**Impact of Novel Antidepressants on Cardiac Metaiodobenzylguanidine (mIBG) Uptake: Experimental Studies in SK-N-SH Cells and Healthy Rabbits**

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## ABSTRACT

**Background**: $^{123}$I-metaiodobenzylguanidine (mIBG) provides independent prognostic value for risk stratification among heart failure patients, but the use of concomitant medication should not impact its quantitative information. We aimed to evaluate the four most-prescribed antidepressants currently used as a first-line treatment for patients with major depressive disorder (MDD) and their potential on altering mIBG imaging results. **Methods**: The inhibition effect of four different types of antidepressants (desipramine, escitalopram, venlafaxine and bupropion) for MDD treatment on $^{131}$I-mIBG uptake was assessed by *in-vitro* cell uptake assays using human neuroblastoma SK-N-SH cells. The half maximal inhibitory concentration ($IC_{50}$) of tracer uptake was determined from dose-response curves. To evaluate the effects of IV pretreatment with desipramine (1.5 mg/kg) and escitalopram (2.5, 15 mg/kg) on mIBG cardiac uptake, *in-vivo* planar $^{123}$I-mIBG scans in healthy New Zealand White Rabbits were conducted. **Results**: The $IC_{50}$ values of desipramine, escitalopram, venlafaxine and bupropion on $^{131}$I-mIBG cellular uptake were 11.9 nM, 7.5 μM, 4.92 μM, and 12.9 μM, respectively. At the maximum serum concentration ($C_{max}$, as derived by previous clinical trials), the inhibition rates of $^{131}$I-mIBG uptake were 90.6 % for desipramine, 25.5 % for venlafaxine, 11.7 % for bupropion and 0.72 % for escitalopram. A low inhibition rate for escitalopram in the cell uptake study triggered investigation of an *in-vivo* rabbit model: with dosage considerably higher than clinical practice, the non-inhibitory effect of escitalopram was confirmed. Furthermore, pretreatment with desipramine led to a marked reduction of cardiac $^{123}$I-mIBG uptake. **Conclusions**: In the present *in-vitro* binding assay and *in-vivo* rabbit study, the selective-serotonin reuptake inhibitor escitalopram had no major impact on neuronal cardiac mIBG uptake within therapeutic dose ranges, while other types of first-line antidepressants for MDD treatment led to a significant decrease. These preliminary results warrant further confirmatory clinical trials regarding the reliability of cardiac mIBG imaging, in particular, if the patient's neuropsychiatric status would not tolerate withdrawal of a potentially norepinephrine interfering antidepressant.



*Keywords*: depression; $^{123}$I-mIBG; antidepressant; cardiac sympathetic nerve system; MDD; major depressive disorder; myocardial sympathetic innervation imaging

## INTRODUCTION

Providing a semi-quantitative score for mortality risk stratification in heart failure, the guanethidine analog $^{123}$I-metaiodobenzylguanidine (mIBG) has recently been Food and Drug Administration-approved after extensive early-phase use (*1-3*). Sharing similar pathways as norepinephrine including entering adrenergic cells through the uptake-1 pathway and storage in presynaptic vesicles, the pharmacokinetics of mIBG mainly depend on NE recycling tissues (*4,5*). However, intake of prescribed or over-the-counter medications in patients envisaged for cardiac mIBG studies inherits potential risks, e.g. artificially lower uptake leading to misinterpretation of cardiac innervation status, or undesirable clinical consequences in case of medication withdrawal (*6*). Hence, the referring physician must carefully judge between potential harm from withdrawing concomitant medications vs. the potential benefit of accurate sympathetic nerve assessment by mIBG imaging (*6,7*).

Widespread availability of safer antidepressant classes has led to a significant increase of antidepressant prescriptions in the last few years. Primary care providers in the United States prescribe double the total number of antidepressants than psychiatrists instead of referring out for cognitive-behavioral therapy (*8,9*). Due to the superior safety profile over previously used tricyclics (TCA), the *Work Group on Major Depressive Disorders* (MDD) recommends three different classes of next generation antidepressants seen as optimal for an initial first-line treatment in patients with MDD. These are selective serotonin reuptake inhibitor (SSRI), serotonin-norepinephrine reuptake inhibitor (SNRI), and norepinephrine-dopamine reuptake inhibitor (NDRI) (*10,11*).

