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Radioiodination and Biological Evaluation of Epinephrine as a Possible Adrenergic Receptors Imaging Agent

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Authors' contributions

This work was carried out in collaboration between all authors. Author AMA designed the study, wrote the protocol and managed the analyses of the study. Author SMA did the practical section and managed the literature searches. Author ITI performed the statistical analysis and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

An adopted method for the preparation of high radiochemical purity [^{125}I] iodoepinephrine was developed in order to characterize the binding properties of adrenergic receptors. Direct radioiodination of epinephrine was carried out using chloramine-T as oxidizing agent. The reaction proceeds well within 15 min at ambient room temperature up to 25±1ºC and afforded a radiochemical yield up to 94%. Different chromatographic techniques (electrophoresis and paper chromatography) were used to evaluate the radiochemical yield and purity of the labeled product. Biodistribution studies were carried out in normal Albino Swiss mice and the results showed rapid and high cardiac uptake of ^{125}I-epinephrine. The result indicates the possibility of using radioiodinated epinephrine as myocardial imaging agent.

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1. INTRODUCTION

There are many types of receptors such as dopamine receptors [1,2], σ-receptors [3], acetylcholine receptors [4-6], serotonin and 5HT2A receptors [1,7], adrenergic receptors (8-11), estrogen receptors [12,13] and steroid receptors [14].

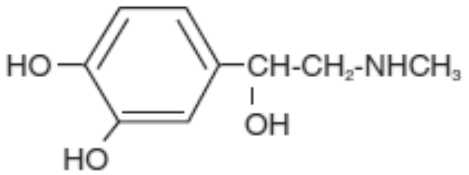
Basically, one approach to the development of a new radioiodinated myocardial agent is the radioiodination of drug analogues that exert cardio selective pharmacological action (i.e. competitive β-adrenoceptors blockade drugs). This is due to the fact that β-adrenergic receptors which are cell membrane sites control fat cell metabolism and fat cell tissue growth [15].

Recently, radioiodination of organic compounds and biomolecules has been the subject of interest of many investigators [16,17]. This 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 chloramine-T (N-chloro-p-toluene sulfonamide sodium salt) must normally be present to oxidize I_2 to a better electrophile (i.e. iodonium ion I^+) [20,21].

The adrenergic (or sympathetic) nervous system (ANS) play central roles [22,23]. The ANS exerts a wide variety of cardiovascular effects, including heart rate acceleration (positive chronotropy), increase in cardiac contractility (positive inotropy), accelerated cardiac relaxation (positive lusitropy), accelerated atrioventricular conduction (positive dromotropy), decrease in venous capacitance, and constriction of resistance and cutaneous vessels. All of these effects aim to increase cardiac performance to prepare and enable the body for the so-called “fight or flight response.” ANS activation in the cardiovascular system translates into release of the two catecholamines that mediate its effects, i.e., norepinephrine (NE or noradrenaline) and epinephrine (Epi or adrenaline) [24]. Epi released into the circulation by the adrenal medulla, affecting both the myocardium and peripheral vessels, and local release of NE and Epi by various peripheral adrenergic nervous systems that can synthesize and release these catecholamines in an autocrine/paracrine manner and are located in blood vessels and in cardiac myocytes themselves [25,26]. The ANS neurotransmitters NE and Epi mediate their effects in cells and tissues by binding to specific cell surface Adrenergic receptor (ARs).

The principal role of Beta-Adrenergic receptors (βARs), a family of prototypical G-protein-coupled receptor (GPCRs), in the heart is the regulation of cardiac rate and contractility in response to NE and Epi. The human heart contains all three βAR subtypes [26]. The receptors play critical roles in the regulation of cardiovascular [27] and pulmonary function [28], as well as other physiological processes. βAR subtypes differ in their affinities for some agonists and antagonists and thus may potentially impart different cellular effects based on this ligand-binding specificity [29]. Epinephrine has a higher affinity for the $beta_2$ receptors when compared to the $alpha_1$ receptors as shown in Fig. 1. Therefore, the effect of epinephrine is dependent on which type of receptor is occupied. The receptor occupancy is dependent on the concentration of a drug and its equilibrium dissociation constant. At low doses, epinephrine can selectively stimulate $beta_2$ receptors, thus producing muscle relaxation and a decrease in peripheral resistance. At high doses, epinephrine produces contraction of vascular smooth muscle and an associated increase in peripheral resistance [30].

