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Prostaglandin PGE_2 and $PGF_{2\alpha}$ levels in nasal secretions from healthy subjects by liquid chromatography and radioimmunological assay*

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Introduction

Prostaglandins (PGs) are involved in the process of allergic rhinitis [1, 2]. Recent studies have focused on the variation of PGD_2 levels in nasal secretions with regard to other chemical mediators, such as histamine and leukotriene C_4, after nasal airway challenge with an allergen [2–4]. In all cases, PG measurements were carried out using only direct RIA techniques [1, 4].

This paper describes the application of the combined HPLC–RIA methodology for quantitative prostaglandin E_2 (PGE_2) and prostaglandin $F_{2\alpha}$ profiling studies in nasal secretions of healthy subjects.

Experimental

Subjects

Ten healthy male and female volunteers (age 28–40 years) were studied. Individuals did not have a history of rhinitis, asthma or allergy and none had taken any anti-inflammatory drug during the previous four weeks.

Nasal washes

With the head tilted slightly backwards 5 ml of saline were instilled in one nostril. After 5–10 s, the subjects expelled nasal secretions and the mixture was stored on an ice-bath until assay. Aspirin (500 mg) was immediately administered to each subject and a second nasal lavage was carried out 1 h later.

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Extraction of PGs

For extraction of PGs from nasal secretions a modification of Powell's method was used [5]. After centrifugation (1500 **g**) at 4°C for 10 min, 2.5 ml aliquots of nasal secretion were adjusted to pH = 3.15 with HCl 0.1 M and passed through a reversed-phase octadecylsilica C18 Sep-Pak cartridge (Waters Ass., Mildford, MA, USA). The cartridge was washed with water (10 ml) and petroleum ether (20 ml) and finally the PGs eluted with methyl formate (7 ml).

PG residues obtained after evaporation of the eluates to dryness under a helium stream were redissolved in 200 μl of acetonitrile–water (35:65, v/v) adjusted to pH 3.4 with acetic acid for subsequent HPLC analysis.

HPLC–RIA analyses of PGs

Sample enrichment for RIA determinations was carried out on a 30 cm × 3.9 mm i.d. μ-Bondapak C18 reversed-phase column (Waters Ass., Milford, MA, USA) eluted isocratically at 1.5 ml min^{-1} with acetonitrile–water (35:65, v/v) adjusted to pH = 3.4. For this purpose two model 414 pumps (Kontron Analytical, Zurich, Switzerland) coupled to a model 200 gradient controller were used to deliver the mobile phases.

Biological samples (100 μl) were injected through a Rheodyne 7125 and the eluent fractions were collected at the authentic retention times previously established for $^3HPGE_2$ (160 Ci $mmol^{-1}$) and $^3HPGF_{2\alpha}$ (180 Ci $mmol^{-1}$) by the use of RayTest Ramona (Isomess, Straubenhardt, FRG) radioactivity detector, directly coupled to the HPLC system. Finally eluates were lyophilized and redissolved in Tris-buffer for radioimmunological assay [6]. RIA determinations in duplicate were carried out using antiserum to PGE_2 and $PGF_{2\alpha}$ commercially available from the Pasteur Institute.

Results and Discussion

Figure 1 shows the HPLC separation obtained with tritiated 6-oxo-prostaglandin $F_{1\alpha}$ (6-oxo-$PGF_{1\alpha}$), $PGF_{2\alpha}$, PGE_2 and PGD_2, which is accomplished in 15 min. The use of acetonitrile–aqueous acetic acid as mobile phase facilitates the lyophilization of the

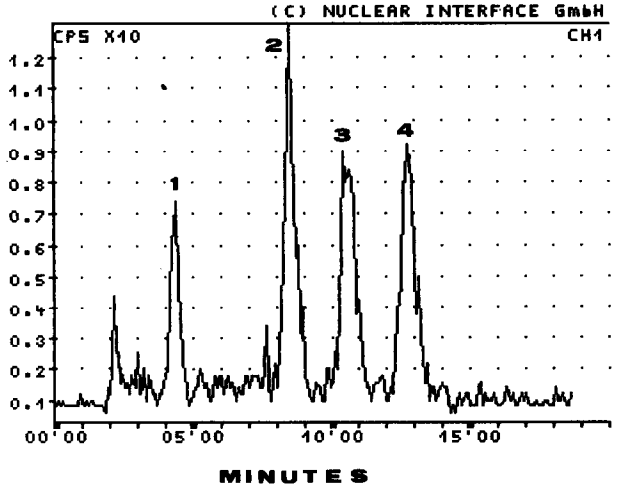

Figure 1
HPLC profile obtained with tritiated 6-oxo $PGF_{1\alpha}$ (1), $PGF_{2\alpha}$ (2), PGE_2 (3) and PGD_2 (4).

collected eluates, avoiding salt precipitation problems. Lyophilizability of the buffer is a necessary condition when HPLC is followed by RIA.

Recoveries of tritiated PGE_2 and $PGF_{2\alpha}$ after Sep-Pak C18 extraction and HPLC separation are shown in Table 1. RIA values of PGE_2 and $PGF_{2\alpha}$ determined in nasal secretions from 10 normal subjects were corrected in accordance to individual recoveries of 84.8 and 87.9%, respectively.

Table 2 illustrates the PGE_2 and $PGF_{2\alpha}$ levels found in nasal secretions from healthy subjects before and after a 500 mg aspirin dose administration. An important inhibitory effect of aspirin on the PG released by nasal mucosa was evident. PG decreases were found to be statistically significant in all cases. This observation confirms the reliability of the PG basal levels found [7].

In conclusion, solid phase extraction combined with HPLC–RIA techniques described here, provides a specific, sensitive and accurate method for the analyses of PGE_2 and $PGF_{2\alpha}$ in nasal secretions. Biological importance of PGS dealing with nasal physiology in health will be addressed elsewhere.

Table 1
PG recoveries (%) after Sep-Pak extractions and HPLC separation

	Sep-Pak	HPLC	Sep-Pak + HPLC	*N*
$PGF_{2\alpha}$	91.9 ± 2.0	95.9 ± 1.9	87.9 ± 2.3	9
PGE_2	90.1 ± 2.7	93.7 ± 3.98	84.8 ± 3.5	11

Table 2
$PGF_{2\alpha}$ and PGE_2 levels (pg ml^{-1}) in nasal secretions from healthy subjects

Sex	Age	$PGF_{2\alpha}$ basal	Aspirin	PGE_2 basal	Aspirin
M	28	216	52	68	32
F	30	101	35	93	19
F	35	67	26	55	85
F	30	85	30	89	56
M	38	94	35	42	20
M	40	77	49	70	30
F	33	120	57	95	27
F	29	21	9	15	9
M	32	28	26	19	22
F	28	50	21	87	26
$\bar{X}$ ± DS		85.9 ± 55.4*	34.0 ± 14.9	63.3 ± 29.8**	32.6 ± 22.0

*Basal versus aspirin $P < 0.005$, *t* matched pairs.
**Basal versus aspirin $P < 0.01$, *t* matched pairs.

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