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Multiple Postsynaptic Dopamine Receptors and Behavioral Manifestation

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FUJITA, N , A HIRATA, K SAITO AND H YOSHIDA *Multiple postsynaptic dopamine receptors and behavioral manifestation* PHARMAC BIOCHEM BEHAV **16**(3) 437–440, 1982 —Previously we demonstrated the existence of non- and positive-cooperative dopamine receptors in rat striatum in [^{3}H] apomorphine binding experiments Non-cooperative sites were sensitive to the inhibition of sulpiride while cooperative sites were not In the present study results dealing with the involvement of those two types of postsynaptic dopamine receptors in different behavioral manifestations is shown employing lisuride hydrogen maleate (LHM) LHM elicited contralateral turnings in 6-hydroxydopamine lesioned rats unilaterally in the striatum whereas it caused ipsilateral turnings in kainic acid lesioned rats as was observed following the administration of apomorphine Furthermore, the effect of LHM on rotating behavior was abolished by the pretreatment with sulpiride On the other hand, LHM inhibited apomorphine induced stereotyped behavior whereas sulpiride failed to block it These results suggested the dual action of LHM on multiple postsynaptic dopamine receptors The results also indicated that non-cooperative postsynaptic dopamine receptors are involved in rotating behavior while cooperative receptors participate in the elicitation of stereotypy

Dopamine receptors Stereotyped behavior Rotating behavior

BINDINGS of [^{3}H] haloperidol, [^{3}H] spiroperidol or [^{3}H] apomorphine to striatal membranes were stereospecifically inhibited by butaclamol isomers The stereospecific bindings of these ligands showed characteristics of dopamine receptors in central nervous system (CNS) being concentrated in the striatum and inhibited by dopaminergic agonists and antagonists [2–4, 15, 16, 18] Thus, we previously demonstrated the existence of two distinct postsynaptic dopamine receptors in rat striatum in [^{3}H] apomorphine binding experiments, one of which gave a simple bimolecular reaction with [^{3}H] apomorphine and was inhibited by sulpiride, while the other one exhibited cooperativity and was insensitive to the inhibition of sulpiride [5,6] In behavioral studies it is well known that brain dopamine is involved in expressing many different types of behaviors Perhaps the most typical behavioral responses observed following the administration of dopaminergic agonists are locomotor activity and stereotyped behavior It was also shown that administration of apomorphine to 6-hydroxydopamine (6-OHDA) lesioned rats unilaterally in the striatum reveals contralateral rotating behavior It is of interest to correlate two distinct postsynaptic dopamine receptors with the expression of these behaviors In the present study, results indicating the involvement of cooperative and non-cooperative dopamine receptors in stereotypy and rotating behavior, respectively, are shown employing lisuride hydrogen maleate (LHM), an ergot derivative which is shown to possess both agonistic and antagonistic activities for multiple dopamine receptors

METHOD

Butaclamol (Ayerst Res Lab) sulpiride (Fujisawa) LHM (Schering AG) and methysergide (Sandz AG) were kindly given to us [^{3}H] Apomorphine (38 6 Ci/mmol) was obtained from New England Nuclear 6-OHDA and kainic acid (KA) were obtained from Regis Chemical and Sigma Chemical, respectively

Male Sprague-Dawley rats (280–300 g, Charles River Lab , Japan) were anethetized with Nembutal (40 mg/kg) and mounted in a David Kopf stereotaxic apparatus Eight μg of 6-OHDA in 2 μl saline or 2 5 μg KA in 1 μl saline were unilaterally injected into striatum according to Pellegrino and Cushman's coordinate (A 2 2, L 2 8, V 4 8) [12] at the rate of 1 μl/2 min

Rats were decapitated and their striata were homogenized in 25 vols of 25 mM Tris-HCl buffer (pH 7 4) containing 5 mM Na_2EDTA and 0 02% ascorbate The homogenate was centrifuged at 100,000×G for 10 min The supernatant was discarded and the pellet was resuspended in 25 vol of homogenization medium

