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Pharmacology, Biochemistry and Behavior 75 (2003) 869–879
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Behavioral effects of neuropeptide Y in F344 rat substrains with a reduced dipeptidyl-peptidase IV activity

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Received 6 March 2003; received in revised form 2 June 2003; accepted 2 June 2003

Abstract

Dipeptidyl-peptidase IV (DPPIV/CD26) is involved in several physiological functions by cleavage of dipeptides with a *Xaa*-Pro or *Xaa*-Ala sequence of regulatory peptides such as neuropeptide Y (NPY). Cleavage of NPY by DPPIV results in NPY_{3-36}, which lacks affinity for the Y_1 but not for other NPY receptor subtypes. Among other effects, the NPY Y_1 receptor mediates anxiolytic-like effects of NPY. In previous studies with F344 rat substrains lacking endogenous DPPIV-like activity we found a reduced behavioral stress response, which might be due to a differential degradation of NPY. Here we tested this hypothesis and administered intracerebroventricularly two different doses of NPY (0.0, 0.2, 1.0 nmol) in mutant and wildtype-like F344 substrains. NPY dose-dependently stimulated food intake and feeding motivation, decreased motor activity in the plus maze and social interaction test, and exerted anxiolytic-like effects. More important for the present hypothesis, NPY administration was found to be more potent in the DPPIV-negative substrains in exerting anxiolytic-like effects (increased social interaction time in the social interaction test) and sedative-like effects (decreased motor activity in the elevated plus maze). These data demonstrate for the first time a differential potency of NPY in DPPIV-deficient rats and suggest a changed receptor-specificity of NPY, which may result from a differential degradation of NPY in this genetic model of DPPIV deficiency. Overall, these results provide direct evidence that NPY-mediated effects in the central nervous system are modulated by DPPIV-like enzymatic activity.

Keywords: Dipeptidyl-peptidase IV; CD26; Neuropeptide Y; F344; Social interaction test; Anxiety; Elevated plus maze; Sedation; Food intake; Feeding motivation

1. Introduction

The enzyme and binding protein dipeptidyl-peptidase IV (DPPIV) belongs to a class of membrane-associated peptidases (De Meester et al., 1999). The ectopeptidase is identical to the leukocyte differentiation marker CD26 and is involved in T-cell dependent immune responses (Kahne et al., 1999) and in cell adhesion (Mentlein, 1999; Shingu et al., in press). Due to its unique ability to liberate *Xaa*-Pro and *Xaa*-Ala dipeptides from the N-terminus of regulatory peptides, important substrates include neuropeptides such as neuropeptide Y (NPY), peptide YY (PYY), and endomorphin (Hildebrandt et al., 2000; Mentlein, 1999). Further substrates are glucagon-like peptide 1 (GLP-1), GLP-2, enterostatin, substance P and various chemokines (De Meester et al., 2000). In the adult nervous system, DPPIV is found primarily in the circumventricular organs and on leptomeningeal cells. The enzyme has direct contact to neuropeptides such as NPY in the cerebrospinal fluid (Mentlein, 1999) and also to endothelial cells of blood vessels including those contributing to the blood brain barrier (Hildebrandt et al., 2000). In addition, a soluble form of DPPIV exists in the blood plasma (Mentlein, 1999).

Because of these pleiotropic effects of DPPIV, we previously investigated the role of the DPPIV-like activity on behavioral and physiological processes in a mutant rat model of DPPIV deficiency. Spontaneous mutations in the *Dpp4* gene of F344 substrains from breeding colonies of Charles River (CR) in Sulzfeld, Germany (Thompson et al., 1991), and Atsugi, Japan (Tiruppathi et al., 1993; Watanabe et al., 1987) result in a nearly complete lack of DPPIV-like activity in these animals. This DPPIV deficiency does not exist in wildtype-like F344 rats from

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doi:10.1016/S0091-3057(03)00154-0

CR breeding colonies in Portage, USA (Karl et al., in press (a)). In a systematical behavioral and physiological characterization of the different substrains, we observed differences in the mutant, DPPIV-deficient F344 rats showing increased water intake, improved glucose homeostasis, blunted natural killer cell function, reduced anxiety-like behaviors, and increased sensitivity for the sedative effect of ethanol (Karl et al., in press (a,b)). We hypothesized that at least some of these differences may be due to a differential metabolism of NPY.

The neurotransmitter NPY was discovered in 1982 (Tatemoto et al., 1982) and is a member of the pancreatic family of peptides. Significant NPY levels were found in most brain regions including the cerebral cortex, hippocampus, thalamus, hypothalamus, and brainstem (Allen et al., 1983; Colmers and Wahlestedt, 1993). The neuropeptide is a 36 amino acid peptide with a large number of tyrosine residues. Based on this amino acid sequence, NPY is cleaved by DPPIV. It has a high affinity to the enzyme and is also metabolized in human serum by DPPIV (De Meester et al., 2000). Among several other physiological responses NPY especially affects feeding behavior and anxiety (Kalra et al., 1999, 2002; Wettstein et al., 1995). The neurotransmitter binds to several NPY receptor subtypes (Y_1, Y_2, Y_4, and Y_5) in rats, which belong to the large superfamily of G-protein-coupled receptors. They are widely distributed in the brain (Blomqvist and Herzog, 1997) and mediate regulatory effects of NPY (Parker and Herzog, 1999). The NPY Y_1 receptor is one of the major receptor subtypes expressed in the rat brain (Dumont et al., 1998) and is particularly involved in the regulation of anxiety (Kask et al., 2002). Importantly, DPPIV removes the first two N-terminal amino acid residues (Tyr-Pro) of NPY with high turnover rates and generates the C-terminal fragment NPY_{3-36}. NPY_{3-36} has a markedly reduced affinity to the NPY Y_1 receptor subtype, while being as potent as the native peptide on the NPY Y_2 and Y_5 receptor subtypes. Possibly, the previously described behavioral and physiological differences of the two DPPIV-deficient F344 substrains from Japan, F344/DuCrj(DPPIV −), and Germany, F344/Crl(Ger/DPPIV −), on the one hand, and the wild-type-like substrain from USA, F344/Crl(Por), on the other hand (Karl et al., in press (b)), could be due to a faster degradation of endogenous NPY to NPY_{3-36} in the control substrain. This would result in a relatively reduced endogenous NPY Y_1 receptor-like tone. Such a mechanism could sufficiently explain the relatively reduced anxiety-like behaviors in the mutant, DPPIV-deficient substrains.

