Atypical antipsychotic effects of quetiapine fumarate in animal models

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KEY WORDS quetiapine fumarate；clozapine；haloperidol；antipsychotic agents；phencyclidine；swimming；depression；schizophrenia and disorders with psychotic features；basal ganglia diseases

ABSTRACT

AIM：To evaluate the effect of quetiapine fumarate in animal models of schizophrenia and its possibility to induce extrapyramidal side effects (EPSE). **METHODS**：The enhancement of immobility in a forced swimming test of mice induced by repeated treatment with phencyclidine and amphetamine swimming "normalization" test of mice were used as animal models of negative and positive symptoms of schizophrenia，respectively. The paw test of rats was used to evaluate the possibility by quetiapine fumarate to induce EPSE. **RESULTS**：After treatment with phencyclidine (10 mg·kg^{-1}·d^{-1}，sc，14 d)，the immobility time in the forced swimming test of mice was increased ($P < 0.01$). Quetiapine fumarate (20，40，and 80 mg·kg^{-1}，ig) and clozapine (10 and 30 mg·kg^{-1}，ig) attenuated the enhanced immobility in the forced swimming test induced by repeated treatment with phencyclidine ($P < 0.01$)，whereas haloperidol (0.3 and 1 mg·kg^{-1}，ig) had no effect. In amphetamine swimming "normalization" test，quetiapine fumarate ameliorated the disorder induced by amphetamine in a dose-dependent manner. In paw test，quetiapine fumarate was much less effective in increasing the forelimb retraction time (FRT) than the hindlimb retraction time (HRT). The minimal effective dose (MED) of HRT (MED_{HRT}) and FRT (MED_{FRT}) of quetiapine fumarate was 20 mg·kg^{-1} and 100 mg·kg^{-1}，respectively，and the ratio of MED_{FRT} to MED_{HRT} was 5. **CONCLUSION**：The effects of quetiapine fumarate in these models indicated its clinical effect on schizophrenia with a reduced liability to produce EPSE.

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Received 1999-04-19 Accepted 1999-06-22

INTRODUCTION

Schizophrenic symptoms may be grouped into positive (eg，hallucinations，delusions，disordered thinking and paranoia) and negative (eg，apathy，manifested as deficits in social interaction，emotional expression and motivation) symptoms[1]. Classical antipsychotics such as haloperidol are poorly efficacious in treating the negative symptoms of chronic schizophrenia，while atypical antipsychotics such as clozapine are more efficacious. The preliminary clinical studies suggest that 2-(2-[4-(dibenzo[b，f][1，4]thiazepin-11-yl)-piperazin-l-yl] ethoxy) ethanol (quetiapine fumarate)，may have therapeutic efficacy with respect to the negative symptoms[2，3]. In the present study，we investigated the possible therapeutic activity of quetiapine fumarate to alleviate the negative symptoms of the schizophrenia，using a new animal model of schizophrenia. Furthermore，we investigated the activity of quetiapine fumarate on amphetamine swimming "normalization" test and its propensity to induce extrapyramidal side effects (EPSE) using paw test that is regarded as a useful model for detecting atypical neuroleptic drugs.

MATERIALS AND METHODS

Chemicals Quetiapine fumarate [c-98-1-7 (B)] and phencyclidine hydrochloride were synthesized by Shanghai Institute of Pharmaceutical Industry. Clozapine was purchased from Suzhou № 6 pharmaceutic factory. Haloperidol was the product of Haipu pharmaceutic factory. Phencyclidine，haloperidol，and *d*-amphetamine were dissolved in 0.9 % saline solution. Quetiapine fumarate and clozapine were suspended in saline containing 1 % (w/v) carboxymethyl cellulose sodium salt (CMC).

Animals Kunming mice，♂，weighing (22 ± *s* 3) g；Wistar rats，♂，weighing (240 ± *s* 15) g and

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Mar；21 (3)
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Kunming mice, ♀, weighing (19 ± *s* 2) g at the beginning of experiments were obtained from Shanghai Experimental Animal Center, Chinese Academy of Sciences (Grade Ⅱ, Certificate № 02-49-2). The animals were housed in plastic cages and were kept at a temperature of 20 – 22 ℃ with a 12 h-light∶12 h-dark cycle (light on at 8 ∶00). Water and rat chow were available *ad lib*.

