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Dual Effect of Isoprostanes on the Release of [^{3}H]D-Aspartate from Isolated Bovine Retinae: Role of Arachidonic Acid Metabolites

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The effect of 8-isoprostanes on potassium (K^+)-depolarization-evoked release of [^{3}H]D-aspartate from bovine isolated retinae was investigated. Isolated bovine retinae were prepared for studies of K^+-evoked release of [^{3}H]D-aspartate using the Superfusion Method. Low concentrations of 8-*iso*$PGF_{2\alpha}$ (1–100 nM) inhibited whereas higher concentrations of this 8-isoprostane (100 nM–30 μM) enhanced K^+-induced [^{3}H]D-aspartate overflow. The excitatory effect of 8-*iso*$PGF_{2\alpha}$ was mimicked by thromboxane receptor agonist, U-46619 and blocked by thromboxane receptor antagonist, SQ 29,548 (10 μM). Pretreatment of tissues with the cyclooxygenase (COX) inhibitor, flurbiprofen unmasked an inhibitory effect of high concentrations of 8-*iso*$PGF_{2\alpha}$ (1–30 μM) on [^{3}H]D-aspartate release that was attenuated by AH 6809 (10 μM). In conclusion, 8-*iso*$PGF_{2\alpha}$ exhibits a dual regulatory effect on K^+-induced [^{3}H]D-aspartate release in isolated bovine retinae. The inhibitory action caused by 8-*iso*$PGF_{2\alpha}$ is due to the activation of EP_1/EP_2 receptors while the excitatory effects are due to the activation of thromboxane receptors.

KEY WORDS: Aspartate; excitatory; inhibitory; isoprostanes; receptors; retina.

INTRODUCTION

Isoprostanes are a series of prostaglandin (PG)-like compounds formed in abundance *in vivo* by a non-enzymatic, free radical catalyzed peroxidation of arachidonic acid independent of the cyclooxygenase (COX) enzyme (1) and to a minor extent, by the inducible COX-2 catalyzed peroxidation of arachidonic acid (2). Of the 8-isoprostane family, 8-*iso*-$PGF_{2\alpha}$ has been detected in all tissues and human biological fluids (3). In addition to 8-*iso*-$PGF_{2\alpha}$, other isoprostanes formed *in vivo* include PGD_2-like (8-*iso*PGD_2), PGE_2-like (8-*iso*PGE_2), PGA_2-like (8-*iso*PGA_2) and thromboxane-like (8-*iso*-Txs). Unlike eicosanoids formed by COX, isoprostanes are formed *in situ* on esterified phospholipids and are released preformed presumably by phospholipases. 8-Isoprostanes are a reliable marker of oxidative stress because they are stable products whose production increases with exposure to oxidative injury in both *in vivo* and *in vitro* animal models (4–7).

Isoprostanes have been reported to exert pharmacological actions in various tissues through activation of prostanoid or prostanoid-like receptors (8–10).

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In the eye, Awe et al. observed that 8-*iso*PGE_2 inhibited electrically evoked noradrenaline release while 8-*iso*$PGF_{2\alpha}$ enhanced the release of this neurotransmitter from isolated human iris-ciliary bodies (11). On the contrary, both 8-*iso*PGE_2 and 8-*iso*$PGF_{2\alpha}$ were reported to enhance the release of field-stimulated norepinephrine release from bovine isolated irides (10). There appears to be species and tissue difference in the nature of receptors that mediate the pharmacological actions of 8-isoprostanes. There is evidence that isoprostanes may act via prostaglandin FP receptors because 8,12-*iso*P-III can activate FP receptors in neonatal rat ventricular myocytes (12). Recently, Nakamura et al. reported that unlike 8-*iso*-$PGF_{2\alpha}$ whose inhibitory effect on evoked norepinephrine release was mediated through thromboxane receptors, the inhibitory effects of 8-*iso*PGE_2 was mediated via EP_3 receptors in isolated rat stomach (13). We have evidence that isoprostanes can produce both thromboxane receptor-mediated excitatory effects and a non-thromboxane receptor mediated inhibitory effects on sympathetic neurotransmission in isolated bovine (10) and human (11) iris-ciliary bodies. Clearly, the exact nature of receptors that mediate the pharmacological actions of isoprostanes is yet to be fully determined.

In the retina, there is evidence that free-radical catalyzed peroxidation of arachidonic acid leads to formation of isoprostanes in human (14) and bovine tissues (15). Recently, Hou et al. reported that 8-*iso*-$PGF_{2\alpha}$ and its metabolite caused vasoconstriction of pig retinal and brain microvessels via an endothelin-dependent, thromboxane A_2 synthesis (16). We have previously shown that oxidant stress induced by hydrogen peroxide can modulate glutamate release from bovine retinae both *in vitro* and *ex vivo* (17,18). It is, however, unclear whether as free radical derived metabolites of the arachidonic acid pathway, isoprostanes can also affect the release of glutamate from mammalian retinae. The aim of the present study was, therefore, to investigate: (i) effect of different series of isoprostanes on the release of glutamate (using [^{3}H]D-aspartate as a marker) from mammalian retina and (ii) role of arachidonic acid metabolites and their receptors on the isoprostane response. Parts of this study have been communicated in an abstract form (19).