To test this hypothesis, it was investigated whether NPY applied intracerebroventricularly produces differential effects in DPPIV-deficient substrains. Thus, we characterized the effect of intracerebroventricular administration of different doses of NPY in the F344/DuCrj(DPPIV −), F344/Crl(Ger/DPPIV −), and F344/Crl(Por) rats on various behavioral tasks aiming at a characterization of feeding and anxiety-like behaviors.

2. Materials and methods

2.1. Animals

For clarity, animal groups were coded as previously described (Karl et al., in press (a)): F344 rats derived from breeding colonies of CR in Atsugi, Japan, were called F344/DuCrj(DPPIV −), animals from breeding colonies in Sulzfeld, Germany, F344/Crl(Ger/DPPIV −), and wild-type-like rats obtained from colonies in Portage, USA, F344/Crl(Por).

All F344 rats of the three different substrains have been housed and bred at the Central Animal Facility of the Medical School Hannover as previously described (Karl et al., in press (a)). Animals were maintained in a separated minimal barrier sustained facility and kept in macrolon type III cages with a standard bedding (Altromin, Lage, Germany). Food (Altromin Standard Diät 1320: Altromin) and water were available ad libitum. Environmental temperature was automatically regulated at 21 ± 1 °C and relative humidity was 60% with an air change rate of 15 times per hour. The animal rooms were operated with a positive pressure of 0.6 Pa. Rats were maintained under a 12:12-h light regime (light onset at 4:00 a.m.). They underwent routine cage maintenance once a week. Routine microbiologic monitoring according to FELASA recommendations (Rehbinder et al., 2000) did not reveal any evidence of infection with common murine pathogens except for *Pasteurella pneumotropica* and *Staphylococcus aureus*. All research and animal care procedures were approved by the Review Board for the Care of Animal Subjects of the district government, Hannover, Germany, and performed according to international guidelines for the use of laboratory animals.

2.2. Determination of DPPIV-like enzymatic activity

All test animals were characterized in regard to their DPPIV-like enzymatic activity as previously described (Karl et al., in press (a)). For determination of plasma activity of F344 rats a microplate-based fluorescence assay was used. EDTA-plasma samples were kept at − 80 °C until use. DPPIV enzyme activity of the different rat substrains was determined by monitoring the release of 4-Amino-7-Methylcoumarin (AMC) from the substrate Gly-Pro-AMC at 360/480 nm (Ex/Em) and 30 °C using the Novostar fluorescence microplate reader (BMG, Offenburg, Germany). The assay consists of 20 μl plasma sample, 100 μl H_2O and 100 μl HEPES buffer pH 7.6 and 50 μl Gly-Pro-AMC. Activity was calculated from the linear slope using a factor of 3.116×10^{-4} μmol/l calculated from an AMC standard curve and the sample dilution. One unit is defined as the enzyme activity, which cleaves 1 μmol Gly-Pro-AMC per minute. The assay is selective for DPPIV-like activities. It has been proved that the substrate is cleaved by DPPIV, by DP II, and by attractin. Probably, they are also substrates for DP8 and DP9. Importantly, the chromophores are not

released by other proline-specific peptidases, such as prolidase, prolyl endopeptidase or aminopeptidase P.

2.3. Surgery of intracerebroventricular cannulation

For surgery, animals were anesthetized with intramuscular ketamine hydrochloride (0.1 ml/100 g body weight; Albrecht, Aulendorf, Germany) and dormitor (0.01 ml/100 g body weight; Pfizer, Karlsruhe, Germany). The intracerebroventricular cannulation technique was identical to a previous report (von Horsten et al., 1998a). After placement of the rat in a Kopf stereotactic apparatus (model 900: David Kopf Instruments, Tujunga, USA), the incisor bar was adjusted on position zero and the ear bars were adjusted to equal positions so that the rat's head was fixed in the apparatus. The eyes were protected against drying with eye-salve (Bepanthen Augen- und Nasensalbe: Hoffmann-La Roche, Grenzach-Wyhlen, Germany). The skull was exposed by a midline incision, the periost was removed, the bone surface was dried, and the position of the bregma was identified. Three stainless steel ancor screws (Breitfeld and Schliekert, Karben, Germany) were secured to the skull and a stainless steel guide cannula (Plastics One, Roanoke, USA) was implanted in the right lateral ventricle and cemented in place with dental cement (Durelon Maxicap: Espe Dental, Seefeld, Germany). The coordinates for the lateral ventricle were 0.7 mm caudal and 1.6 mm lateral to the bregma, with the guide cannula (Plastics one) extending 3.4 mm ventral to the skull surface. Flow of small amounts of 0.9% saline (Braun Melsungen, Melsungen, Germany) through the protracted injection (internal) cannula (Plastics One) was used to verify that the guide cannula was positioned shortly above the ventricular system. The guide cannula was then fitted with a dummy cannula (Plastics One) of the same length to prevent leakage of cerebrospinal fluid. Animals were housed individually after surgery. The anatomical position of the cannulation was verified by post mortem intracerebroventricular dye application (Berlin blue) and inspection of third ventricular staining in randomly chosen rats. The animals of the three F344 substrains F344/DuCrj(DPPIV −), F344/Crl(Ger/DPPIV −), and F344/Crl(Por) were operated in the age of 95 (± 5) days. After a recovery phase of at least 10 days we started with the observation of the animals' behavior in different behavioral test paradigms (an overview is given in Fig. 1).

2.4. Drugs, intracerebroventricular injections, and dosages

A stock solution of human/rat NPY (2 mol; Polypeptide, Wolfenbüttel, Germany) was adjusted under sterile conditions to final concentrations (0.2 nmol/5 μl and 1 nmol/5 μl) using 0.9% saline. The final concentrations were made 24 h before the different experiments.

