

Scintigraphic imaging of neuroadrenergic cardiac function: An in vitro and in-vivo study

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Introduction

Radiolabelled metaiodobenzylguanidine (MIBG), a monoamine uptake and storage tracer, is used for the scintigraphic detection of tumors deriving from the neural crest [1,2] as well as for assessing the functional status of the sympathetic nerve endings in the human heart and lungs [3, 4]. While there have been several publications dealing with the clinical applications of this tracer, less is known about the mechanisms underlying its uptake by noradrenergic nerve endings; moreover the findings that have emerged from the few studies that have been conducted are often discordant, in part due to the different experimental models used [5-9].

A greater understanding of the mechanisms underlying the uptake of this tracer is essential since a number of controversial issues recently emerged, especially in cardiac studies: (1) the frequent uptake abnormalities in normal subjects [10-12]; (2) the unclear interference of various drugs [4,13-14]; (3) the influence of the specific activity of the tracer preparation on cardiac scintigraphies [15-17]. Indeed the pharmacokinetic behaviour of MIBG in humans depends not only on the specific uptake by noradrenergic neurons (which forms the basis for its use in scintigraphic studies), but also on the non-specific neuronal uptake and non-neuronal uptake [18-20]. A selective model is needed, one capable of focusing exclusively on the true specific carrier-mediated uptake mechanism by sympathetic nerve terminals in order to better understand the pharmacokinetic behaviour of MIBG, as compared to noradrenaline (NA), and to study in-vitro the effects of the presence and degree of pharmacologic interaction of various drugs on the MIBG uptake.

Synaptosomes are nerve terminals isolated from rat cerebral cortex; they are a well validated in-vitro pharmacokinetic model [21-22]. In this study we aimed at verifying the feasibility of the use of synaptosomes as an in-vitro model for studying MIBG specific uptake and at evaluating the uptake analogies of MIBG and NA in neuroadrenergic tissues by examining the ability of MIBG to inhibit the uptake of NA.

On the other hand an in-vivo model is also needed in order to confirm in-vitro data and to provide more convincing evidence of the interference of medical therapies currently delivered to patients with cardiac diseases. To this aim in this study we used Wistar rats and we verified the possibility: 1) to image the rats heart by standard nuclear medicine imaging equipment using ¹²³I-MIBG (tracer of the adrenergic terminals) and ²⁰¹Thallium (tracer of myocardial perfusion); 2) to obtain quantitative data on the MIBG uptake under baseline conditions and under pharmacologic challenge.

Material and Methods

Synaptosomes

Crude cortical synaptosomes were prepared essentially according to Gray and Whittaker [23]. Briefly, adult male wistar rats (200-250 g) were sacrificed by decapitation; the cortices were rapidly removed and homogenized in 40 vol of 0.32 M sucrose buffered at pH 7.4 with phosphate. The homogenate was centrifugated (5 min, 1000 x g) and synaptosomes were isolated from the supernatant by centrifugation at 12000 x g for 20 min. Synaptosomes were then resuspended in 0.32 M glucose, and the suspension was diluted 1:5 in a physiological medium with the following composition: 125 mM NaCl; 3 mM KCL; 1.2 mM MgSO₄; 1.2 mM CaCl₂; 1 mM NaH₂PO₄; 22 mM NaHCO₃; (PH 7.4), and aerated with 95% O₂; and 5% CO₂. The final protein concentration of the synaptosomal suspension was approximately 400 µg/100 µl. Samples of the suspension (500 µl final volume) were preincubated for 10 min at 37°C with various concentrations of MIBG (ranging from 0.01 µM to 1 µM); 100 µl of ³H-NA (0.04 µM and 0.2 µM) were then added and the incubation was continued for 5 min. At the end of the incubation period the synaptosomal suspension was transferred to a Wathman Glass microfiber filter (GF/C), washed with 3x2 ml of medium at room temperature under moderate vacuum conditions; the filters were counted for radioactivity.

