PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 161, 128–135 (1979)

An Analysis of Postvagal Inotropic Responses[1] (40505)

JEROD M. LOEB,[2] JOHN X. THOMAS, JR.,[2] DAVID K. MURDOCK, DAVID E. EULER, AND WALTER C. RANDALL

Department of Physiology, Loyola University of Chicago, Stritch School of Medicine, Maywood, Illinois 60153

The transient sinus tachycardia which follows the cessation of vagal stimulation (postvagal tachycardia) has been well studied (1–4). In addition to the positive chronotropic effects, some investigators have noted a rebound in contractile force following the administration of intracoronary acetylcholine (5) or following the termination of vagal stimulation (6). These positive inotropic effects have been attributed to the release of a positive inotropic mediator (5), the presence of adrenergic fibers within the vagosympathetic trunk (6) or an "unmasking" of adrenergic activity after cessation of vagal stimulation (7). Vagal stimulation after atropine administration has also been shown to produce a positive inotropic effect during the stimulation period (8). However, other investigators have failed to document the presence of positive postvagal inotropic responses. Thus, Stanton and Vick (9), in the chloralose anesthetized dog, did not observe any rebound increases in force following vagal stimulation during ventricular pacing.

Therefore, the purpose of this investigation was to further characterize mechanisms underlying the inotropic responses following vagal stimulation. Specifically, factors such as vagal hypotension, sympathetic fibers in the vagus and adrenergic and cholinergic involvement were examined.

Materials and methods. A. *Preparation.* Mongrel dogs of either sex weighing 15–20 kg were preanesthetized with fentanyl and droperidol (Innovar, 0.3 ml; iv) and anesthetized with α-chloralose (75 mg/kg; iv). Both the femoral vein and artery were catheterized for administration of fluids as well as measurement of arterial pressure, respectively. The trachea was cannulated and the animal ventilated with 40% O_2 via a Bird Mark 7 respirator. Both vagi were transected in the cervical area. A thoracotomy was performed on the left side at the fifth intercostal space. A pericardial cradle was constructed and Walton-Brodie strain gauge arches (13 mm in length) were sewn to the epicardium of the right ventricular conus and left ventricular anterior surface. In some animals, gauges were also sewn to both atria. The gauges were oriented parallel to the epicardial fibers. A small bipolar electrode was sewn to the right or left atrium to record an atrial electrogram and an additional electrode was placed on the right ventricle for pacing. All tracings were displayed on a Grass Model 7 polygraph.

B. *Protocol.* The peripheral end of the right or left vagus was stimulated in the neck using a bipolar hook electrode connected to a Grass Model 5 stimulator. The stimulation parameters used were: frequency 20/sec, duration 5.0 msec and voltage 5–10 v. The stimuli were applied for 15–60 sec. In some experiments the stellate ganglia on both sides were transected in order to eliminate sympathetic influences and the vagal stimulations were repeated. In those experiments requiring occlusion of the inferior vena cava, the vessel was isolated immediately caudal to the pericardial entrance of the vessel.

Drugs used in this study were dissolved in 0.9% saline and administered via the femoral vein catheter. The drugs included: atropine sulfate (Sigma, Inc.); propranolol hydrochloride (Ayerst Labs); and guanethidine (CIBA-Geigy).

Results. A typical response to stimulation of the peripheral end of the right cervical vagus after transection of both stellate ganglia is shown in Fig. 1. The vagus was stimulated for 30 sec during the period indicated by an upright shift of the time reference (second trace). Note that arterial pressure during the stimulation fell toward zero due to a paucity

[1] Supported by USPHS Grant No. 08682.

[2] During the tenure of this investigation, Dr. Loeb and Dr. Thomas were Research Fellows of the Chicago Heart Association.

