Effect of tienoxolol, a new diuretic β -blocking agent, on urinary prostaglandin excretion in the rat

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1 The effects of tienoxolol, (ethyl 2-[3-[(1,1-dimethylethyl)amino]-2-hydroxypropoxy]-5-[(2-thienylcarbonyl) amino] benzoate, hydrochloride), a novel drug exhibiting both diuretic and β -adrenoceptor blocking properties, were investigated on urinary 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-PGF_{1 α}) and PGE₂ excretion in the rat and compared to those of reference diuretic (furosemide) and β -adrenoceptor antagonists (acebutolol, propranolol). Since tienoxolol was shown to bind to A₁ and A₂ adenosine receptors, the action of theophylline was also evaluated.

2 Tienoxolol (8–128 mg kg⁻¹, p.o.) induced a dose-related increase of 6-keto-PGF_{1 α} excretion from 32 mg kg⁻¹ but a significant elevation of urinary PGE₂ levels was only reached after administration of 128 mg kg⁻¹. However, renal prostaglandin concentrations were not modified by tienoxolol.

3 Furosemide (32 mg kg⁻¹) displayed a strong diuretic activity but did not enhance 6-keto-PGF_{1 α} excretion. Likewise, the latter was unaffected by acebutolol and propranolol (128 mg kg⁻¹) and no significant diuresis was observed following administration of these two β -blocking agents. Theophylline (64 mg kg⁻¹), like tienoxolol, was able to induce both diuresis and urinary prostaglandin excretion. Furthermore, they bound with similar affinities to A₁ and A₂ adenosine receptors. This led to the suggestion that a relationship between P₁-purinoceptors, prostaglandin release, diuresis and natriuresis could exist.

4 Oral co-administration of NECA (0.2 mg kg⁻¹) with tienoxolol markedly reduced the urinary 6-keto-PGF_{1 α} excretion observed when tienoxolol was administered alone. However, neither diuresis nor natriuresis were modified, demonstrating that the proposed relationship was untenable.

5 In conclusion, PGI₂ probably does not participate in the diuretic and natriuretic activity of tienoxolol. The increase of urinary 6-keto-PGF_{1 α} excretion may result not only from the haemodynamic properties of the drug but also from the rise of the urinary flow induced by tienoxolol.

Keywords: Diuretic drug; diuresis; β -adrenoceptor antagonist; natriuresis; adenosine receptor antagonist; urine; PGE₂; kidney; 6-keto-PGF_{1 α}

Introduction

Prostaglandins are formed in both the renal medulla and cortex, and participate in several aspects of renal physiology. Prostacyclin (PGI₂) mainly produced in the glomerulus, modulates cortical functions (maintenance or increase of renal blood flow, regulation of glomerular filtration rate, renin secretion) (Anderson *et al.*, 1976; Weber *et al.*, 1976; Dunn & Hood, 1977; Whorton *et al.*, 1977; Terragno *et al.*, 1978). Prostaglandin E₂ (PGE₂), synthesized primarily in the papilla, regulates medullary functions (water, sodium and chloride excretion) (Anderson *et al.*, 1976; Fine & Trizna, 1977; Stokes, 1979; Sraer *et al.*, 1982).

Diuretic-induced hypotension and salt and water loss are considered to be, at least in part, mediated by prostaglandins (Gullner *et al.*, 1980; Tannenbaum *et al.*, 1975).

Tienoxolol (Figure 1) is a novel drug, combining in the same molecule diuretic, natriuretic and β -adrenoceptor blocking properties which have previously been demonstrated not only in normotensive conscious rats and monkeys after oral dosing (Cloarec *et al.*, 1984; Bouley *et al.*, 1986), but also in man (Berdeaux *et al.*, 1988). Furthermore, tienoxolol binds to A₁ and A₂ adenosine receptors (previous unpublished observations).

Since the mechanism(s) underlying the diuretic and natriuretic effects of this compound is (are) not yet known, the aim of the present study was to investigate whether urinary prostaglandin excretion and binding to adenosine receptors could be involved in tienoxolol-induced diuresis and natriuresis.

