Experimental Physiology

Kinetics of thyroxine (T_4) and triiodothyronine (T_3) transport in the isolated rat heart

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The dynamics and kinetics of thyroid hormone transport in the isolated rat heart were examined using the modified unidirectional paired tracer dilution method. The uptake of ^{125}I-thyroxine (^{125}I-T_4) and ^{125}I-triiodothyronine (^{125}I-T_3) from the extracellular space into heart cells was measured relative to the extracellular space marker ^{3}H-mannitol. The thyroid hormone maximal uptake was 54.4 % for ^{125}I-T_4 and 52.15 % for ^{125}I-T_3. The thyroid hormone net uptake was 25.69 % for ^{125}I-T_4 and 25.49 % for ^{125}I-T_3. Backflux from the intracellular space was 53.17 % for ^{125}I-T_4 and 61.59 % for ^{125}I-T_3. In the presence of unlabelled thyroid hormones, ^{125}I-T_4 and ^{125}I-T_3 maximal uptakes were reduced from 10.1 to 59.74 % and from 34.6 to 65.3 %, respectively, depending on the concentration of the unlabelled hormone, suggesting a saturable mechanism of the thyroid hormone uptake by the heart cells, with $K_{m(T4)}$= 105.46 μM and the maximal rate of ^{125}I-thyroid hormone flux from the extracellular space to heart cells ($V_{max(T4)}$) = 177.84 nM min^{-1} for ^{125}I-T_4 uptake, and $K_{m(T3)}$ = 80.0 μM and $V_{max(T3)}$ = 118.5 nM min^{-1} for ^{125}I-T_3 uptake. *Experimental Physiology* (2001) **86.1**, 13–18.

Thyroid hormones are the iodized derivatives of the amino acid tyrosine and are synthesized by the thyroid gland. Thyroxine (T_4) and triiodothyronine (T_3) regulate the processes of general metabolism (oxidative phosphorylation), growth, development and specific gene expression (Oppenheimer & Schwartz, 1987; Samuels, 1988). About 90 % of the thyroid production is T_4 while 10 % is T_3. It is thought that T_4 has little, if any, biological activity, and it is considered as a prohormone, which becomes activated upon conversion into T_3 in peripheral tissues. About 80 % of the daily T_3 production is generated via this process while the remaining 20 % is directly secreted from the thyroid (Hennemann, 1987).

To respond to thyroid hormone signals, cells have to contain specific thyroid hormone receptors (nuclear proteins) and thyroid hormone response elements (TRE) (specific DNA sequences upstream of regulated genes) (Brent *et al.* 1989). Thyroid hormone exerts its actions at a cellular level by binding to specific thyroid hormone receptor (TR) and binding of TR to TRE, in order to stimulate or inhibit the rate of transcription of specific genes (Franklyn & Gammage, 1996). Up to now, two major classes of thyroid hormone nuclear receptors have been described: thyroid hormone receptor-α (TRα) and thyroid hormone receptor-β (TRβ) (Franklyn & Gammage, 1996; Nagaya *et al.* 1996). Several isoforms (α_1, α_2, α_3, β_1 and β_2) are created by alternative splicing of TRα and TRβ gene (Nagaya *et al.* 1996) and they display differences in terms of their tissue distribution and functional properties (Franklyn & Gammage, 1996). It has been shown that TRα_1, TRα_2 and TRβ_1 are expressed in the myocardium, and that TRα_1 and TRβ_1 bind the ligand (thyroid hormone), while TRα_2 is non-ligand binding variant which may exert a 'dominant negative' influence on the action of α_1 and β_1 thyroid hormone receptor proteins (Brent *et al.* 1991; Franklyn & Gammage, 1996).

