All-trans-retinoic acid distribution and metabolism in vitamin A-marginal rats

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VITAMIN A AND ITS ACTIVE METABOLITES are essential for normal immune competence, and vitamin A deficiency is well-known to increase the severity of infectious diseases (59, 60). Conversely, retinoids [all-trans-retinoic acid (RA), cis isomers, metabolites, and synthetic analogs] have been studied extensively for their ability to treat or prevent numerous disease conditions. cis and trans isomers of RA are used therapeutically for diseases of the skin, such as cystic acne and hyperkeratotic disorders (43), and all-trans-RA has proved successful in the treatment of acute promyelocytic leukemia (23, 41). Similarly, retinoids may reduce the risk of other cancers (40). Moreover, RA may possess immune adjuvant properties, suggesting that it could be administered in conjunction with vaccines to prevent or reduce the severity of various infections (3, 31, 32). Surprisingly, however, few studies have addressed the effects of disease states on the metabolism of RA.

Under normal physiological and dietary conditions, RA is present in the circulation at very low levels, in the range of 4–14 nM in humans (8, 12) and rats (62), which is 0.2–0.7% of plasma retinol levels. Even when RA is administered in pharmacological doses, plasma RA remains quite low (42, 46) and turns over rapidly with a turnover time of ~2 min in vitamin A-adequate rats (25) and a first-order terminal half-life of ~45 min in rhesus monkeys given large doses of either all-trans-RA or 9-cis-RA and in humans (1, 37, 58). Despite the low concentration and rapid turnover of RA, plasma RA contributes significantly to tissue retinoid pools in a manner that is organ specific. In rats studied under steady-state conditions, the liver derived more than 75% of its RA pool from plasma, suggesting that the delivery of RA from plasma to liver is an important process for maintaining RA concentrations in the normal, homeostatically regulated range (25). The liver and biliary system also play central roles in the catabolism and excretion of retinoids (57, 68). RA is capable of inducing its own metabolism (65–67), and therefore RA turnover is likely to increase during RA therapy.

The acute-phase response to inflammation results in marked alterations in hepatic protein synthesis and energy metabolism, which in turn, widely affect other organ systems. The levels of albumin, retinol-binding protein (RBP), and other nutrient transport proteins are reduced (4), whereas other plasma proteins are elevated during inflammation (44). Previous studies of vitamin A homeostasis during inflammation have established that inflammation induces a state of low plasma retinol (hyporetinolemia), similar to that observed during normal infections, including measles and diarrhea (22, 39, 55). In rats with LPS-induced inflammation, the level of RBP mRNA in the liver was reduced prior to the reduction in the concentration of the RBP-retinol complex in plasma (50). In studies of similar design, hepatic retinol was increased (19), suggesting that inflammation impairs the transport of retinol from the liver to plasma. Moreover, in rats with marginal vitamin A status the reduction in plasma due to marginal vitamin A status was further compounded by inflammation (51). However, studies have not yet addressed the effects of acute inflammation on the metabolism of plasma RA, the active metabolite of retinol that mediates the majority of physiological functions of vitamin A.

In the present study, we have used LPS-induced inflammation in rats with marginal vitamin A deficiency, a model of subclinical vitamin A deficiency that affects many children in the developing world where infection and inflammation are also widespread (61), to study the effects of acute, nonseptic inflammation on the distribution and catabolism of plasma RA. We hypothesized that LPS would alter the distribution of RA between plasma and tissues, especially the liver, which is centrally involved in retinoid metabolism (53, 54). Because RA itself can offset the effects of vitamin A deficiency (10, 26), significantly alter the metabolism of retinol (33, 69), and induce its own catabolism in vitro and in vivo (11, 48, 63), we
designed a 2 × 2 factorial study to examine the effects of RA alone, LPS-induced inflammation alone, and both in combination on RA homeostasis. The results of these studies demonstrate that RA and LPS independently affect the distribution and the metabolism of RA in a tissue-specific manner and that RA and LPS are, for the most part, independent factors in the regulation of RA homeostasis.

