

Promoter-independent regulation of cell-specific dopamine receptor expression

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Abstract Here we describe the construction of recombinant adenoviruses expressing dopamine D2 and D4 receptors, and their ability to mediate high levels of heterologous expression in a variety of cell types *in vitro* and *in vivo* for at least 7 days post infection. These experiments demonstrated that maximum receptor expression is achieved generally within 24 h and remains constant thereafter. Maximum expression levels were highly variable between cell lines and dependent on infection efficiency and promoter strength. Correction for these two variables revealed differences in relative expression levels between cell lines varying by two orders of magnitude. Our results indicate that in addition to gene transcription, post-transcriptional mechanisms play a dominant role in determining dopamine receptor levels in this system.

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Key words: Dopamine; Adenovirus; Expression; D2 dopamine receptor; D4 dopamine receptor; Cell line; Ligand binding; Cyclic AMP

1. Introduction

In its simplest form receptor expression levels are the result of *de novo* synthesis and protein degradation. There is an increased understanding of several aspects of these processes and how they are altered in response to receptor activation, but it is less clear to what extent different cellular processes modulate steady-state levels of G protein-coupled receptors [1–10]. To our knowledge there are no reports that have provided information on the relative contribution of the sum of all post-transcriptional processes in steady-state level receptor expression. In general it is assumed that the level of activation of the genes for G protein-coupled receptors are the predominant factors in receptor expression and that the various post-transcriptional processes play a modulatory role in receptor expression [7,11–16].

We examined cell-specific dopamine receptor expression by efficiently introducing the G protein-coupled dopamine D2 and D4 receptors in a controlled manner into a variety of cell lines. Subsequent evaluation of steady-state receptor levels in relation to gene expression mediated by the constitutively active Rous sarcoma virus long terminal repeat promoter enhancer (RSV-LTR) and efficiency of delivery of the expression

vector allowed for the comparison of the relative contribution of these different processes to receptor expression.

To achieve an efficient and controlled delivery of the dopamine receptor expression cassettes in a wide host range of cells, we used a recombinant adenovirus. Type 5 adenoviruses have been successfully used to transfer several different transgenes, both *in vitro* and *in vivo*, to a wide host range of replicating and non-replicating cells [17], including the dopamine D2 receptor [18,19].

In this study, we created first generation recombinant adenoviral vectors expressing either the D2 or D4 dopamine receptors. The recombinant vectors were characterized in a variety of cell lines and in rat brain with respect to their ability to induce dopamine receptor expression. We demonstrate that recombinant adenoviruses are excellent viral vectors for expressing dopamine receptors *in vitro* and *in vivo*. Further, the recombinant vectors mediate receptor expression in many, but not all cell types. Receptor expression is not only dependent on infectivity and promoter strength, but is highly dependent on post-translational events that vary strongly between different cell lines.

2. Materials and methods

2.1. Cell culture

CHO-K1 cells (ATCC, Rockville, MD) were grown in α -MEM supplemented with 2.5% fetal bovine serum and 2.5% horse serum (Gibco BRL). HEK-293, COS-7, mouse Ltk⁻, and HeLa cells (ATCC, Rockville, MD) were grown in α -MEM supplemented with 10% FBS. GH4-C1 and SKNMC cells were cultured in Ham's F10 medium supplemented with 8% FBS.

2.2. Recombinant adenoviruses

First generation recombinant adenoviruses expressing the dopamine D2 (AdRSVD2), D4.4 (AdRSVD4.4) and the firefly luciferase gene (AdRSVLuc) under control of the RSV-LTR promoter were constructed as previously described [20]. Briefly, the shuttle plasmid pXCJL-2, containing the transgene of interest, was cotransfected with the plasmid pJM17 into HEK-293 cells and overlaid with agarose (plasmids pXCJL-2 and pJM17 were a kind gift of Dr. F. Graham, McMaster University, Hamilton, Ont.). Plaques were picked, verified by restriction digestion and subjected to plaque purification followed by two rounds of CsCl density ultracentrifugation. Virus was dialyzed using a Pierce Dialysis Cassette (Pierce) and viral stocks were aliquoted and stored at -70°C . Titers were determined with a HEK-293 plaque assay. Stocks were assayed for wild type adenoviruses using HeLa plaque assays and PCR with E1 specific primers [21,22].

2.3. Histochemical β -galactosidase staining

Recombinant adenovirus expressing the β -galactosidase gene under control of the CMV promoter (AdCMVLacZ, a gift from Dr. F. Graham, McMaster University, Hamilton, Ont.) were detected by

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histochemical staining using X-Gal. To detect β -galactosidase, culture medium was aspirated and the cells were fixed with 2% formaldehyde/0.2% glutaraldehyde (10 min at room temperature), followed by staining with X-Gal staining solution (100 mM sodium phosphate, 1.3 mM MgCl_2 , 3 mM $\text{K}_3\text{Fe}(\text{CN})_6$, 3 mM $\text{K}_4\text{Fe}(\text{CN})_6$, and 1 mg/ml X-Gal) at 37°C, overnight.

