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Behavioural Subsensitivity Induced by Long-term Administration of a Low Dose of Haloperidol to Rats

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Abstract

This study examines the effects on open-field and stereotyped behaviour of rats of abrupt withdrawal from repeated treatment with a low (0·03 mg kg^{-1}) dose of haloperidol.

Single administration of this low dose of haloperidol significantly increased open-field locomotion without modifying apomorphine (0·5 or 2·0 mg kg^{-1})-induced stereotyped behaviour. Forty-eight hours after abrupt withdrawal from 0·03 mg kg^{-1} haloperidol (twice daily for 15 days) a significant decrease in locomotion frequency was observed, but no change was observed in apomorphine-induced stereotypy.

Our results suggest that dopamine autoreceptor supersensitivity might be evaluated in a behavioural situation of absence of postsynaptic dopamine receptor supersensitivity.

It has repeatedly been observed that mixed D_1/D_2 and selective D_2 dopamine receptor agonists affect motor activity in both a dose-dependent and a biphasic manner in rodents. At high doses, these dopamine agonists cause hyperactivity and stereotypy (Johnson et al 1976; Frussa-Filho & Palermo-Neto 1988), whereas at low doses they inhibit locomotor activity (Di Chiara et al 1976; Strömbom 1976; Conceição & Frussa-Filho 1996). A biphasic effect of mixed and selective D_2 dopamine receptor blockers on motor activity of mice and rats has also been reported. Thus, moderate and high doses of neuroleptics reduce spontaneous locomotion frequency and apomorphine-induced stereotyped behaviour (Bernardi et al 1981; Frussa-Filho & Palermo-Neto 1991), whereas at low doses they attenuate the motor suppression caused by low doses of dopamine agonists and increase spontaneous locomotion frequency (Di Chiara et al 1976; Strömbom 1977; Ögren et al 1984). Whereas the stimulant and depressant motor effects of high doses of dopamine receptor agonists and antagonists, respectively, are considered to be a result of their action on postsynaptic dopamine receptors (Kelly et al 1975; Puech et al 1978), the effects of low doses of these agents (motor depression and stimulation, respectively) are considered to be a consequence of modifications of synaptic levels of dopamine mediated by a preferential action on functionally defined presynaptic dopamine autoreceptors (Carlsson 1975; Ögren et al 1984).

Long-term treatment of rats and mice with moderate and high doses of neuroleptic drugs might result in increased sensitivity of central postsynaptic dopamine receptors similar to the denervation supersensitivity observed in the peripheral nervous system after long-term interruption of neuronal transmission (Gianutsos et al 1974). Abrupt withdrawal from long-term haloperidol (Bernardi et al 1981; Vital et al 1995), metoclopramide (Frussa-Filho & Palermo-Neto 1988) and sulpiride (Frussa-Filho & Palermo-Neto 1990) treatment not only enhanced the general activity of rats observed in the open field, but also their responses to apomorphine-induced stereotyped behaviour. These effects have been considered to be a consequence of the development of a supersensitivity of central dopamine pathways (Frussa-Filho & Palermo-Neto 1988). According to Klawans (1973), the induction of striatal dopamine receptor supersensitivity by repeated administration of neuroleptics in rodents might be related to the emergence of drug-induced extrapyramidal side effects such as tardive dyskinesia in man. As with dopamine postsynaptic receptor supersensitivity, the development of dopamine autoreceptor supersensitivity after long-term treatment with neuroleptics is supported by substantial biochemical (Martress et al 1977), eletrophysiological (Gallager et al 1978) and behavioural (Verimer et al 1980; Hansan & Leonard 1981; Hietala et al 1987) evidence. Behaviourally, dopamine autoreceptor supersensitivity corresponds to enhancement of the locomotor inhibitory effects of apomorphine after withdrawal from long-term treatment with neuroleptics at moderate or high doses (Verimer et al 1980; Hietala et al 1987). The interpretation of these behavioural data requires caution, however, because the basal level of locomotor activity was usually enhanced as a consequence of the concomitant development of dopamine postsynaptic receptor supersensitivity. Indeed, in some experiments the relative ability of apomorphine to suppress locomotor activity was enhanced in neuroleptic-treated animals although, in absolute terms, the inhibitory effect of apomorphine was very close to that seen in the control group (Hietala et al 1987).

