Flupenthixol and cefotiam: effects on vitamin A metabolism in rats

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We examined the alterations in vitamin A metabolism as a result of flupenthixol or cefotiam administration. The impact of these drugs on indices of vitamin A status was evaluated in Brown Norway and Long–Evans rats. Intramuscular drug administration for 28 d resulted in a decline in systemic retinol. Changes in circulating retinol with time for chronic dosing showed drug treatment (P<0.001) and time (P<0.003) to be significant factors, but rat strain (P=0.33) was not a significant factor. Flupenthixol was the most active retinol-lowering compound (P<0.005). At the end of the 28 d period, hepatic retinyl ester hydrolase activity was greater in drug-treated rats than in controls (P<0.05). With regard to effects on liver reserves: (1) flupenthixol treatment resulted in vitamin A depletion (P<0.05); (2) cefotiam treatment stimulated vitamin A accumulation; (3) distinctive patterns of retinol and its esters were seen in response to treatment. It is reasonable to assume that the drugs interfere with vitamin A in at least two ways: (1) lowering of plasma retinol, an early event in the interaction, may be caused by inhibition of hepatic holo-retinol-binding protein secretion or stimulation of clearance, or both; (2) when plasma retinol levels are persistently low, and as the hepatic deposits of the xenobiotics build up, there are changes in the vitamin A pool size and composition of the liver. Candidate enzymes are retinyl ester hydrolase and cytochrome P450. The relationship between these two events will be studied in further detail.

Flupenthixol–vitamin A interaction: Cefotiam–vitamin A interaction: Retinyl ester hydrolase

Interactions between pharmacological agents and micronutrients, especially vitamin A, have gained wide interest. This is because vitamin A promotes general growth, epithelial cell differentiation, visual function, reproduction and immunocompetence in human subjects (Sporn et al. 1984). In human subjects, physico-chemical interactions between drug ingredients and vitamin A may arise as a consequence of a number of mechanisms, and are known to occur in situ at the site of entry and in transit or at storage sites. Examples include the reduction in bioavailability of lipid-soluble vitamins due to drug–bile acid complex formation (cholestyramine, neomycin, kanamycin and p-aminosalicylic acid) or pancreatic lipase inhibition (tetrahydrolipstatin) (Samuel et al. 1965; Faloone et al. 1966; Barnard & Heaton, 1973; Fernández & Borgström, 1990; Favaro et al. 1994). The lowering of serum retinol levels is another example of drugs altering vitamin A metabolism (Haubold et al. 1953; Smith et al. 1992). This secondary depletion of circulating retinol levels may contribute to night blindness, which has been reported after procaine penicillin or N-(4-hydroxyphenyl)retinamide (Fenretinide) administration (Haubold et al. 1953; Smith et al. 1992). Interference with the detoxifying system represents an alternative mode of action by which drugs interact with retinol metabolism at the microsomal level. Several therapeutic agents, including phenobarbital, methylprednisolone, prednisone, hydrocortisone, methodone, phenothiazine, phenytoin and cyclosporin A, have been shown to be among the group of drugs known to induce hepatic vitamin A depletion in human subjects as well as in experimental animals (Leo et al. 1984, 1987; Azais et al. 1987). Part of the efficacy of some of the drugs mentioned earlier (i.e. phenobarbital and phenothiazine) in this context is probably due to induction of some forms of cytochrome P450 (CYP), a supergene family of haemoprotein enzymes that catalyse oxidation of xenobiotics, including vitamin A (Leo et al. 1984, 1987). With regard to the biotransformation of vitamin A, most studies have focused on CYP, but these vitamin A-metabolising enzymes are not the only ones in the decomposition pathway of retinyl esters. In addition, the liver contains several retinyl ester-splitting enzymes capable of hydrolysing chylomicron-derived (exogenous) retinyl ester or generating free retinol (vitamin A alcohol) from its storage form as esters (for review, see Harrison, 1998). The liberated retinol acts as precursor for re-esterification for storage (Blaner et al. 1985; Blomhoff et al. 1985), as a ligand for secretion with retinol-binding protein into plasma (Smith et al. 1998), as an intermediate for biosynthesis of retinoic acid (the final morphogenic derivative of vitamin A; Napoli & Race, 1990) and as

Abbreviations: BN, Brown Norway; CYP, cytochrome P450; LE, Long–Evans; REH, retinyl ester hydrolase; RL, retinyl linoleate; RM, retinyl myristate; RO, retinyl oleate; RP, retinyl palmitate; RS, retinyl stearate.