Thyroid hormones have strong effects on the heart and circulation. It is considered that thyroid hormones perform direct and indirect actions on the heart. Thus, direct thyroid hormone actions on the heart involve regulation of transcription of a number of functionally relevant genes in the myocardium (nuclear mechanism). These include the myosin heavy chain contractile proteins (Ojamaa & Klein, 1993; Dubus *et al.* 1993), Na^+,K^+-ATPase (Liu *et al.* 1993; Huang *et al.* 1994), Ca^{2+}-ATPase and phospholamban (Kimura *et al.* 1994), which modulate the activity of calcium translocator (Klein & Ojamaa, 1996). But some effects, like the thyroid hormone effects on calcium uptake by the myocyte, are mediated by direct action on the plasma

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membrane (extranuclear mechanisms) (Gotzsche, 1994; Franklyn & Gammage, 1996). Further studies in the heart showed that thyroid hormones up-regulated the development of β-adrenergic receptors (Pracyk & Slotkin, 1992) and in this way had an indirect effect on the heart, mediated by possible altered sensitivity to catecholamine action (Klein & Ojamaa, 1996).

To perform most of their biological actions, thyroid hormones have to be translocated through the cell membrane and reach a cell nucleus of the target cells. It was shown that the transport of thyroid hormones through the cell membrane is a carrier-mediated process (Hennemann *et al.* 1987; Samson *et al.* 1996). Further studies showed that the membrane transport of thyroid hormone is a stereo-specific, facilitated or active process in many tissues and cell types (Samson *et al.* 1996). However, the molecular mechanisms of thyroid hormone transport into target cells are not quite clear and they are of great interest.

In this study we have investigated the dynamics and kinetics of ^{125}I-thyroid hormone (T_3 and T_4) cellular uptake during a single capillary passage through the isolated perfused rat heart.

METHODS

Thyroid hormone uptake by the isolated rat heart was investigated using the modified unidirectional paired tracer dilution method and Langendorff´s technique for the isolated heart with constant perfusion flow. The uptake of ^{125}I-T_3 and ^{125}I-T_4 from the extracellular space was determined relative to the passage of a reference marker molecule ^{3}H-mannitol.

Materials

The Krebs-Henseleit buffer contained (mM): NaCl 118, KCl 4.7, $NaHCO_3$ 25, $MgSO_4.7H_2O$ 1.7, $CaCl_2$ 2.5, KH_2PO_4 1.2 and D-glucose 11 (Merck, Darmstadt, Germany).

The scintillation fluid contained POPOP (0.05 g 1.4-bis(2-(5-phenyloxazoly)) benzene) and PPO (6 g 2.5-diphenyloxazole) per litre of toluene (Sigma Chemical Co., USA).

The specific radioactivities for ^{125}I-triiodothyronine, ^{125}I-thyroxine and ^{3}H-mannitol were 280–480, 200–310 and 955 mCi mM^{-1}, respectively. The radioisotopes ^{125}I-triiodothyronine and ^{125}I-thyroxine were obtained from the Institute of Nuclear Science 'Vinca' (prepared using the chloramin-T-method; Hunter & Greenwood, 1962), Belgrade, Yugoslavia, and ^{3}H-mannitol was purchased from Amersham International, UK. Unlabelled triiodothyronine and thyroxine were obtained from Sigma Chemical Co.

Methods

Isolation and perfusion of the rat heart. Wistar rats of both sexes, weighing between 180 and 200 g, were killed by cervical dislocation (according to Schedule 1 of the Animals, Scientific procedures, Act 1986, UK), and the heart was excised and then retrogradely perfused using Langendorff's constant perfusion flow technique, with a peristaltic pump at 3 ml min^{-1} (g wet heart weight)$^{-1}$. The perfusate was a modified Krebs-Henseleit buffer equilibrated with 95 % O_2–5 % CO_2 and the pH was adjusted to 7.4 at 37 °C. Cardiac functions, heart rate and contractility, were observed and registered using a PIC-Digital Recording System (ECM, Kragujevac, Yugoslavia). The heart rate and contractility were within physiologicals limits.

