# Effects of Topical *alpha*-Substituted Anandamides on Intraocular Pressure in Normotensive Rabbits

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***Purpose:*** Anandamides have been observed to lower intraocular pressure in the rabbit eye, preceded by a period of hypertension. Amidases are thought to catabolize these compounds into their component parts, including arachidonic acid. Direct application of arachidonic acid has been observed to cause a marked rise of intraocular pressure. Thus, anandamide analogs resistant to catabolism were thought possibly devoid of this initial hypertension, and their effects on rabbit IOP investigated.

***Methods:*** A series of chiral *alpha*-substituted anandamides were synthesized and studied for their effect on the intraocular pressure (IOP) of normotensive pigmented rabbits. Each test compound was dissolved in an aqueous 2-hydroxypropyl-β-cyclodextrin solution (containing 3% polyvinyl alcohol) and administered (62.5 μg) unilaterally to the eye.

***Results:*** The most promising compounds caused a statistically significant reduction of IOP (vs. vehicle) in the treated eyes. Of these, the R-*alpha*-isopropyl compound exhibited the best activity tested. Unlike the *alpha*-unsubstituted analogs previously studied, hypotensive effects were not preceded by an initial elevation of IOP and indomethacin pre-treatment (12.5 mg, s.c.) did not eliminate the IOP response, as demonstrated by administered R-*alpha*-isopropyl anandamide.

***Conclusions:*** Catabolism of *alpha*-unsubstituted anandamides may account for their observed intraocular hypertensive effects. The physiological mechanism by which *alpha*-substituted anandamides work apparently differs from that of the more easily metabolized *alpha*-unsubstituted compounds.

**KEY WORDS:** anandamides; intraocular pressure; cyclodextrin; indomethacin; rabbit.

## INTRODUCTION

It was originally observed by Hepler and Frank (1) that subjects who smoked marijuana developed a reduced intraocular pressure (IOP). Human experiments (2,3) using pure *delta*-9-tetrahydrocannabinol (THC) subsequently pinpointed this compound, the main psychoactive ingredient in *Cannabis sativa* L., as a responsible constituent, resulting in numerous studies exploring cannabinoids as possible anti-glaucoma drugs (4). Other cannabinoid receptor agonists of dissimilar chemical structure have also been reported (5) to possess a similar IOP lowering effect.

Devane *et al.* (6) first reported in 1992, that a compound isolated from lipid fractions of porcine brain was found to displace a radiolabeled probe ([$^3$H]HU-243) for the cannabinoid (CB1) receptor. Further purification of these fractions yielded a compound they named "anandamide" (arachidonoylethanolamide, AEA). Its structure was determined, confirmed by synthesis and subsequently found to possess cannabimimetic pharmacological activity. Two additional anandamides have been found (7), suggesting that this class of compounds may form an entire family of chemical mediators in the brain.

Abadji *et al.* (8) first reported the synthesis and pharmacological testing of R-*alpha*-methyl anandamide, demonstrating its higher potency and metabolic stability to the action of endogenous amidases, *versus* the prototype compound, the S-enantiomer and either *beta*-methyl enantiomers. Desarnaud *et al.* has reported (9) the enzymatic lability of anandamide analogs according to their fatty acid carbon chain length and degree of saturation.

Pate *et al.* (10) recently published evidence that topical AEA lowers IOP in the eyes of normotensive albino and pigmented rabbits and demonstrated a relationship between the administered dose and IOP profile. Devane *et al.* (11) had shown a qualitatively similar effect, based on their preliminary evidence. Earlier studies disclosed that an indomethacin pretreatment, which inhibits prostaglandin synthesis, influences the effects of ocularly applied arachidonic acid (14,15) on IOP. The mechanism by which AEA produces its hypotensive effect in the rabbit eye is unknown, but subsequent work by Pate *et al.* (12) implicated the cyclo-oxygenase system and revealed the influence of alkylamide length and terminal moiety substitution.