EXPERIMENTAL PROCEDURE

Studies were performed with cow eyeballs obtained from the local slaughterhouse (J.F.O 'Neill Packing Co. and All American Meats, Inc.). Eyeballs from freshly sacrificed cows were transported to the laboratory in an ice bucket. Eyes were quickly enucleated under light and the cornea, lens and vitreous humor were carefully removed. The remaining eyecup was everted and placed in a vial containing Krebs buffer solution.

Potassium-Induced [^{3}H]D-Aspartate Release. The methodology employed for studies of [^{3}H]D-aspartate release was essentially the same as described by us (20) and others (21). Briefly, isolated mammalian retinas were incubated in oxygenated Krebs buffer solution containing 200 nM of [^{3}H]D-aspartate at 37°C for 1 h. The Krebs buffer solution was composed of the following (millimolar): sodium chloride, 118; potassium chloride, 4.8; calcium chloride, 1.3; potassium dihydrogen phosphate, 1.2; sodium bicarbonate, 25; magnesium sulfate, 2.0; and dextrose, 10 (pH 7.4). After incubation, tissues were rinsed, mounted between nylon mesh cloth and placed in thermostatically-controlled Superfusion Chambers (Warner Instrument Corp., CT). Tissues were superfused at 1.5 ml/min with oxygenated Krebs solution, with superfusates collected every 6 min. After an initial 60 min of superfusion to establish a stable baseline of spontaneous tritium efflux, release of [^{3}H]D-aspartate was evoked by K^+ (50 mM; iso-osmotic replacement of Na^+ with K^+) stimuli applied at 90–102 min (S_1) and 138–150 min (S_2) after the onset of superfusion. K^+-induced [^{3}H]D-aspartate release was estimated by subtraction of the extrapolated basal tritium efflux from total tritium released in the 24-min period after the onset of stimulation. Under this experimental condition, [^{3}H]D-aspartate has been reported to be a valid marker of endogenous glutamate in retinal neurons which use these amino acids as transmitters (21–23). Basal (unstimulated) tritium efflux was assumed to decline linearly between pre-stimulation and post-stimulation fractions. All tissues received two stimulations (S_1 and S_2) 36 min apart. To examine the effect of isoprostanes on [^{3}H]D-aspartate release, each drug was present in the Krebs buffer solution applied to tissues 12 min before and during S_2. When used, antagonists were present in the buffer solution 30 min before S_1 and also during S_2.

Data Analysis. Results for superfusion studies are expressed as the absolute (area under the peaks) S_2/S_1 ratios. Except where indicated otherwise, values given are means ± standard error of the mean (SEM). Significance of differences between S_2/S_1 values levels obtained in control and agent-treated preparations was evaluated using analysis of variance followed by Dunnett's test (GraphPad Software, CA). Differences with P values < 0.05 were accepted as statistically significant.

Drugs and Chemicals. The following drugs were used: 8-*iso*-PGA_1, 8-*iso*PGE_1, 8-*iso*PGE_2, 8-*iso*$PGF_{2\alpha}$, 8-*iso*(15R)$PGF_{2\alpha}$, flurbiprofen, SQ 29,548, U-46619, AH 6809 (Cayman Chemical, Ann Arbor, MI USA); caffeic acid (SIGMA, St. Louis, MO); ozagrel hydrochloride (Tocris Cookson Inc., Ellisvilee MO; [^{3}H]D-aspartate (1000Ci/mmol) Dupont NEN, Boston, MA.

RESULTS

Biphasic Effect of 8-*iso*$PGF_{2\alpha}$ on [^{3}H]D-Aspartate Release

As illustrated in Fig. 1 (panel a), application of an iso-osmotic Krebs solution containing K^+ (50 mM) for two successive collection periods elicited an overflow of [^{3}H]D-aspartate from isolated,