Analysis of β -galactosidase expression in brain tissue was done on cryostat sections (20 μm thickness) from frozen rat brain. Sections were placed on glycerine-coated slides, and dried. Brain slices were fixed in 2% paraformaldehyde/0.2% glutaraldehyde at 4°C for 15 min, and then stained with X-Gal solution at 37°C overnight.

2.4. Luciferase assays

Luciferase assays were done as described previously [23]. Lysate of infected cells (200 μl) was combined with 15 μl of luciferase cocktail (750 nM Tris/MES, pH 7.8; 15 mM MgOAc and 40 mM ATP) in plastic cuvettes and loaded into a luminometer (BioOrbit 1250, Pharmacia) where 200 μl of 1 mM luciferin dissolved in 5 mM potassium phosphate, pH 7.5 was automatically dispensed. Peak light emission was recorded by a chart recorder.

2.5. Ligand binding analysis

Infected cells were collected and homogenized at 4°C in binding buffer (50 mM Tris-HCl, pH 7.4, 5 mM EDTA, 1.5 mM CaCl_2 , 5 mM KCl and 120 mM NaCl). Homogenates were centrifuged at 39 000 $\times g$ for 15 min and the pellets were resuspended in binding buffer at a concentration of 1 mg/ml. For saturation binding analysis, 500 μl of homogenate was incubated in duplicate with increasing concentrations (10–1000 pM) of [^3H]spiperone (120 $\mu\text{Ci}/\text{mmol}$). For competition binding analysis, 200–250 pM [^3H]spiperone was co-incubated with increasing concentrations (10–1000 pM) of competing ligands (in duplicate), either in the presence or in the absence of 200 μM Gpp(NH)p. Non-specific binding was determined by co-incubation of [^3H]spiperone with 30 μM of dopamine or 10 μM of butaclamol. The samples were incubated in a final volume of 1.5 ml for 2 h at room temperature and filtered using a cell harvester (Skatron Instruments, Norway) and counted by liquid scintillation counting. All binding experiments were done in duplicate or triplicate. Densities (B_{max}) and the dissociation constants (K_d) of the various ligands were determined by Scatchard analysis. Ligand binding data were analyzed using the non-linear least-square curve-fitting program LIGAND. Soluble protein concentrations were determined by the Bradford method [24].

2.6. Measurement of intracellular cyclic AMP levels

CHO-K1 cells were plated on 6-well plates 1 day prior to infection, and infected at a multiplicity of infection (moi) of 100. One day later, cells were washed once with phosphate buffered saline (PBS) followed by a wash with 1 ml of HBBS/well (118 mM NaCl, 4.6 mM KCl, 1 mM CaCl_2 , 1 mM MgCl_2 , 10 mM D-glucose, 20 mM HEPES and 0.3 mM isobutyl-1-methyl-xanthine (IBMX), pH 7.2). Cells were stimulated with 10 μM of forskolin and incubated with various concentrations of dopamine and quinpirole ranging from 0.1 pM to 100 μM . Cells were incubated for 20 min at 37°C in a final volume of 1 ml and then harvested in 1 ml of permeabilization buffer (0.05% (v/v) Triton X-100 in HBBS buffer). Samples were vortexed and centrifuged for 5 min at 13 000 rpm and the supernatant was collected and frozen at -80°C for cAMP measurement by radioimmunoassay (RIA). The experiments described were done in triplicate.

2.7. cAMP radioimmunoassays

Measurement of intracellular cAMP levels was performed as described previously using a cyclic AMP specific antiserum (Sigma)[25]. The cAMP RIAs were performed in triplicate for each experiment.

2.8. Infection of cell lines and rat brain tissue

For expression studies in cell lines, medium was aspirated from 150 mm plates. 15 ml of medium containing recombinant adenovirus at the appropriate moi was added to the cells. The cells were incubated for 24 h at 37°C (5% CO_2). Next, the infection medium was removed, cells were rinsed with PBS and medium was added. This point in time of the experimental protocol was defined as time zero post infection.

Microinjection of recombinant adenovirus into brain tissue was performed using standard stereotactic neurosurgical procedures, under anesthesia with a single injection of sodium pentobarbital (55

mg/kg i.p.), and buprenorphine (0.01–0.05 mg/kg). The animals were then placed in a stereotactic frame (KOPF 900-David Kopf Instruments, CA). At the chosen coordinates (Paxinos and Watson, 1986) a small opening was drilled in the skull to permit insertion of a fine (32 gauge) injection needle, connected to a 10 μl Hamilton syringe containing the viral preparation. Each injection consists of a single injection of 1–5 μl (10^9 – 10^{10} plaque forming units (pfu/ml)) injected over a 5–15 min interval using an automated delivery system (Razel Syringe Pump, Razel Scientific Instruments). The needle was withdrawn slowly over 30 s, the skull defect filled with bone wax, and the scalp sutured.

