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Review Article

New agents for imaging the cardiac nervous system?

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CONTENTS

Abstract

Technetium-99m (^{99m}Tc) is the radionuclide of choice for nuclear medicine imaging. However, some radiotracers are still labeled with radioiodine, which has various detrimental characteristics. Among these agents is the radioiodine- labeled guanethidine analogue MIBG (metaiodobenzylguanidine), which is used for the study of cardiac innervation. This scintigraphic depiction of the innervation patterns is of clinical importance to evaluate the function of the cardiac sympathetic nervous system. This paper reviews the possibilities of developing a ^{99m}Tc-labeled radiotracer that has adrenergic specificity. This search has resulted in four agents which, with some effort, can be labeled with ^{99m}Tc and have the potential to be used for diagnostic purposes: epinephrine, metahydroxyepinephrine, desipramine and atomoxetine. In order to avoid pharmacological effects, the final product after labeling should be (nearly) carrier-free.

Introduction

Noninvasive imaging of the heart began to develop rapidly in the seventies with the advent of thallium-201 (^{201}Tl) for myocardial perfusion imaging. This radionuclide was considered to be extremely useful for the diagnosis of coronary artery disease, risk stratification and prognosis. In the eighties, single photon emission computed tomography (SPECT) became available and ^{201}Tl was gradually replaced with compounds labeled with technetium-99m (^{99m}Tc), which has more favorable characteristics for nuclear medicine procedures. Among these compounds were ^{99m}Tc-sestamibi and tetrafosmin (1). The adrenergic agent metaiodobenzylguanidine (MIBG), labeled with either iodine-131 (^{131}I) or iodine-123 (^{123}I), emerged early in the eighties (2). MIBG is an analogue of the false neurotransmitter guanethidine and is taken up by adrenergic neurons (Fig. 1). MIBG bears some similarity with noradrenaline (Fig. 2). This agent proved to be a successful radiopharmaceutical for the study of cardiac innervation in disorders such as ischemic heart disease, heart failure and diabetes (3). For radiochemical reasons, however, it can only be labeled with radioiodine, which has drawbacks with regard to image quality due to the release of radioiodine after *in vivo* application, as well as being expensive to produce. Moreover, the released radioiodine rapidly accumulates in the thyroid gland unless effectively blocked, leading to unwanted radiation burden to this organ.

The radionuclide ^{99m}Tc offers many advantages for scintigraphic imaging and more than 85% of all nuclear medicine procedures are performed with radiopharmaceuticals tagged with this isotope. Technetium-99m is available on site by elution from a molybdenum-99 generator in which ^{99m}Tc is constantly formed from the mother isotope by radioactive decay. Technetium-99m has a half-life of approximately 6 h and emits photons of 140 keV, which are well suited for imaging with current gamma cameras. Additionally, ^{99m}Tc is about 100 times

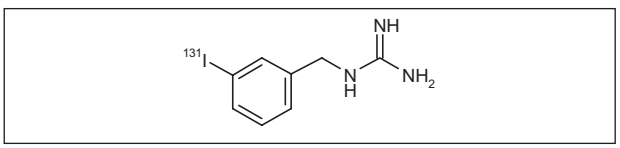


Fig. 1. Structure of MIBG.

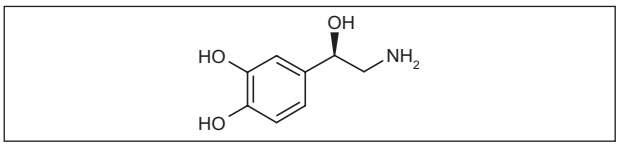


Fig. 2. Structure of noradrenaline.

cheaper than ^{123}I and allows for better binding than radioiodine to biomolecules.

These superior characteristics of ^{99m}Tc over radioiodine encouraged us to look into the possibilities of developing a ^{99m}Tc-labeled adrenergic tracer. This paper reviews the pros and cons of these potential agents and, briefly, the (patho)physiological aspects of cardiac innervation.

