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Antipsychotic-Like Profile of Alstonine

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COSTA-CAMPOS, L., D. R. LARA, D. S. NUNES AND E. ELISABETSKY. *Antipsychotic-like profile of alstonine*. PHARMACOL BIOCHEM BEHAV **60**(1) 133–141, 1998.—Although recently developed drugs have brought significant improvement, the treatment of psychotic disorders still presents serious drawbacks. Because inherent complexity and lack of satisfactory understanding of the underlying pathophysiology impose limits for rational drug design, resourceful approaches in the search for antipsychotics are pertinent. This article reports pharmacological properties of alstonine, a heteroyohimbine-type alkaloid, which exhibited an antipsychotic-like profile, inhibiting amphetamine-induced lethality, apomorphine-induced stereotypy, and potentiating barbiturate-induced sleeping time. Atypical features of alstonine were the prevention of haloperidol-induced catalepsy and lack of direct interaction with D_1, D_2 and 5-HT_{2A} receptors, classically linked to antipsychotic mechanism of action. © 1998 Elsevier Science Inc.

Alstonine Antipsychotic Serpentine alkaloids Dopamine

THE recent accretion of a considerable body of knowledge has challenged the classic dopamine centered understanding of the pathophysiology of schizophrenia (3). However, the still limited comprehension of psychotic disorders constrains rational drug design and motivates innovative approaches in the search for antipsychotics. Typical antipsychotics have apparent therapeutic limitations, especially concerning effectiveness on the so-called negative symptoms and induction of autonomic and extrapyramidal side effects (13,14). It has long been established that typical antipsychotic (Fig. 1) clinical potency is dependent on their ability to block dopamine D_2 receptors (8,25). In contrast, atypical antipsychotics (Fig. 1), such as clozapine and risperidone, which have proved more effective in controlling positive and negative symptoms, as well as presenting diminished extrapyramidal side effects (4), are associated with a less potent blockade of D_2 receptors, combined with 5-HT_2 antagonism (17). The multiple mechanism of action of clozapine as well as its clinical advantages have been well documented (7).

Natural products are inextricably linked to the history of schizophrenia. In 1931, the use of *Rauwolfia serpentina* in the treatment of insanity was reported (27). Reserpine (Fig. 1) is a major alkaloid in the plant root and was developed as the first antipsychotic drug. Amphetamine, a congener of ephedrine, the active compound of the Chinese drug *Ma Huang*, was the basis for the first model of psychosis (1).

Ethnopharmacology has been defined as "the interdisciplinary scientific exploration of biologically active agents traditionally employed or observed by men" (11). In Nigeria, two-thirds of health practitioners are traditional healers (20), the medical setting is pluralistic, and there are distinct categories of healers including traditional birth attendants, traditional psychiatrists, herbalists, and traditional pharmacists (19,20,21,23, 28), An ethnopharmacological study in Nigeria has lead to the investigation of a plant-based extract used by traditional psychiatrists with anecdotal antipsychotic-like effects. This extract was later found to bear an antipsychotic-like profile (Elisabetsky et al., unpublished results) using a behavioral approach similar to the present study. Phytochemical studies have identified alstonine as one of the major components of this extract.

The following study investigates the putative antipsychotic profile of alstonine using behavioral and neurochemical strategies.

METHOD

Animals

Male Swiss albino adult (20–35 g) mice (CS_1 strain) and male Wistar adult rats were used for behavioral and binding experiments, respectively. Animals were housed at 22 ± 2°C with food and water ad lib and 12 L:12 D cycles. Behavioral exper-

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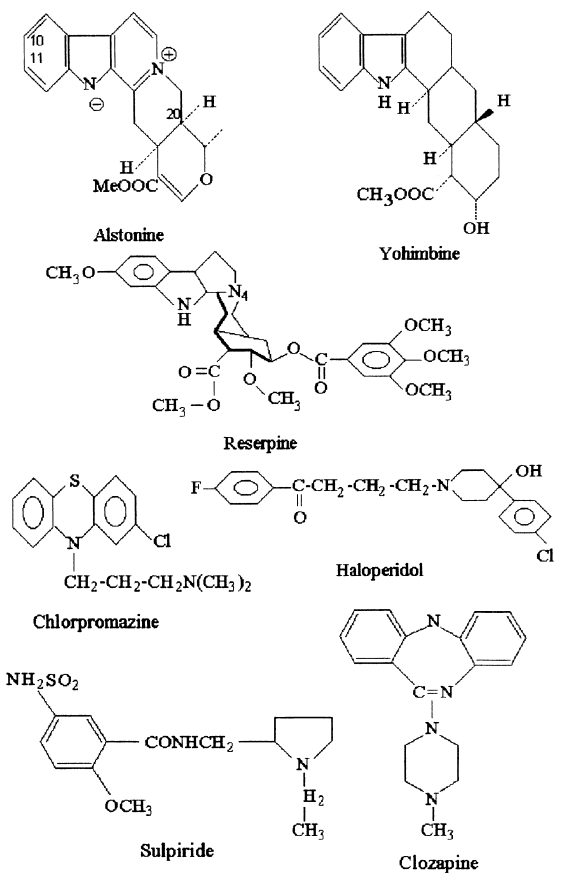

FIG. 1. Chemical structures of serpentine-like alkaloids (alstonine and reserpine), classical (chlorpromazine and haloperidol), and atypical antipsychotics (clozapine and sulpiride).

iments were conducted in mice due to the limited source of alstonine and to allow for comparison with the original extract.

Materials

Alstonine (HCl) was generously provided by Prof. Maurice Iwu, University of Nigeria at Nsukka, and Walter Reed Institute (USA). Chlorpromazine, clozapine, sodium pentobarbital, diazepam, and apomorphine were purchased from Sigma Chemical Co. (St. Louis, MO), sulpiride, reserpine, and ritanserine from Research Biochemicals International (Natick, MA), and haloperidol used from commercial Haldol® (Janssen Farmacêutica Ltda, São José dos Campos, SP, Brazil). [^{3}H]Spiperone (109 μCi/mmol) and [^{3}H]SCH23390 (83 μCi/mmol) were purchased from Amersham (Buckinghamshire, UK).

