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Communications to the Editor

Mechanism of Steroid Anesthetic Action: Interactions of Alphaxalone and Δ^{16}-Alphaxalone with Bilayer Vesicles

Sir:

Traditionally, the potency of anesthetics has been correlated with some of their general physical properties, primarily their lipid solubility.[1] However, for anesthetic steroids, this does not hold true. Small changes in steroid structure can lead to large differences in anesthetic activity.[2,3] For example, 3α-hydroxy-5α-pregnane-11,20-dione (alphaxalone) acts as an anesthetic and is used clinically as the main active component in the anesthetic Althesin, while 3α-hydroxy-5α-pregnan-16-ene-11,20-dione (Δ^{16}-alphaxalone), which differs from alphaxalone by having a double bond in the C_{16} position (Figure 1), lacks anesthetic activity.

Recently, we found that although the two steroids incorporated equally in membrane preparations,[4] the anesthetic alphaxalone inhibited anion transport in human erythrocytes to a much greater extent than its nonanesthetic, Δ^{16} analogue.[5] These results led us to the idea that the considerable difference in activity exhibited by the two analogues may be due to differences in their interactions with cellular membranes. The present investigation based on NMR experiments examines the interactions of the two steroids with phosphatidylcholine bilayer vesicles used as model membranes.

Figure 2 depicts ^{1}H NMR spectra of three different vesicle preparations at 70 °C. One sample was made up of egg lecithin while the other two were egg lecithin preparations into which alphaxalone and Δ^{16}-alphaxalone were incorporated. As can be seen, no resonances corresponding to Δ^{16}-alphaxalone can be detected in the ^{1}H NMR spectrum of lecithin vesicles containing this steroid. In contrast, ^{1}H NMR signals for alphaxalone are observed in the corresponding vesicle preparation. These resonances were assigned to the acetyl methyl ($\nu_{1/2}$ = 2.5 Hz) and C_{18} angular methyl ($\nu_{1/2}$ = 10 Hz) resonances by analogy, from the corresponding ^{1}H NMR spectrum of alphaxalone in $CDCl_3$. As expected, the ^{1}H resonances of alphaxalone became progressively broader at lower temperatures, reflecting the decreasing mobility of the steroid in the bilayer (Figure 3).

The ^{13}C NMR results were consistent with the ^{1}H NMR data. No ^{13}C resonances due to Δ^{16}-alphaxalone, could be observed in its phosphatidylcholine vesicle preparations. On the other hand, ^{13}C NMR spectra of vesicles into which alphaxalone was incorporated showed several resonances corresponding to this steroid (Figure 4). The observed alphaxalone ^{13}C resonances were identified as being due to carbons with relatively long relaxation times. These included the nonprotonated carbons that relax inefficiently due to the absence of directly attached ^{1}H dipoles and methyl group carbons that experience slow relaxation because of internal rotation.

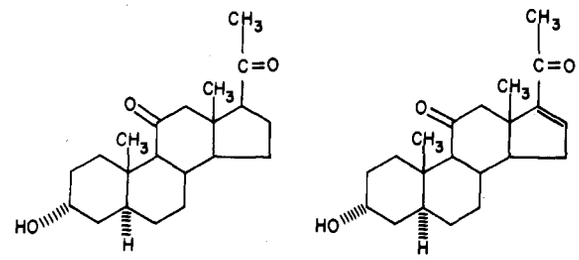

Figure 1.

Since the ^{1}H and ^{13}C resonances corresponding to Δ^{16}-alphaxalone were too broad to be detected in the presence of the overwhelmingly larger lecithin signals, no direct quantitative comparisons of the motions of the two steroids in the bilayer were possible. To obtain such information, we selectively deuterated alphaxalone and Δ^{16}-alphaxalone (>95%) in the acetyl methyl position[6] and recorded the ^{2}H NMR spectra of the steroids incorporated in lecithin vesicles. Both steroids gave ^{2}H NMR spectra with a signal that was unencumbered by the phospholipid resonances (the natural abundance of ^{2}H is only 0.0156%). As shown in Figure 5, the ^{2}H acetyl methyl resonance of Δ^{16}-alphaxalone ($\nu_{1/2}$ = 50 Hz) was found to be more than fourfold broader than the corresponding resonance of alphaxalone ($\nu_{1/2}$ = 12 Hz).

