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Paradoxical Induction of Tyrosine Aminotransferase by Progesterone *in Vivo*: Potentiation of Effects of Low Levels of Glucocorticoid Hormone (40660)

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The ability of a variety of natural and synthetic steroids to antagonize glucocorticoid hormone action at the receptor level is now well established (1, 2). A number of such antiglucocorticoid compounds have been studied in detail by Tomkins and his coworkers, who postulated that they have affinity for a different conformational form of the cytoplasmic glucocorticoid receptor from that to which true glucocorticoids bind (2), such that the complex formed between receptor and antiglucocorticoid is incapable of activation and subsequent transfer to the nucleus (3).

A diversity of antiglucocorticoid hormonal effects have now been examined in *in vitro* studies (1-8), the majority of which have employed progesterone. It has been shown that, in keeping with its ability to bind to the cytosolic glucocorticoid receptor (2) and the inability of the resulting steroid-receptor complex to be translocated to the nucleus (3), progesterone is an effective antagonist to a number of well characterized responses to glucocorticoids *in vitro*. Thus progesterone or other antiglucocorticoids have been shown to block glucocorticoid-induced stimulation of tyrosine aminotransferase (TAT) synthesis by hepatoma cells in tissue culture (1), to antagonize glucocorticoid-mediated cytolysis (7) or inhibition of glucose uptake (5) in thymus cells, to reverse the induction of alkaline phosphatase in HeLa cells by prednisolone (6), and, most recently, to overcome the glucocorticoid hormone-induced inhibition of hepatoma cell proliferation *in vitro* (8).

Despite considerable interest in the subject, virtually no studies thus far have examined possible antiglucocorticoid effects of progesterone *in vivo*. Although, because of its other biological effects, there might well prove to be problems associated with the use of progesterone as a therapeutic antiglucocorticoid, the possibility that at the very least it might prove of use as an experimental pharmacologic tool made it of interest to explore its potential properties as an antiglucocorticoid in intact animals. In this paper we report the results of studies initially undertaken to examine the effects of progesterone as a potential *in vivo* antagonist to the well-defined phenomenon of glucocorticoid-induced tyrosine aminotransferase synthesis in rat liver. In contrast to the anticipated antagonistic effect, we were surprised to find that progesterone exerts a striking synergism upon the induction of this enzyme by exogenous glucocorticoid, and in addition markedly stimulates the synthesis of TAT in response to endogenous circulating glucocorticoid. These observations, as well as an investigation of possible mechanisms by which this potentiation is mediated, form the basis for this report.

Materials and methods. Materials. Studies were performed on male Sprague-Dawley rats weighing between 130 and 150 g. Normal animals were maintained on food and water *ad libitum*, and adrenalectomized rats on the same regimen except for supplementation of their drinking water with 0.9% NaCl. Adrenalectomies were performed by the Zivic-Miller Laboratories, Inc. (Allison Park, Pa.), and subsequent studies were performed between 5 and 10 days after adrenalectomy. Hydrocortisone, progesterone, and dexamethasone were all obtained from the Sigma Chemical Company (St. Louis, Mo.).

Administration of steroids and preparation of samples for TAT assay. All steroids (in doses expressed per 100 g of body weight) were administered in solution by intraperitoneal injection in 0.3 ml dimethylsulfoxide (DMSO). Control animals received an equal volume of DMSO alone. All groups contained four to six animals each. Rats were injected between 9 and 10 AM and were sacrificed by stunning and exsanguination exactly 4 hr later. Typical dose-response relationships for the induction of TAT by hydrocortisone and dexamethasone are shown in

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Figs. 1 and 2, respectively. DMSO by itself was repeatedly shown to have no effect on liver TAT activity.

Following sacrifice, livers were immediately removed and homogenized individually in 4 vol of chilled 0.14 *M* KCl, and all subsequent steps were performed at 0–4° unless otherwise noted. Homogenization was accomplished by means of 20 strokes in a Dounce-type, hand-driven, all-glass homogenizer equipped with a loosely fitting pestle (Blaessing Glass Specialties, Rochester, N.Y.). Homogenates were centrifuged at 30,000 *g* for 30 min, and the resulting clear supernatant fractions decanted into individual chilled tubes prior to assay for TAT activity.

