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COMPARISON OF THE EFFECTS OF 6-CHLORO-2-AMINOTETRALIN AND OF Org 6582, A RELATED CHLOROAMPHETAMINE ANALOG, ON BRAIN SEROTONIN METABOLISM IN RATS

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Abstract—Org 6582 and 6-chloro-2-aminotetralin can be viewed as *p*-chloroamphetamine analogs having rigid conformation. Because *p*-chloroamphetamine exerts multiple actions on brain serotonin neurons, we compared the effect of these rigid analogs on certain parameters of brain serotonin metabolism in rats to determine the extent of dissociation of these multiple actions. 6-Chloro-2-aminotetralin resembled *p*-chloroamphetamine in lowering brain levels of tryptophan hydroxylase, serotonin and 5-hydroxyindoleacetic acid (5-HIAA) in rat brain, though its effects were less than those of *p*-chloroamphetamine and were short-lasting in contrast to the permanent neurotoxic effects of *p*-chloroamphetamine. Org 6582 did not lower tryptophan hydroxylase or serotonin levels in rat brain; its lowering of 5-HIAA levels can be attributed to its inhibition of serotonin re-uptake. *In vitro*, Org 6582 was a slightly stronger inhibitor of serotonin uptake than was 6-chloro-2-aminotetralin but a distinctly weaker inhibitor of monoamine oxidase. Inhibition of uptake into serotonin neurons *in vivo* was evaluated by measuring the ability of 6-chloro-2-aminotetralin and of Org 6582 to antagonize the neurotoxic effects (lowering of serotonin levels or lowering of serotonin uptake) produced by *p*-chloroamphetamine. In these experiments, Org 6582 but not 6-chloro-2-aminotetralin was a potent inhibitor of uptake into serotonin neurons *in vivo*, in agreement with the findings of Goodlet *et al.* [I. Goodlet, S. E. Mireylees and M. F. Sugrue, *Br. J. Pharmac.* **56**, 367P (1976)] and of Sugrue *et al.* [M. F. Sugrue, I. Goodlet and S. E. Mireylees, *Eur. J. Pharmac.* **40**, 121 (1976)] on Org 6582.

p-Chloroamphetamine and related halogenated amphetamines have been known to affect brain serotonin metabolism since the initial studies of Pletscher *et al.* [1, 2] on *p*-chloromethamphetamine and Fuller *et al.* [3, 4] on *p*-chloroamphetamine. Studies of these and similar compounds led to the recognition of multiple effects of *p*-chloroamphetamine on brain serotonin neurons (inhibition of tryptophan hydroxylation, inhibition of serotonin re-uptake, release of serotonin from storage granules, inhibition of monoamine oxidase attack on serotonin, and toxic destruction of serotonin neurons) and to the suggestion that these multiple actions are potentially dissociable [5]. In previous structure–activity studies with chlorinated amphetamine derivatives, we had found that 6-chloro-2-aminotetralin (an analog of *p*-chloroamphetamine having a rigid conformation) lowered brain serotonin levels slightly and 5-hydroxyindoleacetic acid (5-HIAA) to a greater extent in rats [6]. 6-Chloro-2-aminotetralin was less effective than *p*-chloroamphetamine in lowering brain levels of these 5-hydroxyindoles and produced only transient effects in contrast to the long-lasting apparently neurotoxic action of *p*-chloroamphetamine. Recently Goodlet *et al* [7] and Sugrue *et al.* [8] have described a compound (*dl*-8-chloro-11-anti-amino-benzo(b)-bicyclo[3.3.1]nona-3,6a(10a) diene hydrochloride) referred to as Org 6582, which they found to be a potent and specific inhibitor of serotonin uptake. Org 6582 contains the *p*-chloroamphetamine nucleus as does 6-chloro-2-aminotetralin and can be viewed as 6-chloro-2-aminotetralin having a third ring structure incorporated into the molecule (Fig. 1). We were therefore interested in comparing Org 6582 and 6-chloro-2-aminotetralin in regard to their effects on 5-hydroxyindole metabolism.