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substrate for oxidative reactions in the pathway for elimination of vitamin A from the body (Roberts et al. 1980). Thus, CYP isozymes and free retinol-generating enzymes function in sequence to catalyse the conversion of retinyl esters to more polar compounds.

As mentioned earlier, phenothiazine and procaine penicillin, members of two distinctly different therapeutic classes, have been recognised as vitamin A antagonists (Haubold et al. 1953; Leo et al. 1984). We wondered whether structural analogue compounds from both classes might also interfere with normal vitamin A metabolism in vivo. Against this background, flupenthixol (a psychotropic—neuropysic agent) and cefotiam (a cephalosporin antibiotic) were selected to assess whether these compounds also adversely affect vitamin A status at doses comparable to those in clinical use. The thioxanthene drug, flupenthixol, has structural characteristics related to those of the phenothiazines and cefotiam shares structural features with penicillins. Flupenthixol, like other thioxanthene compounds, is used in the therapy of schizophrenia, organic psychoses and other idopathic psychotic illnesses and is often administered for long periods; therapeautic efficasy is maximal 3–4 weeks following initial administration (Dollery, 1991). Due to the high tissue binding of flupenthixol, the serum level is slow relative to the tissue concentration. The greatest flupenthixol levels have been found in liver, lungs, intestine and kidneys. Like other phenothiazine derivatives, flupenthixol is extensively metabolised in the liver before being excreted (Dollery, 1991). Besides flupenthixol, in our present study we also utilised cefotiam for comparative purposes. The pharmacological effect of cephalosporins is analogous to that of penicillins. This explains the wide use of cephalosporins in clinical practice. Once given by injection, they are rapidly circulated and penetrate tissues. Following intramuscular administration, 50–70% of the drug is excreted unchanged in the urine (Brogard et al. 1989). As a therapeutically relevant compound, cefotiam is the drug of choice for treatment of infections caused by susceptible Gram-positive and Gram-negative bacteria (e.g. treatment of peritonitis associated with continuous abdominal peritoneal dialysis, biliary tract infections and perinatal infections). In cases of severe infection, the use of a cephalosporin antibiotic for up to 20 d is recommended (Perry & Brogden, 1996). One of its advantages is that adverse reactions are rare; side effects include occasional reports of renal function abnormalities (Riegel & Hörl, 1993).

Rats are the most common laboratory model for investigating vitamin A metabolism. However, inter-strain differences in both vitamin A metabolism and responsiveness to drug administration are known to exist among rats, making extrapolation of data from one strain to another difficult (Pirovino et al. 1990; Seifert et al. 1991; Lahmame & Armario, 1996; Tuutioke et al. 1996; Schindler et al. 2002). Therefore, another factor considered when designing the present study was the rat strain. Since there are distinct differences in the expression of non-specific carboxylesterase isozymes among rat strains, experiments were conducted on two strains of laboratory rat: Brown Norway (BN) and Long–Evans (LE) (Bender et al. 1984; Hedicin, 1990).

Non-specific carboxylestases are catalysts involved in hepatic processing of xenobiotics (Mentlein & Heymann, 1984), and as detoxifying enzymes they operate in concert with CYP isozymes (see earlier). Two members of the non-specific carboxylesterase family have been shown to hydrolyse retinyl esters (i.e. bile-acid-independent carboxylesterase-4 and bile-acid-dependent retinol ester hydrolase (REH)) (Mentlein & Heymann, 1987; Schindler et al. 1998). Of the two retinyl ester-splitting carboxylestases, it is REH that will receive attention in the present paper, since flupenthixol and cefotiam are known to be in vitro REH inhibitors (Schindler, 2001). The objective of these experiments with BN and LE rats was to investigate the individual effects of flupenthixol and cefotiam on the metabolism of vitamin A in vivo. We report: (1) the sequential changes in the plasma retinol concentration during the 28 d exposure period; (2) the treatment-related effects on REH activity; (3) the hepatic vitamin A pool size and composition; (4) the amount of vitamin A within the kidneys.

