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ORIGINAL INVESTIGATION

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FAUC 213, a highly selective dopamine D_4 receptor full antagonist, exhibits atypical antipsychotic properties in behavioural and neurochemical models of schizophrenia

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Abstract *Rationale:* 2-[4-(4-Chlorophenyl)piperazin-1-ylmethyl]pyrazolo[1,5-a]pyridine (FAUC 213) is a highly selective antagonist at the dopamine D_4 receptor subtype. It was designed as a derivative of two partial antagonists and has been proven to be a complete antagonist in mitogenesis assay. *Objectives:* In the present study, FAUC 213 was examined for antipsychotic properties in animal models of behavioural neurobiology and neurochemistry. *Methods:* Different concentrations of FAUC 213 were screened for effects on spontaneous, as well as amphetamine-induced, locomotor activity and apomorphine-induced prepulse disruption. The liability of causing extrapyramidal side effects was investigated in models of catalepsy and by high-performance liquid chromatography (HPLC) detection of dopamine turnover in several brain regions. The application schedule was validated, and the bioavailability of the compound determined, by means of a HPLC-pharmacokinetic study. *Results:* A significant effect in both the reduction of amphetamine-induced locomotor hyperactivity and the restoration of apomorphine-disrupted prepulse inhibition was found at 30 mg/kg. This dose proved not to be high enough to induce catalepsy or to increase dopamine turnover in the dorsal striatum, nucleus accumbens and medial prefrontal cortex. The selective D_4 antagonist FAUC 213, therefore, is not believed to mediate the above-mentioned effects via D_2 receptor antagonism, but a partial involvement of 5-HT_2- and α_1-receptors cannot be ruled out at present. *Conclusions:* We have gathered evidence that FAUC 213 exhibits atypical antipsychotic characteristics.

Keywords Dopamine D_4 selective · FAUC 213 · Complete antagonist · Prepulse inhibition · Locomotor activity · Catalepsy · Dopamine turnover · Atypical antipsychotic

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Introduction

Since the dopamine D_4 receptor was identified in 1990 by Van Tol et al., there has been increasing interest in this receptor subtype as a putative target for the treatment of schizophrenia. The D_4 receptor belongs to the D_2-like receptor family (D_2, D_3 and D_4) that is characterised by structural and functional similarities, such as a certain degree of homology and congeneric G-protein coupling. As the clinical potencies of typical neuroleptics, like haloperidol, seem to be intimately correlated with their D_2 receptor affinities (reviewed by Snyder 1996), the fact that some of these drugs also exhibit remarkable binding properties towards the other D_2-like receptors also aroused fundamental interest in the D_3 and D_4 receptor subtype. Moreover, the considerable pharmacological profile of the atypical antipsychotic clozapine has focused special attention on the D_4 receptor, since it shows a moderate 2-fold to 5-fold preference for D_4 over D_2 (Chabert et al. 1994; Roth et al. 1995; Newman-Tancredi et al. 1997; Löber et al. 2001) and good correlations between the clinically efficacious dose and its D_4 affinity (Seeman 1995; Seeman and Van Tol 1995). It also shows superior efficacy in refractory schizophrenia and negative symptoms such as amotivation and social withdrawal, as well as a minimal risk or even absence of side effects such as the extrapyramidal symptoms (EPS) and elevation of the prolactin level (reviewed by Remington and Kapur 2000).

The clinical use of clozapine is unfortunately restrained by the approximate 1% incidence rate of agranulocytosis, a presumably immune-mediated fatal lack of white blood cells. The challenge is, therefore, to develop novel atypical antipsychotics mimicking the unusual properties of clozapine. Due to the binding profile of clozapine, there are several approaches reflecting the different views about the receptor basis of this atypical drug action (reviewed by Seeman et al. 1997). These various hypotheses include low affinity for D_2 as exclusive drug action, concurrent antagonism at D_2 and muscarinergic receptors, balanced blocking of D_2 and 5-HT_{2A} receptors, and selective antagonism at D_4 receptors. With respect to the D_4 research, numerous investigations have focused on receptor distribution in different brain regions, involving autoradiographic labelling of receptor binding sites (Primus et al. 1997; Lahti et al. 1998; De la Garza and Madras 2000), neuroanatomical detection of receptor protein using specific antibodies (Mrzljak et al. 1996; Ariano et al. 1997; Defagot et al. 1997) and localisation of D_4-mRNA by means of Northern blot/reverse-transcription polymerase chain reaction (RT-PCR) (Van Tol et al. 1991; Matsumoto et al. 1995, 1996; Mulcrone and Kerwin 1997). These studies suggest, in a largely consistent manner, that D_4 receptors are preferentially located in limbic or cortical structures such as the amygdala and hippocampus or the prefrontal cortex, respectively. These are known to be involved in the regulation of mood and cognition and are suspected of playing a part in the aetiology of schizophrenia, although it remains controversial whether D_4 levels are increased in schizophrenic brains (Seeman et al. 1993, 1995) or not (Reynolds and Mason 1995). Also, lower D_4 levels were found, by comparison, in the striatum and other regions modulating motor functions, suggesting a small probability of causing EPS. Taken together, this evidence indicated that selective D_4 antagonists could be valuable in further investigations of the relevance of this receptor subtype in the treatment of schizophrenia. Now, after several of these compounds have been tested and published, the issue remains controversial (Bristow et al. 1997; Patel et al. 1997; Mansbach et al. 1998). For instance U-101958, a prominent D_4 selective antagonist, was found to exhibit some agonistic activity (Gazi et al. 1998, 1999). In contrast, 2-[4-(4-chlorophenyl)piperazin-1-ylmethyl]pyrazolo[1,5-a]pyridine (FAUC 213) was shown to be not only highly D_4 selective (Table 1), but also a complete antagonist with less than 3% agonistic activity compared with the full agonist quinpirole (Löber et al. 2001). FAUC 213 was therefore considered worthy of having its biological profile examined with methods of behavioural neurobiology and neurochemistry as described in this article.

Table 1 Receptor binding data of FAUC 213 and FAUC 113 compared with clozapine at human dopamine, porcine serotonin and α_1 receptors according to Löber et al. (2001). K_i values are the means of two to four independent experiments ±SEM using eight different concentrations, each in triplicate

Compound	K_i [nM] ± SEM									
	[^{3}H]Spiperone				[^{3}H]8-HO-DPAT	[^{3}H]Ketanserin	[^{3}H]Prazosin	Receptor selectivity		
	$hD_{2(long)}$	$hD_{2(short)}$	hD_3	$hD_{4.4}$	p5-HT_{1A}	p5-HT_2	$p\alpha_1$	$hD_{2(short)}/hD_{4.4}$	p5-HT2/$hD_{4.4}$	$p\alpha1/hD_{4.4}$
FAUC 213	3400±450	6300±1900	5300±720	2.2±0.23	1200±100	520±80	270±20	2900	240	120
FAUC 113	3200±400	4300±650	5000±650	3.6±0.87	1400±130	380±140	240±10	1200	110	67
Clozapine	41±1.5	28±0.50	960±45	16±0.50	460±120	12±1.0	n.d.	1.8	0.8	n.d.