METHODS

6-Chloro-2-aminotetralin hydrochloride was synthesized in the Lilly Research Laboratories by Dr. Bryan B. Molloy and his associates. *p*-Chloroamphetamine hydrochloride was purchased from the Regis Chemical Co. Org 6582 was kindly supplied by Organon Laboratories Ltd. through the courtesy of Dr. M. F. Sugrue. All drugs were racemic mixtures.

Male Wistar rats from Harlan Industries, Cumberland, IN. weighed about 150 g at the time of use. The rats were housed in hanging wire cages in a controlled-temperature room with 12 hr light:dark cycles and were given food and water *ad lib.* Drugs were given by intraperitoneal injection in aqueous solutions. Rats were killed by decapitation, and whole brains were quickly excised and frozen on dry ice, then stored frozen prior to analysis. Serotonin and 5-HIAA levels were determined spectrofluorometrically [9]. Serotonin uptake into synaptosomes was measured *in vitro* by a previously described procedure using [^{14}C]serotonin [10]. Rat brain mitochondrial monoamine oxidase was assayed with [^{14}C]serotonin as substrate as described previously [11]. Tryptophan

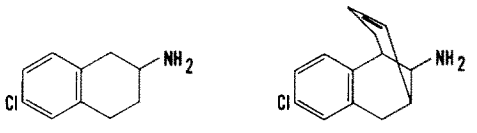

Fig. 1. Structures of 6-chloro-2-aminotetralin and Org 6582.

hydroxylase was assayed by a spectrofluorometric method [12].

RESULTS

Effect on monoamine oxidase in vitro. Figure 2 compares the effects *in vitro* of 6-chloro-2-aminotetralin and Org 6582 on mitochondrial monoamine oxidase from rat brain, with serotonin as the substrate. 6-Chloro-2-aminotetralin had about the same activity as a monoamine oxidase inhibitor as we had reported earlier for *p*-chloroamphetamine [11], whereas Org 6582 was only about one-thirtieth as active.

Effect on tryptophan hydroxylase and serotonin levels. Table 1 shows the effect of 6-chloro-2-aminotetralin and Org 6582 on levels of tryptophan hydroxylase, serotonin and 5-HIAA in rat brain. *p*-Chloroamphetamine was included in this experiment for comparison. 6-Chloro-2-aminotetralin, like *p*-chloro-amphetamine, lowered tryptophan hydroxylase and serotonin levels as reported earlier [6, 13]. In contrast, Org 6582 had no significant effect on tryptophan hydroxylase or serotonin levels at this time interval. Sugrue *et al.* [8] have likewise observed no lowering of brain serotonin by Org 6582 at other time points. The effects of 6-chloro-2-aminotetralin were less than those of *p*-chloroamphetamine; in this experiment the lowering of serotonin levels by 6-chloro-2-aminotetralin was not statistically significant due to variation in the control group, but other experiments have established the ability of this compound to lower brain serotonin (see Table 4, [6]). The levels of 5-HIAA were significantly reduced by all three compounds. In the case of Org 6582, this effect may have been due entirely to inhibition of serotonin re-uptake [14] and was less than the effects of the other two compounds, whose inhibition of 5-hydroxyindole synthesis and of monoamine oxidase probably contributed to their lowering of 5-HIAA levels.

