Functional expression of the murine D_2, D_3 and D_4 dopamine receptors in *Xenopus laevis* oocytes

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Abstract The different murine D2-type dopamine receptors (D_{2L}, D_{2S}, D_{3L}, D_{3S}, and D_4) were expressed in *Xenopus laevis* oocytes. The D2-type receptors were all similarly and efficiently expressed in *Xenopus* oocytes and were shown to bind the D2 antagonist [^{125}I]sulpride. They were all shown to activate Cl^- influx upon agonist stimulation. Using the diagnostic inhibitor bumetanide, we were able to separate the $Na^+/K^+/2Cl^-$ cotransporter component of the Cl^- influx from the total unidirectional Cl^- influx. The D_{3L} subtype was found to operate exclusively through the bumetanide-insensitive Cl^- influx whereas the other D2-type receptors acted on the $Na^+/K^+/2Cl^-$ cotransporter as well. The pertussis toxin sensitivity of the receptor-activated chloride influx via the $Na^+/K^+/2Cl^-$ cotransporter varied between the various D2-type receptors showing that they may couple to different G proteins, and activate different second messenger systems.

Key words: Murine D2 dopamine receptor (D_2, D_3, D_4); Bumetanide; Dopamine; *Xenopus* oocyte; $Na^+/K^+/2Cl^-$ cotransporter

1. Introduction

Two types of dopamine receptor, named D1 and D2, have for many years been known to transmit the action of the CNS neurotransmitter dopamine. These two classes of dopamine receptors belong to the G protein-linked receptor superfamily, and differ in their pharmacological and biochemical properties (reviewed in [1,2]). A multiplicity of dopaminergic receptors have recently been revealed by cloning studies. Each receptor arises from a different gene, but still adheres to the original D1/D2 classification. Thus, D1-like features are exhibited by the cloned D_1 and D_5 receptors, while the D_2, D_3 and D_4 receptors all display D2-like properties [3]. The D_2 and D_3 receptor subtypes have furthermore been found to be alternatively spliced in the coding region, yielding functional short and long receptor isoforms [4–8]. These splice variants differ by a stretch of amino acids (29 for the D_2 receptor, 21 for the D_3 receptor) located in the putative third cytoplasmic loop. These stretches of amino acids are either present or absent in the alternatively spliced isoforms thereby giving rise to the D_2 and D_3 'long' and 'short' subtypes accordingly (D_{2L}, D_{2S}, D_{3L} and D_{3S}).

The cloned D_2, D_3 and D_4 receptors have been expressed in various cell lines, and functional studies have revealed their ability to couple to a range of second messenger pathways [9–11]. Amino acid sequence comparison shows these three receptors to be most diverse in their third cytoplasmic domains, the region highlighted for its role in G protein coupling and second messenger activation. In addition, for both the D_2 and D_3 receptors, alternative splicing occurs in this domain. This has led to much speculation as to whether the functional differences between the various dopamine receptors may lie in their G protein specificity and, hence, signal transduction pathways.

We have previously cloned and sequenced the murine D_2, D_3 and D_4 receptors [6,12]. In this study, we have expressed these cloned murine receptors in *Xenopus laevis* oocytes in order to examine and compare some of the functional characteristics of the D_{2L}, D_{2S}, D_{3L}, D_{3S} and D_4 receptors. The oocyte of *Xenopus laevis* represents a powerful tool for studying many of the functions of cells using microinjection of foreign mRNAs [13] and cDNAs [14].

In the oocyte, most of the Cl^- transport is mediated by the Cl^- channels (which are directly activated by Ca^{2+}) and the bumetanide-sensitive $Na^+/K^+/2Cl^-$ cotransporter, regulated by protein kinase C (PKC) and protein kinase A (PKA) [15]. Since the $Na^+/K^+/2Cl^-$ cotransporter is regulated by PKA, activation or inhibition of adenylate cyclase will affect the $Na^+/K^+/2Cl^-$ cotransporter, but not the Cl^- channels.

Presented here is a functional assay for receptors linked via G proteins to the signal transduction pathways involving cAMP. The assay, which utilizes the unidirectional Cl^- influx mediated by the endogenous $Na^+/K^+/2Cl^-$ cotransporter as a reporter for functional receptors, is used to demonstrate that the various D2-type receptors modulate differentially chloride influx following expression in *Xenopus laevis* oocytes. This may further support the notion that functional diversity of dopamine receptors is also a consequence of the signal transduction pathway that they stimulate.

