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Intrinsic Activity Determinations at the Dopamine D2 Guanine Nucleotide-Binding Protein-Coupled Receptor: Utilization of Receptor State Binding Affinities

R. A. LAHTI, L. M. FIGUR, M. F. PIERCEY, P. L. RUPPEL, and D. L. EVANS

CNS Diseases Research (R.A.L., L.M.F., M.F.P., D.L.E.), and Biostatistics (P.L.R.), Upjohn Laboratories, Kalamazoo, Michigan 49001

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SUMMARY

Guanine nucleotide-binding protein-coupled receptors have been shown to exist in both a high affinity agonist (HiAg) and a low affinity agonist (LowAg) state. The formation of the HiAg state is promoted by agonists, and the formation of this state of the receptor appears to be a critical factor in the generation of the effector-activating complex $G_\alpha \cdot GTP \cdot Mg^{2+}$ and in the production of a stimulus. The magnitude of the difference in the affinity a compound has for the HiAg versus the LowAg state of the receptor has been related to the intrinsic activity of the compound. In this paper the HiAg and LowAg affinities (K_i) of full and partial dopamine agonists of varying levels of intrinsic activity were determined using membranes from Chinese hamster ovary cells stably transfected with the $D2_i$ receptor. The HiAg state was defined using the recently described dopamine agonist ligand [^{3}H]U-86170, and the LowAg state was defined using [^{3}H] raclopride plus 600 μM GTP. The LowAg/HiAg ratios for apomorphine (43), HW-165 (12.5), (−)-3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine [(−)-3-PPP] (4.5), terguride (1.6), SDZ-208-911 (1.2), and SDZ-208-912 (0.3) were found to correlate well with their electrophysiologically derived intrinsic activities (r = 0.92). Using this relationship, the intrinsic activity for compounds such as (+)-3-PPP (112%), quinpirole (104%), U-68553B (102%), and U-86170 (95%) was predicted to be high (>90%); (−)-apomorphine (73%) was of high/moderate intrinsic activity, HW-165 (52%), (+)-apomorphine (51%), and (−)-3-PPP (34%) were in the intermediate range, and terguride (16.5%), SDZ-208-911 (11.7%), and SDZ-208-912 (−12%) were at the lower end of the intrinsic activity spectrum. The receptor state binding-determined intrinsic activity values for quinpirole (100%), U-86170F (94.8%), HW-165 (52.1%), (−)-3-PPP (34.3%), SDZ-208-911 (11.7%), and SDZ-208-912 (−12%) were found to correlate well (r = 0.908) with their maximum response (intrinsic activity), as determined using ATP-mediated increases in arachidonic acid release from CHO-$D2_i$ cells. In addition, the maximal effect of several of these compounds on rat striatal homovanillic acid (HVA) levels was determined. The drug-induced changes in tissue HVA levels were found to be consistent with the affinity-derived intrinsic activities of the drugs. For example, high intrinsic activity agonists such as quinpirole and U-86170F (104% and 95% intrinsic activity, respectively) reduced HVA, whereas compounds with intermediate intrinsic activity, such as HW-165 and (−)-3-PPP (52% and 34% intrinsic activity, respectively), exerted little effect on HVA levels. Low intrinsic activity agonists like SDZ-208-911 (10.4%) increased HVA, and SDZ-208-912, which is nearly devoid of intrinsic activity, caused an even larger increase in HVA. These results demonstrate that the intrinsic activity of agonists active at guanine nucleotide-binding protein-coupled receptors may be estimated by using a ^{3}H-agonist ligand to determine the affinity of a compound at the HiAg state and an ^{3}H-antagonist plus GTP to determine its affinity at the LowAg state and that these values are predictive of effects on *in vitro* and *in vivo* biological systems. There exist important clinical implications of these measures, perhaps critical to guiding drug selection for dopamine-related illnesses like Parkinson's disease and schizophrenia.

The biological activity of a compound is dependent upon a number of different parameters. Two of the most important are the affinity of the compound and its intrinsic activity. Affinity is a measure of the attraction a molecule has for the receptor, and intrinsic activity may be thought of as the power of a molecule to produce an action (1, 2). In the words of Ariens, "if the drug that interacts with the receptor has intrinsic activity, a stimulus is produced" (p. 5). The size of the stimulus produced by a compound may be directly related to its intrinsic activity and the environment (i.e., the concentration of the endogenous ligand) of the receptor (3). The size of the stimulus also varies with the agonist properties of the compound, with full agonists having more intrinsic activity than partial agonists.