Inhibition of serotonin uptake. The inhibition of serotonin uptake *in vitro* by rat brain synaptosomes was inhibited by Org 6582 slightly more effectively than by 6-chloro-2-amino-tetralin. The IC_{50} value estimated by interpolation from data at three concentrations of each inhibitor was 2.3×10^{-6} M for Org 6582 and 3.6×10^{-6} M for 6-chloro-2-aminotetralin. The inhibition of uptake into serotonin neurons *in vivo* was evaluated by antagonism of the neurotoxic effects of *p*-chloroamphetamine, which requires active uptake in order to destroy serotonin neurons [15, 16]. Table 2 shows the results obtained when *p*-chloroamphetamine neurotoxicity was evaluated by measuring serotonin uptake *in vitro* 72 hr after *p*-chloroamphetamine injection. Pretreatment with Org 6582 significantly antagonized *p*-chloroamphetamine's action, but 6-chloro-2-aminotetralin did not have a significant effect. Table 3 shows the results obtained when *p*-chloroamphetamine neurotoxicity was evaluated by measuring serotonin levels in brain. 6-Chloro-2-aminotetralin at doses of 10, 20 or 40 mg/kg did not

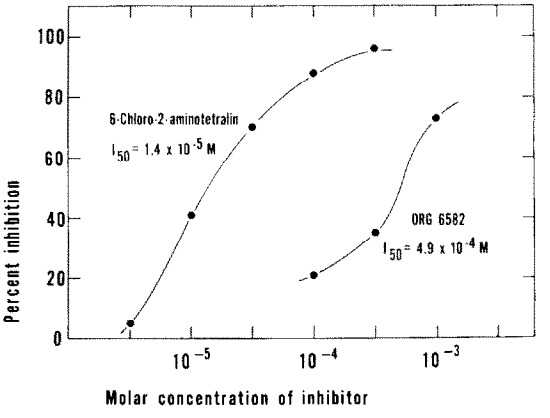

Fig. 2. Inhibition of monoamine oxidase *in vitro*. [^{14}C]-Serotonin was substrate at a 100 μM concentration. The concentration producing 50 per cent inhibition (I_{50}) was estimated by interpolation.

Table 1. Effect of *p*-chloroamphetamine, 6-chloro-2-aminotetralin and Org 6582 on tryptophan hydroxylase, serotonin and 5-hydroxyindoleacetic acid levels in rat brain*

Treatment group	Tryptophan hydroxylase (μg/g/hr)	Serotonin (μg/g)	5-HIAA (μg/g)
Saline	20.3 ± 0.6†	0.57 ± 0.03†	0.50 ± 0.03†
p-Chloroamphetamine	12.6 ± 0.5‡ (62%)	0.30 ± 0.002‡ (53%)	0.30 ± 0.01‡ (60%)
6-Chloro-2-aminotetralin	15.3 ± 0.6‡ (75%)	0.50 ± 0.01† (88%)	0.30 ± 0.01‡ (60%)
Org 6582	20.8 ± 1.2† (103%)	0.55 ± 0.02† (96%)	0.36 ± 0.01‡ (72%)

* Test compounds were injected i.p. in aqueous solution at a dose of 0.1 m-mole/kg 6 hr before the rats were killed. Mean values ± standard errors for five rats per group are shown.

† Did not differ from the control group at the P = 0.05 level of significance.

‡ Significant differences from the saline-treated control group (P < 0.001).

Table 2. Antagonism of the *p*-chloroamphetamine-induced neurotoxic decrease in serotonin uptake capacity by 6-chloro-2-aminotetralin and Org 6582 in rats*

Treatment group	Serotonin uptake (pmoles/mg protein)
Control	7.10 ± 0.34
p-Chloroamphetamine alone	3.51 ± 0.43†
p-Chloroamphetamine after 6-chloro-2-aminotetralin	4.54 ± 0.47†
p-Chloroamphetamine after Org 6582	6.30 ± 0.14‡

* 6-Chloro-2-aminotetralin hydrochloride and Org 6582 were injected i.p. at 10 mg/kg 30 min before *p*-chloroamphetamine hydrochloride (10 mg/kg, i.p.). Rats were killed 72 hr after *p*-chloroamphetamine injection. Synaptosomes from cerebral cortex were prepared for measurement of serotonin uptake. Mean values ± standard errors for five rats per group are shown.

† Significantly different from control group, $P < 0.05$.