Line-width ($\nu_{1/2}$) measurements can provide information on the slower components of motion in anisotropic model membrane systems through their relationship to spin–spin relaxation times (T_2).[7a,b] Our NMR experiments with vesicle preparations show that in alphaxalone different nuclei located in different positions of the molecule have consistently longer T_2 values than in the Δ^{16} analogue. Since no difference in vesicle size was observed for the steroid-incorporated vesicles at the concentration used, the

(1) Seeman, P. *Pharmacol. Rev.* **1972**, *24*, 583–655.
(2) Atkinson, R. M.; Davis, B.; Pratt, M. A.; Sharpe, H. M.; Tomich, E. G. *J. Med. Chem.* **1965**, *8*, 426–432.
(3) Phillips, G. H. *J. Steroid Biochem.* **1975**, *6*, 607–613.
(4) Fesik, S. W. Ph.D. Thesis, University of Connecticut, Storrs, CT, 1981.
(5) Makriyannis, A.; Fesik, S. W. *J. Neurosci. Res.* **1980**, *5*, 25–33.
(6) Selective deuterations were performed by base-catalyzed exchange with ^2H_2O at 50 °C using Me_2SO as the solvent.
(7) (a) Sheetz, M. P.; Chan, S. I. *Biochemistry* **1972**, *11*, 4573–4581. (b) Horwitz, A. F.; Horsley, W. J.; Klein, M. P. *Proc. Natl. Acad. Sci. U.S.A.* **1972**, *69*, 590–593.

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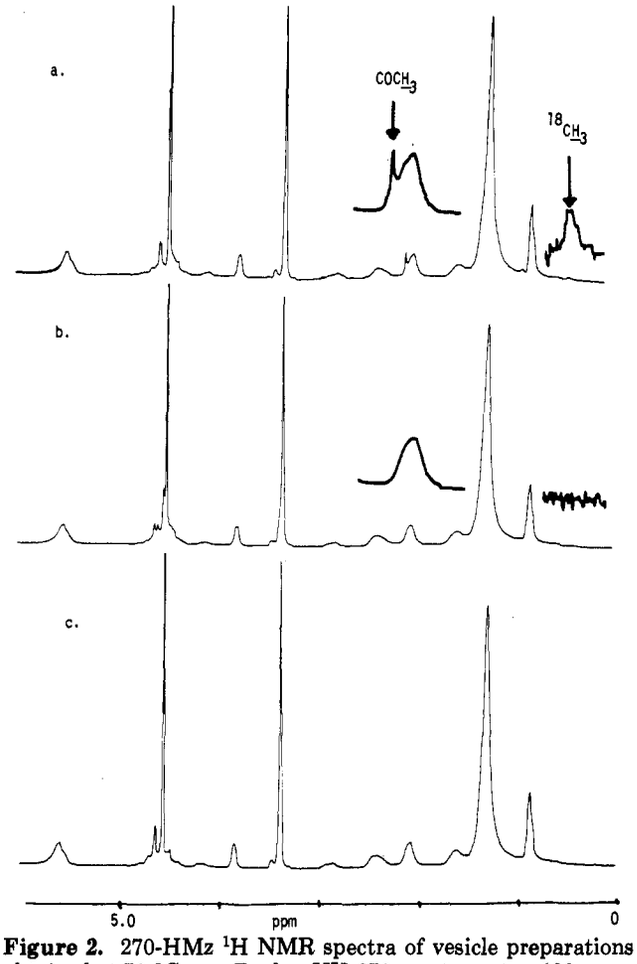

Figure 2. 270-HMz ^1H NMR spectra of vesicle preparations obtained at 70 °C on a Bruker HX-270 spectrometer; 100 accumulations with a repetition time of 10 s were used for each spectrum: (a) alphaxalone (4 mM) and lecithin (20 mM purified egg lecithin, Sigma Chemical Co.; L-α-phosphatidylcholine, Type XI-E); (b) Δ^{16}-alphaxalone (4 mM) and lecithin (20 mM); (c) lecithin (20 mM).

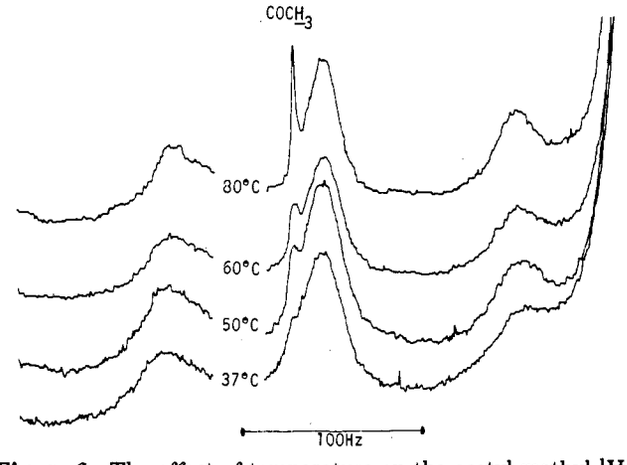

Figure 3. The effect of temperature on the acetyl methyl ^1H resonance of alphaxalone.

different steroid line widths must be due to differences in the local motions and/or anisotropic behavior of the steroid in the bilayer rather than to differences in the overall tumbling of the lipid vesicles.[8]

The results obtained from the NMR experiments described in this communication are consistent and suggest

(8) Gent, M. P. N.; Prestegard, J. H. *Biochemistry* **1974**, *13*, 4027–4033.

