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Dopaminergic and α_1-Adrenergic Properties of B-HT920 Revealed in Morphine-Dependent Rats

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VAN DER LAAN, J. W. *Dopaminergic and α_1-adrenergic properties of B-HT920 revealed in morphine-dependent rats.* PHARMACOL BIOCHEM BEHAV **26**(2) 265–269, 1987.—B-HT920 is known to be a selective α_2-adrenoceptor agonist, and has been used in a study on morphine-withdrawal in rats. In accordance with other α_2-agonists B-HT920 was found to potentiate "jumping" and to reduce "body shakes." However, B-HT920 did not suppress body weight loss. Furthermore, it induced strong salivation and prevented ptosis (described for the α_1-adrenergic agonist ST-587). Rearing and locomotor activity appeared to be enhanced, an effect shared by dopamine-agonist lisurid. The effects of B-HT920 have been specified using the α-adrenergic antagonists yohimbine and prazosin and the dopamine antagonist haloperidol. Yohimbine could not antagonize any of the actions of B-HT920. However the increase in rearing and locomotion was blocked by haloperidol. The induction of salivation was prevented by prazosin. Pretreatment with prazosin showed a decrease in the loss of body weight caused by B-HT920, while pretreatment with yohimbine showed that B-HT920 induced an increased loss in body weight. These data suggest that B-HT920 under certain conditions exerts dopamine-agonistic actions in stimulating locomotor activity and α_1-adrenergic actions in inducing salivation and enhanced loss of body weight.

Morphine-withdrawal behaviour B-HT920 Lisurid Haloperidol Prazosin Yohimbine

B-HT920 (2-amino-6-allyl-5,6,7,8 tetrahydro-4H-thiazolo [4,5-d]-azepine dihydrochloride) was synthesized in a series of compounds exhibiting selective action on α_2-adrenergic receptors [7]. It appeared to be a very potent compound, equipotent to clonidine with a much higher affinity for the α_2-receptor compared to the alpha$_1$-receptor and thus more selective than clonidine [14,18]. With respect to the cardiovascular system *in vivo*, B-HT920 can be a valuable tool as a receptor specific drug. However, with respect to behavioural patterns, B-HT920 has an anomalous effect on open field behaviour in being far more potent than expected when compared to other α_2-agonist sensitive models [23]. It has been reported that B-HT920, in contrast to the chemically related compound azepexole (B-HT933), can stimulate dopamine autoreceptors similar to low doses of apomorphine [1]. Also, in our research on the effects of α_2-agonists on morphine-withdrawal behaviour, B-HT920 appeared to have several effects different from those of other α_2-agonists. In this paper we report data indicating that under special circumstances B-HT920 can act as an α_1-adrenergic and dopaminergic agonist.

METHOD

Animals

Male albino rats (150–200 grams) randomly selected from a random bred Wistar strain (CPB-TNO, Zeist, the Netherlands) were used in all experiments. The animals were initially housed six in a wire cage with food and water ad lib. Lights were kept on from 6.00 a.m. till 6.00 p.m. The experiments were performed between 9.00 a.m. and 4.00 p.m. in a room with white noise and a temperature of 22–24°C.

Drugs

The following drugs were used: B-HT920 (2-amino-6-allyl-5,6,7,8 tetrahydro-4H-thiazolo [4,5-d]-azepine dihydrochloride; Boehringer, Ingelheim, FRG), lisurid maleate (Schering Berlin, FRG), yohimbine·HCl (Sigma, St. Louis, MO), prazosin·HCl (Pfizer, NY), haloperidol (Janssen, Beerse, Belgium), naloxone·HCl (Endo, NY), morphine·HCl (OPG, Utrecht, The Netherlands) and morphine (base) (Diosynth, Apeldoorn, The Netherlands). Haloperidol was dissolved in a few drops of glacial acetic acid and the solution was diluted with saline until the appropriate concentration was obtained. When necessary the pH was adjusted to 5–6 with 2 N NaOH.