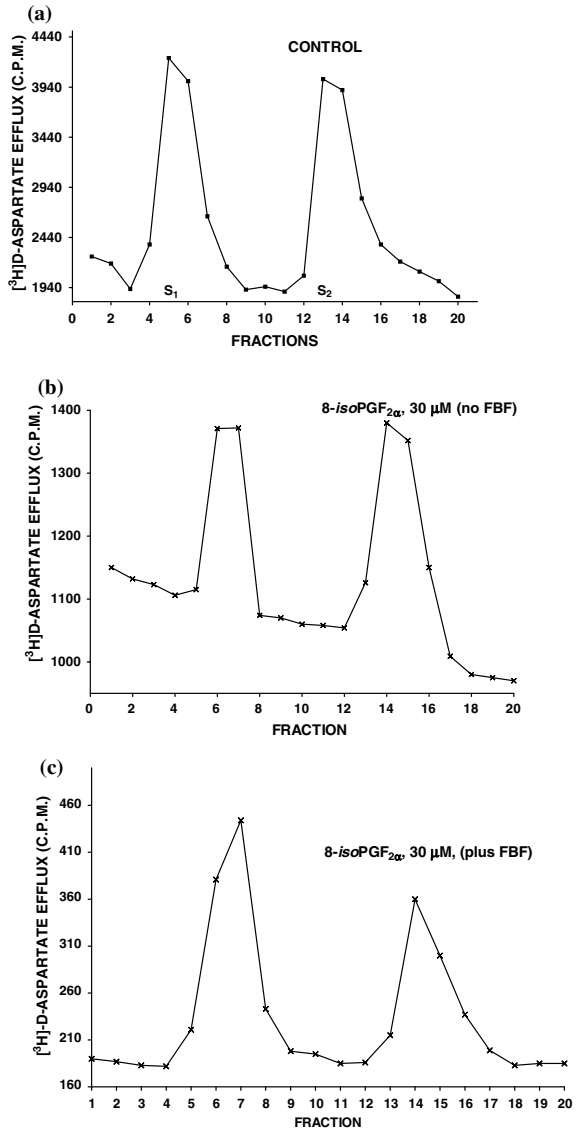

Fig. 1. Effect of 8-*iso*prostaglandin $F_{2\alpha}$ (8-*iso*$PGF_{2\alpha}$) on KCl (K^+, 50 mM)-induced release of [^{3}H]D-aspartate from isolated, superfused retinas. K^+ stimuli were applied at fractions 5/6 (S_1) and 13/14 (S_2). Panel A, control (no agent present). Panels B and C, 8-*iso*$PGF_{2\alpha}$ (30 μM) applied 12 min before S_2 in absence and presence of flubiprofen (FBF), respectively. Fractions of the superfusate containing [^{3}H]D-aspartate were collected at 6-min intervals and analyzed for radioactivity as described in the Methods section.

superfused bovine retinae yielding S_2/S_1 ratios of 1.05 ± 0.04 ($n = 8$). Application of 8-*iso*$PGF_{2\alpha}$ (30 μM) 12 min prior to second K^+ stimulus caused an enhancement of K^+ -induced [^{3}H]D-aspartate overflow without affecting basal tritium overflow (Fig. 1, panel b). In the presence of flurbiprofen (3 μM), the same concentration of 8-*iso*$PGF_{2\alpha}$ exhibited an inhibitory response (Fig. 1, panel c). In the concentration range, 1 nM to 30 μM, 8-*iso*-$PGF_{2\alpha}$ exhibited a biphasic response, being inhibitory (maximum inhibition of 25% at 0.1 μM concentration) at lower concentrations and excitatory at higher concentrations (Fig. 2). Since most of the pharmacological actions of isoprostanes can be mimicked by thromboxane receptor agonists, we examined the effects of U-496619 on K^+-induced [^{3}H]D-aspartate release. As observed with higher concentrations of 8-*iso*$PGF_{2\alpha}$, U-46619 (10 μM) caused an enhancement of K^+-induced [^{3}H]D-aspartate release (Fig. 3).

Role of Thromboxane. Due to the susceptibility of isoprostane responses to inhibition by thromboxane receptor antagonists, we examined the effect of SQ 29,548 on enhancement of K^+-induced [^{3}H]D-aspartate release induced by 8-*iso*$PGF_{2\alpha}$ and U-46619. It was interesting to note that while SQ 29,548 (10 μM) on its own had no effect on K^+-induced [^{3}H]D-aspartate release, it attenuated the effect of both 8-*iso*$PGF_{2\alpha}$ (30 μM) and U46619 (10 μM) on this excitatory neurotransmitter in isolated bovine retinae (Fig. 3). To determine whether endogenous thromboxanes could mediate 8-*iso*-$PGF_{2\alpha}$ –mediated enhancement of K^+ -induced [^{3}H]D-aspartate release, tissues were pretreated with the thromboxane synthetase inhibitor, ozagrel. As illustrated in Fig. 3, ozagrel (10 μM) attenuated the excitatory effect of 8-*iso*$PGF_{2\alpha}$ (30 μM) on K^+-induced [^{3}H]D-aspartate release.