2.9. Autoradiography of dopamine receptors in rat brain

D2-like receptors were detected by autoradiography using [^3H]spiperone (200 pM) (Amersham Life Science) [26]. Non-specific binding was determined by inclusion of 200 nM haloperidol. Slides were pre-incubated in buffer (50 mM HEPES, 1 mM EDTA, 5 mM KCl, 1.5 mM CaCl_2 , 4 mM MgCl_2 , 120 mM NaCl) for 30 min, and then immersed in buffer containing [^3H]spiperone for 2 h at 20°C. Next, the slides were washed twice for 5 min with pre-incubation buffer at 4°C, followed by a rinse with distilled water at 4°C, and dried. The slides were exposed to X-ray film (^3H Hyperfilm, Amersham Life Science) for 2–3 weeks. Relative optical density (ROD) of the autoradiographic signals was measured by image analysis as described previously [26].

3. Results

3.1. Pharmacological analysis of D2 and D4 expressing recombinant adenovirus

Competition binding analyses were performed in CHO-K1 cells infected at an moi of 100 with either the AdRSVD2 or AdRSVD4.4 adenoviruses. The results of these studies are listed in Table 1 and demonstrate the appropriate rank order of affinity for the different ligands for the receptors [27]. The agonists, dopamine and quinpirole, bound with high affinity to the D4 receptor. Inclusion of the non-hydrolyzable GTP analogue Gpp[NH]p in the binding assay lowered the affinity for agonists. D2 receptors bound the agonists only with low affinity that was insensitive to the addition of Gpp[NH]p. Saturation binding analysis of the different cell lines (CHO-K1, COS-7, HeLa, SKNMC and GH4-C1) infected with these viruses demonstrated that D2 and D4.4 (as well as D4.2 and D4.7 isoforms; data not shown) bound [^3H]spiperone with an affinity of about 75 pM and 150 pM, respectively. Infection of the different cell lines with AdRSVLuc did not result in detectable [^3H]spiperone binding.

Table 1

Pharmacological analysis of dopamine D2 and D4.4 receptors expressed by adenoviral vectors in CHO-K1 cells

	Affinity (K_i) (nM)	
	AdRSVD2	AdRSVD4.4
Nemonapride	0.1	0.1
Haloperidol	1.4	0.9
Raclopride	43	3400
Clozapine	444	14
Dopamine	> 5000	8.2
Dopamine+G	> 5000	21.0
Quinpirole	> 2000	5.0
Quinpirole+G	> 2000	12.4

Competition binding analysis of various dopamine receptor ligands, using [^3H]spiperone, for dopamine D2 and D4.4 receptors expressed in CHO-K1 cells with the adenoviral vectors AdRSVD2 and AdRSVD4.4, respectively. The average affinities (K_i) were calculated with the non-linear least-square curve fitting program LIGAND.

