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Preparation of Labelled dopamine by ^{125}I using Lactoperoxidase and N- Bromosuccinamide

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Abstract

The present study was carried out to prepare ^{125}I- dopamine Tracer, using oxidizing agents such as Lactoperoxidase and N-Bromosuccinamide , which was purified by column sephadex G-75 and used to study the biodistribution in normal Albino Swiss mice and the results indicate the possibility of using [^{125}I] iodo dopamine as myocardial imaging agent. The conditions of radioiodination such as concentration of oxidizing agent, reaction time, concentration of Atenolol and effect of pH were studied to get maximum yield. Stability study of dopamine tracer was carried out.

Keywords: *Radioiodine-^{125}/ dopamine Molecule as β_1 1-Receptor / Lactoperoxidase/ N- bromosuccinamide.*

1 Introduction

There are many types of receptors such as dopamine receptors [1,2,3], acetylcholine receptors [4-6], serotonin and 5HT2A receptors [1,7], adrenergic receptors [8-11], estrogen receptors [12,13], and steroid receptors [14] Basically, one approach to the development of a new radioiodinated myocardial agent is the

radioiodination of drug analogues that exert cardio selective pharmacological action (i.e. competitive adrenoceptors blockade drugs). That is due to those adrenergic receptors which are cell membrane sites control fat cell metabolism and fat cell tissue growth [15].

Recently, radioiodination of organic compounds and bimolecular have been the subject of interest of many investigators [16, 17]. That is due to that iodine atom occupies a similar volume to that of methyl or ethyl group and can substitute for an alkyl group in an organic molecule without unduly perturbing the steric or polar configuration [18, 19]. On the other hand, carbon-iodine bond has similar polarities to carbon-carbon bond. An oxidizing agent such as Lactoperoxidase and N- Bromosuccinamide must normally be present to oxidize I2 to a better electrophile (i.e. iodonium ion I+) [23,24].

In this work, a simple method for radioiodination of dopamine has been investigated. dopamine is used in nuclear medicine a selective β_1-adrenoceptor blocking drug due to its interaction with these receptors afforded a significant effect on cardiac rhythm and automaticity. The radioiodine atom was incorporated in the dopamine molecule (Fig. 1) via it s aromatic ring which facilitate the electrophilic radioiodination process [22].

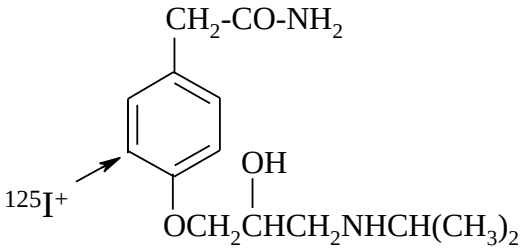


Fig. 1: Structure formula of Dopamine

2 Materials and Methods

All chemicals and reagents used in this work obtained from sigma Chemical Co., USA, Ethanol, dopamine , / Lactoperoxidase , N- Bromosuccinamide Sephadex, Disodium hydrogen phosphate, Sodium chloride, Sodium iodide Na125I (5 mCi/50 ul) was delivered from (Isotop, Budapest) pH 7-11 . Animals: Albino Swiss types weighing 22 g were used for the biodistribution studies.

2.1 Preparation of ^{125}I-dopamine

a: Lactoperoxidase method

The procedure of Fraker and Speck (1978) was used for iodination. The method for preparation of ^{125}I- dopamine is summarized as follows: to an Eppendorf tube, 10 µl phosphate (0.05M, pH 7,4) containing 10 µg dopamine was dispensed followed by addition of 20 µl 0.5 M phosphate buffer (pH 7.4). This mixture was mixed with 10µl/Na ^{125} I (1 mCi) and the reaction was started by the addition of 10 µl solid phase lactoperoxidase 1:10 followed by 10 µl H_2O_2 according to Jyotsna etal., 1986. The reaction mixture was vortexed , and 10 µl H_2O_2 solution was added after 10 minutes. The iodination vial was vortex, every 5 minutes throughout the reaction . After 30 minutes the reaction was stopped by the addition of 100 µl 0.05M phosphate buffer (pH7,4) containing 0.1 sodium azide and 1% bovine. The separation and purification using sephadex G-75 column.

