Influence of dopamine infusion on plasma prolactin released by kidney capsule transplanted anterior pituitaries[1]

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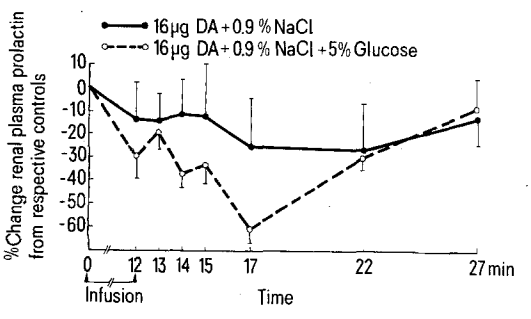
Summary. The infusion of dopamine into the renal artery resulted in decreased prolactin release from 3 anterior pituitary glands transplanted under the kidney capsule. Prolactin levels continually decreased over a 5 min period after DA infusion was terminated and thereafter approached preinfusion levels by the end of 10 min.

The catecholamine dopamine (DA) has been shown to influence the secretion of prolactin from the anterior pituitary[3]. It is believed that dopamine inhibits prolactin release either by acting directly on the pituitary[4] or by influencing the activity of a prolactin inhibiting factor[5]. This study was designed to investigate: a) the effect of DA infusion on the release of prolactin by the kidney capsule transplanted anterior pituitary and b) the time required for prolactin, suppressed by DA infusion, to return to initial values.

Materials and methods. Female Sprague-Dawley rats (Spartan Research Animals, Inc., Haslett, Mich. USA) were ovariectomized when they were 200–250 g in body weight. 2 weeks later, 3 rat anterior pituitary glands were hemisected and transplanted under the left kidney capsule using ether anesthesia. 1–2 weeks after transplantation the animals were anesthetized using methoxyflurane (Metofane, Pitman-Moore, Inc., Washington Crossing, N. J., USA). This anesthetic does not release prolactin in the ovariectomized rat[6]. A midline ventral incision was made and the right iliolumbar vein, vena cava, descending aorta, left kidney and renal artery were exposed. After clamping the aorta 1 cm anterior to the renal artery, the vessel was punctured 0.2 cm anterior to the renal artery with a 23 gauge needle. PE 10 tubing (Intramedic Clay-Adams I.D. = 0.28 mm, O.D. = 0.61 mm) was stretched to a fine diameter using heat, filled with 0.9% NaCl and inserted into the renal artery via the puncture in the aorta. The exposed portion of the catheter was secured to the wall of the aorta directly beneath it with 6–0 suture. When secured in this manner the reduction in blood flow to the kidney was minimal. After removing the clamp 0.9% NaCl was infused at the rate of 3.93 µl/min using a Harvard infusion pump. A catheter of PE 50 tubing (Intramedic polyethylene 0.58 × 0.965 mm) containing 0.9% NaCl and 5 units of heparin/ml was inserted into the aorta anteriad in the region of the common iliac arteries and secured. After ligating the right iliolumbar vein 1.5 cm from the vene cava, PE 50 tubing bent 90° at the tip and containing heparinized saline was inserted into it through an incision made with microscissors. The catheter was then passed into the vena cava to the region of the left renal vein where the bent end of the catheter was inserted into the renal vein. The catheter was then secured to the iliolumbar vein using a ligature. After completing the above procedure blood sampling and infusions proceeded as follows: Heparinized saline (0.2 ml) was injected into the aortic catheter to prevent clotting. 2 initial blood samples (0.3 ml) were withdrawn from the vein and aorta, respectively. All venous samples were withdrawn over a period of 20 sec to prevent vena cava blood from mixing with renal vein blood. Blood volume was replaced with an equal volume of heparinized saline via the aortic catheter for all samples. The infusion solution consisted of either 0.9% NaCl alone, 0.9% NaCl–5% glucose alone, a total of 16 µg dopamine in 0.9% NaCl, or a total of 16 µg DA in 0.9% NaCl–5% glucose. Infusion into the renal artery at a rate of 39.3 µl/min began at time 0:00 and ended at time 0:12, at which time the catheter was removed from the renal artery into the aorta. Venous samples were collected 0:12, 13, 14, 15, 17, 22 and 27 min after the start of infusion. Arterial samples were collected at times 0:12 and 27. After obtaining the last blood sample the animal was sacrificed using an overdose of anesthetic. Serum prolactin levels were determined by radioimmunoassay using NIAMDD-RP-1 as the reference standard. The details of the assay have been published elsewhere[7]. Statistical analysis of the data was accomplished using a two-way analysis of variance[8] with computer assistance.

Results. The initial renal venous and aortic arterial plasma prolactin levels prior to DA infusion were 144.1 ± 33.5ng/ml and 78.8 ± 18.7 ng/ml respectively; the prolactin level in ovariectomized animals without pituitary transplants was 16.4 ± 0.7 ng/ml.



The influence of dopamine (DA) infusion on renal venous plasma prolactin levels. The percent decrease in prolactin was corrected for the decrease observed for saline and saline-glucose control infusions. Vertical lines represent the standard error of the mean.

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3 R. M. MacLeod, in: Frontiers in Neuroendocrinology, vol. 4, p. 169. Ed. L. Martini and W. F. Ganong. Raven Press, New York 1976.
4 R. M. MacLeod and J. E. Lehmeyer, Endocrinology *95*, 1202 (1974).
5 J. Meites and J. A. Clemens, Vitam. Hormo. *30*, 165 (1972).
6 M. G. Subramanian, D. M. Lawson and R. R. Gala, Life Sci. *18*, 305 (1976).
7 E. Y. H. Kuo and R. R. Gala, Biochem. biophys. Acta *264*, 462 (1972).
8 W. J. Dixon and F. J. Massey, jr, in: Introduction to Statistical Analysis, 2nd ed., p. 163. McGraw-Hill Book Co., New York 1958.

