procedure required for the preparation of cycasin aglycone is complex (*4, 10*), this host-mediated method may be advantageous for further study of cycasin and related compounds with additional microbial indicators.

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Prostaglandin Stimulation of Rat Corticosteroidogenesis

Abstract. *Prostaglandins and their C20:ω6 fatty acid precursors are present in rat adrenal glands. Small doses of prostaglandins (PGE_1, PGE_2, or $PGF_{1\alpha}$, 1.4 to 2.4 micromolar) increased steroidogenesis in the superfused adrenal glands obtained from hypophysectomized rats. This effect was mimicked in part by both adrenocorticotropin and its postulated intracellular intermediate adenosine 3′,5′-cyclic monophosphate; all three responses were inhibited by cycloheximide.*

The suggestion that prostaglandins may regulate the intracellular concentration of adenosine 3′,5′-cyclic monophosphate (cyclic AMP), the postulated intracellular intermediate of hormonal action (*1*), was made at the Nobel Symposium on prostaglandins (*2*) and this hypothesis was based on the finding that prostaglandin efflux from adipose tissue was directly associated with hormonal stimulated lipolysis (*3*), a mechanism known to involve cyclic AMP and which can be inhibited by PGE_1 (*4*); PGE_1 was known to inhibit other hormonal responses, namely, the permeability response of toad bladder (*5*) and kidney tubules to vasopressin (*6*).

In adipose tissue a hormone-activated lipase was believed to be involved in prostaglandin release; this released fatty acids including bis-homo-γ-linolenic acid, and this C20:ω6-polyenoic acid underwent cyclization and oxidation by the microsomal prostaglandin synthetase to yield PGE_1 (*7*); PGE_1 then acted back to prevent further formation of cyclic AMP, thus serving as a feedback regulator of the lipolytic mechanism. This hypothesis was subsequently extended to the rat gastric mucosa where the hormonal response (acid secretion) was also believed to be mediated via cyclic AMP. Prostaglandin and free fatty acid release from the mucosa was detected, and reapplication of PGE_1 resulted in inhibition of the acid secretion induced by nervous, drug, or hormonal stimulation (*8*).

As in most tissues, prostaglandins are present in the rat adrenal gland (*9*), but unexpectedly the concentrations of prostaglandins present and released from the gland were reduced on adrenocorticotropin (ACTH) stimulation of the adrenal. However, more prostaglandins may be obtained from the superfusate than can be directly extracted from the gland. Furthermore, there are relatively large concentrations of the C20:ω6 precursor of PGE_2 and $PGR_{2\alpha}$ present in the form of cholesterol arachidonate (*10*).

In this report we now present data on the effect of prostaglandins on rat corticosteroidogenesis when a continuous superfusion method is used (*11*).

Female Sprague-Dawley rats (120 to 140 g) were obtained 3 to 6 hours after acute hypophysectomy by the parapharyngeal route (Charles River Breeding Laboratories, Inc.). The adrenal glands were removed, trimmed of fat, bisected, and decapsulated. Glands from not less than ten rats were used for each incubation; approximately 20 minutes elapsed from the time of killing to the beginning of the incubation during which time the glands were kept in ice-cold Krebs-Ringer bicarbonate solution. The bisects were superfused, in a volume of 2 ml at 0.4 ml/min, with Krebs-Ringer bicarbonate solution plus glucose (200 mg/100 ml). The medium was gassed with a mixture of oxygen and carbon dioxide (95:5), and the vessel was agitated in a Dubnoff shaker at 37°C. The effluent was collected every 30 minutes in an automatic fraction collector. One experiment consisted of two incubations (test and control), each lasting 5 to 6 hours. Corticosterone, the principal steroid secreted by the rat adrenal cortex, was assayed fluorimetrically (*12*) with an Aminco-Bowman fluorimeter. The contribution of nonspecific fluorescence when incubation media are assayed in vitro is almost negligible (*13*). Neither PGE_1, PGE_2, nor $PGF_{2\alpha}$ interfered with the corticosterone assay. Prostaglandins, cyclic AMP, ACTH, and cycloheximide were prepared in the superfusing medium.

During the first 1 to 2 hours a small but significant increased release of corticosterone was always observed (Fig. 1, open circles), which may be due to residual ACTH, since the phenomenon was not seen in rats that had been hypophysectomized 48 hours earlier. The rise may also be a consequence of the change from Krebs solution at 4°C to 37°C. The basal release of corticosterone was about 0.4 μg per rat per hour. The preparation responded to graded doses of ACTH and cyclic AMP with increased steroid output.

The compounds PGE_1 (2.8 μM), $PGF_{2\alpha}$ (2.8 μM), and PGE_2 (1.4 to 2.8 μM) increased steroidogenesis, PGE_2 being the most potent of these. Introduction of PGE_2 (2.8 μM) into the medium caused a rapid and highly significant increase in steroidogenesis which was maximal within 1 hour ($P < .01$). This stimulation was not sustained, and 3 hours after the start of the PGE_2 perfusion there was no significant difference in corticosterone secretion although the PGE_2 was in contact with the adrenals (Fig. 1).

The steroidogenic effect of PGE_2 was dose dependent. The maximum steroid response to 2.8 μM PGE_2 was 1.2 μg per rat per hour (Fig. 1) and that to 1.4 μM PGE_2 was 0.8 μg per rat per hour (Fig. 2). The lower limit for a detectable response was 0.28 μM. The response elicited by PGE_2, in contrast to that obtained by equipotent doses of ACTH and cyclic AMP, was transitory. The decay of the steroidogenic response to PGE_2 is clearly seen in Fig. 1 (2.8 μM) and in Fig. 2 (1.4 μM), where, in contrast to ACTH (0.5 milli-international unit per milliliter) and cyclic AMP (1.0 μM), PGE_2 did not exert a significant steroidogenic effect after 4 hours.

