The effects of the new antipsychotic, sertindole, on latent inhibition in rats

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Latent inhibition (LI) is a measure of retarded conditioning to a previously presented non-reinforced stimulus, that is impaired in schizophrenic patients and in rats treated with amphetamine. Neuroleptic drugs are known to produce two effects in this paradigm: to antagonize amphetamine-induced disruption of LI, and to facilitate the development of LI when administered on their own. The present experiments tested the effects on LI of the new neuroleptic, sertindole. The experiments used a conditioned emotional response procedure in rats licking for water, consisting of three stages: pre-exposure, in which the to-be-conditioned stimulus (a tone) was repeatedly presented without being followed by reinforcement; conditioning, in which the pre-exposed stimulus was paired with reinforcement (a foot shock); and test, in which LI was indexed by degree of suppression of licking during tone presentation. In Experiment 1 the effects of 0.31, 1.3 and 5.0 mg/kg sertindole were assessed following pre-exposure to 40 non-reinforced tones. Experiment 2 tested the effects of 5 mg/kg on LI following pre-exposure to 10 non-reinforced tones. Experiment 3 investigated antagonism of amphetamine-induced disruption of LI by 5.0 mg/kg sertindole. The results demonstrated that sertindole (5.0 mg/kg) possesses a neuroleptic-like profile in the LI model; it facilitates the development of LI and antagonizes amphetamine-induced disruption of LI.

Keywords: Latent Inhibition – Pre-exposure – Rat – Sertindole

INTRODUCTION

The dopamine (DA) hypothesis of schizophrenia suggests a hyperactivity in limbic/cortical dopaminergic pathways to be involved (see reviews, see Seeman, 1987; Wyatt et al., 1988). Accordingly, it is hypothesized that the therapeutic effects of antipsychotic drugs are mediated by blockade of DA receptors in these areas, whereas blockade of DA receptors in the nigrostriatal system is responsible for the development of extrapyramidal side effects. This differentiation can be demonstrated using neurophysiological techniques: subchronic treatment with classical antipsychotics that induce extrapyramidal side effects (e.g. haloperidol) inactivates both systems, whereas clozapine (which does not induce extrapyramidal side effects) selectively inactivates limbic/cortical DA neurons in the ventral tegmental area (VTA; Bunney, 1984; Skarsfeldt, 1988).

Using this well-validated animal model the unique properties of sertindole [5-chloro-1-(4-fluorophenyl)-3-[N-[2-(2-imidazolidin-1-yl)ethyl]-4-piperidyl]-1H-indole] were discovered. After 3 weeks of daily treatment sertindole inactivates spontaneously active VTA DA neurons at doses more than 100 times lower than those inactivating nigral DA neurons (Skarsfeldt and Perregaard, 1990; Skarsfeldt, 1992). Sertindole has high affinity for DA D2 receptors, 5-HT₂A receptors and α₁-adrenoceptors in vitro, with weak or no affinity for a variety of other neurotransmitter receptors, and in particular, no antimuscarinic activity. A potent and long-lasting inhibitory effect on 5-HT₂A receptors is observed in vivo, whereas acute DA blocking activity is weak or absent depending on the test model (Sanchez et al., 1991; Arnt, 1992; Hyttel et al., 1992). Accordingly sertindole does not induce catalepsy and does not inhibit stereotyped behaviour induced by dopaminergic drugs, but does antagonize d-amphetamine-induced hyperactivity (Sanchez et al., 1991; Arnt, 1992; Arnt et al., 1992; Hyttel et al., 1992). Initial clinical studies have confirmed the antipsychotic activity of sertindole (McEvoy et al., 1993).

In the present experiments sertindole was studied in an animal model that simulates attentional deficit, a cognitive dysfunction that is considered characteristic of schizophrenia and in which limbic DA mechanisms are apparently involved (Weiner, 1990; Gray et al., 1991; Feldon and Weiner, 1992). The model is based on the paradigm of latent inhibition (LI), in which non-reinforced pre-exposure to a stimulus retards subsequent conditioning to
that stimulus when it is paired with a reinforcer (Lubow, 1973). For example, if an animal is pre-exposed to a series of tones, these tones are less readily associated with other stimuli, such as shock, or responses such as avoidance. This decremental process is considered to reflect a process of learning not to attend to, or to ignore irrelevant stimuli (Lubow, 1973, 1989; Mackintosh, 1973, 1975, 1983; Moore, 1979; Lubow et al., 1981). Initial development of the LI model of schizophrenia focused on the analogy between amphetamine-induced disruption of LI in rats and the well-known psychomimetic effects of this drug in humans (Solomon et al., 1981; Weiner et al., 1981, 1984, 1988). Subsequently, it was shown that LI is disrupted in normal human volunteers given oral amphetamine, and in acute schizophrenics tested within the first week of a schizophrenic episode (Baruch et al., 1988; Gray et al., 1991).