For the procedure of intracerebroventricular injection, animals were habituated to experimental handling daily within 7 days prior to the start of the first experiment. For the intracerebroventricular administration animals were taken out of the home cage and the dummy cannula was replaced by the internal cannula. Peptide or 0.9% saline were injected intracerebroventricularly in a volume of 5 μl over 20 s through the internal cannula extending 4.4 mm ventral to the skull surface. The internal cannula was attached to a microsyringe (Hamilton Bonaduz, Bonaduz, Switzerland) with approximately 30 cm of a polyethylene tubing (Plastics One), which allowed the animal to move freely during the intracerebroventricular injection. Then the internal cannula was replaced by the dummy cannula again and the rat was placed back into the home cage. The experiment started 15 min after the administration procedure. During the habituation phase the handling procedure was exactly the same except for the application of the compound.

Two different doses of NPY were used for this study and a 3 (substrain) × 3 (treatment) experimental design was set up. Thus, F344 rats of each substrain were divided into three treatment groups each ($n = 7$), which were treated with 0.9% saline (vehicle: 0.0 nmol/5 μl), 0.2/5 μlnmol, or 1 nmol/5 μl NPY.

2.5. Experimental designs of the behavioral test paradigms

2.5.1. Feeding behavior

The feeding response (latency to start eating and the overall food consumption) following an intracerebroventricular administration of NPY were measured for 2 h. Two

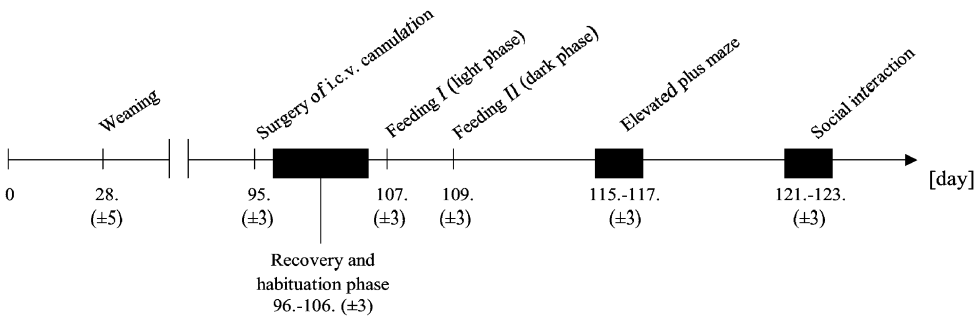

Fig. 1. Test setting. Test biography of rats of the three different substrains F344/Crl(Por), F344/DuCrj(DPPIV −), and F344/Crl(Ger/DPPIV −); the animals' age in the various tests is shown.

experiments were performed: in experiment I, the feeding response after intracerebroventricular treatment was recorded in the light phase; in experiment II, 60 h later, the same response was measured 1 h after onset of the dark phase (Kushi et al., 1998; Marsh et al., 1998).

2.5.2. Anxiety

In early studies various authors defined tasks like the open field and the elevated plus maze as tests for emotionality (Archer, 1973; Denenberg, 1969; Walsh and Cummins, 1976). In more recent years, the same paradigms have been discussed in regard to their potential to provide indicators of anxiety, and the construct "emotionality" has been less frequently used (File and Seth, 2003; Mechiel Korte and De Boer, 2003; Prut and Belzung, 2003). Therefore, we use the term "anxiety-like behavior" in regard to these tests.

2.5.2.1. Elevated plus maze. The elevated plus maze (EPM) represents the natural conflict of rats between the tendency to explore a novel environment and the tendency to avoid a brightly lit open area (Handley and Mithani, 1984; Montgomery, 1958). The behavior is also influenced by thigmotaxis and the fear of heights (Treit and Fundytus, 1988). The time spent on open arms of the EPM as well as the percentage of open arm entries (ratio of open to total arm entries) are inversely related to anxiety (Hogg, 1996; Pellow et al., 1985; Pellow and File, 1986). The number of enclosed and total arm entries reflects the general motor activity (File, 1986). An automated EPM (TSE, Bad Homburg, Germany) with ledges (Fernandes and File, 1996) for rats was used. The animal was placed onto the center platform facing an open arm. In the following 5 min the entries into open/enclosed arms, the time spent on open/enclosed arms or on the center platform, behaviors like *rearing* and *self-grooming* and the defecation score were recorded on video (Baldwin and File, 1986; Lister, 1987). Illumination was dim (10 lx) and the experiment started 1 h after onset of the dark phase. A rat was taken to have entered an arm when all four paws were on this arm. After each session the apparatus was cleaned with 70% ethanol. To avoid influences on the animals' endogenous rhythm on behavioral activity, tests were performed on three consecutive days with the animals of all substrains being grouped by dose of intracerebroventricular NPY treatment.

2.5.2.2. Social interaction. Pairs of rats, placed in a novel environment, engage in active social interactions, which include a variety of social behaviors (File, 1988; File and Hyde, 1978). The rat social interaction test is used widely to measure anxiety-like behaviors (File, 1988) and to detect anxiogenic and anxiolytic-like effects of drugs (Kask et al., 2001a). The overall active social interaction (SI) time is inversely related to the anxiety of the animals (File, 1980; File and Hyde, 1978; Karl et al., 2003), which is confirmed by the observation that the maximum active SI time is found when rats are tested in a familiar test arena with low level of illumination (File and Hyde, 1978). Furthermore, a decrease in SI time is correlated with an increase in other behaviors indicating increased anxiety: defecation, *freezing*, and displacement activity (i.e., eating of nonedible objects by rats that are not food deprived or *self-grooming*). Therefore, the decrease in social behaviors is consistent with behavioral indications of increased arousal or anxiety and is not explained by any changes in other competing behaviors such as exploration (File, 1980, 1988). The apparatus used was a squared steel open field ($50 \times 50 \times 50$ cm) that was placed inside a sound isolation box. The floor was divided into 25 squares by a cross grid (Kask et al., 2001a). Two rats of similar substrains and NPY treatment were exposed to the area. In the following 10 min the total duration and the frequency of the different behaviors *anal sniffing*, *following*, *allo grooming*, *walking over*, *crawling under*, and *nosing* of each rat were recorded. Additionally, the frequency of *self-grooming* and the motor activity (traveled distance—by recording the total number of square entries) were analyzed. The behavior was monitored online using a video camera placed above the open field inside the isolation box. The arena was brightly illuminated (180 lx) and experiments started 1 h after onset of the dark phase. After each session the apparatus was cleaned with 70% ethanol. As in the EPM test, rats were tested on three consecutive days to avoid influences on the rats' endogenous rhythm.