Tyrosine aminotransferase activity. This was determined by the method of Diamondstone (9) with minor modification. One unit of tyrosine aminotransferase activity was defined as that quantity of enzyme resulting in the formation of 1 nmole of product per minute at 37° under saturating substrate conditions; a "working extinction coefficient" of 19,900 M^{-1} for *p*-hydroxybenzaldehyde at 331 nm was used (9).

Results. Initial studies in normal animals: "Induction" of TAT by progesterone alone. Prior to examination of progesterone as a possible antagonist to the induction of TAT by exogenous glucocorticoid, the effects of graded doses of progesterone alone were examined in normal animals (adrenal glands intact). A surprising finding was that, instead of being without effect, or possibly even causing a fall in liver enzyme activity due to a peripheral antagonism to endogenous glucocorticoid (corticosterone), the administration of progesterone by itself resulted in a striking rise in hepatic TAT activity (Fig. 3). The effect was fully evident at doses of progesterone as low as 0.2 mg, showing no increase even with doses an order of magnitude higher.

Studies in adrenalectomized animals: Potentiation of low doses of exogenous glucocorticoid by progesterone. Because of the unex-

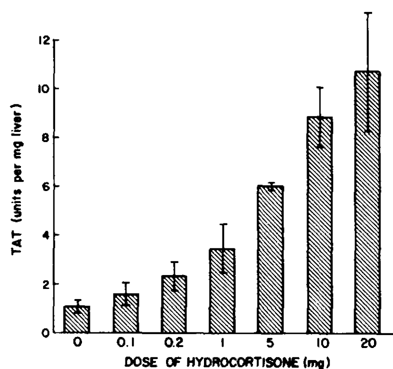

FIG. 1. Induction of tyrosine aminotransferase (TAT) as a function of hydrocortisone dose. Hydrocortisone in the doses indicated on the abscissa was administered to adrenalectomized rats and hepatic TAT activity determined 4 hr later as described under Methods. Maximal induction is achieved at a dose of 10 mg hydrocortisone.

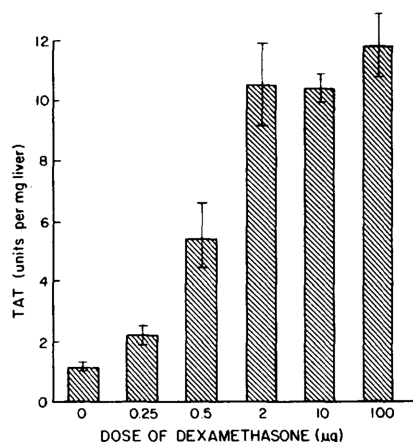

FIG. 2. Relationship between dexamethasone dose and TAT induction in adrenalectomized rats. Maximal induction is observed at a dose of 2 μg dexamethasone.

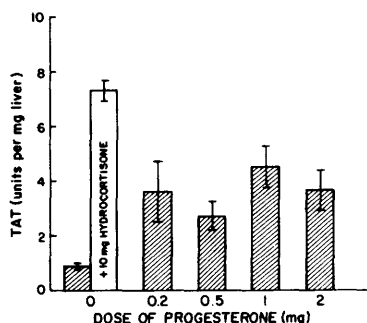

FIG. 3. Induction of TAT in normal rats by the administration of progesterone. The dose of progesterone is given on the abscissa, and, for comparison, the effect of a maximally inducing dose of hydrocortisone (10 mg) is included. Note that about half-maximal induction is achieved by the administration of as little as 0.2 mg progesterone, and that increasing this dose by a factor of 10 is without further effect.

pected finding that progesterone was a potent inducer of TAT in normal rats, the possibility that progesterone might itself possess intrinsic glucocorticoid activity *in vivo* was tested and excluded by experiments in adrenalectomized animals. Figure 4 shows that, in contrast to the effects of progesterone administration in intact animals (cf. Fig. 3), even large doses of progesterone, when administered to adrenalectomized rats, were found to be entirely without effect upon liver TAT activity. What was of special interest, however, was that, while inactive in these latter animals when given by itself, progesterone—even at low doses—markedly potentiated the induction of enzyme by submaximal doses of simultaneously administered glucocorticoid. In the presence of progesterone, a dose of 0.2 mg hydrocortisone—itself barely sufficient to cause a significant elevation in enzyme activity—is seen to result in a level of induction achieved only with a maximally inducing (50-fold higher) dose of hydrocortisone alone (see also Fig. 1).

