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Size Heterogeneity of Rat Prolactin Secreted *in Vitro*: Effect of Incubation Time and Dopamine (40987)

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Abstract. Culture medium harvested at various times (1, 2, and 6 hr) during the incubation of hemipituitary fragments from ovariectomized estrogen-treated rats was subjected to Sephadex G-100 gel filtration chromatography and the radioimmunoassayable prolactin was determined in the eluted fractions. Three heterogeneous forms of the hormone were found in the medium. As incubation time progressed, the level of the two larger components decreased. Dopamine (1×10^{-6} *M*) added to the incubation medium either during the first or third to sixth hours of incubation had no effect on the relative quantities of the heterogeneous forms being released into the medium.

We (1) and others (2, 3) have previously shown that the rat anterior pituitary contains at least three components of immunoreactive prolactin. We have also demonstrated that the prolactin released into plasma *in vivo* following several stimuli, which cause enhanced secretion, is predominately the smallest form found in the pituitary (1). Dombroske *et al.* (2) have shown that all three components of pituitary prolactin are released into incubation medium *in vitro* and in the same proportions as found in sonicates of pituitaries. The objective of the present study was to confirm this earlier work and to examine what effect, if any, dopamine, a known inhibitor of *in vitro* prolactin secretion (4–6), has on the release of the heterogeneous forms.

Materials and methods. Adult female Sprague–Dawley rats (Spartan Research Animals, Inc., Haslett, Mich.) were used in these studies. Animals were housed two per cage in a limited access room with controlled light (lights on 0600–2000 hr.) and temperature (23°) and were ovariectomized after 2–4 weeks. In addition, all rats were treated sc with 500 μg polyestradiol phosphate (Estradurin, Ayerst Laboratories) 5–7 days before sacrifice which was by stunning and decapitation at 0700–0800 hr. Pituitaries were quickly removed; the neural lobe excised, and the anterior pituitaries were bisected and placed in Medium 199 (Microbiological Associates) containing 28m*M* HEPES and 11 m*M* $NaHCO_3$ (pH 7.4). Two hemipituitaries from separate rats were weighed and placed into 20-ml scintillation vials containing 1 ml of fresh Medium 199 buffered as described above. Hemipituitaries from four rats were allocated to vials in each group described below. Approximately 30 min elapsed between the time the pituitaries were removed and the time when the incubation was started. Incubation of the pituitaries was done at 37° under a 95% O_2–5% CO_2 atmosphere (gas flow, 300 ml/min) in a metabolic shaker bath (Aquatherm, New Brunswick Corporation). Medium was harvested at the end of the first, second, and sixth hours of incubation. Fresh Medium 199 was added at the beginning of the second and third hours. To one group of vials 1×10^{-6} *M* dopamine was added at the beginning of the incubation. Dopamine (1×10^{-6} *M* was added to a second group of vials at the beginning of the third hour. Medium harvested from four vials at each period was pooled and an aliquot was removed and stored for subsequent radioimmunoassay, the details of which are presented elsewhere (1). The remainder of each pool was subjected to gel filtration chromatography.

Gel filtration of the pooled samples of incubation medium was carried out on 2.6 × 90–95-cm columns of Sephadex G-100 or G-150 equilibrated and eluted with the radioimmunoassay buffer (PBS, 0.14 *M* NaCl, 0.01 *M* Na_2HPO_4–NaH_2PO_4, pH 7.6) containing 0.1% bovine serum albumin and 0.01% merthiolate as a preservative. A

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large molecular weight blue dextran (Dextran 2000, Pharmacia; 2.5 mg/ml) was used to mark the void volume of the column at each run. One hundred 4- to 5-ml fractions collected from the beginning of the blue dextran peak were assayed for prolactin by radioimmunoassay using NIAMDD-RP-1 (11 IU/mg) as standard. The prolactin concentrations expressed as nanograms per milliliter of fraction were plotted against the elution volume of each fraction (V_e) corrected for the void volume of the column (V_0) which varied slightly between columns.

