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B-HT 958 stimulates dopamine autoreceptors but blocks noradrenaline autoreceptors in the brain

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B-HT 958 (2-amino-6-(*p*-chlorobenzyl)-4H-5,6,7,8-tetrahydrothiazolo 5,4-d azepine) blocked the γ-butyrolactone-induced increase in the synthesis of dopamine and slowed down the α-methyltyrosine-induced disappearance of dopamine in the mouse brain by haloperidol-sensitive mechanisms. In reserpine-treated mice, B-HT 958 produced at most a weak locomotion and no change in the apomorphine-induced increase in motor activity. The motor activity of normal mice was reduced by B-HT 958. At high doses, B-HT 958 accelerated the α-methyltyrosine-induced disappearance of noradrenaline and it inhibited the effects of clonidine on the turnover and on the synthesis of noradrenaline in the mouse brain. The findings indicate that the dopamine autoreceptors can be selectively stimulated by B-HT 958 but that the α_2-adrenoceptors can be blocked following high doses.

B-HT 920 (2-amino-6-allyl-4H-5,6,7,8-tetrahydrothiazolo 5,4-d azepine) potently stimulates dopamine (DA) autoreceptors in the brain judging from its effects on DA synthesis and turnover and on motor activity (Andén et al 1982a). At high doses, however, B-HT 920 also retards the turnover of noradrenaline (NA) in the brain (Andén et al 1982a). The latter finding indicates that a high dose of B-HT 920 can stimulate α_2-adrenoceptors, in agreement with previous findings from the peripheral sympathetic nervous system (Kobinger & Pichler 1980; van Meel et al 1981). The chemically related compound B-HT 958 (2-amino-6-(*p*-chlorobenzyl)-4H-5,6,7,8-tetrahydrothiazolo 5,4-d azepine) has been reported to be both agonist and antagonist at peripheral α_2-adrenoceptors (Pichler et al 1982). These findings prompted us to study the effects of B-HT 958 on DA and NA receptors in the mouse brain.

MATERIALS AND METHODS

Male mice of the NMRI strain, 24–28 g, were kept in cages, each containing 10 animals. They had free access to food and water. The mice were placed in a room illuminated between 7 pm and 7 am. All experiments were performed between 8 am and 6 pm. Care was taken to prevent drug-induced changes in the body temperature.

Biochemistry

The turnover of the NA and DA in the mouse brain was studied by means of a supramaximal dose of the tyrosine hydroxylase inhibitor DL-α-methyl-tyrosine methylester (α-MT, H 44/68; 250 mg kg^{-1} i.p., 4 h before killing) (Spector et al 1965; Corrodi & Hanson 1966; Andén et al 1966). A possible effect of B-HT 958 on DA receptors and α_2-adrenoceptors was tested by means of the DA receptor antagonist haloperidol (2·0 mg kg^{-1} i.p.) and the α_2-receptor agonist clonidine (0·1 mg kg^{-1} i.p.), respectively.

A stimulatory effect of B-HT 958 on presynaptic DA receptors in the corpus striatum and in the limbic system of mice was studied when the nerve impulse flow in the DA neurons was eliminated following treatment with γ-butyrolactone (GBL; Roth et al 1973; Walters & Roth 1976; Gianutsos et al 1976). Such a blockade of the nerve impulse flow presumably interrupts the stimulation of the DA receptors on the DA nerve terminals by the DA normally released and leads subsequently to enhanced synthesis of DA (Kehr et al 1972). The synthesis of DA in the two above mentioned structures of the mouse brain was estimated as the accumulation of dopa following inhibition of the dopa decarboxylase by 3-hydroxybenzylhydrazine (NSD 1015; 100 mg kg^{-1} i.p., 30 min before killing) (Carlsson et al 1972).

