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Comparison of Antipsychotic Activity and Discriminative Stimulus Effects of the Novel Acylprolyltyrosine Containing Compound, GZR-123, and Sulpiride

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ASMAKOVA L. S., T. S. KALININA, R. U. OSTROVSKAYA, T. A. GUDASHEVA, N. I. ZAITSEVA, N. A. BONDARENKO, T. A. VORONONA, AND S. B. SEREDENIN. *Comparison of antipsychotic activity and discriminative stimulus effects of the novel acylprolyltyrosine containing compound, GZR-123, and Sulpiriride.* PHARMACOL BIOCHEM BEHAV **64**(2) 359–362, 1999.—The present experiment has been performed to determine the pharmacological profile of a newly synthesized systematically active prolyltyrosine containing compound, caproyl-L-Pro-L-Tyr methyl ester (GZR-123), and to compare it with that of the standard atypical benzamide neuroleptic, sulpiride. GZR-123 demonstrated antagonistic activity on apomorphine-induced climbing and on L-DOPA–dependent extrapolatory behavior in dose ranging between 0.4–4.0 mg/kg IP. It did not provoke a cataleptogenic effect or lethality, even at doses much higher than those causing antidopamine effects (more than 500 mg/kg). The effective doses of sulpiride in the above-listed antidopamine tests were shown to be 17.5 and 16.0 mg/kg IP correspondingly. Although these doses of sulpiride did not demonstrate cataleptogenic effects, an increase of the dose level to 120 mg/kg induced pronounced catalepsy. Both GZR-123 (6 mg/kg) and sulpiride (40 and 60 mg/kg) were investigated by training rats to discriminate each of them from saline in a two-lever, water-reinforcement operant procedure. Both GZR-123 and sulpiride demonstrated weak discriminability in this task. GZR-123 increased drug-associated lever selection when administered in doses of 2 and 6 mg/kg, for sulpirirde these doses were demonstrated to be 25–60 mg/kg. In contrast to GZR-123, which did not provoke a sedative effect, sulpiride in higher discriminable doses caused sedation, which stems probably from the motivational, but not from the motor deficit. © 1999 Elsevier Science Inc.

Antipsychotics Acylprolyltyrosines GZR-123 Sulpiride Antidopamine activity Drug discrimination

THE precise neural mechanisms underlying the pathophysiology and pharmacology of psychotic disorders was considered for more than 20 years as a consequence of dopamine hyperactivity. But as basic research has progressed, it has become clear that dopamine (DA) hyperactivity cannot be considered the single cornerstone of this pathology, and that other chemical messengers are involved, interacting with DA at pre- and postsynaptic sites. Among the other neurotransmitters are glutamic acid and GABA (1); among the regulatory peptides—neurotensin (NT) (8,13).

A novel approach that is developing in the Institute of Pharmacology Russian Academy of Medical Sciences (Moscow) is based on both the structure of NT and on the structure of the atypical benzamide neuroleptic sulpiride. This approach is based on the assumption that sulpiride can be supposed as a nonpeptidic analogue of the active NT fragment, responsible for its neuroleptic-like activity. Superimposition of L-Pro-L-Tyr-R and sulpriride (using Dreiding molecular models) revealed the overlap of pyrrolidine cycles and phenyl rings of both molecules in unstrained conformations. The sequence of Pro-Tyr corresponds to the neurotensin fragment NT (10–11), which is present in the major biologically active metabolite of neurotensin, NT (8–13). Based on this approach, a group of tripeptoid NT analogues was synthesized. The study of the substituted N-acylprolyltyrosines revealed a pronounced neuroleptic-like activity in the apomorphine-induced climbing test and in L-DOPA–dependent extrapolatory behavior (7).

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The aims of this study were to continue this investigation by studying the antidopamine activity of the novel representative of this group, caproylprolytyrosine methyl ester—GZR-123 (Fig. 1)—in comparison to sulpiride, and the analyze their discriminative properties.

METHOD

The following methods have been used for the evaluation of the pharmacological profile of the substances under study.

Inhibition of Apomorphine-Induced Climbing Behavior in Mice

Male mice (C57BL6), weighing 22–25 g, were placed in wire mesh cages (12 cm diameter, 14 cm high) and were allowed 1 h for adaptation and exploration of the new environment. Apomorphine was dissolved immediately before the experiments in saline containing 0.1% ascorbic acid, and injected at a dose of 5.0 mg/kg SC. The test compound was dissolved in saline containing 3% Tween 80, and was injected IP 10 min prior to apomorphine. For evaluation of climbing 10 min after apomorphine, 30 readings were taken for 1 h (scoring scale: four paws on bottom, no climbing—0; one paw on the wall—1; two paws on the wall—2; three paws on the wall—3; four paws on the wall, full climbing—4). Mice which were consistently climbing before apomorphine injection have been discarded. The climbing scores were individually totaled (maximum score: 120 per mouse) and the total score of the active control (vehicle IP—apomorphine SC) was set to 100% (6). ED_{50} values with 95% confidence limits were calculated using 8–16 mice per dose by Litchfield and Wilcoxon (11).