Over the last decade, it has become easy to measure the affinity of a compound for a receptor, using receptor binding

ABBREVIATIONS: G protein, guanine nucleotide-binding protein; CHO, Chinese hamster ovary; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid; HVA, homovanillic acid; LowAg state, low affinity agonist state; HiAg state, high affinity agonist state; 3-PPP, 3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine.

techniques. The proliferation, over the last few years, of radioligands for many different receptors has made the task straightforward, sensitive, and quite accurate. The more recent cloning of several G protein-coupled receptors and their transfection into receptor-naive host cells have provided an even more powerful, selective, and exacting tool for the determination of the affinity of a compound for a receptor (4–7). The measure of the affinity of a drug is of immense value for understanding the pharmacological activity of that compound.

On the other hand, determinations of intrinsic activity have not been so facile. In the case of the G protein-coupled receptors, elevations or decreases in cAMP or phosphatidylinositol have been most useful in quantifying intrinsic activity (8). However, these measurements are laborious, time consuming, and difficult. Additionally, more elaborate *in vivo* measurements of electrophysiological, biochemical, endocrine, or behavioral end-points have also been used successfully for intrinsic activity. Numerous attempts have also been made to use GTP-induced shifts in agonist affinity to characterize a compound as a full agonist, partial agonist, or antagonist (9, 10). In contrast to what is reported here, previous methods used ^{3}H-labeled antagonist ligands, in the absence and presence of GTP, to determine affinities at the high and low affinity agonist states, respectively. Although useful to some extent, these GTP shifts using ^{3}H-antagonists as the sole ligand do not have the power necessary for more refined determinations. Consequently, important intrinsic activity determinations have not been available to complement the determination of the affinity of a compound for a receptor.

Several investigators (10–12) have provided considerable insight into the mechanism by which the G proteins, their associated receptors, ligands, guanine nucleotides (GTP and GDP), and magnesium interact to produce a stimulus. Evidence suggests that, when an agonist binds to the LowAg state of the receptor, it induces coupling of the receptor-agonist-complex to the G protein and then, in the presence of Mg^{2+}, forms the HiAg state of the receptor. The HiAg state of the receptor is broken down by GTP, forming the $G_\alpha \cdot GTP \cdot Mg^{2+}$ complex that activates the effector (i.e., adenylate cyclase) molecule and produces a stimulus.

Because this mechanism produces a receptor-mediated function and the LowAg and HiAg states of the receptor are the critical parts of the cyclic process, the binding affinities at these two states could predict the intrinsic activity of a compound (10, 11). In contrast to other studies, this series of studies defines the affinity of a compound for the HiAg state of the dopamine D2 receptor by using a dopamine agonist ligand, [^{3}H]U-86170 (13, 14), and the affinity at the LowAg state has been determined by using an antagonist ligand, [^{3}H]raclopride, in the presence of high concentrations of GTP. This dual ^{3}H-ligand approach provides a more accurate measure of the affinities of a compound at these two states of the receptor. This report describes studies that predict intrinsic activity from carefully defined receptor binding data. Correlations of these *in vitro* derived intrinsic activity values with *in vitro* and *in vivo* observed responses are presented to support this method of quantifying intrinsic activity.

Experimental Procedures

Cell culture and receptor binding. Receptor binding studies were conducted in membranes prepared from CHO cells stably transfected with the $D2_i$ (7) cloned receptor. CHO-$D2_i$ cells were grown to near-confluence before harvesting. Cells were then rinsed twice with a buffer containing 25 mM Tris, 1 mM EDTA, and 1 mM EGTA, pH 7.5 (TEE buffer) and were dislodged from the culture flasks into the same buffer, flasks were rinsed with TEE buffer containing 10 mM $MgCl_2$, and cells were disrupted using a Polytron at setting 6 for 10–15 sec. Ruptured cells were centrifuged at 18,000 × *g* for 15 min, resuspended in TEE buffer containing 10 mM $MgCl_2$, via the Polytron, centrifuged as before, and resuspended in a buffer containing 20 mM HEPES, 1 mM EDTA, 150 mM NaCl, and 10 mM $MgCl_2$, pH 7.5. Aliquots were frozen at −20° until further use.

For [^{3}H]U-86170 binding studies, the membrane preparations were thawed and diluted to approximately 20–40 μg of protein/ml in 20 mM HEPES, 10 mM $MgSO_4$, pH 7.4. The concentration of [^{3}H]U-86170 in these studies was approximately 2 nM, and 1 μM U-86170F was used to determine nonspecific binding. Parallel [^{3}H]raclopride (15) binding studies were conducted, using approximately 1 nM [^{3}H]raclopride with 1 μM haloperidol as the nonspecific binding determinant, in both the absence and the presence of 600 μM GTP, in a buffer composed of 50 mM Tris, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, and 1 mM $MgCl_2$, pH 7.4.

