Pharmacology Biochemistry & Behavior, Vol. 25, pp. 897–901, 1986. © Ankho International Inc. Printed in the U.S.A. 0091-3057/86 $3.00 + .00

Spontaneous Chewing Movements in Rats During Acute and Chronic Antipsychotic Drug Administration

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Received 20 March 1986

GUNNE, L. M., U. ANDERSSON, U. BONDESSON AND P. JOHANSSON. *Spontaneous chewing movements in rats during acute and chronic antipsychotic drug administration.* PHARMACOL BIOCHEM BEHAV **25**(4) 897–901, 1986.— Single intraperitoneal doses of various antipsychotic drugs (clozapine 6, 12, 25 mg/kg, sulpiride 100 mg/kg, haloperidol 0.5, 1.0, 2.0 mg/kg, fluphenazine 0.5, 1.0, 2.0 mg/kg) induced a depression of the spontaneous chewing movement (SCM) rate in rats during the first 6–8 hours. Haloperidol and fluphenazine elicited a rebound increase in SCM on day 2–5, while clozapine and sulpiride did not. Atropine (5 mg/kg) reduced the SCM rate. During chronic administration for 10 months clozapine (50 mg/kg/day) caused no changes in the SCM rate. Sulpiride (120 mg/kg/day) gave a marginal rise above control levels, while thioridazine (40 mg/kg/day), chlorpromazine (30 mg/kg/day), fluphenazine (0.6 mg/kg/day) and haloperidol (0.4 mg/kg/day) produced highly significant increases in SCM rates. It is suggested that the present animal model may prove useful for monitoring the risk of tardive dyskinesia with individual drugs.

Spontaneous chewing movements Antipsychotic drugs Chronic administration Acute administration Tardive dyskinesia

CHRONIC administration of haloperidol has been shown to induce an increased rate of spontaneous chewing movements (SCMs) in rats [7, 9, 20]. This behavior became apparent after 3–4 months of chronic drug administration and following discontinuation it disappeared only very slowly over a time period of five months [7]. To further study this potential model for tardive dyskinesia (TD), we have administered other antipsychotic drugs on a chronic basis applying doses in ratio to the therapeutic doses used in the control of schizophrenia. The drugs tested in the present experiments were clozapine, sulpiride, thioridazine, chlorpromazine, fluphenazine and haloperidol. In addition we have also studied the acute effects on SCM rate of various single doses of clozapine, sulpiride, fluphenazine and haloperidol. In one experiment, in which these drugs had induced an increase in chewing rate, the influence of atropine was tested.

METHOD

A total of 160 Sprague-Dawley rats were used (80 male, 80 female) weighing 180–220 g at the beginning of the experiment. They were housed under standard conditions of lighting (12 hr light/dark cycle) and temperature (21±1°C). Each experimental group consisted of equal numbers of male and female animals, but since there were no obvious sex-related differences the results were combined.

Acute Administration

Drug-naive animals were used throughout. The following drugs and doses were administered intraperitoneally to groups of 6 rats each: clozapine 6, 12, 25 mg/kg, sulpiride 25, 50 and 100 mg/kg, fluphenazine 0.5, 1.0, 2.0 mg/kg, and haloperidol 0.5, 1.0, 2.0 mg/kg. Before injection all drugs (bases) were dissolved in 0.1 M hydrochloric acid and pH adjusted to 6.0 with 0.1 M NaOH. Twelve control rats were given saline.

Assessment of jaw movements was carried out with each individual animal in a Plexiglas tube (i.d. 58 mm) which was placed under a sound-insulated cover. The rat head profile was inspected via a closed circuit TV camera with a close-up lens and jaw movements were counted during 2 min sessions after the animal had adjusted to the situation for 2 min. Observers were unaware of the treatments given. Measurements of SCMs were made before injections and at 1, 2, 3, 5, 8, and 24 hr, and thereafter on day 3, 5 and 7.

Repeated Administration

Six rats were given haloperidol 1 mg/kg IP once daily for 3 consecutive days and saline on day 4. Jaw movements were counted before and at 1, 2, 3, 5, 8 hours after each injection. On day 5–12 there were daily measurements of SCMs.