Effect of quetiapine fumarate on enhancement of immobility in a forced swimming test by repeated treatment with phencyclidine The test has been described by Noda Y *et al*[1]. In short, on d 1, each of the 110 male Kunming mice was individually placed in a transparent glass cylinder (20 cm high and 8 cm in diameter) with water 8 cm deep and was forced to swim for 3 min. The immobility time in the forced swimming was measured and the mice were randomized into 11 treatment groups according to the result. The mice were considered to be immobile when they remained floating on the water making only those movements necessary to stay afloat. From d 2 to d 15, the control group was injected sc with saline (10 mL·kg^{-1} mice body weight per day), and other groups were injected sc with phencyclidine at 10 mg·kg^{-1}·d^{-1}. On d 16, the control group and the vehicle group were administrated with saline (10 mL·kg^{-1} mice body weight, ig) and other groups were dosed with either haloperidol (0.3, 1 mg·kg^{-1}, ig), clozapine (3, 10, and 30 mg·kg^{-1}, ig) or quetiapine fumarate (10 to 80 mg·kg^{-1}, ig). One hour later, each mouse was placed into water again for 3 min and the immobility time was recorded.

Amphetamine swimming "normalization" test The test was carried out as previously described[4]. Briefly, 180 female Kunming mice were arranged randomly into 9 treatment groups. Mice were fasted for approximately 24 h and then dosed with either saline (10 mL·kg^{-1} mice body weight, ig), clozapine (2.5, 5, and 10 mg·kg^{-1}, ig) or quetiapine fumarate (5 to 80 mg·kg^{-1}, ig) respectively. Thirty minutes later *d*-amphetamine sulfate was administered ip at 2.5 mg·kg^{-1}. The amphetamine swimming "normalization" test was performed 30 min after the injection of amphetamine. Mice were placed into an 8.75 cm wide circular swimming channel that contained water at 25 ℃ to a depth of 5.5 cm. Marks were placed on the floor and on the side of the tank 180 degrees apart. A "swim" was scored each time a mouse swam from one mark to the other. The number of swim for 3 min was recorded for each mouse.

Paw test The apparatus employed in this study was described in detail previously[5,6]. All experiments were performed between 10∶00 AM and 16∶00 PM. On the day of experiment, the rat was injected ip with either saline (1 mL·kg^{-1} rats body weight), haloperidol (0.1 to 1 mg·kg^{-1}), clozapine (5 to 100 mg·kg^{-1}) or quetiapine fumarate (5 to 100 mg·kg^{-1}). One hour later, the rats were placed on the paw test platform, their limbs were put into the limb holes and the forelimb retraction time (FRT) and the hindlimb retraction time (HRT) were recorded. The minimum and maximum time of FRT and HRT was set at 1 s and 30 s. Each dose of haloperidol, clozapine or quetiapine fumarate (as well as saline control) was tested in a group of 8 drug-naive rats. The minimal effective dose (MED) was defined as the lowest dose that induced a great increase compared with saline.

Statistical analysis Results were expressed as $\bar{x} \pm s$ and analyzed using ANOVA followed by *t*-test.

RESULTS

Effect of quetiapine fumarate on enhancement of immobility in a forced swimming test by repeated treatment with phencyclidine The immobility time of repeated saline-treated mice in the forced swimming test described above was (50 ± 24) s. A great-prolonged immobility time was observed in the repeated phencyclidine (10 mg·kg^{-1}·d^{-1}, sc, 14 d)-treated mice. When such mice received classical antipsychotic haloperidol (0.3 and 1 mg·kg^{-1}, ig), the immobility time was not affected. But when atypical antipsychotic clozapine (10 and 30 mg·kg^{-1}, ig) was administered in such repeatedly phencyclidine-treated mice, the enhancing effect of phencyclidine on the immobility was greatly attenuated. The effect of quetiapine fumarate on the immobility time was similar to that of clozapine. At the dose of 20, 40 and 80 mg·kg^{-1}, quetiapine fumarate did reduce the immobility enhanced by repeated treatment with phencyclidine ($P < 0.01$), although it failed to reduce it at the lowest dose tested (10 mg·kg^{-1}) (Fig 1).

