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SHORT COMMUNICATIONS

Association between the Membrane-Fluidizing Properties and Porphyrin-Inducing Activity of Alfaxolone and Related Steroids

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SUMMARY

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Alfaxolone and four related steroids were tested for porphyrin-inducing activity in chick embryo liver cells. Alfaxolone (3α-hydroxy-5α-pregnane-11,20-dione) and 3α-hydroxy-5α-pregnane-20-one which have both anesthetic and membrane-fluidizing properties were found to exhibit high potency in inducing porphyrin biosynthesis. The 3β-hydroxy compounds (3β-hydroxy-5α-pregnane-11,20-dione and 3β-hydroxy-5α-pregnane-20-one) and Δ16-alfaxolone, which are nonanesthetic and produce less disorder of phospholipid bilayers, were considerably less potent as porphyrin-inducing compounds. These findings provide strong support for the concept that at least some porphyrin-inducing drugs act by disorganizing membrane lipids.

INTRODUCTION

Many drugs produce an elevation of δ-aminolevulinic acid (ALA) synthetase activity in chick embryo liver cells (1, 2). The elevation of activity has been shown to result from increased synthesis of the enzyme (3, 4). As a result of elevated ALA-synthetase activity porphyrins accumulate and the level of porphyrins is therefore used as an approximate index of enzyme activity (1). It is of considerable interest to determine the mechanism of action of these drugs. In a previous publication, we postulated that porphyrin-inducing drugs act, at least in part, by disorganizing or "fluidizing" membrane lipids (5). This hypothesis grew out of the observation that compounds possessing porphyrin-inducing activity (2, 6) and compounds causing maximum "fluidization" of model phospholipid bilayer membranes (7–10) display similar structural features, viz., a certain degree of lipophilicity and an uncharged polar group. In the previous study (5) we measured the porphyrin-inducing activity of 11 lipophilic, uncharged analogs of adamantane and benzene all of which contained a polar group and were known to "fluidize" a model lipid bilayer system. All 11 analogs induced porphyrin accumulation. In this communication we wish to report on the porphyrin-inducing properties, in cultured chick embryo liver cells, of a series of steroid molecules possessing structurally specific anesthetic activity.

Alfaxolone (3α-hydroxy-5α-pregnane-11,20-dione, Fig. 1a) and 3α-hydroxy-5α-pregnane-20-one (Fig. 1b) have anesthetic activity while the 3β-hydroxy compounds (Figs. 1c and d) are inactive. Δ16-Alfaxolone (3α-hydroxy-5α-pregn-16-ene-11,20-dione, Fig. 1e), which differs from alfaxolone by a double bond in the D ring of the steroid nucleus, is nonanesthetic (11). More importantly for our purposes those steroids which are active as anesthetics (Figs. 1a and b) cause fluidization of a model lipid bilayer while those that do not (Figs. 1c–e) produce considerably less disordering of a lipid bilayer (11). In our initial publication (5) fluidization referred to the capacity of a given molecule to increase phospholipid fatty acyl chain motion in dipalmitoylphosphatidylcholine liposomes at a temperature below the normal transition temperature for that lipid. Lipid chain motion was monitored by the spin-label technique. In the present study we have used the bilayer "fluidization" data of Lawrence and Gill (11). Fluidization in the study of Lawrence and Gill (11) refers to the capacity of a given steroid to increase lipid chain motion in phosphatidylcholine–cholesterol liposomes as monitored by incorporated spin-labeled dipalmitoylphosphatidylcholine. The steroid analogs provide a highly sensitive tool for testing our hypothesis. If the hypothesis was correct we anticipated finding marked porphyrin-inducing potency in the anesthetic steroids which fluidized bilayers. On the other hand we anticipated finding considerably less porphyrin-inducing activity in the nonanesthetic steroids which produced less disordering of lipid bilayers. Our objective was to determine whether this was the case.

