Corticosteroid Production Rates in Strain 2 Guinea Pigs following L2C/NB Leukemia Transplantation


SUMMARY

Production rates of cortisol, 2α-hydroxycortisol, and 6β-hydroxycortisol were determined (by isotope dilution technics) in individual adult strain 2 guinea pigs following transplantation of the guinea pig lymphoblastic leukemia L2C/NB. There was a statistically significant (P < 0.01, 0.02, and 0.01) 2.0-, 1.4-, and 1.9-fold higher production of cortisol, 2α-hydroxycortisol, and 6β-hydroxycortisol, respectively, in leukemia guinea pigs in comparison with similarly treated controls. No statistically significant change was observed in the fraction of the total cortisol production converted peripherally to 2α- and 6β-hydroxycortisol. The results indicate that in leukemic animals there was an increased adrenal secretion of 6β-hydroxycortisol but not of 2α-hydroxycortisol.

INTRODUCTION

In a previous study with two transplantable strain 2 guinea pig tumors (L1B leukemia and a liposarcoma) it was demonstrated that during the late stages of these malignant diseases the urinary excretion of cortisol, 2α-hydroxycortisol, and 6β-hydroxycortisol increased nearly 3-fold (12). While this study strongly suggested an enhancement in adrenocortical activity in tumor-bearing animals, conclusive proof was lacking since strongly suggested an enhancement in adrenocortical activity in tumor-bearing animals, conclusive proof was lacking since similar treated guinea pigs, and data were lacking on the magnitude and variability of the effect among individual animals.

Unfortunately, when methods became available to study production rates (by isotope dilution technics) in individual animals (5), the L1B leukemia and liposarcoma used in the first study became extinct. This paper reports the effect of a related transplantable guinea pig leukemia (L2C/NB (6, 9, 10, 11, 13) on the production rates of cortisol, 2α-hydroxycortisol and 6β-hydroxycortisol. In the guinea pig, 2α- and 6β-hydroxycortisol are cortisols hydroxylation products of both the adrenal and the liver (1, 2). In addition to the total production rates of these corticosteroids, data are also presented on the fractions secreted by the adrenal and formed peripherally from cortisol.

MATERIALS AND METHODS

Production rates were determined from the cumulative specific activities of the extensively purified urinary corticoids, following the simultaneous injection of tracer doses of cortisol-4,14C and tritiated 2α- and 6β-hydroxycortisol, using the Porter-Silber reaction for the determination of quantity and liquid scintillation spectrometry for the simultaneous determination of 14C and 3H in a manner similar to that previously detailed (2, 5).

Chromatography was done in tanks 120 cm long, using a solvent saturating device (8). Radioactivity was determined in a Packard Tri-Carb liquid scintillation spectrometer (Model 314 E), which was adjusted to efficiencies of tritium and 14C of 11 and 43%, respectively. Sufficient counts were accumulated to bring the counting error to below 3%. Samples for counting were added in 0.1 ml of methanol to 10 ml of toluene containing 0.4% 2,5-diphenyloxazole and 0.01% of 1,4-bis-(5,phenyloxazolyl) benzene (both obtained from Pilot Chemicals, Watertown, Massachussetts).

The radioactive steroids used (cortisol-4,14C, 45 mc/m mole; 2α-hydroxycortisol-1,2-3H, 950 mc/m mole; and 6β-hydroxycortisol-1,2-3H, 13.8 mc/m mole) were at least 95% pure as checked by scans of radioactivity on paper chromatograms and by reverse isotope dilution and purification in several systems. The cortisol-4,14C contained no material with radioactivity of the mobility of either 2α- or 6β-hydroxycortisol. The preparation and properties of the tritiated steroids have been described (3). The radioactivity injected varied from 6 X 10⁶ to 4 X 10⁸ dpm cortisol-4,14C, from 1 X 10⁶ to 9 X 10⁸ dpm 2α-hydroxy cortisol-1,2-3H, and from 1 X 10⁶ to 3 X 10⁸ dpm 6β-hydroxycortisol-1,2-3H.

The total production rates were calculated from the radioactivity injected and the cumulative specific activity of the purified respective steroids according to the expression:

\[ P_T = \frac{R}{\tau \times t} \]

where \( P_T \) = production rate, \( R \) = radioactivity injected, \( \tau \) = cumulative specific activity = the specific activity of the urinary steroid isolated from urine collected during the time \( t \) which is sufficient for the excretion of 95% or more of the radioactivity in the form of the steroid studied (15). Urine collection for 24 hours was sufficient for the achievement of the latter condition with guinea pigs.

