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Prostaglandin $F_{2\alpha}$ Receptor in Ovine *corpora lutea*

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Evidence is presented for the existence of a prostaglandin-$F_{2\alpha}$ receptor in ovine *corpora lutea*. Prostaglandin $F_{2\alpha}$ was specifically bound to a particulate fraction of this tissue with a dissociation constant of 0.1 μM. Prostaglandins $F_{1\alpha}$, E_1 and E_2 had considerably less affinity for the receptor whereas prostaglandins A_2 and B_2 had little or no affinity. From a comparison of the binding constants of various prostaglandins and related compounds it could be concluded that the carboxyl group, the three hydroxyl groups and the 5,6-*cis* double bond (but not the 13,14-*trans* double bond) of prostaglandin $F_{2\alpha}$ were all important for binding to the receptor.

The uterus is required for normal cyclic regression of *corpora lutea* in a number of mammalian species [1, 2]. Pharriss and Wyngarden first postulated that prostaglandin $F_{2\alpha}$ was responsible for this phenomenon [3] and subsequent experiments have shown that this prostaglandin is luteolytic in several animals [2]. Conclusive evidence was recently presented that prostaglandin $F_{2\alpha}$ is a luteolytic hormone in the sheep [4]; it was released in relatively high concentrations from the uterus at the time of luteal regression and was transferred from the utero-ovarian vein to the ovarian artery by a counter-current mechanism [4].

The mechanism of the luteolytic effect of prostaglandin $F_{2\alpha}$ is unknown. It has been proposed that prostaglandin $F_{2\alpha}$ acts luteolytically by restricting the blood flow through the ovary [3], but subsequent experiments [5, 6] have not substantiated this hypothesis. Prostaglandin $F_{2\alpha}$ has been found to depress cholesterol ester synthetase activity in rat ovaries and it was suggested [7] that this might be the mechanism for the reduction in progesterone synthesis caused by prostaglandin $F_{2\alpha}$ *in vivo* [2–4]. In bovine *corpora latea* slices prostaglandins E_1, E_2, $F_{2\alpha}$ and A_1 (decreasing order of effectiveness) stimulated rather than inhibited progesterone biosynthesis [8]. However, it was recently reported that prostaglandin $F_{2\alpha}$ decreased the synthesis of progesterone by sections of rabbit *corpora lutea* in a modified organ culture [9].

It seemed probable that an early event in the luteolytic action of prostaglandin $F_{2\alpha}$ in the sheep would be interaction with a receptor in the *corpus luteum*. We now wish to report evidence for the existence of a prostaglandin $F_{2\alpha}$-specific receptor in ovine *corpora lutea*.

Abbreviation. Me_3Si, trimethylsilyl.

Trivial Names. Prostaglandin $F_{1\alpha}$, 9α,11α,15(*S*)-trihydroxyprost-13-*trans*-enoic acid; prostaglandin $F_{2\alpha}$, 9α,11α,15(*S*)-trihydroxyprosta-5-*cis*,13-*trans*-dienoic acid; prostaglandin $F_{2\beta}$, 9β,11α-15(*S*)-trihydroxyprosta-5-*cis*,13-*trans*-dienoic acid; prostaglandin E_1, 11α,15-(*S*)-dihydroxy-9-ketoprost-13-*trans*-enoic acid; prostaglandin E_2, 11α,15(*S*)-dihydroxy-9-ketoprosta-5-*cis*,13-*trans*-dienoic acid; prostaglandin A_1, 15(*S*)-hydroxy-9-ketoprosta-10,13-*trans*-dienoic acid; prostaglandin A_2, 15(*S*)-hydroxy-9-ketoprosta-5-*cis*,-10,13-*trans*-trienoic acid; prostaglandin B_2, 15(*S*)-hydroxy-9-ketoprosta-5-*cis*,8(12),13-*trans*-trienoic acid.

MATERIALS AND METHODS

Ovaries were collected from 1 to 5-year-old sheep on the island of Gotland during October and November and were frozen within 1 h of slaughter. Prostaglandins were obtained from Dr J. Pike of the Upjohn Company (Kalamazoo, Mich.) unless otherwise mentioned. Protein was determined by the method of Lowry *et al.* [10].