The more extended use of mIBG imaging outside controlled clinical trials (*1,12,13*) and the interfering potential of these drugs with norepinephrine transporters (NET) (*14-17*) raise concern of inhibitory effects on cardiac mIBG uptake (*6*). Previously established, TCA and SNRI lead to significant reduction of mIBG uptake (*4,18*). However, substantial evidence on the NET interference of other first-line antidepressants for MDD treatment, such as SSRI and NDRI, is still lacking (*6*). Using an *in-vitro* binding assay and an *in-vivo* rabbit model which mimics uptake 1 mediated norepinephrine clearance comparable to the human heart (*19*), we aimed to explore the four most-prescribed antidepressants for MDD and their potential on altering mIBG imaging results.

## MATERIALS and METHODS

### Radiopharmaceuticals

$^{123}$I-mIBG (AdreView$^{TM}$) and $^{131}$I-mIBG were both purchased from GE Healthcare (Freiburg i. Breisgau, Germany; specific activity, $^{123}$I-mIBG: 270.1 GBq/mmol, $^{131}$I-mIBG: 11.1 – 55.5

GBq/mmol). $^{3}$H-labeled norepinephrine hydrochloride ($^{3}$H-norepinephrine) was purchased from Perkin-Elmer (Rodgau, Germany). $^{18}$F-fluorodeoxyglucose ($^{18}$F-FDG) was produced as previously described (*20*).

**In-vitro-cell Uptake Assay – $^{131}$I-mIBG**

Human neuroblastoma SK-N-SH cells and desipramine were obtained from Sigma-Aldrich (Munich, Germany). Escitalopram was received from Lundbeck (Hamburg, Germany), venlafaxine from betapharm (Augsburg, Germany) and bupropion from neuraxpharm (Langenfeld, Germany). An overview of the investigated antidepressants is given in Fig. 1. SK-N-SH cells were grown in minimum essential medium with 2 mM L-glutamine and fetal bovine serum (10%). Cells were cultured in 75 cm$^2$ flasks and later transferred to 12 well-plates 1 day before uptake assay. Cells were incubated with $^{131}$I-mIBG. Increasing concentrations of antidepressants and $^{131}$I-mIBG (3.7kBq/well) were added and incubated at 37 °C for 1 hour. Antidepressants and treating doses were as follows: desipramine (TCA) at 10 pM - 10 µM, escitalopram (SSRI) at 10 pM - 100 µM, venlafaxine (SNRI) at 10 pM - 10 µM and bupropion (NDRI) at 10 pM – 1 mM. Following incubation for 1 hour, cells were washed twice with ice-cold phosphate buffered saline and solubilized with 0.1N NaOH. Radioactivity associated with the cells was measured using a γ-counter (FH 412; Frieseke & Höpfner, Erlangen, Germany) expressing the results in counts per minute (cpm). $^{131}$I-mIBG cpm were normalized by dividing cpm of co-treatment with $^{18}$F-FDG. As a physiological reference, the inhibition effect of desipramine and escitalopram on $^{3}$H-norepinephrine uptake was assessed in the same assay system. Assays were carried out in medium containing inhibitors of catecholamine metabolism (100 µM pargyline, 20 µM pyrogallol). Since norepinephrine uptake in SK-N-SH cells was peaked earlier than in mIBG, we have adopted 30 min as the preferred tracer incubation time for the norepinephrine uptake assay. The radioactivity associated with the cells was measured using a liquid scintillation counter.

For both in-vitro cell uptake studies, non-specific uptake was determined in the presence of 10 µM desipramine, and specific cellular tracer uptake (%) was calculated by subtracting nonspecific uptake from total uptake. The inhibition rate by each antidepressant at each dose was calculated as a percentage of control. Dose-response curves were plotted to determine the half maximal inhibitory concentration ($IC_{50}$) values and % inhibition at reported maximum concentration ($C_{max}$) using ImageJ software (ImageJ software, version 1.47v; National Institutes of Health) (*21*). $C_{max}$ was defined as maximum concentrations of a drug achieved after dosing as determined in clinical trials (*21*).

