ENDOGENOUS DOPAMINE IN CARDIOVASCULAR TISSUES OF RABBIT

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SUMMARY: Present study involved the quantitative estimation of dopamine in cardiovascular tissues of rabbit. Tissues of heart, carotid and femoral arteries and aortae as well as blood were included in this study. Among all the tissues, carotid arteries showed highest dopamine contents while among the tissues of heart auricles contained highest concentration of Dopamine. The results suggested a possible physiological role of dopamine at the level of carotid arteries.

However, further studies are required to establish the role of cardiovascular dopamine receptors in the over all cardiovascular homeostasis.

Key Words: Dopamine, cardiovascular, quantitative estimation, physiological role.

INTRODUCTION

The third biogenic amine Dopamine (DA), 2-(2, 4 dihydroxy phenyl ethyl amine) has been extensively studied regarding its anatomical distribution biochemistry and pharmacology in the central nervous system. Chemically synthesized in 1910 by Mannich and Jacobsohn (1), was recognized as a vital intermediate in the biosynthesis of Noradrenaline by Blaschko in 1939 (2). Now it has been confirmed as a putative neurotransmitter on both sides of blood brain barrier, with the help of both biochemical and electrophysiological approaches. It is also proved that dopamine has in fat got its own receptors and neuronal tracts (3,4). CNS content of dopamine constitutes more than 50 percent, while on the peripheral sites it represents 1-5 percent of total catecholamine pool in sympathetically innervated tissues like atrium, mesenteric artery, etc., (5). Although, a lot of work has been reported in cardiovascular system related to receptor pharmacology of dopamine, but there is a limited information on quantitative estimation of DA in the cardiovascular tissues. Much of the earlier work on the quantitative analysis of DA involved only the nervous tissues and brain (6, 7). The work reported on blood, plasma and urine dopamine contents was with reference to change in DA levels during various pathological states. Abnormally low plasma NA: DA ratios were reported in patients with essential hypertension (8). Others have also reported significant elevation of urinary DA concentration during sodium loading (9). Dopamine reported in plasma and urine was found to be peripheral in origin since small amounts of catecholamines that may leak out of the brain are converted into metabolites before entering the peripheral circulation (10).

Role of dopamine in the biological system and its mode of action can be established by estimating the actual quantity of DA in the cardiovascular tissues.

The present study involved the quantitative estimation of dopamine in cardiovascular tissues of rabbit. The study was designed with an object to provide a preliminary work encompassing complete quantitative data of dopamine in cardiovascular tissues of a single species.

By estimating the actual quantity of dopamine in these tissues, its role in vascular system and heart could be suggested both from physiological and pathological point of view.

MATERIAL AND METHODS

Chemical methods are widely used for assay of extremely small amounts of circulating catecholamines (11, 12). Fluorimetric technique offer required sensitivity and is among the highly sophisticated and sensitive technique, used for the detection of

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catecholamines in the biological material. The dopamine extraction from the tissues and blood was carried out by the solvent extraction method of Brownlee and Spriggs (3), using butanol as a solvent.

GENERAL METHOD

Healthy rabbits 1.00-2.00 kg., were selected for experiments. They were divided into five batches, having five rabbits in each. Each batch was used for single experiment. Food and water was given to them *ad-libitum*.

Animals were killed by a blow on the neck and the cardiovascular tissues (Aorta, right ventricle, left ventricle, Auricle, right and left carotids, right and left femorals) were removed blotted on filtre paper and their weight was noted. 10 ml blood was also withdrawn.

Tissues and blood were immediately frozen at -20°C, for storage purpose and analyzed when required.

SOLVENT EXTRACTION METHOD

The frozen tissues were homogenized in 30 ml butanol. This homogenate was then transferred to a 125 ml, stoppered bottle and 2 ml of 0.01 N HCl and 5 g sodium chloride were added. The bottle was then shaken at room temperature with a speed of 50 strokes per minute for one hour. The mixture was then centrifuged at 2000 rpm for five minutes.

The 25 ml of the organic phase was withdrawn into another stoppered bottle 50 ml of n-heptane and 7 ml of 0.01 HCl were added; then the bottle was shaken for five minutes. At this stage catecholamines had passed into the aqeous phase. The mixture was centrifuged for two minutes at 2000 rpm and the aqueous phase was withdrawn.

Assay of Dopamine

To 2.5 ml of the 0.01 N HCl acid extract (aqeous phase) was added, 0.5 ml of 0.1 M phosphate buffer (pH 6.5). The pH was then adjusted to 6.46-6.45 by dropwise addition of 0.5 M and 0.1 M potassium carbonate. Adjustment of pH is critical. Aliquots of 2x1.2 ml were placed into two seperate test tubes, one for the test and other for the blank.