Prostaglandin $F_{2\beta}$

A mixture of prostaglandin $F_{2\alpha}$ + prostaglandin $F_{2\beta}$ was prepared by reduction of prostaglandin E_2 with $NaBH_4$ [11] and the pure β-epimer was isolated by preparative thin-layer chromatography [12]. Analysis of the methyl ester, 9,11,15-tris(*O*-Me_3Si) derivative by gas-liquid chromatography-mass spectrometry (LKB 9000) gave a *C*-value of 23.5 on SE-30 [11] and a mass spectrum which was similar to that of the 9α-epimer (*cf.* [13]).

1,9α,11α,15(S)-Tetrahydroxyprosta-5-cis,13-trans-diene

Prostaglandin $F_{2\alpha}$ (5 mg) was treated with diazomethane and then dissolved in 30 ml dry diethyl

ether. 30 mg $LiAlH_4$ was added and the suspension was heated under reflux for 2 h. After addition of ethyl acetate and water, the mixture was acidified to pH 3 and extracted with diethyl ether. The product was purified by preparative thin-layer chromatography with benzene—dioxane—acetic acid (25:15:1, v/v/v) as the solvent system ($R_F = 0.23$; yield, 4 mg). Gas-liquid chromatography-mass spectrometry of the 1,9,11,15-tetrakis(*O*-Me_3Si) derivative gave a *C*-value of 24.3 (SE-30) and major ions at *m/e* 628 (*M*), 613 (*M*-15; loss of $\cdot CH_3$), 557 (*M*-71; loss of $\cdot (CH_2)_4CH_3$), 538 (*M*-90; loss of Me_3SiOH), 467 (*M*-(90+71); loss of $Me_3SiOH + \cdot (CH_2)_4CH_3$), 448 (*M*-2×90; loss of 2 Me_3SiOH), 441, 377 (*M*-(2×90+71), loss of 2 Me_3SiOH plus $\cdot (CH_2)_4CH_3$), 361, 358, 353, 352, 351, 339, 332, 327, 287, 261, 243, 237, 217 ($[Me_3SiO{=}CH{-}CH{=}CH{-}OMe_3Si]^+$), 199, 197, 191 ($[Me_3SiO{=}CH{-}OMe_3Si]^+$), 173 ($[Me_3SiO{=}CH(CH_2)_4CH_3]^+$) and 129.

Table 1. *Dissociation constants for the binding of prostaglandins and related compounds to a particulate fraction from ovine* corpora lutea

Prostaglandin or related compound	Amount added	K_d
	ng	μM
Prostaglandin $F_{1\alpha}$	500	2.0
Prostaglandin $F_{2\alpha}$	—	0.10
Prostaglandin $F_{2\beta}$	500	7.3
1,9α-11α,15(*S*)-Tetrahydroxy-prosta-5-*cis*,13-*trans*-diene	5000	38
13,14-Dihydroprostaglandin $F_{2\alpha}$	50	0.23
13,14-Dihydro-15-ketoprostaglandin $F_{2\alpha}$	1000	11
15-Methyl prostaglandin $F_{2\alpha}$	20	0.18
Prostaglandin E_1	1000	34
8-*Iso*prostaglandin E_1	5000	260
Prostaglandin E_2	500	2.7
Prostaglandin A_2		>260
Prostaglandin B_2		>260

Preparation of a Particulate Fraction with Prostaglandin-$F_{2\alpha}$-Binding Activity

Ovine *corpora lutea* were dissected free from surrounding ovarian tissue and minced in 2.5 volumes of Tris-saline buffer (0.01 M Tris-HCl, pH 7.5, 0.15 M NaCl). The mixture was homogenized using a Potter-Elvehjem homogenizer and centrifuged at $1000 \times g$ for 20 min. The supernatant was strained through cheesecloth and recentrifuged at $100000 \times g$ for 60 min. The pellet was resuspended in 0.8 volumes (ml/g *corpora lutea*) of Tris-saline buffer with a Potter-Elvehjem homogenizer. This suspension was used for the binding experiments.

Binding Assay

The resuspended particulate fraction (0.2 ml) was added to a solution of 2—20 ng of [9β-^{3}H]-prostaglandin $F_{2\alpha}$ (1 Ci/mmol) in Tris-saline buffer (10 μl) plus either ethanol (7 μl) or an unlabeled compound dissolved in ethanol (7 μl). This amount of ethanol reduced the binding of [9β-^{3}H]prostaglandin $F_{2\alpha}$ by 10—15%. The mixture was incubated for 40 min at 23 °C unless otherwise mentioned, diluted with buffer (0.2 ml) and applied to a 2-ml column of Sephadex G-50 fine. After the level of the suspension had fallen below the surface of the Sephadex, buffer (0.85 ml) was added and the eluate (bound prostaglandin $F_{2\alpha}$) was collected in a Packard vial. Unbound prostaglandin $F_{2\alpha}$ was then eluted from the column with a further 3.5 ml buffer. The radioactivity in the first and second fractions was determined with a liquid scintillation spectrometer (Packard Tri-Carb, model 3385) after the addition of Instagel® (10 ml). All values were corrected for non-specific binding, which was determined by measuring the binding of [9β-^{3}H]-prostaglandin $F_{2\alpha}$ in the presence of an excess (1 or 5 μg) of unlabeled prostaglandin $F_{2\alpha}$.

