vision, and also rejects any hypothesis which ascribes polarization sensitivity to some dichoric or birefringent element of the eye's preretinal optical apparatus common to all photoreceptors under all adaptation conditions[24].

The 90° periodicity of polarization sensitivity (Fig. 1*c*) also argues against any single preretinal 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. 1*c* 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. 4*a* the thresholds of Fig. 1*c* 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 square; 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 square in Fig. 4 establish the presence of a polarization-opponent neural mechanism. This result suggests, by analogy with the role of colour-opponent coding[25], 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. 4*b*). The thresholds for all targets with polarization contrasts $\geq 20\%$, 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 $\geq 20\%$.

Perhaps the most striking general conclusion to be drawn from our results 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[26]). 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 polarize 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 D_1/D_2 receptor synergism

Daniele Piomelli, Catherine Pilon, Bruno Giros, Pierre Sokoloff, Marie-Pascale Martres & Jean-Charles Schwartz

Unité de Neurobiologie et Pharmacologie (U. 109) de l'INSERM, Centre Paul Broca, 2ter rue d'Alésia, 75014 Paris, France

UNDERSTANDING the actions of the neurotransmitter dopamine in the brain is important in view of its roles in neuropsychiatric illnesses[1]. Dopamine D_1 receptors, which stimulate both adenylyl cyclase[2] and phospholipase C[3], and D_2 receptors, which inhibit them[4,5], can nevertheless act synergistically to produce many electrophysiological and behavioural responses[6]. Because this functional synergism can occur at the level of single neurons, another, as yet unidentified, signalling pathway activated by dopamine has been hypothesized[7]. We report here that in Chinese hamster ovary (CHO) cells transfected with the D_2 receptor complementary DNA, D_2 agonists potently enhance arachidonic acid release, provided that such release has been initiated by stimulating constitutive purinergic receptors or by increasing intracellular Ca^{2+}. In CHO cells expressing D_1 receptors, D_1 agonists exert no such effect. When D_1 and D_2 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[8] suggest that facilitation of its release may be implicated in dopaminergic responses, such as feedback inhibition mediated by D_2 autoreceptors, and may constitute a molecular basis for D_1/D_2 receptor synergism.

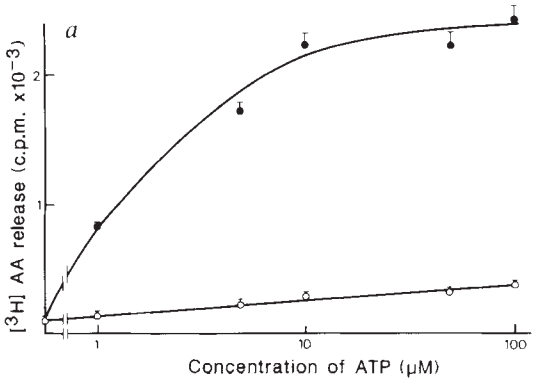
The effect of D_2 receptor stimulation on the release of arachidonic acid (AA) from membrane phospholipids was studied in CHO cells, transfected with rat D_2 receptor cDNA ($CHO(D_2)$) and labelled by incubation with [^{3}H]AA (Fig. 1). The D_2 receptor agonist quinpirole did not affect [^{3}H]AA release when applied alone (Fig. 1; Table 1). By contrast, the drug strongly potentiated [^{3}H]AA release when this was evoked by stimulating purinergic receptors with ATP (1–100 μM; Fig. 1*a*)[9]. Potentiation of ATP-induced [^{3}H]AA release was half-maximal at 34 ± 3 nM quinpirole (mean ± s.e.m., $n = 4$) and maximal at 250 nM (Fig. 1*b*). Because the stimulation of constitutive