Methods

Collection of urine and kidneys

Male CD rats (Charles River France) weighing 130–140 g were fasted overnight with free access to water. Tienoxolol (8–128 mg kg⁻¹), acebutolol (128 mg kg⁻¹), propranolol (128 mg kg⁻¹), furosemide (32 mg kg⁻¹) and theophylline (64 mg kg⁻¹) were administered orally (0.5 ml 100 g⁻¹ body weight).

All the animals received an intraperitoneal water load of 25 ml kg⁻¹, and were then housed in groups of two in metabolism cages. Six hours later, urine samples were collected with aspirin 11.5 mM and frozen until PGE₂ and 6-keto-PGF_{1 α} determination. Urine volume was measured in each metabolism cage.

Rats were also treated by oral co-administration of 5'-(N-ethylcarboxamido) adenosine (NECA) (0.2 mg kg⁻¹) with tienoxolol (128 mg kg⁻¹) or theophylline (64 mg kg⁻¹). At the end of the 6 h period, urine was collected either in the presence of aspirin 11.5 mM to determine prostaglandin levels or in the absence of aspirin to measure natriuresis by flame

Figure 1 Chemical structure of tienoxolol.

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photometry. Thirty min, 2 h, 4 h and 6 h after oral administration of tienoxolol (8, 32 and 128 mg kg⁻¹), rats were killed. Whole kidneys were quickly excised and frozen until PGE₂ and 6-keto-PGF_{1α} extraction.

Prostaglandin extraction

PGE₂ and 6-keto-PGF_{1α} extraction procedure was carried out as previously described (Powell, 1980) with slight modifications. Kidneys were thawed at room temperature and homogenized in 10 volumes (w/v) of ice-cold buffer (50 mM Tris HCl, 11.5 mM aspirin, pH 7.4). Homogenization was performed with a tissue disrupter Ultra-Turrax (Janke & Kunkel, Stauffen, Germany) for 30 s at 4°C (60–70% of the maximal speed), followed by a glass-teflon potter homogenizer (Prosciencs, Paris, France) (setting 6). Aliquots (1 ml) of the homogenate were mixed with absolute ethanol (3 ml) and the mixtures were allowed to stand for 5 min. Distilled water (16 ml) was then added and the mixtures were centrifuged at 480 g for 20 min at 4°C (centrifuge J2-21 M/E, Beckman, Gagny, France). The supernatant was removed, transferred to another tube, and the pH was adjusted to 3–4 with 0.1 N HCl (250 µl).

An octadecylsilyl silica column (Amersham, Les Ulis, France) was used for PGE₂ and 6-keto-PGF_{1α} extraction. The column was first conditioned with 20 ml absolute ethanol followed by 20 ml distilled water. The acidified supernatant was then introduced to the column and eluted sequentially with 20 ml ethanol/water (15:85), 20 ml petroleum ether and 5 ml methyl formate. The methyl formate fraction was evaporated under a stream of nitrogen and the dried extract was dissolved in 500 µl absolute ethanol and 500 µl distilled water. The dissolved extract was stored at –20°C until prostaglandin determination.

Since the use of unextracted urine samples has been validated by many authors (Granstrom & Kindahl, 1978; Quilley *et al.*, 1986), urinary PGE₂ and 6-keto-PGF_{1α} concentrations were measured in unextracted samples.

Prostaglandin radioimmunoassay

PGI₂ was measured with a radioimmunoassay kit for 6-keto-PGF_{1α}, the stable hydrolysis product of PGI₂. Briefly, 100 µl aliquots of urine or renal tissue extract, [¹²⁵I]-6-keto-PGF_{1α} (≈33000 c.p.m.), 6-keto-PGF_{1α} antiserum (cross-reactivity of less than 1% with PGE₂, PGF_{2α}, PGE₁, PGF_{1α}, TXB₂) and buffer (0.05 M phosphate buffer, pH 6.8, with 0.05% bovine serum albumin) were incubated in a total volume of 500 µl overnight at 4°C. Standards covered the range 3.1 pg up to 400 pg of 6-keto-PGF_{1α}. Bound and free antigen were separated by means of 500 µl of donkey anti-rabbit second antibody bound to magnetizable polymer particles. Then, separation was carried out by application of a magnetic field and the supernatant was removed. The radioactivity in the pellet was determined by solid scintillation spectroscopy using a Kontron Gamma Counter at a counting efficiency of 76%. Each assay was performed in duplicate.

