Neurochemical Research, Vol. 21, No. 12, 1996, pp. 1521–1526

Effects of the Antidepressant Pirlindole* and Its Dehydro-Derivative on the Activity of Monoamine Oxidase-A and on $GABA_A$ Receptors

A. E. Medvedev,[1] V. I. Shvedov,[2] T. M. Chulkova,[1] O. A. Fedotova,[2] E. Saederup,[3] and R. F. Squires[3,4]

(Accepted June 27, 1996)

The effects of pirlindole and dehydro-pirlindole on $GABA_A$ receptors and MAO-A activity were investigated in vitro. Pirlindole was inactive as a GABA antagonist. Dehydro-pirlindole exhibited partial and selective blockade of a subset of $GABA_A$ receptors with an EC_{50} of 12μM and maximum reversal (ΔB_{opt}) of 42%. Inhibition of rat brain and human placenta MAO-A by both compounds was much more potent (with IC_{50} range 0.3–0.005 μM). Their effects on MAO-A activity were partially reversible in vitro. In contrast to pirlindole, dehydro-pirlindole may act not only as MAO-A inhibitor but also as a clozapine-like selective $GABA_A$ receptor blocker, preferentially blocking a subset of $GABA_A$ receptors that are not sensitive to DMCM or Ro 5-4864.

KEY WORDS: Pirlindole; dehydroperlindole; clozapine; antidepresant/antipsychotic; monoamine oxidase; GABA receptors.

INTRODUCTION

Pirlindole (2,3,3a,4,5,6-hexahydro-8-methyl-1-H-pyrazino [3,2,1-j,k]carbazole hydrochloride) is clinically effective in the treatment of depression as well as some forms of schizophrenia (1,2). Pirlindole is known to be a selective inhibitor of monoamine oxidase (MAO) type A (3). It also exhibits some pharmacological effects that are independent of its MAO inhibitory activity (4). After administration in vivo pirlindole may also block $GABA_A$ receptors in the brain and cause convulsions in mice (5) and rats (6) that can be blocked by diazepam. However, in vitro, pirlindole did not reverse the inhibitory effect of 1 μM GABA on [^{35}S]TBPS binding, even at a concentration of 100 μM [7]. The apparent discrepancy between in vivo and in vitro activity might be due to the biotransformation of pirlindole in vivo to metabolites with $GABA_A$ receptor blocking activity.

Recently we found that clozapine and 21 other antidepressant/antipsychotic drugs, including dehydro-pirlindole (DHP), that partially reverse the inhibitory effect of 1 μM GABA on ^{35}[S]TBPS binding, yielded additive reversals when combined pairwise with DMCM (methyl 6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate) or Ro 5-4864 (8). DMCM (9) is highly potent and selective (EC_{50} = 9 ± 4.1 nM, ΔB_{opt} = 40 ± 7.1%, n = 7). Ro 5-4864 was earlier reported to be a less potent, but partial, $GABA_A$ antagonist in this system (EC_{50} = 710 ± 130 nM, ΔB_{opt} = 49 ± 1.5%, n = 3) (10). Together, DMCM (100 nM) and Ro 5-4864 (5 μM) reverse 1 μM GABA 75 ± 4.8% n = 5. The reversing effects of both

[1] Institute of Biomedical Chemistry, Russian Academy of Medical Sciences, Moscow, Russia.
[2] Centre of Drug Chemistry—Institute of Pharmaceutical Chemistry, Moscow, Russia.
[3] Nathan Kline Institute for Psychiatric Research, Orangeburg, NY.
[4] Address reprint requests to: R. F. Squires, The N. S. Kline Institute, 140 Old Orangeburg Rd., Orangeburg, NY 10962. Tel.: (914) 359-8308; fax: (914) 365-6107.
* Pirlindole is the generic name of the drug pyrazidol.

