

Effects of Several Sera on Prolactin and GH Release from Rat Pituitary Cells *in Vitro* (41344)

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Abstract. To study the acute effects of serum and albumin on prolactin (PRL) and growth hormone (GH) release from pituitary cells, 10, 25, and 50% of normal rat (NRS), horse (HS), fetal calf (FCS), or normal human serum (NHS) or 1, 2.5, and 5% of bovine serum albumin (BSA) was administered to dispersed rat pituitary cells (DC) and to clonal rat pituitary mammothrophs (2B8). Further, to obtain information on the nature of the serum factors influencing pituitary hormone release TRH or serum from hypophysectomized rats or heated, or dialyzed rat serum was also added into the culture medium containing DC, or 2B8 cells. Twenty-five and fifty percent of NRS, HS, and NHS consistently stimulated PRL and GH release from DC cultures. In contrast, FCS and BSA had no effect on the release of either hormone. 2B8 cells showed significant increases in the release of PRL during incubation with NRS, HS, NHS, or FCS at all of the concentrations of serum employed. However, the 2B8 cells showed no change in PRL release when BSA was added to the culture medium. The PRL and GH releasing activities of 10^{-7} M TRH or 25% of hypophysectomized rat serum were much smaller than those obtained with the addition of 25% NRS to DC or 2B8 cells. Moreover, dialysis of NRS (which removes molecules below a mol wt of 12,000) significantly reduced the releasing activities for PRL both in DC and 2B8 cultures, while heat treatment of NRS did not change the activities in either cell system. These results indicate that (i) mammalian sera possess PRL- and GH-releasing activities, (ii) TRH is probably not a factor in this response although other hypothalamic releasing or release-inhibiting hormones may be involved, and (iii) these factors are heat stable and some of the PRL-releasing factors are molecules below a mol wt of 12,000.

It is well known that serum contains a number of stimulatory factors which are needed for the growth of most mammalian cells in culture (1). Several sera have been commonly used as supplements to synthetic media in cell cultures of pituitary cells (2, 3). However, the acute effects of these sera, or serum proteins such as albumin, on pituitary hormone release have not been studied. Therefore, we examined the effects of rat, horse, calf, or human serum or bovine serum albumin on prolactin (PRL) and growth hormone (GH) release from dispersed rat anterior pituitary cells (DC) and from clonal rat pituitary mammo-

trophs (4). In addition, we have made preliminary studies on the nature of the factors influencing the release of these hormones using TRH or several types of treated sera, i.e., serum from hypophysectomized rats, and heated, or dialyzed rat serum. A preliminary report of our findings has been published (5).

Materials and Methods. *Dispersed cell cultures.* Male Sprague-Dawley (SD) rats (Charles River Corp., Wilmington, Mass.), weighing 200-250 g, were used as donors for the pituitary cells. For each experiment 10 or 20 animals were killed by decapitation. The pituitary glands were removed aseptically under a laminar flow hood, the posterior lobe was discarded, and the remaining tissue was placed into a sterile petri dish and minced to the size of 1 mm³ in Hank's balanced salt solution without Ca²⁺ or Mg²⁺. The minced pituitary tissue was washed gently with Hank's solution five times to remove blood cells. Then, 5 ml of

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0.1% trypsin without Ca^{2+} or Mg^{2+} (Grand Island Biological Co., GIBCO, Grand Island, N.Y.), solubilized in Hank's solution containing 0.1% BSA was added to the petri dish, and the tissue was then transferred to a 25-ml Erlenmeyer flask. These solutions were agitated gently by a stirrer for 10 min, and the supernatant, which contained the DC, was collected in an ice-cooled 50-ml Erlenmeyer flask after filtration through lens paper. Then, 5 ml of Ham's F-10 medium supplemented with 10% fetal calf serum (FCS) and 2.5% horse serum (HS) was added to the flask to block the action of the trypsin. The trypsinization and cell collection procedures were repeated five times. Finally, the cell suspension was centrifuged for 5 min at 1000 rpm, and resuspended with Ham's F-10 medium. These cells were cultured in T-25 flasks at 37° for 2 days in Ham's F-10 medium containing 10% FCS and 2.5% HS in an atmosphere of 5% CO_2 and 95% air. The number of cells per flask was similar in each experiment and varied from 7.5 to 23.6×10^4 in different experiments. The cell viability as determined by the trypan blue dye exclusion method was always over 93% before and after incubation.