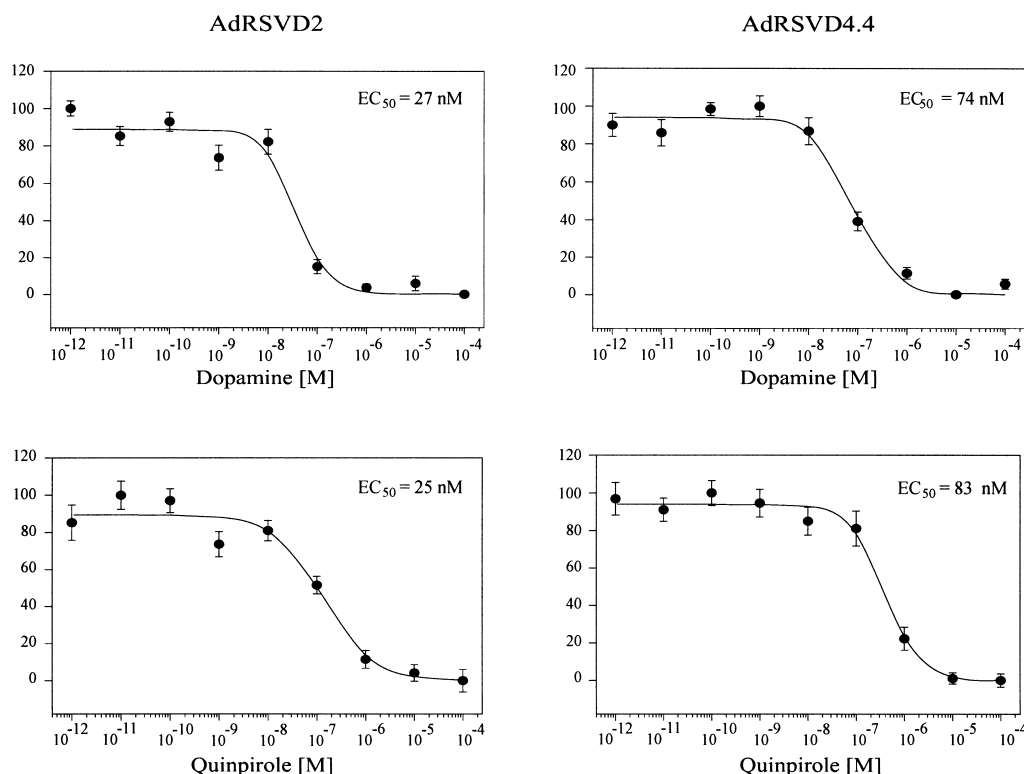


Fig. 1. Inhibition of forskolin-stimulated cAMP levels by dopamine and quinpirole of CHO-K1 cells infected with the adenoviral dopamine D2 and D4 expression vectors AdRSVD2 and AdRSVD4.4, respectively. The percentage inhibition is standardized by setting forskolin-stimulated cAMP levels at 100% and maximal inhibition by dopamine at 0%. The percentage of inhibition by dopamine was on average 90%. The curves presented are a composite of at least nine independent experiments in which the individual points of the experiments are given as the mean $\pm$ S.E.M. The EC₅₀ displayed is the mean of values obtained from the individual experiments.

3.2. Functional analysis of D2 and D4 expressing recombinant adenovirus

Functional studies were performed in parallel with saturation and competition binding analyses in CHO-K1 cells infected with either AdRSVD2 or AdRSVD4.4 at an moi of 100. Fig. 1 illustrates the results of our functional assays measuring the inhibition of forskolin-stimulated cAMP accumulation via either the D2 or D4 receptors in the presence of different concentrations of the agonists dopamine or quinpirole.

The percentage inhibition of forskolin-stimulated cAMP levels in the CHO-K1 infected cells was about 90% for both D2 and D4 receptors. The EC₅₀ for dopamine and quinpirole was 20–80 nM.

3.3. Adenoviral-mediated dopamine receptor expression in vitro and in vivo

To explore the host range and factors that modulate the expression levels of the dopamine receptors various estab-

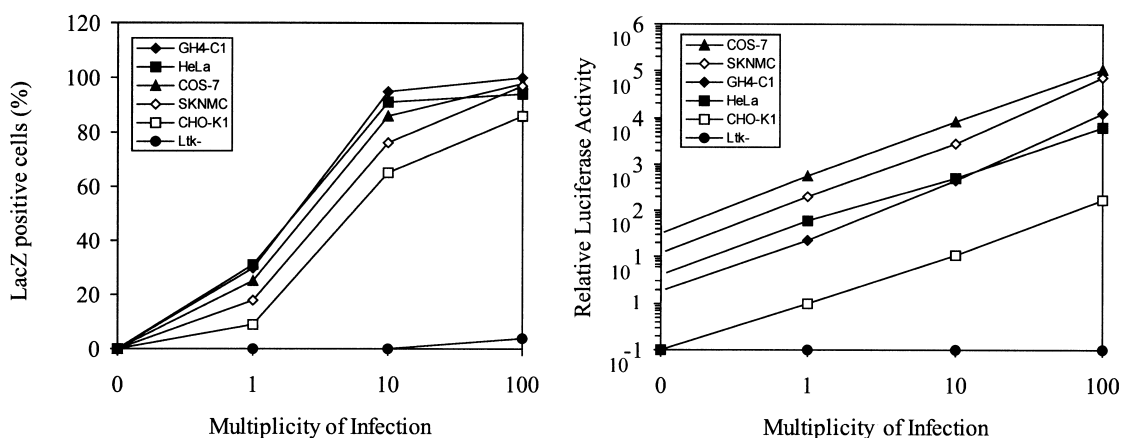


Fig. 2. Infectivity and expression of the adenoviral β -galactosidase and luciferase expression vectors AdCMVLacZ and AdRSVLuc in different cell lines. The left panel depicts the relationship between multiplicity of infection and percentage of β -galactosidase (LacZ) expressing cells of different cell lines infected with AdCMVLacZ. The right panel shows the relationship between multiplicity of infection and luciferase activity for the different cell lines infected with AdRSVLuc. The individual points in the graphs represent the average of at least five independent experiments.