Measurement of ^{125}I-thyroid hormone uptake. The thyroid hormone uptake was measured using a rapid dual-isotope dilution technique (Yudilevich & Mann, 1982). Following 20–30 min equilibration with Krebs-Henseleit buffer, a 100 μl bolus was injected into the perfusion system containing ^{3}H-mannitol as an extracellular reference tracer, or ^{125}I-thyroid hormone (T_3 or T_4) as a test molecule. The first 30 drops and one cumulative 4 min sample of venous effluent were sequentially collected. This collection time was shown by preliminary experiments to give more than 90 % recovery of reference tracer (Rosic *et al.* 1987; Mitrovic *et al.* 1991). The samples were prepared for scintillation counting (Rackbeta, LKB-Wallac counter) by addition of 2 ml 98 % ethanol and 2 ml scintillation fluid.

The purity of the iodinated components used, before (in the perfusion medium) and after the experiments (in the effluent), was higher than 98 % following sephadex G-25 chromatography (Hunter & Greenwood, 1962).

The radioactivity of each isotope in the venous effluent sample (as a percentage of the injected dose) was plotted against the collection time, in order to obtain venous concentration–time curves, i.e. dilution profiles for both test and reference tracer.

The cellular uptake of ^{125}I-thyroid hormone was estimated directly from the venous dilution profiles using Eqn (1), where ^{125}I-thyroid hormone (^{125}I-TH) and ^{3}H-mannitol (^{3}H-man) represent radioactivity (in counts min^{-1}) recovered in successive effluent samples:

$$\text{Uptake (\%)} = (1 - {}^{125}\text{I-TH}/\,{}^{3}\text{H-man}) \times 100, \quad (1)$$

The uptake is derived from the difference between the mannitol value and that of thyroid hormone recovery in each drop. The maximal uptake (U_{max}) of thyroid hormone is the mean of the uptake values taken over the time period during which the uptake has reached a plateau.

U_{net} is the mean uptake over the whole period, i.e. the recovered activity in all drops. The net cellular uptake (U_{net}) was calculated as:

$$U_{net}\,(\%) = (1 - \text{total recovered } {}^{125}\text{I/total recovered } {}^{3}\text{H}) \times 100, \quad (2)$$

The backflux (BF) of thyroid hormone from intracellular to extracellular space could be calculated as:

$$\text{BF (\%)} = (\,U_{max} - U_{net})/U_{max}, \quad (3)$$

To determine the kinetics of the thyroid hormone uptake, isolated hearts were perfused with different concentrations of the unlabelled thyroid hormone.

The inhibition of U_{max} in the presence of various concentration of the unlabelled thyroid hormone was calculated as:

$$\text{Inhibition (\%)} = [(U_{max,control} - U_{max})/U_{max,control}\,] \times 100, \quad (4)$$

The unidirectional transport or flux (V) of the thyroid hormone from the extracellular space was estimated (in nM min^{-1}) from the maximal fractional tracer uptake (U_{max}), the perfusion rate (F, ml min^{-1}) and the concentration of unlabelled thyroid hormone in perfusate (C, μM), as described by Yudilevich & Mann (1982):

$$V = -F \ln(\,1 - U_{max})C, \quad (5)$$

From the rate of thyroid hormone uptake, in the presence of different concentrations of unlabelled thyroid hormone, the Michaelis-Menten constant (K_m) and V_{max} (the maximal rate of ^{125}I-thyroid hormone transport from the extracellular space to heart cells) were calculated using Eadie-Hoffstee analysis (Hoffstee, 1959).

Statistics

The data were analysed using Student's *t* test; a *P* value of < 0.05 was considered as statistically significant.

RESULTS

In the first group of experimental animals we investigated the dynamics and kinetics of ^{125}I-T_4 transport from the extracellular space into the heart cells, during a single capillary ^{125}I-T_4 passage through the coronary circulation.

Dilution profiles for ^{3}H-mannitol and ^{125}I-T_4 (obtained from the coronary sinus effluent after the bolus injection of tracers into the aorta) are illustrated in Fig. 1. H^3 and I^{125} radioactivities, expressed as a percentage of the total injected dose, in each drop of venous effluent (labelled % Recovered dose) were plotted against the collection time. The dilution profile curve for ^{125}I-T_4 shows lower values than the dilution profile curve for ^{3}H-mannitol, which demonstrates ^{125}I-T_4 uptake by the heart cells.