MATERIALS AND METHODS

Animals and diet. All animal procedures were approved by the Institutional Animal Use and Care Committee of the Pennsylvania State University. Female Sprague-Dawley (Charles River Laboratories, Boston, MA) rats with litters of 12 female pups were fed a vitamin A-deficient diet from the time of arrival to reduce the accumulation of vitamin A from milk by their nursing offspring (18). The rats were housed in a room maintained at 22°C with a 12:12-h light-dark cycle, and food and water were available ad libitum. When the pups were 21 days old, they were weaned in pairs and fed a nutritionally complete AIN-93G diet (45) modified to contain 0.412 mg of retinyl palmitate per kilogram of diet (Research Diets, New Brunswick, NJ). This amount of vitamin A was shown previously to be adequate for normal growth but results in plasma retinol levels about half that of control-fed rats (51).

Experimental design. When the rats were between 54 and 60 days of age, they were randomly assigned to one of four treatment groups (n = 9/group): control, RA alone, LPS alone, or RA + LPS. Rats treated with RA were given an oral dose of 500 μg all-trans-RA (Sigma-Aldrich, St. Louis, MO) prepared in of the albumin-bound [3H]RA, prepared as described below, was removed immediately after treatment until the end of the 12-h experiment. When the rats were between 54 and 60 days of age, they were randomly assigned to one of four treatment groups (n = 9/group): control, RA alone, LPS alone, or RA + LPS. Rats treated with RA were given an oral dose of 500 μg all-trans-RA (Sigma-Aldrich, St. Louis, MO) prepared in of the albumin-bound [3H]RA, prepared as described below, was removed immediately after treatment until the end of the 12-h experiment period (50). Twelve hours posttreatment, the rats were lightly anesthetized by isoflurane-oxygen inhalation, and 0.15 ml/100 g BW of the albumin-bound [3H]RA, prepared as described below, was injected into the exposed left common iliac vein. The incision was closed with a sterile surgical staple, and the rats were allowed to recover from the anesthesia. Rats were killed either 10 min (n = 5/group) or 30 min (n = 4/group) postinjection by carbon dioxide asphyxiation. Blood was drawn from the inferior vena cava in heparinized syringes, and the liver, lung, spleen, and small intestine were quickly removed and weighed, and aliquots were frozen in liquid nitrogen for storage at −80°C before subsequent analysis. Because RA is rapidly metabolized, it is important to collect tissues quickly (11, 47); thus each rat was treated on a defined schedule so that all tissues could be collected and frozen within 5 min or less after euthanasia. The 10- and 30- min time periods chosen for this experiment were based upon previous reports (25) and pilot work (data not shown) which showed that >95% of the [3H]RA dose was cleared from plasma within 10 min and that metabolism of [3H]RA to polar metabolites could be readily detected by 30 min.

Dose preparation. All procedures involving retinoids were carried out in either dim or yellow light. The doses of [3H]RA for intravenous injection were prepared fresh immediately prior to injection. To prepare a 1-ml aliquot, 10 μCi of [3H]RA ([11,12(N)-3H]retinoic acid, specific activity 1.96 TBq/nmol; from PerkinElmer, Boston, MA) was combined with 0.23 μmol of all-trans-RA, and the solvent was evaporated under argon until <10 μl remained. Next, 5 μl of Tween 20 (Sigma-Aldrich) and 0.9 ml of 0.1% rat serum albumin (diluted in sterile PBS; Calbiochem, San Diego, CA) were added, and the mixture was vortexed. Then, 0.10 ml of whole rat plasma was added to the sample and immediately vortexed. The final concentra-
RESULTS

Inflammation- and RA-induced changes in organ weight and plasma retinol. Rats grew normally and appeared in good health, and groups did not differ in body weight at the time of treatment (Table 1). Twelve hours later, after treatment and an overnight fast, all groups lost ≤8.2 g BW (Table 1). There were no differences due to treatment in the relative weights of the lungs and small intestine. The relative weight of the spleen was higher in LPS-treated rats, indicative of mild inflammation, and the relative weight of the liver of rats treated with RA + LPS was slightly increased (Table 1). There was no difference in liver vitamin A, which averaged from 17 to 21 nmol/g (Table 1). Plasma retinol (Table 1) was measured as an indicator of vitamin A status and to confirm the effect of LPS-induced inflammation on plasma retinol. Plasma retinol averaged 0.54 μM in the control group, which was similar to previous studies of rats with marginal vitamin A deficiency (51), but it was only 42% of that in vitamin A-sufficient rats (50). Plasma retinol was further reduced by 47% in rats with LPS-induced inflammation compared with the marginally vitamin A-deficient control group, whereas RA treatment did not affect plasma retinol in this 12-h study (Table 1). There was no difference due to time (10 min vs. 30 min) in the sum of the total $^3$H-labeled retinoids recovered in the plasma, liver, lung, and small intestine (data not shown). However, LPS decreased the sum of the total $^3$H-labeled retinoids recovered in the plasma, liver, lung, and small intestine compared with RA-treated rats (Table 1).