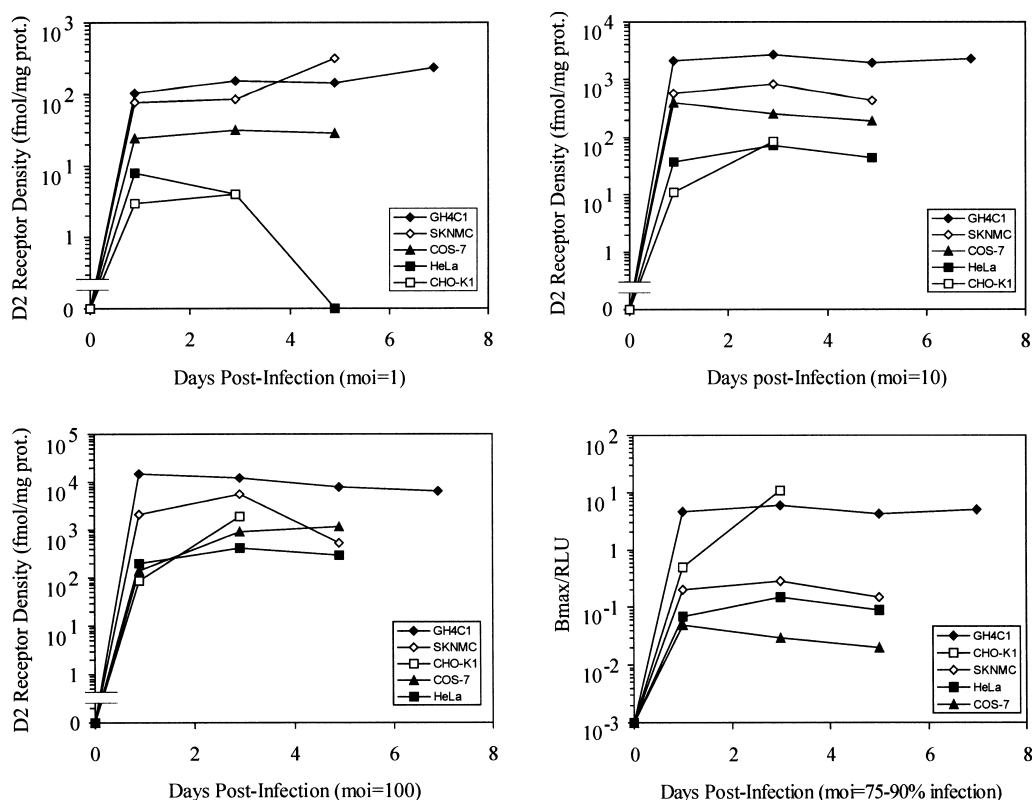


Fig. 3. Relationship between dopamine D2 receptor expression and time post infection with AdRSVD2 at moi of 1 (A), 10 (B), 100 (C). The relationship between receptor expression independent from promoter-mediated expression ($B_{\max}$ /relative luciferase activity (RLU)) and time at moi with equivalent infection rates (75–90%) is shown in D. The individual data points in the graphs are the average densities determined by a 12 point Scatchard plot performed in duplicate from at least two independent experiments.

