trols; presumably the number or reactivity of parental lymphoid cells was insufficient to kill the hybrid and a state of tolerance to the hybrid ensued. It is possible, therefore, that transfusion, by some mechanism, was able to swing the balance toward tolerance and recovery in several additional doubtful cases. In the strain combination used, anemia due to the graft-*vs*-host reaction was mild, in the range of 30-40%. In other strain combinations, and in other species, in which a more severe immunologic anemia might occur, transfusions would be more useful in eliminating this as a cause of death, but again it seems likely that most animals would inevitably succumb, for the basic cause of death in homologous disease is still an enigma.

Summary. (1) Transfusion of the hybrid mouse in parabiosis with a parental strain partner was followed by shunting of blood to the latter. The hybrid mouse was not benefited. (2) When the hybrid mouse was separated from the parental strain partner 10 days after union, the hybrid mouse showed a temporary weight gain, followed by progressive wasting and death. Transfusion of the hybrid mouse to a normal hematocrit level did not alter this course of events. (3) When separation and transfusion of the hybrid were carried out 6 days after parabiosis, there was a greater gain in weight than in nontransfused controls, and a reduction in mortality from 11/12 to 6/8. In both transfused and nontransfused groups survival time was significantly prolonged, compared to hybrid mice remaining in parabiosis. (4) Transfusion of hybrid mice suffering from homologous disease resulted in a reduction of mortality from 8/10 to 6/10 but otherwise the course of the disease was not altered. (5) Parabiosis intoxication, in the strain combination employed, appears to involve the effects of both anemia and the graft-*vs*-host reaction. (6) Syngeneic blood transfusion is of little value in treatment of the graft-*vs*-host reaction in the strain combination employed.

1. Hilgard, H. R., Cornelius, E. A., Dalmasso, A. P., Martinez, C., Good, R. A., J. Exp. Med., 1964, v119, 567.

2. Billingham, R. E., Sci., 1959, v130, 947.

3. Kaplan, H. S., Rosston, B. H., Stanford Med. Bull., 1959, v17, 77.

4. Porter, K. A., Brit. J. Cancer, 1960, v14, 66.

5. Eichwald, E. J., Lustgraaf, E. C., Strainer, M., J. Nat. Cancer Inst., 1959, v23, 1193.

6. Hall, C. E., Hall, O., J. Exp. Med., 1956, v103, 263.

7. Eichwald, E. J., Lustgraaf, E. C., Fuson, R. B., Pfaff, J. P., Ann. N. Y. Acad. Sci., 1960, v87, 119.

8. Siskind, S., Leonard, L., Thomas, L., ibid., 1960, v87, 452.

9. Hilgard, H. R., Dalmasso, A. P., Martinez, C., Good, R. A., Proc. Soc. Exp. Biol. and Med., 1963, v112, 935.

10. Martinez, C., Smith, J. M., Aust, J. B., Good, R. A., ibid., 1958, v97, 736.

11. Page, L. B., Culver, P. J., Harvard Univ. Press, Cambridge, Mass., 1960, chap. 6.

Received June 4, 1965. P.S.E.B.M., 1965, v120.

Influence of Phenoxybenzamine on Vascular Responses to Vasoactive Agents.* (30555)

MICHAEL N. ROMANO† AND LERNER B. HINSHAW

Department of Physiology, University of Oklahoma Medical Center, and Physiology Laboratories, Civil Aeromedical Research Institute, Oklahoma City

Phenoxybenzamine (Dibenzyline) has been reported to be effective in increasing survival and altering the typical responses observed in endotoxin, traumatic, and hemorrhagic shock(8,9,10,11). One explanation of the protection afforded by phenoxybenzamine in endotoxin shock is that it suppresses the action of pressor agents such as epinephrine and norepinephrine, thus increasing blood flow to

* Research supported in part by grant from Am. Heart Assn. and Tulsa County (Oklahoma Heart Assn.).

† Submitted in partial fulfillment for the degree of Master of Science, February, 1965.

critical vascular beds(3,8,12,14,15,16). Serotonin (5-hydroxytryptamine), histamine and acetylcholine may also be implicated(5,6,14, 15), although it is not known if phenoxybenzamine alters the action of these agents during severe hypotension.

The purpose of the present study was to investigate which of several endogenous vasoactive agents are blocked by phenoxybenzamine and to demonstrate the relationship between concentrations of phenoxybenzamine and its potency in antagonizing the action of acetylcholine, histamine, norepinephrine, serotonin and epinephrine.

