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The Effect of Various Prostaglandins and a Prostaglandin Synthetase Inhibitor on Rat Anterior Pituitary Cyclic AMP Levels and Hormone Release *in Vitro* (38475)

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It has been suggested that prostaglandins (PGs) might mediate the effects of some hormones on the adenyl cyclase activity of their target organs (1, 2). It has already been demonstrated that several prostaglandins stimulate pituitary adenyl cyclase and increase cAMP concentrations in the intact gland (3, 4), while they have also been shown to increase GH release *in vitro* (5–7). Furthermore, it has been reported that 7-oxa-13 prostanoic acid, a prostaglandin antagonist, inhibits both TSH (8) and GH (9) release *in vitro*.

In the present experiments, we studied the effects of prostaglandins E_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$, and the prostaglandin synthetase inhibitor, 1-(p-chlorobenzoyl)-5-methoxy-2-methylindole-3 acetic acid (indomethacin), on rat anterior pituitary cyclic AMP levels and concomitant release of luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin (Prl), thyroid-stimulating hormone (TSH) and growth hormone (GH) *in vitro*.

Material and Methods. Male Simonsen rats weighing 200–250 g were killed by decapitation and their pituitaries were halved and randomized between several Erlenmeyer flasks containing 2 ml of Krebs–Ringer bicarbonate buffer, 0.1% glucose, 0.1% bovine serum albumin (KRBG). Each flask contained the equivalent of two glands.

The pituitaries were preincubated for 30 min and then transferred to the appropriate test medium. They were then incubated for 4 hr in a Dubnoff metabolic shaker at 37°, in an atmosphere of 95% O_2 and 5% CO_2. At the end of each incubation, the medium was removed and the content of LH, FSH, Prl, TSH and GH was measured using specific radioimmunoassays (RIA).[2] LH was measured by the method of Niswender *et al.* (10). LH concentrations (μg/ml) were expressed in terms of NIH-LH-S1 reference preparation. FSH, prolactin and TSH were all measured using the method and RIA reagents generously supplied by NIAMD. FSH values were expressed as μg/ml of NIAMD-FSH-RP-1 standard. Prolactin concentrations (μg/ml) were expressed in terms of NIAMD-PRL-RP-1, while TSH values were expressed in terms of NIAMD-rat-TSH-RP-1 (μg/ml). GH was measured using the reagents supplied by NIAMD with modifications suggested by Dr. G.T. Peake. Results were expressed in μg/ml of a NIAMD-rat-GH-RP-1. After incubation, the pituitaries were immediately frozen on dry ice and lyophilized. After being weighed, the glands were homogenized in 0.5 ml of 5% trichloroacetic acid (TCA). The homogenates were then centrifuged and the supernatant extracted five times with 2 vol of ether acidified with *N* HCl. Cyclic AMP concentrations were then measured according to the method of Gilman (11), using the reagents supplied by Diagnostic Products Corp., Culver City, CA. Results are expressed in terms of pM of cyclic AMP per mg of dry gland.

The prostaglandins E_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$ were generously supplied by Dr. J.E. Pike of the Upjohn Company, Kalamazoo, MI. The prostaglandins were introduced into the KRBG solution using a dimethyl sulf-

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TABLE I. EFFECT OF PROSTAGLANDINS E_1, E_2, $F_{1\alpha}$ AND $F_{2\alpha}$ ON BASAL HORMONE RELEASE *IN VITRO*.[a]

	μg hormone/ml/4 hr				
	LH	FSH	PRL	TSH	GH
Experiment 1:					
Control	0.21 ± 0.03	5.5 ± 0.2	16.4 ± 1.1	30 ± 5	50 ± 4
PGE_1 0.03 m*M*	0.18 ± 0.01	5.8 ± 0.2	16.4 ± 0.9	30 ± 7	173 ± 15**
Experiment 2:					
Control	0.17 ± 0.02	6.1 ± 0.3	14.4 ± 0.5	27 ± 1	34 ± 3
PGE_2 0.015 m*M*	0.18 ± 0.01	6.3 ± 0.5	16.3 ± 0.7	26 ± 1	166 ± 12**
$PGF_{1\alpha}$ 0.015 m*M*	0.20 ± 0.02	6.9 ± 0.3	15.2 ± 1.5	24 ± 1	88 ± 14*
$PGF_{2\alpha}$ 0.015 m*M*	0.19 ± 0.02	6.2 ± 0.4	15.6 ± 1.3	24 ± 3	84 ± 3**