Our interest, therefore, was to evaluate the development of dopamine autoreceptor supersensitivity in a behavioural condition of absence of postsynaptic dopamine receptor supersensitivity. Thus, we studied the effects of abrupt withdrawal from repeated treatment with a 0·03-mg-kg^{-1} dose of haloperidol on rats' open-field locomotion. Because this low dose of haloperidol was found to increase locomotor activity of rats after a single administration, it was expected to produce a selective long-term blockade at dopamine autoreceptors.

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Materials and Methods

Animals

Genetically similar male Wistar rats, 220–300 g, 90 days old, were used. The animals arrived in the experimental laboratory 7 days before the beginning of the experiment and were immediately housed in wire mesh cages (16 × 30 × 19 cm) at 20–23°C, on a 12-h light-dark cycle (lights on at 0700 h) with free access to food and water. Each animal was used for one experiment only.

Drugs

Haloperidol (Haldol injectable solution 5 mg mL^{-1}; Johnson & Johnson) was diluted with distilled water. Apomorphine (Merck) was dissolved in a 0·2% solution of ascorbic acid. Saline was used as control solution for haloperidol and the 0·2% solution of ascorbic acid was used as control solution for apomorphine.

Open-field studies

The open-field apparatus was constructed as described by Broadhurst (1960). Hand-operated counters were used to score locomotion frequency (number of floor units entered). The rats were placed individually in the open-field arena and locomotion frequency was observed for 6 min. Before placement of the animals the open-field was washed with water-alcohol (19 : 1) to obviate possible bias as a result of odour clues left by previous subjects. To minimize possible effects of circadian changes on open-field behaviour, experimental and control observations were alternated.

Stereotypy studies

The animals were observed for stereotyped behaviour in their home cages free from water and food. Stereotypy was quantified every 10 min after apomorphine administration according to the scoring system proposed by Setler et al (1976). Briefly, scores from 0 to 6 were attributed to an animal's behaviour by an observer who was unaware of the drug treatment. The grading system was: 0, asleep or stationary; 1, active; 2, predominantly active but with bursts of stereotyped sniffing, rearing or head bobbing; 3, constant stereotyped activity such as sniffing, rearing or head bobbing but with locomotor activity still present; 4, constant stereotyped activity maintained in one location; 5, constant stereotyped activity but with bursts of licking or gnawing and biting, or both; 6, continual licking or gnawing, or both, of cage grids. In each group, the total sum of stereotypy scores for each animal was used for statistical analysis.

Determination of a stimulant dose of haloperidol in open-field locomotion of rats

Forty-four rats previously habituated to the open field (6 min exposure 24 h before the test session) were divided at random into one control and three experimental groups of 11 animals each, acutely and intraperitoneally treated with control solution (1·0 mL kg^{-1}) or haloperidol (0·03, 0·1 or 0·3 mg kg^{-1}), respectively. Thirty minutes after these single treatments, the rats were placed individually in the centre of the open-field arena, and locomotion frequency was quantified for 6 min.

Effects of a single dose of 0·03 mg kg^{-1} haloperidol on apomorphine-induced stereotyped behaviour

Thirty rats were randomly divided into five groups of six animals each: saline + ascorbic acid, saline + apomorphine 0·5 mg kg^{-1}, haloperidol 0·03 mg kg^{-1} + apomorphine 0·5 mg kg^{-1}, saline + apomorphine 2·0 mg kg^{-1} and haloperidol 0·03 mg kg^{-1} + apomorphine 2·0 mg kg^{-1}. The animals received saline or 0·03 mg kg^{-1} haloperidol intraperitoneally. Thirty minutes later they were given a 0·2% solution of ascorbic acid, 0·5 mg kg^{-1} apomorphine or 2·0 mg kg^{-1} apomorphine, subcutaneously, and observed for stereotyped behaviour.

