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Stimulation of postsynaptic D_2^- dopamine receptors by B-HT 958 is revealed by co-treatment with the D_1^- receptor agonist SKF 38393

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Abstract—The motor activity of reserpine-treated mice was used to study effects of B-HT 958 (2-amino-6-(*p*-chlorobenzyl)-4H-5, 6, 7, 8-tetrahydrothiazolo-[5, 4-d]-azepine) on postsynaptic dopamine and noradrenaline receptors. The motor activity was only slightly stimulated by B-HT 958 or by the D_1^- receptor agonist SKF 38393 but it was markedly increased by the two drugs given in combination. The effect of B-HT 958 peaked earlier following low rather than high doses. The enhanced motor activity was inhibited by the D_2^- receptor antagonist sulpiride or the D_1^- receptor antagonist SCH 23390, indicating that it was caused by stimulation of both receptor types. The results suggest that B-HT 958 stimulates postsynaptic D_2^- receptors in addition to D_2^- autoreceptors and that its blockade of postsynaptic α_2-adrenoceptors is of no importance for the motor activity.

The motor activity of mice and rats is inhibited by B-HT 920 (2-amino-6-allyl-5, 6, 7, 8-tetrahydro-4H-thiazolo-[4, 5-d]-azepine) (Andén et al 1982, 1983; Hinzen et al 1986). This effect might be caused by stimulation of D_2^- dopamine (DA) receptors on DA neurons, i.e., DA autoreceptors. In contrast, B-HT 920 increases rather than decreases the motor activity of mice and rats when given in combination with the D_1^- DA receptor agonist SKF 38393 (Andén & Grabowska-Andén 1987, 1988; Grabowska-Andén & Andén 1987; Hjorth & Carlsson 1987; Pifl & Hornykiewicz 1988). This stimulatory effect of B-HT 920 is blocked by sulpiride indicating that it is the result of stimulation of postsynaptic D_2^- DA receptors.

B-HT 920 stimulates α_2-adrenoceptors in addition to D_2^- receptors (Kobinger & Pichler 1980; Andén et al 1982, 1983). Recently, it has been claimed that stimulation of α_2-adrenoceptors might be the mechanism behind behavioural effects of B-HT 920 (Ferrari et al 1988; Johansen et al 1988). The 6-*p*-chlorobenzyl analogue B-HT 958 blocks rather than stimulates α_2-adrenoceptors but stimulates D_2^- receptors similarly to B-HT 920 (Grabowska-Andén & Andén 1984; Hörtnagel et al 1985). In the present investigation, B-HT 958 was given in combination with SKF 38393 in order to study the importance of α_2-adrenoceptors for the increased motor activity observed after combined stimulation of D_1^- and D_2^- receptors.

Materials and methods

Male mice of the NMRI strain, 22–25 g, were kept in cages, each containing 10 animals. The mice had free access to food and water up to the measurement of the motor activity. They were placed in a room illuminated between 7 am and 7 pm. The experiments were performed between 8 am and 6 pm. The mice were used only once. Care was taken to prevent drug-induced changes in the body temperature by placing the mice under a lamp with infrared light.

Motor activity. The motor activity of groups of three mice was measured by means of two matched electronic motility meters (Motron Products, Stockholm, Sweden) (Modigh 1972; Engström et al 1974). One meter consisted of a cage (20 × 32 × 25 cm) with 40 photocells in a 5 × 8 array in the floor. The cage was placed in a sound-proof and fan-ventilated box with a 25 W lamp in the ceiling. The motility was recorded every 15 min during 2–6 h. The apparatus did not monitor the amplitude of small movements such as those of the tail and head.