The present study investigates the effects of another class of topical anandamide analogs on the IOP of normotensive rabbits. The prototype anandamide was divided into three regions (Table I) in the manner suggested by Walpole *et al.* (13) for their similar capsaicin analogs. The effects of modification to AEA at their designated "B" region, primarily to the *alpha*-carbon site of the ethanolamide, are reported here, including each of the optical isomers for any resultant chiral compounds. The low aqueous solubility of anandamides was overcome through the use of cyclodextrins (CDs), which have been used to increase the solubility of many organic compounds (16).

## MATERIALS AND METHODS

### Synthesis of Anandamide Analogs

Arachidonoyl ethanolamide was purchased from Organix Inc. (Woburn, MA, USA). The other anandamides were synthesized by the method of Devane *et al.* (6) and their structures confirmed by IR and MS. Arachidonic acid was obtained from Nu-Chek Prep (Elysian, MN, USA) and the various substituted amine precursors were purchased from Sigma (St. Louis, MO, USA), Aldrich (Milwaukee, WI, USA), Fluka (Ronkonkoma, NY, USA) or Merck (Darmstadt, Germany). Hydroxypropyl-β-cyclodextrin (2-HP-β-CD, Encapsin®, mw = 1297.4) was purchased from Janssen Biotech (Olen, Belgium) and polyvinyl alcohol (PVA, mw = 124,000 – 186,000) obtained from Aldrich (Milwaukee, WI, USA).

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### Preparation of Instilled Eyedrops

An ethanol solution of the candidate anandamide was evaporated under a stream of nitrogen, and the compound was redissolved in an aqueous 15–30% 2-HP-β-CD solution containing 3% PVA, which was used to increase the residence time of the eyedrop solution on the precorneal area, giving more time for the CD-drug inclusion complex to disassociate before its clearance (17). The vehicle control contained 20% CD and 3% PVA. The pH of these solutions were then adjusted to 7.4 with NaOH and made isotonic with sodium chloride. The final concentration of anandamides (Table I) was 2.5 mg/ml (25 μl dose = 62.5 μg).

### Preparation of Indomethacin Solutions

Indomethacin (Sigma, St. Louis, MO, USA) was dissolved (5.0 mg/ml) in an aqueous 20% 2-HP-β-CD solution. Solution pH was then adjusted to 7.4 with NaOH and made isotonic with sodium chloride. Each rabbit in the indomethacin study received 12.5 mg (i.e., 4.0–6.5 mg/kg) of indomethacin by subcutaneous injection 30 min before ocular treatment.

### Intraocular Pressure (IOP) Measurements

The experimental animals used in this study were normotensive pigmented rabbits of either gender (2.4–3.2 kg, n = 10), bred from NZW and Dutch Belted parental stock. The rabbits were housed singly in cages under standard laboratory conditions: 10 hr dark/14 hr light cycle, 20.0 ± 0.5°C, 55–75% relative humidity. They were given food and water *ad libitum* except during the test. All animals were treated in accordance with the ARVO Resolution on the Use of Animals in Research.

To perform each evaluation, the rabbits were placed in plastic restraining boxes located in a quiet room. A single drop (25 μl) of the test solution was instilled unilaterally on the upper corneoscleral limbus. During instillation, the upper eyelid was slightly pulled away from the globe. IOP was measured using a BioRad (Cambridge, MA, USA) Digilab Modular One Pneumatonometer. Before each measurement, one or two drops of oxybuprocaine (0.06%) were applied to the cornea before tonometry to eliminate discomfort. The upper and lower eyelids were gently retracted, and the applanation sensor was brought into contact with the center of the cornea. For every determination, at least two readings were taken from each treated (ipsilateral) and untreated (contralateral) eye and the mean of these readings used. The IOP of the rabbits was measured at 2, 1 and 0 h before, and at 0.5, 1, 2, 3, 4 and 5 h after, eyedrop administration. IOP at the time of eyedrop administration (0 h) was used as a baseline value. Baseline IOPs ranged between 19.4–31.4 mmHg (n = 10).

All studies were set up using a masked and randomized crossover design. At least 72 h of wash-out time was allowed for each rabbit between dosings.

### Analysis of Data

Results are presented as a change in IOP (mmHg), mean ± S.E. (standard error). A one-factor analysis of variance (ANOVA) for repeated measurements was used to test the statistical significance of differences between groups. Significance in the differences of the means was tested using the Fisher's Protected Least Significant Difference (PLSD) method at a 95% confidence level.