In a parallel experiment 6 rats were given similar haloperidol injections for 3 days followed by atropine sulfate 5 mg/kg IP on day 4, instead of saline.

Chronic Administration

Groups of 8 rats were given therapeutically equivalent doses of clozapine, sulpiride, thioridazine, chlorpromazine,

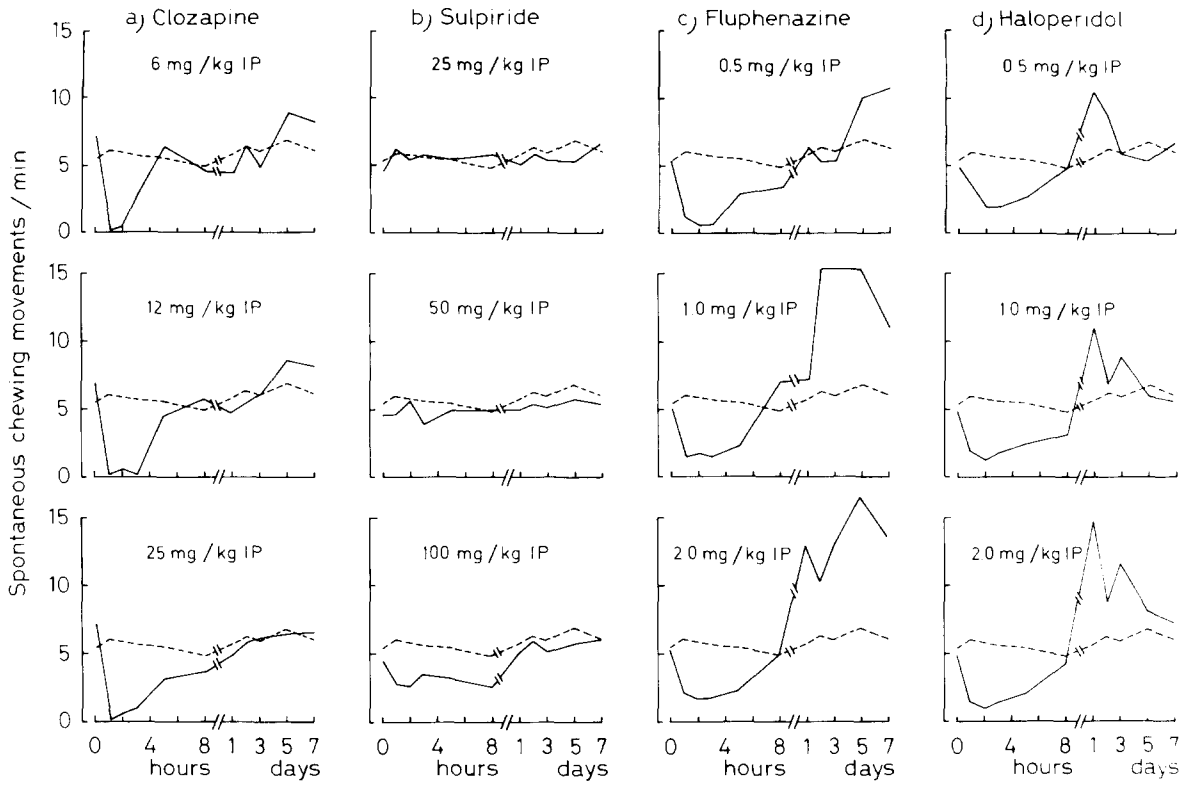

FIG. 1. Solid lines: SCM response to single IP injections at time point zero of (a) clozapine 6, 12, 25 mg/g, (b) sulpiride 25, 50, 100 mg/kg, (c) fluphenazine 0.5, 1.0, 2.0 mg/kg and (d) haloperidol 0.5, 1.0, 2.0 mg/kg. Broken lines: saline injected controls.

