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MONOAMINE OXIDASE INHIBITION BY NOVEL ANTIDEPRESSANT TETRINDOLE

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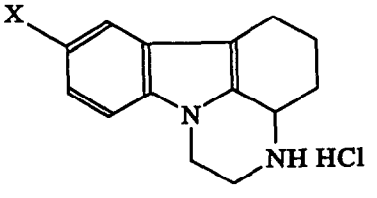
Abstract—The novel antidepressant tetrindole (2,3,3*a*,4,5,6-hexahydro-8-cyclohexyl-1*H*[3,2,1-*j*,*k*] carbazole) was found to be a selective inhibitor of monoamine oxidase A (MAO A). *In vitro* it inhibited rat brain mitochondrial MAO A in a competitive manner with K_i value of 0.4 μM. A 60 min preincubation did not change the competitive mode of interaction between enzyme and tetrindole (K_i value was 0.27 μM). The inhibition of rat brain mitochondrial MAO B was of mixed type with K_i value of 110 μM. Dilution or dialysis of mitochondrial suspension did not restore MAO A activity after inhibition by tetrindole both *in vitro* and *in vivo*, whereas inhibition of MAO B *in vitro* was completely reversible. Oral administration of tetrindole inhibited rat brain and liver mitochondrial MAO A by 80% within 0.5–1 hr and the onset of recovery of enzyme activity became evident after 24 hr. A small inhibition of MAO B (−20–30%) was observed in isolated brain and liver mitochondria within 1–6 hr and enzyme activity had completely recovered after 16 hr. The data obtained indicate that antidepressant activity of tetrindole may be explained by selective inhibition of MAO A, however an apparent discrepancy between competitive manner of MAO A inhibition *in vitro* and poor recovery of enzyme activity *in vivo* does not allow us to decide whether tetrindole is a "tight-binding" reversible inhibitor or a selective irreversible inhibitor of MAO A.

Many antidepressants employed clinically act as inhibitors of monoamine oxidase [monoamine:oxygen oxidoreductase (deaminating) (flavin-containing) EC 1.4.3.4; MAO‡) [1–4]. Modern biochemical strategy for the development of a new generation of antidepressants is based on selective and reversible inhibition of MAO type A [3, 4]. In this connection one of a prospective class of basic compounds for the development of such antidepressants might be pyrazinocarbazole. Previously it was shown that some derivatives of pyrazino [3,2,1-*j*,*k*] carbazole possess properties typical of antidepressants [5] and the most potent agent of this series, already used in clinical practice, is pirlindole (2,3,3*a*,4,5,6-hexahydro-8-methyl-1*H*-pyrazino [3,2,1-*j*,*k*] carbazole hydrochloride) (Fig. 1) [2, 6]. Pirlindole is a highly selective and reversible inhibitor of MAO A [2, 7]. The data on its inhibitory properties suggest that the effect of pirlindole on the catalytic activity of MAO A reflects a specific interaction of the drug with the active site of MAO A rather than a structural resemblance to biogenic indole amine derivatives [7].

Recently it has been shown that the novel derivative of pyrazino [3,2,1-*j*,*k*] carbazole, tetrindole (2,3,3*a*,4,5,6-hexahydro-8-cyclohexyl-1*H*-pyrazino [3,2,1-*j*,*k*] carbazole hydrochloride) (Fig. 1) shows an antidepressant activity, which exceeds that of pirlindole [8]. In this study we have investigated the inhibitory effects of tetrindole on the activity of MAO.

MATERIAL AND METHODS

Tetrindole was originally synthesized at the Centre for Drug Chemistry (Moscow, Russia) [8]. ^{14}C-Labelled 5-hydroxy[side chain 2-^{14}C]tryptamine (5-HT) creatinine sulphate, 2-phenyl [1-^{14}C]ethyl-



(a) X = CH_3 ——

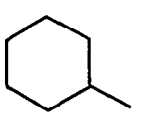
(b) X = 

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

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‡ Abbreviations: 5-HT, 5-hydroxytryptamine; IC_{50}, concentration required for 50% inhibition of enzymatic activity; MAO, monoamine oxidase; PEA, 2-phenylethylamine.