‡ Significantly different from group treated with *p*-chloroamphetamine alone, $P < 0.001$.

protect against *p*-chloroamphetamine neurotoxicity. In contrast, Org 6582 at doses of 5, 10 and 20 mg/kg caused a dose-dependent antagonism of the *p*-chloroamphetamine effects. We had chosen the 72-hr time interval after *p*-chloroamphetamine so that the slight and short-lasting effects of 6-chloro-2-aminotetralin on serotonin levels [6] would not complicate the interpretation. The results of these experiments done at 72 hr (Table 2 and 3) indicated that 6-chloro-2-aminotetralin did not protect against *p*-chloroamphetamine neurotoxicity. We know from earlier work [16, 17] that *p*-chloroamphetamine requires continual re-uptake over a prolonged period in order to cause permanent destruction of serotonin neurons, so we thought that the absence of any effect *in vivo* indicating uptake inhibition of 6-chloro-2-aminotetralin might have been due to the long (72 hr) time interval. Since 6-chloro-2-aminotetralin was almost as effective in inhibiting serotonin uptake *in vitro* as Org 6582, we had expected some evidence for uptake inhibition *in vivo*. For these reasons we did another experiment in which we looked for antagonism of the short-term (6 hr) depletion of brain serotonin by *p*-chloroamphetamine (Table 4). At this 6 hr time interval, 6-chloro-2-aminotetralin itself caused slight depletion of serotonin, yet it significantly antagonized the effects of *p*-chloroamphetamine. Org 6582, which alone did not alter serotonin levels, completely blocked the effects of *p*-chloroamphetamine. These results suggest that 6-chloro-2-aminotetralin does inhibit serotonin uptake *in vivo* but that its effect is not sufficiently great or of sufficient duration to prevent *p*-chloroamphetamine neurotoxicity. In contrast, Org 6582 effectively prevents both the short-term and long-term effects of *p*-chloroamphetamine.

Behavioral effect. A comparison of the central nervous system (CNS) stimulatory effects of 6-chloro-2-aminotetralin, Org 6582 and *p*-chloroamphetamine is shown in Table 5. *p*-Chloroamphetamine caused pronounced effects on all of the parameters measured, whereas 6-chloro-aminotetralin had somewhat less stimulatory action. The effects of Org 6582 were minimal.

DISCUSSION

p-Chloroamphetamine inhibits serotonin uptake [10, 18], lowers tryptophan hydroxylase [19] and 5-hydroxyindole levels [4], inhibits monoamine oxi-

Table 3. Antagonism of the *p*-chloroamphetamine-induced neurotoxic depletion of brain 5-hydroxyindole levels by 6-chloro-2-aminotetralin and Org 6582 in rats*

Treatment group	Serotonin (μg/g)	5-HIAA (μg/g)
Saline-treated control	0.63 ± 0.02†	0.43 ± 0.02†
p-Chloroamphetamine alone	0.27 ± 0.02‡	0.18 ± 0.01‡
p-Chloroamphetane after 6-chloro-2-aminotetralin		
10 mg/kg	0.26 ± 0.01‡	0.18 ± 0.01‡
20 mg/kg	0.29 ± 0.01‡	0.21 ± 0.01‡
40 mg/kg	0.27 ± 0.01‡	0.20 ± 0.01‡
6-Chloro-2-aminotetralin alone, 40 mg/kg	0.53 ± 0.01†,‡	0.38 ± 0.01†
p-Chloroamphetamine after Org 6582		
5 mg/kg	0.43 ± 0.02†,‡	0.29 ± 0.02†,‡
10 mg/kg	0.53 ± 0.02†,‡	0.30 ± 0.01†,‡
20 mg/kg	0.59 ± 0.02†	0.39 ± 0.01†
Org 6582 alone 20 mg/kg	0.59 ± 0.02†	0.42 ± 0.03†

* 6-Chloro-2-aminotetralin hydrochloride and Org 6582 were injected i.p. at the doses indicated 73 hr before the rats were killed. *p*-Chloroamphetamine hydrochloride was injected i.p. at 0.1 m-mole/kg (20.6 mg/kg) 72 hr before the rats were killed. Mean values ± standard errors for five rats per group are shown.