[a] Each value represents the mean ± S.E. of four flasks per experimental group.
* = $P < 0.05$; ** = $P < 0.01$ vs the respective control.

oxide (DMSO) vehicle. Appropriate concentrations of DMSO were also added to the control and stimulated flasks such that the final DMSO concentration was 0.1 %. 1-(p-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid (indomethacin) was generously supplied by Merck, Sharp and Dohme, West Point, PA. Indomethacin was also introduced into the incubation medium in DMSO solution. In some experiments, the prostaglandins or indomethacin were allowed to absorb to the albumin in the KRBG in order to avoid the use of a solvent. A partially purified ovine hypothalamic extract (HE) was prepared by dilute acetic acid extraction and was purified by ultrafiltration and Sephadex gel filtration. It was found to contain significant amounts of the activities corresponding to luteinizing hormone-releasing factor (LRF), follicle-stimulating hormone-releasing factor (FRF) and prolactin-inhibiting factor (PIF). Stimulation of hormone release by K^+ was accomplished by replacing NaCl with an equivalent concentration of KCl.

In each experiment, means and standard errors were determined by pooling the results from all flasks having identical treatments. Levels of significance were determined by application of Student's *t* test. The significance levels for standard treatments, such as the addition of HE, increased [K^+], or synthetic LRF (sLRF),[3] have been omitted from the tables in order to avoid confusion with the significance of the experimental manipulations being studied.

Results. Table I shows the effect of PGE_1 (.030 m*M*) and PGE_2, $F_{1\alpha}$ and $F_{2\alpha}$ all at 0.015 m*M* on LH, FSH, Prl, TSH and GH release after a 4 hr incubation period. While all of the prostaglandins significantly increased the release of GH, there were no significant effects observed on either LH, FSH, Prl or TSH release. In experiment 2, PGE_2 was shown to be twice as effective as either PGs $F_{1\alpha}$ or $F_{2\alpha}$ in releasing growth hormone.

Since the PGs had no effect on the basal release of LH, FSH, prolactin or TSH, it was decided to examine their effects on glands which were under the influence of a hypothalamic extract. These results are shown in Table II. In experiment 1, the HE (1.6 mg/ml) was found to increase significantly the release of LH, FSH, TSH and GH, while it significantly inhibited the release of prolactin and induced a small decrease in the intracellular concentration of cAMP, of borderline significance. In this experiment, the prostaglandins were added directly to the incubation medium bound to bovine serum albumin, such that their final concentration was 2 m*M*. All of the prostaglandins were found to potentiate significantly the release of both GH and TSH, with a parallel increase in tissue cAMP levels. They had no significant effects on either LH, FSH or prolactin release, although they did appear to inhibit slightly HE-stimulated LH release.

[3] We are indebted to Dr. J. M. Stewart (University of Colorado Medical Center) for the gift of sLRF.

TABLE II. EFFECT OF PROSTAGLANDINS E_1, E_2, $F_{1\alpha}$ AND $F_{2\alpha}$ ON HE OR sLRF-STIMULATED HORMONE RELEASE AND INTRACELLULAR cAMP LEVELS.[a]