Physiology of cardiac innervation

The autonomic nervous system, consisting of the sympathetic and parasympathetic systems, plays a major role in the regulation of myocardial contractility, heart rate and perfusion. Autonomic tone is controlled by various centers in the brain which interact with chemoreceptors, baroreceptors and other receptors in various body parts, including the skin and the viscera. The innervation of the heart occurs via fibers which originate from the cardiac plexus and travel in the subendocardium following the coronary arteries from the base to the apex.

The main neurotransmitter of the sympathetic system is noradrenaline (norepinephrine), which is released into the synaptic cleft from the vesicles in the presynaptic area, followed by binding to postsynaptic (α and β) adrenoceptors. Once activated, β_1-adrenoceptors mediate cardiac stimulation, whereas β_2-adrenoceptors control smooth muscle relaxation. Increased levels of noradrenaline may lead to diminished expression and/or desensitization ("downregulation") of the adrenoceptors. Also, α_2-adrenoceptors at the presynaptic side modulate the release of noradrenaline. On the postsynaptic side, the α_1-adrenoceptor enhances cardiac contractility by increasing the responsiveness of the myofilaments to calcium ions (see Fig. 3).

Overall, the activation of the sympathetic (adrenergic) system leads to stimulation of the heart, resulting in increased heart rate (chronotropic effect), stronger contraction (inotropic effect) and enhanced electrophysiological properties (dromotropic effect).

Autonomic neuropathy

Various conditions including cardiac ischemia, congestive heart failure and diabetes mellitus are thought to affect the function of the sympathetic nervous system.