The antipsychotic action of the typical neuroleptic, haloperidol, is characterized by two effects in the LI model: it restores LI in amphetamine-treated animals (Weiner et al., 1990; Warburton et al., 1994), and it facilitates LI when given on its own (Weiner and Feldon, 1987; Weiner et al., 1987; Christison et al., 1988; Feldon and Weiner, 1988, 1991a,b). The facilitatory effect of neuroleptics on LI is particularly marked under conditions which do not yield LI in control animals, namely when the number of non-reinforced stimulus pre-exposures is low (Weiner and Feldon, 1987; Feldon et al., 1990; Feldon and Weiner, 1991b). The utility of the LI model as a screening test for neuroleptic compounds is underscored by the fact that it is also sensitive to atypical neuroleptics such as sulpiride (Feldon and Weiner, 1991b) and clozapine (Weiner et al., 1994), and the finding that LI is restored in medicated schizophrenics after their psychoses diminishes with treatment (Baruch et al., 1988).

The aim of the present experiments was to test whether sertindole produces a neuroleptic-like facilitatory effect on LI. LI was assessed using a conditioned emotional response (CER) procedure in rats licking for water, consisting of three stages: pre-exposure, in which the to-be-conditioned stimulus (a tone) is repeatedly presented without being followed by reinforcement; conditioning, in which the pre-exposed stimulus is paired with reinforcement (a foot shock); and test, in which LI is indexed by the degree of suppression of licking during tone presentation.

Experiment 1 assessed the effects of 0.31, 1.3, and 5.0 mg/kg sertindole on LI following 40 tone pre-exposures. These doses were chosen because of the different effects they exert on DA and 5-HT receptors: at the lower doses, serotoninergic inhibition dominates, whereas higher doses produce a more balanced effect on DA and 5-HT receptors, and are necessary in order to show effects in DA-related models (Sánchez et al., 1991; Arnt et al., 1992). We were particularly interested in the effects of the 5 mg/kg dose, because at this dose sertindole shows maximal blockade of amphetamine-induced hyperactivity (Arnt et al., 1992; J. Arnt, personal communication). The lower doses were also interesting because serotonergic blockade is known to disrupt LI (Cassaday et al., 1993). Since LI was obtained in all drug conditions in Experiment 1, Experiment 2 tested the effects of only the highest (5.0 mg/kg) dose on LI, and used a more sensitive procedure for detecting facilitatory drug effects, namely non-reinforced pre-exposure to only 10 tones, which does not produce LI in control animals. In this experiment clear facilitation of LI was obtained. Therefore, Experiment 3 investigated whether 5 mg/kg sertindole can also antagonize the disruptive effects of amphetamine on LI.

**METHODS**

**Subjects**

A total of 240 male Wistar rats, approximately 4 months old, were used, housed one to a cage under reversed cycle lighting for the duration of the experiment. Upon delivery, subjects were maintained on freely available food and water for 3 weeks. On the 22nd day, animals were weighed and placed on a 23 h water restriction schedule which continued throughout the experiment.

**Apparatus**

The apparatus consisted of four Campden Instruments Rodent Test Chambers (Model 410), each housed in a ventilated sound-attenuating Campden Instruments Chest (Model 412). A drinking bottle could be inserted into the chamber through a 0.5 cm diameter hole located in the centre of the left wall of the chamber, 2.5 cm above the grid floor. When the bottle was not present, the hole was covered by a metal lid. Licks were detected by a drinkometer circuit (Campden Instruments drinkometer Model 453). The pre-exposed, to-be-conditioned stimulus was a 2.8 kHz tone produced by a Sonalert module (Model SC 628). Shock was supplied by a Campden Instruments shock generator (Model 521/C) and shock scrambler (Model 521/S) set at 0.75 mA. Equipment programming and data recording were computer controlled.