In this work, a simple method for radioiodination of epinephrine has been investigated. Certain reaction parameters affecting the rate of the reaction including the effect of the hydrogen ion concentration, the substrate and oxidizing agent amounts and the reaction time have been investigated. The method afforded a high radiochemical yield of pure ^{125}I-epinephrine.



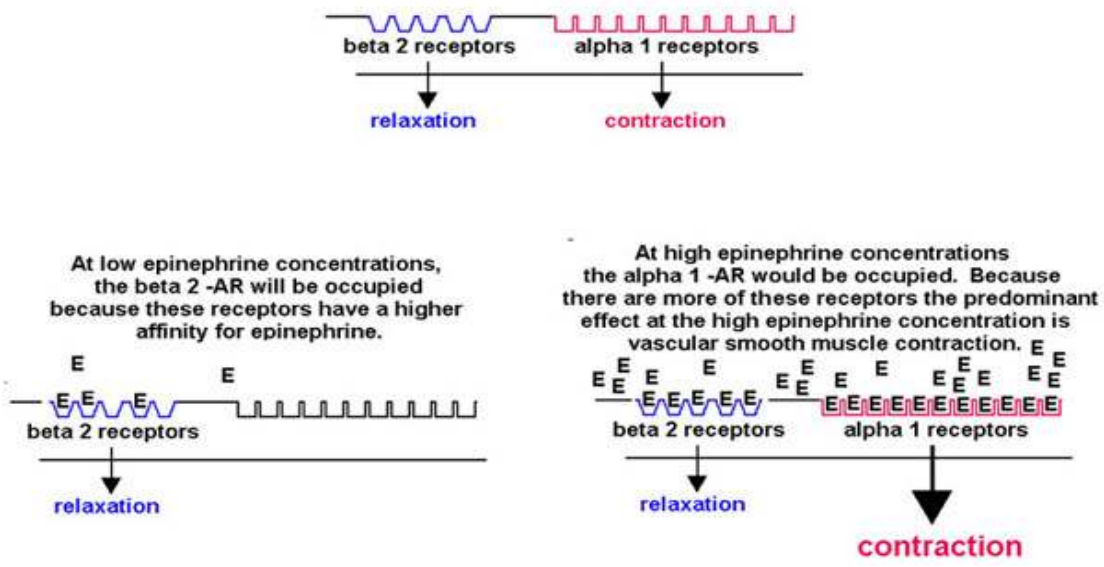


Fig. 1. The structure of epinephrine and its receptors occupancy[29]

2. EXPERIMENTAL

2.1 Materials

- All chemicals and reagents used in this work were of analytical grade and used without further purification.
- Sodium iodide $Na^{125}I$ (185 MBq / 0.1 ml) was delivered from Nordion (Belgium) as a carrier free and reductant free solution. Solution pH was adjusted to pH 7 before use.
- *Animals:* Albino Swiss type mice weighing 30±2 g were used for the biodistribution studies.

2.1.1 Initial method of radioiodination of epinephrine for optimization of the factors affecting the Yield %

In a screw caped reaction vial, an appropriate ethanolic solution of the substrate with the desired concentration and a suitable activity of $Na^{125}I$ (7–15MBq) were added, followed by the addition of the oxidizing agent (CAT).The pH value of the reaction mixture (pH2-11) was varied using different buffer systems. The reaction was allowed to proceed for a chosen interval of time at different temperatures, after which the reaction was terminated by the addition of 50µl of aqueous solution of $Na_2S_2O_5$ (20 mg/ml) to ensure that all the unreacted iodine is in a reduced form before chromatographic analysis.

