13. Burton, K., Biochem. J. **62**, 315 (1956).
14. Lowry, O. H., Roberts, N. R., Leiner, K. Y., Wu, M., and Farr, A. L., J. Biol. Chem. **207**, 1 (1954).
15. Webb, J. M. and Lindstrom, H. V., Arch. Biochem. Biophys. **112**, 273 (1965).
16. Bray, G. A., Anal. Biochem. **1**, 279 (1960).
17. Prosky, L., Roberts, B., Jr., O'Dell, R. G., and Imblum, R. L., Arch. Biochem. Biophys. **126**, 393 (1968).

Received Feb. 19, 1969. P.S.E.B.M., 1969, Vol. 131.

The Cardiovascular Response to Intrapulmonary Pressure Inflation in Dogs Treated with Reserpine, Propranolol, and Phenoxybenzamine (33936)

K. David Hayashi,[1,2] (Introduced by Paul N. Yu)
(With the technical assistance of Mrs. Karen Brumm, Mr. Edward Hunt, Mr. Joseph Jones, and Miss Deborah Kuehnert)
Cardiology Unit, Department of Medicine, University of Rochester School of Medicine and Dentistry, and Strong Memorial Hospital, Rochester, New York 14620

Abnormal responses to the Valsalva maneuver have been described in derangement of the autonomic nervous system (1, 2), in individuals treated with ganglionic blocking agents or guanethidine (3, 4), and in conditions in which the adrenergic neurotransmitter norepinephrine is depleted from myocardium, such as congestive heart failure (5) or after reserpine treatment (5, 6). Since the introduction of newer classes of pharmacological agents, it is now possible to study the specific alpha- or beta-adrenergic functions (7, 8).

In the present study an attempt was made (a) to demonstrate the role of specific alpha- or beta-adrenergic function, and (b) to describe the effect of altered myocardial norepinephrine metabolism after reserpine administration, as reflected by arterial blood pressure and heart rate responses to intrapulmonary pressure inflation (IPPI) in anesthetized dogs.

Materials and Methods. Nineteen mongrel dogs weighing 9–20 kg were studied. Pentobarbital sodium (Nembutal) anesthesia was employed initially in a dose of 25–40 mg/kg. Additional small doses were given, when necessary, to maintain the desired light-to-moderate anesthetic depth. The technique for IPPI and recording femoral arterial and endotracheal pressures, and ECG were described previously (9).

Eight dogs were studied before and after alpha- and/or beta-adrenergic blockade. In 2 dogs, propranolol was administered intravenously in a dose of 0.25 mg/kg. In 4 dogs, intravenous phenoxybenzamine (PBZ) was given in a dose of 2.5 mg/kg. In one dog, PBZ (1.25 mg/kg) was given first, followed by administration of propranolol. In another dog, intravenous injection of propranolol was followed by PBZ (1.25 mg/kg). Observations were made every 5–30 min following drug administration for about 2 hr in this group of dogs.

Eleven dogs were given reserpine. These dogs were put into two groups: Group A, acute treatment group; 4 dogs received reserpine 0.5 mg/kg intravenously on the day of experiment and responses to IPPI were observed over 4–5 hr. Two dogs were given 0.1 mg/kg and were similarly studied. Group S, subacute treatment group; 2 dogs received 0.5 mg/kg intravenously 18 hr prior to the experiment. One dog received 0.5 mg/kg subcutaneously 18 hrs prior to the experiment. Two dogs received 0.5 mg/kg subcutaneously each at 24 and 48 hr prior to the experiment. Group A dogs before reserpine administration served as controls for the cardiovascular response to IPPI.

[1] Supported in part by U.S. Public Health Service grants HE-03966 and HE-5500 and the Genesee Valley Heart Association.

[2] Work performed during tenure of a U.S. Public Health Service Traineeship in Cardiology.

TABLE I. Response to PBZ.[a]

Dog no.	ΔHR		ΔMBP	
	Control period	PBZ	Control period	PBZ
1	41	19	40	4
2	64	19	27	—3
3	30	16	26	—5
4	22	3	26	—7

[a] Values after PBZ were obtained 60 min after intravenous injection.

The increase in heart rate (beats per minute) in phase 2[3] over that in control phase was expressed as ΔHR. Mean arterial blood pressure (MBP) was estimated by the sum of diastolic pressure and one-third of pulse pressure. The increase in MBP (mmHg) in phase 4[3] over control phase was expressed as ΔMBP. The MBP in phase 4 was obtained from the maximum value within 15 sec after the cessation of IPPI. The effects of propranolol were analyzed 15 min and those of PBZ 60 min after drug administration. The *t* test for paired samples was employed when the study group served as its own control. Otherwise, the *t* test for independent samples was employed.