Prazosin was dissolved in deionized water containing 5% (w/v) glycerol and 5% (w/v) glucose. Yohimbine was suspended in 1% tragacanth. Morphine base was used as a slow release suspension (SRM) and was suspended in a mixture of 0.75 ml mannide mono-oleate (Arlacel A, Sigma), 4.25 ml paraffin oil, and 5 ml saline [3] in a concentration of 30 or 60 mg/ml. The other drugs were dissolved in saline. Yohimbine

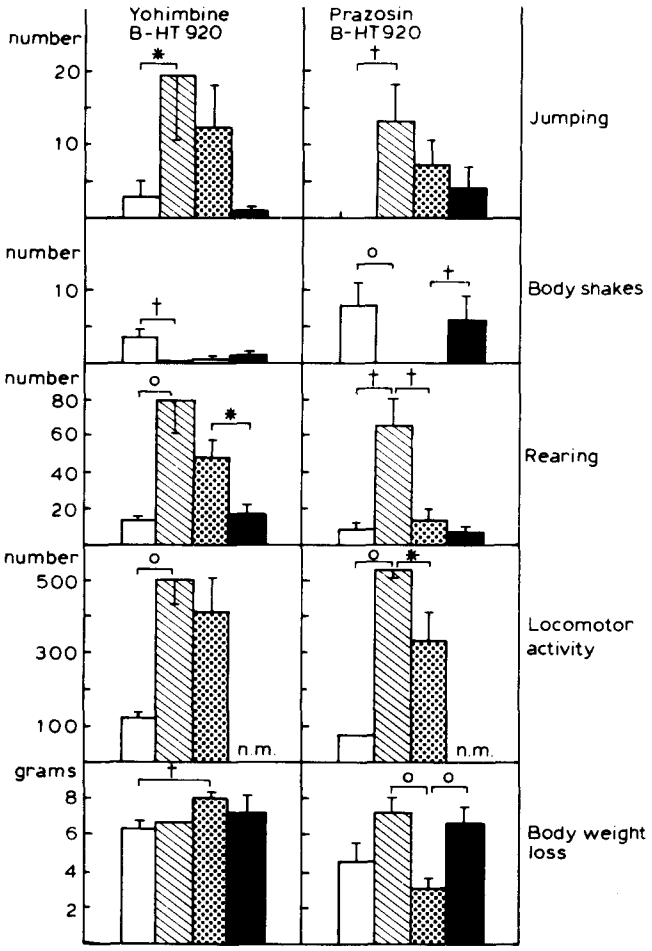

FIG. 1. Interaction between B-HT920 and α-antagonists with respect to their effects on naloxone-precipitated behaviour. Rats were treated with morphine SRM 300 mg/kg at day 1 with SRM 600 mg/kg at day 4. On day 8 they received an injection of naloxone (0.5 mg/kg IP) and subsequently they were observed during 30 min. Yohimbine (3 mg/kg PO) and prazosin (1 mg/kg IP) were injected 45 min before injection with B-HT920 (400 μg/kg SC) and naloxone (0.5 mg/kg IP). White areas: control treatment, injection of antagonist- and agonist-vehicle; striped areas: treatment with antagonist vehicle and B-HT920; stippled areas: treatment with yohimbine (left) or prazosin (right) and B-HT920; black areas: treatment with yohimbine (left) or prazosin (right) and agonist-vehicle. Each group consisted of 6 animals. Data are given as the mean±S.E.M. *$p<0.05$; †$p<0.02$; ○$p<0.005$; Mann-Whitney-U-test. n.m.=not measured.

was given orally whereas prazosin, haloperidol, lisurid and naloxone were administered intraperitoneally·B-HT920, morphine·HCl and the SRM-preparations were injected subcutaneously.

