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2-Bromolisuride, an Ergot Derivative, With Dopamine Antagonistic and Serotonin Agonistic Properties

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FINK, H , R MORGENSTERN AND T OTT *2-Bromolisuride, an ergot derivative, with dopamine antagonistic and serotonin agonistic properties* PHARMACOL BIOCHEM BEHAV **38**(2) 321–325, 1991 —The open-field test was used to study the involvement of dopaminergic and serotonergic mechanisms in the effects of 2-bromolisuride on locomotor activity in the rat 2-Bromolisuride produced a dose-dependent inhibition of spontaneous locomotor activity This is most likely due to an antagonistic action at postsynaptic dopamine receptors Low doses of 2-bromolisuride potentiated apomorphine-induced hypermotility This potentiating effect was not mediated by a blockade of presynaptic dopamine receptors, because it was not prevented by 6-OHDA lesion of the nucleus accumbens The potentiating effect of 2-bromolisuride was completely blocked by the serotonin antagonists cyproheptadine and ritanserin It is suggested that 2-bromolisuride possesses dopamine antagonistic and serotonin agonistic properties

Apomorphine 2-Bromolisuride Dopamine antagonist Lisuride Locomotor activity Rat Serotonin agonist

THE derivative of lisuride, 2-bromolisuride (Br-LIS) is the first ergot compound with antidopaminergic properties. This was evident from investigations assessing dopamine receptor blocking actions of drugs. Thus Br-LIS induces catalepsy in mice, antagonizes dopamine agonist-induced stereotypies in rats and mice, inhibits locomotor activity in rats, antagonizes dopamine agonist-induced locomotor hyperactivity in rats and inhibits apomorphine-induced hypothermia in mice (21,22).

Further, Br-LIS stimulates the dopamine biosynthesis and DOPAC formation in rat striatum and limbic system, reverses the apomorphine-induced inhibition of DOPA accumulation in these structures of γ-butyrolactone-pretreated rats and enhances the prolactin secretion in male rats and man (9, 10, 20, 21)

In most tests Br-LIS is approximately as potent as haloperidol. Taken together, all findings indicate that Br-LIS blocks pre- and postsynaptic dopamine receptors like a classical neuroleptic (11). The parent substance of Br-LIS, lisuride, is known to act as a central dopamine agonist at pre- and postsynaptic receptors and to display serotonin agonistic properties, too. This complex action of lisuride was shown in a large number of electrophysiological, neurochemical and behavioral studies (1, 3, 7, 8, 14–18, 23–25) Therefore, the 2-brominated lisuride derivative, Br-LIS, was expected to influence central serotonergic mechanisms, too.

In previous papers we were able to demonstrate that locomotor activity is a suitable parameter to reflect interactions between the serotonergic system and the mesolimbic dopaminergic system and to characterize psychotropic drugs with respect to the transmission system primarily involved in their actions (4, 5, 12). Therefore, it was of interest to investigate Br-LIS-induced locomotor effects regarding a complex involvement of both serotonergic and dopaminergic functions.

METHOD

Animals

The experiments were performed on male Wistar rats (VEB Versuchstierproduktion Schonwalde) weighing 140–160 g. They were housed in groups of 10 animals per cage at a room temperature of 22 ± 2°C and a 12-h light-dark schedule. The rats received food and water as required

Measurement of Locomotor Activity

The experiments were carried out between 8:00 a.m and 11 00 a.m. and between 2 00 p.m. and 4 00 p m. in a soundproof room. Prior to the experiment the animals were adapted to that room for at least 2 h Locomotor activity of rats in a new environment was measured in an open-field, consisting of a 1 × 1 m

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area surrounded by a 40 cm high wall. An array of 10 infrared light beams divided this area into 36 equal squares The detector signals, produced by interrupting these photobeams, were fed into a digital logic providing a selective automatic counting of squares, crossed by the animal during a 5-min observation period. Repetitive interruptions of one photobeam induced by stereotyped behavior, e.g., sniffing, rearing, or rotations were not registered Rats were used only once.