0037-9727/79/060128-08 $01.00/0

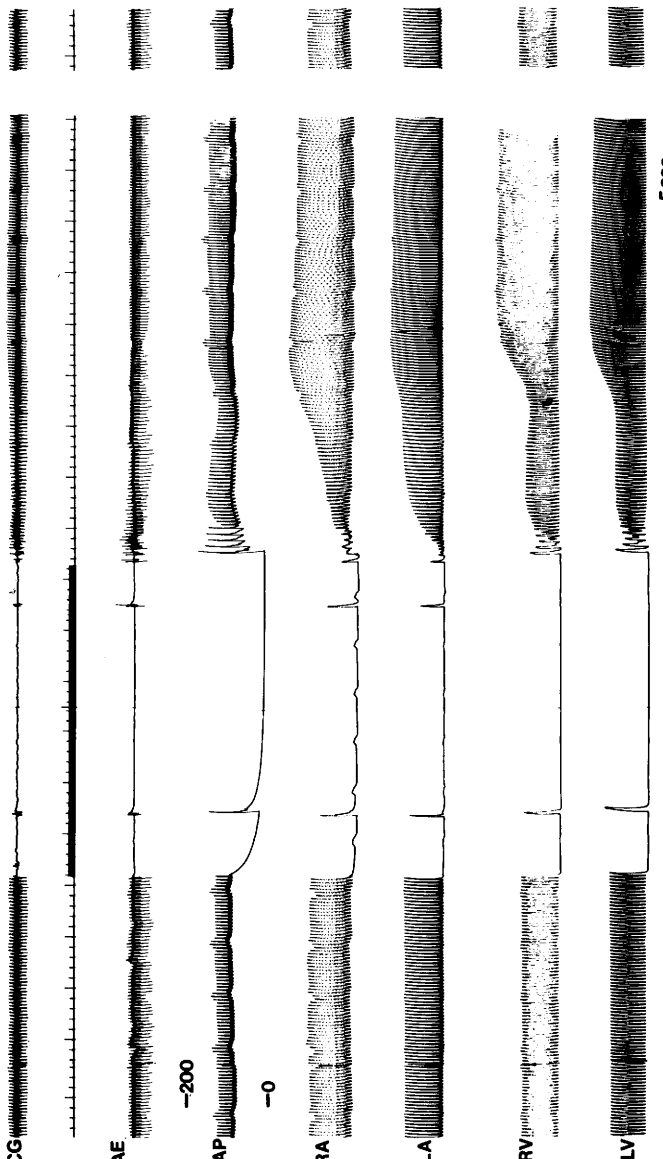

FIG. 1. Effect of right vagal stimulation. The traces show from above down: electrocardiogram (ECG), right atrial electrogram (RAE), aortic pressure (AP), right atrial contractile force (RA), left atrial contractile force (LA), right ventricular contractile force (RV) and left ventricular contractile force (LV). The efferent end of the right cervical vagus was stimulated for 30 sec as indicated by the time marker. Note the profound hypotension during stimulation and after termination of stimulation, a return of contractility toward the control level (but not exceeding control) followed by a secondary positive inotropic effect.

of escape beats. Following termination of stimulation, contractility in each of the chambers returned gradually toward the control, but did not immediately exceed that level. However, approximately 15 sec after the stimulation, contractility rose above the control level in all four chambers. This positive inotropic effect lasted for about 30 sec and only then began to return toward the control. Similar responses were noted in the eight animals studied. Prior to transection of the sympathetic nerves, stimulation of the right vagus was followed only by an immediate positive inotropic response (with respect to control contactility) with no further delayed increase. Figure 2 shows the contractile responses to stimulation of the peripheral end of the left cervical vagus after section of both stellate ganglia. It is important to note that the arterial pressure was only slightly reduced during stimulation of the left vagus. In addition pulse pressure appeared to be increased. Following the termination of stimulation, contractility in both ventricles returned only to the control level and did not exceed it. Only when left vagal stimulation significantly reduced heart rate with a concomitant fall in arterial pressure did a positive postvagal inotropic response occur.