Development of Dependence

Rats were treated subcutaneously at day 1 with morphine HCl (5 mg/kg SC) 10–15 min before injection of the SRM-suspension (morphine base 300 mg/kg, 30 mg/ml). At day 4 the rats received a second injection of SRM containing a twofold dose (morphine base 600 mg/kg, 60 mg/ml). Both injections were given into the back of the animal (on a 4–5 cm distance from the neck to allow for subsequent SC injection of drugs into this region). During this time the animals were singly housed with food and water ad lib.

TABLE 1

EFFECTS OF B-HT920 AND α-ANTAGONISTS ON PTOSIS AND SALIVATION DURING NALOXONE-PRECIPITATED MORPHINE WITHDRAWAL BEHAVIOR

	Ptosis Incidence	p	Salivation Incidence	p	n
Saline	11		0		17
B-HT920	1	0.032	15	3.10^{-7}	18
Yohimbine	4		1		5
Prazosine	3		0		5
B-HT920 + Yohimbine	2		4		5
B-HT920 + Prazosine	1		0	0.0003	6

Rats were treated with morphine SRM 300 mg/kg at day 1 and with SRM 600 at day 4. On day 8 they received an injection of naloxone (0.5 mg/kg IP) and subsequently they were observed during 30 minutes. Yohimbine (3 mg/kg PO) and prazosin (1 mg/kg IP) were given 45 minutes before injection with B-HT920 (400 μg/kg SC) and naloxone. These animals were the same as these in Fig. 1.

Precipitation of Withdrawal and Behavioural Observation

On the eighth day each rat was placed in a perspex/glass box (box area 25×30 cm, height 25 cm) with sawdust bedding and a macrolon cage as a lid. Shortly thereafter animals received a subcutaneous injection of B-HT920 followed directly by an IP injection of naloxone. Lisurid, haloperidol, yohimbine or prazosin were given 45 min before naloxone. Subsequent behaviour was continuously observed over 30 min. Four animals were observed simultaneously. The following signs were scored as present or absent: salivation, diarrhea, ptosis, teeth chattering, hunchback posture and pilo-erection.

"Escape jumping" (all feet off the bottom), "wet dog shakes" (body shakes involving trunk and shoulders), head shakes and "rearing" (the two forepaws off the bottom against the wall or in the air) were counted. Shortly before and directly after the observation period the animals were weighed.

Horizontal activity was measured using photocell activity meters in which the activity was measured as the number of crossings of a line parallel to the longest sides of the cage through the centre of the cage.

Statistics

The Mann-Whitney U-test [17] was used for evaluating the results of the behavioural studies. Differences with a probability equal to or lower than 5% (one-tailed) were considered to be statistically significant. Differences between incidences were tested using the Fisher's exact probability test.

RESULTS

Effects of B-HT920 and Interaction With Alpha-Antagonists

B-HT920 (0.4 mg/kg SC) given 1 min before nalxone (0.5 mg/kg IP) induced an increase in jumping, rearing and hori-