RA alone and in combination with LPS increases the total radioactivity in plasma and the proportion of [3H]RA metabolites. In control rats, greater than 95% of the injected dose was eliminated from plasma within 10 min (Fig. 1A). In comparison, total $^3$H-labeled retinoids were higher in plasma in the RA, LPS, and RA + LPS groups at this time. Using two-way ANOVA, we showed both RA and LPS to be significant factors, but there was no interaction. By 30 min, total $^3$H-labeled retinoids in plasma were similar in the control group and the LPS-treated group but were higher in the RA group (Fig. 1B). Furthermore, rats treated with RA + LPS had significantly more (8.5%) of the injected $^3$H dose in plasma, which was approximately twice the amount in the control group ($P < 0.01$).

Total $^3$H-labeled polar metabolites in plasma, representing lipid-soluble metabolites of RA and its aqueous metabolites combined, were determined on a pooled sample from each treatment group at each time. $^3$H-labeled polar metabolites were increased by RA, compared with the control group, at both 10 and 30 min. At 10 min, about 60% of the total $^3$H-labeled retinoids recovered in plasma already consisted of

Table 1. Body weights, relative organ weights, and plasma retinol concentrations in RA- and LPS-treated rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>RA</th>
<th>LPS</th>
<th>RA + LPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW at dosing, g</td>
<td>233.8±9.80</td>
<td>224.2±6.87</td>
<td>212.9±5.74</td>
<td>217.9±7.36</td>
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<tr>
<td>Change in BW, g/12 h</td>
<td>−6.8±1.0</td>
<td>−6.4±0.9</td>
<td>−8.0±1.0</td>
<td>−8.2±1.0</td>
</tr>
<tr>
<td>Liver weight, % BW</td>
<td>3.64±0.06</td>
<td>3.76±0.12</td>
<td>3.91±0.11</td>
<td>4.03±0.10</td>
</tr>
<tr>
<td>Lung weight, % BW</td>
<td>0.51±0.02</td>
<td>0.53±0.01</td>
<td>0.54±0.02</td>
<td>0.53±0.02</td>
</tr>
<tr>
<td>Spleen weight, % BW</td>
<td>0.22±0.01</td>
<td>0.22±0.01</td>
<td>0.27±0.01</td>
<td>0.29±0.01</td>
</tr>
<tr>
<td>Small intestine weight, % BW</td>
<td>2.29±0.09</td>
<td>2.41±0.09</td>
<td>2.43±0.13</td>
<td>2.43±0.14</td>
</tr>
<tr>
<td>Plasma retinol, μmol/l</td>
<td>0.54±0.06</td>
<td>0.44±0.06</td>
<td>0.30±0.04</td>
<td>0.29±0.04</td>
</tr>
<tr>
<td>Liver total retinol, nmol/g</td>
<td>17±11</td>
<td>21±6</td>
<td>18±5</td>
<td>14±7</td>
</tr>
<tr>
<td>Total $^3$H-labeled retinoids, % ID†</td>
<td>30.0±1.23</td>
<td>33.3±1.38</td>
<td>26.5±1.16</td>
<td>28.1±1.15</td>
</tr>
</tbody>
</table>

Values are means±SE; n = 9/group. RA, retinoic acid; LPS, lipopolysaccharide; BW, body wt. *For liver total retinol, values are means±range of two representative rats per group. †For total $^3$H-labeled retinoids, values are sums of plasma, liver, lung, and small intestine total $^3$H-labeled retinoids, expressed as percent injected dose (ID). *Statistically different from control rats ($P < 0.05$). †Statistically different from RA-treated rats ($P < 0.05$).
3H-labeled polar metabolites, which increased to 75% at 30 min (Fig. 1, C and D). In the plasma of LPS-treated rats, 3H-labeled polar metabolites were elevated 10 min postinjection but equal to the control level by 30 min. In rats treated with the combination of RA + LPS, the proportion of 3H-labeled polar metabolites was highest (Fig. 1, C and D), representing ~68% and 82% of total 3H-labeled retinoids at 10 and 30 min, respectively. Thus RA and the combination of RA + LPS resulted in a two- to threefold increase in plasma total 3H, compared with the control group, and the increase was mostly in the form of polar metabolites.