b: N - Bromosuccinamide method

The procedure was carried out according to Reay (1982) . Into a micro Eppendorf tube, 10µl of (0.5M) $PO4^-$ (pH= 7.4) and 0.5 mCi ofNa- ^{125}I were added succesively to 10 µl of dopamine (20µg). The reaction was initiated by adding 10µl of N-bromosuccinamide (50µg) as oxidizing agent. It was allowed to proceed for 3 min and the reaction was quenched by using 200 µl of 0.5M phosphate buffer or 10 µl of $Na_2S_2O_5$ (300 µg). The labelled hormone was transefered to gel filtration on a sephadex column to separate the iodine and any damaged ^{125}I- dopamine

2.2 Initial Method of Radioiodination for Centralization of the Factors Affecting the Yield %

Exactly weighed 100 µg dopamine in 0.1 ml absolute ethanol was transferred to a v-shaped bottom reaction vial then 10 µl Na^{125}I (9.25 MBq) was added,10µl solid phase lactoperoxidase 1:10 followed by 10µl H_2O_2. The reaction vial was left at ambient room temperature up to 25 $\pm$ 1^{o}C for 30 min, the reaction was stopped by the addition of 100µ l 0.05M phosphate buffer (pH7,4) containing 0.1 sodium azide and 1% bovine then subjected to chromatographic and electrophoresis analysis as the following :

2.2.1 Chromatographic analysis:

Thin layer chromatographic analysis (TLC)

Thin layer chromatography (TLC) was used for determination of radiochemical yield and radiochemical purity. TLC plates : Silica Gel G-60, Solvent : chloroform : water : ammonia [90 : 8 : 2 (v/v/v)] and Rf of [^{125}I] iodo dopamine = 0.7

2.2.2 Electrophoresis

Electrophoresis process consists of applying a radioactive sample on a paper or polyacrylamide gel soaked in a suitable buffer and applying an appropriate voltage across the paper of gel for certain period of time. The components of the sample move to different positions along the paper or gel medium, depending on their charge and ionic mobility [23].

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2.2.3 Electrophoresis conditions

Electrophoresis was done with EC 3000 p-series programmable (E.C. apparatus corporation) power and chamber supply units using cellulose acetate strips. These strips were moistened with 0.05 M phosphate buffer pH 7.2 and then were introduced in the chamber. Samples (5 μl) were applied at a distance of 10 cm from cathode. Standing time and applied voltage were continued for one and half hours. Developed strips were dried and cut into 1 cm segments and counted by a well-type NaI scintillation counter. The radiochemical yield was calculated as the ratio of the radioactivity of the labeled product to the total radioactivity.

$$\text{Radiochemical yield}\,(\%) = \frac{\text{Peak activity of the product}}{\text{Total activity}} \times 100$$

2.2.4 In-vivo Evaluation of [^{125}I] iodo dopamine

In-vivo experiments were carried out in male Albino Swiss mice. At time 0, animals were injected intravenously in tail vein with [^{125}I] iodo dopamine (0.2 MBq/g). Animals were euthanized from 0-2 h after injection of the tracer, mice were sacrificed by cervical dislocation. Blood samples were obtained by heart puncture. The tissues were removed, washed, weighted and counted; heart, lung, liver, spleen, kidney, stomach, intestine, thyroid, brain, bone and sample from muscle. injected dose per gram tissue.

3 Results and Discussion

3.1 Effect of Oxidizing Agent Amount

The results obtained revealed that electrophilic radioiodine generated by Lactoperoxidase and N- Bromosuccinamide as shown in Fig. (2). At higher amount of oxidizing agent, the radiochemical yield decreases may be due to iodinated side products formed.

Analysis of the The counting tubes including standard equivalent to 1 % of injected dose were assayed in a well gamma counter and the results were evaluated as percent if reactions produced from the reaction by electrophoresis results in three peaks, one corresponding to free iodide which moved towards the anode with 20 cm distance at the condition mentioned earlier. The second remained at spoting point and the third fraction was also migrated towards the anode to a lesser extent equals to 10 cm. The species that stayed at the spoting point was found to be identical to that of [^{125}I] iodo dopamine under the same electrophoresis condition developing system consists of 0.05 M phosphate buffer of pH 7.2

The work presents here indicates that the incorporation of iodonium ion (^{125}I+) occurs in the C-3 position of the reactive ring of the dopamine molecule which may be categorized as electrophilic substitution reaction proceeds well at ambient room temperature up to 25oC. The reaction takes place by using iodinating agents, Na^{125}I, N- Bromosuccinamide and Lactoperoxidas. Certain parameters affecting the electrophilic substitution reaction were investigated to achieve the desired radiochemical yield (%) of [^{125}I] iodo dopamine lyophilized solution of Na^{125}I.

