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Internalization of radiolabelled [DTPA^0]octreotide and [DOTA^0,Tyr^3]octreotide: Peptides for somatostatin receptor-targeted scintigraphy and radionuclide therapy

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Summary

We compared the internalization of [^{90}Y-DOTA^0,Tyr^3]octreotide and [^{111}In-DOTA^0,Tyr^3]octreotide with that of [^{125}I-Tyr^3]octreotide and [^{111}In-DTPA^0]octreotide in the subtype 2 somatostatin receptor (sst_2)-positive rat pancreatic tumour cell lines CA20948 and AR42J and in the somatostatin receptor-negative human anaplastic thyroid tumour cell line ARO. We demonstrated that [^{111}In-DTPA^0]octreotide, [^{90}Y-DOTA^0,Tyr^3]octreotide and [^{111}In-DOTA^0,Tyr^3]octreotide are internalized by a receptor-specific, time- and temperature-dependent process. The amount of [^{90}Y-DOTA^0,Tyr^3]octreotide internalized was higher than that of [^{111}In-DOTA^0,Tyr^3]octreotide and [^{111}In-DTPA^0]octreotide. (© 1998 Chapman & Hall Ltd.)

Introduction

The somatostatin analog [^{111}In-DTPA^0]octreotide consists of the octapeptide octreotide, the chelator DTPA [diethylenetriamine pentaacetic acid (enabling radiolabelling with a radiometal)] and the radionuclide ^{111}In. We have described its use for scintigraphic imaging of somatostatin receptor-positive lesions [1]. A new and interesting application is the use of this radiolabelled octreotide for radionuclide therapy. Promising results have been reported in humans [2]. However, the Auger electron emitter ^{111}In is probably not the optimal radionuclide for radiotherapy. A β^- particle-emitter like ^{90}Y with a maximum β^- energy of 2.3 MeV and a half-life of 64 h appears more suitable. As the ^{90}Y-DTPA complex is not stable, we recently derivatized octreotide with the chelator DOTA (tetraazacyclododecane tetraacetic acid), enabling stable radiolabelling with ^{90}Y. In addition, Phe^3 in octreotide was replaced by Tyr to increase hydrophylicity. *In vivo* experiments in rats with this compound radiolabelled with either ^{111}In or ^{90}Y showed favourable biodistribution characteristics [3]. Although accumulations of ^{111}In and ^{90}Y in somatostatin receptor-positive organs was found [3], it has not been demonstrated that radiolabelled [DOTA^0,Tyr^3]octreotide is internalized. The success of the therapeutic strategy relies, however, on the amount of radioactivity concentrated within tumour cells, which is determined by the rate of internalization of the radioligand and by intracellular retention of the radionuclide. For [^{125}I-Tyr^3]octreotide [4] and [^{111}In-DTPA^0]octreotide [5], internalization into somatostatin receptor-positive cells has been described recently. We have also studied the internalization of ^{111}In- and ^{90}Y-labelled [DOTA^0,Tyr^3]octreotide in addition to that of [^{111}In-DTPA^0]octreotide and [^{125}I-Tyr^3] octreotide in the subtype 2 somatostatin receptor (sst_2)-positive rat pancreatic tumour cell lines CA20948 and

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AR42J and in the somatostatin receptor-negative human anaplastic thyroid tumour cell line ARO. This allowed us to discriminate between the effects caused by different chelators (DTPA *vs* DOTA), different radionuclides (^{111}In *vs* ^{90}Y) and Phe^3 to Tyr replacement in octreotide.

Materials and methods

Labelling of octreotide derivatives

DTPA-octreotide and $^{111}InCl_3$ were provided by Mallinckrodt Medical (Petten, The Netherlands), and octreotide by Sandoz (Basel, Switzerland). $^{90}YCl_3$ was obtained from Nordion (Canada). [^{125}I-Tyr^3]octreotide [6], [^{111}In-DTPA^0]octreotide [7], [^{111}In-DOTA^0,Tyr^3]octreotide and [^{90}Y-DOTA^0,Tyr^3]octreotide [3] were prepared as described previously.

Cell culture

AR42J cells were grown in RPMI-1640 (Gibco, Grand Island, NY), CA20948 cells were grown in DMEM (Gibco), and ARO cells were grown in DMEM/F12 (Gibco). All media were supplemented with 2 mM glutamine and 10% fetal calf serum. Before the experiment, sub-confluent cell cultures were transferred to 6- or 24-well plates.

