ELSEVIER

European Journal of Pharmacology 301 (1996) R11–R13

ejp

Rapid communication

Affinities and intrinsic activities of dopamine receptor agonists for the hD_{2l} and $hD_{4.4}$ receptors

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Received 7 February 1996; accepted 9 February 1996

Abstract

The affinity and intrinsic activity of dopamine receptor agonists were determined at the human dopamine hD_{2l} and $hD_{4.4}$ receptors. (−)-3-Hydroxy-*N*-*n*-propylpiperidine ((−)3-PPP) had an intrinsic activity of 46% and 83%, whereas (+)-*N*-propylnorapomorphine ((+)-NPA) had intrinsic activities of 61% and 58% at the dopamine hD_{2l} and $hD_{4.4}$ receptors, respectively. Affinities also varied. A single, or multiple, dopamine D_2-type receptor(s) may be involved in schizophrenia and agonists are being tested as therapy. Understanding their properties at the individual dopamine D_2-family receptors is important.

Keywords: Dopamine hD_{2l} receptor; Dopamine $hD_{4.4}$ receptor; Intrinsic activity

Antipsychotic drugs block dopamine D_2 receptors. There are now at least three dopamine D_2-type receptors, the D_2, D_3 and D_4 (Civelli et al., 1991). The dopamine D_4 receptor (Van Tol et al., 1991) may provide an explanation for the atypical behavior of clozapine and (Lahti et al., 1993) dopamine D_4 receptor occupancy may be associated with antipsychotic activity, whereas extrapyramidal side effects may be importantly associated with dopamine D_2 receptor occupancy. An important issue is to which receptor(s) should a drug's action be directed.

Partial dopamine receptor agonists are of interest as therapeutic agents since they can reduce the functioning of a system to a desired level. Dopamine receptor agonists have been evaluated as treatments for schizophrenia (Tamminga et al., 1978, 1994). These intrinsic activities were determined using in vivo measures, or in in vitro systems with tissue samples having mixed receptor populations. The issue of binding affinities and intrinsic activities of agents at the different dopamine receptors has generally been ignored.

Using receptor state binding affinities (Lahti et al., 1992) we determined the intrinsic activity of dopamine receptor agonists at the cloned dopamine hD_{2l} and the $hD_{4.4}$ receptors. Chinese hamster ovary-$hD_{4.4}$ (CHO-$hD_{4.4}$) cells were grown in a 1 l suspension culture using α-minimum essential medium (MEM; Sigma) containing 50 ml/l of heat inactivated fetal calf serum (Sigma), penicillin-streptomycin (Pen-Strep) and amphotericin. Cells were harvested by centrifugation at $10000 \times g$ for 10 min, and processed as previously described (Lahti et al., 1992). CHO-hD_{2l} cell membranes were prepared as before (Lahti et al., 1992).

Using [^{3}H]U-86170, a dopamine receptor agonist, the drug affinities were determined at the high affinity agonist state of the dopamine hD_{2l} receptor, and using [^{3}H]raclopride, a dopamine receptor antagonist, in the presence of 600 μM GTP (Lahti et al., 1992) the drug affinities were determined at the low affinity agonist state of the receptor. Using [^{3}H](±)-2-(*N*-propyl-*N*-2-thienylethylamino)-5-hydroxytetralin ([^{3}H]N-0437) (0.6 nM) in 50 mM Hepes, 10 mM $MgSO_4$, pH 7.4, an agonist at the dopamine $hD_{4.4}$ receptor, the drug affinities were determined at the high affinity agonist state of the $hD_{4.4}$ receptor, and using [^{3}H]YM-09151-2, a dopamine $hD_{4.4}$ antagonist, in the presence of 600 μM GTP the drug affinities were determined at the low affinity agonist state of the dopamine $hD_{4.4}$ receptor. Incubation was for 1 h at room temperature, samples were filtered, and counted. The non-

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PII S0014-2999(96)00127-6

specific binding determinant was 3 μM pimozide. Non-linear regression analysis derived IC_{50} values were converted to K_i values using standard methods, and where more than one determination was made, the results were combined. The K_d values at the dopamine $hD_{4.4}$ receptor were 0.37 nM for [^{3}H]YM-09151-2 and 0.8 nM for [^{3}H]N-0437, and at the dopamine hD_{2l} receptor were 2.25 nM for [^{3}H]raclopride and 0.51 nM for [^{3}H]U-86170.