Effects of withdrawal from long-term administration of 0·03 mg kg^{-1} haloperidol on open-field locomotion of rats

Twenty-three rats were divided into one control group and one experimental group of 11 or 12 animals each. The rats of the experimental group were injected twice daily (0700 h and 1800 h) for 15 days, intraperitoneally, with haloperidol (0·03 mg kg^{-1}). Control animals were similarly injected with saline. Thirty minutes after the last administration of drug or control solution, the animals were observed for open-field locomotion. Other test sessions were held 24, 48, 72 and 96 h after withdrawal.

Effects of withdrawal from long-term administration of 0·03 mg kg^{-1} haloperidol on apomorphine-induced stereotyped behaviour

Twenty-four rats were randomly divided into two control groups and two experimental groups of six animals each and submitted to long-term treatment with haloperidol (0·03 mg kg^{-1}) or saline, as described above. Forty-eight hours after withdrawal, rats of one control group and one experimental group were injected with 0·5 mg kg^{-1} apomorphine, subcutaneously, whereas animals of the other control and experimental groups received 2·0 mg kg^{-1} apomorphine, subcutaneously. All the animals were then observed for stereotyped behaviour.

Statistical analysis

Bartlet's test indicated that our open-field data were non-parametric. Consequently, Kruskal–Wallis analysis of variance then the Mann–Whitney *U*-test were used to analyse both open-field and stereotypy data.

Results

As can be seen in Table 1, acute administration of 0·03 mg kg^{-1} haloperidol significantly increased ($H = 9·61$, $P < 0·05$) the locomotion frequency of rats observed in the open field. In contrast, the open-field locomotion of animals treated with 0·1 and 0·3 mg kg^{-1} haloperidol was not significantly different from control.

Table 2 shows that a single (0·03 mg kg^{-1}) dose of haloperidol did not significantly modify apomorphine (0·5 or 2·0 mg kg^{-1})-induced stereotyped behaviour ($P > 0·05$). The absence of difference between saline- and haloperidol-treated animals was not because stereotypy was already maximum or minimum. Indeed, the stereotypy intensities induced by 0·5 mg kg^{-1} apomorphine were significantly higher than those of rats treated with ascorbic acid solution instead of

Table 1. Effects of single low doses of haloperidol on open-field behaviour of rats.

Haloperidol dose (mg kg^{-1})	Locomotion frequency
0·0	30·3 ± 4·5
0·03	48·2 ± 6·2*
0·1	24·5 ± 6·7
0·3	23·3 ± 5·9

Values are given as means ± s.e.m. (n = 11). *$P < 0·05$ when compared with control (saline) animals (Kruskal–Wallis analysis of variance, Mann–Whitney U-test).

Table 2. Stereotypy intensity in rats treated with 0·2% ascorbic acid solution or 0·5 or 2·0 mg kg^{-1} apomorphine after single administration of saline or haloperidol (0·03 mg kg^{-1}) or withdrawal from long-term administration.

Pretreatment	Treatment	Stereotypy intensity (sum of scores)
Single		
Saline	Ascorbic acid	3·8 ± 0·4
Saline	0·5 mg kg^{-1} apomorphine	25·8 ± 1·8*†
Haloperidol	0·5 mg kg^{-1} apomorphine	19·2 ± 2·4*†
Saline	2·0 mg kg^{-1} apomorphine	34·3 ± 1·4†
Haloperidol	2·0 mg kg^{-1} apomorphine	35·2 ± 3·4†
Long-term		
Saline	0·5 mg kg^{-1} apomorphine	21·8 ± 2·0*
Haloperidol	0·5 mg kg^{-1} apomorphine	21·7 ± 2·3*
Saline	2·0 mg kg^{-1} apomorphine	36·8 ± 3·4
Haloperidol	2·0 mg kg^{-1} apomorphine	45·5 ± 10·9

Values given are the means ± s.e.m. (n = 6). *Statistically significant difference from animals treated with 2·0 mg kg^{-1} apomorphine (Kruskal–Wallis analysis of variance, Mann–Whitney U-test). †Statistically significant difference from animals treated with 0·2% ascorbic acid solution instead of apomorphine (Kruskal–Wallis analysis of variance, Mann–Whitney U-test).

apomorphine and significantly lower than those induced by 2·0 mg kg^{-1} apomorphine in rats treated once with either saline or haloperidol ($H = 23·5$, $P < 0·05$).