Incubation was for 1 hr at room temperature, samples were filtered, using a cell harvester, over Schleicher and Schuell no. 24 filters that had been presoaked in 0.05% polyethylenimine, and filters were rinsed three times with approximately 5 ml of cold 50 mM Tris buffer, pH 7.4. Radioactivity on the filters was determined by using standard liquid scintillation techniques.

Drugs were tested using a minimum of six different concentrations, in triplicate, and the experiments were repeated a minimum of two times. IC_{50} values were determined using appropriate receptor binding programs,[1] and IC_{50} values were converted to K_i values using previously described methods (16).

HVA determinations. Rat striatal HVA levels were determined using high performance liquid chromatography techniques, as described previously (17). Male Sprague-Dawley rats (150–170 g) were used. Four rats comprised a treatment group. Drugs were administered via the intraperitoneal route, and rats were sacrificed 45 min after dosing. Striata were rapidly removed and stored at −20° until they were analyzed. HVA levels in control rats were 154 ± 9 ng/g of tissue.

Arachidonic acid release. ATP-mediated arachidonic acid release from CHO-$D2_i$ cells was determined using a combination of procedures described by others (18–20). CHO-$D2_i$ cells, which were grown in α-minimum essential medium supplemented with 10% fetal calf serum, 1% glutamine (200 mM), 1% Pen-Strep (Irvine Scientific), and 1% G418 (80 mg/ml), in T-150 tissue culture flasks, were dissociated with a nonenzymatic cell dissociation solution. The cells were pelleted and resuspended at 1 × 10^6 cells/ml. These cells were then plated in 24-well tissue culture plates (1 ml/well) and incubated for 18–24 hr at 37° with 5% CO_2. For the assay, medium was removed and 0.5 μCi of [^{3}H] arachidonic acid, in 1 ml of α-minimum essential medium containing 1% glutamine and 0.5% bovine serum albumin, was added and allowed to incubate for 3 hr. After the cells were preloaded with [^{3}H]arachidonic acid, the medium was aspirated off, the cells were rinsed twice with medium, and 1 ml of medium containing 12 μM ATP and drug was added. The cells were then incubated for 30 min, at which time 0.5 ml of medium was removed and the amount of [^{3}H]arachidonic acid released was measured by using standard scintillation counting techniques. Using the response of quinpirole as 100%, the dose-dependent [^{3}H]arachidonic acid release was plotted as a percentage response, and the maximum test drug response was considered as a measure of the intrinsic activity of the drug.

Results

Binding affinities as determinants of intrinsic activity. Table 1 shows the comparison between the activity of dopamine agonists, partial agonists, and antagonists and their

[1] P. Ruppel and P. Berzins, personal communication.

inhibition of [^{3}H]U-86170 binding and [^{3}H]raclopride binding, in the presence and absence of 600 μM GTP. This allows the comparison of similarities and differences in affinity ratios of an agonist ligand versus an antagonist ligand in the presence of GTP or of an antagonist without or with GTP, the traditional GTP shift. For a full agonist, such as dopamine, the use of an agonist ^{3}H-ligand gives the highest affinity (16.8 nM), whereas an antagonist ^{3}H-ligand plus GTP gives the lowest affinity (4012 nM). For partial agonists, such as HW-165 and (−)-3-PPP, the use of an agonist ^{3}H-ligand also gives higher affinity values for the D2 receptor than does an antagonist ^{3}H-ligand plus GTP, although the difference is not as great as it is for full agonists such as dopamine. In a comparison of the two sets of ratios of affinities for HW-165 and (−)-3-PPP, the traditional GTP shifts (^{3}H-antagonist/^{3}H-antagonist plus GTP), as shown under C/B in Table 1 (2.2 and 2.4, respectively), do not distinguish between the two drugs, which are known to have different intrinsic activities (21, 22). On the other hand, with the agonist/antagonist plus GTP ratios (LowAg/HiAg ratio), the ratio of C/A in Table 1, a separation is seen between HW-165 and (−)-3-PPP (ratios of 12.4 and 4.5, respectively) and also between (−)-3-PPP and SDZ-208–911 (23) (ratios of 4.5 and 1.2, respectively). Compounds with low intrinsic activity, i.e., SDZ-208–912 (23), and antagonists such as chlorpromazine are the most difficult to separate with this technique.