Table 1. Inhibition of rat brain and liver mitochondrial MAO by tetrindole

Organ	IC_{50} (M) MAO A	IC_{50} (M) MAO B	Ratio of IC_{50} MAO A:MAO B
Brain	4.0×10^{-7}	1.3×10^{-4}	0.0031
Liver	4.5×10^{-8}	6.3×10^{-5}	0.0007

Tetrindole was preincubated with brain and liver mitochondria at 37° for 30 min before assay of MAO activity. Data represent means of three independent experiments, the SD in all cases never exceeded 10%.

amine (PEA) HCl, [7-^{14}C]tyramine HCl were obtained from Amersham Radiochemical Centre (Amersham, U.K.). Cold 5-HT, PEA and tyramine were purchased from the Sigma Chemical Co. (St Louis, MO, U.S.A.). Other chemicals of the highest grade available were produced by Reakhim (Moscow, Russia).

Male albino rats weighing 180–200 g were used in the experiments. Tetrindole was administered in a single dose 25 mg/kg (76 μmol/kg) per os, control rats received the same volume of water. The animals were killed 0.5–36 hours after administration of tetrindole (or water). Whole brains and livers were homogenized in 0.3 M sucrose. Rat brain and liver mitochondria were prepared as described in Refs 9 and 10, respectively.

The activity of MAO was assayed radiometrically [11] with minor modifications [12] using the following substrates: 100 μM [^{14}C]5-HT (sp. act. 4 Ci/mol),

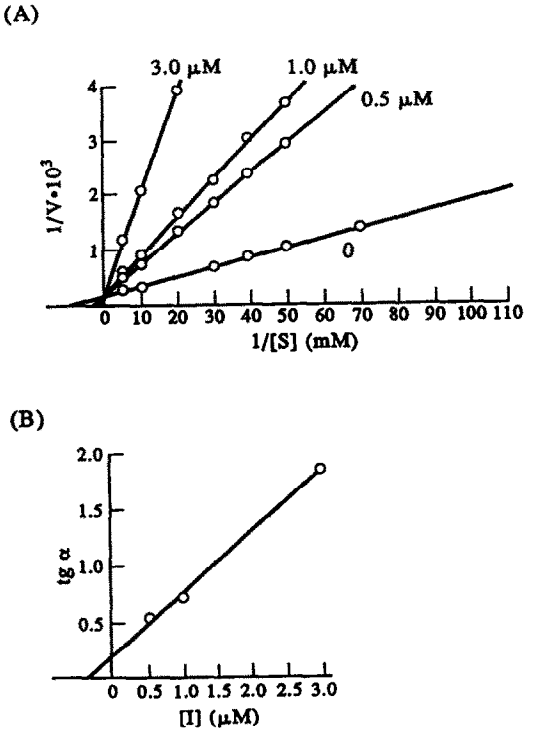

Fig. 2. Double reciprocal (A) and secondary (B) plots of MAO A inhibition by tetrindole. Activity of rat brain mitochondrial MAO A was assayed in the absence (0) and the presence of 0.5–3 μM tetrindole without preincubation of enzyme with inhibitor. (A) ordinate: 1/(initial velocity in cpm/min/mg of protein); abscissa: 1/(5-HT concentration in mM). (B) slope in arbitrary units versus inhibitor concentration (in μM). All values are means of determinations in three mitochondrial fractions.

