NOTE

Effects of Prostaglandin E₁ and Indomethacin on ACTH, Prolactin, GH and LH from Rat Pituitary *in Vitro*

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Synopsis

The effects of prostaglandin E₁ (PGE₁) and indomethacin (IDM) on the release of several pituitary hormones from the rat pituitary were investigated *in vitro*.

An addition of 2 μ g/ml of PGE₁ to the medium elicited the release of growth hormone (GH) and prolactin, but not of adrenocorticotropin (ACTH) and luteinizing hormone (LH). Although the addition of 1 μ g/ml of IDM alone resulted in no effect on the basal release of these hormones, IDM diminished the release of ACTH induced by crude rat hypothalamic extracts (HE) or lysine-8-vasopressin (LVP), and LH induced by HE or luteinizing hormone-releasing hormone (LH-RH).

These findings implicate that a part of PGE₁ action might be a direct one on the pituitary gland and PGE₁ might release GH and prolactin, whereas IDM might have a direct action on the pituitary gland, and that blunt the release of these pituitary hormones induced by several stimuli.

It is well known that prostaglandins (PGs) exist in most parts of living tissues (Shaws *et al.*, 1971; Butcher and Baird, 1968) and have hormone-like action which may be mediated by activation of adenylcyclase-cyclic AMP system in various endocrine glands (Zor *et al.*, 1970; Marsh, 1971; Kaneko *et al.*, 1969; Flack *et al.*, 1969; McLeod and Lehmeier, 1970).

It is also reported that PGs are present in the hypothalamus and are released into the ventricle of the brain by several stimuli (Harms *et al.*, 1973), and that those of the E series may be potent inhibitors of sympathetic neuro-effector transmission (Hedqvist, 1973). Thus, it is conceivable that PGs might be involved in regulating the release of pituitary hormones.

Indomethacin (IDM) is known to inhibit

the synthesis of PGs (Vane, 1971), and to affect on endocrine function is several glands.

The purpose of the present study is to ascertain the action of prostaglandin E₁ (PGE₁) and IDM on the release of pituitary hormones from rat pituitary *in vitro*.

Materials and Methods

Male Sprague-Dawley rats (180-200 g) were used as pituitary donors. The pituitaries were obtained by decapitation and after removal of the posterior pituitary they were cut in half and a hemipituitary was placed in each beaker with 2 ml of Krebs-Ringer bicarbonate medium containing 200 mg/dl glucose and 0.25% bovine serum albumin (KRBG-BSA). Four beakers were used for each sample. After 45-min preincubations two times the medium was replaced by 1 ml of KRBG-BSA and incubated for 30 minutes. The medium was decanted. Then 1 ml KRBG-BSA or KRBG-BSA containing the test material was added to the beaker and incubated for 30 minutes. The amounts of ACTH, prolactin, GH

and LH released into the medium during the second 30-min incubation were compared with those released during the first 30-min incubation and expressed as a percentage.

ACTH concentration in the medium was determined by radioimmunoassay as described by Ratcliffe (1971). Prolactin, LH and GH were determined by radioimmunoassay using NIAMDD rat prolactin, LH and GH kits, respectively.

PGE₁ was obtained from Ono Pharmaceutical Ltd. and IDM from Merck Co. Ltd.. PGE₁ was dissolved in KRBG-BSA just before use. IDM was dissolved in 0.1 M phosphate buffer pH 9 in high concentration and then diluted with KRBG-BSA to an appropriate concentration. Lysine-8-vasopressin (LVP) was kindly supplied by Sandoz Co. Ltd., and crude rat hypothalamic extracts (HE) by NIAMDD.

Duncan's new multiple range test was used for the comparison of the mean release of ACTH, prolactin, LH or GH in each beaker (Steel and Torrie, 1966).

Results

Effects of PGE₁ and IDM on the release of ACTH.

An addition of equivalent dose of 0.5

crude rat HE or 25 mU/ml or LVP to the medium significantly elicited ACTH release as compared with the control group. When 2 µg/ml of PGE₁, 10, 100 or 1000 ng/ml of IDM was added to the medium, ACTH release was not altered (Table 1, 2, 3).