Drugs. The following drugs were used: B-HT 958 (2-amino-6-(*p*-chlorobenzyl)-4H-5, 6, 7, 8-tetrahydrothiazolo-[5, 4-d]-azepine 2 HCl; Boehringer Ingelheim*, Ingelheim am Rhein, FRG), SKF 38 393 ((±)-7, 8-dihydroxy-1-phenyl-2, 3, 4, 5-tetrahydro-1H-3-benzazepine HCl; Research Biochemicals, Wayland, MA, USA), reserpine (CIBA-Geigy*, Mölndal, Sweden), (±)-sulpiride (ICFI*, Milan, Italy), SCH 23390 ((*R*)-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2, 3, 4, 5-tetrahydro-1H-3-benzazepine maleate; Schering*, Kenilworth, NJ, USA). Reserpine and sulpiride were dissolved in a drop of glacial acetic acid and 5·5% glucose was added to the volume. The other compounds were dissolved in 0·9% NaCl, sometimes after acidification and heating. The doses refer to the forms indicated here. The injections of B-HT 958 and SKF 38393 were performed 5 min before the start of the motor activity recordings.

Statistical analysis. All values were transformed ($\sqrt{(\text{counts}+0{\cdot}5)}$) and they are expressed as arithmetic means with s.e.m. The statistical significances were calculated by the F-test following a two-factor experiment with repeated measures on one factor (Winer 1970).

Results

After pretreatment with reserpine (10 mg kg^{-1} s.c., 4 h), the spontaneous motor activity was very low (Fig. 1). The motor activity of the reserpine-treated mice was only slightly increased by B-HT 958 (100 mg kg^{-1} i.p.) or SKF 38393 (6 mg kg^{-1} s.c.).

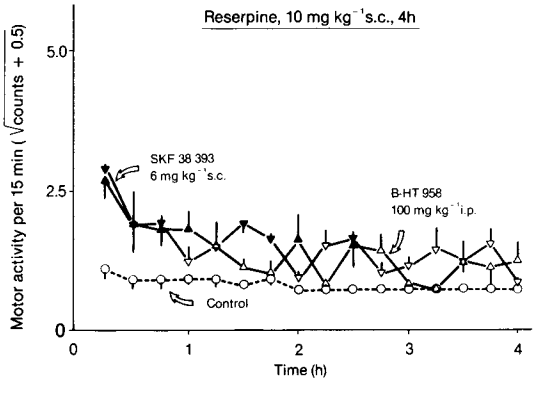

FIG. 1. Effect of B-HT 958 and SKF 38393 (6 mg kg^{-1} s.c.) on the motor activity of mice pretreated with reserpine (10 mg kg^{-1} s.c. 4 h previously). The values are means and s.e..m. of transforms. The filled symbols indicate statistically significant differences ($P < 0{\cdot}05$) from the reserpine-treated control group.

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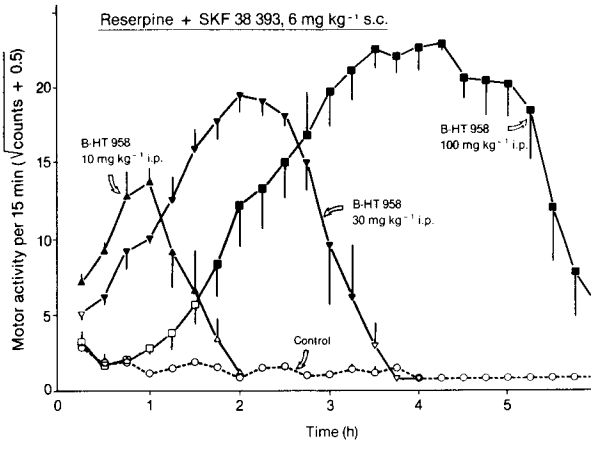

FIG. 2. Effect of B-HT 958 (▲ 10, ▼ 30, ■ 100 mg kg^{-1} i.p.) on the motor activity of mice treated with reserpine (10 mg kg^{-1} s.c., 4 h) and SKF 38393. The values are means and s.e.m. of transforms. The filled symbols indicate statistically significant differences ($P < 0.05$) from the control group treated with reserpine and SKF 38393.