Reserpine was diluted in Tween 80 (TWE). Clozapine and sulpiride were solubilized in HCl (0.1 N), and the pH was adjusted (7.0 and 5.0, respectively) with NaOH (1 N) for behavioral studies. Chlorpromazine and apomorphine were diluted in saline. Haloperidol, sodium pentobarbital, and alstonine hydrochloride were diluted in distilled water. Diazepam was dilute in PEG 20%. Control groups received saline (NaCl 0.9%, SAL), or polyethylene glycol (20%, PEG) or Tween 80 (20%, TW) as appropriate. For binding studies, ritanserine was dissolved in 4% methanol.

Behavioral Experiments

Protection of lethality induced by amphetamine in grouped mice. Mice were divided in groups of 10 and received amphetamine (12.5 mg/kg, IP) before being placed in small boxes (19.0 × 9.0 × 9.5 cm). Test drugs were given IP 30 min before amphetamine. The degree of lethality was noted 24 h after amphetamine administration (2). Fisher's test was used for statistical analysis.

Antagonism of stereotypic behavior induced by amphetamine in mice. Stereotypic behavior induced by 16.0 mg/kg of amphetamine was observed after 10, 30, 45, 60, 90, and 120 min after amphetamine in small (9.5 × 9.0 × 9.5 cm) Plexiglas boxes. Test drugs were injected 30 min before amphetamine. Stereotypy was graded from 0 to 3 according to the following criteria: 0 = absence of stereotypy; 1 = presence of stereotyped movements of the head and intermittent sniffing; 2 = sniffing and chewing; and 3 = chewing and intense licking (2). Significant differences were determined by Kruskal–Wallis followed by Mann–Whitney test.

Antagonism of stereotypic behavior induced by apomorphine in mice. Apomorphine 1.0 mg/kg SC was administered 30 min after test materials. Mice were observed for stereotypic behavior as for amphetamine at 15, 30, 45, and 60 min postapomorphine (2,31). The model was validated with haloperidol. Significant differences were determined by Kruskal–Wallis followed by Mann–Whitney test.

Interaction with haloperidol-induced catalepsy in mice. Catatonic behavior was measured by the bar test, which consists of placing the animal's forepaws on a horizontal glass rod of 0.6 mm diameter, raised 6 cm above the floor by two wooden blocks. The intensity of catatonic behavior was measured at 20-min intervals up to 2 h (6). Results were analyzed by means of Kruskal–Wallis followed by Mann–Whitney test.

Potentiation of barbiturate sleeping time in mice. Thirty minutes after treatment with test materials groups of eight mice were treated IP with 30.0 mg/kg of sodium pentobarbital. After the barbiturate injection the sleeping time (time elapsed between loss and recuperation of righting reflex) was recorded. Criterion for recuperation of righting reflex is that animals have to regain their normal posture for three consecutive times when challenged to remain on their backs (33). The model was validated with diazepam, and the data analyzed by means of ANOVA test.

Neurochemistry

The methods for membrane preparation and dopamine receptor binding were adapted with minor changes from Schettini et al. (25,26).

Membrane preparation. Rats were sacrificed by decapitation and either the striata or frontal cortex was excised (22), pooled, and homogenized in ice-cold 50 mM Tris-HCl buffer, pH 7.4 (1:40 wt/vol). The homogenate was centrifuged at 40,000 × *g* for 15 min. The pellet was rehomogenated in Tris buffer and centrifuged again. The final pellet was resuspended in 50 mM Tris-HCl buffer, pH 7.4 containing 120 mM NaCl, 5 mM KCl, 1 mM MgCl, 1 mM CaCl, and 0.01% ascorbic acid.

Radioligand binding assays. All experiments were performed in triplicate in 250 μl volume containing 0.5 mg of tissue (which was added last) for D_1 and D_2 receptors and 0.7 mg for the 5-HT_{2A} receptor. Tubes were incubated for 30 min at 37°C, filtered on Whatmann GF/B glass fiber filters, and washed once with 10 ml of 50 mM Tris-HCl buffer, pH 7.4. After drying the filters for 15 min at 80°C, radioactivity was counted in vials containing 3 ml of scintillation liquid.

D_1 receptor. 0.5 nM [^{3}H]SCH23390 was used on striatal membranes. Nonspecific binding (11–14%) was obtained using 1 μM SCH23390. Saturation studies in our laboratory indicated a K_d of 0.41 ± 0.05 nM and a B_{max} of 87.8 ± 4.8 pmol/g. Control experiments were run with clozapine (100 μM).

D_2 receptor. [^{3}H]Spiperone (0.4 nM) was used on striatal membranes. Nonspecific binding (24–28%) was obtained using 10 μM sulpiride. Saturation studies indicated a K_d of 0.23 ± 0.02 nM and a B_{max} of 48 ± 2.7 pmol/g. Control experiments were run with chlorpromazine (10 μM) and clozapine (100 μM).

5-HT_{2A} receptor. 5-HT_{2A} receptor binding using [^{3}H]spiperone was developed in our laboratory as follows. Because spiperone binds preferentially to D_2, 5-HT_{2A}, and 5-HT_{1A} receptors (10,18), we chose to access 5-HT_{2A} receptors specifically by using tissue from frontal cortex, which has high density of this receptor (9) and negligible concentrations of 5-HT_{1A} (9) and D_2 receptors (16). A similar strategy is used in vivo with the analogue [^{3}H]-3-N-methylspiperone in positron emission tomographic (PET) studies in humans to study 5-HT_{2A} and D_2 receptors simultaneously (29). [^{3}H]Spiperone (0.4 nm) was used on frontal cortex membranes (700 μg/tube). Nonspecific binding (33–36%) was obtained using 10 μM chlorpromazine (which also binds to the 5-HT_{2A} receptor). Ritanserine, which selectively binds to 5-HT_2 receptors, eliminated specific binding, whereas sulpiride, selective to D_2 receptors, had no effect (less than 10% inhibition at 100 μM—data not shown). Therefore, [^{3}H]spiperone specific binding on frontal cortex reflects its interaction with 5-HT_{2A} receptors. Saturation experiments indicated a K_d of 0.42 ± 0.06 nM and a B_{max} of 23.8 ± 3.9 pmol/g. Control experiments were run with ritanserine (10 μM) and clozapine (100 μM).