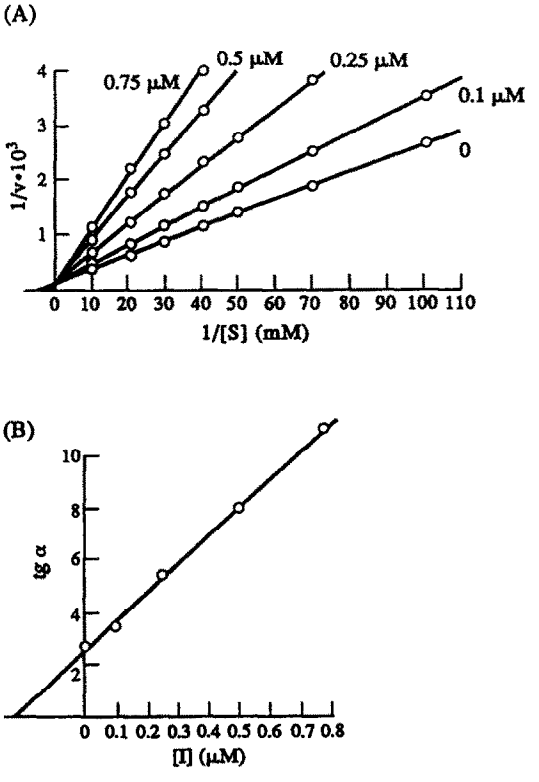

Fig. 3. Double reciprocal (A) and secondary (B) plots of MAO A inhibition by tetrindole. Activity of rat brain mitochondrial MAO A was assayed in the absence (0) and the presence of 0.1–0.75 μM tetrindole after preincubation for 60 min at 37° of enzyme with inhibitor. (A) ordinate: 1/(initial velocity in cpm/min/mg of protein); abscissa: 1/(5-HT concentration in mM). (B) slope in arbitrary units versus inhibitor concentration (in μM). All values are means of determinations in three mitochondrial fractions.

5 μM [^{14}C]PEA (sp. act. 5 Ci/mol) or 100 μM [^{14}C]-tyramine (sp. act. 4 Ci/mol). These concentrations of 5-HT and PEA are selectively deaminated by MAO A and MAO B, respectively, whereas tyramine is a common substrate for both MAO A and MAO B [13, 14].

The inhibitory effect of tetrindole on the activity of MAO A and MAO B *in vitro* was tested using the same concentration of [^{14}C]5-HT and [^{14}C]PEA, respectively. These concentrations, which are either close to or below K_m value allow competitive inhibition to be detected. IC_{50} (the concentration required for 50% inhibition of enzymatic activity) values of MAO inhibition by tetrindole were calculated from their inhibition curves, using a range of concentrations from 10^{-10} to 10^{-3} M. The reversibility of the effect of tetrindole on MAO A was tested using Ackermann–Potter's method [15]. The recovery of MAO activity after inhibition by tetrindole *in vitro* and *in vivo* was also examined by dialysis of brain mitochondria (2 mL, protein concentration 1.5 mg/mL) for 24 hr at 4° against 500-fold excess of 0.05 M phosphate buffer (pH 7.4) with change of buffer after 1, 2, 4 and 7 hr. Protein content was determined by the method of Lowry *et al.* [16] using bovine serum albumin as standard. The enzyme activity is expressed as cpm/min/mg of protein.

RESULTS

Incubation of rat brain and liver mitochondria with tetrindole for 30 min at 37° caused selective inhibition of MAO A. The IC_{50} value for inhibition of MAO A by tetrindole is three orders of magnitude less than that obtained for MAO B (Table 1). In our preliminary experiments a 30-min preincubation of brain preparations with tetrindole increased inhibition of both MAO A and MAO B, however the decrease in the ratio IC_{50} MAO A:MAO B from 0.008 (without preincubation) to 0.002 (after 30-min preincubation) suggests that time-dependent inhibition by tetrindole is more characteristic of MAO A (Medvedev *et al.*, unpublished). Double reciprocal plots of the initial velocities determined in the presence of tetrindole without preincubation showed that the drug inhibits the activity of MAO A competitively in rat brain mitochondria with K_i (slope) value of 0.4 μM (Fig. 2). Preincubation of brain mitochondria with tetrindole for 60 min at 37° had no effect upon the kinetic characteristics of MAO A inhibition (Fig. 3): in double reciprocal plots of MAO A inhibition by tetrindole, all straight lines crossed on the $1/v$ axis at the same point suggesting that the inhibition is still fully competitive, with K_i (slope) value of 0.27 μM.

In contrast to MAO A the inhibition of MAO B after 30 min preincubation of brain mitochondria with tetrindole was of mixed type (Fig. 4) with K_i (slope) of 110 μM. This is consistent with the notion that selective inhibition of MAO A by another pyrazino-carbazole-derived antidepressant pirlindole is due to specific interaction with the active site of the enzyme rather than to a structural similarity to indoleamine substrates [7].