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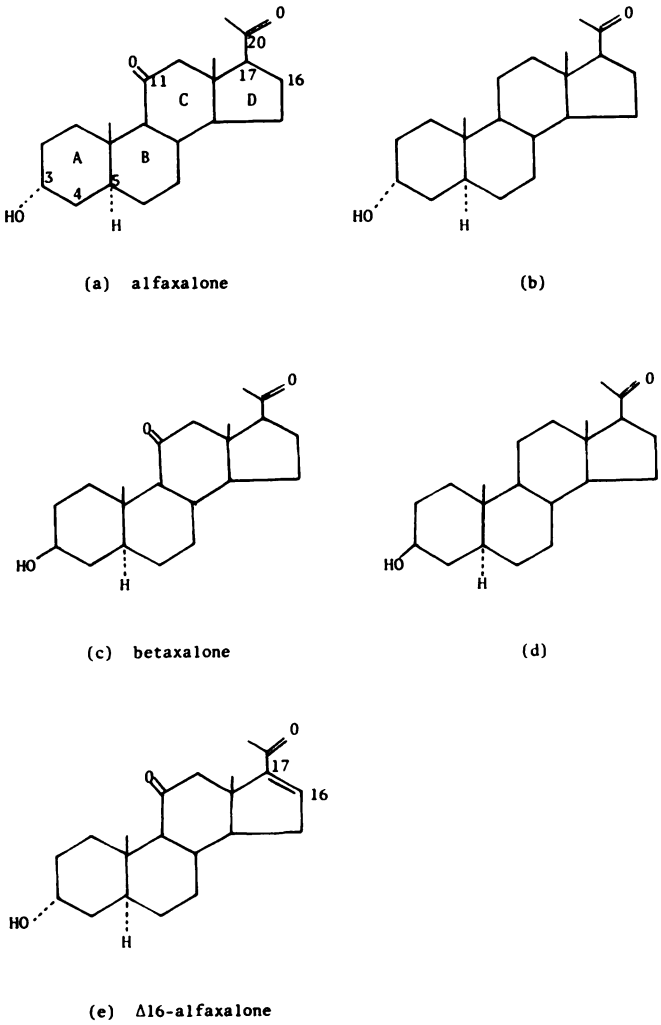

FIG. 1. Structures of steroid anesthetics and nonanesthetics

METHODS

Source of compounds. Alfaxolone and derivatives were obtained from Glaxo (Greenford, Middlesex, England). Waymouth MD 705/1 medium was purchased in powder form from Grand Island Biological Company, Grand Island, New York. Insulin (bovine pancreas, 24 IU/mg), L-thyroxine sodium pentahydrate (T_4),[1] bovine serum albumin (fraction V powder), bovine serum albumin (crystallized and lyophilized, for protein standard), collagenase (type I, 460 NF units/mg), penicillin G sodium, and streptomycin sulfate were purchased from Sigma Chemical Company (St. Louis, Mo.). The Folin–Ciocalteau phenol reagent was purchased from Fisher Scientific.

Cell culture technique. The details of the cell culture technique have been previously described (12, 13). The cells were maintained in 6-cm-diameter disposable plastic petri dishes (Falcon Plastics, Oxnard, Calif.) containing 5 ml of Waymouth MD 705/1 medium supplemented with 60 mg penicillin G, 100 mg streptomycin sulfate, 1.0 mg insulin, and 1.0 mg T_4 per liter. After an initial incubation period of 24 h, the medium was discarded and replaced with fresh medium. Chemicals, dissolved in 95% ethanol (10 μl), were added to the cell cultures, and the dishes reincubated. Porphyrin content of cells and medium and protein content of cells were measured quantitatively 24 h later (1). Porphyrins were measured by a fluorometric procedure. For this purpose a standard fluorescence curve was constructed using 1,2,3,4, and 5 ng/ml standard of coproporphyrin I in 1 M perchloric acid: ethanol (1:1 v/v). Results are expressed as nanograms of porphyrins per milliliter of protein. Each point in Figs. 2–5 represents the mean of five determinations ± SEM.

[1] Abbreviation used: T_4, L-thyroxine sodium pentahydrate.

RESULTS AND DISCUSSION

Alfaxolone (Fig. 1a), one of two steroid components of Alfathesin, a new anesthetic agent, has been reported to have porphyrin-inducing activity in chick embryo liver cells comparable to that of thiopental (14). This finding has been confirmed (Fig. 2). A closely related analog, Δ16-alfaxalone (Fig. 1e), is nonanesthetic and antagonizes the depressant action of alfaxolone on synaptic transmission in the guinea pig olfactory cortex (15). Since Δ16-alfaxalone is considerably less active than alfaxalone in fluidizing lipid layers (11) it was anticipated that it would be less potent as a porphyrin-inducing steroid; this was indeed found to be the case (Fig. 2).

3α-Hydroxy-5α-pregnane-20-one (Fig. 1b) differs from alfaxalone by lacking an 11-oxo group. Since this analog retains anesthetic activity and the ability to fluidize lipid bilayers (11) it was anticipated that it would have considerable potency as a porphyrin-inducing agent. In fact its porphyrin-inducing activity was indistinguishable from that of alfaxalone (Fig. 3).

