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RAPID ELEVATION OF RAT SERUM PROLACTIN CONCENTRATION BY CYCLOSPORINE, A NOVEL IMMUNOSUPPRESSIVE DRUG

Stephan B. Cardon, Douglas F. Larson, and Diane Haddock Russell

Department of Pharmacology
University of Arizona College of Medicine
Tucson, AZ 85724

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Within one hr of the administration of cyclosporine to rats, there was a 4-fold elevation in the serum prolactin concentration. Doses of 0.12, 1.2, and 12 μg/100 g body weight cyclosporine significantly elevated the serum prolactin level. Higher doses, 120 or 1200 μg/100 g body weight cyclosporine resulted in small but insignificant elevations of the serum prolactin concentration. Bromocriptine, a dopamine agonist which inhibits prolactin release from the anterior pituitary, completely blocked the elevation in serum prolactin in response to cyclosporine alone. These data suggest that the ability of cyclosporine to suppress immune function may involve its ability to rapidly produce hyperprolactinemia.

Prolactin may be the pituitary hormone of primary importance in the regulation of vertebrate growth and development (1). Prolactin is coupled to altered macromolecular synthesis in a variety of mammalian tissues including spleen, thymus, kidney, liver, adrenals, heart, and reproductive tissues such as mammary gland and prostate (2-4). The administration of prolactin to rats induces ornithine decarboxylase (ODC), the rate-limiting enzyme in polyamine biosynthesis, and, in certain tissues such as kidney, results in an elevated ribosomal RNA content (3,5). We have demonstrated recently the presence of specific [^{125}I]prolactin binding sites on human peripheral blood T- and B-lymphocytes (6,7). Binding of [^{125}I]prolactin to these receptors could be inhibited in a dose-dependent manner by the administration of cyclosporine. Total inhibition of binding of the radiolabel occurred at a concentration of cyclosporine known to suppress organ transplant rejection in humans (8). This suggested to us that the mechanism by which cyclosporine alters immune function might include its ability to selectively inhibit prolactin receptors. Prolactin is a unique hormone in that its circulating level exerts a negative feedback on its release from

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lactotrophs in the anterior pituitary, and this further suggested that cyclosporine administration might alter the circulating level of prolactin. We found a 4-fold elevation of serum prolactin within 1 h of administration of 12 µg/100 g cyclosporine (66.3 vs. 16.8 ng/ml serum in the cyclosporine-treated vs. vehicle controls, $p < 0.0025$, Fig. 2). The elevation in serum prolactin could be totally blocked by bromocriptine, a dopamine agonist that inhibits release of prolactin at the level of the prolactin-secreting cells in the anterior pituitary. These data are the first report of the ability of cyclosporine to alter the serum concentration of prolactin. Further, cyclosporine does this in a dose-dependent manner.

Materials and Methods

Materials. Sprague-Dawley rats (male, 4 weeks of age) were obtained from the University of Arizona Division of Animal Resources. Cyclosporine (CsA, Sandimmune™) was obtained from Sandoz Pharmaceuticals (East Hanover, NJ). Sesame oil and 2-bromo-α-ergocryptine methane sulfonate was purchased from Sigma Chemical Co. (St. Louis, MO) and [^{125}I]iodine was obtained from ICN (Irvine, CA). Rat prolactin and rat prolactin antiserum (rabbit) were obtained from the NIADDK. Anti-rabbit gamma globulin was obtained from TLC Antibodies (Texas Tech Medical Center, Lubbock, TX). Iodo-beads were from Pierce Chemical Co. (Rockford, IL).

Methods. CsA was dissolved in EtOH (120 mg/ml) to make a stock solution which was stored under refrigeration. The stock stolution was mixed with sesame oil for injection; bromocriptine was suspended in sesame oil for injection. Rats were fed laboratory rodent chow and water ad libitum and were on a 0600-1900 h photoperiod. All rats were used at approximately 4½ weeks of age. For the dose response study, vehicle control rats received sesame oil (100 µl, i.p.) and other rats received 1200, 120, 12, 1.2, or 0.12 µg per 100 g body weight CsA in 100 µl sesame oil, i.p. For the CsA/bromocriptine study, rats received sesame oil (200 µl, i.p.) as the vehicle control or 12 µg CsA/100 g body weight and/or 75 µg bromocriptine/100 g body weight in a total sesame oil volume of 200 µl, i.p. Bromocriptine was given 10 min prior to CsA when both were given. One h after injection, rats were rapidly killed by decapitation and bled. Blood was allowed to clot at room temperature and after centrifugation, serum was removed, frozen and stored until assay. Iodination of prolactin for radioimmunoassay was done with Pierce Iodo-beads using a procedure modified from Markwell (9). The serum prolactin levels were determined by radioimmunoassay using the method of the NIADDK.

Results

Effect of various doses of cyclosporine on serum prolactin levels in the rat

Serum prolactin levels exhibited a bell-shaped dose response curve to CsA. A dose of 0.12 µg/100 g body weight CsA elevated the serum prolactin significantly to a level 2.9-fold above vehicle controls ($p < 0.025$, Fig. 1).