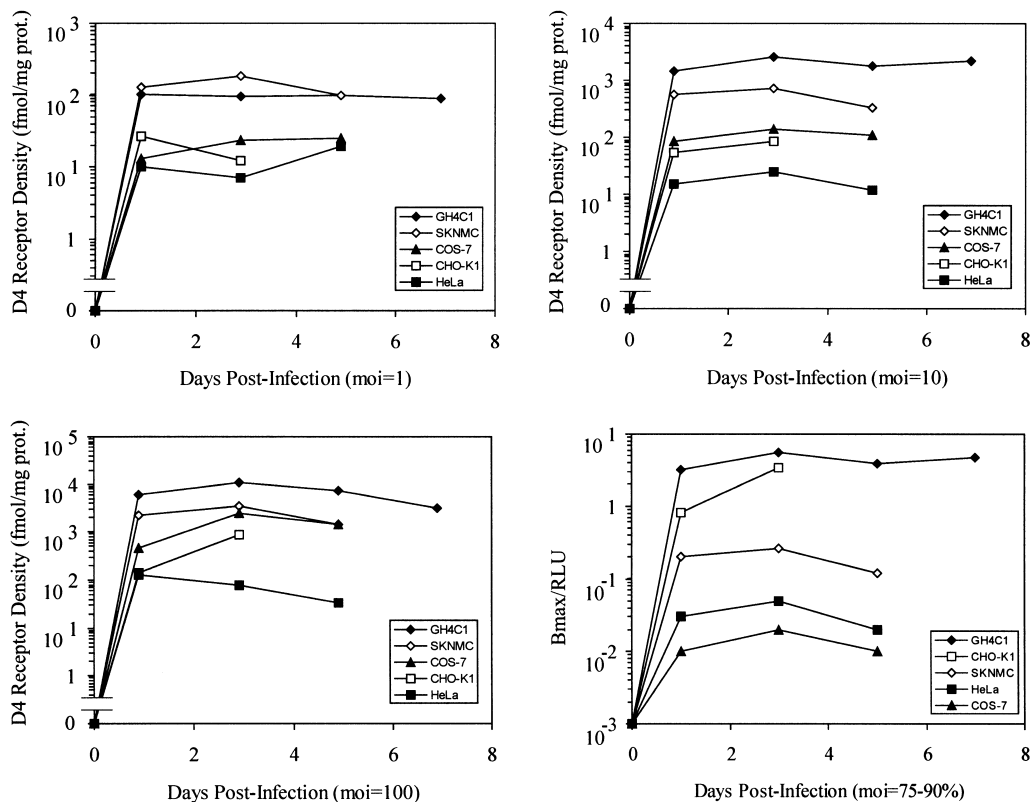


Fig. 4. Relationship between dopamine D4 receptor expression and time post-infection with AdRSVD4.4 at moi of 1 (A), 10 (B), 100 (C). The relationship between receptor expression independent from promoter-mediated expression ($B_{\max}$ /relative luciferase activity (RLU)) and time at moi with equivalent infection rates (75–90%) is shown in D. The individual data points in the graphs are the average densities determined by a 12 point Scatchard plot performed in duplicate from at least two independent experiments.

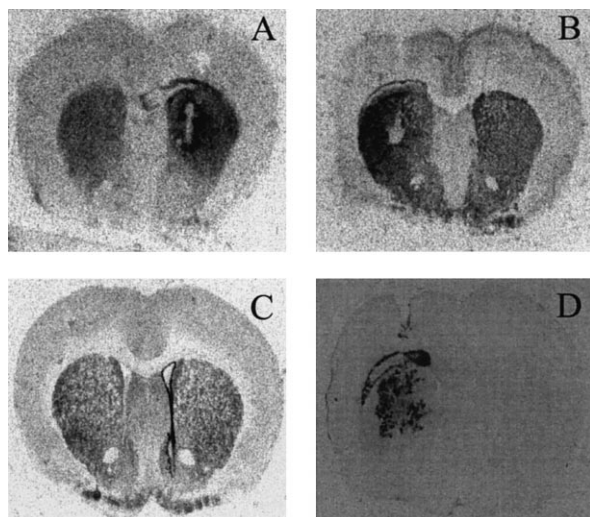


Fig. 5. Autoradiographic detection of D2 and D4 receptors expressed by AdRSVD2 and AdRSVD4, respectively, through stereotactic delivery of these adenoviral expression vectors in rat brain. The dopamine receptors were visualized by in vitro autoradiography using [3 H]spiperone at 7 days post infection. A: Autoradiographic detection of [3 H]spiperone binding sites in a coronal section through rat brain infected with AdRSVD2 in right striatum. B: Autoradiographic detection of [3 H]spiperone binding sites in a coronal section of rat brain infected with both AdRSVD4.4 and AdCMVLacZ on the same site. C: Autoradiographic detection of [3 H]spiperone binding sites in a coronal section of rat brain infected with AdRSVD4.4 in the right ventricle. D: The consecutive brain section shown in B stained for β -galactosidase expression.

lished cell lines (Figs. 2–4) and brain tissues (Fig. 5) were infected with AdRSVD2, AdRSVD4.4, AdRSVLuc and AdCMVLacZ. Saturation binding and Scatchard analyses were performed on CHO-K1, COS-7, HeLa, SK-N-MC and GH4-C1 cell lines infected with AdRSVD2 or AdRSVD4.4 at moi of 1, 10 and 100. Receptor density measurements ($B_{\max}$) for each receptor at post-infection times of 1, 3 and 5 days are presented in Figs. 3 and 4. We observed an almost linear relationship between moi and receptor density. Maximum re-

ceptor density is reached in most experiments within 24 h post infection. Both D2 and D4 receptors were expressed at highest levels in GH4-C1 and SKNMC cells. Even higher levels of expression can be detected in HEK293 cells (~ 50 pmol/mg protein; data not shown) which are, in contrast to the other used cell lines, replication permissive for adenoviral vectors.

To analyze the contribution of infection rate and promoter strength to receptor expression we analyzed in parallel the expression of AdCMVLacZ and AdRSVLuc in these cell lines at different moi, 24 h post infection (Fig. 2). There is a non-linear relationship between moi and the percentage of cells with detectable β -galactosidase levels, and a linear relationship between moi and luciferase activity in all cell lines, except in mouse Ltk $^-$ cells which fail to express either reporter gene. The rank order of infectivity, as measured by percentage of β -galactosidase expressing cells, and rank order of RSV promoter strength, as measured by luciferase activity, differ with the rank order for adenoviral-mediated dopamine receptor expression of the cell lines. Relative dopamine receptor expression at 24 h, controlled for equal infectivity (75–90%) and corrected for relative promoter strength in the different cell lines, exhibits large differences in relative expression between the examined cell lines (Fig. 6).

