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Short communication

Positron emission tomography shows high specific uptake of racemic carbon-11 labelled norepinephrine in the primate heart

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Abstract. (–)-Norepinephrine is the predominant neurotransmitter of the sympathetic innervation of the heart. Racemic norepinephrine was labelled with carbon-11 and injected i.v. into Cynomolgus monkeys. Five minutes after injection there was a more than tenfold higher radioactivity in the heart than in adjacent tissue. Pretreatment with the norepinephrine reuptake inhibitor desipramine reduced the uptake by more than 80%. The high specific uptake of racemic [^{11}C]norepinephrine indicates that enatiomerically pure (–)-[^{11}C]norepinephrine has promising potential for detailed mapping of the sympathetic innervation of the human myocardium.

Key words: Norepinephrine – Positron emission tomography – Heart– Monkeys

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Introduction

The autonomic nervous system regulates performance of the heart. The ventricular myocardium is primarily innervated by sympathetic fibres which branch into multiple plexus terminals. (–)-Norepinephrine is the predominant transmitter of the sympathetic innervation. Following nerve stimulation, (–)-norepinephrine is released into the synaptic cleft. A small amount activates receptors on the myocytes whereas a major fraction is removed by neuronal reuptake (uptake I) [1, 2] and stored in vesicles.

Positron emission tomography (PET) is a suitable technique for non-invasive quantitative examination of the heart [3]. Before the advent of PET, racemic [^{11}C]norepinephrine was prepared from [^{11}C]cyanide and the uptake was determined in the canine heart by dissection and counting [4]. Another useful line of development has been to develop analogues such as [^{18}F]fluorometaraminol and [^{11}C]hydroxyephedrine which, unlike norepinephrine, are not metabolised by the enzymes monoamine oxidase and cathecol-*O*-methyltransferase [5]. (–)-Norepinephrine is the naturally occurring enantiomer. However, the neuronal uptake of norepinephrine is not stereoselective [6, 7]. In a recent study a high uptake has been demonstrated by PET for both (+)- and (–)-[^{18}F]6-fluoronorepinephrine in the baboon heart [8].

Radiolabelled (–)-norepinephrine may be advantageous to analogues in the sense that endogenous (–)-norepinephrine will be tracked. In the present exploratory study the uptake of racemic [^{11}C]norepinephrine was examined in the primate heart.

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Materials and methods

Racemic [^{11}C]norepinephrine was prepared with high specific radioactivity (>1000 Ci/mmol) from [^{11}C]nitromethane [9].

Two male Cynomolgus monkeys (weight 4 and 4.5 kg) were obtained from the National Laboratory of Bacteriology, Solna, Sweden. They were anaesthetised with ketamine (Ketalar, 5 mg/kg per hour i.m.). For PET imaging the monkeys were positioned recumbent on the right side. Radioligand was injected through a cannula which was inserted into a sural vein.

The PET system used was Scanditronix PC2048-15B with a spatial resolution of 4.5 mm full width at half maximum. The attenuation correction was determined from a transmission scan. Radioactivity was measured by PET for 63 min according to a preprogrammed sequence of frames. Blood samples (2 ml) were drawn from a femoral vein at 4, 10, 20 and 30 min after radioligand injection for measurement of radioactivity in plasma and for analysis of [^{11}C]norepinephrine metabolism in plasma by high-performance liquid chromatography (HPLC) [10].

In each monkey a first experiment was performed in which [^{11}C]norepinephrine was injected i.v. (20–40 MBq). In one of the monkeys a second experiment was performed on the same day to determine the specificity of binding. Desipramine (3 mg/kg i.v.), a reuptake inhibitor, was injected i.v. 1 h before [^{11}C]norepinephrine [11].

Regions of interest (ROIs) were drawn for the ventricular myocardium and lung on the PET images. Radioactivity in an ROI was determined for each sequential frame and plotted versus time.

Results

After i.v. injection of [^{11}C]norepinephrine there was rapidly a high uptake of radioactivity in the heart (Figs. 1, 2). In both monkeys radioactivity of the myocardium was more than 10 times higher than in adjacent lung tissue and more than 5 times than in plasma.

Following pretreatment with the norepinephrine reuptake inhibitor desipramine, the uptake of radioactivity in the myocardium was markedly reduced (Figs. 1, 2). This observation indicates that a major proportion of the radioactivity in the heart represents specific binding to the norepinephrine transporter. In addition, a part of the radioactivity may represent radioligand which has been transported into the sympathetic neuron. Pretreatment with desipramine had no evident effect on radioactivity in lung or plasma. More than 90% of plasma radioactivity represented unchanged ligand 30 min after injection (Fig. 3).

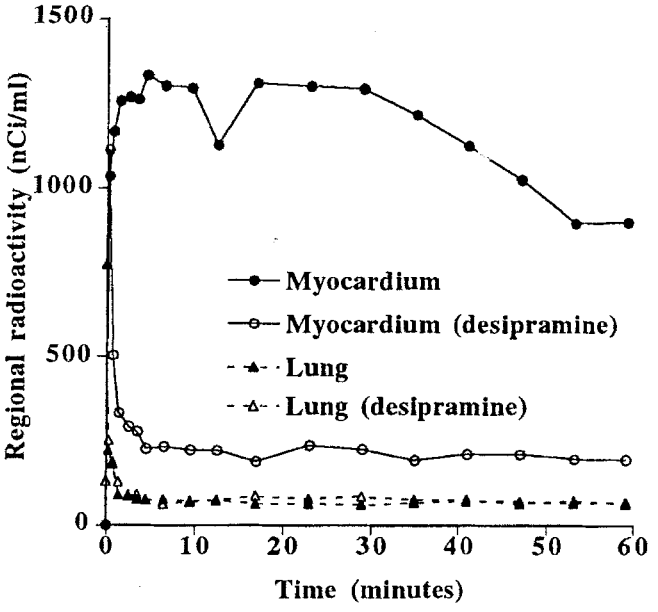

Fig. 1. Time curves for radioactivity in the myocardium and lung of a Cynomolgus monkey following i.v. injection of [^{11}C]norepinephrine in a control experiment (*filled symbols*) and pretreatment experiment with desipramine (3 mg/kg) (*open symbols*)