A biopsy of the right atrial appendage (RAA) was obtained from dogs treated with PBZ or reserpine at the termination of the experiment and the sample was kept frozen until assay. The biopsy sample was analyzed for norepinephrine (NE) and epinephrine (E) by a spectrophotofluorometric assay, using the method described by Maynert and Klingman (10). Values for NE and E taken from RAA of a different series of normal dogs served as controls.

Results. Table I shows ΔHB and ΔMBP before and after PBZ administration. The PBZ produced a significant decrease in ΔHR ($p<0.05$) and ΔMBP ($p<0.001$). Table II shows the responses to propranolol, which produced a significant decrease in ΔHR ($p<0.02$). Propranolol, however, did not change ΔMBP. Dogs which received PBZ and propranolol consecutively showed essentially the same response as that observed after each medication. The NE concentration of RAA in PBZ-treated dogs was 0.67 μg/g ± 0.07 (mean ± SE). It was significantly lower than that of 10 normal controls (2.18 ± 0.28).

The data on ΔHR, ΔMBP and NE concentration in RAA in reserpine-treated dogs are summarized in Fig. 1. Compared with control dogs there was a significant decrease in ΔHR ($p<0.05$), but not in ΔMBP in Group A dogs. Significant decrease in ΔMBP as well as in ΔHR was observed ($p<0.01$) in Group S dogs in comparison with Group A dogs. A complete absence of phase 4 overshoot was noted in all the Group S dogs.

There was a significant depletion in NE concentration of RAA in both Groups A and S. In Group S dogs virtually no NE was recovered. Time course of ΔHR and ΔMBP following intravenous reserpine administration is shown in Fig. 2.

Discussion. The selective beta-adrenergic blockade by propranolol produced a significant reduction in the magnitude of phase 2 reflex tachycardia induced by IPPI. This is in keeping with the generally accepted concept that the drug counteracts chronotropic action of the cardiac sympathetic nervous system (7). A possibility exists that propranolol may reduce phase 4 arterial blood pressure overshoot by blocking the inotropic effect of the sympathetic nervous system (6). However, since there was no appreciable suppression of the overshoot, the inotropic

TABLE II. Response to Propranolol.[a]

Dog no.	ΔHR		ΔMBP	
	Control period	Propranolol	Control period	Propranolol
1	24	10	34	42
2	25	3	22	16
3	45	3	46	15
4[b]	54	10	10	4

[a] Values after propranolol were obtained 15 min after intravenous injection.

[b] PBZ had been given intra-arterially 35 min before propranolol was given.

[3] The four phases of the response to IPPI are defined after the Valsalva phenomenon in an analogous fashion.

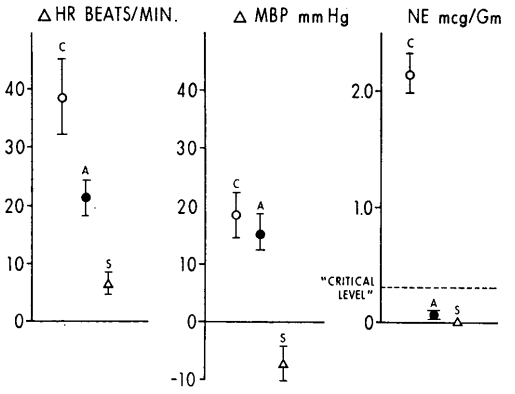

FIG. 1. Reflex changes in heart rate (△HR), mean blood pressure (△MBP) and right atrial appendage NE concentration in 3 groups: (C), controls; (A), acute reserpine-treated group; and (S), subacute reserpine-treated group. Points = the mean; and vertical bars = the standard error of the mean.

influence of the cardiac sympathetic nervous system appears to be of rather minor importance.

The PBZ completely abolished phase 4 overshoot in response to IPPI. Therefore, phase 4 overshoot appears to be largely due to alpha-adrenergic function. The PBZ also produced a significant reduction in the magnitude of phase 2 reflex tachycardia induced by IPPI. Whether this reduction can be explained on the basis of alpha-adrenergic blocking effect of the drug remains to be determined. PBZ inhibited net uptake of tagged NE into catecholamine storage granules *in vivo* (11) as well as *in vitro* (12). We found that NE concentration of RAA in the PBZ-treated dogs was significantly reduced. On the other hand, myocardial NE depletion, whether induced by administration of reserpine (13) or by experimental congestive heart failure (14), has been associated with decreased chronotropic response. Therefore, it would appear quite possible that the lack of full phase 2 heart-rate response in dogs after PBZ administration may be associated with myocardial NE depletion. Whether myocardial NE depletion, per se regardless of how it was produced, or inhibition of NE uptake into the storage granules, abnormalities in NE storage or release is responsible for the decreased chronotropic response remains to be shown.