To explore whether the recombinant D2 and D4 expressing adenoviruses can express the receptors in vivo we injected AdRSVD2 and AdRSVD4.4 by themselves or in combination with AdCMVLacZ ($\sim 2 \times 10^7$ pfu per injection (2–5 μ l) for each virus) in caudate putamen and the third ventricle. Seven days post injection expression of the dopamine receptors is detected by in vitro autoradiography in the injected areas (Fig. 5) and corresponds to the region stained for β -galactosidase activity when co-injected with AdCMVLacZ. Expression of the receptors was higher than the corresponding non-injected contralateral sites, but no differences were observed when only AdCMVLacZ or AdRSVLuc were injected. In striatum AdRSVD2- and D4.4-mediated dopamine receptor expression was measured by comparing the ROD of the injected site was about 110% (D4.4) and 30% (D2) increased compared to the contralateral site as measured by

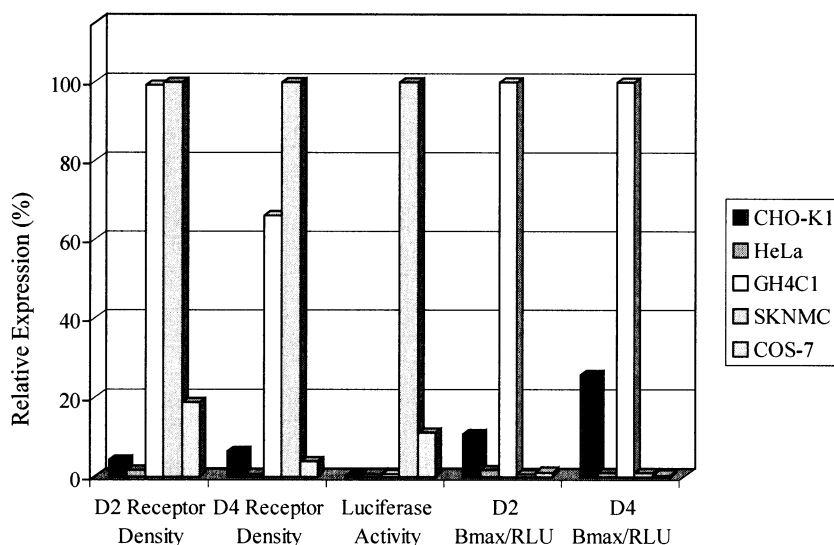


Fig. 6. Relative expression of D2 and D4 receptors and luciferase in different cell lines infected at 75–90% infection efficiency with AdRSVD2 (A), AdRSVD4.4 (B) and AdRSVLuc (C) 24 h post infection. The relative expression of the receptors corrected for promoter-mediated contribution ($B_{\max}$ /relative luciferase activity (RLU)) is shown for D2 (D) and D4.4 (E).

[³H]spiperone binding. Injection of the receptor expressing viruses into the third ventricle resulted in a high [³H]spiperone signal in the ependymal cell layer lining the ventricles with an average 3.6-fold higher level of [³H]spiperone binding than seen in striatum (Fig. 5).

4. Discussion

The data presented demonstrate that first generation recombinant adenoviruses are suitable vectors to mediate high levels of dopamine D2 and D4 receptor expression in a wide variety of established cell lines and in rat brain. The human D2_{long} and D4.4 receptors [27–29] were expressed from the RSV-LTR promoter cloned into the E1a region of adenovirus serotype 5. These replication-deficient recombinant viruses were successfully grown in HEK293 cells at titers approaching 10¹¹ pfu/ml. The viral stocks contained no detectable levels of wild type virus as determined by both HeLa plaque assays and PCR.

The pharmacological profiles and functional activity of the dopamine receptors expressed by the recombinant adenoviruses were tested in CHO-K1 cells. These cells have previously been shown to mediate functional dopamine D2 and D4 receptor expression when stably transfected with expression vectors using the RSV-LTR promoter [25]. The virally expressed dopamine receptors display the appropriate pharmacological profile characterized by the low affinity of raclopride and relatively higher affinity of clozapine for D4 receptors as compared to D2 receptors (Table 1) [27]. In contrast to the D4 receptor expressing cells, the D2 expressing cells do not display high affinity binding for either dopamine or quinpirole (Table 1). However, both receptor subtypes expressed by the adenoviral vector can almost completely reverse forskolin-stimulated cAMP increases when stimulated by dopamine or quinpirole (Fig. 1). This indicates that both receptors are functional and the difference in percentage of high affinity agonist binding sites may be due to the levels of expression of the receptors in relation to the available pool of interacting G proteins [30,31], indicating that the two receptors interact preferentially with different G proteins.