Relationship of intrinsic activity to electrophysiological effects. Several approaches were taken to verify the meaning of the LowAg/HighAg ratios for dopamine agonists. First, the LowAg/HiAg ratios obtained for the agonists and partial agonists were compared with their reported levels of intrinsic activity in an electrophysiological model (24–26) (Fig. 1). A robust correlation (r = 0.98) exists between the electrophysiologically determined intrinsic activity of a compound and the logarithm of the LowAg/HiAg ratio for that compound, as defined by the inhibition of [^{3}H]U-86170 binding and the inhibition of [^{3}H]raclopride plus GTP binding. As presented in Fig. 1 (*ACTUAL*), the electrophysiologically derived intrinsic activity of 100% and a binding ratio of 43 for apomorphine were used to establish the upper limit of the curve. To accommodate the higher LowAg/HiAg ratio of dopamine (238) and other compounds, it was necessary to normalize the fit so that the LowAg/HiAg ratio of 238 for dopamine defined the 100% intrinsic activity level. This is also presented in Fig. 1 (*NORMAL*).

To characterize this ratio further, a number of standard compounds were selected and their binding (LowAg/HiAg) ratios were determined. Using the characteristics of the regression line from Fig. 1 and the compound binding ratios, the percentage intrinsic activity for each compound was estimated (Table 2). The percentage intrinsic activity for the standards begins around 100% for full agonists such as quinpirole and dopamine and drops to 73% for apomorphine, 52% for HW-165, 43% for (−)-3-PPP, 17% for terguride, 12% for SDZ-208–911, and 5% or less for chlorpromazine (5.3%) and SDZ-208–912 (−12%). These later values suggest that estimates of intrinsic activity at this very low end of the scale are not too informative. The (+)- and (−)-enantiomers of 3-PPP are found to have different levels of intrinsic activity (112% and 43%, respectively), as do the (−)- and (+)-enantiomers of apomorphine (73.1% and 51.4%, respectively). The partial agonist activity of (+)-apomorphine, and several other (*S*)-(+)-aporphines, has recently been described (27).

Relationship of intrinsic activity to effects on arachidonic acid release. The results presented in Fig. 2 demonstrate that the intrinsic activity of a drug is predictive of its effect on arachidonic acid release. U-86170 and quinpirole exert the largest effect on arachidonic acid release, and their effect is the maximum effect (100%) obtainable. The effect of HW-

TABLE 1

Inhibitory effect (K_i mean ± standard deviation) of various compounds on agonist ([^{3}H]U-86170) and antagonist ([^{3}H] raclopride) binding at the dopamine CHO-D2$_L$ receptor

[^{3}H]U-86170 was used at a concentration of approximately 2 nM and 1 μM U-86170F was used as the nonspecific binding determinant. [^{3}H]Raclopride binding was measured in parallel with [^{3}H]U-86170 binding; [^{3}H]raclopride was used at 1 nM, with 1 μM haloperidol being used to determine nonspecific binding. Drugs were tested at six different concentrations, in triplicate. Incubation was for 1 hr at room temperature; samples were then filtered, rinsed, and counted. IC_{50} values were converted to K_i values by using standard methods.

Compound	K_i			Ratio	
	[^{3}H]Raclopride				
	[^{3}H]U-86170 (A)	Without GTP (B)	With GTP (C)	C/A	C/B
	nM				
Dopamine	16.8 ± 12.6	615 ± 38	4012 ± 2025	238	6.5
HW-165	12.4 ± 1.8	70.5 ± 7.4	154 ± 13	12.4	2.2
(−)-3-PPP	82.5 ± 31.0	156 ± 0.7	374 ± 223	4.5	2.4
SDZ-208-911	2.0 ± 0.5	2.4 ± 0.2	2.3 ± 0.3	1.2	0.96
Chlorpromazine	0.6 ± 0.1	1.0 ± 0.03	0.5 ± 0.05	0.8	0.5
SDZ-208-912	2.2 ± 0.3	0.6 ± 0.04	0.6 ± 0.03	0.3	1.0