Disappearance of progesterone effect at large doses of exogenous glucocorticoid. The observation that the induction of TAT by progesterone alone is completely blocked by antecedent adrenalectomy, and the further finding that inducibility can be restored by

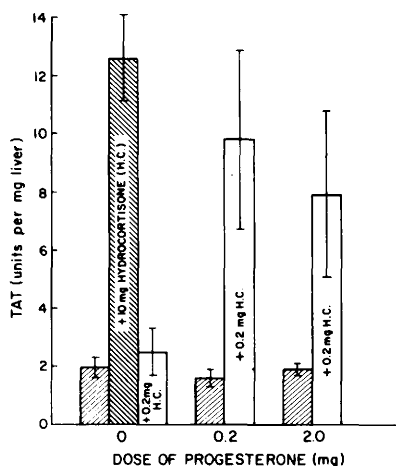

FIG. 4. Effects of progesterone on TAT induction in *adrenalectomized* animals. While progesterone by itself is seen to be entirely without effect upon TAT activity in adrenalectomized rats, it strikingly potentiates the induction of enzyme by low doses of hydrocortisone (H.C.; see text). The effect of a maximally inducing dose of hydrocortisone is again included for comparison.

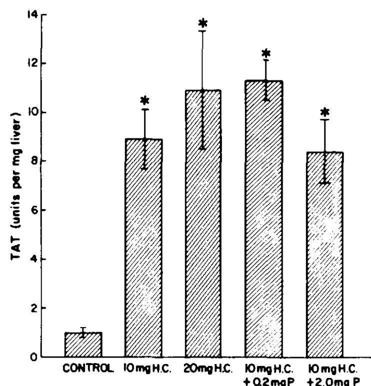

FIG. 5. Absence of a potentiating effect of progesterone (P) at high (maximally inducing) doses of hydrocortisone (H.C.) in adrenalectomized rats. None of the mean values for bars marked with asterisks differ significantly from each other ($P > 0.1$).

simultaneous repletion with a small dose of glucocorticoid that by itself has only a minimal effect on enzyme induction, suggested a number of possible interpretations. One such possibility was that the rise in TAT level induced by progesterone, rather than reflecting a stimulation of enzyme synthesis, might instead be attributable to a reduction in the rate of which TAT, once synthesized, is degraded. Although unlikely *a priori* (the increase in TAT activity following progesterone administration is large in magnitude and occurs rapidly with respect to the normal half-life of the enzyme in liver (10)), this possibility was nevertheless tested by examining the influence of progesterone administration on the levels of TAT induced by large doses of hydrocortisone. If, rather than stimulating TAT synthesis, the administration of progesterone interfered with the degradation of TAT by some independent mechanism, the potentiating effect of the hormone observed at low doses of glucocorticoid should be observed at large doses of glucocorticoid as well. That this is not the case is seen in Fig. 5, which shows that at large doses of hydrocortisone the simultaneous administration of progesterone is entirely without additional effect upon hepatic TAT activity.

Possible relation of observed synergism to competitive binding of progesterone and natural glucocorticoids by transcortin: Contrasting absence of synergism between progesterone and dexamethasone. An additional mechanism

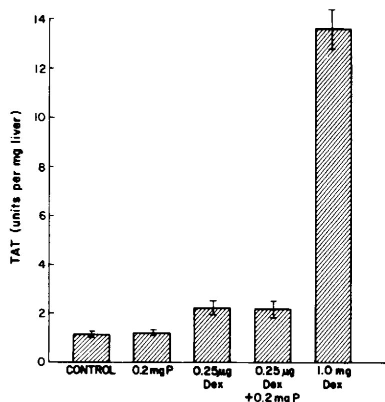

FIG. 6. Lack of synergism between progesterone (P) and dexamethasone (Dex). The absence of synergism between progesterone and dexamethasone contrasts with the potentiation by progesterone of enzyme induction in response to endogenous corticosterone (Fig. 3) and exogenous hydrocortisone (Fig. 4).