2. Materials and methods

2.1. Materials

All reagents were of analytical grade, and were purchased from Sigma, St. Louis, MO, unless otherwise stated. Bumetanide was added to the oocytes from a 7.5 mM stock solution in ethanol. Dopamine was prepared fresh as a stock solution of 100 mM in a Ca^{2+} free modified frog Ringer's solution (ORi: 107 mM NaCl, 2.5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM Na_2HPO_4, 5 mM HEPES, pH 7.6) supplemented with penicillin G (10 units/l) and streptomycin sulfate (10 mg/l) with 1% ascorbic acid. ^{36}Cl, [^{125}I]sulpride and [^{35}S]methionine were obtained from Amersham, UK.

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Abbreviations: BSA, bovine serum albumin; CNS, central nervous system; DAG, diacylglycerol; G protein, GTP-binding protein; HEPES, 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid; Ins-1,4,5-P3, inositol-1,4,5-trisphosphate; PKC, protein kinase C; PKA, protein kinase A; PLC, phospholipase C; PTX, pertussis toxin

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2.2. Frogs and oocytes

Ovarian lobes were surgically removed from *Xenopus laevis* anesthetized with 3-aminobenzoic acid ethyl ester (0.1%). The ovaries were disrupted into small clumps and oocytes released by incubation with vigorous agitation at room temperature for 2 h with collagenase (type IA; 2 mg/ml) in a Ca^{2+} free ORi. Complete release of follicular cells required subsequent incubation for 10 min in Ca^{2+} free ORi. Defolliculated oocytes of oogenesis stages V and VI were manually selected and placed in 3 ml Petri dishes (Nunclon, Denmark). The following day the oocytes were injected with cDNA encoding the D_{2L}, D_{2S}, D_{3L}, D_{3S} and D_4 receptors inserted into the eukaryotic expression vector pcDNAI/Amp (InVitrogen). The cDNAs were dissolved in H_2O, final concentration of cDNA was 0.20 ng/nl, and a volume of 9.2 nl was injected per oocyte. Injections were performed as described by Colman [14]. Oocytes were maintained for 3 days at 19°C in ORi supplemented with penicillin G (10 mg/l) and streptomycin sulfate (10 mg/l) and the medium was changed daily.

2.3. Antibody preparation

The D_2-specific antibody directed against peptide SPPERTRYSPIPPS corresponding to residues 288–301 of the murine D_2 dopamine receptor was elicited and purified as described previously [16].

2.4. [^{35}S]Methionine labeling and immunoprecipitation

Eight oocytes injected with D_{2S} or D_{2L} cDNAs were labeled for 3 h at 21°C, in the presence of 220 μCi of [^{35}S]methionine in 1 ml ORi. The oocytes were manually homogenized in 65 mM Tris-HCl pH 7.5, 1 mM sodium vanadate and 10 mM EDTA, in the presence of the protease inhibitors phenylmethylsulfonyl fluoride (PMSF; 1 mM), leupeptin (5 μg/ml), soybean trypsin inhibitor (5 μg/ml) and benzamidine (15 μg/ml). Yolk proteins were removed by 10 min centrifugation at 25 000×g. Extracts were solubilized with 2×immunoprecipitation buffer (130 mM Tris-HCl, pH 7.5, 150 mM NaCl, 5% Triton X-100, 100 mM NaH_2PO_4, 1 mM sodium vanadate, 10 mM EDTA, and 10 mg/ml BSA with the protease inhibitors as mentioned above) for 10 min on ice with intermittent vortexing, and spun for 10 min at 12 000×*g*. The supernatants were pre-absorbed to protein A-agarose (Immobilized rProtein A, Repligen), pre-swollen in solubilization buffer for 30 min at 4°C. Antibodies were pre-bound to protein A-agarose by their incubation for at least 1 h at 4°C. The solubilized lysates were incubated with the protein A-agarose-bound antibody overnight at 4°C, with shaking. When peptide inhibition of the immunoprecipitation was performed, the peptide was included at a final concentration of 20 μM. Upon immunoprecipitation, beads were washed 5 times at 4°C in 50 mM Tris-HCl, pH 8.0, 0.1% SDS, 0.1% Triton X-100, 5 mM EDTA, supplemented with the above mentioned protease inhibitors, with decreasing concentrations of NaCl starting at 150 mM. The adsorbed material was eluted from the beads with sample buffer (25 mM Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 5% β-mercaptoethanol, 0.01% bromophenol blue) at 37°C for 30 min, and electrophoresed on SDS-polyacrylamide gels as previously described [16].

