EFFICACY OF POTENTIAL ATYPICAL ANTIPSYCHOTIC LEK-8829 ON BEHAVIORAL EFFECTS IN RAT MODEL OF CATALEPSY AND INHIBITION OF AMPHETAMINE INDUCED PSYCHOMOTOR STIMULATION

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Summary: D2 antagonist drugs, such as haloperidol, are potent antipsychotic drugs but unfortunately also have a high propensity to cause unwanted extrapyramidal syndrome (EPS). LEK-8829 is a dopamine D2 receptor antagonist with intrinsic activity at dopamine D1 receptors. Here we speculate that the intrinsic activity of LEK-8829 at dopamine D1 receptors may reduce its propensity to provoke EPS. The experimental rats were thus behaviorally evaluated in sessions performed before and after the 21 day treatment period with haloperidol or LEK-8829, for the induction of catalepsy or for the inhibition of amphetamine-induced locomotor activity in the open field by the same dose of the respective drug as was used for 21 daily injections. We found that after 21 day treatment with LEK-8829 or haloperidol, both drugs retained or even increased their potential for the induction of catalepsy. However, while LEK-8829 retained its efficacy for the inhibition of amphetamine-induced psychomotor stimulation, the efficacy of haloperidol was significantly diminished. We conclude that LEK-8829 retains its antipsychotic efficacy during prolonged treatment and possess lower propensity for the induction of EPS as compared to typical neuroleptic haloperidol.

Key words: LEK-8829; dopamine D1 agonist; dopamine D2 antagonist; haloperidol; subchronic treatment

Introduction

Patients with schizophrenia exhibit positive, negative and cognitive symptoms that arise from an imbalance between the brain dopamine (DA) pathways mediating D2 and D1 receptor signaling (1, 2). Both hypo functioning and hyper functioning DA systems thus likely coexist in schizophrenia, albeit in different brain regions (4). Subcortical increase of DA, leading to hyperstimulation of D2 receptors, would give rise to the positive symptoms, while a concomitant cortical deficit of DA, leading to hypostimulation of D1 receptors, would give rise to the negative and cognitive symptoms. There is indeed robust evidence that DA hypofunction and altered D1 receptor signaling within the prefrontal cortex (PFC) play a central role in the induction of working memory deficits, suggesting that a reduced D1 receptor neurotransmission might cause cognitive impairments in schizophrenia (3). Although the blockade of dopamine D2 receptors (4) is a prerequisite characteristic of all clinically effective antipsychotic drugs, the excessive blockade of dopamine D2 receptors within the basal...
ganglia often provokes unwanted extrapyramidal syndrome (EPS) (5,6,7,8,9), characterized by parkinsonism, akathisia, catalepsy, and, after long-term treatment, tardive dyskinesia (7,8). A major disadvantage of typical antipsychotic (neuroleptic) drugs is their propensity for the production of EPS (8,9,10,11). LEK-8829 (9,10-didehydro-N-methyl-(2-propynyl)-6-methyl-8-aminomethylergoline) has been initially developed in order to be more effective and/or produce fewer side effects than typical antipsychotics drugs, such haloperidol. In vitro radioligand binding studies revealed that LEK-8829 possess low affinity for rat striatal 3H-SCH23390-labeled dopamine D1 binding sites and high affinity for striatal 3H-spiperone-labeled D2 and cortical 3H-ketanserin labeled serotonin-2 (5-HT2) sites. The ratio of pKi values 5-HT1/D2 was 1.11 (closer to that of clozapine than haloperidol) (12). Based on these experiments it has been concluded that the drug may be considered to have atypical antipsychotic potential (8). However, further behavioral, gene-expression and pharmacological studies in unilateral animal models of striatal dysfunction and cocaine self-administration have revealed that LEK-8829 also possess a considerable intrinsic activity at dopamine D1 receptors (13, 14). It has been therefore proposed that such pharmacological profile of antipsychotic drugs could alleviate both negative and positive symptoms of schizophrenia (15).

Catalepsy may be considered to represent an animal model for neuroleptic-induced parkinsonism in humans (16,17) and is often used for the evaluation of the liability of newly synthesized potentially antipsychotic drugs for the production of EPS (18). Amphetamine elevates dopamine release and in animal models reproduces the positive-like symptoms of schizophrenia. Inhibition of amphetamine-induced motor hyperactivity and behavioral disinhibition by antipsychotic drugs is used to evaluate their efficacy for the amelioration of positive symptoms (19, 20,21, 22).