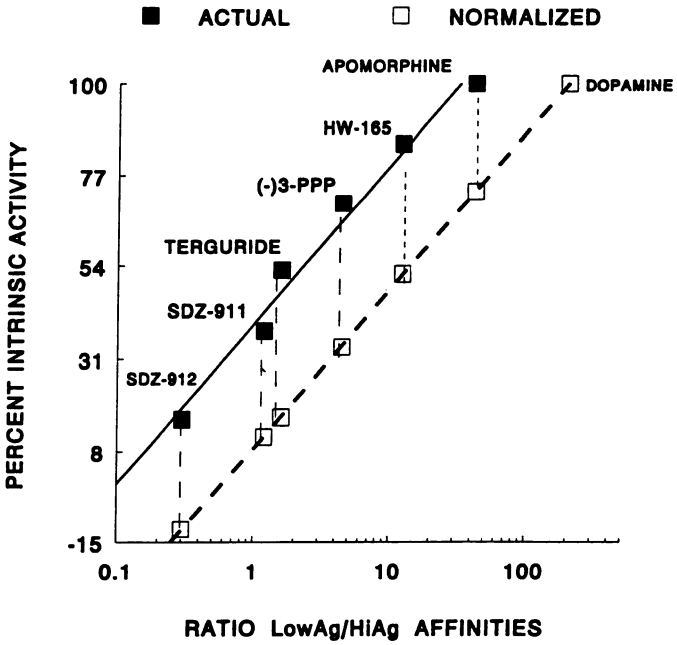

Fig. 1. Relationship between LowAg/HiAg state affinities for the D2 receptor and electrophysiologically determined intrinsic activity. K_i values for the LowAg and HiAg states were determined as described in Experimental Procedures and presented in Table 2. Electrophysiologically derived intrinsic activity values (apomorphine, 100%; HW-165, 85%; (−)-3-PPP, 70%; SDZ-208–911, 38%) were those producing a reduction in firing rate of rat substantia nigra pars compacta neurons (19, 20). The *regression line* describing the relationship of these two parameters was determined and normalized to fit the properties of dopamine, which was considered to have 100% intrinsic activity and was determined to have a LowAg/HiAg ratio of 238. The equation describing the linear regression line was $y = 39.6x + 39.0$, with correlation coefficient r = 0.98.

TABLE 2

Intrinsic estimations and receptor binding affinities determined for various dopaminergic agents at the dopamine D2 receptor

K_i values were determined using [^{3}H]U-86170 binding or [^{3}H]raclopride plus 600 μM GTP at the CHO-D2$_i$ receptor. Six drug concentrations were used to determine the K_i values. The ratio LowAg/HiAg was defined as K_i for [^{3}H]raclopride plus GTP/K_i for [^{3}H]U-86170 inhibition of binding. Intrinsic activity values were calculated using the definition of the regression line generated by plotting the logarithm of the ratio versus the electrophysiologically determined (19, 20) intrinsic activity of standards and setting dopamine at 100%, as in Fig. 1.

Compound	Intrinsic activity	Ratio, LowAg/HiAg	K_i [^{3}H]U-86170
	%		nM
(+) 3-PPP	112.6	423	8.5
Quinpirole	104.3	263	30.5
Dopamine	100.0	238	16.8
U-68553B	101.6	222	0.5
U-86170F	94.8	151	1.8
(−)-Apomorphine	73.1*	43.0	2.7
HW-165	52.1*	12.4	12.4
(+)-Apomorphine	51.4	12.0	31.9
(−)-3-PPP	34.3*	4.5	82.5
Terguride	16.5	1.6	0.8
SDZ-208-911	11.7*	1.2	3.1
Bromergocryptine	6.8	0.9	42.4
Chlorpromazine	5.3	0.8	1.8
SDZ-208-912	−11.9	0.3	3.6

* Electrophysiologically determined.

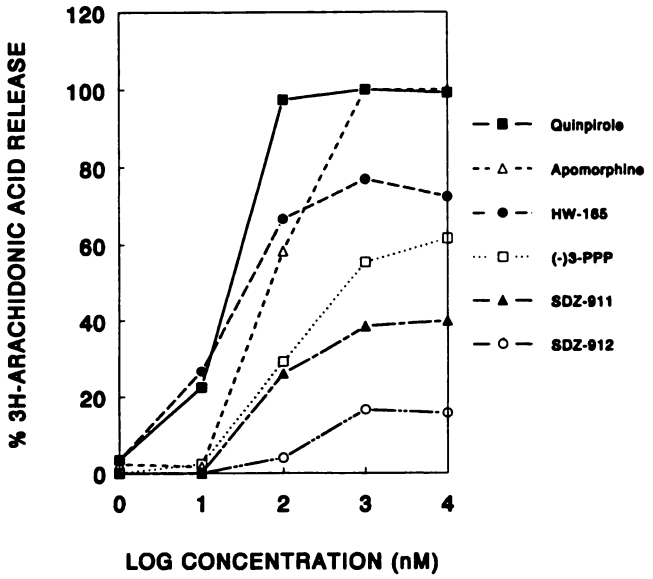

Fig. 2. Effect of dopamine agonists on ATP-mediated [^{3}H]arachidonic acid release from CHO-D2$_i$ cells. Maximal release of arachidonic acid was defined as the maximum amount released by the full agonist quinpirole. The responses of doses of other drugs were calculated as a percentage of the maximum quinpirole response. In a typical experiment, approximately 450,000 cpm of [^{3}H]arachidonic acid were added to the cells, unstimulated cells secreted 511 ± 87.5 cpm of [^{3}H]arachidonic acid, in the presence of 12 μM ATP the amount released increased to 7,246 ± 543.2 cpm, and in the presence of both ATP and 10 μM quinpirole the amount released increased to 15,440 ± 310 cpm. Thus, quinpirole-released [^{3}H]arachidonic acid was 8,194 cpm or 55.5% of the total released.

