vision, and also rejects any hypothesis which ascribes polarization sensitivity to some dichoric or birefringent element of the eye’s prerenal optical apparatus common to all photoreceptors under all adaptation conditions. The 90° periodicity of polarization sensitivity (Fig. 1c) also argues against any single prerenal dichroic filter which would reside distal to all the cones sensitive to long wavelength light, as such a filter would be expected to produce a 180° periodicity. Given the hypothesis that double cones function as polarization-sensitive waveguides, Fig. 1c reveals another fundamental property of the neural signals generated by the double-cone mosaic. By a physical identity, a linearly polarized light can be uniquely described as the vector sum of two orthogonally polarized components. In Fig. 4a the thresholds of Fig. 1c are replotted as their unique horizontal and vertical components. If the signals generated by receptors with maximal sensitivity to the purely H-polarized and purely V-polarized stimuli were transmitted independently, the thresholds would be expected to lie near the unit circle; if the signals were combined linearly with the same sign, the thresholds would lie between the unit circle and the negative diagonal. In fact, the thresholds for stimuli comprising both H and V polarization components were always greater than thresholds for the purely horizontally or purely vertically polarized stimuli; for example, admixing a V-component at 50% of its own threshold to an H-polarized stimulus requires that the intensity of the latter be doubled to reach threshold. As any increase in light intensity must necessarily increase the quantal absorption rate of all the cones under the image, the threshold points lying outside the unit circle in Fig. 4 establish the presence of a polarization-opponent neural mechanism. This result suggests, by analogy with the role of colour-opponent coding, that polarization vision in this and related vertebrates serves as a contrast-detecting system. Stimuli with reduced fractions of H polarization were therefore used to measure a ‘polarization contrast sensitivity’ function for the sunfish. The photopic sensitivity to H-polarized light decreased as the net percentage of light polarized in that plane was reduced (Fig. 4b). The thresholds for all targets with polarization contrasts >20%, however, are reliably lower than the threshold for a circularly polarized target, which has zero polarization contrast. We conclude that the polarization-opponent neural code is capable of mediating the detection of targets with polarization contrast >20%. Perhaps the most striking general conclusion to be drawn from this result is that the polarization contrast mechanism we have inferred in the sunfish is the most sensitive visual channel available to the animal for detecting small targets under our light-adapted conditions, in the long-wavelength region of the visible spectrum (the region where underwater scattering is least contrast-degrading). Our 12.5° stimulus, for example, subtends the same visual angle as does a sunfish at 0.5 m, whose scales we have found (unpublished observations) to partially polarize reflected light. Polarization contrast thus seems likely to be used by this vertebrate (and the many others possessing similar double-cone mosaics) as a mechanism for object detection. Certainly, a polarization contrast visual system will improve the visibility of objects that partially polarized reflected light in an environment that has much scattered light with random polarization.

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Dopamine activation of the arachidonic acid cascade as a basis for D1/D2 receptor synergism

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UNDERSTANDING the actions of the neurotransmitter dopamine in the brain is important in view of its roles in neuropsychiatric illnesses. Dopamine D1 receptors, which stimulate both adenyl cyclase and phospholipase C, and D2 receptors, which inhibit them, can nevertheless act synergistically to produce many electrophysiological and behavioural responses. Because this functional synergism can occur at the level of single neurons, another, as yet unidentified, signalling pathway activated by dopamine has been hypothesized. We report here that in Chinese hamster ovary (CHO) cells transfected with the D2 receptor complementary DNA, D2 agonists potentiate enhance arachidonic acid release, provided that such release has been initiated by stimulating constitutive purinergic receptors or by increasing intracellular Ca2+. In CHO cells expressing D1 receptors, D1 agonists exert no such effect. When D1 and D2 receptors are coexpressed, however, activation of both subtypes results in a marked synergistic potentiation of arachidonic acid release. The numerous actions of arachidonic acid and its metabolites in neuronal signal transduction suggest that facilitation of its release may be implicated in dopaminergic responses, such as feedback inhibition mediated by D2 autoreceptors, and may constitute a molecular basis for D1/D2 receptor synergism.

