The Nature of Pressor Substances in Pheochromocytomas

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The actions of aqueous extracts of two pheochromocytomas are compared before and after the administration of the adrenergic blocking agent, Dibenamine. One tumor contained predominantly a substance which, like norepinephrine, had pressor actions after Dibenamine. The other tumor contained chiefly an epinephrine-like substance. The significance of the presence of norepinephrine in pheochromocytomas and its role in epinephrine metabolism are discussed.

Several investigators have detected norepinephrine in various tissues\(^1\)\textsuperscript{-}\textsuperscript{4} and in Epinephrine, U.S.P., derived from the adrenal glands of cattle.\(^7\)\textsuperscript{-}\textsuperscript{9} The suggestion has been made repeatedly that norepinephrine may be a chemical mediator at certain adrenergic neuroeffector junctions.\(^1\)\textsuperscript{-}\textsuperscript{4}, \textsuperscript{7}, \textsuperscript{10} There have appeared during the past year the first, and almost simultaneous, reports\(^5\)\textsuperscript{-}\textsuperscript{14}, \textsuperscript{15} of the presence of varying amounts of norepinephrine in the human adrenal medulla and in pheochromocytomas. In a preliminary paper,\(^16\) published at virtually the same time, we reported the presence of a large amount of a "pressor substance other than epinephrine" in a pheochromocytoma. In each of these studies different chemical and biologic methods of identification were used. We have recently assayed a second pheochromocytoma. Methods similar to ours have been used by Beyer\(^17\) in the study of two adrenal medullary tumors.

There were distinct differences in the clinical aspects of our two cases. The first tumor (hereafter referred to as tumor A) was removed from a 13 year old white boy who had severe sustained arterial hypertension. The clinical features resembled those seen in essential hypertension, and this emphasizes the necessity for considering pheochromocytoma in the differential diagnosis of arterial hypertension.

Case Reports

Case J. F. L., a 13 year old white boy, had complained of difficult vision for one year. He was examined by an ophthalmologist who noted changes in the ocular fundi compatible with those of an hypertensive retinopathy. He was referred to Doernbecher Memorial Hospital on Nov. 11, 1948. The only other symptoms were transient headaches associated with exertion, occasional episodes of sweating and intermittent pain in the lumbar region.

He was a ruddy complexioned, alert and well developed boy who appeared well. The arterial blood pressure measured in the arms was 212/154, the pulse rate was 120, full and regular. There was marked edema of the optic discs and in the posterior poles of the retina with numerous large white exudates and striate hemorrhages. There was generalized constriction of the retinal arteries with well marked segmentation. The left cardiac border was displaced slightly to the left; the second aortic and pulmonic sounds were loud and snapping. The remainder of the physical examination was negative.

Routine studies of the blood, urinalyses, phenolsulphathalein test for kidney function, dextrose tolerance test, electrocardiograms, intravenous pyelograms and laminograms of both kidney areas were normal.

On Nov. 15, 1948, N-piperidino-methyl-benzodioxane (20 mg.) was administered intravenously. Within two minutes after beginning the injection, the arterial blood pressure fell from 178/138 to 152/100 and then rose in the next 15 minutes to 198/158 mm. Hg. On Nov. 17, 1948, 2 mg. per Kg. of body weight of the adrenergic blocking agent, N,N dibenzyl beta-chloroethylamine (Dibenamine), was injected intravenously. The arterial blood pressure fell from the resting level of 228/174 to 184/144 three minutes after beginning the injection and had fallen to 130/100 six and one-half hours after Dibenamine where it remained for six hours (fig. 1). This result suggested that the major part of the patient's hypertension was due to circulating epinephrine or an epinephrine-like substance. On Nov. 18, 1948, under basal avertin anesthesia supplemented with nitrous oxide and oxygen, a tumor of the left adrenal gland weighing 28 Gm., was removed and identified histologically as a pheochromocytoma. Benzodioxane and Dibenamine injections postoperatively have produced no significant changes in the
arterial blood pressure while the patient remained in the supine position. He was last seen in December, 1949, at which time he was asymptomatic and his arterial blood pressure was 132/88.

The second case (hereafter referred to as tumor B) exhibited the symptoms more commonly associated with pheochromocytomas.

