5. Spolter, P. D., Harper, A. E., *Fed. Proc.*, 1962, v21, 8.

6. ———, *J. Nutr.*, in press.

7. Hubbell, R. B., Mendel, L. B., Wakeman, A. J., *ibid.*, 1937, v14, 273.

8. Spolter, P. D., Harper, A. E., PROC. SOC. EXP. BIOL. AND MED., 1961, v106, 184.

9. Spolter, P. D., Baldridge, R. C., *J. Biol. Chem.*, 1963, in press.

10. Lowry, O. H., Rosebaugh, N. J., Farr, A. L., Randall, R. J., *J. Biol. Chem.*, 1951, v193, 265.

11. Schneider, W. C., *ibid.*, 1945, v161, 293.

12. ———, in S. P. Colowick and N. O. Kaplan, Ed., *Methods in Enzymology*, Academic Press, Inc., New York, 1957, Vol. III, 680.

13. Stekol, J. A., Mody, U., Perry, J., *J. Biol. Chem.*, 1960, v235, PC59.

14. Farish, P. T., Salmon, W. D., Sauberlich, H. E., *J. Nutr.*, 1961, v73, 23.

15. Rubin, E., Hutterer, F., Gall, E. Cs., Popper, H., *Nature*, 1961, v192, 886.

16. Hutterer, F., Rubin, E., Singer, E. J., Popper, H., *Cancer Res.*, 1961, v21, 206.

17. Cohen, G. N., Gros, F., *Ann. Rev. Biochem.*, 1960, v29, 533.

18. Villa-Trevino, S., Farber, E., *Biochem. Biophys. Acta*, 1962, v61, 649.

19. Baldridge, R. C., *J. Biol. Chem.*, 1958, v231, 207.

20. Baldridge, R. C., Burket, R., *Fed. Proc.*, 1960, v19, 4.

21. McIssac, W. M., Page, I. H., *Nature*, 1961, v190, 347.

22. Whitehead, R. G., Arnstein, H. R. V., *ibid.*, 1961, v190, 1105.

23. Rao, D. R., Deodhar, A., Subramanian, H. D., *Biochem. Biophys. Res. Comm.*, 1963, v10, 243.

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Antagonism of Hydrocarbon Anesthetic-Epinephrine Arrhythmias in Dogs by Nethalide, a Dichloroisoproterenol Analogue. (28389)

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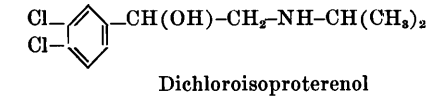
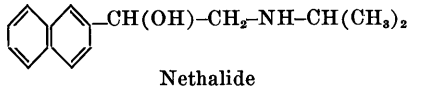
The synthesis of nethalide,* an analogue of the specific beta receptor antagonist dichloroisoproterenol(1), which differs from the latter in being free of intrinsic sympathomimetic activity(2), makes available a better pharmacological tool for studying adrenotropic receptors of the beta type(3). It was the purpose of the present study to utilize this compound in an effort to assess the importance of beta receptors in the mediation of hydrocarbon anesthetic-epinephrine arrhythmias.



Dichloroisoproterenol



Nethalide

Methods. Unpremedicated dogs weighing 10-15 kg were given sufficient thiopental sodium (15-25 mg/kg) intravenously to allow tracheal intubation to be performed. An anesthetic mixture was then administered by means of a semi-closed system with a to and fro carbon dioxide absorber (recharged every 2 hours) and the animals allowed to breathe spontaneously throughout the experiment. With cyclopropane a flow rate of 600 ml/min (500 ml oxygen + 100 ml cyclopropane) was maintained, giving a concentration of approximately 16%. Concentrations of trichloroethylene and halothane (Fluothane†) which would produce myocardial sensitization were experimentally determined but actual vapor concentrations were not measured. For a 2 l/min oxygen flow rate, these were

* Nethalide is the generic name for (2-isopropylamino-1-[2-naphthyl]ethanol HCl) which was earlier referred to as I.C.I. 38,174 and now also has the trade name Alderein by Imperial Chemical Industries, Ltd., England. In addition, Ayerst Laboratories, who generously supplied the drug for this study, have employed the designation AY-6204.

† Ayerst Laboratories.

found to be the minimum setting of a Foregger vinethene vaporizer containing trichloroethylene which just allowed bubbling to occur, and settings of 1.5-2.4 of an F-N-S(4) vaporizer charged with halothane. The sensitization produced by these agents as measured by epinephrine challenge was quantitatively similar to that obtained with cyclopropane. Intravenous injections were given in a foreleg vein through a percutaneously placed indwelling needle or polyethylene catheter kept patent by a slow saline infusion. On an Offner Dynograph was continuously recorded a lead 2 electrocardiogram and blood pressure in a femoral artery from a percutaneously placed polyethylene catheter attached to a Statham pressure transducer.

The method employed to produce cardiac arrhythmias was based on that described by Cummings and Hays(5). Thirty to 60 minutes of equilibration with the anesthetic agent under study was allowed prior to a series of 2-3 control epinephrine challenges at 15 minute intervals. An experimental run consisted of determining the total μg/kg of epinephrine that had been administered intravenously at the time of first appearance of multiple ventricular ectopic beats. Epinephrine solutions were freshly prepared for each animal by dilution of Adrenalin 1:1000‡ with distilled water to contain 1.0 μg/kg/ml and 10 μg/kg/ml. The procedure was standardized by administering the epinephrine solutions at a rate of 3 ml/min with the more dilute solution being infused first up to a total volume of 10 ml, and then if the end point was not reached immediately followed by up to 10 ml of the more concentrated epinephrine solution at the same infusion rate. In those experiments where a marked blockade was demonstrated, 1:1000 Adrenalin was then slowly infused. The nethalide HCl solution in a concentration of 10 mg/ml was administered intravenously as soon (usually 3-5 minutes) as the animal returned to its pre-epinephrine EKG pattern and blood pressure levels, and followed 15 minutes later by an epinephrine challenge. No dog was used in more than one experiment.