As illustrated in Fig. 1, long-term treatment of rats with 0·03 mg kg^{-1} haloperidol significantly reduced open-field locomotion 48 h after abrupt withdrawal, compared with untreated animals.

Table 2 also shows that 48 h after withdrawal from long-term treatment there was no significant difference ($P > 0·05$) in apomorphine-induced stereotyped behaviour between rats treated with 0·03 mg kg^{-1} haloperidol or those treated with saline. As expected, the stereotypy intensities induced by 2·0 mg kg^{-1} apomorphine were significantly higher than those induced by 0·5 mg kg^{-1} apomorphine in rats treated long-term with either saline or haloperidol ($H = 16·5$, $P < 0·05$).

Discussion

Apomorphine is a direct-acting dopamine agonist that, at stereotypic doses, is thought to produce its effects by stimulation of postsynaptic dopamine receptors (Ernst 1967). In contrast, spontaneous locomotion in the open field depends on the action

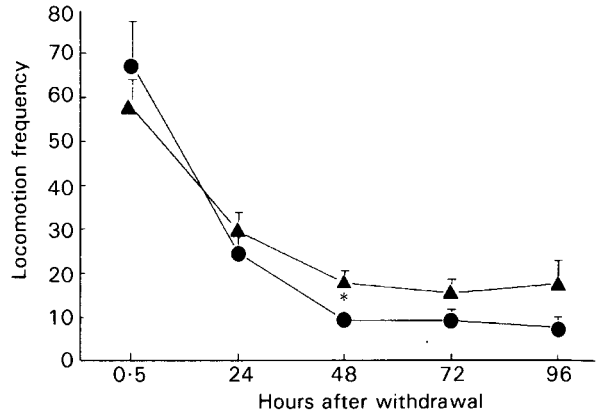

FIG. 1. Effects on open-field locomotion of rats of withdrawal from long-term treatment with 0·03 mg kg^{-1} haloperidol. ▲ Saline, ● haloperidol. Results are expressed as means ± s.e.m. *$P < 0·05$ compared with values for control animals (Mann–Whitney U-test).

of endogenous dopamine on these receptors and, therefore, also on dopamine release, re-uptake and many other factors, including dopamine autoreceptors.

Our results show that a single administration of 0·03 mg kg^{-1} haloperidol increased open-field locomotion in rats, suggesting preferential blockade of dopamine autoreceptors at this dose. In further support of this assumption, 0·03 mg kg^{-1} haloperidol did not modify apomorphine-induced stereotyped behaviour. Even more important, withdrawal from long-term administration of 0·03 mg kg^{-1} haloperidol resulted in a significant reduction in open-field locomotion without modifying apomorphine-induced stereotypy. These findings suggest, in turn, that long-term administration with 0·03 mg kg^{-1} haloperidol induced supersensitivity of dopamine autoreceptors without affecting the plasticity of postsynaptic dopamine receptors.