Amphetamine swimming "normalization" test Drug-naive mice could swim around the swim channel in a normal manner and their number of swims for 3 min was approximately 21, while *d*-amphetamine-treated mice failed to swim normally. Instead, they stood in one place and pawed the tank wall or made abortive swims, and their numbers of swims decreased in comparison with

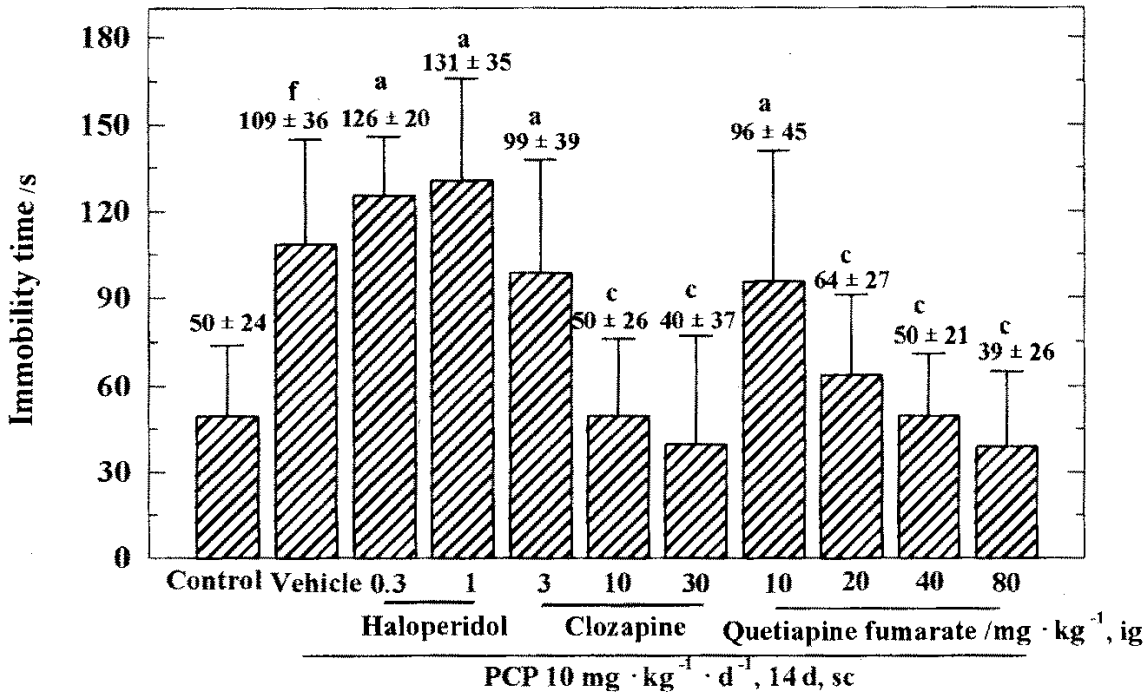

Fig 1. Effects of quetiapine fumarate, haloperidol, and clozapine on the phencyclidine-induced enhancement of immobility in mice. In the repeated phencyclidine-treated mice, the animals performed the measurement of immobility 1 h after vehicle, haloperidol (0.3 and 1 mg·kg^{-1}), clozapine (3 to 30 mg·kg^{-1}), and quetiapine fumarate (10 to 80 mg·kg^{-1}) were administered ig. $n = 10$ experiments. $\bar{x} \pm s$. $^{a}P > 0.05$, $^{b}P < 0.05$, $^{c}P < 0.01$ *vs* vehicle-treated repeated phencyclidine-treated group. $^{f}P < 0.01$ *vs* control group.

the control group ($P < 0.05$). After ig clozapine or quetiapine fumarate, the abnormal swimming of mice was ameliorated and their scores of swims for 3 min were increased in a dose-dependent manner (Tab 1).

Tab 1. Effects of quetiapine fumarate and clozapine in the amphetamine swimming "normalization" test in mice. $n = 20$ mice. $\bar{x} \pm s$. $^{a}P > 0.05$, $^{b}P < 0.05$, $^{c}P < 0.01$ *vs* vehicle-treated controls.

Treatment	Dose/ mg·kg^{-1}, ig	Swimming score
Vehicle		6 ± 9
Quetiapine fumarate	5	13 ± 8^{b}
	10	14 ± 9^{b}
	20	20 ± 10^{c}
	40	21 ± 14^{c}
	80	30 ± 14^{c}
Clozapine	2.5	3 ± 4^{a}
	5	15 ± 14^{b}
	10	19 ± 7^{c}

Paw test Control injection of saline induced only a very small FRT [(1.13 ± 0.13) s] and a small HRT [(3.9 ± 1.0) s]. Classical antipsychotic haloperidol produced a dose-dependent prolongation of the FRT and the HRT (Fig 2A). It had equal MED (0.15 mg·kg^{-1}) for both the FRT and the HRT, so the ratio of MED_{FRT} to MED_{HRT} was 1. Atypical antipsychotic clozapine led to a dose-dependent increase of the HRT with an MED of 10 mg·kg^{-1}. At the highest dose tested (100 mg·kg^{-1}), clozapine did not induce a great increase of FRT, so its MED_{FRT} was large than 100 mg·kg^{-1} therefore bringing the ratio of MED_{FRT} to MED_{HRT} to be more than 10 (Fig 2B). Similar to clozapine, quetiapine fumarate was much less effective in increasing the FRT than HRT. The MED_{HRT} and MED_{FRT} of quetiapine fumarate were 20 mg·kg^{-1} and 100 mg·kg^{-1} (Fig 2C) and thereby the ratio of MED_{FRT} to MED_{HRT} was 5 (Tab 2).