Competition studies were conducted with six concentrations of alstonine up to 100 μM. Statistic significance was defined as $p < 0.05$.

RESULTS

Alstonine significantly protected grouped mice from amphetamine-induced lethality in a bell-shaped dose-dependent fashion (Fig. 2). The effective dose range was 0.5 to 2.0 mg/kg; 0.1 and 20 mg/kg did not prevent mice lethality. All antipsychotics tested were active, whereas reserpine did not show effect. Alstonine did not alter the intensity of amphetamine-induced stereotypic behavior (Fig. 3c), whereas haloperidol, chlorpromazine, and sulpiride were effective (Fig. 3a and b). Concerning apomorphine-induced stereotypic behavior alstonine (0.1–1.0 mg/kg) significantly reduced behavioral scores (Fig. 4c). The highest dose (2.0 mg/kg) was ineffective. Haloperidol completely prevented the effects of apomorphine. Chlorpromazine, sulpiride, and clozapine also were effective at given doses and times of observations (Fig. 4a and b). Alstonine significantly diminished haloperidol-induced catatonic time (Fig. 5c). Chlorpromazine was ineffective (Fig. 5a), whereas clozapine and sulpiride had consistent effects at all doses (Fig. 5b). Alstonine also potentiated pentobarbital-induced sleeping time (Fig. 6).

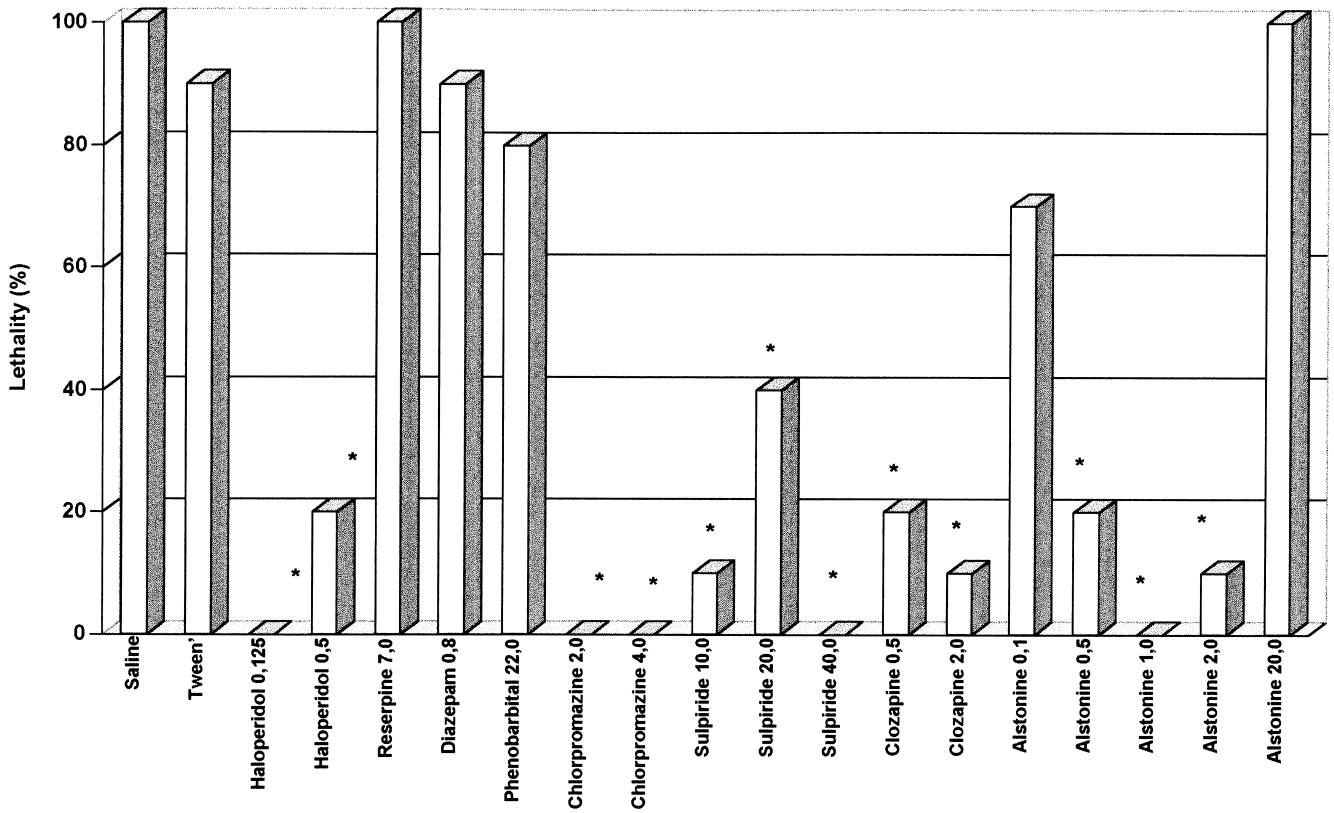

FIG. 2. Effects of classical (haloperidol and chlorpromazine) and atypical antipsychotics (clozapine and sulpiride), reserpine, barbiturate (pentobarbital), and benzodiazepine (diazepam), alstonine, and vehicles (saline, tween) on amphetamine-induced lethality in grouped mice. Doses in mg/kg. Drugs administered IP. *$p < 0.05$, Fisher.