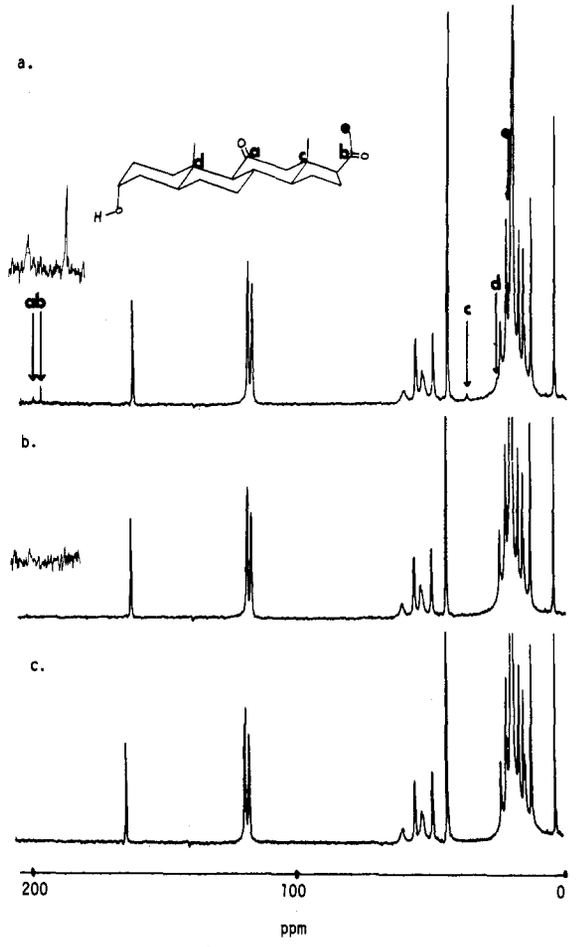

Figure 4. 22.6-MHz ^{13}C NMR spectra of vesicle preparations obtained at 37 °C on a Bruker WH-90 spectrometer; 20 000 accumulations with a repetition time of 2 s were used: (a) alphaxalone (52 mM) and lecithin (260 mM); (b) Δ^{16}-alphaxalone (52 mM) and lecithin (260 mM); (c) lecithin (260 mM).

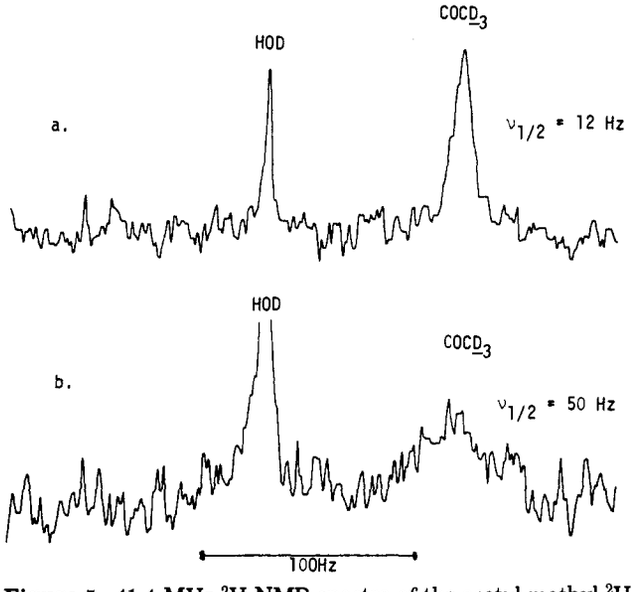

Figure 5. 41.4-MHz ^2H NMR spectra of the acetyl methyl ^2H resonance of (a) alphaxalone (4 mM) and (b) Δ^{16}-alphaxalone (4 mM) incorporated in lecithin (20 mM) bilayers. Spectra were obtained at 70 °C on a Bruker HX-270 spectrometer; 4000 accumulations with a repetition time of 1 s were used for each spectrum.

that alphaxalone is considerably more mobile in the phospholipid bilayer than Δ^{16}-alphaxalone. The data also

suggest that the acetyl group is probably moving faster than the steroid nucleus, since the observed ^{13}C and ^{1}H resonances of the $COCH_3$ side chain are consistently narrower than the ^{13}C frequencies of the rigid ring system or the ^{1}H frequencies of the methyl groups directly attached to it.