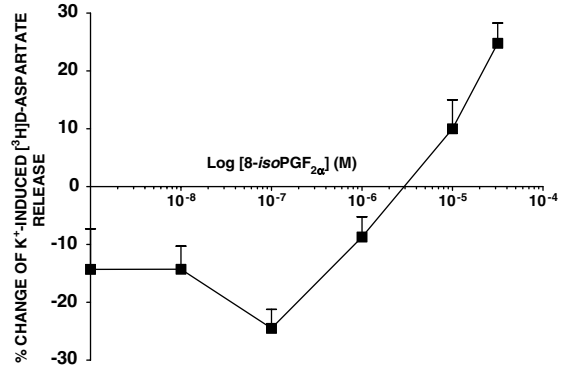

Fig. 2. Effect of 8-*iso*prostaglandin $F_{2\alpha}$ (8-*iso*$PGF_{2\alpha}$) on KCl (K^+, 50 mM)-induced release of [^{3}H]D-aspartate from isolated, bovine retinas. Data shown are derived from ratios of S_2/S_1 peaks as described in Methods section. Vertical bars represent means ± SEM of four to eight determinations.

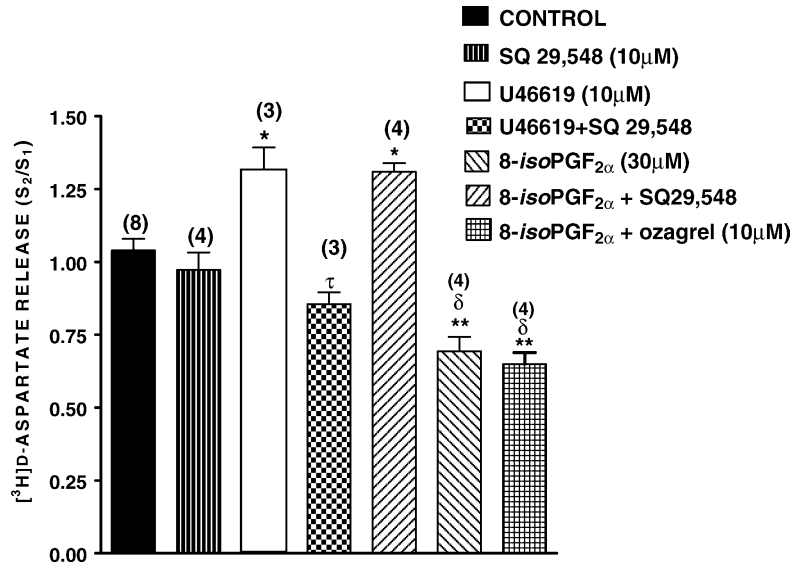

Fig. 3. Effect of SQ 29,548, U-46619 8-*iso*prostaglandin $F_{2\alpha}$ (8-*iso*$PGF_{2\alpha}$) and ozagrel on KCl (K^+, 50 mM)-induced release of [^{3}H]D-aspartate from isolated, bovine retinas; control and in the presence of SQ 29,548 (10 μM), U-46619 (10 μM), U-46619 plus SQ29,548, 8-*iso*$PGF_{2\alpha}$ (10 μM), 8-*iso*$PGF_{2\alpha}$ plus SQ 29,548 and 8-*iso*$PGF_{2\alpha}$ plus ozagrel (10 μM). Vertical bars represent means ± SEM. Number of observations is in parenthesis. $^*P < 0.01$; $^{**}P < 0.001$, significantly different from untreated control. $^{\Gamma}P < 0.001$, significantly different from U-46619 alone. $^{\delta}P < 0.001$, significantly different from 8-*iso*$PGF_{2\alpha}$ alone.

Inhibitory Effect of Isoprostanes on [^{3}H]D-Aspartate Release: Role of Prostaglandins

In a series of experiments, we investigated the role of other products of the COX pathway (i.e., prostaglandins) in the response produced by 8-*iso*-$PGF_{2\alpha}$ on [^{3}H]D-aspartate release. In the presence of the COX inhibitor, flurbiprofen (3 μM), 8-*iso*-$PGF_{2\alpha}$ elicited a concentration-dependent inhibition of evoked [^{3}H]D-aspartate release (Fig. 4). Based upon the inhibition exhibited by 8-*iso*$PGF_{2\alpha}$ in presence of flurbiprofen, we further examined the effect of other isoprostanes, 8-*iso*PGA_1, 8-*iso*PGE_1,

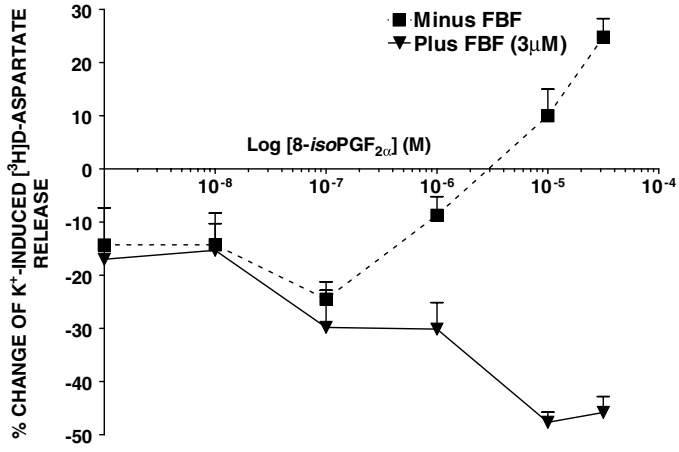

Fig. 4. Effect of 8-*iso*prostaglandin $F_{2\alpha}$ (8-*iso*$PGF_{2\alpha}$) on KCl (K^+, 50 mM)-induced release of [^{3}H]D-aspartate from isolated, bovine retinas; in the presence (solid line) absence (dotted line) of flurbiprofen (FBF;3 μM). Data shown are derived from ratios of S_2/S_1 peaks as described in Methods section. Vertical bars represent means ± SEM of four to eight determinations.