The effect of D2 receptor stimulation on the release of arachidonic acid (AA) from membrane phospholipids was studied in CHO cells, transfected with rat D2 receptor cDNA (CHO(D2)) and labelled by incubation with [3H]AA (Fig. 1). The D2 receptor agonist quinpirole did not affect [3H]AA release when applied alone (Fig. 1; Table 1). By contrast, the drug strongly potentiated [3H]AA release when this was evoked by stimulating purinergic receptors with ATP (1–100 μM; Fig. 1a). Potentiation of ATP-induced [3H]AA release was half-maximal at 34 ± 3 nM quinpirole (mean ± s.e.m., n = 4) and maximal at 250 nM (Fig. 1b). Because the stimulation of constitutive

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purinergic receptors produced a transient increase in intracellular Ca\(^{2+}\) concentration, \([\text{Ca}^{2+}]\) (effector concentration for half-maximal (EC\(_{50}\)) at 5 \(\mu\)M, as determined with Fura-2), we examined the effects of quinpirole on the release of \(^{3}H\)AA evoked by the Ca\(^{2+}\) ionophore A23187. Potentiation of A23187-induced \(^{3}H\)AA release was half-maximal at 30 \(\pm\) 10 nM quinpirole, and maximal at 50 nM (Fig. 1b). Similarly, quinpirole enhanced the release of \(^{3}H\)AA evoked either by \(\alpha\)-thrombin, which enhances \([\text{Ca}^{2+}]\), and activates phospholipase A\(_2\) (PLA\(_2\)) in CHO by a receptor-mediated mechanism,\(^b\), or by ionomicyn (not shown). By contrast, D\(_2\) receptor stimulation did not affect either A23187-induced formation of \(^{3}H\)inositol phosphates, catalysed by phosphatidyl inositol-specific phospholipase C (ref. 10) or A23187-induced release of \(^{3}H\)choline, catalysed by both phospholipases C and D (ref. 11) (not shown).

Potentiation of \(^{3}H\)AA release by quinpirole resulted from occupation of D\(_2\) receptors. In agreement with this is: (1) the response was antagonized by the D\(_2\) receptor blocker haloperidol (Fig. 1c); (2) dopamine as well as other D\(_2\) receptor agonists enhanced A23187-induced \(^{3}H\)AA release (EC\(_{50}\) = 14 \(\pm\) 2 nM for dopamine \((n = 8)\) and 6 \(\pm\) 1 nM for pergolide \((n = 16)\)); (3) the D\(_2\) receptor agonist SKF 38393 was ineffective (Fig. 1b); and (4) wild-type CHO cells did not respond to quinpirole plus ATP (Table 1) or to quinpirole plus A23187 (not shown).

The ability to affect \(^{3}H\)AA release was selective for the D\(_2\) receptor subtype. In CHO clones expressing D\(_2\) receptors, neither SKF 38393 nor quinpirole affected ATP-induced \(^{3}H\)AA release (Table 1). But SKF 38393 did enhance the accumulation of cyclic AMP (from 0.8 \(\pm\) 0.1 to 11.3 \(\pm\) 0.9 pmol per well, \(n = 4\)), indicating that D\(_2\) receptors were coupled to adenyl cyclase. In addition, only a small effect of quinpirole (blocked by haloperidol) was seen in CHO cells expressing human D\(_2\) receptor.\(^c\) (Table 1). D\(_2\) receptors are not (or only weakly) linked to adenyl cyclase inhibition when expressed in CHO (ref. 13 and our unpublished results), suggesting that the appropriate G protein may be lacking in these transfected clones.

Inhibition of adenyl cyclase activity by D\(_2\) receptors often involves a G-protein member of the G\(_i/G\(_\alpha\) family, and is inhibited by pertussis toxin (PTX).\(^2\) Similarly, in CHO(D\(_2\)) cells, PTX prevented the response to quinpirole without affecting A23187-induced AA release (Fig. 1d), indicating that a PTX-sensitive G protein, linked to the D\(_2\) receptor, may regulate AA release (possibly by enhancing the sensitivity of PLA\(_2\) to intracellular \([\text{Ca}^{2+}]\)).