Figure 2 illustrates the uptake plot obtained using the equation for uptake (Eqn (1)), as explained in Methods. The maximal cellular uptake (U_{max}) was determined from the maximal values in this plot, and it appears 38–66 s after the bolus was given.

The dynamics of ^{125}I-T_4 transport from the extracellular space into heart cells, during a single capillary passage through the coronary circulation, is summarized in Table 1, which includes calculated values of U_{max}, U_{net} and backflux (BF) of

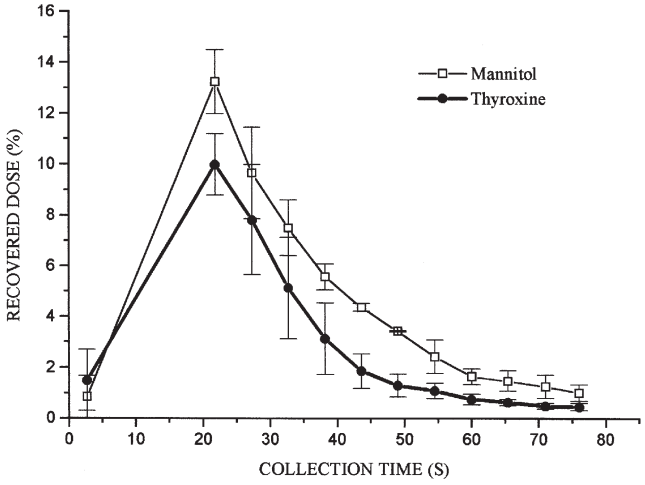

Figure 1

The recovery of ^{3}H-mannitol and ^{125}I-thyroxine as a percentage of injected radioactivity plotted against collection time (s). The lower recovery of ^{125}I-thyroxine relative to ^{3}H-mannitol indicates thyroxine uptake by the heart cells.

Table 1. Dynamics of ^{125}I-triiodothyronine and ^{125}I-thyroxine transport from the extracellular space into the heart cells

	U_{max} (%)*	U_{net} (%)*	BF (%)*	*n*
^{125}I-thyrox.	54.4 ± 0.58	25.69 ± 3.50	53.17 ± 8.50	7
^{125}I-triiodo.	52.15 ± 0.76	25.49 ± 10.6	61.59 ± 14.26	5

*Student's *t* test; $P < 0.05$. Values are means ± S.E.M; *n*, number of experiments.

^{125}I-T_4 from the intracellular space. The control U_{max} value for ^{125}I-T_4 was 54.4 ± 0.58 %, and U_{net} was 25.69 ± 3.50 %. The backflux of ^{125}I-T_4 from the intracellular space was 53.17 ± 8.50 %.

To determine the kinetics of ^{125}I-T_4 transport from the extracellular space into heart cells, various concentrations of unlabelled T_4 were added in the perfusion medium. At the lowest concentration used, 1 μM, U_{max} was 48.9 ± 1.04 % (Table 2.). For the other concentrations used, from 10 μM to 275 μM, the maximal uptake values were from 40.8 ± 1.44 % to 21.9 ± 2.90 %. These results show that maximal cellular uptake of ^{125}I-T_4 was reduced from 10.1 ± 2.9 % to 59.74 ± 8.85 %, depending on the concentration of unlabelled hormone (T_4) (Table 2)

The rate of unidirectional ^{125}I-T_4 transport or flux in the presence of increasing concentrations of unlabelled T_4 was estimated using Eqn 4 (Methods) and is shown in Fig. 3.

The Michaelis-Menten constant (K_m) and V_{max} were then estimated as previously described in Methods. The K_m was 105.46 ± 15.69 μM and V_{max} was 177.84 ± 11.50 nmol min^{-1}.

The dynamics and kinetics of ^{125}I-T_3 transport from the extracellular space into the heart cells, during a single capillary T_3 passage through the coronary circulation, were investigated in the second group of experimental animals.