0364-3190/96/1200-1521$09.50/0

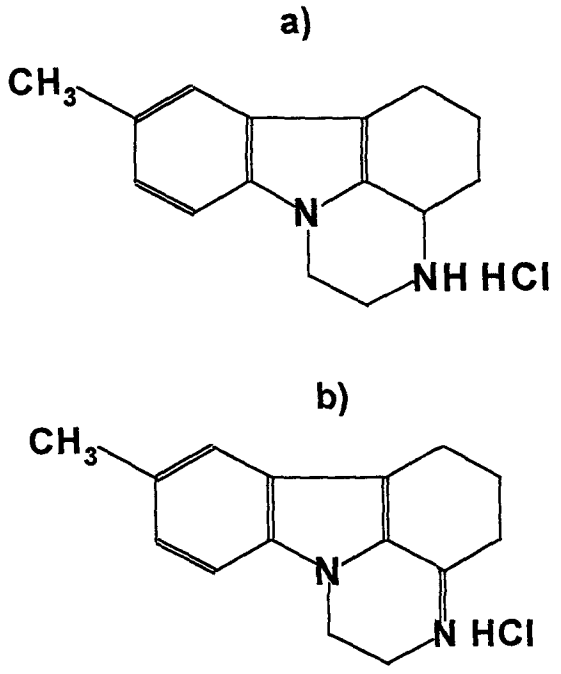

Fig. 1. Structural formulae of pirlindole (a) and dehydro-pirlindole (b)

DMCM and Ro 5-4864 can be blocked by triazolam (<200 nM) and other potent benzodiazepines (BZ), suggesting that these 2 compounds antagonize GABA indirectly by acting on two separate classes of BZ binding sites with about 10% overlap. The remaining 25% of the $GABA_A$ receptors measured by the present method (consisting mainly of high affinity $GABA_A$ receptors) are not sensitive to DMCM or Ro 5-4864 (8). Like the 20 other antidepressant and antipsychotic drugs tested (8) DHP preferentially blocks this fraction of $GABA_A$ receptors and exhibits additive reversals together with DMCM (100 nM) and Ro 5-4864 (5 μM).

It has recently been shown that another antidepressant, tetrindole (2,3,3a,4,5,6-hexahydro-8-cyclohexyl-1-H-pyrazino [3,2,1-j,k]carbazole hydrochloride), structurally related to pirlindole, inhibited MAO-A in a competitive and fully reversible manner even after prolonged 60 min incubation (11). However after administration of tetrindole in vivo, MAO-A activity was not recovered after 24 h dialysis, whereas MAO-B activity under these conditions was completely restored (11).

Some evidence suggests that in vitro MAO-A catalyses the oxidative conversion of pirlindole into its dehydro-derivative (12), which may be a more potent, slowly reversible, inhibitor of MAO-A. This might explain the persistence of MAO-A inhibition observed after pirlindole injection, not only in brain and liver homogenate, but also in mitochondria isolated after administration of the drug to rats (13,14).

In the present study we have compared the effects of pirlindole and DHP as $GABA_A$ receptor blockers as well as on the activity of MAO-A. We have also investigated the reversibility of MAO-A inhibition by these compounds.

EXPERIMENTAL PROCEDURES

Pirlindole and DHP were originally synthesized at the Centre for Drug Chemistry (Moscow, Russia) (15). ^{14}C-Labeled 5-hydroxy[side chain 2-^{14}C]tryptamine (5-HT) creatinine sulphate, 2-phenyl[1-^{14}C]ethylamine (PEA) HCL were obtained from Amersham Radiochemical Centre (Amersham, UK). [^{3}H]Pargyline and ^{35}S-TBPS were obtained from Du Pont NEN Products (Boston, MA, U.S.A.). Non-radioactive 5-HT and PEA were purchased from Sigma Chemical Co. (S. Louis, MO, U.S.A.). Other chemicals of the highest grade available were produced by Reakhim (Moscow, Russia) or Fisher Scientific (U.S.A.).

Rat brain, liver and human placenta mitochondrial fractions were isolated by conventional differential centrifugation (11,16) in 0.1 M phosphate buffer, pH 7.5. The activity of MAO-A was assayed radiometrically as described in (11). The recovery of MAO after inhibition by pirlindole and DHP was examined by mitochondria wash and by investigating [^{3}H]pargyline binding. In the first case the percent inhibition values were calculated with respect to the corresponding control values after washing. Specific binding of [^{3}H]pargyline to human placenta mitochondria was performed as described previously (17). At saturating [^{3}H]pargyline concentrations, non-specific binding determined in the presence of 2 mM non-radioactive pargyline did not exceed 10%.