Experimental procedures

Sources of pharmacological agents, chemicals and experimental animals

Therapeutic agents were obtained from manufacturers as follows: flupenthixol dihydrochloride from Troponwerke (Köln, Germany) and cefotiam dihydrochloride from Takeda Pharma (Stolberg, Germany). With the exception of retinyl palmitate (RP), which was a generous gift from Hoffman–La Roche (Basel, Switzerland), all specific reagents (i.e. stearoyl chloride, linoleoyl chloride, myristoyl chloride, oleoyl chloride) were purchased from Sigma (Deisenhofen, Germany). The suppliers of other reagents and chemicals have been reported earlier (Schindler et al. 2002). All chemicals and solvents were of analytical grade.

Male rats of the strains BN (body weight 243–4–346·8 g) and LE (body weight 337·9–452·9 g) were purchased from Zentralinstitut für Versuchstierzucht (Hannover, Germany). Rats were housed singly, in cages (150 × 270 × 430 mm) with mesh bases, in a temperature- and humidity-controlled environment with a 12 h light–dark cycle. During experiments, they were given free access to water and a standard rat chow (rodent diet no. 1314; Altromin, Lage, Germany) that contained adequate amounts of vitamin A (15·77 μmol retinol/kg diet). On days 0–28, individual body weights were monitored daily. Care and treatment of the rats was approved by the Christian-Albrechts-University of Kiel Institutional Animal Care and Use Committee.

Experimental design

Three separate groups (six rats per group) of BN and LE rats were studied in a randomised experiment. One group, the placebo group, received an equivalent volume (i.e. 1·5 ml/kg body weight) of the dosing vehicle (i.e. saline, 150 mmol NaCl/l) only, the second group received flupenthixol and the third group received cefotiam. The pharmacological agents chosen for the present study were sufficiently soluble in physiological saline.
(150 mmol/l NaCl) to be used in our present experiments. Solutions of the drugs were made fresh, protected from exposure to light, and stored at 4°C. The formulations were injected into the musculus quadriceps femoris daily for 28 d (Fig. 1) and the animals were observed until termination. The route of administration was based on clinical practice. Only one dosage of each drug (Table 1) was given to each animal. The doses of the test compounds that were chosen in the present study span the range used for therapeutic purposes. To illustrate this, we compiled corresponding data for the study drugs from a review of the available literature (Table 1; Dollery, 1991; Mutschler & Schäfer-Korting, 1996). The doses administered to test animals were calculated from the recommended therapeutic doses for a human subject corrected to relative body surface area, according to Löschler et al. (1991). At the end of the 28 d study period the rats were killed with diethyl ether, and blood, liver and kidneys were collected. The organs were rinsed with ice-cold saline (150 mmol/l NaCl) containing 2·5 mmol ascorbate/l and 1·7 mmol EDTA/l, blotted and weighed. The frozen plasma and tissue samples were stored in light-proof tubes at less than −20°C for subsequent analyses.

Determination of retinyl ester hydrolase activity and protein concentration

Portions of liver (2 g) were homogenised with a Potter-Elvehjem homogeniser (Braun, Melsungen, Germany) in 5 vol. 0·1 mM-Tris-maleate buffer, pH 7·0. The homogenate was then centrifuged at 50 000 g for 5 min at 4°C. Portions of the supernatant fraction were analysed for REH activity by a retinol-releasing assay detailed by Schindler et al. (1998). The reaction mixture consisted of 0·5 ml Triton X-100 (2 mg/ml), 3-(3-cholamidopropyl)dimethylammonio)-1-propane-sulfonate (200 mmol/l), RP (50 μmol/l), Tris-maleate (0·1 mol/l), pH 7·2. The 60 min reaction at 37°C was started with the addition of the enzyme sample (2·4 mg protein). After stopping the reaction with the addition of 0·5 ml cold ethanol, the product was extracted from the reaction mixtures with 2 ml n-hexane containing butylated hydroxytoluene (1 g/l). The upper phase was then evaporated under N₂, dissolved in chloroform–methanol (1·5:4·0, v/v) and analysed for retinol by reverse-phase HPLC as described later for plasma vitamin A determination. In this assay, the reaction rate was linear with regard to time and enzyme concentrations. In each assay, blank tubes (without enzyme) were used to correct for spontaneous hydrolysis of substrate. To prevent photo-decomposition of vitamin A, incubations were performed in light-protected, Teflon-lined, screw-capped tubes, under N₂. An average of duplicate or triplicate extracts of each sample was used for this enzymic assay. Enzyme results are presented as specific activity. The protein content in the supernatant fraction was measured according to Bradford (1976) using bovine serum albumin as the standard.