The time-course of the action of B-HT 958 on the DA receptors was estimated by means of the accumulation of dopa (see above) in reserpine-pretreated mice (10 mg kg^{-1} i.p., 24 h before killing). Such a pretreatment was also used in combination with clonidine (0·1 mg kg^{-1} i.p.) in order to test a possible effect of B-HT 958 on α_2-adrenoceptors in NA-predominant regions, i.e. the brain stem and the hemispheres (Andén et al 1982a). The mice were decapitated and the brains quickly removed. In the

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dopa experiments, the corpus striatum, the limbic system, the brain stem (hypothalamus, thalamus, mesencephalon, pons, medulla oblongata) and the hemispheres (cerebral cortex lateral to the rhinal fissure, cerebellum) were dissected on an ice-cooled Petri dish under an operation microscope (Andén et al 1976). The tissue was homogenized in ice-cooled perchloric acid containing $Na_2S_2O_5$, Na_2-EDTA and an internal standard (100 ng 3,4-dihydroxybenzylamine in the amine experiments or 100 ng α-methyldopa in the dopa experiments). The catechols were purified by means of alumina and reverse-phase, high-performance liquid chromatography and determined electrochemically (Felice et al 1978; Andén et al 1982a).

Motor activity

The motor activity of groups of three mice was measured using two matched 'M/P 40 Fc electronic motility meters' (Motron Products, Stockholm, Sweden) (Modigh 1972; Engström et al 1974). One meter consisted of a cage in the floor of which there were 40 photocells in a 5 × 8 array with a distance of 40 mm between the centres of the photocells. Each meter was placed under a 25 W lamp in a soundproof, fan-ventilated chamber. The apparatus did not record the movements of the tail and other small amplitude movements.

Drugs

The following drugs were used: B-HT 958 (2-amino-6-(*p*-chlorobenzyl)-4H-5,6,7,8-tetrahydrothiazolo 5,4-d azepine 2HCl; Boehringer Ingelheim*, Ingelheim am Rhein, FRG), B-HT 920 (2-amino-6-allyl-4H-5,6,7,8-tetrahydrothiazolo 5,4-d azepine 2HCl; Boehringer Ingelheim, Ingelheim am Rhein, FRG), apomorphine HCl 1/2 H_2O (Sandoz, Basle, Switzerland), clonidine HCl (Boehringer Ingelheim*, Stockholm, Sweden), reserpine (CIBA-Geigy*, Mölndal, Sweden), DL-α-methyltyrosine methylester HCl (α-MT, H 44/68; Hässle*, Mölndal, Sweden), haloperidol (Leo*, Helsingborg, Sweden), 3-hydroxybenzylhydrazine HCl (NSD 1015; Department of Pharmacology, University of Gothenburg, Sweden), γ-butyrolactone (GBL; Merck, Darmstadt, FRG), 3,4-dihydroxybenzylamine (Sigma, St Louis, MO, USA), L-α-methyl-dopa (MSD*, Rahway, NJ, USA). Reserpine and haloperidol were dissolved in a few drops of glacial acetic acid and 5·5% glucose was added to volume. The other drugs were dissolved in 0·9% NaCl. The doses refer to the forms indicated.

RESULTS

Utilization of dopamine and noradrenaline

The concentrations of DA and NA in the whole brain of mice were lowered 4 h after the administration of α-MT by 63 and 42%, respectively (Fig. 1). The α-MT-induced disappearance of DA was retarded by B-HT 958 at 3 mg kg^{-1} and higher doses (Fig. 1).

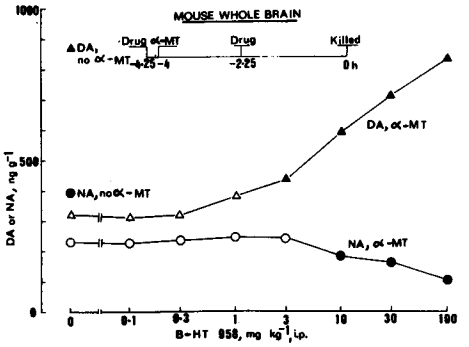

FIG. 1. Concentrations of dopamine (DA) and noradrenaline (NA) in the mouse whole brain following α-methyltyrosine (α-MT, 250 mg kg^{-1} i.p., 4 h before killing) plus different doses of B-HT 958 (two doses i.p., 4½ and 2½ h before killing). The concentrations without treatment with α-MT and B-HT 958 are also shown (no α-MT). The values are means from 3–10 determinations. The filled symbols indicate statistically significant differences from the group treated with only α-MT ($P < 0·05$; one-way analysis of variance followed by Dunnett's *t*-test).