which, in contrast to the preceding one, might account for a true potentiation of glucocorticoid-induced TAT synthesis by progesterone, and which would be consistent with all of the preceding experimental observations, would be occupation of plasma glucocorticoid binding sites by progesterone: Progesterone, a hormone with known high affinity for transcortin,[1] might exert its effect by binding to the carrier protein and thereby either displacing previously bound endogenous corticosterone or preventing the binding of exogenous hydrocortisone with a resulting marked rise in the concentration of free (unbound) glucocorticoid in the plasma, and a corresponding rise in intracellular glucocorticoid concentration. This possibility is directly testable, for it leads to a specific experimental prediction. If indeed the mechanism by which progesterone potentiates the effect of exogenous hydrocortisone or endogenous corticosterone is attributable to a competition for glucocorticoid binding sites on transcortin and a consequent rise in free glucocorticoid concentration, one would predict that the phenomenon of potentiation should be *absent* in the instance of a glucocorticoid such as dexamethasone which, in contrast to the former two glucocorticoids, does not bind to transcortin (2, 15). The effects of progesterone given simultaneously with a dose of dexamethasone by itself sufficient to result in a definite but only modest increase (a doubling) of enzyme activity were accordingly examined. Figure 6 shows the result. In marked contrast to its synergism both with exogenously administered hydrocortisone in the adrenalectomized animal (Fig. 4) and with circulating endogenous corticosterone in the normal animal (Fig. 3), progesterone was found to be entirely without effect upon the induction of TAT by dexamethasone.

Discussion. The initial unexpected finding that progesterone (an antiglucocorticoid hormone virtually devoid of glucocorticoid activity *in vitro* (2)) was a potent inducer of TAT in normal rats suggested several possibilities: (i) that progesterone, *in vivo*, might indeed possess intrinsic glucocorticoid activity; (ii) that the observed rise in enzyme activity in response to progesterone was not in fact due to an increased rate of TAT synthesis but rather to a decreased rate of enzyme degradation; or (iii) that the observed induction of TAT, rather than reflecting a direct action of progesterone itself, was instead an indirect effect of the exogenous hormone mediated by endogenous glucocorticoid. Such latter (indirect) effects might, for example, include a nonspecific (e.g., stress-mediated) secretion of corticosterone in response to progesterone (a possibility which would be of only trivial interest), or instead a true potentiation of enzyme induction in response to preexisting circulating endogenous glucocorticoid. The possibility that progesterone itself or one of its metabolites might possess intrinsic glucocorticoid activity *in vivo* was tested and excluded by experiments in adrenalectomized animals. In these latter animals progesterone was nevertheless shown to markedly potentiate the effects of a defined dose of exogenous glucocorticoid, suggesting that the observed induction of TAT by progesterone alone in the intact animal might similarly be mediated by a potentiation of the effects of a preexisting level of endogenous glucocorticoid rather than by a progesterone-induced stimulation of endogenous glucocorticoid secretion. The possibility that the synergistic effect of progesterone was attributable to a decreased rate of enzyme degradation was

[1] The affinities of progesterone, corticosterone, and hydrocortisone for transcortin are 90, 30, and 30 μM^{-1}, respectively (11).

not only argued to be implausible from a priori considerations, but was further rendered unlikely by the observation that the synergistic effect of progesterone upon TAT induction disappeared at high doses of exogenous glucocorticoid.

