Effects of Prostaglandin E_2 on the Uptake of ^{3}H-Thymidine in Pregnant Mice

Despite the widespread clinical use of certain prostaglandins for the termination of pregnancy and the induction of labor, little attention has been directed to the effects of these substances on fetal and maternal metabolism. Prostaglandin E_2 (PGE_2) administered to pregnant rats[1] and mice[2] induced a high incidence of fetal death and resorptions. The mechanism for this lethal action is uncertain. We report here the effects of PGE_2 on the uptake of ^{3}H-thymidine in maternal and fetal tissues of mice.

Materials and methods. Prostaglandin E_2 (50 or 100 µg) was administered s.c. to pregnant Swiss Webster mice from day 8 through 12 of gestation. Another group of mice received the solvent alone and served as controls. The animals were killed on the last day of treatment 1 h after an i.v. administration of 1 µCi/g ^{3}H-thymidine (New England Nuclear; Specific Activity: 6.7 C/m*M*). Selected maternal tissues and the conceptuses were excised under a dissecting microscope and processed subsequently to determine the concentration of radioactivity. The tissue samples were pooled and weighed immediately after removal, dissolved in NCS and neutralized with acetic acid. 10 ml of a scintillation cocktail (5 g PPO; 0.5 g POPOP; 250 ml ethylene glycol monomethyl ether; 750 ml toluene) was added and the samples were counted three times for 10 min in a Beckman LS 150 Liquid Scintillation Counter.

Uptake of ^{3}H-thymidine in pregnant mice treated with prostaglandin E_2

Tissues	Control	Prostaglandin-treated	
		50 µg	100 µg
Maternal			
Brain	303.2 ± 50.4	210.0 ± 11.5	152.5 ± 10.1
P[b]		< 0.05	< 0.01
Lung	387.6 ± 68.8	252.4 ± 54.6	211.3 ± 31.1
P		< 0.05	< 0.05
Liver	653.7 ± 122.5	591.1 ± 101.4	520.2 ± 98.3
P		NS	< 0.05
Kidney	451.5 ± 75.3	303.4 ± 82.7	401.4 ± 90.5
P		< 0.05	NS
Spleen	1881.2 ± 183.4[a]	1555.8 ± 190.3	922.0 ± 167.9
P		< 0.05	< 0.01
Embryos	662.9 ± 81.1	324.6 ± 53.5	
P		< 0.01	

[a] Mean counts per min (cpm) per mg wet tissue weight. Standard error of the mean on the basis of 10 min counts; [b] *P*, significance of difference from the control; NS, not significant.

Results and discussion. A summary of our findings is presented in the Table. The uptake of ^{3}H-thymidine in maternal and embryonic tissues was significantly reduced following pre-treatment with the prostaglandin.

Inhibition of ^{3}H-thymidine uptake in maternal liver, brain and spleen showed a dose dependent relationship. The uptake of ^{3}H-thymidine was markedly reduced in the embryos. However, a correlation between the uptake of ^{3}H-thymidine in the 2 treatment groups was not possible, since all fetuses had been resorbed at the higher dose level.

These preliminary findings suggest that PGE_2 is capable of inhibiting DNA synthesis, either directly or indirectly. This inhibition could account for some of PGE_2 effects on the fetus and placenta during pregnancy[3,4]. Further studies of ^{3}H-thymidine, ^{3}H-uridine, and ^{3}H-leucine at different gestational periods are in progress.

Zusammenfassung. Die Behandlung von trächtigen Mäusen mit Prostaglandin E_2 führt zu einer Verminderung des Einbaus von ^{3}H-Thymidin im mütterlichen und fötalen Gewebe.

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The Response of the Adrenal Gland to Hypoglycaemia in the Conscious Calf

A technique has recently been devised which permits collection of the whole of the effluent blood from the innervated right adrenal gland in the conscious unrestrained calf[1,2]. The present paper describes experiments in which this technique has been employed to investigate the changes in glucocorticoid and catecholamine output from the gland during insulin hypoglycaemia.

Materials and methods. Insulin was injected i.v. at doses of 0.1, 0.5 or 4.0 units/kg 14–24 h after surgery. Adrenal blood flow was estimated gravimetrically and the outputs of steroids and catecholamines were then calculated from the adrenal venous plasma concentrations. Adrenaline and noradrenaline were estimated by a modification of von Euler and Lishajko's fluorimetric technique[3], glucocorticoids by competitive protein binding assay[4] and glucose enzymatically.

Results and discussion. Results of a typical experiment illustrating the characteristic responses to insulin hypoglycaemia at each dose are compared in the Figure.