For assay of [^{3}H] apomorphine binding, the membrane suspension was incubated at 37°C for 5 min in 2 ml of medium consisting of 25 mM Tris-HCl buffer (pH 7 4), 5 mM Na_2EDTA, 0 02% ascorbate and [^{3}H] apomorphine in the presence of 10^{-6} M (−)- or (+)-butaclamol as reported previously [5,6] The reaction was terminated by filtering the incubation medium through Whatman glass filters (GF/F) The filters were then washed 4 times with 2 ml of homogenization medium and radioactivity was counted in a liquid scintillation counter The difference in the amount of [^{3}H] apomorphine bound in the presence of 10^{-6} M (−)- and (+)-butaclamol was designated as specific [^{3}H] apomorphine binding to striatal membranes

Stereotyped behavior was measured according to the

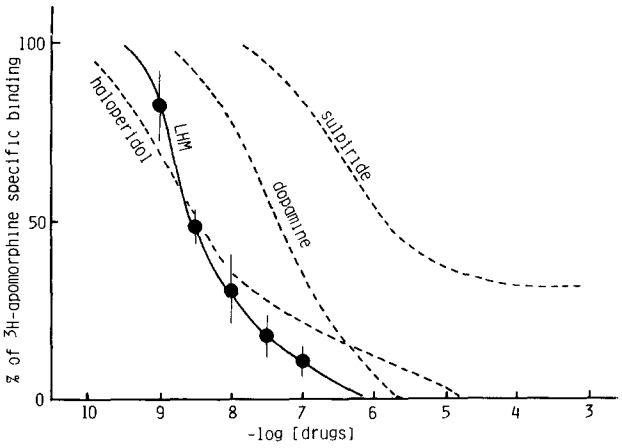

FIG 1 Inhibition of [^{3}H] apomorphine binding to rat striatal membranes by LHM (solid line) Inhibition of [^{3}H] apomorphine binding by dopamine, haloperidol or sulpiride was shown for reference Bars indicate S D of three experiments

TABLE 1
EFFECTS OF LHM AND SULPIRIDE ON [^{3}H] APOMORPHINE BINDING

	Specific [^{3}H] apomorphine binding % of control
Control	100
LHM (3×10^{-9} M)	49 7 ± 4 2
Sulpiride (10^{-5} M)	31 5 ± 3 7
LHM (3×10^{-9} M) plus Sulpiride (10^{-5} M)	33 8 ± 3 9

The specific [^{3}H] apomorphine binding was measured in the presence of 6 nM ligand Each value is the mean ± S D for three experiments of triplicate assays

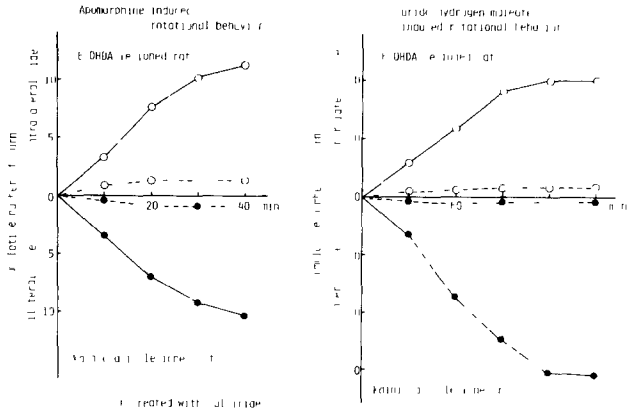

FIG 2 Effect of apomorphine and LHM on 6-OHDA and KA lesioned rats in striatum Rotating behavior was measured 2 weeks following the treatment with neurotoxins Apomorphine (200 μg/kg)(A) or LHM (50 μg/kg)(B) was given to 6-OHDA and KA lesioned rats Effects of sulpiride (50 mg/kg) on apomorphine or LHM induced rotational behavior was also examined (dashed lines in figures)

scoring system of Costall *et al* [1] by injecting 500 μg/kg apomorphine (SC) to rats