TABLE 1

RELATIVE WATER CONSUMPTION AND MEAN DOSE LEVELS OBTAINED DURING 10 MONTHS OF DRUG ADMINISTRATION

Drug	Concentration mg/ml	Water intake % of untreated	Dose mg/kg/day
None	—	100	—
Clozapine	0.5	60	52
Sulpiride	1.0	98	119
Thioridazine	0.5	45	38
Chlorpromazine	0.5	42	28
Fluphenazine	0.005	73	0.55
Haloperidol	0.005	73	0.41

fluphenazine or haloperidol with their drinking water. In addition 16 rats were untreated controls. Drug solutions were prepared fresh every week. Table 1 shows that the addition of drugs reduced the intake of water to varying degrees. It also shows actual dose (the rats were weighed weekly and their water consumption was monitored).

These experiments lasted for 17 months. During the first 2 months all groups were undrugged. Drugs were given for 10 months, whereafter the animals were observed during another 5 months washout period.

Assessment of SCMs were made at time point 0 and then monthly in all animals. In these experiments jaw movements were counted with the animal in an observation cage as described earlier [9].

Statistics

In acute experiments split-plot ANOVAs were used to test the differences between neuroleptic-treated and control groups at 1, 2 and 3 hours. To verify the subsequent rebound increase, comparisons were made by *t*-tests at 1 and 2 days for the haloperidol-injected and at 3, 5 and 7 days for the fluphenazine-injected rats. The effects of atropine on day 4 after 3 daily haloperidol injections was tested using split-plot ANOVAs at 1, 2 and 3 hours after atropine or saline injections.

In the chronic experiments the main effects of drugs and phases were tested in a two-way (split-plot design) ANOVA with subsequent Tukey HSD tests [14]. For statistical purposes the measurement of SCMs were subdivided in 4 different phases: (1) three monthly baseline measurements before drug administration, (2) the first 2 months of drug administration, (3) from the 3rd to 10th month of drug administration and (4) during 5 months washout after discontinuation of drugs. Results within the untreated control groups were divided into corresponding time periods. One-way ANOVAs were performed to test the differences between drugs within each phase. Unless otherwise stated, differences were significant on at least a 5 percent level.

RESULTS

Acute Experiments

Single injections of clozapine (6, 12 and 25 mg/kg) and the highest sulpiride dose (100 mg/kg) produced significant de-

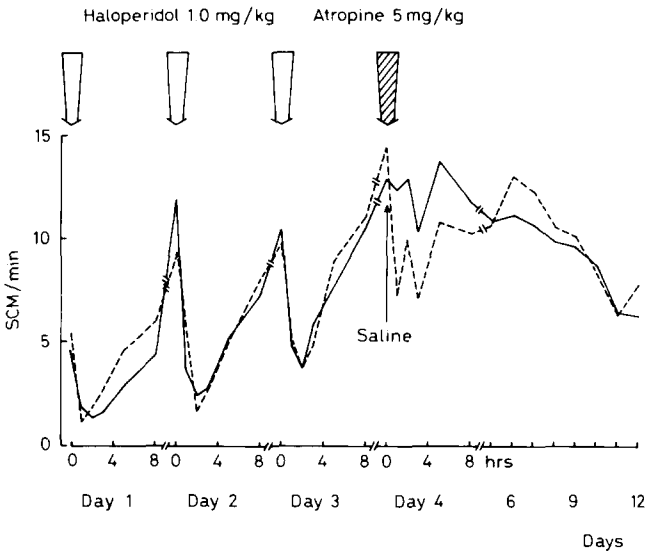

FIG. 2. SCM response in two groups of rats to haloperidol 1.0 mg/kg administered daily on 3 consecutive days. On day 4 one group received saline (solid line) the other atropine sulphate 5 mg/kg (dotted line).