Experimental procedures

Animals

A total of 94 male adult Wistar rats (Zur:Wist[HanIbm]; Research Unit Schwerzenbach, Schwerzenbach, Switzerland), weighing 300–350 g at the time of the experiments, were used in this study. The animals were housed in groups of four per cage under a reversed light-dark cycle (lights on: 1900–0700 hours) in a temperature- (21 ±1°C) and humidity- (55±5%) controlled room with free access to food (Kliba 3430, Klibamühlen, Kaiseraugst, Switzerland) and water. Beginning 3 days before the experiments, and continuing throughout the study, all rats were handled daily. Behavioural testing was carried out during the dark phase of the cycle. All experiments were conducted in accordance with Swiss regulations for animal experimentation.

Drugs

All drug solutions were freshly prepared on the day of experiment. FAUC 213 was synthesised at the Department of Medicinal Chemistry of the Friedrich-Alexander University Erlangen-Nürnberg and prepared for peroral application as a 7.5, 15 and 30-mg/ml solution in PEG400/200 mM acetate-buffer (pH~3.9) = 40/60 with the final pH adjusted to 4.3. D-Amphetamine sulfate (Sigma Chemical Company, St. Louis, USA) was dissolved in a 0.9% NaCl solution for intra-peritoneal injection in order to obtain a concentration of 1.0 mg/ml calculated as the salt. For increased stability, R(−)-apomorphine hydrochloride (Sigma Chemical Company) was dissolved in 0.9% NaCl containing 0.1% ascorbic acid giving a 0.1-mg/ml solution for subcutaneous administration. Haloperidol was diluted from ampoules with 5-mg/ml solvent (Janssen-Cilag, Baar, Switzerland) with 0.9% saline to a concentration of 0.3 mg/ml and adjusted to pH 5.5 by addition of Na_2CO_3 to enable intra-peritoneal injection.

Apparatus and procedures for behavioural testing

Detection of locomotor activity

Locomotor activity was measured in 15 stations, each being a clear plastic compartment 25 cm wide × 40 cm long × 40 cm high and completely embedded within an individual sound-attenuating wooden cabinet. One end wall of each compartment was made of wood, and the floor consisted of a black removable pan containing a thin layer of dark absorbent, autoclaved earth. A large drop-down door in the front wall of every compartment allowed easy access to the animal. A fan mounted on the wall of each cabinet provided adequate ventilation and a camera was mounted in the ceiling, centred approximately 49 cm above the compartment floor. The field of vision of this camera included the entire area in which the animal was freely able to move. The stations were located in a well-ventilated, temperature-, humidity- and sound-controlled room used only for this experiment. All the cameras were connected to a 16-channel multiplexer (Sony model YS-DX216CE) located in an adjacent room, and the multiplexer was connected in turn to a Dell OptiPlex GXpro computer running image-analysis software (Visual Basic program based on an NIH Image Analysis script). Each white Wistar rat was monitored against a darker background. An image from each of the stations was taken every second and compared with the previous one. The activity or animal's displacement was measured as pixel changes (from light to dark and vice versa) per second. One-second activity values ranged from 0% (no movement) to approximately 7.5%. The multiplexed images from the 15 stations could be recorded simultaneously on one videotape with a Sony SVT-1000P video recorder and viewed on a single monitor.

Prepulse inhibition

Prepulse inhibition (PPI) testing was conducted in four ventilated startle chambers (SR-LAB, San Diego Instruments, San Diego, CA), each containing a transparent Plexiglas tube (diameter 8.2 cm, length 20 cm) mounted on a Plexiglas frame. Acoustic pulses and prepulses were presented via a speaker mounted 24 cm above the tube. Motion inside the tube was detected by a piezoelectric accelerometer below the frame. The amplitude of the whole body startle to an acoustic pulse was defined as the average of 100 1-ms accelerometer readings collected from pulse onset. Delivery of the acoustic stimuli and recording of startle responses were controlled by a computer. The animals were tested in squads of four with startle chambers counterbalanced across the different experimental groups. After placing the animals inside the tubes, the startle session began with a 5-min acclimatisation period during which only a 68-dB(A) background noise was presented. This background noise continued throughout the session. After the acclimatisation period, four startle pulses of 120 dB(A), each of 30 ms duration, were presented. These served to achieve a relatively stable level of startle reactivity for the remainder of the test session since the most pronounced habituation of the startle reflex occurs within the first few startle-pulse presentations (Geyer et al. 1990; Koch 1999). To measure PPI, six blocks of 11 trials each were presented. The 11 trials in each block included: two "pulse-alone" trials, one "prepulse followed by pulse" trial for each of four prepulse intensities, one "prepulse alone" trial for each of four prepulse intensities, and one "no stimulus" trial. Prepulses were broad-band bursts of 20 ms duration and intensity of either 72, 76, 80, or 84 dB(A). The time interval between prepulse offset and pulse onset was always 80 ms. The different trial types were presented pseudo-randomly with an intertrial interval of 10–20 s (average 15 s). Altogether, a complete test session lasted about 23 min.

Catalepsy bar test

The animals were tested in a Perspex box measuring 26×17×17 cm containing a bar mounted 10 cm above the floor. Following a habituation period of 1 min, the animals were gently placed with their front paws on the bar while their hind paws remained on the floor. The latency for the rat to remove both front paws from the bar was measured, up to a maximum of 120 s.

Catalepsy paw test

A 30×30-cm Perspex plate mounted on plastic feet 20 cm above the floor was utilised. The plate contained two holes of 4 cm diameter for the forelimbs and two holes of 5 cm diameter for the hindlimbs, as well as an opening for the tail. The rat was held behind the forelimbs and gently placed in the holes, first with the hindlimbs and then with the forelimbs. The latency for the rat to retract one of the front paws was measured from the moment the rat retracted one of the hind paws (up to a maximum of 120 s).