Determination of Dissociation Constants

In order to determine the dissociation constants for the binding of prostaglandin $F_{2\alpha}$, [9β-^{3}H]prostaglandin $F_{2\alpha}$ (2, 4, 10 and 20 ng) was incubated with the particulate fraction as described above. Dissociation constants for other prostaglandins and related compounds were determined from Scatchard plots [14] of the data obtained by incubating the above amounts of [9β-^{3}H]prostaglandin $F_{2\alpha}$ together with a fixed amount of the unlabeled compound with the particulate fraction. The amount of unlabeled compound added in each case is given in Table 1. All experiments were carried out in triplicate.

Large-Scale Separation of Bound and Free Prostaglandin $F_{2\alpha}$

[9β-^{3}H]Prostaglandin $F_{2\alpha}$ (400 ng) was incubated with the resuspended particulate fraction (5 ml; 22 mg protein/ml) for 40 min at 23 °C. After the addition of Tris-saline buffer (5 ml) the suspension was applied to a column (14×2.1 cm) of Sephadex G-50 fine. The column was eluted with the same buffer. Fractions of 1.6 ml were collected and the radioactivity of aliquots (0.1 ml) of every second fraction was determined (Fig. 1). Fractions 13—22 (bound prostaglandin $F_{2\alpha}$) and 29—45 (free prostaglandin $F_{2\alpha}$) were combined separately, diluted with 5 volumes of ethanol, filtered and concentrated *in vacuo* to about 10 ml. Water (40 ml) and unlabeled prostaglandin $F_{2\alpha}$ (10 μg) were then added to each solution. After acidification to pH 3 prostaglandins were extracted with diethyl ether

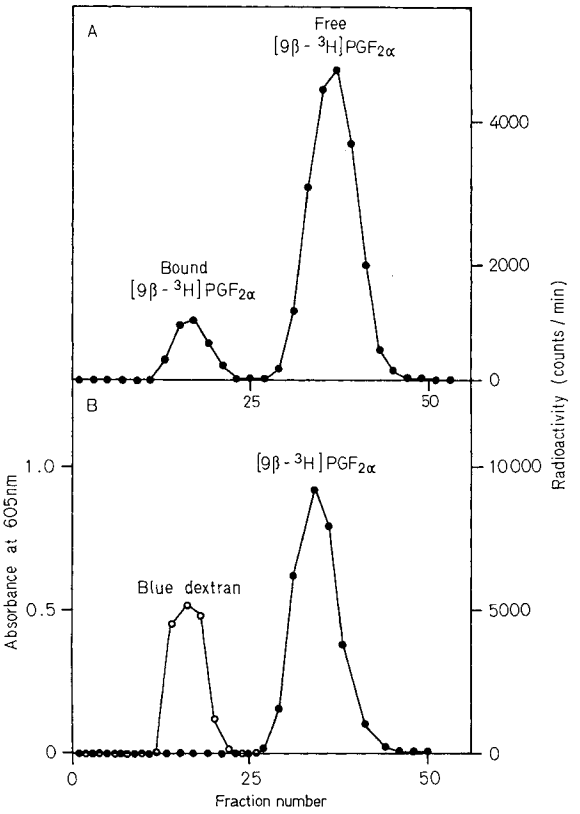

Fig. 1. *Separation of bound and free [9β-³H]prostaglandin $F_{2\alpha}$ (A) and blue dextran and [9β-³H]prostaglandin $F_{2\alpha}$ (B).* A 50-ml column of Sephadex G-50 fine was used for the chromatography as described in the text. Fractions of 1.6 ml were collected and the radioactivity in 0.1 ml aliquots was determined. (○) Absorbance at 605 nm; (●) radioactivity in 0.1 ml aliquots. PG, prostaglandin

(3×50 ml). The ether extract was washed with water and concentrated to dryness *in vacuo*. The purity of the bound and free prostaglandin $F_{2\alpha}$, after methylation with diazomethane, was determined by thin-layer chromatography using the organic phase of an ethyl acetate—methanol—water (16:1:10, v/v/v) mixture as solvent [13].