The reversibility of MAO inhibition by tetrindole

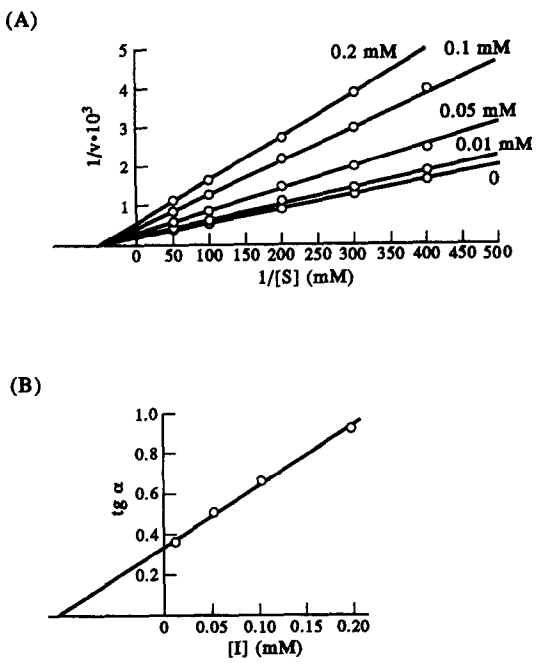

Fig. 4. Double reciprocal (A) and secondary (B) plots of MAO B inhibition by tetrindole. Activity of rat brain mitochondrial MAO B was assayed in the absence (0) and the presence of 0.01–0.2 mM tetrindole after preincubation for 30 min at 37° of enzyme with inhibitor. (A) ordinate: 1/(initial velocity in cpm/min/mg of protein); abscissa: 1/(PEA concentration in mM). (B) slope in arbitrary units versus inhibitor concentration (in mM). All values are means of determinations in three mitochondrial fractions.

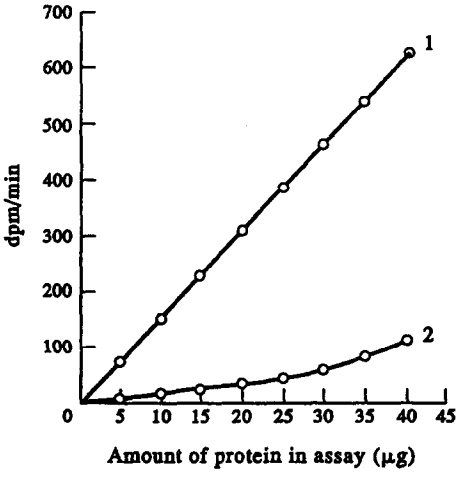

Fig. 5. Ackermann–Potter's plot of inhibition of 5-HT oxidation by tetrindole. The MAO A concentration was varied by altering the amount of rat brain mitochondria in the assay mixture. Mitochondria were preincubated with tetrindole for 30 min at 37°: 1—without tetrindole; 2—with 4×10^{-7} M tetrindole. Data represent means of determinations in three mitochondrial fractions.

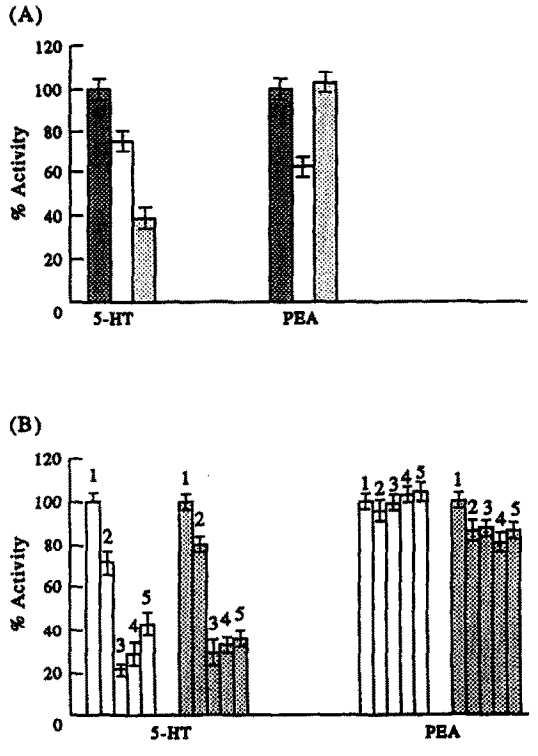

Fig. 6. Recovery of MAO activity in dialysed rat brain mitochondria after *in vitro* (A) or *in vivo* inhibition (B) by tetrindole. Vertical axis: MAO activity (% of control). (A) dark columns: initial MAO activity in mitochondria preincubated without inhibitor; open columns: MAO activity after preincubation of mitochondria for 30 min at 37° with 5×10^{-5} M (in the case of MAO B) or 4×10^{-7} M (in the case of MAO A) tetrindole; light columns: MAO activity after dialysis against 0.05 M phosphate buffer pH 7.4 for 24 hr at 4°. (B) open columns: MAO activity before dialysis; light columns: MAO activity after dialysis against 0.05 M phosphate buffer pH 7.5 for 24 hr at 4°. 1—control; time after tetrindole administration (hr): 2—0.5; 3—16; 4—24; 5—36. Data represent means ± SD of five experiments.

