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Aminoalkylindoles: atypical dopamine antagonists

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The neuropharmacological profile of a series of aminoalkylindole compounds (AHR-1229-(3-[2-(3-indolyl)-ethyl]-butylamino-1-phenyl-pyrrolidine), AHR1771-(1-[2-(2-methyl-3-indolyl)ethyl]-4-phenyl-3,4-dehydropiperidine), AHR1806-(1-[2-(5-chloro-3-indolyl)-ethyl]-4-phenyl-3,4-dehydropiperidine), AHR1858-(1-[2-(3-indolyl)ethyl]-4-(4-fluoro-phenyl)-1,2,3,6-tetrahydropyridine), AHR1859-(1-[2-(1-methyl-3-indolyl)ethyl]- 4-phenyl-1,2,3,6-tetrahydropyridine), AHR1709-(1-[2-(3-indolyl)ethyl]-4-phenyl- 1,2,3,6-tetrahydropyridine) was determined in comparison with the classical neuroleptic agents haloperidol and oxypertine, the latter being of similar indole structure. The indole analogues were shown to antagonize amphetamine-induced toxicity in aggregated mice, to indicate a 'tranquillizing' action but, in contrast to haloperidol and oxypertine, showed weak or no activity in other classical behavioural tests for neuroleptic action, catalepsy induction and stereotypy antagonism. In further contrast to haloperidol or oxypertine, the indole derivatives failed to displace [^{3}H]spiperone in radioligand binding assays and failed to increase prolactin levels. However, similarly to both typical and atypical neuroleptic agents, the indole derivatives were shown to inhibit the behavioural hyperactivity resulting from the intracerebral administration of dopamine into the mesolimbic nucleus accumbens of rat. The dissociation of an ability to antagonize a dopamine action in the mesolimbic system from classical neuroleptic actions involving other cerebral dopamine systems is the most important finding of the present study.

The search for neuroleptic agents to inhibit dopamine function has detected but few compounds of differing activity spectra, and it is acknowledged that future developments in this area must look for improved pharmacokinetic profile, lower toxicity or increased selectivity of action to a particular dopamine system. Two preliminary studies have thus drawn attention to a series of aminoalkylindoles (Welstead et al 1967) of which a number of agents were shown to inhibit isolation-induced aggressive behaviour in mice and, from this series, one agent, AHR1709, was later shown to specifically inhibit the increased behavioural activity caused by intrastriatal or intra-accumbens dopamine (Costall et al 1978). Therefore, the present studies aim to analyse the neuroleptic profile of these compounds to define any specificity of action.

MATERIALS AND METHODS

Male Sprague-Dawley rats (250–300 g) were used in catalepsy, stereotypy and intra-accumbens dopamine hyperactivity tests, and in [^{3}H]spiperone binding assays. Catalepsy was tested as the time of maintenance of an abnormal imposed position with the front limbs extended over a 10 cm high bar, and this was converted to a score: 0 = no catalepsy, 1 = 0·1–2·5 min, 2 = 2·6–5·0 min, 3 = 5·1–10·0 min, 4 = 10·1–20·0 min, 5 = 20·1 min—∞. Stereotyped behaviour (induced by 10 mg kg^{-1}) (+)-amphetamine, 1 h pretreatment) and its antagonism was assessed using the scoring system: 0 = no stereotypy, 1 = periodic sniffing and/or repetitive head and limb movements, 2 = continuous sniffing and/or repetitive head and limb movements, 3 = periodic gnawing, biting or licking, 4 = continuous gnawing, biting or licking. Rats were prepared for the intra-accumbens dopamine hyperactivity test using standard stereotaxic techniques to implant bilateral guides to allow subsequent (10–14 days recovery) intra-accumbens injection of dopamine (50 μg in 1 μl delivered over 60 s 2 h after 100 mg kg^{-1} nialamide) (see Costall & Naylor 1976). Hyperactivity was assessed using individual photocell boxes; potential antagonists were given i.p. 2 h after dopamine when hyperactivity was established.