The steroidogenic response to graded

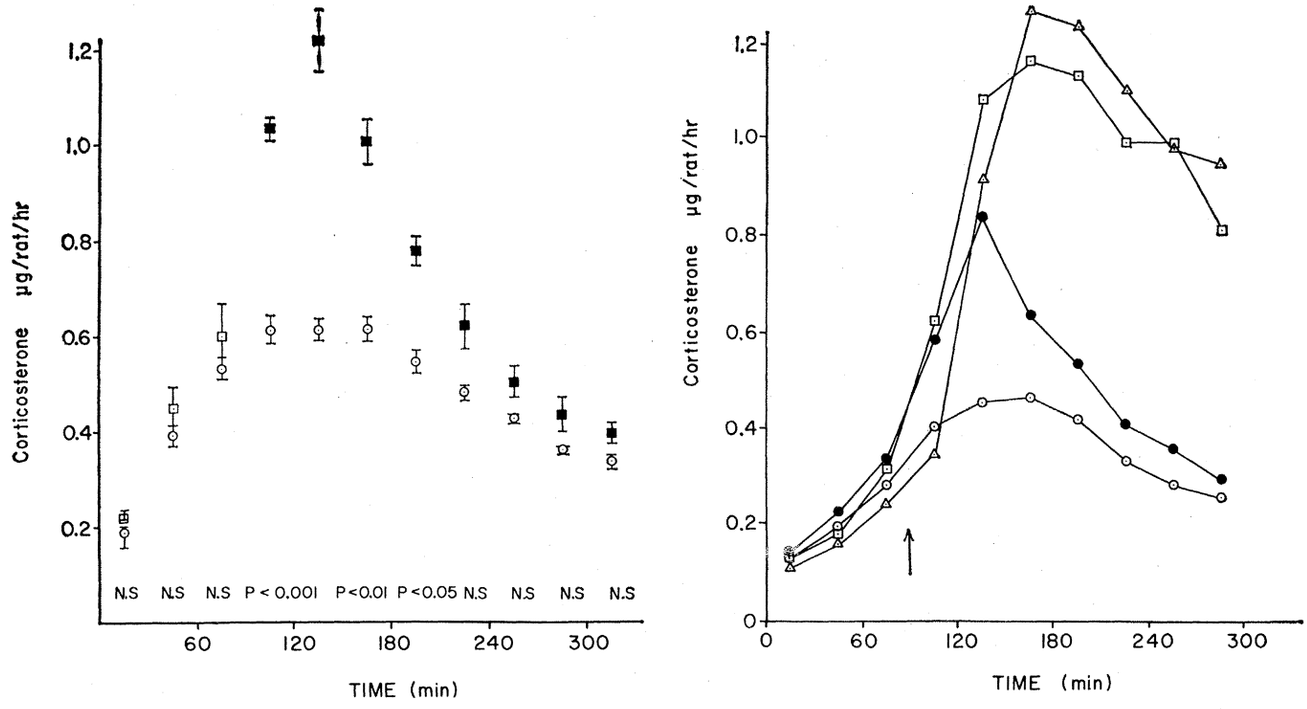

Fig. 1 (left). Stimulation of corticosteroidogenesis by PGE_2. (○, ⊡) Krebs-Ringer bicarbonate solution plus glucose; (■) PGE_2 (2.4 μM). Each point is the mean ± standard error of four experiments. Differences between the two groups were tested by Student's *t*-test and the probability levels are shown along the abscissa; N.S., not significant. Fig. 2 (right). The effect of PGE_2 (1.4 μM) (●), ACTH (0.5 milli-international unit per milliliter) (⊡), and cyclic AMP (1.0 mM) (△) on corticosteroidogenesis. The mean of the three control incubations is shown by open circles. The remaining points are single observations. Drugs were perfused continuously from the time indicated by the arrow.

doses of PGE_2 was also clearly evident in rats hypophysectomized 48 hours earlier. Thus the response could not be ascribed to residual ACTH attached to binding sites (*14*). Moreover, the response to PGE_2 was inhibited by cycloheximide (1 μM). The steroidogenic responses to ACTH and cyclic AMP are both inhibited by cycloheximide (*15*), and in consequence it is reasonable that PGE_2, like ACTH and cyclic AMP, is acting prior to the formation of pregnenolone (*16*), since cycloheximide is believed to inhibit the formation of a protein controlling this part of the steroid biosynthetic pathway (*17*).

Preliminary work has shown that PGE_2 in vivo, like ACTH and cyclic AMP, significantly increases ($P < .001$) plasma (+ 70 percent) and adrenal (+ 40 percent) corticosterone concentrations. The maximum effect was observed within 15 minutes of administering PGE_2 (1 mg/kg intraperitoneally) into female rats hypophysectomized 3 to 6 hours earlier. This effect was unlikely to be due to the hypotension, diarrhea, and other effects of PGE_2, since the steroidogenic effect was also elicited in vitro.

The physiological role of the prostaglandins is still obscure in spite of the intensive work of the last few years. There are at least two major hypotheses: (i) that prostaglandins released on stimulation reduce platelet aggregation and act as functional vasodilating substances, and (ii) that prostaglandins regulate the action of hormones by modifying intracellular cyclic AMP concentrations. It is clear from the above results that the prostaglandins may mimic the action of hormones and added cyclic AMP on some target tissues as well as inhibit hormone effects on other tissues. Recent work of Butcher and his colleagues (*4*) indicates that PGE_1 may increase as well as decrease cyclic AMP formation, depending upon the tissue to which the prostaglandin is applied. In consequence, there now exists the possibility that prostaglandins may serve a positive or negative feedback role in regulating cyclic AMP formation.

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