**FIG. 1** Quinpirole facilitates \(^{3}H\)AA release from prelabelled CHO(D\(_2\)) cells. a. Effect of quinpirole on the release of \(^{3}H\)AA evoked by stimulating constitutive purinergic receptors with ATP. Cells were incubated with ATP (1–100 \(\mu\)M) in the absence (C) or in the presence of quinpirole at 0.5 \(\mu\)M (○). When applied alone, 100 \(\mu\)M ATP stimulated \(^{3}H\)AA release to 343 \(\pm\) 14% of control (\(P < 0.05\), Student's t-test). b. Concentration-response curves for the potentiating effect of quinpirole on \(^{3}H\)AA release evoked either by ATP (100 \(\mu\)M) (○) or by Ca\(^{2+}\) ionophore A23187 (4 \(\mu\)M) (△). The figure also demonstrates the lack of effect of SKF 38393, a D\(_2\) receptor agonist, on ATP-induced (○) or A23187-induced (△) \(^{3}H\)AA release. The small response at 10 \(\mu\)M SKF 38393 was probably due to the stimulation of D\(_2\) receptors, because it was blocked by the selective D\(_2\) receptor antagonist, raclopride (0.5 \(\mu\)M). c. Haloperidol inhibits the potentiation by quinpirole of A23187-evoked \(^{3}H\)AA release. CHO(D\(_2\)) cells were incubated with quinpirole (1 \(\mu\)M–10 \(\mu\)M) plus A23187 (4 \(\mu\)M), without (○) or with haloperidol (10 nM (●) or 100 nM (□). d. Potentiation by quinpirole of A23187-evoked \(^{3}H\)AA release is prevented by incubating CHO(D\(_2\)) cells with pertussis toxin (PTX). Cells were incubated with various concentrations of PTX (1–10,000 ng ml\(^{-1}\)) before exposing them to quinpirole (1 \(\mu\)M) plus A23187 (4 \(\mu\)M).

METHOIDS. Transfection of CHO cells with rat D\(_2\) receptor (also termed D\(_2\))\(^{27}\) cDNA has been described elsewhere.\(^3\) Wild-type and transfected CHO clones were maintained in monolayer culture in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum. CHO(D\(_2\)) cells expressed 1.3 \(\times\) 10\(^5\) D\(_2\) receptors per cell. Cells (24-well plates) were labelled by incubation with \(^{3}H\)AA (Amersham, 219 Ci mmol\(^{-1}\), 0.5 \(\mu\)Ci ml\(^{-1}\)) in DMEM (1 ml) containing 0.5% BSA for 2 h at 37 °C. To eliminate unincorporated radioactivity, cells were washed twice with 0.5 ml DMEM plus BSA before incubating them for 30 min at 37 °C in 1 ml of the same medium, containing final concentrations of the appropriate drugs. \(^{3}H\)AA release was determined by liquid scintillation counting in samples (0.5 ml) of the incubation medium. No significant difference in \(^{3}H\)AA labelling of cell lipids was observed among CHO clones in any experiments. Incubation with PTX did not affect \(^{3}H\)AA labelling. Analysis by thin-layer chromatography revealed that free \(^{3}H\)AA constituted more than 90% of the released radioactivity in both control and stimulated samples. Results represent means \(\pm S.E.M.\) of 8–12 separate determinations. In b and c s.e.m. bars are omitted for clarity. In each experiment, results were calculated as percentage of either A23187-treated or ATP-treated cells. \(^{3}H\)AA release from CHO(D\(_2\)) cells incubated with 4 \(\mu\)M A23187 was 672 \(\pm\) 47 c.p.m. (\(n = 72\)) and for cells incubated with 100 \(\mu\)M ATP 302 \(\pm\) 25 c.p.m. (\(n = 32\)). Control cells released 105 \(\pm\) 5 c.p.m. of \(^{3}H\)AA (\(n = 80\)). Curves were fitted to the experimental data using the least squares method.
In agreement with their opposing effects on adenyl cyclase activity, D₁ and D₂ receptors have antagonistic roles in the regulation of some neural functions. In many cases, however, these receptors also exert synergistic actions. In the striatum for example, where about 30% of neurons may express both subtypes, there is a synergistic inhibition of \((\text{Na}^+ + \text{K}^+)\) ATPase activity by D₁ and D₂ receptor coactivation.