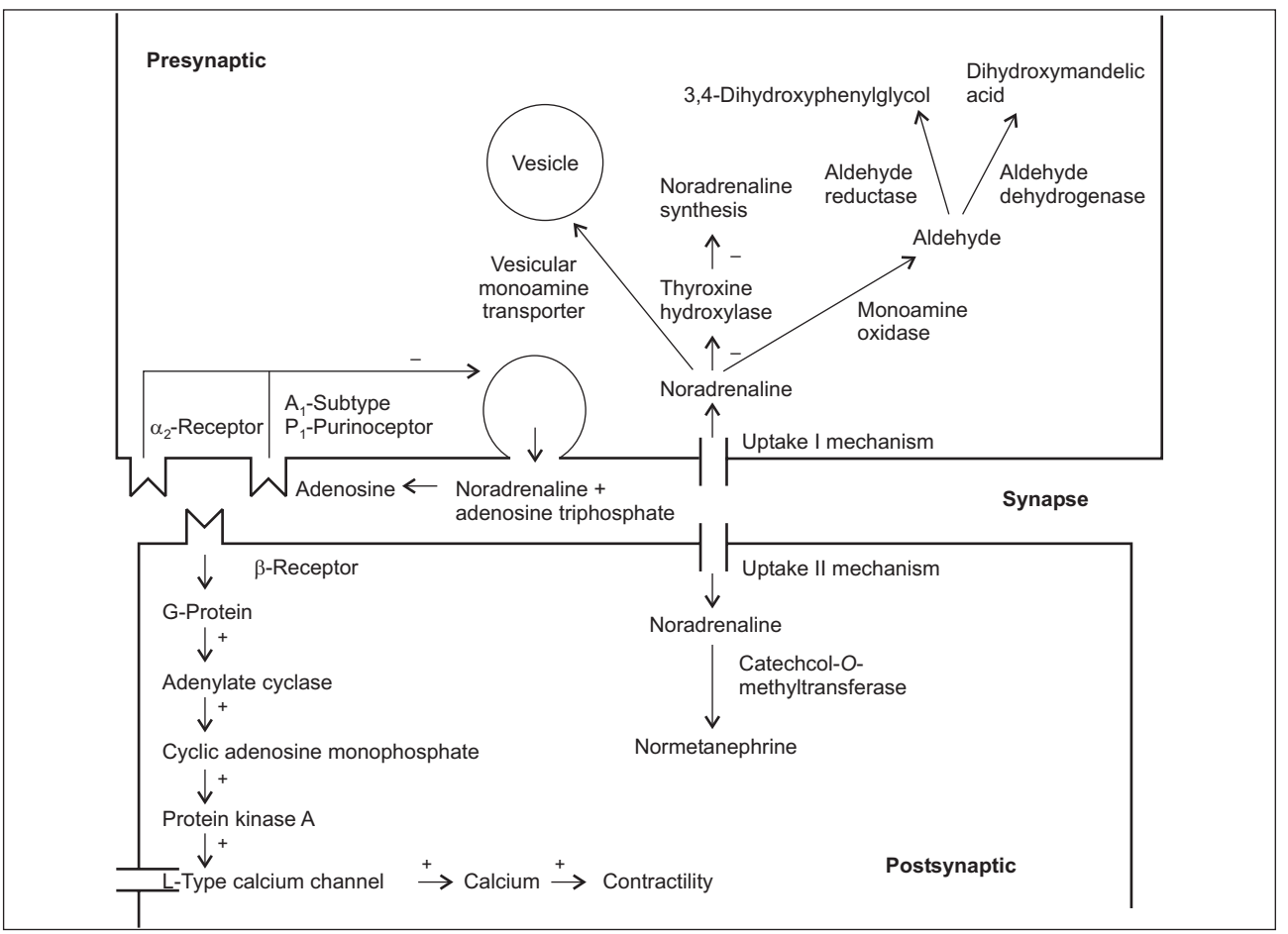


Fig. 3. Sympathetic neurotransmission and biochemical pathways.

Myocardial ischemia influences the integrity of the cardiac neurons. Presumably, the lack of local arterial blood supply decreases the local concentration of energy substrates. Together with a local release of harmful substances, particularly oxygen free radicals, this change in chemical milieu results in impairment of the ventricular nerves and, eventually, cardiac function (4).

Congestive heart failure is associated with enhanced activity of the sympathetic nervous system in order to support the cardiovascular system. In addition, systemic arterial constriction serves to preserve blood pressure and organ perfusion. However, prolonged exposure to increased levels of noradrenaline has deleterious effects, such as desensitization of postsynaptic β-adrenoceptors, which further contribute to cardiac malfunction (5).

A similar effect occurs in patients suffering from diabetes. Activation of the sympathetic nervous system takes place already at the early stage of this disease (6). Chronic exposure to noradrenaline causes downregulation of adrenoceptors. Cardiac innervation is also disturbed by hyperglycemia and insulin deficiency (7). Diabetic autonomic neuropathy often leads to altered myocardial blood flow regulation, ventricular dysfunction and silent ischemia or infarction (8).

Studying cardiac adrenergic innervation

Autonomic neuropathy may lead to cardiovascular symptoms such as dizziness, faintness, blackouts and tachycardia during rest, and postural hypotension. Often these phenomena are accompanied by electrocardiographic changes and catecholamine imbalance. The latter can be measured by determining the plasma levels of noradrenaline, which requires invasive catheterization of the coronary artery system. Another test measures the cardiac noradrenaline spillover after intravenous application of [^{3}H]-noradrenaline (9). Besides the invasiveness of these investigations, no information is obtained on the regional distribution of adrenergic innervation.

At present, scintigraphy is the only method for imaging innervation patterns. This nuclear medicine test uses radiolabeled catecholamine analogues to visualize the cardiac nervous system. As mentioned above, this is achieved with radioiodine-labeled MIBG for planar and SPECT imaging. In addition, various agents allow imaging with positron emission tomography (PET). Scintigraphic studies with PET are carried out with tracers that are radiolabeled with 511 keV-emitting radionuclides, such as carbon-11 (^{11}C) and fluorine-18 (^{18}F). These agents include [^{18}F]-fluorometaraminol, [^{11}C]-metahydroxyephedrine and [^{11}C]-phenylephrine. The utility of these radiotracers for the study of cardiac innervation has been reviewed by Raffel and Wieland (10).

Although interesting from a (patho)physiological standpoint, the main limitation of these PET tracers is their availability. The production of the radionuclides ^{11}C and ^{18}F requires a cyclotron and the chemical synthesis and pharmaceutical quality control require advanced expertise and instrumentation. Overall, the production of PET tracers is a costly affair. Nevertheless, these agents provide a wealth of information on the integrity of (human) cardiac innervation, especially as they allow the quantitative measurement of rate constants associated with presynaptic sympathetic function. Moreover, PET imaging offers better spatial resolution and higher photon sensitivity than planar or SPECT modalities.