2B8 cell cultures. Pituitary clonal (2B8) cells which have been shown to produce only PRL (4) were used in this study. These cells were cultured by the same method employed with the dispersed cells. The 2B8 cells were incubated at 37° for 3 days in Ham's F-10 medium containing 10% FCS and 2.5% HS. The number of cells per flask was similar in each experiment and varied from 2 to 22×10^5 in different experiments.

Experiment 1: Effects of several sera or bovine serum albumin. After 2–3 days of cell culture, the medium was discarded and 10, 25, or 50% of normal male rat serum (NRS), horse serum (HS; GIBCO), fetal calf serum (FCS; GIBCO), or normal adult human serum (NHS), or 1, 2.5, or 5% of bovine serum albumin (BSA; Sigma Chemical Co., St. Louis, Mo.) dissolved in Ham's F-10 serum-free medium (pH 7.7) was added to DC and 2B8 cell cultures. Cells incubated in serum-free Ham's F-10 medium were used for the controls. After 6 hr of incubation in the presence of the different sera or BSA

the media were collected. All media samples were kept frozen at -27° until assayed.

Experiment 2: The effects of serum of hypophysectomized rats. To determine whether hypothalamic hormones might participate in serum-mediated pituitary hormone release 25% of hypophysectomized female rat serum was added to the culture medium containing DC or 2B8 cells. The effects produced by this serum were compared to those obtained when serum from intact female rats was used. The serum mentioned above was obtained from 75-day-old female SD rats, which were hypophysectomized at 45 days of age.

Experiment 3: The effects of heated or dialyzed rat serum. To obtain information on the nature of the serum factors influencing pituitary hormone release NRS was heated at 60° for 30 min, or dialyzed at 4° against distilled water for 24 hr using Fisher dialysis tubing (Cat. No. 6-667E, exclusion at mol wt of 12,000). The effects of these treated sera were compared with those of NRS.

RIA of rat PRL and GH. Rat PRL was measured in duplicate by radioimmunoassay (RIA) methods previously described (6). Rat GH was also measured by double antibody RIA procedures using materials supplied by NIAMDD. Results were expressed in terms of the NIH standards, PRL-RP-1 and GH-RP-1.

Intra- and interassay coefficients of variation were 2.1 and 4.8% for PRL, and 5.9 and 7.5% for GH, respectively. The pooled NRS used in these experiments contained 70 ng/ml of PRL and 50 ng/ml of GH, and the values of PRL and GH in NRS were subtracted from the experimental values obtained. HS, FCS, NHS, and BSA did not show any cross-reactivity in the rat PRL and GH RIA systems.

Statistical analysis. The data were subjected to analysis of variance followed by the Student/Newman-Keul's test for comparing the differences between group means.

Results. Basal hormone secretion. After 2 or 3 days of culture of DC or 2B8 cells, the media were collected and PRL and/or GH content was measured. Generally, there were no significant differences in PRL and

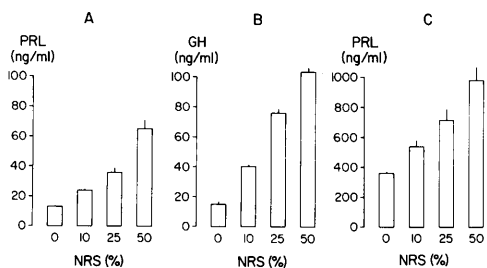


FIG. 1. Effects of addition of increasing concentrations of normal rat serum to Ham's F-10 culture medium on the release of PRL and GH into the medium during a 6-hr incubation. A and B show release of PRL and GH from DC cultures; C shows release of PRL from 2B8 cells.