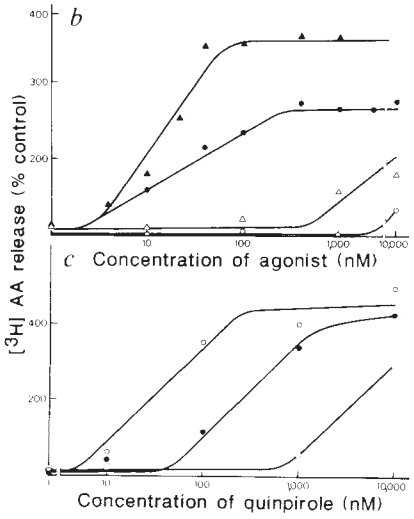
purinergic receptors produced a transient increase in intracellular Ca^{2+} concentration, $[Ca^{2+}]_i$ (effector concentration for half-maximal (EC_{50}) at 5 μM, as determined with Fura-2), we examined the effects of quinpirole on the release of [^{3}H]AA evoked by the Ca^{2+} ionophore A23187. Potentiation of A23187-induced [^{3}H]AA release was half-maximal at 30 ± 10 nM quinpirole, and maximal at 50 nM (Fig. 1*b*). Similarly, quinpirole enhanced the release of [^{3}H]AA evoked either by α-thrombin, which enhances $[Ca^{2+}]_i$ and activates phospholipase A_2 (PLA_2) in CHO by a receptor-mediated mechanism[9], or by ionomycin (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. 1*c*); (2) dopamine as well as other D_2 receptor agonists enhanced A23187-induced [^{3}H]AA release (EC_{50} = 14 ± 2 nM for dopamine (n = 8) and 6 ± 1 nM for pergolide (n = 16)); (3) the D_1 receptor agonist SKF 38393 was ineffective (Fig. 1*b*); 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_1 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 ± 0.1 to 11.3 ± 0.9 pmol per well, n = 4), indicating that D_1 receptors were coupled to adenylyl cyclase. In addition, only a small effect of quinpirole (blocked by haloperidol) was seen in CHO cells expressing human D_3 receptor[12] (Table 1). D_3 receptors are not (or only weakly) linked to adenylyl 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 adenylyl cyclase activity by D_2 receptors often involves a G-protein member of the G_i/G_o family, and is inhibited by pertussis toxin (PTX)[5]. Similarly, in CHO(D_2) cells, PTX prevented the response to quinpirole without affecting A23187-induced AA release (Fig. 1*d*), 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 Ca^{2+})[14].





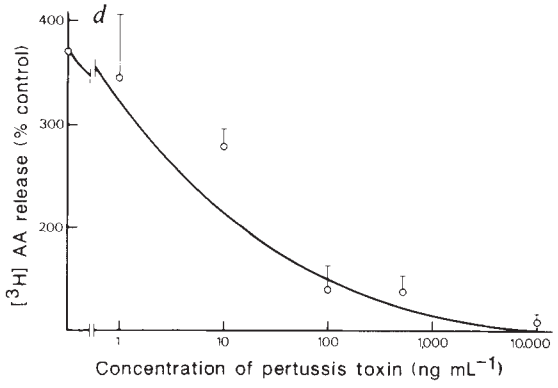


FIG. 1 Quinpirole facilitates [^{3}H]AA release from prelabelled CHO(D_2) cells. *a*, Effect of quinpirole on the release of [^{3}H]AA evoked by stimulating constitutive purinergic receptors with ATP. Cells were incubated with ATP (1–100 μM) in the absence (○) or in the presence of quinpirole at 0.5 μM (●). When applied alone, 100 μM ATP stimulated [^{3}H]AA release to 343 ± 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 μM) (●) or by Ca^{2+} ionophore A23187 (4 μM) (▲). The figure also demonstrates the lack of effect of SKF 38393, a D_1 receptor agonist, on ATP-induced (○) or A23187-induced (△) [^{3}H]AA release. The small response at 10 μM 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 μM). *c*, Haloperidol inhibits the potentiation by quinpirole of A23187-evoked [^{3}H]AA release. CHO(D_2) cells were incubated with quinpirole (1 nM–10 μM) plus A23187 (4 μM), without (○) or with haloperidol at 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 for 4.5 h with various concentrations of PTX (1–10,000 ng ml^{-1}) before exposing them to quinpirole (1 μM) plus A23187 (4 μM).

