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Effects of microinjection of 2-chloro-11 (2-dimethylaminoethoxy)-dibenzo[b,f]-thiepine (zotepine), thioridazine and haloperidol into the striatum and nucleus accumbens on stereotypic behaviour and motor activity

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The tricyclic compound 2-chloro-11-(2-dimethylaminoethoxy)-dibenzo[b,f]-thiepine (zotepine) is being developed in Japan as a neuroleptic (Fukuhara et al 1979; Noda et al 1979; Uchida et al 1979). In human studies, zotepine has been found to be a potent neuroleptic with few extrapyramidal side effects (Fujisawa Company, personal communication). In animal experiments, it has been shown to block apomorphine- and methamphetamine-induced gnawing behaviour and body-turning, with ED50 values similar to those of chlorpromazine. It also causes catalepsy and blocks apomorphine-induced vomiting in dogs. These results imply that zotepine has an inhibitory effect on the dopaminergic systems in the brain. Its antipsychotic effect is probably due to its blocking action on the dopaminergic systems. In this paper, we report the results of some preliminary studies on the psychopharmacology of zotepine. We microinjected zotepine directly into the striatum and nucleus accumbens of rats and studied the effects of apomorphine-elicited stereotypic behaviour and motor activity. The effects are compared with those of two well-known neuroleptics, thioridazine and haloperidol.

Methods and materials

Male Sprague-Dawley rats from Tyler Lab, Washington, 250–300 g, were housed in a vivarium under a 12-h light-dark cycle and provided with free access to food and water. Guide cannulae (23 g) were implanted bilaterally in the skulls by stereotaxic technique, with the tip 1 mm below the cortical surface. Five to seven days after implantation (to allow recovery from surgery), the drugs to be tested were injected bilaterally with a 30 g cannula inserted through the guide. The coordinates of injection were: AP + 7·4; L ± 2·6; DV + 2·0 and AP + 9·0; L ± 1·0; DV 0 for the striatum and nucleus accumbens, respectively (de Groot 1959). The rate of injection was 2 μl min^{-1} and the volume was 2 μl for the striatum and 1 μl for the nucleus accumbens. We left the injection cannula in the brain for an additional 30 s after the injection to allow the drug to diffuse and to prevent backflow. At the end of each experiment, the brain was removed, sectioned, and examined for location of drug injection.

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Zotepine, thioridazine, and haloperidol were injected into the striatum and nucleus accumbens of test animals. In control animals 0·9% NaCl (saline) was injected instead. In some experiments, α-methyl-*p*-tyrosine methyl ester (AMPT, 250 mg base kg^{-1} i.p.) was injected into rats 2 h before the intracerebral drug injection.

Measurement of stereotypic behaviour and motor activity. Five minutes post-intracerebral injection of drugs, apomorphine was injected (1 mg kg^{-1}, solution containing 1 mg ml^{-1} of L-ascorbic acid) subcutaneously in the flank. Five minutes later, motor activity was measured in a Stoelting Electronic Activity Meter for 1 h, with sensitivity adjusted such that only gross body movement was recorded. The meter was placed in a sound-proofed box. Behaviour was observed through a one-way mirror and scored at 0, 15, 30, 45 and 60 min during the course of motor activity measurement. The scoring system modelled after Iversen's (1977) 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 behaviour for each animal is reported as the sum of the scores in the five observations.

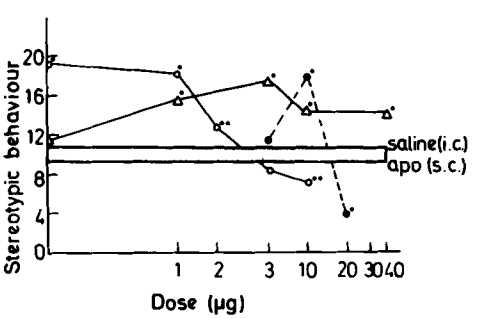

FIG. 1. Log-dose response curves of the effect of bilateral injection of zotepine (●) thioridazine (△) and haloperidol (○) into the striatum on apomorphine elicited stereotypic behaviour. i.c. = intracerebral injection; s.c. = subcutaneous injection. *$P < 0.01$; **$P < 0.05$ different from the i.c.-saline/s.c. apomorphine injected animals.