Experimental design

Locomotor activity

Spontaneous and amphetamine-induced locomotion was assessed in the 15 test boxes with 30 rats on two successive days. On day 1, each rat was placed in one of the boxes and left there for 60 min to measure spontaneous (baseline) activity. The rats were then allocated to five treatment groups (VEH+VEH, VEH+AMPH, F7.5+AMPH, F15+AMPH and F30+AMPH) consisting of n=6 animals per group matched for baseline locomotor activity. On day 2 (treatment day), each rat was perorally pretreated with FAUC 213 in three different drug doses (7.5, 15 and 30 mg/kg bodyweight) or

vehicle (made-up of the identical solute as used to dissolve FAUC 213: PEG400/100mM-acetate-buffer(pH~3.9)=40:60 adjusted to a pH~4.3), and then immediately placed in the same box as the previous day for 60 min to monitor the influence of FAUC 213 versus VEH on spontaneous locomotion. Following this, all rats were injected intra-peritoneally with 1.0 mg/kg amphetamine or 0.9% saline and instantly replaced in their boxes for another 105 min to monitor the effects of FAUC 213 pretreatment versus VEH on AMPH-induced hyperactivity.

PPI experiments

Thirty-nine naive rats were used for the PPI experiment, conducted on two successive days. On day 1, all rats were subjected to baseline PPI testing and were then matched according to their PPI and startle response in four treatment groups. On day 2, the effect of pretreatment with FAUC 213 at dose levels of 15 mg/kg and 30 mg/kg versus VEH pretreatment was tested on apomorphine-disrupted PPI. Pre-treatment was applied perorally 45 min prior to the subcutaneous injection of 0.1 mg/kg APO versus VEH (0.1% ascorbic acid in 0.9% saline). Since APO penetrates quickly into the brain, the animals were placed in the PPI-boxes approximately 5 min after the injection. The four differently treated groups had sizes as follows: VEH+APO: n=9; VEH+VEH, F15+APO and F30 +APO: n=10.

Catalepsy experiments

The experimental protocol consisted of three groups of eight animals. Haloperidol (0.3 mg/kg, i.p.), FAUC 213 (30 mg/kg, p.o.) or vehicle (PEG400/100mM-acetate-buffer=40:60-adj. to pH~4.3, p.o.) was applied 45 min before testing. The rats were first monitored in the catalepsy bar test and then in the catalepsy paw test, as previously described.

Neurochemical and pharmacokinetic experiments

Postmortem HPLC analysis of dopamine metabolism

After the catalepsy testing procedure, the animals were taken to a separate room where they were sacrificed by decapitation exactly 60 min after the application of FAUC/HAL/VEH. The brains were removed rapidly, quickly frozen and stored at −20°C until preparation.

Tissue preparation

The frozen brains were placed ventral side up in a rat brain matrix (Harvard Apparatus, South Natick, MA, USA) on an ice-chilled plate. Double-edge blades were used to prepare coronal sections approximately 2 mm thick. The partially frozen slices were placed on an ice-cold dissection plate for the removal of discrete brain regions using a 2-mm diameter punch tip together with the MP-600 micropunch system (Harvard Apparatus). Tissue punches from the right and left hemispheres were taken together for subsequent neurochemical analysis. Three structures were prepared according to the atlas of Paxinos and Watson (1986): the medial prefrontal cortex (mPFC) was prepared from the first slice (Bregma 4.80 to 2.80), the nucleus accumbens (NAcc) from the next slice (Bregma 2.80 to 0.80), and the dorsal striatum (dStr) from the adjacent slice (Bregma 0.80 to −1.20). The punching was accomplished as shown in Fig. 1A for the mPFC, 1B for the NAcc and 1C for the dStr. The tissue samples were weighed and placed in 1.5-ml plastic tubes containing ice-cooled perchloric acid (300 µl, 0.4 M), homogenised for 10 s using ultrasound and centrifuged for 20 min at 15000 g and 4°C. The supernatant was passed through a 0.2-µm filter and kept at 4°C until high-performance liquid chromatography (HPLC) analysis.

Determination of dopamine and metabolites

Dopamine and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were analysed using reversed-phase ion-pair chromatography combined with electrochemical detection under isocratic conditions. The detector potential was set at +750 mV using a glassy carbon electrode and a Ag/AgCl reference electrode. The mobile phase (34.88 mM citric acid, 54.37 mM sodium acetate, 0.67 mM ethylene diamine tetraacetic acid disodium salt, 0.69 mM 1-octanesulfonic acid, 4% methanol in HPLC grade water, final pH 4.10) was delivered at a flow rate of 1.0 ml/min at 30°C onto the reversed-phase column [100×3.0 mm with guard column 10×3.0 mm filled with Chromsher 5B (Varian ChromSep, Palo Alto, USA)]. Aliquots (20 µl) were injected by an autosampler with cooling module set at 4°C. Data were calculated using an external standard calibration.

Pharmacokinetic study

Twenty male Wistar rats were treated with FAUC 213 (30 mg/kg, p.o.) 30, 45, 60, 90 or 120 min before being sacrificed by decapitation. Four untreated animals were also decapitated to enable calibration of the extraction process by spiking and extracting comparable brain

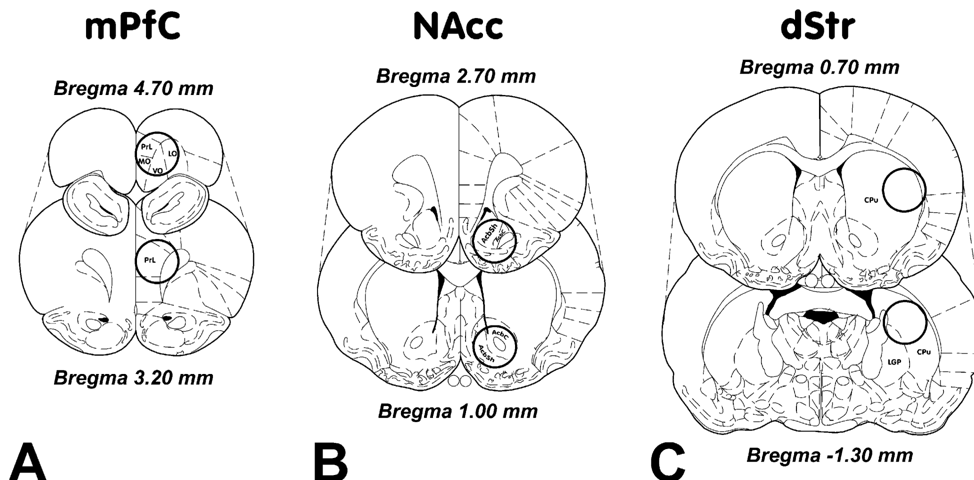

Fig. 1 Silhouette drawings of micropunchings onto representative sections of the rat brain. Localisation of the micropunchings for the medial prefrontal cortex (mPFC) (**A**), the nucleus accumbens (NAcc) (**B**), and the dorsal striatum (dStr) (**C**) are depicted by black circles 2 mm in diameter. The three tissue slice preparations are represented by one layer drawing close to the upper border and one close to the lower border, chosen according to Paxinos and Watson (1986): slice A (4.80–2.80 mm bregma), slice B (2.80–0.80 mm bregma), slice C (0.80 mm to −1.20 mm bregma)

tissue under analogous conditions. All 24 brains were rapidly removed, quickly frozen and stored at −80°C until further analysis.