To examine whether D₁ and D₂ receptors may interact in regulating \(^{3}\text{H}\)AA release, we used CHO cells transfected with both receptor cDNAs (CHO(D₁ + D₂)). In these cells, express 10 times more D₂ than D₁ receptor, the overall effect of D₁/D₂ receptor stimulation by dopamine was to enhance cAMP formation (Fig. 2c), as previously shown in striatal slices. This response was blocked by the D₁ antagonist SCH 23390, while an underlying D₂-dependent inhibition was evidenced using the D₂-receptor blocker raclopride (Fig. 2c).

In \(\text{CHO(D₁ + D₂)}\) cells, quinpirole potentiated A23187-evoked release of \(^{3}\text{H}\)AA, whereas SKF 38393 was ineffective (Fig. 2a). Response to quinpirole was strongly enhanced, however, in the presence of 0.5 \(\mu\text{M}\) SKF 38393 (Fig. 2a) (an effect which was half-maximal at 30 \(\mu\text{M}\), and maximal at 0.5 \(\mu\text{M}\), not shown). Dopamine, which acts both on D₁ and on D₂ receptor subtypes, enhanced A23187-induced \(^{3}\text{H}\)AA release in \(\text{CHO(D₁ + D₂)}\) cells with seven-fold greater potency than in \(\text{CHO(D₁)}\) cells. 

\[ E_{\text{Ca}^2+} \text{in } \text{CHO(D₁ + D₂)} = 2.3 \pm 0.2 \text{ nM versus } 14 \pm 2 \text{ nM in } \text{CHO(D₁)} \]  
\[ n = 8 \]  

The greater potency of dopamine may be attributed to a synergistic contribution of D₁ receptors to the D₂ response. In agreement, raclopride inhibited completely the response to dopamine, whereas SCH 23390 reduced its potency by about 10-fold (Fig. 2b). In the presence of SCH 23390, the \(E_{\text{Ca}^2+}\) for dopamine was 28 ± 1.4 nM, a value similar to that obtained in \(\text{CHO(D₁)}\) cells.

Two observations suggest that cAMP formed through D₁ receptor stimulation may participate in the synergistic AA response. First, in \(\text{CHO(D₁ + D₂)}\) cells the overall effect of dopamine was to enhance cAMP formation (Fig. 2c). Second, in \(\text{CHO(D₂)}\) cells the cAMP analogue 8-bromo-cAMP (0.1 mM) enhanced by 272 ± 43% \((n = 4)\) the effect of 1 \(\mu\text{M}\) quinpirole on \(^{3}\text{H}\)AA release (evoked by 4 \(\mu\text{M}\) A23187). Interestingly, the synergistic inhibition of \((\text{Na}^+ + \text{K}^+)\) ATPase activity in striatal neurons by D₁ and D₂ receptor stimulation may also involve

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**TABLE 1** Effects of D₁ and D₂ receptor agonists on basal or ATP-induced \(^{3}\text{H}\)AA release in transfected CHO cells

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>ATP + quinpirole (µM)</th>
<th>Quinpirole (µM)</th>
<th>SKF 38393 (µM)</th>
<th>A23187 (µM)</th>
<th>SKF 38393 (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHO (wild)</td>
<td>112 ± 13</td>
<td>90 ± 7</td>
<td>87 ± 16</td>
<td>111 ± 2</td>
<td>114 ± 7</td>
</tr>
<tr>
<td>CHO (D₁)</td>
<td>106 ± 19</td>
<td>68 ± 8</td>
<td>78 ± 19</td>
<td>114 ± 16</td>
<td>141 ± 19</td>
</tr>
<tr>
<td>CHO (D₂)</td>
<td>330 ± 93</td>
<td>90 ± 23</td>
<td>98 ± 22</td>
<td>119 ± 10</td>
<td>131 ± 19</td>
</tr>
<tr>
<td>CHO (D₁, D₂)</td>
<td>267 ± 34</td>
<td>90 ± 14</td>
<td>103 ± 24</td>
<td>142 ± 13</td>
<td>ND</td>
</tr>
<tr>
<td>CHO (D₂)</td>
<td>178 ± 3</td>
<td>103 ± 5</td>
<td>102 ± 2</td>
<td>142 ± 8</td>
<td>139 ± 5</td>
</tr>
</tbody>
</table>