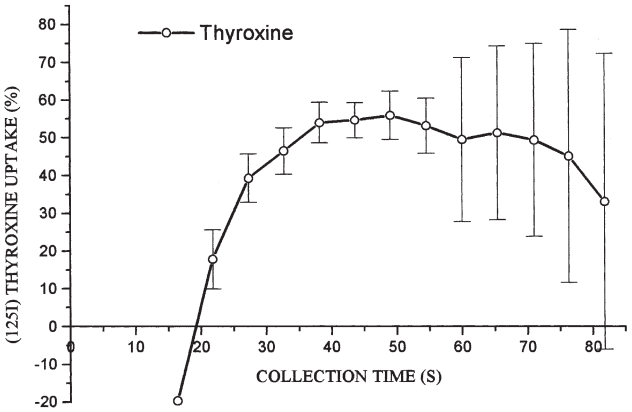

Figure 2

Uptake of ^{125}I-thyroxine relative to ^{3}H-mannitol plotted against collection time (s).

Table 2. Maximal ^{125}I-thyroxine uptake by heart cells in the presence of various concentrations of unlabelled thyroxine

Unlabelled T_4 (μM)	U_{max} (%)*	Inhibition (%)*
Control	54.40 ± 0.58	—
1	48.9 ± 1.04	10.1 ± 2.9
10	40.8 ± 1.44	24.98 ± 6.3
34.37	45.64 ± 3.04	16.09 ± 5.6
70	39.43 ± 2.78	27.53 ± 5.1
137.5	39.24 ± 1.18	27.87 ± 5.2
206	28.15 ± 2.1	48.25 ± 6.4
275	21.9 ± 2.90	59.74 ± 8.85

* Student's t test; $P < 0.05$. Values are mean ± S.E.M.

Table 3. Maximal ^{125}I-triiodothyronine uptake by heart cells in the presence of various concentrations of unlabelled triiodothyronine

Unlabelled T_4 (μM)	U_{max} (%)*	Inhibition (%)*
Control	52.15 ± 0.76	—
35	34.1 ± 5.6	34.61 ± 10.7
70	25.54 ± 4.4	51.04 ± 8.46
140	28.95 ± 8.65	44.49 ± 16.58
211	23.75 ± 6.4	54.45 ± 9.15
282	18.1 ± 4.88	65.26 ± 9.37

* Student's t test; $P < 0.05$. Values are mean ± S.E.M.

Figure 4 illustrates dilution profiles for ^{3}H-mannitol and ^{125}I-T_3. H^3 and I^{125} radioactivities, expressed as a percentage of the total injected dose, in each drop of venous effluent were plotted against the collection time. The dilution profile curve for ^{125}I-T_3 shows lower values than the dilution profile curve for ^{3}H-mannitol, which demonstrates ^{125}I-T_3 uptake by the heart cells.

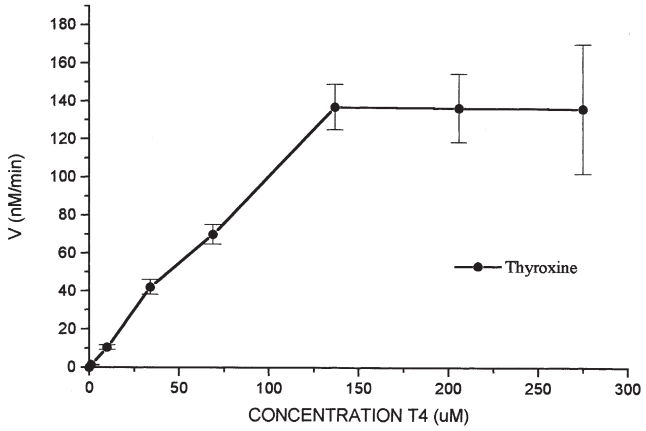

Figure 3

The rate of unidirectional ^{125}I-thyroxine transport or flux (V).