Interpretations of the importance of dopamine autoreceptor supersensitivity in the general phenomenon of neuroleptic supersensitivity are contradictory. Gordon et al (1987) speculated that, once initiated, increased dopamine autoreceptor sensitivity could become a self-sustained phenomenon, resulting in both the development and the maintenance of a chronic receptor supersensitivity at both pre- and postsynaptic receptor sites. According to this author, once the presynaptic dopamine autoreceptor increases their sensitivity, less dopamine would be required to activate these inhibitory autoreceptors, resulting in a chronic state of reduced release or synthesis, or both, of dopamine. This chronic reduction in the release of dopamine, once established, would in turn result in a reduction in the down-regulation of both pre- and postsynaptic dopamine receptors by endogenous dopamine. Conversely, Taminga (1981) proposed that if both auto and postsynaptic dopamine receptors became equally supersensitive, the synaptic activity would remain balanced and, potentially, extrapyramidal motor symptoms of tardive dyskinesia would perhaps be absent or reduced. Our results seem to be more consistent with Taminga's interpretation. Indeed, whereas withdrawal from long-term administration of high, depressant, 'postsynaptic' doses of haloperidol produces marked increases in open-field locomotion (Bernardi et al 1981; Vital et al 1995), withdrawal from repeated treatment with a low, sti-

mulant, 'autoreceptor' dose of the neuroleptic induced a transient though significant reduction in locomotion frequency.

Whereas a significant increase in locomotion frequency was observed 30 min after a single 0·03 mg kg^{-1} dose of haloperidol, it should be noted that after repeated administration this haloperidol dose did not significantly increase open-field locomotion in the first observation session (which was held 30 min after the last injection). This result suggests the development of tolerance to the stimulant effect of this haloperidol dose. Interestingly, tolerance also develops to the depressant effect of higher doses of this neuroleptic on open-field locomotion (Bernardi et al 1981).

From another standpoint it is worth remarking that the autoreceptor hypothesis to explain the depressant and stimulant effects of low doses of dopamine agonists and antagonists, respectively, has been questioned by several research groups. For example, Ståhle & Ungerstedt (1989) concluded that dopamine agonist-induced hypolocomotion was not related to reduced extracellular levels of dopamine because it could be elicited by dopamine agonists in rats treated with amphetamine at doses that were shown to increase extracellular levels of dopamine. These authors suggested that dopamine agonist-induced hypolocomotion might be mediated by stimulation of populations of particular postsynaptic dopamine receptors whose sensitivity to dopamine agents might be considerably higher than the sensitivity of the postsynaptic dopamine receptors mediating, for example, stereotyped behaviour and hyperlocomotion. Although our experimental design did not enable us to distinguish between the alternative hypotheses, our results suggest that, whether they are autoreceptors or particular postsynaptic receptors, the receptors mediating the stimulant effect of low doses of neuroleptics become supersensitive after long-term blockade. More important, our findings indicate that this supersensitivity might be independent of alterations in the sensitivity of dopamine receptors mediating the depressant effect of high doses of neuroleptics.

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References

Bernardi, M. M., De Souza, H., Palermo-Neto, J. (1981) Effects of single and long-term haloperidol administration on open-field behavior of rats. Psychopharmacology 73: 175–188

Broadhurst, P. L. (1960) Experiments in psychogenetics. In: Eysenck, H.J. (ed.) Experiments in Personality, Vol. 1. Routledge Kegan Paul, London.

Carlsson, A. (1975) Receptor-mediated control of dopamine metabolism. In: Usdin, E., Snyder, S. (eds) Pre- and Postsynaptic Receptors. Dekker, New York, pp 49–63

Conceição, I. M., Frussa-Filho, R. (1996) Effects of microgram dose of haloperidol on open-field behavior in mice. Pharmacol. Biochem. Behav. 53: 833–838

Di Chiara, G., Porceddu, M. L., Vargiu, L., Argiolas, A., Gessa, G. L. (1976) Evidence for dopamine receptor mediating sedation in the mouse brain. Nature 264: 564–567

Ernst, A. (1967) Mode of action of apomorphine and dexamphetamine on gnawing compulsion in rats. Psychopharmacologia 10: 316–324

Frussa-Filho, R., Palermo-Neto, J. (1988) Effects of single and long-term metoclopramide administration on open-field and stereotyped behaviour of rats. Eur. J. Pharmacol. 149: 323–329

Frussa-Filho, R., Palermo-Neto, J. (1990) Effects of single and long-term administration of sulpiride on open-field and stereotyped behavior of rats. Braz. J. Med. Biol. Res. 23: 463–472