Sample preparation

The frozen brains were weighed before being placed in 50-ml plastic tubes and covered with 15 ml chloroform. The brain tissue was homogenised in a cooling room at 4°C using an Ultra-Turrax T25 (Janke&Kunkel IKA-Labortechnik, Staufen i. Br.) at ~20000 rpm for 60 s and stored afterwards on ice for 120 s. A 5-N NaOH-solution (200 µl) was then added, and this mixture was homogenised two further times for 60 s each, storing it for another 60 s on ice in-between. An additional 15 ml chloroform was used to wash the adherent homogenate from the ultraturrax into the tube. These were stored on ice until re-mixing the tubes for 1 min by shaking and then centrifuging at 3000 rpm for 15 min. The organic layer was subsequently transferred into a round glass flask and evaporated to dryness at 474 mbar and 40°C. The residue was redissolved and sonicated for 15 min in 3 ml chloroform, passed through a 0.2-µm filter and evaporated under a stream of nitrogen at 70°C. Finally, it was redissolved again by a 15-min ultrasound treatment in 500 µl of a 60:40 (v/v) mixture of acetonitrile-water (containing 0.1% formic acid) and filtered through a membrane filter and stored at −80°C until HPLC analysis.

Detection of FAUC 213 brain levels

FAUC 213 was analysed using reversed-phase ion-pair chromatography combined with UV detection at 258-nm wave length under gradient-elution conditions. The mobile phase (mph1: acetonitrile/mph2:25 mM KH_2PO_4, 5 mM pentanesulfonic acid in HPLC grade water, final pH 7.00) was delivered at a flow rate of 1.0 ml/min at 40°C onto the reversed-phase column [250×3.0 mm with guard column 40×3.0 mm filled with LiChrosorb RP18 (Merck KG, Darmstadt, Germany)] of a particle size of 7 µm or 10 µm, respectively. The composition of the mobile phase was controlled by a gradient-time program consisting of five time periods: 0–15 min: plateau at 60% mph1/15–20 min: ramp from 60% to 90% mph1/20–30 min: plateau at 90% mph1/30–40 min: ramp from 90% to 60% mph1/40–60 min: plateau at 60% mph1 (to restore starting conditions). Fifty microliter aliquots were injected. Data were calculated by an external standard calibration. Brain tissue of untreated animals was spiked with different concentrations of FAUC 213 in the range of 500 ng to 6000 ng per sample.

Data analysis

All statistical analyses were performed with the StatView software system (Abacus Concepts, Inc., Berkeley, CA, 1992) using analysis of variance (ANOVA). Data are presented as means. In the text and in bar plots, the variability is indicated by the standard error of the mean (SEM). Fisher's protected least significant difference test was used for post-hoc comparisons following a significant main effect or interaction revealed by ANOVA. Significant differences were accepted at $P<0.05$.

Locomotor activity experiment

The locomotor activity of the rat was calculated as mean percentage pixel-change for each 5-min block of testing. A 5×12 ANOVA consisting of a between-subjects factor of treatment (VEH+VEH, VEH+AMPH, F7.5+AMPH, F15+AMPH, F30+AMPH) and a repeated-measurements factor of 12 5-min blocks was used to analyse the 1-h baseline activity and also the 1-h activity following injection of vehicle or FAUC 213 at doses of 7.5, 15 and 30 mg/kg. A similar 5×21 ANOVA was used to examine the 105 min following AMPH injection.

PPI experiment

The percentage of PPI (%PPI) induced by each prepulse intensity was calculated as: [100–(100×startle amplitude on prepulse followed by pulse-trial)/(startle amplitude on pulse-alone trial)] from the data taken during the six test blocks to measure PPI. The overall mean %PPI was calculated for the four prepulse intensities, whereas the mean startle amplitude was calculated as the average response to the 16 "pulse-alone" trials. The analyses consisted of three-way ANOVAs with a between-subjects factor of treatment (VEH+VEH, VEH+APO, F15+APO, F30+APO) and two within-subjects factors of day (baseline day, treatment day) and either the

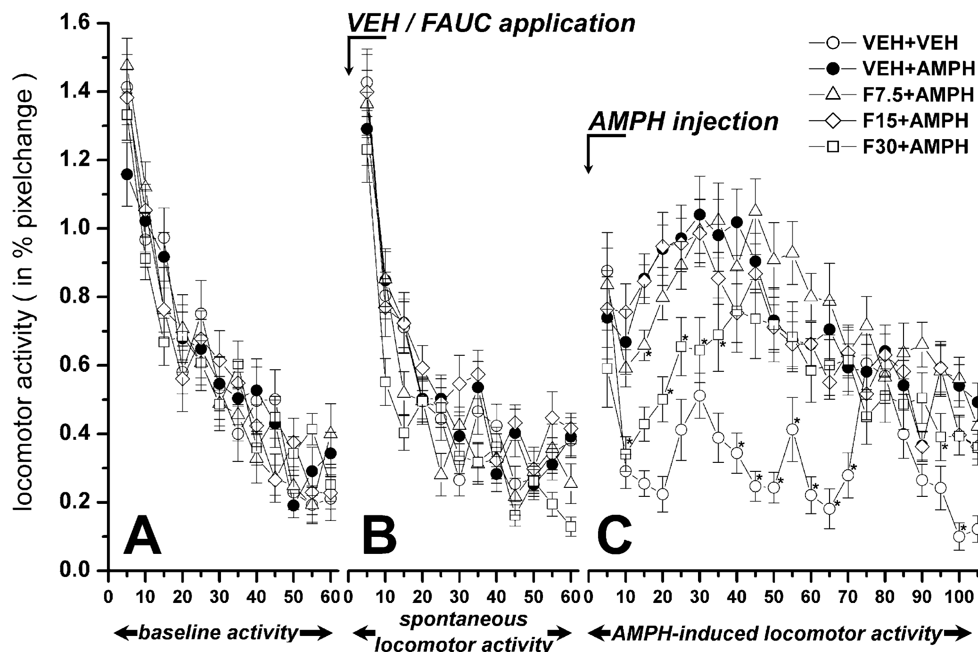

Fig. 2 Spontaneous and amphetamine-induced locomotor activity in the open field after peroral application of different doses of FAUC 213. Baseline activity (**A**) was recorded for 60 min 1 day before treatment. Spontaneous locomotor activity (**B**) was measured for 60 min following peroral application of FAUC 213 at doses of 7.5, 15 and 30 mg/kg or vehicle (n=6 rats per group). Amphetamine (1.0 mg/kg i.p.) was then injected and the induced hyperactivity was recorded for 105 min (**C**). All data are given as mean values of 5-min bins±SEM. *Asterisks* denote only the minimum effective dose compared with vehicle/amphetamine-treated animals (*$P<0.05$)

16 “pulse-alone” presentations (startle responses) or the four prepulse intensities (%PPI).

