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DRUG-INDUCED PORPHYRIN BIOSYNTHESIS—XI.
EFFECT OF SKF 525-A ON THE ACTIVITY OF PORPHYRIN-INDUCING DRUGS IN CHICK EMBRYOS, CHICKENS AND RATS*

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Abstract—Hexobarbital (100 mg/kg) induces a marked elevation of hepatic δ-aminolevulinic (ALA)-synthetase and porphyrin levels in the 17-day-old chick embryo but is inactive in the 18-day-old chicken. Propylisopropylacetamide (PIA, 100 mg/kg) induces a marked elevation of ALA-synthetase activity in the 17-day-old chick embryo but only a small elevation in the 18-day-old chicken. After pretreatment of 18-day-old chickens with 2-diethylaminoethyl-2,2-diphenylvalerate hydrochloride (SKF 525-A), hexobarbital and PIA (100 mg/kg) induce a marked elevation of hepatic ALA-synthetase and porphyrin levels. The hepatic level of unchanged PIA was significantly higher in SKF 525-A-pretreated than in untreated chickens. Chick embryo liver is more sensitive to porphyrin-inducing drugs than chicken liver. However, after blockade of drug metabolism by SKF 525-A, the lower sensitivity of the chicken liver is compensated for by high levels of unchanged drug. After pretreatment of the 17-day-old chick embryo with SKF 525-A, the porphyrin-inducing activity of 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine (DDC) and of PIA is increased while that of allylisopropylacetamide (AIA) and 3,5-diethoxycarbonyl-2,4,6-trimethylpyridine (Ox-DDC) is not affected. PIA (300 mg/kg) which did not produce an increase in hepatic ALA-synthetase activity in rats elicits a slight but significant increase in the SKF 525-A-pretreated rat.

A variety of drugs precipitate acute attacks of hepatic porphyria, and it is important to determine which drugs may be safely administered to patients with the clinically latent hereditary trait [1]. A variety of animals and cultured liver preparations have been used to test drugs for porphyrin-inducing activity, e.g. chick embryo liver cell cultures [2], 17-day-old chick embryos [3], chickens [4], rats [5], mice [6] and rabbits [7]. Frequently a drug will induce hepatic porphyrin accumulation in one test system but not in another [3], and the problem arises in deciding which system allows the best prediction to be made of the results to be expected in the porphyric patient. If the reason for the variability was understood, an appropriate test system could be selected more readily. Brodie *et al.* [8] have suggested two possible reasons for species variation in response to a drug: (1) variation in drug metabolism resulting in differences in the amounts of drug at the site of action, and (2) variation in sensitivity of receptor sites. It is thought that the variability in drug activity in different species is due in large part to different rates of drug metabolism [9]. Thus, the rate of drug metabolism in a particular species might be so rapid that its pharmacological activity, observed in a second species, might not be recognized. 2-Diethylaminoethyl-2,2-diphenylvalerate hydrochloride (SKF 525-A) is an inhibitor of hepatic drug metabolism, and it has been suggested by Mannering [9] that the use of this inhibitor in conjunction with a new drug being investigated would eliminate or minimize variability of drug response in different species. The objective of the present study was to determine whether the use of SKF 525-A in conjunction with porphyrin-inducing drugs would minimize the variability of response previously observed in different test systems.

The first drug selected for study was hexobarbital, as it was active in the 17-day-old chick embryo but inactive in the 18-day-old chicken [10]. The second drug selected for study was propylisopropylacetamide (PIA), as it was considerably more active in the 17-day-old chick embryo than in the 18-day-old chicken [11]. The question we sought to answer was the following: Would SKF 525-A pretreatment of the chicken result in a slower rate of drug metabolism and thus increase the responsiveness of the chicken to these drugs? PIA is more potent that allylisopropylacetamide (AIA) in chick embryo liver cell cultures [12]. In the 17-day-old chick embryo, PIA although less potent than AIA induces high levels of hepatic δ-aminolevulinic (ALA)-synthetase activity. On the other hand, PIA is reported to be inactive in the rat, while AIA exhibits marked activity in this species [13]. The second question we sought to answer was the following: Would PIA exhibit porphyrin-inducing activity in the SKF 525-A-pretreated rat comparable to that observed in the 17-day-old chick embryo and isolated chick embryo liver cells? The sensitivity of the 17-day-old chick embryo to some porphyrin-inducing drugs, e.g. 3,5-diethoxycarbonyl-2,4,6-trimethylpyridine, is considerably less than that of chick embryo liver cell culture [11, 14, 15]. The final question we sought to answer was: Could the sensitivity of the 17-day-old chick embryo to porphyrin-inducing drugs be increased by pretreatment with SKF 525-A?

