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0014-4754/88/010043-03$1.50 + 0.20/0

Luteolytic potency of 16-phenoxy-derivatives of prostaglandin $F_{2\alpha}$

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Summary. The binding of 16-phenoxy derivatives of prostaglandin (PG) $F_{2\alpha}$ to rat luteal membranes, and also their abortifacient potency in pregnant rats, have been studied. Competitive binding studies with various PG-analogues were performed in ovaries of juvenile rats pretreated with PMSG and HCG, and in parallel studies the abortifacient potency of these substances was tested in pregnant rats. It was observed that this class of derivatives bound to the $PGF_{2\alpha}$ receptor as well as, or even better than the parent compound $PGF_{2\alpha}$. Modifications in the carboxyl group at C-1 yielded derivatives with a higher affinity for the receptor, in decreasing order of effectiveness as follows:- COOR > COOH > OH. The data obtained from the binding studies also compared well with data on the abortifacient potency in pregnant rats. It is concluded that the addition of a phenoxy group to either the lower or upper side chain of $PGF_{2\alpha}$ may augment the binding to the receptor as well as the biological responses induced by the post receptor effect.
Key words. Prostaglandin; rat luteal membrane; receptor binding affinity; abortifacient potency; luteolytic potency.

The short duration of action of the naturally-occurring prostaglandins is a result of their fast metabolism by prostaglandin (PG)-15-OH- dehydrogenase [1,2]. One of the major goals of prostaglandin research has therefore been the generation of compounds possessing a higher affinity for the receptor molecule as well as a longer duration of action. Such a derivative should preferably retain specifically selected porperties of the natural prostaglandin. One generally used approach for the synthesis of long-acting prostaglandin is the introduction of bulky groups in the neighborhood of the 15-hydroxyl group of $PGF_{2\alpha}$ [3].

Previous experiments have shown that the introduction of such bulky groups, for example an epoxide at C-15, reduced the affinity for the receptor, whereas the addition of lipophilic substituents in the lower and upper side chain augmented binding [4]. It was then concluded that the contribution of the various substituents to the binding affinity is additive.

In this investigation, we planned to test the binding characteristics of 16-phenoxy-derivatives of $PGF_{2\alpha}$ to membrane particles isolated from superovulated rat ovaries, and if possible to correlate them with abortifacient potency in pregnant rats.

Materials and methods. Substances: (9β-^{3}H) $PGF_{2\alpha}$ (sp. act. 5.5×10^{11} Bq/mmol) was purchased from the Radiochemical Centre, Amersham. Prostaglandin derivatives were supplied by Schering AG, Berlin/Bergkamen and C-Pfizer, Inc. Groton, Connecticut. Human chorionic gonadotrophin, HCG, (2350 IU/mg) and pregnant mare serum gonadotrophin, PMSG, (2577 IU/mg) were obtained from Schering AG, Berlin and dissolved in 0.9% NaCl. Indomethacin was supplied by Boehringer, Mannheim, W. Germany. All other substances were of analytical grade.

Animals: Immature female rats (Hans-Wistar-Schering) were kept in groups of 10–12 animals, and mature rats weighing 180–200 g were kept in groups of 8–10 animals in large plastic cages. They were housed in air conditioned rooms under a controlled light regimen. The animals were provided with a standard diet of Altromin® and water ad libitum. To obtain a single well-defined generation of corpora lutea, 22-day-old female rats were injected s.c. with 50 IU PMSG, followed 72 h later by 25 IU HCG. The day of HCG administration was defined day 0 of pseudopregnancy.

