A Preliminary Study of Urinary Catecholamines in Hypertension

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Introduction

Clinical manifestations in some patients suffering from essential hypertension are suggestive of sympathetic noradrenaline (NAD) determination is that the increased activity of the sympathetic nervous system in hypertension, if any, is associated with a greater release of NAD into the blood, followed by an increase in its metabolism and excretion.

Initial attempts to investigate sympathetic nervous activity more directly by studing the urinary excretion of catecholamines and their metabolites in hypertensive patients failed to reveal any consistent indications of hyperfunction of the sympathetic nervous system in most cases of essential hypertension. In earlier studies, urinary excretion of NAD, AD, VMA and Normetanephrine (NM) were found to be normal, decreased or elevated (De-Champlain, 1972). When expressed in terms of incidence, only a small portion of essential hypertensive patients, between 5 (De-Quattro, 1971) and 10 to 33 per cent (De-Champlain, 1972; Hoeldlke, 1974; Januszewcz and Wocial, 1975) has been shown to have a clear or a more or less evident increase in catecholamine excretion.

Taking into the view of this controversy in results preliminary investigations of he level of catecholamines in hypertensive patients, have been taken.

Material and Methods :

Collection of 24 hr. urine specimen was done at pH 4 to inhibit the oxidation of catecholamines by using approximately 5 ml of H_2SO_4 of 3 mol/Lit. This acidified urine was referigerated at 4°C for the storage purposes and analysed when required.

In all, 20 hypertensive and 10 normal subjects were studied. Specimen of urine of hypertensive patients were collected from local hospitals alongwith relevant data. All these patients were under treatment with various antihypertensive agents.

Solvent-Extraction Method :

The method for the extraction of catecholamines was that of Brownlee and Spriggs (1965), which was itself a modification of the method of Shore and Olin (1958), and used one quarter of the volume of all reagents.

A 20 ml of the 25 hours specimen of urine, which was collected in acid solution ($p^H = 4.0$), is brought to $p^H = 8.5$ with 1N sodium hydroxide solution. The precipitated phosphates were removed by centrifugation of specimen at the speed of 2000 r.p.m. A 10 ml of urine specimen was thn transferred in a conical flask, 30 ml Butanol, 2 ml of 0.01N HC1 and 5 gm solid NaC1 were added to the sample. The mixture was then shaked by a shaker at medium speed for 10 mints. The mixture was centrifuged at 2000 r.p.m., for five minutes and 25 ml of the organic phase was withdrawn into another conical flask. 50 ml of N-heptane and 7 ml of 0101N HC1 were added and mixture was shaken for five minutes. At ths stage, catecholamines had passed into the aqueous phase. The mixture was then centrifuged for five minutes and the aqueous phase was withdrawn.

Estimation of Catecholamines :

Two test tubes were taken for Nor-adrenaline and two tubes for adrenaline, one was for the test solution, and the other was for the blank for each amine. 1.5 ml of aqueous phase was

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tkaen into each tube.

0.5 ml of 2M acetate buffer of p^H = 5.0 and p^H = 3.0 was added to both the tubes for nor-adrenaline and adrenaline respectively. 0.05 ml of iodine solution was added to the tubes for the test solution and 0.1 ml of sodium thiosulphate solution was added to both the tubes for the blank solutions of adrenaline and noradrenaline. After six minutes the above order was reversed and 0.05 ml of iodine solution was added to the blanks and 0.1 ml of sodium thiosulphate solution was added to the test solutin (of both). 0.5 ml of freshly prepared alkaline ascorbate solution was then added to each of the test thbes an allowed to act for five minutes. Finally, 0.5 ml of 5N acetic acid was added to the test and the blank solutions to stop the reaction.

After 20–30 minutes the fluorescence of the resulting products was read with JASCO-550 Spectrofluorimeter, at 385 mu (excitation) and 485 mu (emission) wavelength for nor-adrenaline and at 410 mu (excitation) and 490 mu (emission) for adrenline.

Results :

During this study, 20 hypertensive patients and 10 normotensive subjects were evaluated for the catecholamine contents in urine. The data of thse subjects is given in Table-1 (hypertensives) and Table-2) (normotensives).

Total catecholamines wee measured in ug/100 ml of urine, NAD was the major component. In hypertensive patients the highest value of catecholamines obtained was 23.5 ug/100 ml and the lowest one was 0.4 ug/100 ml. The mean value obtained of total catecholamines in 100 ml urine was 3.48 ± 1.15 ug.

