treated (*18*) transiently transfected HeLa TK$^-$ or 293 cells. Every experiment was repeated with c-Jun proteins expressed in both cell lines. The results were essentially identical for both cell types, except the effect of TPA treatment was clearly detectable only with the material derived from 293 cells. Transfected cells were lysed by the addition of lysis buffer [6 M guanidinium-HCl and 0.1 M Na_2HPO_4-NaH_2PO_4 (pH 8.0), with 1 ml per 10-cm dish], and the overexpressed protein was purified by nickel-chelate affinity chromatography as described (*6*) and brought into dialysis buffer [30 mM Pipes KOH (pH 6.0), 10% glycerol, 0.1% lauryldimethylamine oxide, 10 mM KCl, 2 mM $MgCl_2$, 0.2 mM EDTA, 1 mM dithiothreitol (DTT), 2 mM phenylmethylsulfonyl fluoride (PMSF), E-64 (4 μg/ml), aprotinin (5 μg/ml), pepstatin (2 μg/ml), and leupeptin (2 μg/ml)]. The yield of both wild-type and mutant cmv-cJun per plate of HeLa TK$^-$ or 293 cells was ~1 μg per dish.

9. As a source of c-Fos and v-Fos E300 proteins, a combined in vitro transcription-translation system was used. A plasmid that contained a rat cDNA encoding the wild-type c-Fos (*26*) or pTZE300 encoding v-Fos (*20*) was transcribed (after linearization with Hind III) essentially as suggested by the supplier with either SP6 (c-Fos) or T7 (v-Fos) RNA polymerases (Promega). The resulting mRNAs (from 6 μg of template DNA) were used in a translation reaction (50 μl) that contained nuclease-treated rabbit reticulocyte lysate (40 μl; Promega) in the presence of 6 μl of L-[^{35}S]methionine (>1000 Ci/mmol; Amersham).
10. PAP as a suspension in $(NH_4)_2SO_4$ solution (Grade I, Boehringer Mannheim) was prepared as described (*6*); 0.05 U (in 0.5 μl) of enzyme were used in a reaction (10 μl) that contained either wild-type or mutant c-Jun protein (8 μl, ~50 ng) in dialysis buffer (*8*) or in vitro–translated c-Fos or v-Fos [2 μl (*9*)] and a final concentration of 25 mM Pipes KOH (pH 6.0), 2 mM PMSF, E-64 (4 μg/ml), aprotinin (5 μg/ml), pepstatin (2 μg/ml), and leupeptin (2 μg/ml). Digestions were performed at 25°C for 1 hour in the presence or absence of a mixture of competitive and noncompetitive phosphatase inhibitors [100 mM NaF, 15 mM Na_2MoO_4, 20 mM (Na_2)-*p*-nitrophenyl phosphate, and 10 mM (Na_2)-bis-glycerophosphate].
11. The pHJmet5 vector was used to express (from a promoter recognized by T7 RNA polymerase) the complete human c-Jun protein in *E. coli* strain BL21 starting at Met^5, which is the probable initiation codon utilized in vivo (*16*). The ec-cJun protein was also brought into dialysis buffer (*8*) before PAP treatment.
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Retinoids Selective for Retinoid X Receptor Response Pathways

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Retinoids have a broad spectrum of biological activities and are useful therapeutic agents. Their physiological activities are mediated by two types of receptors, the retinoic acid receptors (RARs) and the retinoid X receptors (RXRs). RARs, as well as several related receptors, require heterodimerization with RXRs for effective DNA binding and function. However, in the presence of 9-*cis*-retinoic acid, a ligand for both RARs and RXRs, RXRs can also form homodimers. A series of retinoids is reported that selectively activates RXR homodimers but does not affect RAR-RXR heterodimers and thus demonstrates that both retinoid response pathways can be independently activated.

Retinoic acid (RA) and its natural and synthetic analogs (retinoids) affect a wide array of biological processes. Retinoids are used in the treatment of many skin diseases (*1*) and are promising drugs for several cancers (*2*). Consistent with their broad biological activity pattern, retinoids have a variety of side effects, including teratogenicity, that limit their therapeutic potential. Specific retinoids with restricted biological activities may have fewer side effects. We have designed and synthesized several retinoids that selectively activate RXRs by inducing the formation of RXR homodimers. Although it was previously thought that both RARs and RXRs function as homodimers, evidence now indicates that RARs require heterodimerization with RXRs for effective DNA binding and function (*3–9*). RXRs also bind several other ligand-regulated receptors, including thyroid hormone receptors and vitamin D_3 receptor (*3–9*). In addition, in the presence of 9-*cis*-retinoic acid (9-*cis*-RA), RXRs form homodimers that have response element specificities that are distinct from those of RAR-RXR heterodimers (*10*), indicating that the two RA response pathways activate distinct sets of genes. Because it binds both RARs and RXRs with high affinity (*11*), 9-*cis*-RA is a very potent activator of RAR-RXR heterodimers (*10*). Thus, 9-*cis*-RA should elicit very broad biological responses. Here, we define a class of retinoids that activates only the RXR homodimer.

