Catecholamine-Releasing Agent Recovered from Plasma of Subjects with Arterial Hypertension (34846)

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The assessment of renal venous and peripheral plasma renin activity has become an important aid in the diagnosis of renovascular hypertension and primary aldosteronism.

In a series of 98 patients in which renin activity was assessed by the method of Boucher (1, 2), we have observed nine cases in which aberrant responses in the arterial pressure of the assay rat indicated the presence of material other than angiotensin. This material was recoverable from both plasma and urine and the studies reported here indicate that the nonangiotensin pressor response observed in the rat bioassay is attributable to an adrenal catecholamine-releasing component bearing certain similarities to bradykinin.

Methods. The blood collection and processing methods used were as described by Boucher (1, 2). We do not routinely subject the angiotensin eluate from the Dowex column to paper chromatography, but utilize this fraction in the rat bioassay after acidification, evaporation to dryness, and removal of all traces of ammonium-acetate by repeated redesolving in ethanol (six–eight times) and evaporation to dryness under high vacuum. The dry ammonium-acetate-free residue is taken up in 1 ml of saline and assayed in the nephrectomized, ganglion-blocked rat using the arterial pressure response to synthetic valine-5 angiotensin II, aspartic β-amide (Hypertensin, Ciba) as a standard for comparison. The volumes used for the bioassay ranged from 0.05 to 0.1 ml.

Drugs utilized in the evaluation of the nature of arterial pressure responses in the rat were propranolol (Inderal I.C.I.) and phenoxybenzamine (Dibenzyline, Smith, Kline and French). Pentolinium tartrate (Ansolysen, Wyeth), 1–2 mg sc, was administered to institute blockade of the sympathetic ganglia. Criteria for alpha and beta receptor blockade were the abolition of the arterial pressure response to norepinephrine (1 μg/kg, iv) and isoproterenol (1 μg/kg, iv) by phenoxybenzamine and propranolol, respectively. In experiments in which the adrenals were to be removed, the rats were subjected to laparotomy prior to the experimental procedures. The adrenals were removed at the appropriate stage in the sequence of responses and the mid-line incision reclosed by clips.

Results. The typical pressor responses of an assay rat to synthetic angiotensin and to the renal venous plasma extract of a patient with renal artery stenosis are shown in Fig. 1 (*a, c*). The time course and general character of these responses are quite comparable. Also shown is the initial small depressor and protracted pressor tracing which characterizes the plasma of nine patients in our series with aberrant pressor responses (Fig. 1*b*). These responses are not attributable to procedural artifacts since two of these patients were later re-evaluated and the aberrant pressor response was shown to still typify their plasma extracts. Furthermore, in over 125 dog experiments in which we have processed plasma by the Boucher technique, we have never observed such responses. Twenty of these dogs represented chronic preparations with experimentally induced renal artery stenosis of varying extent and serial plasma renin levels were followed over several months.

Neither phenoxybenzamine nor proprano-

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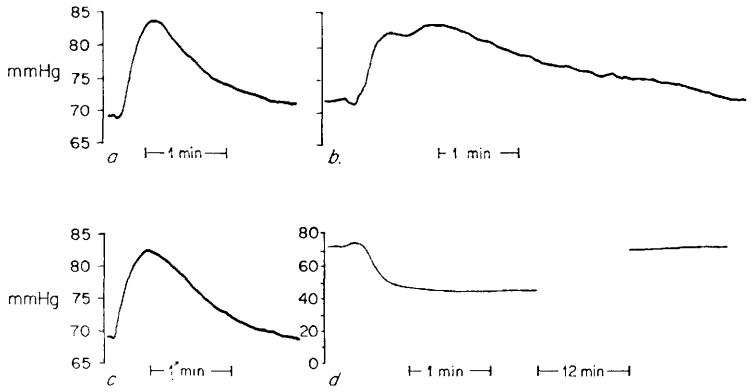

FIG. 1. Pressor responses of assay rat. *a*, 2 ng angiotensin II; *b*, atypical pressor response; *c*, angiotensin-like response from renal vein plasma of a patient with renal artery stenosis; *d*, effect of phenoxybenzamine on the response shown in *b*.

lol significantly affected the pressor response to angiotensin II. When the atypical plasma was utilized, however, phenoxybenzamine blocked the pressor response, resulting in a protracted depressor effect (Fig. 1*d*); propranolol did not affect the depressor response observed after alpha blockade. This indicates that the depressor component is not dependent on a beta adrenergic mechanism.

Removal of the adrenal glands induced substantially the same response as phenoxybenzamine, indicating that the pressor component is adrenal-dependent (Fig. 2).

In five of the nine patients with atypical plasma extracts, bilateral renal venous plasma was also analyzed. The abnormal pressor responses of the rat were similar for all plasma samples in these patients. Untreated urine, and urine processed by the Boucher technique from two different patients resulted in the same aberrant pressor response when injected into the bioassay animal.