GH from flask to flask, and the coefficient of variation in each experimental group was less than 4 and 7% for PRL and GH, respectively.

Effects of sera or BSA on PRL and GH release. In DC cultures 10, 25, or 50% of NRS brought dose-response increases in PRL and GH release. At any concentration, these increases were significant ($P > 0.01$) when compared to the control value, except for the PRL value at 10% NRS which was not statistically significant (Figs. 1A, B). 2B8 cells also showed dose-response increases in PRL release with the addition of NRS ($P < 0.05$ at 10%, $P < 0.01$ at 25% and 50% of NRS) (Fig. 1C).

DC cultures also showed dose-response increases in PRL and GH release with the administration of 10, 25, and 50% of HS. Except for the GH value at 10% HS, all other values were statistically significant (P

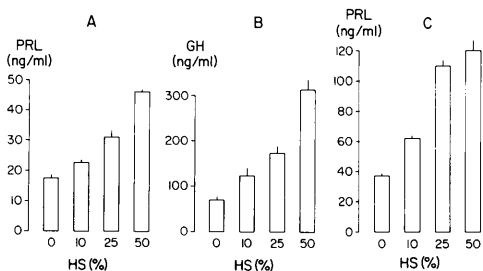


FIG. 2. Effects of addition of increasing concentrations of commercial horse serum to Ham's F-10 culture medium on the release of PRL (A) and GH (B) from DC cultures during a 6-hr incubation. C shows release of PRL from 2B8 cells.

$< 0.05 \sim 0.01$; Figs. 2A, B) from their respective controls. Similarly, 2B8 cells showed significant PRL increases for each of the three concentrations of HS ($P < 0.01$; Fig. 2C).

Although FCS and BSA had no stimulatory effect on either PRL or GH secretion from DC cultures, FCS, but not BSA, induced clear PRL releases from 2B8 cells ($P < 0.01$ at each concentration) (Table I).

After the incubation of DC cultures with NHS, release of PRL and GH was significantly stimulated at all concentrations employed ($P < 0.01$) except for GH at 10% NHS (Figs. 3A, B). NHS had also a clear stimulatory action on PRL release from 2B8 cells ($P < 0.01$; Fig. 3C).

Effect of TRH on PRL and GH release from DC cultures. Although TRH increased the PRL and GH released into the medium, those values failed to reach statistical significance by Student/Newman-Keul's test (Figs. 4A, B) (By use of Student's t test both increases were significant to the probability level of < 0.001 for PRL and < 0.05 for GH, respectively.) The PRL and GH releasing activities of 25% NRS were much greater than those of TRH ($P < 0.05$ for PRL; $P < 0.01$ for GH). However, when TRH was administered with 25% of NRS, PRL and GH release were significantly enhanced compared to the single administration of 25% NRS ($P < 0.01$; Figs. 4A, B).

Effect of hypophysectomized rat serum or heated or dialyzed serum on PRL and GH release. Hypophysectomized rat serum had significantly lower activity for PRL and GH release from DC or 2B8 cells than serum from normal rats (NRS) ($P < 0.05 \sim 0.01$) (Figs. 5A, B, C). Although the serum from hypophysectomized rats did not significantly stimulate the release of PRL from DC cultures (Fig. 5A), it had a significant stimulatory effect on PRL release from 2B8 cells ($P < 0.01$; Fig. 5C) and GH release from DC cultures ($P < 0.01$; Fig. 5B).

