine-unsusceptible (apo-unsus) rats [6].

In 1985, a breeding program was started with both groups of rats resulting in two pharmacogenetically selected rat lines. Further characterization revealed that apo-sus rats are not only more susceptible to apomorphine but also to phenylephrine, a noradrenergic agonist. This en-

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hanced phenylephrine responsiveness is accompanied by a reduced concentration of noradrenaline in the nucleus accumbens measured with immunostaining [6].

Additional studies showed a marked difference in behavioral responses between rats of both lines. The presence of an aggressive rat in a defeat test triggered in apo-sus rats a fleeing rather than a freezing response as displayed by apo-unsus rats. Furthermore, exposure to novelty resulted in a 50% longer track distance and thus a higher locomotor activity in apo-sus rats. These behavioral differences in response to stress were accompanied by distinct differences in stress-induced activity of the hypothalamic-pituitary-adrenal (HPA) system. Apo-sus rats showed a prolonged and enhanced novelty-induced activation of the HPA system, due to corticosteroid feedback resistance and/or an enhanced central drive [22,29].

Since the pharmacogenetic selection was based on susceptibility to apomorphine we postulated that apo-sus rats displayed neurochemical modifications in central dopamine systems. In order to test this hypothesis we compared the following parameters for the activity of the major central dopamine systems. First, tyrosine hydroxylase (TH) mRNA levels in A₉, A₁₀ and A₁₂ dopaminergic cell bodies and A₆ noradrenergic cell bodies were measured using a quantitative *in situ* hybridization procedure. Second, dopamine D₁ as well as D_{2/3} receptor binding sites were determined in the terminal areas of the dopaminergic pathways with quantitative receptor autoradiography and a membrane binding assay. Third, D₁ and D₂ mRNA levels were determined in these brain areas using *in situ* hybridization.

Results of the present study demonstrate that apo-sus rats are marked by significantly higher TH mRNA, D₁ receptor mRNA and D_{2/3} receptor binding sites, selectively in the dopaminergic A₉ nigrostriatal and A₁₂ tuberoinfundibular pathways but not in the A₁₀ mesolimbic pathway in comparison with apo-unsus rats.

2. Materials and methods

2.1. Animals

Adult male Wistar rats of two pharmacogenetically selected rat lines, weighing 120–140 g at arrival, were obtained from the Department of Psycho- and Neuropharmacology of the University of Nijmegen, The Netherlands. Selection of the two rat lines was previously made on the basis of their behavioral responsiveness to the dopamine agonist apomorphine [6]. One rat line is susceptible for apomorphine, apo-sus line, and thus shows a high gnawing score. In contrast, rats of the other line (apo-unsus rats), show only a minimal gnawing response and are thus apomorphine-unsusceptible.

The selection procedure is described in detail by Cools et al. [6]. Briefly, a group of 60 male and 60 female rats of a normal Wistar population was injected with 1.5 mg/kg

apomorphine s.c. which induced a stereotypic gnawing behavior. These rats were divided into three groups according to their gnawing response; group A consisted of rats with a low gnawing score (less than 10 per 45 min); the apo-unsus rats (27% of the original population); group B consisted of rats with a high gnawing score (more than 500 per 45 min); the apo-sus rats (23% of the original population); and group C which consisted of rats with a score of 10–500 per 45 min. Nine pairs of rats of group A and nine pairs of rats of group B were used to start the two breeding lines. After weaning, at the age of 30 days, males and females were separated and grouped together (two or three animals per cage per sex per selection line). Retention of genetic selection was tested in rats of the first litter of each generation. At the age of 60 days, these rats were injected with apomorphine and tested in the same procedure as described above. Apo-unsus rats with the lowest scores and apo-sus rats with the highest scores were selected and used for subsequent development of the next generation, brother-sister crossing was not allowed. Rats of the second and the third litter of each generation were used for experiments. In the present experiments rats of the 12–13th generation were used. Mean gnawing scores of the first litters of these generations were, 8 ± 4.5 for apo-unsus and 807 ± 100 for apo-sus rats. Rats were housed under controlled conditions of temperature (21°C) and lighting (12 h light/12 h dark cycle, lights on at 07.00 h) with food and water available *ad libitum*.

