Potentiation of prostaglandin E2-induced release of LH by the prostaglandin analogue, 7-oxa-13-prostynoic acid

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Abstract. The prostaglandin (PG) analogue 7-oxa-13-prostynoic acid (7-OPA) was infused into a lateral ventricle of the brain of adult male rats and the effect of the analogue on a subsequent stimulation of LH release by intraventricular infusion of PGs was determined. Pretreatment of the animals with 44–132 μg of 7-OPA potentiated the stimulatory effect of 2 μg PGE2 on the release of LH but the analogue alone had no effect on the hormone secretion. The minimal effective dose of PGE2 was determined to be within the range 0.01–0.05 μg and it was found that priming with 132 μg of 7-OPA caused a formerly sub-threshold dose (0.01 μg) of PGE2 to become an effective stimulus for the release of LH. In contrast to its potentiating effect on PGE2-induced LH release 7-OPA did not alter the stimulatory action of PGF2α (2 μg) on the secretion of LH. 7-OPA had no effect on LRH-induced release of LH indicating that the PG analogue acts at a suprapituitary site to enhance PGE2-induced LH release. The potentiating effect of 7-OPA may be exerted at a binding site for PGE2 in the brain and the results suggest the existence of a different binding site for PGF2α. The possibility also exists that 7-OPA inhibit metabolic inactivation of PGE2.

A substantial amount of evidence indicates that prostaglandins (PGs) are involved in the neural regulation of pituitary gonadotrophin secretion (Hedge 1977). The primary site of action appears to be in the hypothalamus (Harms et al. 1974; Eskay et al. 1975, 1977; Chobsieng et al. 1975; Drouin et al. 1976) where PGs stimulate the release of luteinizing hormone releasing hormone (LRH) but the mechanism whereby this effect is exerted has not been resolved. Prostaglandin receptors have been demonstrated in various tissues including thymocytes (Schaumburg 1973), lipocytes (Kuehl & Humes 1972), and corpus luteum (Powell et al. 1974, 1975), and it has recently been suggested (Warberg et al. 1976) that PGs might also bind to a receptor site in the brain as an event prior to their stimulatory effect on the release of LRH and LH. This hypothesis was based on the observation that PGs with certain functional groups (previously found to be important for receptor activation) (Powell et al. 1974, 1975) were particularly potent stimulators of LH release in male rats.

The present investigation was undertaken to elucidate the role of a central PG binding site for PG-induced release of LRH. For this reason, the PG analogue 7-oxa-13-prostynoic acid (7-OPA) which is believed to interfere with receptor binding of PGs (Fried et al. 1969; Kuehl & Humes 1972; Eakins & Sanner 1972) was infused intraventricularly into adult male rats and the effect on subsequent stimulation of LH release by PGE2 or PGF2α was determined.

Materials and Methods

Adult male rats of the Wistar strain (350–400 g, bred at the Panum Institute) were anaesthetized with pentobarbitral injected ip (6–8 mg/100 g body weight). The animals were ventilated with oxygen through an endotracheal tube connected to a rodent respirator.

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7-OPA was dissolved in a solution consisting of 1 vol of TRIS buffer (50 mM, pH 7.4) and 1.35 vol of 95% ethanol. The solvent for 7-OPA was used as a control for the analogue. The PG's were dissolved in a solution consisting of 1 vol of 95% ethanol and 9 vol of 0.02 Na₂CO₃ in 0.15 M NaCl. A trace quantity of lissamine green was added to the PG solution as a marker dye, and the pH was adjusted to pH 7.4 using 10 M NaOH. The diluent for PG's served as the control solution. Synthetic LRH (Beckman) was dissolved in TRIS buffer (50 mM, pH 7.4).