The binding of [^{35}S]TBPS to EDTA/water dialysed rat forebrain membranes was performed as described previously (7,10,18,19). Non-specific [^{35}S]TBPS binding, defined as the binding obtained in the presence of 100 μM picrotoxin, did not exceed 10–15% of total binding. One μM GABA was added to suppress specific [^{35}S]TBPS binding to about 35% of control. Concentrations of DHP were chosen so that, about half of the concentrations reversed the inhibitory effect of 1 μM GABA less than 50% of maximal (optimum) reversal, the other half greater than 50% of maximum reversal. Plateaus were used to estimate ΔB_{opt} as defined previously (7,10,19). DHP was also tested together pairwise with DMCM, Ro 5-4864 and clozapine, to determine the pattern of additivities with these compounds (8).

RESULTS

In agreement with earlier results (7), pirlindole was a relatively weak inhibitor of [^{35}S]TBPS binding with an IC_{50} value of 265 ± 21 μM (n = 3) and did not reverse the inhibitory effect of 1 μM GABA on ^{35}S-TBPS binding. The concentration-response curve was rather steep with a Hill coefficient near 1.9. In contrast to pirlindole, DHP exhibited partial blockade of $GABA_A$ receptors (Fig. 2). The concentration of DHP required to achieve

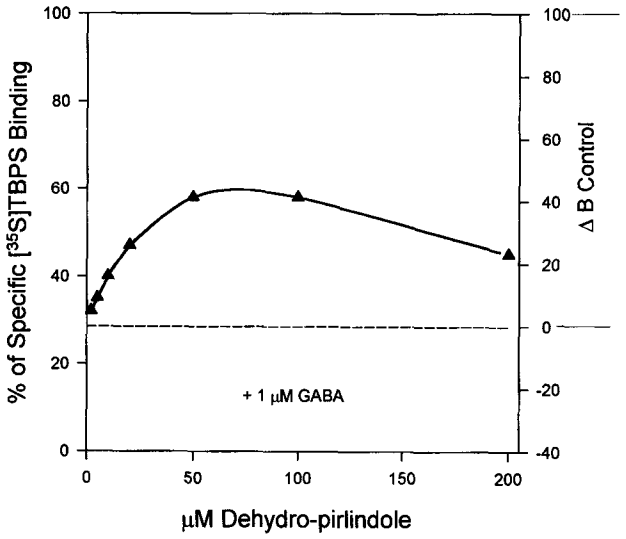

Fig. 2. Concentration-response curve for derhydro-pirlindole in reversing the inhibitory effect of 1 μM GABA on ^{35}S-TBPS binding. The dashed line represents the specific ^{35}S-TBPS binding in the presence of 1 μM GABA.

50% maximum reversal of 1 μM GABA was 12 ± 4.8 μM (EC_{50} value), with ΔB_{opt} = 42 ± 5.5% and a Hill coefficient 1.4, n = 4. Thus DHP is slightly less potent, but more selective, than clozapine (EC_{50} = 7.5 ± 0.75 μM, ΔB_{opt} = 50 ± 1.7%, n = 3) as a $GABA_A$ receptor blocker (19). When tested pairwise with DMCM (100 nM), Ro 5-4864 (5 μM) and clozapine (30 μM), DHP was found to be non-additive with clozapine but partially additive with DMCM (22%) and Ro 5-4864 (33%) (Table II). For comparison, the antidepressant and convulsant (see Metralindole = Inkasan, Merck Index, 11th Ed.), Inkasan, which is structurally related to DHP, was tested at a concentration (5 μM) that only reversed the inhibitory effect of GABA 23%, together with DMCM (100 nM) and Ro 5-4864 (5 μM). Under these conditions DMCM and Ro 5-4864 gave essentially complete additivities with Inkasan (Table II). In contrast, clozapine yielded only partial additivities with DMCM and Ro 5-4864, when tested at a high concentration giving ΔB_{opt} reversal. Tested at a lower concentration (10 μM) giving 34% reversal, clozapine was almost fully additive with DMCM and Ro 5-4864 (Table II).

Inhibition of rat brain and human placenta MAO-A by pirlindole was more potent than its effect on $GABA_A$ receptors (Fig. 3,a,b) with IC_{50} values of 0.32 ± 0.07 and 0.09 ± 0.01 μM, respectively. DHP exhibited much more potent inhibition of MAO-A from these sources with IC_{50} values 6.4 ± 0.9 and 4.5 ± 0.5 nM, respectively.