In order to eliminate the hypotension characteristic of right vagal stimulation, the right ventricle was paced during the stimulation period. Figure 3 shows an example of the results obtained. It is clear that ventricular drive (and thus maintenance of mean pressure), eliminated the marked positive inotropic effect that was evident in Fig. 1 (same animal). Similar results were found in all eight animals. In another approach, since previous studies had suggested that sympathetic fibers within the vagosympathetic trunk were responsible for the positive inotropic effects (8), atropine (0.1 mg/kg) was next administered in order to eliminate parasympathetic effects. Such blockade would also be expected to eliminate the vagally-induced hypotension. After atropine administration, vagal stimulation was never followed by rebound increases in contractility. However, as has been previously described in atropinized preparations (4, 8, 10), during the period of vagal stimulation, slight positive chronotropic and inotropic responses were evident.

In another group of animals, propranolol (1.5 mg/kg) was administered in order to delineate further the mechanism of the positive inotropic effect. After beta blockade, control contractility was reduced by about 50% but in no instance was vagal stimulation followed by a biphasic contractile response. Figure 4 shows graphically, the results thus far presented. For the purposes of clarity, only data for left ventricular contractility are shown. A significant reduction in the magnitude of the delayed positive inotropic effect was produced by simultaneous ventricular pacing during the stimulation period ($p < 0.001$). In addition, the magnitude of the late positive inotropic response was significantly reduced following left vagal stimulation ($p < 0.001$). Beta blockade with propranolol also produced a significant reduction in the late positive inotropic effect after vagal stimulation ($p < 0.001$).

The results presented thus far indicate a role for catecholamines in postvagal inotropic responses. One of the major remaining questions is the source of the catecholamines. This is especially important in view of the fact that postvagal chronotropic responses have been attributed to catecholamine release from other than sympathetic endings (1–4). Two approaches were utilized in this section. It was reasoned that if adrenal medullary catecholamine release was responsible for the positive postvagal inotropic response, then temporarily clamping the inferior vena cava immediately after the vagal stimulation should delay the inotropic response by preventing medullary catecholamines from reaching the heart. Figure 5 shows an example of the results obtained. The right vagus was stimulated for 30 sec. Simultaneous with the termination of stimulation, a Satinsky clamp was placed on the inferior vena cava and kept in place for a period exceeding that necessary for the production of the secondary positive inotropic response in the control state (about 15 sec) and then released. As is clearly apparent, a secondary rebound positive inotropic effect is present at approximately 15 sec after release of the clamp. Similar results were found in three animals tested with this