LPS and RA independently affect the total amount and distribution of 3H-labeled retinoids in liver. At 10 min postinjection, the liver of control rats contained ~24% of the injected dose as total 3H-labeled retinoids (Fig. 2A). The liver of LPS- and RA + LPS-treated rats contained less of the total dose, suggesting that LPS-induced inflammation may delay the uptake of 3HRA by the liver. By 30 min, total 3H-labeled retinoids fell by 4.3% in the control group (Fig. 2B), and a similar reduction was observed in each treatment group (Fig. 2, A and B). Using two-way ANOVA, we showed that LPS reduced the total 3H-labeled retinoids at both 10 and 30 min (Table 2; Fig. 2, A and B).

The proportion of unmetabolized [3H]RA and 3H-labeled polar retinoids was determined on liver samples from individual rats. LPS reduced the amount of unmetabolized [3H]RA in liver at both 10 and 30 min (Fig. 2C; Table 2). Although there was no effect of RA on unmetabolized [3H]RA in liver at 10 min, unmetabolized [3H]RA was much lower in the liver of all RA-treated rats (RA alone and RA + LPS), compared with the control group, at 30 min (Fig. 2D; Table 2). The percent of total 3H-labeled polar metabolites did not differ among treatments at 10 min (Table 2), although the ratio of total 3H-labeled polar metabolites to [3H]RA was greater in all RA- and LPS-treated rats compared with the control group. By 30 min, total 3H-labeled polar metabolites increased in the liver of all RA-treated rats, regardless of inflammation (Fig. 2D; Table 2). At this time, total 3H-labeled metabolites represented ~82% of the total radioactivity present in the liver in both of these groups. Thus, although LPS significantly affected the total amount of 3H present in the liver at both times, only RA affected the amount of 3H-labeled polar metabolites in the liver (Table 2).

RA metabolism in extrahepatic tissues. Next, we examined two extrahepatic tissues, the lungs and small intestine, both of which are intimately involved in vitamin A homeostasis (53, 54), to determine their response to RA- and LPS-induced inflammation (Fig. 3). Because to the relatively small amount of total 3H recovered in the lungs and small intestine (see below), total 3H-labeled retinoids were only partitioned into an organic phase and an aqueous phase; thus the organic phase may contain unmetabolized [3H]RA and 3H-labeled polar metabolites, whereas all 3H in the aqueous phase has undergone metabolism. Less than 1% of the injected dose of [3H]RA was recovered in the lungs of control rats at either time. However, this still represented an enrichment of 1.34 ± 0.12 and 1.25 ± 0.05 at 10 and 30 min, respectively, based on the relative weight of the lung. RA treatment did not affect total 3H-labeled polar metabolites at 10 min (Table 2), although the ratio of total 3H-labeled polar metabolites to [3H]RA was greater in all rat groups. Thus, although LPS significantly affected the total amount of 3H present in the liver at both times, only RA affected the amount of 3H-labeled polar metabolites in the liver (Table 2).

Table 2. Two-way ANOVA of liver, lung, and small intestine radioactivity 10 and 30 min postinjection