From the 14C to 3H ratio in the urinary 2α- and 6β-hydroxycortisol and that in the injection solution, one may arrive at the fraction of the total cortisol production which is converted into...
the respective hydroxylated derivatives of cortisol peripherally—the cortisol to 2α-hydroxycortisol and the cortisol to 6β-hydroxycortisol conversion factors $\rho_{2α-OHF}$ and $\rho_{6β-OHF}$, respectively, where $\rho = \frac{[\text{C}]}{[\text{H}]}$ in urinary steroid)/[[[\text{C}]/[\text{H}]]$ in injection solution] (7). From the $\rho$ values it is possible to arrive at the adrenal and peripheral contributions of the total production rates of 2α- or 6β-hydroxycortisol, assuming that there is no source of these steroids other than adrenal secretion or peripheral formation from circulating cortisol. Thus, if the total production, peripheral production, and adrenal secretion of 2α-hydroxycortisol and the total production of cortisol are designated $P_{2α}^2$, $P_{2α}^2$, $P_{2α}^a$, and $P_{2α}^p$ respectively: $P_{2α}^a = P_{2α}^p \times \rho_{2α-OHF}$ $P_{2α}^p = P_{2α}^p - P_{2α}^a$.

The animals used were descendants of inbred Strain 2 guinea pigs obtained from the Animal Production Section, NIH, and bred at the Worcester Foundation for Experimental Biology. They were fed Purina guinea pig chow (Ralston Purina Co.) ad libitum and fresh green vegetables.

The origins of the leukemia used, its subsequent history, aspects of its biology, and its present designation as L2C/NB have been described (6, 9, 10, 11, 13). The L2C/NB leukemia has now been maintained as a stable line in inbred Strain 2 guinea pigs at the Worcester Foundation for Experimental Biology for 2 years (since its transfer from the National Cancer Institute). At the commencement of this study, the L2C/NB leukemia was approximately in its 30th consecutive passage.

L2C/NB leukemia transplantation was done by injecting intraperitoneally 0.5 ml whole citrated or heparinized blood from donors which exhibited a positive blood smear for lymphoblastic leukemia (13). The positive leukemic animals studied died of leukemia 2–3 days later and exhibited greatly enlarged spleens, enlarged livers, and mesenteric lymph nodes as previously described for the related L2B leukemia (10).

EXPERIMENTAL DESIGN

The leukemic guinea pigs did not die of bacterial infection, and the morphologic effects that accompany endotoxin were not present. The adrenal enlargement in acute leukemia was of a different nature than that observed with acute infection or endotoxin given on long or short term. The exact chain of events leading to death is not completely understood, but it seems to involve a massive infiltration of the brain with tumor lymphocytes particularly near the respiratory centers and the hypothalamus, which provides the basis for eventual death with possible preceding stimulation of the adrenal pituitary axis. For these reasons it was felt that the most appropriate controls would be similarly treated normal animals.

Since there is as great a variability in the production rates (unpublished observations with several strains) between individual guinea pigs as that found in the same individuals on different days, no particular advantage existed in using the same animals during their pretransplantation period as controls. Since such a design would also have the drawback of interference by uncontrollable factors present on certain days and not on others, the experiments reported here were designed to allow comparison between leukemic and similarly treated controls studied simultaneously.

Adult Strain 2 guinea pigs 3–5 months old (weighing 500–780 gm) were randomly divided into control and experimental groups, each group containing an equal number of animals with ±50 gm of weight and ±1 month of age. Two separate studies were done with a total of 10 animals in Study I and 20 animals in Study II. (One leukemic animal of the experimental group died during urine collection in Study II.) The control and experimental animals were treated in precisely the same fashion except that the controls were injected (i.p.) with 0.5 ml of normal Strain 2 guinea pig blood, while the experimental animals received an equal volume of freshly drawn leukemic blood from Strain 2 guinea pig carriers.

Prior to leukemia transplantation (or normal blood injection) all animals were accustomed to the production rate determination procedure by simulated runs twice weekly for 2 weeks.

Since not all experimental animals became leukemic at the same time, production rates were determined in experiments in which a leukemic and a normal control animal (chosen at random) were studied simultaneously. Injection of tracer was done between 10 and 11 a.m.

RESULTS

The total production rates of cortisol, 2α-hydroxycortisol, and 6β-hydroxycortisol determined in normal controls and leukemic animals obtained in the 2 separate studies are summarized in Table 1. There was a statistically significant increase in the production rates of all 3 steroids studied. In Table 2 are given the fractions of the total cortisol production converted peripherally to 2α- and 6β-hydroxycortisol $\rho_{2α-OHF}$ and $\rho_{6β-OHF}$. There was no statistically significant difference ($P > 0.5$) in the $\rho$'s between leukemic and control animals.

The mean adrenal secretion ($P_a$) and the peripheral production from cortisol ($P_p$) of 2α- and 6β-hydroxycortisol are summarized in Table 3, from which it appears that in the leukemic animals there was approximately a twofold increase in both the adrenal secretion and the peripheral formation (from cortisol) of 6β-hydroxycortisol. Whereas the peripheral formation of 2α-hydroxycortisol from cortisol also increased twofold in the leukemic animals, the mean adrenal secretion of this steroid remained unchanged.