Discussion. In 9 of 98 patients with systemic arterial hypertension undergoing clinical screening for hypertension, plasma processed by the method of Boucher *et al.* (1, 2) induced an atypical pressor response during the assay procedure. In contrast to the short-duration pressor response seen with angiotensin II, the aberrant response consisted of a brief initial depressor phase followed by a prolonged pressor response. This suggests

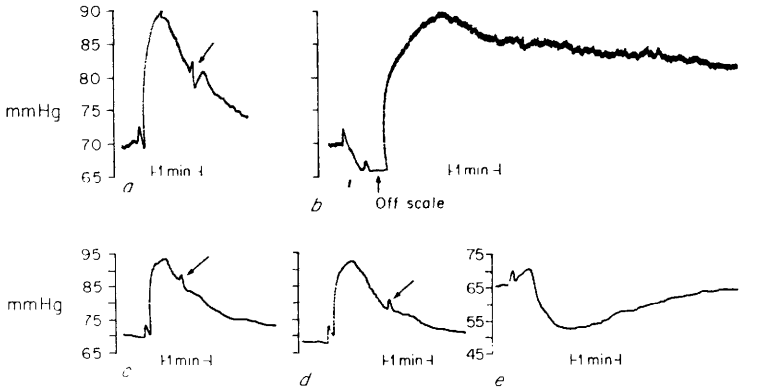

FIG. 2. Pressor responses of assay rat. a–b, prior to bilateral adrenalectomy; *c–e*, after bilateral adrenalectomy; *a*, 5 ng angiotensin II; *b*, atypical pressor response; *c*, 5 ng angiotensin II; *d*, extract of renal vein plasma from a dog with experimental renovascular hypertension; *e*, same material as in *b*. Arrows indicate artifacts induced by flushing venous catheter.

that the plasma and the urine of these subjects contains either a pressor substance or an agent capable of releasing naturally occurring pressor materials.

As concerns the nature of the agent, we can state, with fair certainty, that it is not tyramine, epinephrine, or norepinephrine, since these agents, when added to blood, are not recovered by the Boucher technique. The vasopressor lipid reported by Khairallah and Page (3) cannot be responsible for the aberrant response since this material is not absorbed on the Dowex column utilized in the isolation procedure. The pressor component is adrenal-dependent and the material, therefore, cannot be identified with the pressor material recovered from incubated human plasma by Croxatto and Diaz (4).

Since it is probable that some angiotensin is recovered along with the unidentified material, and although angiotensin has been reported to release epinephrine from the adrenals under certain circumstances (5, 6) this potential action of angiotensin cannot be the expalantion for the depressor component as it is not blocked by beta blocking agents. If, indeed, some angiotensin is recovered from the column, it is apparent that its pressor action is masked by the activity of the aberrant material. There are, however, certain basic similarities in these responses and those obtained with bradykinin in the ganglion-blocked, nephrectomized rat. Bradykinin, at levels of 1–2 μg, produces a depressor, followed by a pressor response. The latter is abolished by adrenalectomy (7) or alpha receptor blockade with phentolamine (8), but not by propranolol (4). We have also verified these observations. These similarities are compatible with the possibility that the material recovered from our "atypical" series represents a plasmakinin with an action similar to that of bradykinin. There is some doubt, however, that it is bradykinin, *per se*, since Boucher reported that this agent is not recovered by his method (1). Like Boucher, we fail to recover bradykinin in the isolation procedure when up to 1 μg/ml is added to plasma. The material is recoverable in similar quantities from peripheral or renal venous blood and, therefore, it is unlikely to be specifically of renal origin.

Sandler *et al.* (9) reported that, of 224 hypertensive patients treated with guanethidine, control was unsatisfactory in 38 (17%). The arterial pressures of 13 out of 16 hypertensives previously resistant to guanethidine were controlled for prolonged periods by the addition of phenoxybenzamine. It would be of value to know whether these patients exhibited a plasma activity of the type reported here.

We suggest that hypertensive patients exhibiting this bradykinin-like activity in peripheral plasma should, in the future, be evaluated for circulating and urinary catecholamine levels as well as their arterial pressure response to alpha adrenergic blocking agents. Whether such patients represent a "class" of as yet undescribed hypertensives remains to be demonstrated.

Summary. In 9 of 98 hypertensive patients undergoing clinical screening, plasma processed by the Boucher method induced atypical pressor responses in the assay procedure. Similar atypical responses were obtained from urine of two of these patients. The response was bradykinin-like in nature and the pressor component was blocked by dibenzylene as well as bilateral adrenalectomy of the assay rat. The agent can not be bradykinin, however, since this agent is not recovered by the Boucher method. The material is recoverable in similar quantities from peripheral or renal venous blood and, therefore, it is unlikely to be specifically of renal origin. Patients with such atypical plasma components may represent a "class" of as yet undescribed hypertensives.

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