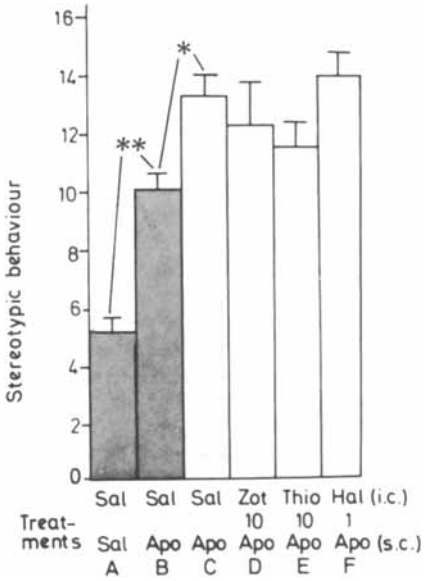

FIG. 2. Effects of intrastriatally injected zotepine, thioridazine and haloperidol on apomorphine elicited stereotypic behaviour after AMPT pretreatment (Bars C-F). i.c. = intracerebral injection; s.c. = subcutaneous injection. ** $P < 0{\cdot}001$; * $P < 0{\cdot}007$.

Stereotypic behaviour was analysed by the 2-tailed Mann-Whitney U-test. Data on motor activity were analysed by the 2-tailed Student's *t*-test. Each datum point represents averaged data from 4–10 animals.

Results

Apomorphine significantly enhances stereotypic behaviour and motor activity in the rats ($P < 0{\cdot}001$) (data not shown). Fig. 1 shows log-dose response curves of the effects of intrastriatally administered zotepine, thioridazine, and haloperidol on apomorphine elicited stereotypic behaviour. Zotepine and haloperidol enhanced the stereotypic behaviour at low doses but suppressed it at high doses. Thioridazine significantly enhanced the apomorphine-elicited stereotypic behaviour at all the doses tested. Owing to the low solubility of thioridazine in saline, no higher dosages of thioridazine were injected.

In addition, we also injected atropine ($10\,\mu g/2\,\mu l$) bilaterally into the striatum of some animals and studied its effect on apomorphine-elicited stereotypic behaviour. The stereotypic behaviour score of the intrastriatally atropine-injected rats was $12{\cdot}2 \pm 1{\cdot}3$, which is not significantly different from controls.

To further investigate the enhancing effect of thioridazine, haloperidol and zotepine on the apomorphine-elicited stereotypic behaviour, we pretreated some animals with AMPT. Fig. 2 shows that apomorphine injection enhances stereotypic behaviour (cf. bar A with bar B) and AMPT pretreatment itself could further enhance apomorphine-elicited stereotypic behaviour (cf. bar C with bar B). However, the pretreatment eliminated the enhancing effects of zotepine ($10\,\mu g$), thioridazine ($10\,\mu g$) and haloperidol ($1\,\mu g$) (cf. bars D, E and F with bar C).

Fig. 3 shows the effects of injection of zotepine ($5\,\mu g$), thioridazine ($5\,\mu g$) and haloperidol ($5\,\mu g$) into the nucleus accumbens on motor activity induced by apomorphine. All three drugs significantly attenuated the motor activity.

Discussion

The blocking effect of zotepine injected into the nucleus accumbens on apomorphine-elicited motor activity (Fig. 3) is in agreement with its action as a dopamine antagonist, since injection of neuroleptics at this site is known to attenuate motor activity. The antipsychotic effect of zotepine is probably due to a blocking effect on the mesolimbic dopaminergic system; this system has been implicated in the aetiology of schizophrenia (Carlsson 1976).

On the other hand, the enhancing effect of intrastriatally injected zotepine, thioridazine and haloperidol on apomorphine-elicited stereotypic behaviour (Fig. 1) was unexpected, because injection of neuroleptics into animals generally blocks stereotypic behaviour. Uchida et al (1979) found that zotepine blocks stereotypic gnawing induced by apomorphine and turning behaviour in rats; the finding would imply a blocking effect on a striatal dopaminergic functions. A blocking effect by thioridazine and haloperidol on striatal dopaminergic functions has also been suggested: they suppressed dopamine-sensitive adenylate cyclase (Karobath & Leitichi 1974; Miller et al 1974) and thioridazine displaced [^{3}H]haloperidol from binding sites in striatal tissue (Creese et al 1976).

