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BRIEF COMMUNICATION

Effects of Repeated Testing on the Incidence of Haloperidol-Induced Catalepsy in Mice

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IWATA, S., K. IZUMI, T. SHIMIZU AND T. FUKUDA. *Effects of repeated testing on the incidence of haloperidol-induced catalepsy in mice*. PHARMACOL BIOCHEM BEHAV **33**(3) 705–707, 1989.—Effects of repeated testing on the incidence of haloperidol-induced catalepsy were investigated in mice. The incidence of catalepsy, evaluated with the forelimbs or hindlimbs placed on a standard horizontal bar, increased in three successive tests in mice injected with haloperidol. Catalepsy was not provoked by repeated testing in animals with saline. In a subsequent study, mice were examined for catalepsy in the forelimbs in the first two trials and then in the hindlimbs. In this procedure, the incidence of catalepsy did not increase with repeated testing. These results suggest that repeated testing increases the incidence of haloperidol-induced catalepsy but does not influence the cataleptogenic potency of the drug.

Catalepsy Haloperidol Mouse Repeated-testing

CATALEPSY is defined as an immobile state in which an animal keeps a constant, bizarre posture. Although the observation of catalepsy induced by drugs is a fundamental method in pharmacology, its assessment and interpretation appear to vary from one investigator to another. When time-course effects of a drug are examined, for example, a number of tests for catalepsy are required using the same animal. In such experiments, it is often unclear whether the results obtained are due to drug effects or merely adaptation effects to the test procedure. Some investigators have reported that catalepsy induced by haloperidol, a dopamine receptor blocker, was enhanced by repeated testing in rats or mice (1, 5–7, 10), and this enhancement was considered to be due to an increase in cataleptogenic ability of the drug during trials (6), whereas another report concluded that such enhancement derived from "training" effects (7).

We found in this study that haloperidol-induced catalepsy in mice was intensified after repeated testing, but that this phenomenon may not be connected with the cataleptogenic potency of the drug.

METHOD

Male ddY mice (Kuroda Junkei Dohbutsu, Ltd., Japan), weighing 29–56 g, were used. The animals were housed with free access to standard food (Clea Japan Inc.) in an air-conditioned room with a temperature of 22–24°C and humidity of 60–70% and maintained under a constant 12-hr light-dark cycle (light on 7:00 a.m.). Haloperidol (Yoshitomi Pharmaceutical Co.) was dissolved in 0.9% saline, and was administered intraperitoneally in a volume of 0.1 ml/10 g. Mice were individually placed in a clear, acrylic box (30 × 45 × 25 cm). Thirty min were allowed before drug injection for adaptation to a new environment. All experiments were performed in the box between 1000 and 1700. Catalepsy was assessed by placing forelimbs or hindlimbs on a horizontal steel bar of a 2-mm diameter at a height of 5 cm. A 60-sec cut-off time was applied to avoid fatigue. Catalepsy was defined as positive when the animals sustained the posture for over 30 sec.

Experiment 1

Effects of repeated or single testing on the incidence of catalepsy were investigated with the forelimbs placed on the bar. In repeated testing, catalepsy was evaluated at 10, 20, and 30 min following administration of haloperidol. Control animals receiving 0.9% saline were manipulated in the same way as test animals. In single testing, catalepsy was examined only once at 30 min after the drug injection.

Experiment 2

Effects of repeated or single testing on the incidence of catalepsy were investigated with the hindlimbs placed on the bar. In the first series of repeated testing (Experiment 2A), catalepsy was evaluated with hindlimbs at 10, 20, and 30 min following the injection of haloperidol. In the second series of repeated testing

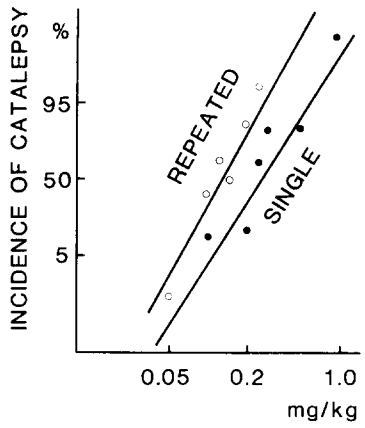

FIG. 1. Effects of repeated or single testing on the incidence of catalepsy in mice determined with the forelimbs placed on the bar. In the repeated (○) testing, catalepsy was evaluated with the forelimbs at 10, 20, and 30 min following administration of haloperidol. In the single (●) testing, catalepsy was examined with the same posture only once at 30 min after the drug injection. The percentage of animals that showed catalepsy determined at 30 min after the drug injection is plotted on the probability scale graph. Each point represents a percentage of 6–9 animals per dose.

