

PROLACTIN LEVELS AND AMPHETAMINE-INDUCED BEHAVIOURAL CHANGES FOLLOWING FLUPHENAZINE DECANOATE ADMINISTRATION

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Abstract

1. A single injection of fluphenazine decanoate (FD) antagonized effects of acute d-amphetamine (2.5 mg/kg) for a variable period of time (4 to 28 days), depending upon the dosage of the neuroleptic used (2.5 or 5.0 mg/kg) and the behavioural parameter(s) monitored.
2. Locomotion and rearing were antagonized for a longer duration than was sniffing. Normal locomotor response to amphetamine was attained 12 and 28 days following the administration of 2.5 and 5.0 mg/kg FD, respectively. However, the 2.5 mg/kg FD group displayed significantly more locomotor activity on days 20 and 24 post-treatment. A similar supersensitive response was not demonstrable with the higher dose of FD (5.0 mg/kg), or with the other behavioural measures.
3. The prompt and pronounced elevation of serum prolactin in response to the neuroleptic returned to within the normal range by days 4 and 14, following administration of 2.5 and 5.0 mg/kg FD, respectively.
4. These results indicate that the behavioural paradigm is more sensitive in monitoring the effects of FD and could serve as a useful model in investigating the dose- and time-related effects of other long-acting neuroleptics.

Key words: antagonism of amphetamine-induced behavioural changes, fluphenazine decanoate, prolactin, supersensitivity

Introduction

It is believed that neuroleptics dis inhibit prolactin release from the anterior pituitary by blocking the dopamine receptors functionally influenced by the activity of the tuberoinfundibular tract (Meites and Clemens, 1972; Meltzer et al., 1976). Thus the study of serum prolactin alterations in response to neuroleptic drugs has the potential of reflecting the extent of dopamine receptor blockade on the tuberoinfundibular-pituitary dopamine system, which could possibly serve as a convenient model of monitoring indirectly the extent of neuroleptic blockade on other dopaminergic systems, such as the mesolimbic and nigrostriatal tracts (Meltzer and Fang, 1976).

Amphetamine elicits a behavioural syndrome characterized by purposeless and repetitive movements which in the rodents take the form of stereotyped locomotion, sniffing, rearing and biting. According to current views, these stereotyped behaviours result from stimulation of dopamine receptors of the nigrostriatal and the mesolimbic systems (Iversen and Koob, 1977; Morelli et al., 1980; Mumford et al., 1979). The objectives of the present investigation were to study the time-course and dose-effect of fluphenazine decanoate (FD) on 1) the tuberoinfundibular-pituitary dopamine system (as reflected by changes in serum prolactin) and 2) the nigrostriatal and/or mesolimbic dopamine systems (as reflected by antagonism of amphetamine-induced behavioural changes).

Method

Animals. Male Sprague-Dawley rats (224±11 g) with free access to food and water, were housed in an environment maintained at 24°C, 60% relative humidity and with 12 hr of light (6 a.m. to 6 p.m.).

Behavioural Testing. This portion of the study consisted of two experiments in which the antagonistic effects of two doses of FD (2.5 mg/kg and 5.0 mg/kg) upon behavioural changes induced by d-amphetamine (2.5 mg/kg), were monitored. The experimental chambers for behavioural observation were identical to the rats' home cage (clear polycarbonate; 43 x 23 x 15 cm) except for the following modifications: the floor was elevated, marked off into six equal sections and had seven holes (2.5 cm diameter).

Procedure. Twelve rats received FD (5.0 mg/kg; i.m.) whereas the control group (12 rats) were given an equal volume (0.1 ml/100 g) of the vehicle (sesame oil). Each rat was observed consecutively for 1 min duration, every 6 min, for the entire observation session. The behavioural parameters being monitored were operationally defined as follows: 1) Sniffing: nose and whiskers moving; 2) poking: rat's nose visibly protruding through one of the holes on the floor; 3) Rearing: standing on hindlegs, with forelimbs off the floor; 4) Locomotion: hindlegs crossing into another section of the floor; 5) Gnawing: rats biting on anything in the cage. Once in their experimental chambers, the rats were allowed 0.5 hr of acclimatization period, following which all rats received a saline (placebo) injection and were observed and rated for the subsequent 0.5 hr period. Immediately following this all rats were injected with d-amphetamine (2.5 mg/kg; i.p.) and the behaviours rated for 2.5 hr, as described above. A similar experiment was conducted using a smaller dose of FD (2.5 mg/kg).