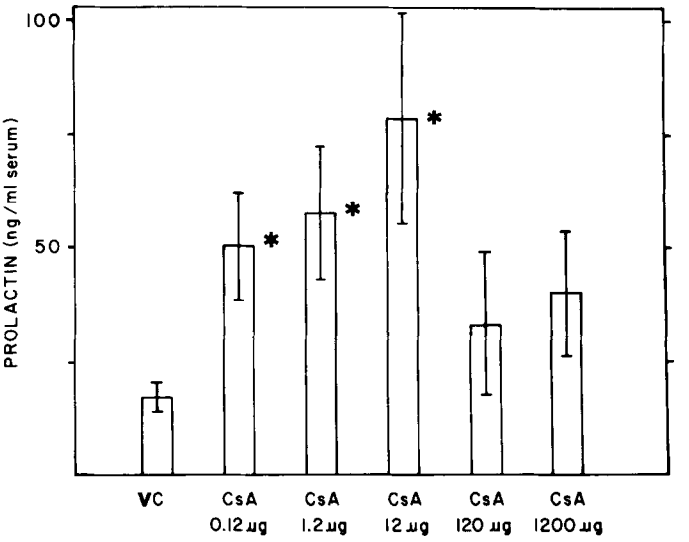

Figure 1. Serum prolactin concentrations in rats in response to cyclosporine (CsA) doses of 0.12, 1.2, 12, 120 and 1200 µg/100 g body weight. N = 4 for each group. *Data differ from vehicle controls, $p < 0.025$.

Maximal serum prolactin concentration occurred in response to 12 µg/100 g body weight CsA, a level 4.5-fold greater than controls ($p < 0.025$). Higher concentrations of CsA, 120 or 1200 µg/100 g body weight, resulted in slight but insignificant elevations in serum prolactin levels.

Effect of bromocriptine on serum prolactin concentrations in the presence and absence of cyclosporine

Bromocriptine alone (75 µg/100 g body weight) produced a slight but nonsignificant decrease in serum prolactin 1 h postinjection (Fig. 2). Control rats without injection had 12.3 ± 2 ng/ml serum detectable prolactin, vehicle alone controls, 16.8 ± 3.9 ng/ml, and bromocriptine-injected rats, 8.8 ± 0.8. The marked elevation in serum prolactin in response to CsA administration (66.3 ± 13.5 ng/ml serum) was abolished totally by the administration of bromocriptine (9.4 ± 2.2 ng/ml serum).

Discussion

These data strongly suggest that the ability of CsA to suppress immune function may be related not only to its ability to inhibit prolactin binding in lymphoid tissues (6,7), but also to its ability to rapidly produce hyper-

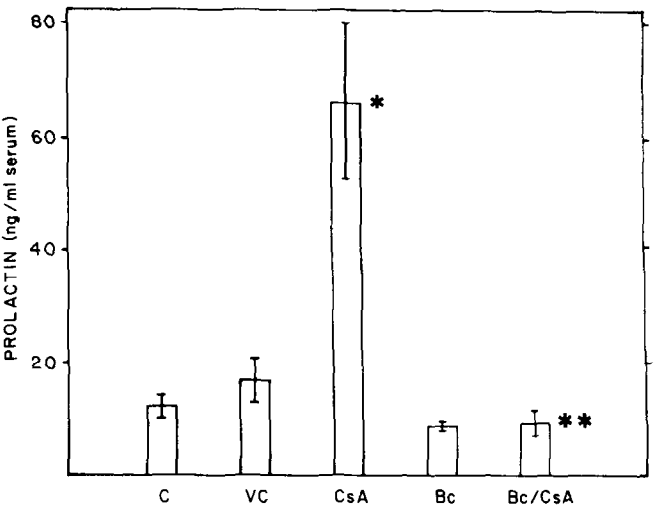

Figure 2. Effect of bromocriptine (Bc) and Bc plus cyclosporine (CsA) on serum prolactin levels in rats. N = 5 for uninjected control (C), Bc and Bc/CsA groups. N = 9 for vehicle controls (VC) and CsA alone groups. *Data differ from vehicle controls,, $p < 0.0025$. **Data differ from CsA alone, $p < 0.005$.

prolactinemia. Suppressed immune responses in hypophysectomized rats can be selectively restored by administration of prolactin (10). On the other hand, prolactin can result in immunosuppression during pregnancy and lactation (11), both conditions in which the circulating levels of prolactin are extremely high. Thus, hypo- and hyperprolactinemia appear to result in a compromised immune state. However, since other major hormones such as thyroid hormone, glucocorticoids, and sex steroids influence prolactin secretion as well as action, careful studies are required to elucidate the meaning of altered circulating prolactin (12-15).

Further studies of the effects of prolactin on lymphoid tissues are required to determine what gene products may be regulated by this hormone. That specific gene products may be regulated seems likely since prolactin specifically regulates the initiation of casein and lactalbumin synthesis in mammary gland (16). This, however, requires the sequential exposure of mammary tissue from pregnant mice to insulin, hydrocortisone, and then prolactin (17,18) suggestive of the precision of the interplay of hormones required for proper differentiative function.

That prolactin may have important regulatory effects on immune function now seems rather certain. Our lack of knowledge in this area is underscored by a quotation by Riddle (1) in a review on prolactin published in 1963: "The failure of more than two decades of fevered investigation to recognize that prolactin resides at the center -- not the periphery -- of an atypical order of pituitary regulation, which in turn has far-reaching implications in over-all vertebrate organization, has been and remains an expensive biological and medical experience."

Acknowledgements

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