2.5. Chloride uptake

$^{36}Cl^-$ was used as tracer. The uptake of $^{36}Cl^-$ was measured by incubation of the oocytes in ORi with 1% (w/v) ascorbic acid. The $^{36}Cl^-$ uptake is linear for at least 2 h (data not shown). The total Cl^- uptake was initiated by the simultaneous addition of $^{36}Cl^-$ (800.000 Bq/ml) and dopamine (100 μM), and followed for 15 min. The uptake was terminated by 5 washes in ice-cold Na^+ free stop solution (107 mM *N*-methyl-D-glucamine Cl^-, 2.5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM Na_2HPO_4, 5 mM HEPES, 100 μM bumetanide, pH 7.6). The bumetanide-insensitive Cl^- uptake was measured in the presence of 30 μM bumetanide. The bumetanide-sensitive Cl^- influx was then calculated as the difference between the total influx and the bumetanide-insensitive flux. The oocytes were then transferred in groups of five into scintillation vials containing 1 ml of 10% SDS in 0.2 M NaOH, and samples were shaken for 3 h. Radioactivity of the cell lysates was determined by liquid scintillation counting. The unidirectional Cl^- influxes, from several batches of oocytes, are calculated from the radioactivity of the cell lysates, and the specific activities of the incubation medium, and presented as nmol/h/oocyte (mean ± S.D.) with the number of experiments using different batches of oocytes in parentheses.

2.6. [^{125}I]Sulpride binding

cDNA injected oocytes were labeled with [^{125}I]sulpride. Briefly, oocytes were incubated at 22°C in ORi containing 1% (w/v) ascorbic acid, 0.1% (w/v) BSA (fraction V), 0.01% (w/v) cytochrome *c* and 0.5 nM [^{125}I]sulpride (10–100 mCi/ml) for 90 min, followed by four rapid washes with this buffer (without isotope). The oocytes were tested under the microscope, and any ruptured oocytes were discharged at this stage. The oocytes were counted in groups of five. Aliquots of the final wash were counted to test the efficiency of the washing procedure: these never exceeded 20 cpm and were used as background. Non-specific binding was determined using non-injected oocytes. The radioactivity of the oocytes was determined by γ-radiation counting (Packard System). The [^{125}I]sulpride binding from several batches of oocytes is presented as fmol/oocyte (mean ± S.D.), with the number of experiments using different batches of oocytes in parentheses, calculated from the radioactivity of the oocytes and the specific activities of [^{125}I]sulpride.

3. Results and discussion

We have employed *Xenopus laevis* oocytes to express the various murine D2-like receptors (D_{2L}, D_{2S}, D_{3L}, D_{3S} and D_4) following microinjection of the corresponding cDNAs. In order to determine the level of expression of each receptor subtype, oocytes were injected with cDNA encoding the murine D_{2L}, D_{2S}, D_{3L}, D_{3S} or D_4 receptors, and groups of oocytes for each receptor subtype were taken for binding analysis using the D_2-specific ligand [^{125}I]sulpride. All five D2 subtypes exhibited significant [^{125}I]sulpride binding (Table 1), and this binding was inhibited when performed in the presence of the competitive ligand, butaclamol (data not shown). While the level of expression of each receptor varied slightly from one batch of oocytes to the other, averages of several experiments showed that all five subtypes were expressed at approximately the same level, with [^{125}I]sulpride binding ranging from 2.44 to 3.16 fmol/oocyte (Table 1).