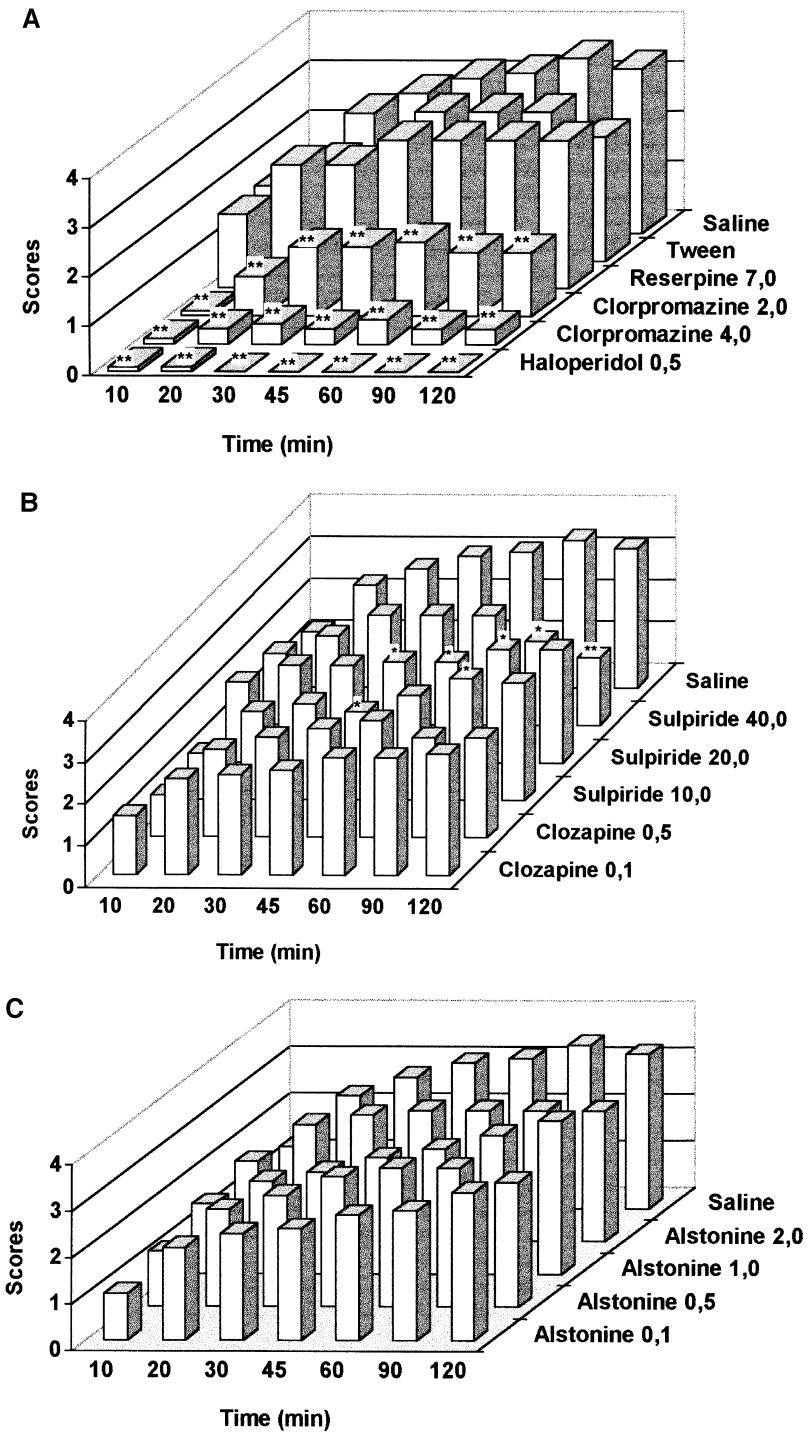

FIG. 3. (a) Effects of classical antipsychotics (haloperidol and chlorpromazine), reserpine and respective vehicles (saline, tween) on amphetamine-induced stereotypy. Doses in mg/kg. Drugs administered IP. $*p < 0.05$, $**p < 0.01$, Mann–Whitney. (b) Effects of atypical antipsychotics (clozapine and sulpiride) and vehicle (saline) on amphetamine-induced stereotypy. Doses in mg/kg. Drugs administered IP. $*p < 0.05$, $**p < 0.01$, Mann–Whitney. (c) Effects of alstonine and vehicle (saline) on amphetamine-induced stereotypy. Doses in mg/kg. Drugs administered IP. $*p < 0.05$, $**p < 0.01$, Mann–Whitney.