Dialyzed rat serum exhibited a significantly reduced PRL releasing activity from DC cultures ($P < 0.01$) and 2B8 cells ($P < 0.01$) compared to NRS (Figs. 6A, B). Although, compared to the control value, the PRL value obtained with the dialyzed serum was not statistically different in DC

TABLE I. EFFECT OF FCS OR BSA ON PRL AND GH SECRETION FROM DC AND 2B8 CELLS

	DC		2B8
	PRL (ng/ml)	GH (ng/ml)	PRL (ng/ml)
Control	21.4 ± 0.3 ^a	46.2 ± 0.7	51.1 ± 1.8 ^a
FCS (10%)	20.6 ± 0.5	42.2 ± 1.1	105.6 ± 3.4*
FCS (25%)	22.9 ± 0.8	46.6 ± 3.0	161.2 ± 5.4*
FCS (50%)	22.3 ± 0.5	48.2 ± 4.1	187.7 ± 4.8*
Control	50.0 ± 2.2	22.0 ± 1.8	48.4 ± 1.1 ^a
BSA (1%)	49.6 ± 1.9	23.3 ± 0.6	46.9 ± 2.1
BSA (2.5%)	49.5 ± 3.3	24.2 ± 1.3	49.4 ± 1.0
BSA (5%)	49.4 ± 1.5	24.4 ± 0.3	47.2 ± 1.0

^a The number of flasks used was 10; for all other groups the number was 5. The values represent mean ± SEM of 5 or 10 samples.

* $P < 0.01$ compared to control; all other values were not statistically different from their controls.

cultures, this serum still had a significant PRL-releasing activity for 2B8 cells ($P < 0.05$). On the other hand, heated rat serum still had an activity to elevate PRL release from DC cultures ($P < 0.05$) and 2B8 cells ($P < 0.01$) compared to controls, and the mean values were not significantly different from those obtained with NRS (Figs. 6A, B).

Discussion. In this study, NRS, HS, and NHS showed clear stimulatory effects on PRL and GH release from primary cultures of dispersed rat pituitary cells and on PRL release from clonal rat mammothrophs. In contrast, FCS had an effect only on 2B8 cells, and BSA did not show any effect on either PRL or GH release from DC or 2B8 cells. It should be noted that the stimulatory effects of the sera in these 6-hr incubations probably involve changes in hormone

synthesis as well as in the release of PRL or GH from the cells. However, since we did not determine the amount of hormone synthesized during the 6-hr incubation the term hormone *release* seems more appropriate than hormone secretion.

The 2B8 cells used in this study were derived by Ishikawa from fetal rat pituitary anlage and at early passage levels the majority of the cells exhibited a normal diploid karyotype (4). However, we have recently found that late passage 2B8 cells, such as were used in these experiments, exhibit aneuploidy and a number of characteristics of transformed cells (7). Consequently, in this study it was of interest to compare the responses of 2B8 cells to those of primary cultures of dispersed rat anterior pituitary cells. We found that, with the ex-

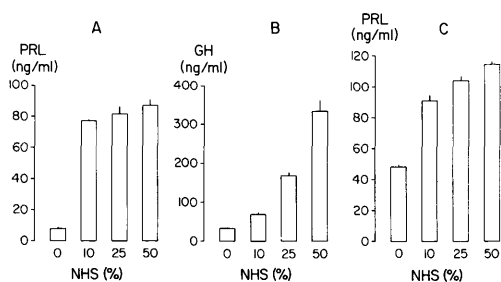


FIG. 3. Effects of addition of increasing concentrations of normal human serum to Ham's F-10 culture medium on the release of PRL (A) and GH (B) from DC cultures during a 6-hr incubation. C shows release of PRL from 2B8 cells.

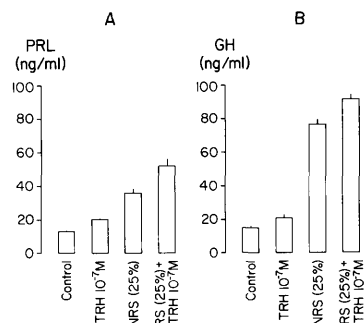


FIG. 4. Effects of addition of TRH, 25% NRS, or TRH in combination with 25% NRS to Ham's F-10 culture medium on the release of PRL (A) or GH (B) from DC cultures during a 6-hr incubation.