	cAMP (pM/mg)	LH	FSH	PRL	TSH	GH
Experiment 1:						
Control	32 ± 1.4	0.17 ± 0.03	3.5 ± 0.1	11.3 ± 0.8	28 ± 4	27 ± 4
HE 1.6 mg/ml	28 ± 0.8*	1.20 ± 0.02	15.5 ± 0.7	7.4 ± 0.2	95 ± 11	73 ± 2
HE + PGE_1 2 m*M*	58 ± 10.1*	1.08 ± 0.09	16.1 ± 0.9	7.2 ± 0.7	169 ± 7**	139 ± 5**
HE + PGE_2 2 m*M*	69 ± 15.0*	1.17 ± 0.05	15.2 ± 0.8	7.4 ± 0.1	161 ± 17*	150 ± 5**
HE + $PGF_{1\alpha}$ 2 m*M*	42 ± 7.4	1.14 ± 0.14	15.0 ± 0.8	7.8 ± 0.6	162 ± 24*	147 ± 11**
HE + $PGF_{2\alpha}$ 2 m*M*	37 ± 5.0	1.12 ± 0.04	16.7 ± 0.7	7.4 ± 0.2	136 ± 2*	160 ± 10**
Experiment 2:						
Control		0.07 ± 0.01	5.0 ± 0.2	17.6 ± 1.7	29 ± 0.3	30 ± 5
sLRF 100 ng/ml		1.37 ± 0.08	32.5 ± 1.1	16.2 ± 1.4	29 ± 4.0	36 ± 4
sLRF + PGE_1 0.01 m*M*		1.23 ± 0.07	32.3 ± 1.4	18.6 ± 1.4	45 ± 7.0	146 ± 19**
sLRF + PGE_2 0.01 m*M*		1.23 ± 0.15	29.8 ± 0.9	15.7 ± 2.4	34 ± 4	148 ± 11**
sLRF + $PGF_{1\alpha}$ 0.01 m*M*		1.14 ± 0.12	29.7 ± 1.7	17.0 ± 1.8	30 ± 6	65 ± 7*
sLRF + $PGF_{2\alpha}$ 0.01 m*M*		1.14 ± 0.06	28.8 ± 1.5*	14.6 ± 0.8	32 ± 3	91 ± 6**
Experiment 3:						
Control		0.15 ± 0.03	3.9 ± 0.1	12.5 ± 0.5	34 ± 4	31 ± 4
HE 1.3 mg/ml		0.54 ± 0.08	8.1 ± 0.6	7.2 ± 0.7	83 ± 5	49 ± 4
HE + $PGF_{2\alpha}$ 0.05 m*M*		0.49 ± 0.04	8.1 ± 0.3	7.6 ± 0.4	142 ± 7**	87 ± 7*

[a] Each value represents the mean ± S.E. of four flasks per experimental group.
* = $P < 0.05$; ** $P < .01$ vs the stimulated group in that experiment.

In order to clarify further the effects of the prostaglandins on releasing factor-stimulated gonadotropin release, glands were incubated in 100 ng/ml of sLRF in the presence and absence of PGs E_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$, all of which were dissolved in DMSO (Exp. 2). As expected, sLRF stimulated FSH and LH release while failing to influence release of the other hormones. In this experiment, $PGF_{2\alpha}$ significantly inhibited sLRF-stimulated FSH release. While all of the PGs released GH, they had no effect on the basal release of TSH in the presence of sLRF.

A third experiment was conducted with a higher dose of $PGF_{2\alpha}$ (0.05 m*M*) added to flasks containing 1.3 mg/ml of HE. While GH and TSH release were again significantly potentiated, no alterations were observed in HE-stimulated LH, FSH or prolactin release.