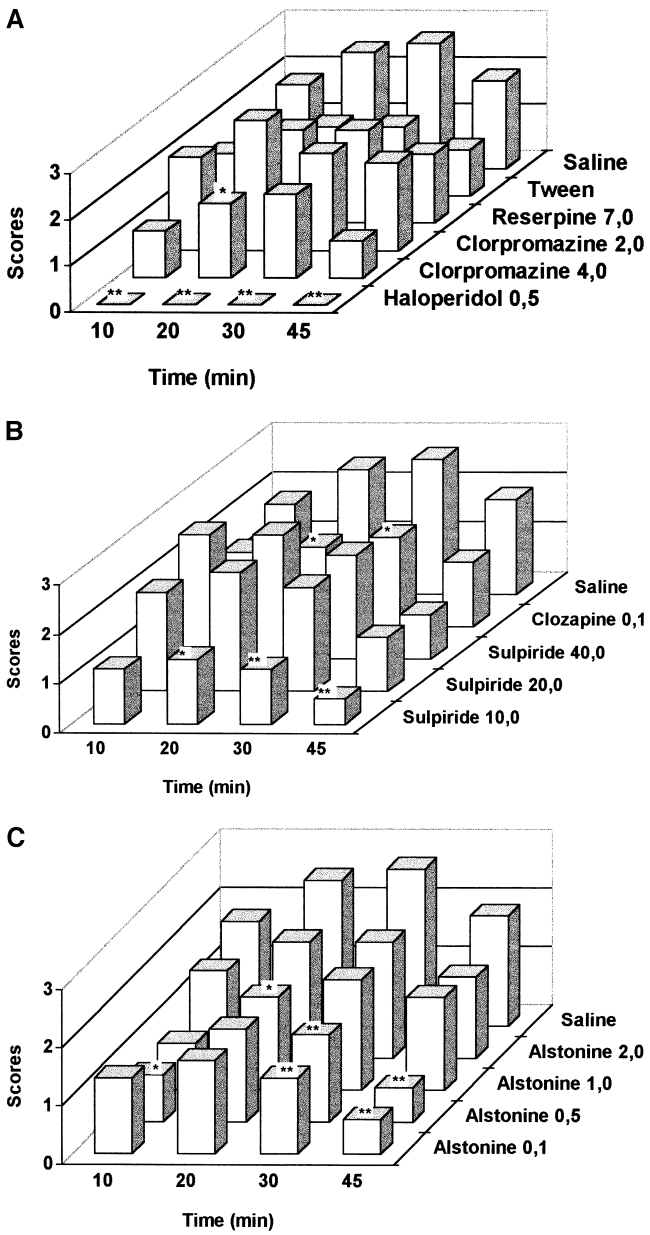

FIG. 4. (a) Effects of classical antipsychotics (haloperidol and chlorpromazine), reserpine, and respective vehicles (saline, tween) on apomorphine-induced stereotypy. Doses in mg/kg. Drugs administered IP. $*p < 0.05$, $**p < 0.01$, Mann–Whitney. (b) Effects of atypical antipsychotics (clozapine and sulpiride) and vehicle (saline) on apomorphine-induced stereotypy. Doses in mg/kg. Drugs administered IP. $*p < 0.05$, $**p < 0.01$, Mann–Whitney. (c) Effects of alstonine and vehicle (saline) on apomorphine-induced stereotypy. Doses in mg/kg. Drugs administered IP. $*p < 0.05$, $**p < 0.01$, Mann–Whitney.

Regarding binding studies, clozapine (100 μM) completely inhibited radioligand binding to D_1, D_2, and 5-HT_{2A} receptors, chlorpromazine (10 μM) abolished D_2 radioligand binding, and ritanserine (10 μM) eliminated binding to 5-HT_{2A} receptors (Table 1). These results indicate that our assays are able to identify ligands to these receptors. Competition studies with [^{3}H]Spiperone and [^{3}H]SCH23390 revealed a lack of interaction of alstonine (up to 100 μM) with D_1, D_2, and 5-HT_{2A} receptors (Fig. 7).

DISCUSSION

The study of antipsychotics has been centered at drugs that modulate dopaminergic activity, with the understanding that benefits of antipsychotic drugs are associated with blockade of mesolimbic D_2 receptors, whereas side effects result from D_2 receptors blockade at the nigrostriatal system (24). A detailed understanding of specific roles of dopamine nuclei, projections, receptor subtypes, as well as their relationship with other neurotransmitters, has opened new ways of looking at the effects of antipsychotics, including the newer atypical compounds.

Notwithstanding the limitations of the in vivo models used in this study in terms of face, construct, and predictive validity (32), alstonine does present in mice an antipsychotic profile analogous to clinically useful compounds. The increased lethality induced by amphetamine in grouped mice is prevented by antipsychotics, but not other tranquilizers (e.g., barbiturates and benzodiazepines) (Fig. 2). This effect is related to the ability of antipsychotics to block D_2 receptors (2). Alstonine prevented amphetamine-induced lethality, with active dosages within the range of 0.5 and 2.0 mg/kg in a bell-shaped dose–response curve. To our knowledge, this pattern has not been reported by any typical or atypical antipsychotic effective in preventing amphetamine-induced lethality. This bell-shaped dose-effect pattern is therefore unique, and argues against a mechanism of action based on D_2 receptor blockade. The lack of interaction of alstonine with the D_2 receptor subtype in the binding studies adds to this hypothesis.

Ellenbroek (1993) (8) discusses the correlation of preventing stereotypes induced by amphetamine and apomorphine and the induction of extrapyramidal symptoms in patients. Typical and atypical antipsychotics had different effects on these models; alstonine reduced apomorphine but not amphetamine-induced stereotypy (Figs. 4 and 5). Interestingly enough, in this regard alstonine differs from the original plant extract (Elisabetsky et al., unpublished results), because this was effective against amphetamine-induced stereotypy and produced only an initial response against apomorphine-induced stereotypy. This discrepancy may be due to the fact that the extract is a complex mixture, containing a variety of other agents.

Several classes of drugs can prevent haloperidol-induced catalepsy, such as anticholinergics, D_1 and D_2 agonists, 5-HT agents, and NMDA antagonists (9,12,17). In this model clozapine, sulpiride and alstonine were active, whereas chlorpromazine was not (Fig. 5). This is an interesting result, especially if interpreted in the context of face and/or predictive validity in regard to effectiveness in schizophrenia negative symptoms (6). In accordance with most antipsychotics, alstonine exhibited sedative properties, evidenced by potentiation of barbiturate-induced sleeping time (Fig. 6). Interestingly, descriptions of the effects of plant preparation containing alstonine by traditional healers in Nigeria emphasized in regard to its potent hypnotic activity.

The lack of interaction of alstonine with D_2, 5-HT_{2A}, and D_1, (Fig. 7) usually associated with antipsychotics mechanisms of action, challenges a simplistic explanation for alstonine behavioral profile in mice. Given the similar general response of mice and rats to dopamine agents, the fact that in vitro studies were done in rats and in vivo in mice is unlikely to be the rea-