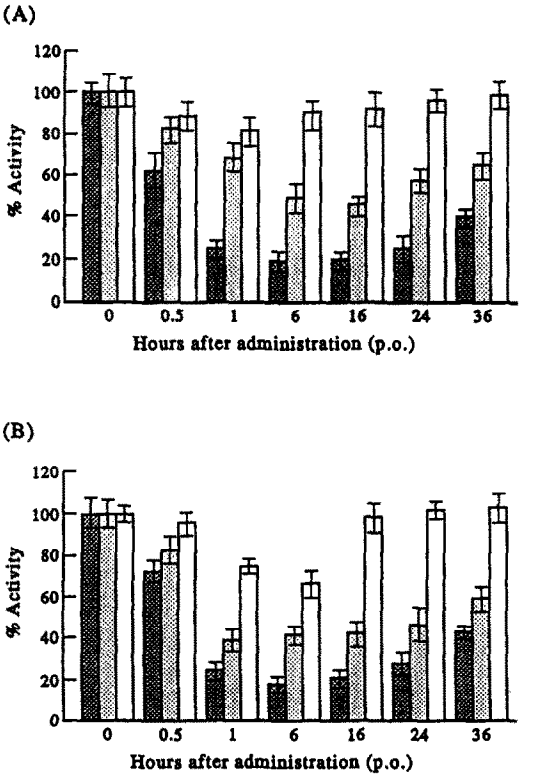

Fig. 7. Time course of MAO inhibition in brain of rats administered tetrindole [25 mg (76 μg)/kg p.o.]. The activity of MAO was assayed in homogenate (A) and isolated mitochondria (B) with 5-HT (dark columns), tyramine (light columns) or PEA (open columns) as substrate. Data are expressed as per cent of control (means ± SD). Five animals were used for each experimental point.

was examined by two methods. Figure 5 shows the degree of MAO A activity as a function of protein concentration determined after 30-min preincubation of brain mitochondria at 37° with or without tetrindole (Ackermann–Potter's plot). Only control samples incubated without the drug demonstrate straight linear dependence of initial velocity versus protein concentration. This suggests that inhibition of MAO A by tetrindole is not completely reversible. The data on dialysis experiments of brain mitochondria preincubated with tetrindole are given in Fig. 6A. Dialysis for 24 hr at 4° against a 500-fold excess of 0.05 M phosphate buffer led to complete recovery of only MAO B activity, whereas activity of MAO A was even less than after preincubation of the enzyme and the drug.

Oral administration of tetrindole to rats caused inhibition of MAO activity in brain and liver. The most potent inhibition was observed with selective MAO A substrate 5-HT. The effect of tetrindole was less prominent with tyramine, which is a common substrate for MAO A and MAO B. The deamination of MAO B substrate PEA was only slightly influenced by the drug. These data, which are in agreement with our *in vitro* experiments, suggest that tetrindole administration to animals selectively inhibits MAO A.

The time course of MAO inhibition in brain and liver is shown in Figs 7 and 8, respectively. Tetrindole produced marked inhibition of MAO A (substrate: 5-HT) already 30 min after administration. The maximum inhibition, roughly 80%, was observed within 1 hr and the onset of recovery of enzyme activity became evident only after 24 hr, but even after 36 hr the activity of brain and liver MAO A was still only about 50% of initial activity. The inhibition of MAO A was the same for the enzyme activity measured in brain and liver homogenate and isolated mitochondria.

The MAO B activity (substrate PEA) was less sensitive to tetrindole: maximum inhibition 20–30% was observed in brain and liver within 1–6 hr after