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EXPERIMENTAL

Experimental animals

Fertilized eggs used were of White Leghorn strains obtained from Archer's Poultry Farm, Brighton, Ontario. They were stored at 10 for no longer than 7 days prior to incubation at 38 . The age of the embryo was taken as the number of days from onset of incubation. Chickens were raised from eggs hatched in our incubator. They were fed Co-op Supplement (United Cooperatives of Ontario, Feed Department, Weston, Ontario) and water *ad lib.* Chickens were 18 days old and weighed approximately 100 g at the time they were utilized. Wistar male rats (150–170 g) were obtained from Bio-Breeding Laboratories, Ottawa. The rats were housed in wire cages for at least 5 days prior to drug administration and were fed mouse/rat diet (Teklad Mills, Winfield, Iowa); water was available *ad lib.* The rats and chickens were starved for 24 hr prior to intraperitoneal injection of drugs.

Source of compounds

Allylisopropylacetamide (AIA) was supplied by Hoffman La Roche, Montreal, and allylisopropylacetamide-[2-^{14}C], activity 7·1 μCi/mg, was obtained through the courtesy of Hoffman La Roche, Basle, Switzerland. AIA-[^{14}C], activity 0·104 μCi/mg, was prepared for use in our experiments from the above sample as previously described [16]. Propylisopropylacetamide (PIA) and propylisopropylacetamide-[2-^{14}C] (PIA-[^{14}C], activity 0·047 μCi/mg) were prepared from AIA and AIA-[^{14}C] as previously described [16]. 3,5-Diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine (DDC) and 3,5-diethoxycarbonyl-2,4,6-trimethylpyridine (Ox-DDC) were prepared as described by Marks *et al.* [17] and 2-diethylaminoethyl-2,2-diphenylvalerate hydrochloride (SKF 525-A) was obtained from Smith, Kline & French, Montreal.

Administration of drugs

Chick embryos. A small hole was made in the egg shell above the air sac. Drugs were injected through this hole into the fluids surrounding the embryo with a sterile 1 inch 20 gauge needle attached to a 2-ml micrometer syringe. The hole in the shell was sealed with Cello-tape and the eggs were returned to the incubator. Embryos were injected with 0·3, 1, 3, 5 and 10 mg hexobarbital in DMSO (0·1 ml). After 12 hr, the embryos were removed from the incubator for the assay of hepatic ALA-synthetase activity and porphyrin production [11]. SKF 525-A (0·3 mg) dissolved in saline (0·1 ml) was injected into one group of ten chick embryos and saline (0·1 ml) was injected into a second group of five chick embryos. After incubation for 1 hr, DDC (0·2 mg) in DMSO (0·1 ml) was injected into the saline-pretreated group and five embryos of the SKF 525-A-pretreated group. DMSO (0·1 ml) was injected into the remaining five SKF 525-A-pretreated embryos. Saline (0·1 ml) was injected into five embryos, and 1 hr later DMSO (0·1 ml) was injected. The embryos were returned to the incubator and removed 12 hr later for ALA-synthetase and porphyrin estimation. The above experiment was repeated with the modification that AIA (0·5 mg) in DMSO (0·1 ml), PIA (1 mg) in DMSO (0·1 ml) or Ox-DDC (4 mg) in DMSO (0·1 ml) was substituted for DDC (0·2 mg) in DMSO (0·1 ml). Moreover, experiments were conducted with some of the above drugs using 3-, 6-, and 18- instead of 12-hr incubation periods following 1 hr after SKF 525-A pretreatment.