## RESULTS

### IOP Studies

Table I shows chemical structures of the studied anandamides, as well as their effects on IOP in the treated eye. Detailed data for ocularly administered doses of anandamides that decreased IOP, when compared to drug-free vehicle, are shown in Table II. Compounds I and XIV were administered to an earlier cohort of rabbits, so absolute quantitation of their resulting data, while suggestive, is not directly comparable to the accompanying compounds.

The effect of anandamides on rabbit IOP was dependent on the particular compound administered. The general observation was that, unlike most of the previous analogs which were modified by altering the length and terminus of the alkylamide substituent (12), unilateral administration of these *alpha*-substituted anandamides caused no initial increase of IOP (except XIV) before inducing hypotension in the treated eye. The maximum reduction in IOP usually occurred at 2–3 h after treatment. While these reductions seem modest, they were statistically significant. The opthalmic drug dexmedetomidine (18) has shown a more dramatic fall in IOP with hypertensive vs. normotensive rabbits. Thus, the effects of *alpha*-substituted anandamides on IOP warrant further study in a more sensitive model.

In the untreated (contralateral) eye, a significant decrease of IOP was either not observed or it occurred only at 5 h (except XIII and XIV).

### Structure-Activity Relationships in Treated Eyes

The IOP effects of the modified anandamides were compared (Table I) with those of the AEA prototype (Compound I). The single most notable influence of *alpha*-substitutions is the disappearance of a very characteristic initial hypertensive peak preceding hypotension. This influence increased with steric bulk of the substituent to a maximum for the isopropyl (Compounds VI and VII).

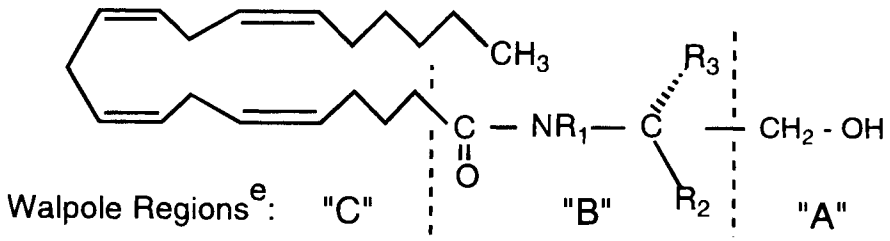
Increasing the length of the *alpha* moiety decreased their hypotensive action. This was evident in comparing the relative activities of the methyl (Compounds II and III) vs. ethyl (Compounds IV and V) and isopropyl (Compounds VI and VII) vs. isobutyl (Compounds VIII and IX) substitutions.

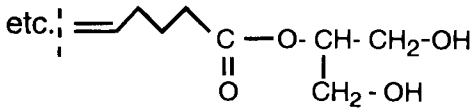
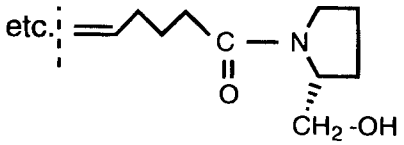
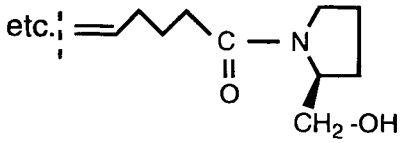
Neither enantiomer series consistently demonstrated the most hypotensive activity. However, the R-*alpha*-isopropyl compound (VI) exhibited the most sustained effect. Further increasing the steric bulk of this substituent by adding a methyl to form the *alpha-tert*-butyl compounds (X and XI), diminished its hypotensive action. Similarly, a phenyl or benzyl group in that position also proved deleterious (data not shown).

Tertiary methyl substitution at the amide nitrogen of AEA (Compound XIII) produced effects similar to that of methyl substitution at the *alpha*-carbon, except for a more pronounced initial hypotension with the former compound.

