

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

ABSTRACT

An adopted method for the preparation of high radiochemical purity [¹²⁵I] iodoepinephrine was developed in order to characterize the binding properties of adrenergic receptors. Direct radioiodination of epinephrine was carried out using chloramine-T as an oxidizing agent. The reaction proceeds well within 15 min at ambient room temperature up to 25 ± 1°C 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 [¹²⁵I] iodoepinephrine. The result indicates the possibility of using [¹²⁵I] iodoepinephrine as myocardial imaging agent.

Key Words: Radioiodine-125 / ephinephrine / heart / Chromatographic Analysis / Biodistribution.

INTRODUCTION

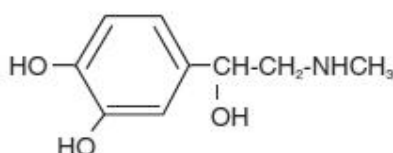
There are many types of receptors such as dopamine receptors ^(1, 2), σ-receptors ⁽³⁾, acetylcholine receptors ⁽⁴⁻⁶⁾, serotonin and 5HT_{2A} receptors ^(1,7), adrenergic receptors ⁽⁸⁻¹¹⁾, estrogen receptors ^(12,13), and steroid receptors ⁽¹⁴⁾.

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). That is due to that β-adrenergic receptors which are cell membrane sites control fat cell metabolism and fat cell tissue growth ⁽¹⁵⁾.

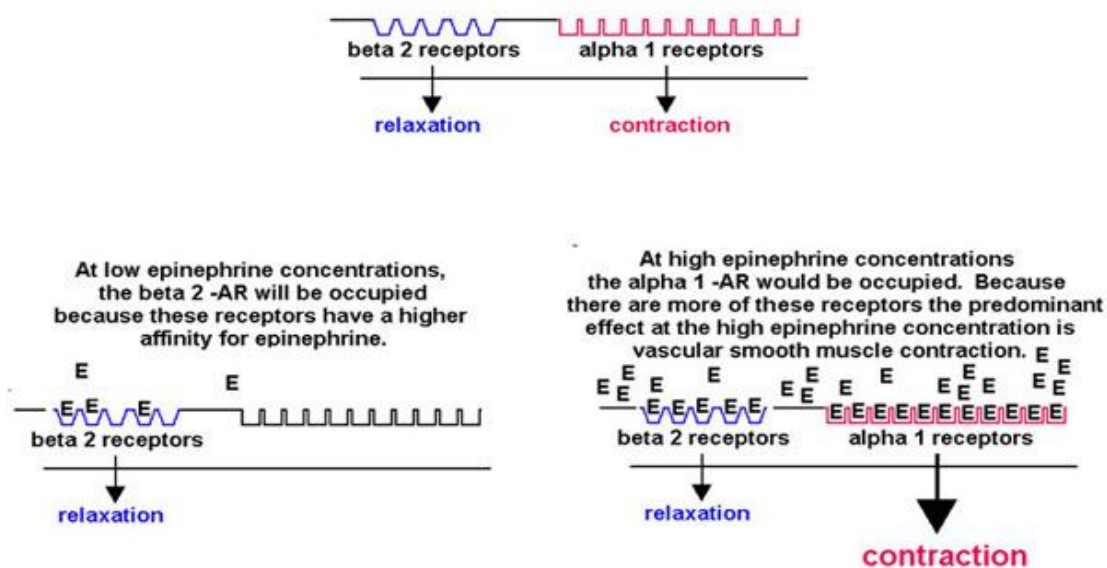
Recently, radioiodination of organic compounds and biomolecules have been the subject of interest of many investigators ^(16, 17). That is due to that iodine atom occupies a similar volume to that of methyl or ethyl group and can substitute for an alkyl group in an organic molecule without unduly perturbing 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 chloramine-T (N-chloro-p-toluene sulfonamide sodium salt) must normally be present to oxidize I₂ to a better electrophile (i.e. iodonium ion I⁺) ^(20,21).