Extraction of tissue vitamin A (retinol plus retinyl esters)

The estimation of vitamin A in portions of liver (2 g) or whole kidneys was carried out by homogenising each tissue in an equal volume of ice-cold saline (150 mmol NaCl/l) containing ascorbate (2·5 mmol/l) and EDTA (1·7 mmol/l) with an Ultra-Turrax homogeniser (Braun; detailed by Schindler et al. 1987). The emulsion was dried under vacuum and the residue was extracted four times with 5 ml chloroform–methanol (1:0:1, v/v) containing butylated hydroxytoluene (230 μmol/l). Before being loaded on an analytical HPLC column a sample of the combined chloroform–methanol layer was diluted 3-fold with chloroform–methanol (1:0:3·2, v/v). In the case of the kidneys, vitamin A was extracted with 5 vol. n-hexane containing butylated hydroxytoluene (230 μmol/l). Following solvent removal, samples were resuspended in 500 μl chloroform–methanol (1:7·4:0, v/v) and subjected to HPLC using the same procedure as described for

### Table 1. Extravascular drug administration protocols

<table>
<thead>
<tr>
<th>Application route</th>
<th>Study group</th>
<th>Treatment duration (d)</th>
<th>Drug dosage regimen</th>
<th>Dose administered (mg/kg body weight)† ‡</th>
<th>Clinical daily dose (regimen) † ‡</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>i.m.</td>
<td>Vehicle control§</td>
<td>28</td>
<td>0·5 ml once per d</td>
<td>–</td>
<td>3 × 10 mg (p.o.)</td>
<td>Dollery (1991)</td>
</tr>
<tr>
<td></td>
<td>Flupenthixol</td>
<td>28</td>
<td>0·5 ml once per d</td>
<td>4·0 (42 mg)‡</td>
<td>3–6 g (i.m.)</td>
<td>Mutschler &amp; Schäfer-Korting (1996)</td>
</tr>
<tr>
<td></td>
<td>Cefotiam</td>
<td>28</td>
<td>0·5 ml once per d</td>
<td>350·0 (3·7 g)‡</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Daily therapeutic dose corresponding to the average 65 kg adult human subject.
† Vehicle fluid for dose solution preparation was physiological saline (150 mmol NaCl/l).
‡ Comparison of the therapeutically active doses as adapted from the literature (Dollery, 1991; Mutschler & Schäfer-Korting, 1996).
§ The animals were weighed once per d, and the dose was adjusted if necessary.
plasma samples. The amount of unesterified retinol + retinyl esters (= total vitamin A) in the samples was expressed as retinol equivalents per g wet tissue.

Estimation of plasma and tissue vitamin A levels
Blood samples (500 µl) were taken by tail clip using sodium heparin as an anticoagulant, and plasma was separated by centrifugation at 1500 g for 20 min at 4°C. Systemic retinol was measured in the hexane-soluble plasma fraction after first precipitating protein with ethanol (500 g/l; Schindler et al. 1985). A sample of the hexane layer was evaporated under N₂ and the residue was resuspended in chloroform–methanol (1:5:4:0, v/v). The retinol extracts were then analysed by reverse-phase HPLC using a fluorescence detector, with excitation and emission wavelengths of 325 and 480 nm, respectively, and a Bio-Tek Kontron spherisorb ODS analytical HPLC column (5 mm internal diameter × 250 mm; Bio-Tek Kontron, Neufahrn, Germany). Isocratic elution was performed at a flow rate of 1.8 ml/min using methanol.

Results
All animals completed the administration protocol. No adverse effects of either drug on the general health or appearance of the rats, or on the morphology of the major organs, were observed throughout the study.