Case 2.* W. C., a 26 year old white rancher, was admitted to the Veterans Administration Hospital, Portland, Oregon, on Aug. 19, 1949. For one year he had noted frequent episodes of transient weakness, "knotting" in his stomach, palpitations, sweating, and headache. These attacks were induced by activity and were relieved by rest. On one occasion the attack seemed to be related to emotional stress. He was first seen at the Veterans Hospital as an outpatient. At that time his arterial blood pressure was reported to be within normal limits. The administration of 0.05 mg. of histamine base was followed by an elevation of the systolic blood pressure to 230 mm. Hg.

Physical examination revealed no abnormal findings. The arterial blood pressure was 120/80 mm. Hg.

Laboratory studies: Hematology, urinalyses and serology were normal. The fasting blood glucose was 145 mg. per 100 cubic centimeters. The basal metabolic rate was +4 per cent. The dextrose tolerance tests were within normal limits.

During "attacks" the arterial blood pressure was not observed to exceed 150–160/90–100. On Aug. 29, 1949, a tumor weighing 9 Gm. was removed from the area medial and adjacent to the right adrenal gland. The tumor was identified histologically as a pheochromocytoma.

**METHODS**

The method of bioassay for each tumor was as follows: The arterial blood pressure of mongrel dogs, anesthetized with sodium pentobarbital, was recorded directly by means of a mercury manometer. Within two hours following surgical removal of the pheochromocytomas, aqueous extracts of the ground tumor tissue were prepared in the proportions of 10 cc. of distilled water per 1 Gm. of tumor tissue. The brei was then filtered through number 2 filter paper. An estimation of the pressor activity of the tumor extract was obtained by comparison with l-epinephrine solution of known concentration (Parke-Davis & Co., Adrenalin Chloride). Dibenamine was then injected slowly intravenously during a 12 to 15 minute period. The initial assays of tumor A were done using a Dibenamine dose of 30 mg. per Kg. of body weight. A reduction of blood pressure was obviated in the subsequent assays by using a dose of 15 mg. per Kg. of Dibenamine. Thirty minutes or more after completion of Dibenamine administration, the tumor extracts and known concentrations of epinephrine were again injected.

**RESULTS**

(1) Effects of the Tumor Extracts upon Arterial Blood Pressure. The extract of tumor A had marked pressor potency prior to Dibenamine administration; the pressor potency of 0.03 cc. of the extract was approximately equal to 1.0 cc. of 1:100,000 l-epinephrine. Following 30 mg. per Kg. of Dibenamine, the pressor response produced by the tumor extract was undiminished compared with the response prior to Dibenamine. These results are illustrated in figure 2. Epinephrine produced the fall of blood pressure that is observed after Dibenamine, and the degree of fall in pressure was greater the larger the dose. Injections of the tumor extract repeated within a short period of time produced qualitatively and quantitatively similar pressor responses. This is illustrated in figure 3. Injection of the tumor ex-

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* The complete case report will be published by Drs. Jarvis Gould and A. L. Mundal, Veterans Administration Hospital, Portland, Oregon.
tract into an unanesthetized dog with a Thiry fistula of the jejunum caused complete inhibition of intestinal motility. This is shown in figure 4. The pressor potency of the refrigerated extract and of a freshly prepared extract of the refrigerated specimen was somewhat reduced 24 hours later, and was almost absent by the fifth day.

Aqueous extracts of tumor B produced pressor responses prior to Dibenamine. An amount of 0.05 cc. of the extract was equivalent in pressor potency to 1.0 cc. of 1:50,000 epinephrine. Following 15 mg. per Kg. of Dibenamine, glands of 2 normal dogs were assayed separately. The extracts of both types of tissues produced pressor responses before Dibenamine. Following Dibenamine there was, in every instance, either no pressor effect or a fall of blood pressure similar to that occurring after the injection of 1-epinephrine.

FIG. 2. Comparison of effects of extracts of tumor A and of epinephrine upon the blood pressure of a dog before and after the administration of 240 mg. per Kg. of Dibenamine (30 mg. per Kg.). At the third mark, the drum remained stationary for approximately 45 minutes.