Effects on (+)-amphetamine lethality were determined according to Burn & Hobbs (1958). Logarithmically spaced doses of test compounds were given to groups of 10 adult, female ICR mice (Flow Labs, Dublin, VA) aggregated in wire-mesh cages (15 × 30 × 10 cm). After 30 min each animal received 21 mg kg^{-1} (+)-amphetamine (LD50 under

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aggregated, but not isolated, conditions) and the number of dead animals in each cage determined after 24 h. Protective ED50 values were calculated (Litchfield & Wilcoxon 1949) and defined as the dose preventing death in 50% of animals.

For the [^{3}H]spiperone binding assays rats were killed by cervical dislocation and their brains rapidly removed. The striata were dissected over ice, homogenized (Polytron PT-10, setting 5 for 10 s) in 100 vol. of ice cold Tris-HCl buffer and centrifuged twice at 50 000 *g* for 10 min with 3 further rehomogenizations in fresh buffer. Final resuspension was in Tris-HCl, 50 mM pH 7·4 at 37 °C, containing 5 mM Na_2EDTA. Binding was determined by incubating 500 µl of tissue suspension (5 mg tissue, equivalent to approximately 300 µg protein, Lowry et al 1951) with 1 nM [^{3}H]spiperone in the presence of buffer or agent under investigation in a total incubate volume of 1·1 ml at 37 °C for 15 min. Samples were rapidly filtered under vacuum through Whatman GF/B filters and washed with two 5 ml rinses of ice-cold buffer. Filters were vigorously shaken for 30 min in Insta-Gel (Packard Instruments) and the radioactivity measured by liquid scintillation spectrometry (efficiency 45%).

Prolactin assays were carried out on female Sprague-Dawley rats (Flow Labs, Dublin, VA, 200–300 g); they were housed individually at 22 ± 3 °C on a 12 h day-night cycle with food (Rat-Mouse Diet, Wayne Lab Blox) and water available ad libitum. Three weeks before dosing, daily vaginal smears were made with 0·15 M sodium chloride and examined microscopically to determine the oestrous stage and cyclic pattern of the rat. Animals were then selected and dosed 2 or 3 days after the appearance of keratinized cells; 2 h after drug injection the animals were decapitated and blood samples taken. Serum was separated from the cells and frozen in a mixture of dry ice and acetone, and kept at −20 °C. Serum prolactin was determined according to the standard procedures supplied with the radioimmunoassay kit obtained from the National Institute of Arthritis, Metabolism and Digestive Diseases, National Institute of Health, Bethesda, Maryland, U.S.A. A log transformation was made of the serum prolactin values and mean differences compared by Dunnett's 2-sided *t*-test at the 5% probability level.

AHR1229 (3-[2-(3-indolyl-)ethyl]-butylamino-1-phenylpyrrolidine). HCl, AHR1771 (1-[2-(2-methyl-3-indolyl)ethyl]-4-phenyl-3,4-dehydropiperidine), AHR1806 (1-[2-(5-chloro-3-indolyl)ethyl]-4- phenyl-3,4-dehydropiperidine), AHR1858 (1-[2-(3-indolyl)ethyl]-4-(4-fluoro-phenyl)-1,2,3,6-tetrahydropyridine), AHR1859 (1-[2-(1-methyl-3-indolyl) ethyl]-4-phenyl-1,2,3,6-tetrahydropyridine). HCl, AHR1709 (1-[2-(3-indolyl)ethyl]-4-phenyl-1,2,3,6-tetrahydropyridine) and oxypertine HCl (Sterling-Winthrop Research Institute) were dissolved in the minimum quantity of *NN*-dimethyl formamide, haloperidol (Janssen) in the minimum quantity of tartaric acid made up to volume with distilled water, nialamide (Sigma) in a minimum quantity of hydrochloric acid made up to volume with distilled water and (+)-amphetamine. SO_4 (Sigma) in distilled water. Administrations were made i.p. in a volume of 1 ml kg^{-1}. For the prolactin studies, the indole agents were suspended in 0·25% methylcellulose and administered in a volume of 5 ml kg^{-1}. For the (+)-amphetamine lethality study, compounds were dissolved or suspended in deionized water (by means of a surfactant and sonification) and given in a volume of 10 ml kg^{-1}. Dopamine HCl (Koch-Light) was prepared for intracerebral administration in nitrogen-bubbled distilled water neutralized with sodium bicarbonate.