METHODS. Transfection of CHO cells with rat D_{2A} receptor (also termed D_{2L})[27] cDNA has been described elsewhere[13]. 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 × 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 μCi 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[28] revealed that free [^{3}H]AA constituted more than 90% of the released radioactivity in both control and stimulated samples. Results represent means ± 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 percentge of either A23187-treated or ATP-treated cells. [^{3}H]AA release from CHO(D_2) cells incubated with 4 μM A23187 was 672 ± 47 c.p.m. (n = 72) and for cells incubated with 100 μM ATP 302 ± 25 c.p.m. (n = 32). Control cells released 105 ± 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 adenylyl cyclase activity, D_1 and D_2 receptors have antagonistic roles in the regulation of some neural functions[6]. In many cases, however, these receptors also exert synergistic actions[6]. In the striatum for example, where about 30% of neurons may express both subtypes[15], there is a synergistic inhibition of $(Na^+ + K^+)$ ATPase activity by D_1 and D_2 receptor coactivation[7].

To examine whether D_1 and D_2 receptors may interact in regulating [^{3}H]AA release, we used CHO cells transfected with both receptor cDNAs (CHO($D_1 + D_2$)). In these cells, which express 10 times more D_2 than D_1 receptor, the overall effect of D_1/D_2 receptor stimulation by dopamine was to enhance cAMP formation (Fig. 2*c*), as previously shown in striatal slices[16]. This response was blocked by the D_1 antagonist SCH 23390, while an underlying D_2-dependent inhibition was evidenced using the D_2-receptor blocker raclopride (Fig. 2*c*).

In CHO($D_1 + D_2$) cells, quinpirole potentiated A23187-evoked release of [^{3}H]AA, whereas SKF 38393 was ineffective (Fig. 2*a*). Response to quinpirole was strongly enhanced, however, in the presence of 0.5 μM SKF 38393 (Fig. 2*a*) (an effect which was half-maximal at 30 nM, and maximal at 0.5 μM, not shown). Dopamine, which acts both on D_1 and on D_2 receptor subtypes, enhanced A23187-induced [^{3}H]AA release in CHO($D_1 + D_2$) cells with seven-fold greater potency than in CHO(D_2) cells (EC_{50} in CHO($D_1 + D_2$) was 2 ± 0.2 nM versus 14 ± 2 nM in CHO(D_2), $n = 8$) (Fig. 2*b*). The greater potency of dopamine may be attributed to a synergistic contribution of D_1 receptors to the D_2 response. In agreement, raclopride inhibited completely the response to dopamine, whereas SCH 23390 reduced its potency by about 10-fold (Fig. 2*b*). In the presence of SCH 23390, the EC_{50} for dopamine was 28 ± 4 nM, a value similar to that obtained in CHO(D_2) cells.

TABLE 1 Effects of D_1 and D_2 receptor agonists on basal or ATP-induced [^{3}H]AA release in transfected CHO cells

Cell	ATP + quinpirole	Quinpirole (1 μM)	SKF 38393 (1 μM)	ATP (25 μM)	ATP + SKF 38393
CHO (wild type)	112 ± 13	90 ± 7	87 ± 16	111 ± 2	114 ± 7
CHO (D_1)	106 ± 19	68 ± 8	78 ± 19	114 ± 16	141 ± 19
CHO (D_{2A})	330 ± 93*	90 ± 23	88 ± 22	119 ± 10	131 ± 19
CHO (D_{2B})	267 ± 34*	90 ± 14	103 ± 24	142 ± 13	ND
CHO (D_3)	178 ± 3*	103 ± 5	102 ± 2	142 ± 8	139 ± 5

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(D_1), 8×10^3; CHO(D_{2A}), 1.1×10^5; CHO(D_{2B}), 1.4×10^5; CHO(D_3), 1×10^5. [^{3}H]AA labelling and incubation with drugs were done as described (Fig. 1 legend). In these experiments, the concentration of ATP used (25 μM) produced only a weak stimulation of [^{3}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 174 ± 8 cpm of [^{3}H]AA and similar control release was obtained in all clones tested (not shown). [^{3}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).