FIG. 3. A continuation of the record shown in figure 2, illustrating the undiminished effect of repeated injections of the extract.
The pressor responses produced by the tumor extracts may be attributed to a substance or substances either of sympathomimetic or nonsympathomimetic nature. The use of Dibenamine to differentiate these two classes of compounds rests upon its ability to block the pressor activity of sympathomimetic compounds without blocking the pressor responses to nonsympathomimetic compounds. The pressor effects of nonsympathomimetic compounds such as angiotonin and Pitressin are not blocked by Dibenamine. Presumably, the site of action of these compounds is directly upon the contractile mechanism.

(2) Effects of 1-Epinephrine and 1-Norepinephrine upon Arterial Blood Pressure. Dibenamine produces a blockade at certain excitatory adrenergic neuroeffector junctions, but it does not block inhibitory adrenergic functions. Following the administration of Dibenamine, according to the doses and time relations indicated by Nickerson and Goodman, epinephrine produces a typical depressor response. This is due, presumably, to the "unmasking" of the vasodilator action of epinephrine. Their earlier studies indicated that the pressor effects of norepinephrine are blocked by Dibenamine, but that little or no reversal occurs because of the weak vasodilator action of norepinephrine. A later report by Nickerson indicated that the time required for Dibenamine "fixation" in the tissues was probably nearer 90 minutes than 30 minutes; and epinephrine given within a 90-minute period after Dibenamine reduced the effectiveness of the blockade, possibly by competing with Dibenamine for its site of action.

The assays of each tumor were begun 30 to 40 minutes after the completion of Dibenamine administration. Multiple injections of epinephrine were given both before and after Dibenamine. The dissimilar results obtained in the bioassay of the tumors, and unanticipated inconsistencies in the action of Dibenamine suggested that the pressor responses observed in tumor A after Dibenamine were, in reality, due to incomplete adrenergic blockade by Dibenamine. This led to an additional series of experiments in which the effects of 1-epinephrine and 1-norepinephrine upon the arterial blood pressure were compared after varying time and dose relations of Dibenamine.

The first group of experiments was done upon 4 dogs anesthetized with sodium pentobarbital. Two dogs were given 15 mg. per Kg. of Dibenamine and 2 were given 30 mg. per Kg. Equipressor doses of 1-epinephrine and 1-norepinephrine were injected alternately before Dibenamine and 30 to 40 minutes after the completion of Dibenamine administration. In the 2 dogs in which 15 mg. per Kg. of Dibenamine was used, 1-norepinephrine produced a distinct pressor response in one dog with the second injection of 1-norepinephrine after Dibenamine. Subsequent doses of 1-norepinephrine ranging from 1 cc. of 1:1000 to 1 cc. of 1:50,000 concentration consistently produced pressor responses. In the second dog, a distinct pressor response occurred with the sixth injection of 1-norepinephrine after Dibenamine. Of the 2 dogs in which 30 mg. per Kg. of Dibenamine was used, one animal failed to show pressor responses to 3 successive injections of 1-norepinephrine 30 minutes after Dibenamine in doses as large as 10.0 cc. of 1:50,000 concentration. In the second dog, 2 cc. of 1:50,000 1-norepinephrine was given as the initial injection 30 minutes after Dibenamine, and produced a small but distinct pressor response. Further injections of 1-norepinephrine resulted in pressor responses of the same magnitude.
In each of the four dogs, a wide range of doses of l-epinephrine produced depressor responses.

In two dogs, no injections were given prior to Dibenamine or less than two to three hours following the administration of 15 mg. per Kg. of Dibenamine. Injections of l-epinephrine and l-norepinephrine were then given alternately. Two hours after Dibenamine administration, the first and second injections of l-norepinephrine (1 cc. 1:50,000 and 1 cc. 1:1000 respectively) produced slight reductions of blood pressure. The third injection of l-norepinephrine (0.5 cc. 1:50,000) and subsequent injections each produced a pressor response. This is shown in figure 5. In the second dog, the first injection given after the three hour interval was 0.5 cc. 1:50,000 l-norepinephrine. A definite pressor response was obtained. In both dogs, l-epinephrine produced only depressor responses.