It is not known why AMPT pretreatment enhanced the apomorphine-elicited stereotypic behaviour (Fig. 2, bar B vs bar C). Costall & Naylor (1973) have also

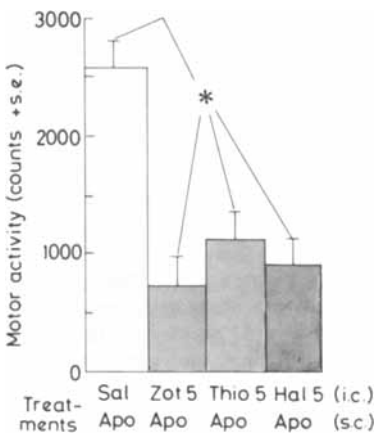

FIG. 3. Effects of injection of zotepine thioridazine and haloperidol into the nucleus accumbens on apomorphine elicited motor activity. I.c. = intracerebral injection; s.c. = subcutaneous injection. * $P < 0{\cdot}01$.

reported a similar observation. However, pretreatment with AMPT attenuated the enhancing effects of zotepine, thioridazine and haloperidol (Fig. 2). It would thus appear that the enhancing effect involves release of dopamine from the nigrostriatal dopamine nerve terminals rather than direct action of the drugs on postsynaptic sites. An explanation for the enhancing effect is that in addition to their postsynaptic effects, these neuroleptics can also block presynaptic dopamine receptors (autoreceptors) in the nigrostriatal system. This property of neuroleptics has been documented by Nowycky & Roth (1978) and Walter & Roth (1976). Blockade of these receptors leads to increased release of dopamine (Seiger et al 1976; Westfall et al 1976) and thus can facilitate the effect of apomorphine. The net effect of these neuroleptics on apomorphine-elicited stereotypic behaviour depends on their relative potencies in blocking pre- and postsynaptic dopamine receptors. With zotepine and haloperidol, the presynaptic effect predominated when low doses of the neuroleptics were injected; thus behavioural facilitation was observed. When high doses of the drugs were injected into the striatum, postsynaptic blockade predominated and attenuation of the apomorphine-elicited stereotypic behaviour was seen. With thioridazine, the dosage injected was not high enough to achieve blockade of the postsynaptic receptors. Since zotepine has a steep dose response relationship (Fig. 1), to make sure that the attenuation effect of AMPT pretreatment was not due to a slight shift of the dose-response curve, we also investigated the effect of injection of 5 μg of zotepine into the striatum on apomorphine-elicited stereotypic behaviour. No enhancement of stereotypy was observed at this lower dose of zotepine when compared with controls (i.e. Fig. 2 bar C). (The stereotypic behaviour score was 12·2 $\pm$ 1·2).

The action of neuroleptics on presynaptic dopamine receptors would counteract their antischizophrenic effect, which is presumed to be achieved by a blockade of the postsynaptic receptors. If a neuroleptic's presynaptic effect counteracts its postsynaptic effect at the striatum, the extrapyramidal side effects will be lessened, since these syndromes are thought to result from a blockade of the postsynaptic receptors. This view would also explain the relatively low incidence of extrapyramidal side effects seen in patients chronically treated with zotepine (Fujisawa Company, personal communication) and thioridazine (Waldrop et al 1961; Gerlach & Simmelsgaard 1978). Thus, the antipsychotic potency and the incidence of extrapyramidal side effects of a neuroleptic would depend on the drug's relative potencies in blocking the pre- and postsynaptic receptors in the mesolimbic and nigrostriatal dopaminergic systems, respectively. A drug with high blocking potency at the striatal presynaptic receptors and mesolimbic postsynaptic receptors would be especially suited to the treatment of schizophrenia.

Thioridazine (Miller & Hiley 1974; Laudron & Leysen 1978) and zotepine (Fujisawa Company, personal communication) have been shown to have anti-acetylcholine effects. Moreover, systemic injection of anti-acetylcholine drugs into animals is known to enhance stereotypic behaviour (Arnfred & Randrup 1968; Scheel-Krüger (1970). Therefore, the enhancing effect of zotepine and thioridazine on apomorphine-elicited stereotypic behaviour could be due to their anti-acetylcholine properties. To test this hypothesis, we injected 10 μg of atropine bilaterally into the striatum of animals and studied its effect on apomorphine-elicited stereotypic behaviour. We observed no effect (see results section), but we saw an intense increase in rearing and motor activity when atropine alone was injected into the striatum. Thus, the effect on behaviour elicited by intrastriatally injected atropine differs from that elicited by either zotepine and thioridazine.

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