RESULTS

Prostaglandin $F_{2\alpha}$ was found to be specifically bound by a crude particulate fraction obtained from ovine *corpora lutea*. The bound and free prostaglandins were conveniently separated by chromatography on small (2 ml) Sephadex G-50 columns. The particulate fraction containing the bound prostaglandin was eluted in the first 0.85 ml buffer whereas free prostaglandin $F_{2\alpha}$ was eluted with an additional 3.5 ml buffer. When blue dextran was chromatographed under similar conditions it appeared exclusively in the first fraction

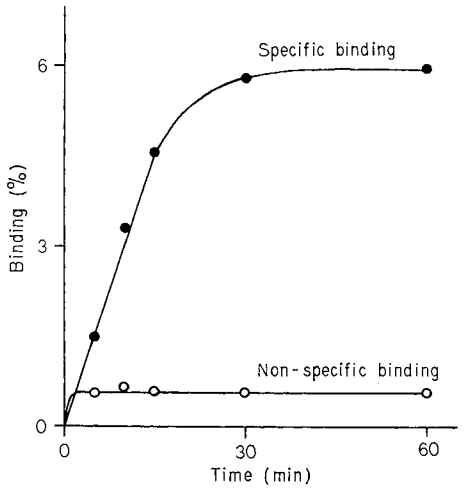

Fig. 2. *Time course of the binding of [9β-³H]prostaglandin $F_{2\alpha}$ to a particulate fraction of ovine* corpora lutea. The protein concentration was 15 mg/ml; for further details see text. (●) Specific binding; (○) non-specific binding

whereas [9β-³H]prostaglandin $F_{2\alpha}$ occurred only in the second fraction. Fig. 1 shows the results of a larger scale experiment in which bound and free prostaglandin $F_{2\alpha}$ were separated on a 50 ml Sephadex column with dimensions proportional to those of the smaller columns. The radioactive material in the two peaks was extracted and methylated. Thin-layer chromatography revealed that the radioactive material from both peaks was homogeneous and had the same R_F value as prostaglandin $F_{2\alpha}$ methyl ester.

The binding of prostaglandin $F_{2\alpha}$ was maximal when the incubation temperature was 23 °C. It was somewhat reduced at 37 °C and considerably reduced at 0 °C. Fig. 2 shows the time course of the binding reaction at 23 °C. Maximum binding was obtained after 30 min. The non-specific binding of prostaglandin $F_{2\alpha}$ was also determined as a function of time (Fig. 2) and was found to be complete in 5 min. Fig. 3 shows the results of an experiment in which the particulate fraction was preincubated with [9β-³H]prostaglandin $F_{2\alpha}$ for 40 min at 23 °C. At this time a 100-fold excess of unlabeled prostaglandin $F_{2\alpha}$ was added and the amount of bound [9β-³H]prostaglandin $F_{2\alpha}$ was determined after various time intervals. In this way the specific binding of prostaglandin $F_{2\alpha}$ was found to be a reversible process.

Prostaglandin $F_{2\alpha}$ binding activity was found only in the particulate fraction of *corpora lutea*. There was no significant binding of prostaglandin $F_{2\alpha}$ by the $100000 \times g$ supernatant fraction of *corpora lutea* or by either the particulate or the $100000 \times g$ supernatant fraction of ovaries from which the *corpora lutea* had been removed.

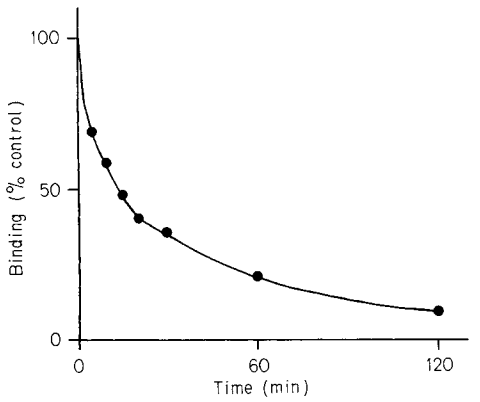

Fig. 3. *Time course of the dissociation of the [9β-^{3}H]prostaglandin $F_{2\alpha}$-particulate fraction complex in the presence of a 100-fold excess of unlabeled prostaglandin $F_{2\alpha}$.* Protein concentration: 23.5 mg/ml; see text for experimental details