Chickens. All drugs were administered intraperitoneally with a sterile $\frac{5}{8}$ inch 25 gauge needle. Solutions of SKF 525-A (6·25 mg/ml) in saline and hexobarbital (50 mg/ml) in DMSO were prepared. Six 18-day-old chickens were injected with aliquots of the SKF 525-A solution (0·4 ml; 25 mg/kg) and after 45 min hexobarbital (0·2 ml; 100 mg/kg) was administered. As a control, the experiment was repeated with the modification that saline alone (0·4 ml) was injected in place of SKF 525-A. Further controls were carried out as follows. A group of six chickens were injected with SKF 525-A solution (0·4 ml) and 45 min later DMSO (0·2 ml) was injected; a second group of six chickens received saline (0·4 ml) instead of the SKF 525-A solution. Twelve hr later the chickens were sacrificed and portions of the livers excised for ALA-synthetase and porphyrin estimation [11]. The basal levels of ALA-synthetase and porphyrins were determined for chickens of this age. A solution of PIA (50 mg/ml) in DMSO was prepared, and the above set of experiments was repeated with PIA (100 mg/kg) substituted for hexobarbital (100 mg/kg).

SKF 525-A (2·5 mg) dissolved in saline (0·4 ml) was injected into one group of four 18-day-old chickens and saline (0·4 ml) was injected into a second group of four chickens. After 45 min, PIA-[^{14}C] (10 mg in 0·2 ml DMSO; 0·007 μCi/mg, 69·83 μmoles) was injected into both groups of chickens. Twelve hr later all the chickens were sacrificed and the livers removed for extraction by a procedure described below.

An accurately weighed amount of AIA-[^{14}C] (0·0022 μCi/mg) was dissolved in DMSO to give a concentration of 30 mg/0·1 ml (212 μmoles). This volume was administered to 18-day-old chickens. Groups of four chickens were decapitated after 1, 6 and 18 hr, and the livers were removed and extracted by the procedure described below. The above experiment was repeated using PIA-[^{14}C] (30 mg/0·1 ml of DMSO, 209 μmoles; 0·0025 μCi/mg) instead of AIA-[^{14}C].

Rats. Solutions of SKF 525-A (16·5 mg/ml) in saline and AIA (165 mg/ml) in DMSO were prepared for intraperitoneal injection. Four rats received SKF 525-A (40 mg/kg) and after 45 min AIA (200 mg/kg). The following control experiments were carried out. The experiment was repeated with the modification that saline (0·4 ml) was injected in place of SKF 525-A. Four rats received SKF 525-A (40 mg/kg) and after 45 min DMSO (0·2 ml). Four rats received saline (0·4 ml) and 45 min later DMSO (0·2 ml). Sixteen hr later the rats were sacrificed and portions of the livers excised for ALA-synthetase [18] and porphyrin [3] estimation. A solution of PIA (165 mg/ml) in DMSO was prepared. The above set of experiments was repeated with the modification that PIA (200 mg/kg) was substituted for AIA (200 mg/kg).

Extraction of drugs from the liver and radioactivity determination

The method used to extract AIA and PIA from the liver was a modification of that used by Racz and Marks [14]. Chicken liver was homogenized in methanol (10 ml) in a Potter-Elvehjem apparatus.

After centrifugation, the supernatant was removed and the residue resuspended in methanol (10 ml) for a second homogenization and centrifugation. The two supernatants were combined and the volume was made up to 20 ml with methanol. Two 1-ml aliquots were placed in counting vials, Aquasol (10 ml) was added and the samples were counted in a liquid scintillation counter using an external standard method to correct for quenching. All samples were counted for 10 min after storage at 4° for 24 hr in a Nuclear Chicago Mark II liquid scintillation counter. The remaining extract (18 ml) was evaporated in a stream of nitrogen at 37° and the residue dissolved in 0·3 ml of an ethanol–chloroform mixture (1:1). AIA and PIA were separated from their metabolites by thin-layer chromatography as described previously [16]. The Silica gel was divided into 1-cm portions and each portion placed in a liquid scintillation vial containing water (3 ml). Aquasol (10 ml) was added, and the samples were thoroughly shaken and counted for radioactivity.

Estimation of δ-aminolevulinic acid synthetase activity and porphyrin accumulation

ALA-synthetase activity and porphyrin accumulation in chick embryo and chicken livers were determined as described previously [11]. The method of Marver *et al.* [18] was used to measure ALA-synthetase activity in rat liver, and the method of Racz and Marks [3] used to measure porphyrin levels in rat liver.