Despite these developments, MIBG remains the "workhorse" of cardiac sympathetic neuronal imaging (10). MIBG uptake occurs through the noradrenaline transporter and vesicular binding, often referred to as the uptake-1 mechanism, at active transport sites (11) (Fig. 3). The uptake-2 mechanism is non-neuronal and is likely due to passive diffusion. According to experiments by Dae *et al*. (12), however, this extraneuronal uptake does not play an important role in the human heart. An important issue is that the neuronal transport of clinically used MIBG is carrier-mediated. This emphasizes the need for low-carrier or carrier-free MIBG preparations for clinical purposes.

Imaging with radioiodinated MIBG

One hour prior to the intravenous administration of 185 MBq (5 mCi) [^{123}I]-MIBG, the patient receives an oral dose of sodium iodide (20 droplets of a 120 mg/ml solution) to block the thyroid gland. In order to minimize the adrenaline level in the body, the patients rest half an hour before the MIBG injection. In this way, the occupancy of adrenergic receptors is low, resulting in high MIBG uptake in the heart. In our experience, optimal diagnostic information is obtained 4 h after the administration of the radiopharmaceutical. Figure 4 shows typical examples of a normal MIBG distribution and an abnormal distribution lacking uptake in an infarcted zone.

Other adrenergic tracers?

Other radioiodinated adrenergic compounds

It is known that polar complexes undergo rapid renal excretion. Vaidyanathan *et al.* considered the use of MIBG analogues with polar subunits such as –H, –OH and –NH_2 in order to improve the target-to-nontarget ratio by lowering the nontarget accumulation (13, 14). Figure 5 shows the various compounds that were tested. In their experiments in mice, it appeared that only the CIBG analogue demonstrated higher cardiac uptake. However, blocking the noradrenaline transporter with desipramine resulted in a reduced uptake of up to 48% in the case of MIBG and up to 58% in the case of CIBG, indicating the lower adrenergic specificity of the latter compound. In the wake of these disappointing results, no further studies on MIBG analogues have been reported in the literature.

A recent investigation into another adrenergic compound that can be labeled with radioiodine was reported by Kiyono *et al*. (15). Figure 6 shows the molecular struc-

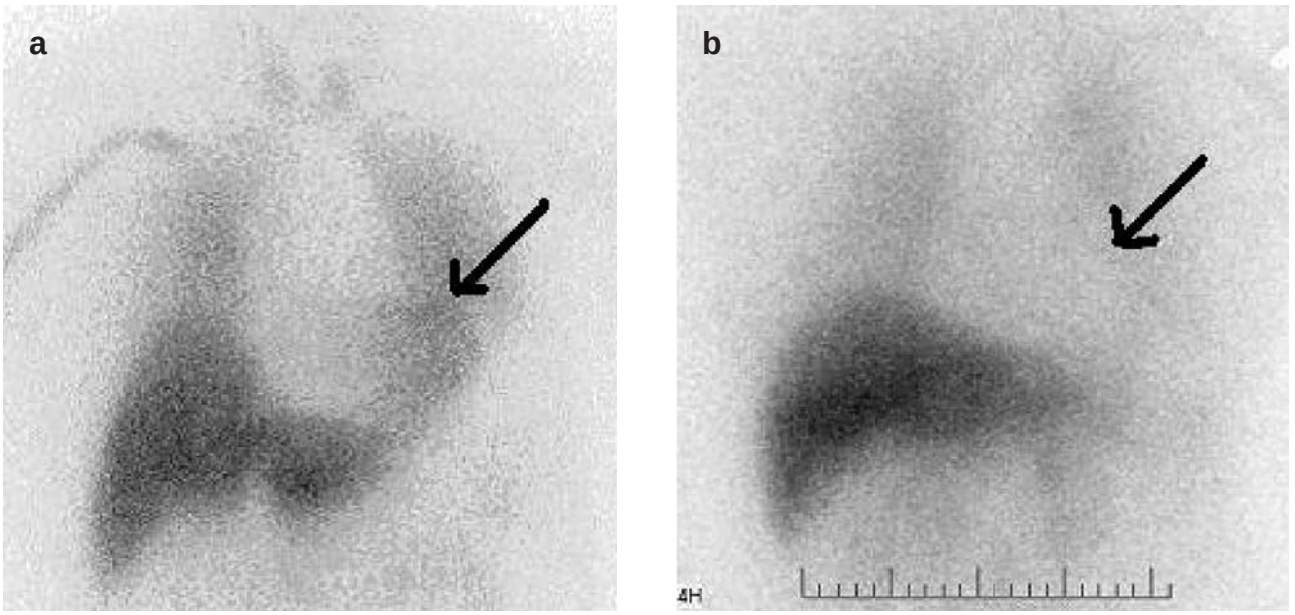
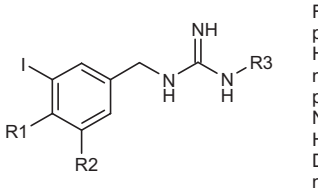