Methods. Twenty adult mongrel dogs averaging 15 kg in weight were anesthetized with sodium pentobarbital (30 mg/kg) intravenously. Blood pressures were continuously monitored from the femoral artery and portal vein by means of Statham pressure transducers and registered on a Sanborn direct writing recorder. Acetylcholine (chloride salt) was administered at spaced intervals in doses of 10, 50, 100 μg/kg followed by histamine (histamine acid phosphate, 1 isomer), norepinephrine (1-norepinephrine bitartrate), serotonin (serotonin sulfate), and epinephrine (1-epinephrine bitartrate) in 1, 5, 10 μg/kg doses. Following this series of drugs, phenoxybenzamine,‡ in 100 cc saline, was then infused. There was a 40-minute waiting period following the phenoxybenzamine infusion after which time the drugs were administered again. The doses of all drugs were expressed as the salt form and were given in 4 series of 5 dogs each with the first series serving as a control group. The second series was given 10 mg/kg phenoxybenzamine, the third series 5 mg/kg and the fourth series 1 mg/kg. The control series received the drugs in the absence of phenoxybenzamine, a suitable time interval was allotted, then the drugs were administered again.

Pressure responses of the sytemic artery and portal vein are expressed as maximum changes in pressure (mm Hg). Means and standard errors were obtained by a standard procedure(1).

Results. There were no significant differences ($p<.05$) in the responses to any of the drugs given in the control series except to 50 μg/kg of acetylcholine. Area changes in the responses to the drugs were calculated in addition to maximum pressure changes. Area changes were not reported because they exhibited the same degree of significance as the pressure alterations.

Tables I, II, III show the portal vein and systemic artery pressure responses to acetylcholine, histamine (Table I), serotonin (Table II), norepinephrine and epinephrine (Table III) before and after the infusion of phenoxybenzamine. The asterisk indicates responses that were significantly different ($p<.05$). Acetylcholine and histamine show the greatest number of significant attenuations with 5 mg/kg, serotonin with 10 mg/kg, epinephrine and norepinephrine with 5 and 10 mg/kg of phenoxybenzamine.

Discussion. Results indicate that the action of phenoxybenzamine is not confined to adrenergic blockade since it also decreases the vasoconstrictive properties of agents other than the catecholamines. When phenoxybenzamine is used with acetylcholine, histamine, and serotonin a greater number of significant differences are obtained in the responses of the portal vein than in the femoral artery. When used with epinephrine and norepinephrine, phenoxybenzamine produces almost the same number of significant differences in the portal vein as in the femoral artery.

Phenoxybenzamine's blocking action of the portal vein responses to all the vasoconstrictive agents used is noteworthy. Endotoxin produces a rise in portal vein pressure, and is believed to release vasoactive agents into the vascular system(5,13,15). The definitive mechanisms responsible for the rise in portal pressure have not been demonstrated but phenoxybenzamine is known to eliminate this rise(2). Phenoxybenzamine is also particularly effective in increasing blood flow in areas of increased sympathetic vasoconstrictor activity(4). Venous return is maintained near control levels when phenoxybenzamine is administered before endotoxin(7). This report has demonstrated that phenoxybenzamine attenuates the action of vasoactive agents on the portal vein. It is therefore suggested that

‡ Dibenzyline, kindly supplied through the courtesy of Smith, Kline & French Laboratories.

TABLE I. Portal Vein and Systemic Artery Pressure Responses* to Acetylcholine (Ach.) and Histamine (Hist.) Before and After Phenoxybenzamine (Phb.).

