The Role of Angiotensin II in a Pressor Response to Nicotine after Phenoxybenzamine[1] (37010)

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The pharmacology of nicotine is quite complex. One facet of its action was first noted by Hazard and Cheymol in 1942 (1). Injecting the alkaloid into atropinized, adrenalectomized dogs treated with the α-adrenergic blocker ergotamine, they found a reversal of blood pressure followed by a postreversal hypertensive effect. These authors offered no explanation for either phenomenon. Recently, however, Belej *et al.* (2), using the same experimental conditions except for the substitution of phenoxybenzamine for ergotamine, demonstrated the reversal phase to be mediated by endogenous norepinephrine released by nicotine.

Investigating the increase in blood pressure (BP) which follows the reversal, Papacostas and Jackson (3) used atropinized dogs treated with the noncompetitive α-adrenergic blocker phenoxybenzamine (PBZ). Hemodynamic studies during the secondary pressor response showed total peripheral resistance to be significantly elevated, accounting wholly for the increased BP, while cardiac output decreased. These results indicated a vasoactive mechanism as the cause of the hypertensive effect and consideration of various endogenous pressor substances pointed to angiotensin II (AT II) as the most likely mediator. The work reported here is an investigation of this hypothesis.

Materials and Methods. Adult mongrel dogs of either sex weighing 12–21 kg were used for all experiments. Animals were anesthetized with sodium pentobarbital (32 mg/kg iv, supplemented as required) and were maintained on positive pressure ventilation with room air (200 ml/kg/min). Atropine sulfate (0.5 mg/kg iv) was administered prior to beginning each experiment and repeated every 2 hr.

Most animals had the left common carotid artery cannulated for blood sampling. Systemic blood pressure was monitored from the left femoral artery with a pressure transducer (Statham P23Db) connected to a polygraph (Gilson Medical Electronics). The left femoral vein was used for the injection of all drugs. Dogs subjected to 60 sec carotid occlusion had the right femoral artery catheterized for blood sampling. Cardiac outputs in some of these animals were determined by the indicator dilution method of Hamilton *et al.* (4). A catheter inserted through the left external jugular vein to the right atrium was used for dye injections. The right femoral artery was used for densitometric measurement of the dye (indocyanine green, Cardio-Green). No blood samples were taken from those animals used for cardiac output measurements during carotid occlusion. Heart rate was monitored with a standard lead II electrocardiogram on the polygraph. Cardiac output was calculated according to the method of Williams, O'Donovan and Wood (5). Stroke volume and total peripheral resistance (TPR) were calculated from the cardiac output, blood pressure, and heart rate. TPR is expressed in arbitrary units obtained by dividing the BP by the cardiac output.

All drugs were either dissolved in or diluted with 0.9% saline. The injection of each drug was followed by 3–5 ml normal saline. The following drugs were administered as the salts in the doses indicated: nicotine salicylate, 200 μg/kg; hexamethonium chloride di-

[1] Supported by U.S. Public Health Service, NIH Training Grant No. 5T01HE05362 from National Heart and Lung Institute.

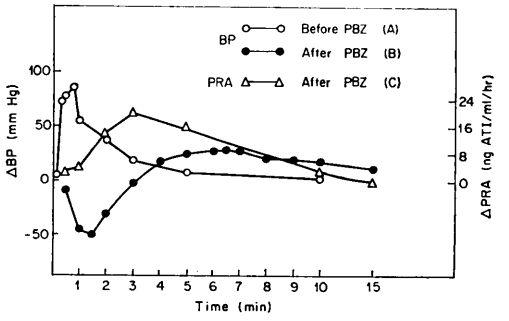

FIG. 1. Blood pressure changes in response to an injection of nicotine in the presence and absence of phenoxybenzamine. Also shown is the change in PRA in response to nicotine in the presence of phenoxybenzamine. PRA did not change in the absence of α-adrenergic blockade. Each point represents the mean of 6–10 animals. The peak pressor responses to nicotine are changes from control 89.4 ± 7.8 (SE) mm Hg in the absence of PBZ (curve A) and 28.9 ± 3.5 (SE) mm Hg in the presence of the blocker (curve B). The peak increase in PRA (curve C) is 19.7 ± 6.2 (SE) ng AT I/ml/hr. All changes are significant ($p < 0.01$).

hydrate, 10 mg/kg; phenoxybenzamine HCl (Dibenzyline, Smith, Kline and French), 10 mg/kg. The required dose of the last compound was diluted to 20 ml and infused over a 20-min period.