The infusion of 16 µg DA in 0.9% NaCl–5% glucose resulted in a significant ($P < 0.01$) decrease in plasma prolactin (figure). A continued decrease in renal plasma prolactin was observed for 5 min after infusion was terminated. At the end of this time a rebound in prolactin secretion was observed reaching the initial value at the end of 10 min. A significant decrease of lower magnitude ($p < 0.05$) in renal plasma prolactin levels was also observed when 16 µg DA in 0.9% saline was infused (figure). The decrease in renal plasma prolactin using DA saline-glucose solution was significantly greater ($p < 0.05$) than that observed for the DA saline solution.

Discussion. It has recently been reported that there was no difference in plasma prolactin levels between the renal and jugular veins for rats with a single pituitary transplanted to the kidney capsule[9]. We have observed a similar finding when only one pituitary was grafted, however, when 3 or more pituitaries were transplanted to the kidney capsule the concentration of plasma prolactin for the renal plasma blood was significantly greater than that for aortic blood (Gala, unpublished observations). We have found a similar result in this study with 3 grafted pituitaries.

The infusion of either saline or saline-glucose without DA resulted in some decrease in renal plasma prolactin (approx. 14%). The reason for the decrease is not known but could reflect some alteration in renal blood flow as a result of infusion of the solutions. In order to visualize the effect of DA on suppressing prolactin release, the percent decrease due to saline or saline-glucose was subtracted from that of DA for the respective carrier solution. It is believed that the suppression of prolactin secretion by DA infusion was due to an action directly on the transplanted pituitary because of the magnitude of the decrease and because the transplanted pituitary contributed the major portion of the prolactin in circulation. It can not be discounted, however, that some of the effect observed may be on the in situ pituitary since the amount of DA administered has been reported in long term infusion studies to suppress prolactin release by the in situ pituitary[10]. The addition of glucose to the carrier solution amplified the inhibitory effects of DA on prolactin release and confirms the observation of others who infused DA into the hypophysial portal vessels[11]. It was suggested that the glucose retards the autooxidation of DA in solution[11].

Our purpose in examining the rebound of prolactin release after DA suppression was to see how rapidly it would occur. It appears that within 1 min after termination of DA infusion (saline-glucose group) there was a slight increase in renal plasma prolactin which was followed by a subseqent decrease over a 5 min period. From this point of maximum inhibition (approx. 50%), 10 min was required for prolactin to reach preinfusion levels. The strongest in vivo evidence for a releasing factor comes from the rapid 2–3 min increase in plasma prolactin induced by ether anesthesia[12,13]. We believe that the initial 1 min increase in prolactin release reflects an increase in vascular flow due to the removal of the catheter from the renal artery. It cannot be completely discounted that the rapid effects of ether on prolactin release may be due to alterations in vascular blood flow to the pituitary. It was demonstrated a number of years ago that ether may induce an initial ($2^1/_2$ min) 40% increase in blood flow[14]. Thus, it may be possible to explain the increase in prolactin to ether without the necessity of a releasing factor. Further, the increase in prolactin induced by ether is about 15–25 ng/ml[12,13], which is comparable to the 17.2 ng/ml change which we have observed here. Once maximum suppression of prolactin is observed, however, approximately 10 min is required for prolactin levels to return to normal, a time compatible with most observations of prolactin release by physiologic stimuli or drug administration. Thus, it may be possible to explain both the rapid increase in prolactin release due to ether and the slower increase due to suckling and drugs by only a hypothalamic inhibitory factor.

9 P. C. O. Lam, W. K. Morishige and I. Rothchild, Proc. Soc. exp. Biol. Med. *152*, 615 (1976).
10 C. A. Blake, Endocrinology *98*, 99 (1976).
11 J. Takahara, A. Arimura and A. V. Schally, Endocrinology *95*, 462 (1974).
12 K. Ajika, S. P. Kalra, C. P. Fawcett, L. Krulich and S. M. McCann, Endocrinology *90*, 707 (1972).
13 J. Terkel, C. A. Blake and C. H. Sawyer, Endocrinology *91*, 49 (1972).
14 H. Goldman, Endocrinology *72*, 588 (1963).

PRO EXPERIMENTIS

A simple technique for scintillation counting of cell preparations on coverslips[1]

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Summary. A simple, rapid and versatile technique for scintillation counting of cells on coverslips is described. The cells are conserved for subsequent staining and autoradiography so that other data can be collected from the same specimen.

A simple scintillation counting technique which avoids the destruction of a specimen of cultured cells and allows further data to be obtained from precisely the same cell population has evident benefits of accuracy and versatility. Errors due to collating results from parallel or analogous cultures are removed, and extensive data can be obtained from relatively little material. There follows a description of such a technique which we are employing in our laboratories, involving scintillation counting of tritium labelled cells grown on coverslips. The cells are conserved for subsequent staining for morphological examination, accurate cell counts, microspectrophotometry and autoradiography.

Materials and methods. Cells are plated out in replicates at a density of 10^5/ml in Leighton tubes containing 11 × 22 mm coverslips and are fed routinely with Eagle's MEN supplemented with 20% foetal bovine serum. When the cultures are semi-confluent and in the log phase of growth (2–4 days), labelled precursor (tritiated thymidine – ^{3}H-TdR) is added to the growth medium for the exposure period, before the cells are harvested by rinsing with saline to remove residual medium and fixing in 95% ethanol. Alternatively, the coverslips may be removed to fresh tubes containing labelled precursor, leaving approximately half of the culture growing on the glass of the Leighton tube, which permits serial sampling