PGE₂ was measured with a radioimmunoassay kit for methyl oximated PGE₂ obtained by conversion of PGE₂ by methoxamine hydrochloride. Following methyl oximation of PGE₂ contained in extracted or unextracted samples, the latter (100 µl) were incubated for 2 h at room temperature with [¹²⁵I]-PGE₂ (methyl oximate derivative) (≈35000 c.p.m.) and PGE₂ antiserum specific for the methyl oximate derivative (cross-reactivity of less than 1% with PGD₂, PGF_{2α}, 6-keto-PGF_{1α}, TXB₂), in a total volume of 300 µl. Standards covered the range 1.25 pg up to 160 pg of methyl oximated PGE₂. Separation of bound and free antigen was then achieved by using 250 µl of donkey anti-rabbit serum coated onto magnetizable polymer particles. The remainder of the experiment was similar to that described for 6-keto-PGF_{1α} radioimmunoassay.

Preparation of brain membranes for adenosine binding assays

To study A₁ adenosine receptors, crude rat brain membranes were prepared according to a previously described method (Schwabe & Trost, 1980). Male CD rats (Charles River France), weighing 180 to 280 g, were killed. Whole brains were quickly removed and homogenized in 25 volumes of ice-cold 50 mM Tris HCl, pH 7.4, using an Ultra-Turrax (30 s at 70% maximal setting). The homogenate was centrifuged at 1000 g for 10 min at 4°C and the resulting supernatant was centrifuged at 48000 g for 20 min at 4°C. The pellet was resuspended in 4 volumes of ice-cold homogenization buffer, incubated with adenosine deaminase (1 µg ml⁻¹) for 30 min at room temperature and centrifuged as before. The final pellet was resuspended in 10 volumes of ice-cold 50 mM Tris-HCl, pH 7.4, and membranes were used either immediately or after storage at –20°C.

A₂-adenosine receptors were studied in rat striatal membranes prepared as previously described (Bruns *et al.*, 1986). Briefly, striata were disrupted in 10 volumes of ice-cold buffer (50 mM Tris-HCl, 10 mM MgCl₂, pH 7.7) for 30 s with an Ultra-Turrax (70% maximal setting). The homogenate was centrifuged at 50000 g for 10 min at 4°C. The pellet was resuspended in 10 volumes of buffer, incubated with adenosine deaminase (1 µg ml⁻¹) for 30 min at room temperature and finally centrifuged as before. The resulting pellet was resuspended in 5 volumes of the same buffer and frozen at –70°C.

Radioligand binding assays

The measurement of (–)-N₆-R-[G-³H]-phenylisopropyl-adenosine ([³H]-PIA) binding to whole brain membranes was performed as described elsewhere (Schwabe & Trost, 1980; Lohse *et al.*, 1984) with slight modifications. Incubations (2 ml) were carried out at 20°C for 30 min in polystyrene tubes containing incubation buffer (50 mM Tris-HCl, pH 7.4), 400 µl of the membrane suspension, 2.5 nM [³H]-PIA and various concentrations of the competing drugs. The reaction was terminated by the addition of 3 ml of cold incubation buffer, followed by rapid filtration through Whatman GF/B glass fibre filters. The filters were immediately washed twice with 3 ml of ice-cold Tris-HCl buffer, transferred into liquid scintillation vials and 10 ml of scintillation fluid were added. Radioactivity was counted by liquid scintillation spectroscopy (Kontron Beta Counter) at a counting efficiency of 56%. Non specific binding was defined as that remaining in the presence of 10 µM N₆-(L-2-phenylisopropyl)-adenosine.