RESULTS

In the previous report [6], we have shown that dopamine inhibits the [^{3}H] apomorphine binding to striatal membranes monophasically with the Hill coefficient of 0 9, whereas haloperidol, a dopaminergic antagonist, displays biphasic inhibition with the coefficients of 0 9 and 0 4 Sulpiride inhibited [^{3}H] apomorphine binding only the portion which was reduced by haloperidol with the Hill coefficient of 0 9 (Fig 1, dashed line) In the present study, it was shown that LHM also inhibits [^{3}H] apomorphine binding with the same pattern as was observed by haloperidol (Fig 1) Thus, the inhibitory effect of LHM with higher affinity (IC_{50}=1 3 nM) was observed by 70% with the Hill coefficient of 1 2 and that with lower affinity (IC_{50}=47 nM) was 30% with the coefficient of 0 6 Furthermore, 3×10^{-9} M LHM, inhibiting only the former portion, and 10^{-5} M sulpiride were not additive in inhibiting [^{3}H] apomorphine binding (Table 1), suggesting that both of them at those concentrations block same [^{3}H] apomorphine binding sites Therefore, the former may correspond to the inhibition of sulpiride sensitive non-cooperative [^{3}H] apomorphine binding while the latter is equivalent to that of sulpiride insensitive cooperative binding sites [6]

Effect of LHM on 6-OHDA and KA lesioned rats in the striatum was examined As shown in Fig 2, LHM induced contralateral turnings in 6-OHDA lesioned rats whereas it caused ipsilateral turnings in KA lesioned rats as was observed following the administration of apomorphine Furthermore, the effect of LHM and apomorphine on rotating behavior was abolished by the pretreatment of rats with sulpiride

Effect of LHM on apomorphine induced stereotyped behavior was also examined It was observed that LHM inhibits apomorphine induced stereotyped behavior (Fig 3) Subcutaneous injection of 500 μg/kg apomorphine induced intense stereotyped behavior Pretreatment of rats with 100 μg/kg LHM suppressed apomorphine induced stereotyped behavior (Fig 3A) Further increase of the amount of LHM to 500 μg/kg completely inhibited apomorphine induced stereotyped behavior (Fig 3B) On the other hand, methysergide, an ergot derivative which has antagonistic activity for serotonin receptors, failed to block apomorphine induced stereotyped behavior even with the doses of 17 mg/kg (not shown) Sulpiride failed to inhibit apomorphine induced stereotyped behavior (Fig 3C) LHM alone (100 μg/kg or 500 μg/kg) induced exploratory activity with periodic sniffing More predominantly, LHM enhanced locomotor activity which is blocked by sulpiride (not shown)

DISCUSSION

Our result showing the induction of rotating behavior in 6-OHDA lesioned rats by LHM is in good agreement with