**Animal Pretreatment**

Female healthy New Zealand White Rabbits (Charles River Laboratories) weighing 2330 ±

825 g were used. Animal protocols were approved by the local Animal Care and Use Committee and conducted according to the Guide for the Care and Use of Laboratory Animals (*22*). Rabbits were assigned to one of the following pretreatment protocols, which were administered via ear vein injection: (1) TCA desipramine 1.5 mg/kg intravenously (IV, n=3), (2) SSRI escitalopram 2.5 mg/kg (n=5) or 15 mg/kg IV (n=4) and (3) saline IV treated controls (n=4).

### $^{123}$I-mIBG Scintigraphy

Ten minutes after preliminary pharmaceutical administration, the radiotracer $^{123}$I-mIBG (50 MBq) was injected IV, and scans were performed 2.5 h after tracer injection with a dual-head gamma camera (Symbia E, Siemens Healthcare, Erlangen, Germany) equipped with medium energy collimator. For imaging, animals were maintained under anesthesia with 2% isoflurane and placed in a prone position on one detector head of the dual-headed gamma camera. Chest scintigraphic images with ventral (anterior) views of 10 min were acquired in 256 × 256 matrices with a zoom factor of 1.0 and an energy window set at 20 % of the 159 keV $^{123}$I photopeak. Cardiac tracer accumulation was quantified according to the Guidelines of the American Society of Nuclear Cardiology (*23*): heart-to-mediastinum ratios (HMR) were calculated by dividing the average count density of the manually drawn region of interest on the left ventricle by that on the mediastinal region of interest on anterior chest images.

### Determination of Antidepressant Drug

Serum concentrations were determined using high-performance liquid chromatography, as previously described (*24*).

### Statistical Analysis

All results are displayed as mean ± standard deviation. The two-tailed paired Student's t-test was used to compare differences between two dependent groups, and the two-tailed independent Student's t-test for differences between independent groups. A P-value of less than *0.05* was assumed to be statistically significant. Statistical analysis was done with StatMate III (ATMS Co., Ltd).

## RESULTS

### In-vitro Study

The $IC_{50}$ values of desipramine, escitalopram, venlafaxine and bupropion on $^{131}$I-mIBG uptake were 11.9 nM, 7.5 μM, 4.92 μM, and 12.9 μM, respectively (Fig. 2). The inhibition

rates of $^{131}$I-mIBG uptake at $C_{max}$ (as derived by clinical studies, (*21*)) were calculated as 90.6 % for desipramine, 0.72 % for escitalopram, 25.5 % for venlafaxine, and 11.7 % for bupropion. The dose ranges of each antidepressant were 33.8 nM - 237 nM for desipramine, 17.9 nM - 94.9 nM for escitalopram, 122 nM - 1.26 µM for venlafaxine and 75.1 nM - 526 nM for bupropion. As a reference, the $IC_{50}$ values of desipramine and escitalopram on $^{3}$H-norepinephrine uptake in the same assay system were 4.03 nM and 3.06 µM, respectively. The inhibition rates of $^{3}$H-norepinephrine uptake at $C_{max}$ were calculated as 99.2 % for desipramine and 8.62 % for escitalopram.

**In-vivo Study**

Focal $^{123}$I-mIBG uptake was identified in all animals indicating cardiac tracer accumulation (HMR, 1.94 ± 0.22, Fig. 3). Pretreatment with the potent neuronal uptake-1 blocking agent desipramine 1.5 mg/kg IV diminished cardiac $^{123}$I-mIBG uptake (HMR, 1.23 ± 0.18, $p<0.001$ vs. controls).