2.2 Chromatographic Analysis

2.2.1 Paper chromatographic analysis (TLC)

Paper chromatography was used for the determination of radiochemical yield. On Whatman paper sheet (1 cm width and 13 cm length), 1–2 µl of the reaction mixture was placed 2 cm above the lower edge and allowed to evaporate spontaneously. For development a fresh mixture of chloroform: water: ammonia [90: 8: 2 (v/v/v)] was used as a mobile phase. After complete development, the paper sheet was removed, dried, and cut into strips, each strip is 1 cm width, and then each strip was counted in a well type γ-counter where radioiodide (I^-) remained near the origin (R_f=0–0.1), while the ^{125}I-epinephrine moved with the solvent front (R_f=0.8).

2.2.2 Electrophoresis

Electrophoresis was done using cellulose acetate strips. These strips were moistened with 0.05M phosphate buffer pH 7.2+0.2 and then were introduced in the chamber. Samples (5 µl) were applied at a distance of 10cm 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. The components of the

sample move to different positions along the paper, depending on their charge and ionic mobility [31].

Radiochemical yield (%) =

$$\frac{\text{Peak activity of the product}}{\text{Total activity}} \times 100$$

3. RESULTS AND DISCUSSION

This work presents the incorporation of iodonium ion ($^{125}I^{+}$) in the C-3 position of the reactive ring of the epinephrine molecule which may be categorized as electrophilic substitution reaction proceeding well at ambient room temperature up to 25±1°C. The reaction takes place by using epinephrine, $Na^{125}I$ and chloramine-T. Certain parameters affecting the electrophilic substitution reaction were investigated to achieve the desired radiochemical yield (%) of ^{125}I-epinephrine.

3.1 Effect of Oxidizing Agent Amount

The results obtained revealed the effect of the amount of oxidizing agent (chloramine-T) on the radiochemical yield of ^{125}I-epinephrine under the same reaction conditions (Table 1). At 100µg CAT maximum labeling yield was obtained. At a concentration of 25 µg of chloramine-T, the yield of ^{125}I-epinephrine was 80% and this may be attributed to the insufficiency of chloramine-T to oxidize all iodide ions to iodonium ions which are the reactive ions. Also, a slight decrease in the radiochemichal yield of ^{125}I- epinephrine was observed while increasing chloramine-T concentration up to200 µg. The excess of chloramine-T concentration undesirable to avoid non-reacted oxidized species which interfere with the labeling reaction giving rise to undesirable side products.

3.2 Effect of Reaction Time

The reaction was carried out at certain time intervals at ambient room temperature up to 25±1ºC. The obtained results are illustrated in Table 2. From this table, It can be concluded that the reaction is fast as the yield of ^{125}I-epinephrine after 1-minute reaction time was 86.9%. The reaction proceeds well by increasing the reaction time up to equilibrium (15 min) with a maximum radiochemical yield ~ 95 %. Upon increasing the reaction time to 60 minutes, the radiochemical yield showed small decrease. This can be attributed to the long exposure of epinephrine to the harsh oxidation conditions of chloramine-T which causes chlorination [32], polymerization and denaturation of substrate [33,34]. So there is no need to increase the reaction time over 15 minutes.

3.3 Effect of Epinephrine Amount

The quantity of epinephrine used during this study varied between 25 to 200 µg. The radiochemical yield was found to increase with increasing the substrate amount as shown in Table 3. This can be attributed to increase in the interaction between the molecules of the substrate and the radioactive iodine atoms till certain extent after which equilibrium was obtained. The data presented in the table clearly show that the optimum radiochemical yield of ^{125}I- epinephrine was obtained when 75 µg of epinephrine was used. By increasing the amount of epinephrine up to 200µg, the yield of ^{125}I-epinephrine decreased.