Inhibition of Extrapolatory Behavior Impairment Induced by L-DOPA in Rats

Male Wistar rats, weighing 180–200 g, were used in this experiment. A rat put into a cylinder plunged into water with the temperature of 22°C should solved an "extrapolatory task," i.e., dive under the cylinder's edge within 2 min, thus escaping this unusual situation. The animals were taken out of water immediately after the solution of the task. Eighty to 90% of the control rats solved the task. One day later 0.9% NaCl solution (control group) or a suspension in 3% Tween 80 of Madopar (100 mg/kg L-DOPA + 25 mg/kg benzerazide) (active control group) were administered IP 60 min prior the rat being put into the cylinder. GZR-123 or sulpiride dissolved in saline + 3% Tween 80 were injected IP 10 min prior to Madopar (test groups). The number of animals with successful escape responses was registered (2). ED_{50} values with 95% confidence limits were calculated using eight rats per dose by Litchfield and Wilcoxon (11).

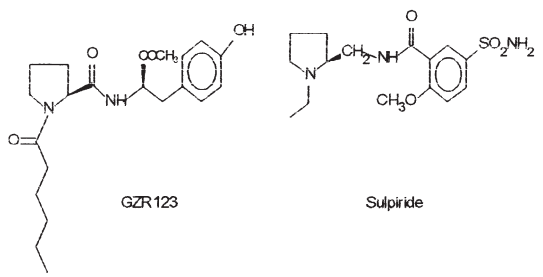

FIG. 1. The comparative chemical structures of GZR-123 and sulpiride.

Catalepsy in Rats

The ability to cause catalepsy was tested in adult male Wistar rats (200–250 g) 0.5, 1.0, 1.5, and 2 h after IP administration of the test compound according to the Morpurgo test (12). The rats were placed with their forepaws onto a bar situated 7 cm above the floor of a jar (22 × 38 × 16 cm), and tested for catalepsy according to a rating score (time resting upon the bar: <10 s—0; 10–20 s—1; 20–30 s—2; >30 s—3). Testing was repeated for each animal at 15-min intervals for 2 h.

Pull-Up Test

Male Wistar rats (250–300 g) were put on the horizontal bar by forepaws; the ability to perform the traction with a hind paws and to hold on the wire was registered for 1 min.

Open-Field Test

Male Wistar rats (300–400 g) were used. The apparatus consisted of a square wooden open field (60 × 60 cm), surrounded by a 25-cm high wooden wall. The square open field was divided into nine small squares with four holes in each. GZR-123 was injected 15 min and sulpiride 30 min before the observation. Each rat was placed in the middle of the central square. The total ambulation score, number of rears, and explorations of holes were evaluated for 2 min. The sum of all these parameters was taken as the "total activity."

Drug Discrimination in Rats

Male Wistar rats (weighting 220–250 mg at the beginning of the experiments) were trained in six operant chamber (Lafayette Instrument Co., IN) with sound-attenuating cages (model 800001), interface (model 118-01) and PC Apple IIe running the study. Each operant chamber was equipped with two levers, liquid dispensers, and an electroshock floor. Only one lever was operational on any particular session. Rats were trained to discriminate drug for saline under a two-lever water reinforced drug discrimination procedure. The daily session duration was 15 min. Initially, after 48 h water deprivation, either lever choice was reinforced with a drop of water, and the ratio of responses per reinforcer was gradually increased to 10. Then rats were trained to press one lever after drug injection and the other following vehicle treatment. To overcome an initial preference for the right or left lever, each group of animals was divided into two subgroups: those that were reinforced for left lever pressing and those that were reinforced for right lever pressing. Rats of all three groups were treated with either drug or saline in the daily sequence SDDSS-DSSDD (D = drug, S = saline). There were three groups of 12 rats. The first group of animals was treated with GZR-123 at doses of 2 mg/kg IP, 15 min before the training. The schedule requirement was FR10 (10 lever presses were required for each reward). After 20 sessions, however, it became apparent, that the animals were not acquiring a discrimination, and the dose was increased to 6 mg/kg. The rats of the second group were treated with sulpiride 25 mg/kg IP, 30 min prior to training sessions, and trained according to the FR10 schedule. After 27 sessions, as in the case with GZR-123, it became clear that the animals were not acquiring a discrimination, and the dose was increased to 40 mg/kg. To facilitate acquisition of the sulpiride discrimination, the animals of the third group were trained according to the FR1 (one lever press was required for each drop). These animals were treated with sulpiride 60 mg/kg IP 30 min prior to training sessions, and after 23 sessions the rats demonstrated the high lever of sulpiride dis-

crimination. However, special test session (3 min without reinforcement of any responding) revealed the lever of choice 50%. This prompted us to increased fixed ration from FR1 to FR6.