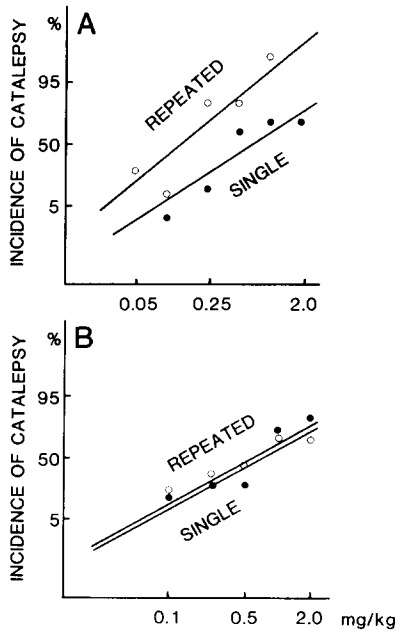

FIG. 2. Effects of repeated or single testing on the incidence of catalepsy in mice determined with the hindlimbs placed on the bar. In the first series of repeated (○) testing, (A), catalepsy was evaluated with the hindlimbs at 10, 20, and 30 min following administration of haloperidol. In the second series of repeated (○) testing (B), catalepsy was examined in the forelimbs twice at 10 and 20 min after the haloperidol injection. On the final trial at 30 min, animals were tested for catalepsy in hindlimbs. In the single (●) testing (A, B), catalepsy was measured in the hindlimbs only once at 30 min after the injection. The percenage of animals that showd catalepsy determined at 30 min after the drug injection is plotted as shown in Fig. 1. Each point represents a percentage of 7–8 animals per dose.

(Experiment 2B), catalepsy was examined with the forelimbs in the first two trials at 10 and 20 min after the haloperidol injection. On the final trial at 30 min, the mice were tested for catalepsy in the hindlimbs (handstand posture). Control animals receiving 0.9% saline were examined similarly as test animals in Experiments 2A and 2B. In the single testing, catalepsy was measured in the hindlimbs only once at 30 min after the injection.

The ED_{50} and 95% confidence limits were obtained by the graphic method (8) in which the percentage of animals that showed catalepsy determined 30 min following administration of haloperidol was plotted on the probability scale graph.

RESULTS

The ED_{50} (95% confidence limits) for catalepsy measured in the forelimbs at 30 min following the haloperidol injection was 0.26 mg/kg (0.12 to 0.34 mg/kg) in single testing and 0.13 mg/kg (0.11 to 0.16 mg/kg) in repeated testing (Fig. 1). The calculated potency ratio (P.R.) was 2.0 and the factor for P.R. ($f_{P.R.}$) was 1.42, indicating that repeated testing significantly increased the incidence of catalepsy ($p<0.05$). Saline-treated mice did not produce catalepsy in their forelimbs under repeated testing.

The ED_{50} for catalepsy measured in the hindlimbs under single and repeated testing was 0.64 mg/kg (0.33 to 1.44 mg/kg) and 0.13 mg/kg (0.08 to 0.23 mg/kg), respectively (Fig. 2A). The P.R. of 4.9 exceeded $f_{P.R.}$ of 2.5, indicating that repeated testing of the hindlimbs also significantly increased the incidence of haloperidol-induced catalepsy ($p<0.05$). Control mice which had received saline did not show catalepsy. Figure 2B compares the incidence of catalepsy between mice examined once in the hindlimbs, and mice tested repeatedly with the forelimbs in the first two trials and then with the hindlimbs. The two dose-response curves are almost the same. The calculated ED_{50} in single testing was 0.64 mg/kg (0.31 to 1.30 mg/kg), and that in repeated testing was 0.62 mg/kg (0.31 to 1.22 mg/kg). There is no significant difference between these values, indicating that repeated testing using different procedures does not intensify catalepsy.