Intracerebroventricular infusion of 7-OPA, PG's or LRH

7-OPA, PG's or LRH were infused into a lateral ventricle of the brain via a metal cannula positioned according to the coordinates previously described by Warberg et al. (1976). The head of the animal was firmly fixed using a holder (Porter et al. 1970) and the cannula was inserted by means of a micromanipulator to ensure an accurate and reproducible placement. All solutions were infused at a rate of 2 μl/min for 2.5 min. 7-OPA or the control solution were infused during the 0- to 2.5-min interval and PG's or LRH were administered during the 3- to 5.5-min period. In the experiments where the minimal effective dose of PGE₂ was determined the prostaglandin (or diluent) was infused during the 0- to 2.5-min interval. Heparinized blood samples (1 ml) were withdrawn from a cannulated femoral artery immediately before and 15, 30, 60 and 90 min after the onset of the intraventricular infusion of 7-OPA or its solvent. Following centrifugation of each blood sample, the plasma was removed and the cellular precipitate was suspended in an equal volume of 0.15 M NaCl. The suspended cellular elements were re-injected into the donor animal within 10 min after obtaining the blood sample. At the end of each experiment the rat was killed by decapitation and the distribution of marker dye (lissamine green) in the brain was determined to confirm the intraventricular infusion.

Hormone determinations

LH in the plasma was measured by radioimmunoassay as outlined by the NIAMDD Rat Pituitary Hormone Distribution Program. LH-RP-1 served as the reference preparation. The least detectable quantity of LH was 1–2 ng per tube and half maximal binding of [¹²⁵I]iodo-LH was observed when the reaction tubes contained 7–9 ng of reference preparation. Serum from ovariectomized rats was used when determining the assay precision. When aliquots of this serum were added to tubes containing [¹²⁵I]iodo-LH and antibody to LH the binding curve obtained was parallel to that found when using LH-RP-1 standards. Within-assay coefficient of variation determined at the lower, middle or upper sensitivity range of the standard curve was 9.3, 9.3 or 12.0%, respectively (N = 15 for each estimate). Between-assay coefficient of variation which was determined at the mid range portion of the standard curve (3–20 ng LH-RP-1) and based on 12 consecutive assays was 10.8%. To eliminate this inter-assay variation all samples from each experiment were run (in duplicate) in the same assay.

Statistical analysis

The differences between the means of treatment groups were analyzed by Student's t-test (Documenta Geigy, 1970) and the difference between initial and post-infusion LH concentrations within a treatment group was evaluated using the paired t-test.

Results

Effect of lateral ventricle infusion of 7-OPA on PGE₂-induced release of LH

When 2 μg of PGE₂ was infused into a lateral ventricle subsequent to administration of the solvent for 7-OPA a marked release of LH was induced as indicated by an increase in the concentration of LH in arterial plasma (Fig. 1). The concentration of LH continued to increase for 60 min and remained elevated during the 60–90 min period. At 60 min the concentration of LH in plasma was about 8-fold greater than that observed prior to the infusion.

When 88 or 132 μg of 7-OPA was infused intraventricularly before the administration of 2 μg PGE₂ the stimulatory effect of PGE₂ was potentiated almost equally by the two doses of 7-OPA (Fig. 1). At 90 min the LH concentration of animals primed with 88 or 132 μg 7-OPA was significantly higher (P < 0.02 and P < 0.01, respectively) than that of control rats which received the 7-OPA vehicle. When 44 μg of 7-OPA was infused the LH concentration observed during the 60–90 min period was higher than that found at a corresponding time in the control animals but the difference was not significant (P > 0.05). However, at all doses of 7-OPA administered, the LH concentration in plasma continued to increase throughout the experimental period. This pattern of LH secretion was in contrast to that observed in the control animals where the LH concentration came to a level at 60 min. 7-OPA alone had no effect on LH secretion as seen when the analogue was infused intraventricularly followed by infusion of the diluent for PGE₂ (Fig. 1). The slight increase in the plasma concentration of LH seen in this experiment at 15 min was only marginally significant (P = 0.047) and hardly attributable to an agonistic
Concentration of LH in arterial plasma following administration of 7-OPA and PGE₂ to adult male rats. All solutions were infused into a lateral ventricle of the brain at a rate of 2 μl/min. 7-OPA was infused during the 0- to 2.5-min interval (Infusion 1) and PGE₂ during the 3- to 5.5-min period (Infusion 2). The number of animals in each treatment group is shown in parentheses. Each point corresponds to the mean plasma LH concentration and the vertical bars represent the magnitude of the standard error of the mean. The key depicting the composition of the solutions infused is given in the inset.