Prostaglandin binding: Superovulated ovaries were homogenized with an Ultra Turrax in Tris-buffer, pH 7.4, containing 10^{-5} mol/l indomethacin. Indomethacin was added to prevent any endogenous prostaglandin synthesis [5]. Large particles and connective tissue were separated by spinning at 1000 × g for 20 min. The supernatant was filtered through a double layer of cheesecloth and the filtrate recentrifuged in a Beckman Ultracentrifuge at 105,000 × g for 60 min at 4 °C. The pellet was washed several times and resuspended to obtain a final protein concentration of approximately 1 mg/ml. Aliquots of the particulate fraction were incubated for 60 min at 37 °C with a constant amount of labeled $PGF_{2\alpha}$ (10 pmol/0.2 ml) and increasing concentrations of unlabeled $PGF_{2\alpha}$ or derivatives. Bound and free prostaglandins were separated on small Sephadex columns as described earlier [6]. The displacement of radioactively labeled ligand by non-radioactive material was generally plotted as percent binding versus the log molar concentration. Protein content of tissue preparations was determined according to Lowry [7].

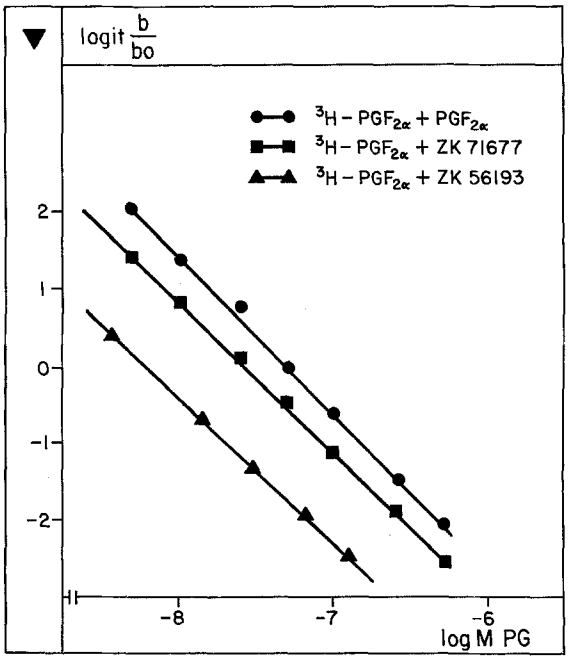
Abortifacient effect of prostaglandins: Vaginal smears were examined daily at 08.00–10.00 h. When required, female Wistar rats were caged with males on the night following proestrus, and the day on which sperm was detected in the smear was designated day 1 of pregnancy. Various doses of prostaglandins or derivatives, dissolved in 0.4 ml benzylbenzoate and castor oil (1:4), were injected s.c. between days 4 and 7 of pregnancy. Plasma progesterone levels were determined on days 3, 5, 7 and 9, and autopsy was performed on day 9 of pregnancy. Animals were tested for pregnancy by counting the number of implantation scars. For each dose, groups of 5 animals were used. Abortifacient potency was then calculated by determining the smallest dose of test substance that induced luteolysis and labor in relation to $PGF_{2\alpha}$.

Radioimmunoassay for progesterone: The antibody against progesterone was raised in our laboratory by immunizing

rabbits with progesterone-6α-succinoyloxy-bovine serum albumin. The dilution of the antiserum used in the assay was such that it bound approximately 50% of added ^{3}H-progesterone in the absence of non-radioactive progesterone. The only steroids with significant cross-reactivity were Δ^5-pregnenonlone and 5α-pregnane-3β-ol, -20-on. The assay was carried out as described by Orczyk et al.[8].

Results. The sigmoidal displacement curves obtained by the binding of a constant amount of ^{3}H-$PGF_{2\alpha}$ in the presence of increasing concentrations of unlabeled $PGF_{2\alpha}$ or derivatives, were linearized by a logit/log transformation (fig.). Determination of the shift of the parallel lines allows the calculation of the relative affinity of ZK 71677 and of ZK 56193 as described by Rodbard[9]. Competition factors of 1.40 and 10 were calculated for ZK 71677 and ZK 56193 respectively ($PGF_{2\alpha} = 1$).