In this study we propose that the likelihood for the development of EPS by the blockade of dopamine D2 receptors with LEK-8829 may be modified by its intrinsic activity at dopamine D1 receptors. We have therefore compared the effects of the prolonged treatment by LEK-8829 or by the reference neuroleptic drug, a dopamine receptor D2 antagonist haloperidol that does not possess intrinsic activity at dopamine D1 receptors, on their liability for the production of EPS and on their efficacy for the treatment of the positive symptoms of schizophrenia.

Materials and methods

Animals

A total adult 104 male Wistar rats (MEC, Ljubljana) with 270-300 g initial body weight were used in all experiments. The rats were housed four per cage under constant temperature and humidity, under 12 h light/dark cycles (on 8 am - off 8 pm). Food and water were continuously available ad libitum in home cages. The animals have been on adaptation in laboratory conditions for 14 days (before being used in experiments). All experiments were approved by Veterinary Administration of the Republic of Slovenia (34401-53/2012/7).

Drugs

LEK-8829 (Lek, Ljubljana, Slovenia), was calculated as the free base and dissolved in 0.9 % saline. Haloperidol (Haldol ampules, 5 mg/ml; Krka-Jenssen, Krka Novo Mesto, Slovenia) solution was diluted to appropriate concentration with 0.9% saline. LEK-8829 or haloperidol were injected subcutaneously (s.c.) in the neck region and administered dissolved in the volume of 2 ml/kg body weight, respectively. Amphetamine (RBI, Natick, MA, USA) was dissolved in 0.9% saline and injected 2 mg/kg and administered s.c.

Catalepsy

Catalepsy was performed by the method described by Krisch (12). It was assessed by the placement of the front limbs of the rats over a horizontal bar (11 cm above the floor). Catalepsy was scored every 20 min for 260 min. The scoring started 20 min after the s.c. injection of the test compounds (LEK-8829 or haloperidol). The score was assigned on the basis of the duration of the cataleptic posture (i.e. until one forepaw touched the floor or the hind legs left the floor to climb onto the bar as follows: score 1 - between 15 and 29 s; score 2 -between 30 and 59 s; and score 30 - 60 s or more.
Inhibition of amphetamine-induced locomotor activity

Locomotor activity in the open field was measured in individual boxes (60cm x 40cm). Rats were given s.c. injection of test compounds (LEK-8829 or haloperidol 2 mg/kg and 0.2 mg/kg, respectively) and were put in the open field. After 20 min in the open field they were given an injection of amphetamine (2 mg/kg). Locomotor activity for individual rats was measured as distance moved in 120 min period using video tracking system for automation of behavioral experiments Noldus Ethovision Pro Version 3.0 (Noldus Information Technology, Wageningen, Netherlands).

Experiment 1

Seven groups of eight animals were used. All groups have been treated once daily for 21 days with drugs or 0.9% saline. The first group was treated with 0.9% saline. The second and the third group were treated with LEK-8829 (2 mg/kg, 0.2 mg/kg). The fourth and the fifth group were treated with haloperidol (2mg/kg, 0.2 mg/kg). In these five groups the catalepsy response was measured on day 1 and on day 21 of the experiment after injection with the same dose of the respective drug that was used for daily treatments. On all other days, the animals were put back into home cages immediately after drug injection.

The sixth group was treated with 0.9 % saline. The seventh group was treated with LEK-8829 (2 mg/kg). However, in these two groups the catalepsy response was evaluated on day 1 and on day 28 of the experiment so that between day 21 and day 28 the rats no received drug treatment.

Experiment 2

Six groups of eight animals were used. Animals have been treated once daily for 21 days. The first group was treated with 0.9 % saline. The second and the third group were treated with LEK-8829 (2 mg/kg, 0.2 mg/kg). The fourth group was treated with 0.9 % saline. The fifth and the sixth group were treated with haloperidol (2 mg/kg, 0.2 mg/kg). They were tested for the inhibition of amphetamine-induced locomotion with LEK-8829 (2 mg/kg, 0.2 mg/kg) one week before and one week after the prolonged drug treatment.