Gaffney and others (13) described a critical level (0.3 μg/g) of tissue NE when they studied the chronotropic response to cardioaccelerator nerve stimulation in reserpine-treated dogs. Close inspection of the data presented in their Fig. 2 shows relatively normal response in severely NE-depleted animals (<0.3 μg/g) when the response was tested earlier than 6 hr after administration of reserpine. Almost complete suppression of the response was noted in all dogs tested 8 hr after resperine administration. These findings suggest that not only the myocardial NE depletion *per se,* but also the duration of the state of depletion, is crucial in producing functional abnormality. The above findings may be explained by a very efficient mechanism by which the body utilized the already depleted NE store (13) and the nonuniformity of intracellular NE localiza-

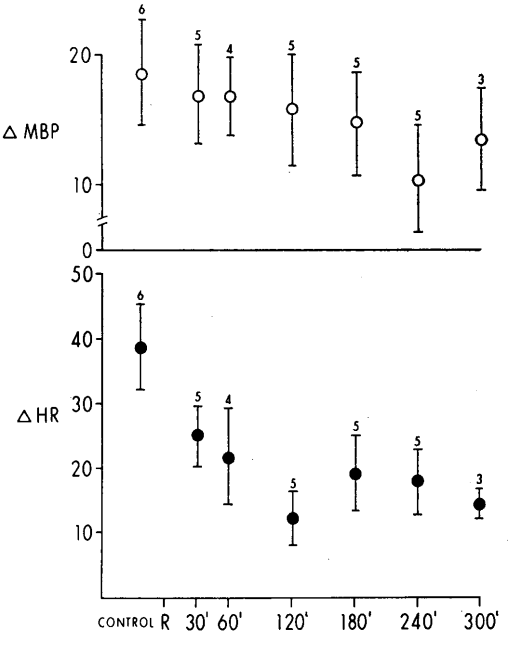

FIG. 2. Response to intrapulmonary pressure inflation during 4–5 hr after acute reserpine treatment: numerals in the diagram indicate number of dogs in which observations were made within 5 min of the indicated time; control values were taken within 10 min prior to reserpine injection; vertical bars = the standard error of the mean; other symbols and abbreviations are the same as those used in Fig. 1.

tion (15).

Evidence has been presented that reserpine blocks the granular uptake of dopamine (DA) (15, 16), which is the immediate precursor to NE. The time delay of the maximum effect of this DA-uptake inhibition would allow, in Group A dogs, some new NE synthesis. In Group S dogs DA uptake would already be depressed to allow no further significant NE synthesis (17). There is suggestive evidence that the newly synthesized NE may be preferentially utilized (18).

Other investigators demonstrated that these nerve granules are transported down neuronal axons by an axoplasmic flow (19, 20). It is possible that a continued supply of these partially NE-depleted granules allows some function in Group A dogs, whereas less reserve would be available in Group S dogs. After reserpine therapy the early recovery of NE fluorescence in nerve endings of the short neurons when compared with the long neurons seems to be consistent with this possibility (21).

Andén and associates (22) showed that state of myocardial NE uptake mechanism following reserpine treatment was correlated better with rat ocular response to sympathetic nerve stimulation than myocardial NE concentration. Good correlation was observed between depression of ^{3}H-NE uptake and body temperature after reserpine treatment in mice (23). Almost simultaneous recovery of body temperature and ^{3}H-NE uptake took place despite severe depletion of tissue NE stores.

Although these findings point to the importance of the uptake mechanism for the integrity of adrenergic nervous function, other factors such as NE synthesis and axoplasmic flow have not been excluded. Stjärne (24) reported a 60% reduction in NE uptake in rabbit heart 1 min after reserpine administration and of more than 90% a little over 4 hr after reserpine. Lundborg and Stitzel (23) observed that granular NE uptake mechanisms are almost completely inhibited in mice within the first 30 min after reserpine treatment. Andén and associates (22) demonstrated almost complete inhibition of ^{3}H-NE uptake in rat heart as early as 3 hr after reserpine administration. These reports of early uptake inhibition do not explain fully the more abnormal function in Group S dogs. Moreover, it was observed in Group A dogs that ΔHR declined, but ΔMBP remained relatively unaltered during the study period. If one assumes that NE tissue uptake mechanism is the major factor that determines the sympathetic nervous function, it is difficult to explain this disparity of ΔHR and ΔMBP responses.