	Ach., μg/kg	Max Δ press., mm Hg Before	After	Phb. inf., mg/kg	Hist., μg/kg	Max Δ press., mm Hg Before	After
Femoral artery response	10	—50 ± 6	—44 ± 5		1	—36 ± 4	—26 ± 3
	50	—80 ± 14	—54 ± 5	1	5	—65 ± 1	—48 ± 5
	100	—84 ± 6	—64 ± 5†		10	—74 ± 3	—60 ± 5
	10	—64 ± 5	—50 ± 7		1	—41 ±22	—33 ± 6
	50	—89 ± 7	—70 ± 5†	5	5	—65 ± 5	—38 ± 5†
	100	—94 ± 6	—72 ± 8†		10	—76 ± 2	—53 ± 7†
	10	—46 ± 7	—32 ± 7		1	—24 ± 7	—15 ± 4
	50	—79 ± 10	—68 ± 7	10	5	—60 ± 5	—26 ± 3†
	100	—99 ± 12	—72 ± 9		10	—75 ± 5	—46 ± 9†
Portal vein response	10	+2 ± 1	+ .6± 0		1	+ 2 ± .5	+2 ± .5
	50	+5 ± 2	+2 ± 1	1	5	+ 5 ± 1	+5 ± 1
	100	+6 ± 1	+2 ± 1†		10	+ 6 ± .5	+7 ± 1
	10	+3 ± .5	+1 ± 0†		1	+ 5 ± .5	0 ± 0 †
	50	+6 ± 1	+2 ± 1†	5	5	+ 8 ± 1	+3 ± 1 †
	100	+6 ± 1	+3 ± 1†		10	+12 ± 2	+5 ± 2 †
	10	+2 ± 0	+1 ± 1		1	+ 3 ± 1	+0 ± 1
	50	+6 ± .5	+1 ± 1†	10	5	+ 8 ± 0	+1 ± .5†
	100	+8 ± 2	+1 ± 1†		10	+ 7 ± 1	+4 ± 1 †

Max Δ press. — Maximum change in pressure. Phb. inf. — Phenoxybenzamine infusion.
* Mean pressures ± S.E. of 5 dogs in each infusion series. † $p < .05$

the maintenance of venous return, when phenoxybenzamine is used after endotoxin, might be due to its blocking effect on some or all of the agents studied in this report.

The object of this study was to demonstrate the relative effectiveness of phenoxybenzamine in its ability to attenuate the action of several vasoactive agents. Perhaps the most important fact demonstrated by this study is that phenoxybenzamine is not only an adrenergic blocking agent, but also has the ability to block other vasoactive agents that have very different chemical structures and variable vasoactive properties.

Summary. Acetylcholine, histamine, norepinephrine, epinephrine and serotonin were injected intravenously in varying doses into 20 adult mongrel dogs. Phenoxybenzamine (Phb.) was then administered by intravenous infusion to 15 dogs. One hour after the infusion, the drugs were again administered in the same dosages. Portal vein and systemic artery pressure responses to all drugs were monitored and degree of Phb. blockade was evaluated for each drug used. Results indicate that the greatest number of significantly blocked responses are seen with 5 mg/kg of Phb. for acetylcholine and histamine; with

TABLE II. Portal Vein and Systemic Artery Pressure Responses* to Serotonin (Ser.) Before and After Phenoxybenzamine (Phb.).

Ser., μg/kg	Max Δ press., mm Hg Before	After	Phb. inf., mg/kg
	Femoral artery response		
1	+ 3 ± 5	+11 ± 3	
5	+ 1 ± 6	— 3 ± 6	1
10	+20 ±10	+ 8 ±10	
1	+15 ± 8	+ 1 ± 6	
5	+ 5 ± 6	+ 0 ± 5	5
10	+17 ±10	— 7 ± 2	
1	— 7 ± 3	0 ± 0†	
5	+22 ± 9	+10 ± 1	10
10	+35 ±10	+14 ± 2	
	Portal vein response		
1	0 ± 0	+ .2± 0	
5	+ 1 ± 0	0 ± 0	1
10	+ 2 ± 0	+ 1 ± 1	
1	0 ± 0	0 ± 0	
5	+ 2 ± 1	0 ± 0†	5
10	+ 7 ± 2	0 ± 0†	
1	0 ± .0	0 ± 0	
5	+ 2 ± .5	0 ± 0†	10
10	+ 4 ± 0	+ 1 ± .5†	

Max Δ pressures — Maximum change in pressure. Phb. inf. — Phenoxybenzamine infusion.
* Mean pressures ± S.E. of 5 dogs in each infusion series.
† $p < .05$

TABLE III. Portal Vein and Systemic Artery Pressure Responses* to Epinephrine (Epi.) and Norepinephrine (Nepi.) Before and After Phenoxybenzamine (Phb.).

