Pharmacology Biochemistry & Behavior, Vol. 13, pp. 397–401. Printed in the U.S.A.

Effects of Thioridazine on Apomorphine-Elicited Stereotypic Behavior and Motor Activity

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Received 4 January 1980

LAI, H., M. A. CARINO, R. SPERRY AND A. HORITA. *Effects of thioridazine on apomorphine-elicited stereotypic behavior and motor activity*. PHARMAC. BIOCHEM. BEHAV. 13(3) 397–401, 1980.—Bilaterally injected thioridazine (10 μg) into the striata of rats augmented the stereotypic behavior elicited by apomorphine. The enhancing effect was attenuated by pretreatment with α-methyl-p-tyrosine. At 48 hr postinjection of thioridazine (1.0 mg/kg, IP), motor suppression from a low dose of apomorphine (0.2 mg/kg, IP) was enhanced; however, motor response to a high dose of apomorphine (1 mg/kg, SC) was not affected. Possible mechanisms of action of thioridazine are discussed.

Thioridazine Striatum Apomorphine Stereotypic behavior Motor activity

THIORIDAZINE has been used in the therapeutic treatment of schizophrenia. Its potency in suppressing psychotic symptoms and improving motor behavior, affect, and speech has been reported to be similar to that of chlorpromazine. Extrapyramidal side-effects, such as Parkinsonism and induction of tardive dyskinesia, are significantly less than those produced by other neuroleptics, such as chlorpromazine and haloperidol [10,27]. Many reported findings suggest that thioridazine may exert its neuroleptic effects by blocking dopamine receptors in the brain. Like other dopamine antagonists, it increases accumulation of HVA in the striatum and the limbic system [3, 22, 29]. It can also displace ^{3}H-haloperidol from binding sites on calf caudate tissue [7] and inhibit dopamine-sensitive adenylate cyclase [14,19]. Furthermore, chronic treatment with thioridazine enhances apomorphine-induced stereotypic behavior in rats, apparently owing to the development of supersensitivity in the dopaminergic system [25].

In this paper we report (1) the effects of direct microinjection of thioridazine into the striata of rats on apomorphine-elicited stereotypic behavior; and (2) the effects of thioridazine on hypoactivity and hyperactivity elicited by a low and a high dosage of apomorphine, respectively.

METHOD

Male Sprague-Dawley rats (250–300 g) obtained from Tylers Lab, WA, were used in these experiments. They were housed in a temperature-controlled vivarium maintained under 12-hr light-dark cycle and provided with food and water ad lib. All experiments were run between 10 a.m. and 4 p.m.

Microinjection of Drugs into the Brain

Rats were anesthetized with sodium pentobarbital (30–40 mg/kg, IP). Guide cannulae (23 ga) were then stereotaxically implanted bilaterally in the skull with the tip 1 mm below the surface of the cortex. Five to seven days after implantation to allow recovery from surgery, thioridazine HCl (Sandoz) dissolved in physiological saline was injected with a 30 ga cannula into the striatum (2 μl) at the rate of 2 μl/min. The injection cannula was held in place for an additional 30 sec in order to minimize backflow of the drug solution. The coordinates of injection were A-P 7.4 mm; L ± 2.0 mm and DV + 2.0 mm for the striatum [11]. In some experiments, animals were pretreated with α-methyl-p-tyrosine methyl ester (α-MT, 250 mg base/kg, IP) 2 hr before the intracerebral injection. Control animals were injected with physiological saline. Five minutes after intrastriatal injection of thioridazine, apomorphine (1 mg/kg, solution contained 1 mg/ml of L-ascorbic acid) was injected subcutaneously in the flank of the animal. The animal was then put in a sound-proof box and behavior was observed through a one-way mirror starting 5 min postinjection of apomorphine. Stereotypic behavior was scored by an experienced experimenter unaware of the treatment of the animals at 0, 5, 30, 45 and 60 min. The scoring system modelled after Iversen's [13] was as follows: 0—asleep; 1—awake, but largely immobile; 2—moving with short bursts of sniffing; 3—moving over the area of the cage with continuous sniffing and rearing; 4—some/no movement and continuous sniffing with head directed down; 5—as 4, but licking, biting and gnawing.