The binding of 5'-N-ethylcarboxamido [8(n)-³H]-adenosine ([³H]-NECA) to striatal membranes was achieved according to the method described by Bruns *et al.* (1986) with minor modifications. Membranes (100 µl) were incubated at 25°C during 1 h with 4 nM [³H]-NECA, various concentrations of the test compounds and incubation buffer (50 mM Tris-HCl, 10 mM MgCl₂, 0.117 µM N₆-cyclopentyladenosine (CPA), pH 7.7) in a total volume of 2 ml. CPA was included in the medium to eliminate A₁ receptor binding. The reaction was terminated by the addition of 3 ml of ice-cold buffer (50 mM Tris-HCl, 10 mM MgCl₂, pH 7.7) and filtration through Whatman GF/B glass fibre filters. The subsequent washing and counting procedures were as described above. Non specific binding was determined by measurement of the amount of radioactivity retained on filters when incubations were performed in the presence of 5 µM unlabelled NECA.

Data analysis

Data are shown as mean ± s.e.mean.

The one-way analysis of variance was used to identify statistically significant differences between prostaglandin levels in control and treated groups. When a significant difference was reached, comparisons were carried out with

Student's *t* test for unpaired data. A *P* value of 0.05 or less was considered to be significant.

Radioligand binding data were analysed by the non linear regression programme LIGAND (Munson & Rodbard, 1980), adapted for an IBM-PC (McPherson, 1985) and obtained from Elsevier-Biosoft (Cambridge, England). The concentration of unlabelled drug causing 50% displacement of [³H]-PIA or [³H]-NECA from binding sites (IC₅₀ value) was calculated by log-logit linear regression analysis of data (EBDA). Then, the latter were analysed assuming models of one and two independent binding sites. A two-site model was accepted over a one-site fit only if it was preferred *P* < 0.05 using the partial *F*-test of the programme. Inhibition constant value (*K_i*) was calculated according to the Cheng-Prusoff equation (1973). The average *K_i* values were determined from two to four values obtained from independent experiments carried out with different whole brain or striatal membrane preparations.

Materials

Propranolol, PIA, NECA, CPA and adenosine deaminase type VI were obtained from Sigma Chemical Co. (St Louis, MO, U.S.A.). [³H]-PIA (35–60 Ci mm⁻¹), [³H]-NECA (20–40 Ci mm⁻¹), [¹²⁵I]-6-keto-prostaglandin F_{1α} and [¹²⁵I]-prostaglandin E₂ radioimmunoassay kits were purchased from Amersham International (Amersham, England).

In all *in vivo* studies, tienoxolol (hydrochloride salt, Carpiem, Rueil-Malmaison, France), furosemide (Hoechst, Paris-La Défense, France), acebutolol (Rhône-Poulenc Santé, Vitry, France), propranolol, theophylline (Coopération Pharmaceutique Française, Melun, France) and NECA were prepared as suspensions in aqueous solution (0.1% sodium chloride, 1% arabic gum and 0.01% Tween 80). In radioligand binding studies, drugs were dissolved in dimethylsulphoxide/ethanol (DMSO)/(EtOH) (80/20, v/v).

Aspirin (115 mM) was prepared in a 5% sodium bicarbonate solution then diluted in 50 mM Tris-HCl buffer, pH 7.4, in order to obtain 11.5 mM (final concentration).

Absolute ethanol, petroleum ether and methyl formate were supplied by Prolabo (Paris, France).

Results

Effects of tienoxolol on urinary PGE₂ and 6-keto-PGF_{1α} excretion

The effects of various single oral doses of tienoxolol relative to a vehicle control group on urinary PGE₂ and 6-keto-PGF_{1α} levels are shown in Figure 2. Tienoxolol induced a dose-related increase of urinary 6-keto-PGF_{1α} concentration which was statistically significant from 32 mg kg⁻¹ (+100%, *P* < 0.001). At 64 mg kg⁻¹ and 128 mg kg⁻¹, urinary 6-keto-PGF_{1α} excretion increase was 200% and 284%, respectively. On the other hand, PGE₂ excretion was enhanced only at the highest dose (+115%, *P* < 0.01).

Effects of tienoxolol on PGE₂ and 6-keto-PGF_{1α} concentrations in the kidney

The effects of various single oral doses of tienoxolol, at different times, on renal tissue PGE₂ and 6-keto-PGF_{1α} levels are shown in Figure 3. Tienoxolol did not affect prostaglandin concentrations in the kidney.

