strated in the bioassay using P II sterilized females as hosts (table 1, E), indicates that the cessation of JH synthesis caused by P II is a relatively slow process[6]. The production of only 10% supernumerary nymphs by implantation of CC-CA from P II sterilized female MWB (F) corroborates reports that the damage caused by P II to the CA is irreversible[10,12]. These results indicate that less JH is necessary for the induction of supernumerary nymphs than for stimulation of yolk deposition in adult females. However, 5th instar MWB are only sensitive to implanted CA for about the 1st 13 h after ecdysis[17].

Most of the 5th instar nymphs receiving P II injections required more than the usual 6–7 days to molt. Slama[18] has stressed that the inhibition of growth and the delay of ecdyses are probably due to the antifeedant property of the precocenes. In contrast to the permanent damage done to the CA of sensitive species by P II, it has been our experience that the antifeedant effects of P II are usually temporary; once insects are removed from contact with P II, feeding resumes. It has recently been found that MWB 5th instar nymphs maintained by starvation just below a critical weight will never molt while those that surpass this weight always molt 6–7 days later[19]. Changes in the median neurosecretory cells (MNC) of the brain following P II treatment have been noticed in the MWB[6,20] and there is evidence that the MNC in Hemiptera stimulate food consumption[21]. However, the changes in the MNC of P II treated *Oncopeltus* can be reversed by the application of JH, suggesting that the inhibition of these cells is not permanent and may be due to the absence of a positive feedback from the CA[20].

The results presented here demonstrate a direct action of P II on the CA in vivo rather than on the neural mechanisms of CA regulation. The inactivation of CA by P II in vitro suggests that the parent molecule does not require bioactivation in other tissues. However, since only those CA which are producing JH are susceptible to inactivation it is possible that the cytotoxic effect of P II on the CA results from a specific in situ activation linked to the metabolic activity of the gland.

1 The authors wish to thank Patricia Ferugia for rearing the insects; Dr D. Soderlund, Department of Entomology, New York State Agricultural Experiment Station for helpful discussions; and Dr A.O. Lea, Department of Entomology, University of Georgia for technical advice. To whom reprint requests should be addressed

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## Influence of pinealectomy on corticotropin (ACTH)[1]

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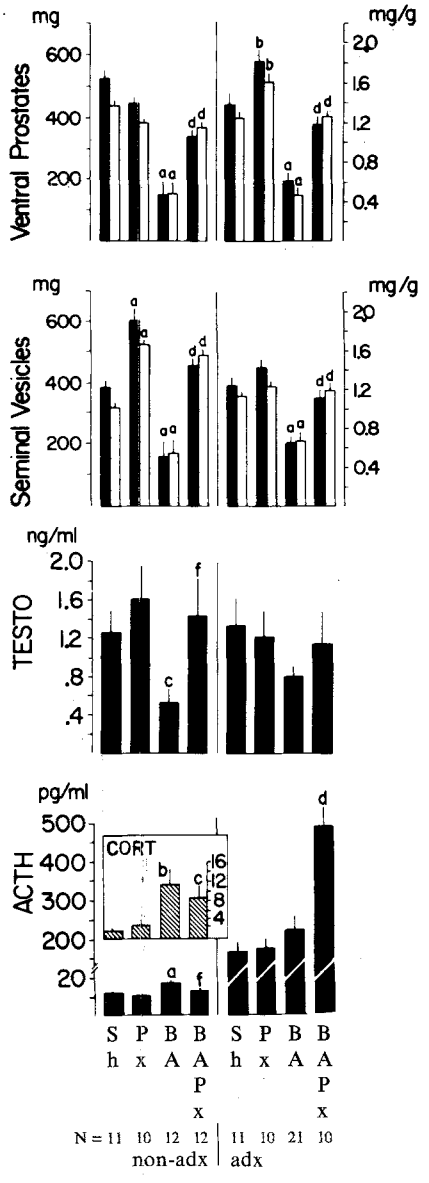
*Summary.* Sensory deprivation produced by removing the eyes and olfactory bulbs in male rats allowed pinealectomy to markedly augment the post-adrenalectomy elevation of ACTH levels. Pineal removal or sensory deprivation separately did not have this effect. Thus, intact sensory input and an intact pineal gland are independently capable of restricting the post-adrenalectomy rise in ACTH levels.

Blinding and anosmia (dual sensory deprivation) in the rat is an established model for pineal-induced suppression of the reproductive system, best reflected in reduced weight of gonadal-dependent structures[3]. The anti-reproductive effect of dual sensory deprivation depends entirely upon an intact pineal gland, with few exceptions[4]. As yet, very little is known about the relationship between blinding-anosmia (BA) and pinealectomy (Px) with respect to the ACTH-adrenal axis.