Catalepsy bar and paw test

The cataleptic behaviour of the rat was scored from 1 to 5 according to the (square root transformed) retraction times (min): 1=t<0.09, 2=t<0.36, 3=t<0.81, 4=t<1.44, 5=t≥1.44 min. ANOVAs consisting of the between-subjects factor of treatment (VEH, FAUC 213, HAL) were used for the bar test as well as the FRT (forelimb retraction time) and HRT (hindlimb retraction time) of the paw test.

Postmortem HPLC-analysis of dopamine metabolism

Dopamine turnover was calculated as ratio of the concentrations (ng/mg tissue) of (HVA + DOPAC)/DA for the separately analysed samples of the three different brain regions: NAcc and dStr. A 3×3 ANOVA with a between-subjects factor of treatment (VEH, FAUC 213, HAL) and a within-subjects factor of region (NAcc and dStr) was performed to evaluate the effects of FAUC 213 versus haloperidol and vehicle on dopamine metabolism.

Results

Activity during the 60 min on the baseline day

Baseline locomotor activity decreased over the 12 5-min bins ($F_{11,275}$=72.93, P<0.001), with no significant difference detected between the treatments. This outcome reflected habituation to the apparatus (Fig. 2A).

Activity during the 60-min monitoring spontaneous locomotion after FAUC/VEH application

Locomotor activity decreased over the 12 5-min bins ($F_{11,275}$=75.18, P<0.001), again reflecting habituation to the apparatus. No significant differences between treatments were found, indicating that spontaneous locomotor activity is not affected by the application of FAUC 213 alone (Fig. 2B).

Activity during the 105-min monitoring locomotor hyperactivity following amphetamine injections

The analysis of amphetamine-induced locomotor hyperactivity yielded a significant main effect of treatment ($F_{4,25}$=2.78, P<0.05), a significant effect of 5-min bins ($F_{20,500}$=8.24, P<0.001) and a significant effect of treatment × 5-min bin interaction ($F_{80,500}$=1.33, P<0.001). Post-hoc tests revealed that, compared with VEH injection, all four amphetamine-injected groups showed a significant elevation in activity (all P<0.001). Only pretreatment with FAUC 30 mg/kg was able to significantly reduce this elevation in AMPH-induced locomotor hyperactivity (P<0.001) (Fig. 3C and Fig. 4).

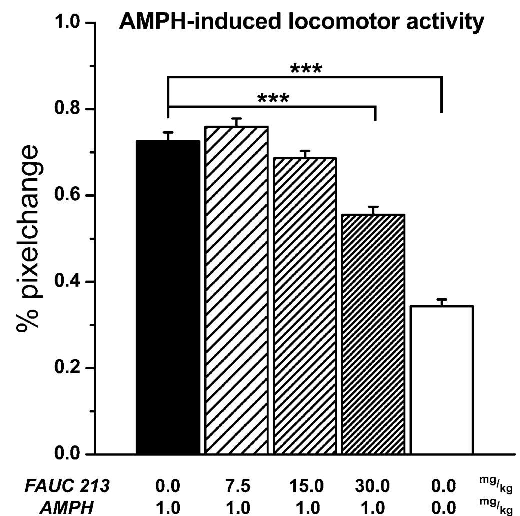

Fig. 3 Inhibition of amphetamine-induced locomotor activity in rats. *Bar plots* are calculated as the mean values of 5-min bins as shown in **C** (±SEM) of n=6 rats per group. *Asterisks* denote the significant differences compared with vehicle/amphetamine-treated animals (***P<0.001)

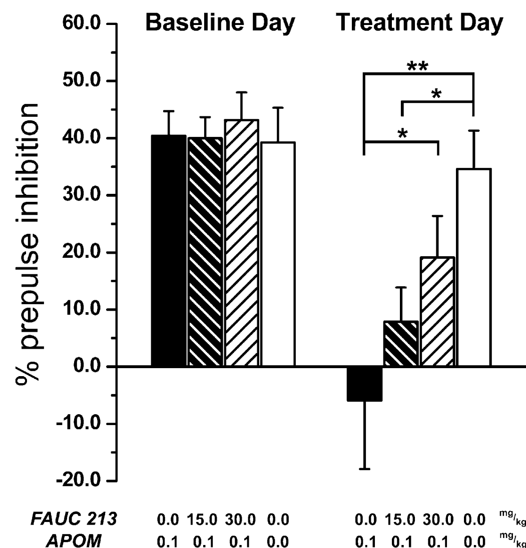

Fig. 4 Effects of pretreatment with FAUC 213 (15 mg/kg or 30 mg/kg, p.o.) on prepulse inhibition disruption by apomorphine (0.1 mg/kg, s.c.) in Wistar rats. Pretreatment was applied 45 min prior to apomorphine. Values are represented as means±SEM of n=9–10 rats per group. *Asterisks* denote significant differences between treatments as revealed by Fisher’s PLSD post-hoc tests (**P<0.01, *P<0.05)

Effects of apomorphine and apomorphine + FAUC 213 (15 mg/kg and 30 mg/kg) on startle response

There was no significant difference in the startle response amplitude of the animals between the different treatment groups. A habituation of the startle response amplitude over the 16 “pulse-alone” presentations was apparent ($F_{15,525}$=19.76, P<0.001). In addition, the significant effect of day ($F_{1,35}$=11.09, P<0.0025) and the interaction of day × pulse presentation ($F_{15,525}$=2.52, P<0.002)

reflected the steeper habituation on day 2 compared with day 1 (data not shown).