Stereotypic behavior for each animal is reported as the sum of the scores of the five observations.

Effects of Thioridazine on Apomorphine-Elicited Motor Activity

Rats were injected with either thioridazine (1.0 mg/kg, IP) or an equal volume of physiological saline. At 10 min or 48 hr

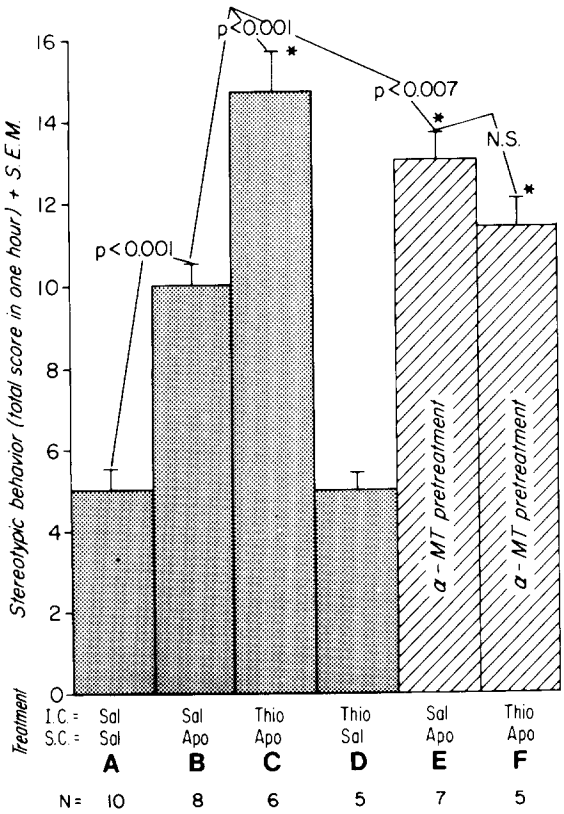

FIG. 1. Stereotypic behavior in different drug-treatment conditions. SC=Subcutaneous injection; IC=Intracerebral injection. *Significantly different from control (IC-saline; SC-saline) at $p<0.01$.

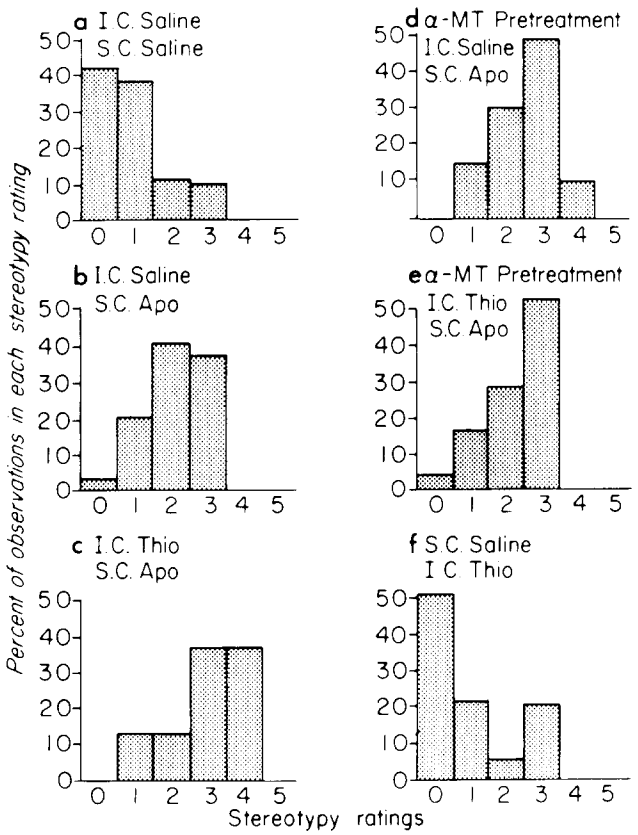

FIG. 2. Percent of observations in each stereotypy rating under different drug-treatment conditions. SC=Subcutaneous injection; IC=Intracerebral injection.