**Procedure**

**Handling.** Prior to the beginning of the experiment, animals were handled for 7 days, for about 2 min each day.

**Baseline.** On each of 5 days, each animal was placed in the experimental chamber and allowed to drink for 20 min.

**Pre-exposure.** With the bottle removed, each animal was placed in the experimental chamber. The pre-exposed (PE) animals received 40 (Experiment 1 and 3) or 10
(Experiment 2) 10 s tones with a variable inter-stimulus interval (ISI) with a mean of 50 s. The non-pre-exposed (NPE) animals were confined to the chamber for an identical period of time but did not receive the tone.

Conditioning. With the water bottle removed, each animal received two tone-shock pairings. Tone parameters were identical to those used in pre-exposure. The 0.75 mA, 1 s shock immediately followed tone termination. The two tone-shock pairings were given 5 and 10 min after the start of the session. After the second pairing, animals were left in the experimental chamber for a further 5 min.

Rebaseline. Each animal was given a drinking session identical to the baseline sessions. Latency to the first lick and the total number of licks were recorded for each subject.

Test. Each subject was individually placed in the chamber and allowed to drink from the bottle. When the subject completed 75 licks the tone was presented, and lasted 5 min. The following times were recorded: time to first lick, time to complete licks 1-50, time to complete licks 51-75 (pre-tone) and time to complete licks 76-100 (tone-on).

The amount of suppression of licking was measured using a suppression ratio, A/A + B, where A is the period prior to the presentation of the stimulus (licks 51-75) and B is the period of the stimulus presentation (licks 76-100). A suppression ratio of 0.01 indicates complete suppression (no LI), and a ratio of 0.50 indicates no change in response time from the period prior to the presentation of the stimulus to the period of stimulus presentation (LI).

The stages of pre-exposure, conditioning, rebaseline and test were given 48 h apart. Each subject was run throughout the experiment in the same chamber.

Experimental design and drug treatment

Experiment 1. One hundred and forty-four animals were randomly assigned to eight experimental groups in a 2 x 4 factorial design with main factors of pre-exposure (0, 40) and drug (vehicle, 0.31 mg/kg, 1.3 mg/kg and 5 mg/kg sertindole). The experiment was run in three identical replications, each using 48 subjects. Sertindole was dissolved in 0.1 M acetic acid (20 mg/ml) and diluted with distilled water to reach the appropriate concentration. The final pH of the 5 mg/kg solution was 4.6. The appropriate drug dose was administered s.c. 2 h prior to the start of pre-exposure and prior to the start of conditioning. The 2 h pretreatment time is typically used in most comparable screening tests (Sanchez et al., 1991). The rebaseline and test stages were conducted without drugs. Data from one subject (from the vehicle-PE group) were lost due to apparatus failure. Thus, the final analyses were performed on data from 143 subjects.

Experiment 2. Forty-eight animals were randomly assigned to four experimental groups in a 2 x 2 factorial design with main factors of pre-exposure (0, 10) and drug (vehicle, 5.0 mg/kg sertindole). Drug treatment was identical to that of Experiment 1.

Experiment 3. Forty-eight animals were randomly assigned to eight experimental groups in a 2 x 2 x 2 factorial design with main factors of pre-exposure (0, 40), sertindole dose (0.0, 5.0 mg/kg) and amphetamine dose (0.0, 1.0 mg/kg). Each subject received two injections prior to pre-exposure and prior to conditioning. The first injection consisted of either 5.0 mg/kg sertindole or an equivalent volume of vehicle administered s.c. 2 h prior to the start of pre-exposure and 2 h prior to conditioning. The second injection consisted of either 1.0 mg/kg d-amphetamine sulphate (Sigma) dissolved in saline or an equivalent volume of saline, administered 10 min prior to the start of pre-exposure and 10 min prior to conditioning.

RESULTS

Experiment 1

A 3 x 2 x 4 ANOVA with main factors of replication (1, 2, 3), pre-exposure (0, 40) and drug (0.0, 0.31, 1.3, 5.0 mg/kg sertindole) performed on both A periods and suppression ratios yielded no significant effects of replication or interactions with this factor (all F < 1.0). Consequently, the data of the three replications were combined for the purpose of statistical analysis.

