sues are likely to be chronically exposed to elevated circulating ALA levels, as there is elevated urinary excretion even in latent asymptomatic cases. Therefore quite high tissue contents of ALA are probably reached, which will be maintained over long periods of time.

These results then contribute further to the accumulating evidence that ALA could be a factor in the production of the clinical manifestations of acute intermittent porphyria.

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Sex difference in the phospholipid composition of rat liver microsomes

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Male rats have a higher level of drug-metabolizing activity in the liver than females [1–10]. There is also a sex difference in phospholipid metabolism of the rat liver [11–15]. Since there is an abundance of evidence to demonstrate that phospholipids play a role in drug metabolism [16–23], it seems possible that the sex difference in drug-metabolizing activity and phospholipid metabolism might be related. However, a sex-dependent association between microsomal phospholipids and drug metabolism has not yet been studied. In previous papers [16, 17, 24], we have demonstrated that changes in drug-metabolizing processes are accompanied by corresponding and parallel alterations in microsomal phospholipids. We decided, therefore, to study whether or not a difference exists in the level and composition of microsomal phospholipids between male and female rats which may be similar to changes of drug metabolism induced by drugs [16, 17, 24].

Two groups of four male and four female Wistar albino rats of approximately equal age and weighing 132–141 g and 105–118 g, respectively, were killed on two separate days. Liver microsomes were prepared [16] and analyzed for protein [25] and phospholipid content [16]. Extraction and separation of the individual phospholipids by thin-layer chromatography were carried out as described previously [16].

The activity of MT-ase* was determined in a medium (2·0 ml) containing Tris buffer, pH 8, 250 μmoles; adenosine triphosphate sodium, 8 μmoles; $MgCl_2$, 20 μmoles; L-[^{14}C-Me]methionine, 0·8 μCi (sp. act. 33·3 μCi/μmole); microsomes, 0·3 ml (protein content 4·5 to 7·4 mg/ml) and supernatant, 0·3 ml (protein content 12·0 to 16·5 mg/ml) equivalent to 75 mg wet liver tissue. Incubation was carried out at 37°, and the reaction was stopped after 0, 10 and 20 min by the addition of a 0·5-ml aliquot to 9·5 ml chloroform–methanol mixture (2:1, by volume) containing 2·0 ml of 0·73% saline; the [^{14}C-Me]-labeled phospholipid was then

* Abbreviations used: MT-ase, *S*-adenosyl-methionine microsomal-phospholipid methyl transferase; PA, phosphatidic acid; PE, phosphatidylethanolamine; PME, phosphatidylmonomethylethanolamine; PDE, phosphatidyldimethylethanolamine, PC, phosphatidylcholine; PI, phosphatidylinositol; PS, phosphatidylserine; LPC, lysophosphatidylcholine; and SM, sphingomyelin.

Table 1. Measurements of liver weight, hepatic microsomal protein and phospholipid of male and female rats*†

Sex	Relative liver wt (% of body wt)	Microsomal Protein (mg/g liver)	Microsomal Phospholipid (μmoles P/g liver)
Male	5·04 ± 0·04	17·54 ± 0·97	9·60 ± 0·21
Female	5·07 ± 0·10	17·10 ± 1·05	8·22 ± 0·20‡

* Description of animals and methods is given in the experimental section.
† Results represent the mean ± S. E. of eight rats in each group.
‡ Values differ significantly from males, $P < 0·05$.

Table 2. Microsomal phospholipid composition in the liver of male and female rats*†

Sex	Total	Recovered	Phospholipid (μmoles P/g liver) PA	PE	PC + PI	SM	LPC + PS	Other
Male	9·60 ± 0·21	8·98 ± 0·26	0·36 ± 0·05	2·24 ± 0·12	4·87 ± 0·10	0·57 ± 0·03	0·50 ± 0·03	0·42 ± 0·05
Female	8·22 ± 0·20‡	7·69 ± 0·25‡	0·22 ± 0·01‡	1·93 ± 0·10	4·36 ± 0·14‡	0·50 ± 0·02	0·36 ± 0·02‡	0·39 ± 0·03

* Description of animals and methods is given in the experimental section.
† Results represent the mean ± S.E. of eight rats in each group.
‡ Values differ significantly from males, $P < 0.05$.

extracted. The excess of [^{14}C-Me]methionine was removed from the organic phase with the upper phase of chloroform–methanol–0·58% saline solution (3:48:47, by volume). The radioactivity of [^{14}C-Me]phospholipid was determined by Buhler solution, using a Packard Tri-Carb liquid scintillation spectrometer. The phospholipid products of the reaction extract were separated by thin-layer chromatography [16], hydrolyzed to isolate the bases [26] and then the bases were separated by paper chromatography [27]. The majority of the radioactive label was thus found to be incorporated into PME, PDE, PC, LPC and some into SM.

No differences were found between male and female rats with respect to the relative liver weight or microsomal protein content, although the body weights were different. However, total phospholipids were significantly higher in males than in females (Table 1). Among the individual phospholipids, PA, PC + PI and LPC + PS were higher in male rats; PE was the same in both sexes (Table 2). In subsequent work, PC and PI were separated, and it was found that the PI content was 0·2 to 0·8 μmole/g of liver accounting for only 8–15 per cent of the combined PC + PI fraction. Thus, the difference in this fraction mainly represented differences in PC level. Under the conditions applied LPC was mixed with PS. This latter component was separated in another chromatographic system, and it was found that rat liver microsomes contained 0–0·25 μmole PS/g or about 0–50 per cent of the combined LPC + PS fraction. Because of the variability of PS, the conclusion drawn for the significant difference in LPC content might only be valid regarding the total LPC and PS mixture.

