THE IN VIVO EFFECT OF INDOMETHACIN AND PROSTAGLANDIN E_2 ON ACTH AND DBCAMP-INDUCED STEROIDOGENESIS IN HYPOPHYSECTOMIZED RATS

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Summary: The level of plasma corticosterone attained in hypophysectomized rats stimulated with ACTH was significantly reduced by pretreatment with indomethacin, an inhibitor of prostaglandin synthesis. This effect was not seen in animals stimulated with dibutyryl cyclic AMP. Intraperitoneal injection of prostaglandin E_2 to indomethacin treated rats restored the normal response to ACTH stimulation. However, PGE_2 itself did not have any significant effect on plasma corticosterone levels. These findings suggest that prostaglandins are involved, perhaps in an allosteric fashion, in the mechanism of action of ACTH.

In attempting to define a physiological role for prostaglandins in hormone action, investigators have focused on the relationship between these biologically active lipids and cyclic 3',5' - adenosine monophosphate (CAMP).

Prostaglandins generally stimulate CAMP production in a variety of tissues with the exception of rat epididymal fat cells where they inhibit hormonally-induced CAMP formation (1). Since adrenocorticotropin (ACTH) is thought to act at its target site through the activation of the adenylate cyclase system (2), the effects of prostaglandins on adrenal steroid biosynthesis have also been investigated. Flack et al. (3) reported that prostaglandin E_2 (PGE_2) and to a lesser extent prostaglandins E_1 (PGE_1) and $F_{2\alpha}$ increased rat adrenal corticosterone secretion in a superfusion system. In addition, these workers found that PGE_2 significantly stimulated the in vivo production of corticosterone in 3 to 6 hour hypophysectomized rats. Saruta and Kaplan (4) using outer beef slices also demonstrated a direct effect of PGE_1 and PGE_2 on adrenal steroidogenesis. In contrast, Blair-West et al. (5) in studying the effects of PGE_1 on the autotransplanted adrenal of the sodium deficient sheep, were unable to assign a definite role for

this prostaglandin in steroidogenesis. Similarly, Peng et al. (6) were unable to show any direct in vivo effect of PGE_1 on adrenal steroid activity in the 24 hour hypophysectomized rat and came to the conclusion that PGE_1 acts indirectly on the adrenal by causing the release of ACTH from the pituitary.

In light of these conflicting reports we have attempted to evaluate the relationship between prostaglandins and adrenal steroidogenesis using a different approach. Accordingly, we administered indomethacin [1-(p-chlorobenzoyl)-5-methoxy-2 methyl indole-3-acetic acid], an inhibitor of prostaglandin synthesis (7-8), to rats and subsequently measured the in vivo production of corticosterone in response to ACTH, dibutyryl cyclic AMP (DBCAMP), and PGE_2. Hypophysectomized rats were used throughout to eliminate any indirect actions of PGE_2 on the adrenal gland that are mediated through the pituitary.

MATERIALS AND METHODS

Female Holtzman rats weighing approximately 200 grams were hypophysectomized intra-aurally using a Hoffman-Reiter hypophysectomy instrument (H. Neuman and Co., Skokie, Illinois). Four hours after hypophysectomy, rats received their first intraperitoneal (ip) injection of indomethacin[1] at a dose of 2 mg/rat in 0.5 ml of 0.1M sodium phosphate buffer, pH 8, or 0.5 ml phosphate buffer alone. Twenty hours after hypophysectomy, a second similar dose of indomethacin or buffer alone was administered.

At 24 hours after hypophysectomy, rats were injected ip with either PGE_2[2] at a dose of 200 µg/rat in 0.2 ml of a mixture of 95% ethanol and 20% aqueous sodium carbonate solution (1:9 v/v) or carbonate solution alone. The rats were then anesthetized with ether and 15' after having been injected with PGE_2 or carbonate buffer, rats were in-

[1] Indomethacin, kindly supplied by Merck, Sharp and Dohme, West Point, Pa. through the courtesy of Dr. Carl Stevenson.

[2] PGE_2 kindly supplied by the Upjohn Co., Kalamazoo, Mich. through the courtesy of Dr. John E. Pike.

Table 1. Effect of Indomethacin on ACTH and DBCAMP-Induced Steroidogenesis in Hypophysectomized Rats

Group	Treatment	No. of Rats	Plasma Corticosterone (µg/100 ml) Means ± SEM
1	-	7	6.7±0.8
2	ACTH	7	63.7±4.3
3	DBCAMP	7	39.2±2.5
4	Indomethacin	7	5.3±0.6
5	Indomethacin + ACTH	7	38.6±4.5
6	Indomethacin + DBCAMP	6	37.1±2.8

ACTH administered at a dose of 8 U/rat, DBCAMP at 7.0 mg/rat, and Indomethacin, twice, at 2 mg/rat. Significance levels: 1 vs 4, N.S., 2 vs 5, $p < 0.005$, 3 vs 6, N.S.

jected with either 0.1 ml sterile saline, 8 U ACTH gel (Organon) or with 7 mg DBCAMP (Sigma) into the jugular vein. Fifteen minutes later, following decapitation, blood was collected from the trunk and the corticosterone levels determined by a modification of the fluorometric method of Silber et al. (9).