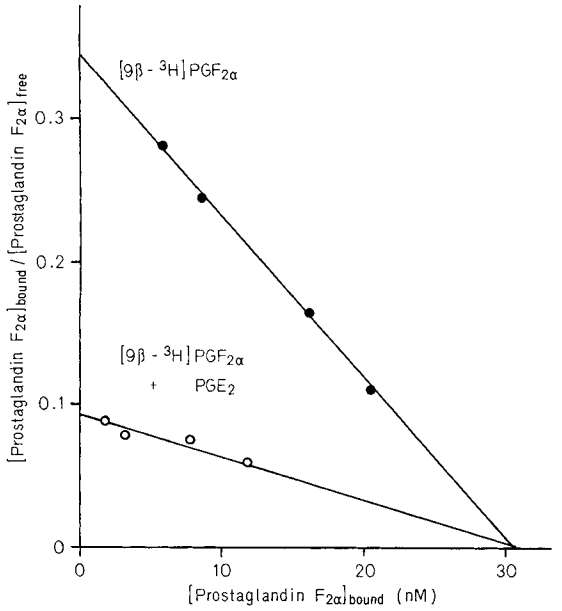

Fig. 4. *Scatchard plots for the interaction of [9β-^{3}H]prostaglandin $F_{2\alpha}$ with the ovine* corpus luteum *receptor.* Protein concentration: 27.5 mg/ml; see text for experimental details. (●) [9β-^{3}H]prostaglandin $F_{2\alpha}$ (2, 4, 10 and 20 ng); (○) [9β-^{3}H]prostaglandin $F_{2\alpha}$ (2, 4, 10 and 20 ng) + prostaglandin E_2 (500 ng). PG, prostaglandin

The binding of prostaglandin $F_{2\alpha}$ was not affected by the addition of 500-fold excesses of arachidonic acid, palmitic acid, linoleic acid, testosterone or progesterone or 1000-fold excesses of prostaglandin A_1, prostaglandin A_2, prostaglandin B_2, 3′:5′-AMP, dibutyryl 3′:5′-AMP, 3′:5′-GMP, ATP or GTP. The binding was inhibited by preincubation of the particulate fraction with trypsin, protease, phospholipase A or *N*-ethylmaleimide.

The dissocation constants of a number of prostaglandins and related compounds were determined from Scatchard plots (Fig. 4) as described above. The results are shown in Table 1. The dissociation constants of prostaglandins A_2 and B_2 were not determined because of their very low affinity for the receptor.

Table 2. *Relative importance of prostaglandin functional groups for the binding reaction*
PG, prostaglandin

Functional group modified	K_d of compound without this group / K_d of corresponding compound with this group	
CO_2H	380	(1,9α,11α,15(*S*)-tetrahydroxyprosta-5-*cis*,13-*trans*-diene/$PGF_{2\alpha}$)
9-OH	27	($PGE_2/PGF_{2\alpha}$)
	17	($PGE_1/PGF_{1\alpha}$)
	73	($PGF_{2\beta}/PGF_{2\alpha}$)
11-OH + 10,11-CH_2CH 11-OH + 8,12-CHCH	96	(PGA_2/PGE_2 or PGB_2/PGE_2)
15-OH	48	(13,14-dihydro-15-keto-$PGF_{2\alpha}$/13,14-dihydro $PGF_{2\alpha}$)
5,6-CH=CH	20	($PGF_{1\alpha}/PGF_{2\alpha}$)
	13	(PGE_1/PGE_2)
C-8 stereochemistry	8	(8-*iso*PGE_1/PGE_1)
13,14-CH=CH	2.3	(13,14-dihydro$PGF_{2\alpha}/PGF_{2\alpha}$)
15-H	1.8	(15-methyl$PGF_{2\alpha}/PGF_{2\alpha}$)

DISCUSSION

Prostaglandin $F_{2\alpha}$ was specifically bound to a particulate fraction from ovine *corpora lutea* with a dissociation constant of 0.1 μM and a concentration of binding sites of about 1.3 pmol/mg protein. The dissociation constant found is 10 to 1000-fold higher than reported for many other hormone-receptor reactions. Only the interaction of noradrenaline with a canine ventricular receptor also had a dissociation constant of 0.1 μM [15]. We found no evidence for a higher affinity prostaglandin $F_{2\alpha}$ receptor in ovine *corpora lutea* when using [17,18-$^3H_2$]prostaglandin $F_{2\alpha}$ of high specific activity (22.5 Ci/mmol). This was also true when the experiments were repeated in the presence of indomethacin (0.1 mM) or 5,8,11,14-eicosatetraynoic acid (0.1 mM) to prevent endogenous prostaglandin synthesis during the experimental procedures.