Values are presented as the mean ± standard error of the mean (SEM). Drug effects were assessed using Mann-Whitney U-test or Kruskal-Wallis H-test.

Measurement of Stereotyped Behavior

Stereotypies induced by apomorphine were recorded according to Wachtel et al. (22). One hour before experimentation individual rats were placed into acrylic glass cages (15 × 25 × 15 cm). Animals were given saline or Br-LIS 30 min prior to apomorphine. Ten min after apomorphine or saline the animals were observed for 2 min for the presence of stereotyped behavior (continuous sniffing, licking or gnawing movements for more than 30 s within this time) by an experienced observer unaware of the previous treatment. Statistical significance was evaluated using two-way contingency table analysis.

Drugs

The following drugs were used· apomorphine hydrochloride (SPOFA), 2-bromolisuride (Br-LIS) (Schering AG, Berlin), cyproheptadine hydrochloride (Sharp & Dohme), desipramine hydrochloride (AWD), 6-hydroxydopamine hydrochloride (6-OHDA) (Fluka), (SPOFA), pargyline hydrochloride (Fahlberg-List), ritanserin (Janssen) Ritanserin, 2-bromolisuride, and cyproheptadine were generous gifts from the respective drug companies. All drugs were dissolved in 0.9% NaCl, except 6-OHDA, which was dissolved in 0.1% ascorbic acid. All doses were calculated as the salt The injection volume of intraperitoneally (IP) administered drugs was 10 ml/kg body weight. The interval between the injection of drug and the commencement of testing was 7 min for apomorphine, and 30 min for the other drugs.

6-OHDA Administration

Rats were anesthetized with sodium hexobarbital (120 mg/kg IP) and positioned in a stereotactic apparatus after pretreatment with desipramine (25 mg/kg IP) and with pargyline (50 mg/kg IP). The neurotoxin 6-OHDA (8 μg) or 0.9% NaCl were administered by means of a Hamilton μl syringe (CR 700–20) in volume of 0.5 μl over a period of 2 min. The injection cannula had an outer diameter of 0.23 mm. The coordinates of the cannula tip were, according to Konig and Klippel for the nucleus accumbens A = 9.4, L = 1.2, DV = −1.0. Lesion was performed bilaterally One week after surgery the behavioral experiments were carried out Rats were killed after the experiment for histological examination Data were discarded if the lesion was not in the nucleus accumbens.

RESULTS

Figure 1 shows that Br-LIS induced a dose-dependent decrease of locomotor activity of rats. The minimum dose of Br-LIS effective in depressing locomotion was 0.06 mg/kg ($p<0.05$). The effect of this dose of Br-LIS on apomorphine-induced locomotor effects is illustrated in Fig. 2. Apomorphine induced in low doses a weak locomotor inhibition, in higher doses a strong locomotor stimulation. A further increase of doses of apomorphine led to a decrease of locomotor stimulation due to the occurrence of competing behavior. The rats showed stereotypies, e.g., rearing, grooming and sniffing. Br-LIS produced a potentiation of the locomotor effects induced by higher doses of apomorphine

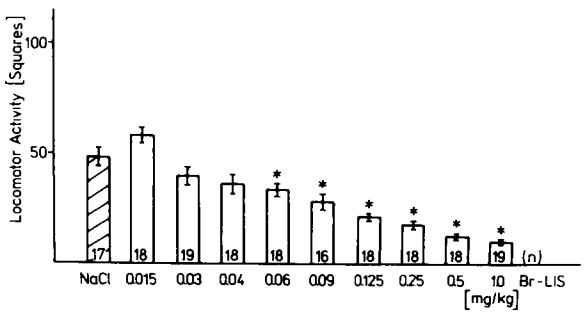

FIG 1 Dose-dependent inhibition of locomotor activity produced by increasing doses of Br-LIS Crossed squares of an open-field were counted for 5 min Bars represent means ± SEM n = number of rats *$p<0.05$ versus control (NaCl)

To determine the dose range of Br-LIS that influences the apomorphine hypermotility, the effect of increasing doses of Br-LIS on hypermotility induced by 2 mg/kg apomorphine was investigated. As shown in Fig. 3 Br-LIS in doses of 0.03 mg/kg and 0.06 mg/kg potentiated the apomorphine hypermotility, whereas doses above 0.09 mg/kg inhibited the apomorphine hypermotility.