Two additional mechanisms which might have accounted for a true potentiation of glucocorticoid-induced TAT synthesis by progesterone, and which would have been consistent with the preceding experimental observations, were the following: (i) The synergistic effect of progesterone in the presence of either endogenous corticosterone or low doses of exogenous hydrocortisone might be attributable to a reduction in the rate of metabolic clearance of glucocorticoid (e.g., by competition of progesterone for a pathway normally resulting in glucocorticoid inactivation) and a consequent rise in total plasma glucocorticoid concentration; or (ii) more interestingly, progesterone, a hormone with known high affinity for transcortin (11), might compete with corticosterone and hydrocortisone for glucocorticoid binding sites on the carrier protein and thereby induce a rise in the concentration of free (unbound) glucocorticoid. The first explanation would appear to be unlikely to account for the rapid induction of TAT in intact animals by progesterone alone since the endogenous secretory rate for corticosterone is on the order of only about 20 μg/100 g body wt per hour (12, 13), and even in the absence of negative feedback mechanisms an inhibition of its degradation would not be expected to lead to a large elevation in plasma level within the relatively short duration of an experimental period. (The time-course of TAT induction is such that TAT levels 4 hr after the administration of an inducing agent are largely dependent upon glucocorticoid-initiated transcriptional events occurring during the first 2 hr (14).) The second explanation, in addition to being of intrinsically greater interest, was directly testable, for it led to the specific prediction that the phenomenon of potentiation observed in the instance of both exogenous hydrocortisone and endogenous corticosterone should be absent in the instance of dexamethasone, a glucocorticoid which, in contrast, does not bind to transcortin (2, 15). The demonstrated absence of potentiation in the instance of dexamethasone not only confirmed this prediction, but further showed that the potentiation observed in the instance of hydrocortisone was not to be ascribed to some heretofore undescribed "permissive" action of progestins on the expression of glucocorticoid effects.

Despite the numerous examples which can be drawn from different *in vitro* systems (1–8), progesterone—a steroid with clearly defined antiglucocorticoid properties *in vitro* (2)—has yet to be demonstrated to have an antiglucocorticoid action in any *in vivo* system. The possible pitfalls in the interpretation of such experiments are illustrated by the present set of observations which show that the administration of progesterone can, in fact, potentiate the effects of both endogenously secreted and exogenously administered natural glucocorticoids in the induction of TAT. That the potentiating effect of progesterone on glucocorticoid-induced enzyme synthesis is not due to any peripheral synergism at the cellular level, but rather is attributable to a competition for glucocorticoid binding sites on transcortin, is supported by the fact that transcortin is known to have a higher affinity for progesterone than it has for either hydrocortisone or corticosterone (11), and by the demonstration that induction in response to dexamethasone is entirely unaffected by progesterone administration. Since progesterone competes potently with both corticosterone and hydrocortisone for binding to transcortin, whereas in contrast it competes only weakly with either of these hormones for the cytosolic glucocorticoid receptor (2, 16), its effects on glucocorticoid-induced phenomena *in vivo* may be expected to be complex and at times "paradoxical." In contrast to the readily demonstrable antiglucocorticoid effects of progesterone *in vitro*, effects observed in the intact animal may reflect a balance between true antiglucocorticoid effects of the hormone at the cytosol receptor level and potentially obscuring "glucocorticomimetic" effects due to concomitant rises in free glucocorticoid levels brought about by displacment of endogenous glucocorticoid from its major storage site in transcortin.

Summary. Progesterone, a steroid hormone with well-defined antiglucocorticoid proper-

ties *in vitro*, is shown, paradoxically, to be a potent inducer of hepatic tyrosine aminotransferase in normal but not in adrenalectomized rats. It is further shown that progesterone, in adrenalectomized animals, markedly potentiates the induction of TAT by low doses of hydrocortisone, but that this synergism disappears at high doses of the latter hormone. In contrast, there is no synergism between progesterone and the synthetic steroid dexamethasone, which, unlike both hydrocortisone and the natural endogenous glucocorticoid corticosterone, does not bind to transcortin. These observations suggest that both the induction of TAT in the normal animal, as well as the potentiation of the inducing effects of low doses of exogenous hydrocortisone in the adrenalectomized animal, are attributable to the displacement by progesterone of endogenous corticosterone and exogenous hydrocortisone, respectively, from transcortin and the consequent rise in concentration of free circulating glucocorticoid. Although progesterone competes strongly with natural glucocorticoids for transcortin, it does so only weakly for the cytosolic glucocorticoid receptor. In contrast to its purely antiglucocorticoid effects *in vitro*, the effects of progesterone in the intact animal may thus reflect a balance between true antiglucocorticoid effects at the cytosol receptor level and other "glucocorticomimetic" effects due to a rise in free glucocorticoid level resulting from the displacement of endogenous glucocorticoid from plasma binding sites.

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