Epinephrine (also known as adrenaline) is a hormone and a neurotransmitter. ⁽²²⁾ It increases heart rate, constricts blood vessels, dilates air passages and participates in the fight-or-flight response of the sympathetic nervous system ⁽²³⁾. In chemical terms, adrenaline is one of a group of monoamines called the catecholamines. It is produced in some neurons of the central nervous system, and in the chromaffin cells of the adrenal medulla from the amino acids phenylalanine and tyrosine ⁽²⁴⁾. epinephrine has a higher affinity for the beta₂ receptors when compared to the alpha₁ receptors as shown in **Figure 1**. Therefore, the effect of epinephrine is dependent on which type of receptor is occupied. Recall that receptor occupancy is dependent on the concentration of a drug and its equilibrium dissociation constant. At low doses, epinephrine can selectively stimulate beta₂ 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⁽²⁵⁾. Beta-Adrenergic receptor (βAR), a family of prototypical G-protein-coupled receptor (GPCRs), can be activated by two endogenous ligands NE and Epi. The receptors play critical roles in the regulation of

cardiovascular⁽²⁶⁾ and pulmonary function⁽²⁷⁾, as well as other physiological processes. β AR 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⁾. Epi is primarily released from adrenal gland to the circulating plasma. The distinct releasing routes suggest preferential activation of a β AR subtype by an individual ligand. This notion is consistent with our recent observations that β_1 AR and β_2 AR have distinct subcellular distribution along the adrenergic synapses between sympathetic ganglion neurons and cardiac muscle cells⁽³⁰⁾. Meanwhile, it is also speculated that Epi might activate the same β AR subtype to initiate distinct signaling for the same physiological responses such as heart contraction. The changes in β -adrenergic receptor density can be associated with cardiac diseases such as congestive heart failure, myocardial ischemia and infarction, cardiomyopathy, diabetes, or thyroid-induced heart muscle disease⁽³¹⁻³⁵⁾.



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Figure 1. The structure of epinephrine and its receptors occupancy⁽²⁵⁾.

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

EXPERIMENTAL

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 weighing 30 ± 2 g was used for the biodistribution studies.

Initial Method of Radioiodination of epinephrine for optimization of the factors affecting the Yield %:

In a screw capped 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 50 μl of aqueous solution of $\text{Na}_2\text{S}_2\text{O}_5$ (20 mg/ml) to ensure that all the unreacted iodine is in a reduced form before chromatographic analysis.

Chromatographic Analysis :

1) Paper chromatographic analysis (TLC)

Paper chromatography was used for 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) Electrophoresis

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 ± 0.2 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. The components of the sample move to different positions along the paper, depending on their charge and ionic mobility⁽²³⁾.

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

RESULTS AND DISCUSSION

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

Effect of Oxidizing Agent Amount

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

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

Values represent the mean $\pm$ SEM n = 6

* Significantly different from the initial values using student's t- test ($P < 0.05$).

$\dagger$ Significantly different from the previous values using student's t- test ($P < 0.05$).

The results obtained revealed that electrophilic radioiodine generated by oxidizing agent (chloramine-T) as shown in **Table1** under the same reaction conditions. At 100 μg 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 ion to iodonium ion which is the reactive ion. Also, a slight decrease in the radiochemical yield of ^{125}I -epinephrine was observed while increasing Chloramine-T concentration up to 200 μg at pH 7. The over excess of Chloramine-T concentration an undesirable aim to avoid non-reacted oxidized species which interfere with the labeling reaction gives rise to undesirable side products.

Effect of Reaction Time

The reaction was carried out at certain time intervals at ambient room temperature up to $25 \pm 1^\circ\text{C}$. 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 time of exchange up to equilibrium (15 min) with a maximum radiochemical yield % $\simeq 95$ %. Upon increasing the reaction time to 60 minutes, the radiochemical yield showed small decrease. This can be attributed to long exposure of epinephrine to the harsh oxidation condition of Chloramine-T, so there is no need to increase the reaction time over 15 minutes.

Table (2): Effect of reaction time on the % labeling yield of ^{125}I - adrenaline

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

Values represent the mean $\pm$ 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 ($P < 0.05$)

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 in the substrate amount as shown in **Table 3**. This can be explained by increasing the interaction between molecules of 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 100 μg or more, the yield of ^{125}I - epinephrine decreased

Table (3): Effect of epinephrine content on the labeling yield.

Ephedrine (μg)	% Labeled compound	% Free iodide
25	80.4 ± 0.31	191.6 ± 0.41
50	$89.0 \pm 0.36^*$	11.0 ± 0.25
75	$94.7 \pm 0.18^*$	5.3 ± 0.28
100	$94.5 \pm 0.40^{*\dagger}$	5.5 ± 0.35
200	$94.5 \pm 0.26^*$	5.5 ± 0.30

Values represent the mean $\pm$ 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 ($P < 0.05$)

Effect of pH of Reaction Mixture

The influence of the pH of the reaction medium on the radiochemical yield of ^{125}I - epinephrine was studied. The reaction was done at pH range from 1 to 11 using the appropriate buffers. The data of this study are presented in **Table 4** which show that a high radiochemical yield of ^{125}I - epinephrine was obtained at pH 7 (95%). This may be due to the fact that Chloramine-T worked 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% at pH equal to 1. Also, towards alkaline side, the yield decreased drastically reaching 79 % at pH equal to 11.

