Pergamon

Pharmacology Biochemistry and Behavior, Vol. 49, No. 2, pp. 309–312, 1994

0091-3057/94 $6.00 + .00

0091-3057(94)E0123-Y

Tardive Dyskinesia: Behavioral Effects of Repeated Intracerebroventricular Haloperidol Injections in Rats Do Not Confirm the Kindling Hypothesis

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Received 13 October 1993

ANDREASSEN, O. A. AND H. A. JØRGENSEN. *Tardive dyskinesia: Behavioral effects of repeated intracerebroventricular haloperidol injections in rats do not confirm the kindling hypothesis.* PHARMACOL BIOCHEM BEHAV 49(2) 309–312, 1994.—The development of tardive dyskinesia (TD) has been claimed to be the result of a kindling-like mechanism. This hypothesis is based on studies suggesting that intermittent neuroleptic treatment may increase the risk of irreversible TD in humans and persistent vacuous chewing movements (VCM) in rats. We investigated the effect of daily intracerebroventricular (ICV) injections of haloperidol 37.5 μg/kg for 12 weeks in rats. Behavior was recorded immediately after the first injection, with 2 weeks intervals during treatment, and 3 weeks after drug withdrawal. In a separate experiment, rats received one injection of haloperidol IP. The acute effect of haloperidol ICV was an immediate increase in VCM and a reduction in motor activity that lasted for approximately 2 h. The behavioral response to haloperidol IP was similar, but of much longer duration. No significant behavioral changes were observed either 24 h after the ICV injections during haloperidol treatment or 3 weeks after drug withdrawal. The results did not support the hypothesis that kindling is important for the development of TD.

Tardive dyskinesia Haloperidol Intracerebroventricular injections Rats Vacuous chewing movements

TARDIVE dyskinesia (TD) is a frequent and serious adverse effect of chronic neuroleptic treatment. The repetitive involuntary movements involve predominantly the muscles in the face, mouth, and tongue. In most patients the dyskinesias fade away after drug withdrawal, but they may be irreversible. The pathophysiological substrate of TD is not known. Several hypotheses have been proposed, among these the kindling hypothesis (15,16).

In the original kindling model, repeated electrical stimulation of various brain sites eventually evokes behavioral and convulsive responses to an initially subthreshold current. The responses may become stimulus independent (9). The kindling concept has later been extended to include pharmacological as well as psychological stimulation (15).

The application of the kindling concept to the development of TD is mainly based on the assumptions that there is a resemblance between electrical potentiation in kindling and drug sensitization during an intermittent neuroleptic treatment regimen, and that drug sensitization is relevant to the development of TD (6–8,15,16). Support for the kindling hypothesis for the development of TD has emerged from studies showing that discontinuous treatment may play a role in the development of neuroleptic-induced oral movements in rats and the development of persistent TD in humans (2,5–7,10,17,20).

Neuroleptic-induced dopaminergic (DA) supersensitivity, that traditionally has been related to the development of oral dyskinesias, has, however, been found after both continuous and discontinuous treatment (1,3,12,13). Furthermore, the importance of DA supersensitivity in the development of TD has been questioned by several authors (18,19).

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The aim of the present study was to further investigate the kindling hypothesis for the development of TD. A rat model of TD was used, studying the development and persistence of neuroleptic-induced oral movements (4,11,18). To achieve a discontinuous treatment with a strong but very short-lasting neuroleptic effect, haloperidol was injected intracerebroventricularly (ICV) daily for 12 weeks, expecting this type of drug exposure to be the best way of imitating the conditions of the original kindling model. To compare the effect of haloperidol administered IP and ICV, a separate experiment injecting haloperidol IP was performed.

METHOD

Animals

Female Sprague-Dawley rats (Mol : SPRD, Møllegaard, Denmark), weighing 225–250 g at the start of the experiment, were used. The rats were housed individually with free access to water. The food was limited to 15 g of pellets per animal per day. The light phase lasted from 0800 to 2000 h. Ambient temperature was 22–24°C. The behavioral recordings took place between 0800 and 1200 h. The animals were treated in accordance with the guidelines of the Norwegian Committee for Experiments on Animals.

Surgery

The rats were anaesthetized with 2.0 ml/kg SC of a mixture of Hypnorm (Janssen) and Dormicum (Roche) diluted with distilled water (the mixture contained fentanyl, 0.05 mg/ml; fluanizone, 2.5 mg/ml; and midazolam 1.25 mg/ml). Using a standard stereotaxic method and the following coordinates (mm from bregma): AP = +1.0; L = ±1.5; H = −2.5 (14), 39 rats were implanted with a guiding cannula (23 gauge). To keep the cannula open throughout the treatment period, a steel wire was placed inside the cannula and a plastic cap closed the cannula between the drug injections. During the experimental period of 15 weeks, 12 animals were removed because they lost their cannulas or developed signs of infection.

