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Susceptibility of Flinders Sensitive and Resistant Rats to Pharmacologically Induced Seizures

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MILLAN, M. H., O. PUCILOWSKI AND D. H. OVERSTREET. *Susceptibility of Flinders sensitive and resistant rats to pharmacologically induced seizures.* PHARMACOL BIOCHEM BEHAV **50**(4) 505-508, 1995.—The Flinders sensitive (FSL) and Flinders resistant (FRL) line rats have been selectively bred for hyper- and hyposensitivity to the hypothermic effect of cholinergic agonists respectively. In this study, pilocarpine (250 mg/kg) and physostigmine (0.8 mg/kg) doses that are subconvulsant to outbread Sprague-Dawley rats were systemically injected to the FSL and FRL rats and a heterogenous F2 cross. All of the FRL rats developed severe motor limbic seizures in response to pilocarpine, while none of the FSL animals did. The F2 crosses showed intermediate reaction. The FRL rats were also more affected by physostigmine than the other two groups. However, the FSL rats were confirmed to be more sensitive to the hypothermic effects of pilocarpine (20 mg/kg) and physostigmine (0.6 mg/kg). Picrotoxin and kainic acid produced similiar responses in the both lines, i.e., induced clonic convulsions in some animals when applied in subtreshold doses (2 and 10 mg/kg, respectively). Thus, the normally cholinergic-insensitive rats are more sensitive to the convulsant effects of high doses of cholinergic agonists, but this increased sensitivity does not extend to noncholinergic convulsants.

Pilocarpine Physostigmine Kainate Picrotoxin Seizures Flinders Line rats

HIGH doses of cholinergic agents directly or indirectly activating muscarinic receptor induce motor limbic seizures and neuronal damage in many brain areas (4,5).

The Flinders Sensitive (FSL) and Flinders Resistant (FRL) line rats have been established by selective breading for difference in sensitivity to the anticholinestetase, diisopropyl fluorophosphate (DFP) (10). The line supersensitive to DFP, the FSL, is also more sensitive to physostigmine and direct muscarinic agonists, arecoline, and oxotremorine than the FRL rats (7,9). These differences have been demonstrated for the hypothermic and several other behavioral responses (6,7,9,12). Oxotremorine-induced hypothermia is currently used as a routine screening to assign animals to the FSL and FRL groups: the FSL rats typically exhibit a 2.5°C drop in temperature and FRL rats typically exhibit only a 0.7°C drop in temperature (6).

In the present study, high but subconvulsant doses [in the Sprague-Dawley (S-D) rat] of pilocarpine and physostigmine were injected systemically to both FSL and FRL rats as well as to a heterogenous group of F2 crosses. It was anticipated that the hypersensitive FSL rats would develop seizures in response to subconvulsant doses of muscarinic agonists and normally hyposensitive FRL rats would not. For comparison, sensitivity to convulsants involving other brain neurotransmitter systems (GABA and excitatory amino acids) was also tested.

METHOD

Animals

Male rats from colonies of FSL, FRL, and F2 cross rats (obtained by brother and sister mating of F1 cross, which was obtained by inermating the FSL and the FRL rats) maintained in the Skipper Bowles Center for Alcohol Studies, University of North Carolina School of Medicine were used. They were approximately 120 days old and their average weight was 425

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TABLE 1

SCORING SCALES USED TO ASSESS THE INTENSITY OF SEIZURES

Pilocarpine and Physostigmine	Picrotoxin	Kainate
1 Gnawing	Myoclonic body jerks	Staring
2 Tremor, splayed position	Forelimb clonus	Wet dog shakes
3 Head bobbing, walking backwards	Clonic-tonic seizure	Head/forelimb clonus
4 Rearing + forelimb clonus		Whole body clonus
5 Rearing + falling		
6 Myoclonic body jerks		
7 Clonic-tonic seizures		

± 10 g for FRL, 385 ± 10 g for FSL, and 430 ± 10 g for F2 crosses, respectively (mean ± SE). The lower body weight of adult FSL rats is a typical trait of this selectively bred line (6).