Comparative effects of tienoxolol, theophylline, furosemide and β-blocking agents on urinary PGE₂ and 6-keto-PGF_{1α} excretion

Urinary 6-keto-PGF_{1α} excretion was weakly enhanced by furosemide (+23%) and propranolol (+39%), while theophylline (+77%, *P* < 0.01) produced significant effects on

Figure 2 Effects of tienoxolol on urinary prostaglandin E₂ (PGE₂) and 6-keto-PGF_{1α} excretion. Tienoxolol was given by gavage at doses ranging from 8 to 128 mg kg⁻¹. Results represent the accumulation of PGE₂ (horizontally lined columns) and 6-keto-PGF_{1α} (solid columns) in urine at the end of a 6 h period. Values are expressed as mean ± s.e.mean from 6 metabolites cages for PGE₂; from 6 cages (16 and 64 mg kg⁻¹), 12 cages (8 and 32 mg kg⁻¹) and 18 cages (128 mg kg⁻¹ and vehicle) for 6-keto-PGF_{1α}. ***P* < 0.001, ****P* < 0.001 as compared to control group, Student's *t* test using residual variance.

Figure 3 Effects of tienoxolol on prostaglandin E₂ (PGE₂) and 6-keto-PGF_{1α} concentrations in the kidney. Results represent the renal tissue levels of PGE₂ and 6-keto-PGF_{1α}, 30 min, 2 h, 4 h or 6 h after oral administration of tienoxolol (8, 32 and 128 mg kg⁻¹) or vehicle. Values are expressed as mean ± s.e.mean from 6 rats (time 30 min) or 12 rats (other times).

6-keto-PGF_{1α} excretion (Figure 4). The most marked effect was observed with tienoxolol, whilst acebutolol was completely inactive.

Since tienoxolol and theophylline were able to stimulate 6-keto-PGF_{1α} excretion, the effects of both compounds were investigated on PGE₂ excretion. The latter was markedly increased by theophylline (+269%, *P* < 0.001) as compared to tienoxolol (+115%, *P* < 0.01) (Figure 4). Theophylline produced much more pronounced effects on urinary excretion of PGE₂ than of 6-keto-PGF_{1α}, whereas the reverse was observed with tienoxolol.

Affinity of tienoxolol for A₁ and A₂ adenosine receptors in rat brain homogenates

Tienoxolol and theophylline bound to A₁ and A₂ adenosine receptors, with some selectivity for A₁ binding sites (Table 1). The two compounds exhibited similar affinities on both receptor subtypes although a slightly greater selectivity was observed with tienoxolol (*K_iA₂/K_iA₁* = 8) than for theophylline (*K_iA₂/K_iA₁* = 5). Acebutolol bound to A₁ binding sites with a very modest affinity (*K_i* = 165 ± 43 μM), while propranolol and furosemide did not displace [³H]-PIA and [³H]-NECA binding.

Figure 4 Comparative effects of oral (b) tienoxolol (128 mg kg⁻¹), (c) theophylline (64 mg kg⁻¹), (d) furosemide (32 mg kg⁻¹), (e) propranolol (128 mg kg⁻¹) and (f) acebutolol (128 mg kg⁻¹) on urinary prostaglandin E₂ (PGE₂) and 6-keto-PGF_{1α} excretion; (a) = control. Results represent the accumulation of PGE₂ (horizontally lined columns) and 6-keto-PGF_{1α} (solid columns) in urine at the end of a 6 h period. Values are expressed as mean ± s.e.mean (vertical bars) from 6 metabolism cages for PGE₂, from 5 cages (propranolol), 6 cages (acebutolol), 12 cages (furosemide and theophylline) and 18 cages (tienoxolol and control) for 6-keto-PGF_{1α} excretion. The effects of acebutolol, propranolol and furosemide were not investigated on urinary PGE₂ excretion. ***P* < 0.01, ****P* < 0.001 as compared to control group, Student's *t* test using residual variance.