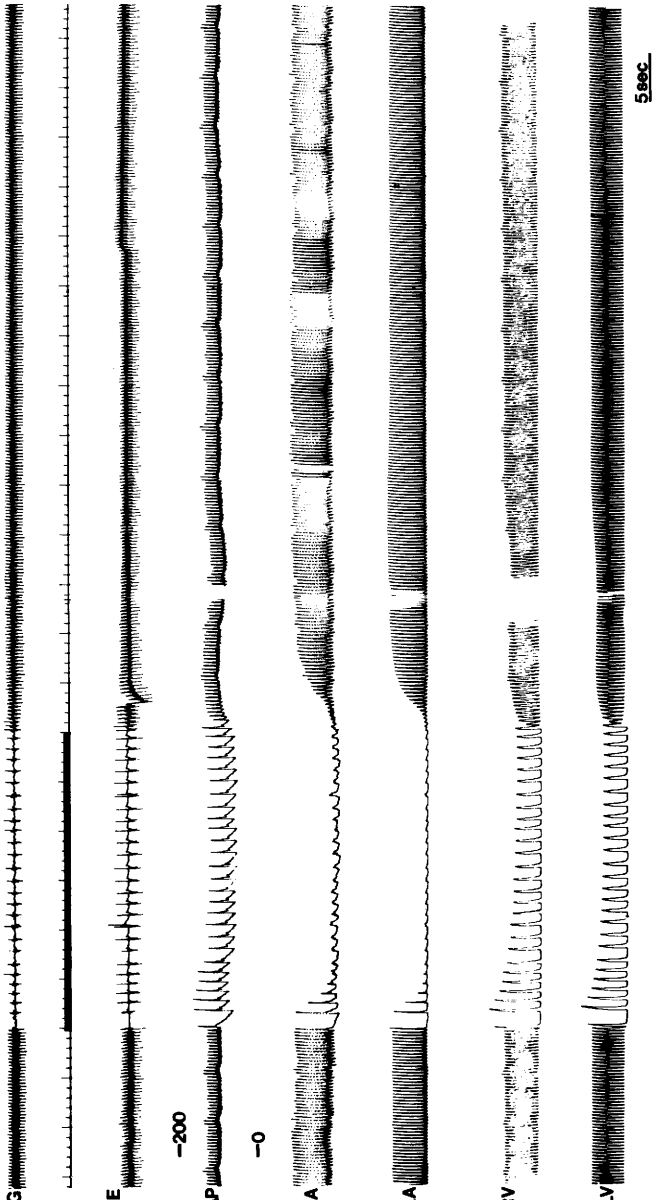

FIG. 2. Effect of left vagal stimulation. The lettering is identical to Fig. 1. The efferent end of the left cervical vagus was stimulated for 30 sec as shown by the time marker. Note only a slight arterial pressure drop during stimulation and the absence of a two-component postvagal inotropic response.