<table>
<thead>
<tr>
<th>Organ</th>
<th>10 min</th>
<th></th>
<th>30 min</th>
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<tbody>
<tr>
<td></td>
<td>RA</td>
<td>LPS</td>
<td>RA</td>
<td>LPS</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total 3H</td>
<td>0.23</td>
<td>0.002</td>
<td>0.87</td>
<td>0.93</td>
</tr>
<tr>
<td>Organic-phase 3H</td>
<td>0.03</td>
<td>0.0001</td>
<td>0.99</td>
<td>0.0001</td>
</tr>
<tr>
<td>Aqueous-phase 3H</td>
<td>0.004</td>
<td>0.05</td>
<td>0.42</td>
<td>0.0001</td>
</tr>
<tr>
<td>[3H]RA*</td>
<td>0.04</td>
<td>0.0002</td>
<td>0.89</td>
<td>0.0001</td>
</tr>
<tr>
<td>3H-labeled polar</td>
<td>0.46</td>
<td>0.32</td>
<td>0.54</td>
<td>0.0001</td>
</tr>
<tr>
<td>metabolites†</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total 3H</td>
<td>0.63</td>
<td>0.01</td>
<td>0.28</td>
<td>0.02</td>
</tr>
<tr>
<td>Organic-phase 3H</td>
<td>0.03</td>
<td>0.23</td>
<td>0.64</td>
<td>0.0001</td>
</tr>
<tr>
<td>Aqueous-phase 3H</td>
<td>0.009</td>
<td>0.06</td>
<td>0.43</td>
<td>0.0001</td>
</tr>
<tr>
<td>Small intestine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total 3H</td>
<td>0.16</td>
<td>0.0004</td>
<td>0.11</td>
<td>0.01</td>
</tr>
<tr>
<td>Organic-phase 3H</td>
<td>0.21</td>
<td>0.03</td>
<td>0.34</td>
<td>0.01</td>
</tr>
<tr>
<td>Aqueous-phase 3H</td>
<td>0.10</td>
<td>0.21</td>
<td>0.96</td>
<td>0.005</td>
</tr>
</tbody>
</table>

* Amount of 3H that eluted with an all-trans-RA standard during solid-phase extraction (SPE). †Amount of 3H that eluted with a 4-oxo-RA standard during SPE.
The amount of LPS administered (50 μg/100 g BW) was similar to that used in previous experiments to elicit a moderate inflammatory response in rats, which included a significant reduction of ~7.5% in body weight over 24 h, a moderate increase in body temperature (0.6°C), and significant hyporetinolemia (50, 51). However, the LPS treatment protocol we have used does not induce endotoxemia or septic shock, as are induced by 10- to 20-fold higher doses of LPS (24, 29). Inflammation-induced hyporetinolemia was confirmed in our experiment by a 47% reduction in plasma retinol. Inflammation was also evident by a small but significant increase in relative spleen weight (Table 1), which is consistent with immune cell activation by LPS as shown in other studies (36, 64). The dose of RA used in this study was shown previously to increase the expression of several retinoid-responsive genes in both vitamin A-deficient and vitamin A-sufficient rats (65) and, therefore, would be expected to have similar effects in vitamin A-marginal rats. By examining the distribution of intravenously administered [3H]RA in the plasma, liver, and extrahepatic organs of vitamin A-marginal rats, we determined that [3H]RA is cleared from plasma and metabolized very rapidly and that each organ differs in its pattern of RA metabolism. RA and LPS each significantly modified the metabolism of [3H]RA. However, for the most part, RA and LPS were independent determinants of RA metabolism.

Plasma retinoid metabolites are predominantly regulated by RA. Reports prior to this study had established that under normal physiological conditions, the plasma pools of RA turns over rapidly in both rats and humans (1, 25, 37, 58). Several investigators have shown that following the clearance of all-trans-RA from plasma, this retinoid subsequently appears as various polar metabolites, including 4-hydroxy-RA, 4-oxo-RA, and retinolyl β-glucuronide (11, 17, 57). In the present study, greater than 95% of the injected dose of [3H]RA was cleared from plasma within 10 min, confirming the rapid clearance of RA from plasma observed in rats and other species. Although treatment with RA resulted in a higher percentage of [3H] in plasma, especially at 30 min, analysis of retinoids after reverse-phase SPE showed that the increase is accounted for by the retinoids present in lung at 10 min, but it significantly decreased the amount of total [3H]-labeled retinoids at 30 min (Fig. 3, A and B; Table 2). In contrast, lung total [3H]-labeled retinoids were higher in LPS rats at both 10 and 30 min (Fig. 3, A and B; Table 2). Partitioning of the lipid extract showed that organic-phase radioactivity was lower and [3H]-labeled aqueous-phase metabolites were higher in the lungs of RA rats at both times (Fig. 3, A and B; Table 2). LPS treatment did not affect the amount of [3H]-labeled aqueous-phase metabolites in the lungs at either time (Fig. 3). Thus, although LPS affected total [3H] in the lung at both times, only RA increased the amount of [3H]-labeled aqueous-phase metabolites.