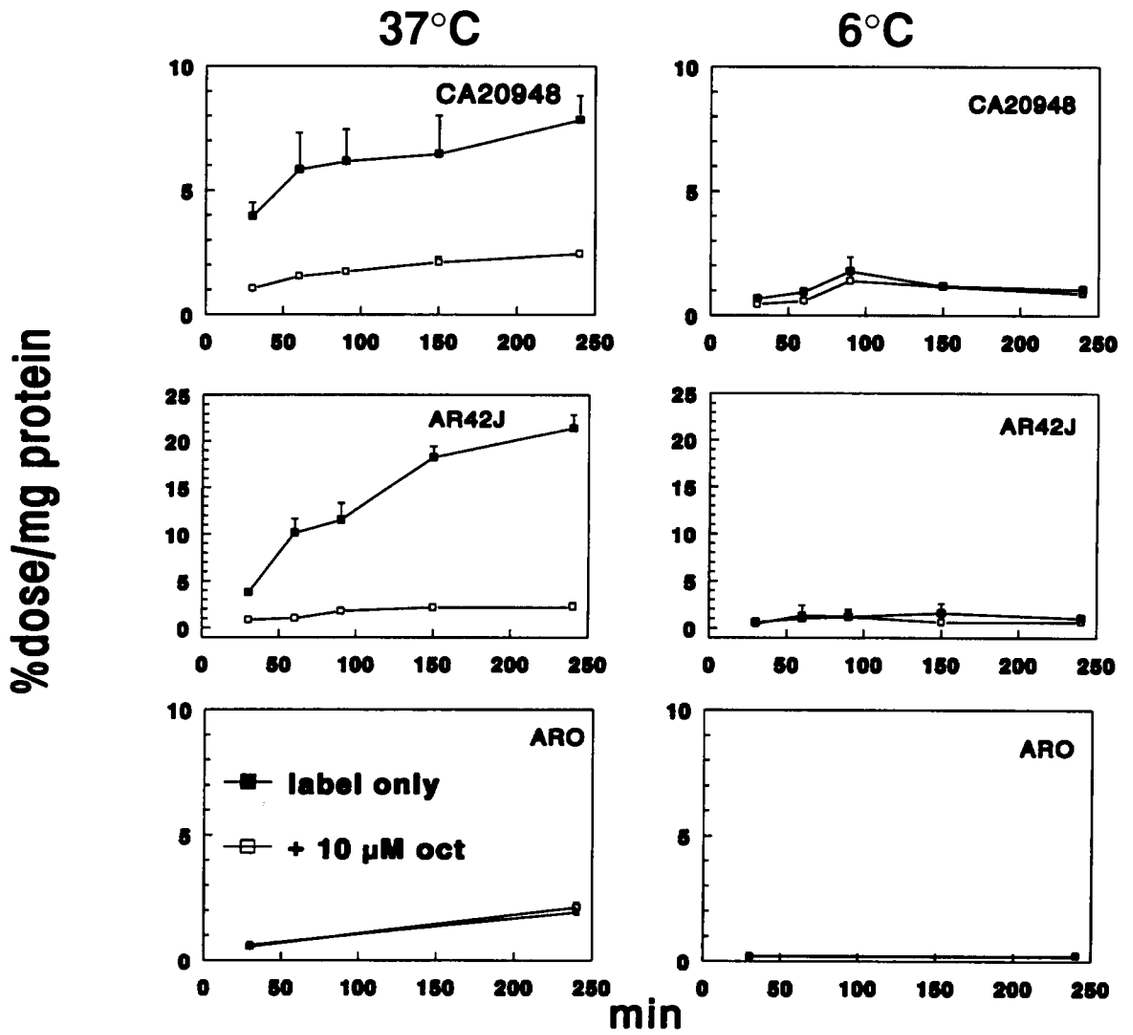

Fig. 1. Temperature dependence of the internalization process of [^{111}In-DTPA^0]octreotide (10 nM). Blockade by competition with 10 µM unlabelled octreotide. Left panels: experiments performed at 37°C. Right panels: experiments performed at 6°C.