We used the TREpal-tk reporter gene (*12*), which is activated by both RAR-RXR heterodimers and RXR homodimers (*10*), in a transient transfection assay (*13*) to evaluate compounds for the induction of RXR activity. When the RXR expression vector was cotransfected with the TREpal-tk reporter gene into African green monkey kidney (CV-1) cells, all-*trans* RA did not efficiently activate the reporter, whereas 9-*cis*-RA did (*10*). Preliminary evaluation of a series of retinoids indicated that several showed activity with cotransfected RXR. The pharmacophoric elements of these retinoid structures were then combined and further modified to produce a subset of retinoids (Fig. 1A) whose activation profiles for RXR were similar to that of 9-*cis*-RA (Fig. 2A). Although none of these compounds revealed activity at 10^{-8} M, the two most potent retinoids, SR11217 and SR11237, showed activities similar to 9-*cis*-RA at 10^{-7} M. The induction profiles were similar to that of a reporter gene carrying CRBPII [the RA response element

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(RARE) (*14*) of the cytoplasmic retinol-binding protein II], which is activated by RXR homodimers but not RAR-RXR heterodimers (*10*) (Fig. 2B). Thus, our synthetic retinoids appear to be effective activators of RXRα. Because ligand binding correlates well with transactivation (*11*), it can be expected that SR11217 and SR11237 also bind to RXR with high affinity.

These retinoids activated both TREpal and CRBPII reporter genes. The ketal SR11237 was the most active, followed by the isopropylidenyl retinoid SR11217, the hemithioketal SR11235, and the thioketal SR11234. The dithiane SR11203 and dioxane SR11236 were the least active. Conformational analysis (Fig. 1B) indicates that the spatial orientations of the lipophilic head and carboxyl terminus of these retinoids were similar to those of 9-*cis*-RA and that activity could be related to the length and volume of the substituent group (CRR′) linking the tetrahydronaphthalene and phenyl ring systems. A ring size of five or fewer atoms was necessary for optimal activity and receptor selectivity. Lengthy substituent groups reduced activity and smaller groups reduced receptor selectivity (*15*). Although the isopropylidene group of SR11217 was perpendicular to the plane of the ketal ring of SR11237, the lengths of both groups were similar. The methyl group of SR11217 that points toward the tetrahydronaphthalene ring overlapped the 19-methyl of 9-*cis*-RA.

The ligand 9-*cis*-RA specifically activates RXRα by inducing RXRα homodimer

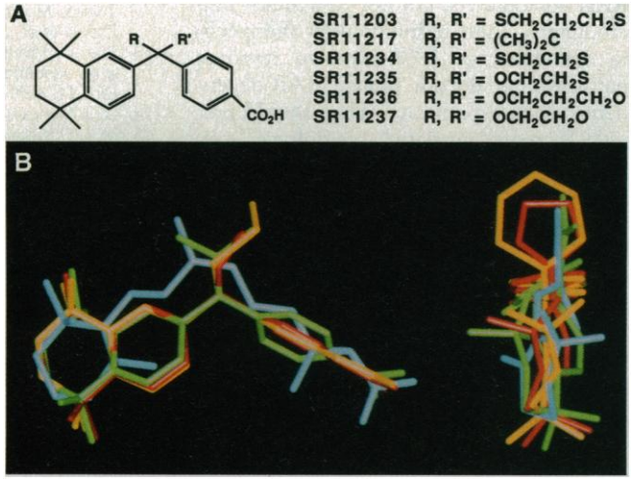

Fig. 1. RXR homodimer-selective retinoids. (**A**) Structures. (**B**) Orthogonal views of structural overlaps of 9-*cis*-RA (blue) and SR-11203 (yellow), SR11217 (green), and SR11237 (red). We generated the conformations by using RANDOMSEARCH in SYBYL, 5.5; Tripos Associates, St. Louis, Missouri. Minimum-energy conformations were compared by superimposition of similar atoms using the program's FIT option.