Effects of apomorphine and apomorphine + FAUC 213 (15 mg/kg and 30 mg/kg) on PPI

The analysis of PPI yielded a nearly significant main effect of treatment ($F_{3,35}$=2.65, P<0.065), a significant main effect of day ($F_{1,35}$=40.95, P<0.001) and a significant interaction of treatment × day ($F_{3,35}$=4.25, P<0.015). This reflected the fact, that while apomorphine in its own right completely abolished the PPI response, FAUC 213 dose dependently reduced this disruption (Fig. 4). Post-hoc comparisons revealed that on the treatment day (day 2) only FAUC 30 mg/kg was able to significantly restore the PPI reduction caused by the APO treatment (P<0.04), to the extent that this group did not differ significantly from the VEH group (P>0.16). A significant effect of prepulse intensity ($F_{4,105}$=29.02, P<0.001) reflected the increased effectiveness of higher intensities of the prepulse stimulus to induce stronger PPI.

Effects of FAUC 213 (30 mg/kg) vs haloperidol (0.3 mg/kg) and vehicle on catalepsy bar and paw test

A significant effect of treatment was found for the bar test ($F_{2,21}$=108.97, P<0.0001), as well as for the forelimb retraction time ($F_{2,21}$=12.02, P<0.001) and the hindlimb retraction time ($F_{2,21}$=145.80, P<0.0001) of the paw test. Post-hoc comparisons revealed that the mean scores of FAUC 213-treated animals did not differ from those of the VEH group, but haloperidol-treated animals had significantly elevated scores (all P<0.001) (Fig. 5).

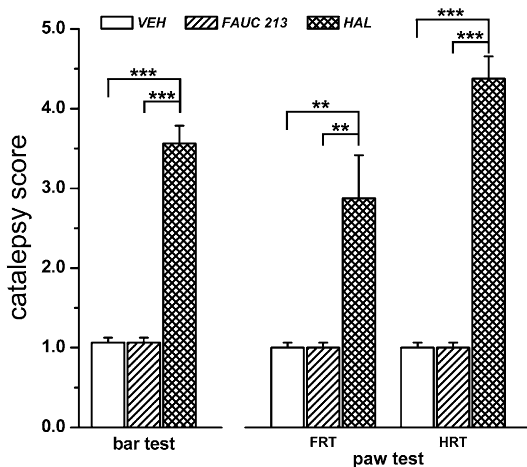

Fig. 5 Catalepsy scores of FAUC 213 (30 mg/kg, p.o.) versus vehicle and haloperidol (0.3 mg/kg, i.p.). Values are means±SEM of n=8 rats per group. FRT/HRT indicates the time for an animal to remove one fore or hindlimb in the paw test. All measured times are scored according to a square root transformed time scale: t<0.09=1, t<0.36=2, t<0.81=3, t<1.44=4, t≥1.44=5 (***P<0.0001, **P<0.001)

Effects of FAUC 213 (30 mg/kg) vs haloperidol (0.3 mg/kg) and vehicle on dopamine metabolism in mPFC, NAcc and dStr

Analysis of dopamine turnover showed a highly significant effect of treatment for the NAcc ($F_{2,21}$=12.68, P<0.001) and the dStr ($F_{2,21}$=63.72, P<0.0001), with a tendency towards significance for the mPFC ($F_{2,21}$=3.22, P<0.065). Post-hoc comparisons revealed that the ratio of dopamine turnover was significantly increased by haloperidol compared with both vehicle and FAUC 213 (NAcc: P<0.001; dStr: P<0.0001; mPFC: P<0.05), whereas FAUC 213-treated animals had a dopamine turnover virtually identical to that of vehicle-treated animals (Fig. 6).

Pharmacokinetic profile of FAUC 213

As shown in Fig. 7, brain levels of FAUC 213 determined 30, 45, 60, 90 and 120 min after peroral application (30 mg/kg) produced a “bateman-like” kinetic curve typical for this kind of drug administration. FAUC 213 penetrated rapidly into the brain, where it reached its highest concentration (2.09±0.63 µg/g) after 45 min and stayed at this level for about 15 min. This time period was in good agreement with the PPI experimental schedule. In addition, there was an interesting correlation between the decline of substance concentration in the brain after about 100 min, to less than half of C_{max}, with the convergence of the 30-mg/kg FAUC curve to the VEH+AMPH curve in amphetamine-induced locomotor activity monitoring at about the same time. The calibration curve was calculated by linear regression of the detected amount of FAUC 213 against the spiked amount and yielded r2~0.994.

Discussion

As the treatment of both positive and negative symptoms of schizophrenia, together with a necessarily good compliance achievable by the absence of adverse side effects such as extrapyramidal motoric symptoms or agranulocytosis, remains an unsolved challenge, many investigative efforts have been made to understand the basis of receptor interaction responsible for the atypical properties of clozapine. Due to the considerable affinities it shows to a number of pharmacological targets like D_2, D_4, 5-$HT_{2A/C}$, 3_1 and M_1 (for D_2, D_4 and 5-$HT_{2A/C}$ see Table 1, $K_i(\alpha_1)$=3.7 nM and $K_i(M_1)$=0.98 nM as reviewed by Arnt and Skarsfeldt 1998), the value of each single receptor, or possible combination of receptors, is hard to determine. As a consequence, different working hypotheses were established following the strategy of imitating one or more elements of that binding profile to gain the same superior clinical quality without the liability of causing the rare, but severe, side effect of agranulocytosis. Some of these have focused on balanced 5-HT_2/D_2-affinity often also including high α_1-affinity, as shown by risperidone, or affinity

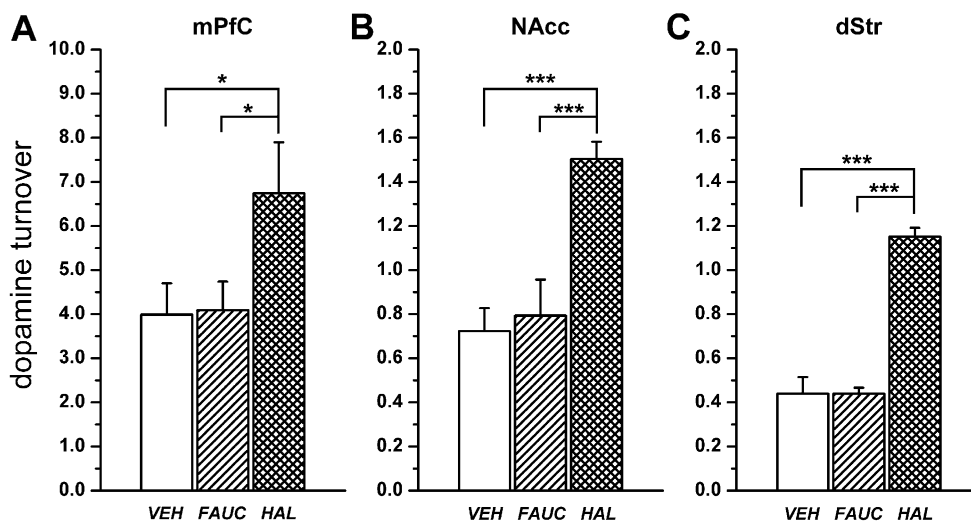

Fig. 6 Effects of FAUC 213 (30 mg/kg, p.o.) versus haloperidol (0.3 mg/kg, i.p.) and vehicle on the levels of dopamine turnover in the medial prefrontal cortex (**A**), the nucleus accumbens (**B**) and the dorsal striatum (**C**). Dopamine turnover is calculated as (DOPAC + HVA)/DA. Data are expressed as means±SEM of n=8 rats per group (***P<0.001, * P<0.05)