	Nepi., μg/kg	Max Δ press., mm Hg Before	After	Phb. inf., mg/kg	Epi., μg/kg	Max Δ press., mm Hg Before	After
Femoral artery responses	1	+ 51 ± 5	+28 ± 8†		1	+ 27 ± 6	—16 ± 7†
	5	+ 90 ± 6	+73 ±11	1	5	+ 73 ±11	—35 ±12†
	10	+102 ± 9	+71 ± 7†		10	+101 ± 8	—31 ±14†
	1	+ 53 ± 9	+ 9 ± 3†		1	+ 28 ± 6	—43 ± 7†
	5	+ 96 ± 9	+22 ± 1†	5	5	+ 78 ±10	—40 ± 6†
	10	+113 ±11	+22 ± 4†		10	+102 ±16	—45 ± 6†
	1	+ 59 ± 9	+15 ± 4†		1	+ 58 ±18	—35 ± 9†
	5	+ 96 ± 6	+20 ± 5†	10	5	+ 91 ± 9	—51 ± 8†
	10	+116 ±12	+22 ± 5†		10	+102 ± 4	—60 ± 8†
Portal vein response	1	+ 3 ± 1	+ 2 ± .5		1	+ 6 ± 1	+ 2 ± 0†
	5	+ 6 ± 1	+ 4 ± 1	1	5	+ 9 ± 1	+ 7 ± 2
	10	+ 7 ± 1	+ 6 ± 2		10	+ 12 ± 2	+ 9 ± 2
	1	+ 3 ± 0	0 ± 0†		1	+ 5 ± 0	+ 1 ± 0†
	5	+ 8 ± 1	+ 3 ± 0†	5	5	+ 12 ± 2	+ 4 ± 1†
	10	+ 10 ± 3	+ 4 ± 1†		10	+ 14 ± 2	+ 4 ± 1†
	1	+ 3 ± 0	+ 1 ± .5†		1	+ 4 ± 1	+ 2 ± 1†
	5	+ 7 ± 1	+ 3 ± 2†	10	5	+ 10 ± 1	+ 4 ± 1†
	10	+ 9 ± 1	+ 4 ± 2†		10	+ 14 ± 1	+ 3 ± 7†

Max Δ press. — Maximum change in pressure. Phb. inf. — Phenoxybenzamine infusion.
* Mean pressures ± S.E. of 5 dogs in each infusion series. † $p < .05$

10 mg/kg Phb. for serotonin, and 5 and 10 mg/kg Phb. for epinephrine and norepinephrine. The portal venous responses to acetylcholine, histamine and serotonin have a greater number of significant differences than the arterial responses. However, all the drugs tested were partially blocked by phenoxybenzamine. The results of these experiments emphasize the lack of specificity exhibited by this adrenergic blocking agent. The action of Phb. in blocking the portal vein pressure responses to the drugs used in the present study may explain the maintenance of venous return due to prevention of portal vein pressure rise when Phb. is administered before endotoxin.

1. Arkin, H., Colton, R., Statistical Methods, College Outline Series, Barns & Noble, Inc., N. Y., 1958.
2. Brake, C., Hinshaw, L., Emerson, T., Am. J. Physiol., 1964, v207, 149.
3. Freeman, N., Freedman, H., Miller, C., ibid., 1941, v202, 545.
4. Goodman and Gilman, Pharmacological Basis of Therapeutics, Macmillan Co., N. Y., 1955, 588.
5. Hinshaw, L., Vick, J., Physiologist, 1960, v3, 170.
6. Hinshaw, L., Vick, J., Jordan, M., Wittmers, L., Am. J. Physiol., 1962, v202, 103.
7. Hinshaw, L., Gilbert, R., Kuida, H., Visscher, M., ibid., 1958, v195, 631.
8. Lillehei, R., Longerbeam, J., Block, J., Am. J. Cardiol., 1963, v12, 599.
9. Lillehei, R., MacLean, L., Arch. Surg., 1959, v78, 116.
10. Nickerson, M., J. Mich. State Med. Soc., 1955, v54, 45.
11. Nickerson, M., Carter, S., Canadian J. Biochem. and Physiol., 1959, v37, 1161.
12. Nykiel, F., Galviano, V., J. Appl. Physiol., 1961, v166, 348.
13. Reid, G., J. Physiol., 1952, v118, 435.
14. Rosenburg, J., Lillehei, R., Longerbeam, J., Zimmerman, B., Ann. Surg., 1961, v154, 611.
15. Thomas, L., Zweifach, B., Benacerraf, B., Trans A. Am. Physicians, 1957, v70, 54.
16. Yard, A., Nickerson, M., Fed. Proc., 1956, v15, 502.

Received July 7, 1965. P.S.E.B.M., 1965, v120.