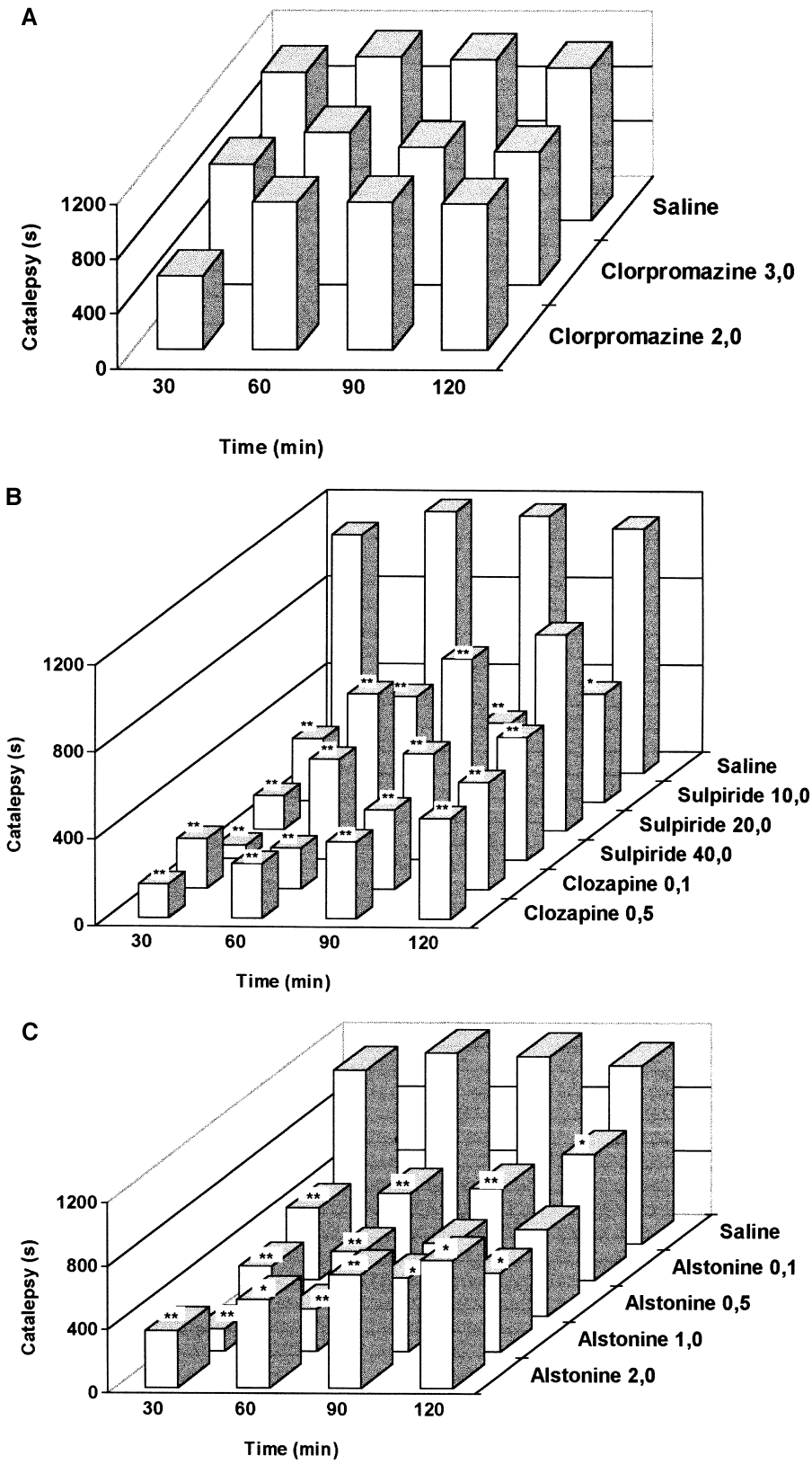

FIG. 5. (a) Effects of classical antipsychotic (chlorpromazine) and vehicle (saline) on haloperidol-induced catalepsy. Doses in mg/kg. Drugs administered IP $*p < 0.05$, $**p < 0.01$, Mann–Whitney. (b) Effects of atypical antipsychotics (clozapine and sulpiride) and vehicle (saline) on haloperidol-induced catalepsy. Doses in mg/kg. Drugs administered IP $*p < 0.05$, $**p < 0.01$, Mann–Whitney. (c) Effects of alstonine and vehicle (saline) on haloperidol-induced catalepsy. Doses in mg/kg. Drugs administered IP. $*p < 0.05$, $**p < 0.01$, Mann–Whitney.

