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REVERSAL OF PROSTAGLANDIN E_2 EFFECT ON ADRENAL CATECHOLAMINE RELEASE AFTER HYPOPHYSECTOMY

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Prostaglandin E_2 induced increased catecholamine release *in vitro* from adrenals of hypophysectomized rats, while in adrenals of intact rats catecholamine release was suppressed by prostaglandin E_2.

Introduction Catecholamine release from adrenergic nerve terminals has been shown to be modulated by α-adrenoceptor agonists as well as by prostaglandins, mainly prostaglandin E (Hedqvist, 1970; Langer, 1974; Starke & Endo, 1976). We have reported that a similar modulation can be demonstrated in the adrenal medulla, i.e. inhibition of catecholamine release, in an *in vitro* incubated adrenal, by the addition of either phenylephrine or prostaglandin E_2 (Boonyaviroj & Gutman, 1975; Gutman & Boonyaviroj, 1977). These effects were found both in rat and in human adrenal medulla slices, incubated *in vitro* (Boonyaviroj & Gutman, 1977). However, when catecholamine release from slices of human phaeochromocytoma, incubated *in vitro*, was studied, prostaglandin E_2 consistently caused augmentation of catecholamine release rather than inhibition (Gutman & Boonyaviroj, 1976). The opposite effect of prostaglandin E on catecholamine release from human adrenal medulla and from phaeochromocytoma was also observed when both tissues were obtained from the same patient (Gutman & Boonyaviroj, 1976). This 'reversal' of prostaglandin effect therefore required elucidation.

Phaeochromocytoma cells differ in two parameters from normal adrenal medulla cells: they are not innervated, and their blood supply does not derive from the adrenal cortex (Winkler & Smith, 1972).

Therefore, we set out to mimic the lack of adrenal cortical blood supply in phaeochromocytoma by reducing the corticosteroid supply to the normal adrenal medulla. This can be achieved by hypophysectomy, which abolishes adrenocorticotrophic hormone (ACTH) secretion and, thereby, reduces corticosteroid release from the adrenal cortex. The effect of prostaglandin E_2 on catecholamine release from adrenals of hypophysectomized rats was therefore studied.

Methods Male rats of the Hebrew University strain, weight 180–200 g, were used throughout. Hypophysectomy was carried out under anaesthesia with ether-oxygen mixture, through a transpharyngeal approach. A 1 mm hole was drilled into the basophenoid bone and the hypophysis was aspirated by suction. Postoperatively the rats were maintained on Purina-chow, and the drinking fluid contained 0.45% NaCl and 2.5% glucose. The adrenal glands were removed for incubation *in vitro* 12 days after the operation.

Rats were killed by dislocation of the neck and the adrenal glands were immediately taken out. Incubation of adrenal glands was carried out as previously described (Boonyaviroj & Gutman, 1975; Gutman & Boonyaviroj, 1977). Each gland was cut into two halves and was placed in a 50 ml conical flask containing 10 ml of Locke solution of the following composition (mM): NaCl 154, KCl 5.6, $MgCl_2$ 5, $CaCl_2$ 0.5, $NaHCO_3$ 1.8 and glucose 5. Incubation was carried out at 37°C in a thermostatic bath, with constant shaking, for 10 minutes. At the end of incubation the glands were separated from the incubation medium. Both the glands and the incubation medium were acidified, with $HClO_4$ (final concentration 0.4 M). Catecholamines were adsorbed on alumina columns, eluted with 0.05 M perchloric acid and then passed through Bio-rex 70 columns (Feuerstein, Boonyaviroj & Gutman, 1977). Adrenaline and noradrenaline were assayed by the trihydroxyindole method using an Aminco-Bowman spectrofluorimeter (Feuerstein *et al.*, 1977).

Acetylcholine chloride (ACh) was purchased from Sigma Chemical Co., St. Louis, Mo. and prostaglandin E_2 was a generous gift of Dr J. Pike, The Upjohn Co., Kalamazoo, Mi.

Results Figure 1 shows that the addition of prostaglandin E_2 (at 10^{-7} M) to rat adrenal glands incubated *in vitro* caused a substantial inhibition of catecholamine release, as we have previously reported, both for rat and for human adrenal medulla (Boonyaviroj & Gutman, 1977). Furthermore, prostaglandin E_2 also inhibited the release of catecholamines caused by ACh. However, when the adrenal glands were obtained from rats after hypophysectomy, the addition of prostaglandin E_2 to the incubation medium caused a significant increase of catecholamine release. This corroborates the effect of prostaglandin E_2 on catecholamine release from human phaeochromocytoma, as previously reported (Gutman & Boonyaviroj, 1976). When catecholamine

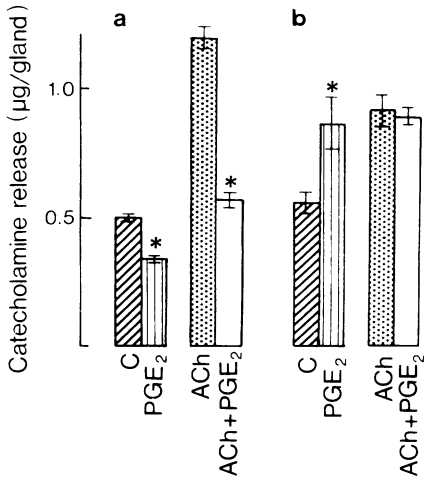

Figure 1 Effect of prostaglandin E_2 on catecholamine release from adrenal glands of control and hypophysectomized rats. C—incubation of adrenal glands without stimulation; PGE_2-incubation of adrenal glands in the presence of prostaglandin E_2 10^{-7}M. ACh—incubation of adrenal glands in the presence of acetylcholine 10^{-4} M. (*a*) Adrenal glands obtained from intact rats. C $n=20$; PGE_2 $n=20$; ACh $n=10$; ACh + PGE_2 $n=10$. (*b*) Adrenal glands obtained from hypophysectomized rats. C $n=20$; PGE_2 $n=22$; ACh $n=21$; ACh + PGE_2 $n=23$.

*$P<0.01$ for the difference between adjacent columns.

release from adrenals of hypophysectomized rats was stimulated by ACh, prostaglandin E_2 did not inhibit the release (Figure 1). However, there was also no significant increase of catecholamine release above that caused by ACh. This could be due to maximal stimulation of release by ACh, so that no additional release could be observed.

Discussion The blood supply of the adrenal medulla derives from the adrenal cortex and, therefore, contains a substantially higher concentration of steroids than blood perfusing other organs. Hypophysectomy abolishes the supply of ACTH to the adrenal cortex and, therefore, reduces the synthesis and release of corticosteroids. Thus, the supply of steroids to the adrenal medulla in hypophysectomized rats is reduced. A similar situation occurs in phaeochromocytoma, where the tumour has a blood supply that does not derive from the adrenal cortex. Under both these conditions, phaeochromocytoma and hypophysectomy, the effect of prostaglandin E_2 on catecholamine release was a significant increase, while in adrenal glands from intact rats or normal human adrenal medulla, prostaglandin E_2 caused a substantial inhibition of catecholamine release.

The supply of corticosteroids could, therefore, be a determining factor in the type of response to prostaglandin E_2, in which case, it would be an interesting example of the modification by steroids of a membrane response. Several possibilities for the mechanism of such a modification may be proposed, e.g. there could be a direct effect of the steroids on membrane composition and/or structure, or the steroids could initiate an intracellular synthetic process which would result in the production of a different receptor incorporated into the membrane. These possibilities are now being studied.

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