Statistical analysis

Data were analyzed using the SPSS computer program (SPSS 19.0 for Windows, Chicago, Illinois, USA). The statistical significance between catalepsy scores was compared by using non-parametric statistical analysis. The Mann-Whitney U Test was used to test for the differences between the treatments on catalepsy score of LEK-8829 vs. haloperidol on the first day. The Wilcoxon Signed Rank Test was used to test for the differences between the effects of treatments on catalepsy score on the first vs. the last day of the treatment. To evaluate if there was a significant difference between treatment groups in regard the effects of acute treatments on the distance moved parameter, we performed one-way ANOVA followed with Scheffe’s multiple comparison test, separately for the test performed before and after the 21 day treatment period, respectively. A paired Student t-test was used for the analysis of statistical significances between treatments on distance moved parameter before and after the 21 day treatment. Statistical significance was set at p < 0.05 for all statistical analyses.

Results

Catalepsy (experiment 1)

Mann-Whitney U Test revealed no significant difference between the catalepsy scores induced by LEK-8829 2mg/kg and haloperidol 0.2 mg/kg on the first day of the treatment (Fig. 1 - compare A - day 1 vs. C - day 1). Wilcoxon Signed Rank Test revealed a statistically significant increase of catalepsy score induced by of 2 mg/kg LEK-8829 on the last day vs. the first day of the treatment (Fig. 1, A - compare day 21 vs. day 1), but no statistically significant differences between catalepsy scores induced by 2mg/kg of LEK-8829 on the first vs. the last day of the treatment (Fig. 1, B - compare day 1 vs. day 28) or of the catalepsy scores induced by 2 mg/kg or by 0.2 mg/kg on the firs vs. the last day of the treatment (Fig 1, C, D – compare day1 vs. day 21). The treatment with saline or with 0.2 mg/kg of LEK-8829 for 21 did not induce catalepsy (i.e. the average catalepsy score during 260 min testing session was below 1) (Fig. 1, D, E).
Figure 1: Effect of prolonged treatment with LEK-8829, haloperidol or 0.9% saline on catalepsy

A - LEK-8829 2mg/kg, catalepsy on day 1 and day 21. B - LEK-8829 2mg/kg, catalepsy on day 1 and on day 28. C - haloperidol 0.2 mg/kg group, catalepsy test on day 1 and day 21. D - haloperidol 2 mg/kg group, catalepsy test on day 1 and day 21. E - 0.9 % saline group, catalepsy test on day 1 and day 28. F - LEK-8829 0.2 mg/kg group, catalepsy test on day 1 and on day 21.

Note: on the first treatment (i.e. in drug naïve animals) the dose of 2mg/kg of LEK-8829 was approximately equipotent with the dose of 0.2 mg/kg of haloperidol (compare day 1, A and C). There was a statistically significant increase of catalepsy in LEK-8829 2mg/kg group on the last day vs. the first day of the treatment (compare day 21 vs. day 1, A) that reverted to previous levels after one week of LEK-8829-free period (compare day 28 vs. day 1, E). No catalepsy was observed in LEK-8829 0.2 mg/kg group (catalepsy score ≤ 1), (F) compare also with absence of catalepsy in 0.9 % saline group (E).
Inhibition of the amphetamine-induced locomotion (experiment 2)

Both LEK-8829 (2 mg/kg) and haloperidol (2 mg/kg, 0.2 mg/kg) statistically significantly inhibited locomotor activity induced by 2 mg/kg of amphetamine both one week before and one week after the 21 day treatment period with the respective drug (One way ANOVA with Scheffe’s post hoc analysis, n = 8, p < 0.05). However, the inhibition of amphetamine-induced locomotor activity after the 21 day treatment by 2 mg/kg of LEK-8829 was equally effective than the inhibition achieved before the 21 day treatment, while the inhibition by haloperidol after the 21 day treatment by 2 mg/kg haloperidol was significantly less effective than before the 21 day treatment. (Two-tailed paired Student’s t, n = 8, p < 0.05). LEK-8829 in the dose of 0.2 mg/kg did not have a significant inhibitory effect when tested neither one week before or one week after the 21 day treatment period and there was also no statistically significant difference between the inhibitory effect of haloperidol in the dose of 0.2 mg/kg one week before and one week after the 21 day treatment period with the respective dose of haloperidol.

Discussion

In this study we have examined for the first time the effects of prolonged treatment by D2 antagonist/D1 agonist LEK-8829 on its efficacy in behavioral experiments. In preliminary experiments we found that for the induction of catalepsy in drug naive rats the dose of 2 mg/kg of LEK-8829 was approximately equipotent with the dose of 0.2 mg/kg of haloperidol, as scored by behavioral observation during four hours after the injection of the drugs. We have thus assumed that the occupancy of dopamine D2 receptors achieved by these doses of LEK-8829 and haloperidol were approximately equal so that we could then compare the effect of prolonged blockade of dopamine D2 receptors achieved by these doses of LEK-8829 or haloperidol on the efficacy of these two D2 antagonist drugs for the production of catalepsy and for the inhibition of amphetamine-induced locomotor activity.