Drug Administration

The ICV injections were performed using a 30 gauge injection cannula that protruded 1 mm below the guiding cannula. The injection cannula was attached to a 25 μl hand-driven microsyringe with a polyethylene tube. Haloperidol 12.5 μg (Janssen) dissolved in 10 μl of distilled water was delivered in the lateral ventricle over a 45-s period. The injection cannula was kept in place for additional 15 s. During injection the rats were hand-held with minimal restraint. Control rats received 10 μl of saline.

The correct localization of the cannulas was verified by brain dissection of six anesthetized control rats injected ICV with methylene blue. The immediate behavioral effect of the haloperidol injections also indicated a proper function of the cannulas.

In the IP injection experiment, haloperidol 1.0 mg/kg or an equivalent volume of saline was used. The rats were handled for 7 days before drug injection.

Behavioral Observations

During observation, the rats were kept in a clear perspex cage (25 × 11 × 13 cm) placed in an observation chamber equipped with mirrors allowing videorecording of the rat from all sides simultaneously (details of the apparatus will be published separately). The behavior of the animal was videorecorded for 3 min after 1 min of adaptation to the cage. At the end of the study, the recorded behavior was rated by a trained observer unaware of the treatment of the rats. By means of a computer program, the following behavioral categories were analyzed: vacuous chewing movements (VCM; single, purposeless mouth openings in the vertical plane, with or without tongue protrusion), jerking (sudden whole body jerks), jaw tremor (high amplitude fasciculations of the mouth or jaw), immobility, moving horizontally, rearing, and grooming. For the two first behavioral categories, the frequencies were counted. For the other categories the durations were recorded.

Protocol

After surgery, the animals were handled daily for 2 weeks before the start of the drug injections using a procedure only leaving out the injection. Then, 20 rats were injected with haloperidol ICV daily for 12 weeks, while 19 rats were injected with saline. Behavioral observations were performed 3.5 min and 2 h after the first ICV injection. Later, the observations were performed every second week, 24 h after the last injection. Three weeks after termination of treatment, the behavior was recorded in seven animals from each group, randomly chosen.

To compare the behavioral effects of haloperidol administrated ICV with conventional IP injections, a separate experiment was performed where rats received one injection of haloperidol or saline IP (n = 12 per group). The behavior was recorded 1 h after injection.

Statistics

Nonparametric tests were used. Between-group differences were analyzed by Kruskal-Wallis ANOVA by ranks and Mann-Whitney U-test, within-group differences across weeks were tested by Wilcoxon matched pairs test and Friedman ANOVA by ranks test.

RESULTS

Three and a half minutes after ICV injection, the animals receiving haloperidol had a statistically significant higher number of VCM compared to animals receiving saline. The difference was reduced, but still significant 2 h after the injection (Fig. 1). The haloperidol-treated rats also showed a statistically significant increase in immobility 3.5 min after injection, but 2 h later this difference was no longer significant (Fig. 1). A statistically significant increase in tremor and a decrease in rearing were observed 3.5 min after ICV injection in animals receiving haloperidol. These differences were no longer significant 2 h after injection. For the other behavioral categories, no significant group differences appeared either 3.5 min or 2 h after injection (data not shown).

One h after IP injection, there was a statistically significant increase both in VCM and immobility in rats injected with haloperidol compared to controls (Fig. 2). The increase in VCM was still present 24 h after the injection. The haloperidol-treated group also showed a statistically significant increase in number of jerks and a significant decrease in rearing 1 h after injection. No significant differences between haloperidol and control groups were observed in any of the other behavioral categories (data not shown).

When the observations were performed 24 h after ICV injections, no significant differences were found between groups in any of the behavioral categories observed either during the 12 weeks treatment period or 3 weeks after drug withdrawal.