DISCUSSION

The present study showed that a small dose of haloperidol increased catalepsy in mice under repeated testing. The incidence of catalepsy increased equally with forelimb and hindlimb testing. This effect cannot be due to repeated manipulation since control mice receiving saline did not show catalepsy on repeated testing. Our results are consistent with the data of Stanley and Glick (9) who first reported that rats tested repeatedly had greater catalepsy scores than animals tested once after haloperidol treatment. Other investigators reported similar observations in rats and mice (1, 2, 6, 7), although Costall *et al.* (4) claimed that they observed no difference in the intensity of haloperidol-induced catalepsy between rats tested frequently and on one occasion.

We performed catalepsy tests by different procedures in Experiment 2B in order to clarify whether repeated testing is able to potentiate the cataleptogenic ability of haloperidol. If the cataleptogenic effect of haloperidol is enhanced by repeated testing, the incidence of catalepsy would increase even if the method of subsequent observation were different. As illustrated in Fig. 2B, repeated testing with the forelimbs on the bar did not reveal the increased incidence of catalepsy in the handstand posture. This suggests that repeated testing in a different posture does not enhance catalepsy.

We conclude that repeated testing apparently enhances the incidence of haloperidol-induced catalepsy, but when different repeating procedures are used, catalepsy is not intensified. This suggests that the cataleptogenic potency of haloperidol is probably not influenced by repeated testing. In other words, our results support the view that the enhancement of drug-induced catalepsy by repeated testing is due to experience or training which may alter the susceptibility of animals to catalepsy.

REFERENCES

1. Ahlenius, S.; Hillegaart, V. Involvement of extrapyramidal motor mechanisms in the suppression of locomotor activity by antipsychotic drugs: A comparison between the effects produced by pre- and post-synaptic inhibition of dopaminergic neurotransmission. Pharmacol. Biochem. Behav. 24:1409–1415; 1986.
2. Antelman, S. M.; Kocan, D.; Edwards, D. J.; Knopf, S.; Perel, J. M.; Stiller, R. Behavioral effects of a single neuroleptic treatment grow with the passage of time. Brain Res. 385:58–67; 1986.
3. Campell, A.; Herschel, M.; Cohen, B. M.; Baldessarini, R. J. Tissue levels of haloperidol by radioreceptor assay and behavioral effects of haloperidol in the rat. Life Sci. 27:633–640; 1980.
4. Costall, B.; Hui, S.-C. G.; Naylor, R. J. Correlation between multitest and single test catalepsy assessment. Neuropharmacology 17:761–764; 1978.
5. Davies, J. A.; Williams, J. The effect of baclofen on α-flupenthixol-induced catalepsy in the rat. Br. J. Pharmacol. 62:303–305; 1978.
6. Hillegaart, V.; Ahlenius, S.; Magnusson, O.; Fowler, C. J. Repeated testing of rats markedly enhances the duration of effects induced by haloperidol on treadmill locomotion, catalepsy, and a condition avoidance response. Pharmacol. Biochem. Behav. 27:159–164; 1987.
7. Klemm, W. R. Neuroleptic-induced catalepsy: A D_2 blockade phenomenon? Pharmacol. Biochem. Behav. 23:911–915; 1985.
8. Litchfield, J. T., Jr.; Wilcoxon, F. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther. 96:99–113; 1949.
9. Stanley, M. E.; Glick, S. D. Interaction of drug effects with testing procedures in the measurement of catalepsy. Neuropharmacology 15:393–394; 1976.
10. Worms, P.; Willigens, M.-T.; Continsouza-Blanc, D.; Lloyd, K. G. The effect of different types of cortical lesions on drug-induced catalepsy in rats: A pharmacological analysis. Eur. J. Pharmacol. 113:53–59; 1985.