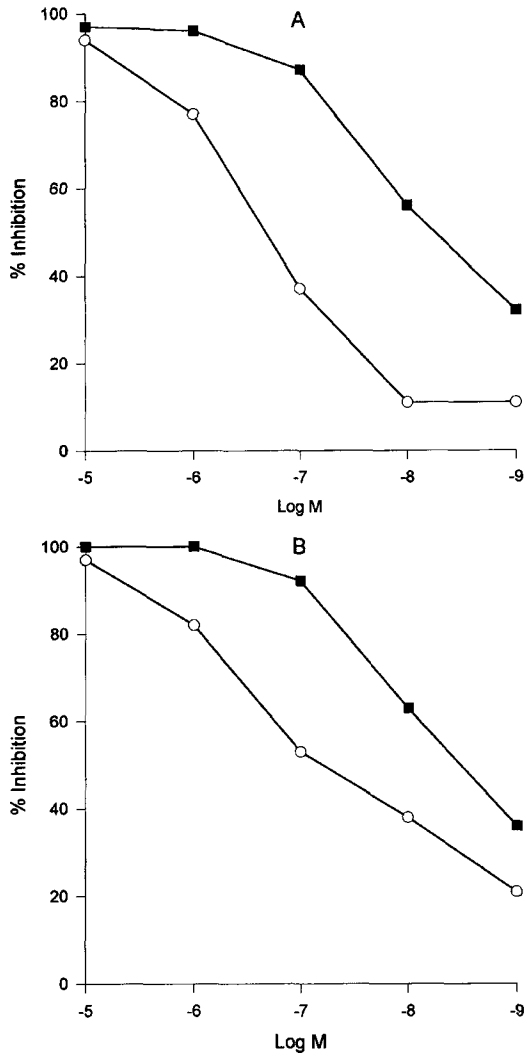

Fig. 3. Inhibition of rat brain (a) and human placenta MAO-A (b) activity by pirlindole (open symbol) and dehydro-pirlindole (closed symbol).

The reversibility of the MAO-A inhibition was investigated by two independent methods. Table I shows that 1 μM pirlindole and DHP incubated with rat liver mitochondria for 1 h at 37°C inhibited MAO-A activity by 73 and 92%, respectively. Subsequent wash of these inhibitors from the mitochondrial fraction results in partial reactivation of MAO-A by 28–29%. Under these conditions, the classical MAO inhibitors pargyline and clorgyline form covalent adducts with the flavin component of MAO (20) irreversibly inhibiting enzyme activity, which did not recover after washing the mitochondrial preparation. The reversibility of MAO-A inhibition was also studied by investigating [^{3}H]pargyline specific binding to human placenta mitochondria

Table I. Effect of MAO Inhibitors on Rat Liver Mitochondrial MAO-A Activity

Inhibitor	Concentration (μM)	Inhibition (%)	Inhibition after wash (%)	Reactivation
Pirlindole	1	69 ± 4	45 ± 3	27 ± 4*
Dehydro-pirlindole	1	92 ± 3	64 ± 3	28 ± 2**
Pargyline	10	95 ± 2	100	0
Clorgyline[a]	1	99	100	0

Rat liver mitochondria were preincubated with or without the compounds 1h at 37°C in 0.1 M phosphate buffer, pH 7.5. MAO-A activity was determined after resedimentation of the mitochondrial fraction.
*P<0.05; **P<0.02. Data are presented means of 3-4 separate experiments (± SEM) except [a] - where result of a single experiment is given.
The P-values were calculated using Student's *t*-test.

Table II. Additivities of Dehydro-Pirlindole with DMCM and Ro 5-4864 as GABA Antagonists

Drug	conc.	n	alone	n	+ 30 μM Clozapine 51 ± 6.8% n = 38	n	+ 100 nM DMCM 40 ± 5.6% n = 49	n	+ 5 μM Ro 5-4864 45 ± 5.6% n = 40
Dehydro-pirlindole	50 μM	8	37 ± 8.6	3	33 ± 5.0[a]	3	59 ± 2.9[a]	3	70 ± 12[a]
Inkasan	5 μM	3	23 ± 4.5		-	3	62 ± 4.0[a]	3	63 ± 4.6[a]
Clozapine	30 μM	38	51 ± 6.8		-	7	75 ± 5.1[a]	3	76 ± 10[a]
	10 μM	3	34 ± 3.0			3	74 ± 2.0[a]	3	75 ± 8.3[a]