Figures 4 and 5 show that cyproheptadine and ritanserin suppressed completely the potentiating effect of Br-LIS on apomorphine hyperlocomotion. The used doses of cyproheptadine and ritanserin did not alter spontaneous locomotor activity as well as apomorphine hyperlocomotion.

Table 1 demonstrates that Br-LIS in doses of 0.03 mg/kg and 0.06 mg/kg failed to influence stereotyped behavior induced by apomorphine in doses of 1 mg/kg and 2 mg/kg. Figure 6 shows that after chemical lesion of dopaminergic terminals within the nucleus accumbens by 6-OHDA both spontaneous locomotor activity and apomorphine hyperlocomotion were slightly increased. Locomotor hyperactivity induced by apomorphine plus Br-LIS

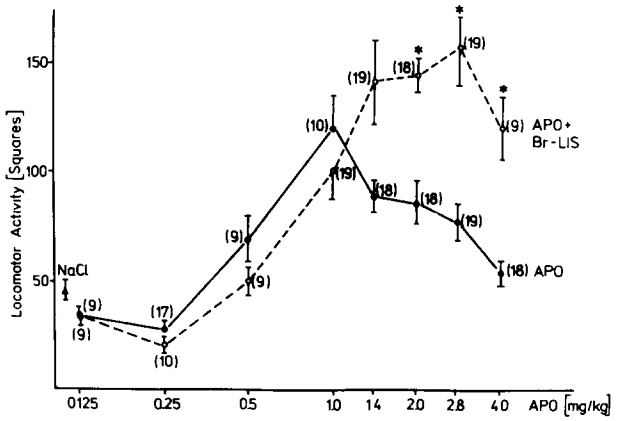

FIG 2 Influence of Br-LIS (0 06 mg/kg) on dose-response curve for apomorphine (APO)-induced locomotor effects. Crossed squares of an open-field were counted for 5 min. Data represent means ± SEM ● Apomorphine, ○ apomorphine plus Br-LIS Number of rats used are shown in the figure *$p<0.01$ versus corresponding apomorphine values

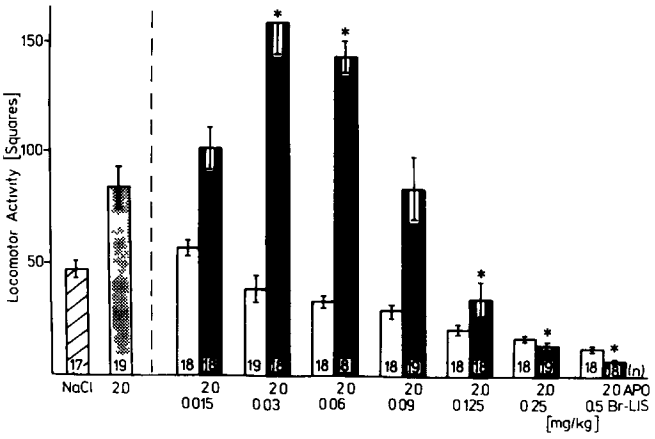

FIG 3 Influence of increasing doses of Br-LIS on hyperlocomotion induced by apomorphine (APO) (2 mg/kg) Crossed squares of an open-field were counted for 5 min Bars represent means ± SEM Stippled bar = apomorphine, open bars = Br-LIS, solid bars = Br-LIS plus apomorphine, n = number of rats *$p<0.01$ versus apomorphine