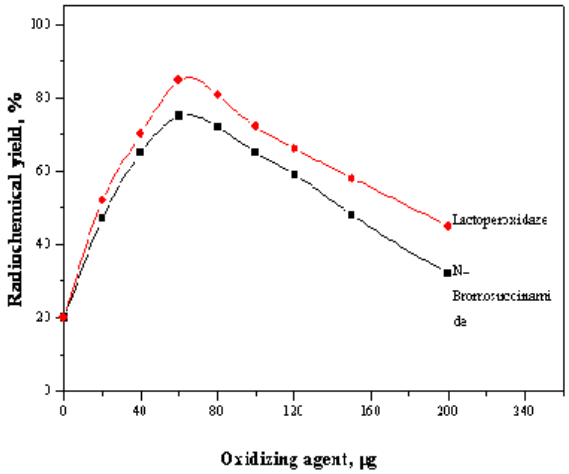


Fig. (2) : Variation of radiochemical yield of [^{125}I] iodo dopamine as a function of oxidizing agent amount (Lactoperoxidase and N- Bromosuccinamide).

3.2. Effect of Reaction Time

The reaction was carried out at certain time intervals at ambient room temperature up to 25^{o}C. The reaction proceeds well by increasing the time of exchange up to equilibrium (40 min and 3 minutes) with a radiochemical yield 92 %and 88% for Lactoperoxidase and N- Bromosuccinamide (Fig. 3 & 4) respectively.

3.2.1.Effect of dopamine Amount

The radiochemical yield was found to increase with increasing in the substrate amount as shown in Fig. (5). This can be explained by increasing the interaction between molecules of substrate and radioiodine till certain extent after which equilibrium was obtained.

3.2. 2.Effect of pH of Reaction Mixture

The initial results obtained revealed that the radiochemical yield increases gradually up to 93% and 88 % at pH 7in case of Lactoperoxidase and N-Bromosuccinamide) respectively then, decreases dramatically on increasing the pH (Fig.6). This is due to that increasing of the alkalinity leds to formation of hypoiodite ion, where the later being transformed to iodate and iodide ions.

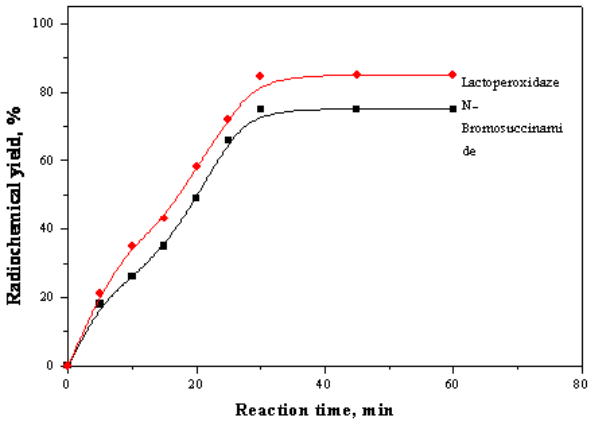


Fig. (3): Variation of radiochemical yield of [^{125}I] iodo dopamine with reaction Time using Lactoperoxidase and n- bromosuccinamide .

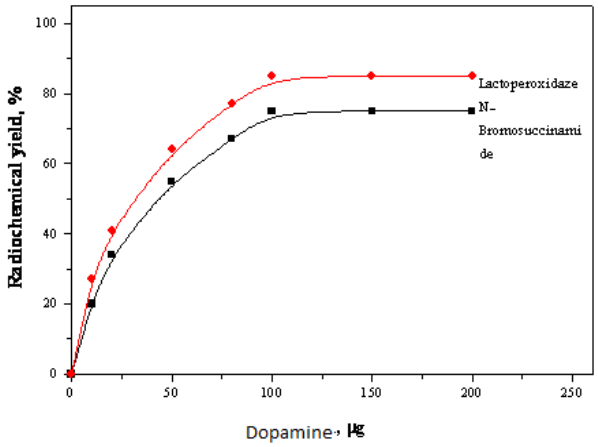


Fig. (4): Variation of radiochemical yield of [^{125}I] iod dopamine with substrate Amount (atenolol) using N- Bromosuccinamide and lactoperoxidase.