The ^3H -amine uptake at 0°C (unspecific uptake) was determined and subtracted from the values obtained at 37°C (= active uptake). The uptake was expressed as pmol/mg protein over 5 min. The protein content was determined as described by Lowry et al [24].

The Dixon method was used for calculation of the inhibition constant of MIBG on ^3H -NA (K_i value) [25].

The ^3H -Noradrenaline (specific activity 36 Ci/mmol) was obtained by Amersham Radiochemical Centre (Buckinghamshire, England); MIBG was obtained by Sorin Biomedica (Saluggia, Italy).

Rats

In different sessions, wistar rats (adult male animals, 230-250 g. body weight) were injected in the authorized laboratory of our Institution with 123I-MIBG and/or 201Thallium in the tail vein under deep anaesthesia by intraperitoneal administration of a mixture of diazepam and ketamine chloridrate (10mg/Kg and 35 mg/Kg, respectively). At different post-injection times, the rats were sacrificed during ongoing anaesthetic treatment. Thoracotomy was soon performed and the heart was excised; the left ventricle was isolated and cut as in Figure 1. The specimens were weighted and than imaging was soon performed using an Elscit 409 single.head gamma-camera (Haifa, Israel) equipped with an high resolution collimator. The samples were positioned on the gamma camera bed; the gamma camera collimator was positioned at 10 cm from the specimens. Planar scintigraphic images were acquired on a 256x256 digital matrix; the analyser window was set at $\pm 25\%$ of the main photopeak of each radionuclide; acquisition time, unless differently specified was 180 seconds. Counts were obtained by regions of interest (ROIs) manually drawn around the specimens on scintigraphic images. The background counts were also recorded before and after the specimens measurements using the same gamma camera configuration and ROI technique described above. Also the syringes' counts were recorded both before and after the injections using the same gamma camera and ROI technique. All obtained counts were corrected for physical decay and for background and were normalized for the effective injected dose and for body weight; resulting counts were expressed as counts per second per pixel (c/sec/pix).

Experiment 1

Experiment 1 was performed in order to verify the possibility to image the rat heart and to quantitatively assess the left ventricular uptake of 201Thallium and of 123I-MIBG. Overall 12 rats were studied and injected in a tail vein with 0.1-0.2 ml of the following radionuclides; 6 were injected with 100 microCi of 201Thallium and were sacrificed after 45 minutes; 6 were injected with 100 microCi of 123I-MIBG and were sacrificed after 45 minutes. Radiopharmaceuticals were produced by Sorin-Biomedica, Saluggia, Italy.

Experiment 2

Experiment 2 was performed in order to verify whether and to what extent MIBG cardiac uptake is reduced by Reserpine, a well known depletor of the norepinephrine storage granules in the presynaptic terminals. As MIBG uptake early after the injection is partly unspecific, the experiment was planned to be carried out in an early and a late phase postinjection. Ten rats have been pre-treated with reserpine (5 mg/Kg intra-peritoneally for 7 days); 5 were sacrificed 1 hour post MIBG injection; 5 were sacrificed 4 hours post MIBG injection. Ten control rats (not treated with reserpine) were also injected with 123I-MIBG: 5 of them were sacrificed 1 hour postinjection, 5 of them 4 hours postinjection. MIBG injected dose was 100 microCi.

Results

Synaptosomes

Figure 2 shows MIBG inhibition of ^3H -NA uptake by the synaptosomes according to the Dixon method [25]. The reciprocal of the uptake value ($1/v$), expressed in picomol/mg protein/5 min, and plotted against MIBG concentrations keeping the ^3H -NA concentration constant, provides a straight line. The line obtained using the same procedure with a second ^3H -NA concentration intersects the first at a point on the left of the vertical axis, which represents K_i . Our findings indicate that MIBG produces competitive inhibition of NA uptake by cortical synaptosomes ($K_i = 0.52 \mu\text{M}$). Since this value is very close to the K_m for ^3H -NA uptake in the cortex ($K_m = 0.4 \mu\text{M}$) according to Iversen et al[26], the same carrier-mediated transport system seems to be used for both substances in this tissue.