8-*iso*PGE_2, and 8-*iso*(15R)$PGF_{2\alpha}$ on K^+-induced [^{3}H]D-aspartate release. At an equimolar concentration of 0.1 μM, all isoprostanes tested except 8-*iso*(15R)$PGF_{2\alpha}$ exhibited an inhibitory effect on K^+-induced [^{3}H]D-aspartate release with the following rank order of activity: 8-*iso*$PGF_{2\alpha}$ > 8-*iso*PGA_1 > 8-*iso*PGE_1 > 8-*iso*PGE_2 (Fig. 5).

Effect of Lipoxygenase Inhibitor, Caffeic Acid. To determine whether products of the lipoxygenase pathway are involved in the observed inhibitory response to 8-*iso*$PGF_{2\alpha}$, tissues were exposed to the lipoxygenase inhibitor, caffeic acid. Caffeic acid (10 μM) had no significant effect on both K^+-induced [^{3}H]D-aspartate release and 8-*iso*$PGF_{2\alpha}$ (0.1–1 μM)-mediated inhibition of K^+-induced [^{3}H]D-aspartate release (Table I).

Effect of EP1/EP2 Receptor Antagonist, AH 6809. To determine the role of prostaglandin receptors in the observed inhibitory response to 8-*iso*-$PGF_{2\alpha}$, tissues were pretreated with AH 6809. As shown in Fig. 6, AH 6809 (10 μM) on its own had no significant effect on K^+-induced [^{3}H]D-aspartate release. On the other hand, the same concentration of AH 6809 attenuated the effect of 8-*iso*$PGF_{2\alpha}$ (0.1–1 μM) (Fig. 6).

Table I. Effect of Caffeic Acid on 8-Isoprostaglandin $F_{2\alpha}$ (8-*iso*-$PGF_{2\alpha}$)–Mediated Inhibition of Potassium Chloride (K^+, 50 mM)-Induced Release of [^{3}H]D-Aspartate from Isolated, Superfused Retinas

Experiment	S_2/S_1	
	Minus caffeic acid	Plus caffeic acid (10 μM)
Control	1.05 ± 0.04 (8)	1.04 ± 0.08 (4)
+ 8-*iso*$PGF_{2\alpha}$ (0.1 μM)	0.739 ± 0.07 (7)*	0.770 ± 0.02 (4)*
+ 8-*iso*$PGF_{2\alpha}$ (1 μM)	0.712 ± 0.07 (4)*	0.750 ± 0.03 (4)*

Values are means ± SEM; Numbers of observations in parenthesis; The 8-isoprostanes were added 12 min before S_2; *P < 0.01, significantly different from untreated control.

Effect of Thromboxane Receptor Antagonist, SQ 29548. To determine the role of thromboxane receptors on the isoprostane-mediated inhibition of K^+-induced [^{3}H]D-aspartate release, tissues were pretreated with the thromboxane receptor antagonist, SQ 29,548. SQ 29,548 (10 μM) did not significantly alter K^+-induced [^{3}H]D-aspartate release nor did it reverse the inhibitory effect of 8-*iso*$PGF_{2\alpha}$ (10 μM) on K^+-induced [^{3}H]D-aspartate release (Table II).

DISCUSSION

It is now established that in addition to being markers of oxidative stress *in vivo* and *in vitro*,

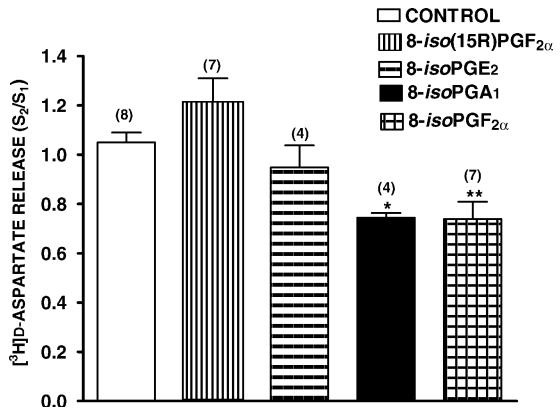

Fig. 5. Effect of the isoprostanes, 8-*iso*(15R)prostaglandin $F_{2\alpha}$ (8-*iso*(15R)$PGF_{2\alpha}$), 8-*iso*prostaglandin E_2 (8-*iso*PGE_2), 8-*iso*prostaglandin A_1 (8-*iso*PGA_1) and 8-*iso*prostaglandin $F_{2\alpha}$ (8-*iso*$PGF_{2\alpha}$) on K^+-evoked [^{3}H]D-aspartate release: control and in the presence of 0.1 μM concentration of the isoprostanes. Vertical bars represent means ± SEM Number of observations is in parenthesis. *P < 0.05; **P < 0.01, significantly different from untreated control.