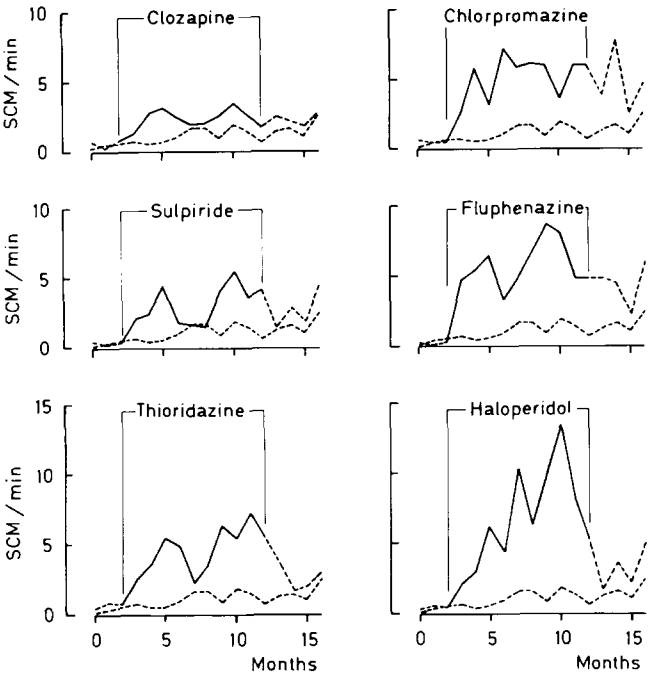

FIG. 3. SCMs during chronic oral administration of clozapine, sulpiride, thioridazine, chlorpromazine, fluphenazine and haloperidol in rats (for doses see Table 1). Solid lines during drug administration, broken lines during control conditions. All curves of experimental groups have been contrasted by the SCM curve for untreated controls.

pressions in SCM rate compared to controls ($p<0.001$). Haloperidol (0.5, 1.0 and 2.0 mg/kg) and fluphenazine (0.5, 1.0 and 2.0 mg/kg) had a dual action. Both produced an initial depression for 6–8 hours, followed by a rebound increase (t-tests yielded $p<0.001$, except for the 0.5 mg/kg doses which gave $p<0.002$) with its maximum for haloperidol at 24 hours and fluphenazine 2–7 days after the injection (Fig. 1 a–d).

Injection of haloperidol (1 mg/kg) for 3 consecutive days caused repeated depressions of SCMs, but each day from a somewhat higher starting level. Administration of atropine sulfate (5 mg/kg) on day 4 reduced the SCM rate, $F(1,10)=7.51$, $p<0.02$, in similarly treated animals (Fig. 2).

Chronic Experiments

The chewing rates produced by each drug are illustrated in Fig. 3. In the untreated control group there was a gradual increase in SCM rate during the 17 months of experiment, $F(3,248)=4.76$, $p<0.01$, indicating a rise in chewing rate with increasing age. All groups had a low starting level (phase 1) with no significant differences between groups. During phase 2 one-way ANOVA yielded a significant treatment effect, $F(6,119)=3.43$, $p<0.01$, and for two drugs (fluphenazine and chlorpromazine) Tukey-tests showed significant increases from these drugs compared to water. In phase 3 there was a significant treatment effect, $F(6,497)=24.32$, $p<0.0001$, and subsequent Tukey-tests showed that haloperidol differed from thioridazine, chlorpromazine, sulpiride, clozapine and water. Thioridazine, chlorpromazine and fluphenazine differed from clozapine and water (fluphenazine also from sulpiride) $p<0.01$. Sulpiride differed from water ($p<0.05$), whereas clozapine did not.

After 300 days of neuroleptic treatment all rats were subjected to a washout (phase 4), when they only got tap water. The frequency of SCM gradually declined during this phase, although in different degrees for different drugs. The ANOVA yielded significant differences between the groups, $F(6,239)=3.33$, $p<0.01$, and Tukey-tests showed that chlorpromazine and fluphenazine remained significantly above control levels.

DISCUSSION

The slow and gradual development of increased chewing behavior during chronic administration of TD-producing neuroleptics in rats, as well as the slow reduction of this phenomenon after drug discontinuation, suggests a parallelism to the emergence and disappearance of TD in the clinic. However, for two drugs (fluphenazine and chlorpromazine) the SCM rise occurred earlier (within 2 months) than what is regularly seen with TD. Such early signs have contributed to some interpretational difficulties and raised questions whether we are actually dealing with a TD model, or if this chewing behavior is rather related to a different clinical syndrome (acute dystonia, AD) [16]. It should be pointed out that both in the clinic and in simian TD models [8] AD and TD may occur in the same individuals.