(3) Effects of Dibenamine upon Arterial Hypertension Produced by Constant Intravenous Infusions of l-Epinephrine and l-Norepinephrine. A pheochromocytoma which produces a sustained hypertension presumably does so by the more or less constant liberation of epinephrine or an epinephrine-like substance into the circulation. If the injection of epinephrine during or soon after the administration of Dibenamine decreases the effectiveness of the adrenergic blockade, as has been suggested by Nickerson and supported by the experiments described above, the explanation of the neutralization by Dibenamine of the major part of the hypertension associated with pheochromocytomas is not readily apparent. Acute experiments were done in an attempt to simulate the sustained hypertension associated with some pheochromocytomas, and to study the effect of Dibenamine upon such hypertension.

Constant intravenous infusions of either l-epinephrine (1:25,000) or l-norepinephrine were given to three dogs by means of a power-driven syringe. The rate of injection was adjusted until the desired level of hypertension was obtained. A dose of 15 mg. per Kg. of Dibenamine was then injected intravenously during an eight minute period. Two of the 3 dogs received l-norepinephrine infusions and the third dog received an infusion of l-epinephrine. In each instance the drug infusions began 10 to 20 minutes prior to the injection of Dibenamine, and were continued throughout the period of Dibenamine administration. One l-norepinephrine infusion was continued for 60 minutes after Dibenamine, and the remaining l-norepinephrine and l-epinephrine infusions were continued for 20 minutes after Dibenamine. Within five minutes after the beginning of the injection of Dibenamine, the arterial blood pressure declined steadily to the pre-hypertensive level, in the case of the l-norepinephrine infusions, and to below the pre-hypertensive level in the l-epinephrine infusion. If the infusions were momentarily discontinued 1 to 10 minutes after completion of Dibenamine injection, the arterial blood pressure returned to the pre-hypertensive level. When the l-epinephrine infusions were started again at the same rate, the arterial blood pressure increased but only one-third as much as it did before Dibenamine. When the l-epinephrine infusion was started again, the arterial blood pressure fell below the pre-hypertensive level. Single injections of l-epinephrine (1 to 2 cc. 1:50,000) produced pressor responses 20 minutes after Dibenamine and slight reductions of arterial blood pressure 40 to 60 minutes after Dibenamine. Single injections of l-norepinephrine (2 cc. 1:50,000) produced consistent pressor responses when injected 20 to 60 minutes after Dibenamine.
DISCUSSION

The results of these experiments indicate that, with the time and dose relations of Dibenamine which were used, the pressor response to l-norepinephrine is not always blocked, and its effects upon the arterial blood pressure after Dibenamine are unpredictable. The failure of Dibenamine to block the pressor activity of l-norepinephrine in dosages which are considerably greater than those necessary to block and to reverse the pressor response to all doses of epi- nephrine, indicates that Dibenamine cannot be used to differentiate pressor responses due to sympathomimetic and nonsympathomimetic compounds when norepinephrine is involved. Various other adrenergic blocking agents such as N-piperdino-methylbenzodioxane (933 F), yohimbine, ergotamine, and ergotoxine are known to be less effective in blocking the pressor activity of l-norepinephrine than of epinephrine. In accordance with the observations of Nickerson, epinephrine injected during the administration of Dibenamine, or soon after, decreases the effectiveness of the adrenergic blockade.

The findings in tumor A are compatible with the reports of the presence of norepinephrine in pheochromocytomas to the extent that the actions of the pressor substance after Dibenamine resembled those of norepinephrine rather than epinephrine. The consistent depressor responses to extracts of tumor B after Dibenamine indicate that the major pressor principle in this tumor was epinephrine, but the presence of norepinephrine is not excluded since the assay method is not quantitatively accurate. The absence of a pressor response to extracts of tumor B after Dibenamine, in contrast to tumor A, is similar to the results of Beyer who has recently bioassayed two pheochromocytomas by the same method. The pressor actions of the extracts of these tumors were abolished after incubation with amine oxidase. He calculated that the norepinephrine content of the two pheochromocytomas was approximately 50 per cent and 90 per cent.