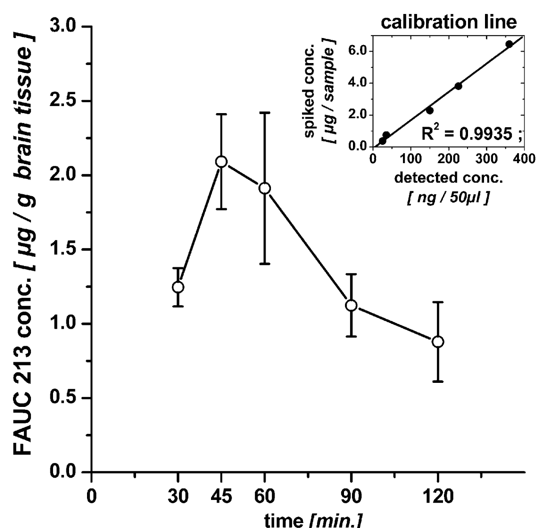

Fig. 7 Brain levels of FAUC 213 (30 mg/kg, p.o.) at five different times after application. Animals were decapitated after 30, 45, 60, 90 and 120 min. Concentrations of FAUC 213 were determined by reversed-phase high-performance liquid chromatography analysis using ultraviolet detection after extraction from the brain matrix. The system was calibrated by spiking brain tissue of untreated animals yielding a linear regression of r^2~0.9935. The *y-axis* represents relative concentrations of FAUC 213 in µg/g wet brain tissue (weighted in frozen condition). Concentrations are presented as mean values±SEM of n=3–4 animals for each time point

for muscarinic receptors, like olanzapine (reviewed by Arnt and Skarsfeldt 1998). But, because of these multiple receptor interactions (often also including 5-HT_{2C} or H_1), these substances produce a number of other side effects such as weight gain, sedation, dizziness, postural hypotension or dry mouth (Rowley et al. 2001). Another branch of research was the evaluation of highly selective substances acting at only one target. Due to the moderate selectivity of clozapine for D_4 versus D_2 receptors and the finding that D_4 receptors seem to be preferentially located in limbic or cortical structures suspected of being associated with schizophrenia, whereas only very low levels are found in the striatum and other regions modulating motor functions, the dopamine D_4 receptor subtype was considered to be one of the most interesting targets. With regard to this, FAUC 213 was designed by studies of regioisomers using FAUC 113 and FAUC 299 as lead structures (Löber et al. 2001) and was shown to be a complete antagonist in [^{3}H]thymidine incorporation as evidence for mitogenetic activity in contrast to its partial agonist precursors (Fig. 8). In addition, FAUC 213 had superior binding properties relative to FAUC 113, a higher affinity towards D_4 (K_i=2.2 nM vs 3.6 nM), as well as an increased selectivity of about 2900-fold for $D_{4.4}$ vs D_2, 240-fold for $D_{4.4}$ vs 5-HT_2 and 120-fold for $D_{4.4}$ vs α_1 (Table 1). In order to study the putative antipsychotic effects of FAUC 213, it was examined in several well-known models of behavioural neurobiology and neurochemistry, including pharmacokinetic validation of the applied parameters and doses. Throughout the whole study, male Wistar rats were used as subjects of experiment, as FAUC 213 showed binding affinities at rat-D_2 and D_4-receptors [$K_i(rD_2)$=1.1 nM and $K_i(rD_4)$ >1000 nM], which are in line with the binding affinities at the human D_2 and D_4 receptors (Table 1).

Stimulant-induced hypermotility, as a common model of in vivo screening of putative antipsychotics, was employed to determine effective doses of FAUC 213 when given perorally to rats. Pilot studies suggested, that adequate doses of FAUC 213 should start at about 5–10 mg/kg. We therefore chose 7.5 mg/kg as the lowest dose, and in addition used 15 mg/kg and 30 mg/kg by doubling the dose for dose–response studies. No significant effects on modification of spontaneous locomotor activity were observed at doses up to 30 mg/kg (p.o.). This served as a control that the drug itself does not exhibit unspecific, especially sedating, effects. In contrast, a dose of 30 mg/kg FAUC 213 significantly decreased AMPH-induced hyperactivity. Such an attenuation of hyperactivity is shown both by typical (like haloperidol), as well as atypical antipsychotics (such as clozapine or risperidone). It is therefore believed that direct D_2 antagonism may

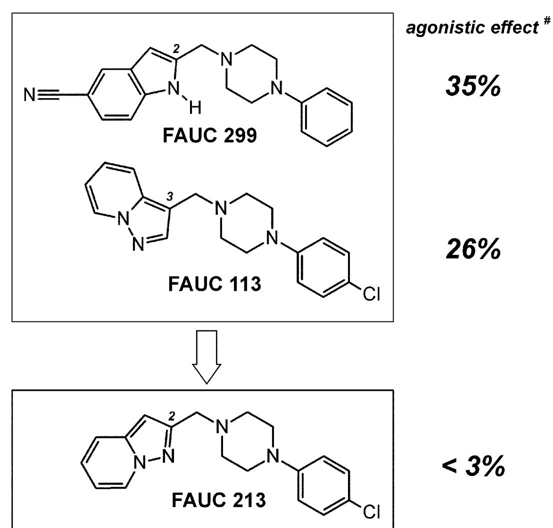

Fig. 8 Chemical structures of the two D_4-selective partial antagonists FAUC 113 and FAUC 299 as precursors of the D_4-selective complete antagonist FAUC 213. #Agonistic effects are given by the rate of [^{3}H]-thymidine incorporation as evidence for mitogenetic activity related to the full agonistic effect of quinpirole (100%), according to Hübner et al.(2000) and Löber et al. (2001)

contribute much to this effect, although other receptors (D_4, α_1, 5-$HT_{2A/C}$, etc.) undoubtedly have some impact.