The times (in seconds) to complete licks 51-76 (A period) in the eight experimental groups were: vehicle-NPE: 4.18; vehicle-PE: 8.59; 0.31 mg/kg sertindole-NPE: 7.44; 0.31 mg/kg sertindole-PE: 5.06; 1.3 mg/kg sertindole-NPE: 5.78; 1.3 mg/kg sertindole-PE: 6.79; 5.0 mg/kg sertindole-NPE: 19.59; 5.0 mg/kg sertindole-PE: 7.24. The longer A period exhibited by the 5.0 mg/kg sertindole-NPE group was reflected in the results of a 2 x 4 ANOVA with main factors of pre-exposure (0, 40) and drug (0.0, 0.31, 1.3, 5.0 mg/kg sertindole), in which the main effect of drug [F(3,135) = 2.47, p < 0.07] and the pre-exposure x drug interaction [F(3,135) = 2.54, p < 0.06] both approached significance.

Figure 1 presents the mean suppression ratios of the pre-exposed and non-pre-exposed groups in each of the four drug doses. As can be seen, LI, i.e. lower suppression of the PE groups in comparison with the NPE groups, is evident in all four drug conditions. The results of a 2 x 4 ANOVA with main factors of pre-exposure (0, 40) and drug (0.0, 0.31, 1.3, 5.0 mg/kg sertindole) carried out on the suppression ratios yielded only a significant main effect of pre-exposure [F(1,135) = 21.74, p < 0.001].
Experiment 2
The times (in seconds) to complete licks 51-76 (A period) in the four experimental groups were: vehicle-NPE: 6.62; vehicle-PE: 5.59; 5.0 mg/kg sertindole-NPE: 4.85; 5.0 mg/kg sertindole-PE: 4.13. The shorter A periods exhibited by the 5.0 mg/kg sertindole groups were reflected in the results of a 2 x 2 ANOVA with main factors of pre-exposure (0, 10) and drug (0.0, 5.0 mg/kg sertindole), which yielded a main effect of drug \( F(1,44) = 4.44, p < 0.05 \).

Figure 2 presents the mean suppression ratios of the pre-exposed and non-pre-exposed groups in the two drug conditions. As can be seen, LI, i.e. lower suppression of licking in the PE compared with the NPE group, was present in all drug conditions except in the vehicle-amphetamine condition. Thus, LI was abolished by amphetamine but this abolition was antagonized by the concomitant administration of sertindole. These outcomes were supported by a 2 x 2 x 2 ANOVA with main factors of pre-exposure (0, 40), sertindole dose (0.0, 5.0 mg/kg) and amphetamine dose (0.0, 1.0 mg/kg) which yielded a significant main effect of pre-exposure \( F(1,40) = 10.41, p < 0.005 \) and a significant pre-exposure x sertindole x amphetamine interaction.

Experiment 3
The 2 x 2 x 2 ANOVA conducted on A periods, with main factors of pre-exposure (0, 40), sertindole dose (0.0, 5.0 mg/kg) and amphetamine dose (0.0, 1.0 mg/kg), yielded no significant outcomes. The times (in seconds) to complete licks 51-76 (A period) in the eight experimental groups were: vehicle-NPE: 5.15; vehicle-PE: 12.76; amphetamine-NPE: 9.50; amphetamine-PE: 6.38; sertindole-NPE: 6.42; sertindole-PE: 12.77; sertindole-amphetamine-NPE: 4.64; sertindole-amphetamine-PE: 15.47.

Figure 3 presents the mean suppression ratios of the pre-

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**FIG. 1.** Suppression ratios (mean + standard errors) for the pre-exposed (PE) and non-pre-exposed (NPE) groups in the four drug conditions: vehicle, 0.31 mg/kg, 1.3 mg/kg, and 5.0 mg/kg of sertindole. Forty pre-exposures were used.

**FIG. 2.** Suppression ratios (mean + standard errors) for the pre-exposed (PE) and non-pre-exposed (NPE) groups in the vehicle and the 5.0 mg/kg sertindole conditions. Ten pre-exposures were used.