Effect of lateral ventricle infusion of 7-OPA on the secretory response of LH to a sub-threshold dose of PGE₂

To determine the dose level at which PGE₂ became an ineffective stimulus for the release of LH small amounts of PGE₂ were administered intraventricularly. In this series of experiments the 15 min blood sample was omitted. When 0.01, 0.05, 0.1, or 1 μg of PGE₂ was infused into a lateral ventricle a dose-related release of LH occurred (Fig. 2). The smallest dose of PGE₂ (0.01 μg) had no effect on LH release whereas 0.05 μg PGE₂ caused a significant rise (P < 0.01) in the LH concentration at 30 min. Thus, under the present conditions the minimal effective dose of PGE₂ was within the range of 0.01–0.05 μg.

When 132 μg of 7-OPA was administered intraventricularly prior to the infusion of 0.01 μg of PGE₂ (a sub-threshold dose) a rapid rise in the concentration of LH in arterial plasma was observed (Fig. 3). At 15 min the LH concentration was significantly higher (P < 0.01) than that observed in control rats. After 15 min the concentration of LH declined gradually but it remained elevated throughout the experimental period. In control rats which were pre-treated with 7-OPA diluent it was found that 0.01 μg of PGE₂ had no stimulatory effect on the release of LH. One animal
Concentration of LH in arterial plasma following administration of PGE₂ which was infused during the 0- to 2.5-min interval. For details, see Fig. 1.

However, even if those data were included it was found that the LH concentration of primed rats was significantly ($P < 0.05$) elevated over that of control animals during the 60–90 min period.
Fig. 4.
Concentration of LH in arterial plasma following infusion of 132 μg 7-OPA and 2 μg of PGF$_2$α. For details, see Fig. 1.

Fig. 5.
Concentration of LH in arterial plasma following administration of 7-OPA and LHRH. 132 μg of 7-OPA was infused during the 0- to 2.5-min interval (Infusion 1) and 50 ng of LHRH was infused during the 3- to 5.5 min period (Infusion 2). For details see Fig. 1.
**Effect of lateral ventricle infusion of 7-OPA on PGE$_{2\alpha}$-induced LH release**

When 2 µg of PGE$_{2\alpha}$ was infused intraventricularly after the administration of 7-OPA solvent the plasma concentration of LH increased rapidly and continued to increase throughout the experimental period (Fig. 4). By 90 min the LH concentration was 4–5-fold greater than the pre-infusion level. Administration of 132 µg 7-OPA had no effect on PGE$_{2\alpha}$-induced LH release.

**Effect of lateral ventricle infusion of 7-OPA on LRH-induced LH release**

When 132 µg of 7-OPA was infused into a lateral ventricle prior to intraventricular infusion of 50 ng of LRH it was found that the time course of LH release and the amount of hormone secreted were identical to that observed in control experiments where LRH was administered without 7-OPA priming (Fig. 5).

**Discussion**

The results of this investigation show that the PG analogue 7-OPA potentiates the stimulatory action of PGE$_{2\alpha}$ on LH secretion in vivo. Furthermore, 7-OPA caused a formerly sub-threshold dose of PGE$_{2\alpha}$ to become an effective stimulus for LH release. In addition, it was found that the minimal amount of PGE$_{2\alpha}$ necessary for the stimulation of LH release in male rats was within the dose range 0.01–0.05 µg. These threshold values have not previously been reported.