Table 1. Effect of chloramine-T (CAT) content on the radiochemical yield of ^{125}I-epinephrine

CAT (µg)	% Labeled compound	% Free iodide
25 µg	80.0±0.40	20±0.3
50 µg	85.5± 0.50*	14.5±0.75
100 µg	94.3±0.04*†	5.7±0.06
150 µg	94.7±0.04*	5.3±0.06
200 µg	89.5 ±0.32*†	11.5 ±0.35

Values represent the mean ± SEM n=6; Significantly different from the initial values using student's t- test (P<0.05);† Significantly different from the previous values using student's t- test (P<0.05)*

Table 2. Effect of reaction time on the % radiochemical yield of ^{125}I- epinephrine

Time/min.	% Labeled compound	% Free iodide
1	86.9±0.36	13.1±0.55
5	87.7±0.29*	23.3±0.9
15	94.8±0.35*	5.2±0.15
30	95.8±0.35*	4.2±0.15
60	92.7±0.14*†	5.3 ±0.25

Values represent the mean±SEM n=6 ; Significantly different from the initial values using unpaired student's t-test (P<0.05) ;† Significantly different from the previous values using unpaired student's t-test (P<0.05)*

3.4 Effect of pH of the Reaction Mixture

The influence of the pH of the reaction medium on the radiochemical yield of ^{125}I- epinephrine

was studied. The reaction was carried out in the pH range from 1 to 11 using the appropriate buffers. The data of this study are presented in Table 4 which shows that a high radiochemical yield of ^{125}I- epinephrine was obtained at pH 7 (95.5 %). This may be due to the fact that chloramine-T works well as oxidizing agent around pH 7. Also, the good labeling yield of ^{125}I-epinephrine around pH 7 may be due to the protonation of the ring at this pH value giving H+ which was substituted by the active iodonium ion I+. When the pH of the reaction medium was shifted towards the acidic side, the yield was very poor reaching 18.4% at pH 1. Also, the yield decreased drastically reaching 79 % at pH 11.

Table 3. Effect of epinephrine content on the radiochemical yield

Epinephrine (µg)	% Labeled compound	% Free iodide
25	80.4±0.31	191.6±0.41
50	89.0±0.36*	11.0±0.25
75	94.7±0.18*	5.3±0.28
100	94.5±0.40*†	5.5±0.35
200	94.5±0.26*	5.5±0.30

Values represent the mean ± SEM n = 6
** Significantly different from the initial values using unpaired student's t-test (P<0.05)*
† Significantly different from the previous values using unpaired student's t-test (P<0.05)

Table 4. Effect of pH of the reaction medium on the radiochemical yield of ^{125}I-epinephrine

pH value	% Labeled compound	% Free iodide
1	18.40±0.11	81.6±0.15
4	94.0±0.30*	6.0±0.2
7	95.5±0.30*	4.5±0.20
9	95.6±0.44*†	4.6 ±0.25
11	79.0±0.20*†	21.0±0.4

Values represent the mean±SEM n=6
** Significantly different from the initial values using unpaired student's t-test (P<0.05)*
† Significantly different from the previous values using unpaired student's t-test (P<0.05)

4. ELECTROPHORESIS ANALYSIS

Electrophoresis analysis of the reaction mixture at optimum labeling conditions indicated that ^{125}I- epinephrine is a neutral complex. This is due to the remaining of ^{125}I- epinephrine compound at the point of spotting,while the free iodide moved 11 cm towards the anode, depending on its charge and ionic mobility. These results are presented in Fig. 2.