Table 1 Affinity for A₁ and A₂ adenosine receptors of diuretics, β-blockers and theophylline in rat brain homogenate

	Inhibition of [³ H]-PIA binding A ₁ receptor K _i (μM)	Inhibition of [³ H]-NECA binding A ₂ receptor K _i (μM)
Tienoxolol	2.64 ± 0.07	20.49 ± 1.67
Theophylline	5.55 ± 0.34	30.50 ± 0.37
Acebutolol	165 ± 43	> 1000
Propranolol	> 1000	> 1000
Furosemide	> 1000	> 1000

The K_i values represent mean ± s.e.mean from 2 to 4 values obtained from independent experiments carried out on each drug with different brain membrane preparations. In a given experiment, K_i values were determined from at least 6 concentrations of each compound as triplicate incubations. Propranolol and furosemide were tested only at 1 mM on both receptor subtypes.

Effects of simultaneous administration of NECA with tienoxolol and theophylline on urinary PGE₂ and 6-keto-PGF_{1α} excretion

Co-administration of vehicle with tienoxolol led to a marked increase in 6-keto-PGF_{1α} levels (19.3 ± 1.9 ng 6h⁻¹, *P* < 0.001) which was comparable with that measured when tienoxolol

Figure 5 Effects of oral co-administration of 5'-(N-ethylcarboxamido)adenosine (NECA, 0.2 mg kg⁻¹) with tienoxolol (128 mg kg⁻¹) (b), theophylline (64 mg kg⁻¹) (c) or vehicle (control) (a) on urinary prostaglandin E₂ (PGE₂) and 6-keto-PGF_{1α} excretion. In the absence of NECA (open columns for both PGE₂ and 6-keto-PGF_{1α}), rats received orally first the vehicle, then the drug (tienoxolol or theophylline) (vehicle-drug group), or two successive administrations of vehicle (control). In the presence of NECA (horizontally lined columns for PGE₂, solid columns for 6-keto-PGF_{1α}), animals received orally first NECA, then the drug (tienoxolol or theophylline) (NECA-drug group), or NECA followed by the vehicle (control). Results represent the accumulation of PGE₂ and 6-keto-PGF_{1α} in urine at the end of a 6 h period. Values are expressed as mean ± s.e.mean from 6 metabolism cages. ****P* < 0.001 indicates a statistically significant difference between the NECA-drug group value and the corresponding vehicle-drug group value.

was administered alone (11.9 ± 0.8 ng 6h⁻¹, *P* < 0.001). Likewise, theophylline given orally with vehicle induced similar effects to that observed when the drug was administered alone, although increase of urinary 6-keto-PGF_{1α} excretion was not statistically significant after co-treatment with theophylline and vehicle (Figure 5).

A very strong and highly significant PGE₂ urinary excretion was induced by simultaneous administration of vehicle with theophylline (+ 388%, *P* < 0.01). Urinary PGE₂ levels were also enhanced by tienoxolol but to a lesser degree (+ 156%). This result was similar to that obtained when tienoxolol was given alone by mouth (+ 115%). In the presence of NECA, tienoxolol-induced urinary 6-keto-PGF_{1α} excretion was dramatically reduced (– 72%, *P* < 0.001) while PGE₂ was only slightly reduced (– 31%). On the other hand, NECA impaired more significantly excretion of PGE₂ induced by theophylline (– 36%) than that of 6-keto-PGF_{1α} (– 11%) (Figure 5).

Effects of simultaneous administration of NECA with tienoxolol and theophylline on diuresis and natriuresis

Diuresis and natriuresis were both increased after simultaneous oral administration of tienoxolol and theophylline with vehicle. When the latter was replaced by NECA, tienoxolol and theophylline-induced diuresis and natriuresis remained unchanged (Table 2).

Discussion

It is generally recognized that urine PGE₂ is mostly of renal origin (renal medulla) (Frölich *et al.*, 1975) while urinary

Table 2 Effects of oral co-administration of 5'-(N-ethylcarboxamido)adenosine (NECA) with tienoxolol, theophylline and vehicle on diuresis and natriuresis

		Control	Tienoxolol (128 mg kg ⁻¹)	Theophylline (64 mg kg ⁻¹)
Diuresis ml 6 h ⁻¹	Vehicle	8.8 ± 0.4	14.5 ± 0.7	10.6 ± 0.7
	NECA (0.2 mg kg ⁻¹)	7.7 ± 0.5	16.1 ± 0.8	10.2 ± 0.5
Natriuresis mEq 6 h ⁻¹	Vehicle	0.10 ± 0.03	0.76 ± 0.10	0.83 ± 0.11
	NECA (0.2 mg kg ⁻¹)	0.02 ± 0.01	0.72 ± 0.07	0.86 ± 0.10