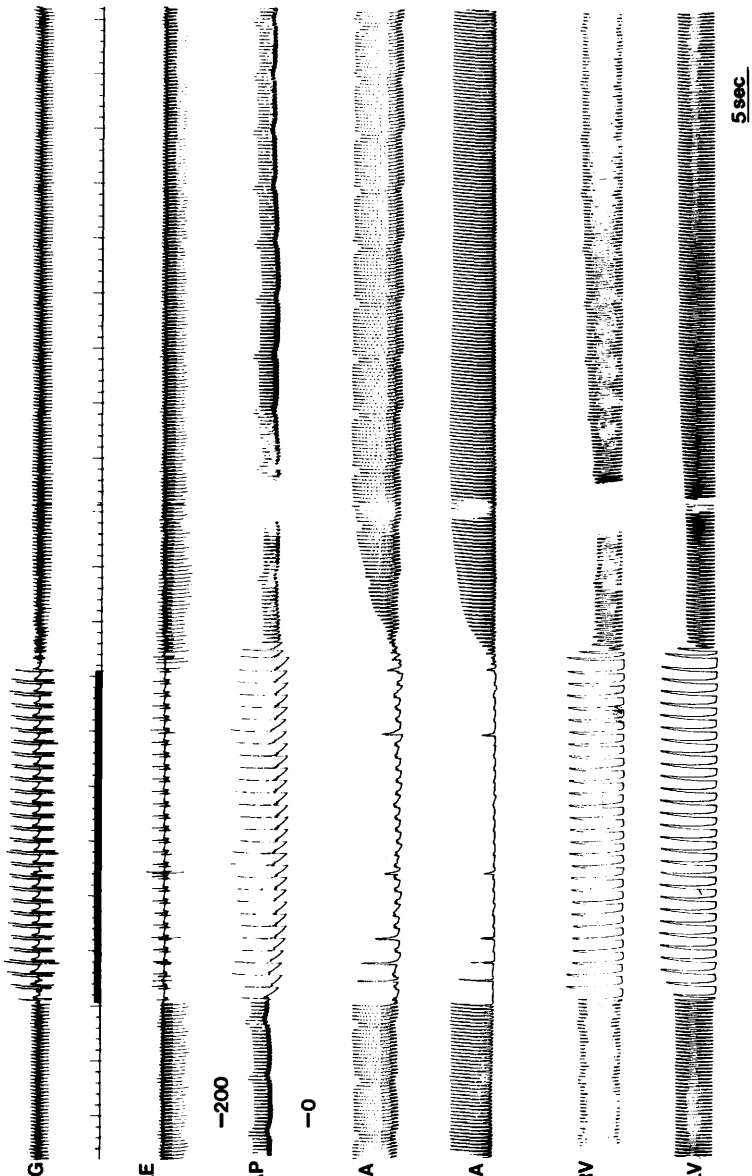

FIG. 3. Effect of right vagal stimulation in the presence of ventricular drive. The lettering is identical to Fig. 1. The right cervical vagus was stimulated for 30 sec as shown by the time marker. Note the maintenance of arterial pressure during vagal stimulation and the consequent elimination of the secondary positive inotropic effect while the initial postvagal negative inotropic effect is unaltered.

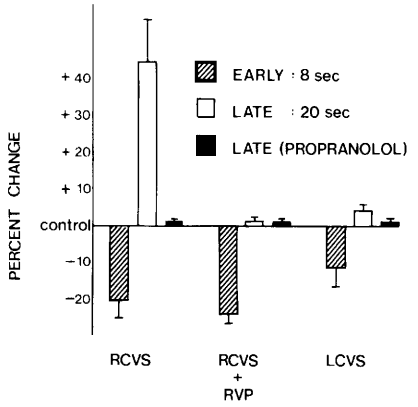

FIG. 4. Contractility changes following vagal stimulation. The cross-hatched bars represent left ventricular contractility measured at 8 sec after vagal stimulation, the empty bars represent left ventricular contractility measured at 20 sec after vagal stimulation and the filled bars represent left ventricular contractility measured at 20 sec after vagal stimulation in the presence of propranolol. RCVS = right cervical vagal stimulation; RVP = right ventricular pacing and LCVS = left cervical vagal stimulation.

protocol. In another approach, in two animals, guanethidine was administered systemically in order to block the release of catecholamines from sympathetic endings but not the adrenal medulla. Under these conditions, vagal stimulation was followed by the usual two component inotropic response. Guanethidine had no effect on the secondary increase in contractility above control levels.

Discussion. Since positive inotropic as well as positive chronotropic changes follow the termination of vagal stimulation, it is necessary to delineate whether a common mechanism can be used to explain both phenomena. It is well established that the vagus nerve in the cervical region contains abundant sympathetic fibers (11). Thus, after muscarinic blockade with atropine, only effects mediated via sympathetic fibers should be evident upon vagal stimulation. This is indeed the case since many investigators have reported both positive inotropic and chronotropic responses under these conditions (4, 8, 10, 12, 13). However, it has also been reported that the positive chronotropic responses persist after chemical sympathectomy with 6-hydroxydopamine (3, 14). Therefore, the situation is far from clarified. The present experiments were directed toward an analysis of the inotropic events which follow a period of cervical vagal stimulation. Most of the mechanisms proposed to explain contractility or rate changes during vagal stimulation following atropine are at least theoretically possible in order to explain postvagal inotropic changes. Baroreceptor mediated catecholamine release has been shown not to play a role in postvagal tachycardia (1); however, such a mechanism may be involved in postvagal inotropic changes. Thus, the present experiments have clearly shown that ventricular pacing during the vagal stimulation period, can eliminate the secondary positive inotropic rebound evident after vagal stimulation. In addition, in a few animals, the administration of guanethidine, a drug that blocks the release of catecholamines from sympathetic endings but not from the adrenal medulla, had no effect on the postvagal positive inotropic response. This experiment would also rule out an acetylcholine-mediated release of norepinephrine from sympathetic endings, a mechanism that has been previously proposed for postvagal tachycardia (15).