To each test tube 0.5 ml of Iodine-potassium iodide solution was added. After five minutes 0.5 ml alkaline sulphite solution was added to the test and 0.5 ml of 2.5 N Sodium-hydroxide was added to the blank. After another five minutes 0.06 ml of 2.5 N acetic acid was added to both test and the blank and these were then irradiated vertically under ultraviolet lamp for 20 minutes, then 0.5 ml water was added to the test 0.5 ml 1 M sodium sulphite solution to the blank. The fluorescence was measured for dopamine at 325 m (excitation) and 378 (emission) wavelength respectively on Jasco FP-550 spectrophotometer. Recovery ranged between 70-80 percent. Mean recovery value was found to be 78 percent throughout the study.

Table 1: Showing concentration of dopamine (μg/g) in the cardiovascular tissues of rabbit.

		Concentration dopamine (μg/g)					
S.No	Type of tissues	1	2	3	4	5	Mean±SEM
1	Carotid artery	(0.710) 0.633	(0.70) 0.625	(0.73) 0.509	(0.80) 0.750	(0.70) 0.540	(0.728) 0.6114±0.037
2	Femoral artery	(0.710) 0.400	(0.70) 0.465	(0.73) 0.432	(0.73) 0.500	(0.75) 0.655	(0.728) 0.718±0.490
3	Aorta	(0.710) 0.364	(0.73) 0.477	(0.70) 0.305	(0.70) 0.382	(0.70) 0.357	(0.708) 0.377±0.025
4	Auricles	(0.710) 0.209	(0.730) 0.173	(0.68) 0.181	(0.70) 0.164	(0.69) 0.156	(0.702) 0.177±0.008
5	Right ventricle	(0.700) 0.056	(0.70) 0.047	(0.70) 0.059	(0.70) 0.058	(0.70) 0.053	((0.700) 0.055±0.002
6	Left ventricle	(0.700) 0.039	(0.700) 0.047	(0.700) 0.042	(0.68) 0.042	(0.70) 0.053	(0.702) 0.0446±0.002
7	Blood	(0.680) 0.024	(0.73) 0.026	(0.68) 0.015	(0.73) 0.037	(0.70) 0.024	(0.704) 0.025±0.003

SEM: Standard eror of mean.

Values in parenthesis represent values for control (Iug/ml).

RESULTS

Table 1 shows the complete data of dopamine contents in cardiovascular tissues and blood of rabbit (n=5). Dopamine was measured in μg/g and μg/ml for tissues and blood respectively. Among all the tissues, blood vessels represent the highest dopamine contents (0.611±0.04 g/ml), while the blood of rabbit shows the lowest value (0.025±0.003 μg/ml) within the blood vessels, carotid arteries represents highest percentage ranging between 77-99 percent with the mean value of 83.9 percent, while among the tissues of heart analysed auricles contain the higher concentration of dopamine 19.2-29.4 percent with mean of 25.2 percent.

DISCUSSION

In the present study we used rabbit for quantitative estimation. Both *in vivo* and *in vitro* studies have shown the existence of specific DA receptors in coronary cerebral, hepatic, femoral, mesenteric carotid and splenic blood vessels, (14-17). The blood vessels chosen for study were carotids, femoral arteries and aortae, since those are the major blood vessels that are involved in circulatory homeostasis.

The results when compared showed that the DA contents were higher in vessels as compared to that of heart tissues of rabbit.

Among all the tissues the carotid arteries have the highest DA (0.6114±0.37μg/g).

Carotid bodies supplying DA to carotid arteries are provided with dopaminergic interneurons. They are involved in the control of the sensitivity of the nerve terminals supplying to the carotid bodies. Our findings are in agreement with the studies reported by Roberts (18). He demonstrated that hypertensive responses to Nicotine and bilateral carotid occlusion were potentiated by Clebopride (DA antagonist), suggesting the possible involvement of DA and DA receptors on sympathetic ganglia and carotid bodies. Type I cells of carotid body chemoreceptors contain high levels of DA (19). After prolonged electrical stimulation of carotid sinus nerve, rabbit carotid body was estimated for catecholamines and it showed significant increase of DA and Adrenaline as well as of all their metabolites (20).

It might be concluded that carotid arteries are having high dopomine contents because of carotid body DA stores, which releases DA in response to chemoreceptor stimulation or by a tonic sympathetic discharge. The high contents of DA and presence of both types of DA receptors strengthens the possibility of Dopamine's physiological role in carotid arteries.

Femoral arteries represents DA content less than that of carotid arteries (0.49±0.039 μg/g) but was significantly greater than that of heart tissues.