DISCUSSION

In guinea pigs with the L2C/NB leukemia there was a statistically significant increase of 2.0-, 1.4-, and 1.9-fold in the total production rates of cortisol, 2α-, and 6β-hydroxycortisol, respectively. The fraction of cortisol converted to 2α- and 6β-hydroxycortisol peripherally was not significantly affected, which indicates no significant change in the total peripheral enzymatic activities responsible for hydroxylatation of cortisol at C-2α and C-6β (mostly hepatic). However, since in the leukemic animals there is a highly enlarged liver which is also infiltrated with leukemic cells (10), the possibility exists that no alteration in the actual concentration (on unit weight basis) of enzymatic activity had occurred. There was an increased adrenal secretion of cortisol and 6β-hydroxycortisol but not of 2α-hydroxycortisol, suggesting a specific enhancement of adrenocortical activity in the leukemic animals. The lack of an increased adrenal secretion of 2α-hydroxycortisol may point to a leukemia-caused decrease in the adrenal enzymatic hydroxylation of cortisol at C-2α.
Leukemia and Corticosteroids in Guinea Pigs

TABLE 1
Production Rates of Cortisol, 2α-, and 6β-Hydroxycortisol in Control and Leukemic Guinea Pigs

<table>
<thead>
<tr>
<th>Guinea pig no.</th>
<th>Controls</th>
<th>Leukemic</th>
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<tbody>
<tr>
<td></td>
<td>F</td>
<td>2α-OFH</td>
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<tr>
<td></td>
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<td></td>
</tr>
<tr>
<td>1</td>
<td>120</td>
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<td>15</td>
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<td>360</td>
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<tr>
<td>Mean ± S.D.</td>
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<td>Significance of leukemic vs. control, P</td>
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</table>

*Expressed as μg/24 hr.

The data summarize 2 separate studies done with Animals 1-5 and 16-20 (Study I) and Animals 6-15 and 21-29 (Study II). In each study controls were injected (i.p.) with 0.5 ml of normal Strain 2 guinea pig blood, while the experimental animals received 0.5 ml of freshly drawn leukemic blood from a single Strain 2 leukemic animal. The experimental Animals 16-29 became leukemic and were studied together with randomly chosen controls 14, 10, 14, 17, 10, 13, 13, 12, 19, 18, 12, 12, and 13 days, respectively, following leukemia transplantation. One animal (No. 30) in Study II died of leukemia during urine collection.

F, cortisol; 2α-OFH, 2α-hydroxycortisol; 6β-OFH, 6β-hydroxycortisol.

TABLE 2
Cortisol to 2α-Hydroxycortisol and Cortisol to 6β-Hydroxycortisol Conversion Factors (PF-2α-OFH and PF-6β-OFH)* following Leukemia Transplantation (mean ± S.D.)*

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Leukemic</th>
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<tr>
<td></td>
<td></td>
<td></td>
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<tr>
<td>PF-2α-OFH</td>
<td>0.083 ± 0.010</td>
<td>0.077 ± 0.014</td>
</tr>
<tr>
<td>PF-6β-OFH</td>
<td>0.045 ± 0.022</td>
<td>0.048 ± 0.016</td>
</tr>
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</table>

*F, cortisol; 2α-OFH, 2α-hydroxycortisol; 6β-OFH, 6β-hydroxycortisol.

The data are a summary of the p values obtained in the same study described in Table 1. The controls and leukemic groups represent 15 and 14 animals, respectively. The differences between control and leukemic were not statistically significant (P > 0.5).

ever, further studies with hepatic and adrenal tissue and leukemic cells from leukemic animals in vitro are necessary to obtain a definitive answer to these questions.

The biologic activity of the hydroxylated cortisol derivatives of cortisol is not certain. Although 2α- and 6β-hydroxycortisol have been found to be inactive in the usual (glycogen deposition and sodium retention) tests (unpublished), a claim has been made that 6β-hydroxycortisol inhibits the effect of cortisol on rat liver tryptophan pyrrolase induction (14). Whether the increased levels of 6β-hydroxycortisol counteract in vivo the effect of the enhanced cortisol productions remains to be determined.

The results of this study are in agreement with the previous study (12), in which an elevated adrenocortical function in leukemic (L2B) animals was suggested from urinary data.

The variability observed in the production rates in the Strain 2 animals as judged from the coefficients of variation (approximately 40%) was similar in magnitude to that previously described for the urinary excretion of these corticosteroids in several
other strains (4), indicating that the latter may actually be a reflection of the variability of adrenal secretion in this species and not of renal effects. Despite the enhanced production in the leukemic animals, the variability of the latter did not appear to differ significantly from that found in the similarly treated controls, both groups probably reflecting similar reactions to environmental and internal "noises." The mechanism of the elevated adrenocortical function in leukemia remains unexplained. Although the possibility that the widely spread infiltration of leukemic cells in the brains of leukemic animals has been the cause of a hypothalamic stimulation of adrenocorticotropic hormone release is interesting, the data do not permit exclusion of so-called nonspecific stress observed in animals near death.

ACKNOWLEDGMENTS

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Corticosteroid Production Rates in Strain 2 Guinea Pigs following L₂C/NB Leukemia Transplantation

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