Effects of Adrenergic Blockade on the Vasoconstrictor Action of Angiotensin in the Perfused Hind Limb[1] (34625)

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Recently it has become apparent that in some vascular beds the observed pressor response to administered angiotensin is the result of both direct stimulation of vascular smooth muscle as well as neuronally mediated effects. It has been reported that angiotensin can stimulate the central nervous system (1–4), peripheral autonomic ganglia (5–7), and can cause the release of catecholamines from the adrenal medulla (8, 9). Some experimental evidence indicates that angiotensin may interact with adrenergic nerve endings either to cause release of catecholamines (10–12) or inhibit the rate of their reuptake (13, 14). The following experiments were designed primarily to test whether angiotensin could cause the release of catecholamines from adrenergic nerve endings innervating blood vessels in the perfused canine hind limb.

Methods. Seventeen mongrel dogs (12.1–18.1 kg) of either sex were anesthetized with 30–35 mg/kg iv sodium pentobarbital. Each was intubated with a cuffed endotracheal tube and was ventilated with a Harvard respirator. A partial or complete laminectomy was performed at L5 or L6 in order to expose the epidural space and a 3-ft length of polyethylene tubing (PE 160) was threaded anteriorly into the epidural space until the tip rested at approximately T1. Both femoral arteries were exposed distal to the inguinal ring for about 5 cm and were freed from their connective tissue sheaths. An electromagnetic flow probe was positioned around the right femoral artery and flow was measured using a Medicon-K 2000 electromagnetic flowmeter. Volume flow in this vessel was estimated using calibration curves previously determined for each probe. The exposed left femoral artery was occluded proximally and distally and an arteriotomy was performed. Inflow and outflow cannulas of the perfusion system were inserted in the proximal and distal segments of the artery, respectively, and were tied in place. Perfusion was established and a Harvard No. 1202 pump was set to deliver the same flow measured in the right femoral artery. Animals were heparinized (3.0 mg/kg) 5 min prior to cannulation and supplemental heparin (8.33 mg) was administered hourly. The polyvinyl chloride tubing (½ × ⅜ in.) in the perfusion system had a dead space of approximately 100 ml and was filled with heparinized saline prior to cannulation. An extracorporeal flow probe was inserted in the perfusion system distal to the pump. A three-way stopcock penetrated the flow probe and was used for drug injections as well as measurement of perfusion pressure. Both common carotid arteries were exposed through a median longitudinal incision in the neck. The left common carotid was cannulated and a catheter was passed caudally into the thoracic aorta for measurement of aortic pressure. Aortic and perfusion pressure were measured using Statham transducers. Pressure records, heart rate, and flow in either the right femoral artery or the perfusion system were displayed on an Offner type S dynograph. The animal and perfusion system were covered to minimize heat loss. The right common carotid was occluded for 30 sec to assess baroreceptor mediated reflex alterations in perfusion pressure. Epidural blockade was induced

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after the method of Flacke (15) by slow infusion of 0.5% lidocaine HCl through the polyethylene tube into the epidural space. After infusing approximately 1 ml/min for 30 min, the carotid clamping procedure was repeated as a test of completeness of epidural blockade. In almost all cases alterations in perfusion pressure were absent, and in those animals where changes in pressure persisted, continued infusion of lidocaine abolished them. Total quantity of lidocaine infused never exceeded 50 ml.

Other drugs used in this study were: angiotensin (Hypertensin, CIBA), phenoxybenzamine HCl (Dibenzyline, Smith, Kline & French), propranolol (Inderal, Ayerst), isoproterenol (K & K Labs., Inc.), and *l*-phenylephrine (K & K Labs., Inc.). Injections of drugs were made rapidly in a 1.0-ml volume into the perfusion system unless otherwise noted. All drugs except angiotensin were made up daily in 0.9% NaCl. Angiotensin was made up in 0.9% NaCl to 10 μg/ml and frozen in 10-ml portions. Suitable amounts were unfrozen prior to each experiment. Mean perfusion pressure and time from injection to the point maximum pressure was achieved were obtained for various trials from the Offner records, and analysis was accomplished with a Control Data Corp. 3600 computer using the analysis of covariance (16).

Results. From initial experiments in two dogs, it was found that 0.5 μg/kg would be a suitable dose to use in these studies. This dose fell on the linear portion of the dose–response curve; it increased perfusion pressure 50–90 mm Hg; and tachyphylaxis did not occur when doses were given at 20-min intervals.