Blood samples for the assay of plasma renin activity (PRA), 6 ml each, were drawn into chilled Vacutainers containing tripotassium ethylenediaminetetraacetate. After centrifugation of the blood, the plasma was removed to a second Vacutainer and frozen at —20° until assay. Measurement of PRA was accomplished by a radioimmunoassay for angiotensin I (AT I) (Schwarz/Mann, Inc.), after the method of Haber *et al.* (6). Details of the procedure used in this work may be found elsewhere (7). PRA was calculated from measurements of AT I generated *in vitro* and expressed as nanograms of AT I formed per milliliter per hour.

Statistical analyses were performed by Student's *t* test for paired comparisons (8). *p* values < 0.05 were taken to indicate significant differences.

Results. Figure 1 shows the change in plasma renin activity in relation to the BP responses to nicotine in phenoxybenzamine-treated animals. Animals given nicotine in the absence of phenoxybenzamine showed no change in plasma renin activity ($p > 0.1$ at all sampling times), while plasma renin activity is significantly elevated from 1 to 8 min after the nicotine injection in the presence of PBZ, with a peak at approximately 3 min. The peak ΔPRA occurs shortly before the peak increase in BP. A time lag of this sort would be expected in view of the several steps involved between the release of renin and the actual formation of AT II. The duration of both the increased PRA and increased BP is quite important, as renin, being enzyme, continues to result in the formation of AT II.

Figure 1 also contrasts the pressor responses to nicotine in the absence and presence of PBZ. Distinct qualitative differences can be seen—the response with no PBZ is fast, large and short-lived, while that in the presence of the blocker is slower, smaller and longer.

Table I shows the results of cardiac output studies during bilateral carotid occlusion in both the presence and absence of PBZ. In the latter case, a significant increase ($p < 0.05$) in TPR is mainly responsible for the increased BP. In the presence of PBZ, this parameter shows no significant change. This lack of response in the presence of PBZ is strong evidence for the efficacy of α-adrenergic blockade by this drug, for bilateral occlusion of the carotid arteries provides a powerful reflex stimulus to the sympathetic nervous system.

Finally, Table II shows that ganglionic blockade with hexamethonium prevents any significant change in BP in response to nicotine. These results indicate that the action of nicotine which results in a pressor response in the presence of PBZ is an indirect one, mediated via a component of the autonomic nervous system.

Discussion. Having hypothesized an angiotensin mechanism for the secondary pressor response to nicotine, the first objective was the demonstration of an increased activity of the renin–angiotensin system during this pressor effect. That an injection of nicotine in the presence of PBZ results in a large increase in PRA temporally related to the secondary increase in BP indicates an elevated activity

TABLE I. Hemodynamic Responses to Carotid Occlusion Before and After Phenoxybenzamine (Demonstrating the Efficacy of α-Adrenergic Blockade.[a]

	Before PBZ					After PBZ				
	Control		Exp.			Control		Exp.		
Expt. no.	CO (liters/min)	TPR	CO (liters/min)	TPR	ΔBP	CO (liters/min)	TPR	CO (liters/min)	TPR	ΔBP
1						1.58	3.79	1.42	4.45	+5
2						1.32	3.32	1.55	2.79	8
3						1.36	2.77	1.41	2.79	7
4	2.43	2.71	2.97	2.83	+30	3.68	0.81	2.76	1.02	−3
5	2.71	3.16	2.31	4.68	37	2.33	2.84	2.01	3.68	13
6	2.26	3.58	2.22	4.68	38	1.48	3.36	1.52	3.28	0
7	1.87	3.52	1.72	4.65	23	1.31	4.13	1.17	4.64	0
Mean	2.32	3.24	2.31	4.21[b]	32[c]	1.87	3.00	1.69	3.29	4.23
SE	0.18	0.20	0.26	0.46	3.49	0.33	0.41	0.20	0.45	2.11

[a] CO = cardiac output (liters/min); TPR = total peripheral resistance (arbitrary units); BP = mean blood pressure (mm Hg).

[b] $p < .05$.