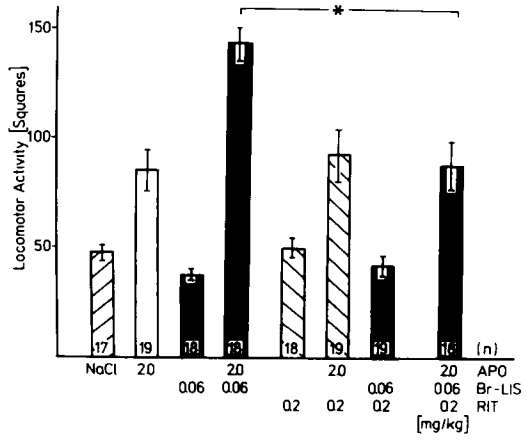

FIG 5 Influence of ritanserin (RIT) (0 2 mg/kg) on Br-LIS-induced potentiation of apomorphine (APO) hypermotility Crossed squares of an open-field were counted for 5 min Bars represent means ± SEM, n = number of rats *$p<0.01$ as indicated

was strongly increased in comparison to sham-lesioned rats. Thus locomotor effects induced by apomorphine and Br-LIS were not altered in quality by this lesion, i.e., the potentiating effect induced by Br-LIS was not affected

DISCUSSION

In the open-field test Br-LIS produced a dose-dependent inhibition of exploratory locomotor activity very similar to classical neuroleptics. As compared with our previously published data, on a molar base, Br-LIS was approximately as potent as haloperidol and somewhat more potent than phenothiazines (13). Our results are in a very good agreement with findings of Wachtel et al. (22) on the locomotor inhibitory effect of Br-LIS. This reduction in spontaneous locomotor activity and also the reduction of apomorphine-induced hyperlocomotion by higher doses of Br-LIS seem to be due to the blockade of postsynaptic dopamine receptors in the mesolimbic system, as it has been demonstrated in both neurochemical and behavioral studies (11, 21, 22). However, the present study has shown that low doses of Br-LIS induced a potentiation of apomorphine-induced hypermotility. In previous studies, using the same experimental design, we investigated the effect of the classical neuroleptic haloperidol in a low dose, which itself did not influence spontaneous locomotor activity, on apomorphine-induced hypermotility. In contrast to Br-LIS, haloperidol was found to induce a parallel shift of the dose-response curve of apomorphine without any potentiation of apomorphine hypermotility (12).

The potentiating effect of Br-LIS appears unlikely to be mediated by the antagonist action of Br-LIS at postsynaptic mesolimbic dopamine receptors. One explanation for this effect of Br-LIS may be its induction by blocking presynaptic dopamine receptors in the mesolimbic system.

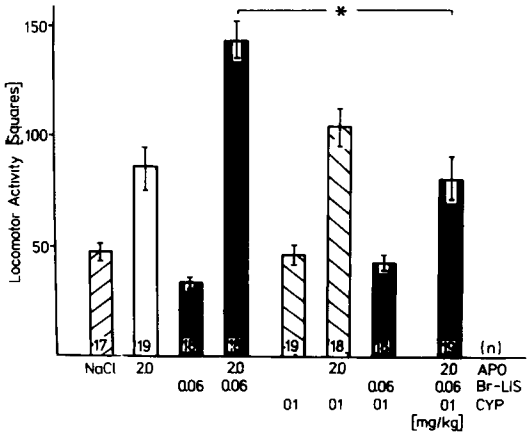

FIG 4 Influence of cyproheptadine (CYP) (0 1 mg/kg) on Br-LIS-induced potentiation of apomorphine (APO) hypermotility. Crossed squares of an open-field were counted for 5 min Bars represent means ± SEM, n = number of rats *$p<0.01$ as indicated

TABLE 1

INFLUENCE OF Br-LIS ON STEREOTYPED BEHAVIOR INDUCED BY APOMORPHINE

Treatment	Stereotyped Behavior
NaCl	0/20
Br-LIS (0 03 mg/kg)	0/20
Br-LIS (0 06 mg/kg)	0/20
Apomorphine (1 mg/kg)	10/20
Apomorphine (1 mg/kg) + Br-LIS (0 03 mg/kg)	12/20
Apomorphine (1 mg/kg) + Br-LIS (0 06 mg/kg)	10/20
Apomorphine (2 mg/kg)	28/30
Apomorphine (2 mg/kg) + Br-LIS (0 03 mg/kg)	20/20
Apomorphine (2 mg/kg) + Br-LIS (0 06 mg/kg)	18/20