Catalepsy was proposed to represent an animal model for neuroleptic-induced parkinsonism in humans and it is used as a standard preclinical test to predict antipsychotic activity and motor side-effect liability (17, 23, 24). It is well known that antipsychotics induce catalepsy in rodents (17, 25, 26, 27, 28) and have a dose-dependent propensity to induce EPS in humans and in animals (24). Catalepsy induced by antipsychotics presumably develops due to excessive blockade of dopamine D2 receptors within the basal ganglia. It is characterized by akinesia, the maintenance of even in unusual body posture (9) with waxy rigidity of the limbs, mutism, and complete inactivity, regardless of outside stimuli and may thus be considered to be analogous to sensory neglect, lack of movement and muscle rigidity, such as observed in parkinsonism. A full catalepsy can be induced by blocking either D1 or D2 receptors (17). Coadministration of the D1 and D2 antagonists
has a synergistic effect on catalepsy. However, catalepsy is not a simple motor inactivation due to the blockade of dopaminergic transmission within the basal ganglia, but rather a complex ‘active immobility’ response (17) that seems to involve more than one neurotransmitter system.

In the first experiment we evaluated the changes in the efficacy of LEK-8829 and of haloperidol for the induction of catalepsy that developed during the prolonged treatment with the respective dose of each drug. We found that only LEK-8829 significantly increased its potential for the induction of catalepsy. Namely, on the last day of the treatment with 2 mg/kg of LEK-8829, the latency for the induction of catalepsy decreased, while the maximal catalepsy score significantly increased and become approximately equipotent with 10X higher dose of haloperidol in drug naive rats (i.e. 2 mg/kg). By comparison, we have speculated that the effect of 21 day treatment with haloperidol on catalepsy might reveal the loss of its efficacy for the induction of catalepsy (26, 29, 30, 31), however, this was also not the case. We speculate that the above results may be explained by the “catalepsy sensitization effect” that develops due to context conditioned learning phenomenon inherent to most of the catalepsy measurement protocols (32, 33). However, when we tested the LEK-8829-treated animals after 7 days of drug-free period, the latency to the onset of catalepsy was decreased, but the catalepsy score reverted to the level induced by 0.2 mg/kg of haloperidol in drug naive animals. We thus speculate that the above results in LEK-8829 treated groups could be better explained by the pharmacokinetic build up and elimination of LEK-8829 in the brain during 21 day treatment period and during the following drug-free period, respectively.

The second experiment showed that in drug naive rats, both LEK-8829 (2 mg/kg) and haloperidol (0.2 mg/kg and 2 mg/kg) almost completely inhibited the locomotor behavior induced by amphetamine (2 mg/kg). However, after the prolonged treatment with the respective drug, only LEK-8829 retained its inhibitory properties, while the inhibition by 2 mg/kg of haloperidol was significantly reduced.