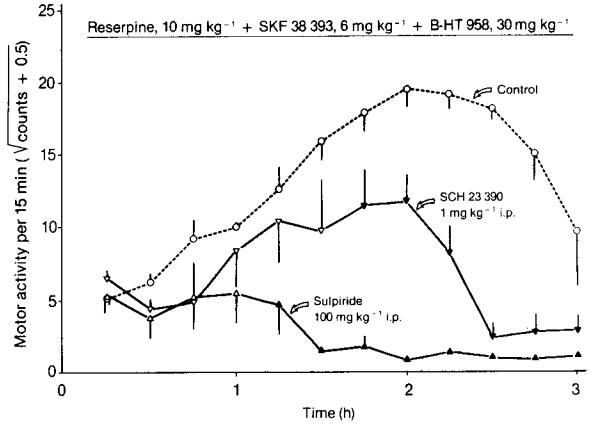

FIG. 3. Effect of sulpiride (100 mg kg^{-1} i.p.) and SCH 23390 (1 mg kg^{-1} i.p.) on the motor activity of mice treated with reserpine (10 mg kg^{-1}) plus SKF 38393 (6 mg kg^{-1}) plus B-HT 958 (30 mg kg^{-1}). The values are means and s.e.m. of transforms. The filled symbols indicate statistically significant differences ($P < 0.05$) from the control group treated with reserpine plus SKF 38393 plus B-HT 958.

The motor activity of mice treated with reserpine and SKF 38393 (6 mg kg^{-1} s.c.) was enhanced by B-HT 958 (Fig. 2). The movements were coordinated and forward-directed similarly to those produced by B-HT 920. The peak activity occurred earlier following 10 than 30 and, particularly, 100 mg kg^{-1} B-HT 958. In addition, it was somewhat smaller.

The motor activity of the mice treated with reserpine plus SKF 38393 (6 mg kg^{-1}) plus B-HT 958 (30 mg kg^{-1}) was inhibited by the D_1^- receptor antagonist SCH 23390 (1 mg kg^{-1} i.p., 30 min before the start of the recording) and was almost completely inhibited by the D_2^- receptor antagonist sulpiride (100 mg kg^{-1} i.p., 60 min before the start of the recording) (Fig. 3).

Discussion

B-HT 958 or SKF 38393 by themselves only slightly enhanced the motor activity of reserpine-treated mice, but they markedly increased it when given in combination. In these respects, B-HT 958 is similar to B-HT 920. Since these two compounds influence the central α_2-adrenoceptors in different directions, their effects on this receptor should not be of any major importance for their stimulation of motor activity.

The stimulatory effect of B-HT 958 when given in combination with SKF 38393 peaked earlier following low rather than high doses. In this respect also, B-HT 958 is similar to B-HT 920 (Andén & Grabowska-Andén 1988). Therefore, this action should not be the result of an effect on α_2-receptors. The reason for the long latency after the high doses is unknown.

The hyperactivity produced by B-HT 958 plus SKF 38393 was inhibited by the D_1^- receptor antagonist SCH 23390 as well as by the D_2^- receptor antagonist sulpiride. These findings indicate that the effect is caused by a simultaneous stimulation of both D_1^- and D_2^- receptors postsynaptically, in agreement with several recent studies (Braun & Chase 1986; Jackson & Hashizume 1986; Waddington 1986; Arnt et al 1987; Clark & White 1987).

The inhibitory effect on the motor activity by B-HT 920 is probably not caused by the simultaneous activation of α_2-adrenoceptors since the α_2-adrenoceptor antagonist yohimbine does not reduce the inhibition (Andén et al 1982) and since the same inhibitory effect is observed after B-HT 958 blocking α_2-adrenoceptors (Grabowska-Andén & Andén 1984; Hörtnagel et al 1985). Thus, both the inhibitory and excitatory effects of B-HT 920 on the motor activity seem to be independent of the α_2-adrenoceptor stimulation produced by this drug.