Table (4): Effect of pH of the reaction medium on the labeling yield of ^{125}I - adrenaline.

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

Values represent the mean $\pm$ 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 ($P < 0.05$)

Electrophoresis analysis

Electrophoresis analysis of the reaction mixture at optimum labeling conditions was 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 was moved 11 cm towards the anode, depending on its charge and ionic mobility. These results are represented in **Figure 2**.

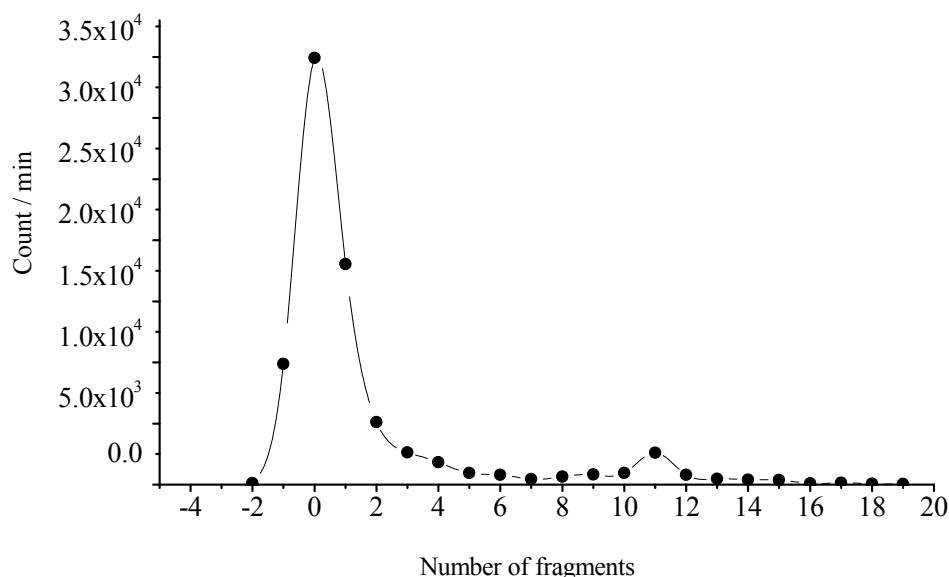


Figure 2. Electrophoretic pattern of ^{125}I - epinephrine compound at optimum labeling conditions (75 μg epinephrine, 100 μg CAT, about 3.7MBq Na^{125}I , pH 7at 25°C for 15min.). **Electrophoresis conditions:** solvent: 0.2M phosphate buffer pH 7, paper type: cellulose acetate; 300 V; running time:90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

***In-vivo* Biodistribution**

The results of *in-vivo* biodistribution studies carried out in Albino Swiss mice at different time intervals after administration of [^{125}I] iodo 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 was increased slowly by time which may be due to the hepatobiliary excretion pathway of the drug. The heart tissue uptake was high as cleared from the table and hence [^{125}I] iodo epinephrine 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 lung uptake was increased by time from 15 min – 2 h indicating that [^{125}I] iodoepinephrine binds to beta-2-adrenergic receptors on bronchiole muscle cells. The brain uptake was low indicating that this tracer can cross 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	12.2	8.7	5.1
Bone	1.7	2.2	2.1	1.5
Muscle	0.7	0.8	0.9	0.9
Liver	5.2	7.5	4.7	2.5

Intestine	3.5	5.4	9.8	5.1
Stomach	4.2	5.0	6.6	4.2
Lung	5.6	3.6	3.0	1.1
Spleen	3.1	3.6	3.0	3.3
Heart	7.8	9.3	12.2	8.5
Thyroid	0.6	0.8	1.5	1.3
Kidney	4.6	8.5	6.9	3.3
Brain	1.5	1.0	0.2	0.1
Urine	5.0	5.8	9.7	13.1

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 $25 \pm 1^\circ\text{C}$ in reasonable time up to 15 min, where chloramine-T appears to be the most effective oxidizing agent..
- 2- An advantage of a new radioiodinated myocardial agent that the ability to estimate the β -adrenergic receptor concentration without blood sampling. Imaging will enable screening of the receptor site location.

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