The rats were housed in groups two to three per cage (25 × 45 × 20 cm), under reversed phase 12 L : 12 D cycles (lights off at 1000 h) in a temperature-controlled room (21 ± 1°), with free acces to food and water.

Rats from each line were randomly assigned to experimental groups. All behavioral assesments were carried out between 1000 and 1400 h under white light illumination in the experimental room kept at 24°C. For determination of behavioral changes, each rat was individually placed in a transparent plastic cage identical to the home cage and left there undisturbed (habituation period) for 30 min prior to the initial temperature measurment or drug injection.

Drugs

Pilocarpine hydrochloride and physostigmine salicylate, both from Sigma (St. Louis, MO), were freshly dissolved in saline and administered IP in doses of 250 mg/kg and 0.8 mg/kg, respectively. These doses were chosen as subtreshold for inducing motor limbic seizures in outbred S-D rats on the basis of pilot experiments and published data (3,4,13). Scopolamine methyl nitrate (1 mg/kg) (Sigma) dissolved in saline was injecteted SC 15 min prior to the cholinomimetics to protect against their peripheral effects. For these experiments, eight rats in each of the groups was used.

Picrotoxin (Sigma) was dissolved in saline and injected SC in the dose of 2 mg/kg (n = 7). Kainic acid hydrochloride (Sigma) was dissolved in 50 μl of 1 N sodium hydroxide, then the solution brought to the final volume with PBS. Kainate was injected IP in 10 mg/kg dose (n = 6).

Seizure Assessment

The rating scales used to score seizure severity are given in Table 1. Additionally, the latency of seizures were recorded. The rats were observed for 60 min following injections, with the exception of the kainate experiment, where the observation time was 180 min. Kruskal-Wallis nonparametric one-way analysis of variance and the Mann-Whitney test were used to analyze the experimental data.

RESULTS

The two Flinders lines are characterized by significantly ($p \leq 0.001$) different, nonoverlapping distributions of temperature decrease scores following oxotremorine test performed at weaning (Table 2). The adult rats showed a similarly different response to the hypothermic effect of pilocarpine (20 mg/kg) and physostigmine (0.8 mg/kg) (Table 2).

Pilocarpine 250 mg/kg induced convulsions in all eight FRL rats, with a mean latency of clonic-tonic seizure of 11 ± 1 min (mean ± SE). None of the FSL rats has developed any kind of epileptic seizure, while only one out of eight F2 rats developed clonic-tonic seizure. The Kruskal-Wallis test on the

TABLE 2

HYPOTHERMIC EFFECT OF CHOLINOMIMETICS IN FLINDERS RATS

Drug (Dose)	Drop in Core Temperature			
	FRL		FSL	
	30 Min	60 Min	30 Min	60 Min
Oxotremorine (0.2 mg/kg)	−0.7 ± 0.1 n = 15		−3.2 ± 0.2* n = 13	
Pilocarpine (20 mg/kg)	−0.9 ± 0.2 n = 6	−0.4 ± 0.2	−2.8 ± 0.1* n = 6	−3.0 ± 0.2*
Physostigmine (0.6 mg/kg)	−0.9 ± 0.05 n = 6	−0.8 ± 0.1	−1.4 ± 0.05* n = 6	−1.5 ± 0.05*

The values represent mean ± SE. All rats were given injections of 1 mg/kg (−)scopolamine methyl nitrate 15 min prior to each cholinomimetic to minimize their peripheral effects. Oxotremorinetests were performed at 3 weeks of age as a routine screening test. Pilocarpine and physostigmine tests were carried out in adult animals 17 weeks of age.