Intrinsic activity estimates, Table 1, were calculated using the ratio of a compound's affinity at the low affinity agonist state to its affinity for the high affinity agonist state. The normalized regression line between intrinsic activity and the log of the ratio of the affinities at the low affinity agonist state/high affinity agonist state was used ($y = 39.4x + 8.6$) for the estimations (Lahti et al., 1992). Affinities presented in Table 1 are those determined using the [^{3}H]agonist ligands.

Interesting differences in intrinsic activity and affinity appear between receptors. (−)-NPA is a full agonist (100%) at the dopamine hD_{2l} receptor and appears to be a partial agonist (57%) at the dopamine $hD_{4.4}$ receptor. (−)-3-PPP is a partial agonist (46%) at the dopamine hD_{2l} receptor and nearly a full agonist (83%) at the dopamine $hD_{4.4}$. The partial (48% intrinsic activity) dopamine receptor agonist activity of (+)-N0437 (Timmerman et al., 1989) receptor is confirmed in this study, as is the full agonist activity of (−)-N0437. (+)-NPA has 6 times higher affinity at the dopamine $hD_{4.4}$ receptor than the hD_{2l} receptor; whereas (−)-N0437 has 14 times higher affinity for the dopamine hD_{2l} receptor than the $hD_{4.4}$ receptor. Dopamine has nearly the same affinity for both receptors.

It is of interest to speculate on the relationship of the above intrinsic activity findings to possible clinical results. If antipsychotic activity is related to dopamine hD_{2l} receptor occupancy, and the level of intrinsic activity, then (−)-3-PPP (46% intrinsic activity) might be better than (+)-apomorphine since its intrinsic activity is lower at the dopamine hD_{2l} receptor. On the other hand if the dopamine $hD_{4.4}$ receptor is responsible for antipsychotic activity one would predict, based upon levels of intrinsic activities that (+)-NPA (58% intrinsic activity) (Baldessarini et al., 1991) would exert a greater effect than would (−)-3-PPP (83% intrinsic activity). If antipsychotic activity is based upon occupancy of both the dopamine hD_{2l} and the dopamine $hD_{4.4}$ receptors then (+)-NPA would be more efficacious since its combined level of intrinsic activity is lowest.

In summary, the affinities and especially the intrinsic activities, of dopamine receptor agonists are not the same among the dopamine D_2 family of receptors. Differences range from full agonist activity at one receptor to low intrinsic activity partial agonist activity at the other, and vice versa. The consequences of such diverse effects in in vivo studies may be of significance to the treatment and understanding of schizophrenia.

Acknowledgements

We acknowledge Hubert H.M. Van Tol (Clarke Institute) for CHO-$hD_{4.4}$ cells, Marc Caron (Duke University) for CHO-hD_{2l} cells, and Richard Hsi (Upjohn Labs) for [^{3}H]U-86170. This work was supported by National Institute of Mental Health Grant 5 ROI MH49667-03.

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Table 1
Affinities and intrinsic activities of dopamine agonists

Compound	hD_{2l} receptor			$hD_{4.4}$ receptor		
	K_i (nM) ± S.D.	Ratio low/high	Intrinsic activity	K_i (nM) ± S.D.	Ratio low/high	Intrinsic activity
Dopamine	4.5 ± 0.6	463	100%	3.9 ± 0.7	471	100%
(−)-Apomorphine	1.5 ± 1.1	124	91%	0.3 ± 0.2	64	80%
(+)-Apomorphine	15.0 ± 9.1	47	75%	3.0 ± 1.5	15	55%
(−)-3-PPP	30.9 ± 13.4	9	46%	12.2 ± 3.1	77	83%
(+)-3-PPP	66.4 ± 12.5	68	81%	12.0 ± 6.5	164	96%
(−)-NPA	0.07 ± 0.04	208	100%	0.6 ± 0.2	17	57%
(+)-NPA	14.0 ± 5.0	21	61%	2.3 ± 2.0	18	58%
(−)-N-0437	0.08 ± 0.04	145	94%	1.1 ± 0.7	106	88%
(+)-N-0437	4.8 ± 2.3	10	48%	7.7 ± 3.4	40	72%

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