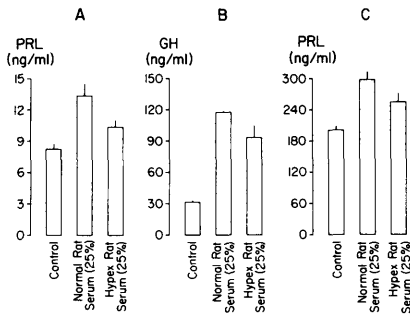


FIG. 5. Effects of addition of 25% NRS or 25% hypophysectomized rat serum to Ham's F-10 culture medium on the release of PRL (A) or GH (B) from DC cultures during a 6 hr incubation. C shows release of PRL from 2B8 cells.

ception of FCS, the responses of 2B8 and DC cultures to the various serum preparations were very similar.

It has been reported that TRH can stimulate both PRL and GH release from the rat pituitary gland (8, 9). However, in this experiment TRH had significantly lower activity than 25% NRS whereas the concentration of TRH used ($10^{-7} M = 36 \text{ ng/ml}$) is thought to be much higher than the physiological concentration of TRH present in rat blood (10–12). It is also known that TRH has a short half-life in serum and is rapidly inactivated by enzymes present in fresh rat serum (13). Therefore, TRH is probably not an important factor in the several sera which were found to stimulate PRL and GH release.

It is worth noting that serum from hypophysectomized rats had a lower activity than NRS for stimulating the release of hormones from DC and 2B8 cells. It was expected that hypophysectomy would induce an elevation of hypothalamic releasing hormones (14, 15). Therefore, if the effects of serum on PRL and GH secretion were due primarily to the presence of hypothalamic releasing factors such as PRF (PRL-releasing factor) and GH-RF (GH-releasing factor) one would expect the serum of hypophysectomized rats to have a greater effect than NRS. We found just the opposite.

Although heat treatment did not change the hormone stimulatory activity, dialyzed rat serum showed a significant loss of releasing activity on PRL secretion from DC

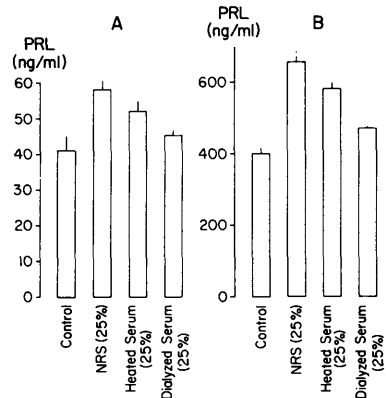


FIG. 6. Effects of addition of 25% NRS, 25% heated NRS, or 25% dialyzed NRS on PRL release from DC (A) on 2B8 cultures (B) during a 6-hr incubation.

and 2B8 cells. In our dialysis of rat serum, molecules with molecular weight smaller than 12,000 should have been removed. From these observations it appears that the serum factors responsible for hormone release are heat stable and some of the factors may be molecules with molecular weight smaller than 12,000.

In rats PRL and GH responses to exogenous stimuli show some similarities. For example, the administration of TRH, serotonin, chlorpromazine, and opioid substances evoke increases in both PRL and GH secretion (8, 9, 16–20), whereas both hormones show decreased responses to DA or the DA agonist, apomorphine, *in vivo* or to somatostatin *in vitro* (17, 21–25). On the other hand, different responses in PRL and GH secretion to other stimuli are also recognized (17, 26–29). Therefore, it might not be unreasonable to assume that serum contains common factors capable of stimulating both PRL and GH release *in vitro*. In fact, it seems likely that the total effect of serum on the acute release of hormones may represent a balance between a number of releasing and release-inhibiting factors present in serum. Some of these factors are probably hypothalamic hormones but a variety of other serum factors may also be involved.

In conclusion, NRS, HS, and NHS all have pronounced releasing activity for PRL and GH from DC and 2B8 cells in culture.

This activity may be influenced by the loss of pituitary function following hypophysectomy and the molecular size of some of the PRL-releasing factors must be below 12,000. In future studies on PRL or GH secretion *in vitro*, investigators should recognize that the effects of serum additives to the culture medium must be carefully analyzed and controlled.

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