The concentration of DA in the brain of mice treated with α-MT and 100 mg kg^{-1} of B-HT 958 did not differ significantly from the control value. B-HT 958, at doses of 10 mg kg^{-1} and higher, significantly accelerated the disappearance of NA (Fig. 1). Haloperidol (2 mg kg^{-1} i.p.) blocked the effect of B-HT 958 (10 mg kg^{-1} i.p.) on the α-MT-induced disappearance of DA, but enhanced that on the α-MT-induced disappearance of NA (Table 1).

Clonidine decelerated the α-MT-induced disappearance of NA markedly (Table 1). B-HT 958 partly inhibited this effect of clonidine on the utilization of NA.

Synthesis of dopamine and noradrenaline

The GBL-induced increase in the accumulation of dopa in the DA-rich corpus striatum was completely antagonized by B-HT 958, 10 mg kg^{-1} (Table 2). haloperidol (2·0 mg kg^{-1}) abolished the changes in the synthesis of DA after B-HT 958. Similar results were obtained in the limbic system (Table 3) where the concentration of DA is 3 times higher than that of NA (Andén et al 1982a). In the limbic system, B-HT 958 reduced ($P < 0·025$) the dopa accumulation

Table 1. Concentrations of dopamine and noradrenaline in the whole brain of mice treated with B-HT 958 (10 or 100 mg kg^{-1} i.p., twice, 4¼ and 2¼ h before killing), DL-α-methyltyrosine methylester (α-MT, 250 mg kg^{-1} i.p., 4 h before killing), haloperidol (2·0 mg kg^{-1} i.p., 4½ h before killing) and clonidine (0·1 mg kg^{-1} i.p., 4⅛ h before killing).

Treatment	Dopamine (ng g^{-1})	Noradrenaline (ng g^{-1})
No drug treatment	862 ± 16·4 (16)	391 ± 4·4 (16)
B-HT 958, 10	901 ± 27·4 (6)	391 ± 9·4 (6)
α-MT	317 ± 11·9 (14)[a]	221 ± 7·0 (14)[a]
B-HT 958, 10 + α-MT	589 ± 17·7 (6)[b]	181 ± 12·5 (6)[f]
Haloperidol + α-MT	229 ± 24·6 (6)[f]	163 ± 7·1 (6)[b]
Haloperidol + B-HT 958, 10 + α-MT	202 ± 23·7 (6)[c]	148 ± 14·3 (6)[g]
Clonidine + α-MT	414 ± 15·0 (6)[b]	330 ± 10·9 (6)[b]
B-HT 958, 100 + α-MT	765 ± 33·1 (6)[b]	93 ± 4·9 (6)[b]
B-HT 958, 100 + Clonidine + α-MT	678 ± 16.7 (6)[d,h]	234 ± 13·0 (6)[d,c]

The values are means ± s.e.m. with the number of experiments in parentheses. Statistical significances were calculated by means of one-way analysis of variance followed by *t*-test ($P < 0·001$ compared with [a] no drug treatment, [b] α-MT, [c] B-HT 958, 10 + α-MT; [d] clonidine + α-MT, [e] B-HT 958, 100 + α-MT; $P < 0·01$ compared with [f] α-MT; $P < 0·05$ compared with [g] B-HT 958, 10 + α-MT, [h] B-HT 958, 100 + α-MT).

below the control level also in the GBL-treated animals.

The inhibitory effect of B-HT 958, 10 mg kg^{-1}, on the synthesis of DA in the corpus striatum and in the limbic system of mice pretreated with reserpine was maximal for about 3 h and lasted for more than 5 h (Fig. 2). An equieffective dose of B-HT 920 (0·1 mg kg^{-1}) had a somewhat shorter duration.