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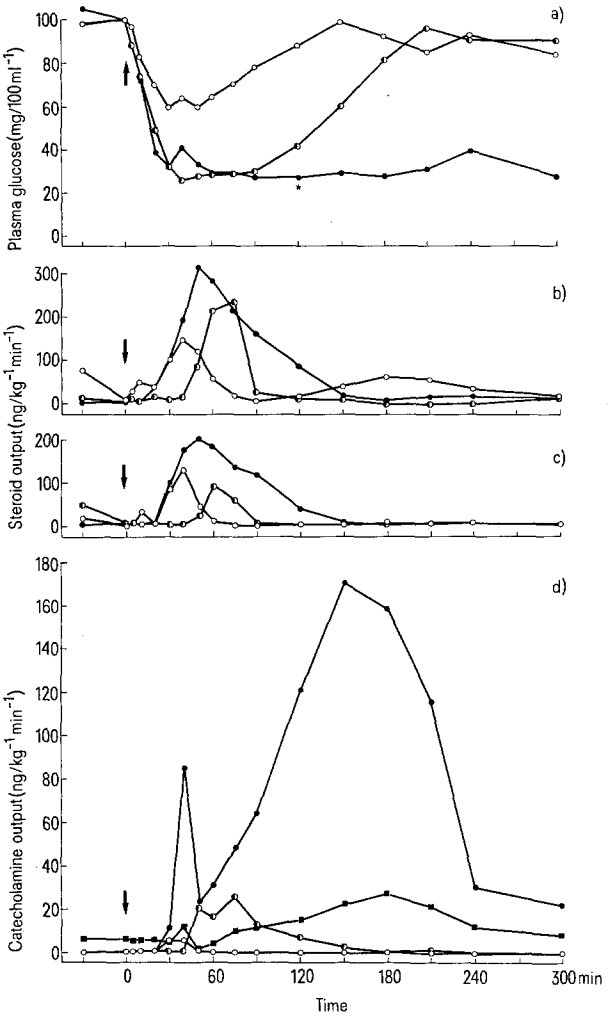
Moderate hypoglycaemia (0.1 unit insulin/kg) invariably produced a transient increase in the output of both cortisol and corticosterone and caused a temporary rise in the concentrations of both steroids in the peripheral arterial plasma. No change in noradrenaline output was detected but a small increase in adrenaline output occurred in some animals between 30 and 60 min after insulin. This response was present whenever a temporary rise in plasma glucose concentration was observed (Figure). Administration of 0.5 unit insulin/kg produced more severe hypoglycaemia but a normal plasma glucose concentration was restored within 3–4 h. By comparison with animals given 0.1 unit insulin/kg, the outputs of cortisol and corticosterone generally rose to higher peak values and were elevated for longer; higher outputs of adrenaline but not noradrenaline were also encountered (Figure). In some, but not all, of these animals a transient increase in adrenal blood flow occurred between 30 and 90 min which was unrelated to any variation in heart rate or blood pressure.



Comparison of the responses of 3 individual conscious calves to insulin at 0.1 (○), 0.5 (◐) and 4.0 units/kg (●, ■). a) Plasma glucose concentration. b) Cortisol output from the right adrenal gland. c) Corticosterone output from right adrenal gland. d) Adrenaline (○, ◐, ●) and noradrenaline (■) outputs from right adrenal gland. Vertical arrows represent administration of insulin. Asterisk represents time at which animal given largest dose of insulin convulsed.

Comparison of these results with those obtained using a still larger dose of insulin (4 units/kg) shows that the increase in glucocorticoid output was transient even though the stimulus provided by severe hypoglycaemia persisted. Intravenous infusions of ACTH produced increased steroid output in the calf at a dose as low as 0.1–0.5 ng/kg^{-1} min^{-1} and higher doses (5.0–50.0 ng/kg^{-1} min^{-1}) are required to increase adrenal blood flow and raise the proportion of corticosterone secreted[1,2]. Increased steroid output during severe hypoglycaemia was generally accompanied by a pronounced rise in adrenal blood flow, together with a fall in the cortisol: corticosterone ratio. The magnitude of these responses is comparable with those to infusion of 5ng ACTH/kg^{-1} min^{-1} [1,2]. Each of these animals collapsed and convulsed 2–3 h after administration of insulin but onset of convulsions was not related to any consistent change in adrenal function. The rise in steroid output was usually associated with increased release of adrenaline, as in animals given smaller amounts of insulin. However, as the glucocorticoid output declined, steadily increasing amounts of catecholamines were secreted. Adrenaline was always the predominant amine released in response to hypoglycaemia and amounted to between 75 and 95% of the total. This is difficult to reconcile with previous findings, in acute experiments under barbiturate anaesthesia in calves of the same age, in which proportionately greater amounts of noradrenaline were consistently secreted in response to splanchnic nerve stimulation or intra-arterial acetylcholine[5,6]. Nevertheless, the absolute amounts of adrenaline released during prolonged hypoglycaemia in the present experiments are in excellent agreement with those which would be predicted from a study of the effects of insulin in conscious calves[7].

Résumé. La sécrétion de glucocorticoides et de catécholamines par la glande surrénale droite fut observée chez des veaux conscients après une injection d'insuline. L'accroissement de la production de stéroides fut passagère malgré une hypoglycémie ininterrompue.