Experiment 1

Good quality scintigraphic images of the left ventricles were obtained with both tracers. Representative images obtained with 123I-MIBG are reported in Fig. 3.

The count statistics per pixel obtained with just 180 seconds acquisition appeared satisfying (201Thallium: 5.30 ± 1.27 c/s/p; 123I-MIBG: 7.76 ± 0.73 c/s/p) and definitely suitable to allow the count quantification required for carrying out experiment #2. An high count image is reported in Fig. 4.

Experiment 2

MIBG uptake 4 hour post-injection in reserpine treated rats was 56% lower than in control rats ($p > 0.0001$) as reported in Table I. Conversely, MIBG uptake value 1 hour postinjection was not significantly different from control rats value.

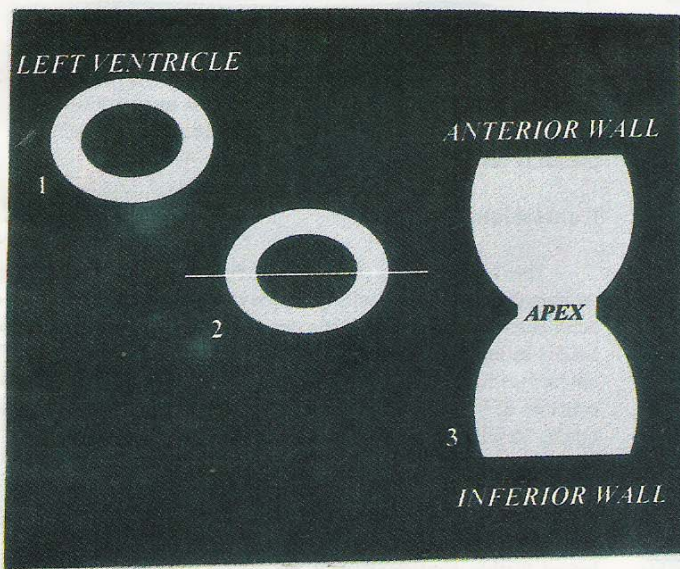
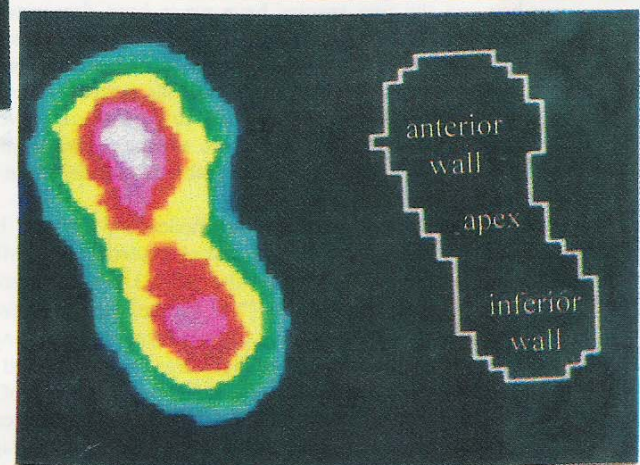
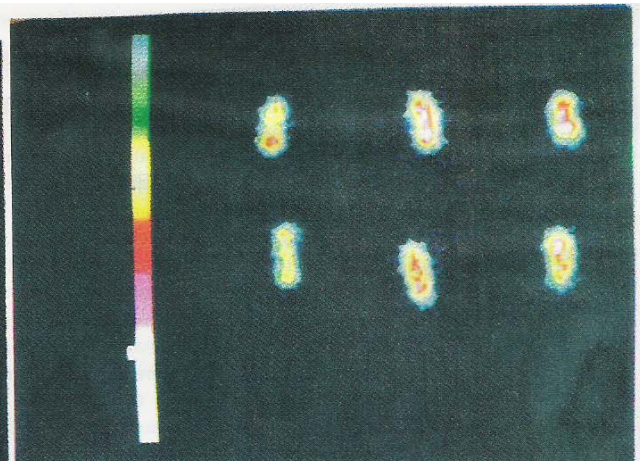