Experimental design

The binding of the radiolabelled peptides to tumour cells and subsequent internalization were studied as described previously [4]. In short, before incubation, cells were washed. Incubation was started by addition of 1 ml internalization medium (internalization medium is culture medium without fetal calf serum, but with 1% bovine serum albumin) with 80 kBq of either radioligand. The peptide concentrations in the different experiments are indicated in the 'Results' section. Cells were incubated at 37°C for the indicated time periods. To determine non-specific internalization, cells were incubated with radioligand and an excess of unlabelled octreotide at concentrations indicated in the 'Results' section. Cellular uptake was stopped by removal of the medium and washing of the cells with 2 ml ice-cold phosphate buffered saline. To discriminate between internalized and non-internalized (surface-bound) ligand, intact cells were incubated with 1 ml 20 mM sodium acetate. The internalized and surface-bound radioactivity were determined in a LKB-1282-Compugammasystem. The internalized fraction was expressed as a percentage of the applied dose per mg cellular protein. The latter was determined using a commercially available kit (BioRad, The Netherlands).

Data are expressed as the mean ± standard deviation for incubations assayed in triplicate, with each experiment performed 2–6 times.

Results

Figure 1 shows the time- and temperature-dependent internalization of [^{111}In-DTPA^0]octreotide in the sst_2-positive CA20948 and AR42J rat pancreatic tumour cells and in the sst_2-negative ARO human anaplastic thyroid tumour cell line. Time-dependent accumulation in the sst_2-positive cells did not reach a plateau within 4 h after incubation at 37°C. Internalization was strongly reduced in the presence of 10 μM unlabelled octreotide, indicating that this process is highly specific, and it was also reduced at 6°C. The acid-removable ('surface-bound') uptake was about 5–10% of the internalized fraction (not shown). The ARO cells were used as negative controls; in these cells, internalization of [^{111}In-DTPA^0]octreotide was low and showed no specific time- or temperature-dependent accumulation. Figure 2 shows the inhibitory effect of increasing concentrations of octreotide on internalization of [^{111}In-DTPA^0]octreotide. Based on these data, we used a concentration of 0.1 nM peptide in the following experiments. To allow a comparison of different experiments, we assessed the internalization of [^{125}I-Tyr^3]octreotide in each group of experiments as a positive control. The internalization of [^{125}I-Tyr^3]octreotide and its blockade by excess octreotide is shown in Fig. 3. Table 1 shows that specific internalization of [^{111}In-DTPA^0]octreotide is about 10% of that of [^{125}I-Tyr^3]octreotide ($P < 0.001$). Figure 4 shows the time- and temperature-dependent internalization of [^{90}Y-DOTA^0,Tyr^3]octreotide in CA20948 and AR42J cells. Accumulation reached a plateau after 2 h incubation at 37°C. Internalization was strongly reduced during incubation in the presence of 1 μM unlabelled octreotide and at 6°C. The acid-removable

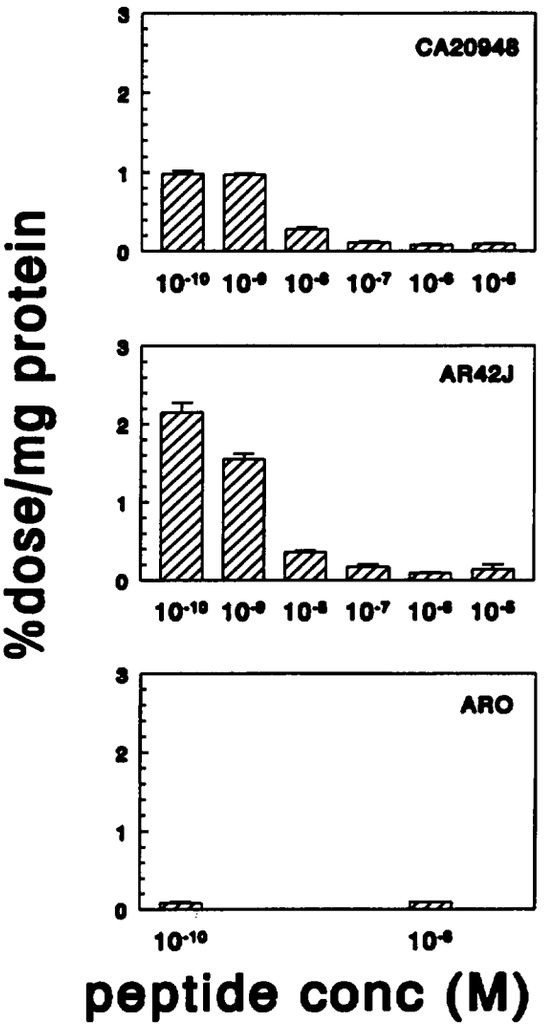

Fig. 2. Internalization of [^{111}In-DTPA^0]octreotide (0.1 nM) after 60 min incubation. The effect of increasing concentrations of octreotide ('peptide') in the medium.