Unlike microsomal phospholipid content, the activity of MT-ase was significantly greater in the liver of females than in males (Table 3).

In our previous papers, it is shown that when female rats are treated with inducers of drug metabolism, the increased drug-metabolizing activity is accompanied by increases in total phospholipid, PE, PC and LPC in the microsomes [16, 17, 24]. In contrast, the same microsomal phospholipids are decreased by hepatotoxic compounds which also reduce the activity of drug-metabolizing enzymes [16, 17, 24]. A comparable occurrence of higher enzyme activity associated with higher phospholipids is seen in the sex difference. Male rats have more drug-metabolizing activity [1, 5, 8, 9, 28] and also higher microsomal phospholipid content (Table 1) than females. The elevated fractions of PC + PI and LPC + PS (Table 2) seen in males as opposed to females are the same ones increased during drug induction of drug-metabolizing enzymes [16, 17, 24]. Certainly this evidence is merely circumstantial; nevertheless, it seems quite possible that in rats the sex difference in phospholipids may well be related to the corresponding sex difference in their drug-metabolizing activity.

Table 3. Activity of [^{14}C-Me]*S*-adenosyl-L-methionine microsomal-phospholipid methyl transferase in the liver of male and female rats*

Sex	Enzyme activity†
Male	0·073 ± 0·009
Female	0·129 ± 0·007‡

* Description of animals and methods is given in the experimental section.
† Results are expressed as μmoles [^{14}C-Me] group from L-[^{14}C-Me]methionine incorporated into microsomal-phospholipid/hr/mg of protein and represent the mean ± S. E. of eight rats in each group.
‡ Values differ significantly from males, $P < 0.001$.

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Effect of clonidine on the synthesis of cerebral dopamine

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A possible interaction between dopamine (DA) and noradrenaline (NA) containing neurons in the brain has been suggested by different studies. The stimulation of dopaminergic receptors by apomorphine [1] or ET 495 [2] increases the turnover of cerebral NA. The disappearance of cerebral NA is about two times faster after inhibition of dopamine β-hydroxylase than after inhibition of tyrosine hydroxylase [1]. These observations can be explained if it is assumed that dopaminergic neurons have a stimulating influence on noradrenergic ones.

In the present work, we have studied the influence of clonidine (ST 155, Catapresan), a NA receptor stimulating agent [3], on the synthesis of cerebral DA. The dose of clonidine used (50 μg/kg) produces a significant fall in the blood pressure of the rat [4] and it reduces the synthesis [5] and release [4] of peripheral NA.

Two groups of fourteen male Charles River rats (220–250 g) were injected in the tail vein with 400 μCi/kg of ^{3}H-TY (L-3,5-^{3}H-tyrosine, 49 Ci/mM, Saclay) and were killed by decapitation 30 min later. The first group were treated with clonidine (50 μg/kg, i.p.) 1 hr before the injection of ^{3}H-TY, the second group serving as the control. The brain was immediately removed and divided into the striatum and telencephalon (the remaining brain from which the brain stem and the hypothalamus have been exluded). The brain areas, pooled two by two, were homogenized in 10 ml of 0·4 N $HClO_4$ containing 0·1% sodium metabisulfite and 0·1% disodium EDTA. Endogenous and ^{3}H-TY and DA were estimated in the striatum and telencephalon: endogenous and ^{3}H-NA were estimated in the telencephalon. DA and NA were separated from TY by chromatography on Dowex 50 WX4 according to the technique of Costa *et al.* [6] DA and NA were purified by adsorption on alumina [7] and TY by chromatography on Dowex 50 WX4 pH 1·5 [6]. Endogenous NA [8], DA [9] and TY [10] were determined fluorimetrically and radioactive products by liquid scintillation counting. An estimate of catecholamine (DA or NA) synthesis was made using the following formula [11]:

$$\text{catecholamine synthesis index (nM/g/30 min)} = \frac{^3\text{H-catecholamine (dis/min/g)}}{\text{Sp. act. } ^3\text{H-TY (dis/min/nM)}}$$

The results, summarized in Table 1, show that the amounts of both endogenous TY and ^{3}H-TY were both reduced by clonidine ($P < 0·05$ in the telencephalon, not significant in the striatum) but the ^{3}H-TY specific activities were not significantly altered. Clonidine treatment reduced ($P < 0·05$) the ^{3}H-DA levels in the striatum and the telencephalon and the endogenous DA levels ($P < 0·05$ in the telencephalon, non significant in the striatum). The ^{3}H-DA specific activities were not significantly modified by clonidine. The DA synthesis index was reduced by 25 per cent in the striatum (not significant, $P = 0·2$) and by 44 per cent in the telencephalon ($P < 0·01$). In the latter, clonidine reduced the ^{3}H-NA levels ($P < 0·01$) and the NA synthesis index by 52% ($P < 0·01$).

The fact that DA synthesis is much more reduced in telencephalon than in striatum can be explained by the presence of large amounts of NA in the telencephalon; consequently, the amount of ^{3}H-DA found in this structure represents at the same time ^{3}H-DA which is stored in dopaminergic neurons [12] and ^{3}H-DA which acts as a precursor of NA. The inhibition of NA synthesis in the telencephalon may account for the reduction in DA synthesis which is observed in this structure. Persson's results [13] differ from ours since he reported that the amount of cerebral ^{3}H-DA synthesized from ^{3}H-TY increased following the injection of clonidine (1–3 mg/kg). This difference may be due to the use of differ-