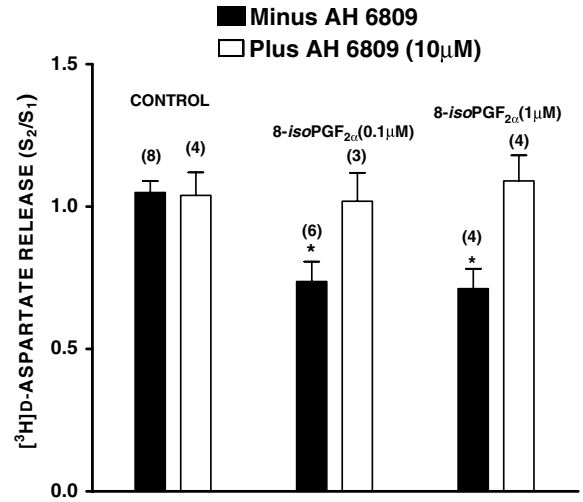

Fig. 6. Effect of AH 6801 on 8-isoprostaglandin $F_{2\alpha}$ (8-*iso*$PGF_{2\alpha}$) mediated inhibition of K^+-evoked [^{3}H]D-aspartate release: control and in the presence of 8-*iso*$PGF_{2\alpha}$ (0.1 μM and 1 μM). Vertical bars represent means ± SEM. Number of observations is in parenthesis. *P < 0.01, significantly different from untreated control.

Table II. Effect of SQ 29,548 on 8-Isoprostaglandin $F_{2\alpha}$ (8-*iso*$PGF_{2\alpha}$)–Mediated Inhibition of Potassium Chloride (K^+, 50 mM)-Induced Release of [^{3}H]D-Aspartate from Isolated, Superfused Retinas

Experiment	S_2/S_1	*n*
Control	1.05 ± 0.04	8
+ SQ 29,548 (10 μM)*	0.973 ± 0.09	4
+ 8-*iso*$PGF_{2\alpha}$ (10 μM)*	0.57 ± 0.04‡	7
+ 8-*iso*$PGF_{2\alpha}$ (10 μM)* + SQ 29,548 (3 μM)†	0.632 ± 0.05‡	4
+ 8-*iso*$PGF_{2\alpha}$ (10 μM)* + SQ 29,548 (10 μM)†	0.728 ± 0.028‡	6

Values are mean ± SEM; *n* = number of observations; *SQ 29,548 and 8-iso$PGF_{2\alpha}$, were added 12 min before S_2; †SQ 29,548 was added throughout the experiment; ‡$P < 0.01$, significantly different from untreated control.

isoprostanes exert pharmacological actions in a variety of tissues. Indeed, 8-*iso*$PGF_{2\alpha}$, the most prominently studied isoprostane in literature, has been reported to be a potent vasoconstrictor in retinal vascular bed (24) kidney (25), lung (26), brain (27) and heart (28) and to modulate acetylcholine (29) and norepinephrine release (13) from isolated guinea pig trachea and isolated rat stomach, respectively. In the mammalian eye, we have previously shown that 8-*iso*$PGF_{2\alpha}$ can regulate sympathetic neurotransmission in bovine and human iris-ciliary bodies. In the present study, we now report, for the first time, that isoprostanes can modulate K^+-induced [^{3}H]D-aspartate release in the isolated bovine retinae. The isoprostane, 8-*iso*$PGF_{2\alpha}$ exhibited an inhibitory action on K^+-induced [^{3}H]D-aspartate release at low concentrations but caused a stimulant effect on the release of this excitatory amino acid at higher concentrations. Interestingly, we reported a similar pattern of dual pharmacological activity for 8-*iso*$PGF_{2\alpha}$ in both bovine and human iris-ciliary bodies (10,11). Taken together, these results indicate that, under our experimental conditions, 8-*iso*$PGF_{2\alpha}$ exhibits a biphasic regulatory action on K^+-induced [^{3}H]D-aspartate release in bovine retinae, *in vitro*. It is possible that the observed biphasic response of 8-*iso*$PGF_{2\alpha}$ on D-aspartate release could be due to an interaction of isoprostanes with amino acid transporters. A review of the literature, however, does not reveal any evidence in support of this hypothesis. The possibility that isoprostanes could interact with amino acid transporters does merit further investigation.