‡ Parke, Davis and Co.

TABLE I. Comparison of Doses (μg/kg) of Epinephrine Producing Arrhythmias Before and 15 Minutes After Intravenous Nethalide Administration to 6 Dogs Anesthetized with Cyclopropane, Trichloroethylene, or Halothane (2 dogs per anesthetic agent).

Anesthetic agent	Nethalide (mg/kg)	Epinephrine dose after nethalide/ Epinephrine dose* before nethalide
Cyclopropane	1.0	9.8/2.5 = 4
"	2.0	35 /3.4 = 10
Trichloroethylene	2.0	35 /3.8 = 9
"	2.0	58 /3.8 = 15
Halothane	2.0	50 /4.8 = 10
"	2.0	40 /2.8 = 14

* Average of 3 control runs except for first dog receiving trichloroethylene in which only 2 controls were obtained (range of individual determinations for all dogs, 1.5-5.5 μg/kg).

Results. The dose of epinephrine required to produce arrhythmias 15 minutes after 2 mg/kg of nethalide was increased with each of the anesthetics to 9-15 times the control value, but only to 4 times in the dog receiving 1 mg/kg (Table I). This blockade persisted for at least one hour in all dogs. All of the animals in this series, except the one receiving only 1 mg/kg of nethalide, subsequently received additional doses of nethalide (up to a total of 7 mg/kg) demonstrating further blockade requiring up to 3 mg of epinephrine to produce the arrhythmia. Despite rapid intravenous administration of up to 10 mg of epinephrine within one hour following even the 2 mg dose of nethalide, ventricular fibrillation was not produced. However, one of the dogs anesthetized with halothane developed ventricular fibrillation during the control period which was readily reversed with an external DC defibrillator allowing the experiment to continue. No attempt was made to defibrillate another dog (not shown in Table I) receiving halothane which also developed ventricular fibrillation and died during the control period. Fibrillation was not encountered with cyclopropane or trichloroethylene. No associated changes were noted on slow intravenous administration of nethalide but a transient (30-60 sec) mild hypotension commonly occurred with rapid injection. This hypotension was usually accompanied by flushing of the skin and occasionally by a decrease in QRS voltage

of the EKG. These latter effects, except for decreased EKG voltage, have been previously reported(2,6).

In 2 additional dogs no effect of nethalide was observed in altering the threshold for electrically induced ventricular fibrillation, thus failing to demonstrate any obvious relationship between adrenergically and electrically induced arrhythmias.

Discussion. Our results have provided evidence that beta receptors are important in the development of epinephrine induced arrhythmias associated with cyclopropane, trichloroethylene, and halothane anesthesia. Blockade of these receptors by nethalide was shown to significantly decrease the sensitization of the dog myocardium to such arrhythmias. The degree of blockade is probably proportional to the dose of the antagonist administered, although more complete dose response studies are needed to substantiate this point. This blockade is in accord with the demonstration by Schull *et al.*(7) that dichloroisoproterenol antagonizes cyclopropane-norepinephrine arrhythmias in dogs. It is also of interest that ventricular fibrillation was never observed even with very large doses of epinephrine after nethalide administration, despite its occurrence with small doses of epinephrine during the control period in 2 of 3 dogs receiving halothane.

While it is interesting to speculate on a possible use of nethalide in clinical anesthesia to prevent the development of cardiac arrhythmias when epinephrine is used with a sensitizing anesthetic, a number of fundamental problems remain to be studied. One of these is the potentiation of the epinephrine pressor response(2), which may require simultaneous administration of an alpha blocking agent to avoid severe hypertension. Also, it may be necessary to administer a vagolytic dose of atropine to avoid parasympathetic dominance of such a pharmacologically sympathectomized heart. The ultimate induction and maintenance of anesthesia and the performance of surgery in an animal or patient with such an obtunded autonomic nervous system may result in more serious problems than were prevented.

Summary. Administration of nethalide, an adrenotropic beta receptor antagonist, to dogs anesthetized with cyclopropane, trichloroethylene, or halothane was found to decrease markedly the sensitivity of the myocardium to epinephrine induced arrhythmias. From 4-15 times the minimum dose of epinephrine causing multiple ventricular ectopic beats during the control period was required to produce this arrhythmia 15 minutes after intravenous nethalide. Ventricular fibrillation was not observed with even large doses of epinephrine after nethalide but did occur during the control period in 2 of 3 dogs receiving halothane. Some possible problems that might be encountered with the use of this agent as an adjunct to anesthesia are discussed.

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1. Powell, C. E., Slater, I. H., *J. Pharm. and Exp. Ther.*, 1958, v122, 480.

2. Black, J. W., Stephenson, J. S., *Lancet,* 1962, v2, 311.

3. Ahlquist, R. P., *Am. J. Physiol.,* 1948, v153, 586.

4. Fabian, L. W., Newton, G. W., Stephen, C. R., *Anesthesiology,* 1958, v19, 284.

5. Cummings, J. R., Hays, H. W., *ibid.,* 1956, v17, 314.

6. Dornhorst, A. C., Robinson, B. F., *Lancet,* 1962, v2, 314.

7. Schull, L. G., Berry, G., Villarreal, R., *Anesthesiology,* 1961, v22, 444.

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