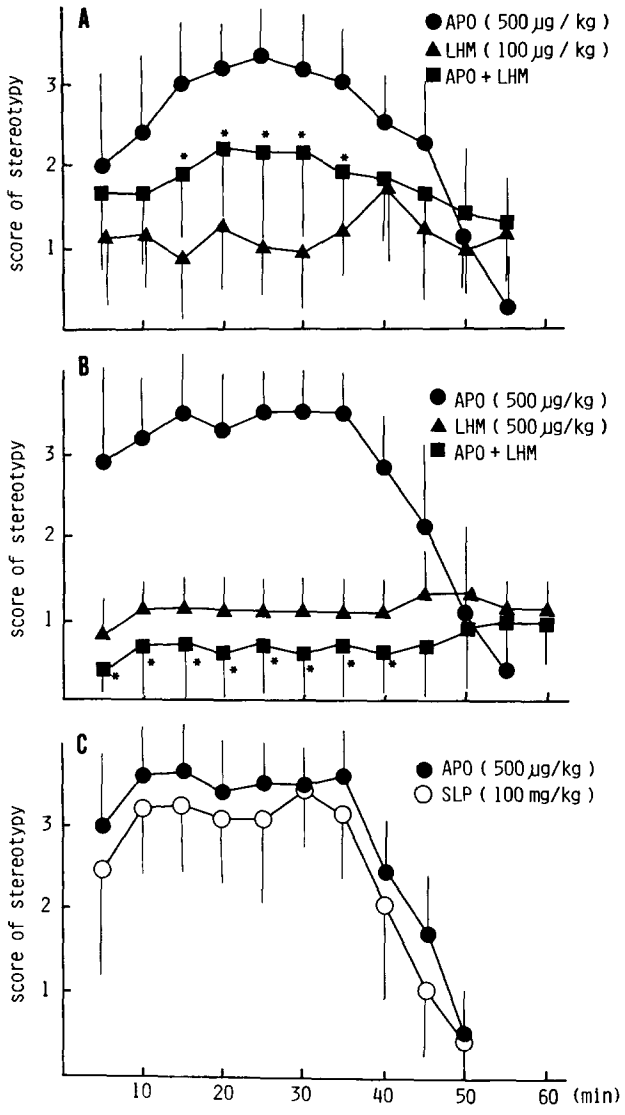

FIG 3 Effect of LHM and sulpiride on apomorphine induced stereotyped behavior Either 100 μg/kg LHM (A) or 500 μg/kg LHM (B) was administered 20 min prior to apomorphine Sulpiride (100 mg/kg) was given 20 min prior to apomorphine (C) *Significantly different from that injected apomorphine at $p<0$ 01 (N=20 in A and 10 in B and C) Bars indicate S D of 20 and 10 experiments in A and B, C, respectively

those reported by Pieri *et al* [13] There are, however, conflicting reports about the exhibition of stereotyped behavior by LHM [9, 10, 17] The behavioral response we have seen following the administration of LHM was mostly locomotor and exploratory activities The exploratory behavior observed with 100 μg/kg LHM was not taken over by biting or gnawing behavior by increasing the dose of LHM to 500 or 1000 μg/kg Therefore, this was not considered typical stereotyped behavior in the present study LHM inhibited apomorphine induced stereotyped behavior instead of eliciting it

Previously, we have shown the existence of sulpiride sensitive non-cooperative and insensitive cooperative [^{3}H] apomorphine binding sites in rat striatum [5,6] In the present study, LHM which has been shown to possess both agonistic and antagonistic activities for multiple dopamine receptors [11] inhibited the binding of [^{3}H] apomorphine to those sites with two distinct Hill coefficients Thus, the possibility that those two sites are severally involved in the exhibition of agonistic or antagonistic activity of LHM was examined

It has been shown that LHM inhibits dopamine sensitive adenylate cyclase activity [14] while sulpiride fails to inhibit it [7] This indicates that LHM may act as an antagonist on sulpiride insensitive cooperative receptors leading to the inhibition of dopamine sensitive adenylate cyclase In our study, LHM inhibited the stimulation of adenylate cyclase by dopamine without affecting on basal activity (pmoles cAMP formed/min/mg protein basal, 208±23, N=6, 10^{-4} M dopamine, 389±19, N=4, 5×10^{-6} M LHM, 213±12, N=3, 10^{-4} M dopamine plus 5×10^{-6} M LHM, 224±21, N=3) Furthermore, the results showing the inhibition of apomorphine induced stereotyped behavior by LHM and the lack of interaction of sulpiride with it indicated that sulpiride insensitive cooperative sites are involved in expressing stereotyped behavior

It has been shown that LHM is very potent in inhibiting prolactin release [8] It has been suggested that the release of prolactin is inhibited by dopaminergic agonists through the activation of dopamine receptors which are not coupled with adenylate cyclase and sensitive to the inhibition of sulpiride (D-2 dopamine receptors, see [11]) In this context, LHM behaves as an agonist on D-2 dopamine receptors in pituitary In the present study, it was also shown that LHM behaves as an agonist for sulpiride sensitive dopamine receptors also in the CNS, inducing rotating behavior and possibly locomotor activity

In conclusion, rotating behavior and locomotor activity may be induced by the activation of non-cooperative sites, while the activation of cooperative sites induces stereotypy Thus, our previous results [5,6] showing higher affinity of [^{3}H] apomorphine to non-cooperative sites than to cooperative site is also in good agreement with behavioral studies In behavioral studies, locomotor activity is observed at lower doses of apomorphine than that necessary to exhibit stereotypy

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