165 is next largest and is 70% of that of quinpirole and U-86710. Following HW-165 are (−)-3-PPP (60%), SDZ-208–911 (50%), and SDZ-208–912 (16%). It is important to note that the rank order of the effect of these compounds on arachidonic acid release is the same as the rank order of intrinsic activity determined from *in vitro* binding affinities and *in vivo* electrophysiological experiments and that a respectable correlation (r = 0.908) exists between the two sets of values.

Relationship of intrinsic activity to effects on rat striatal HVA levels. Because of the known relationship between dopamine agonists and rat striatal HVA levels, in that dopamine agonists decrease rat striatal HVA and low-intrinsic activity partial agonists increase striatal HVA, the relationship between intrinsic activity and rat striatal HVA change was studied further. In Fig. 3 are presented results from the HVA study, which demonstrate the varying effects of dopaminergic agonists on rat striatal HVA levels. In no instance does a 10 mg/kg dose cause a statistically significantly greater effect than does the 3.0 mg/kg dose, demonstrating a maximal agonist effect on HVA, although in the case of SDZ-208–912 the lower dose appears to give a larger response. High intrinsic activity dopamine agonists such as quinpirole, U-86170F, and apomorphine cause significant decreases (−70% to −65% of control) in HVA levels. On the other hand, partial agonists such as HW-165 and (−)-3-PPP do not cause significant changes (−1.7% and +11.9%) from control but result in data significantly different from each other. At the lower intrinsic activity end of the scale, SDZ-208–911 and SDZ-208–912 both cause significant increases (+55% and +142% of control), with SDZ-208–912 causing the largest increase. It is important to note that the maximum decrease in rat striatal HVA is approximately −75% and the maximum increase is +142%.

The observed (10 mg/kg dose) and predicted (based on binding-derived intrinsic activity values) effects of several drugs on HVA levels are presented in Fig. 3. In the case of the all-or-none response, an underlying normal tolerance distribution has been used (1, 28) to characterize the relationship. This is similar to what has long been suggested for quantal bioassays (29). For ease of model fitting, we have used a logistic model to describe the relationship between percentage intrinsic activity and striatal HVA. A logistic model and the probit model, which is based on the underlying normal assumption, both yield very similar

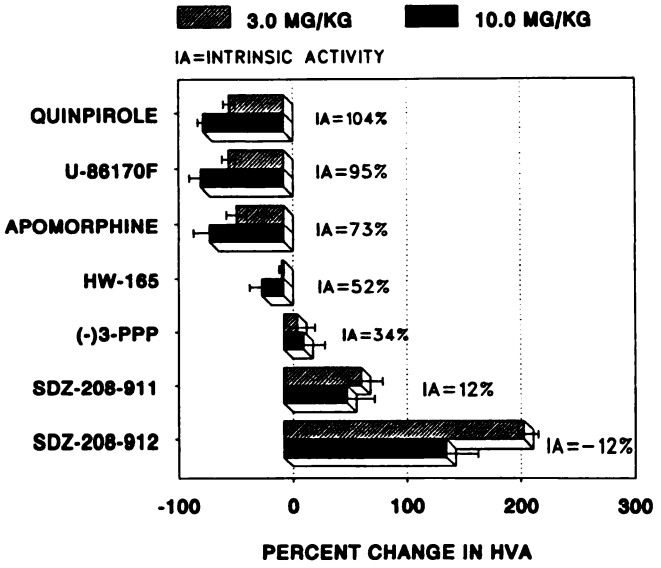

Fig. 3. Effect of 3.0 and 10.0 mg/kg doses of dopamine agonists on rat striatal HVA levels. The effect was determined by administering drug via the intraperitoneal route and sacrificing the animals 45 min after dosing. Striata were rapidly dissected out on ice and kept frozen at −20° until they were analyzed by high performance liquid chromatography and electrochemical detection techniques. Four rats were used for each dose. HVA levels in control rats were 154 ± 9 ng/g of tissue. Intrinsic activity values from Table 2 are displayed.

results, with a very slight difference in the tales of the response curve. The best fitting logistic model was:

$$\% \text{ Intrinsic activity} = \frac{1.0}{1 + e^{(2HVA)}}$$

The correlation between the determined percentage intrinsic activities and the expected percentage intrinsic activities using this HVA model was $r = 0.96$ (Fig. 4). This is a highly significant correlation ($p < 0.0006$).

In Fig. 5 the relationship between the LowAg/HiAg ratio-predicted intrinsic activity, the electrophysiologically determined intrinsic activity, the maximally observed effect of a drug on rat striatal HVA levels, and the maximum release of arachidonic acid is presented. The varied effects exhibit a close correspondence to the *in vitro* binding-derived intrinsic activity results.