In the previous binding assay study, venlafaxine and bupropion showed considerable inhibitory effects on mIBG, whereas escitalopram demonstrated almost no inhibition for mIBG uptake at $C_{max}$. Hence, to further definitely rule out an inhibitory potential, escitalopram was also tested *in-vivo*: Using a dose of 2.5 mg/kg escitalopram, no pharmagological influence on cardiac $^{123}$I-mIBG uptake was observed (HMR, 2.01 ± 0.13). Even after increasing the escitalopram dose to 15 mg/kg, no substantial impact on $^{123}$I-mIBG uptake was recorded (HMR, 2.05 ± 0.19, Fig. 3). Average serum concentrations of escitalopram after IV pretreatment of 2.5 mg/kg and 15 mg/kg were 232 ng/ml and 1067 ng/ml, respectively; thus, approximately 13- to 15- fold higher than standard clinical therapeutic concentrations of 15-80 ng/ml in human (*21*).

**DISCUSSION**

When interpreting results of cardiac mIBG imaging, it is crucial to realize unintended effects of prescribed medications (*6,7,25*). Due to the large variety of drugs directly competing with mIBG for NE transporters, testing of these drugs should meet the following prerequisites: the drug should be widely prescribed, it should follow the norepinephrine metabolic pathway and should be investigated in a robust preclinical model which is relevant clinically and translateable. Based on these principles, we tested the four most-prescribed classes of antidepressants for MDD treatment (*10*). These compounds are also known to interfere with the NET (*6*). The herein used *in-vitro* neuroblastoma cell lines as well as the rabbit model are both well-established methods for evaluating NET-related tracer mediation (*4,26-28*) and the

sequence of investigating cardiac catecholamine analogue tracers by using SK-N-SH cells followed by a subsequent in-vivo rabbit imaging study has been frequently described in the literature (*28,29*). As expected, pretreatment with the TCA desipramine led to a significant reduction of cardiac uptake, whereas venlafaxine and bupropion suggested moderate inhibitory effects. However, the SSRI escitalopram demonstrated no *in-vitro* interaction with mIBG (Fig. 2). Based on these initial findings of our cell assay, escitalopram was also tested in healthy rabbits to definitely rule out its inhibitory potential on mIBG uptake: initial *in-vitro* findings could be further corroborated, as no *in-vivo* interaction with mIBG after escitalopram pretreatment could be observed in the scintigraphy study (Fig. 3).

Tracer uptake via NET forms the backbone for presynaptic sympathetic innervation imaging (*30,31*). The specificity for the presynaptic uptake-1 as well as the mechanism of vesicular packaging inside the nerve terminals differs from species to species and among available radiotracers (*32*). Even though smaller rodents like rats might be easier to handle and more affordable, Rischpler et al. concluded that the rabbit heart might be a more attractive model for the assessment of sympathetic nerve conditions, as the specificity to uptake-1 seems to be higher in rabbits than in rats *(33).* Analogous to previous findings in rabbits, cardiac $^{123}$I-mIBG uptake via neural uptake-1 was re-confirmed with *in-vivo* scintigraphy using the potent uptake-1 blocking agent desipramine (*28*). Hence, as the contribution of norepinephrine clearance to neural uptake-1 is also pronounced in human hearts (*19*), the rabbit heart offers an attractive platform for testing catecholamine analogue tracers. The herein used human neuroblastoma SK-N-SH cells are also established as a robust model for NET-related tracer mediation (*28*): $^{3}$H-norepinephrine reflects the physiological condition of norepinephrine (*34*) and its inhibition rate at $C_{max}$ using desipramine was 99.2 % in the present study.

The SSRI escitalopram is classified as the most potent serotonin transporter selective compound, a property predictive for antidepressant efficacy (*15,35*). However, escitalopram possesses a moderate affinity for the NET (*16,17*), which might inherit potential interactions with mIBG uptake. Utilizing competition assays, escitalopram demonstrated a serotonin transporter selectivity approximately 8000-9000 higher compared to NET (*15*). This is in line with our *in-vitro* results demonstrating very low inhibition rates of $^{131}$I-mIBG uptake (0.72 %) at $C_{max}$. Additionally, $IC_{50}$ of escitalopram was significantly higher than its $C_{max}$, suggesting that the potential maximum concentration may theoretically be reached long before achieving the $IC_{50}$ value (Fig. 2). In addition to that, our *in-vivo* rabbit study revealed no considerable effect of escitalopram on cardiac mIBG uptake, even in plasma blood concentrations considerably higher than clinical norms (*21*).