The expressed receptor could be immunoprecipitated from injected oocytes as shown in Fig. 1 for the D_{2S} receptor. A band of 80 kDa is immunoprecipitated with the specific anti-peptide antibodies, while it is not precipitated in the presence of the relevant peptide, or in non-injected oocytes. This band most probably represents the glycosylated form of the receptor [17,18]. The lower heavy band of about 60 kDa, which appears in all lanes, is not specific.

To analyze the function of the expressed receptors, we have examined and compared second messenger activation between the respective D2 receptor subtypes. The unidirectional Cl^- influx, following dopamine stimulation of D2 receptor-expressing oocytes, was measured (Table 1). The basal Cl^- influx in non-injected oocytes is 16.9 ± 2.9 nmol/h/oocyte ($n = 6$). This influx was increased 5–6-fold upon incubation with dop-

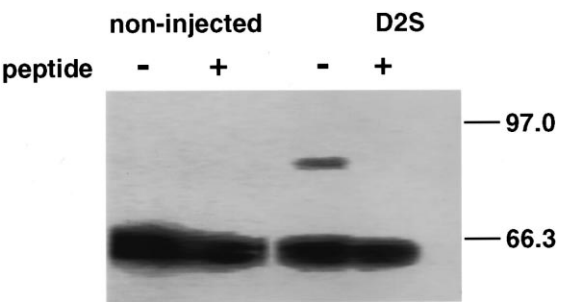

Fig. 1. Immunoprecipitation of exogenous D_{2S} dopamine receptor expressed in *Xenopus laevis* oocytes using the D_2-specific anti-peptide antibodies.

Table 1
Sulpride binding sites, dopamine-induced unidirectional Cl^- influx and normalization of the dopamine-induced unidirectional Cl− influx in *Xenopus* oocytes injected with cDNA encoding the murine D_{2S}, D_{2L}, D_{3S}, D_{3L} or D_4 dopamine receptors

Receptor subtype injected	[^{125}I]Sulpride binding sites (fmol/oocyte) ($n=3$)	Total Cl^- influx (nmol/h/oocyte) ($n=4$–6)	Normalized Cl^- influx (nmol/h/fmol recep) ($n=4$–6)
D_{2L}	2.83 ± 0.56	105.6 ± 28.8	25.2 ± 7.1
D_{2S}	3.16 ± 0.44	102.8 ± 20.5	37.1 ± 11.6
D_{3L}	3.09 ± 0.75	77.8 ± 8.7	29.3 ± 6.5
D_{3S}	2.44 ± 0.26	99.0 ± 21.8	28.1 ± 9.7
D_4	2.47 ± 0.05	77.7 ± 23.0	32.0 ± 10.1
Non-injected	0.07 ± 0.02	16.9 ± 2.9	–

Cell surface binding of [^{125}I]sulpride in cDNA-injected or water-injected oocytes and Cl uptake were measured as described in Section 2. The results are presented as mean ± S.D. from four to six independent experiments. The normalized Cl influx is expressed as the total Cl^- influx (nmol/h/oocyte) per [^{125}I]sulpride binding site (fmol/oocyte) in paired experiments after correction for the influx in control oocytes.

amine, in oocytes expressing the various D2-type dopamine receptors. All five dopamine receptor constructs were found capable of activating a chloride influx. While some variation appeared to exist between the subtypes for the total Cl^- influx, when these values were normalized to account for differences in receptor number, it was shown that all five D2-type receptors produce a similar chloride influx after activation by dopamine (Table 1). Thus, the various dopamine receptors are similarly expressed in *Xenopus* oocytes, and are all capable of acting as functional receptors which activate second messenger pathways upon agonist binding.

The Cl^- influx in *Xenopus laevis* oocytes consists of at least two components which can be distinguished by the use of specific ion transport inhibitors, such as the diuretic agent bumetanide, a specific cotransport inhibitor. Receptors coupled to changes in chloride influx may be operating through either the $Na^+/K^+/2Cl^-$ cotransporter (measured as the bumetanide-sensitive Cl^- influx) and/or the Cl^- channels (bumetanide-insensitive). The endogenous Cl^- channels which are directly activated by Ca^{2+} act as an amplification system for the increase in intracellular Ca^{2+}, thus providing a sensitive, although indirect assay of changes in Ca^{2+} homeostasis. In order to analyze the relative contribution of each of the components of the chloride influx, and to determine whether the respective D2-type dopamine receptors may have differential activities at the $Na^+/K^+/2Cl^-$ cotransporter, we measured the agonist-stimulated Cl^- influx in dopamine receptor-expressing oocytes, in the absence or presence of bumetanide (30 μM). The bumetanide-sensitive Cl^- influx contributed by the cotransporter activity is calculated by subtraction of the bumetanide-insensitive component from the total Cl^- influx. The two components of the dopamine-induced Cl^- influx in D2-expressing oocytes (the bumetanide-sensitive and -insensitive Cl^- influx) are shown in Table 2.