Fig. 4. **a**: Scintigram of the human heart 4 h after intravenous injection of [^{123}I]-MIBG. **b**: [^{123}I]-MIBG scintigram of the human heart after infarction. The absence of MIBG uptake (compared to **a**) represents disturbed innervation.

ture of the compounds that were tested for their adrenergic properties. Radioiodinated *N*-methyl-3-(2-iodophenoxy)-3-phenylpropanamine (MIPP; R = CH_3) was found to possess the highest affinity for the presynaptic noradrenaline transporter. It is noteworthy that the (*R*)-isomer showed higher affinity than the (*S*)-isomer. *In vivo* experiments in rats have demonstrated an unfavorable biodistribution, with high uptake in the lungs, probably due to the high lipophilicity of the agent. This high lung uptake prevents a proper interpretation of cardiac innervation and would presumably also make a [^{99m}Tc]-labeled derivative unsuitable for scintigraphy.

Technetium-99m-labeled adrenergic tracers

Although the radionuclide ^{99m}Tc has many advantages over radioiodine, a major disadvantage is that it cannot easily substitute a hydrogen, carbon, nitrogen or oxygen atom in a biomolecule. Therefore, the linking of ^{99m}Tc requires another methodology taking into account that the physicochemical character influences or dictates the biological fate of the radioagent. This is especially true for the binding of ^{99m}Tc to receptor-specific molecules, which requires the tethering of a [^{99m}Tc]-chelate moiety to a receptor-binding compound. A well-known example was described by Skaddan *et al.* (16) and concerns the labeling of agents for the imaging of estrogen receptor sites. The chelate design starts from the fact that a bifunctional chelator binds the ^{99m}Tc radionuclide without appreciable *in vitro* and *in vivo* dissociation, whereas its structure allows the linkage to the agent of biomedical interest, maintaining maximum biological integrity. Various bifunctional chelators have been used for this purpose. A recent review by Banerjee *et al*. (17) expands upon the recent advances in the synthesis of ^{99m}Tc complexes. These concern the use of N_xS_{4-x} bifunctional chelates, the use of the *N*-oxysuccinimidylhydrazonicotinamide (HYNIC) system and the single amino acid chelates for the $[Tc(CO_3)]^{1+}$ core. An example is given in Figure 7, which illustrates the structure of [^{99m}Tc]-labeled



	R_1	R_2	R_3
MIBG	H	H	H
CIBG	Cl	H	H
FIBG	F	H	H
pAIBG	NH_2	H	H
HIBG	OH	H	H
mINGB	H	NO_2	H
pINGB	NO_2	H	H
NHIBG	H	H	OH
HIMBG	OH	OCH_3	H
DHIBG	OH	OH	H
mAIBG	H	NH_2	H
GIBG	CH_4N_3	H	H
GMIBG	$C_2H_6N_3$	H	H
MeIBG	CH_3	H	H

Fig. 5. Schematic representation of radioiodine-labeled MIBG derivatives.

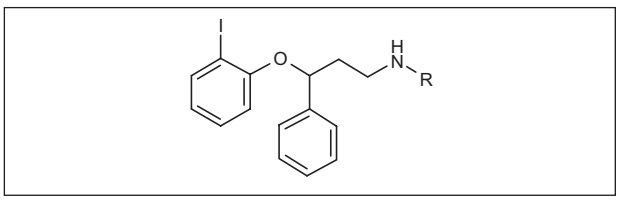


Fig. 6. Schematic representation of radioiodine-labeled analogues of 3-phenoxy-3-phenylpropanamine.