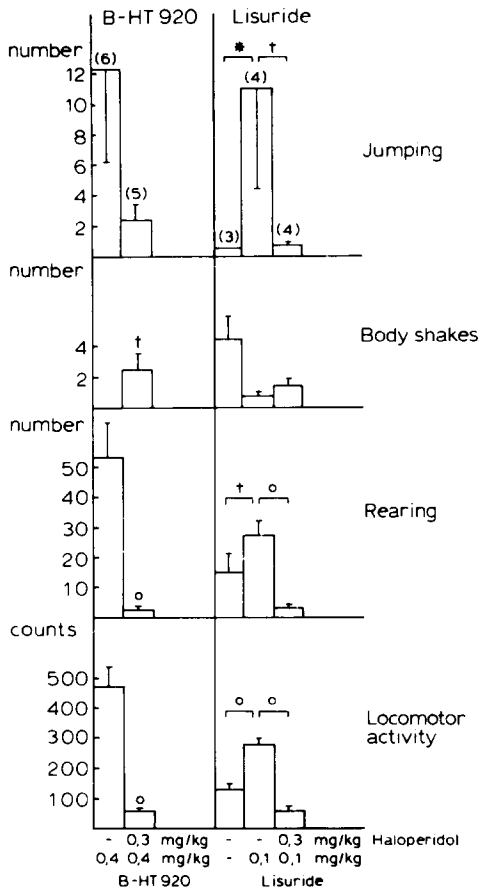

FIG. 2. Interaction between B-HT920 and lisuride with haloperidol. Rats were treated as in Fig. 1. Haloperidol and lisuride were given intraperitoneally 45 min before naloxone in doses given under the columns. B-HT920 was given subcutaneously just before naloxone (0.5 mg/kg IP). Each group consisted of 12 animals in the case of B-HT920 and 8 in the case of lisuride. Data are given as the mean±SEM, *$p<0.05$; †$p<0.02$; O$p<0.005$; Mann-Whitney-U-test. Numbers between brackets represent the number of animals exhibiting jumping.

zontal activity and a decrease in body shakes (Fig. 1). Furthermore it induced strong salivation ($p<0.01$, Fisher) while it prevented ptosis ($p<0.05$, Fisher) (see Table 1). Yohimbine (3.0 mg/kg PO) and prazosin (1.0 mg/kg IP) did not have any effect on morphine-withdrawal symptoms, precipitated by naloxone (Fig. 1). Pretreatment with yohimbine did not change the effects of B-HT920, except in enhancing the body weight loss. Prazosin, however, antagonized the increase in rearing and partly the increase in horizontal activity, while it decreased the body weight loss. Pretreatment with prazosin prevented fully the induction of salivation by B-HT920 ($p<0.01$, Fisher).

Comparison of B-HT920 and Lisurid: Antagonism by Haloperidol

B-HT920 (0.4 mg/kg SC) given 1 min before naloxone (0.5 mg/kg IP) induced an increase in jumping, rearing and locomotor activity and a decrease in body shakes (Fig. 2) similar to the results in the first experiment. Groups with only antagonist- and agonist-vehicle were deleted from the experiment. Treatment of the animals with lisurid (0.1 mg/kg IP) 45 min before naloxone also induced an increase in jumping, when comparing the jumping animals [mean±S.E.M.: 60±0.2 (vehicle-vehicle) vs. 22±12 (vehicle-lisuride) as a score of positive animals]. Rearing and locomotor activity were increased while no significant effects were found on body shakes. Haloperidol (0.3 mg/kg IP) did not have any significant effect on morphine-withdrawal symptoms precipitated with naloxone. It reversed, however, several effects of B-HT920, viz. the decreasing effects on body shakes and the increasing effect on locomotor activity and rearing (Fig. 2). Concurrent treatment with haloperidol and lisurid prevented the effects of the latter compound on jumping (when relating the positive animals 22.3±12.0 (vehicle-lisuride) vs. 1.3±0.3 (haloperidol-lisuride) and on locomotor activity and rearing. For the other symptoms such as body shakes and body weight loss no significant differences between groups were found.