Polonged period of the blockade of dopaminergic transmission induced either by chronic treatment by dopamine D2 antagonist drugs or by the depletion of endogenous dopamine results in the up-regulation of striatal dopamine D2 receptors. The treatment with most antipsychotic agents thus causes an up-regulation of D2 dopamine receptors, an effect that may result in the loss of their efficacy and the production of irreversible motor side effects such tardive dyskinesia (28, 34). For example, decreases in dopamine receptor input produced by the administration of dopamine receptor antagonists, such as haloperidol, have been shown to increase the levels D2 dopamine receptors (35, 36, 37, 38, 39) and D2 receptor mRNA (40, 41). On the other hand, when selective D2 antagonist is combined with the agonist selective for the D1 receptors, both D2 receptor proliferation and behavioral supersensitivity is completely blocked (29). We therefore speculate that in our experiments the intrinsic activity of LEK-8829 at dopamine D1 receptors may have prevented the irreversible motor side effects such tardive dyskinesia (28, 34). For example, decreases in dopamine receptor input produced by the administration of dopamine receptor antagonists, such as haloperidol, have been shown to increase the levels D2 dopamine receptors (35, 36, 37, 38, 39) and D2 receptor mRNA (40, 41). On the other hand, when selective D2 antagonist is combined with the agonist selective for the D1 receptors, both D2 receptor proliferation and behavioral supersensitivity is completely blocked (29). We therefore speculate that in our experiments the intrinsic activity of LEK-8829 at dopamine D1 receptors may have prevented the up-regulation of dopamine D2 receptors, such as occurs after the prolonged blockade of dopamine D2 receptors with haloperidol. Moreover, prolong periods of dopamine depletion, such as occurs in animal models of parkinsonism could lead to the development of denervational dopaminergic hypersensitivity which is characterized by the functional uncoupling of dopamine receptors, so that when this occurs a selective agonists of either type of dopamine receptors induces psychomotor response, such as locomotor stimulation and stereotypies, even when the experimental animals are simultaneously treated with the antagonist of the opposite type of dopamine receptor. In models of parkinsonism with prolonged dopamine depletion in the brain the intrinsic activity of LEK-8829 at dopamine D1 receptors has been revealed by the robust psychomotor response that seems to be unabated by the concurrent blockade of dopamine D2 receptors by the drug or even by cotreatment with haloperidol (11, 42, 43, 44). We therefore assume, that the decreased efficacy of haloperidol for the inhibition of amphetamine-induced locomotor activity indicates dopamine D2 receptor upregulation that has developed during the period of prolonged treatment with haloperidol, while the prolonged treatment with LEK-8829 did not have such effect. Moreover, our results also imply that the prolonged treatment with LEK-8829 in experimental animals with functioning dopaminergic transmission does not result in the development of the functional uncoupling of dopamine receptors, since by this token the intrinsic activity of LEK-8829 on dopamine D1 receptors would by itself stimulate locomotor
behavior, despite the concomitant blockade of dopamine D2 receptors by the drug.

Conclusions

We conclude that LEK-8829 may retain its antipsychotic efficacy during chronic treatment and that pharmacological profile of atypical antipsychotic drugs with intrinsic activity at dopamine D1 receptors may be beneficial for the prevention of tardive dyskinesia. We therefore speculate that prolonged treatment with LEK-8829 does not result in the up-regulation and/or in the functional uncoupling of dopamine D2 receptors.

References

21. Dawe GS, Hwang EH, Tan CH. Pathophy-

22. Seeman P. All roads to schizophrenia lead to dopamine supersensitivity and elevated dopamine D2(high) receptors. CNS Neurosci Ther 2011;17(2): 118–32.


34. Seeman P. Dopamine receptors and the dopamine hypothesis of schizophrenia. Synapse 1987; 1(2): 133–2.


UČINKOVITOST POTENCIALNEGA ATIPIČNEGA ANTIPSIHOTIKA LEK-8829 NA VEDENJSKE UČINKE NA PODGANJIH MODELIH KATALEPSIJE TER Z AMFETAMINOM POVZROČENE PSIHOMOTORIČNE STIMULACIJE

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Povzetek: Antagonisti dopaminskih D2 receptorjev, kot je na primer haloperidol, so učinkovita antipsihotična zdravila, ki pa na žalost pogosto povzročijo neželeni ekstrapiramidni sindrom (EPS). LEK-8829 je antagonist dopaminskih D2 receptorjev z intrinzično aktivnostjo na dopaminskih receptorjih D1. Predpostavili smo, da intrinzična aktivnost LEK-8829 na dopaminskih receptorjih D1 lahko zmanjša nagnjenost te snovi za povzročanje EPS.

V raziskavi smo preučevali učinke 21 dnevnega tretiranja s haloperidolom oziroma z LEK-8829 na izraženost katalepsije ter na zaviranje z amfetaminom povzročene psihomotorične stimulacije v odprtem polju, tako da smo poskusne podgane vedenjsko testirali pred in po omenjenim 21-dnevnim obdobjem tretiranja in po njem, pri čemer smo uporabili enaka odmerka učinkov. Ugotovili smo, da 21 dnevno tretiranje z LEK-8829 oziroma s haloperidolom ni imelo učinka ali pa je celo povečalo njun kataleptični učinek. Po drugi strani pa se je pokazala razlika pri zaviranju z amfetaminom povzročene psihomotorične stimulacije v odprtem polju, pri čemer je bilo zaviranje z LEK-8829 enako učinkovito, zaviranje s haloperidolom pa manj učinkovito kot pri prvem vedenjskem testiranju.

Zato menimo, da bi imel pri zdravljenju shizofrenije potencialni atipični antipsihotik LEK-8829, v primerjavi s tipičnim nevroleptikom haloperidolom, manjšo tendenco za izgubo učinkovitosti ter za povzročanje EPS.

Ključne besede: LEK-8829; dopaminski agonist D1; dopaminski antagonist D2; haloperidol; subkronično tretiranje