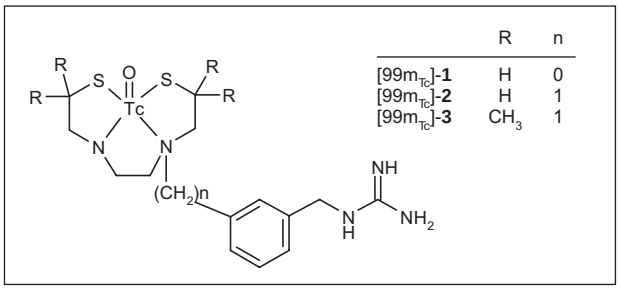


Fig. 7. Technetium-99m-labeled "MIBG" through an N_2S_2 core structure.

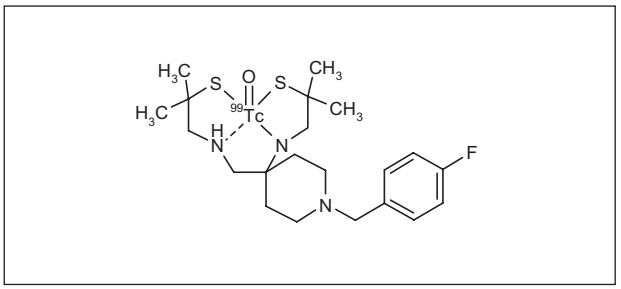


Fig. 8. Structure of [^{99m}Tc]-FBPBAT in which ^{99m}Tc is bound to the agent through an N_2S_2 structure.

"MIBG" on the basis of an N_2S_2 complex. Neither of the tested compounds, however, showed better adrenergic properties than MIBG labeled with ^{123}I (18).

Sammick and coworkers (19) published a study on another compound known to bind to adrenergic structures. This agent, 1-(4-fluorobenzyl)-4-(2-mercapto-2-methyl-4-azapentyl)-4-(2-mercapto-2-methylpropyl-amino)piperidine (FBPBAT) (Fig. 8), could also be labeled with ^{99m}Tc using an N_2S_2 core, which "traps" the radionuclide *in vitro,* and could be prepared in a carrier-free way. However, the high lipophilisity gave rise to uptake in the lungs, thereby obscuring cardiac accumulation. Moreover, this [^{99m}Tc]-labeled compound appeared to bind to β_1-adrenocaptors and much less to α_1-adrenoceptors, both in the postsynaptic area. Unlike MIBG, which is taken up by the presynaptic sympathetic neurons, this new agent reflects noradrenaline uptake in postsynaptic cells and cannot replace MIBG.

Future [^{99m}Tc]-labeled tracers?

The investigations on PET tracers labeled with ^{11}C or ^{18}F have led various research groups to look further into the possibility of tagging these compounds with ^{99m}Tc. The [^{11}C]-labeled compounds epinephrine and metahydroxyephedrine have demonstrated high affinity for the presynaptic noradrenaline transporter. [^{11}C]-Epinephrine reflects both uptake and vesicular storage, whereas metahydroxyephedrine reflects only uptake.

Would it be possible to label these compounds with ^{99m}Tc? Apart from the above-mentioned labeling methods using bifunctional chelators, another direct method is available whereby reduced ^{99m}Tc binds to atoms with electron pairs. In general, the formation of a stable ^{99m}Tc complex requires atom bridging between the ligand and a lower valence state Tc atom, which is achieved by a reducing agent such as stannous chloride. Under such reducing conditions, Tc(VII) is reduced to Tc(IV), but depending on the complexing agent and the reaction conditions, Tc(III) and Tc(V) may also be produced. Thus, in principle, in molecules with free $-NH_2$ or similar groups it is possible that a complex with reduced technetium could be formed. It may be that a stable complex would require more than one ligand and Tc-labeled compounds with two complexing agents are no exception. This may not have a negative effect on the biological characteristics, as long as the molecular part comprising the biological features is properly exposed. Needless to say, the direct labeling method often results in a poorly defined labeled molecule and further experiments (*e.g.*, with mass spectrometry) are necessary to elucidate the molecular structure of the ^{99m}Tc complex. Moreover, a labeling procedure which results in a stable compound and a labeling yield of > 95% is often only obtained after time-consuming "trial and error" methods. The advantage of this method is that the final molecule does not have a bulky N_2S_2 ligand containing ^{99m}Tc (as is shown in Figs. 7 and 8), which might hamper effective binding to the transporter.