RESULTS

The response of the liver of the 17-day-old chick embryo to increasing doses of hexobarbital is shown in Fig. 1. A significant elevation of ALA-synthetase activity was observed with hexobarbital (7·5 mg/kg). After the administration of hexobarbital (25 mg/kg), a significant elevation of both ALA-synthetase activity and porphyrins was observed. The maximum response occurred with hexobarbital (125 mg/kg).

Control values of ALA-synthetase activity and porphyrin levels in chickens receiving saline (0·4 ml) followed 45 min later by DMSO (0·2 ml) were 37·5 ± 3·8 nmoles ALA/g of liver/hr and 0·18 ± 0·03 μg porphyrin/g of liver when measured 12 hr later. Chickens receiving SKF 525-A (25 mg/kg) in saline (0·4 ml) followed 45 min later by DMSO (0·2 ml) had levels of 35·5 ± 4·6 nmoles ALA/g of liver/hr and 0·33 ± 0·06 μg porphyrin/g of liver when measured 12 hr later. Hexobarbital did not produce a significant elevation of ALA-synthetase activity nor of porphyrin levels in the liver of the 18-day-old chickens (Fig. 2). However, after SKF 525-A pretreatment, hexobarbital produced a marked elevation of both ALA-synthetase activity and porphyrin levels (Fig. 2). PIA (100 mg/kg) produces a small elevation of ALA-synthetase activity and of porphyrin levels in the liver of the 18-day-old chicken (Fig. 2). However, after pretreatment with SKF 525-A, a marked elevation of both ALA-synthetase activity and porphyrin levels was noted when compared to the results obtained with PIA alone.

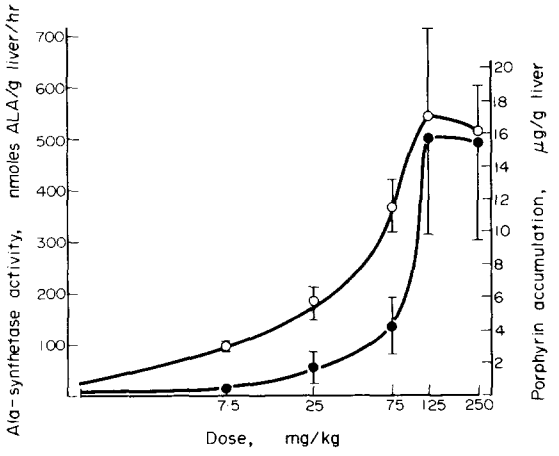

Fig. 1. ALA-synthetase activity (○——○) and porphyrin accumulation (● ——●) in the livers of chick embryos 12 hr after administration of increasing doses of hexobarbital. Each point represents the mean of five to six determinations (±S. E. M.).

Control values of ALA-synthetase activity and porphyrin levels in 17-day-old chick embryos receiving saline (0·1 ml) followed 1 hr later by DMSO (0·1 ml) were 20·0 ± 2·04 nmoles ALA/g of liver/hr and 0·43 ± 0·03 μg porphyrin/g of liver when measured 12 hr later. Chick embryos receiving SKF 525-A (0·3 mg) in saline (0·1 ml) followed 1 hr later by DMSO (0·1 ml) had levels of 70·1 ± 5·6 nmoles ALA/g of liver and 0·51 ± 0·03 μg porphyrin/g of liver when measured 12 hr later. The effect of SKF 525-A pretreatment on AIA-, PIA-, OxDDC- and DDC-induced ALA-synthetase activity and porphyrin accumulation in the livers of 17-day-old chick embryos is shown in Figs. 3 and 4. After SKF 525-A pretreatment, a significant elevation of both ALA-synthetase activity and porphyrin levels was noted in the DDC-treated embryos (Fig. 4) but not in the AIA- and Ox-