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A high dose of MPTP overcomes the protective effect of selegiline against dopaminergic neurotoxicity

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Abstract—1-Methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) at 90 mg kg^{-1} s.c., a dose lethal in non-pretreated mice, was well tolerated in selegiline ((−)-deprenyl)-pretreated mice and produced persistent depletion of striatal dopamine and its metabolites one week after the last of four daily injections. The protective effect of selegiline against dopaminergic neurotoxicity of MPTP can thus be overridden by a high dose of MPTP that would be lethal without selegiline pretreatment.

1-Methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) was discovered to cause parkinsonian symptoms in human drug abusers who inadvertently self-administered it (Langston et al 1983) and then was shown to cause selective neurotoxicity to nigrostriatal dopamine neurons, accompanied by severe motor dysfunction, in non-human primates (Burns et al 1983; Langston et al 1984). In mice, MPTP causes persistent depletion of striatal dopamine and its metabolites (Heikkila et al 1984a), loss of dopamine uptake capacity in the striatum (Wallace et al 1984; Sershen et al 1984), and histologic evidence of cell loss in the substantia nigra (Gupta et al 1984). The neurotoxic effect of MPTP toward nigrostriatal dopamine neurons requires metabolic conversion of MPTP to 1-methyl-4-phenyl-pyridinium (MPP+) via monoamine oxidase type B (MAO-B) (Chiba et al 1984), and selegiline ((−)-deprenyl) and other inhibitors of MAO-B prevent the neurotoxicity in primates (Langston et al 1984) and in mice (Heikkila et al 1984b).

Recently we found that the acute lethality of MPTP in mice is also prevented by inhibitors of MAO-B (Fuller et al 1988). In selegiline-pretreated mice, high doses of MPTP that would otherwise be lethal could be given without lethality or overt signs of toxicity. The study described here was done to see if a high dose of MPTP could override the protective effect of selegiline against striatal neurotoxicity. The results show that a 90 mg kg^{-1} dose of MPTP, which is lethal ordinarily, is well tolerated in selegiline-pretreated mice and produces persistent depletion of striatal dopamine and its metabolites analogous to that seen with lower doses of MPTP in non-pretreated mice.

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Male CRL/CFW mice, 20–30 g, were purchased from Charles River Breeding Laboratories (Portage, MI). MPTP hydrochloride was synthesized by Dr David W. Robertson in the Lilly Research Laboratories, and selegiline was a gift from Professor J. Knoll, Semmelweis School of Medicine, Budapest, Hungary. Mice were killed by cervical dislocation. Brains were removed and dissected, then striata were frozen on dry ice and stored at −60°C. Dopamine and its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were measured by liquid chromatography with electrochemical detection (Fuller & Perry 1977; Perry & Fuller 1979).

Table 1 gives the concentrations of dopamine and its metabolites in mouse striatum after several treatment paradigms. MPTP at 30 mg kg^{-1} caused marked depletion of striatal dopamine and slightly less depletion of DOPAC and HVA. Four doses of selegiline, before each of the four MPTP doses, completely prevented the depletion of dopamine and its metabolites. A higher dose of MPTP, 90 mg kg^{-1}, which is ordinarily lethal (Fuller et al 1988) but which could be given to selegiline (4)-pretreated mice without any lethality or severe effects that could be observed, caused significant depletion of dopamine and its metabolites, though less than that produced by a 30 mg kg^{-1} dose of MPTP given without selegiline. When the 90 mg kg^{-1} dose of MPTP was given to mice pretreated with only one dose of selegiline (1 h before the first dose of MPTP), it also was well tolerated by the mice and caused more marked depletion of dopamine and its metabolites, approaching the magnitude of the depletion seen with the 30 mg kg^{-1} dose of MPTP given alone. Neither of the selegiline treatment regimens alone caused any effects on dopamine or its metabolites one week later.

The current findings show that a high dose of MPTP, which would be lethal in non-pretreated mice but which is well tolerated in selegiline-pretreated mice, can override the protective effects of selegiline against selective neurotoxicity toward nigrostriatal dopamine neurons. Because selegiline is an irreversible inhibitor of MAO-B causing inhibition of that enzyme for as long as 14 days (Fuller et al 1988), a single dose of selegiline