In any individual trial the accuracy of lever selection was assessed as the percentage of correct lever responses to the total amount of the pressing on both levers. The number of the presses per minute was designated as a rate of responding. The difference in the percentage of the responding on the drug-associated lever for drug-pretreated rats and saline-pretreated rats was analyzed by means of Student's *t*-test.

RESULTS

GZR-123 diminished the degree of apomorphine-induced climbing in the dose range of 0.4–4.0 mg/kg IP with an ED_{50} of 0.45 mg/kg. Sulpiride demonstrated an antiapomorphine effect at an ED_{50} dose as high as 17.5 mg/kg (Table 1). The same difference in the level of the effective doses for GZR-123 and sulpiride was revealed in another test, typical for antipsychotics of various chemical structures (2)—antagonism to L-DOPA–dependent impairment of extrapolatory escape. GZR-123 in doses up to 500 mg/kg induced neither catalepsy nor acute toxicity. Higher doses have not been tested. Sulpiride provoked catalepsy in doses between 100–140 mg/kg and acute toxicity in doses of 250 mg/kg.

Data on acquisition of the discriminative stimulus induced by GZR-123 are presented in Fig. 2. It is clear that animals treated with GZR-123 at a dose of 2 mg/kg after 20 trials selected the drug-associated lever with an accuracy of only 55–65%. The increase of the dose to 6 mg/kg enhanced this index to 65–75%. Acquisition of the discriminative stimulus properties induced by sulpiride is shown in Fig. 3. When administered at a dose of 25 mg/kg, sulpiride induced a level of discrimination of 65–80%. The increase of the dose to 40 mg/kg led to a small rise of this index, but 75% of the animals died during the 5-month-long experiment. The rats treated with sulpiride at 60 mg/kg did not acquire the discrimination with the FR1 schedule. That prompted us to change the schedule to FR6. In these conditions a rather high accuracy of lever selection (70–80%) was achieved (Fig. 4). This level was reached in a 2-month-long experiment without lethal outcomes.

GZR-123 in discriminable doses did not decrease the rate of operant respondings. Sulpiride decreased the rate of respondings (to 68 and 54% for doses of 40 and 60 mg/kg correspondingly). Long-term administration of sulpiride (60 mg/kg) attenuated the total open-field activity in rats (up to 38%). Judging by preservation of the pulling up on the horizontal bar, sulpiride's sedative effect manifested itself in the decrease of the responses rate is not mediated by myorelaxation. It comes probably from a motivational deficit. Single administration of sulpiride in doses 60–80 mg/kg did not change the rate of operant responding or open-field activity. If the increase of the sedation in the case of long-term (2–5 month) sulpiride administration is caused by accumulation of sulpiride or by the sensitization to this drug, it not quite clear.

TABLE 1

PHARMACOLOGICAL PROFILE OF GZR-123 IN COMPARISON TO SULPIRIDE

	ED_{50}, mg/kg 95%(Confidence Limits)		
Drugs	Apom*	DOPA†	Cata‡
GZR-123	0.45 (0.33–0.61)	0.35 (0.23–0.53)	>500
Sulpiride	17.5 (11.3–27.1)	16.5 (9.11–24.3)	⩾120

*Inhibition of apomorphine-induced climbing in mice.

†Antagonism of extrapolatory behavior impairment induced by L-DOPA in rats.

‡Induction of catalepsy in rats.

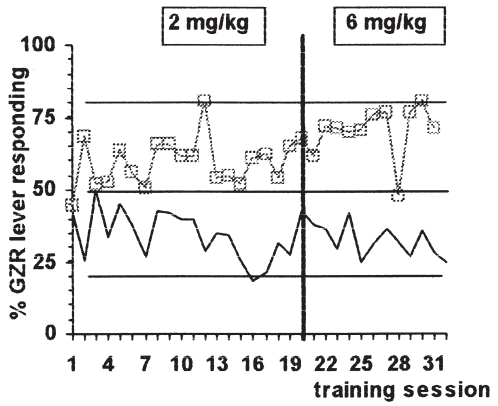

FIG. 2. Acquisition of the discriminative stimulus properties of GZR 123. Data represent the amount of the responding on the GZR-123–associated lever; the upper curve for GZR-123-pretreated sessions, the lower curve for saline-pretreated sessions. Each point is averaged for 12 rats.