There are no literature data available on the ^{99m}Tc labeling of epinephrine or metahydroxyephedrine and it is uncertain whether this is a good or a bad sign. Other interesting compounds for which no ^{99m}Tc labeling methods have been described are desipramine and atomoxetine (Figs. 9 and 10), both in use as antidepressants. Both drugs show high-affinity binding to the presynaptic noradrenaline transporter and no or virtually no affinity for other transporters and receptors.

It should be noted that the above-mentioned compounds show marked pharmacological actions. The pre-

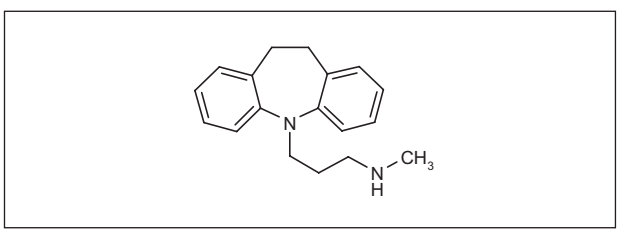


Fig. 9. Structure of desipramine.

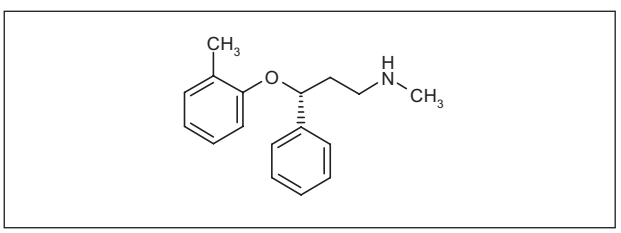


Fig. 10. Structure of atomoxetine.

viously mentioned advantages of a carrier-free preparation of the labeled compound are therefore a prerequisite which increases the degree of difficulty with regard to chemical synthesis. However, a “carrier-free” preparation would at best still contain 10^{12}-10^{14} atoms of ^{99m}Tc and the amount of carrier (the drug) may be many times larger. Extensive toxicity studies with this type of labeled compound may therefore be warranted.

Conclusions

Cardiac innervation controls heart function and heart rate. Ischemic disorders affect not only the perfusion of the myocardium, but often also the innervation. This is true especially for transmural infarction, in which the innervation is more disturbed than is suggested by the size of the infarcted zone. For this condition, but also in the case of, *e.g.,* diabetes, heart transplantation and drug-induced cardiotoxicity, it is of diagnostic and prognostic value to evaluate cardiac innervation. Needless to say, disturbed innervation eventually leads to heart failure, arrhythmia and possibly “sudden death”.

Scintigraphic investigation remains the only way to study the heterogeneity of cardiac sympathetic innervation. MIBG was developed on the basis that guanethidine is a potent neuron-blocking agent that acts on sympathetic nerve endings. The uptake of [^{123}I]-labeled MIBG, occurring through the energy-dependent uptake-1 mechanism, allows the visualization of cardiac neurotransmission by planar or tomographic (SPECT) imaging. For a number of physical and biological reasons, it would, however, be advantageous to develop a ^{99m}Tc-labeled adrenergic compound. Our search has resulted in four potential scintigraphic agents that could be labeled with ^{99m}Tc. Two of these agents have demonstrated presynaptic adrenergic uptake (epinephrine and metahydroxyephedrine) and have been tested *in vitro* and *in vivo* in PET investigations. The other two agents are used as potent antidepressants (desipramine and atomoxetine). So far, there are no studies published on the ^{99m}Tc labeling of these four agents and this may well be due to the degree of difficulty. In spite of this, our own experience with biomolecules (20) indicates that persistent attempts and efforts may yield the desired result, which would place these new drugs in the mainstream of cardiac imaging.

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