2.6. Statistical analysis

The analysis of the various behavioral data was assessed using a two-way ANOVA (factor: Substrain × Treatment) and by one-way ANOVA (factor: substrain or treatment—split by the corresponding factors) followed by the Fisher–PLSD test for post hoc comparison, if appropriate. Differences were regarded as statistically significant if $P < .05$. Results within the text in the Results section present the *degrees of freedom*, F values, and P values of one-way ANOVAs, while in figures and tables the P values of the corresponding post hoc tests (Fisher–PLSD test) are provided. In most cases the number of animals per substrains and treatment group was $n = 7$. Presenting the *degrees of freedom* indicates exceptions from this. Significant post hoc effects for the factor substrain vs. the control animals of the F344/Crl(Por) substrain are indicated by asterisks ($^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$), whereas significant differences between the two DPPIV-deficient rat substrains F344/DuCrj(DPPIV −) and F344/Crl(Ger/DPPIV −) are presented by double crosses ($^{\#}P < .05$, $^{\#\#}P < .01$, $^{\#\#\#}P < .001$). Significant post hoc effects for the factor treatment vs. the vehicle-treated animals are indicated by crosses ($^{+}P < .05$, $^{++}P < .01$, $^{+++}P < .001$), whereas significant differences between the two different NPY doses 0.2 nmol and 1.0 nmol are presented by "x" ($^{x}P < .05$, $^{xx}P < .01$, $^{xxx}P < .001$). All data are presented as means ± S.E.M.

3. Results

3.1. DPPIV-like activity

One-way ANOVA of the DPPIV-like activity of all animals exhibited significant differences between substrains (Fig. 2). A nearly complete lack of enzymatic-like activity was found in the two mutant substrains F344/DuCrj(DPPIV −) and F344/Crl(Ger/DPPIV −) and a wild-type-like pattern of DPPIV-like activity was found in the rats from the F344/Crl(Por) substrain [$F(2,61) = 400.4$; $P < .0001$].

3.2. Feeding behavior

Two-way ANOVA revealed a significant treatment effect on the food intake in the dark phase [factor treatment: $F(2,55) = 54.7$; $P < .0001$] as well as in the light phase [factor treatment: $F(2,55) = 116.8$; $P < .0001$]. Each of the different substrains exhibited a dose-dependent increase of overall food consumption after intracerebroventricular administration of NPY (data not shown). Neither significant effects for the factor substrain nor any significant interactions were observed, although mutant, DPPIV-negative rats showed a trend for reduced food intake stimulation in the light phase. Similarly, two-way ANOVA revealed that intracerebroventricular treatment shortened the latency to eat dose-dependently [factor treatment: in dark phase: $F(2,55) = 13.1$; $P < .0001$; one-way ANOVA for F344/DuCrj(DPPIV −): $F(2,18) = 17.6$; $P < .0001$, while in light phase: $F(2,55) = 26.9$; $P < .0001$; one-way ANOVA for F344/Crl(Por): $F(2,18) = 10.1$; $P = .0011$; one-way ANOVA for F344/DuCrj(DPPIV −): $F(2,18) = 4.8$; $P = .02$; one-way ANOVA for F344/Crl(Ger/DPPIV −): $F(2,19) = 13.6$; $P = .0002$]. However, neither significant differences for the factor substrain nor significant Substrain × Treatment interactions were found (Table 1).

Table 1
Effects of intracerebroventricular NPY on the latency to eat

	F344/Crl (Por)	F344/DuCrj (DPPIV −)	F344/Crl (Ger/DPPIV −)
Latency to eat during the light phase [s]			
Vehicle	77.1 ± 20.4	60.3 ± 21.2	85.7 ± 18.2
0.2 nmol NPY	12.7 ± 2.3^{++}	12.7 ± 1.1^{+}	17.5 ± 3.3^{+++}
1.0 nmol NPY	10.6 ± 1.6^{+++}	13.3 ± 4.3^{+}	8.0 ± 0.8^{+++}
Latency to eat during the dark phase [s]			
Vehicle	51.6 ± 18.5	80.6 ± 15.3	54.3 ± 16.1
0.2 nmol NPY	39.8 ± 14.8	17.5 ± 3.4^{+++}	38.2 ± 14.0
1.0 nmol NPY	10.3 ± 1.5	12.3 ± 1.3^{+++}	14.2 ± 1.4

Latency to start eating in F344 substrains with a differential DPPIV-like enzymatic activity after intracerebroventricular administration of different doses of NPY. Animals' feeding behavior was recorded following the injections of the different doses of NPY. Data represent means ± S.E.M. Significant post hoc effects for the factor treatment vs. the vehicle-treated animals are indicated by crosses.

$^{+}$ $P < .05$.
$^{++}$ $P < .01$.
$^{+++}$ $P < .001$.

3.3. Anxiety

3.3.1. Elevated plus maze

Two-way ANOVA revealed no significant effect for the factor substrain on the ratio between open arm entries and total arm entries as an indicator of anxiety [$F(2,51) = 1.1$; $P = .34$; Fig. 3], although this ratio increased dose-dependent significantly after intracerebroventricular treatment with NPY (factor treatment: $F(2,51) = 10.5$; $P < .0001$). Additionally, NPY administration increased significantly

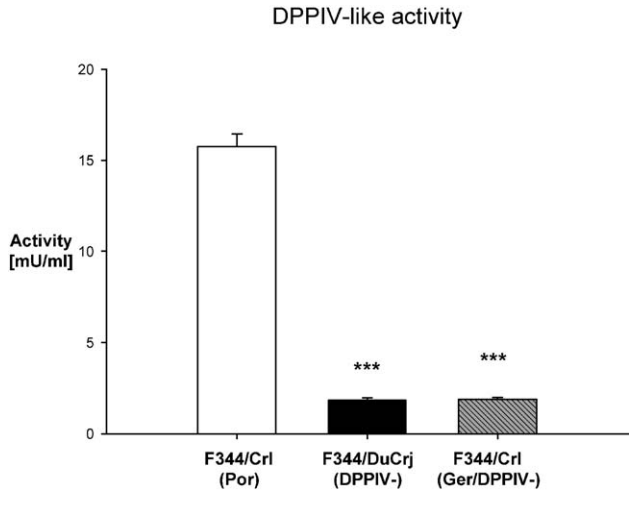

Fig. 2. DPPIV-like activity. DPPIV-like activity [mU/ml] of all test animals was screened; blood from the tail vain was sampled and analyzed as described above. Data represent means ± S.E.M. Asterisks (*** $P < .001$) reflect significant differences vs. F344/Crl(Por)—detected in the Fisher–PLSD test.