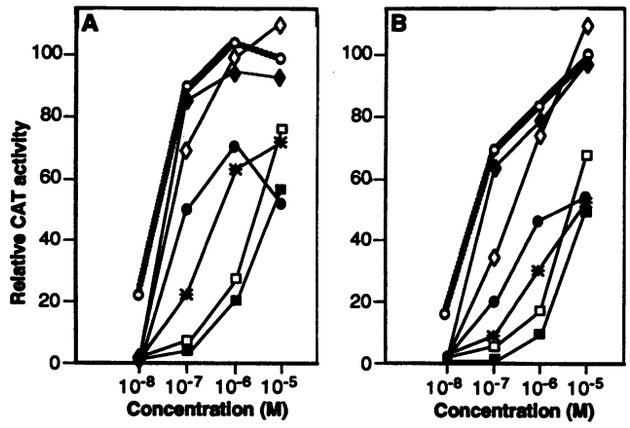

Fig. 2. Profiles of RXRα-dependent transactivation of reporter constructs (**A**) *TREpal-tk-CAT* (*10*) and (**B**) *CRBPII-tk-CAT* (*10*) by (○) 9-*cis*-RA or retinoids (□) SR11203, (◇) SR-11217, (*) SR11234, (●) SR11235, (■) SR11236, and (◆) SR11237. Results of a representative experiment are shown. In four independent experiments, induction profiles did not vary significantly. We normalized CAT activity for transfection and harvesting efficiency by measuring the enzymatic activity derived from the cotransfected β-galactosidase expression plasmid (pCH110, Pharmacia).

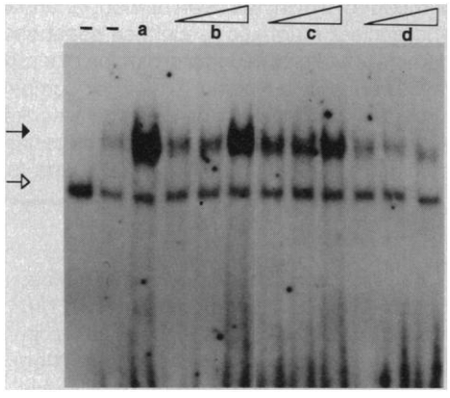

Fig. 3. Gel retardation assays. Retinoids SR11217 and SR11237 induce RXR homodimer binding to TREpal. In vitro synthesized RXRα was incubated (first column contains no RXRα) in the presence of ^{32}P-labeled TREpal either without retinoid (–) or with (a) 9-*cis*-RA (10^{-7} M) or (b) SR11217, (c) SR11237, or (d) SR11231 (each at concentrations 10^{-8}, 10^{-7}, 10^{-6} M, indicated by rising slope) for 30 min and then analyzed. Retinoid SR11231 neither induces RXR homodimer binding nor functions as a transcriptional activator of RXR and served here as negative control. Solid arrow indicates the specific RXR homodimer complex. Open arrow marks nonspecific band observed with unprogrammed retriculocyte lysate (visible in all lanes).

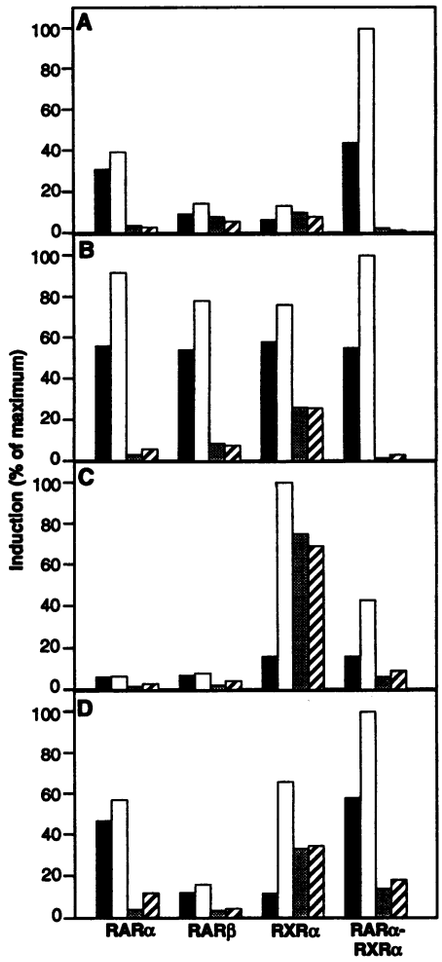

Fig. 4. Transcriptional activation by RARα, RARβ, RXR-homodimers, and RARα-RXRα heterodimers induced by RA (filled bar), 9-*cis*-RA (open bar), SR11217 (shaded bar), and SR11237 (striped bar). CV-1 cells were cotransfected with 100 ng of reporter plasmid (**A**) CRBPI-tk-CAT, (**B**) βRARE-tk-CAT, (**C**) CRBPII-tk-CAT, and (**D**) apoAI-tk-CAT. Retinoids were applied at 5×10^{-7} M. Results of a representative experiment are shown. In four independent experiments, induction profiles did not vary significantly. CAT activity was normalized to β-galactosidase activity.