A further problem of interpretation is connected with the

SCM response to atropine. In accordance with earlier results atropine was shown to reduce SCM frequency in rats, while physostigmine had the opposite effect [9,15]. In a majority of clinical cases of TD atropine has been known to aggravate TD (while reducing or abolishing AD) and hence the anticholinergic response of our rat model is AD- rather than TD-like. Conversely, occasional TD cases have been reported to respond with amelioration of symptoms on atropine and aggravation on physostigmine [2, 10, 12]. The present rat model therefore cannot be discarded as a TD model simply due to its response to anticholinergic drugs.

The withdrawal phase after discontinuation of chronic drug treatment in most instances showed a gradual reduction of SCMs. However, only in fluphenazine- and chlorpromazine-treated rats did the SCM rates decrease slowly enough for this model to qualify as a TD model. TD in the clinic is not necessarily irreversible, but during periods of low dosing or complete withdrawal some cases show a gradual reduction of symptoms over observation periods of years [4]. When the symptoms disappear within three months after discontinuation of neuroleptics the condition should be described as withdrawal dyskinesia (WD) rather than TD [17]. Using the same terminology for our animal model, some drugs (thioridazine and haloperidol) seem to have induced only WD. However, since WD regularly antedates and forbodes TD, the two conditions are closely interrelated.

The findings of both increases and decreases in SCM rates in the present acute experiments are not easily reconcilable with clinical experiences. A chewing behavior in drug-naive animals modifiable by neuroleptic drugs has not been described in primates. In humans with manifest TD, however, symptoms are temporarily reduced following each new neuroleptic drug administration and this may be followed by a rebound increase when drugs are discontinued. Similar observations have also been made in a monkey made dyskinetic through chronic haloperidol administration [6].

The repeated administration of haloperidol once daily for three consecutive days caused similar depressions of SCM activity each time, though the starting level before the injections was gradually increased. This seems to reflect a shift in the balance between SCM depressing and augmenting effects, which may eventually result in a lasting rise of SCM rates after long-term administration.

The results of SCM measurements both in acute and chronic experiments in rats have thus revealed specific response patterns, which may or may not be relevant for the study of TD. An important question is whether this animal model can be used to predict the inherent risk of TD with individual drugs. Unfortunately, at the present time, there are not too many drugs with a documented lower TD risk, but clozapine is considered to be such an antipsychotic [1, 13, 18] and sulpiride may be another [5,18]. It is therefore of interest that in the present chronic experiments clozapine did not differ from untreated controls and sulpiride caused only marginally increased SCMs compared to controls. The other neuroleptics (haloperidol, fluphenazine, chlorpromazine and thioridazine), which have all been reported to cause TD, increased SCM rates well above sulpiride, clozapine and controls.

The doses of the present chronic experiments were chosen to correspond approximately with doses employed in the clinic [3,19]. Still, when drugs are compared in this way, a broad spectrum of chronic doses has to be studied for each drug. We have carried out such studies for two drugs [11]. SCM rises were found to be dose-dependent for haloperidol in doses between 0.07–0.24 mg/kg/day whereas clozapine failed to induce changes in a range of chronic doses between 10–50 mg/kg/day.

Our conclusion is that SCM rate measurements during chronic administration of antipsychotic drugs in rats may provide an instrument to predict the risk of TD with individual drugs. It is still too early to express an opinion as to whether acute experiments might serve the same purpose, though even after a single administration clozapine and sulpiride appeared to have an SCM response discernible from haloperidol and fluphenazine.

ACKNOWLEDGEMENTS

This study was supported by a Swedish MRC grant No. 4546. We thank Delagrange for sulpiride, Janssen Leo Pharma AB for haloperidol, Leo Rhodia for chlorpromazine, Sandoz Inc. for clozapine and thioridazine and Squibb Inc. for fluphenazine.