*Materials and methods.* 5-week old male Sprague-Dawley rats (Madison, Wisconsin) were randomly divided into groups according to the surgical procedures received: Sh, sham-pinealectomy; Px, pinealectomy[5]; BA, removal of the eyes and olfactory bulbs[6]; and BAPx, combined blinding-anosmia and pinealectomy. The BA group was additionally sham-pinealectomized. Rats were then exposed to a cycle of 12 h light (beginning at 06.00 h) and 12 h dark with food and tap water available until 9 weeks of age, when bilateral adrenalectomy (adx) was performed on a portion of each group. All surgical procedures were done under ether anesthesia by us. Adx animals were given 1% NaCl in water (containing oxytetracycline 1 mg/ml for the 1st 7 days) to drink instead of tap water. 21 days after adx, the rats were sacrificed by guillotine at 07.30 h within 90 sec after the animal room was entered under basal c inditions. At autopsy, all previous surgical procedures were seen to be complete. Ventral prostates and seminal vesicles were excised and weighed wet. Trunk plasma was subjected to radioim-




Influence of sham-pinealectomy (Sh), pinealectomy (Px), blinding-anosmia (BA), combined BAPx, and bilateral adrenalectomy (adx) on weight of testicular-dependent organs and on plasma testosterone (TESTO) and plasma ACTH (mean ± SE). The number of rats per group (N) is indicated below the group symbols near the bottom of the figure. The inset in the ACTH graph, labelled CORT, represents corticosterone concentrations in µg/dl. For organ weights, absolute values (dark bars) are referred to the left axis, and values relative to body weight (open bars) are referred to the right axis of each graph. Significance levels are indicated above the bars: a, $p < 0.001$ vs Sh; b, $p < 0.01$ vs Sh; c, $p < 0.05$ vs Sh; d, $p < 0.001$ vs BA; f, $p < 0.05$ vs BA, comparisons being between groups with like adrenal status (non-adx or adx).

munoassay of ACTH[7], corticosterone (Endocrine Sciences, Tarzana, California), and testosterone[8]. Statistical analysis was accomplished using analysis of variance and t-test for several means[9].

*Results.* Suppression of the reproductive system in BA rats, with or without adx, was documented by small ventral prostates and seminal vesicles (figure). These effects were prevented in the BAPx groups. Testosterone levels were suppressed in the non-adx rats after BA unless they had also been pinealectomized (BAPx). There was a higher basal ACTH after BA in the non-adx animals, reversed in the BAPx group. Basal corticosterone levels were also higher in the BA and BAPx non-adx rats. There was a much greater post-adx elevation of ACTH levels in BAPx than in Sh, Px or BA rats.

*Discussion.* As far as we are aware, this is the 1st report of an effect of the pineal gland on measured ACTH levels, although previous studies[10,11] indirectly suggested that such an effect might be possible. In the present study, there was no observed effect of Px alone in rats with or without intact adrenal glands. The effect of the pineal is seen by comparing the BA and BAPx groups (figure). In the presence of the adrenals, the small rise in ACTH in BA rats was prevented by combined BAPx. BA alone in the present study resulted in higher morning levels of corticosterone about 2 months after surgery, in contrast to the results of Dunn et al.[12] who found suppression of these levels about 3 weeks after BA. Based on what is known about blinded rats[13], it is possible that the length of time following BA may explain the discrepancy, in that light deprivation for different lenghts of time might allow different amounts of phase-shift in a free-running rhythm with an altered period in the BA rats. However, the 24-h pattern of corticosterone and ACTH secretion of the BA rat has not yet been characterized. Although Px alone raised morning corticosterone levels in 1 study[14], our failure to demonstrate this is compatible with the results of others[15-17].

Interestingly, in rats without the restrictive influence of the adrenals, combined BAPx removed a significant residual inhibitory influence still present after adx alone. That is, the inhibitory influence of the pineal could be uncovered by observing ACTH levels in adx blind-anosmic animals either with (BA) or without (BAPx) the pineal intact. Similar comparison between Px and BAPx groups of adx rats allows observation of the inhibitory influence of intact sight-smell. In contrast, intact sight-smell was supportive of the reproductive system (Sh vs BA) by preventing the inhibitory influence of the pineal (BA vs BAPx) on testicular-dependent organs, with or without intact adrenals. As far as we know, this is the 1st report of suppression of testosterone concentrations in BA rats, also prevented by additional Px. Why the tendency toward lower testosterone levels was not statistically significant in the BA group after adx is not explained. An unproven speculation might be one suggesting that the high post-adx levels of ACTH might counteract the inhibitory pineal influence on testosterone control mechanisms. Whether a longer interval between adx and sacrifice would then allow some actual recrudescence of testicular-dependent organs in BA adx animals is not yet known.

In the present study, the largest effect of Px on ACTH was observed after adx and in conjunction with BA. These data suggest a role for both the pineal and sight-smell in control of ACTH. Future studies, employing more sampling points throughout the day and night, should determine whether absence of the pineal and of sight-smell modifies ACTH levels primarily by altering tonic secretion or by changing rhythmic phasing of hormone levels. For Px, the latter explanation is unlikely, because Px does not affect the corticosterone rhythm of rats[16].