Effect of epidural blockade on the pressor response to angiotensin. Forty-eight control injections were made in 12 epidurally blocked dogs and were compared to 11 angiotensin injections made in three nonepidurally blocked animals. Following epidural blockade it became apparent that the level of initial perfusion pressure in the hind limb was in part responsible for the magnitude of the change in perfusion pressure following

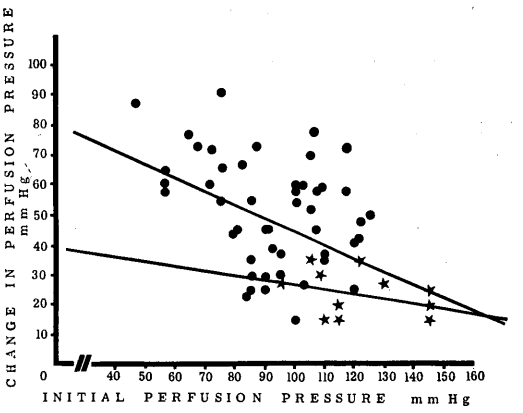

FIG. 1. Response of the perfused femoral vascular bed of the dog to injection of angiotensin (0.5 μg/kg: (*), angiotension before epidural blockade; (●), angiotensin following epidural blockade. Linear regression lines common to each group of data points are indicated.

administration of angiotensin or other pressor agent (Fig. 1). There was variability in the magnitude of the pressor response at different initial perfusion pressure levels and, therefore, the analysis of covariance was applied to both groups of data. After adjustment with covariance a highly significant F value (8.57, $p < 0.01$) indicates that real differences exist between treatment means of epidurally blocked and nonepidurally blocked animals (Table IA).

Effect of alpha adrenergic blockade on the pressor response to angiotensin in epidurally blocked dogs. Results of 24 angiotensin injections in 6 dogs were compared to 48 control injections in 12 dogs. Alpha adrenergic blockade was induced with phenoxybenzamine HCl (3–4 mg/kg iv). Completeness of this blockade was tested for by administration of *l*-phenylephrine (30 μg/kg) before and 45 min after administration of phenoxybenzamine. After adjustment with covariance so that treatments could be compared from a common initial pressure level, no significant difference in the magnitude of the pressor response to angiotensin was found in alpha blocked animals from that obtained in control animals (Table IB).

Effect of beta adrenergic blockade on the pressor response to angiotensin in epidurally

TABLE I. Effects of Angiotensin Injection on Perfusion Pressure in the Canine Hind Limb Before and After Adjustment with Covariance.

Treatment	IPP	MAXPP	AMAXPP	
A. Control (48)	91.7 ± 2.79	142.9 ± 3.07	143.8 ± 2.29	$F(1,59) = 8.57$ $p<0.01$
Nonepidurally blocked[a] (11)	121.4 ± 5.31	145.4 ± 5.10	128.4 ± 5.70	
B. Control (48)	91.7 ± 2.79	142.9 ± 3.07	143.8 ± 2.29	$F(1,69) = 2.10$ ns
Alpha blocked (24)	95.8 ± 4.71	152.2 ± 5.24	150.2 ± 3.61	
C. Control (48)	91.7 ± 2.79	142.9 ± 3.07	143.8 ± 2.29	$F(1,65) = 0.17$ ns
Beta blocked (20)	83.8 ± 5.86	138.0 ± 8.41	142.9 ± 4.34	
		TMP	ATMP	
D. Control (47)	91.1 ± 2.78	121.6 ± 4.47	122.4 ± 6.17	
Alpha blocked (24)	95.7 ± 4.71	133.4 ±14.18	128.8 ± 8.71	$F(2,87) = 0.53$ ns
Beta blocked (20)	83.8 ± 5.86	115.9 ± 5.45	119.3 ± 9.58	

[a] All other groups are epidurally blocked; values represent the mean ± SE; number of injections in parentheses; IPP, initial perfusion pressure (mm Hg); MAXPP, maximum perfusion pressure (mm Hg); AMAXPP, adjusted maximum perfusion pressure (mm Hg); TMP, time to peak response (sec); ATMP, adjusted time to peak response (sec); ns, not significant.

blocked dogs. The results of 20 angiotensin injections in 5 beta blocked dogs were compared to 48 control angiotensin injections in 12 dogs. Beta adrenergic blockade with propranolol was found to be transient and it was necessary to supplement the initial dose (2 mg/kg iv) with 1 mg/kg iv, 5 min prior to each subsequent angiotensin injection. Completeness of the blockade was tested for by administration of 5 μg/kg isoproterenol before and after propranolol. After adjustment with covariance no significant difference in the pressor response to angiotensin was observed between beta blocked and control animals (Table IC).