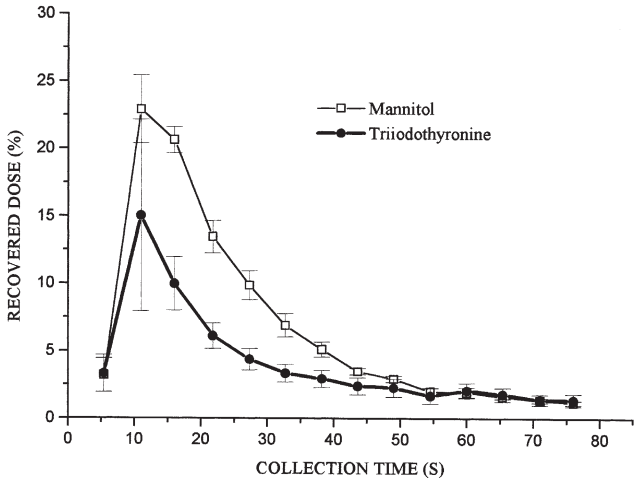

Figure 4

The recovery of ^{3}H-mannitol and ^{125}I-triiodothyronine as a percentage of injected radioactivity plotted against collection time (s). The lower recovery of ^{125}I-triiodothyronine relative to ^{3}H-mannitol indicates triiodothyronine uptake by the heart cells.

The uptake plot for ^{125}I-T_3 is shown in Fig. 5. Maximal cellular ^{125}I-T_3 uptake (U_{max}) was determined from the maximal values in this plot, and it appears 13.7–35.45 s after the bolus injection.

The basic nature of T_3 transport during a single passage through the coronary circulation is summarized in Table 1, which includes calculated values of U_{max}, U_{net} and backflux (BF) of T_3 from the intracellular space.

The kinetics of T_3 transport from the extracellular space into heart cells was measured with various concentrations of unlabelled T_3 present in the perfusion medium. At the lowest concentration used, 34.9 μM, U_{max} was 34.1 ± 5.6 % (Table 3). These results show that maximal cellular uptake of ^{125}I-T_3 was reduced (see Table 3) depending on the concentration of the unlabelled T_3 hormone.

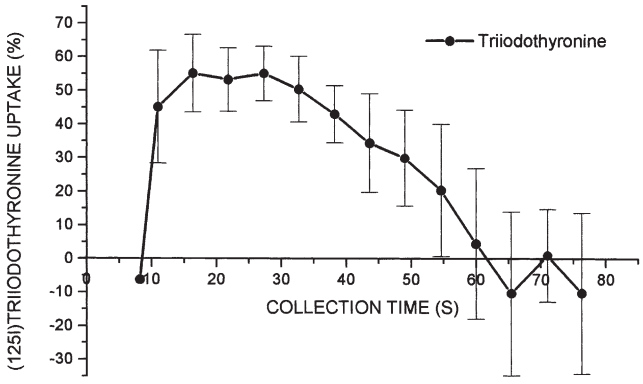

Figure 5

Uptake of ^{125}I-triiodothyronine relative to ^{3}H-mannitol plotted against collection time (s).

The rate of unidirectional ^{125}I-T_3 transport or flux in the presence of increasing concentrations of the unlabelled T_3 was estimated using the Eqn (4) (Methods) and shown in Fig. 6.

The Michaelis-Menten constant (K_m) was 80.0 ± 14.04 μM and V_{max} was 118.5 ± 22.57 nM min^{-1}.

DISCUSSION

To examine the dynamics and kinetics of ^{125}I-thyroxine and ^{125}I-triiodothyronine transport from the extracellular space into heart cells, the modified unidirectional paired tracer dilution method (Rosic *et al.* 1996) and Langendorff's technique for the isolated heart with constant perfusion flow were used. In these experiments T_4 and T_3 uptake by the cells of intact hearts were measured. ^{125}I-T_4 and ^{125}I-T_3 uptake by the heart cells was measured relative to ^{3}H-mannitol, as a reference tracer. Mannitol is a marker molecule and its recovery in coronary venous effluent represents a simple diffusion into and out of the extracellular space (Mann & Yudilevich, 1984; Samuels, 1988; Preston & Segal, 1992*a*,*b*; Kostic *et al.* 1995*)*, whereas the lower values of thyroxine (Fig. 1) and triiodothyronine (Fig. 4) recovery reflect cellular uptake.