*$p \leq 0.001$ vs. FRL, two-tailed Student's t-test.

rating scores yielded an H-value of 14.16, indicative of highly significant ($p \leq 0.001$) group difference overall. Follow-up Mann-Whitney test confirmed the difference between FSL and FRL rats ($p \leq 0.01$), but showed also that the F2 group response was significantly ($p \leq 0.05$) higher than the FSL group (Fig. 1). The dose of physostigmine chosen for the present experiment, 0.8 mg/kg did not precipitate clonic-tonic seizures in any of the groups studied. However five out of eight FRL rats developed clonic seizures while only one out of eight FSL rats did. In the F2 group, 50% of animals exhibited clonic convulsions to physostigmine. Therefore, the Kruskal-Wallis test on the rating scores confirmed that there was significant ($H = 7.59, p \leq 0.025$) difference in responses (Fig. 1).

The convulsant response to picrotoxin and kainate is shown in Fig 2. There were no significant differences between the FSL and FRL in the intensity of response. Three rats of each line seized following picrotoxin injection, while four FRL and three FSL rats exhibited clonic seizures after kainate injection. However, the average latencies of the maximum responses were consistently shorter in FRL than in FSL rats: 24 ± 3 min vs. 35 ± 1 min for picrotoxin; 68 ± 3 min vs. 101 ± 13 for kainate.

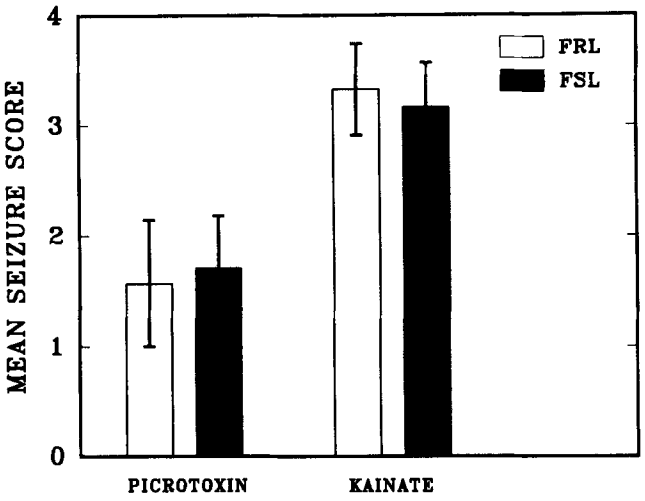

FIG. 2. Intensisty of behavioral response to picrotoxin (2 mg/kg; scale 0-3) and kainic acid (10 mg/kg; scale 0-4) in FRL and FSL rats. Columns represent mean values (± SEM) from seven rats (picrotoxin) and six rats (kainate) per group, Mann-Whitney U-test.

DISCUSSION

The results of this study show paradoxically that the FRL rats develop very potent limbic seizures in response to subconvulsant dose of pilocarpine, while the FSL rats do not (Fig. 1). This paradoxical increased sensitivity of the FRL rats to the high dose of pilocarpine occurred in spite of their being typically resistant to the hypothermic effects of low doses of oxotremorine and pilocarpine (Table 2). The responses of the FSL and FRL rats to the lower doses of cholinergic agonists are, therefore, consistent with all the previous literature on these rats (6,7,9,12-14), but the increased sensitivity of the FRL rats to a high dose of pilocarpine is not.

All the FRL rats developed maximum intensity (clonic-tonic) limbic seizures shortly after injection of pilocarpine. The same dose did not induce seizures in the FSL group, while in the F2, only one animal developed clonic-tonic seizure. As shown in a previous study, the 250 mg/kg dose of pilocarpine did not induce seizures in S-D rats (3) used to derive Flinders Line rats (10).