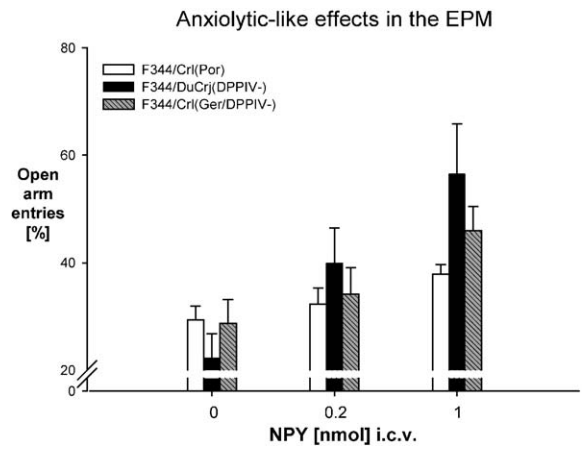

Fig. 3. Anxiolytic-like effects in the EPM. The number of arm entries of the test animal into open and enclosed arms were recorded for 5 min; the ratio of open arm entries to total arm entries serves as a measure for anxiety [%]. Data represent means ± S.E.M.

the time spent on open arms [two-way ANOVA; factor treatment: $F(2,51)=8.6$; $P=.0006$; data not shown]. But since in both parameters no significant Substrain × Treat-Treatment interactions or significant substrain differences were found, these results indicate that the three substrains did not respond differentially to the anxiolytic-like effects of intracerebroventricular NPY as determined by the EPM.

However, the motor activity (total arm entries) was significantly reduced in the mutant F344/DuCrj(DPPIV −) and F344/Crl(Ger/DPPIV −) animals [two-way ANOVA; factor substrain: $F(2,51)=6.4$; $P=.0032$] compared to the wildtype-like rats after intracerebroventricular administration of 1 nmol NPY [one-way ANOVA for 1 nmol: $F(2,16)=10.2$; $P=.0014$; Fig. 4A]. Overall, NPY treatment significantly decreased motor activity, especially in the DPPIV-deficient rats of both substrains [two-way ANOVA; factor treatment: $F(2,51)=4.9$; $P=.012$]. In addition, the frequency of enclosed arm entries was significantly reduced after intracerebroventricular treatment with NPY [two-way ANOVA; factor treatment: $F(2,51)=12.1$; $P<.0001$; one-way ANOVA for F344/DuCrj(DPPIV −): $F(2,18)=5.8$; $P=.01$; one-way ANOVA for F344/Crl(Ger/DPPIV −): $F(2,19)=4.7$; $P=.02$; Table 2], although the difference between the substrains failed to be statistically significant [two-way ANOVA; factor substrain: $F(2,51)=2.3$; $P=.11$]. Furthermore, a significant Substrain × Treatment interaction regarding the time spent on open arms per entry was found [two-way ANOVA; Substrain × Treatment: $F(2,51)=2.6$; $P<.05$; Fig. 4B]. Although one-way ANOVA revealed no significant differences regarding the factor substrain within the differential NPY treatments, the animals, which lack DPPIV-like activity were found to stay longer on the open arms per entry than the control F344/Crl(Por) rats (Fig. 4B). NPY treatment significantly increased the time spent on open arms per entry [two-way ANOVA; factor treatment: $F(2,51)=7.7$; $P=.0012$].

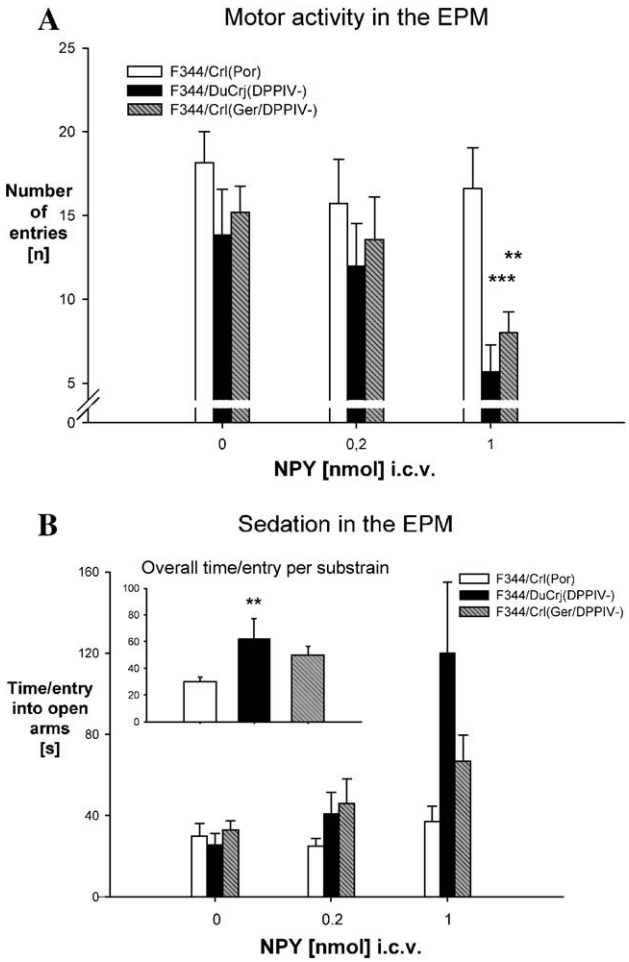

Fig. 4. (A) Motor activity in the EPM. The total number of arm entries of the test animal was recorded for 5 min; number of total arm entries serves as a measure for motor activity [*n*]. Data represent means ± S.E.M. Asterisks (* * * $P<.001$; * * $P<.01$) reflect significant differences vs. F344/Crl(Por)—detected in the Fisher–PLSD test. (B) Sedation in the EPM. The time spent on the open arms per entry was recorded [s]; additionally, the overall time spent on the open arms per entry in each substrain is presented. Data represent means ± S.E.M. Asterisks (* * $P<.01$) reflect significant differences vs. F344/Crl(Por)—detected in the Fisher–PLSD test.