formation (*10*). We investigated the retinoid-induced RXR homodimer binding to the TREpal by gel retardation assay (*16*). In the absence of 9-*cis*-RA, RXR did not bind to this response element (Fig. 3). Retinoids SR11217 and SR11237 induced RXR homodimer binding to the response element in a concentration-dependent manner. Retinoid SR11203, which behaved as a weak activator in the transient transfection assays, induced weak RXR binding (*17*), whereas the strongest activators, SR11217 and SR11237, induced homodimer binding very effectively, as judged from the strength of the band induced (Fig. 3). Retinoid SR11231, which did not activate the RXR homodimer (*17*), was not able to induce RXR homodimer binding, either. Similar results were obtained with the CRBPII-RARE and the apolipoprotein AI (apoAI)–RARE (*17*).

To analyze whether these retinoids were selective for RXR homodimers, we used reporter constructs carrying either the rat cytoplasmic retinol-binding protein I (CRBPI) gene RARE (*18*), which is only bound and activated by RAR-RXR heterodimers (*10*); the RARβ2 gene promoter RARE (*19*), which is most effectively bound by heterodimers but also activated to some degree by RXR homodimers (*10*); the CRBPII-RARE, which is activated only by RXR homodimers (*10*) and on which RAR represses RXR activity (*14, 17*); or the apoAI gene RARE (*20*), which is bound and activated by RAR-RXR heterodimers as well as by RXR homodimers. Each reporter construct was cotransfected with RARα, RARβ, RXRα, or RXRα and RARα together (*13*). The retinoids were analyzed at a concentration of 5×10^{-7} M (a dose shown to yield almost full induction, Fig. 2). The retinoids activated only RXR homodimers, but not RAR-RXR heterodimers (Fig. 4). Like 9-*cis*-RA, both SR11217 and SR11237 were strong activators of the CRBPII-RARE. However, in contrast to 9-*cis*-RA, they did not induce the CRBPI-RARE, which is activated only by the RAR-RXR heterodimer (Fig. 4). Thus, although SR11217 and SR11237 behaved very similarly to 9-*cis*-RA on the CRBPII-RARE, they showed no response on the CRBPI-RARE, on which 9-*cis*-RA is the optimal activator. The βRARE was slightly activated by SR11217 and SR11237, consistent with the relatively low affinity of RXR homodimers for this response element (*10*). The apoAI-RARE was most effectively activated by RAR-RXR heterodimers in the presence of 9-*cis*-RA, as observed previously (*10*). In addition to the activity found in CV-1 cells, a significant and RXR-specific activation by retinoids SR11217 and SR11237 was seen in various other cell lines, including Hep G2 cells, where a high response was seen. When cotransfected alone, RARα, RARβ, and RARγ were not activated significantly by any of the synthetic retinoids on any of the response elements tested (Fig. 4) (*17*). Heterodimers that RARα and RARβ form with endogenous RXR-like proteins in CV-1 cells were also unresponsive to these retinoids.

These retinoids thus specifically induce RXR homodimer formation and activate RXR homodimers, but not RAR-RXR heterodimers. These retinoids allow the specific activation of RXR-selective response pathways but do not induce RAR-dependent response pathways. They should provide a more restricted physiological response than previously available RA isomers and may be useful for elucidation of retinoid response pathways. Pathological conditions and biological pathways that are only affected by pharmacological doses of RA (*21*), where RA may induce RXR homodimer formation (*10*), could also be responsive to this class of retinoids.

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Isolation and Structure of a Brain Constituent That Binds to the Cannabinoid Receptor

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Arachidonylethanolamide, an arachidonic acid derivative in porcine brain, was identified in a screen for endogenous ligands for the cannabinoid receptor. The structure of this compound, which has been named "anandamide," was determined by mass spectrometry and nuclear magnetic resonance spectroscopy and was confirmed by synthesis. Anandamide inhibited the specific binding of a radiolabeled cannabinoid probe to synaptosomal membranes in a manner typical of competitive ligands and produced a concentration-dependent inhibition of the electrically evoked twitch response of the mouse vas deferens, a characteristic effect of psychotropic cannabinoids. These properties suggest that anandamide may function as a natural ligand for the cannabinoid receptor.

The psychoactive constituent of cannabis, Δ^9-tetrahydrocannabinol (Δ^9-THC) (*1*), binds to a specific G protein–coupled receptor in the brain (*2*). Sequence information on the cannabinoid receptor is available from cloned rat (*3*) and human (*4*) genes, but thus far it has not provided insight into the protein's physiological role(s). The abundance and anatomical localization of the receptor in the brain (*5*), together with the behavioral effects of Δ^9-THC (*6*), are consistent with roles in the control of