DISCUSSION

A major problem in evaluating atypical antipsychotics is the lack of adequate animal models for testing the negative symptoms of schizophrenia. The

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Mar；21 (3)
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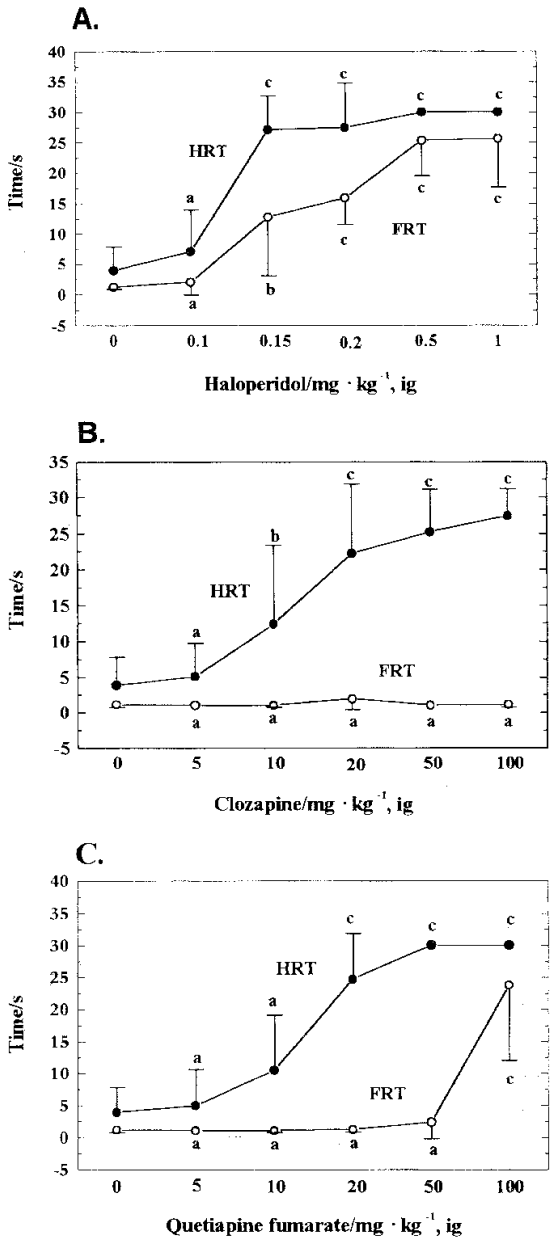

Fig 2. Effects of increasing doses of haloperidol, clozapine, and quetiapine fumarate on FRT and HRT in paw test. $n = 8$ mice. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control group.

amphetamine-induced social isolation in monkeys[6] and the phencycli-dine-induced enhancement of immobility in a forced swimming test of mice[1] seem to represent two of the few animal models which have been used for the evaluation of atypical antipsychotics for negative symptoms of schizophrenia. The test on mice might be a relatively simple one. Our results showing that repeated treatment with phencyclidine in mice prolonged the immobility time in the forced swimming test 24 h after the final injection andthat quetiapine fumarate and clozapine attenuated the phencyclidine-induced enhancement support the suggestion that this model could be used as a model for the negative symptoms. The mechanisms by which the negative symptoms are induced in phencyclidine psychosis have yet to be elucidated. The 5-hydroxytryptaminergic system has been suggested to be involved in the negative symptoms of schizophrenia. Phencyclidine has been shown to interact with the 5-hydroxytryptaminergic system, since phencyclidine inhibits [^{3}H] spiperone binding to 5-HT_{2A} receptors[7] and phencyclidine-induced behaviours are inhibited by 5-HT_{2A} receptor antagonists such as ritanserin[8]. Quetiapine fumarate is a novel dibenzothiazapine antipsychotic drug with low potency on dopamine D_2 receptor and relatively high potency on 5-HT_2 receptor[9]. In the present study, the enhancing effect of phencyclidine on immobility is antagonized by quetiapine fumarate and clozapine, both of which show 5-HT_2 receptor antagonistic properties. All these results suggest that the effect of phencyclidine on the immobility in the forced swimming test is mediated, at least in part, via 5-HT_2 receptors. However, other mechanisms involved in the effects of phencyclidine remain to be elucidated since phencyclidine is also known to interact with several other binding sites in the brain, including a phencyclidine binding site within the *N*-methyl-*D*-aspartate (NMDA) receptor complex and a sigma binding site.