In the brain stem and the hemispheres where NA is the predominant catecholamine, clonidine, but not

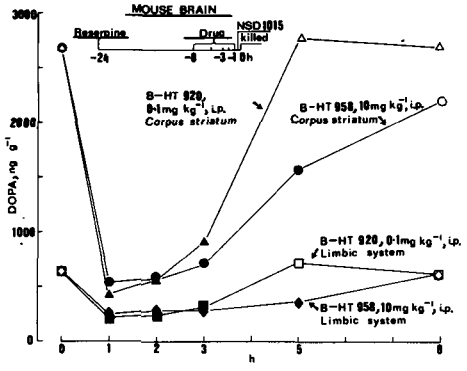

FIG. 2. Time course of the effects of B-HT 958 and B-HT 920 on the synthesis of dopamine in the corpus striatum and the limbic system of mice. The synthesis was estimated as the dopa accumulation following reserpine (10 mg kg^{-1} i.p., 24 h before killing) and 3-hydroxybenzylhydrazine (NSD 1015; 100 mg kg^{-1} i.p., 30 min before killing). The times indicate the intervals between the i.p. injection of B-HT 958 or B-HT 920, and killing. The values are means from 3–6 determinations. The filled symbols indicate statistically significant differences from the 0 time group ($P < 0·05$; one-way analysis of variance followed by Dunnett's *t*-test).

Table 2. Accumulation of dopa in the corpus striatum and the limbic system of mice induced by γ-butyrolactone (GBL, 750 mg kg^{-1} i.p., 35 min before killing) and 3-hydroxybenzylhydrazine (NSD 1015, 100 mg kg^{-1} i.p., 30 min before killing). Haloperidol (2·0 mg kg^{-1} i.p.) and B-HT 958 (10 mg kg^{-1} i.p.) were injected 55 and 40 min before killing, respectively.

Treatment	Dopa concentration, ng g^{-1} *Corpus striatum*	*Limbic system*
NSD	971 ± 61·4 (5)	391 ± 27·3 (5)
GBL + NSD	2357 ± 97·5 (6)[a]	580 ± 52·7 (6)[a]
B-HT 958 + GBL + NSD	957 ± 30·1 (6)[b]	275 ± 5·3 (6)[b]
Haloperidol + GBL + NSD	2628 ± 77·4 (5)	543 23·9 (5)
Haloperidol + B-HT 958 + GBL + NSD	2461 ± 151·4 (6)	503 ± 28·1 (6)

The values are means ± s.e.m. with the number of experiments in parentheses. Statistical significances were calculated by means of one-way analysis of variance followed by *t*-test ($P < 0·001$ compared with [a] NSD, [b] GBL + NSD).

B-HT 958, markedly reduced the accumulation of dopa following inhibition of the dopa decarboxylase (Table 3). This effect of clonidine was almost completely reversed by B-HT 958.

Motor activity

The locomotor activity of normal mice was reduced from 416 ± 37·9 (mean ± s.e.m., n = 6) to 213 ± 18·1 (n = 6; $P < 0·001$) and 274 ± 41·8 (n = 6; $P < 0·05$) counts per 30 min when B-HT 958 was given immediately before the start of the recording at an intraperitoneal dose of 1 and 10 mg kg^{-1}, respectively. The effect of 10 mg kg^{-1} B-HT 958 was somewhat more pronounced if it was given 60 min earlier (191 ± 30·0, n = 6; $P < 0·001$).

In reserpine-pretreated mice, B-HT 958 at a dose of 10 mg kg^{-1} induced a transient, very slight increase in the locomotor activity and some sniffing (Table 4). These signs occurred only in some of the

Table 3. Accumulation of dopa in the hemispheres and the brain stem of mice. The animals were treated with reserpine (10 mg kg^{-1} i.p., 24 h before killing) and 3-hydroxybenzylhydrazine (NSD 1015, 100 mg kg^{-1} i.p., 30 min before killing). B-HT 958 (100 mg kg^{-1} i.p.) and clonidine (0·1 mg kg^{-1} i.p.) were injected i.p. 90 and 75 min before killing, respectively.

Treatment	Dopa concentration, ng g^{-1} Hemispheres	Brain stem
Reserpine + NSD	135 ± 5·7 (6)	218 ± 19·4
Reserpine + clonidine + NSD	44 ± 2·9 (6)[a]	122 ± 9·5[a]
Reserpine + B-HT 958 + NSD	105 ± 5·4 (6)[a]	262 ± 12·7
Reserpine + B-HT 958 + clonidine + NSD	90 ± 2·4 (5)[b,c]	274 ± 19·3[b]

The values are means ± s.e.m. with the number of experiments in parentheses. Statistical significances were calculated by means of one-way analysis of variance followed by *t*-test ($P < 0·001$ compared with [a] reserpine + NSD, [b] reserpine + clonidine + NSD; $P < 0·05$ compared with [c] reserpine + B-HT 958 + NSD).