Frussa-Filho, R., Palermo-Neto, J. (1991) Effects of single and long-term droperidol administration on open-field and stereotyped behaviour of rats. Physiol. Behav. 50: 825–830

Gallager, D. W., Pert, A., Bunney, W. E. J. (1978) Haloperidol-induced presynaptic da supersensitivity is blocked by chronic lithium. Nature 273: 309–312

Gianutsos, G., Drawbaugh, R. B., Hynes, M. D., Lal, H. (1974) Behavioural evidence of dopaminergic supersensitivity after haloperidol. Life Sci. 14: 887–898

Gordon, J. H., Clopton, K., Curtin, J. C., Koller, W. C. (1987) Chronic autoreceptor blockade and neuroleptic-induced dopamine receptor hypersensitivity. Pharmacol. Biochem. Behav. 26: 223–228

Hansan, F., Leonard, B. E. (1981) Studies on the action of sulpiride on dopamine receptors in the rat brain in vivo. Neuropharmacol. 20: 1327–1330

Hietala, J., Macdonald, E., Syvalahti, E., Scheinin, M. (1987) Alterations in peripheral and central dopamine receptor sensitivity after subchronic treatment with fluphenazine and sulpiride. Pharmacol. Toxicol. 61: 48–52

Johnson, A. M., Loew, D. M., Vigouret, J. M. (1976) Stimulant properties of bromocriptine on central dopamine receptors in comparison to apomorphine, (+)-amphetamine and L-DOPA. Br. J. Pharmacol. 56: 59–68

Kelly, P. H., Seviour, P. W., Iversen, S. D. (1975) Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens, septi and corpus striatum. Brain Res. 94: 507–522

Klawans, H. L. (1973) The pharmacology of tardive dyskinesias. Am. J. Psychiat. 130: 82–86

Martress, M. P., Constentin, J., Baudry, M., Marcais, H., Protais, P., Schwartz, J. C. (1977) Long-term changes in the sensitivity of pre and postsynaptic dopamine receptors in mouse striatum evidenced by behavioural and biochemical studies. Brain Res. 136: 319–337

Ögren, S. O., Rodebjer, A., Angeby, K. (1984) The 'presynaptic' blocking potency of sulpiride and haloperidol in the rat is age-dependent. Neurosci. Lett. 46: 203–207

Puech, A., Simon, P., Boissier, J. R. (1978) Benzamides and classical neuroleptics: comparison of their actions using six apomorphine-induced effects. Eur. J. Pharmacol. 50: 261–300

Setler, P., Sarau, H., McKenzie, J., (1976) Differential attenuation of some effects of haloperidol in rats given scopolamine. Eur. J. Pharmacol. 39: 117–126

Ståhle, L., Ungerstedt, U. (1989) Yawning and suppression of exploration in amphetamine-treated rats, incompatibility with the autoreceptor hypothesis. Psychopharmacology 97: 553–560

Strömbom, U. (1976) Catecholamine receptor agonists: effects on motor activity and tyrosine hydroxylation in mouse brain. Naunyn-Schmiedeberg's Arch. Pharmacol. 292: 167–176

Strömbom, U. (1977) Antagonism by haloperidol of locomotor depression induced by small doses of apomorphine. J. Neural Transm. 40: 191–194

Taminga, C. A. (1981) Tardive dyskinesia and dopamine receptors. In: Usdin, E., Bunney, W. E. J., Davis, J. M. (eds) Neuroreceptors, Basic and Clinical Aspects. John Wiley & Sons, Chichester, pp 231–241

Verimer, T., Goodale, D. B., Long, J. P., Flynn, J. R. (1980) Lithium effects on haloperidol-induced pre- and postsynaptic dopamine receptor supersensitivity. J. Pharm. Pharmacol. 32: 665–666

Vital, M. A. B. F., Frussa-Filho, R., Palermo-Neto, J. (1995) Effects of monosialoganglioside on dopaminergic supersensitivity. Life Sci. 56: 2299–2307