EFFECTS OF DEXAMETHASONE 17-ESTERS ON ADRENAL WEIGHT AND HYDROLYSIS OF GLUCOCORTICOID 17-ESTERS IN RAT FETUSES

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Prednisolone was detected in the brain of rat fetus subcutaneously administered prednisolone 17-acetate. After the subcutaneous injection of betamethasone 17-propionate or dexamethasone 17-propionate to rat fetus, the unchanged steroid was detected mainly in the brain.

The hydrolytic rates of prednisolone 17-acetate, betamethasone 17-esters (acetate, propionate) and dexamethasone 17-esters (acetate, propionate, valerate) in the livers of rat fetuses were studied. Prednisolone 17-acetate was hydrolyzed to prednisolone very rapidly. Betamethasone 17-esters and dexamethasone 17-esters were hydrolyzed more slowly. The hydrolytic rate of dexamethasone acetate among the dexamethasone 17-esters was the most rapid, followed by propionate and valerate.

The adrenals of rat fetuses became significantly atrophied after subcutaneous administration of dexamethasone and its 17-esters. The simultaneous administration of betamethasone 17, 21-dipropionate and its metabolite betamethasone impaired the hypertrophic effect of the former on the adrenal weights of rat fetuses. The effects of the hydrolytic rate of these glucocorticoid 17-esters and of C16-methyl conformation on the hypothalamo-pituitary-adrenal system in rat fetuses are discussed.

Keywords—betamethasone 17, 21-dipropionate; betamethasone 17-acetate, propionate; prednisolone 17-acetate; dexamethasone 17-acetate, propionate, valerate; glucocorticoid effect; metabolites in rat fetus brain; hydrolysis

Usually, glucocorticoids such as dexamethasone (DM), betamethasone (BM) or prednisolone (Pred) induce adrenal atrophy in rat fetuses at late-pregnancy. However, the synthetic anti-inflammatory glucocorticoid BM 17, 21-dipropionate (BMDP) produces adrenal hypertrophy in rat fetuses upon administration to pregnant mothers or fetuses in utero. The main metabolite of BMDP, BM 17-propionate, induces adrenal hypertrophy in rat fetuses. Other 17-esterified BM, BM 17-acetate and BM 17-valerate, also produce adrenal hypertrophy, but Pred 17-acetate or 17-propionate behave as ordinary glucocorticoids on rat adrenals. However, as to whether the 16α-methyl epimers of BM 17-esters, DM 17-esters, induce such adrenal hypertrophy is not known.

Since steroid esters are hydrolyzed in the liver, the mother compounds BM and Pred, produced by the hydrolysis of these glucocorticoid 17-esters, may modulate the effects of the latter on the hypothalamo-pituitary-adrenal (HPA) system. Therefore, metabolites should appear in the brain following administration of 17-esterified corticosteroids and investigations on rates of hydrolysis of these steroids in fetal tissues are important to clarify the difference between their peculiar hypertrophic effect and atrophic one on adrenals of rat fetuses.

In this study, effects of DM 17-esters on adrenal weights of rat fetuses were investigated to evaluate the role of the 16-methyl conformation on the effects of glucocorticoid 17-esters on the HPA system. We also determined the metabolites
which appeared in the fetal brain after the administration of Pred 17-acetate, BM 17-propionate and DM 17-propionate and examined the rates of hydrolysis of these glucocorticoid 17-esters.

MATERIALS AND METHODS

Materials — Betamethasone 17-acetate (BMA), betamethasone 17-propionate (BMP), betamethasone 17-valerate (BMV), prednisolone 17-acetate (PMA), dexamethasone 17-acetate (DMA), dexamethasone 17-propionate (DMP) and dexamethasone 17-valerate (DMV) used for studying the hydrolysis and 17-hydroxydeoxycorticoesterone as an internal standard for high-performance liquid chromatography were synthesized at our laboratory. Dexamethasone (DM), cortisol and deoxycorticosterone were purchased from Sigma Chem. Co., USA. Betamethasone (BM), betamethasone 17,21-dipropionate (BMDP) and prednisolone (Pred) were obtained from Schering Corp., USA.