Tables 1 and 2 show the relative binding of $PGF_{2\alpha}$ and its 16-phenoxy-derivatives as well as their abortifacient potency in pregnant rats. This class of compounds includes substances with a higher intrinsic activity than the parent compound $PGF_{2\alpha}$. The highest level of binding was observed with the hydrophobic biphenylester of the phenoxy-derivate (ZK 56193 and ZK 62474). The 15-ethylene ketal had reduced affinity, but was still in the range of $PGF_{2\alpha}$ (ZK 71677 and ZK 56440). The introduction of chlorine, a space-filling substituent, into the phenyl ring led to a reduction in binding (ZK 64519). The fluorine atom, as in ZK 64520, has about the size of a hydrogen atom and the derivative exhibits the same affinity as ZK 56440. Very high binding was still achieved when the configuration at C_{15} was changed



Relative binding of $PGF_{2\alpha}$, ZK 71677 and ZK 56193 to membrane particles of rat corpora lutea after logit/log transformation, whereby logit $B/B_0 = \ln \frac{B/B_0}{1 - B/B_0}$.

Table 1

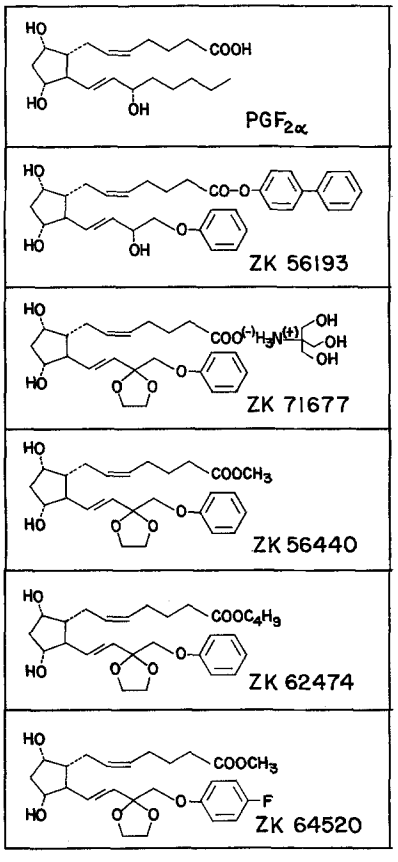
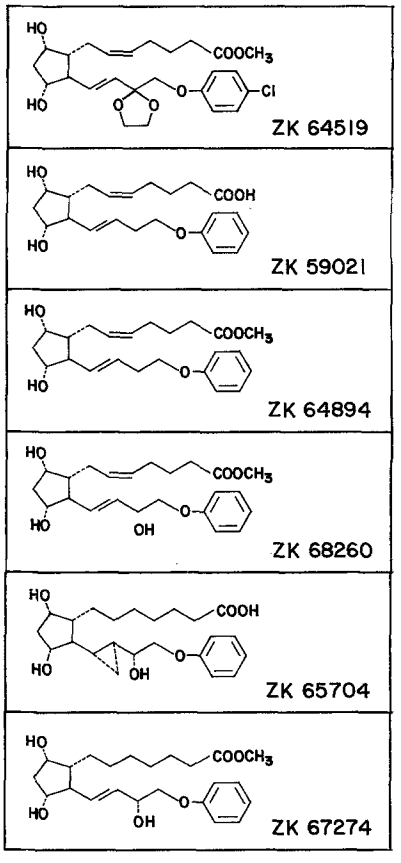


Table 2



Tables 1 and 2. Relative binding of $PGF_{2\alpha}$ and 16-phenoxy derivatives of $PGF_{2\alpha}$ to rat luteal membranes, and relative abortifacient potency of these compounds in pregnant rats.

(ZK 68260). Binding was markedly reduced when the 15-hydroxyl group was omitted (ZK 64894). $PGF_{1\alpha}$ (without double bond in upper side chain) together with the phenoxysubstituents, also showed good binding, which was decreased when the double bond in the lower side chain was substituted with a methylene ring (ZK 65704). The binding affinity of the substances listed in tables 1 and 2 compared well with the abortifacient potency of these compounds.