Most of experiments carried out on the femoral arteries in anaesthetized dogs or on isolated preparations of animals have shown the existence of presynaptic DA inhibitory receptors on the sympathetic nerves supplying them. DA acts on these receptors to cause neurogenic vasodilation (14, 16). These receptors were found to have no physiological role (21). In aorta the lowest DA contents were present among the three vessels, estimated and there is no evidence of DA receptors in aorta. It might be suggested that there may be no function of dopamine in aorta. The small amount of dopamine in aorta, may be released from the sympathetic nerves innervating it. The quantity of DA is less in heart tissues as compared to that in blood vessels. In order of concentration, auricles represent highest DA contents within heart tissues.

Auricles > Right ventricle > Left ventricle.

The sympathetic innervation of heart is through cardiac sympathetic ganglia and cardioaccelerator nerve.

Both *in vitro* and *in vivo* studies using cat and dog heart have demonstrated that when presynaptic DA receptors are stimulated, there is an inhibition of positive chronotropic responses, produced by stimulating the right postganglionic cardioaccelerator nerve (22-24). Thus, DA has an indirect action in heart. It can be suggested that the sympathetic nerves and cardiac ganglia where it is stored with Noradrenaline. Neurogenic mechanism of action of DA has been reported in cat atrial slices (25). Bell (5) compared endogenous DA: NA ratios in different animals, the ratios for heart tissues showed little difference which suggests a rapid turnover rate of NA in cardiovascular tissues.

Thus DA may be having some physiological role in ganglionic transmission of heart, which is not yet clear, but it has no direct role in the myocardium and there is no dopaminergic innervation to the heart. It should also be considered here that the main neurotransmitter in heart is Noradrenaline and there is a dominating population of β-1 receptors in heart, while no DA receptors have been reported. The DA administered in large doses under some clinical situations (CHF, Shock) act as a direct ionotropic agent through receptors.

Another point worth considering here, is the rate limiting step of NA biosynthesis from hydroxylation of tyrosine to hydroxylation of DA in NA nerve terminals of heart (26, 27). The reduction in the number of adrenoreceptors with subsequent increase in DA receptors have also been reported in hypertrophied rat heart. These findings reflect possible pathophysiological role of DA in maintaining opti-

mal left ventricular output during hypertrophy and hypertension (28).

Our results showed the lowest concentration of DA in blood. This reflect various possibilities. Firstly, after the liberation from sympathetic nerves or dopaminergic nerves DA is partially destroyed by MAO and COMT and is taken back into the nerves, therefore, the circulating levels of DA are low. Under physiological conditions plasma DA levels are significantly low to exert any effect.

Plasma DA level may be served as a diagnostic parameter for certain diseases. It has found to be elevated in response to certain afferent stimuli haemorrhage and restraint (5).

Clinicaly heart failure in advanced stage is characterized by increased plasma levels of NA and DA (29). Takashaki *et al.* (30) studied plasma DA levels in various types of hypertension. There were also elevated DA levels reported in Pheochromocytoma, primary Aldosteronism and cystic fibrosis (31).

Although it is not yet clear, what role dopaminergic system play in cardiovascular homeostasis and pathophysiology of cardiovascular disorders the evidence suggested various possibilities.

DA in vascular area may have a role in controlling transmitter release and turnover, since DA antagonist increased NA out flow vascular area (32). It can also be suggested that under conditions where Noradrenaline release is excessive such as during contineous nerve stimulation DA release from sympathetic nerves is also elevated, then it may regulate neurotransmitter release through inhibitory presynaptic DA-2 receptors.

The tissues in which DA contents are low, there is a possibility that in these tissues DA receptors are present in low quantity and/or may not have an active role under physiological conditions. On the contrary DA at all levels suggests its neurogenic mechanism of action, through presynapic DA receptors in sympathetic nerves and ganglia (in peripheral sites). However, under clinical conditions such as hypertension cardiac Ischemia and CHF the DA concentration in plasma was found to be significantly high (30), suggesting a definite role of DA in cardiovascular homeostasis for which further studies are required.

REFERENCES

1. Mannich C, Jaccobsohn W : Über oxyphenyl-alkylamine and dioxyphenyl alkylamine. ber Deut Chem Ges, 43:189, 1910.

2. Blacshko H : The specific action of L-DOPA decarboxylase. J Physiol (London), 96:50, 1939.

3. Libet B : The role of SIF cells play in ganglionic transmission. In: Nonstraital dopaminergic neurons. Adv Bichem Psychopharmacol, ed by E Costa, GL Gessa Raven press, New York, 16:541, 1977.

4. Stoof JC, Kababian JW : Two dopamine recoptors: biochemistry, physiology and pharmacology. Life Sci, 35:2281, 1984.

5. Bell C : Dopamine: Precursor or Neurotransmitter in sympathetically innervated tissues. Blood vessels, 24:234, 1987.

6. Laverty R, Sharman DF : The estimation of small quantities of 3,4 dihydroxyphenyl ethylamine in tissues. Brit J Pharmac, 24:538, 1965.