Various established cell lines were infected with the recombinant adenoviral vectors at different moi for various lengths of time to evaluate the influence of time and moi on dopamine receptor expression. All cell lines tested that were efficiently infected by the recombinant adenovirus expressed the dopamine receptors. Ltk⁻ cells could not be infected efficiently with adenovirus. In general, it was observed that maximum expression for the dopamine receptors was reached within 24 h post infection in the various cell lines, and the levels of expression were proportional to the moi. Further, expression levels stayed relatively constant after 24 h for the duration of the culture to a maximum of 7 days. The duration of culture post infection was determined by the ability to grow without having to split the cells, and thus was directly determined by the cell doubling time. The cells continued to grow normally after infection, although at the highest moi some cell toxicity was observed. In absolute terms the somatomammotrophic cell line GH4-C1 and the neuroepithelioma SKNMC expressed the highest levels of dopamine receptors. For the D4 receptors the maximum recorded levels in GH4-C1 cells were more than 10-fold higher than reported in any other expression system used. The relatively constant levels of ex-

pression for the duration of culture suggests that dilution of the expression vector through cell doubling is of minor influence, possibly reflecting the relatively long half-life of the receptors.

The recombinant adenoviral vector mediates good expression of the dopamine D2 and D4 receptors in both striatum and ependymal cells lining the ventricles. In addition we were able to express these two receptors in primary cultures of anterior pituitary cells (data not shown). Similar findings have recently been reported by Ikari et al. [18], who achieved increased *in vivo* expression of D2 receptors in striatum, using an adenoviral vector. This vector mediated a change in rotational behavior after unilateral injection of the vector in striatum, indicating that their vector is functionally active and can be used to alter behavioral responses [19]. This vector of Ikari et al. [18] employs the cytomegalovirus (CMV) immediately early promoter enhancer rather than the RSV-LTR promoter of our studies. In time course experiments they could achieve maximum receptor expression in striatum of about 50% over endogenous D2 expression in that region at day 3 post injection. The expression decreased to 20% at day 7 to undetectable after 3 weeks post injection. Our D2 expressing vector achieved a 30% increase in expression of D2 receptors in striatum at 7 days post injection, which is in agreement with Umegaki et al. [19]. However, our D4 expressing vector mediated levels of expression that were 110% over endogenous D2 levels in striatum. Whether this is inherent to the D4 receptor or normal variation requires a comparative analysis study. The *in vivo* time course studies done by Umegaki et al. [19] suggest that maximum D2 receptor expression occurs rapidly after infection, but contrary to our *in vitro* studies a rapid loss in expression was also observed, possibly involving a combination of cellular immune responses, cytotoxicity [32,33] and promoter silencing [34].

The difference in expression levels between the cell lines can be attributed to several factors, which include infection efficiency and RSV-LTR promoter strength. Differences in efficiency of infection were most striking for the mouse Ltk⁻ cells, which apparently cannot be infected with adenovirus. This cell line is known to use the employed viral promoters from DNA transfection assays using expression constructs with these promoters. Comparison of the expression of the receptors at equivalent infection levels between different cell lines still reveals large differences in expression. In part these differences are due to differences in strength at which the RSV-LTR promoter is used in the different cell lines, as can be seen from the differences in expression of the reporter vector AdRSVLuc. The SKNMC and COS-7 cells, in contrast to the CHO-K1 cells, display very efficient expression of AdRSVLuc (Fig. 6). However, dopamine receptor expression levels in the different cell lines do not correlate with the expression of the luciferase reporter vector. When the dopamine receptor expression is adjusted for infection efficiency and corrected for promoter strength, differences in dopamine receptor expression of more than two orders of magnitude can still be observed between the different cell lines (Fig. 6). These analyses indicate that the high levels of dopamine receptor expression in SKNMC cells is strongly determined by the promoter strength, while high levels of expression in GH4-C1 and CHO-K1 cells are more strongly determined by post-transcriptional regulation. These data indicate that factors other than infection efficiency and promoter strength play

an important role in the expression of these receptors. Recently, much progress has been made in the understanding of how agonist-mediated processes modulate receptor synthesis, internalization, sequestration and down-regulation, but little is known about how cells regulate their steady-state levels of expression. Potential factors underlying these differences may be related to which signal transduction pathways the receptors are coupled to, and mechanisms that control agonist-mediated receptor regulation. However, it should be noted that both the D2 and D4 receptor show very similar profiles in expression levels in the different cell lines, despite observed differences in functional coupling between the two receptors [23]. Cellular factors that modulate RNA stability, the efficiency of translation and transport and turnover of non-stimulated dopamine receptors have not been described, but appear to be strong determinants for cell-specific expression. This adenovirus-mediated expression system provides a valuable resource for the examination of such processes.

In conclusion, recombinant adenoviral vectors are excellent vehicles to mediate high expression of D2 and D4 receptors *in vitro* and *in vivo*. Expression of the receptors is, as expected, dependent on factors that determine viral tropism as well as the strength of the employed promoter. Most importantly, these studies demonstrate that steady state levels of receptor expression are strongly dependent on post-transcriptional processes which can contribute to large differences, up to two orders of magnitude, in receptor expression in different cell types.

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