While alfaxalone (Fig. 1a) is a potent anesthetic, be-

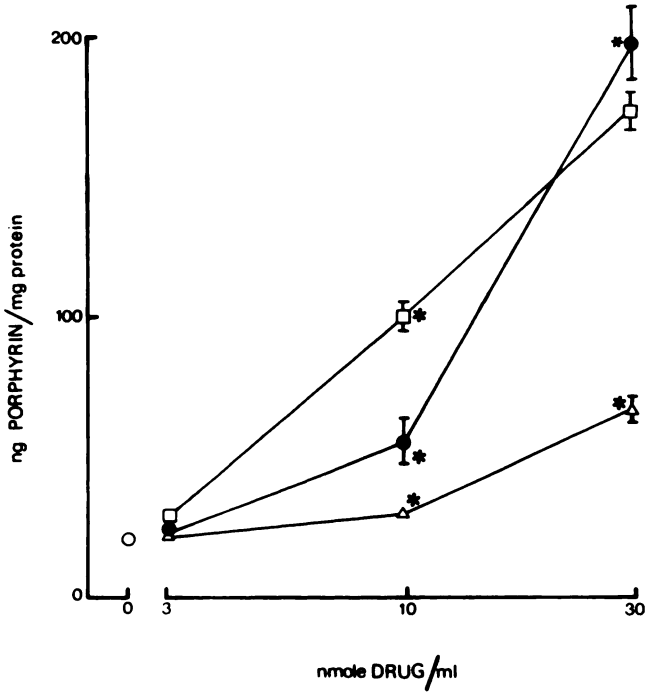

FIG. 2. *Porphyrin accumulation in response to increasing doses of alfaxolone* (●), *Δ16-alfaxolone* (△), *and thiopental* (□)

Each point represents the mean of five determinations ± SEM. Asterisks denote a significant difference in porphyrin accumulation ($P < 0.05$) between dishes treated with the same dosage of different drugs.

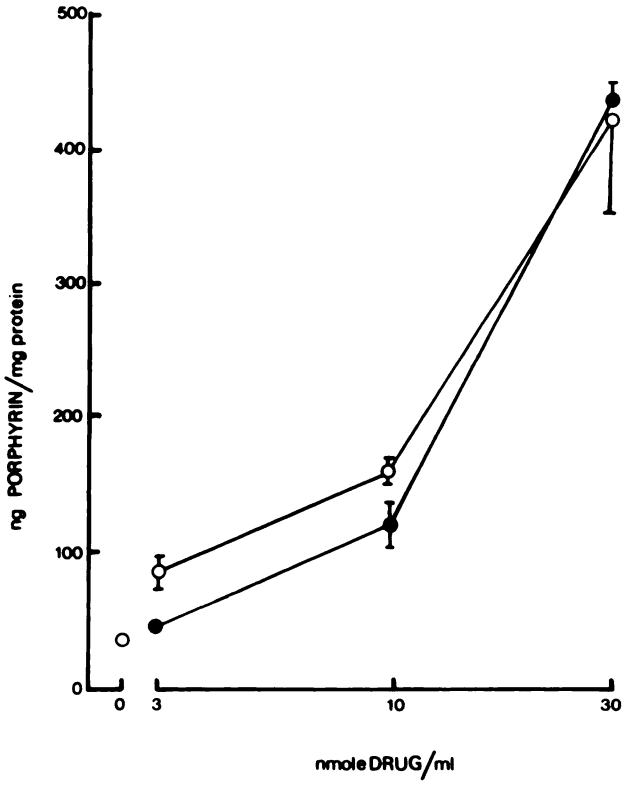

FIG. 3. *Porphyrin accumulation in response to increasing doses of alfaxolone* (●) *and 3α-hydroxy-5α-pregnane-20-one* (○)
Each point represents the mean of five determinations ± SEM.

taxalone, the isomeric 3β-hydroxy compound (Fig. 1c), is inactive. A similar, though smaller, difference has been reported between 3α-hydroxy-5α-pregnane-20-one (Fig. 1b) and the isomeric 3β-hydroxy compound (Fig. 1d).