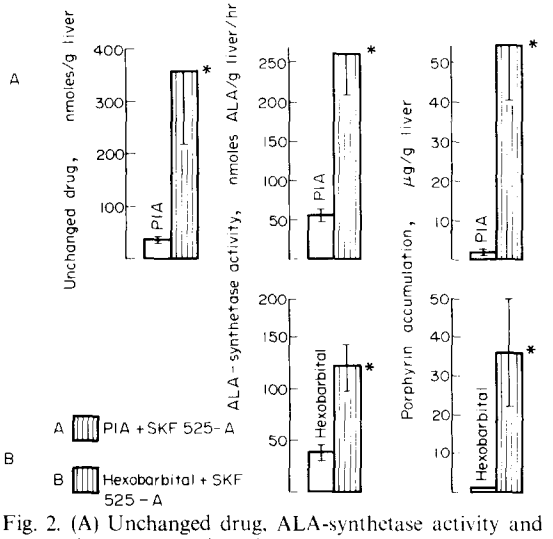

Fig. 2. (A) Unchanged drug, ALA-synthetase activity and porphyrin accumulation in the livers of 18-day-old chickens 12 hr after administration of PIA (100 mg/kg), without SKF 525-A pretreatment (open bars); with SKF 525-A (25 mg/kg) pretreatment (hatched bars). (B) ALA-synthetase activity and porphyrin accumulation in the livers of 18-day-old chickens 12 hr after administration of hexobarbital (100 mg/kg), without SKF 525-A pretreatment (open bars); with SKF 525-A (25 mg/kg) pretreatment (hatched bars). The results in A and B represent the mean of four determinations for unchanged drug and the mean of six determinations for ALA-synthetase activity and porphyrin accumulation (±S.E.M.). The asterisk denotes significance at the 0·05 level.

DDC-treated chick embryos (Fig. 3). After SKF 525-A pretreatment, a significant elevation of ALA-synthetase activity but not of porphyrin levels was noted in the PIA-treated embryos (Fig. 3).

After administration of saline (0·4 ml) followed 45 min later by DMSO (0·2 ml), the following control levels were determined 16 hr later in rat liver: ALA-synthetase activity, 19·3 ± 1·5 nmoles ALA/g of liver/hr; porphyrins, 0·37 ± 0·03 μg porphyrin/g of liver. Injection of SKF 525-A (40 mg/kg) into rats followed 45 min later by DMSO (0·2 ml) produced no significant change in these control values measured after 16 hr. AIA (200 mg/kg) produced a marked elevation of ALA-synthetase activity and porphyrin accumulation in rat liver (Fig. 5). Pretreatment of rats with SKF 525-A resulted in a reduction in AIA-induced ALA-synthetase activity and porphyrin levels (Fig. 5). However, in neither case was the reduction significant at the 0·05 level. PIA (200 mg/kg) did not cause a significant elevation of ALA-synthetase activity nor of porphyrin levels. However, after SKF 525-A pretreatment, a small but significant elevation of ALA-synthetase activity and porphyrin levels was noted as compared to treatment with PIA alone (Fig. 5).

In our experiments, we have administered radioactive drugs to 18-day-old chickens and have measured the total radioactivity in the liver at various time intervals. This radioactivity represents unchanged drug and metabolite(s). We have converted our results from dis. min/g of liver into nmoles of drug/g of liver. In the ensuing discussion when referring to drug and metabolite(s) we shall use the term "total drug." The term "unchanged drug" will be used to designate that portion of the drug that has not undergone metabolic transformation. Amounts of total drug and unchanged drug in chicken livers at various time intervals after AIA and PIA administration are shown in Fig. 6. After injection of AIA, total drug reached a maximum in the liver after approximately 1 hr and then dropped slowly to approximately 22 per cent of the peak level after 18 hr. The level of total drug after administration of PIA remained stable for a period of 6 hr and then dropped slowly to about 28 per cent of the peak level after a period of 18 hr. The amount of unchanged drug in chicken livers after administration of AIA was significantly higher at 1- and 6-hr time periods than after administration of PIA (Fig. 6). No significant amount of unchanged AIA or PIA was detected in the livers 18 hr after drug administration.