2.2. Tissue preparation

Apo-sus and apo-unsus rats (7–8 weeks of age) were killed by decapitation between 09.00 and 13.00 h. For the *in situ* hybridization and *in vitro* autoradiography experiments brains were frozen on dry ice, sectioned in a cryostat (coronal plane, 20 μ m) and mounted on poly L-lysine (Sigma) coated slides.

For the striatal membrane preparation, brains were rapidly removed and the striatum was dissected bilaterally according to the method described previously [9].

2.3. *In situ* hybridization

2.3.1. Tyrosine hydroxylase mRNA

A synthetic oligonucleotide DNA probe encoding rat tyrosine hydroxylase (TH, 45 nucleotides: 1442–1487) was prepared on an Applied Biosystems DNA synthesizer. The probe was 3' end labelled using α -[³⁵S]deoxyadenosine triphosphate (specific activity greater than 1000 Ci/mmol, Amersham) and terminal deoxynucleotidyl-transferase (Boehringer Mannheim). The hybridization procedure as described by Nicot et al. [17] was used. The control study consisted of an RNase A digestion (20 μ g/ml) prior to hybridization with the probe.

Sections taken at the hypothalamic, mesencephalic and locus coeruleus level [19] were fixed with 4% formal-

dehydrate in phosphate-buffered saline, pH 7.4, for 10 min, rinsed twice in PBS (phosphate-buffered saline) and once in $4 \times$ SSC. Subsequently, the sections were placed in 0.25% acetic anhydride in 0.1 M triethanolamine (TEA) for 10 min to reduce nonspecific binding. This prehybridization procedure was completed with a rapid wash with water and dehydration in ethanol and chloroform. The sections were subsequently air-dried. The probe was diluted in a hybridization buffer containing 50% formamide, $4 \times$ SSC, $1 \times$ Denhardt, 1% sarcosyl, 10 mM dithiothreitol, 0.1 M potassium phosphate at pH 7.4, 250 μ g/ml yeast tRNA, 250 μ g/ml herring sperm DNA and 100 μ g/ml polyadenylic acid. Hybridization buffer (85 μ l) was applied to each slide and covered with a coverslip which was sealed with rubber cement. For overnight hybridization, which was performed at 42°C, the slides were placed into moist containers in order to prevent dehydration. The next day the coverslips were removed and the sections were washed at 45°C in $4 \times$ SSC containing 0.02% SDS for 30 min, twice in $1 \times$ SSC for 30 min and two times in $0.1 \times$ SSC each for 30 min. Finally, the sections were dehydrated in ethanol and air-dried before being exposed to a β_{\max} hyperfilm (Amersham, France) for 14 days at room temperature.

2.4. D_1/D_2 dopamine receptor mRNA

Different RNA probes were transcribed in vitro from the full-length receptor cDNA encoding D_1 and D_2 dopamine receptor, which had been subcloned into pBlue-script II KS (+) using appropriate polymerase chain reaction (PCR) primers according to the sequences published by Bunzow et al. [5] and Zhou et al. [30], PCR-amplified with the specific primers flanking the T7 and SP6 RNA polymerase promoters, gel purified and used as a template for in vitro transcription. After transcription the template was degraded with RNase-free DNase I. The [35 S]UTP (Amersham, specific activity greater than 1000 Ci/mmol) labelled anti-sense cRNA probes were synthesized according to the protocol provided by the manufacturer of the T7/SP6 RNA polymerases (Promega, Madison, WI, USA) with 200 ng of PCR fragment as the template. Sense cRNA probes were used as a control for labelling specificity. Preceding the hybridization step sections were fixed, treated with acetic anhydride, dehydrated and defatted as described before [12]. Sections were hybridized with the [35 S]UTP-labelled probe ($1 \cdot 10^6$ cpm per slide) complementary to brain tissue D_1 or D_2 mRNA. After overnight hybridization at 50°C sections were washed, incubated with RNase A, air-dried and exposed to Kodak X-OMAT films for 14 days.

2.5. D_1 receptor binding

Dopamine D_1 receptor binding sites were analyzed using in vitro autoradiography as described earlier [13].