Effect of adrenergic receptor blockade on the time for development of the pressor response to angiotensin. The correlation between the initial perfusion pressure and the time for the development of the pressor response to angiotensin was low (0.288) but significant ($p < 0.01$). Comparison was, therefore, made among the following groups after adjustment with covariance: (i) control, (ii) alpha blocked, and (iii) beta blocked. All of these animals were epidurally blocked. No significant differences were observed among these three groups in the time for the development of the pressor response to angiotensin (Table ID). Typical records of perfusion pressure following angiotensin administration are shown in Fig. 2.

Discussion. It has been reported that close arterial administration of angiotensin can enhance the action of postganglionic sympathetic nerve stimulation in some vascular beds. In an attempt to explain these observations Hertting and Suko (17) suggested that the decrease in flow associated with the vasoconstrictor action of this peptide would allow more time for catecholamines released by nerve stimulation to interact with vascular adrenergic receptors. Others believe that angiotensin may itself cause release of catecholamines from adrenergic nerve endings (10–12) or affect the rate of their reuptake (13, 14). Since postganglionic adrenergic neurons can be presumed to be virtually inactive in this preparation, little if any neurotransmitter should be released, and hence no conclusion can be made as to the ability of angiotensin to affect reuptake by adrenergic nerve endings. However, if angiotensin can cause the release of catecholamines from adrenergic nerve endings, catecholamine-produced effects should be superimposed on the effects related to the vasoconstrictor action of this peptide. Magnitude and possibly rate of development and duration of the pressor response would be altered by

catecholamine-produced effects. However, since angiotensin can cause release of catecholamines from the adrenal medulla (8, 9), and thus might prolong the response following intra-arterial injection, the duration of the pressor response was not examined in these studies. The local effects of angiotensin would be most clearly evident in a vascular bed in which reflexly induced and central hemodynamic actions of this peptide have been eliminated. It should be possible to modify the catecholamine-dependent portion