The dissociation constants for the binding of various prostaglandins and related compounds to the particulate fraction described above are given in Table 1. Table 2 shows the relative importance of different functional groups of prostaglandins for the binding reaction. It is evident that the carboxyl group is quite essential for binding since its replacement by a hydroxymethyl group [1,9α,11α,-

15(*S*)-tetrahydroxyprosta-5-*cis*,13-*trans*-diene] increased the dissociation constant about 400-fold. Oxidation of the 9-hydroxyl group of prostaglandin $F_{2\alpha}$ to a keto group (prostaglandin E_2) or inversion of the stereochemistry about C-9 (prostaglandin $F_{2\beta}$) also reduced the affinity for the receptor considerably, thus indicating the importance of the 9α-hydroxyl group for the binding reaction. The weak or non-existent binding of prostaglandin A_2 and prostaglandin B_2 suggests that the 11-hydroxyl group is rather important in the prostaglandin-receptor interaction although the effect of the annular double bond of either of these compounds cannot yet be deduced. Conversion of the secondary alcohol group at C-15 in 13,14-dihydroprostaglandin $F_{2\alpha}$ to a keto group increased the dissociation constant about 50-fold. Reduction of the 5,6-double bond resulted in 10 to 20-fold higher dissociation constants for the reduced species (prostaglandin $F_{1\alpha}$ *versus* prostaglandin $F_{2\alpha}$ and prostaglandin E_1 *versus* prostaglandin E_2). The stereochemistry about C-8 also affected the binding as 8-*iso*prostaglandin E_1 had a dissociation constant eight-times higher than that of prostaglandin E_1. On the other hand, reduction of the Δ^{13}-*trans* double bond (13,14-dihydroprostaglandin $F_{2\alpha}$) or substitution of a methyl group for the hydrogen atom at C-15 (15-methylprostaglandin $F_{2\alpha}$) had little effect on binding activity.

The results obtained in this investigation can be compared with the results of experiments *in vivo* on the luteolytic effects of different prostaglandins. Most of these experiments have been carried out with prostaglandin $F_{2\alpha}$ (*cf.* Introduction). Administration of prostaglandin $F_{1\alpha}$ to sheep also resulted in a marked drop in progesterone secretion by the ovaries whereas prostaglandin E_1 had a much smaller effect [16]. Prostaglandin E_2 [17] and, to a lesser extent, prostaglandin E_1 [18] induced luteolysis in hamsters but these prostaglandins were less potent than prostaglandin $F_{2\alpha}$. These results are consistent with the dissociation constants shown in Table 1. Of the prostaglandins tested, prostaglandin $F_{2\alpha}$ had the greatest affinity for the receptor. Prostaglandins $F_{1\alpha}$ and E_2 each had dissociation constants 20 to 30-times higher than prostaglandin $F_{2\alpha}$ whereas the dissociation constant of prostaglandin E_1 was over 300-times higher.

The presence of a prostaglandin E receptor in rat adipocytes was recently reported [19]. In this case the relative affinities of some prostaglandins were as follows: prostaglandin $E_1 \approx$ prostaglandin E_2 $>$ 13,14-dihydroprostaglandin $E_1 \gg$ prostaglandin $A_1 \approx$ prostaglandin $F_{2\alpha} >$ 13,14-dihydro-15-keto-prostaglandin E_1. Both the prostaglandin E receptor from adipose tissue and the prostaglandin $F_{2\alpha}$ receptor from ovine corpora lutea required an alcohol group rather than a keto group at C-15 of the prostaglandin. On the other hand the 5,6-*cis* double bond was much more important for the interaction of prostaglandins with the *corpus luteum* receptor (see Table 2) than it was for the interaction of prostaglandins with the adipocyte receptor. The latter receptor did not differentiate appreciably between prostaglandin E_1 and prostaglandin E_2.

The nature of the prostaglandin $F_{2\alpha}$ receptor is largely unknown. However, it was labile to digestion with trypsin, protease and phospholipase A as well as to treatment with *N*-ethylmaleimide. Further studies on the properties of the membrane-bound prostaglandin $F_{2\alpha}$ receptor are in progress.

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