Slide-mounted sections were incubated with 2.0 nM [3 H]SCH 23390 (Amersham specific activity 83 Ci/mmol) in 50 mM Tris buffer pH 7.5 at 25°C, containing 0.1% ascorbic acid, 120 mM NaCl, 5 mM κ Cl, 1 mM CaCl_2 and 1 mM MgCl_2 . As SCH 23390 has been reported to bind serotonergic sites, 1 μ M mianserin was added to the incubation buffer to block these serotonergic sites. Following a 60-min incubation period at room temperature, the slides were drained, dipped in distilled water (4°C), washed two times for 10 min in 50 mM Tris, pH 7.6 at 4°C, and subsequently briefly dipped two times into distilled water (4°C). Finally the sections were rapidly air-dried using a portable hair dryer set to 'cool'. Non-specific binding was evaluated by treating a parallel set of slides with the same concentration of tritiated ligand with a 10^{-5} M final concentration of unlabelled piflutixol. Sections were exposed to a 3 H-hyperfilm (Amersham) at room temperature for 14 days.

2.6. Dopamine D_2/D_3 receptor binding

2.6.1. Membrane binding assay

Striatal tissue was collected in 10 ml of ice-cold 50 mM Tris buffer at pH 7.4 and homogenized using a polytron homogenizer for 10 s. The crude homogenates were centrifuged for 35 min at 60,000 g. The supernatant was discarded and the pellet re-homogenized with the polytron homogenizer in 10 ml of 50 mM Tris buffer. After centrifugation for 35 min at 60,000 g, the supernatant was removed and the pellet re-suspended in 2.5 ml Tris buffer. Aliquots of 250 μ l containing ± 600 μ g protein were rapidly frozen in liquid nitrogen. For the apo-sus and apo-unsus rats two and three different pools of striatal membranes of five rats, respectively, were used.

Striatal membranes (20 μ g) were incubated in incubation buffer: 50 mM Tris-HCl buffer, pH 7.0, containing 120 mM NaCl, 5 mM KCl, 1 mM CaCl_2 , 1 mM MgCl_2 , 5.7 mM ascorbic acid and 0.1 nM [125 I]iodosulpride (specific activity greater than 2000 Ci/mmol, Amersham) in a final volume of 100 μ l for 75 min at room temperature. Non-specific binding was determined after addition of 1 μ M haloperidol. Labelled membranes were recovered by centrifugation at 12,000 rpm at 4°C. After removing the supernatant, the pellet was washed in 500 μ l of the incubation buffer and another centrifugation step (12,000 rpm, 4 min at 4°C) was subsequently performed. Finally the pellet was counted in a gamma counter after removal of the supernatant. The amount of bound radioligand was less than 5% of the total radioactivity added. Protein content of membrane suspensions was determined by a BIO-RAD protein assay.

2.6.2. In vitro autoradiography

Dopamine $D_{2/3}$ binding sites have been studied using in vitro autoradiography as described earlier [24]. Briefly, 20 μ m cryostat sections, at striatal and mesencephalic

levels according to the atlas of Paxinos and Watson [19], were incubated for 30 min at room temperature with 150 μ l of 0.1 nM 125 I-labelled idosulpride per section in the same incubation medium as used in the membrane binding assay. For the non-specific binding, 2 μ M haloperidol was added to the incubation medium. After incubation, the sections were washed 2 times, each for 4 min, with the incubation medium, one time with autoclaved millipore water at 4°C and then air-dried. Sections were exposed to a β_{max} hyperfilm (Amersham) at room temperature, for 4 days in case of striatal sections and 15 days for the mesencephalon sections.

2.7. Computer-assisted image analysis

Optical density of the autoradiograms was quantified with a computerized image analysis system HistoRAG, BIOCOM (Les Ulis, France) [21]. The film background was subtracted after shading correction. Quantification occurred on the basis of a standard curve calculated from a set of 125 I-microscales for [125 I]idosulpride binding, and 3 H-microscales for [3 H]SCH 23390 binding. For 35 S-labelled probes 14 C-microscales, which are comparable with 35 S-scales, were used (Amersham, United Kingdom). From each brain, 10 sections were quantified by outlining the area of interest.

2.8. Statistical analysis

The paired Student's *t*-test was applied for statistical evaluation of differences in TH, D_1 , D_2 mRNA and dopamine D_1 and D_2/D_3 receptor binding between rats of the two lines. Results are expressed as mean \pm SEM with *n* the number of animals used for statistical analysis.