Drugs were given simultaneously with vehicle or NECA, and diuresis and natriuresis were determined at the end of a 6 h period. Values are expressed as mean ± s.e.mean from 9 metabolism cages (diuresis) or 3 cages (natriuresis).

excretion of 6-keto-PGF_{1α}, the stable metabolite of prostacyclin, could reflect not only renal synthesis of PGI₂ (renal cortex) but also its own arterial blood levels or those of PGI₂ (Rosenkranz *et al.*, 1981; Zipser & Martin, 1982).

Tienoxolol stimulates urinary 6-keto-PGF_{1α} excretion at doses similar to those required for diuresis and natriuresis (Cloarec *et al.*, 1984), whereas PGE₂ levels are significantly enhanced only at the higher doses. Theophylline also causes diuresis and natriuresis accompanied by more pronounced effects on urinary excretion of PGE₂ than of 6-keto-PGF_{1α}. Since PGI₂ and PGE₂ are diuretic and natriuretic (Anderson *et al.*, 1976; Bolger *et al.*, 1978), it is tempting to speculate that both prostaglandins are involved in tienoxolol and theophylline-induced diuresis and natriuresis. Furthermore, as the principal renal sites of PGI₂ and PGE₂ synthesis are not the same, the differing effects of the two compounds on prostaglandin excretion suggest that their renal distribution is different. Tienoxolol may be mainly distributed in the cortex and also at the vascular level, but poorly in the medulla, which could stimulate 6-keto-PGF_{1α} excretion. On the other hand, theophylline may be mainly distributed in the medulla and could activate PGE₂ excretion. The different physical molecular characteristics and pharmacokinetic properties of tienoxolol and theophylline may explain such a tissue distribution.

Surprisingly, tienoxolol does not change prostaglandin levels in the kidney which seems to be inconsistent with data obtained in urine. This apparent discrepancy could be explained by the fact that an accumulation of prostaglandins is measured in urine, while 6-keto-PGF_{1α} and PGE₂ levels are determined only at a given time in the renal tissue. Moreover, prostaglandin extraction is performed from whole kidney so that a slight, but significant, rise of 6-keto-PGF_{1α} and/or PGE₂ in a well defined renal area could be masked. Therefore, it would be of a great interest to evaluate the effects of tienoxolol on 6-keto-PGF_{1α} and PGE₂ concentrations in the cortical and medullary tissues.

Propranolol induces a weak diuresis (+23% at 128 mg kg⁻¹) as compared to tienoxolol (+90% at 128 mg kg⁻¹), but does not stimulate 6-keto-PGF_{1α} excretion in urine. Acebutolol (128 mg kg⁻¹) modifies neither diuresis nor urinary excretion of prostaglandins. Consequently, in this study, tienoxolol is the only β-adrenoceptor antagonist able to promote both diuresis and prostaglandin excretion.

Furosemide (32 mg kg⁻¹) produces a marked diuresis but does not change the urinary excretion of 6-keto-PGF_{1α}. Although furosemide markedly enhances urinary prostaglandin excretion both in animals and man (Abe *et al.*, 1977; Oliw & Anggård, 1979; Patak *et al.*, 1979), results obtained in this study corroborate those of other authors (Halushka *et al.*, 1979; Tan & Mulrow, 1977) showing urinary prostaglandin excretion to remain unchanged when long collection periods are used. Prostaglandin excretion is maximal within 15 to 40 min returning to control excretion rates 1 to 2 h following either oral or intravenous doses of furosemide (Scherer & Weber, 1979; Wilson *et al.*, 1982), this gives an unchanged net excretion rate when urine is collected for more than 2 h. These data confirm that factors such as urinary volume are able to affect prostaglandin excretion (Kirschenbaum & Serros, 1980) and, on the other hand, suggest that the time course of urinary prostaglandin excretion induced by tienoxolol could be different from that of furosemide. Furthermore, the latter is a much more potent diuretic agent than tienoxolol so that urinary volume probably does not play a role as important as with furosemide.