The use of bumetanide revealed some differential properties in functional activity between the various D2 dopamine receptor subtypes. While the stimulated Cl^- influx in oocytes expressing the D_{3L} receptor was virtually unaffected by bumetanide, showing that this receptor subtype has no stimulatory effect on the $Na^+/K^+/2Cl^-$ cotransporter, the Cl^- influxes in oocytes expressing the other receptor subtypes were reduced in the presence of bumetanide. This shows that these receptor subtypes may act on both the $Na^+/K^+/2Cl^-$ cotransporter and the bumetanide-insensitive Cl^- transport systems. Although D2 dopamine receptors are thought to operate through inhibition of adenylate cyclase, they may also inhibit phospholipase C, leading to reduced PKC activity [19]. Such reduced PKC activity may lead to activation of the $Na^+/K^+/2Cl^-$ cotransporter as previously suggested [15]. Alternatively, the cotransporter activity might be explained by activation of D_2 and D_4 (but not D_3) dopamine receptor by type II adenylate cyclase, through a release of βγ subunits of the G protein as reported recently [20].

Concerning the bumetanide-insensitive component of the Cl^- influx, it should be noted that in measuring Cl^- influx in the presence of flufenamic acid, an inhibitor of Ca^{2+}-activated Cl channels [21], no change in the Cl influx in oocytes expressing either of the dopamine receptors has been observed (data not shown). This suggests that the bumetanide-insensitive Cl influx is not contributed by the Ca^{2+}-activated Cl channel in this expression system. The precise nature of the bumetanide-insensitive Cl^- influx needs further investigation.

The variability in second messenger activity between the receptor subtypes suggest that the various receptors may in-

Table 2
Effect of dopamine on the $Na^+/K^+/2Cl^-$ cotransport activity in *Xenopus* oocytes injected with cDNA encoding the murine D_{2S}, D_{2L}, D_{3S}, D_{3L} or D_4 dopamine receptors

Receptor subtype	Cotransporter (nmol/h/oocyte) (bumet.-sensitive Cl^- influx)	Other Cl− transporter (nmol/h/oocyte) (bumet.-insensitive Cl^- influx)	Inhibition of total Cl^- influx by bumetanide (%)
D_{2L}	25.7 ± 5.0	97.7 ± 29.2	21
D_{2S}	52.4 ± 23.8	62.4 ± 7.8	46
D_{3L}	0.9 ± 0.9	98.1 ± 15.4	1
D_{3S}	53.7 ± 16.4	81.5 ± 25.8	40
D_4	38.3 ± 10.7	54.7 ± 9.3	41
Non-injected	4.7 ± 1.4	9.8 ± 2.4	32

Cl^- uptake was measured as described in Section 2, in the absence and presence of bumetanide. The Cl^- influx via the $Na^+/K^+/2Cl^-$ cotransporter is calculated as the Cl^- influx in the presence of bumetanide subtracted from the Cl^- influx in the absence of bumetanide. The Cl^- influx via the $Na^+/K^+/2Cl^-$ cotransporter is presented as the bumetanide-sensitive Cl^- influx. The bumetanide-insensitive uptake of Cl^- was measured in the presence of 30 μM bumetanide. The results are presented as the mean ± S.D. from four to six experiments.