Two observations suggest that cAMP formed through D_1 receptor stimulation may participate in the synergistic AA response. First, in CHO($D_1 + D_2$) cells the overall effect of dopamine was to enhance cAMP formation (Fig. 2*c*). Second, in CHO(D_2) cells the cAMP analogue 8-bromo-cAMP (0.1 mM) enhanced by 272 ± 43% ($n = 4$) the effect of 1 μM quinpirole on [^{3}H]AA release (evoked by 4 μM A23187). Interestingly, the synergistic inhibition of $(Na^+ + K^+)$ ATPase activity in striatal neurons by D_1 and D_2 receptor stimulation may also involve

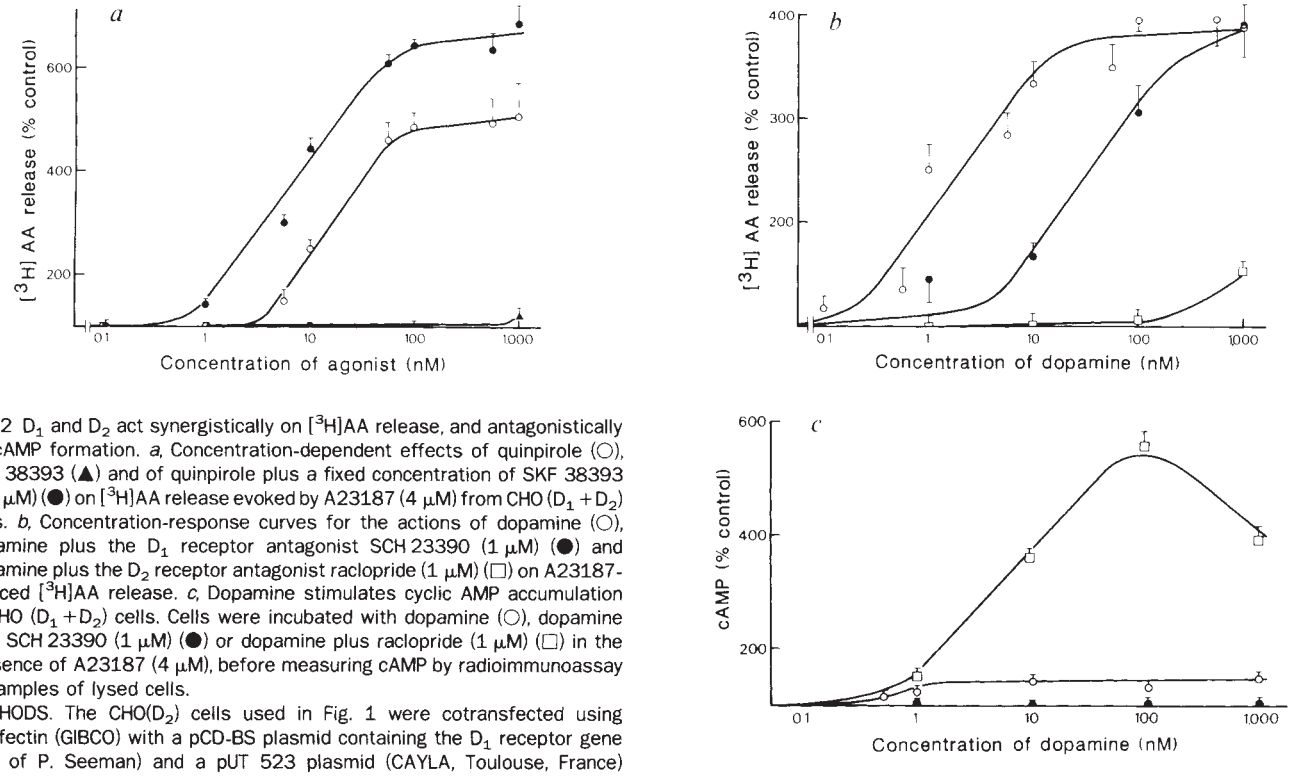


FIG. 2 D_1 and D_2 act synergistically on [^{3}H]AA release, and antagonistically on cAMP formation. *a*, Concentration-dependent effects of quinpirole (○), SKF 38393 (▲) and of quinpirole plus a fixed concentration of SKF 38393 (0.5 μM) (●) on [^{3}H]AA release evoked by A23187 (4 μM) from CHO ($D_1 + D_2$) cells. *b*, Concentration-response curves for the actions of dopamine (○), dopamine plus the D_1 receptor antagonist SCH 23390 (1 μM) (●) and dopamine plus the D_2 receptor antagonist raclopride (1 μM) (□) on A23187-induced [^{3}H]AA release. *c*, Dopamine stimulates cyclic AMP accumulation in CHO ($D_1 + D_2$) cells. Cells were incubated with dopamine (○), dopamine plus SCH 23390 (1 μM) (●) or dopamine plus raclopride (1 μM) (□) in the presence of A23187 (4 μM), before measuring cAMP by radioimmunoassay in samples of lysed cells.

METHODS. The CHO(D_2) cells used in Fig. 1 were cotransfected using Lipofectin (GIBCO) with a pCD-BS plasmid containing the D_1 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^{-1} phleomycin and clonal cell lines screened in binding experiments with ^{125}I-labelled SCH 23982 (NEN), a selective D_1 receptor radioligand[26]. CHO($D_1 + D_2$) cells used in these experiments expressed about 1.2×10^4 D_1 receptors per cell. Expression of D_2 receptor was not affected by D_1 receptor cotransfection. [^{3}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}H]AA release experiments depicted in Fig. 2*b*. 