GABA (1 μM) was present throughout.
All values are reversals given as percent of ΔB control (control ^{35}S-TBPS binding minus binding in the presence of 1 μM $GABA_A$ ave ± S.D.). Dehydro-pirlindole (50 μM), clozapine (30 μM), DMCM (100 nM), and Ro 5-4864 (5 μM) all produced nearly maximal reversals. Inkasan fully reverses the inhibitory effect of 1 μM GABA (ΔB_{opt} = 110 ± 3.0%, EC_{50} = 14 ± 1.5 μM, n = 3) (7). At 5 μM, Inkasan reverses 23% and is nearly fully additive with DMCM and Ro 5-4864.
DMCM (100 nM) together with Ro 5-4864 (5 μM) reversed 1 μM GABA 75 ± 4.8%, n = 5 (7).
n = not significant (p > 0.2)
[a] = p<0.002, all compared to the corresponding drug alone. All p-values were calculated using Student's *t*-test.

which contain only MAO-A. It was found that the specific binding of [^{3}H]pargyline was 20–40% higher in the presence of DHP or pirlindole, compared with the irreversible MAO-A inhibitor clorgyline (data not shown). This finding also supports the conclusion that both pirlindole and DHP interact reversibly with MAO-A, and is consistent with the finding that MAO activity returned to normal within 24 h after in vivo administration (21).

DISCUSSION

The results presented here suggest that, in contrast to pirlindole, DHP may act as a clozapine-like partial $GABA_A$ receptor blocker in vitro. The weak inhibitory effect of pirlindole on ^{35}S-TBPS binding is probably not pharmacologically relevant. It was previously shown that the incubation of pirlindole with mouse brain mitochondria, containing catalytically active MAO-A, caused a shift of its u.v. absorption spectrum, which became similar to that of standard chemically synthesized DHP. Although the product of MAO(A)-dependent redox conversion of pirlindole has not been identified, some indirect data suggest that it may be DHP (12).

DHP is a more potent inhibitor of MAO-A than pirlindole and possible irreversible inhibition might explain the persistence of MAO-A inhibition after isolation of mitochondria following pirlindole administration in vivo (12,13). However, by analogy with such MAO inhibitors as moclobemide and brofaromine (22,23), the latter phenomenon may also be due to tight (but not covalent) binding of pirlindole to MAO-A and slow dissociation.

If the MAO-dependent conversion of pirlindole into DHP actually occurs in vivo the possibility of its interaction with $GABA_A$ receptors, as suggested by its convulsant properties, would require dissociation of the MAO-A-DHP complex. Using two independent approaches we have demonstrated partial reversibility of MAO-A inhibition by pirlindole and DHP. This suggests

that DHP may interact in the brain with other biological targets including $GABA_A$ receptors. This might explain the potentiation of clonidine-induced aggression by pirlindole, as well as by incasan (24), which fully reversed the inhibitory effect of GABA on ^{35}S-TBPS binding in vitro (7) Inkasan (also spelled Incasan, Incazan, is called Metralindole in the USA, see Merck Index, 11th ed.) is structurally related to DHP and also has antidepressant, as well as convulsant, properties (30).

Perhaps the selective blockade of $GABA_A$ receptors by DHP represents a mechanism of antidepressant action in addition to MAO-A inhibition. DHP can be added to the growing list of antidepressant and antipsychotic drugs that appear to preferentially block a fraction of $GABA_A$ receptors that are not sensitive to DMCM or Ro 5-4864 (8). For a review of evidence suggesting that selective blockade of certain $GABA_A$ receptors is involved in antidepressant and antipsychotic drug action, see Refs. 7 and 19.

Although DHP is slightly less potent than clozapine, it is slightly more selective as a GABA antagonist. Regarding the mechanism of action of these drugs it should be kept in mind that high affinity for a particular site does not guarantee pharmacological or therapeutic relevance. For example, clozapine exhibits very high affinity for dopamine D_4, serotonin $5HT_{2A}$ and $5HT_{2c}$, muscarinic m_2, α adrenergic α_1 and α_2 as well as H_1 receptors (K_i values <10 nM) (25). However, at least three separate studies indicate that, in clozapine-responding psychiatric patients, blood plasma clozapine concentrations must exceed 1 μM to obtain a therapeutic effect and concentrations of 3 μM are not unusual (26,27,28). In rat, and most likely also in human, the concentration of clozapine is about 24-fold higher in brain than in blood plasma (29) making the concentrations of clozapine required to reverse GABA in our TBPS system therapeutically relevant. These findings would seem to rule out a therapeutic role for clozapine acting on any receptor, alone, for which it has a K_d value in the low nanomolar range, although it is possible that a dual action, one on a high affinity site, the other on a lower affinity $GABA_A$ receptor, is required for antipsychotic/antidepressant activity. Similarly, the concentrations of pirlindole and DHP in blood plasma and brain, required for therapeutic effects, will ultimately determine the pharmacological and therapeutic relevance of the various binding sites for pirlindole and DHP. Like clozapine, pirlindole is usually administered in doses of several hundred milligrams per day. The pharmacological activities of pirlindole and DHP were reported to be similar in experimental animals suggesting that the clinical doses of DHP required for therapeutic effects may be about the same as for pirlindole.