3. Results

3.1. TH mRNA

In situ hybridization revealed that apo-sus rats contain significantly higher ($P < 0.01$) levels of TH mRNA in the substantia nigra pars compacta (A_9 cell body region) and the arcuate nucleus (A_{12} cell body region) as compared to the apo-unsus rats (Table 1) (28% and 52% increase in substantia nigra and arcuate nucleus, respectively). In contrast, the hybridization signal measured in the ventral tegmental area (VTA, the A_{10} dopamine cell group) and the locus coeruleus (A_6 noradrenergic cell group) did not show a significant difference in the two rat lines. Representative autoradiograms of sections hybridized with the 35 S-labelled TH oligonucleotide probe are shown in Fig. 1A (substantia nigra and VTA), 1B (arcuate nucleus) and 1C (locus coeruleus).

Table 1

TH mRNA levels in four catecholamine cell body regions of apo-sus and apo-unsus rats. Data are expressed as mean \pm S.E.M. in arbitrary units of optical density

	Apo-sus	Apo-unsus
Substantia nigra (A_9)	42.4 \pm 2.0 **	33.1 \pm 2.0
VTA (A_{10})	31.9 \pm 2.0	28.1 \pm 3.0
Locus coeruleus (A_6)	37.0 \pm 2.3	32.7 \pm 1.8
Arcuate nucleus (A_{12})	96.6 \pm 4.5 **	63.6 \pm 2.7

Ten sections per brain were measured. ** $P < 0.01$ apo-sus vs apo-unsus. A_9/A_{10} ($n = 13$ – 14 animals) and A_6/A_{12} ($n = 5$ – 6 animals) were measured in independent hybridization procedures.

3.2. Dopamine D_1 receptor binding

The density of D_1 binding sites was quantified in the medial and lateral part of the caudate-putamen and the nucleus accumbens, the projection areas of the nigrostriatal and mesolimbic dopamine pathways. Table 2 shows that no significant differences were found in [3 H]SCH 23390 binding between apo-sus and apo-unsus rats, in any of the fields measured.

3.3. D_1 receptor mRNA

Dopamine D_1 receptor encoding mRNA levels, however, were significantly higher in the lateral part of the caudate putamen of apo-sus rats compared with the same area of apo-unsus rats (33% increase) (Table 3). In the medial part adjacent to the lateral ventricle and in the nucleus accumbens no line differences in D_1 receptor expression were measured.

3.4. Dopamine D_2/D_3 receptor binding

3.4.1. Membrane binding

The specific and non-specific binding of [125 I]idosulpride, in a concentration range of 0–50 μ g/100 μ l buffer, to striatal membranes, was determined. Specific binding was found to be linear up to at least 50 μ g tissue protein in 100 μ l medium. For the subsequent experiments, we used 20 μ g protein in a total volume of 100 μ l. [125 I]idosulpride was used at a concentration of 0.1 nM, corresponding to 8–12% occupancy of the total number of binding sites as reported by Martres et al. [14]. At this concentration the specific binding of [125 I]idosulpride to striatal membrane homogenates was 4.21 \pm 0.5 and 2.22 \pm 0.59 fmol/mg protein in apo-sus and apo-unsus rats, respectively. Values were the mean of five independent experiments. The twofold higher [125 I]idosulpride binding in apo-sus rats was statistically significant ($P < 0.05$).

3.5. In vitro autoradiography

In vitro autoradiography of brain tissue sections was used to establish the specific localization of the difference