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 means ± s.e.m. of 4–12 separate determinations. The cAMP levels are expressed as percentge of A23187-treated cells (1.46 ± 0.08 pmol per well, $n = 8$). A23187 did not significantly affect cAMP levels (not shown).

cAMP[7]. As AA and its cytochrome P_{450} metabolites are potent inhibitors of $(Na^+ + K^+)$ ATPase[17], their formation may account for the synergism described in striatal neurons.

We have shown here that D_2 agonists enhanced Ca^{2+}-stimulated AA release at nM concentrations and with EC_{50} 20- to 30-fold lower than the corresponding K_i for receptor binding[13]. This suggests that partial D_2 receptor occupation is sufficient to produce a robust AA response. It will be important to determine whether this facilitatory action, described here for transfected CHO cells, occurs in brain areas, such as the striatum, where comparable D_2 receptor densities are found and receptor-stimulated AA release has been demonstrated[18].

The facilitatory effect of D_2 receptors on Ca^{2+}-dependent AA release reported here suggests that, in presynaptic terminals, the Ca^{2+} increase which follows depolarization and triggers dopamine release may, at the same time, enable D_2 autoreceptors[19] to release AA and its metabolites. These may, in turn, participate in the autoinhibitory actions of dopamine, for example by modulating K^+ or Ca^{2+} channel activities[20–23] or protein phosphorylation[24]. Finally, as costimulation of a Ca^{2+}-mobilizing receptor revealed an unexpected amplification by dopamine of AA release, more neurotransmitters than previously thought may operate through the AA cascade by a similar mechanism.