ACKNOWLEDGMENT

We thank Jene White for preparing the manuscript. This work was supported by the Russian State Program "Design of New Drugs" (Grant No. 040103), and New York State Office of Mental Health.

REFERENCES

1. Mashkovsky, M. D., and Andreva, N. I. 1981. Pharmacological properties of 2,3,3a,4,5,6-hyxahydro-8-methyl-1-H-pyrazino [3,2,1-j,k]carbazole hydrochloride (pirlindole) a new antidepressant. Arnzneimittelforsch Drug Res 31:75–79.
2. Mashkovsky, M. D. 1993. Drugs: Reference book for clinicians. Vol. I, pp. 110–116, Meditzina, Moscow.
3. Gorkin, V. Z. 1985. Studies on the nature and specific inhibition of monoamine oxidase, pages 9–14 *in* Neuropharmacology, Kelemen K, Magyar, K. and Vizi E. S. (eds).
4. Simon, P., Poncelet, M., and Chermat, R. 1985. Persistence of central effects of pirlindole, a short-acting monoamine oxidase inhibitor, in the presence of monoamine oxidase inhibitor. Drug Develop Res 5:323–326.
5. Martorana, P. A., Heucke, O., and Nitz, R.-E. 1979. The new antidepressant pirlindole. Antagonism of acute overdose in the mouse. Arnzneimittelforsch Drug Res 29:950–952.
6. Verstakova, O. L., Gerchikov, L. N., Chicherina, L. A., Sharova, S. A., and Liberman, S. S. 1985. Toxicological characteristics of the Sovient antidepressant pyrazidol. Farmakol. Toksikol. 48:57–60.
7. Squires, R. F., and Saederup, E. 1988. Antidepressants and metabolites that block GABA-A receptors coupled to ^{35}S-t-butylbicyclophosphothionate binding sites in rat brain. Brain Res 441: 15–22.
8. Squires, R. F., and Saederup, E., 1997. Neurochem. Res. (in press).
9. Braestrup, C., Schmiechen, R., Neef, G., Nielsen, M., and Petersen, E. N. 1982. Interaction of convulsive ligands with benzodiazepine receptors. Science 216:1241–1243.
10. Squires, R. F., and Saederup, E. 1987. $GABA_A$ receptor blockers reverse the inhibitory effect of GABA on brain specific [^{35}S]TBPS binding. Brain Res 414:357–364.
11. Medvedev, A. E., Kirkel, A. Z., Kamyhanskaya, N. S., Moskvitina, T. A., Axenova, L. N., Gorkin, V. Z., Andreva, N. I., Golovina, S. M., and Mashkovsky, M. D. 1994. Monoamine oxidase inhibition by novel antidepressant tetrindole. Biochem. Pharmacol. 47:303–308.
12. Baumanis, E. A., Birska, I. A., Reikhman, G. O., Shvedov, V. I., and Gorkin, V. Z. 1987. On the mechanism of modification by pyrazidol of the catalytic activity of mitochondrial monoamine oxidase. Probl. Med. Chem. N6:90–96.
13. Mashkovsky, M. D., Gorkin, V. Z., Veryovkina, I. V., Asnina, V. V., and Tupikina, S. M. 1981. Selective inhibition of type A monoamine oxidase by pyrazidol. Bull. Eksper. Biol. Med. N2:169–171.
14. Medvedev, A., Gorkin, V., Shvedov, V., Fedotova, O., Fedotova, I., and Semiokhina, A. 1992. Efficacy of pirlindole, a highly selective reversible inhibitor of monoamine oxidase type A, in the prevention of experimentally induced epileptic seizures. Drug Invest. 4:501–507.
15. Dvoryantseva, G. G., Pol'shakov, V. I., Sheikner, YuN, Shvedov, V. I., Grynev, A. N., Mashkovsky, M. D., Karapetyan, H. A., and Strukov, Y. T. 1985. Structure-activity relationship in pyrazino[3,2,1-j,k] carbazole derivatives. Eur. J. Med. Chem-Chem. Ther. 230:414–418.
16. Cesura, A. M., Bos, M., Galva, M. D., Imhof, R., and DaPrada, M. 1990. Characterization of the binding of [^{3}H]Ro 41-1049 to