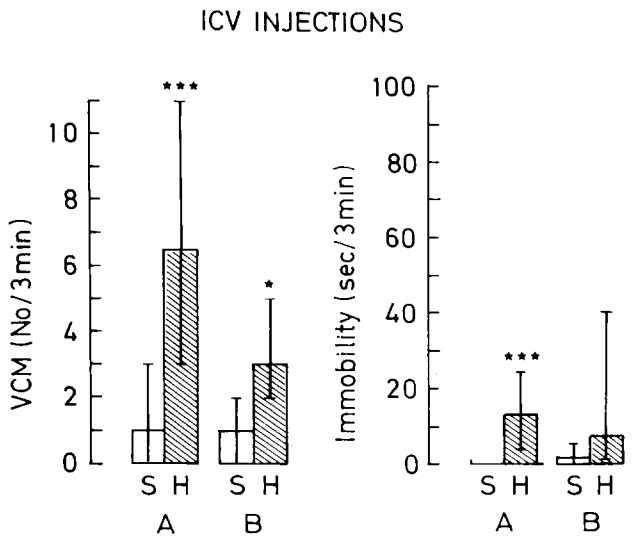

FIG. 1. Number of VCM and time spent immobile following ICV injection of haloperidol (H) 37.5 μg/kg (n = 20) and saline (S; n = 19). Medians with 25th percentile and 75th percentile are presented. Behavioral data were collected in periods of 3 min, starting 3.5 min after injection (A), and 2 h after injection (B). *$p < 0.05$, **$p < 0.005$, ***$p < 0.0005$ (Mann-Whitney U-test).

DISCUSSION

The essential result of the present study is that long-term intermittent treatment with haloperidol, using daily ICV injections for 12 weeks, seems unable to increase VCM beyond the period of acute drug effect. This is in contrast to earlier results (6,7) indicating that an intermittent treatment regimen is essential for the persistent increase in VCM after drug withdrawal. The present results seem to challenge the kindling hypothesis for development of the assumed TD analogue VCM in rats.

The term pharmacological kindling arises from a model of drug sensitization in which repetition of IP injections of DA agonists induced abnormal behavior in rats (16). In this model, an adequate drug dose, intermittent stimulation, and appropriate drug-free intervals are claimed to be the critical variables (16). In the present study, both ICV and IP injections of haloperidol had significant behavioral effects, and the ICV injections of haloperidol increased the number of VCM as much as the IP injections did. However, while the abnormal behavior subsided approximately 2 h after the ICV injections, it lasted 24 h after the IP injections. Based on these findings, daily ICV injections seem to be the best model for pharmacological kindling with haloperidol.

It is possible that the drug exposure was insufficient in inducing the necessary drug sensitization. This is difficult to test because a firm relationship between VCM and a particular transmittersystem has not yet been established. However, the acute behavioral drug effect was clearly present in connection with the injections throughout the treatment period.

In most studies using continuous neuroleptic treatment of rats, an increase in VCM has been found, also for varying periods of time after drug withdrawal (11,18). In a recent study (7), haloperidol was given in the drinking water (approximately 1 mg/kg/day) either every day or 2 days a week for 15 weeks. During treatment, both types of treatment increased VCM, but only the discontinuously treated animals had increased VCM after drug withdrawal. The results are clear, but with a stimulus duration of 48 h or more it seems difficult to understand these results within the framework of kindling.

In models of drug-induced epilepsy using repeated injections of proconvulsants, EEG changes are recorded. In other models of pharmacological kindling the similarity between drug sensitization by intermittent drug administration and kindling by intermittent electrical stimulation is, to our knowledge, not based on any observed neurophysiological changes, but on observations of behavior (7,15,16).

In the present study, the general characteristics of the pharmacological stimulus approach those of the electrical stimulus in the original kindling model, being powerful but short lasting and repeated every 24 h. In this model, the target behavior could not be achieved and the results do not support the hypothesis that kindling is an important mechanism for the development of the assumed TD analogue VCM in rats.

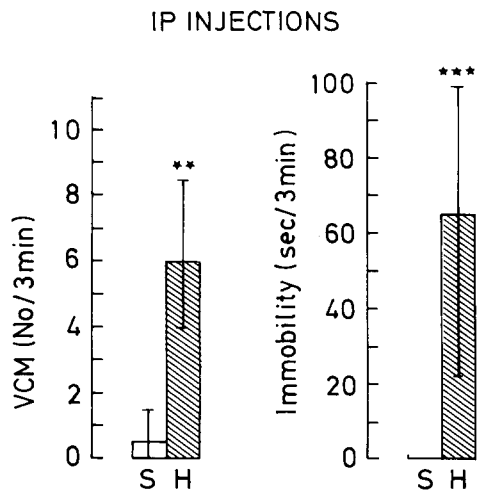

FIG. 2. Number of VCM and time spent immobile following IP injection of haloperidol (H) 1 mg/kg and saline (S). Medians with 25th percentile and 75th percentile are presented, n = 12 per group. Behavioral data were collected in a period of 3 min starting 1 h after injection. *$p < 0.05$, **$p < 0.005$, ***$p < 0.0005$ (Mann-Whitney U-test).

ACKNOWLEDGEMENTS

The authors wish to thank Torhild F. Sunde for excellent technical assistance and Professor Kjell Hole for helpful discussions. The research was supported by Gerda Meyer Nyquist Gulbranson and Gerdt Meyer Nyquist's fund and by the Norwegian Research Council.

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