One point of importance is the fact that the postvagal inotropic and chronotropic responses follow different time courses. The tachycardia reaches a maximum soon after the cessation of stimulation (4), while the inotropic effect has been shown here to be delayed by approximately 20 sec. This would suggest the possibility of a mechanism that could involve a circulating neuromediator, presumably from the adrenal medulla. Supporting this are the experiments reported here in which the inferior vena cava was occluded immediately following the stimulation period. This occlusion (15–20 sec) delayed the onset of the positive inotropic response for an equivalent period of time thus supporting the idea of adrenal medullary catecholamines as a requirement for the response. Another possibility made unlikely by this series of experiments is a Bowditch-type frequency-force relationship. Since the postvagal tachycardia is not eliminated via ventricular drive (1), frequency related increases in force could still be expected. However, in no case, was ventricular drive during vagal stimulation followed by a postvagal positive inotropic response.

One of the areas of considerable interest is

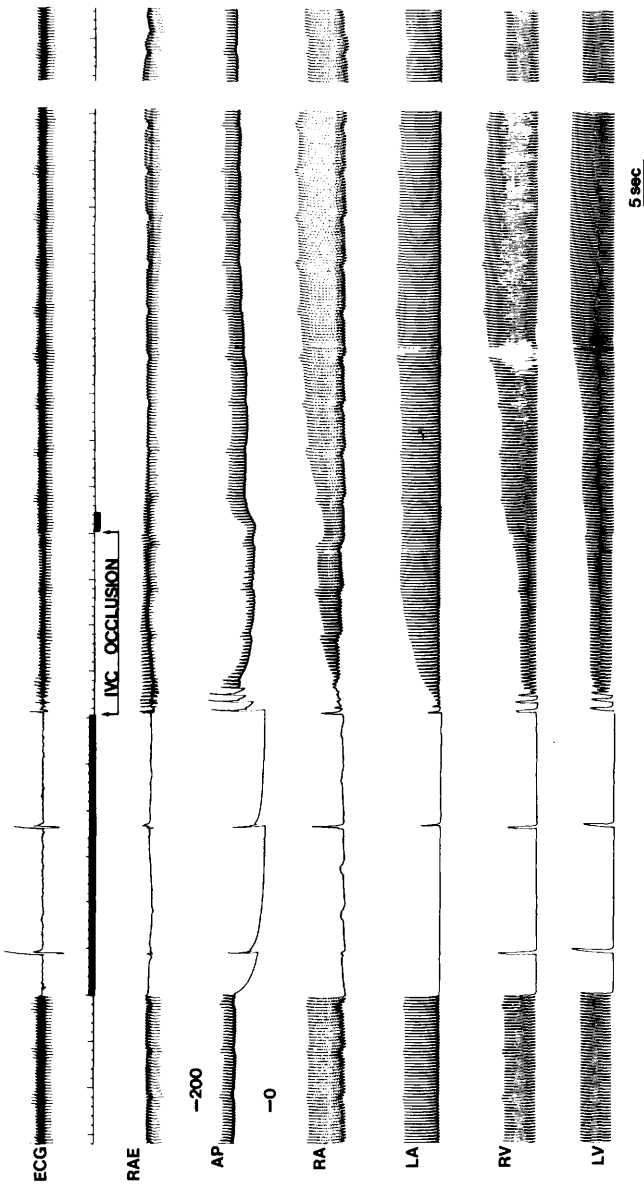

FIG. 5. Effect of right vagal stimulation followed by inferior vena caval occlusion. The lettering is identical to Fig. 1. The right cervical vagus was stimulated for 30 sec as shown by the time marker. Immediately following the stimulation the inferior vena cava was occluded with a Satinsky clamp and held for 15 sec. Following release of the clamp, a positive inotropic effect is evident after a delay of approximately 15 sec. Note that the initial negative inotropic effect is unaltered.