Approximately 3.5% of the injected dose of [3H]RA was recovered in the small intestine of control rats at 10 and 30 min (Fig. 4, A and B), representing an enrichment of 1.15 ± 0.07 and 1.92 ± 0.15, respectively. Treatment with RA increased the amount of [3H] recovered in the small intestine at 30 min (Fig. 4, A and B; Table 2), whereas LPS increased the total amount of [3H] present in the small intestine only at 10 min (Fig. 4, A and B; Table 2). The amount of [3H]-labeled aqueous-phase metabolites in the small intestine was significantly increased by RA, but not by LPS, at 30 min (Fig. 4B; Table 2). In fact, [3H]-labeled aqueous-phase metabolites recovered in the small intestine of RA-treated rats exceeded the total amount of radioactivity recovered from each of the other groups examined at either time (Fig. 4, A and B). Therefore, although LPS affected the total [3H] in the small intestine at 10 min, only RA affected both total [3H] and [3H]-labeled aqueous-phase metabolites at 30 min.

DISCUSSION

The present study was conducted to better understand the regulation of RA homeostasis during inflammation. To this end, we designed our study to investigate the effects of RA and LPS each alone, as well as in combination, on the distribution and metabolism of a dose of [3H]RA in vitamin A-marginal rats, a model of subclinical vitamin A deficiency relevant to human populations in which inflammation is also prominent.

Fig. 3. RA and LPS independently affect total radioactivity and [3H]-labeled aqueous-phase metabolites present in the lungs. Lung was collected 10 (n = 5; A) or 30 (n = 4; B) min after injection of [3H]RA, and organic-phase and aqueous-phase radioactivity was determined. Bars are means ± SE. Different letters above bars within panels indicate significant differences (P < 0.05, a < b < c).

Fig. 4. RA and LPS independently affect total radioactivity and [3H]-labeled aqueous-phase metabolites present in the small intestine. Small intestine was collected 10 (n = 5; A) or 30 (n = 4; B) min after injection of [3H]RA, and organic-phase and aqueous-phase radioactivity was determined. Bars are means ± SE. Different letters above bars within panels indicate significant differences (P < 0.05, a < b < c).
combination of \(^{3}\)H-labeled organic and aqueous-phase metabolites (Fig. 1). It is evident that plasma \(^{3}\)H]-RA was maintained within a narrow range regardless of pretreatment, whereas plasma \(^{3}\)H]-labeled metabolites were rapidly formed and were present at a higher level in RA-treated rats. In contrast, LPS had a modest (at 10 min) or no (at 30 min) effect on total \(^{3}\)H and \(^{3}\)H]-labeled organic metabolites in plasma. Thus \(^{3}\)H]-RA delivered into the plasma compartment was rapidly metabolized by all rats, but \(^{3}\)H]-RA metabolism was accelerated in RA-treated rats, regardless of concurrent inflammation.

Inflammation-induced changes in hepatic \(^{3}\)H]-RA metabolism. The liver is directly involved in the maintenance of retinol homeostasis by regulating its storage, mobilization, and utilization (52). Previous work has established that hepatic retinol mobilization is impaired during acute inflammation (19, 50). Since RA mediates most of the physiological functions of vitamin A, we were interested in understanding the effects of LPS-induced inflammation on the uptake and metabolism of \(^{3}\)H]-RA by liver. Significant amounts of RA are taken up by liver from plasma and presumably utilized or recycled (25). On average, 24% of the injected dose was recovered in the liver of control rats at 10 min (Fig. 2A). This represents an enrichment of \(6.48 \pm 0.50\) based on the relative weight of the liver. The amount of total \(^{3}\)H]-labeled retinoids recovered in liver was lower at 30 min than at 10 min in all four groups (Fig. 2). This reduction could be due in part to the loss from liver back into plasma of the parent compound \(^{3}\)H]-RA or more polar metabolites such as retinoyl \(\beta\)-glucuronide (2). Alternatively or additionally, the decline in liver radioactivity could be caused by the formation and excretion of polar and aqueous-phase metabolites of RA, which appear in the bile of normal rats 2 h after RA administration (11, 57, 62). In our study, LPS significantly reduced the total amount of \(^{3}\)H present in liver at both 10 and 30 min (Fig. 2A and B). We speculate that the observed reduction in liver radioactivity could be a consequence of three primary causes. First, LPS-induced inflammation increases vascular permeability, which could alter organ blood flow and thus influence the uptake, utilization, and recycling of RA by the liver (7, 13). Second, acute inflammation is known to diminish the expression of fatty acid transport protein (FATP) and fatty acid translocase (FAT), both of which are involved in the uptake of long-chain fatty acids from plasma (14, 34). Similarly, LPS administration reduces the expression of cellular fatty acid binding protein, L-FABP (35). While it is currently believed that RA readily diffuses across plasma membranes in a nonfacilitated manner, the effects of inflammation on different fatty acid transporters suggest that these or similar changes could affect RA homeostasis as well. Third, specific transporters involved in the uptake and secretion of chemicals by the liver are altered following LPS administration (5). Thus vascular alteration coupled with changes in membrane-bound transporters involved in fat and xenobiotic homeostasis, known to be affected by LPS, may have contributed to the LPS-induced decrease in liver \(^{3}\)H]-labeled retinoid in our LPS-treated rats.