Discussion. The development of prostaglandin analogues possessing a higher luteolytic activity is of considerable importance because of their potential for use in reproductive physiology [10,11]. Although the exact mechanism of action of the prostaglandins remain undefined, a first step may be binding to specific receptors. Receptors for $PGF_{2\alpha}$ have been identified in corpora lutea of various species including man [6]. Time-course studies on superovulated rat ovaries have shown that receptors for $PGF_{2\alpha}$ reached peak levels on days 7 and 9 of pseudopregnancy [12]. In this investigation, radioligand binding studies in rat corpora lutea were performed with $PGF_{2\alpha}$, on day 7 after HCG administration. Information about the structural requirements of $PGF_{2\alpha}$ for receptor binding was established by comparing displacement curves for a wide variety of $PGF_{2\alpha}$ derivatives. It was found that modifications in the carboxyl group at C-1 gave compounds with high affinity for the receptor in decreasing order of effectiveness as follows:- COOR > COOH > OH. Spacefilling substituents at C-15 reduced binding, but a lipophilic group at C-1 increased it, as in the case of the biphenyl ester, compound (ZK 56193), which was found to be about 10 times as potent as $PGF_{2\alpha}$ itself. The contribution of the various substituents to the intrinsic binding affintiy is additive. A number of compounds with additional substituents in the phenyl ring have found wide application in veterinary medicine [13,14].

The data obtained from the binding analysis generally compared well with the abortifacient potency in pregnant rats. $PGF_{2\alpha}$ induces abortion as a result of its luteolytic effect, together with a direct stimulation of the uterine musculature. $PGF_{2\alpha}$ derivatives that displayed high potency in receptor binding in vitro, were also potent in terminating luteal function and inducing abortion.

In conclusion, the binding characteristics of 16-phenoxy derivatives of $PGF_{2\alpha}$ to rat luteal membranes, as well as their abortifacient potency in pregnant rats have been demonstrated. Contribution of various functional groups to the binding activity was also elucidated. It is thus apparent that the knowledge of the structural requirements of each receptor would allow the development of new compounds with unique combinations of properties and possibilities for novel clinical applications.

Acknowledgments. The experiments were performed in the research laboratories of Schering A.G., Berlin/West Germany, department of biochemical pharmacology, where the author was a research fellow. The author wishes to thank Dr E. Schillinger, Head of Department, for help in designing the experiments.

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0014-4754/88/010045-03$1.50 + 0.20/0

Forced synthesis of trace amounts of juvenile hormone II from propionate by corpora allata of a juvenile hormone III-producing insect

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13 July 1987

Summary. Corpora allata of the cockroach *Diploptera punctata* normally synthesize only the isoprenoid juvenile hormone III (JH III). Only under extreme in vitro conditions (absence of carbon sources other than propionate) do they produce trace amounts of the homoisoprenoid JH II in addition to JH III. The specificity of the in vitro synthesis of JH III by *D. punctata* is thus consistent with the observed lack of homoisoprenoid JHs in this insect.
Key words. Juvenile hormone synthesis; propionate; homoisoprenoid; cockroach; corpora allata.

The synthesis of homoisoprenoid JHs by corpora allata from Lepidoptera is now well established [1]. All non-lepidopteran insect species studied so far produce exclusively JH III, as shown by analysis of the product of in vitro incubations of their corpora allata or by reliable assays of hemolymph and/or whole body titers of JH [1]. For instance, the corpora allata of the viviparous cockroach *Diploptera punctata* synthesize only the isoprenoid JH III in vitro [2] and only JH III can be detected in the hemolymph by GC-MS, the homoisoprenoid JH II, JH I or JH 0 being discriminated at least at the 5×10^4 level [3]. The specificity of JH homolog production is of considerable interest, because many examples of loose substrate specificity in enzymes of JH biosynthesis have been presented [1]. Propionate can serve as precursor of the higher