It is tempting to speculate upon the possible roles of inhibition of NE synthesis and of transport of these granules by axoplasmic flow to explain our findings. A study of the response to IPPI after blocking the NE synthetic pathway using alpha methyltyrosine may clarify this point.

Conclusions. Reflex adrenergic responses to IPPI in anesthetized dogs were studied. Phase 4 arterial blood pressure overshoot was abolished by PBZ but not by propranolol indicating a major alpha-adrenergic contribution in the production of the overshoot. Phase 2 reflex tachycardia was blocked by propranolol, as a manifestation of beta-adrenergic blocking effect. PBZ also suppressed the response, although to a lesser degree. A marked myocardial NE depletion was associated with a significant reduction in phase 2 heart rate response and complete abolition of phase 4 arterial blood pressure overshoot to IPPI, when dogs were pretreated with reserpine more than 18 hr prior to the experiment. But reduction in reflex tachycardia was not as marked and the pressure overshoot was normal if the studies were carried out 4–5 hr following reserpine administration. Several possible factors are offered as possible explanations for the "time factor" and for the difference in ΔHR and ΔMBP responses in Group A dogs.

1. Wilkins, R. W. and Culbertson, J. W., Trans. Assoc. Am. Physicians **60**, 195 (1947).

2. Sharpey-Schafer, E. P., J. Physiol. (London) **134**, 1 (1956).

3. Elisberg, E. I., Miller, G., Weinberg, S. L., and Katz, L. N., Am. Heart J. **45**, 227 (1953).

4. Cudkowicz, L., Respiration **25**, 81 (1968).

5. Chidsey, C. A., Braunwald, E., Morrow, A. G., and Mason, D. T., New Engl. J. Med. **269**, 653 (1963).

6. Am. Physiol. Soc., "Handbook of Physiology, Section 2: Circulation" (W. F., Hamilton, ed.), Vol. 3, p. 1875–1886. Williams and Wilkins, Baltimore, Maryland (1965).

7. Epstein, S. E. and Braunwald, E., New Engl. J. Med. **275**, 1106 (1966).

8. Abboud, F. M., Schmid, P. G., and Eckstein, J. W., J. Clin. Invest. **47**, 1 (1968).

9. Hayashi, K. D., Proc. Soc. Exptl. Biol. Med. **131**, 426-429 (1969).

10. Maynert, E. W. and Klingman, G. I., J. Pharmacol. Exptl. Therap. **135**, 285 (1962).

11. Axelrod, J., Hertling, G., and Potter, L., Nature **194**, 297 (1962).

12. Iversen, L. L., J. Pharm. Pharmacol. **17**, 62 (1965).

13. Gaffney, T. E., Chidsey, C. A., and Braunwald, E., Circulation Res. **12**, 264 (1963).

14. Covell, J. W., Chidsey, C. A., and Braunwald, E., Circulation Res. **19** 51 (1966).

15. Wurtman, R. J., New Engl. J. Med. **273**, 637 (1965).

16. Kirshner, N., Rorie, M., and Kamin, D. L., J. Pharmacol. **141**, 285 (1963).

17. Burack, W. R. and Draskoczy, P. R., J. Pharmacol. Exptl. Therap. **144**, 66 (1964).

18. Kopin, I. J., Breese, G. R., Kraus, K. R., and Weise, V. K., J. Pharmacol. Exptl. Therap. **161**, 271 (1968).

19. Landuron, P. and Belgaire, F., Life Sci. **7**, 1 (1968).

20. Kapeller, K. and Mayor, D., Proc. Roy. Soc. (London) Ser. B **167**, 282 (1967).

21. Owman, O. and Sjoberg, N., Life Sci. **6**, 2549 (1967).

22. Andén, N. E., Magnusson, T., and Waldeck, B., Life Sci. **3**, 19 (1964).

23. Lundborg, P. and Stitzel, R. E., Brit. J. Pharmacol. **33**, 98 (1968).

24. Stjärne, L., Acta Physiol. Scand. **62**, Suppl. 228 (1964).

Received Oct. 21, 1968. P.S.E.B.M., 1969, Vol. 131.