Replacing the terminal methyl of *alpha*-ethyl anandamide with a hydroxy, as well as substituting the amide nitrogen with an oxygen, resulted in 2-arachidonyl glycerol (Compound XIV), which exhibited (Figure 1) an action profile similar

**Table I.** Anandamide Structures and IOP Effects on Rabbits, with Reported CB-1 Receptor Values




| No | Name | $R_1$ | $R_2$ | $R_3$ | IOP Change (Treated Eye)[d] | Reported CB-1 Ki value (nM) |
|---|---|---|---|---|---|---|
| I | Arachidonoyl ethanolamide (Data from Ref. 10) | H | H | H | ↑[a] ↓[b] | 22[f], 89[g] |
| II | R-*alpha*-methyl anandamide | H | $CH_3$ | H | d | 20[h] |
| III | S-*alpha*-methyl anandamide | H | H | $CH_3$ | ↓[b] | 173[h] |
| IV | R-*alpha*-ethyl anandamide | H | $CH_2CH_3$ | H | d | |
| V | S-*alpha*-ethyl anandamide | H | H | $CH_2CH_3$ | d | |
| VI | R-*alpha*-isopropyl anandamide | H | $CH(CH_3)_2$ | H | ↓[b] | |
| VII | S-*alpha*-isopropyl anandamide | H | H | $CH(CH_3)_2$ | ↓[c] | |
| VIII | R-*alpha*-isobutyl anandamide | H | $CH_2CH(CH_3)_2$ | H | d | 5420[f] |
| IX | S-*alpha*-isobutyl anandamide | H | H | $CH_2CH(CH_3)_2$ | ↓[c] | >10000[f] |
| X | R-*alpha*-tert-butyl anandamide | H | $C(CH_3)_3$ | H | d | |
| XI | S-*alpha*-tert-butyl anandamide | H | H | $C(CH_3)_3$ | d | |
| XII | *alpha*-dimethyl anandamide | H | $CH_3$ | $CH_3$ | d | 162[i] |
| XIII | N-methyl anandamide | $CH_3$ | H | H | ↓[b] | 4980[f] |
| XIV | 2-arachidonoyl glycerol |  | | | ↑[a]↓[b] | 472[j], 89[k] |
| XV | Arachidonoyl R-2-pyrrolidine methanol |  | | | ↓[b] | |
| XVI | Arachidonoyl S-2-pyrrolidine methanol |  | | | ↓[b] | |







[a] initial increase in mean IOP ≥ 1.0 mmHg.
[b] significant decrease in IOP when compared to vehicle ($p < 0.05$).
[c] notable decrease of mean IOP, but not statistically significant ($p > 0.05$).
[d] IOP effect equivocal.
[e] Ref. 13.
[f] Ref. 19.
[g] Ref. 20.
[h] Ref. 8.
[i] Ref. 21.
[j] Ref. 22.
[k] Ref. 23.

to that of AEA, but with a greatly exaggerated initial hypertensive phase.

Joining the amide nitrogen with the *alpha*-carbon via a trimethylene bridge to form pyrrolidine compounds (XV and XVI) resulted in less activity compared to corresponding methyl compounds substituted singly at either of these two positions.

## Untreated Eyes

Unilateral ocular administration of the title compounds typically did not cause a statistically significant decrease of IOP in untreated eyes, compared to the vehicle (Table II). However, some statistically significant decreases of IOP in

**Table II.** Intraocular Pressure Changes (mean mmHg ± S.E.) at Predetermined Times (h) in Normotensive Pigmented Rabbits (n = 10) after Unilateral Administration of 25 μl of Anandamide Solution (dose = 62.5 μg)