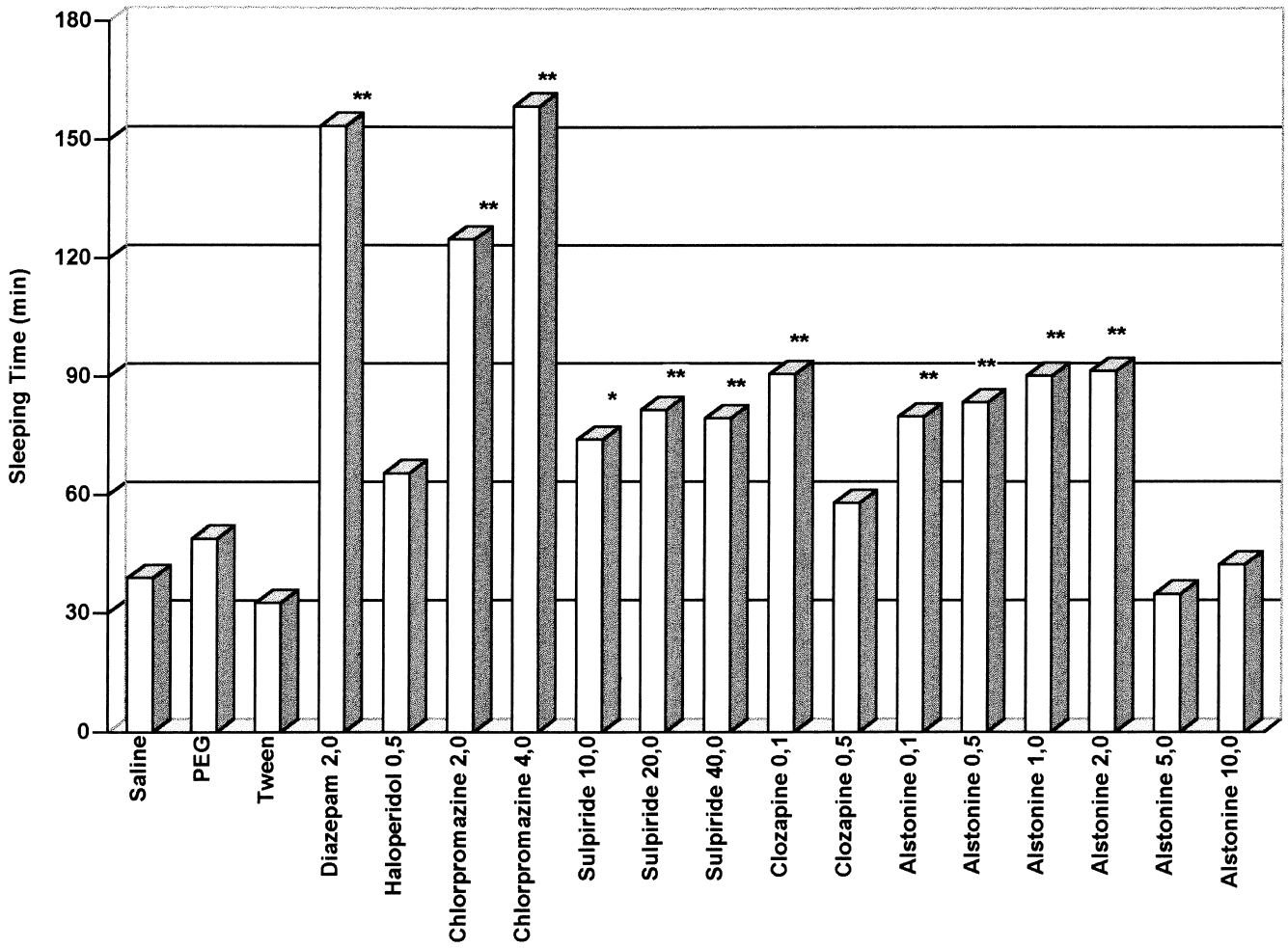

FIG. 6. Effects of classical antipsychotics (haloperidol and chlorpromazine), atypical antipsychotics (clozapine and sulpiride), reserpine, benzodiazepine (Diazepam), alstonine, and respective vehicle (saline, Tween, and PEG) on potentiation of barbiturate sleeping time. Doses in mg/kg. Drugs administered IP. $*p < 0.05$, $**p < 0.01$, Mann–Whitney.

son for these intriguing in vitro results. Interestingly, as observed with alstonine, a bell-shaped dose–response curve has been noticed with putative α_2 antagonists (e.g., idazoxan, efaroxan, and yohimbine) in behavioral (5) and microdialysis studies (30). An effect of alstonine on α_2 receptor may be of relevance because α_2 receptors modulation have been implicated as a possible explanation for the atypical profile of clozapine (15,24). The structural similarities of alstonine and yohimbine (see Fig. 1) argues in favor of testing this hypothesis.

Relevant factors to the understanding of alstonine effects include interactions with other neurotransmitters and/or receptor subtypes at different dose levels, differential brain distribution, and active metabolites. Indeed, if metabolites are the actual active compounds, the modulation on dopamine

TABLE 1

CONTROL BINDING EXPERIMENTS IN RATS WITH KNOWN LIGANDS TO D_1, D_2, AND 5-HT_{2A} RECEPTORS

Receptor	Blank	Clozapine (100 μM)	Chlorpromazine (10 μM)	Ritanserine (10 μM)
D_1	63.8 ± 8.1	3.4 ± 0.9*	—	—
D_2	41.0 ± 3.7	1.7 ± 0.5*	2.1 ± 0.7*	—
5-HT_{2A}	18.6 ± 4.0	2.1 ± 0.7*	nonspecific	0.3 ± 0.1*

Results expressed in pmol/g of protein.
$*p < 0.01$.

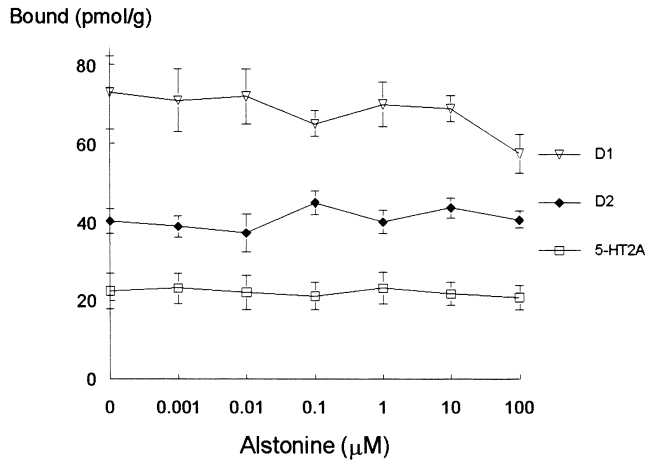

FIG. 7. Influence of alstonine on [^{3}H]SCH23390 specific binding to striatum (for D_2 receptors), [^{3}H]Spiperone specific binding to striatum for D_1 receptors) and [^{3}H]spiperone specific binding to frontal cortex (for 5-HT_{2A} receptors).

and serotonine receptors eventually brought about by alstonine could not have been detected by the binding studies here reported.

In light of the history of alstonine-containing plants used in humans for antipsychotic purposes, its behavioral profile in mice, and lack of direct interaction with receptors associated with antipsychotic actions, further experiments are warranted to clarify the unique pharmacological profile of alstonine.