PPI of acoustic startle in rats, one of the most intensively studied animal models with predictive validity for antipsychotic properties of compounds (reviewed by Geyer et al. 2001), was also used for evaluating FAUC 213. As a model of sensorimotor gating, the PPI paradigm reduces the startle reflex by a weak prepulse preceding the startle pulse, without it having the power to induce a startle reflex by itself. This phenomenon has been shown to be remarkably attenuated in schizophrenic patients. Analogically, it is disruptable in rodents or humans by treatment with, for instance, dopamine agonists (apomorphine) or dopamine releasers (amphetamine, Braff et al. 2001) and can be restored by typical and atypical antipsychotics. In accordance with the results obtained by measuring amphetamine-induced locomotor activity, we decided to perform the experiment at treatment levels of 15 mg/kg and 30 mg/kg (p.o.) FAUC 213 (the highest ineffective and the effective doses). Testing of higher doses was considered to be not appropriate, to the best of our knowledge, as we would lose receptor specificity. A dose-related increase in PPI compared with the vehicle-pretreated apomorphine group was found, with a significant effect at 30 mg/kg. Additionally, no significant effects on startle magnitude were detected, which indicates that the effects on PPI are not influenced by unspecific drug action such as sedation. These results confirm previously seen effects and also seem to concur with other investigations on predominantly D_4 selective compounds (Bristow et al. 1997; Mansbach et al. 1998), which reported significant restoration of the apomorphine-induced PPI deficit only at elevated doses. This raises the question whether receptors other than D_4 play a (major) role in the observed effects. Although mixed D_2/5-HT_2 antagonists were reported to decrease the effect of apomorphine on PPI (reviewed by Geyer et al. 2001), the pure 5-HT_2 antagonist MDL 100,907 restored PPI deficits caused by 5-HT_{2A} agonists, NMDA antagonists and isolation rearing, but not apomorphine (Geyer et al. 1999). Likewise the α_1 antagonist prazosine was shown to have an impact on the phencyclidine, but not apomorphine disruption of PPI (Bakshi and Geyer 1997). As a result of data obtained from knock-out mice (Dulawa et al. 2000), the activation of 5-HT_{1A} receptors is believed to increase PPI. Therefore, the 5-HT_{1A} antagonist (−)-UH-301 decreased PPI, whereas the 5-HT_{1A} antagonist WAY 100,135 showed an effect on neither PPI nor on restoration of PPI disruption caused by the D_2/D_3 agonist 7-OH-DPAT (reviewed by Geyer et al. 2001). All these data suggest that antagonism at neither α_1, 5-HT_{1A}, nor 5-HT_2 receptors is likely to contribute to the observed effects. Because D_2 receptor occupation is known to be correlated to the adverse side effect EPS, we investigated whether D_2 receptors are involved in the drug action at a dose level of 30 mg/kg FAUC 213. Therefore, it was tested in models of catalepsy against 0.3 mg/kg haloperidol as positive control. FAUC 213 clearly had no effect in either the bar test (Kuschinsky and Hornykiewicz 1972) or the paw test (Ellenbroek et al. 1987). As exemplified by haloperidol, typical antipsychotics usually reduce stimulated hypermotility at the same dose levels that induce catalepsy in rats, since both effects are most likely to be caused by D_2 antagonism. Atypical antipsychotics, however, are devoid of cataleptic effects at doses potent in stimulated hypermotility (Arnt and Skarsfeldt 1998). The conclusion is, therefore, that FAUC 213 is not thought to be mediating its effects via a high D_2 receptor occupation.

To further establish this deduction, we investigated the neurochemical effects of FAUC 213 versus haloperidol in three different brain regions known to be involved in antipsychotic drug action by postmortem HPLC analysis of dopamine and its metabolites. In both the NAcc and the dStr, haloperidol (0.3 mg/kg) produced significantly elevated levels of HVA and DOPAC, which contributed mainly to the increase of the dopamine turnover ratio, whereas FAUC 213 did not alter either DOPAC- and HVA- or DA levels. In the mPFC, haloperidol decreased DA levels, but only slightly increased levels of HVA and DOPAC, resulting in a nonetheless higher DA turnover, whereas FAUC 213 again did not alter DA levels and only slightly elevated the sum of DOPAC- and HVA levels. The results for haloperidol correspond to previous investigations (Karoum and Egan 1992; Patel et al. 1997), except for the fact that the increase in the turnover ratio in mPFC was mediated by DA decrease instead of increase of DOPAC+HVA. As FAUC 213 had no effect on dopamine turnover in NAcc and dStr, and only a negligible effect on the DOPAC+HVA levels in mPFC, it is supposed that this is due to a lack of activity at presynaptic D_2 receptors and the absence of presynaptic D_4 receptors. The blocking of the inhibitory effect of such presynaptic receptors on the release of dopamine into the synaptic gap followed by subsequent metabolisation to HVA or DOPAC (Westerink

1985) might not be the only possible explanation for the observed results, but it seems to be more plausible and concrete than the assumption of postsynaptic receptor blockade followed by a positive feedback mechanism on this synapse or on other dopaminergic neurons in this area. The lack of effect on dopamine metabolism shown at a dose of 30 mg/kg of FAUC 213 in this study, together with the inability to cause catalepsy, may account for the fact that, in contrast to haloperidol, there are not enough D_2 receptors occupied in brain regions controlling motor functions in order to cause extrapyramidal side effects.

Furthermore, our pharmacokinetic investigations revealed that the nominally high dose of 30 mg/kg only results in mean C_{max} levels of about 2.1 μg (6.4 nmol) per gram brain tissue. This might be due to weak peroral availability, efficient metabolising mechanisms or poor galenic formulation. In addition, the pharmacokinetic data confirm that the behavioural and neurochemical experiments were conducted when optimal levels of FAUC 213 were present in the brain.

Conclusions

Peroral administration of the dopamine D_4 receptor full antagonist FAUC 213 yielded a significant effect in both the reduction of amphetamine-induced locomotor hyperactivity and in the restoration of apomorphine-disrupted PPI at a minimal effective dose of 30 mg/kg. Although this dose seemed to be nominally high, only a small fraction was found present in brain after a period of 45–60 min. Additionally, this dose level proved not to be high enough to induce catalepsy in rats or to increase dopamine turnover in the dStr, NAcc and mPFC by antagonism of D_2 receptors. In summary, we have gathered evidence, that FAUC 213 exhibits atypical antipsychotic characteristics, although it remains uncertain whether other receptors such as adrenergic or serotoninergic were partially involved in the effects found in this study. The D_4 antagonist FAUC 213 should, therefore, be further examined.

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