† Values differ significantly ($P < 0.005$) from the group treated with *p*-chloroamphetamine alone.

‡ Values differ significantly ($P < 0.005$) from the saline-treated control group.

Table 4. Antagonism by 6-chloro-2-aminotetralin and Org 6582 of the initial depletion of brain serotonin after *p*-chloroamphetamine administration to rats*

Cotreatment	Serotonin (μg/g)	
	Saline-treated	*p*-Chloroamphetamine-treated
None	0.62 ± 0.02	0.25 ± 0.01†
6-Chloro-2-aminotetralin	0.48 ± 0.01‡	0.34 ± 0.02†,§
Org 6582	0.57 ± 0.04	0.63 ± 0.02§

* 6-Chloro-2-aminotetralin hydrochloride and Org 6582 were injected i.p. along with saline or *p*-chloroamphetamine hydrochloride. All drugs were injected at 0.1 m-mole/kg. Rats were killed 6 hr later. Mean values ± standard errors for five rats per group are shown.

† Significant difference from group with same cotreatment but without *p*-chloroamphetamine, $P < 0.01$.

‡ Significant difference from corresponding control group without cotreatment, $P < 0.01$.

§ Significant difference from *p*-chloroamphetamine group without cotreatment, $P < 0.01$.

Table 5. Behavior of rats treated with *p*-chloroamphetamine, 6-chloro-2-aminotetralin or Org 6582*

Behavioral effect	Score			
	Saline	*p*-Chloro-amphetamine	6-Chloro-2-aminotetralin	Org 6582
Exophthalmos	0/0	5/5	5/3	0/0
Piloerection	0/0	5/10	5/8	5/5
Locomotion	0/0	5/10	5/4.5	3/1.5
Sniffing	0/0	5/5	4/3.5	0/0
Searching	0/0	5/15	5/7.5	1/0.5
Alertness	0/0	5/5	5/5	1/0.5
Mean total score per rat	0	10.4	6.4	1.3

* Compounds were injected i.p. at 0.1 m-mole/kg 30 min before behavior was scored in groups of five rats. Results are expressed as number of rats affected/total score for each group. For exophthalmos and alertness, the maximum group score possible was 5; for the other parameters, the maximum group score possible was 15.

dase [11], causes CNS stimulation [4, 20] and apparently causes neurotoxic degeneration of serotonin neurons in rats [21–23]. The rigid conformational analog of *p*-chloroamphetamine, 6-chloro-2-aminotetralin, retains the ability to inhibit serotonin uptake and monoamine oxidase *in vitro* and some of the ability to lower tryptophan hydroxylase and 5-hydroxyindole levels as well as to cause CNS stimulation *in vivo*, though it does not cause long-lasting effects attributable to neurotoxic degeneration of serotonin neurons. All of the effects of *p*-chloroamphetamine except uptake inhibition are virtually eliminated in Org 6582, which has a further restricted conformation by virtue of another ring structure. Org 6582 did not inhibit monoamine oxidase *in vitro*, did not alter tryptophan hydroxylase or serotonin levels *in vivo*, and had minimal CNS stimulatory effects. On the other hand, Org 6582 was a potent inhibitor of serotonin uptake *in vitro* and antagonized both the short-term and long-term effects of *p*-chloroamphetamine on serotonin neurons *in vivo*; this latter action of Org 6582 presumably illustrates its ability to inhibit uptake into serotonin neurons, though other mechanisms such as interference with *p*-chloroamphetamine metabolism cannot be proven to be unimportant. Thus, Org 6582 is a potent inhibitor of uptake into serotonin neurons *in vivo*, as reported earlier by Goodlet *et al.* [7] and Sugrue *et al.* [8], without directly affecting other parameters of serotonin neuronal function.

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