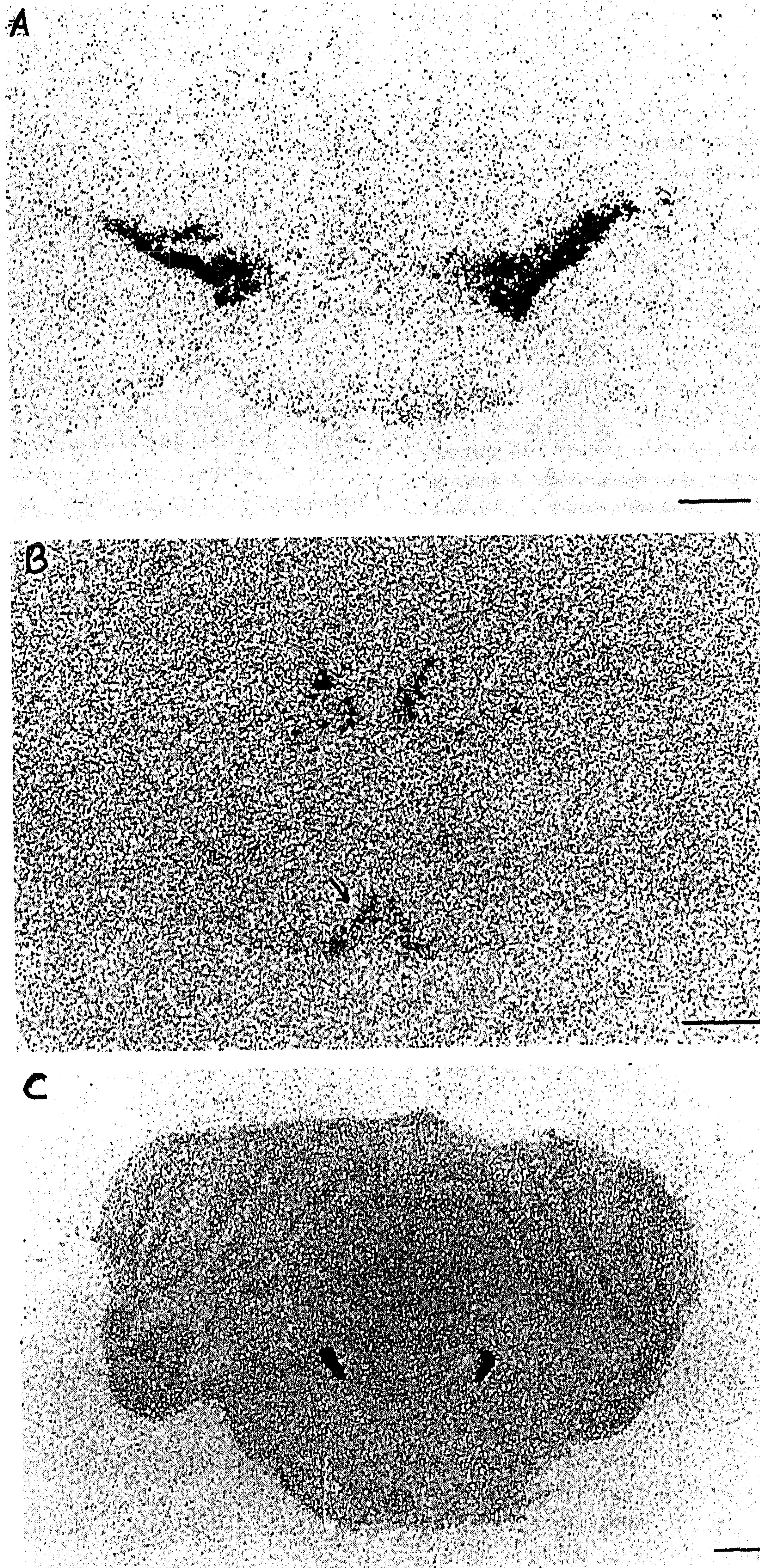


Fig. 1. Autoradiogram of mesencephalon (1A), arcuate nucleus (1B) and locus coeruleus (1C). Sections were hybridized with ³⁵S-labelled tyrosine hydroxylase oligonucleotide probes. Scale bar is equivalent to 500 μ M.

Table 2

Density of ^3H -SCH 23390 binding sites in different brain areas of apo-sus and apo-unsus rats

	Apo-sus	Apo-unsus
Lat. c. putamen	19.9 ± 0.5	19.2 ± 0.9
Med. c. putamen	21.3 ± 0.5	19.2 ± 1.0
Nucl. accumbens	18.9 ± 0.5	17.9 ± 0.6

Data represent mean ± S.E.M. labelling intensity in arbitrary units of optical density. A total of eight sections per rat were measured. $n = 5$ animals per group.