Table 2
Motor activity in the EPM

	F344/Crl (Por)	F344/DuCrj (DPPIV −)	F344/Crl (Ger/DPPIV −)
Vehicle	4.3 ± 0.75	3.9 ± 0.77	3.8 ± 0.87
0.2 nmol NPY	3.1 ± 0.51	2.0 ± 0.85	2.7 ± 0.68
1.0 nmol NPY	2.4 ± 0.41	0.6 ± 0.31 $^{++}$	1.0 ± 0.38 $^{++}$

The number of enclosed arm entries as an additional parameter for motor activity is presented. Data represent means ± S.E.M. Significant post hoc effects for the factor treatment vs. the vehicle-treated animals are indicated by crosses.
$^{++}$ $P < .01$.

3.3.2. Social interaction

Recording the time spent in active social interaction in pairs of rats of the same substrain and treatment as an indicator of anxiety revealed that the time spent in the various behaviors was increased in F344/DuCrj(DPPIV −) rats (trend, not significant) as well as significantly increased in the F344/Crl(Ger/DPPIV −) animals of the 0.2 nmol NPY treatment group [two-way ANOVA; factor substrain: $F(2,48) = 7.8$; $P = .001$; one-way ANOVA for 0.2 nmol: $F(2,16) = 9.4$; $P = .002$; Fig. 5A]. Furthermore, two-way ANOVA revealed a significant NPY-dependent increase in the social interaction time of all animals [factor treatment: $F(2,48) = 11.3$; $P < .0001$; Fig. 5A]. Additionally, we found a significant Substrain × Treatment interaction in the frequency of *anal sniffing* [two-way ANOVA; Substrain × Treatment interaction: $F(2,48) = 3.0$; $P = .026$], which was significantly increased in the F344/Crl(Ger/DPPIV −) rats after treatment with 0.2 nmol NPY [two-way ANOVA; factor substrain: $F(2,48) = 3.5$; $P = .04$; one-way ANOVA for 0.2 nmol: $F(2,16) = 10.9$; $P = .001$; Fig. 5B]. The significantly decreased frequency of *self-grooming* in the DPPIV-deficient substrains disappeared after intracerebroventricular treatment with 1 nmol NPY [two-way ANOVA; factor substrain: $F(2,48) = 7.6$; $P = .001$; one-way ANOVA for vehicle: $F(2,18) = 4.2$; $P = .03$; one-way ANOVA for 0.2 nmol: $F(2,16) = 4.2$; $P = .03$; Fig. 5C]. Additionally, two-way ANOVA revealed a significant reducing effect of NPY administration on the frequency of *self-grooming* [factor treatment: $F(2,48) = 3.8$; $P = .03$]. Interestingly, the sum of frequencies of the various social behaviors (*anal sniffing*, *following*, *allo grooming*, *walking over*, *crawling under*, and *nosing*) exhibited no significant differences between the substrains [two-way ANOVA; factor substrain: $F(2,48) = 2.6$; $P = .09$; Fig. 6], although the overall motor activity of the