The growth curves of the experimental animals are shown in Fig. 2. However, inspection of Fig. 2 indicates that the BN and LE rats used in this series were strikingly different from each other in terms of initial body weight. Therefore, initial body weight was used as a covariate in the statistical ANOVA. Estimated mean values indicated that there were no significant differences in the time course of changes in body weight during the pre-chronic dosing phase, except for flupenthixol-treated LE rats, which weighed 13% less than baseline at the end of the acute treatment period (day 8). In contrast to flupenthixol-treated LE rats, whose body weights increased significantly by ≥13% during chronic drug treatment, the body weights of the other experimental animals remained relatively stable throughout the late study period.

In order to document in more detail the influence of the experimental drugs on systemic retinol, the present study focused on acute (days 0–8; cf. Fig. 3) and more chronic (days 12–28; cf. Fig. 3) changes in plasma retinol concentration during the 28-day drug treatment. Analysis of systemic retinol levels revealed that both flupenthixol and cefotiam treatment produced perturbations in plasma retinol homeostasis. As shown in Fig. 3, plasma retinol concentrations decreased in response to treatment (acute drug effect, P<0.001) independent of gavage-related stress starting on day 0, and remained low relative to baseline throughout the early study period (days 0–8). In addition, main effects of rat type (P<0.003) and time (P<0.001) as well as significant drug × time (P=0.002) and strain × time (P<0.001) interactions were observed. The interaction term in the ANOVA for drug and strain was also significant (P<0.012), i.e. the retinol-lowering effect of the drugs was much more effective in LE rats. Examination of the drug × strain estimated mean values showed that retinol concentrations during the first 8d were lower for flupenthixol- and cefotiam-treated LE rats than for corresponding vehicle-treated controls. Plasma retinol levels in LE rats, however, were also found to be lower in the flupenthixol group compared with the cefotiam group.

The response to chronic treatment (days 12–28) was similar in BN and LE rats (strain effect, P=0.33). Systemic retinol levels in drug-treated rats were lower than in vehicle-treated controls and remained so until day 28. For plasma retinol measured on days 12, 16, 20, 24 and 28, significant main effects of drug (P<0.001) and time (P<0.03), as well as significant interactions of drug × time (P<0.02) and strain × time (P<0.05), were observed. Estimated mean values indicated that the drug effect was
due to lower retinol levels in both flupenthixol- (P = 0·032) and cefotiam- (P = 0·001) treated rats compared with vehicle-treated controls. Furthermore, the observed retinol-lowering effect of flupenthixol was more pronounced than that seen for cefotiam (P = 0·005).

At the end of the study, we tested the possible effects of the two drugs on REH activity, and found that both flupenthixol- (P = 0·05) and cefotiam- (P = 0·03) treated rats had more REH activity than corresponding controls (Table 2). These results establish for the first time that REH activity is up-regulated by flupenthixol and cefotiam.

Because the liver is the major source of plasma retinol, it was also considered important to determine the levels of non-esterified and esterified retinol in the liver.
### Table 2. The influence of intramuscular dosing of flupenthixol or cefotiam on the specific activity of hepatic retinyl ester hydrolase, the size and composition of liver vitamin A stores and the retinoid content of the kidneys in Brown Norway and Long–Evans rats†

(Estimated mean values with their standard errors for six rats per group)