Results. During the first hour of incubation when large quantities of hormone were being released into the medium, three prolactin components were present (Fig. 1A). The very largest of these, which we term the "void volume" component, comprised 9% of the total prolactin activity recovered from the column. A small amount of immunoreactive hormone (3%) eluted in the region that is designated as "big" prolactin whereas most of the activity (88%) was observed in the elution volume of "little" prolactin. These same components were released during the second hour of incubation (Fig. 1B) but the relative amounts of the "void volume" and "big" components had decreased to 5 and 2%, respectively. During the next 3 hr of incubation the "void

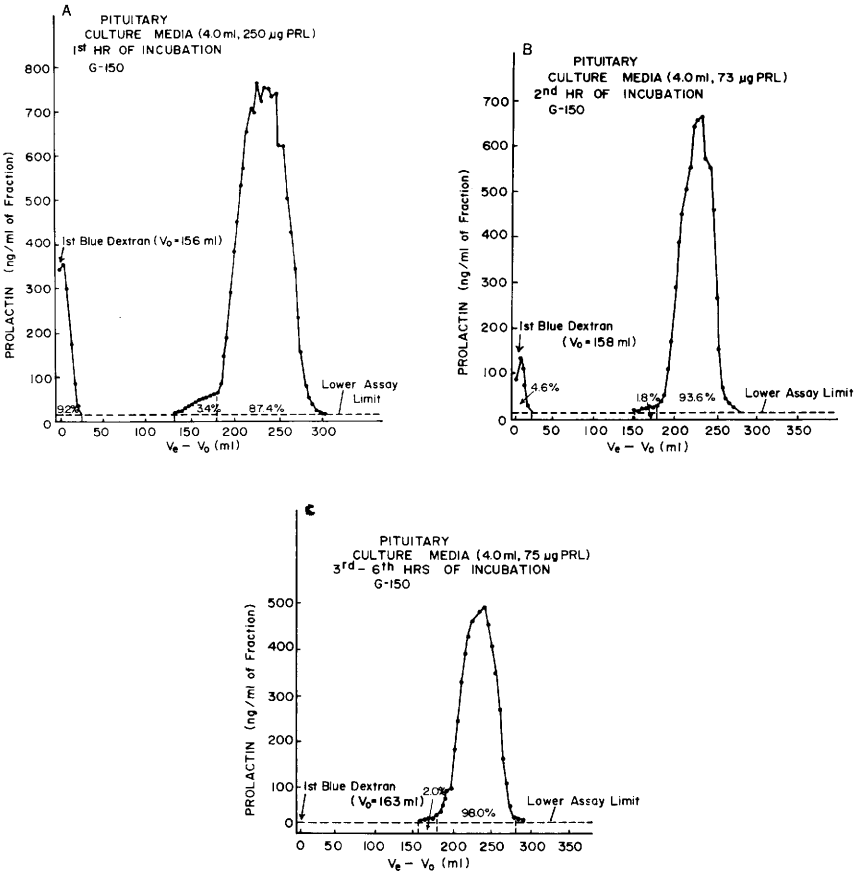


FIG. 1. Sephadex gel filtration patterns of prolactin released into incubation medium by hemipituitaries obtained from ovariectomized, estrogen-treated rats during the first (A), second (B), and third to sixth (C) hours of incubation. Dotted lines represent lower limits of the prolactin radio-immunoassay used to quantitate the levels of hormone in fractions eluted from columns. V_e is the elution volume of the fractions; V_0 is the void volume of the column. The quantity of incubation medium and the amount of prolactin subjected to gel filtration are shown at the top left portion of each panel.