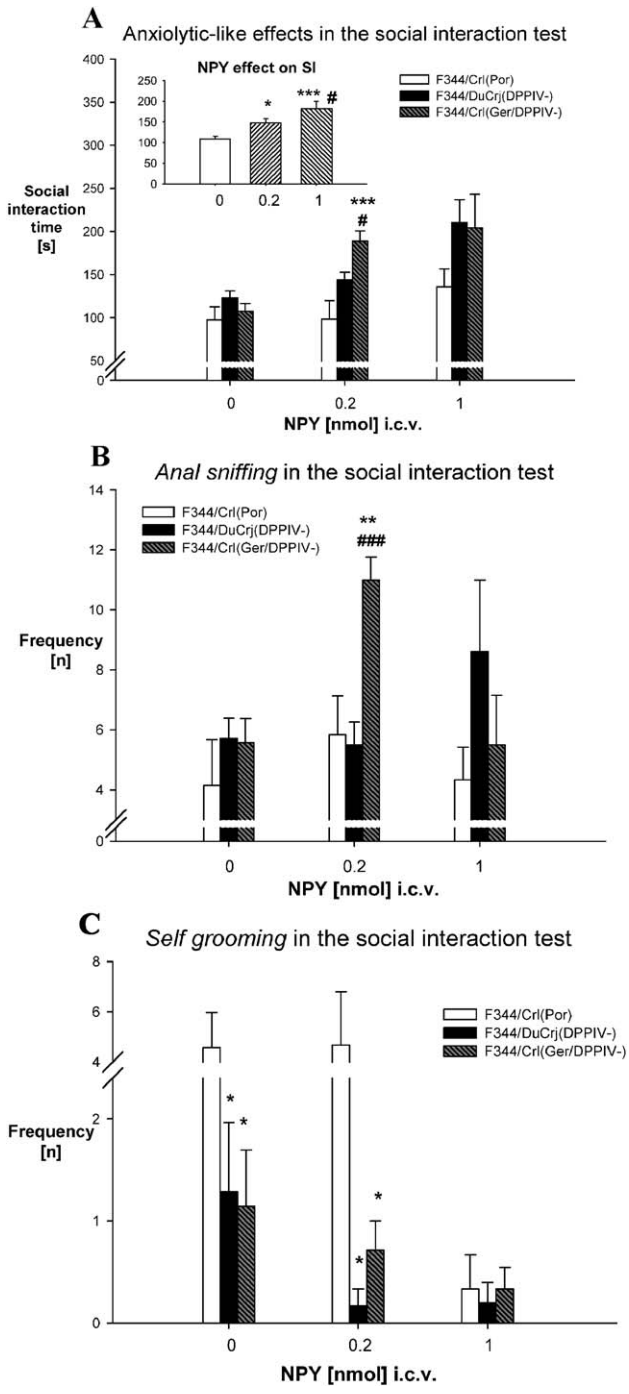

Fig. 5. (A) Anxiolytic-like effects in the social interaction test. The time spent in active SI was recorded; two weight-matched rats of the same substrain and treatment group were exposed to an open field; rats' active social behavior was observed in the following 10 min; additionally, the overall effect of different doses of NPY on SI is presented. Data represent means±S.E.M. Large panel: Asterisks (***P<.001) reflect significant differences vs. F344/Crl(Por), whereas double crosses ($^{\#}P$<.05) display significant differences between F344/DuCrj(DPPIV−) and F344/Crl(Ger/DPPIV−)—detected in the Fisher–PLSD test. Small panel: Significant post hoc effects for the factor "treatment" vs. the vehicle-treated animals are indicated by asterisks (*P<.05; ***P<.001), whereas double crosses ($^{\#}P$<.05) reflect significant differences between the two different doses of NPY as revealed by the Fisher–PLSD test. (B) *Anal sniffing* in the social interaction test. The frequency of *anal sniffing* was recorded during 10 min of observation; rats of different substrains but of the same treatment group were compared. Data represent means±S.E.M. Asterisks (**P<.01) reflect significant differences vs. F344/Crl(Por), whereas double crosses ($^{\#\#\#}P$<.001) display significant differences between F344/DuCrj(DPPIV−) and F344/Crl(Ger/DPPIV−)—detected in the Fisher–PLSD test. (C) *Self-grooming* in the social interaction test. The frequency of *self-grooming* was recorded during 10 min of observation; rats of different substrains but of the same treatment group were compared. Data represent means±S.E.M. Asterisks (*P<.05) reflect significant differences vs. F344/Crl(Por)—detected in the Fisher–PLSD test.

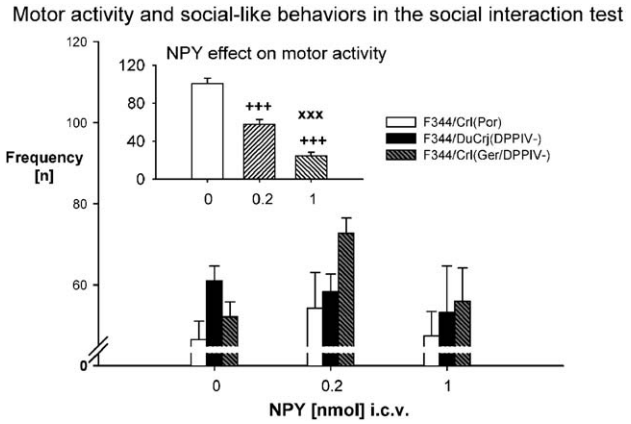

Fig. 6. Motor activity and social-like behaviors in the social interaction test; the summed frequency of social-like behaviors was recorded; additionally, the overall effect of NPY on motor activity is presented by the total number of square entries after intracerebroventricular administration of different doses of NPY. Data represent means ± S.E.M. Significant post hoc effects for the factor treatment vs. the vehicle-treated animals are indicated by crosses ($^{+++}P<.001$), whereas "x" ($^{xxx}P<.001$) reflects significant differences between the two different NPY doses 0.2 nmol and 1.0 nmol—detected in the Fischer–PLSD test.

rats (measured by number of square entries) was significantly reduced by NPY [two-way ANOVA; factor treatment: $F(2,48)=55.2$; $P<.0001$].

4. Discussion

This study shows for the first time a differential response to intracerebroventricular administration of NPY in rats that differ in their endogenous DPPIV-like enzymatic activity. Namely, the different doses of NPY induced significantly more pronounced sedative-like effects on EPM behavior and anxiolytic-like effects on the rat social interaction test in the DPPIV-deficient F344 rats. The study provides further evidence for the orexigenic, potent sedative-, and anxiolytic-like effects of NPY and confirms previously described spontaneous mutations in the *Dpp4* gene in the two F344 rat substrains, which leads to an extreme reduction of DPPIV-like activity in the F344/DuCrj(DPPIV −) and the F344/Crl(Ger/DPPIV −) animals (Karl et al., in press (a); Tsuji et al., 1992).

NPY, as a physiological appetite transducer (Kalra et al., 1999), is the only known peptide that can cause animals to eat until they are obese (Inui, 1999). Injections of NPY either in the third ventricle (Jolicoeur et al., 1991; Levine and Morley, 1984) or the hypothalamus (Schwartz et al., 2000; Stanley et al., 1986), and especially into the paraventricular nucleus (PVN) (Brief et al., 1992; Merlo Pich et al., 1992), exert a powerful stimulatory effect on feeding and drinking behavior of rats. All animals of the different substrains exhibited a dose-dependent increase in food intake after intracerebroventricular administration of NPY. In addition, for the first time this study proved a stimulating effect of intracerebroventricularly administrated NPY on the latency to start eating, which could be discussed as an influence of NPY on the feeding motivation of rodents. However, in contrast to the proposed hypothesis, no pronounced differences in feeding behavior were found between the wildtype-like and mutant F344 substrains. This may be due to the fact that active DPPIV in the wildtype-like animals cleaves NPY to $NPY_{3\text{-}36}$, which results in a loss of Y_1 receptor subtype but not Y_5 (and Y_2) subtype receptor affinity. Importantly, the feeding behavior of rodents is very likely mediated via Y_1 and Y_5 receptors (Inui, 1999; Kalra et al., 1999; Turnbull et al., 2002) and possibly also via Y_2 receptors (Sainsbury et al., 2002). Y_5 agonists stimulate food intake (Bischoff and Michel, 1999) and inhibition of NPY-stimulated food intake has been observed upon central administration of antisense oligonucleotides directed against the Y_5 receptor. Additionally, Y_5 receptor mRNA was detected in abundance in the PVN and the lateral hypothalamus, areas that have been implicated in the control of feeding behavior (Gerald et al., 1996). Antisense oligonucleotides directed against the Y_1 receptor were also reported to inhibit NPY-stimulated food intake (Bischoff and Michel, 1999), and treatment with Y_1 receptor antagonists like BIBO3304 or BIBP3226 blocked NPY-induced food intake (Bischoff and Michel, 1999; Inui, 1999). Also the Y_2 receptor subtype seems to be involved in feeding and body weight regulation (Inui, 2000). Y_2 receptor knockout mice developed increased food intake and body weight (Naveilhan et al., 1999), which indicates an inhibitory role for this receptor subtype in the central regulation of body weight and food intake. After all, the stimulating effect of NPY on feeding seems to be mediated via the Y_1, Y_5, and perhaps the Y_2 receptor, although NPY, Y_1, and Y_5 knockout mice did not show expected impaired feeding and body weight loss (Erickson et al., 1996; Marsh et al., 1998; Pedrazzini et al., 1998). Therefore, it is not surprising that the food intake of all F344 substrains is similar because endogenous native NPY in the mutant substrains and cleaved $NPY_{3\text{-}36}$ in the wildtype-like substrain are both potent mediators for feeding behavior.