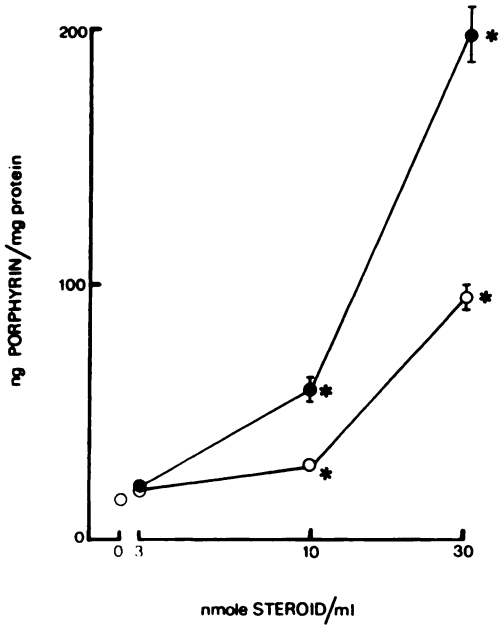

FIG. 4. *Porphyrin accumulation in response to increasing doses of alfaxolone (3α-hydroxy-5α-pregnane-11,20-dione)* (●) *and betaxolone (3β-hydroxy-5α-pregnane-11,20-dione)* (○)
Each point represents the mean of five determinations ± SEM. Asterisks denote a significant difference in porphyrin accumulation between dishes treaed with the same dosage of different steroids.

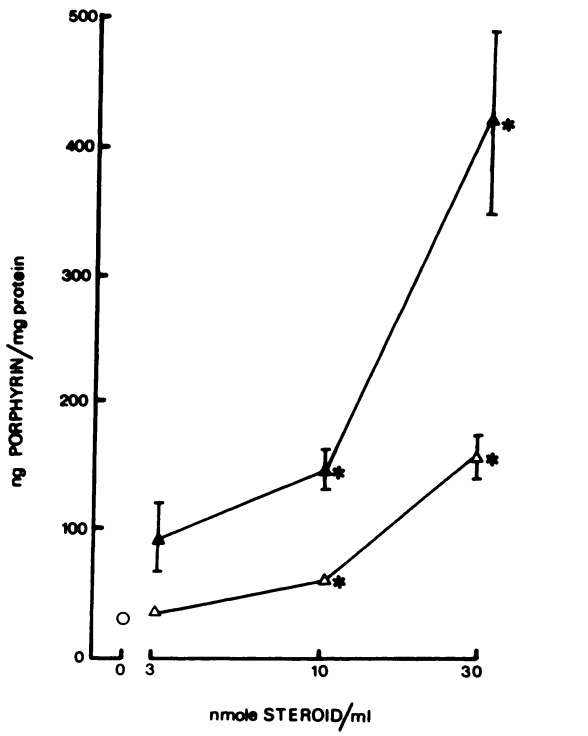

FIG. 5. *Porphyrin accumulation in response to increasing doses of 3α-hydroxy-5α-pregnane-20-one* (▲) *and 3β-hydroxy-5α-pregnane-20-one* (△)
Each point denotes the mean of five determinations ± SEM. Asterisks denote a significant difference in porphyrin accumulation between dishes treated with the same dosage of different steroids.

Since the 3α-hydroxy compounds were more effective in fluidizing lipid bilayers than the isomeric 3β-hydroxy compounds (11) it was anticipated that the 3α-hydroxy compounds would exhibit greater porphyrin-inducing activity than the 3β-hydroxy compounds. This was found to be the case (Figs. 4 and 5).

In summary therefore we have tested five steroid compounds. Two of these steroids have anesthetic and membrane-fluidizing properties and were found to exhibit high potency in inducing porphyrin biosynthesis. The remaining three steroids, which are nonanesthetic and produce less disordering of a lipid bilayer, were considerably less potent as porphyrin-inducing compounds. These findings provide strong support for the concept that at least some porphyrin-inducing drugs act by inducing disorganization in membrane lipids.

According to current ideas, there appear to be two mechanisms for the induction by chemicals of hepatic ALA-synthetase: (1) a direct action on the nucleus to increase the amount of an induction-specific RNA for ALA-synthetase; and (2) an action to deplete a "regulating heme pool" (16–18). Several membranes, for example, those of the nucleus and mitochondrion, are involved in the above two mechanisms. The mitochondrial membrane could represent the membrane site of action for the following reasons. Coproporphyrinogen III is transported from its site of synthesis in cytoplasm into mitochondria for conversion to heme. It is possible that a fluidizing action of a chemical on mitochondrial membranes might hinder the transport of coproporphyrinogen

III into mitochondria. This action, in turn, would lead to diminished mitochondrial heme synthesis, a diminished "regulating heme pool," enhanced ALA-synthetase activity, and porphyrin accumulation.