RESULTS AND DISCUSSION

The effect of indomethacin on ACTH or DBCAMP-induced plasma corticosterone production in hypophysectomized rats is shown in Table 1. Indomethacin administered 16 and 4 hours prior to sacrifice, did not alter the normal dramatic rise in plasma corticosterone seen in hypophysectomized rats stimulated with DBCAMP. However, the same doses of indomethacin clearly inhibited ($\sim$ 39%) the normal response of these animals to maximal ACTH stimulation. Baseline levels of plasma corticosterone remained unaffected by indomethacin treatment.

Since indomethacin has been shown to reduce prostaglandin production both in vivo and in vitro in a number of tissues (7-8), the results obtained above suggested that prostaglandins may be involved in the mechanism

Table 2. Reversal by PGE_2 of the Inhibitory Effect of Indomethacin on ACTH-Induced Steroidogenesis in Hypophysectomized Rats

Group	Treatment	Plasma Corticosterone (µg/100 ml) Means ± SEM	
		Exp. 1	Exp. 2
1	-	8.2±0.9 (5)	5.3±0.5 (8)
2	ACTH	73.9±6.2 (6)	62.9±3.7 (7)
3	PGE_2	9.8±0.8 (7)	6.1±1.1 (7)
4	ACTH+PGE_2	69.2±4.1 (5)	75.3±4.8 (8)
5	Indomethacin	6.5±0.4 (5)	5.2±0.7 (7)
6	ACTH+Indomethacin	37.6±2.3 (7)	40.9±2.6 (7)
7	PGE_2+Indomethacin	9.7±0.8 (5)	4.9±0.3 (7)
8	ACTH+PGE_2+Indomethacin	58.6±4.9 (6)	76.8±4.4 (7)

Numbers in parenthesis show the number of rats in each group. ACTH administered at dose of 8 U/rat, Indomethacin, twice at 2 mg/rat, and PGE_2 at 200 µg/rat. Significance levels: Exp. 1, 2 vs 4, N.S., 2 vs 6, $p < 0.001$, 4 vs 8, N.S., 6 vs 8, $p < 0.005$; Exp. 2, 2 vs 4, N.S., 2 vs 6, $p < 0.001$, 4 vs 8, N.S., 6 vs 8, $p < 0.001$

of action of ACTH. To substantiate this further, additional experiments were carried out in which indomethacin-treated rats were given PGE_2 prior to the administration of ACTH. The results of these studies are shown in Table 2.

Prostaglandin E_2 alone did not significantly affect baseline plasma corticosterone levels of hypophysectomized rats, nor did PGE_2 augment the response of these animals to ACTH stimulation. It is clear, however, that PGE_2 effectively reversed the previously observed inhibitory action of indomethacin on ACTH-induced steroidogenesis. The levels of plasma corticosterone in these animals were not significantly different from those of rats given ACTH without indomethacin pretreatment. It is unlikely that these results were due to indomethacin or PGE_2-induced changes in adrenal blood flow, since it has been shown (10) that adrenal blood flow is not a factor in steroid production when high doses of ACTH (8 U) are employed as

they were in this study. Furthermore, the response to DBCAMP was unaffected by indomethacin pretreatment.

The lack of a direct ACTH-like effect of PGE_2 on the adrenal of the 24 hour hypophysectomized rat supports the similar findings of Peng et al. (6) with PGE_1. The studies of Flack et al. (3) reporting preliminary results of a significant in vivo stimulation of plasma corticosterone levels with PGE_2 differ from the present studies. This difference may be related to their using rats that had been hypophysectomized for only 3 to 6 hours, whereas our studies were carried out 24 hours following hypophysectomy.

A possible interpretation of the present data is that an allosteric control mechanism may be involved. PGE_2, although by itself devoid of any significant stimulatory effect on steroidogenesis, may still be regulating the action of ACTH. This may occur if PGE_2 modifies the binding site for ACTH on the plasma membrane, or if it alters one or several membrane-bound enzymes involved in the formation or metabolism of CAMP, including adenylate cyclase, phosphoprotein phosphatase, and phosphodiesterase. Such an allosteric mechanism has recently been postulated by Johnson and Ramwell (11), based on kinetic data of the interaction of prostaglandins with ATPase and adenylate kinase in human erythrocytes and platelets, rat liver mitochondria, and rabbit skeletal muscle. It may be that prostaglandins are involved in the transmission of the signal arising from ACTH-receptor interaction to the catalytic site of adenylate cyclase, a role also suggested for sialic acid (12). These results also suggest that the endogenous levels of prostaglandins in the adrenal cortex of the hypophysectomized rat are sufficient to allow maximal stimulation of corticosteroidogenesis by ACTH. Only by reducing the levels of prostaglandins by indomethacin treatment, has it been possible to demonstrate a decreased response to ACTH.

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