[c] $p < .01$.

of the renin–angiotensin system under these conditions, and is evidence for a role of this system in the pressor response. The reason for a lack of an increased PRA in response to nicotine in the absence of phenoxybenzamine is not fully understood. One possible explanation is that the large immediate increase in BP under these conditions serves to inhibit renin release by an intrarenal baroreceptor mechanism.

Consideration of the other known endogenous pressor hormones released by nicotine led to the conclusion that AT II is the major, if not the sole, pressor mechanism. A number of factors weigh against a direct vascular action of released catecholamines. Figure 1 shows a classical nicotine pressor response in the absence of PBZ. This response is different from the secondary pressor effect seen in the presence of α-adrenergic blockade. Also, the dose of PBZ used provides a strong and irreversible receptor blockade. This dose of PBZ has been shown to "reverse" the pressor effect of endogenous norepinephrine released by nicotine (2) and to prevent any pressor response from the release of this catecholamine by bilateral carotid occlusion (Table I). Finally, the factor of time is against a role of catecholamines, for these hormones have a half-life of only one circulation time in dogs (9), and the stimulating effect of nicotine is transitory (curve A in Fig. 1).

Another potential mediator for the secondary pressor response is vasopressin. Although nicotine is known to release this peptide from the neurohypophysis (10), at least two factors militate against a significant participation of the hormone in this particular response. The results in Table II indicate that ganglionic blockade completely prevents both the depressor and pressor actions of nicotine. Were nicotine causing the increased BP by releasing vasopressin, the response should be unaffected by hexamethonium, since this quaternary amine does not penetrate the central nervous system (11). A second factor, as with the catecholamines, is time. According to Nash (12) the BP response to an injection of vasopressin looks much like that shown in Fig. 1 for nicotine in the absence of PBZ, *i.e.*, it is rapid, sharply peaked, and short-lived. In addition, Gilmore and Vane (13) have shown vasopressin to have a half-life of approximately 1 min in dogs; therefore it will have virtually disappeared before the nicotine pressor effect in the presence of PBZ has begun.

There are two other known endogenous pressor hormones in dogs, and they also are unlikely to be involved in the secondary pressor response. Nicotine is known to release

TABLE II. Effects of Hexamethonium on BP Responses to Nicotine in Presence of α-Adrenergic Block.[a]

	Nicotine			
	Before hexamethonium; ΔBP		After hexamethonium; ΔBP	
Expt. no.	↓	↑	↓	↑
8	—35	25	—2	3
9	—28	47	0	0
10	—40	17	—3	5
Mean	—34.33	29.67	1.67	2.67
SE	3.48	8.97	0.86	1.46

[a] ↓ = peak depressor response; ↑ = peak pressor response.

serotonin from body stores, but this action is blocked by atropine (14), and our animals were well atropinized. Also, in addition to blocking catecholamines, PBZ is an efficacious blocker of the vascular effects of serotonin (15). The final possibility is one of the prostaglandins, F_{2a}. Although this lipid has a pressor action in dogs (16), the increased BP is the result of an increase in cardiac output, with no appreciable change in total peripheral resistance (17). This mechanism is opposite to that found in this study, *i.e.*, a decreased cardiac output with an increase in TPR.

In conclusion, the factors which eliminate other pressor hormones support the angiotensin hypothesis. Injection of nicotine in the presence of PBZ causes both an increase in circulating epinephrine and a resultant fall in BP. Both these phenomena are known stimuli of renin release (18). We have demonstrated a large and prolonged increase in PRA. Renin, an enzyme, continues to act on its substrate until destroyed. Thus, although AT II, like the other pressor agents discussed, has a short half-life, the circulating renin can continue to generate the peptide, accounting for the prolonged elevation of BP.

Summary. We have attempted to establish the mechanism of a prolonged pressor response to nicotine in anesthetized, atropinized dogs treated with the α-adrenergic blocker, phenoxybenzamine. The increased blood pressure follows the classical epinephrine reversal and is due to an increased total peripheral resistance. The time course and shape of the pressure curve tend to eliminate known endogenous pressor hormones except angiotensin II. A radioimmunoassay for plasma renin activity established that this parameter was in fact significantly increased. We therefore conclude that angiotensin II is likely to be the hormone primarily responsible for the secondary pressor response.

The authors thank Smith, Kline, and French Laboratories, Philadelphia, PA for generous supplies of phenoxybenzamine HCl (Dibenzyline).

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Received July 24, 1972. P.S.E.B.M., 1973, Vol. 142.