Discussion

The primary issue in the development and evaluation of drugs of the partial agonist type is their intrinsic activity. To date, *in vivo* biochemical or electrophysiological measures have been the primary means for such determinations, despite their complex methodology. Receptor binding studies, through the use of GTP shifts for agonists, have been able to categorize agonists and antagonists but cannot adequately rank the partial agonists in order. These earlier receptor binding studies, in which intrinsic activity estimations were made, used antagonist ligands that bind equally well to both receptor states. In the presence of GTP the predominant population of the receptor is the LowAg state, and in the absence of GTP the receptor population exists in a mixture of the LowAg and HiAg states; the amount corresponding to each state is not readily definable using an antagonist ligand. This "GTP shift" technique has been useful but was inherently limited, providing only a rough approximately of intrinsic activity, primarily because the HiAg site was not accurately characterized.

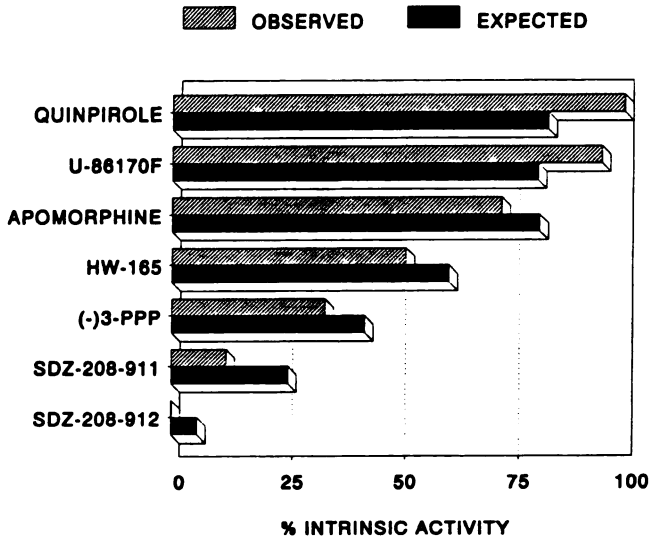

Fig. 4. Observed versus expected intrinsic activity, based on a logistic model for striatal HVA levels. Observed intrinsic activity values are those presented in Table 2. The expected intrinsic activity values were derived using the effect on rat striatal HVA levels for the 10 mg/kg dose of each drug and the relationship percentage intrinsic activity = $1.0/1 + e^{(2HVA)}$. The correlation between the observed and expected percentage intrinsic activity was $r = 0.96$ ($p < 0.0006$).

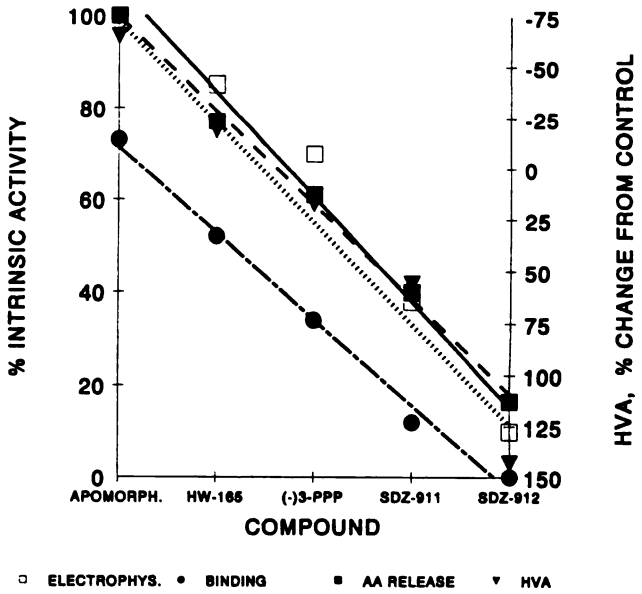

Fig. 5. Relationship between binding-derived, electrophysiologically derived, and [^{3}H]arachidonic acid (*AA*) release-derived intrinsic activity values (*left axis*) and the maximal effect of a compound on rat striatal HVA levels (*right axis*). See Table 2 and Figs. 1, 2, and 4 for details. The maximum decrease in striatal HVA was approximately −75%, and the maximum increase was +142%; therefore, in the figure these values represent the equivalent of 100% and 0%.