RESULTS

Catalepsy induction

Haloperidol (0·25 mg kg^{-1} i.p.) and oxypertine (5–40 mg kg^{-1} i.p.) induced dose-dependent catalepsy in rats (ranging from scores 1 to 5). Of the indole compounds tested AHR1709 and AHR1229 (1·25–40 mg kg^{-1} i.p.) were without cataleptic potential, the only obvious behavioural change being increased alertness and repetitive head movements. AHR1859 was similarly characterized but a score 1 catalepsy accompanied by muscular hypotonia developed at 40 mg kg^{-1} i.p. AHR1806 and AHR1858 (5–40 mg kg^{-1} i.p.) caused inconsistent or low intensity catalepsy which was not dose related. AHR1771 caused clear catalepsy at 40 mg kg^{-1} i.p. (score 2) but a dose-dependency could not be shown (score 1 at 10 and 20 mg kg^{-1} i.p.). In haloperidol interaction studies, the maximal catalepsy induced by 2 mg kg^{-1} haloperidol (1 h) was not reduced by minimally effective, threshold doses (5 mg kg^{-1} i.p.) of AHR1771, AHR1806 or AHR1858. A submaximal cataleptic response to 0·5 mg kg^{-1} i.p. haloperidol (1 h) was not modified by 5 mg kg^{-1} i.p. AHR1771, AHR1806 or AHR1858.

Stereotypy antagonism

Haloperidol (0·025–0·05 mg kg^{-1} i.p.) and oxypertine (2·5–5·0 mg kg^{-1} i.p.), administered as 30 min pretreatments, prevented the development of

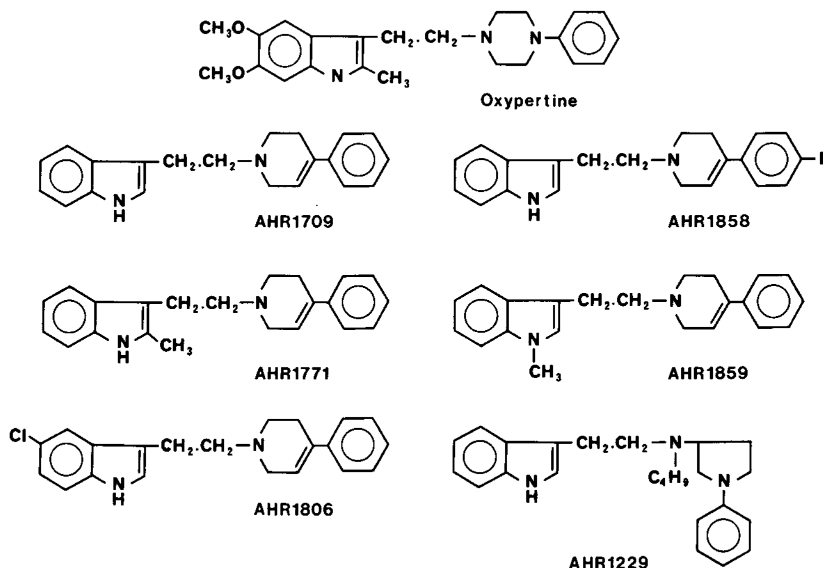

FIG. 1. Chemical structures of oxypertine and the AHR aminoalkylindole derivatives used in the present studies.

stereotyped responding to 10 mg kg^{-1} i.p. (+)-amphetamine. None of the AHR indole derivatives, administered in doses up to 20 mg kg^{-1} i.p. (20 min) had any effect on amphetamine stereotypy.

Antagonism of intra-accumbens dopamine-induced hyperactivity

Haloperidol (0·025–0·2 mg kg^{-1} i.p.) and oxypertine (2·5–10 mg kg^{-1} i.p.) dose-dependently reduced and abolished the hyperactivity response to intra-accumbens dopamine (50 μg, 2 h) in the absence of any non-specific sedative effects. AHR1709, AHR1771 and AHR1858 (1·25–20 mg kg^{-1} i.p.) similarly reduced and then abolished activity, AHR1859 caused a dose-dependent reduction in hyperactivity but was less potent, whilst AHR1229 caused only a modest antagonism, in the order of 40% at 20 mg kg^{-1} i.p., and AHR1806 was inactive at 20 mg kg^{-1} i.p. (Table 1).