Because there is evidence that pharmacological effects of isoprostanes can be mimicked by thromboxane receptor agonists (10), we compared the effect elicited by 8-*iso*$PGF_{2\alpha}$ with those caused by U-46619 on K^+-induced [^{3}H]D-aspartate release in bovine retinae, *in vitro*. Indeed, we have previously reported that the excitatory effects of 8-*iso*$PGF_{2\alpha}$ on sympathetic neurotransmission in bovine iris-ciliary bodies could be mimicked by U-46619 *in vitro* (10). In the present study, U-46619 also caused an enhancement of K^+-induced [^{3}H]D-aspartate release in bovine retinae suggesting that thromboxane receptor stimulation can produce an excitatory action on the release of aspartate from this tissue.

There is evidence that the excitatory effects of 8-*iso*$PGF_{2\alpha}$ at pre- and post-junctional sites can be blocked by thromboxane receptor antagonists (13, 30–31). Due to weak receptor-binding properties of 8-*iso*$PGF_{2\alpha}$ to the thromboxane receptor, it has been proposed that the excitatory effect of isoprostanes is mediated via a unique receptor that is similar to but distinct from the thromboxane A_2/PGH_2 receptor (8,9). In the present study, we found that SQ 29,548 inhibited the enhancement of evoked neurotransmitter release induced by 8-*iso*$PGF_{2\alpha}$, suggesting that this response may be mediated via thromboxane receptors. It is, however, unclear whether the so-called unique isoprostane receptor with thromboxaneA_2-like receptor properties is involved in the observed isoprostane response. It may well be that high concentrations of 8-*iso*$PGF_{2\alpha}$ causes the biosynthesis of thromboxanes which in turn, activate thromboxane receptors to cause the excitatory response. Indeed, in a previous study, we found that the thromboxane synthetase inhibitor, carboxyheptylimidazole abolished the stimulatory effect of 8-*iso*PGE_2 on electrically evoked [^{3}H]norepinephrine release in bovine retinae, suggesting that endogenous thromboxanes mediate this excitatory sympathetic response (10). In this study, pretreatment of retinal tissues with the thromboxane synthetase enzyme inhibitor, ozagrel attenuated the excitatory effect of 8-*iso*$PGF_{2\alpha}$ on evoked neurotransmitter release. It was also interesting to note that SQ 29,548 attenuated the enhancement of K^+-induced [^{3}H]D-aspartate release caused by U-46619. These results are in agreement with our previous

observation in bovine iris-ciliary bodies, where SQ 29,548 attenuated sympathetic excitatory effects of 8-*iso*$PGF_{2\alpha}$, *in vitro* (10). Taken together, these results indicate that the excitatory effect of 8-*iso*-$PGF_{2\alpha}$ on [^{3}H]D-aspartate release may be due to an action on thromboxane receptors. Furthermore, endogenous thromboxanes may contribute to the isoprostane response. It is possible to speculate that there is an endogenous isoprostane tone that modulates excitatory neurotransmitter release in retinal tissues via the excitatory thromboxane or thromboxane-like receptors in retinal tissues. It also appears that the mechanism of stimulatory effect of isoprostanes on excitatory neurotransmitter release is similar to that observed for sympathetic neurotransmission in bovine irides (10).

Since arachidonic acid metabolites have been reported to modulate neurotransmitter release in several tissues (15,32), we investigated the role of these second messengers on 8-*iso*$PGF_{2\alpha}$ effect on K^+-induced [^{3}H]D-aspartate release. In a series of experiments, we pretreated retinal tissues with the COX inhibitor, flurbiprofen prior to exposure to 8-*iso*$PGF_{2\alpha}$. This concentration of flurbiprofen has been reported to be adequate in blocking endogenously generated prostanoids in retina (15). Pretreatment of tissues with flurbiprofen unmasked an inhibitory action of high concentrations of 8-*iso*-$PGF_{2\alpha}$ on K^+-induced [^{3}H]D-aspartate release in bovine isolated retinae. It is pertinent to note that the inhibitory effect of lower concentrations 8-*iso*-$PGF_{2\alpha}$ was independent of inhibition of COX, suggesting that these responses are not mediated by endogenously generated prostanoids. This observation was contrary to that we reported for sympathetic neurotransmitter release in bovine iris-ciliary bodies where the inhibitory effect of low concentration of 8-*iso*$PGF_{2\alpha}$ was diminished following pretreatment with flurbiprofen (10).