7. Stjarne L, Lishajko F : Drug induced inhibition of Noradrenaline synthesis in vitro in bovine splenic nerve tissue, Br J Pharmac Chemother, 27:398, 1966.

8. Alexander RW, Gill JR Jr, Yamabe H, et al : Effects of dietary sodium and of acute saline infusion on the interrelationship between Dopamine excretion and adrenergic activity in man. J Neurochem, 28:677, 1977.

10. Ziegler MG, Lake CR, Wood JH, et al : Relationship between norepinephrine in blood and cerebrospinal fluid in the presence of a blood-cerebrospinal fluid barrier. J Neurochem, 28:677, 1977.

11. Ball SG, Oates NS, Lee MR : Urinary dopamine in man and rat: Effects of inorganic salts on dopamine excretion. Clin Sci Mol Med, 655:167, 1978.

12. Udenfriend S : Tyrosine and metabolites. In: Fluorescence assay in biology and medicine. Ed by S Udenfriend, B Horecker, NO Kaplan, J Marmur, HA Scheraga, Academic press, New York, Vol-I:137, 1969.

13. Brownlee G, Springgs TLB : Estimation of dopamine noradrenaline, adrenaline and 5-Hydroxytryptamine from single rat brains. J Pharm Pharmacol, 17:429, 1965.

14. Goldber LI : Cardiovascular and renal actions of Dopamine: Potential clinical applications. Pharmacol Rev, 24:1, 1972.

15. Goldberg LI, Hsich Y, Resnekov L : Newer catecholamines for treatment of heart failure and shock: an update on Dopamine and a first look on dobutamine. Prog Cardiovasc Dis, 19:327, 1977.

16. Lokhandwala MF, Barrett MF, Barett RJ : Cardiovascular and therapeutic implications. J Auton Pharmac, 3:189, 1982.

17. Amenta D, Cavallotte C, Collier WL, et al : Effects of Dopamine on cyclic adenosine monophosphate generating system in rabbit internal carotid and mid cerebral artery. Arch Int Pharmacodyn, 291:1988.

18. Roberts DJ : The pharmacological basis of the therapeutic activity of clebopride and related subsituted benzamides. Current Ther Res, 31:15, 1982.

19. Gonzalez ER, Rigual SJ, Fidone C : Mechanisms of termination of action of Dopamine in carotid body chemoreceptors. J Autonom Nerv System, 18(3):249, 1987.

20. Armenguad C, Leiternee LM, Surta JF : Changes in monoamine content of rabbit carotid body following prolonged electrical stimulation of carotid sinus. Neuroscience Lett, 78(1):46, 1987.

21. Dupont AG, Lefebre RA, Bogaert MG : Identification and characterization of peripheral neuronal Dopamine receptors in rat. Clin Exp Theory Prac, A9(5 and 6):913, 1987.

22. Lokhandwala MF, Walkins MD, Sobouni MH, Alkhadi KD : The pharmacological analysis of the actions of SKF 82526 on cardiovascular Dopamine receptors. J Pharmacol Exp Ther, 234:337, 1985.

23. Lokhandwala MF, Buckley JD : The effect of L-DOPA on peripheral sympathetic nerve function: Role of peresynaptic Dopamine receptors. J Pharmac Exp Ther, 204:362, 1978.

24. Ilhan M, Long JP, Cannon JG : Dopaminergic activity on non-hydroxylated amminotetralin derivative on cat hearts. Arch Int Pharmacodyn, 271:213, 1984.

25. Langer SZ, Vidal M, Duval N : Presynaptic Dopamine receptors in cardiovascular system. Clin Exp Theory and Prac A9 (5 and 6):837, 1987.

26. Sole MJ, Kamble AB, Hussain MN : A possible change in rate limiting step for norepinaphrine synthesis in the cardiomyopathic sysian hamster. Circ Res, 41:814, 1977.

27. Francis GS : Neurohumoral mechanism involved in congestive heart failure. Am J Cardiol, 55:15A, 1985.

28. Fadda G, Shamas NW, Birbari A, et al : Dopamine receptors in hypertrophied rat heart. Gen Pharmac, 18(6):665, 1987.

29. Ginsburg R, Bristow MR, Billingham ME, et al : Study of the normal and failing isolated human heart: increased response of failing heart to isoproterenol. Am Heart J, 100:535, 1983.

30. Tokahashi M, Muira Y, Sano N, et al : Plasma dopamine concentrations in various types of hypertension. Folia Endocrinol, 62:713, 1986.

31. Martin SH, Turler K, Kaser H, Kreamer K : Plasma and urinary catecholamines in patents with cystic fibroses. Pediatr Res, 19(1):47, 1985.

32. Shikuma R, Yoshimura M, Kambara S, et al : Dopaminergic modulation of salt sensitivity in patients with essential hypertension. Life Sci, 38:915, 1986.

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