Inhibition of (+)-amphetamine lethality in mice

While none of the experimental compounds was as potent as haloperidol in blocking (+)-amphetamine lethality in mice under aggregated conditions, all compounds except AHR1229 were virtually equipotent with oxypertine (Table 2).

Displacement of [^{3}H]spiperone from rat striatal membranes

A preliminary Scatchard analysis of [^{3}H]spiperone binding (using 10 μM dopamine to assess specific binding) indicated a saturable binding, a plateau at 1–2 nM and K_D 0·87 nM. In the displacement studies dissociation constants (K_i values derived by the formula of Cheng & Prusoff 1973) for spiperone and haloperidol of 1·2 and 1·8 nM respectively were obtained for the displacement of 1 nM[^{3}H]spiperone. The indole compounds caused either modest displacement at high micromolar concentrations (AHR1709, AHR1859, AHR1858, AHR1806) or were ineffective even at 10^{-5} M (AHR1229, AHR3219) to preclude a determination of K_i values.

Modification of rat serum prolactin levels

The i.p. injection of vehicle (0·25% methylcellulose) did not alter serum prolactin levels from those recorded after saline injection. Oxypertine (50 mg kg^{-1}) caused a 33 fold increase in serum

Table 1. Effects of haloperidol, oxypertine and some AHR indole derivatives on the hyperactivity response to intra-accumbens dopamine in the rat.

Treatment	Hyperactivity antagonism ED50 (mg kg^{-1} i.p.)
Haloperidol	0·05
Oxypertine	5·0
AHR1709	3·4
AHR1806	>20.0
AHR1858	3·1
AHR1771	8·3
AHR1229	>20·0
AHR1859	15·6

n = 6–8. s.e.m.s on original data <12%.

Table 2. Inhibition of (+)-amphetamine lethality in mice.

Treatment	(+)-Amphetamine antagonism ED50 (mg kg^{-1} i.p.)
Haloperidol	0·1
Oxypertine	2·0
AHR1229	>20.0
AHR1709	0·7
AHR1771	2·3
AHR1806	4·5
AHR1858	1·0

prolactin levels. All AHR indole derivatives, administered at doses 10 × the calculated ED50 value to inhibit the lethal response to (+)-amphetamine in aggregated mice (the inactive agent in the behavioural test, AHR1229, was administered at an arbitrary dose of 50 mg kg^{-1}), failed to modify serum prolactin levels (Table 3).

DISCUSSION

The initial study on the aminoalkylindole compounds showed a potential to antagonize the aggressive behaviour of fighting mice, a broad indication of 'tranquillizing' activity (Welstead et al 1967). A 'tranquillizing' effect was further evidenced by present findings of ability to antagonize amphetamine-induced toxicity in aggregated mice. However, the present studies showed the indole derivatives to have little or no activity in the classical tests for neuroleptic activity, catalepsy induction and stereotypy antagonism, in which the standard neuroleptic, antischizophrenic agents haloperidol and oxypertine, the latter of indole structure, showed marked dose-dependent activity. Indeed, the most obvious behavioural change to AHR1859, AHR1709 and AHR1229 was one of increased alertness and small repetitive head movements, an indication of behavioural excitation. Only AHR1771 induced a consistent catalepsy of modest intensity, its potency being approximately half that of oxypertine, and at least fifty times less than that of haloperidol. The negative activity of the AHR indole analogues is indicative of an inability to block at the classical neuroleptic sites. This was confirmed in the [^{3}H]spiperone binding assay; the indole derivatives either failed to displace [^{3}H]spiperone from rat striatal tissue or were required in such exceptionally high concentrations as to preclude a pharmacological relevance.

Table 3. Effects of oxypertine and some AHR indole derivatives on rat serum prolactin levels.