later, each rat was injected with either apomorphine (1 mg/kg SC or 0.2 mg/kg, IP) or physiological saline. All injection solutions contained 1 mg/ml L-ascorbic acid. Motor activity was measured with a Stoelting Activity Meter (No. 31400M) for 30 min beginning 5 min after the apomorphine injection. Thus, there were three treatment variables and 12 treatment groups in this experiment.

Pretreatment: Thioridazine 1.0 mg/kg, IP; saline. *Time after pretreatment*: 10 min; 48 hr. *Drug injection before motor activity measurement*: 1.0 mg/kg SC apomorphine; 0.2 mg/kg IP apomorphine; saline.

Data Analysis

Motor activity was analyzed by analysis of variance and difference between groups was determined by the Newman-Keuls Multiple Range Test. Stereotypic behavior was analyzed by the 2-tailed Mann-Whitney U-test.

RESULTS

Microinjection of Thioridazine into the Striatum

The effects of the different drug treatments on stereotypic behavior are graphed in Fig. 1. Injection of apomorphine (1 mg/kg, SC) significantly increased stereotypic behavior (cf. bar B with bar A). Bilateral injection of thioridazine (10 μg) into the striata resulted in further enhancement (cf. bar C with bar B). However, injection of thioridazine into the striatum without subcutaneous injection of apomorphine did not change the averaged total stereotypic behavior score as compared with control (saline-injected) animals (cf. bar D with bar A). Pretreating the animals with α-methyl-p-tyrosine (α-MT) also significantly enhanced the effect of apomorphine (cf. bar E with bar B) but attenuated the effect of intrastriatally injected thioridazine (cf. bar E with bar F).

The effects on the different categories of stereotypic behavior under different drug treatment conditions are shown in the distributions graphs in Fig. 2. Apomorphine injection resulted in the occurrence of more "intense" stereotypic behavior (cf. Fig. 2b with 2a). Intrastriatal thioridazine injection augmented stage 4 stereotypy (cf. Fig. 2c with Fig. 2b). However, α-MT pretreatment with thioridazine injection into the striatum eliminated stage 4 stereotypic behavior (cf. Fig. 2d with Fig. 2e). When thioridazine was injected bilaterally into the striatum without subcutaneous injection of apomorphine, stage 3 stereotypic behavior was augmented but stage 1 was decreased (Fig. 2f with 2a). Under this experimental condition, stage 3 behavior always occurred at the beginning of the session (i.e., at 0 time).

Effects of Thioridazine on Apomorphine-Elicited Motor Activity

Figure 3a and b show the effects of thioridazine 10 min and 48 hr postadministration, respectively. Figure 3a shows that 1.0 mg/kg of apomorphine significantly enhanced motor activity whereas 0.2 mg/kg of apomorphine reduced motor activity, compared with that of animals injected with saline (i.e., saline pretreatment+saline injection). Thioridazine at 1 mg/kg decreased spontaneous and also apomorphine-elicited