Fig. 1: Dissection outline of the left ventricle



CEREBRAL CORTEX SYNAPTOSOMES

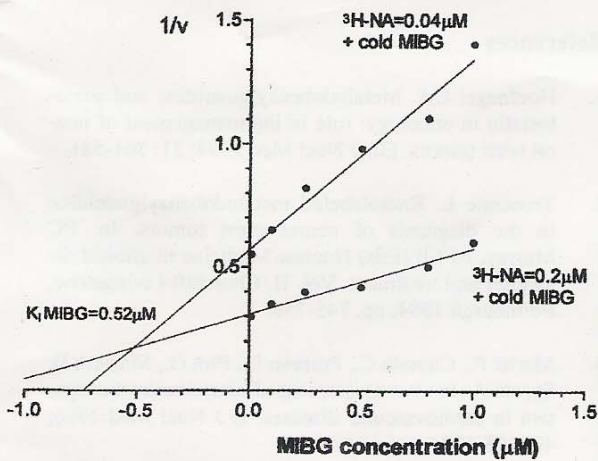


Fig. 2: Dixon plot of MIBG inhibition of $^3\text{H-NA}$ uptake by synaptosomes isolated from the rat cerebral cortex. The rate of $^3\text{H-NA}$ uptake is expressed in picomol/mg protein/5 min incubation. Each point represents the mean of triplicate determinations. The point where the two rect lines intersect is the K_i value (inhibition constant).

In figure 5 a scintigraphic image of the left ventricles of all the animals tested is reported.

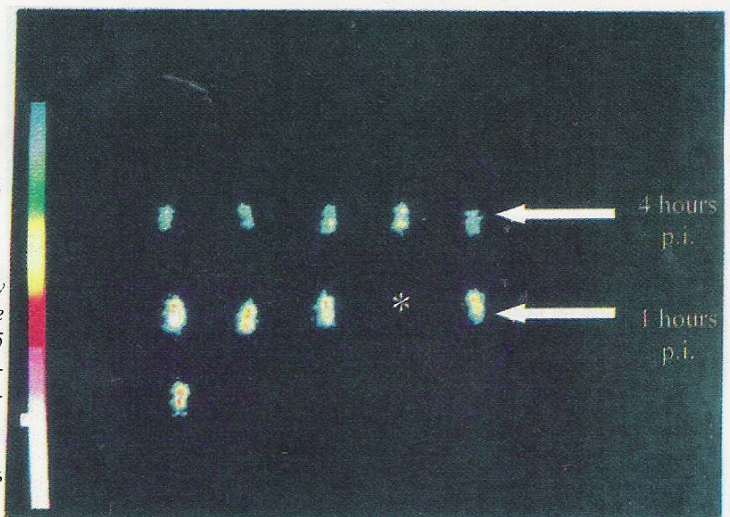


Fig. 3: $^{123}\text{I-MIBG}$ images of the left ventricle of 6 rats obtained with 180 seconds acquisition time

Fig. 4: $^{123}\text{I-MIBG}$ image of the left ventricle a rat obtained with 1 hour acquisition time. The homogeneity of tracer distribution in the anterior wall, in the apex and in the inferior wall can be clearly appreciated.

Fig. 5: $^{123}\text{I-MIBG}$ images of the left ventricle of the rats under reserpine challenge sacrificed 1 hour post-injection and 4 hours post-injection (arrows); reduced signal of the hearts of the latter group is evident. Quantitative data are reported in Table I. (*) represents the site where the background was computed

have been postulated for MIBG uptake (unspecific neuronal and non neuronal [18-20]). This model allows trial runs of different concentrations of NA, MIBG and, eventually, of interfering drugs, mimicking in-vitro different physiological or pathological conditions, such as patients with pheochromocytoma (with high levels of endogenous NA), or the use of different amount of cold MIBG in radiolabelled MIBG preparations [15-17].