| Compound | 0 h | 0.5 h | 1 h | 2 h | 3 h | 4 h | 5 h |
|---|---|---|---|---|---|---|---|
| *Treated Eye* | | | | | | | |
| Vehicle[b] | 0.0 ± 0.0 | 0.5 ± 0.4 | 0.1 ± 0.5 | −0.7 ± 0.4 | −0.1 ± 0.9 | 0.9 ± 0.6 | 1.9 ± 0.8 |
| I[c] | 0.0 ± 0.0 | 2.5 ± 1.3[a] | −3.4 ± 1.0[a] | −5.2 ± 1.3[a] | −2.6 ± 1.6 | −0.9 ± 1.5 | −0.2 ± 1.1 |
| III | 0.0 ± 0.0 | 0.7 ± 1.2 | −0.8 ± 0.8 | −2.9 ± 0.6[a] | −1.2 ± 0.6 | −0.1 ± 0.7 | 0.4 ± 0.5 |
| VI | 0.0 ± 0.0 | −1.7 ± 0.5 | −2.4 ± 0.4[a] | −2.3 ± 0.5 | −2.2 ± 0.6[a] | −0.6 ± 1.0 | 0.4 ± 0.8 |
| VII | 0.0 ± 0.0 | −0.7 ± 0.5 | −1.0 ± 0.7 | −1.6 ± 0.7 | −1.7 ± 1.0 | −0.4 ± 0.6 | 0.0 ± 1.2 |
| XIII | 0.0 ± 0.0 | −1.5 ± 0.5 | −1.7 ± 0.4 | −2.1 ± 0.6 | −1.2 ± 0.8 | 0.1 ± 0.9 | −0.2 ± 0.8[a] |
| IX | 0.0 ± 0.0 | 0.5 ± 0.5 | −0.7 ± 0.7 | −2.5 ± 0.6 | −1.4 ± 0.8 | −0.2 ± 0.5 | 1.5 ± 0.9 |
| XIV[d] | 0.0 ± 0.0 | 6.6 ± 3.2 | 4.9 ± 1.3[a] | −1.6 ± 2.7 | −5.4 ± 1.0[a] | −3.0 ± 0.8[a] | −0.9 ± 0.9 |
| XV | 0.0 ± 0.0 | −0.3 ± 0.6 | −1.0 ± 0.7 | −0.9 ± 0.5 | −1.2 ± 0.6 | −0.9 ± 0.4[a] | −0.4 ± 0.9[a] |
| XVI | 0.0 ± 0.0 | −0.4 ± 0.7 | −0.7 ± 0.8 | −0.2 ± 0.9 | −0.6 ± 0.7 | −0.7 ± 0.9 | −0.4 ± 0.8[a] |
| *Untreated Eye* | | | | | | | |
| Vehicle[b] | 0.0 ± 0.0 | 0.0 ± 0.4 | 0.2 ± 0.1 | −0.3 ± 0.3 | 0.2 ± 0.3 | 0.9 ± 0.5 | 2.0 ± 0.6 |
| I[c] | 0.0 ± 0.0 | −2.1 ± 0.3[a] | −1.6 ± 1.1 | −0.3 ± 1.7 | −0.3 ± 1.5 | 0.3 ± 1.8 | −0.1 ± 1.5 |
| III | 0.0 ± 0.0 | 1.2 ± 0.4 | 0.9 ± 0.3 | 0.5 ± 0.2 | 1.5 ± 0.2 | 2.1 ± 0.3 | 2.7 ± 0.3 |
| VI | 0.0 ± 0.0 | 0.4 ± 0.2 | −1.2 ± 0.4 | −0.3 ± 0.4 | −0.6 ± 0.6 | 0.9 ± 0.6 | 1.9 ± 0.4 |
| VII | 0.0 ± 0.0 | 0.0 ± 0.3 | −0.1 ± 0.3 | 0.0 ± 0.3 | 0.2 ± 0.4 | 1.2 ± 0.4 | 1.9 ± 0.5 |
| XIII | 0.0 ± 0.0 | −1.1 ± 0.3[a] | −1.5 ± 1.1[a] | −1.4 ± 0.4 | −1.0 ± 0.6 | −0.3 ± 0.5 | −0.1 ± 0.6[a] |
| IX | 0.0 ± 0.0 | 1.2 ± 0.3 | −0.2 ± 0.4 | −0.8 ± 0.4 | 0.4 ± 0.4 | 0.8 ± 0.4 | 3.1 ± 0.6 |
| XIV[d] | 0.0 ± 0.0 | −0.9 ± 0.5 | −1.7 ± 0.9 | −1.9 ± 0.7[a] | −1.6 ± 0.6[a] | −0.7 ± 0.4[a] | −0.3 ± 1.1 |
| XV | 0.0 ± 0.0 | 0.0 ± 0.4 | −0.2 ± 0.4 | −1.0 ± 0.2 | −0.6 ± 0.5 | −0.6 ± 0.5 | 0.6 ± 0.6[a] |
| XVI | 0.0 ± 0.0 | 0.3 ± 0.4 | −0.1 ± 0.6 | −0.3 ± 0.4 | 0.6 ± 0.5 | 0.2 ± 0.4 | 0.7 ± 0.4[a] |