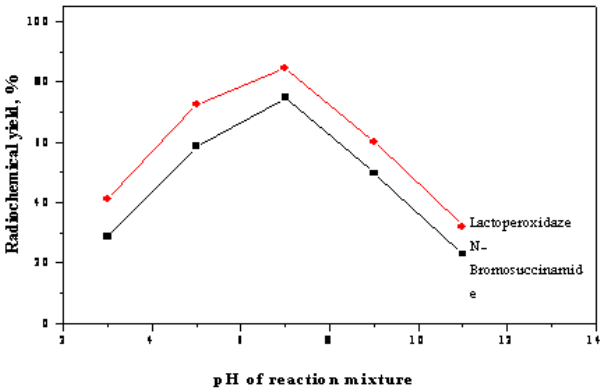


Fig. (5): Variation of radiochemical yield of [^{125}I] iodo dopamine with pH of reaction Mixture using Lactoperoxidase and N- Bromosuccinamide as oxidizing agents.

3.2 .3.Biodistribution in Normal Mice

Organ distribution studies were carried out in a group of 8 male normal Albino Swiss mice. Each animal was injected in the tail vien with 0.2 ml solution containing 8 KBq of [^{125}I] iodo dopamine The mice were put in metabolic cages for the recommended time then sacrificed. The organs as well as other body parts were dissected. Activity in each organ was counted and expressed as a % of the injected activity / organ. The weights of blood, bone and muscles were assumed to be 7, 10 and 40 % of the total body weight, respectively. Correction was made for background radiation and physical decay during the experiment in counts per minutes (CPM) (23).

$$\% \text{ Injected Dose / Organ} = \frac{\text{Organ CPM}}{\text{Standard CPM}} \times 100$$

3.2.4 .In-vivo Biodistribution

The results of in-vivo biodistribution studies carried out in normal Albino Swiss mice at different time intervals after administration of [^{125}I] iodo dopamine were shown in Table (1). The blood uptake was 8.9% at 15 min post injection and decreased to 3.9 % at 2 h, indicating that the labeled compound cleared from the systematic circulation within 2 h after administration. The liver uptake was increased slowly by time which may be due to the hepatobilary excretion pathway of the drug. The heart tissue uptake was high as cleared from the table and hence [^{125}I] iodo dopamine can be used safely as myocardial imaging agent. The renal uptake is higher than that the hepatic uptake indicated that the labeled compound excreted mainly through the urinary system. The thyroid uptake was increased by time from 15 min – 2 h indicating that [^{125}I] iodo dopamine undergoes in-vivo degradation during this time. The brain uptake was low indicating that this tracer can cross blood brain barrier with relatively low percent and so the tracer has low lipid solubility.

Table (1): Biodistribution of [^{125}I] iodo dopamine in normal Albino Swiss mice

Organs & Body fluids	Time post injection (min) / % I. D. / g tissue after intravenous administration			
	15	30	60	120
Blood	8.9	26	13.7	3.9
Bone	0.7	4.2	3.1	3.5
Muscle	0.2	0.8	0.9	0.9
Liver	0.5	0.5	1.7	1.5
Intestene	11	5,4	3.2	2.1
Stomach	6	6.3	6.6	7.0
Lung	3.1	2.6	1.0	0.1
Spleen	7.8	3.6	3.0	3.3
Heart	9.6	5.3	15.2	12.5
Thyroid	3.6	7.9	10.6	16.8
Kidney	1.5	1.0	0.2	0.1
Brain	5.0	5.8	1.7	3.1
Urine	7	8	2	4

4 Conclusion

The obtained results permit the following conclusions:

1- The technique has proven to be useful to incorporate radioiodine radionuclides onto the ring of the dopamine molecule (as a reactive group) at ambient room temperature up to 25^{o}C in reasonable time up to 40 min.
2- An advantage of reactions of this type is the accessibility of classes of compounds that are thermolabile and other biomolecules containing reactive groups such as phenol, imidazoles and indoles without perturbation of initial structure of the substrate molecules.

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