REFERENCES

1. Ackenheil, M. and H. Hippius. Clozapine. In: *Psychotherapeutic Drugs. Part 2*, edited by E. Usdin and I. Forrest. New York: M. Dekker, 1977, pp. 923–956.
2. Casey, D. E. and D. Denney. Pharmacological characterization of tardive dyskinesia. *Psychopharmacology (Berlin)* **54:** 1–8, 1977.
3. Davis, J. M. Comparative doses of costs of antipsychotic medication. *Arch Gen Psychiatry* **33:** 858–861, 1976.
4. Gardos, G., J. O. Cole, A. Perenyi, D. E. Casey, I. Samu, M. Kallos and S. Schnielbolk. Five-year follow-up study of tardive dyskinesia. In: *Chronic Treatments in Neuropsychiatry*, edited by D. Kemali and G. Racagni. *Advances in Biochemical Psychopharmacology*, vol 40. New York: Raven Press, 1985, pp. 37–42.
5. Gerlach, J. and D. E. Casey. Sulpiride in tardive dyskinesia. *Acta Psychiatr Scand [Suppl]* **311:** 93–101, 1984.
6. Gunne, L. M. and S. Barany. A monitoring test for the liability of neuroleptic drugs to induce tardive dyskinesia. *Psychopharmacology (Berlin)* **63:** 195–198, 1979.
7. Gunne, L. M. and J-E. Häggström. Reduction of nigral glutamic acid decarboxylase in rats with neuroleptic-induced oral dyskinesia. *Psychopharmacology (Berlin)* **81:** 191–194, 1983.
8. Gunne, L. M. and S. Barany. Haloperidol-induced tardive dyskinesia in monkeys. *Psychopharmacology (Berlin)* **50:** 237–240, 1976.
9. Gunne, L. M., J. Growdon and B. Glaeser. Oral dyskinesia in rats following brain lesions and neuroleptic drug administration. *Psychopharmacology (Berlin)* **77:** 134–139, 1982.
10. Jeste, D. V. and R. J. Wyatt. In search of treatment for tardive dyskinesia: Review of the literature. *Schizophr Bull* **5:** 251–293, 1979.
11. Johansson, P., D. E. Casey and L. M. Gunne. Dose dependent increases in rat spontaneous chewing rates during long-term administration of haloperidol but not clozapine. *Psychopharmacol Bull*, in press.

12. Jus, A., K. Jus, J. Gaulter, C. Villeneuve, P. Pires, R. Pineau and R. Villeneuve. Studies on the action of certain pharmacological agents on tardive dyskinesia and on the rabbit syndrome. *Int J Clin Pharmacol* **9:** 138–145, 1974.
13. Juul Povlsen, U., U. Noring, R. Fog and J. Gerlach. Tolerability and therapeutic effect of clozapine. *Acta Psychiatr Scand* **71:** 176–185, 1985.
14. Kirk, R. E. *Experimental Design: Procedures for the Behavioral Sciences, 2nd edition.* Belmont, CA: Brooks & Cole, 1969, pp. 245–318.
15. Rupniak, N. M. J., P. Jenner and C. D. Marsden. Cholinergic manipulation of perioral behaviour induced by chronic neuroleptic administration to rats. *Psychopharmacology* **79:** 226–230, 1983.
16. Rupniak, N. M. J., P. Jenner and C. D. Marsden. Pharmacological characterisation of spontaneous or drug-associated purposeless chewing movements in rats. *Psychopharmacology* **85:** 71–79, 1985.
17. Schooler, N. R. and J. M. Kane. Research diagnosis for tardive dyskinesia. *Arch Gen Psychiatr* **39:** 486–487, 1982.
18. Simpson, G. M. and J. H. Lee. A ten-year review of antipsychotics. In: *Psychopharmacology: A Generation of Progress,* edited by A. DiMascio and K. F. Killam. New York: Raven Press, 1978, pp. 1131–1138.
19. Titeler, M. and P. Seeman. Radioreceptor labeling of pre- and postsynaptic dopamine receptors. In: *Long-Term Effects of Neuroleptics,* edited by F. Cattabeni, G. Racagni, P. F. Spano and E. Costa. *Advances in Biochemical Psychopharmacology,* vol 24. New York: Raven Press, 1980, pp. 159–165.
20. Waddington, J. L., A. J. Cross, S. J. Gamble and R. C. Bourne. Spontaneous orofacial dyskinesia and dopaminergic function in rats after 6 months of neuroleptic treatment. *Science* **220:** 530–532, 1983.