Treatment	No.	Dose (mg kg^{-1} i.p.)	Serum prolactin (ng ml^{-1})*
Saline	5	—	8·20 ± 4·20
Methylcellulose	5	—	6·84 ± 3·66
Oxypertine	5	50	227·69 ± 34.63**
AHR1709	5	6·5	9·4 ± 3·8
AHR1806	5	50	6·03 ± 3·95
AHR1858	5	10	8·42 ± 2·71
AHR1771	3***	22·5	10·82 ± 2·76
AHR1229	5	50	2·95 ± 0·73

* Mean ± s.e., ** $P < 0·05$, *** Study restricted by limited amount of drug.

However, with the exception of AHR1806 and AHR1229, the indole agents AHR1709, AHR1859 and AHR1771 all caused an effective antagonism of the locomotor hyperactivity induced by intra-accumbens dopamine similarly to a wide range of antischizophrenic agents. However, the majority of the neuroleptic agents which inhibit a mesolimbic dopamine hyperactivity are also able to effectively block other cerebral dopamine systems (see review by Costall & Naylor 1980). Indeed, all known antischizophrenic agents in clinical use exert particularly marked effects on the tuberoinfundibular dopamine system to modify hormone release. In particular, an ability to increase prolactin secretion has been taken as a reliable index of neuroleptic action; this effect is achieved at low doses of neuroleptic, doses frequently too small to induce any overt changes in motor behaviour (see, for example Van Der Gugten 1976). It is the most important finding of the present work that, notwithstanding the use of the AHR indoles at doses 10 × greater than required to secure a very effective antagonism of the amphetamine toxicity in grouped mice, these agents failed to modify prolactin levels. This contrasts with the ability of oxypertine to cause an approximate thirty fold increase in prolactin levels.

In summary, from a series of aminoalkylindoles having structures similar to that of the classical neuroleptic agent oxypertine, agents were detected which failed to induce catalepsy, failed to antagonize amphetamine stereotypy, failed to displace [^{3}H]spiperone and failed to increase prolactin levels. This suggests a fundamental difference in their mechanism of action from that of the classical neuroleptic agents. However, similar to both typical and atypical neuroleptic agents (Costall & Naylor 1976), the indole analogues could inhibit the hyperactivity caused by intra-accumbens dopamine. Whilst it could perhaps be argued that the ability of the AHR indoles and neuroleptics to antagonize the amphetamine toxicity in aggregated mice could reflect both central and peripheral effects, the antagonism of the hyperactivity induced by intra-accumbens dopamine

is much more suggestive of a specific action. However, this does not necessarily imply that the AHR indole compounds are exerting a direct dopamine receptor blockade. 5-Hydroxytryptamine, for example, can antagonize the dopamine hyperactivity when injected into the nucleus accumbens, and 5-HT and the AHR compounds have the indole structure in common. Whilst the mechanism of action of the indole analogues therefore remains to be established, the present results would encourage a belief that it may be possible to dissociate an ability to antagonise dopamine action in the mesolimbic system from a classical neuroleptic action on other cerebral (pituitary and possibly striatal) dopamine systems.

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REFERENCES

Burn, J. H., Hobbs, R. (1958) Arch. Int. Pharmacodyn. Ther. 113: 290–295

Cheng, Y. C., Prusoff, W. H. (1973) Proc. Natl. Acad. Sci. U.S.A. 73: 4354–4358

Costall, B., Funderburk, W. H., Leonard, C. A., Naylor, R. J. (1978) J. Pharm. Pharmacol. 30: 771–778

Costall, B., Naylor, R. J. (1976) Eur. J. Pharmacol. 35: 161–168

Costall, B., Naylor, R. J. (1980) Rev. Pure Appl. Pharmacol Sci. 1: 3–83

Lowry, D. J., Rosebrough, N. J., Farr, A. L., Randall, R. J. (1951) J. Biol. Chem. 193: 265–275

Litchfield, J. T. Jr., Wilcoxon, F. (1949) J. Pharmacol. Exp. Ther. 96: 99–113

Van Der Gugten, A. A., Sahuleka, P. C., Van Galen, G. H., Kwa, H. G. (1976) J. Endocrinol. 68: 355–368

Welstead, W. J., Da Vanzo, J. P., Helsley, G. C., Lunsford, C. D., Taylor, C. R. (1967) J. Med. Chem. 10: 1015–1021