Table 3

Dopamine D1 receptor mRNA expression in apo-sus and apo-unsus rats

	Apo-sus	Apo-unsus
Lat. c. putamen	68.7 ± 1.5 **	51.5 ± 1.7
Med. c. putamen	40.1 ± 3.0	33.1 ± 3.7
Nucl. accumbens	18.6 ± 1.2	16.1 ± 1.0

Data represent mean ± S.E.M. labelling intensity in arbitrary units of optical density. A total of 10 sections per rat were measured. ** $P < 0.01$ apo-sus vs apo-unsus. $n = 4$ animals per group.

in striatal [^{125}I]iodosulpride binding. The density of binding sites was quantified in the nucleus accumbens, the medial and lateral area of the caudate-putamen, the olfactory tubercle and the substantia nigra. Fig. 2 shows the regional distribution of [^{125}I]iodosulpride binding sites at the level of the caudate-putamen at which their density was quantified. An uneven distribution pattern of these sites was shown with highest binding levels in the lateral part of this nucleus. Apo-sus rats showed an increased specific binding in the range of 30% in both subregions of the caudate-putamen in comparison with those of the apo-unsus rats (Table 4). In the nucleus accumbens, the olfactory tubercle and the substantia nigra pars compacta no significant differences in [^{125}I]iodosulpride binding were measured compared with apo-unsus rats.

Table 4

Density of [^{125}I]iodosulpride binding sites measured in different brain areas of apo-sus and apo-unsus rats

	Apo-sus	Apo-unsus
Lat. c. putamen	9.6 ± 1.6 **	6.4 ± 1.4
Med. c. putamen	5.1 ± 0.9 **	3.7 ± 0.9
Nucl. accumbens	3.0 ± 0.4	2.3 ± 0.7
Olf. tubercle	3.2 ± 0.6	2.8 ± 0.8
Sub. nigra	6.1 ± 0.4	5.7 ± 0.5

Data represent mean ± S.E.M. in arbitrary units of optical density. A total of 10–12 sections per rat was measured. ** $P < 0.01$ apo-sus vs apo-unsus. $n = 5$ –7 animals per group.

3.6. D_2 receptor mRNA

Dopamine D_2 receptor mRNA expression was measured in the lateral and medial part of the caudal caudate nucleus. For the lateral caudate values were 357 ± 19 and 354 ± 41 arbitrary units of optical density for apo-sus and apo-unsus rats, respectively. In the medial part of the caudal caudate nucleus D_2 mRNA levels were 219 ± 25 and 220 ± 14 arbitrary units of optical density for apo-sus and apo-unsus rats, respectively. No significant differences were observed between apo-sus and apo-unsus rats.

4. Discussion

Results of the present study show that apo-sus rats are marked by a higher level of striatal D_2/D_3 dopamine receptor binding and D_1 receptor mRNA level, while D_2 receptor mRNA levels and D_1 binding sites were comparable in rats of both lines. TH mRNA concentration was significantly higher in the A_9 substantia nigra and A_{12} arcuate nucleus of apo-sus rats. These observations support the hypothesis that a potentially elevated capacity of dopamine synthesis and increased striatal dopamine $D_{2/3}$ receptor binding underlies the enhanced apomorphine sus-



Fig. 2. Autoradiogram of [^{125}I]iodosulpride binding sites in coronal sections of rat forebrain. Scale bar is equivalent to 500 μM .

ceptibility of apo-sus rats. Moreover, we found that the increased responsiveness of the dopamine system of apo-sus rats was primarily restricted to the nigrostriatal and tuberoinfundibular pathways.

The vigorous gnawing response of apo-sus rats after apomorphine administration suggested high dopamine receptor responsiveness. This assumption was substantiated by the following findings. Apomorphine is a non-selective dopamine agonist which is able to bind to D_1 as well as to D_2 and D_3 receptors. Hybridization of brain sections with specific probes for D_1 and D_2 receptors showed that only dopamine D_1 receptor mRNA levels were significantly elevated in the lateral part of the caudate putamen of apo-sus rats. D_2 receptor mRNA level was not different between rats of the two lines. However, it has been demonstrated that identical mRNA levels not necessarily need to be reflected in similar receptor properties [12,13]. Therefore, we also determined dopamine D_1 and $D_{2/3}$ receptor binding sites.

[^3H]SCH 23390 binding to dopamine D_1 receptors was similar in rats of the two selection lines. This lack in consistency between D_1 mRNA expression and binding sites in rats of the two lines could be due to differences in translation, receptor processing or posttranslational events like phosphorylation [12,13]. The precise relationships between alterations in one or more of these processes remains to be clarified. Striatal membrane homogenates of apo-sus rats showed a significantly higher level of [^{125}I]iodosulpride binding than a similar membrane preparation of apo-unsus rats. A similar explanation as described above for the D_1 receptor could account for the apparent discrepancy between dopamine D_2 receptor mRNA levels and binding sites in apo-sus compared with apo-unsus rats. Binding experiments were performed at a ligand concentration which occupies only 8–12% of the total number of binding sites [14]. These conditions do not allow discrimination between a difference in dopamine receptor affinity for iodopsulpride or a change in the number of binding sites. Additional Scatchard analysis is needed before either altered receptor affinity or receptor number can be accepted as underlying mechanism for the enhanced susceptibility of apo-sus rats to the dopamine agonist apomorphine.