The present data appears to be in line with the clinical effects of quetiapine fumarate published so far. Wetzel and colleagues, in an open label study, showed that quetiapine fumarate produced a great reduction in total scale for the Assessment of Negative Symptoms scores within 3 weeks[2], a finding also supported by Arvanitis *et*

Tab 2. Results of haloperidol, clozapine, and quetiapine fumarate in paw test. Presented are the minimal effective doses (MED) defined as the lowest dose which led to a great increase in the FRT and HRT.

Drug	MED_{FRT}/ mg·kg^{-1}	MED_{HRT}/ mg·kg^{-1}	Ratio MED_{FRT}/MED_{HRT}
Classical drug			
Haloperidol	0.15	0.15	1.0
Atypical drug			
Clozapine	> 100.0	10	> 10.0
Quetiapine fumarate	100.0	20	5

al in two double-blind trials[3].

Amphetamine-induced disorder of mice resembles to the positive symptoms of acute paranoid schizophrenia by augmenting dopaminergic neurotransmission within the CNS[10]. In amphetamine swimming "normalization" test, quetiapine fumarate and clozapine ameliorate the abnormal swimming of mice and increase their scores of swims for 3 min in a dose-dependent manner. This indicates that quetiapine fumarate, like clozapine, is effective for the positive symptoms of schizophrenia.

In the paw test, classical antipsychotics (like haloperidol, chlorpromazine[5], or fluphenzaine[6]) affect the forelimb retraction time as well as the hindlimb retraction time to a similar extent. Atypical antipsychotics (like clozapine, thioridazine[5], or risperidone[6]) are much more effective in increasing hindlimb retraction time. Neuroleptics-induced prolongation of HRT may betoken their antipsychotic action, while neuroleptics-induced prolongation of FRT may indicate their differential propensity for producing EPSE[5]. Quetiapine fumarate, similar to clozapine, is much less effective in increasing FRT than HRT. So our results, which coincide with the findings of Ellenbroek *et al*[6], may bespeak quetiapine fumarate has a low propensity for inducing EPSE. This is also in line with the preliminary clinical studies[11].

It has been reported that 5-HT_2 receptors play a conditional role in decreasing EPSE: 5-HT_2 antagonists may delay the onset and decrease the severity of EPSE but cannot totally eliminate its occurrence[12]. Furthermore, Ellenbroek *et al* showed that the 5-HT_2 blocking potency of risperidone was responsible for its lack of effect on the FRT[13]. So the similar effect on the FRT of quetiapine fumarate may be mediated, at least in part, via 5-HT_2 receptors since they both have strong affinity for 5-HT_2 receptors and comparatively weaker affinity for D_2 receptors, although further studies are needed.

Our findings, taken together with previous findings, indicate that quetiapine fumarate is an atypical antipsychotic drug with a low propensity to induce EPSE. Moreover, it appears to have an improved therapeutic profile with respect to the negative symptoms of schizophrenia. This profile correlates with the affinity of quetiapine fumarate for 5-HT_2 receptors.

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ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Mar；21 (3)
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富马酸奎的平在动物模型中的非典型抗精神病作用

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关键词 富马酸奎的平；氯氮平；氟哌啶醇；抗精神病药物；苯环利啶；游泳；抑郁症；精神分裂症及有精神病症状的紊乱；基底神经节疾病

目的：评价富马酸奎的平在精神分裂症阴性和阳性症状模型中的作用及导致锥体外系副反应（EPSE）的可能性. 方法：以苯环利啶（10 mg·kg^{-1}·d^{-1}，sc，14 d）引起小鼠在强制性游泳实验中保持不动的时间（immobility time，IT）延长为阴性症状模型，*d*-苯丙胺游泳正常化实验为阳性症状模型，paw test 评价药物导致 EPSE 的可能性. 结果：富马酸奎的平（20，40，80 mg·kg^{-1}，ig）可逆转苯环利啶引起的小鼠在强制性游泳实验中 IT 延长；5 – 80 mg·kg^{-1}，ig 可对抗 *d*-苯丙胺导致的小鼠游泳行为异常；在 paw test 中更易使后肢回缩时间延长，MED_{FRT}/MED_{HRT}比值为 5. 结论：富马酸奎的平是对精神分裂症阴性和阳性症状均有效的非典型抗精神病药物.

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