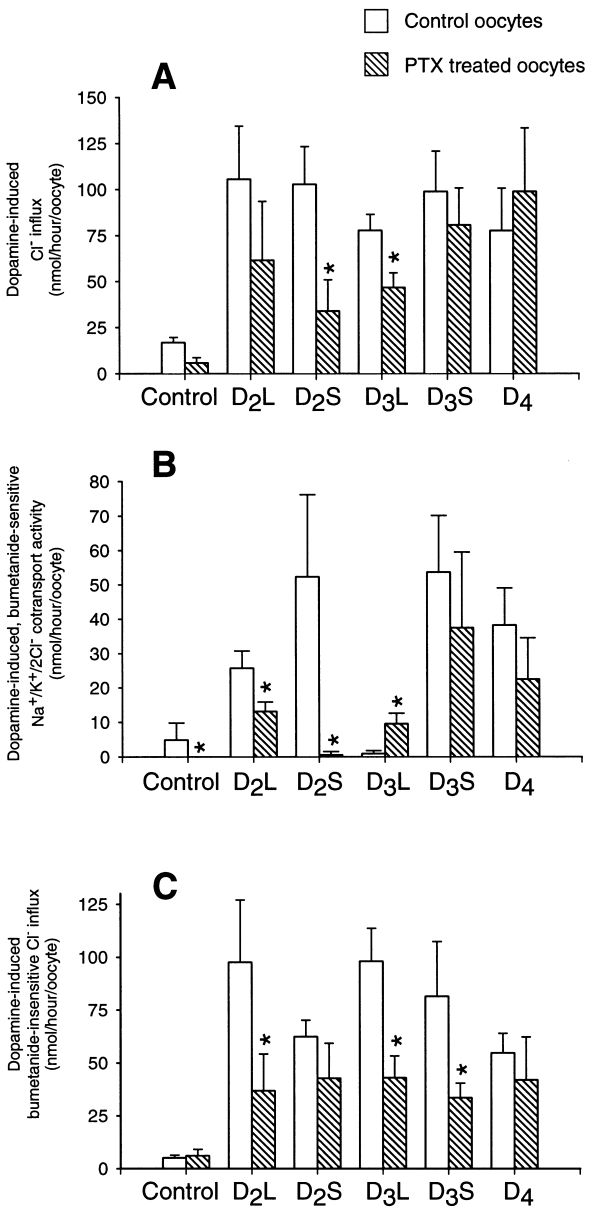

Fig. 2. Effect of pertussis toxin on the dopamine-induced unidirectional Cl^- influx in *Xenopus* oocytes expressing the murine D2-type dopamine receptors. Following 60 h of incubation, *Xenopus* oocytes, microinjected with D2 receptor cDNAs (9.2 nl; 0.2 ng/nl) were treated for 12 h with pertussis toxin (0.5 μg/ml). The uptake of Cl^- was measured by incubation of oocytes in ORi with dopamine (100 μM), pertussis toxin (0.5 μg/ml) and $^{36}Cl^-$, as described in Section 2 and carried out in the absence of inhibitor to give the total Cl^- influx (A), or in the presence of 30 μM bumetanide (C). The Cl^- influx via the $Na^+/K^+/2Cl^-$ cotransporter (B) is calculated as the Cl^- influx in the presence of bumetanide (C) subtracted from the Cl^- influx in the absence of bumetanide (total Cl^- influx, A) and is presented as the bumetanide-sensitive $^{36}Cl^-$ influx. The results are presented as the mean ± S.D. from four to six experiments. Significant differences from the appropriate control oocytes ($P \leq 0.05$, using Student's independent *t*-test) are marked by an asterisk.

teract with different signal transduction pathways to modulate the activity of the $Na^+/K^+/2Cl^-$ cotransporter. This led us to examine the G protein association of these dopamine receptor-activated second messenger pathways. One of the principal advantages of *Xenopus* oocytes, which makes them an attractive system for exogenous expression of G protein-coupled receptors, is the wide range of G proteins they display. Several pertussis toxin (PTX)-sensitive G proteins have been identified in *Xenopus* oocytes [22], and in a recent report, Quick and coworkers [23] described the coexistence of two forms of phospholipase C activated by PTX-sensitive (Go) or PTX-insensitive (Gq) G proteins in the *Xenopus* oocyte. We therefore tested whether the murine dopamine receptors expressed in the oocytes mediate changes in chloride influx through coupling with G proteins sensitive or resistant to PTX.