Note added in proof: A study by Kanterman *et al.*[29] has appeared after submission of the present study, showing that stimulation of D_2 receptors in transfected CHO cells augments A23187-induced [^{3}H]AA release. □

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A role for peptide in determining MHC class II structure

Scheherazade Sadegh-Nasseri & Ronald N. Germain

Lymphocyte Biology Section, Laboratory of Immunology,
National Institute of Allergy and Infectious Diseases,
National Institutes of Health, Bethesda,
Maryland 20892, USA

T LYMPHOCYTES recognize antigen-derived peptides associated with major histocompatibility complex (MHC) class I or class II proteins[1,2]. Peptide is critical in class I heavy-chain folding and/or stable association with β_2-microglobulin[3–6]. Although data exist suggesting a relationship between class II structure and peptide association[7–9], no equivalent positive contribution of peptide to the folding state or stability of class II dimers has yet been demonstrated. We report here that most purified $E\alpha^kE\beta^k$ molecules leaving low pH in the absence of specific peptide lack a compact, stable dimeric structure. Brief exposure to the appropriate peptide just before and during neutralization promotes this specific conformation in proportion to stably bound peptide, indicating that peptide is important in determining class II MHC structure. Our results also indicate that efficient generation of long-lived peptide-class II complexes involves two stages: initial peptide binding in an acidic environment, which enhances the ability of class II to enter a conformation, from which stabilization upon neutralization results in high-affinity binding of previously associated peptide.

When analysed without reduction or boiling, class II molecules show three distinct forms on electrophoresis in SDS-polyacrylamide gels[8]: 'compact' (C) dimers migrating with an apparent relative molecular mass of 56,000 (M_r, 56K), 'floppy' (F) dimers (63–67K) which are partially denatured C dimers, and disassembled α (31–33K) and β (28–30K) chains. To investigate the relationship between peptide binding and the structure of class II, we isolated $E\alpha^kE\beta^k$ molecules[7] and analysed their gel migration under a variety of conditions. Most purified $E\alpha^kE\beta^k$ incubated at pH 7.2 with or without added peptide migrates as C dimers of 56K; a small fraction (5–10%) migrates as free α and β chains (Fig. 1*a* and *b*). Incubation for 30 min at pH 4.5 and 37 °C, followed by neutralization, converts most of the C dimers into either F dimers or free α and β chains. These α and β chains derive from SDS dissociation of 'unstable' (U) dimers, based on co-precipitation by anti-C terminal peptide antisera (data not shown). The presence during acid treatment and neutralization of any of several peptides known to bind to $E\alpha^kE\beta^k$, leads to retention of the C structure and a concomitant diminution in F or U dimers (Fig. 1*a* and *b*). For most $E\alpha^kE\beta^k$ to retain the C form, 50–100 μM peptide is required; 10 μM peptide has barely detectable effects (results not shown). Activity is related to binding to $E\alpha^kE\beta^k$, as peptides lacking this property (hen egg lysozyme (HEL) residues 46–61, interacts with $A\alpha^kA\beta^k$ (ref. 10); ovalbumen

TABLE 1 Specificity of the peptide effect on $E\alpha^kE\beta^k$ conformation

$E\alpha^kE\beta^k$ incubation conditions		Relative protein density*			
		Floppy	Compact	Eα	Eβ
pH 4.5, no peptide	→ pH 7.2	49.0	19.0	8.0	5.0
pH 4.5 + DASP	→ pH 7.2	23.0	48.0	2.0	3.0
pH 4.5 + OVA 323–339	→ pH 7.2	60.0	14.0	4.0	6.0
pH 4.5 + HEL 46–61	→ pH 7.2	48.0	20.0	7.0	3.0

* SDS-polyacrylamide gel stained with silver was scanned for protein density. Results for each $E\alpha^kE\beta^k$ form are expressed as per cent of total protein in the applied sample, as determined by laser densitometry. Less than 25% of total $E\alpha^kE\beta^k$ appears as high molecular weight bands (>90K) in every sample. Experimental conditions for treatment and gel analysis are as described in the legend to Fig. 1*b*. Peptides were used at 100 μM. All peptides were shown to have appropriate bioactivity by stimulation of T-cell hybridomas in the context $E\alpha^kE\beta^k$ (DASP), $A\alpha^dA\beta^d$(OVA 323–339), and $A\alpha^kA\beta^k$ (HEL 46–61).