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Crustecdysone is Without Estrogenic or Antiestrogenic Activity in the Rat

Crustecdysone (β-ecdysone, 20-hydroxyecdysone or ecdysterone) occurs naturally in insects[1–4] and crustaceans[5] and is capable of inducing moult in most arthropod groups[6,7]. It also occurs in plants in very large quantities[8–10]. Ecdysone has been reported to stimulate protein synthesis in mouse liver[11] and to inhibit growth of sarcoma and embryonic mouse fibroblasts[12]. Since the ecdysone in plants and animals is likely to find its way into cattle and humans through their food, it was thought worthwhile to scan this compound for its possible estrogenic and antiestrogenic activity.

An inbred strain of 80–120-day-old, 4-day cycling, adult mature white rats on an LD regime of 12:12, was used for the present study. The animals were maintained on Bengal gram and water supplemented with milk. Estrogenic activity was studied by Allan-Doisy test of vaginal cornification[13] and antiestrogenic activity was studied by the vaginal response method[14]. The animals were spayed and when the endogenous estrogen completely disappeared, as shown by absence of vaginal cycling (a phase contrast microscope was used for examination of the smear), a priming dose of 1 µg of estradiol dipropionate (Ovocyclin, Ciba India) in 0.1 ml olive oil was injected s.c. into each rat. When the estrogen completely disappeared from the blood stream, as shown by vaginal cycling method, a total of 30 µg of a solution of ecdysterone (obtained through the kind courtesy of Dr. D. KING to Dr. G. C. UNNITHAN and from Dr. W. E. ROBBINS) in 0.02 ml of 50% aqueous glycerol was delivered into the vagina of each rat in 2 doses at 10.00 h on 2 consecutive days through a steel canula attached to a syringe, to a group of 10 rats. An equal number of controls were given glycerol only. Smears were taken and read at 10.00 and 17.00 h on the 3rd day. Smears of all the experimental and control animals were found equally negative. Into another group of animals, 0.6 µg of the estrogen contained in 0.1 ml olive oil was injected s.c. at 09.00 h and 2 doses of ecdysterone in 0.02 ml of 50% aqueous glycerol (total dose, 0.5 mg) was administered intravaginally at 09.00 and 17.00 h, whereas the controls received 0.02 ml of 50% aqueous glycerol. Examination of the smear after 56, 64, and 72 h of first administration indicated no difference between the controls and the experimentals, both being positive. Hence it may be seen that ecdysterone does not have either estrogenic or antiestrogenic activity as detectable by the methods employed. The absence of estrogenic activity to ecdysterone is in accord with the structure of estrogenic steroids which have a phenolic A-ring and a carbon atom in position 18 but not in position 19[13].

Zusammenfassung. Es wird mittels Vaginalzytologie bei Ratten gezeigt, dass Ecdyson keine Östrogenaktivität und bei östrogenbehandelten Tieren auch keine Anti-oestrogen-Aktivität entfaltet.

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Activité protéosynthétique des glandes prothoraciques et titre d'ecdysone chez des larves permanentes de *Locusta migratoria* obtenues par irradiation sélective du tissu hématopoïétique

L'irradiation sélective du tissu hématopoïétique de jeunes larves de stade V (dernier stade larvaire) de *Locusta migratoria* bloque le déterminisme endocrine de la mue suivante, les insectes évoluant en larves permanentes; une irradiation témoin de régions ventro-abdominales de surface équivalente n'affecte pas la mue[1]. Dans la présente étude nous avons suivi l'évolution des glandes prothoraciques de larves devenues permanentes à la suite d'une radiolésion du tissu hématopoïétique, en choisissant comme critère la vitesse d'incorporation d'un acide-aminé dans les protéines synthétisées pendant un laps de temps donné. D'autre part nous avons suivi l'évolution du taux d'ecdysone à la suite de la radiolésion du tissu hématopoïétique, en la comparant à celle des larves normales, décrite dans un travail précédent[2].

Des larves normales et des larves à tissu hématopoïétique irradié (25000 R, anticathode en tungstène, durée de l'irradiation: 10 min, tension appliquée 42 kV; intensité du courant de chauffage: 32 mA; distance source-objet: 15 cm) reçoivent une injection intraabdominale de leucine tritiée (activité spécifique: 30 Ci/mMol; injection unique de 30 µl par insecte d'une solution correspondant à une radioactivité totale de 30 µCi par injection); les glandes prothoraciques des insectes en expérience sont prélevées aux temps 0, 10 min, 20 min et 40 min dans de l'acide perchlorique; après addition d'albumine bovine et centrifugation à 4000 *g*, le culot est traité à l'acide perchlorique à chaud en vue de l'extraction des seules protéines. Des lavages répétés à l'éthanol permettent de retirer la leucine radioactive non liée aux protéines. La radioactivité liée

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