The observed differences in mobility between the two steroids in the bilayer can be explained by assuming that the variation in steroid geometry leads to different phospholipid–steroid interactions. We can thus postulate that the inactive Δ^{16}-alphaxalone interacts with the fatty acid chains of phosphatidylcholine bilayer in such a manner that the most stable bilayer geometry is maintained while the interacting steroid is partially immobilized. This interaction may be similar to the interaction of cholesterol with phospholipids, which has been previously described.[9,10] In contrast, the biologically active alphaxalone, because of its slightly different stereochemical features, interacts with the bilayer differently. Presumably, this interaction results in a disruption of the bilayer geometry, which is evidenced by the higher mobility of the incorporated steroid. The perturbation produced by the anesthetic molecule in the lipid region of the membrane could be transmitted to the membrane-associated proteins, resulting in a modification of their functions.[11]

Our results can be used to explain the different biological activities of alphaxalone and Δ^{16}-alphaxalone as anesthetics and as inhibitors of sulfate transport in the red blood cell, both of which are functions associated with a membrane-bound protein. We have recently tested on the phospholipid system described here a number of steroids structurally related to alphaxalone but having widely different anesthetic activity. The results are consistent with the hypothesis that steroid anesthetic activity depends on the ability of the drug to perturb the membrane lipids. This ability appears to be governed by strict geometric requirements.

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Registry No. Alphaxalone, 23930-19-0; Δ^{16}-alphaxalone, 32226-03-2.

(9) Brainard, J. R.; Cordes, E. H. *Biochemistry* 1981, *20*, 4607–4617.
(10) Huang, C.-H. *Lipids* 1977, *12*, 348–356.
(11) Sandermann, H. *Biochim. Biophys. Acta* 1978, *515*, 209–237.

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Articles

Modified Steroid Hormones. 7. 4-Fluoro-17β-estradiol: Carbon-13 Nuclear Magnetic Resonance, Crystal and Molecular Structure, and Biological Activity[1a,b]

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The 4-fluoro analogue of 17β-estradiol was investigated for estrogenic action in rats by determination of uterine hyperemia and [^{3}H]uridine incorporation and was found to be an active estrogen. Antitumor activity of this analogue was demonstrated against 7,12-dimethylbenz[*a*]anthracene (DMBA) induced rat mammary adenocarcinoma. Its ^{13}C NMR spectrum was determined, and all signals were assigned. A detailed X-ray diffraction investigation of 4-fluoro-17β-estradiol was carried out, and it crystallized in triclinic space group $P\bar{1}$ with two steroids and one methanol in a unit cell of a = 7.367 (1), b = 9.363 (6), c = 12.531 (1) Å, α = 89.31 (3), β = 93.38 (1), γ = 109.62 (3)°, V = 812.8 Å^3, and Z = 2. The structure was solved and refined to an R index of 0.062 using 3045 reflections measured on an automated diffractometer. The oxygen atoms at C(3) and C(17) at either ends link the molecules together in a head to tail fashion. The hydroxy groups of the solvent molecules also take part in linking the molecules sideways through the hydroxy groups.

The cancer chemotherapist's armamentarium contains a variety of hormonal agents, such as adrenocorticosteroids, androgens, antiandrogens, estrogens, antiestrogens, antigonadotropic agents, and progestogens.[2,3] The ablation of endocrine glands for removal of endogenous sources of hormones (notably, orchiectomy in prostatic cancer and oophorectomy, adrenalectomy, and hypophysectomy in breast cancer) is practiced, as well as androgen administration in breast cancer, and estrogen administration in breast cancer and in prostatic cancer.[4] The results of

(1) (a) Part 6: Neeman, M.; O'Grodnick, J. S. *Can. J. Chem.* 1974, *52*, 2941–2949. (b) This investigation was supported in part by American Cancer Society Grant P-265 (to M.N.), and Roswell Park Memorial Core Center Grant CA 16056 of the National Cancer Institute, National Institutes of Health. (c) Syracuse Research Corp., Syracuse, NY 13210.
(2) Carter, S. K.; Bakowsky, M. T.; Hellman, K. "Chemotherapy of Cancer"; Wiley: New York, 1977; pp 83–93.
(3) Holland, J. F.; Frei III, E. "Cancer Medicine"; Lea and Febiger: Philadelphia, 1974; pp 889–945.

0022-2623/83/1826-0465$01.50/0