The tests with synaptosomes are repeatable, easy, and inexpensive both in terms of time and sacrificed animals. Moreover the model is extremely sensitive: it allows the detection of variations in concentrations even to 0.01 μM .

The synaptosomes approach has two limitations. The first is that the synaptosomes used were of animal origin; thus the data resulting from the tests could not be automatically extrapolated to humans; however, significant functional differences in catecholaminergic synaptosomes between the two species have never been demonstrated. The second limitation is that heart or lung synaptosomes (peripheral nervous system) would be theoretically preferable to cerebral ones (central nervous system) when the heart or lung uptake of MIBG is to be studied; however, the model would be much more expensive and cumbersome to achieve due to greater technical difficulties in isolating peripheral synaptosomes.

Other animal and human models have so far been employed in order to study the characteristics of MIBG uptake: adrenomedullary bovine cells [31], neuroblastoma cells [14], human platelets [7-8] and cell lines transfected with NA transporter protein [9]; in this paper we attempted to use rats hearts because of their large availability and low cost. We demonstrated that the injection of 100 microCi of ^{201}Tl or ^{123}I -MIBG provides high cardiac count rates 45 minutes post-injection. This allows scintigraphic images of good quality to be recorded using standard nuclear medicine instrumentation in a reasonable time and quantitative uptake measures to be obtained with good count statistics. Such good image quality let us hypothesize that experiments with regional cardiac damage (i.e. myocardial infarction) can be carried out, allowing to compare regional distribution of myocardial perfusion (^{201}Tl) and adrenergic innervation (^{123}I -MIBG).

In experiment #2 we showed that ^{123}I -MIBG cardiac uptake late after tracer administration (4 hours) is significantly reduced under reserpine treatment (-56%), confirming that MIBG acts as analogue of norepinephrine and that the rat heart is a suitable in-vivo model. The lack of uptake reduction early after administration (1 hour) confirms Nakajo et al data [31] about the presence of a significant non-specific uptake of MIBG (non neuronal or extravascular uptake) and suggest uptake measures to be carried out not earlier than 4 hours postinjection in order to focus on specific uptake mechanisms (uptake-1, energy dependent, intra-vesicular).

Our in-vivo approach has also some limitations: possible differences between the human and the rat hearts should be taken into account when trying to refer animal data to human subjects; the different amount of MIBG un-

specific cardiac uptake is one of them. Furthermore, despite recent advances some human conditions are difficult to be replicated in rat: myocardial infarction, for instance, one of the most interesting application fields of ^{123}I -MIBG [3,19,33], require a difficult surgical approach in rats [34,35]. To this aim, in our laboratory a transmural necrotizing injury by freeze thawing lesion technique is being presently tested [36].

Conclusions

The synaptosome model of neuroadrenergic nerve endings has been successfully employed to study the characteristics of MIBG specific uptake; the capability of MIBG to inhibit the carrier mediated uptake of NA in synaptosomes has been demonstrated: it showed an inhibition constant of 0.52 μM (Ki) the rat heart model provided high quality scintigraphic images of the rats left ventricles and high count statistics using standard nuclear medicine techniques: quantitative data on the MIBG heart uptake under baseline conditions and under reserpine challenge were obtained; both methods have shown to be feasible and allowed further confirmation of the qualities of MIBG as a good tracer of noradrenergic nerve endings; they have the potential to be usefully employed to quantitatively assess the pharmacological interferences of various drugs on NA and MIBG uptake.

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Fig. 3: ¹²³I-MIBG images of the left ventricle of 6 rats obtained with 180 seconds acquisition time

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