Indomethacin has been shown to be a potent inhibitor of prostaglandin synthetase of sheep seminal vesicle tissue (12). Because of the stimulatory effect of prostaglandins on GH and TSH release, the effects of indomethacin on the secretion of LH, FSH, GH, TSH and prolactin and on pituitary cyclic AMP levels were examined in this system. The results from these experiments on the effect of indomethacin are shown in Table III. In experiment 1, indomethacin (final concentration 0.1 m*M*) was added in a DMSO carrier to control flasks, flasks containing 2% m*M* $[K^+]$ and to flasks containing HE (1.3 mg/ml). Indomethacin alone significantly inhibited GH release while it had no significant effect on the release of the other hormones. While a 27 m*M* $[K^+]$ concentration significantly released all of the hormones except Prl, the addition of indomethacin was effective in inhibiting only TSH secretion. The HE significantly increased the release of LH, FSH, TSH and GH while it inhibited Prl release. The addition of 0.1 m*M* indomethacin to flasks containing HE had no significant effects on release of any of the hormones investigated, although it again appeared to inhibit TSH and GH release. In experiment 2, indomethacin in a DMSO carrier was added (final concentration 1.0 m*M*) to flasks containing 5 ng/ml of sLRF. Again sLRF significantly increased the release of LH and FSH while it had no effect on the release of the other hormones. In this experiment, indomethacin significantly potentiated sLRF-stimulated LH and FSH release while it had no effect on either Prl, TSH or GH release. In the third experiment, indomethacin was added directly to the KRBG as a complex with BSA to give a final concentration of 2.7 m*M*. Under these circumstances, indomethacin was found to release LH, FSH and TSH, while it inhibited both Prl and GH secretion. The effect of indomethacin (2.7 m*M*) on HE-stimulated (1.6 mg/ml) and K^+ (25 m*M*) stimulated release of LH and FSH was found to be additive. In contrast, no significant modifications of the HE or K^+-stimulated release of TSH or GH were observed. The drug was found to inhibit Prl secretion under the influence of 25 m*M* $[K^+]$ and to further inhibit Prl release under the influence of the HE. In contrast to the prostaglandins, indomethacin was found to have no significant effects on cAMP concentrations within the gland.

Discussion. Although the prostaglandins are capable of increasing the basal release of GH *in vitro*, we have found that they are only capable of potentiating HE-stimulated TSH release. Prostaglandin stimulation of GH release has been well documented *in vitro* (5–7). Two reports have also appeared which suggest a stimulation of TSH release with PGE_1 (8, 13). It has been suggested that the GH secretory process is automatically activated *in vitro* by the removal of hypothalamic influences (14), such that the observed 'direct' effect of PGs on GH release is in fact a potentiation of this already stimulated secretory process. Thus, it would appear that PGs can further augment the release of TSH and GH when this release has already been stimulated.

In several instances, indomethacin significantly inhibited the release of TSH and GH while it had no significant effect on intracellular cyclic AMP levels. This would seem to indicate that a prostaglandin synthesizing enzyme system is operable in the somatotroph and thyrotroph, and that prostaglandins do modify the secretory output of these cells. In fact it has been reported that the prostaglandin antagonist, 7-oxa-13-prostanoic acid, can inhibit PG-induced cyclic

TABLE III. EFFECT OF INDOMETHACIN ON THE BASAL, HE, K^+ AND sLRF-STIMULATED HORMONE RELEASE AND INTRACELLULAR cAMP CONCENTRATIONS.[a]