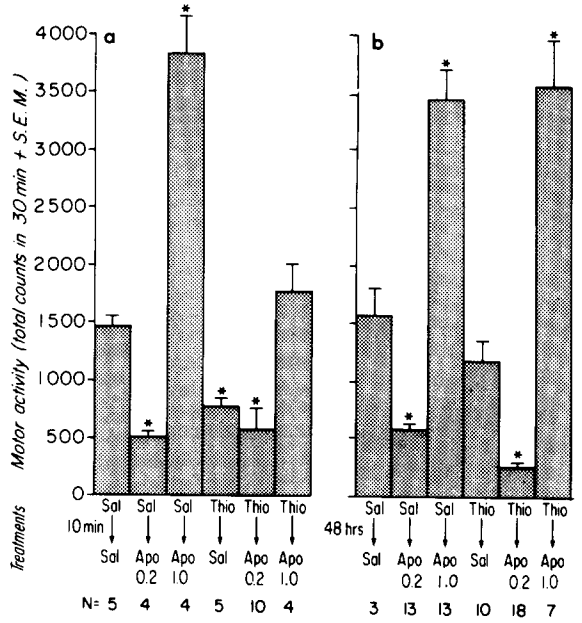

FIG. 3. (a) Motor activity in different drug-treatment conditions measured 10 min after thioridazine pretreatment. (b) Motor activity in different drug-treatment conditions measured 48 hr after thioridazine pretreatment. *Significantly different from control (saline pretreatment+saline injection) $p<0.01$.

motor activity. However, pretreatment with thioridazine (1 mg/kg) did not result in further reduction of motor activity caused by 0.2 mg/kg of apomorphine injection. Figure 3b shows the effect of thioridazine 48 hr postadministration. The responses of animals pretreated with thioridazine to 1.0 mg/kg of apomorphine were similar to those of control animals (i.e., animals pretreated with saline injection). However, the animals pretreated with thioridazine 48 hr previously showed an enhanced response to 0.2 mg/kg of apomorphine (i.e., greater reduction in motor activity) when compared with the animals pretreated with saline ($p<0.05$).

DISCUSSION

Although it has been reported that thioridazine inhibits certain dopaminergic functions (see introduction), it has also been reported that thioridazine given systemically has only a minimal effect on the nigrostriatal dopaminergic system. It has been shown that thioridazine cannot block dopaminergic drug-induced turning behavior in unilaterally nigral-lesioned animals [8,15] and it is not very effective in blocking apomorphine- and amphetamine-elicited stereotypic behaviors [6]. Therefore, one would expect that thioridazine injection into the striatum would either attenuate or not affect apomorphine-elicited stereotypic behavior. We found, however, that intrastriatally injected thioridazine actually enhanced apomorphine-elicited stereotypic behavior (Fig. 1). The mechanism of this enhancement can only be speculated. One possible explanation is that it is due to the anticholinergic property of the drug, since it is well known that thioridazine blocks muscarinic receptors and has a relatively high affinity for cholinergic receptors [6,18]. Furthermore, systemic injection of anticholinergic drugs to animals can actually enhance apomorphine-elicited stereotypic behavior [23], and cholinergic drugs can inhibit amphetamine-induced stereotypic behavior [1].

The effect of direct injection of anticholinergic drugs into the striatum on stereotypic behavior is not clear. It has been reported [5] that anticholinergics directly injected into striatum have no effect on amphetamine-induced stereotypic behavior and therefore it was concluded that the enhancing effect of systemic-administered anti-cholinergics on stereotypic behavior is due to their effect on non-striatal sites in the brain. A recent paper [31], however, reported that atropine injected into the striatum could induce stereotypic behavior. In this instance, rearing behavior was the predominant behavior observed.

To test this hypothesis, we also injected atropine (10 μg/2 μl) bilaterally into the striata of rats but observed no enhancement of apomorphine-elicited stereotypic behavior. The stereotypic behavior score was 12.2 ± 1.3, which is not significantly different from that of animals injected with apomorphine alone. However, we observed an intense increase in motor activity and rearing behavior when atropine (IC) alone was administered. It is significant that the behavior elicited by intrastriatal-injected atropine was quite different from that elicited by injection of thioridazine. Moreover, the hypothesis that thioridazine enhances apomorphine-elicited stereotypic behavior owing to its anticholinergic effect cannot account for the fact that the enhancing effect can be blocked by pretreatment with α-MT. The attenuation of the effect of thioridazine by α-MT could not be due to its sedating effect on the animals since pretreatment with α-MT actually enhanced the apomorphine-elicited stereotypic behavior (Fig. 1, cf. bar B with bar E). It would appear that release of dopamine is required for the enhancement of stereotypic behavior by intrastriatally injected thioridazine.