[a] Significantly different from vehicle value at a 95% confidence level (ANOVA, Fisher's PLSD test).
[b] 20% HP-β-CD + 3% PVA.
[c] AEA data from Reference 10. Differences were statistically significant (*) relative to control solution (NaCl) values cited therein.
[d] Data from separate study (n = 6). Differences were statistically significant (*) relative to control solution (NaCl) values in that study (see Figure 1).

contralateral eyes occurred 5 h after application of compounds XIII (also at 0.5 and 1 h), XV, and XVI. These may represent an inhibition of routine late-afternoon IOP increases, rather than a substantial IOP decrease. For compound XIV, no initial increase of IOP was observed, but a statistically significant decrease of IOP did occur (Figure 1), although it was smaller than that of the treated eye.

### Indomethacin Studies

2-Arachidonoyl glycerol (62.5 μg) produced a large initial elevation of IOP followed by a significant hypotension in the treated eye 30–60 min after administration, compared to the effects of ocular 0.9% NaCl (Figure 1). In the untreated eye, only a hypotensive response was observed after its administration. Indomethacin (12.5 mg), given subcutaneously 30 min before ophthalmic dosing, completely prevented IOP responses to the compound, both in the treated and untreated eyes. This type of pre-treatment, followed by ocular application of 0.9% NaCl, did not affect IOP by itself.

Subcutaneous indomethacin (12.5 mg) did *not* inhibit the IOP effects of R-*alpha*-isopropyl anandamide (Figure 2).

## DISCUSSION

### Structure-Activity Relationships

The present study demonstrates that variations in the *alpha*-carbon substituent cause marked changes in observed IOP effects, although not in a manner consistent with the binding behavior (8,19,21) of identical compounds at the CB1 receptor (Table I). In addition, evaluation of the analogous 2-alkyl series (Walpole "C" section) compounds known to possess potent CB-1 receptor binding effects (19,21), has shown little or no reduction of rabbit normotensive IOP (unpublished data). The results thus far, suggest R-*alpha*-isopropyl anandamide (Compound VI) as the most promising candidate for further testing.

AEA (10) and the other *alpha*-unsubstituted analogs tested previously (12) caused an initial increase of IOP in the treated eye, in contrast to the immediate hypotension caused by the present compounds. It may be possible that this apparent qualitative difference is due to compound potency (10), but the doses employed here are comparable to the hypertension-inducing doses of the aforementioned studies.

### Mechanism of Action

Unilateral administration of the *alpha*-substituted analogues provoked an immediate decrease of intraocular pressure in treated eyes and only a minor hypotensive response in untreated eyes (Table II). We earlier had reported this asymmetry with AEA (10) and other Walpole "A" section analogs (12). These data suggest that the effect of anandamides on IOP is local, rather than mediated via the central nervous system (CNS). The minor contralateral effects of the anandamides may result from systemic absorption of the applied dose via blood circulation (24,25) from where the drug may be transferred to the untreated eye (26). Previous experiments (27) have shown