('surface-bound') uptake was less than 5% of the internalized fraction (not shown). Specific internalization of [^{90}Y-DOTA^0,Tyr^3]octreotide was higher than that of [^{111}In-DTPA^0]octreotide (Table 1, $P < 0.001$), whereas it was about one-quarter of that of [^{125}I-Tyr^3]octreotide ($P < 0.001$). In Fig. 5, the time-dependent internalization of both ^{111}In- and ^{90}Y-labelled [DOTA^0,Tyr^3]octreotide is compared in AR42J cells. From the blockade experiments with unlabelled octreotide at 60 min, it is shown that for both compounds, internalization was highly specific. Furthermore, internalization of [^{90}Y-DOTA^0,Tyr^3]octreotide was higher than that of [^{111}In-DOTA^0,Tyr^3]octreotide from 30 min onwards ($P < 0.01$).

Table 1. Internalization of [^{111}In-DTPA^0]octreotide, [^{111}In-DOTA^0,Tyr^3]octreotide and [^{90}Y-DOTA^0,Tyr^3]octreotide after 60 min incubation at 37°C.[a]

Compound	*Mean ± S.D.*
[^{125}I-Tyr^3]octreotide	100 ± 12
[^{111}In-DTPA^0]octreotide	8.2 ± 0.7*
[^{111}In-DOTA^0,Tyr^3]octreotide	14.6 ± 1.1*
[^{90}Y-DOTA^0,Tyr^3]octreotide	28.8 ± 2.5*

[a] Data for each experiment are expressed as the percentage of specific [^{125}I-Tyr^3]octreotide internalization tested in the same experiment. Data are the means of those obtained in two somatostatin receptor-positive cell lines used.
* $P < 0.001$ versus [^{125}I-Tyr^3]octreotide.

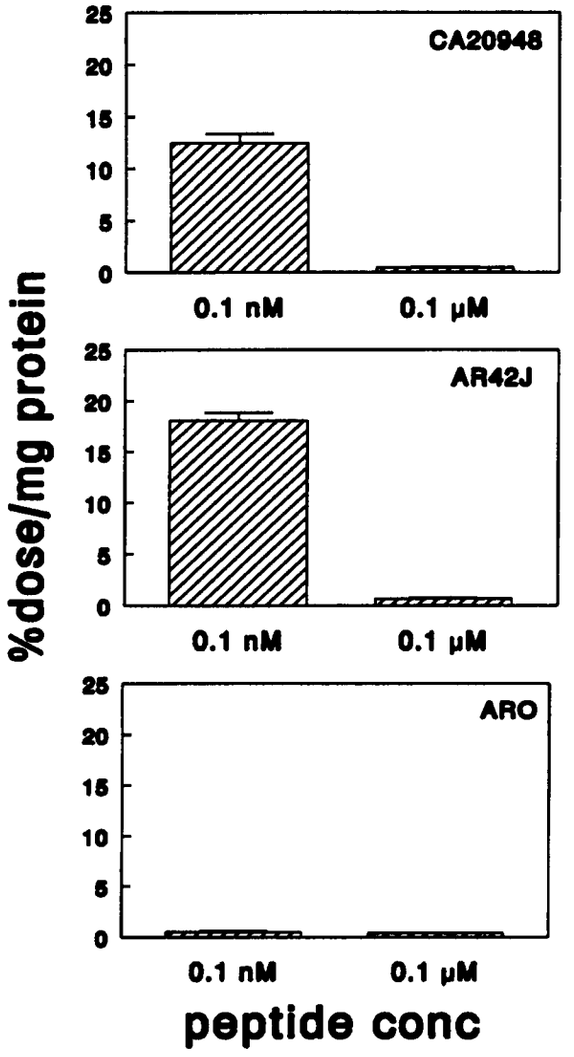

Fig. 3. Internalization of [^{125}I-Tyr^3]octreotide (0.1 nM) after 60 min incubation. Blockade with 0.1 µM octreotide.