Prolactin Studies. Separate experiments were performed to assess the effects of two doses of FD (2.5 and 5.0 mg/kg) on serum prolactin levels. Forty-eight rats were injected with FD (5.0 mg/kg; i.m.) and 18 control rats with an equal volume (0.1 ml/100 g) of the vehicle (sesame oil). Six hours after the first injection (day 0), groups of control (n=6) and FD-treated (n=6) rats were decapitated, with care being taken not to unduly stress the animals. Trunk blood was collected and plasma separated out, and stored frozen until subsequent analysis. Plasma samples from groups of 6 neuroleptic-treated rats were similarly obtained on days 1, 4, 7, 14, 21 and 35 following the administration of fluphenazine decanoate. Samples from 2 additional groups of control animals were obtained about mid-way through (day 21, n=6) and at the end of the experiment (day 35, n=6). The above procedure was repeated on additional 66 rats, using a different dose of FD (2.5 mg/kg).

Prolactin Assay. Prolactin levels were determined in the serum samples in duplicate by a double antibody radioimmunoassay method, using reagents kindly supplied by Dr. A.F. Parlow, through the NIAMDD Rat Pituitary Hormone Distribution Program.

Results

Behavioural Experiments. The raw data of the individual behaviours was collapsed over 0.5 hr intervals, and subjected to analysis of variance with repeated measures.

Globally, the poking and grooming behaviours occurred with extremely low frequency in both the groups, and were excluded from subsequent statistical analysis.

Administration of d-amphetamine markedly stimulated the locomotor activity of the control rats, which diminished gradually over the 2.5 hr observation period. The locomotor response of the neuroleptic-treated rats was significantly lower than that of controls on test days 4, 7, 11, 14, 18, 21 and 28. Although initially the amphetamine-induced locomotor activity remained blunted at every 0.5 hr test interval, with the progression of days significant blockade was only demonstrable during the first and/or second post-amphetamine 0.5 hr bins. Similarly, the frequency of amphetamine-induced rearing was markedly suppressed for up to 28 days following the administration of FD. Amphetamine-induced sniffing was also suppressed by pretreatment with FD. However, this suppression (in contrast to that of locomotion and rearing) was present for a shorter duration (14 days).

With the lower dose of FD (2.5 mg/kg), a similar over-all pattern in the blockade of amphetamine-induced locomotor (Fig. 1), rearing and sniffing activity, could be seen. However, these effects were less pronounced and of a shorter duration than those resulting from the