the active site of human monoamine oxidase A. Mol. Pharmacol. 37:358–366.
17. Anderson, M., and Tipton, K. F. 1994. Estimation of monoamine oxidase concentrations in soluble and membrane-bound preparations by inhibitor binding. J. Neural Transm [Suppl] 41:47–53.
18. Squires, R. F., Casida, J. E., Richardson, M., and Saederup, E. 1983. [^{35}S]t-Butylbicyclo-phosphorothionate binds with high affinity to brain-specific sites coupled to γ-aminobutyric acid-A and ion recognition sites. Mol. Pharmacol. 23:326–336.
19. Squires, R. F., and Saederup, E. 1991. A review of evidence for GABergic predominance/glutamatergic deficit as a common etiological factor in both schizophrenia and affective psychoses: more support for a continuum hypothesis of "functional" psychosis. Neurochem. Res. 16:1099–1111.
20. Singer, T. P. 1985. Inhibitors of FAD-containing monoamine oxidase, pages 219–229, *in* B. Mondovi (ed.) Structure and Functions of Amine Oxidases CRC Press, Boca Raton, FL, 1985.
21. Verevkina I. V., Asnina V. V., Gorkin, V. Z., and Mashkovskii, M. D. 1985. Inhibition of brain monoamine oxidase in the rat by multiple pyrazidol administration. Bull. Exsper. Biol. Med. 99: 583–584.
22. Anderson, M., Waldmeier, P., and Tipton, K. F. 1991. The inhibition of monoamine oxidase by brofaromine. Biochem. Pharmacol. 41:1871–1877.
23. Keller, H. H., Kettler, R., Keller, G., and DaPrada, M. 1987. Short-acting novel MAO inhibitors: In vitro evidence for the reversibility of MAO inhibition by moclobemide and Ro 1605491. Naunyn Schmied Arch Pharmacol. 335:12–20.
24. Kuksgaus, N. E., Golovina, S. M., and Andreeva, N. I. 1991. The influence of the Soviet antidepressants pyrazidol and incazan on the effect of clonidine. Farmacol. Toksikol 54(1):11–14.
25. Bymaster, F. P., Calligaro, D. O., Falcone, J. F., Marsh, R. D., Moore, N. A., Tye, N. C., Seeman, P., and Wong, D. T. 1996. Radioreceptor binding profile of the atypical antipsychotic olanzapine. Neuropsychopharmacology 14:87–96.
26. Perry, P. J., Miller, D. D., Arndt, S. V., and Cadoret, R. J. 1991. Clozapine and Norclozapine plasma concentrations and clinical response of treatment-refractory schizophrenic patients. Am. J. Psychiat. 148:231–235.
27. Hasegawa, M., Gutierrez-Esteinou, R., Way, L., and Meltzer, H. Y. 1993. Relationship between clinical efficacy and clozapine concentrations in plasma in schizophrenia: effect of smoking. J. Clin. Psychopharmacol. 13:383–390.
28. Kronig, M. H., Munne, R. A., Szymanski, S., Safferman, A. Z., Pollack, S., Cooper, T., Kane, J. M., and Lieberman, J. A. 1995. Plasma clozapine levels and clinical response for treatment-refractory schizophrenic patients. Amer. J. Psychiat. 152:179–182.
29. Baldessarini, R. J. Centorrino, F., Flood, J. G., Volpicelli, S. A., Huston-Lyons, D., and Cohen, B. M. 1993. Tissue concentrations of clozapine and its metabolites in the rat. Neuropsychopharmacology 9:117–124.
30. Verstakova, O. L., Gerchikov, L. N., Guskova, T. A., Liberman, S. S., Chicherina, L. A., and Sharova, S. A. 1985. Toxicological study of the Soviet tetracyclic antidepressant inkasan. Farmakol. Toksikol. 48:99–103.