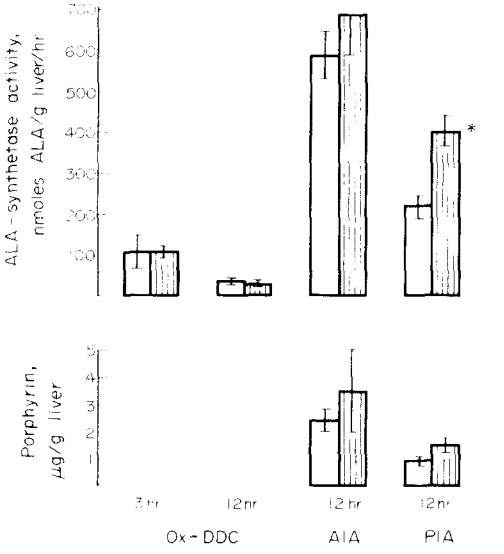

Fig. 3. ALA-synthetase activity and porphyrin accumulation in the livers of 17-day-old chick embryos 12 hr after administration of AIA (0·5 mg), PIA (1 mg) and Ox-DDC (4 mg) and 3 hr after administration of Ox-DDC, without SKF 525-A pretreatment (open bars) and with SKF 525-A (0·3 mg) pretreatment (hatched bars). The results with AIA represent the mean of 15 determinations, with PIA the mean of 40 determinations and with Ox-DDC the mean of 5 determinations (±S. E. M.). The asterisk denotes significance at the 0·05 level.

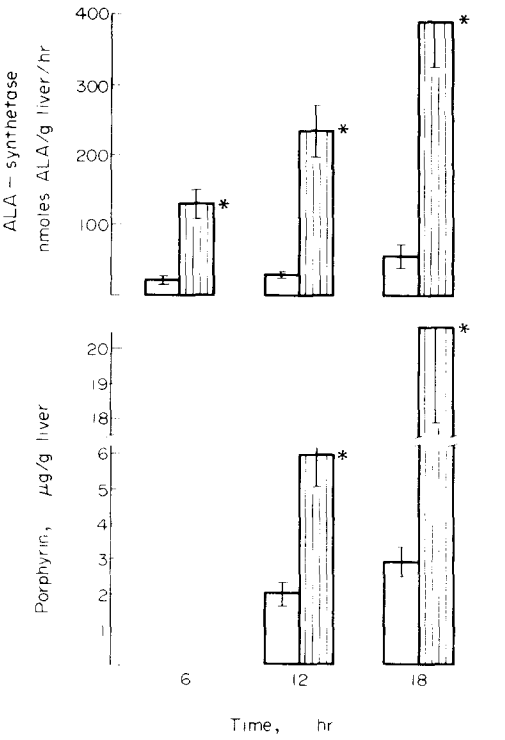

Fig. 4. ALA-synthetase activity and porphyrin accumulation in the livers of 17-day-old chick embryos, 6, 12 and 18 hr after administration of DDC (0·2 mg) without SKF 525-A pretreatment (open bars) and with SKF 525-A (0·3 mg) pretreatment (hatched bars). The results at 12 and 18 hr represent the mean of ten determinations, while the results at 6 hr represent the mean of five determinations (±S. E. M.). The asterisk denotes significance at the 0·05 level.

PIA (69·83 μmoles) was administered to 18-day-old chickens, and 37·3 nmoles of unchanged drug/g of liver was detected 12 hr later (Fig. 2). When the experiment was repeated, after SKF 525-A pretreatment, 359 nmoles of unchanged PIA/g of liver was found.

DISCUSSION

In previous studies, AIA was found to be a more potent porphyrin-inducing drug than PIA in the 18-day-old chicken [11]. Examination of the results in Fig. 6 provides an explanation. Thus, higher levels of ALA-synthetase and porphyrins previously observed [11] can be correlated with higher hepatic levels of unchanged AIA than unchanged PIA.

Hexobarbital induces a marked increase in ALA-synthetase activity and porphyrin levels in chick em-

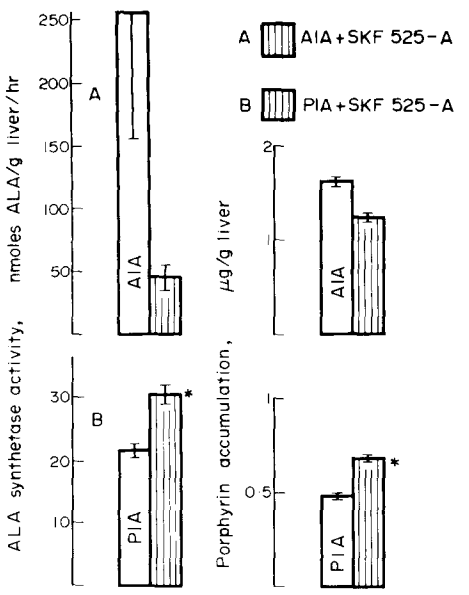

Fig. 5. (A) ALA-synthetase activity and porphyrin accumulation in rat liver 16 hr after administration of AIA (200 mg/kg) without SKF 525-A pretreatment (open bars) and with SKF 525-A (40 mg/kg) pretreatment (hatched bars). The results represent the mean of four determinations (±S. E. M.). (B) The same as above except that PIA was substituted for AIA. The asterisk denotes significance at the 0·05 level.