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Analyte</th>
<th>BN strain</th>
<th>LE strain</th>
<th>Statistical significance of effect $P$</th>
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<td>Liver</td>
<td>REH*</td>
<td>4.08 ± 0.28</td>
<td>4.19 ± 0.27</td>
<td>4.30 ± 0.30</td>
</tr>
<tr>
<td></td>
<td>Vitamin A (nmol/g)§</td>
<td>1720.90 ± 166.49</td>
<td>166.22 ± 161.50</td>
<td>1768.69 ± 178.05</td>
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<tr>
<td></td>
<td>Retinol (%)¶</td>
<td>15.71 ± 1.32</td>
<td>17.66 ± 1.28</td>
<td>17.05 ± 1.41</td>
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<td></td>
<td>RL–RM (%)¶</td>
<td>14.86 ± 1.40</td>
<td>13.40 ± 1.30</td>
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<td></td>
<td>RP–RO (%)¶</td>
<td>53.39 ± 2.19</td>
<td>52.02 ± 2.13</td>
<td>57.34 ± 2.34</td>
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<td></td>
<td>RS (%)¶</td>
<td>16.05 ± 0.56</td>
<td>16.93 ± 0.55</td>
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<tr>
<td>Kidney</td>
<td>Vitamin A (nmol/g)§</td>
<td>1.78 ± 0.21</td>
<td>1.71 ± 0.21</td>
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*REH activity was determined by monitoring the rate of retinol formation (nmol/h per mg protein) at 37°C from 50 μmol retinyl palmitate/l in 0.5 ml buffer (Tris–maleate 0.1 mmol/l, Triton X-100 2 mg/ml, 3-((3-cholamidopropyl)dimethylammonio)-1-propane sulfonate (200 mmol/l), pH 7.2).

†For details of diets and procedures, see Table 1, and Fig. 1 and p. 598.

‡Indicates the statistical analysis of the main effects of drug or strain or their interaction.

§The amount of vitamin A (retinol + retinyl esters) in liver and kidney samples is expressed as nmol retinol equivalents/g fresh tissue.

††Results are expressed as a percentage of the total vitamin A fraction (taken as 100%) of rat liver.

**Table 2.** The influence of intramuscular dosing of flupenthixol or cefotiam on the specific activity of hepatic retinyl ester hydrolase, the size and composition of liver vitamin A stores and the retinoid content of the kidneys in Brown Norway and Long–Evans rats†