To this end, we examined the effect of overnight incubation of the oocytes with PTX (0.5 μg/ml) on the total dopamine-induced Cl^- influx in oocytes expressing the various D2-type receptors (Fig. 2A), on the $Na^+/K^+/2Cl^-$ cotransporter (that is bumetanide-sensitive; Fig. 2B) and on the Cl^- influx that is bumetanide-insensitive, i.e. measured in the presence of bumetanide (Fig. 2C). Using this assay, we have observed some functional differences between the respective receptor subtypes, suggesting that the different receptor subtypes may couple to different G proteins to produce their effect on Cl^- influx.

In contrast to the D_2 and D_3 receptor subtypes, D_4 receptor activity was not significantly altered by the presence of PTX, suggesting that in *Xenopus* oocytes it may couple with one or more PTX-insensitive G proteins to cause changes in Cl^- influx. By contrast, in the case of the D_2 receptor, PTX reduced the total Cl^- influx mediated by the D_{2S} isoform by approximately 67%, and that mediated by the D_{2L} isoform by approximately 40% (Fig. 2A). However, the activities of the two components of this ion influx, namely the bumetanide-sensitive $Na^+/K^+/2Cl^-$ cotransporter and the bumetanide-insensitive Cl^- influx, differed between these two isoforms. While the bumetanide-insensitive Cl^- influx was reduced by approximately 60% by PTX for the D_{2L} isoform, it was only slightly reduced for the D_{2S} isoform (Fig. 2C). Thus, the majority of the inhibitory effect of PTX on the D_{2S}-induced influx was caused by uncoupling from the signalling cascade that activates the $Na^+/K^+/2Cl^-$ cotransporter. On the other hand, the signal transduction pathway that activates the cotransporter after stimulation of the D_{2L} receptor remained relatively unaffected by PTX (Fig. 2B), indicating that D_{2S} and D_{2L} couple to different G proteins when expressed in *Xenopus* oocytes. For the D_3 receptor, both long and short isoforms showed a reduction (50–60%) in the presence of PTX, at the bumetanide-insensitive Cl^- influx (Fig. 2C), whereas for both D_3 receptor isoforms, PTX had little or no effect on the $Na^+/K^+/2Cl^-$ cotransporter, indicated by the bumetanide-sensitive component of the Cl^- influx (Fig. 2B).

The D_4 receptor-produced changes in the Cl^- influx do not appear to result from activation of PTX-sensitive G proteins in *Xenopus* oocytes (Fig. 2C), implying it acts through non-Gi-like proteins. Besides PTX-insensitive G proteins coupled to PLC, such as Gq, a number of other PTX-insensitive G proteins exist, whose second messenger activity is not known. One or more of these G proteins could be activated by the D_4 receptor, and subsequently modulate the Cl^- influx pathways in the *Xenopus* oocyte. In addition, we found that for PTX-treated oocytes expressing the D_{3L} receptor, there even appeared to be an increase in the Cl^- influx via the $Na^+/K^+/2Cl^-$ cotransporter compared to non-PTX-treated oocytes. This raises the possibility that this subtype may normally inhibit the $Na^+/K^+/2Cl^-$ cotransporter via a pathway

which involves a PTX-sensitive G protein. In the presence of PTX, this inhibition would be removed, and the $Na^+/K^+/2Cl^-$ cotransporter activity would appear to increase. Along these lines it has been recently shown that the rat D_4 receptor inhibits forskolin-stimulated cAMP accumulation via the PTX-sensitive $G\alpha_{t2}$ [24]. Furthermore, PKA is involved in the activation of the $Na^+/K^+/2Cl^-$ cotransporter in several cell types [25,26], so a receptor-induced inhibition of adenylate cyclase would be consistent with a suppression of the $Na^+/K^+/2Cl^-$ cotransport activity. Another possibility is that D_{3L} causes activation of protein kinase C, a protein kinase previously demonstrated to inhibit the $Na^+/K^+/2Cl^-$ cotransporter in *Xenopus* oocytes [15].

In this paper, we have presented a rapid and reliable assay for analyzing and comparing properties of related receptors of the family of D2-type receptors. Using this technique, we have shown that the members of this subfamily interact with different G proteins to produce similar effects further down in the signal transduction pathway. This approach should facilitate further analysis of the precise elements involved in the signal transduction pathways of each D2 receptor subtype, and should provide useful insight into the signaling mechanisms of dopamine receptors.

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