Based upon the observed inhibitory action of 8-*iso*$PGF_{2\alpha}$ on [^{3}H]D-aspartate release in the presence of flurbiprofen, we further evaluated the effect of different series of isoprostanes on the release of the excitatory amino acid neurotransmitter. At an equimolar concentration (100 nM), the rank order of activity was as follows: 8-*iso*$PGF_{2\alpha}$ > 8-*iso*PGA_1 > 8-*iso*PGE_1 > 8-*iso*PGE_2. It was interesting to note that 8-*iso*PGE_2, which has been reported to be a potent inhibitor field-stimulated [^{3}H]norepinephrine release in bovine iris-ciliary bodies (15 times more potent than 8-*iso*$PGE_{2\alpha}$) (10) and to reduce intraocular pressure in normotensive rabbits (33) was the least potent of all isoprostane agonists tested in the present studies. In human and canine airway smooth muscle, Jansen et al. also observed that 8-*iso*PGE_2 exhibited a higher potency (10–100 times excitatory effect) than 8-*iso*$PGF_{2\alpha}$ (34). Taken together, these studies reveal that in spite of their similarity in structure, different members of the 8-isoprostane family exhibit specific pharmacological effects in different tissues.

To determine the role of other arachidonic acid metabolites on the observed inhibitory response to 8-*iso*$PGF_{2\alpha}$, we examined the effect of caffeic acid, a lipoxygenase enzyme inhibitor on the isoprostane response. Caffeic acid had no significant effect on 8-*iso*$PGF_{2\alpha}$ –induced inhibition of K^+-induced [^{3}H]D-aspartate release response, suggesting that the lipoxygenase pathway does not play a role on 8-*iso*$PGF_{2\alpha}$ response. Similarly, the thromboxane receptor antagonist, SQ 29,548 failed to reverse the 8-*iso*$PGF_{2\alpha}$-mediated inhibitory effect on the release of the excitatory amino acid neurotransmitter. On the other hand, the prostanoid EP_1/EP_2 receptor antagonist, AH 6809 reversed the effect of 8-*iso*-$PGF_{2\alpha}$, suggesting the involvement of prejunctional EP receptors in the inhibitory isoprostane response. A similar observation was made by Nakamura et al. who reported that 8-*iso*$PGF_{2\alpha}$ inhibited sympathetic neurotransmission by interacting with EP receptors in gastric sympathetic nerve terminals in rats (13). Taken together, these data indicate that 8-*iso*$PGF_{2\alpha}$ acts on prejunctional EP receptors to elicit inhibition of potassium depolarization-evoked release of [^{3}H]D-aspartate. It may well be that these EP receptors are different from the other prejunctional EP receptors that have been described at sympathetic and parasympathetic nerve terminals (35,36).

It was interesting to note that in the absence of flurbiprofen, 8-*iso*$PGF_{2\alpha}$ exhibited a stimulatory effect that was blocked by a thromboxane receptor antagonist. Pretreatment tissues with flurbiprofen not only abolished this effect, but also unmasked an inhibitory response that was reversed by the prostaglandin EP receptor inhibitor, AH 6809. It is possible to speculate that when all the arachidonic acid metabolite pathways are intact, 8-*iso*$PGF_{2\alpha}$ stimulates synthesis of products of the thromboxane synthetase pathway that elicit a response via the SQ 29,548-sensitive receptors. However, inhibition of cyclooxygenase by flurbiprofen leads to a direct effect of 8-*iso*$PGF_{2\alpha}$ on prejunctional EP receptors.

The dual response elicited by 8-*iso*$PGF_{2\alpha}$ on evoked [^{3}H]D-aspartate release indicates that there

is a spectrum of activity whereby at low concentrations, the inhibitory action of this isoprostane could serve a neuroprotective role against glutamate excitotoxicity. At high concentrations, the excitatory action of 8-*iso*$PGF_{2\alpha}$ on evoked [^{3}H]D-aspartate release may counterbalance the inhibitory effects of oxidative stress on the release of glutamate release. Indeed, oxidative stress induced by reactive oxygen metabolites such as hydrogen peroxide has been shown to reduce basal glutamate concentrations in the bovine retina and vitreous humor (17) and K^+-evoked release of [^{3}H]D-aspartate from this tissue (18). Interestingly, LeDay et al. also showed that treatment of isolated bovine neural retina with hydrogen peroxide increased the production of 8-*iso*$PGF_{2\alpha}$ from this tissue (15). It is tempting to speculate that isoprostanes may play an integral role in the maintenance of glutamate content in retinal neurons, an effect that contributes to the overall activity of the amino acid as a neurotransmitter in this tissue. Be that as it may, the fact that isoprostanes can produce direct effects on glutaminergic transmission suggests that these sites could provide a potential target for therapeutic intervention in some of the diseases of the retina that are associated with oxidative stress such as diabetic retinopathy.

We conclude that 8-*iso*$PGF_{2\alpha}$ exhibits a dual regulatory effect on K^+-induced [^{3}H]D-aspartate release in isolated bovine retinae. In low concentrations, 8-*iso*$PGF_{2\alpha}$ produces an inhibitory action presumably via the activation of EP receptors but at high concentrations, products of the thromboxane synthetase pathway may be involved in this excitatory response. Inhibition of cyclooxygenase unmasks an inhibitory effect of high concentrations of 8-*iso*$PGF_{2\alpha}$ that is mediated by a direct action of this 8-*iso*prostane on pre-junctional EP receptors.

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