One µg/ml of IDM blunted the ACTH release induced by LVP ($p < 0.01$) or HE ($p < 0.05$). Although PGE₁ added to the medium did not alter basal ACTH release from the pituitary, it diminished significantly HE-induced ACTH release (Table 3; Exp. A. and B.). Simultaneous addition of HE, PGE₁ and IDM augmented the ACTH release as compared with the control group. However, the amount of ACTH released into the medium was significantly lower when HE was added alone. When both 25 mg/ml of LVP and 1 µg/ml of IDM were added to the medium, the LVP-induced ACTH release was apparently blunted (Table 1).

Table 1. Effect of indomethacin (IDM) on lysine-8-vasopressin-induced ACTH release from rat pituitaries *in vitro*.

Treatment	Dose	Mean ACTH Concentration (pg/ml)		2nd Incubation 1st Incubation $\times 100$	P Value
		1st Incubation	2nd Incubation		
1) Control	...	822.5 $\pm$ 116.3*	722.5 $\pm$ 105.3	87.8 $\pm$ 2.4	1) vs 2): $p < 0.01$
2) Lysine-8-Vasopressin (LVP)	25 mU/ml	763.1 $\pm$ 26.9	1695.0 $\pm$ 244.0	221.8 $\pm$ 29.2	1) vs 3), 4): N.S.**
3) IDM	1 µg/ml	806.9 $\pm$ 117.7	1021.3 $\pm$ 254.2	122.4 $\pm$ 12.8	2) vs 3), 4): $p < 0.01$
4) LVP + IDM	25 mU/ml 1 µg/ml	1146.3 $\pm$ 225.3	1382.5 $\pm$ 210.6	129.8 $\pm$ 20.6	3) vs 4): N. S.

*: Mean $\pm$ S.E.M. **: not significant.

Table 2. Effect of indomethacin on ACTH release from rat pituitaries *in vitro*

Treatment	Dose (ng/ml)	Mean ACTH Concentration (pg/ml)		2nd Incubation 1st Incubation $\times 100$	P Value
		1st Incubation	2nd Incubation		
1) Control	...	972.5 $\pm$ 252.3	792.5 $\pm$ 242.5	77.8 $\pm$ 5.9	...
2) Indomethacin	10	1020.0 $\pm$ 284.1	743.8 $\pm$ 125.6	72.1 $\pm$ 3.5	N.S.*
3) Indomethacin	100	787.5 $\pm$ 23.3	504.3 $\pm$ 118.5	71.2 $\pm$ 21.0	N.S.
4) Indomethacin	1000	783.3 $\pm$ 23.3	544.0 $\pm$ 91.0	69.6 $\pm$ 2.1	N.S.

*: not significant.

Effects of PGE₁ and IDM on prolactin release.

The release of prolactin had a tendency to be decreased following the addition of an equivalent dose of 0.5 HE to the medium. An addition of 2 $\mu\text{g/ml}$ of PGE₁ augmented

the release of prolactin into the medium. The prolactin release induced by PGE₁ was abolished by addition of HE. No effect of 1 $\mu\text{g/ml}$ of IDM added to the medium on the release of prolactin was observed (Fig. 1).

Table 3. Effect of crude hypothalamic extracts (HE), prostaglandin E₁ (PGE₁), and indomethacin (IDM) on ACTH release by rat pituitaries *in vitro*.