[^{125}I]iodosulpride has been reported to bind to D_2 as well as D_3 receptors [18,26]. Both receptor types are present in the olfactory tubercle and striatum [1], however, D_2 receptors are present in much greater density than D_3 receptors [18]. Russell et al. [23] reported a heterogeneous distribution of D_2 receptors subserving different functions in distinct striatal regions. Therefore, we confirmed and extended our membrane binding data by measuring [^{125}I]iodosulpride binding in the different striatal subfields with in vitro receptor autoradiography. Most pronounced differences between the two rat lines occur in the lateral and medial part of the caudate putamen (30% increase in binding). The same trend, but less pronounced, towards

increased receptor binding was found in projection areas of the mesolimbic dopaminergic pathways; the olfactory tubercle and the nucleus accumbens. These observations are in agreement with results of previous studies in rats [7,11] and mice [8,15,25] showing that specifically striatal dopaminergic receptor binding correlates with the intensity of apomorphine-induced stereotypes. Our findings suggest that mainly D_2 receptors, specifically in the caudate-putamen, are involved in selection for susceptibility for apomorphine-induced gnawing behavior.

As an indication for dopamine synthesis TH mRNA levels in the dopaminergic A_9 , A_{10} and A_{12} as well as the noradrenergic A_6 cell groups were measured. Apo-sus rats only display significantly higher TH mRNA levels in A_9 and A_{12} cell bodies of the nigrostriatal and tuberoinfundibular pathway, respectively. The A_{12} dopaminergic neurons are involved in the negative regulation of prolactin release [10,16,20]. Accordingly, from the increased TH mRNA in the arcuate nucleus it can be predicted that the prolactin response in apo-sus rats is more strongly inhibited than in apo-unsus rats. Recently we have indeed shown that apo-sus rats with higher TH mRNA levels in the arcuate nucleus have an attenuated prolactin release during exposure to novelty of after a conditioned emotional response (Rots et al., *Endocrinology*, in press). In fact apo-sus rats did not show a stress-induced increase in prolactin release at all under conditions that their apo-unsus counterparts did show a significantly elevated plasma prolactin release. This finding suggests that the elevated TH mRNA levels in the arcuate nucleus indeed result in a higher level of dopamine synthesis in apo-sus rats as compared with apo-unsus rats. Of course, also in this case caution should be exercised with extrapolation of TH mRNA levels to dopamine neurotransmission.

The differences in TH mRNA occur besides in the A_{12} also in the A_9 cell group, which contains cell bodies of the nigrostriatal dopamine system. Specifically this dopamine pathway is implicated in apomorphine-induced stereotypic behavior [8,15]. The fact that the apo-sus and apo-unsus rat lines show only minor differences in TH mRNA in A_{10} cell bodies is interesting since the mesolimbic pathway is known to be involved in novelty-induced locomotor activity which is significantly elevated in apo-sus rats. Moreover, only a trend towards increased TH mRNA was measured in the A_6 noradrenergic cell group of the locus coeruleus. Whereas, results of a previous study [6] showed a decrease rather than the expected increase in noradrenaline immunostaining in the nucleus accumbens. It might well be that the two rat lines not only differ in TH mRNA, but also in translation and catalytic efficiency of this enzyme. Obviously more work on catecholamine turnover and release is needed to pinpoint the differences in catecholaminergic transmission.

In conclusion, genetic selection of two rat lines based on enhanced behavioral responsiveness to apomorphine is substantiated by increased TH transcription in A_9 and A_{12}

dopaminergic neurons, elevated D₁ receptor gene expression and increased D₂/D₃-receptor binding in the terminal area of the nigrostriatal dopamine projections. Apo-sus rats and their apo-unsus counterparts appear to be an excellent animal model to investigate individual variation in dopamine response patterns linked to function and pathology of the nigrostriatal and tuberoinfundibular dopamine systems.

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