In the present study the affinity of a compound for the two different agonist-associated states of the G protein-coupled receptor has been more accurately defined and determined. This has been accomplished by using a well defined receptor preparation (CHO-D2$_i$ cell membranes), using an agonist ligand such as [^{3}H]U-86170 to define the affinity of a compound for the HiAg state of the receptor, and using an antagonist ligand, in this case [^{3}H]raclopride, in the presence of GTP to define the affinity of a compound for the LowAg state of the receptor. The ratio of these two affinity values provides a reliable measure of the intrinsic activity of a compound and can reliably predict intrinsic activity in the range from 100% to 10%. Below the 10% level, the predictions appear to be less informative and do not separate frank antagonists from very low intrinsic activity agonists. Thus, an *in vitro* method, utilizing receptor binding-determined affinities, has been established for the estimation of the intrinsic activity of most dopamine D2 agonists and partial agonists. The extension of this technique to other similar G protein-coupled receptors is highly reasonable.

The intrinsic activity of a drug appears to be one of the most important determinants of its overall biological activity. A high intrinsic activity is indicative of a frank agonist, whereas compounds with intermediate intrinsic activity may be agonists in high spare receptor (30) or low endogenous ligand environments; low intrinsic activity compounds may be agonists under unusual environmental conditions (high numbers of spare receptors and very low endogenous agonist concentrations), but more often they appear as antagonists. Consequently, by varying the intrinsic activity of a drug one can produce a gradient of responses from activation to inhibition, simply by applying the desired amount of intrinsic activity. Also, spare receptors may account for the observation (Fig. 5) that the *in vitro* measure of intrinsic activity using binding affinities in cell-free systems, where spare receptors are absent, is lower than the *in*

vitro and *in vivo* measures of intrinsic activity, where spare receptors are probably present.

The demonstration of a relationship between the intrinsic activity of a compound, as determined using receptor-state affinities, and the *in vitro* arachidonic acid release from CHO-$D2_i$ cells provides a proximal and direct assessment of the concept that receptor-state binding affinities can be used to estimate the intrinsic activity of a compound. Furthermore the demonstration of a relationship between the intrinsic activity of a compound and its effect on rat striatal HVA levels is also important and may provide additional support for the concept described above. High intrinsic activity agonists, such as apomorphine, cause decreases in HVA, whereas compounds with moderate amounts of intrinsic activity, such as HW-165 and (−)-3-PPP, exert little effect on HVA levels. On the other hand, the low intrinsic activity agonists SDZ-208–911 and SDZ-208–912 both cause increases in striatal HVA. These findings are consistent with the individual reported effects of some of these compounds on striatal HVA levels (21–23, 31). Taken individually these effects on HVA levels may not be striking, but taken together the relationship of the effect on HVA level to intrinsic activity becomes apparent. There appears to be a switch point at about the 50% intrinsic activity level. Compounds with much greater than 50% intrinsic activity (quinpirole and apomorphine) cause decreases in striatal HVA, those in the intermediate range [HW-165 and (−)-3-PPP] cause only small changes (if any) in striatal HVA, and those with much less than 50% intrinsic activity (SDZ 208–911 and SDZ-208–912) cause increases in striatal HVA. This switch point effect on striatal HVA is predictable based upon the concepts set down by Ariens (1), which relate intrinsic activity, receptor occupancy, and stimulus size to each other. Thus, it is possible to predict the effect of a compound on a biological system, i.e., striatal HVA levels, once its intrinsic activity is known, and vice versa. It may also be that dopamine-induced changes in plasma growth hormone levels, striatal acetylcholine levels, and locomotor activity in resperinized mice, emesis, extrapyramidal side effects, parkinsonism, and psychosis reduction are affected differently by compounds of varying intrinsic activity. As an example, in humans the very low intrinsic activity dopamine agonist SDZ-208–912 not only causes a reduction in psychosis but also produces extrapyramidal side effects (32), yet (−)-3-PPP, which has a higher level of intrinsic activity than does SDZ-208–912, may reduce psychosis but is not noted to produce extrapyramidal side effects (33) at behaviorally active doses. It appears that by carefully selecting the level of intrinsic activity it may be possible to produce therapeutic effects with minimal side effects.

The results presented in this report demonstrate that estimations of the intrinsic activity of a compound can be obtained using appropriate agonist and antagonist ^{3}H-ligands to define the affinity of the drug at the LowAg and HiAg states of the receptor. The ratio of these affinities has been shown to correlate well with other intrinsic activity determinations. Intrinsic activity values, in turn, may be used to explain and/or predict the actions of a compound in other biological systems, such as effects on striatal HVA levels, prolactin levels, locomotor activity, emesis, extrapyramidal side effects, or decreases/increases in psychotic behavior. These measures may be able to select optimal drugs for the treatment of diseases like parkinsonism and schizophrenia.

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Send reprint requests to: Robert A. Lahti, Ph.D., CNS Diseases Research, Upjohn Laboratories, Kalamazoo, MI 49001.