	cAMP (pM/mg)	LH	FSH	PRL (μg/ml/4 hr)	TSH	GH
Experiment 1:						
Control		0.17 ± 0.02	6.6 ± 0.2	13.6 ± 0.6	26 ± 3	94 ± 4
Indo. (0.1 m*M*)		0.17 ± 0.02	6.8 ± 0.4	13.9 ± 0.2	29 ± 3	74 ± 5*
[K^+] (27 m*M*)		0.44 ± 0.02	11.5 ± 0.9	12.7 ± 0.9	136 ± 11	313 ± 20
K^+ + Indo.		0.51 ± 0.03	13.2 ± 0.6	13.2 ± 0.4	88 ± 6*	272 ± 29
HE (1.3 mg/ml)		0.79 ± 0.07	15.6 ± 1.0	8.5 ± 0.7	66 ± 3	126 ± 12
HE + Indo.		0.86 ± 0.05	16.6 ± 0.7	10.1 ± 1.0	53 ± 6	105 ± 3
Experiment 2:						
Control		0.12 ± 0.02	4.0 ± 0.3	13.0 ± 1.0	26 ± 1	39 ± 2
sLRF (5 ng/ml)		0.82 ± 0.04	13.7 ± 1.6	15.9 ± 0.8	29 ± 6	39 ± 3
sLRF + Indo. (1.0 m*M*)		1.05 ± 0.05*	19.0 ± 0.5*	15.5 ± .07	45 ± 7	38 ± 5
Experiment 3:						
Control	40 ± 3.0	0.21 ± 0.01	4.7 ± 0.4	12.8 ± 0.1	40 ± 2	73 ± 5
Indo. (2.7 m*M*)	36 ± 3.0	1.55 ± 0.14**	28.1 ± 1.6**	4.9 ± 0.4**	81 ± 11*	50 ± 5*
HE (1.6 mg/ml)	35 ± 2.0	0.88 ± 0.06	10.8 ± 0.4	8.4 ± 0.6	95 ± 10	89 ± 6
HE + Indo.	35 ± 0.4	2.08 ± 0.18**	37.8 ± 0.5**	4.6 ± 0.2*	108 ± 19	68 ± 7
[K^+] (25 m*M*)	41 ± 1.0	0.31 ± 0.02	7.5 ± 0.4	11.6 ± 0.7	163 ± 18	195 ± 16
K^+ + Indo.	39 ± 1.6	1.44 ± 0.24**	25.1 ± 1.7**	6.1 ± 0.7*	146 ± 12	177 ± 27

[a] Each value represents the mean ± S.E. for four flasks per experimental group.
* = $P < .05$; ** $P < .01$ vs the preceding flask.

AMP accumulation, GH release (9) and TSH release (8). Whether or not a prostaglandin synthesis system is under hypothalamic or target feedback control is not yet known.

In contrast to their effects on GH and TSH, the PGs were ineffective in consistently altering *in vitro* either the basal or HE-altered release of LH, FSH or prolactin, even when a parallel increase in intracellular cAMP concentrations was observed. In agreement with these results, Zor *et al.* (3, 4) were unable to observe any effect of various PGs on LH release. The observed potentiation of sLRF-stimulated LH and FSH release by indomethacin and the stimulation of release of these two hormones at high doses of the drug would seem to imply that certain PGs might exert a negative influence on gonadotropin secretion. However, a significant inhibition of gonadotropin release by the particular PGs employed in this work was seldom observed. Prostaglandins have been shown to inhibit adenyl cyclase activity in isolated adipocytes (15). It is possible that their effect on the adenyl cyclase in gonadotrophs might also be inhibitory.

Curiously, a marked inhibition of prolactin secretion was also obtained at high doses of indomethacin along with the parallel increase in LH and FSH. This same dose of indomethacin was included in the radioimmunoassay of all the hormones and not found to interfere with the assay. Excluding any nonspecific effects of the drug, these results would seem to indicate that a PG other than those employed in this study may be involved in stimulating prolactin release.

In conclusion, these studies indicate that several known prostaglandins are capable of influencing anterior pituitary cyclic AMP levels along with GH and TSH release, while they have little effect on LH, FSH or prolactin secretion. Furthermore, studies using indomethacin suggest that a prostaglandin synthesis system is involved in GH, TSH and prolactin secretion, and that a particular prostaglandin might influence gonadotropin secretion in the reciprocal manner. Whether or not this enzyme system does exist in the pituitary and whether it is under hypothalamic or target organ feedback control will remain unanswered until fluctuations in the concentrations of these compounds are measured within the glands under different physiological states.

Summary. (1) Prostaglandins E_1, E_2, $F_{1\alpha}$ or $F_{2\alpha}$ significantly increased the release of GH, with a parallel increase in intracellular cAMP concentrations, while they only potentiated HE-stimulated TSH release.

(2) None of the prostaglandins examined consistently effected either the basal or HE-altered release of LH, FSH or prolactin.

(3) The prostaglandin synthetase inhibitor, indomethacin, inhibited GH and TSH release and, at high doses of the drug, inhibited prolactin release. In contrast, the drug appeared to potentiate both HE and sLRF-stimulated gonadotropin release. It had no significant effect on intracellular cAMP concentration.

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