An alternative explanation for our results is that thioridazine can block presynaptic dopaminergic receptors (autoreceptors) on the nigrostriatal dopaminergic nerve terminals. Interactions of neuroleptics with presynaptic dopamine receptors have been reported. Low dosages of benzperidol, droperidol, haloperidol, pimozide and sulpiride can reverse the hypoactivity and brain DOPAC decrease produced by a low dose of apomorphine [9]. Supersensitivity developed in the presynaptic receptors in the striatal and mesolimbic dopaminergic systems in rats after chronic treatment with fluphenazine decanoate [21]. Haloperidol, clozapine and fluphenazine can further enhance the increase in tyrosine hydroxylase activity in response to supermaximal electrical stimulation of the nigrostriatal tract [20]. Many neuroleptics, including haloperidol, chlorpromazine and thioridazine, can reverse the dopamine synthesis blocking effect of apomorphine in the nigrostriatal system of animals treated with gamma-butyrolactone [20,28]. However, the effect of chlorpromazine and thioridazine are less potent than the butyrophenones [20]. Blockade of the presynaptic receptors causes increase in dopamine synthesis and release [12, 24, 30]. Release of dopamine in the striatum by thioridazine would enhance the stereotypic behavior elicited by apomorphine. The fact that stage 3 stereotypic behavior was observed when thioridazine alone was injected bilaterally into the striatum (Fig. 2f) may also be due to an increase in release of dopamine in the striatum. Little stage 3 behavior was observed when saline was injected into the striatum (Fig. 2a). However, a necessary condition of this hypothesis is that the dopamine released should be more than enough to counteract the postsynaptic blocking effect of thioridazine [14,19]. Other direct proofs e.g. to show that injection of thioridazine into the striatum increases release of dopamine or production of dopamine metabolites, are required to prove this hypothesis.

Furthermore, it is significant to point out here that pretreatment with α-MT has also been reported to potentiate the thioridazine-induced cataleptic response in rats [6] as well as the neuroleptic effect of thioridazine in chronic schizophrenics [4]. In both cases, it seems that a process involving the release of dopamine counteracts the postsynaptic effects of thioridazine. This effect of thioridazine may explain the low incidence of extrapyramidal side effects observed in patients treated with thioridazine [10,27]. The blocking effect of thioridazine on the striatal dopamine postsynaptic receptors is counteracted by its indirect agonist effect acting on the presynaptic sites. A similar view has been proposed for the action of clozapine [2], another neuroleptic that causes relatively less extrapyramidal syndromes. It has been proposed that clozapine causes an enhanced release of dopamine via a neuronal feedback mechanism that reverses its blockade of postsynaptic dopamine receptors in the striatum.

The hypothesis that thioridazine acts on presynaptic dopamine receptors can also explain our finding that 48 hr-thioridazine pretreatment enhanced the motor suppression effect of a low dose of apomorphine (Fig. 3b). It has been suggested that the motor suppression effect of apomorphine is due to its action on the presynaptic receptors [9,26]. The effect of 48 hr-thioridazine pretreatment could not be due to a blockade of postsynaptic dopamine receptors by residual thioridazine left in the brain, since 10 min-thioridazine pretreatment did not affect the motor response of the animals to a low dose of apomorphine (Fig. 3a). A possible explanation of the results is that supersensitivity developed in the presynaptic receptors after the thioridazine pretreatment. In our case, the dosage of thioridazine injected (1 mg/kg) probably also blocks the postsynaptic receptors since 10 min after the injection of thioridazine both spontaneous and apomorphine-elicited motor activities were attenuated (Fig. 3a). However, the fact that response to a high dose of apomoprhine was not affected by the 48 hr-thioridazine pretreatment (Fig. 3b) suggests that the pretreatment did not cause a long-lasting effect on the postsynaptic dopamine receptors.