Animals — Virgin female Sprague-Dawley rats purchased from CLEA Japan, Inc. were mated overnight with the same strain at 8 to 12 weeks of age. The day when spermatozoa were found in vaginal smears was designated as day 0 of pregnancy. The rats were fed commercial chow (CA-1 pellets, CLEA Japan, Inc.) and water ad libitum. All animals were kept in a room lighted from 7 a.m. to 7 p.m. and maintained at 25 ± 1°C with a relative humidity of 55 ± 10%.

Administration of Steroids and Weighing of Adrenals — Steroids suspended in 0.5% gum arabic (vehicle) were subcutaneously injected at a dose of 100 μg/0.02 ml to the fetuses on day 19 of pregnancy in utero through the uterine wall while the mother rats were under ether anesthesia. Two days after the treatment, mother rats were killed with a blow on the head and the fetuses were immediately removed. The left adrenal of the fetus was removed and weighed in a semimicrobalance with a sensitivity of 10 μg.

Detection of Metabolite in the Fetal Brain — Fetuses were removed from the uteri of the mother rat on day 21 of pregnancy and subcutaneously administered with about 400 μg/0.1 ml of PMA, BMP or DMP suspended in 2% polysorbate 80 aqueous solution. Two hours after the administration, the fetuses were decapitated and the brains were placed in ice-cold 10-ml test tubes, and homogenized with a glass rod. The homogenate was extracted twice by vigorous shaking of the test tube following addition of 5 ml of ethyl acetate. The solvent was evaporated and part of the extracts dissolved in methanol was subjected to high-performance liquid chromatography.

Hydrolysis of Glucocorticoid 17-Esters — Fetuses were quickly picked up from the uteri of rats killed by bleeding on day 21 of pregnancy. Fetal livers were collected rapidly in a beaker cooled with ice. They were homogenized with four volumes of ice-cold 1/15 M phosphate buffer (pH 7.3) prepared by mixing 1/15 M Na₂HPO₄ and KH₂PO₄ and centrifuged at 9000 × g for 30 min at below 5°C. One hundred micrograms of glucocorticoid 17-esters dissolved in 100 μl of methanol was incubated at 37°C with 5 ml of the 9000 × g supernatant fraction of the liver. Incubation was carried out with shaking for 2.5 min for PMA and for 15 or 30 min for the other glucocorticoid 17-esters. The reaction was stopped by the addition of 20 ml of ethyl acetate followed by vigorous shaking of the incubation flask. The organic solvent phase was separated and evaporated. Extraction for steroids was done twice in the same manner. The extracts were dissolved in 100 μl of methanol containing the internal standard (IS) then 1 μl each was subjected to high-performance liquid chromatography.

High-performance Liquid Chromatography — A high-performance liquid chromatograph (Varian Model 8500) with a fixed-wavelength UV detector (254 nm) was used. The analytical conditions were as follows: column, μBondapak C₁₈, 2 mm i.d. x 30 cm (Waters packed column); flow rate, 100 ml/hr; mobile phase, H₂O: methanol = 34 : 66 (v/v) for the detection of metabolites in the fetal brain. In the in vitro study of hydrolysis of glucocorticoid 17-esters, the conditions were: column, Micropak NH₂-10, 2 mm i.d. x 25 cm (Varian packed column); flow rate, 130 ml/hr; mobile phase, n-hexane:dichloromethane:
isopropyl alcohol = 78:11:11 (by vol) for BMA, BMP and DMP, 75:12.5:12.5 (by vol) for DMA, 82:9:9 (by vol) for DMV, 87:6.5:6.5 (by vol) for PMA and 64:18:18 (by vol) for Pred. IS was cortisone for BMA, BMP, DMP and Pred, 17-hydroxy deoxycorticosterone for DMA, deoxycorticosterone for PMA. The calibration curve drawn by plotting the peak height ratio of authentic steroids and IS showed a good linearity.