Table 4. Effect of B-HT 958 alone or in combination with apomorphine (3·0 mg kg^{-1} i.p. immediately before the recording) on the motor activity of mice pretreated with reserpine (10 mg kg^{-1} i.p., 4 h before the recording). B-HT 958 was injected intraperitoneally immediately or 60 min* before the start of the recording. The values are means ± s.e.m. with the number of experiments in parentheses. Statistical significances were calculated using Student's *t*-test.

Treatment	Counts per 60 min	Difference
A. Reserpine	0·7 ± 0·33 (6)	
B. Reserpine + B-HT 958, 3·0 mg kg^{-1}	1·7 ± 1·12 (6)	B–A: $P > 0.05$
C. Reserpine + B-HT 958, 10 mg kg^{-1}	61·8 ± 21·89 (6)	C–A: $P < 0.025$
D. Reserpine + B-HT 958, 30 mg kg^{-1}	21·5 ± 4·47 (6)	D–A: $P < 0.001$
E. Reserpine + apomorphine	686·7 ± 21·60 (6)	E–A: $P < 0.001$
F. Reserpine + B-HT 958*, 10 mg kg^{-1} + apomorphine	710·3 ± 20·77 (6)	F–A: $P < 0.001$ F–E: $P > 0.05$

mice. The effects were not more pronounced after 30 or 100 mg kg^{-1} of B-HT 958.

Apomorpine, 3 mg kg^{-1}, increased the locomotor activity of reserpine-pretreated mice (Table 4). B-HT 958, 10 mg kg^{-1}, did not affect the stimulatory effect of apomorphine on locomotor activity.

DISCUSSION

B-HT 958 completely inhibited the GBL-induced increase in the synthesis of DA by a mechanism sensitive to haloperidol. This effect occurred without nerve impulses in the DA neurons. Therefore, it is most likely that B-HT 958 stimulates presynaptic DA autoreceptors on the DA nerve terminals in a manner similar to that found previously for apomorphine and B-HT 920 (Kehr et al 1972; Walters & Roth 1976; Andén et al 1982a). In reserpine-treated mice, high doses of B-HT 958 produced at most weak locomotion and sniffing, indicating no, or only a very slight, stimulatory effect on postsynaptic DA receptors. Thus, the haloperidol-sensitive deceleration of the α-MT-induced disappearance of DA by B-HT 958 is most likely to be also due to stimulation of DA autoreceptors. The deceleration of the α-MT-induced disappearance of DA by B-HT 958 has recently also been reported in an abstract (Hörtnagl et al 1983).

B-HT 958 accelerated the α-MT-induced disappearance of NA, in contrast to that of DA. Furthermore, it antagonized the inhibitory effects of the α_2-receptor agonist clonidine on the turnover of NA and on the accumulation of dopa in NA-predominant regions. Therefore, it is likely that B-HT 958 blocks α_2-receptors in the central nervous system in analogy with previous observations on those from the periphery. It should be pointed out that B-HT 958 influenced the turnover of NA only at higher doses than those effective on the turnover of DA. B-HT 958 can also act as an α_2-adrenoceptor agonist in the periphery (Pichler et al 1982). Such an action does not seem to be of importance in the central nervous system since the α-MT-induced disappearance of NA in the brain and the dopa accumulation in the NA-predominant regions were not slowed down by any dose of B-HT 958.

B-HT 958 did not reduce the apomorphine-induced increase in the motor activity of reserpine-treated mice, indicating that B-HT 958 does not block the classical postsynaptic DA receptors. Therefore, the inhibitory effect of B-HT 958 on the motor activity of mice not treated with reserpine was probably caused by stimulation of DA autoreceptors leading to diminished release of DA. The motor activity was possibly somewhat more inhibited following 1 than 10 mg kg^{-1}. The reason for this difference might be a transient and slight stimulation of postsynaptic DA receptors after the high dose. Another possible explanation is a stronger blockade of the α_2-receptors following 10 than 1 mg kg^{-1} of B-HT 958 since blockade of central α_2-adrenoceptors by yohimbine can enhance the mouse motor activity (Andén et al 1982b).

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