Changes in receptor sensitivity after a single injection of a dopaminergic drug is well documented. Injection of a single dose of haloperidol is followed 2 days later by an increased behavioral responsiveness to apomorphine and a decrease in the level of HVA [17]. These changes are interpreted to be due to a development of supersensitivity in the postsynaptic cells. Similarly, a single injection of apomorphine also causes a marked elevation in behavioral response to low dosages of apomorphine [17]. The latter effect is suggested to be due to the development of a state of hyposensitivity in the presynaptic receptors. In both instances described above, the effects from a single-dose treatment develop fast and follow a long (10–14 days) time course of recovery. In our case, the supersensitivity to a low dose of apomorphine was seen at least 2 days after the acute thioridazine treatment. It may be interesting to see how long this state of supersensitivity lasts.

ACKNOWLEDGMENT

This research was partially supported by a grant from the Sandoz Pharmaceuticals.

REFERENCES

1. Arnfred, T. and A. Randrup. Cholinergic mechanism in brain inhibiting amphetamine-induced stereotypic behavior. *Acta pharmac. tox.* **26:** 384–394, 1968.
2. Bartholini, G., W. Haefely, M. Jalfre, H. H. Keller and A. Pletscher. Effects of clozapine on cerebral catecholaminergic neurone system. *Br. J. Pharmac.* **46:** 736–740, 1972.
3. Bower, M. B., Jr. and A. Rozitis. Regional differences in homovanillic acid concentration after acute and chronic administration of antipsychotic drugs. *J. Pharm. Pharmac.* **26:** 743–745, 1974.
4. Carlsson, A., B. E. Roos, J. Walinder and A. Skott. Further studies on the mechanism of antipsychotic action: Potentiation by α-methyl-tyrosine of thioridazine effects in chronic schizophrenics. *J. Neural Transm.* **34:** 125–132, 1973.
5. Costall, B. and R. J. Naylor. Modification of amphetamine effects by intracerebrally administered anticholinergic agents. *Life Sci.* **11:** 239–253, 1972.
6. Costall, B. and R. J. Naylor. Detection of the neuroleptic properties of clozapine, sulpiride and thioridazine. *Psychopharmacologia* **43:** 69–74, 1975.
7. Creese, I., D. R. Burt and S. H. Snyder. Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. *Science* **192:** 481–483, 1976.
8. Crow, T. J. and C. Gillbe. Dopamine antagonism and antischizophrenic potency of neuroleptic drugs. *Nature, New Biology* **245:** 27–28, 1973.
9. DiChiara, G., M. L. Porceddu, L. Vargiu, A. Argiolas and G. L. Gessa. Evidence for dopamine receptors mediating sedation in the mouse brain. *Nature* **264:** 564–567, 1976.
10. Gerlach, J. and H. Simmelsgaard. Tardive dyskinesia during and following treatment with haloperidol, haloperidol + biperiden, thioridazine and clozapine. *Psychopharmacology* **59:** 105–112, 1978.
11. Groot, J. de. Rat forebrain in stereotaxic coordination. *Verh. K. med. Akad. Wet.* **52:** 11–39, 1959.
12. Iversen, L. L., M. Rogawski and R. J. Miller. Comparison of the effects of neuroleptic drugs on pre- and post-synaptic dopaminergic mechanisms in the rat striatum. *Molec. Pharmac.* **12:** 251–262, 1976.
13. Iversen, S. D. Striatal function and stereotypic behavior. In: *Psychobiology of the Striatum,* edited by A. R. Cools *et al.* Amsterdam: Elsevier/North-Holland Biomedical Press, 1977, pp. 99–118.
14. Karobath, M. and H. Leitichi. Antipsychotic drugs and dopamine-stimulated adenylate cyclase prepared from corpus striatum of rat brain. *Proc. natn. Acad. Sci. U.S.A.* **71:** 2915–2918, 1974.
15. Kelly, P. H. and R. J. Miller. The interaction of neuroleptic and muscarinic agents with central dopaminergic system. *Br. J. Pharmac.* **54:** 115–121, 1975.
16. Laudron, P. M. and J. E. Leysen. Is the low incidence of extrapyramidal side effects of antipsychotics associated with antimuscarinic properties? *J. Pharm. Pharmac.* **30:** 120–122, 1978.
17. Martres, M. P., J. Costentin, M. Baudry, H. Marcais, P. Protais and J. C. Schwartz. Long-term changes in the sensitivity of pre- and post-synaptic dopamine receptors in mouse striatum as evidenced by behavioral and biochemical studies. *Brain Res.* **136:** 319–337, 1977.
18. Miller, R. J. and C. R. Hiley. Antimuscarinic properties of neuroleptics and drug-induced Parkinsonism. *Nature* **248:** 596–597, 1974.
19. Miller, R. J., A. S. Horn and L. L. Iversen. The action of neuroleptic drugs on dopamine-stimulated adenosine cyclic 3′,5′-monophosphate production in rat neostriatum and limbic system. *Molec. Pharmac.* **10:** 759–766, 1974.