Transfections were done as described in Fig. 1 legend. Receptor capacities for the transfected clones (determined in binding experiments described in refs 25 and 26) were the following (receptor/cell): CHO(D₁) 8 × 10⁵; CHO(D₂) 0.9 × 10⁵; CHO(D₁, D₂) 1.4 × 10⁵; CHO(D₂) 1 × 10⁵. \(^{3}\text{H}\)AA labelling and incubation with drugs were done as described (Fig. 1 legend). In these experiments, the concentration of ATP used (25 mM) produced only a weak stimulation of \(^{3}\text{H}\)AA release which, in the absence of quinpirole, was not statistically significant. Similar results were obtained, however, when 100 µM ATP or 4 µM A23187 were used (data not shown). Results represent the mean ± s.e.m. of at least four separate determinations, and are expressed as percentage of unstimulated controls. In control conditions, wild-type CHO cells released 147 ± 8 pmoles of \(^{3}\text{H}\)AA and similar control release was obtained in all clones tested (not shown). \(^{3}\text{H}\)AA release was not statistically different from control samples unless so indicated. *Significantly different from both control and ATP-treated samples. P < 0.05 (Student's t-test).

METHODS. The CHO(D₁) cells used in Fig. 1 were cotransfected using Lipofectin (GIBCO) with a pcD-BS plasmid containing the D₁ receptor gene (gift of P. Seeman) and a pUT 523 plasmid (CAYLA, Toulouse, France) containing a phleomycin-resistance gene. Cells were selected in DMEM containing 50 µg/ml tetracycline and conal cell lines screened in binding experiments with \(^{125}\)I-labelled SCH 23982 (NEN), a selective D₁ receptor radioligand. CHO(D₁) cells used in these experiments expressed about 1.2 ± 10⁶ D₁ receptors per cell. Expression of D₂ receptor was not affected by D₁ receptor cotransfection. \(^{3}\text{H}\)AA release was determined as described in the legend to Fig. 1. Accumulation of cAMP was determined in the same cultures used for the \(^{3}\text{H}\)AA release experiments depicted in Fig. 2b. The cAMP was extracted from cells using 0.1 M HCl and sonication, and cAMP concentration determined using a radioimmunoassay kit (Amersham) after neutralization of the acid extracts. Results represent the mean ± s.e.m. of 4–12 separate determinations. The cAMP levels are expressed as percentage of A23187-treated cells (1.46 ± 0.08 pmol per well, n = 8). A23187 did not significantly affect cAMP levels (not shown).

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A role for peptide in determining MHC class II structure

Scheherazade Sadegh-Nasser & Ronald N. Germain

T lymphocytes recognize antigen-derived peptides associated with major histocompatibility complex (MHC) class I or class II proteins. Peptide is critical in class I heavy-chain folding and/or stable association with β2-microglobulin. Peptide, in combination with the MHC molecule, is essential for binding to the T cell receptor. The specificity of the T cell response is determined by the structure of the peptide-MHC complex. The table below shows the specificity of a peptide epitope for different MHC class II variants. The peptides were designed to recognize the T cell receptor with high affinity and specificity. The results indicate that the peptide epitope is essential for the binding of the T cell receptor to the MHC molecule. The peptide epitope is specific to the MHC class II molecule and can be used to determine the structure of the MHC class II molecule. The results also indicate that the peptide epitope is specific to the T cell receptor and can be used to determine the structure of the T cell receptor.