bryo liver (Fig. 1) but is inactive in the 18-day-old chicken (Fig. 2). After pretreatment of chickens with SKF 525-A, hexobarbital exhibits marked activity (Fig. 2). PIA (100 mg/kg, 4 mg/40 g egg) has been shown to produce a marked elevation of hepatic ALA-synthetase activity (approximately 23 times control) 12 hr after administration to the 17-day-old chick embryo accompanied by a small increase in porphyrin levels [11]. In contrast to this result, PIA (100 mg/kg) produced a small elevation of ALA-synthetase activity (approximately 1 1/2 times control) 12 hr after administration to the 18-day-old chicken accompanied by a small increase in porphyrin levels (Fig. 2). After pretreatment of chickens with SKF 525-A, PIA exhibits marked activity (Fig. 2). The likely interpretation of these findings is that SKF 525-A blocks metabolism of PIA and hexobarbital in the chicken liver resulting in higher levels of unchanged drug in the livers and, therefore, greater activity. This interpretation is supported by the results shown in Fig. 2. In the SKF 525-A-treated chicken, the level of PIA is approximately ten times that observed in the untreated animal. We are, therefore, able to answer the first question posed in the introduction: SKF 525-A pretreatment of the chicken appears to cause a slower rate of metabolism of PIA and hexobarbital and thus increases the responsiveness of the chicken to these drugs.

It appeared possible that the greater responsiveness of the 17-day-old chick embryo compared to the chicken was due to slower drug metabolism in the chick embryo than in the chicken. However, data assembled in Table 1 containing some of our present results with chickens and previous results with chick embryos [16] do not support this idea. In the 18-day-old chicken the ALA-synthetase level is approximately six times control levels, 6 hr after the administration of AIA (300 mg/kg) [11]. At this time the chicken contains 1550 nmoles of unchanged AIA. In the 17-day-old chick embryo, 6 hr after AIA administration the ALA-synthetase activity reaches approximately 11 times control levels [11]. At this time chick embryo liver contains 178 nmoles of unchanged AIA/g of liver [16]. Since ALA-synthetase activity is greater in the chick embryo liver than it is in the chicken liver at a time when there is approximately one-tenth as much unchanged drug in the chick embryo liver, it may be concluded that the greater responsiveness of the chick embryo liver is due to an increased sensitivity to induction by porphyrin-inducing drugs and not to a slower rate of drug metabolism. However, after blockade of drug metabolism by SKF 525-A, the lower sensitivity of the chicken liver is compensated for by the high levels of unchanged drug.

In rats, PIA (200 mg/kg) did not cause a significant elevation of hepatic ALA-synthetase activity nor of porphyrin levels (Fig. 5), a result which agrees with reports by other workers [13]. After SKF 525-A pretreatment, the response elicited by PIA was slightly but significantly enhanced (Fig. 5). The second question posed in the introduction was whether PIA would exhibit porphyrin-inducing activity in the SKF 525-A-pretreated rat comparable to that observed in the 17-day-old chick embryo and isolated chick embryo liver cells. By comparing the results in Fig. 5 with those previously obtained with PIA in the other test systems [11], the answer is that the activity of PIA in the SKF 525-A-pretreated rat is much lower.

The next question that arises is why does SKF 525-A pretreatment markedly enhance PIA activity in the chicken but not in the rat? A possible explanation is the following: SKF 525-A may inhibit the metabolism of a drug in one species but not in another [9]. Thus, SKF 525-A has been shown to inhibit *O*-dealkylation of phenacetin in rat microsomes [9] but not in rabbit microsomes [19]. It is, therefore, possible that the inhibitory effect of SKF 525-A on PIA metabolism is considerably greater in the chicken than in the rat.