Discussion

Somatostatin receptors are membrane glycoproteins, of which five subtypes (sst_{1-5}) have been cloned [8, 9]. Octreotide binds with high affinity to sst_2 and with lower affinity to sst_3 and sst_5, while it does not bind to sst_1 and sst_4 [8, 9]. Octreotide scintigraphy is therefore predominantly based on the visualization of sst_2. Currently, radionuclide therapy of sst_2-positive lesions is being explored. We investigated the effects of radionuclide therapy using [^{111}In-DTPA^0]octreotide on the growth of sst_2-positive tumours in rats. Significantly fewer tumours were found in treated animals than in untreated animals. As regards radionuclide therapy in humans using [^{111}In-DTPA^0]octreotide, we observed positive effects on clinical symptoms, hormone production and tumour proliferation in 12 of 20 end-stage patients. However, ^{111}In is not the most appropriate radionuclide for radionuclide therapy as it lacks the preferred higher energies and larger ranges of β^- particles. ^{90}Y, with its maximum β^- energy of 2.3 MeV and high affinity for the chelator DOTA, is a better candidate.

For successful radionuclide therapy, it is important that the radiopharmaceutical is internalized by the tumour cells. We have reported internalization of [^{125}I-Tyr^3]octreotide in *in vitro* studies using AtT20 mouse pituitary tumour cells [4]. Furthermore, internalization of [^{111}In-DTPA^0]octreotide into human neuroendocrine tumour cells has recently been described [5]. This study confirmed internalization of [^{111}In-DTPA^0]octreotide, but also showed that this internalization process was highly specific and temperature-dependent. The