In normotensive subjects the highest quantity ofcatecholamines obtained in 100 ml of urine was 14.5 ug., and the lowest value of catecholamines was found to be 0.2 ug/100 ml. Mean value of catecholamine contents in normotensives was 3.82 ± 1.42 ug/100 ml. No statistically significant difference was found among the catechola-

TABLE – 1

SHOWING THE DATA OF VARIOUS PARAMETERS OF 20 HYPERTENSIVE PATIENTS

Sl. No.	Sex	Age (Years)	Systolic Blood Pressure (m.m. of Hg.)	Diastolic Blood Pressure (m.m. of Hg.)	Total Catecholamines (ug/100 ml.)
1	Male	30	150	94	0.8
2	”	40	140	90	0.8
3	”	50	180	100	2.8
4	”	50	170	110	2.1
5	”	56	150	100	3.4
6	”	58	180	130	4.1
7	”	58	125	80	1.3
8	”	65	156	100	9.4
9	Female	26	180	110	1.3
10	”	33	130	90	0.8
11	”	35	135	110	2.6
12	”	35	160	105	4.0
13	”	40	180	110	1.5
14	”	45	140	90	5.5
15	”	48	140	90	23.5
16	”	50	190	110	1.4
17	”	50	130	80	0.4
18	”	50	200	110	1.8
19	”	50	180	90	0.9
20	”	62	140	80	1.3

TABLE – 2

SHOWING THE DATA OF VARIOUS PARAMETERS OF 10 NORMOTENSIVE SUBJECTS

Sl. No.	Sex	Age (Years)	Systolic Blood Pressure (m.m. of Hg.)	Diastolic Blood Pressure (m.m. of Hg.)	Total Catecholamines (ug/100 ml.)
1	Male	43	125	80	1.0
2	”	46	130	90	0.3
3	”	48	130	90	2.2
4	”	53	120	80	0.2
5	”	58	110	70	8.0
6	Female	42	110	80	1.0
7	”	52	120	94	14.5
8	”	53	130	90	0.2
9	”	54	110	80	4.6
10	”	65	120	90	5.0

mnes of hypertensive patients and normotensive subjects (Table-3).

Other parameters such as age, systolic and diastolic blood pressure when compared in hypertensive patients with normotensive subjects except age other two parameters were found satisfically significant (< .001 and < .01 respectively).

TABLE – 3

SHOWING THE MEAN VALUES AND PROBABILITY (P) FOR THE AGE, B. P. AND CA'S IN HYPERTENSIVES AND NORMOTENSIVES SUBJECTS MEAN + S. R.

Particulars	Age	Systolic B. P. (m.m. of Hg)	Diastolic (m.m. of Hg)	Catecholamines (ug/100 ml
Hypertensives (n = 20)	46.55 ± 2.42	157.52 ± 5.12	98.95 ± 2.91	3.48 ± 1.15
Nomotensives (n = 10)	51.40 ± 2.21	120+50 ± 2.62	84.40 ± 2.36	3.82 ± 1.42
P	N. S.	< .001	< .01	N. S.

Discussion :

In present studies when CA's were compared between hypertensive patients and normotensive subjects, the mean value of CA's in normotensives was found more than hypertensive patients, though it was not satistically significant (Table-3). These findings confirmed the results of D'Amelio, et al. (1978). They reported "no significant differences were noted in urinary excretion of adrenaline and noradrenaline in standardized basal conditons among normal and hypertensive (essential and renovascular) patients.

On the contrary urinary catecholamines were found increased in borderline and labile hypertensive patients when compared with controls, while in renovascular hupertension urinary Adrenaline was significantly less than normotensives but urinary Nor-adrenaline was found much increased in same (Renovascular) situation when compared with controls (Cuche, et al., 1975; Kuchel, et al., 1975). In present studies though the patients were all hypertensive but definite kind of hypertension was not assigned to them, however, the other diseases with hypertension such as Diabetes, polycystic kidney, parkinsonism etc., were detected and have been noted.

The difficulties encountered in investigating hypertensive subjects result from the fact that arbitrarilly chosen dividing lines between normotension and hypertension place control subjects on one side and subjects with hypertension of various degrees of severity on the other. This could account for the inconsistent reports that urinary catecholamines are normal low or elevated in essential hypertension (Goldstein and Killip, 1965).

The differences between levels of catecholamines in hypertensive patients and normotensive subjects may lead to speculate several reasons as the basis. It is suggested that the hyperactive sympathetic system would release more catecholamine during hypertension than in normal condition; though it will depend on the type of hypertension, severity of hypertension and diseases other than hypertension which may be responsible for the increase of catecholamines such as pheochromocytone, renovascular manifistation and diabetes etc. In the present series of experiments, some patients were found suffering from diabetes malitus, kidney stone, polycystic kidney, parkinsonism (The numbers are 4, 7 8, and 20 respectively in Table - 1).

Drug therapy of hypertensive patients may also effect the rate of production of catecholamines specially NAd. Wwidmann, et al., (1980) showed that adrenergic blockage with Debrisquin decreased plasma NAd by 50% in all groups, while thiazide did not produce any change in basal plasma catecholamine levels. It is also reported that beta blockers reduced supine circulating CA's or NAd levels (De-Champlain, et al., 1978). Contrary to these results DeLeeuew, et al., (1978) showed that in propranolol treated group of essential hypertensive patients NAD secretion was more consistent and enhanced.

The effects of medicine, given to the patients in the prsent series though not observed but the results showing higher catecholamine levels in urine of normotensive subjects than hyperten-

sive patients may be justified on the basis of the treatment. It seems that if there would have not been in any medication the values of CAS might have been higher in hypertensive patients than controls.

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