Our results show that the maximal values of the recovered radioactivity of the thyroid hormones (^{125}I-T_3 and ^{125}I-T_4) and the reference tracer (^{3}H-mannitol) appeared in the same fraction of coronary venous effluent (Figs 1 and 4). The evident parallelism of the curves, obtained in our experiments and the coincident peak of recovered radioactivity indicate that both the test (^{125}I-T_3 and ^{125}I-T_4) and the reference (^{3}H-mannitol) molecules have similar diffusion characteristics. The reliable and reproductive results gained with this method confirms its application, although it gives us no information as to where the hormone was bound in the cell.

The positive values of ^{125}I-thyroid hormone uptake appeared 16 s (T_4) and 8 s (T_3) after the moment of tracer injection into the coronary circulation, and these values were positive within 92 s (for ^{125}I-T_4) and within 60 s (for ^{125}I-T_3) of collection time (Figs 2 and 5).

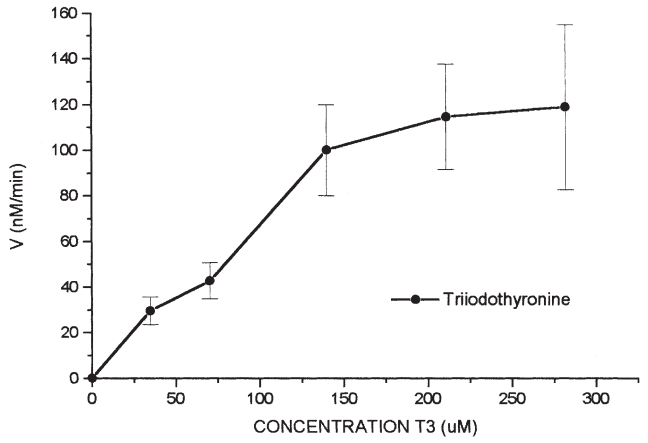

Figure 6

The rate of unidirectional ^{125}I-triiodothyronine transport or flux (V, nM min^{-1}) from the extracellular space into the heart cells.

Maximal values for thyroid hormone uptake, relative to ^{3}H-mannitol, appeared between 38 and 55 s for ^{125}I-T_4 and between 14 and 35 s for ^{125}I-T_3 after the start of sample collection, and they were, respectively, 54.4 ± 0.58 % and 52.15 ± 0.76 % (Table 1.). These mean values for maximal thyroid hormone (^{125}I-T_4 and ^{125}I-T_3) uptake reflect the relatively high ^{125}I-T_4 and ^{125}I-T_3 uptake by the rat heart cells, which is in accordance with values obtained from the isolated perfused sheep choroid plexus (Preston & Segal, 1992*a*).

Furthermore, our results for inhibition of maximal thyroid hormone (^{125}I-T_4 and ^{125}I-T_3) uptake in the presence of unlabelled thyroid hormones suggest a saturable mechanism for T_4 and T_3 uptake by cells in the isolated rat heart, with a higher uptake capacity (V_{max}) for T_4 than for T_3 carriers. The K_m values show no significant difference, suggesting similar affinities of T_4 and T_3 for their respective carriers.

Our results provide relevant and useful information for further investigations which will evaluate whether hyperthyroidism and/or hypothyroidism can affect the membrane uptake of thyroid hormones under experimental conditions.

Conclusions

The results of this study have shown that the modified unidirectional paired tracer dilution method and Langendorff's technique for the isolated heart with constant perfusion flow gave reliable and reproducible results, which support its application in the study of the dynamics and kinetics of thyroid hormone transport from the extracellular space into heart cells.

The results showing inhibition of maximal ^{125}I-T_3 and ^{125}I-T_4 uptake in the presence of the unlabelled T_3 and T_4 indicate that specific carrier transport mechanism(s) for thyroid hormones, with relatively high V_{max} and low K_m values, exist in the heart cells.

Thyroxine carriers in heart cells showed a higher binding capacity (V_{max}) than triiodothyronine carriers, but the carriers showed a similar affinity for thyroxine and triiodothyronine.

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