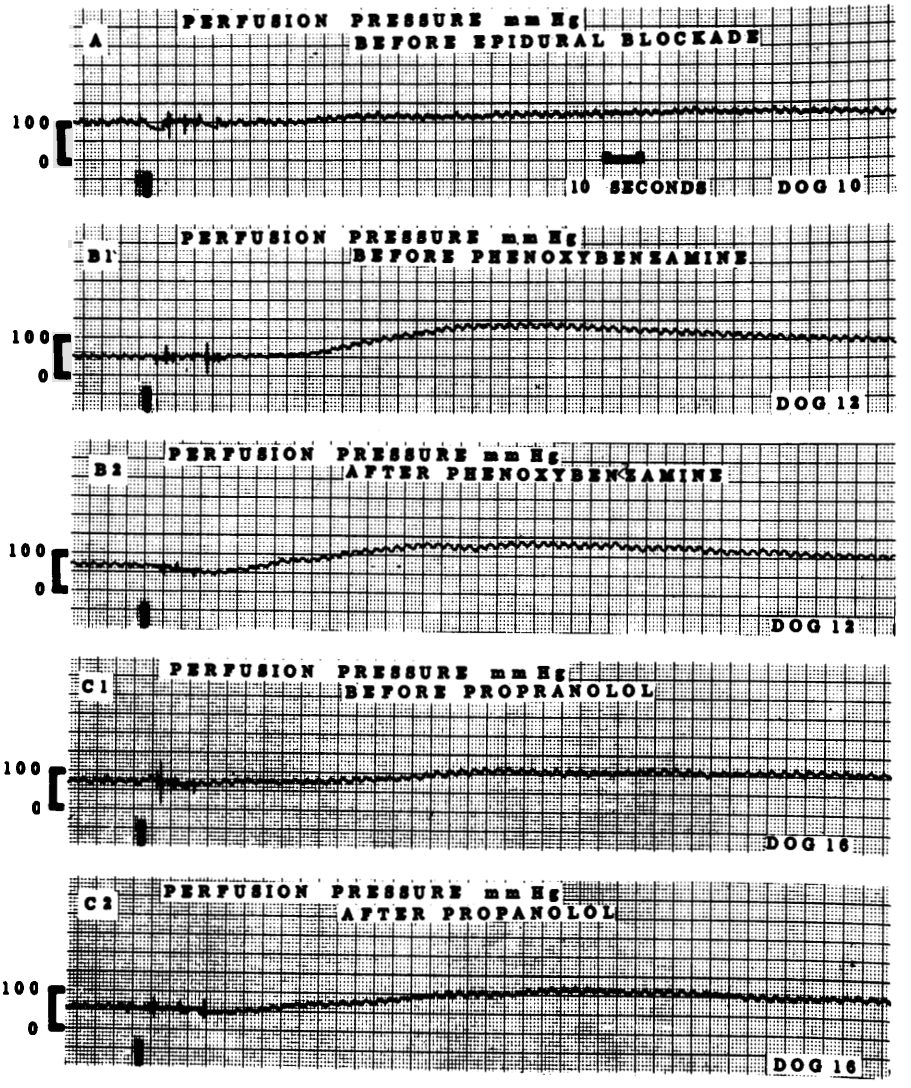

FIG. 2. Pressure response in the perfused canine hind limb following injection of angiotensin (0.5 μg/kg: (A↑), injection of angiotensin before epidural blockade; (B1↑), injection of angiotensin after epidural blockade and before blockade with phenoxybenzamine HCl (3–4 mg/kg). (B2 ↑), injection of angiotensin after epidural blockade and after blockade with phenoxybenzamine HCl. (C1↑), injection of angiotensin after epidural blockade and before blockade with propranolol (2–6 mg/kg). (C2↑), injection of angiotensin after epidural blockade and after blockade with propranolol. Small differences in the magnitude of the pressor response seen in the same animal following alpha or beta blockade can be attributed to different initial perfusion pressure levels as indicated by statistical analysis.

of the pressor response by injection of agents which produce either alpha or beta adrenergic blockade. More rapid development as well as an increase in the magnitude of the pressor response might be expected after beta blockade while after alpha blockade the magnitude of the pressor response should be diminished and its development slowed. In the femoral vascular bed of the dog perfused at constant flow in which reflex regulation was abolished by epidural blockade, angiotensin does not appear to cause the release of catecholamines from adrenergic nerve endings since no significant differences were observed in the magnitude or in the rate of development of the pressor response between control animals and those which had undergone either alpha or beta adrenergic blockade.

The results of these studies may be more clearly interpreted if one considers the work of Glick *et al.* (18). They have indicated that in skeletal muscle of the canine hind limb neuronally released catecholamines are incapable of interaction with beta receptors which are accessible to bloodborne agents. Difficulty was encountered in suppressing alpha receptors located in the vicinity of adrenergic nerve endings, while those which could be reached by bloodborne agents were more easily blocked. The possibility exists that in the present studies neurotransmitter released by angiotensin might always interact with unblocked alpha receptors and might never reach an area where beta receptors exist. If this is so, no change in the magnitude or the duration of the pressor response would be observed following alpha or beta blockade.

An alternative explanation has been proposed by Khairallah (19). He suggested that *in situ* blood vessels in different sites may respond to angiotensin in different ways. Only a direct vasoconstrictor response is exhibited by umbilical arteries whereas renal arteries show an indirect response which is dependent on the presence of sympathetic innervation. Most vascular beds are apparently combinations of both types of vessels. The lack of response observed in these studies could signify that the femoral vascular bed contains only the direct responding type of vasculature.

Recently Davis and Hammond (20) reported that the changes in resistance observed following postganglionic nerve stimulation in a vascular bed perfused at constant flow are much less than when the vascular bed is perfused at constant pressure. Because the femoral vascular bed was perfused at constant flow in these studies, small angiotensin-induced catecholamine effects might not have been detected.

Summary. The ability of angiotensin to cause release of catecholamines from adrenergic nerve endings was studied in anesthetized dogs in which the femoral vascular bed was perfused at constant flow and in which reflexly induced changes in perfusion pressure were abolished by epidural blockade. Magnitude and rate of development of the pressor response to angiotensin were examined prior to blockade and after alpha adrenergic blockade with phenoxybenzamine or beta adrenergic blockade with propranolol. No significant alterations in the pressor response were observed following adrenergic receptor blockade. As no evidence was obtained for release of catecholamines by angiotensin in this preparation, any effects of angiotensin on reuptake of catecholamines would not be tested.

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