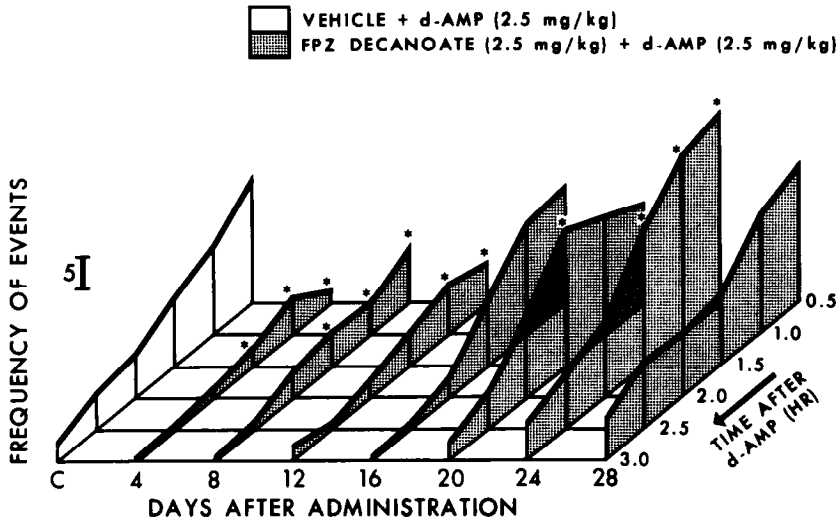


Fig. 1. Time-course of fluphenazine-decanoate (2.5 mg/kg) effect on d-amphetamine-induced locomotor activity. Tridimensional figure: On the ordinate: frequency of the quantified activity; on the horizontal abscissa: days following the administration of fluphenazine decanoate ; on the oblique abscissa: time after d-amphetamine administration. The cumulative profile of vehicle-treated controls is represented by the unshaded area on the extreme left and profiles of fluphenazine decanoate-treated groups are represented by the subsequent shaded areas. $n=6$ for each test session. *Significantly different with respect to controls at $p<0.05$

higher dose of FD. Amphetamine-induced locomotor activity was partially antagonized by FD (2.5 mg/kg) on days 4, 8 and 12. However, on days 20 and 24, the neuroleptic treated rats displayed significantly more locomotor activity (see Fig. 1). With rearing behaviour, a partial blockade could also be seen for the same duration (up to 12 days), following which a reinstatement and maintenance of normal responsiveness was apparent. As in the case with the larger dose, amphetamine-induced sniffing was antagonized for the shortest period. In this instance, antagonism of sniffing was demonstrable mainly on the first day (day 4) and on the second half hour of the next test session (day 8).

Prolactin Studies. There was no statistically significant difference in the prolactin levels of 3 control groups ($n=6$) sacrificed at the beginning (day 0), the middle (day 21) and the end of the experiment (day 35). Administration of FD (5.0 mg/kg) resulted in a pronounced elevation of prolactin (1447% of control) within 6 hours of the injection (day 0). The prolactin level remained significantly elevated, albeit by a smaller magnitude, on sampling days 1, 4 and 7 (see Table 1). By day 14, prolactin level had returned to within the normal range and remained there for the duration of the experiment. With the lower dose of FD (2.5 mg/kg) prolactin concentration increased significantly (by 352%) within 6 hours of administration (day 0). However, this elevation was of a much smaller magnitude than that following the larger dose of the neuroleptic, and was detectable only up to day 1 of sampling.

Discussion

The present results demonstrate that a single injection of FD can antagonize behavioural

Table 1
Effect of fluphenazine decanoate (FD) on plasma prolactin

| | Days Following FD Administration | | | | | | | |
|--------------|----------------------------------|-----------------------|---------------------|---------------------|---------------------|--------------------|-------------------|--------------------|
| | C | 0 | 1 | 4 | 7 | 14 | 21 | 35 |
| | Prolactin levels (ng/ml) | | | | | | | |
| FD 25. mg/kg | 10.7±2.6 (100%) | 37.8±12.2 (352%)* | 24.7±7.5 (230%)* | 8.4±0.9 (78%) | 9.6±1.6 (89%) | 12.3±2.0 (115%) | 10.3±1.7 (97%) | 12.9±1.7 (120%) |
| FD 5.0 mg/kg | 7.6±1.8 (100%) | 109.9±9.3 (1447%)* | 20.0±2.3 (263%)* | 24.0±2.6 (316%)* | 16.3±1.6 (215%)* | 8.9±2.3 (116%) | 6.8±1.5 (89%) | 7.7±2.0 (102%) |

*Significantly different with respect to controls at $p < 0.05$.

effects of acute d-amphetamine (2.5 mg/kg) for a variable period of time (4 to 28 days), depending upon the dosage of the neuroleptic used (2.5 or 5.0 mg/kg) and the behavioural parameter(s) monitored. Globally, the magnitude of behavioural antagonism and its time course were greater with the higher dose of the neuroleptic.