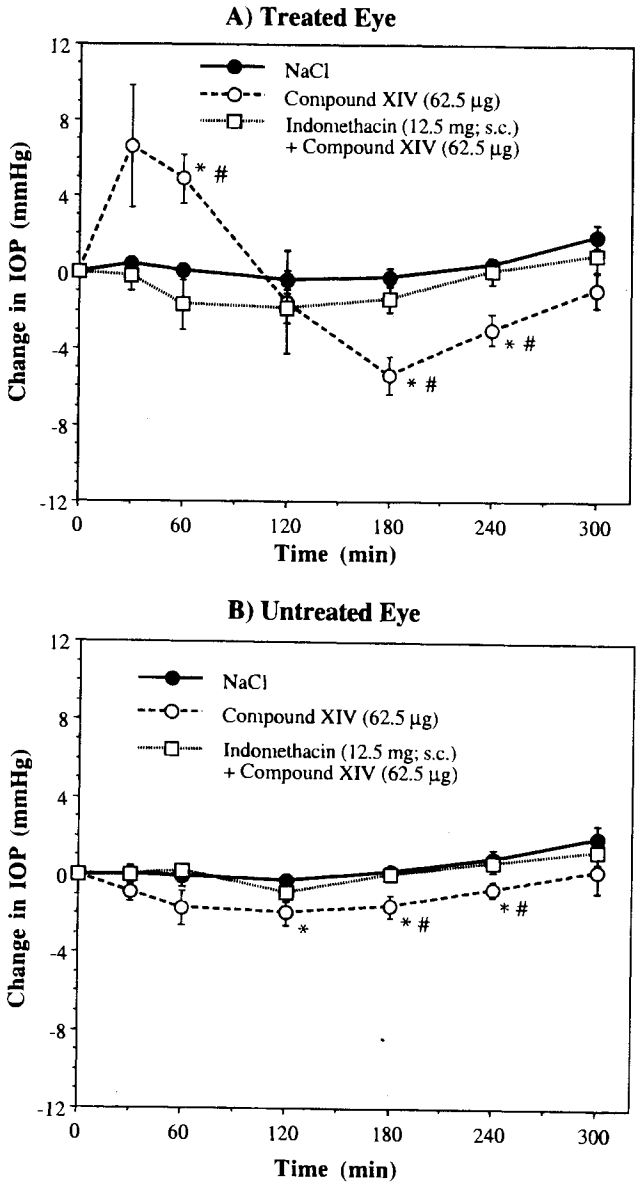



**Fig. 1.** IOP changes (mean ± S.E., n = 10) in treated (A) and untreated (B) eyes of pigmented rabbits after unilateral ocular administration (25 µl) of 0.9% NaCl (●), 62.5 µg Compound XIV without indomethacin pre-treatment (○), or 62.5 µg Compound XIV after pre-treatment (12.5 mg, s.c.) with indomethacin (□). Symbols indicate data significantly different from values for 0.9% NaCl (*), or Compound XIV after indomethacin pretreatment (#), at a 95% confidence level (ANOVA, Fisher's PLSD test).

that although the concentration of a drug in the untreated eye may be substantially lower than that of the treated eye, these lower concentrations are often sufficient to cause some reduction in IOP. It must be noted, however, that the experimental set up and the observed results do not completely preclude the possibility that some of the IOP-lowering effects of anandamides are mediated via the CNS.

Since it has been suggested (14,15) that a prostaglandin, rather than arachidonic acid itself, affects IOP after arachidonic acid treatment, part of our studies has focused upon the question of whether anandamides possess direct IOP influencing properties or possibly serve as precursors for prostaglandin synthesis, either as anandamides or a source of arachidonic acid. The present indomethacin study (Figure 1) of 2-arachidonoyl glycerol (Compound XIV) is consistent with earlier ones showing that IOP effects of arachidonic acid (14,15), AEA (10) and other *alpha*-unsubstituted anandamides (12), can be eliminated via indomethacin pre-treatment, this last compound being a cyclo-oxygenase inhibitor preventing prostaglandin synthesis. In addition, the exaggerated initial hypertensive phase of Compound XIV is similar to that of applied arachidonic acid, indicating that the ester is readily catabolized to produce the latter compound *in situ*, in spite of a chain substitution analogous to the *alpha*-substituted anandamides.