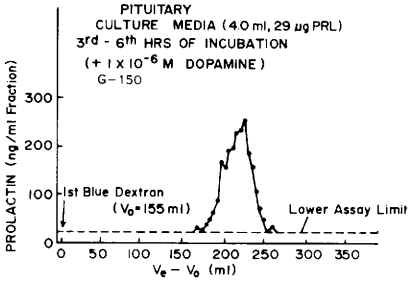
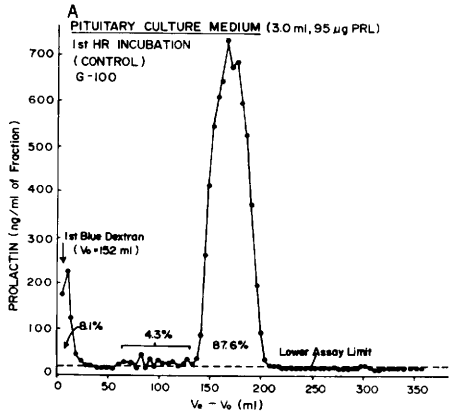


FIG. 2. Sephadex gel filtration pattern of prolactin released into incubation medium by hemipituitaries obtained from ovariectomized, estrogen-treated rats during the third to sixth hours of incubation in the presence of 1×10^{-6} *M* dopamine. For other details see the legend to Fig. 1.



volume" component disappeared (Fig. 1C). When dopamine was added during the last 3 hr of incubation, there was a marked reduction (60%) in the total amount of prolactin being released (Fig. 2) but the elution profile was not different from that observed when no dopamine was added. When dopamine was added during the first hour of incubation (Fig. 3B) there was again a 60% reduction in the amount of prolactin released but no change in the relative proportions of the three components that were noted when dopamine was absent (Fig. 3A).

Discussion. The data presented in this report confirm, in general, the observations of Dombroske *et al.* (2) that multiple forms of prolactin are released by pituitary explants into incubation medium *in vitro*. However, contrary to this earlier report, we observed that the larger forms, especially the "void volume" component, appear to be released in decreasing amounts or not at all as the incubation is continued. In addition, the *in vitro* release does not appear to be in proportion to that observed in the pituitary by us (1) and by Wallis (3). This observation also conflicts with the report of Dombroske *et al.* (2). These differences may be due to the times when medium was harvested or to the physiologic state of the rats which served as pituitary donors.

It is not clear at this time what the heterogeneous forms of prolactin are, but it is possible that they represent multiple pools of pituitary prolactin which have been previously reported (7, 8). There is also some

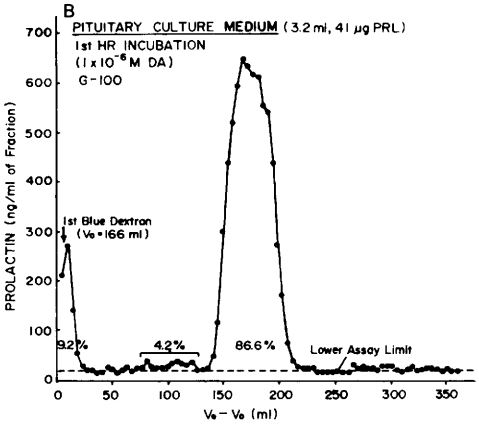


FIG. 3. Sephadex gel filtration patterns of prolactin released into incubation medium by hemipituitaries obtained from ovariectomized, estrogen-treated rats during the first hour of incubation in the absence (A) or presence (B) of 1×10^{-6} *M* dopamine. For other details see the legend to Fig. 1.

indication that the larger components may be the precursors of the smallest form (9). Whatever they are, the heterogeneous forms are all secreted, albeit not in the same proportion as observed in the pituitary. The exact cause for the decline in the secretion of the larger forms is not known. Perhaps the larger forms comprise a pool or pools of prolactin that are rapidly exhausted at high rates of secretion whereas "little" prolactin is released from a pool of greater magnitude. We have observed, in preliminary studies, that only very small quantities of the larger components are found in homog-

enates of pituitary explants from ovariectomized, estrogen-treated rats after 4 hr of incubation (unpublished).

It is of interest that dopamine, at a dose that reduced overall prolactin secretion by 60%, did not alter the relative proportions of the various forms being secreted (Fig. 3B vs 3A). This suggests that all the forms are secreted by a common mechanism or very similar mechanisms that are inhibited by dopamine, or that dopamine affects a common step of processing of prolactin in the lactotroph prior to actual secretion, as has been shown in lactating rats by Grosvenor *et al.* (10).

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