DISCUSSION

N-Acylprolyltyrosine should be considered as a new type of potent antipsychotic agent with the ability to inhibit apomorphine-induced climbing without production of catalepsy even at doses much higher than antidopamine. The tendency to induce catalepsy in rats is known to be an indication of the propensity to cause extrapyramidal side effects (9). That suggests that GZR-123 will lack of extrapyramidal side effects. An important advantage of proline-tyrosine containing

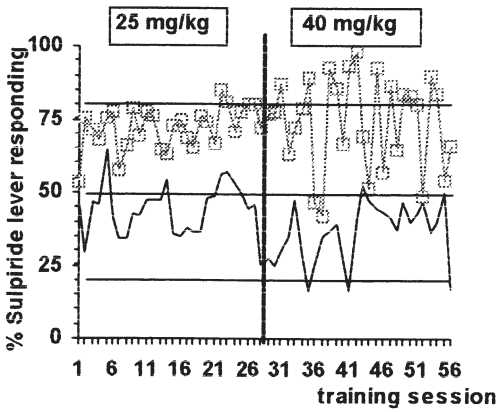

FIG. 3. Acquisition of the discriminative stimulus properties of sulpiride 40 mg/kg. Data represent the amount of the responding on the sulpiride-associated lever; the upper curve for sulpiride-pretreated sessions, the lower curve for saline-pretreated sessions. Each point is based on 12 rats at the first month and from 3 rats at the fifth month due to the 75% mortality induced by chronic sulpiride.

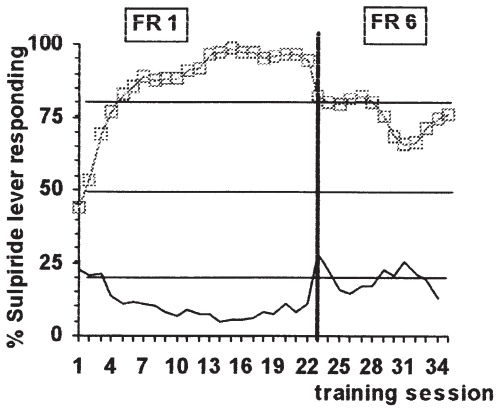

FIG. 4. Acquisition of the discriminative stimulus properties of sulpiride 60 mg/kg. Data represent the amount of the responding on the sulpiride-associated lever; the upper curve for sulpiride-pretreated sessions, the lower curve for saline-pretreated sessions. Each point is based on 12 rats.

dipeptides over the longer ones, including neurotensin and its major fragment, NT (8–13), is that they are effective after systemic administration (7). The results obtained with GZR-123, the newly synthesized compound of this group, confirm its efficiency as a promising antipsychotic substance. The comparison with sulpiride show the greater difference between the doses effective in antipsychotic tests and those for side effects (catalepsy, toxicity).

Catalepsy is a characteristic response to neostriatal dopamine receptor blockade (4), while apomorphine-induced climbing is mediated by enhanced dopaminergic neurotransmission in the mesolimbic system (10), thus GZR-123, like other N-acylprolyltyrosines, probably did not block neostriatal dopamine receptors. Its ability to overcome apomorphine-induced climbing suggests that it blocked of the dopaminergic neurotransmission in the mesolimbic system.

The study of GZR-123 and sulpiride in the drug discrimination procedure demonstrates weak to moderate activity (between 60 and 80%) for both substances, but while GZR-123 demonstrated this effect at doses of 2 and 6 mg/kg, sulpiride appeared to be effective only in doses of 25–60 mg/kg.

Neither long-term nor single administration of GZR-123 in discriminable doses produced a decrease in overall response rates in the drug discrimination procedure and did not influence open-field activity. On the contrary, long-term administration of sulpiride at a dose of 60 mg/kg produced a decrease of responses rate in the drug discrimination procedure and a diminuation of open-field activity. These data are consistent with those obtained by Sanger and Perrault (14) for sulpiride and Cohen et al. (5) for tiapride. The failure of GZR-123 to provoke a sedative side effect represents the important peculiarity of this compound.

GZR-123 did not induced lethality after long-term administration for 4 months, while long-term (for 5 months) sulpiride administration caused high mortality.

Currently available antipsychotic drugs have a number of limitations. The chief shortcoming of typical neuroleptics is the high incidence of side effects. Further aggravation of cognitive disturbances preexisting in schizophrenics may also be considered an important disadvantage of the typical neuroleptics. Sulpiride, like other atypical neuroleptics, does not provoke the pronounced extrapyramidal side effects in therapeutic doses, but its antipsychotic activity manifests itself only in the case of long-term administration, and the effectiveness in respect to negative symptoms is not always high enough (3). This is the reason for studying effective neuroleptics devoid of side effects. The development of a novel group of systemically active N-acylprolyltyrosines is likely to be a certain step forward in this field.

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