DISCUSSION

The present data indicate that the action of B-HT920, known to be an α_2-adrenergic agonist, may involve dopaminergic and α_1-adrenergic receptors. Compared to other α_2-agonists as clonidine and azepexole, B-HT920 could not decrease the body weight loss in naloxone-precipitated morphine-withdrawal. This contrasts with other α_2-agonists such as clonidine and azepexole [21]. On one hand antagonism of the α_2-adrenergic receptor by yohimbine caused an increase in body weight loss by B-HT920, an effect expected to be α_1-adrenergic [13]. On the other hand, pretreatment with prazosin, leaving the α_2-adrenergic action undisturbed, revealed a decrease in body weight loss belonging to an α_2-adrenergic effect. Furthermore, B-HT920 induced salivation, an effect which could be antagonized by prazosin (Table 1). Similar effects on body weight loss and salivation have been described for ST-587, a lipophilic α_1-agonist [21]. This data suggests for the first time that the action of B-HT920 *in vivo* may involve α_1-adrenergic receptors under special conditions. Until now only *in vitro* experiments indicated that B-HT920 in addition to its α_2-adrenergic properties may act under certain conditions as an α_1-adrenergic agonist [10,19]. The decrease in B-HT920-stimulated locomotor activity by prazosin indicate also an α_1-receptor mediated action of B-HT920. However, a dopaminergic action appears to be present simultaneously (see below). The loss of specificity of B-HT920 cannot be due only to the use of high doses since it has been shown that *in vitro* the ratio between α_2 and α_1-effects is much higher (±500) than the ratio of dosage for B-HT920 used here ($20\times ED_{50}$ for a standard α_2 effect; [23]). It can be questioned whether such a high dose of B-HT920 will induce a direct effect on behaviour or induces strong disturbances in blood pressure and other peripheral symptoms causing behavioural disturbances indirectly. Data obtained with clonidine [9,16] however, indicate that the excitatory action of this α_2-agonist does not occur without the simultaneous administration of naloxone. Naloxone seems to activate the organism in any way so that it is more sensitive to these agonists [8,16], e.g., via an alteration in receptor number or an increase in receptor response (adenylate cyclase, [8]). The excitatory action would probably not be the outcome of the inhibition of the sympathetic nervous system since in morphine-withdrawal this system is hyperactive [13]. Furthermore the effect of B-HT920 alone on the behaviour of rats is a dimunution of spontaneous activity [23]. The latter facts suggest that the excitatory

action of B-HT920 seen in the present study again is the result of an interaction with naloxone.

The pharmacological character of the excitatory action of B-HT920 has been studied using single doses of various antagonists. Previous studies have shown that using these doses no excitatory, but rather slight inhibitory, effects of these antagonists are present [21,24]. The absence of potentiation of morphine-withdrawal behaviour by yohimbine is in agreement with this data [21] and may be explained by a maximally active noradrenergic system under the conditions used here. It is remarkable that the potentiation of jumping by B-HT920 could not be antagonized by yohimbine, in contrast to what has been described for the effects induced by clonidine and azepexole [21]. Also the effects of lisuride were not affected by yohimbine (data not shown). The effects observed for lisuride on morphine-withdrawal behaviour fully agree with those described by others [4]. The increase in locomotor activity and rearing induced by B-HT920 was the third anomaly in comparison with the effects of other α_2-agonists [21]. The antagonism of the effects of B-HT920 on locomotor activity and rearing by haloperidol suggests the involvement of dopaminergic receptors in the action of B-HT920. Recently, an *in vivo* dopamine-agonistic action has been described for B-HT920 in rats and mice, in contrast to azepexole [1,22], which is in agreement with the present data. Although it has been reported that lisuride may have a high affinity for the α_2-adrenergic receptor recent data indicate that lisuride is a potent antagonist at this receptor lacking agonistic properties [11,12]. Serotoninomimetic properties for lisuride have been recently described [5]. However data of Samanin *et al.* [15] as well as results of own research [22] have shown that a serotonin agonist, m-chlorophenyl piperazine, suppresses withdrawal jumping.

In conclusion, the action of B-HT920 appears to involve α_1-adrenergic receptors when morphine-withdrawal behaviour is present. The mechanism by which naloxone exerts its interaction is not clear but seems to be related to the precipitation of the withdrawal. Morphine tolerance and withdrawal have been shown to alter the number of various receptors [6, 8, 15]. However, for α_1-receptors no changes in number or affinity have been described [6]. Other mechanisms for changing the receptor response may be possible. In addition, B-HT920 may act via dopaminergic receptors and this action may be responsible for the observed differences with compounds such as azepexole and clonidine.

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