At the level of individual behavioural components, there seemed to be a differential antagonistic effect engendered by this neuroleptic: locomotion and rearing behaviours were antagonized for a much longer duration than was the sniffing response. This differential antagonistic effect may be related to the involvement of distinct neurochemical and/or neuroanatomical mechanisms in the mediation of d-amphetamine-induced behavioural changes. Indeed, in recent years it has become evident that various components of stereotypy do not arise from the same brain area; locomotion seems to depend on stimulation of dopaminergic mechanisms in the mesolimbic system while licking and gnawing arise from the striatum (Iversen and Koob, 1977; Morelli et al., 1980; Kelly et al., 1975). It is thus tempting to suggest that, at the doses employed in this experiment, FD may be exerting a preferential effect on limbic over striatal dopaminergic neurotransmission. An alternative explanation may be gleaned from recent evidence implicating the existence of at least two distinct types of dopamine receptors (Cools, 1977; Titeler et al., 1978). It is thus also possible that the sniffing behaviour may depend more on the stimulation of the dopamine receptors relatively insensitive to fluphenazine effects. Following the apparent reinstatement of normal locomotor response, the 2.5 mg/kg FD group displayed significantly more locomotor activity on days 20 and 24 post-treatment. This reversal in activity lends further credence to the hypothesis that dopamine receptors become supersensitive during withdrawal from chronic exposure to receptor blocking agents. It is of interest to note that a similar supersensitive response was not demonstrable with the higher dose of FD (5.0 mg/kg). This could possibly be attributed to the experimental span and testing intervals, which may not have coincided with the supersensitive state, or perhaps this response may have reversed before effective levels of fluphenazine were gone (Wheeler and Roth, 1980). In contrast to the locomotor activity, supersensitive response was not reflected in rearing and sniffing behaviours. These results also indicate that the monitoring of discrete behavioural components might be more informative than single stereotypy scores obtained from the constellation of behavioural repertoires.

Administration of FD resulted in a prompt and pronounced elevation of serum prolactin level already demonstrable at the time of the first sampling (6 hr post injection). This is concordant with the clinical observation of Nasrallah et al. (1979) who reported that plasma prolactin concentration of patients increased (2-6 fold) between 3 and 6 hr after the injection of FD. These observations are also consistent with the kinetics of fluphenazine after FD administration and may have considerable significance for the use of FD in acute conditions (Curry et al., 1979). Our data also demonstrate that the effects of FD were dose dependent, in that the magnitude of prolactin increase and its duration were greater with the larger dose of the neuroleptic.

In rats administered 2.5 and 5.0 mg/kg dose of FD prolactin levels were within the normal range by days 4 and 14, respectively. In male schizophrenic subjects being treated with FD, the prolactin levels remained elevated for up to 4 weeks (Merali and Lapierre, 1981). Further

ermore, in the behavioural experiments reported here, effects of FD (5 mg/kg) could also be detected for up to 28 days. Normally, prolactin released from the pituitary is pulsatile and it remains possible that the change in prolactin profile (as reflected by the number and/or the magnitude of prolactin peaks) may have revealed a different time-course. However, it is also possible that the continuous interruption of the pituitary dopamine receptors may have rendered them "supersensitive" to dopamine, the physiological prolactin inhibitory factor. Recently, Annunziato et al. (1980) reported that serum prolactin levels, 24 hr after the last neuroleptic (short-acting) injection, were significantly lower in the repeat-treated group than in the acute treated rats. This can be interpreted as an enhanced sensitivity of pituitary dopamine receptors or alternatively, the consequence of an increased turnover of tuberohypophyseal dopamine neurons which follows prolonged elevation of circulating prolactin levels (Annunziato, 1980; Naber et al., 1979).

In conclusion, these data indicate that FD effects are reflected by changes in serum prolactin as well as by antagonism of amphetamine-induced behavioural changes. The time course of FD effect on the nigrostriatal and/or mesolimbic dopamine system(s) (as reflected by antagonism of amphetamine-induced behavioural changes) is longer than that on the tuberoinfundibular-pituitary system (as reflected by circulating prolactin levels). Thus the behavioural paradigm reported here seems more sensitive in detecting the effects of relatively low levels of FD, and suggests that this could serve as a useful model in investigating the dose- and time-related effects of other long-acting neuroleptics.

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