the initial ventricular response after the cessation of vagal stimulation. In the unpaced as well as the paced preparation, immediately following vagal stimulation, the contractility in both ventricles returned towards, but always was significantly less than control levels (see Figs. 1–3, 5). Similarly, in both atria, contractility returned toward control, but remained somewhat less than control in the period immediately following the vagal stimulation. However, ventricular contractility remained depressed for a longer time course than atrial contractility. It has been shown that considerably higher concentrations of cholinesterase are present in atria when compared to the ventricle (16), thus providing a functional substrate for the faster atrial time course. Thus, it is conceivable that the short lasting effect of acetylcholine in the atrium may be mediated in part by the higher concentrations of cholinesterase present in the atrial tissues. An alternative explanation might be that a much greater delay between cholinergic receptor activation and the appearance of a response in the effector cell may exist in the ventricles when compared to the atria. In support of this concept, in the paced preparation, Harman and Reeves (7) observed a much greater latency in the onset of a response in the ventricles compared to the atria. Likewise, when vagal stimulation is terminated, a longer delay may exist in the ventricles between deactivation of the cholinergic receptor and return of the cell to its control state.

Summary. Stimulation of the right cervical vagosympathetic trunk in the chloralose–anesthetized, decentralized dog was followed by a two-component postvagal inotropic response: an initial return of contractility toward the control level, but significantly less than control and a secondary, more delayed, significant positive inotropic effect compared to control contractility. Maintenance of arterial pressure by ventricular pacing during vagal stimulation eliminated the late positive inotropic effect but not the early response. Occlusion of the inferior vena cava for 15–20 sec immediately following vagal stimulation delayed the late positive inotropic effect for a period equal to the duration of the occlusion. Blockade of muscarinic cholinergic receptors (atropine) eliminated the late response as did blockade of beta adrenergic receptors (propranolol). Block of catecholamine release from sympathetic endings (guanethidine) had no effect on the late response. It is concluded that adrenal medullary catecholamines, released presumably as a consequence of vagally-induced hypotension, are responsible for the late positive inotropic effect produced following vagal stimulation.

1. Copen, D. L., Cirillo, D. P., and Vassalle, M., Amer. J. Physiol. **215,** 696 (1968).
2. Vassalle, M., Mandel, W. J., and Holder, M. S., Amer. J. Physiol. **218,** 115 (1970).
3. Holder, M. S., Anolik, M. A., and Vassalle, M., Archiv. Sci. Biol. **55,** 103 (1971).
4. Loeb, J. M., and Vassalle, M., Cardiology **63,** 270 (1978).
5. Hollenberg, M., Carriere, S., and Barger, A. C., Circ. Res. **16,** 527 (1965).
6. Levy, M. N., Ng, N., Martin, P., and Zieske, H., Circ. Res. **19,** 5 (1966).
7. Harman, M. A., and Reeves, T. J., Amer. J. Physiol. **215,** 1210 (1968).
8. Randall, W. C., Priola, D. V., Pace, J. B., and Wechsler, J. S., Proc. Soc. Exp. Biol. Med. **124,** 1254 (1967).
9. Stanton, H. C., and Vick, R. L., Archiv. Int. Pharmacodyn. **176,** 16 (1968).
10. Randall, W. C., Wechsler, J. S., Pace, J. B., and Szentivanyi, M., Amer. J. Physiol. **214,** 1205 (1968).
11. Kabat, H., Amer. J. Physiol. **128,** 246 (1939).
12. Priola, D. V., and Fulton, R. L., Circ. Res. **25,** 265 (1969).
13. Alter, W. A., Weiss, G. K., and Priola, D. V., Eur. J. Pharmacol. **24,** 329 (1973).
14. Alter, W. A., Weiss, G. K., and Priola, D. V., J. Pharmacol. Exp. Ther. **187,** 99 (1973).
15. Smith, D. C., Amer. J. Physiol. **218,** 1618 (1970).
16. James, T. N., and Spence C., Anat. Rec. **155,** 151 (1966).

Received November 29, 1978. P.S.E.B.M. 1979, Vol. 161.