AIA produced a significant elevation of ALA-synthetase activity and a moderate elevation of porphyrin levels in the rat (Fig. 5). After SKF 525-A pretreat-

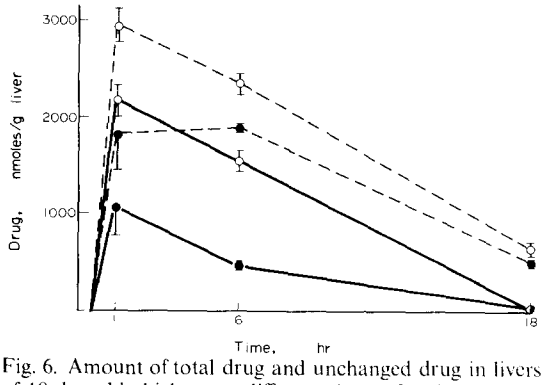

Fig. 6. Amount of total drug and unchanged drug in livers of 18-day-old chickens at different times after injection of AIA[^{14}C] (212 μmoles) and PIA[^{14}C] (209 μmoles). Key: (○—○) total drug AIA[^{14}C]; (●—●) total drug PIA[^{14}C]; (○—○) unchanged drug AIA[^{14}C]; and (●—●) unchanged drug PIA[^{14}C]. Each point represents the mean of four determinations (±S. E. M.).

Table 1. ALA-synthetase activity and amount of unchanged AIA in the livers of 18-day-old chickens and 17-day-old chick embryos after administration of AIA

Time after drug administration (hr)	18-Day-old chicken		17-Day-old chick embryo	
	ALA-synthetase activity (nmoles ALA/g liver hr)	Unchanged drug (nmoles/g liver)	ALA-synthetase activity (nmoles ALA/g liver hr)	Unchanged drug (nmoles/g liver)
1		2325		178
6	205	1550	239	178

ment, the response elicited by AIA was reduced (Fig. 5). However, because of the large S. E., the result is not significant at the 0·05 level. These results are similar to those reported by De Matteis [20] and De Matteis *et al.* [21] who showed that SKF 525-A pretreatment inhibited AIA-induced [20] and DDC-induced [21] ALA-synthetase activity and porphyrin accumulation in rat liver. De Matteis [22] has suggested that AIA and DDC might be converted to active metabolites in rat liver, thus explaining the inhibitory effect of SKF 525-A. However, this suggestion is difficult to reconcile with the findings of Kaufman *et al.* [23] who showed that phenobarbital pretreatment of rats resulted in an increased rate of metabolism of AIA accompanied by a marked reduction in AIA-induced hepatic ALA-synthetase activity. SKF 525-A exerts several effects in the rat in addition to inhibiting cytochrome P-450-dependent drug-metabolizing enzymes [24], and it is possible that one of these additional effects is responsible for the complication in interpretation of the results. The use of other inhibitors of drug metabolism such as piperonyl butoxide might yield more clear-cut results.

In a previous study, 3,5-diethoxycarbonyl-2,4,6-trimethylpyridine (Ox-DDC) was found to exhibit very low porphyrin-inducing activity in the 17-day-old chick embryo while exhibiting high efficacy in isolated chick embryo liver cells [14]. The difference in efficacy was shown to be caused by rapid metabolic inactivation in the 17-day-old chick embryo [14] and slow inactivation in isolated chick embryo liver cells [15]. It was thus anticipated that SKF 525-A pretreatment of the 17-day-old chick embryo would increase the activity of Ox-DDC. This has been found not to be the case (Fig. 3). In a previous study, PIA was found to be a less potent porphyrin-inducing drug than AIA in chick embryo liver [11], and this was attributed to a more rapid rate of metabolism of PIA than AIA [16]. It was, therefore, anticipated that SKF 525-A pretreatment would result in enhanced PIA activity. This was found to be the case (Fig. 3). It was moreover anticipated that it would have little effect on the activity of the slowly metabolized drug AIA. This was found to be so (Fig. 3). Finally, a marked enhancement in the activity of DDC was noted in the SKF 525-A-pretreated chick embryo (Fig. 4). This result suggests that, in the chick embryo, DDC itself rather than a metabolite is active. It is noteworthy that in the rat the activity of DDC is decreased after SKF 525-A pretreatment [21]. The third question posed in the introduction was: Could the sensitivity of the 17-day-old chick embryo to porphyrin-inducing drugs be increased by pretreatment with SKF 525-A? The answer is that the sensitivity to some porphyrin-inducing drugs but not others is enhanced by SKF 525-A pretreatment.

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