Inasmuch as tienoxolol and theophylline bind to A₁ and A₂ adenosine receptors with similar affinities, and although the radioligand binding studies were not performed in the kidney but in the brain, it is proposed that P₁-purinoceptors could be involved in prostaglandin release which could then contribute to diuresis and natriuresis induced by both drugs. This assumption is supported by the fact that NECA, a potent adenosine receptor agonist which affects neither pros-

taglandin excretion nor diuresis and natriuresis at the studied dose (0.2 mg kg⁻¹), markedly reduces tienoxolol-stimulated urinary 6-keto-PGF_{1α} excretion while exhibiting only a moderate effect on urinary PGE₂. Likewise, a decrease, though not significant, of PGE₂ level in urine is observed after co-administration of NECA and theophylline. Considering that tienoxolol and theophylline may have a preferential renal tissue distribution, it is possible that NECA may impair tienoxolol-induced 6-keto-PGF_{1α} excretion in the cortex and theophylline-induced PGE₂ release in the medulla. In other words, NECA may be distributed in both renal areas and compete with each of these drugs for common binding sites, namely adenosine receptors.

However, the impairment of 6-keto-PGF_{1α} and PGE₂ excretion does not modify the diuretic and natriuretic activities of tienoxolol and theophylline. Therefore, prostacyclin and PGE₂ probably do not participate in the diuresis and natriuresis observed. Interestingly, enprofylline, a xanthine derivative with a low affinity for P₁-purinoceptors (Schwabe *et al.*, 1985; Bruns *et al.*, 1986), stimulates prostaglandin excretion at doses that have no effect on urine volume or electrolyte (Takeuchi *et al.*, 1981; Baer *et al.*, 1983). Taken together, these data suggest that adenosine receptor inhibition mediates the prostaglandin excretion observed with tienoxolol and xanthines, but this excretion appears to be unrelated to diuresis and natriuresis. Regarding the effects of tienoxolol on 6-keto-PGF_{1α} excretion, P₂-purinoceptors should also be considered in this pharmacological response since they were shown to modulate prostaglandin formation by Burnstock (1978) and Carter *et al.*, (1988). As suggested above, tienoxolol-induced urinary 6-keto-PGF_{1α} excretion may result from an action of the drug at the vascular level. Prostacyclin is the major prostaglandin produced by most blood vessels and the stimulation of its release from aortic endothelial cells of porcine (Pearson *et al.*, 1983) or bovine (Van Coevorden & Boeynaems, 1984) origin can be obtained by the activation of P_{2Y} receptors (Needham *et al.*, 1987). Furthermore, PGI₂ release may also result from an activation of nucleotide receptors located on smooth muscle cells (Demolle *et al.*, 1988; O'Connor, 1992) and recent studies have demonstrated the presence of P_{2Y}-purinoceptors in the rat renal cortex (Nanoff *et al.*, 1990).

Finally, the increase of PGI₂ biosynthesis induced by different antihypertensive agents could also result initially from the reduction of arterial blood pressure (Frölich, 1990). Such a reduction was previously described following tienoxolol administration in man (Coupez *et al.*, 1989). Moreover, the increase of urinary 6-keto-PGF_{1α} excretion observed after treatment with diuretics may be mainly related to the increased urinary flow (Frölich *et al.*, 1985). It is conceivable therefore that tienoxolol may stimulate 6-keto-PGF_{1α} excretion by these two mechanisms.

To summarize, the hypothesis of a relationship between P₁-purinoceptors, prostaglandin release, diuresis and natriuresis appears to be untenable. A causal role of prostaglandins in the mechanism underlying the diuretic and natriuretic effects of tienoxolol is unlikely, while adenosine receptor inhibition may play a role. In this respect, A₂ adenosine receptors may represent the site of action for the diuretic effects of methylxanthines such as theophylline and caffeine (Freissmuth *et al.*, 1987). The pharmacological significance of urinary 6-keto-PGF_{1α} and PGE₂ excretion evoked by tienoxolol remains to be clarified as does the tissue origin of PGI₂ and the mechanism of its production. However, data obtained in the renal tissue seem to indicate that this enhanced 6-keto-PGF_{1α} is of prerenal origin.

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