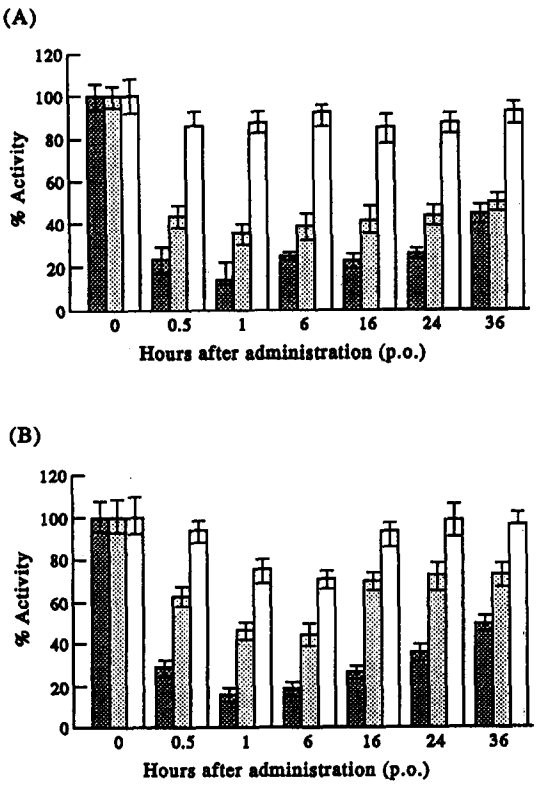

Fig. 8. Time course of MAO inhibition in liver of rats administered tetrindole [25 mg (76 μM)/kg p.o.]. The activity of MAO was assayed in homogenate (A) and isolated mitochondria (B) with 5-HT (dark columns), tyramine (light columns) or PEA (open columns) as substrate. Data are expressed as per cent of control (means ± SD). Five animals were used for each experimental point.

its administration mainly in isolated mitochondria and to a lesser extent in homogenates. The activity of MAO B had recovered completely by 16 hr.

Dialysis of brain mitochondria obtained from tetrindole-treated animals for 24 hr at 4° had not recovered MAO A activity (Fig. 6B) and even slightly decreased MAO B activity, which was indistinguishable from the control before dialysis.

DISCUSSION

The results obtained in this study demonstrate that the novel antidepressant tetrindole behaves as a specific inhibitor of MAO A. This conclusion is mainly supported by the weak inhibitory effect of tetrindole on PEA deamination in brain and liver mitochondria compared to high inhibition of 5-HT deamination both *in vitro* and *ex vivo* after p.o. administration. *In vitro* it acts as a pure competitive inhibitor of MAO A even after 60-min preincubation with brain mitochondria (Figs 2 and 3). However, data on reversibility tests of inhibition *in vitro* and after *in vivo* administration indicate that activity of MAO A had not recovered by dilution or dialysis procedures, whereas inhibition of MAO B was completely reversible. *Ex vivo* inhibition of MAO A was not reversed even 0.5–1 hr after tetrindole administration, whereas *in vitro* experiments 1-hr incubation between enzyme and the drug still demonstrated competitive inhibition of MAO A by tetrindole.

Time-course experiments of MAO inhibition *in vivo* show that recovery of MAO A activity was not complete 36 hr after tetrindole administration. For comparison activity of brain and liver MAO A of rats treated with the modern reversible inhibitors moclobemide and brofaromine had recovered almost completely within 16–24 hr whereas enzyme activity even 48 hr after administration of irreversible MAO inhibitor phenelzine corresponded to 20–35% of control [17].

Thus there is an apparent discrepancy between the competitive mode of MAO A inhibition by tetrindole *in vitro* (even after 60-min preincubation) and the poor recovery of enzyme activity after inhibition *in vitro* and *in vivo*.

Previously it was shown that the inhibition of MAO A by the reversible inhibitor brofaromine was time dependent and reactivation of MAO A pretreated with brofaromine occurred gradually [18]. The recovery of MAO A activity in dialysed homogenates of brain from brofaromine-treated rats was statistically significant, although low, even when samples were dialysed at 37°; in these conditions MAO A activity of brain from moclobemide-treated rats had recovered by 90% [19].

The low IC_{50} value for the inhibition of MAO A by tetrindole and its competitive mode of action could be considered as evidence for characterizing tetrindole as a "tight-binding"-type of inhibitor [18]. However, at the present time we cannot exclude the possibility that *in vivo* it is converted into a more potent (possibly irreversible) inhibitor.

In conclusion, the present study demonstrated that antidepressant activity of tetrindole described recently [8] may be related to the selective inhibition of brain MAO A. The time-course and the potency of tetrindole action is comparable with such modern antidepressants as moclobemide and brofaromine [17]. At the present time the nature of enzyme inhibition is not completely understood and additional experiments are required for elucidation of mechanism(s) of inhibition and recovery of MAO A activity.

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