RA-induced changes in hepatic \(^{3}\)H]-RA metabolism. Pharmacokinetic studies have determined that long-term administration of RA results in a decrease in plasma RA and subsequent increase in RA metabolism (42, 46, 58). Specific cytochrome P450 enzymes are induced in response to high cellular concentrations of RA, which may work in concert to regulate the degradation of RA and to return the concentration of RA to normal cellular levels (17, 28, 48, 49, 65, 66). In our studies, pretreatment of marginally vitamin A-deficient rats with oral RA significantly increased the amount of \(^{3}\)H]-labeled metabolites present in the liver. Furthermore, this induction in RA metabolism occurred irrespective of LPS administration. The inability of LPS to affect the amount of \(^{3}\)H]-labeled metabolites in the liver was surprising since the detoxification capacity of the liver for drugs and xenobiotics is diminished during inflammation (20, 21), evidenced by an LPS-induced reduction in both the expression and enzymatic activity of various members of the cytochrome P450 family (56). Thus the results presented here suggest that the ability of RA to induce its own catabolism is maintained during periods of physiological stress, which would serve to prevent the elevation of RA concentrations when acute or chronic RA treatment is used therapeutically as an adjuvant or to treat different disease conditions.

Inflammation- and RA-induced changes in extrahepatic \(^{3}\)H]-RA metabolism. Similar to liver, both lung and small intestine have been implicated in retinol (6, 38, 53, 54) and RA homeostasis (27, 53). To determine whether LPS-induced inflammation affects these organs in a manner similar to its effects in liver, we examined the small intestine and lung for organic- and aqueous-phase radioactivity. Similar to liver, both lung and small intestine exhibited enrichment of the injected \(^{3}\)H]-RA dose soon after administration, indicating these organs have the capacity to take up and concentrate RA. LPS initially increased the total amount of \(^{3}\)H present in the lung and small intestine, but by 30 min the amount returned to control levels in both organs (Figs. 3 and 4). In contrast, only RA administration increased the amount of aqueous-phase radioactivity at 10 and 30 min in both organs, irrespective of LPS treatment. The increased aqueous-phase radioactivity could be caused by enhanced RA oxidation because both lung and small intestine contain members of the cytochrome P450 family capable of oxidizing RA (27, 30). Interestingly, in the small intestine, the amount of aqueous-phase radioactivity increased in each treatment group over time, suggesting the presence of biliary metabolites of RA in the small intestine by 30 min (11, 57, 62).

In conclusion, the results of our study demonstrate that LPS-induced inflammation affects the organ distribution of RA, which may reflect differences in either the rate of uptake or the recycling of RA in a tissue-specific manner. The greatest effect was observed in the liver, where LPS reduced the amount of radioactivity recovered by nearly 30%. However, despite differences in the initial distribution of RA, LPS did not affect the total amount of \(^{3}\)H]-labeled metabolites present in the liver, lung, and small intestine by 30 min. The interval from 10 to 30 min may have been sufficient for all tissues to metabolize most of the RA into metabolites. In contrast, although RA administration did not influence tissue radioactivity levels, it did cause a significant increase in the recovery of \(^{3}\)H]-labeled metabolites, irrespective of LPS. Taken together, our results suggest that acute inflammation can alter systemic RA homeostasis by disrupting the normal physiological distribution of RA. Nevertheless, the moderate degree of inflammation induced in our rat model did not greatly compromise the metabolism of \(^{3}\)H]-RA, whereas the conversion of \(^{3}\)H]-RA to \(^{3}\)H]-labeled metabolites was significantly increased after treatment with RA.
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GRANTS

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