20. Nowycky, M. A. and R. H. Roth. Dopaminergic neurons: role of presynaptic receptors in the regulation of transmitter biosynthesis. *Prog. Neuro-psychopharmac.* **2:** 139–158, 1978.
21. Nowycky, M. C. and R. H. Roth. Presynaptic dopamine receptors—development of supersensitivity following treatment with fluphenazine decanoate. *Naunyn-Schmeidebergs Arch. Pharmac.* **300:** 247–254, 1977.
22. O'Keefe, R., D. F. Sharman and M. Vogt. Effect of drugs used in psychosis on cerebral dopamine metabolism. *Br. J. Pharmac.* **38:** 287–304, 1970.
23. Scheel-Krügger, J. Central effects of anticholinergic drugs measured by the apomorphine gnawing test in mice. *Acta. pharmac. tox.* **28:** 1–6, 1970.
24. Seiger, A., L. Olston and L. O. Farnebo. Brain tissue transplanted to the anterior chamber of the eye. 4. Drug-modulated transmitter release in central monoamine nerve terminals lacking normal post-synaptic receptors. *Cell Tiss. Res.* **165:** 157–170, 1976.
25. Smith, R. C. and J. M. Davis. Behavioral evidence for supersensitivity after chronic administration of haloperidol, clozapine, and thioridazine. *Life Sci.* **19:** 725–732, 1976.
26. Strömbom, U. Catecholamine receptor agonists. Effect on motor activity and rate of tyrosine hydroxylation in mouse brain. *Naunyn-Schmeidebergs Arch. Pharmac.* **292:** 167–176, 1976.
27. Waldrop, F. N., R. H. Robertson and A. Vourlekis. A comparison of the therapeutic and toxic effects of thioridazine and chlorpromazine in chronic schizophrenic patients. *Comp. Psychiat.* **2:** 96–105, 1961.
28. Walter, J. R. and R. H. Roth. Dopaminergic neurons: An *in vivo* system for measuring drug interaction with presynaptic receptors. *Naunyn Schmiedelbergs Arch. Pharmac.* **296:** 5–14, 1976.
29. Westerink, B. H. C. and J. Korf. Influence of drugs on striatal and limbic homovanillic acid concentration in the brain. *Eur. J. Pharmac.* **33:** 31–40, 1975.
30. Westfall, T. C., M. J. Besson, M. F. Giorguieff and J. Glowinski. The role of the presynaptic receptors in the release and synthesis of ^{3}H-dopamine by slices of rat striatum. *Naunyn-Schmeidebergs Arch. Pharmac.* **292:** 279–287, 1976.
31. Zambo, K., L. Decsi and J. Nagy. Stereotypy after intracaudate injection of atropine in the rat. *Neuropharmacology* **18:** 727–730, 1979.