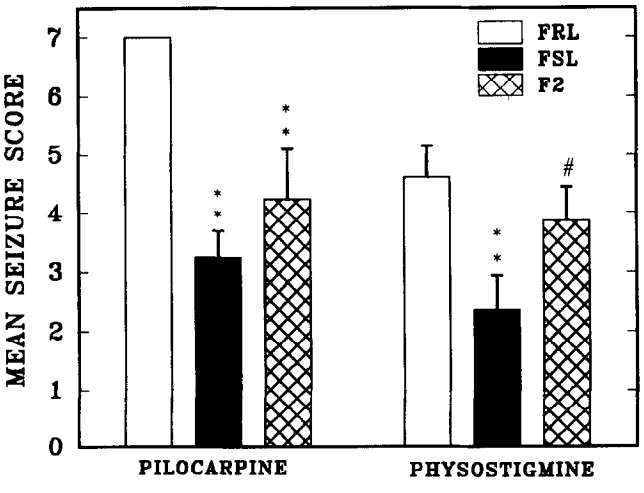

FIG. 1. Intensity of behavioral response of FRL, FSL, and F2 rats to 250 mg/kg of pilocarpine and 0.8 mg/kg of physostigmine IP. Columns represent mean values (± SEM) from eight rats per group. **$p \leq 0.001$ vs. FRL; #$p \leq 0.05$ vs. FSL, Mann-Whitney U-test.

Physostigmine in the subconvulsant dose used in this study did not evoke clonic-tonic seizures in any rat, but the pattern of group response (FRL > F2 > FSL) was similar to that of pilocarpine. In both cases, both FRL and FSL rats were different from the F2 rats, suggesting a two-way selection.

A great majority of the studies carried out on Flinder lines have compared the FSL group only to the FRL group. There are only a few studies that used randomly bred S-D rats or genetically heterogenous crosses. The earlier studies have generally indicated that the randomly bred rats and the FRL rats respond rather similarly to cholinergic challenges (10,14). However, more recent studies have tended to show that both FRL and FSL rats are different from randomly bred S-D or heterogenous crosses (6,9). Thus, the present findings are consistent with the more recent literature, suggesting that both Flinders Lines are different from randomly bred S-D rats.

The paradoxical hypersensitivity of the FRL rats to the subconvulsant dose of pilocarpine was unexpected, and explanations for this outcome are not immediately obvious. It should be pointed out that there is evidence for the involvement of other neurotransmitter systems in pilocarpine-induced seizures. Antagonists of glutamatergic receptors, muscimol (GABA agonist) and dopaminergic D_2 receptor agonists, protect against this kind of seizures (1,3,11). It may, thus, be worthwhile to consider evidence for changes in the function of these systems in the FSL and FRL rats.

It has been reported that the FSL rats are more sensitive to diazepam and muscimol (12), possibly due to higher GABA/benzodiazepine receptor population in the striatum as compared to S-D rats. This abnormality might make the FSL rats less susceptible to seizures. However, the FRL rats did not differ significantly from the S-D rats either in response to the drugs or in the density of the receptors. In addition, it has been shown that the response of FSL rats to the hyperthermic effect of MK-801 were similar in the two lines (13).

The fact that both lines of Flinders rats showed similar

response to kainate, excitatory amino acid agonist, and picrotoxin, a GABA antagonist (Fig. 2), makes it unlikely that differences in these systems between the lines could account for the differential susceptibility to pilocarpine-induced seizures. Few rats in either group developed seizures. The fact that the latency of the occurrence of maximal response was consistently shorter in the FRL rats further proves that this line is more seizure prone than the FSL overall.

There are dissimilarities between the two lines in the dopaminergic system function. The FSL rats are apparently less sensitive than the FRL rats to the stereotypic effects of dopamine agonists, indirectly indicating a subsensitivity of D_2 receptors in the FSL rats (2). The FRL rats, however, did not show abnormalities in the dopaminergic system function.

The discrepancy between sensitivity to hypothermic and convulsant effects of cholinergic agonists in the two lines might also be explained by a possible involvement of different types of muscarinic receptors and their distribution within the brain. However, there is lack of information on the muscarinic receptor subtypes involved in either phenomenon. Previous work has shown that the FSL rats have elevated a number of muscarinic receptors in the striatum and the hippocampus (8), as compared to FRL or S-D rats, but there are no published data on muscarinic receptor subtypes.

In conclusion, the data presented here show a greater seizure susceptibility of the FRL rats as compared to FSL and F2 rats in response to a high dose of pilocarpine. Reference to existing literature does not provide a satisfactory explanation for the hypersensitivity of the FRL rats to high doses of cholinergic agonists. These findings may instigate more thorough investigations, especially concentrating on abnormalities in the FRL rats.

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