**FIG. 3.** Suppression ratios (mean + standard errors) for the pre-exposed (PE) and non-pre-exposed (NPE) groups in the four drug conditions: vehicle (VEH), 1 mg/kg d-amphetamine (AMPH), 5.0 mg/kg sertindole (SERT), and 5.0 mg/kg sertindole + 1 mg/kg d-amphetamine (SERT-AMPH). Forty pre-exposures were used.
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\[ F(1,40) = 4.10, p < 0.05 \]. Post-hoc t-tests based on the error term derived from the ANOVA confirmed that LI was present in the vehicle-vehicle \( t(40) = 3.22, p < 0.01 \), sertindole-vehicle \( t(40) = 2.03, p < 0.05 \) and sertindole-amphetamine \( t(40) = 2.04, p < 0.01 \) conditions.

DISCUSSION

The results of Experiments 2 and 3 showed that two characteristic effects of neuroleptics on latent inhibition—facilitation of LI expression following weak pre-exposure, and attenuation of amphetamine-induced disruption of LI—are found after treatment with 5 mg/kg sertindole. The lack of facilitatory effect at this dose in Experiments 1 and 3, in the vehicle-sertindole condition, is consistent with our experience with both typical and atypical neuroleptics, which indicates that enhancement of LI is more readily detectable when the pre-exposure parameters used are insufficient to produce robust LI in control animals. It is possible that with a high number of pre-exposures a ceiling of inattention to the pre-exposed stimulus is attained which cannot be further increased by neuroleptics.

Sertindole is inactive in classical in vivo models for neuroleptic activity, such as antagonism of methylphenidate-induced gnawing and amphetamine-induced stereotypy (Hyttel, 1991; Sánchez et al., 1991), as well as in acute electrophysiological tests; that is, it does not increase the firing frequency of DA neurons in substantia nigra pars compacta (SNc) or VTA, and does not reverse d-amphetamine- and apomorphine-induced inhibition of firing of these neurons (Skarsfeldt, 1992). Thus, the importance of the present results derives both from the demonstration of neuroleptic properties of sertindole, and from the fact that these properties were detected using an acute administration regime, as found with other neuroleptics tested in LI (Weiner and Feldon, 1987; Christison et al., 1988; Feldon and Weiner, 1991b).

Following acute treatment, sertindole binds to D2 receptors and this effect is more pronounced in the limbic than in striatal areas (Hyttel et al., 1992). In addition, although the marked selectivity of sertindole for inhibiting VTA compared with SNc neurons becomes evident only after prolonged treatment, it potently prevents the hyperactivity response from the beginning of a continuous DA infusion into the nucleus accumbens (Hyttel, 1991). The drug also antagonizes amphetamine-induced hyperactivity which is known to depend on DA release in the nucleus accumbens, having its maximal effects in this test paradigm at the dose shown here as effective for LI facilitation (Arnt et al., 1992). Since the development of LI is apparently subdued by mesolimbic DA mechanisms (Weiner, 1990; Gray et al., 1991), the most likely mechanism for the neuroleptic effect of sertindole on LI is blockade of D2 receptors in the mesolimbic system. In this context it is interesting to point out that although sertindole is a very potent 5-HT2a receptor antagonist, it does not appear to act as such in the LI model, since selective 5-HT2a antagonists disrupt LI (Cassaday et al., 1993). This lack of serotonergic effect is consistent with the claim that serotonergic blockade is not an essential component of antipsychotic drug action (Gerlach, 1991; Seeman, 1992).

The present results have important implications for the LI model as a screening test for compounds with antipsychotic potential. A major problem in the search for antipsychotic drugs is the lack of adequate animal models (Gerlach, 1991). This problem is particularly salient in the case of atypical neuroleptics which are inactive in the classical tests of DA blockade, as detailed above for sertindole. The LI model provides a unique test paradigm for detecting antipsychotic potential of drugs as it is equally sensitive to typical and atypical neuroleptics differing in their in vitro and in vivo pharmacology. Moreover, whereas antagonism of amphetamine-induced disruption of LI is a drug-drug model, the facilitation of LI does not require the administration of a DA agonist, so that the model does not rely on pharmacological means to elicit the behaviour of interest. In addition, the LI model taps antipsychotic potential with both acute (Weiner and Feldon, 1987; Weiner et al., 1987, 1994; Feldon and Weiner, 1988, 1991b; Warburton et al., 1994) and repeated drug administration (Christison et al., 1988; L. Weiner and J. Feldon, unpublished observations). Finally, the LI model of antipsychotic drug action has direct relevance to the clinic because it models an attentional process that is disrupted in schizophrenia and is ameliorated by neuroleptic treatment.

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