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REFERENCES

1. Baldessarini, R. J.: Drugs and the treatment of psychotic disorders. In: Hardman, J. G., Gilman, A. G.; Limbird, L. E. eds., Goodman and Gilmann's The pharmacological basis of therapeutics. New York: McGraw-Hill; 1996:399–430.
2. Bourin, M.; Poisson, L.; Larousse, C.: Piracetam interaction with neuroleptics in psychopharmacological tests. Neuropsychobiology 19:93–96; 1986.
3. Bunney, B. G.; Bunney, W. E., Jr.; Carlsson, A.: Schizophrenia and glutamate, In: Bloom, F. E.; Kupfer, D. J., eds. Psychopharmacology: The fourth generation of progress. New York: Raven Press, Ltd.; 1995:1205–1214.
4. Chouinard, G.; Jones, B.; Remington, G.; Bloom, D.; Addington, D.; MacEwan, G. W.; Labelle, A.; Beauclair, L.; Arnott, W.: A Canadian multicenter placebo-controlled study of fixed doses of respiridone and haloperidol in the treatment of chronic schizophrenic patients. J. Clin. Psychopharmacol. 13:25–40; 1993.
5. Colpaert, F. C.: Effects of putative α-adrenoceptor antagonists and of other compounds on the loss of righting reflex and on exophtalmia induced by xylazine in the rat. Drug Dev. Res. 7:125–131; 1986.
6. Costall, B.; Naylor, R. J.: On catalepsy and catatonia and the predictability of the catalepsy test for neuroleptic activity. Psychopharmacology (Berlin) 34:233–241; 1974.
7. Coward, D. M.: General pharmacology of clozapine. Br. J. Psychiatry 160:1992.
8. Ellenbroek, B. A.: Treatment of schizophrenia: A clinical and preclinical evaluation of neuroleptic drugs. Pharmacol. Ther. 57:1–78; 1993.
9. Ezrin-Waters, C.; Muller, P.; Seeman, P.: Catalepsy induced by morphine and haloperidol: Effects of apomorphine and anti-cholinergic drugs. Can. J. Physiol. Pharmacol. 54:516–519; 1976.
10. Glennon, R. A.; Dukat, M.: Serotonin receptor subtypes. In: Bloom, F. E.; Kupfer, D. J., eds. Psychopharmacology: The fourth generation of progress. New York: Raven Press, Ltd.; 1995:1205–1214.
11. Holmstedt, B.; Bruhn, J. G.: Ethnopharmacology—A challenge. J. Ethnopharmacol. 8:251–256; 1983.
12. Jackson, D. M.; Westlind-Danielsson, A.: Dopamine receptors: molecular biology, biochemistry and behavioral aspects. Pharmacol. Ther. 64:291–369; 1994.
13. Kane, J. M.; Marder, S. R.: Psychopharmacologic treatment of schizophrenia. Schizophrenia Bull. 19:287–302; 1993.
14. Kane, J. M.; Lieberman, J. A.: Adverse effects of psychotropic drugs. New York: Guilford Press; 1992.
15. Litman, R. E.; Hong, W. W.; Weissman, E. M.; Su, T.; Potter, W. Z.; Pickar, D.: Idazoxan, an α_2 antagonist, augments fluphenazine in schizophrenic patients: a pilot study. J. Clin. Psychopharmacol. 13:264–267; 1993.
16. Martres, M. P.; Bouthenet, M. L.; Sales, N.; Sokoloff, P.; Schwartz, J. C.: Widespread distribution of brain dopamine receptors evidenced with ^{125}I-Sulpiride, a highly selective ligand. Science 228:752–755; 1985.
17. Meltzer, H. Y.: Atypical antipsychotic drugs. In: Bloom, F. E.; Kupfer, D. J., eds. Psychopharmacology: The fourth generation of progress. New York: Raven Press, Ltd.; 1995:1277–1286.
18. Meltzer, H. Y.; Matsubara, S.; Lee, J.: Classification of typical and atypical antipsychotic drugs on the basis of dopamine D-1, D-2 and Serotonin2 pKi values. J. Pharmacol. Exp. Ther. 251:238–246; 1989.
19. Moore, N. A.; Blackman, A.; Awere, S.; Leander, J. D.: NMDA receptor antagonists inhibit catalepsy induced by either dopamine D1 or D2 receptor antagonists. Eur. J. Pharmacol. 237:1–7; 1993.
20. Odebiyi, A. I.: Western trained nurses assessment of the different categories of traditional healers in southwestern Nigeria. Int. J. Nurse Stud. 25:333–342; 1990.
21. Oppong, A. C. K.: Healers in transition. Soc. Sci. Med. 28:917–924; 1989.
22. Paxinos, G.; Watson, C: The rat brain in stereotaxic coordinates. New York: Academic Press; 1982.
23. Pearce, T. O.: The assessment of diviners and their knowledge by civil servants in Southwestern Nigeria. Soc. Sci Med. 28:917–924; 1989.
24. Pickar, D.: Prospects for pharmacotherapy of schizophrenia. Lancet 345:557–562; 1995.
25. Seeman, P.; Lee, T.; Chau-Wong, M.; Wong, K.: Antipsychotic drug doses and neuroleptic/dopamine receptors. Nature 261:717–719; 1976.
26. Schettini, G.; Ventra, C.; Tullio, F.; Grimaldi, M.; Meucci, O.; Marino, A.: Modulation by GTP of basal and agonist-mediated striatal adenylate cyclase activity following chronic blockade of D1 and D2 dopamine receptors: involvement of G Proteins in the development of receptor supersensitivity. J. Neurochem. 59:1667–1674; 1992.

27. Shore, P. A.; Giachetti, A.: Reserpine: basic and clinical pharmacology. In: Iversen, L. L., Iversen, S. D.; Snyder, S. H. eds. Handbook of psychopharmacology, vol. 10. New York: Plenum Press: 1978:471–490.
28. Sofowora, A.: Medicinal plants and traditional medicine in Africa. Chichester, UK: John Wiley; 1982.
29. Stockmeier, C. A.; Dicarlo, J. J.; Zhang, Y.; et al.: Characterization of typical and atypical antipsychotic drugs based on in vivo occupancy serotonin-2 and dopamine-2 receptors. J. Pharmacol. Exp. Ther. 266:1374–1384; 1993.
30. Tellez, S.; Colpaert, F.; Marien, M.: The α_2-adrenoceptor antagonist, (+)-efaroxan, enhances acetylcholine release in the rat cortex in vivo. Eur. J. Pharmacol. 277:113–116; 1995.
31. Waddington, J. L.; Gamble, S. J.: Differential effect of aging on distinct features of apomorphine stereotypy in the adult rat. Neurosci. Lett. 20:95–99; 1980.
32. Willner, P.: The validity of animal models of depression. Psychopharmacology (Berlin) 83:1–16; 1984.
33. Yamamoto, I.; Kimura, T.; Tateoka, Y.; Watanabe, K.; Kang Ho, I.: Hypnotic activity of N^3-benzilthymidine on mice. Life Sci. 41:2791–2797; 1987.