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### Discussion

In 1953, a clinically relevant pharmacokinetic interaction involving the antipulmonary drug procaine penicillin with vitamin A in healthy individuals was described by Grabkoth et al. (1953). Another finding of that study was the observation that the liver (the major target organ for vitamin A) was associated with defects in the retina and vitamin A handling. The results of this study suggested that the pharmacokinetics of vitamin A and penicillin were similar (both chemically thiolated and transporters). For these reasons, the present study was undertaken to examine whether administering flupenthixol or cefotiam locally, could modify the levels of vitamin A and penicillin. A model of this study was used to determine whether the pharmacokinetics of vitamin A and penicillin were modified by flupenthixol or cefotiam, thereby allowing for the ability to examine whether administering these drugs locally could modify the levels of vitamin A and penicillin. A model of this study was used to determine whether the pharmacokinetics of vitamin A and penicillin were modified by flupenthixol or cefotiam, thereby allowing for the ability to examine whether administering these drugs locally could modify the levels of vitamin A and penicillin.
weight of flupenthixol-treated LE rats was significantly lower than those of the LE cefotiam-treated group or the LE control group (Fig. 2). Body weight was, however, not significantly correlated to the systemic vitamin A values and, consequently, the weight differences appear not to influence the changes in the retinol level with time (Fig. 3). The growth pattern shown only by the flupenthixol-treated LE rats is again somewhat puzzling. The observed changes in body weight, initially dropping and then returning to pre-treatment values, are not consistent with the hypothesis that administration-related stress is a critical factor in the promotion of growth retardation seen in these animals. In a study of the safety of flupenthixol in male Sprague–Dawley rats, a single intraperitoneal injection (0.3 mg/kg) caused decrements in the free-feeding behaviour and in the locomotor–exploratory activity of the animals (Pitts & Horvitz, 2000). It is therefore likely that the flupenthixol-induced weight loss in young LE rats observed in our present study can be explained by the appetite-suppressive effect of this pharmacovigilant agent. In comparison with LE rats, BN rats were resistant to flupenthixol-induced weight loss. We can only speculate on the reason for this apparent discrepancy. One possibility is that these results reflect a genetically determined difference in susceptibility to flupenthixol anorexia. Genetic factors, such as rat strain, also appear to contribute to the response of plasma retinol levels to stressful situations (e.g. injection-related discomfort, diethyl ether anaesthesia and wounding). In particular, the plasma retinol levels fall within hours and are depressed at day 4 in all blood samples compared with those at time zero (Fig. 3). The fall in plasma retinol in the controls, however, was not as pronounced as that seen in the treatment groups, and at days 8–28 the concentration was significantly increased compared with the value at day 4. The transitory marked dip of plasma retinol in the pre-chronic dosing period, followed by a gradual rise in the chronic dosing period despite continued physiological stress, points to a metabolic adaptation. The difference in the magnitudes of change also indicates that strain differences exist in response to stress, with LE more affected than BN. One proposed mechanism underlying the immediate drop of plasma vitamin A after initiation of treatment is an impaired synthesis and/or secretion of carrier protein (i.e. retinol-binding protein, a negative acute-phase reactant protein; Schindler et al. 2003) from the liver. In view of the short exposure time, it is probable that the experimental drugs reach the liver at a low concentration, which is, however, sufficiently high to exert the retinol-lowering effect found. Another rate-limiting step in the output pathways is at the level of the enzyme-catalysed generation of non-esterified retinol. The liver is thought to express several retinyl ester-splitting enzymes, including REH, that appear genetically, physiologically and biochemically distinct (Harrison, 1998). Because flupenthixol and cefotiam have been shown to inhibit purified liver REH (Schindler, 2001), one might also have expected both agents to inactivate the enzyme in vivo after treatment for 28 d. Surprisingly, the drugs caused an increase in REH activity, suggesting an induction of enzyme protein in these animals. It may be hypothesised that the increased biosynthesis of this hydrolase represents a compensatory mechanism resulting from its inhibition. The small increases in REH activities, however, were not accompanied by increased recovery of systemic retinol to pre-treatment levels. Thus, REH either does not normally contribute substantially to the maintenance of steady-state circulating retinol levels or the changes in REH activity were not of great enough magnitude to cause a corresponding rise in plasma retinol concentrations. However, the lack of a significant rise in intrahepatic retinol concomitant with the increase in REH activity was interpreted to mean that at least in flupenthixol-treated rats retinol expenditure (via elimination route) was favoured. Within this context, one has to remember that flupenthixol is an antipsychotic drug of the thioxanthene class, with pharmacokinetics characterised by an extensive distribution to hepatic tissue and a significant elimination via liver (Dollery, 1991). However, safety testing of phenothiazine neuroleptics, such as phenothiazine, chlorpromazine and promazine indicates that these thioxanthene-like compounds are inducers of certain CYP. CYP enzymes reported to be induced by these drugs include: CYP1A1, CYP1A2 and members of the 2B and 3A series by phenothiazine (Thomas et al. 1983; Tateishi et al. 1999); CYP1A1, CYP1A2, CYP2B1 and members of the 3A subfamily by chlorpromazine (Thomas et al. 1983; Murray, 1992; Tateishi et al. 1999); CYP1A1, CYP1A2 and CYP2B1 by promazine (Murray, 1992). Since these CYP isoforms are thought to play a role in vitamin A oxidation (Leo & Lieber, 1985; Martini & Murray, 1993; Ahmad et al. 2000), such induction may be a link to explain the vitamin A depletion seen in flupenthixol-treated rats. Under conditions in which an excess of retinol enters the degradation pathway, its local availability as ligand for retinol-binding protein may be lowered. If the increase in retinol degradation in rats treated with flupenthixol was accompanied by a temporary loss of intrahepatic retinol, it could also well account for the extremely low plasma retinol levels observed compared with both cefotiam-treated and non-treated rats. Also of interest concerning the fall in hepatic vitamin A concentrations is that flupenthixol-treated LE rats began to gain weight 13 d after initiation of treatment. Therefore, the reduction of vitamin A in the liver of the growing rats may also be the result of an increased metabolic demand for retinoic acid by the extrahepatic tissues.

In contrast to the pharmacokinetics of flupenthixol, the properties of cefotiam include stability against hepatic metabolism (Brogard et al. 1998). Accordingly, a review of the literature failed to reveal any case where cefotiam is considered to be an agent that modulates CYP function or expression. Consistent with this suggestion, the present investigation has revealed that the semi-synthetic parenteral cephalosporin, cefotiam, at the dose selected, failed to influence the changes in the retinol level with time. This observation again supports our suggestion about the mechanism underlying the immediate drop of plasma vitamin A after initiation of treatment (see earlier). Another possibility is that the fall in plasma retinol is due...
References


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