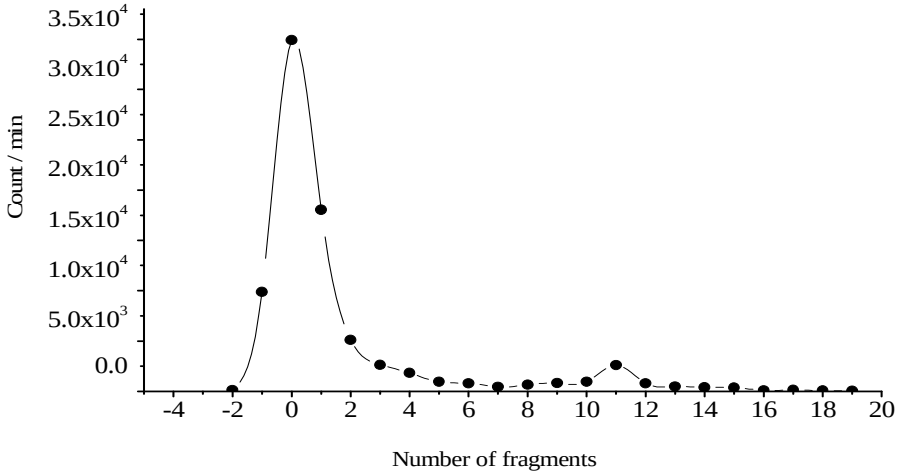


Fig. 2. Electrophoretic pattern of ^{125}I- epinephrine compound at optimum labeling conditions (75µg epinephrine, 100µg CAT, about 3.7MBq Na^{125}I, pH 7at 25°C for 15min.)
Electrophoresis conditions: solvent: 0.2M phosphate buffer pH 7, paper type: cellulose acetate;; running time:90 minutes and 300 V

4.1 *In-vivo* Biodistribution

The results of the *in-vivo* biodistribution studies carried out in Albino Swiss mice at different time intervals after administration of ^{125}I-epinephrine were shown in Table 5. The blood uptake was 18.2 % at 15 min post injection and decreased to 5.1 % at 2 h, indicating that the labeled compound cleared from the systematic circulation within 2 h after administration. The liver uptake decreased slowly by time which may be due to the hepatobiliary excretion pathway of the drug. The heart tissue uptake was high as clear from the table and hence radioiodinated epinephrine can be used safely as myocardial imaging agent. The renal uptake is higher than the hepatic uptake indicating that the labeled compound excreted mainly through the urinary system. The lung uptake decreased by time from 15 min–2h indicating that ^{125}I-epinephrine binds to beta-2-adrenergic receptors on bronchiole muscle cells. The brain uptake was low indicating that this tracer can cross the blood brain barrier with relatively low percent.

Table 5. Biodistribution of [^{125}I] epinephrine in normal albino Swiss mice

Organs & body fluids	Time post injection (min)/ % I. D./g tissue after intravenous administration			
	15	**30**	**60**	**120**
Blood	18.2±1.4	12.2±1.2	8.7±0.8	5.1±0.5
Bone	1.7±0.6	2.2±0.8	2.1±0.7	1.5±0.8
Muscle	0.7±0.2	0.8±0.3	0.9±0.3	0.9±0.4
Liver	5.2±0.9	7.5±0.5	4.7±0.8	2.5±0.7
Intestine	3.5±0.3	5.4±0.6	9.8±0.7	5.1±0.4
Stomach	4.2±0.5	5.0±0.7	6.6±0.5	4.2±0.3
Lung	5.6±0.4	3.6±0.6	3.0±0.1	1.1±0.1
Spleen	3.1±0.6	3.6±0.6	3.0±0.2	3.3±0.2
Heart	7.8±0.4	9.3±0.8	12.2±0.9	8.5±0.6
Thyroid	0.6±0.2	0.8±0.4	1.5±0.4	1.3±0.2
Kidney	4.6±0.7	8.5±0.9	6.9±0.5	3.3±0.5
Brain	1.5±0.3	1.0±0.3	0.2±0.1	0.1±0.2
Urine	5.0±0.8	5.8±0.6	9.7±0.8	13.1±0.6

5. CONCLUSION

The obtained results permit the following conclusions:

1. The technique have proven to be useful to incorporate radioiodine radionuclides onto the ring of the epinephrine molecule (as a reactive group) at ambient room temperature up to 25±1ºC in reasonable time up to 15 min, where chloramine-T appears to be the most effective oxidizing agent.
2. An advantage is the preparation of a new radioiodinated myocardial imaging agent that has the ability to estimate the β-adrenergic receptor concentration without blood sampling. Imaging will enable screening of the receptor site location.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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