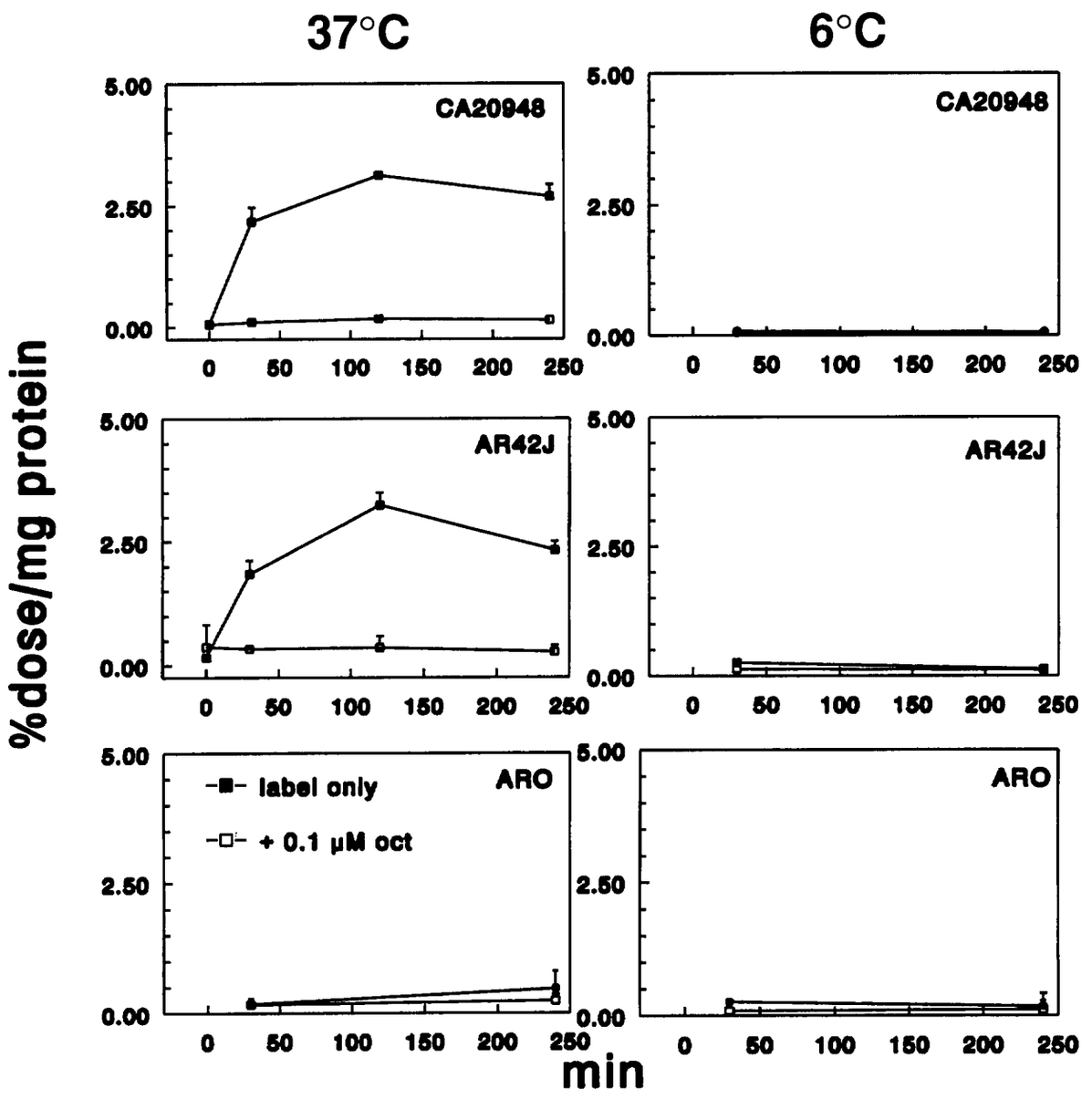

Fig. 4. Time and temperature dependence of the internalization process of [^{90}Y-DOTA^0,Tyr^3]octreotide (0.1 nM). Blockade with 0.1 µM octreotide.

amount of [^{111}In-DTPA^0]octreotide specifically internalized was about 10% of that of [^{125}I-Tyr^3]octreotide, in accordance with the reported difference in affinity of these radioligands for somatostatin receptors in rat brain cortex membranes [7]. In this study, we also observed specific and temperature-dependent internalization of [^{90}Y-DOTA^0,Tyr^3]octreotide, which was greater than the internalization of [^{111}In-DTPA^0]octreotide and [^{111}In-DOTA^0,Tyr^3]octreotide, in accordance with our biodistribution findings *in vivo* in rats [3]. It has recently been shown that this internalization pathway for [^{111}In-DTPA^0]octreotide occurs via endosomes fusing with lysosomes where degradation of the radiolabelled peptide occurs. After dissociation of the ligand, the receptor proteins may return to the plasma membrane, while ^{111}In-DTPA-containing degradation products are retained in the lysosomes, causing the long retention of radioactivity in receptor-positive cells [10]. Assuming the same mechanism for radiolabelled [DOTA^0,Tyr^3]octreotide uptake into receptor-positive

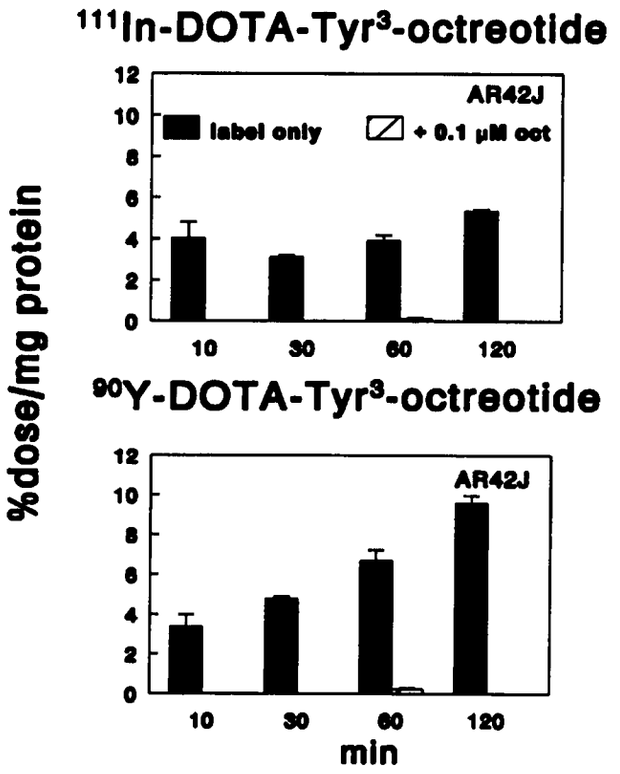

Fig. 5. Comparison of time-dependent internalization of [^{111}In-DOTA^0,Tyr^3]octreotide and [^{90}Y-DOTA^0,Tyr^3]octreotide (both 0.1 nM). Blockade at 60 min with 0.1 µM octreotide.

cells, the difference in internalized radioactivity suggests a shorter retention of cellular radioactivity after incubation with [^{90}Y-DOTA^0,Tyr^3]octreotide and [^{111}In-DOTA^0,Tyr^3]octreotide than after incubation with [^{111}In-DTPA^0]octreotide.

Acknowledgements

The rat pancreatic tumour cell lines AR42J and CA20948, both of which are somatostatin receptor-positive, were obtained from ECACC and cultures from solid tumours [8], respectively. The human anaplastic thyroid tumour cell line ARO was obtained from Professor Juillard (University of California, Los Angeles, CA) via Professor Fenzi (Universita di Medicina e Chirurgica, Naples, Italy). H.M.R. acknowledges support from the Swiss National Science Foundation (31–42516/94).

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