1 This work was supported in part by NIH Grant No. P30 HD 10202. We thank F. Lynd and R.J. Reiter for advice, P. Starr and J. Sackman for technical assistance, and Bess Mitchell for typing.

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## DOCA administration increases renal phospholipase activity in the rat[1]

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*Summary.* The phospholipase activity of renal tissue has been evaluated in controls and in DOCA treated rats. DOCA treated animal showed a higher than normal enzyme activity. Since a phospholipase is the key step in prostaglandin biosynthesis, it is suggested that the increased prostaglandin release promoted by mineraloactive steroids is mediated by an activation of this key enzyme.

Recent studies strongly suggest that interactions capable of modifying renal function occur between the kidney kallikrein-kinin and prostaglandin systems, as well as with mineraloactive steroids. In fact, the kallikrein-kinin system has been shown to influence the release of renal prostaglandins[3,4]. On the other hand, increased urinary kallikrein excretion has been observed in man with primary aldosteronism[5], and in animals receiving sodium retaining steroids[6]. Since the interaction between the kallikrein-kinin system and prostaglandins seems to take place through an activation of a phospholipase $A_2$[7], we have evaluated the phospholipase activity of renal tissue in DOCA-treated rats.

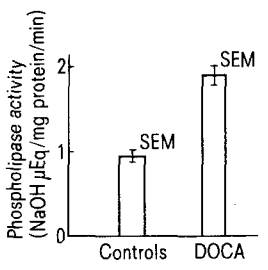
*Experimental.* The study was carried out in 16 male Sprague-Dawley albino rats weighing 230–280 g, which were divided into 2 groups. Group 1 consisted of 8 rats which were injected with 25 mg DOCA acetate in 1 ml sesame oil every 5 days for 20 days, and were fed a standard laboratory diet with free access to 0.9% NaCl as drinking fluid. Group 2 consisted of 8 control animals which were injected every 5 days with sesame oil alone, and were fed the same diet, but had free access to tap water as drinking fluid.

Blood pressure was measured employing a photometric cell and a proximal cuff on the tail of the conscious prewarmed animal. At the end of the study the animals were killed under ether anesthesia. The kidneys were excised, washed with 0.25 M sucrose until the venous effluent was clear, decapsulated, minced with a razor blade, and homogenized in a Potter-Thomas homogenizer using 6 ml sucrose. The homogenates were then centrifuged at 3000 rpm for 10 min in a Sorvall centrifuge at 4 °C, the precipitate was discarded, and the clear supernatant was employed for the determination of protein concentration and phospholipase activity. Protein concentration was determined according to Lowry et al.[8], employing bovine serum albumin as a standard. The assay of phospholipase activity was carried out as previously described[9] and expressed as μeq. NaOH/min/mg protein. The mean protein concentration and phospholipase activity of the 2 kidneys of each rat was considered for statistical analysis which was carried out using the t-test for unpaired variables.

*Results.* The mean increase in body weight during the experimental period was 14±3 SEM g in DOCA treated and 25±5 SEM g in control rats, without a significant difference between the 2 groups (t = 1.5380). Blood pressure in DOCA treated rats was 115±1 SEM mmHg at the start and 136±3 SEM mmHg at the end of the study, and this increase was significant (t = 4.9766; $p < 0.001$). Blood pressure in control rats showed no significant variation (117±1 SEM mmHg at the start and the end of the study t = 0). Mean kidney protein concentration was 65±2 SEM mg in DOCA treated, and 66±3 SEM mg in control rats, without any difference between the 2 groups (t = 0.2514). Mean renal phospholipase activity was 1.916±0.113 SEM μeq/NaOH/min/mg protein DOCA treated, and 0.941±0.065 SEM μeq/NaOH/min/mg protein in control rats; the difference between the 2 groups was highly significant (t = 7.4433; $p < 0.001$).

*Discussion.* It has been repeatedly demonstrated that an excess of mineraloactive steroids increases kallikrein production[5,6,10], and that the generated kinins in turn promote prostaglandin release[4]. Recently Nasjletti et al[11] have confirmed that in DOCA treated rats the increase in prostaglandin production is kallikrein-dependent, since enzyme inhibition lowers prostaglandin release.

However, although the mechanism by which the kallikrein-kinin system influences prostaglandin synthesis has been postulated, it has never been demonstrated. From our data it appears that in DOCA treated rats there is a clear cut increase in renal phospholipase activity, and this enzyme is considered the key step in prostaglandin biosynthesis[7]. This increase in renal phospholipase activity might also affect the renin-angiotensin system, since the liberated lysophospholipids are know to inhibit the renin-angiotensinogen




Shows the kidney phospholipase activity of controls and DOCA treated rats.