Pheochromocytomas generally contain a higher percentage of norepinephrine than does normal adrenal medullary tissue. Goldenberg reported three tumors in which the nor-epinephrine content ranged from 53 per cent to 90 per cent; Holton assayed three tumors which contained from 60 per cent to more than 90 per cent norepinephrine, and Calkins reported a tumor containing approximately 83 per cent norepinephrine. The presence of norepinephrine in both normal and pathologic adrenal medullary tissue suggests that norepinephrine may be a precursor of epinephrine or that norepinephrine may be liberated as such directly into the circulation. Blaschko has proposed that norepinephrine is the precursor of epinephrine, and that the secondary amine, epinephrine, results from the methylation of the primary amine, norepinephrine. Büllbring and Burn perfused suprarenal glands of dogs with heparinized blood and found that there was an increase in the secretion of epinephrine when norepinephrine was added to the perfused blood. Büllbring demonstrated that incubated suspensions of dog and cat adrenal glands are capable of converting norepinephrine to epinephrine. She concluded from her studies that epinephrine is produced from norepinephrine through a transmethylation reaction in the adrenal medulla. The presence of greater amounts of norepinephrine in adrenal medullary tumors suggests the possibility of impairment in the transmethylation reaction.

The possibility of direct liberation of norepinephrine into the circulation has received the attention of several investigators. Following continuous stimulation of the splanchnic nerves in the chloralosed cat, West observed that epinephrine liberation gradually decreases until at the end of one hour 70 per cent of the active material from the adrenal gland is norepinephrine. Büllbring and Burn stimulated the splanchnic nerves of spinal cats from which the viscera had been removed and the renal circulation excluded. Under these conditions splanchnic stimulation liberated from the adrenal gland a mixture of epinephrine and norepinephrine in which the norepinephrine content varied from 20 to 80 per cent. There is little information concerning the possibility that norepinephrine is liberated into the circulation from adrenal medullary tumors although Vogt has recently reported the detection of
circulating norepinephrine in a case of pheochromocytoma. We have been unable to determine by the response of the arterial blood pressure to Dibenamine either the nature or the amounts of pressor substance or substances which are liberated into circulation from pheochromocytomas. The arterial hypertension produced in dogs by the constant intravenous infusion of l-norepinephrine or l-epinephrine was abolished by the injection of 15 mg. per Kg. of Dibenamine. Such a result is difficult to explain in view of the frequent failure of Dibenamine to block the pressor effects of single injections of l-norepinephrine where the conditions for the development of adrenergic blockade are most favorable. The demonstration of the presence of l-norepinephrine in adrenal medullary tumors does not justify the assumption that this is the substance being liberated into the circulation. Attempts to correlate bioassay findings with the clinical features of pheochromocytomas must be deferred until more information is available concerning the nature of the active substance or substances which are liberated by these tumors.

The demonstration of norepinephrine in the adrenal medulla as well as in various adrenergic nerves strengthens the concept that this compound is an intermediary in the production of epinephrine. Two observations indicate that this is the case rather than that epinephrine is the precursor of norepinephrine. First, the epinephrine: norepinephrine ratio is higher in normal than in tumorous adrenal medullary tissue. Second, the more physiologic the conditions under which epinephrine is liberated from the adrenal medulla, the greater the proportion of epinephrine released.

The view may be restated that norepinephrine is concerned with epinephrine metabolism in all of the tissues which produce epinephrine. It is likely that both excitatory and inhibitory adrenergic nerves and the adrenal medulla produce epinephrine by the same steps and that the last step in the series of reactions is the conversion of norepinephrine to epinephrine. It is possible that, from all sources, some norepinephrine enters the circulation and that from some sources under certain conditions the proportion of norepinephrine entering the circulation may be increased. It is probably an oversimplification to consider that excitatory adrenergic nerves liberate only norepinephrine and inhibitory adrenergic nerves liberate only epinephrine.

**SUMMARY AND CONCLUSIONS**

1. Two cases of pheochromocytoma are reported. The tumors were bioassayed by comparing the effects of the tumor extracts upon the arterial blood pressure of dogs before and after the administration of the adrenergic blocking agent, Dibenamine.

2. One tumor contained predominantly a substance resembling norepinephrine. The second tumor contained chiefly an epinephrine-like substance.

3. Dibenamine is similar to other adrenergic blocking agents in its inability to block uniformly the pressor action of norepinephrine.

4. The role of norepinephrine in epinephrine metabolism is discussed.

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