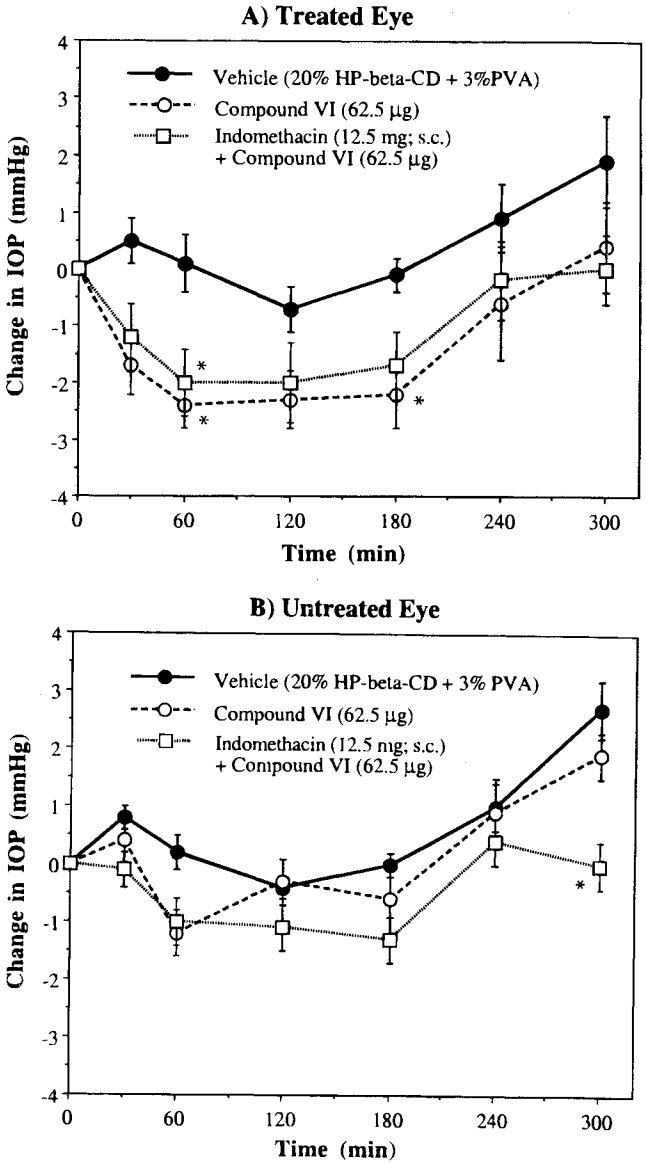



**Fig. 2.** IOP changes (mean ± S.E., n = 10) in treated (A) and untreated (B) eyes of pigmented rabbits after unilateral ocular administration (25 µl) of vehicle (●), 62.5 µg Compound VI without indomethacin pre-treatment (○), or 62.5 µg Compound VI after pre-treatment (12.5 mg, s.c.) with indomethacin (□). Symbols indicate data significantly different from values for vehicle (*) at a 95% confidence level (ANOVA, Fisher's PLSD test).

It seems likely that at least the initial hypertensive phase of *alpha*-unsubstituted anandamides results from their catabolism and consequent release of free arachidonic acid. Whether it is this free acid or perhaps the anandamides themselves that are further metabolized to produce the observed hypotensive action is presently unknown. In contrast, the *alpha*-substituted anandamides do not serve as good amidase substrates (8,19) and therefore, arachidonic acid precursors. Since the hypotensive effect of R-*alpha*-isopropyl anandamide is not neutralized by the presence of indomethacin (Figure 2), prostaglandin synthesis directly from it via the (PGH synthase-1) cyclo-oxygenase pathway would seem not to occur.

Thus, several hypotheses remain to be tested, including the possibility that the IOP influence of *alpha*-substituted anandamides stems either from their direct effects on receptors or from the action of metabolites unrelated to prostaglandins. Investigations of these and other hypotheses are in progress.

## CONCLUSIONS

Individual components of the characteristic bi-phasic IOP effect of anandamides are separable. The initial hypertensive phase can be completely eliminated, and its hypotensive profile modified, by altering the length, steric bulk and enantiomeric position of the *alpha*-carbon substituent within the Walpole "B" region of these molecules. Interestingly, since indomethacin pre-treatment does not eliminate the hypotension of eyes administered R-*alpha*-isopropyl anandamide, a second mechanism of IOP reduction is implied. Clarification of a possible role for cyclo-oxygenase or other metabolic pathways in mediating the IOP influence of anandamides requires further study.

## ACKNOWLEDGMENTS

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