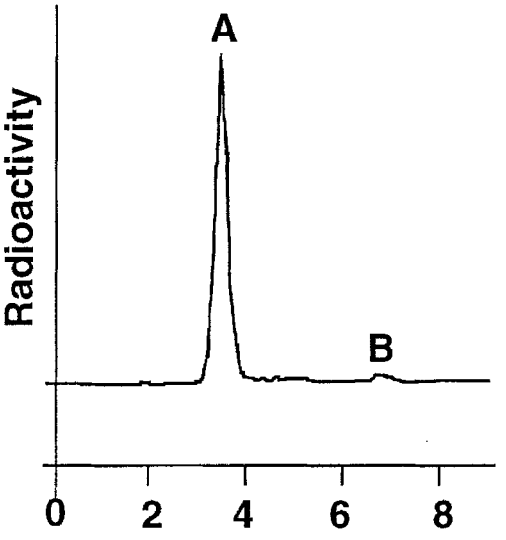

Fig. 3. HPLC chromatogram of plasma from a cynomolgus monkey 20 min after injection of racemic [^{11}C]norepinephrine. *A*, unchanged [^{11}C]norepinephrine; *B*, main radiolabelled metabolite

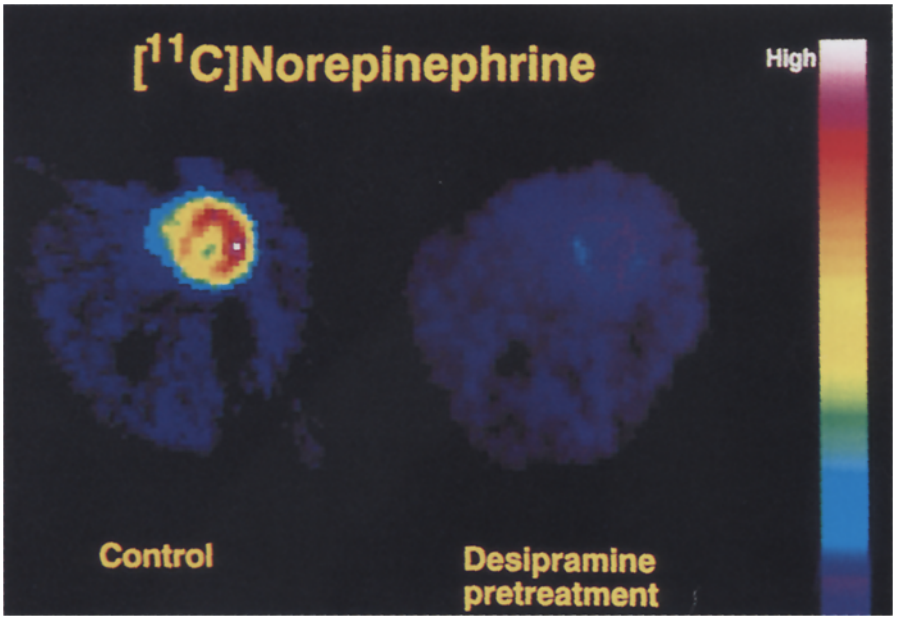

Fig. 2. PET images showing distribution of radioactivity in a horizontal section of the chest of a Cynomolgus monkey following i.v. injection of [^{11}C]norepinephrine in a control experiment (*left*) and a pretreatment experiment with desipramine (*right*)

Discussion

The effect of desipramine, a selective inhibitor of the norepinephrine reuptake, in reducing the high uptake of radioactivity in the myocardium of Cynomolgus monkeys following i.v. injection of racemic [^{11}C]norepinephrine indicates that a major part of the radioactivity visualised by PET represents neuronal uptake of racemic [^{11}C]norepinephrine.

The fact that in both monkeys more than 90% of plasma radioactivity represented unchanged ligand 30 min after injection (Fig. 3) indicates a comparatively slow rate of radioligand metabolism [10] and does not support the view that rapid metabolism may be a major drawback to the use of [^{11}C]norepinephrine as a radioligand for PET.

(–)-Norepinephrine is stored in vesicles and thereby protected from metabolic degradation whereas (+)-norepinephrine is exposed to degradative enzymes like monoamine oxidase within the cytosol. This difference is consistent with the more rapid decline of (+) than (–)-[^{14}C]norepinephrine in the mouse heart [7] and more rapid washout of (+)- than (–)-[^{18}F]6-fluoronorepinephrine in the baboon heart [8]. The use of the (–)-enantiomer may thus allow application of a less complex mathematical model for quantitative interpretation of the uptake curves.

For PET imaging the use of enantiomerically pure (–)-[^{11}C]norepinephrine should be advantageous since myocardial uptake will mimic that of the endogenous neurotransmitter (–)-norepinephrine. On the basis of the present results with racemic [^{11}C]norepinephrine a high specific uptake is expected in future studies with (–)-[^{11}C]norepinephrine. It should accordingly be possible to obtain a detailed mapping of (–)-[^{11}C]norepinephrine uptake in the healthy and diseased myocardium of human subjects.

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