Values indicate number of rats displaying stereotyped behavior

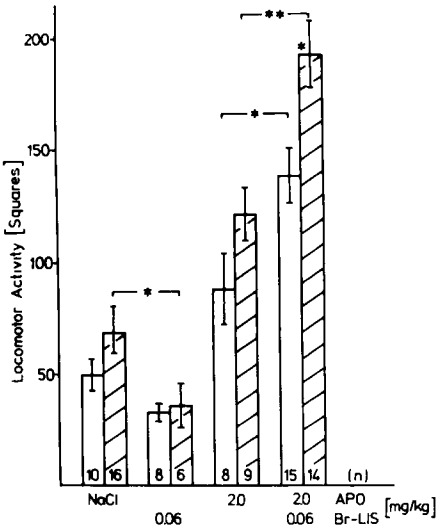

FIG 6 Influence of 6-OHDA lesion of the nucleus accumbens on Br-LIS-induced potentiation of apomorphine (APO) hypermotility Crossed squares of an open-field were counted for 5 min Bars represent means ± SEM. Open bars = sham-operated rats, striped bars = 6-OHDA-lesioned rats, n = number of rats *$p<0$ 05 as indicated and apomorphine plus Br-LIS in sham-operated rats versus apomorphine plus Br-LIS in 6-OHDA lesioned rats, respectively **$p<0$ 005 as indicated

Therefore, 6-OHDA lesion of the nucleus accumbens was performed to destroy the dopamine nerve terminals in this structure, which is known to be mainly involved in the induction and expression of locomotor effects. However, the potentiating effects of Br-LIS remained.

Another explanation for the potentiating effect of Br-LIS may be that Br-LIS affects the nigrostriatal system and antagonizes apomorphine-induced stereotypies and, consequently, reinstates the locomotor activity. Therefore, the influence of Br-LIS in doses, which potentiated apomorphine hypermotility, on apomorphine-induced stereotyped behavior was investigated. Br-LIS failed to influence apomorphine-induced stereotypies. Thus we concluded that the potentiating effect of Br-LIS is mediated by an action on another transmission system.

In previous studies we found that serotonin agonists potentiate apomorphine-induced hypermotility (4,5). It has been demonstrated that serotonin agonists in low doses act preferentially at somatodendritic receptors at serotonergic midbrain raphe neurons, thus reducing activity of the serotonergic system [(2), see (4)].

Serotonergic and dopaminergic systems interact in mesolimbic structures as the serotonergic system inhibits dopaminergic function. Therefore, the consequence of action of serotonin agonists in low doses is an augmentation of dopaminergic-induced hypermotility. Since serotonin agonists and Br-LIS are very similar in their effects a serotonin agonistic action of Br-LIS was assumed

This is also supported by the fact that Br-LIS is a derivative of lisuride, which is known to be one of the most potent serotonin autoreceptor agonists (8,14). To prove this idea the influence of serotonin antagonists on Br-LIS-induced potentiation effect was investigated. Cyproheptadine was used in a dose which has been shown to antagonize serotonergic locomotor effects (6). Additionally, the potent and more specifically acting serotonin antagonist ritanserin was included in this study (19). Both serotonin antagonists suppressed the potentiating effect of Br-LIS on apomorphine hypermotility completely This suggests a serotonin agonist action of Br-LIS. Neurochemical studies are necessary to clarify whether the serotonin agonist action of Br-LIS is due to direct serotonin receptor stimulation or a functional effect.

In summary, our data indicate that the dopamine antagonistic component of Br-LIS appears to predominate under normal conditions, while the serotonin agonistic component becomes apparent under conditions when the mesolimbic dopamine receptors are stimulated. Taken together, behavioral investigations with Br-LIS suggest that this compound represents a lisuride derivative with a more complex neuropharmacological profile.

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