Treatment	Dose	Mean ACTH Concentration (pg/ml)		2nd Incubation 1st Incubation	×100	P Value
		1st Incubation	2nd Incubation			
Exp. A						
1) Control	...	857.5±111.9*	910.0±198.0	102.6±	9.6	1) vs 4): p<0.05
2) HE	0.5 HE/ml	822.5±104.3	3495.0±129.7	449.4±	69.1	1) vs 2), 5), 7): p<0.01
3) PGE ₁	2 μg/ml	1003.8±142.6	1050.0±103.0	106.7±	6.1	1) vs 3), 6): N.S.**
4) HE+FGE ₁	0.5 HE +2 μg/ml	930.0± 56.1	2430.0±163.0	229.7±	15.8	2) vs 4): p<0.01
5) HE+IDM	0.5+1 μg/ml	997.5±107.2	3140.0±310.4	322.6±	40.5	2) vs 5), 7): p<0.05
6) PGE ₁ +IDM	2 μg+1 μg/ml	872.5±218.1	938.8±265.2	106.5±	9.2	3) vs 4): p<0.05
7) HE+PGE ₁ +IDM	0.5 HE +2 μg/ml	650.0± 40.2	2010.0±110.1	313.6±	28.4	3) vs 6): N.S.
Exp. B						
1)' Control	...	1020.8±157.6	956.3±156.8	94.5±	8.8	1)' vs 2)': p<0.01
2)' HE	0.5 HE/ml	822.5±198.2	2585.0±142.0	340.4±	59.7	1)' vs 3)': N.S.
3)' PGE ₁	2 μg/ml	905.0±284.5	696.3±246.6	72.8±	5.9	1)' vs 4)': p<0.05
4)' HE+PGE ₁	0.5 HE +2 μg/ml	846.3±294.0	1395.0±113.5	211.5±	48.4	2)' vs 4)': p<0.05

*: Mean $\pm$ S.E.M.

**: not significant.

Fig. 1. Effects of an equivalent dose of 0.5 crude rat hypothalamic extracts (HE), an/or 2 $\mu\text{g/ml}$ of prostaglandin E₁ (PGE₁) on the release of prolactin from rat pituitaries. Columns represent the mean values and a horizontal bar above the column donates standard error of the mean. Control vs PGE₁: P < 0.01, PGE₁ vs PGE₁ + HE: P < 0.01.

Fig. 2. Effects of an equivalent dose of 0.5 crude rat hypothalamic extracts (HE), 2 μ g/ml prostaglandin E₁ (PGE₁) and/or 1 μ g/ml of indomethacin (IDM) on the release of rat GH from rat pituitaries. Control vs PGE₁: $P < 0.01$.

Fig. 3. Effects of an equivalent dose of 0.5 crude rat hypothalamic extracts (HE), 2 μ g/ml of prostaglandin E₁ (PGE₁) and/or 1 μ g/ml of indomethacin (IDM) on the release of rat LH from rat pituitaries. Control vs HE: $P < 0.01$.

Fig. 4. Effects of 5 ng/ml LH-RH and/or 1 μ g/ml of indomethacin (IDM) on the release of rat LH from rat pituitaries. Control vs LH-RH: $P < 0.01$, LH-RH vs LH-RH + IDM: $P < 0.01$.

Effects of PGE₁ and IDM on the release of GH.

The addition of an equivalent dose of 0.5 HE had a tendency to increase GH release, but it was not statistically significant. When 2 μ g/ml of PGE₁ was added to the medium a significant increase in GH release into the medium was caused. One μ g/ml of IDM had no effect on the release of GH. The amounts of GH released into the medium containing both PGE₁ and HE were less than those observed for PGE₁ alone (Fig. 2).

Effects of PGE₁ and IDM on the release of LH.

HE significantly augmented the release of LH into the medium. However, the addition of 2 μ g/ml of PGE₁ resulted in no stimulation of LH release (Fig. 3). One μ g/ml of IDM had a tendency to blunt the release of LH induced by HE. An addition of 1 μ g/ml of IDM abolished the LH release induced by 5 ng/ml of LH-RH (Fig. 4).

Discussion

These findings demonstrate that PGE₁ may be capable of stimulating the release of GH and prolactin, but not the release of ACTH and LH, at the pituitary level, and that IDM may have no influence on the basal release of GH, prolactin, ACTH and LH; whereas it may blunt the release of ACTH induced by HE or LVP, and LH induced by LH-RH or HE at the pituitary gland.

Peng *et al.* (1970) indicated that the action of PGE₁ on ACTH release is not direct on the anterior pituitary gland, but at some level in the central nervous system, possibly the hypothalamus. Hedge (1971) also reported that PGE₁ injected into the median eminence increased ACTH secretion. On the contrary, Vale *et al.* (1971) reported that PGE₁ acted on the pituitary and released ACTH directly.