REFERENCES

1. Granick, S. The induction *in vitro* of the synthesis of δ-aminolevulinic acid synthetase in chemical porphyria: A response to certain drugs, sex hormones, and foreign chemicals. *J. Biol. Chem.* **241:** 1359–1375 (1966).
2. Marks, G. S. The effect of chemicals on hepatic heme biosynthesis, in *Handbook of Experimental Pharmacology*, Vol. 44: *Heme and Hemoproteins* (F. De Matteis and W. N. Aldridge, eds.). Springer-Verlag, New York, 201–231 (1978).
3. Whiting, M. J. and S. Granick. δ-Aminolevulinic acid synthase from chick embryo liver mitochondria. *J. Biol. Chem.* **251:** 1340–1346 (1976).
4. Whiting, M. J. and S. Granick. δ-Aminolevulinic acid synthase from chick embryo liver mitochondria. *J. Biol. Chem.* **251:** 1347–1353 (1976).
5. Neilson, I. R., F. T. Chaykowski, M. A. Singer, and G. S. Marks. A possible association between membrane-fluidizing properties and porphyrin-inducing activity of drugs. *Biochem. Pharmacol.* **28,** 3589–3593 (1979).
6. Marks, G. S., V. Krupa, F. Murphy, H. Taub and R. A. Blattel. Mechanism of drug-induced porphyrin biosynthesis. *Ann. N. Y. Acad. Sci.* **244:** 472–480 (1975).
7. Jain, M. K., N. Y. Yu and L. V. Wray. Drug-induced phase change in bilayer as possible mode of action of membrane expanding drugs. *Nature* (*Lond.*) **255:** 494–496 (1975).
8. Jain, M. K., N. Yen-Min Wu, T. K. Morgan, Jr., M. S. Briggs and R. K. Murray, Jr. Phase transition in a lipid bilayer. II. Influence of adamantane derivatives. *Chem. Phys. Lipids* **17:** 71–78 (1976).
9. Chaykowski, F. T., J. K. S. Wan and M. A. Singer. Interaction of small molecules with phospholipid bilayer membranes: a spin label study. *Chem. Phys. Lipids* **23:** 111–123 (1979).
10. Singer, M. A. and J. Wan. Interaction of butylated hydroxytoluene (BHT) with phospholipid bilayer membranes: Effect on ^{22}Na permeability and membrane fluidity. *Biochem. Pharmacol.* **26:** 2259–2268 (1977).
11. Lawrence, D. K. and E. W. Gill. Structurally specific effects of some steroid anesthetics on spin-labeled liposomes. *Mol. Pharmacol.* **11:** 280–286 (1975).
12. Fischer, P. W. F., R. O. Morgan, V. Krupa and G. S. Marks. Drug-induced porphyrin biosynthesis. XV. Induction of porphyrin biosynthesis in chick embryo liver cells maintained in serum-free Waymouth medium. *Biochem. Pharmacol.* **25:** 687–693 (1976).
13. Morgan, R. O., P. W. F. Fischer, J. K. Stephens and G. S. Marks. Thyroid hormone enhancement of drug-induced porphyrin biosynthesis in chick embryo liver cells maintained in serum-free Waymouth medium. *Biochem. Pharmacol.* **25:** 2609–2612 (1976).
14. Fischer, P. W. F., A. Ferizovic, I. R. Neilson and G. S. Marks. Porphyrin-inducing activity of alfaxolone and alfadalone acetate in chick embryo liver cells. *Anesthesiology* **50:** 350–352 (1979).
15. Richards, C. D. and T. R. Hesketh. Implications for theories of anaesthesia of antagonism between anaesthetic and non-anaesthetic steroids. *Nature* (*Lond.*) **256:** 179–182 (1975).
16. De Matteis, F. and A. H. Gibbs. Stimulation of the pathway of porphyrin synthesis in the liver of rats and mice by griseofulvin, 3,5-diethoxycarbonyl-1,4-dihydrocollidine and related drugs: Evidence for two basically different mechanisms. *Biochem. J.* **146:** 285–287 (1975).
17. Maxwell, J. D. and U. A. Meyer. Pharmacogenetics in the field of heme metabolism: Drug sensitivity in hereditary hepatic porphyria, in *Handbook of Experimental Pharmacology*, Vol. 44: *Heme and Hemoproteins* (F. De Matteis and W. N. Aldridge, eds.). Springer-Verlag, New York, 239–254 (1978).
18. Sinclair, P. R. and S. Granick. Heme control on the synthesis of delta-aminolevulinic acid synthetase in cultured chick embryo liver cells. *Ann. N. Y. Acad. Sci.* **244:** 509–518 (1975).

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