TABLE I. Effects of Dexamethasone Derivatives on Adrenal Weight of Rat Fetuses

<table>
<thead>
<tr>
<th>Compound</th>
<th>No.</th>
<th>Body weight (g)</th>
<th>Left adrenal weight</th>
<th>Absolute (mg)</th>
<th>Relative (mg/g of body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle (0.5% gum arabic)</td>
<td>19</td>
<td>4.72±0.30</td>
<td>0.78±0.11</td>
<td>0.17±0.03</td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>19</td>
<td>3.45±0.30*</td>
<td>0.57±0.06*</td>
<td>0.17±0.02</td>
<td></td>
</tr>
<tr>
<td>DMA</td>
<td>16</td>
<td>3.76±0.47*</td>
<td>0.59±0.07*</td>
<td>0.16±0.03</td>
<td></td>
</tr>
<tr>
<td>DMP</td>
<td>14</td>
<td>4.44±0.29*</td>
<td>0.71±0.10</td>
<td>0.16±0.02</td>
<td></td>
</tr>
<tr>
<td>DMV</td>
<td>15</td>
<td>3.81±0.21</td>
<td>0.52±0.08</td>
<td>0.14±0.02*</td>
<td></td>
</tr>
</tbody>
</table>

Fetuses were subcutaneously injected with dexamethasone derivatives at a dose of 100 μg on day 19 and killed on day 21 of intrauterine development. Each value represents a mean ± standard deviation. *Significantly different from the vehicle-treated group (p < 0.05).

FIG. 1. Liquid Chromatograms of the Brain Extracts of Rat Fetuses administered Glucocorticoid 17-Esters

(a) Vehicle administration, (c) BMP administration, (b) PMA administration, (d) DMP administration.
RESULTS

Effects of DM 17-Esters on Adrenal Weights of Rat Fetuses

As shown in Table I, body weights and adrenal weights significantly decreased by the administration of DM, DMA and DMV to rat fetuses compared to the vehicle group.

Detection of Metabolite in the Fetal Brain

Fig. 1 shows the chromatograms of brain extracts from rat fetuses sacrificed 2 hours after administration of PMA, BMP or DMP. After PMA administration, no peak corresponding to authentic PMA was detected while that corresponding to authentic Pred appeared. After the administration of BMP or DMP, the peaks corresponding to BM and DM as well as BMP and DMP were detectable. The concentrations of the unchanged steroids were about six to seven times higher than that of the hydrolyzate.

Hydrolysis of Glucocorticoid 17-Esters

Fig. 2 shows the rate of disappearance of glucocorticoid 17-esters from the incubation medium during incubation with the 9000 × g supernatant fraction of rat fetus liver. Incubation of 100 μg of PMA for 25 min abolished the steroid completely, while a peak corresponding to the retention time of Pred appeared in the incubation mixture in a quantity of 100 μg. BM 17-esters and DM 17-esters disappeared at a slower rate than PMA from the incubation medium. BM 17-esters and DM 17-esters bearing a longer side chain of fatty acid at the C-17 position disappeared more slowly. Each BM 17-ester (acetate, propionate) tended to decrease at a higher rate than the corresponding DM 17-ester. Upon incubation of BMA, the peak corresponding to the retention time of authentic BM

![Graph showing the disappearance of the substrate over time for different hormones](image)

FIG. 2. Hydrolysis of Glucocorticoid 17-Esters with the 9000 × g Supernatant Fraction of the Liver in Rat Fetuses

<table>
<thead>
<tr>
<th>TABLE II. Effect of BMDP combined with BM given subcutaneously to 19-day-old Fetuses on Adrenal Weights in Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound</td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
</tr>
<tr>
<td>BMDP 100 μg</td>
</tr>
<tr>
<td>BMDP + BM</td>
</tr>
<tr>
<td>100 μg +100 μg</td>
</tr>
<tr>
<td>BM 100 μg</td>
</tr>
</tbody>
</table>

* Significantly different from the vehicle-treated group (p < 0.05).
Glucocorticoid Effects and Hydrolysis

was detectable. Boiling of the tissue preparation prior to incubation abolished the esterase activity. Effect of BMDP combined with BM on Adrenal Weights

The degree of adrenal hypertrophy caused by the administration of BMDP diminished to the control level by the combined administration of BMDP and BM (Table II).