Although little is known as to whether or not IDM inhibits the ACTH release at the pituitary level, our results that the ACTH release induced by LVP or HE was abolished by the addition of 1 μ g/ml of IDM at the

pituitary level *in vitro* suggest the possibility that IDM might block endogenous PGs synthesis at the ACTH secreting cells and inhibit ACTH release *in vivo*.

In our results, an addition of 2 $\mu\text{g/ml}$ of PGE_1 alone had no effect on the ACTH release. Curiously, PGE_1 blunted the ACTH release induced by HE. These results implicate that PGE_1 might have no stimulatory or inhibitory effect on the ACTH release at the pituitary level, but that it might inactivate corticotropin-releasing factor or activate corticotropin release-inhibiting factor.

Recently, Ojeda *et al.* (1974) reported that the intraventricular injection of PGE_1 markedly increased the rat prolactin release, but not intravenous injection, and PGE_1 injected into the pituitary of ovariectomized rats also slightly stimulated the release of prolactin. Therefore, they suggest that it might stimulate the release of prolactin on both the hypothalamus and the pituitary gland. Our results obtained in this study demonstrated that a small amount of PGE_1 had a direct action on the pituitary gland and stimulated the release of prolactin. This finding implicates the possibility that a part of PGE_1 on the release of prolactin is a direct action on the pituitary gland.

The simultaneous addition of HE and PGE_1 to the medium abolished the prolactin release induced by PGE_1 . Thus, it is conceivable that HE might contain an inhibitory substance which competes with PGE_1 at the pituitary gland. Ojeda *et al.* (1974) found that a small amount of PGE_1 injected into the 3rd ventricle in rats blocked an inhibition of prolactin release induced by dopamine.

Hedqvist (1971) found that PGs of the E series inhibited the release of noradrenalin in response to the nerve stimulation in various tissues. Therefore, considering about the hypothalamus containing catecholamines, it is possible that PGE_1 might inhibit the release of catecholamines on the hypothalamus *in vivo*.

The results that the addition of HE could

not significantly stimulate the release of GH may be explained by the fact that HE contain not only GH-releasing factor but also somatostatin or another GH release-inhibiting factor, and these hormones may cancel out each action at the pituitary gland.

Ratner *et al.* (1973) reported that an *in vitro* addition of PGE_1 over a concentration range of 10^{-7} to 10^{-5} M enhanced the release of GH and 7-oxa-13-prostynoic acid, an antagonist of prostaglandins, reduced the stimulation of GH release found following the addition of PGE_1 . Ito *et al.* (1971) found intravenous drip infusion of PGE_1 in man to induce a marked increase in GH secretion.

Recently, Sundberg *et al.* (1975) also reported that prostaglandins E_1 , E_2 , $\text{F}_{1\alpha}$ or $\text{F}_{2\alpha}$ significantly increased the release of rat GH *in vitro*. Our results on the GH release obtained in rats is consistent with their results.

Several investigators reported that PGs acted in inducing the release of LH-RH (Eskay *et al.*, 1975; Sato *et al.*, 1975), and then caused the release of LH and FSH (Harms *et al.*, 1974; Sato *et al.*, 1974). Harms (1974) found that PGE_1 and PGE_2 might be involved in the neural control of the pituitary gonadotropin release, and PGE_1 had no direct effect on the release of gonadotropins at the pituitary level, but PGE_2 had.

It is generally accepted that inhibitors of PGs synthesis (IDM and aspirin) interfere with the ovulation, and at least a part of the effect is caused at ovarian level (Carlson *et al.*, 1974). Recently, Ojeda *et al.* (1975) reported that inhibitors of PGs synthesis administered at high doses inhibited the LH release in the rat and this effect might be due to a direct effect of drugs on the central nervous system. On the other hand, Sundberg *et al.* (1975) found that IDM did not affect the basal LH release, but that it potentiated gonadotropin release *in vitro* stimulated by both HE and LH-RH.

Although it has not been clarified

whether IDM blocks the release of LH at the level of the anterior pituitary, our result that the addition of 2 μ g/ml of IDM blunted the release of LH induced by both HE and LH-RH *in vitro* raises the possibility that IDM may blunt the release of LH at the pituitary level *in vivo*.

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