NPY is deeply involved in anxiety-like behaviors of rodents (Kask et al., 2002). Intracerebroventricular administration of NPY and Y_1 agonists decreased anxiety in the EPM (Broqua et al., 1995; Heilig et al., 1989) and the open field (von Horsten et al., 1998b). Furthermore, bilateral microinjections of NPY into the dorsocaudal septum increased the social interaction time in the social interaction test (Kask et al., 2001b). In addition, rats treated with antisense oligodeoxynucleotides blocking Y_1 receptors displayed behavioral signs of anxiety (Wahlestedt et al., 1993) and the Y_1 receptor antagonists BIBO3304 and BIBP3226 blocked anxiolytic-like effects of exogenously administered NPY (Kask et al., 1996, 2001b; von Horsten et al., 1998b). These findings strongly suggest a role for the Y_1 receptor in mediating the anxiolytic-like action of NPY (Heilig et al., 1989; Kask and Harro, 2000). But anxiolytic-like effects of NPY are not only mediated via NPY Y_1 receptors in the

amygdala (Sajdyk et al., 1999) and the dorsal periaqueductal gray matter (Kask et al., 1998a) but probably also via Y_2 receptors in the locus coeruleus (Kask et al., 1998b, 2001b) and via Y_5 receptors (Sajdyk et al., 2002). Importantly, also a dose-dependent sedative effect of NPY on locomotor activity in the plus maze has been reported (Broqua et al., 1995; Heilig et al., 1989). In addition, central NPY administration suppressed activity in the open field and in the homecage (Heilig and Murison, 1987), which was interpreted as sedation (Fuxe et al., 1983). Also other studies proved a dose-dependent decrease in the motor activity of rats after intracerebroventricular administration of NPY (Jolicoeur et al., 1995) and NPY antibody treatment increased motor activity (Yamada et al., 1996). This suppression of activity is supposed to be mediated by Y_1 receptors (Heilig et al., 1988; Kask et al., 1999; von Horsten et al., 1998b), which are also involved in the NPY-induced sensitization to sedation (Naveilhan et al., 2001).

In our study the NPY-treated mutant animals of F344/DuCrj(DPPIV −) and F344/Crl(Ger/DPPIV −) exhibited a dose-dependent reduction in motor activity in the EPM. The wildtype-like F344/Crl(Por) rats were unaffected by the sedative effect of NPY, probably because of differences in the NPY catabolism between the F344 substrains with a DPPIV-dependent cleavage of NPY to the Y_1 receptor unspecific NPY_{3-36} only in control rats. Also the dose-dependent increased time spent on open arms per entry in the mutant animals supports a probably Y_1-mediated sedative-like effect of NPY. The differential response of the three F344 substrains to the different doses of NPY in regard to this parameter supports our hypothesis regarding the DPPIV-dependent differential NPY catabolism in wildtype-like and mutant animals. In addition, NPY had an anxiolytic-like effect in all substrains, which was detected by an increase in the ratio of open arm to total arm entries and in the total time spent on open arms. Notably, these possible anxiolytic-like effects of NPY on the total time spent on open arms could also be influenced by the sedative-like effect of NPY. At least in the F344 genetic background, importantly, a very narrow pharmacological window between sedative-like and anxiolytic-like effects of NPY especially in the EPM has to be considered to avoid false positive results. Therefore, not only the time spent on open arms should be recorded as a parameter for detecting anxiolytic-like behaviors in the EPM. Furthermore, various behavioral tests for anxiety have to be used for a complete screening of anxiety-like behaviors in rodents. Therefore, we also applied the social interaction test for recording anxiolytic-like effects of NPY. Interestingly, in this task, the DPPIV-deficient rats exhibited an increased anxiolytic-like response to the NPY administration compared to the wildtype-like animals by an increased SI time. This is supported by the frequency of *anal sniffing* with a significant differential response of the substrains regarding the different NPY treatments. To check for possible sedative-like influences of NPY on the anxiety-like behaviors in this task, we analyzed the overall motor (number of square entries) and social-like (summed frequency of social behaviors) activity of our animals. Motor activity was dose-dependently reduced by NPY treatment, but despite the sedative effect on ambulatory activity, social-like behaviors remained unaffected by NPY. Thus, NPY exerts a clear effect on anxiety as exemplified by an increased SI time in the rat social interaction test in the mutant animals, which was further substantiated by a significantly reduced frequency of *self-grooming* (as a measurement for displacement activity) in the mutant substrains, which disappeared dose-dependently after NPY treatment.

Overall, these data show the suggested differences between the wildtype-like and mutant F344 substrains regarding their NPY catabolism because of the more potent Y_1 receptor-mediated anxiolytic- and sedative-like effects of the neurotransmitter in the mutant, DPPIV-deficient animals. The differences within the two DPPIV-deficient substrains F344/DuCrj(DPPIV −) and F344/Crl(Ger/DPPIV −) in the social interaction test could be due to different compensatory mechanisms during ontogeny, but further studies are necessary focusing on this phenomenon.

In conclusion, this study shows for the first time a differential potency and specificity of NPY between wildtype-like and DPPIV-deficient rat substrains and thereby supports the concept that these animals provide a useful model to study the various behavioral and physiological effects associated with DPPIV-enzymatic activity. Therefore, F344 rat substrains F344/Crl(Por), F344/DuCrj(DPPIV −), and F344/Crl(Ger/DPPIV −) indicate an excellent natural animal model to study the "DPPIV–NPY axis". In particular, the mutant animals exhibited an increased responsiveness to the sedative- and anxiolytic-like effect of intracerebroventricularly administered NPY, which is very likely mediated via a Y_1 receptor-dependent mechanism. Since pharmacological inhibition of DPPIV-like activity has been demonstrated to potentiate the effects of NPY administration in vivo (Dimitrijevic et al., 2002), it seems possible that DPPIV inhibitors specifically targeting the CNS are useful modifiers of the centrally mediated effects of NPY.

Acknowledgements

This work is supported by the DFG (GK705). The critical comments by Sheila Fryk and Jerry Tanda on the manuscript are gratefully acknowledged.

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