DISCUSSION

Usually, glucocorticoids suppress the HPA system of rat fetuses at late stages of pregnancy but BMDP or BMP induce adrenal hypertrophy by stimulating the HPA system in rat fetuses. This adrenal hypertrophy produced by the administration of BMDP or BMP has been suggested to be caused by the same metabolite in the fetal brain, BMP, the main metabolite of BMDP (unpublished). The administration of other glucocorticoid 17-esters, 17-esters of Pred (acetate, propionate) and DM (acetate, propionate, valerate), induced adrenal atrophy of rat fetuses (Table I). The parent compounds of all these steroids, BM, Pred, and DM (Table I), produced adrenal atrophy. Therefore, the rate of hydrolysis of these glucocorticoid 17-esters may modulate the action on the HPA system. Both BM and DM 17-esters were hydrolyzed slowly in fetal liver (Fig. 2). BMP and DMP were detected unchanged in the brains of rat fetuses (Fig. 1). BM and DM were also found. BMP has been detected mainly in the fetal brain after the administration of BMDP to mother rats (unpublished). Moreover, the simultaneous administration of BMDP and the latter metabolite BM diminished the stimulating effect of BMDP on the HPA system (Table II). Based on these results, the concentration of BMP at the active site after the administration to rat fetuses is estimated to be higher than the effective concentration of the mother compound, BM. Thus, unchanged BMP may act on the HPA system. Unchanged DMP also may act on it for the similar reason. Accordingly, the unchanged 17-esters of BM or DM may act on the HPA system of rat fetuses producing the opposite effect on it. PMA was hydrolyzed rapidly to Pred by the incubation with the 9000 × g supernatant fraction of rat fetus livers (Fig. 2) and only the hydrolyzate Pred was detected in the fetal brain following PMA administration (Fig. 1). Therefore, the concentration of Pred at the active site can be speculated to reach a level which induces adrenal atrophy. When PMP is hydrolyzed to Pred at a similar speed, the adrenal atrophy induced by the PMP treatment may be caused by the hydrolyzate, Pred.

The rate of hydrolysis of both 17-esters of BM and DM was extremely slower than that of PMA under incubation with the 9000 × g supernatant of rat fetus livers (Fig. 2). This fact suggests that the methyl group at C16 position serves as a steric hindrance to the attack of the liver esterase at the C17 position of these steroids. The steric hindrance of the C16-methyl substituents against the liver esterase in hydrolysis of glucocorticoid 17-esters is slightly stronger in the α-configuration than in the β one, because of the slightly slower rate of hydrolysis of DM 17-esters than that of BM 17-esters.

BM 17-esters activate the HPA system of rat fetuses but the 16α-methyl epimers, DM 17-esters, suppress it as does the conventional glucocorticoid (Table I). Hasegawa et al. demonstrated that C16-β-methylation of steroid with 17-ester is necessary to cause fetal adrenal hypertrophy because of suppression of the HPA system for Pred 17-esters and its activation for BM 17-esters. This opposite effect between 17-esters of Pred and BM on the HPA system can be explained by the difference between their rate of hydrolysis (Fig. 2). However, the unchanged BM 17-esters and DM 17-esters that are considered to act on the HPA system of rat fetus may have opposite effect on it. This suggests that 16β-methylation of 17-esterified corticosteroids may modify the side-chain conformation of the D-ring required for feedback of the HPA system. In 16-methyl substituted compounds, 16β-CH₃-progesterone and 16α-CH₃-progesterone as well as 16β-methyl-17,21-dihydroxypregn-4-ene-3,20-dione (16β-methyl-cortisolone) and 16α-methyl-cortisolone, the conformation of the
methyl group at C16 modifies the rotation of the side chain about the C17-C20 axis. In 17-esters of BM and DM, introduction of the ester bond at the C17 position will cause further complexity in the rotation of this side chain. Thus, one could expect that DM 17-esters have the side-chain conformation of the D-ring required for feedback to the HPA system, but BM 17-esters do not. Such a difference in the conformation of these esters could partially account for differences in interaction with the receptor. In BM 17-esters, the steroid-receptor complex may be prevented from undergoing the conformational change necessary for activation when the side chain conformation is modified by 16β-methylation and 17α-esterification.

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REFERENCES