The present observations are somewhat surprising because it has earlier been found that 7-OPA has an inhibitory effect on stimulus-induced release of anterior pituitary hormones in vitro (Vale et al. 1971; Ratner et al. 1973; Dowd et al. 1973). Since 7-OPA is considered to be a competitive PG-antagonist presumably acting at the receptor level (Fried et al. 1969, 1971; Kuehl & Humes 1972; Eakins & Sanner 1972) the early investigations indicated that a PG receptor might be involved in the release of adenohypophysial hormones. This contention was supported by Warberg et al. (1976) who found that the LH-releasing activity of PGs depended on structural features (the 5, 6 cis double bond and the 11-hydroxyl group) which were previously shown to be important for receptor binding of PGE$_{2\alpha}$ (Powell et al. 1974, 1975). It was suggested that activation of a PG binding site in the brain was essential for PG-induced release of LH (Warberg et al. 1976). Since PGE$_{2\alpha}$ stimulates the secretion of LH primarily via enhanced release of LRH from the brain (Harms et al. 1974; Eskay et al. 1975, 1977; Chobsieng et al. 1975; Drouin et al. 1976) the present observation that LH release as induced by 50 ng of LRH (an intermediate dose) was unaffected by 7-OPA indicates that the analogue acts at a suprapituitary site to potentiate the effect of PGE$_{2\alpha}$. The cellular localization of such a site is uncertain. Prostaglandins may act directly on the LRH secreting neuron (Harms et al. 1976) but probably not at the axonal terminal as indicated by the observation of Warberg et al. (1977) that PGs had little, if any, effect on the release of LRH from isolated hypothalamic nerve endings (synaptosomes).

In addition to interfering with the binding of PGs 7-OPA also inhibits their synthesis (Fried et al. 1971). However, it appears unlikely that the present findings are attributable to this action of the analogue since blockade of PG synthesis in the brain has been reported to inhibit (and not stimulate) LH release in the rat (Ojeda et al. 1975).

7-OPA has been used mostly in vitro and little information is available about its purported antagonistic activity under in vivo conditions. Hedge & Hanson (1972) who administered 7-OPA to anaesthetized female rats using an analogue/PG ratio of 2.5–100 failed to block PGE$_{2\alpha}$- or stress-induced ACTH release but attributed the failure to an insufficient dose of 7-OPA. In the present experiments where a 7-OPA/PGE$_{2\alpha}$ ratio of 22–66 was used the analogue showed no sign of antagonistic activity. However, the observation that 7-OPA is an antagonist in vitro does not necessarily imply that it acts similarly in vivo. In the living animal a variety of factors influence the activity of 7-OPA (and PGE$_{2\alpha}$) and the potentiating effect of 7-OPA may therefore not only be explained by interaction with a PG receptor. For example, the analogue might serve to inhibit metabolic inactivation of PGE$_{2\alpha}$ in vivo. However, such an activity has not been described in vitro and further experimentation is needed to evaluate this possibility.

As a competitive antagonist mimicking the structure of PG's 7-OPA might interact with a PG receptor to produce a weak agonistic response. Thus, a synergistic effect with PGE$_{2\alpha}$ could be seen leading to an increased response to PGE$_{2\alpha}$ and a
decreased threshold. However, as pointed out earlier there was no evidence of an agonistic activity when 7-OPA was administered alone.

It could be argued that intraventricular infusion of 7-OPA (a hydrophobic carboxylic acid) would lower pH and alter the concentration of ionized Ca\(^+\)\(^+\) in the cerebrospinal fluid (CSF). However, there is no evidence that such changes in the CSF result in an augmented response to PG's. In fact, the LH releasing activity of PGF\(_{2\alpha}\) was not affected by 7-OPA indicating that the potentiating effect on PGE\(_2\) was specific. Furthermore, on the basis of the results of Johnson & Saunders (1971) it appears unlikely that 7-OPA forms micelles (and binds Ca\(^+\)\(^+\)) in solution.

If one assumes that 7-OPA exerts its action at the level of a receptor the present data suggest the existence of separate binding sites for PGE\(_2\) and PGF\(_{2\alpha}\).

The rather surprising findings reported here are not inconsistent with the hypothesis that PGE\(_2\) activates a central receptor site prior to its stimulation of LRH release. However, on the basis of the present observations no final conclusion can be drawn regarding the existence of such a receptor mechanism and further investigations (including binding experiments) are needed to verify the hypothesis.

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References


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