Due to the limited literature on the effect of antidepressants on mIBG pharmacokinetics, the level of evidence on judging the effects of SSRI, SNRI, TCA or NDRI with a specific focus

on cardiac studies is limited (*6*). *Jacobson* and coworkers primarily divided patients using neuropsychiatrics with higher vs. lower potency (for NET interference): the HMR ratios for those patients under high potency neuropsychiatric drugs was significantly lower compared to the lower potency medication group. However, a subanalysis for the influence of specific types of antidepressants on mIBG uptake could not be provided (*25*). Apart from that, research has been particularly conducted using platelets, pheochromocytoma, and neuroblastoma cells: analogous to our findings for the SSRI escitalopram, previous studies revealed that mIBG neuronal uptake is minimally affected by the SSRI fluvoxamine (*6,36,37*). The SNRI milnacipran led to a moderate reduction of mIBG uptake in the heart (*18,38*), which is also similar to our binding assay results (inhibition rate at $C_{max}$ 26% for the SNRI venlafaxine). A brain study using PET agents yielded comparable results for the occupancy of NET under milnacipran in man (*39*). In line with our findings for desipramine, Sisson and coworkers reported on a significant reduction of cardiac mIBG uptake 2h after administration of the TCA imipramine in healthy volunteers (*40*). Recently, mIBG has been also introduced for evaluating Parkinson's Disease (*41,42*). Shimizu et al. investigated the usefulness of myocardial scintigraphy for differentiating dementia with Lewy bodies from Alzheimer's disease and any patients under the NDRI bupropion were excluded (*43*). The present study provides the first evidence of reduced mIBG uptake *in-vitro* (inhibition rate at $C_{max}$, 12%). Hence, in a conservative approach, bupropion might be better discontinued prior to mIBG administration.

However, as a limitation of our *in-vivo* rabbit study, no hemodynamic data including left ventricular function, heart rate and blood pressure were recorded. Due to their anticholinergic properties, the TCA desipramine is known to have several effects on the heart, including decrease of blood pressure, electrophysiological alterations as well as on myocardial contractility (*44*). On the other hand, SSRIs such as fluvoxamine or escitalopram, seems to have only slight effects on the heart rate, which is mainly caused by their pharmacological profile of almost no anticholinergic activity (*44-46*). Hence, given a potential influence on hemodynamics under TCA, one might speculate that desipramine may have led to a further decrease of cardiac mIBG uptake in the present *in-vivo* study. Moreover, a potential gap between healthy conditions and cardiac impairment has to be addressed in future investigations: Albeit we are seeking to provide an overview on the basic influence of antidepressants on cardiac mIBG uptake, an examination in cardiac impaired animal models could also demonstrate whether disease-based alterations are still robustly detectable under escitalopram.

In summary, these findings suggest there may be interference of NET binding by TCA, SNRI and NDRI therapy in patients scheduled to undergo mIBG cardiac imaging.

Conversely, SSRI therapy may be continued without confounding effect in these same patients. Although further confirmation in humans is warranted, a well-designed trial might be difficult to approve due to the absence of a verum arm as a patients' mental status might not tolerate the withdrawal of therapy. Moreover, patients are advised to taper antidepressants, rather than discontinuation in order to reduce the risk of withdrawal symptoms (*47*). This makes it difficult to determine the appropriate time point at which to obtain the mIBG scan.

## CONCLUSION

In the present *in-vitro* binding assay and *in-vivo* rabbit study testing the most-prescribed antidepressant classes for MDD treatment, the SSRI escitalopram had no major interference on the neuronal mIBG uptake at a therapeutic dosage, while other frequently prescribed types of antidepressants for MDD led to a significant decrease in uptake. Further assessment to reveal the potential interference of neuropsychiatric medications on cardiac mIBG uptake is warranted.

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**FIGURES and FIGURE LEGENDS**

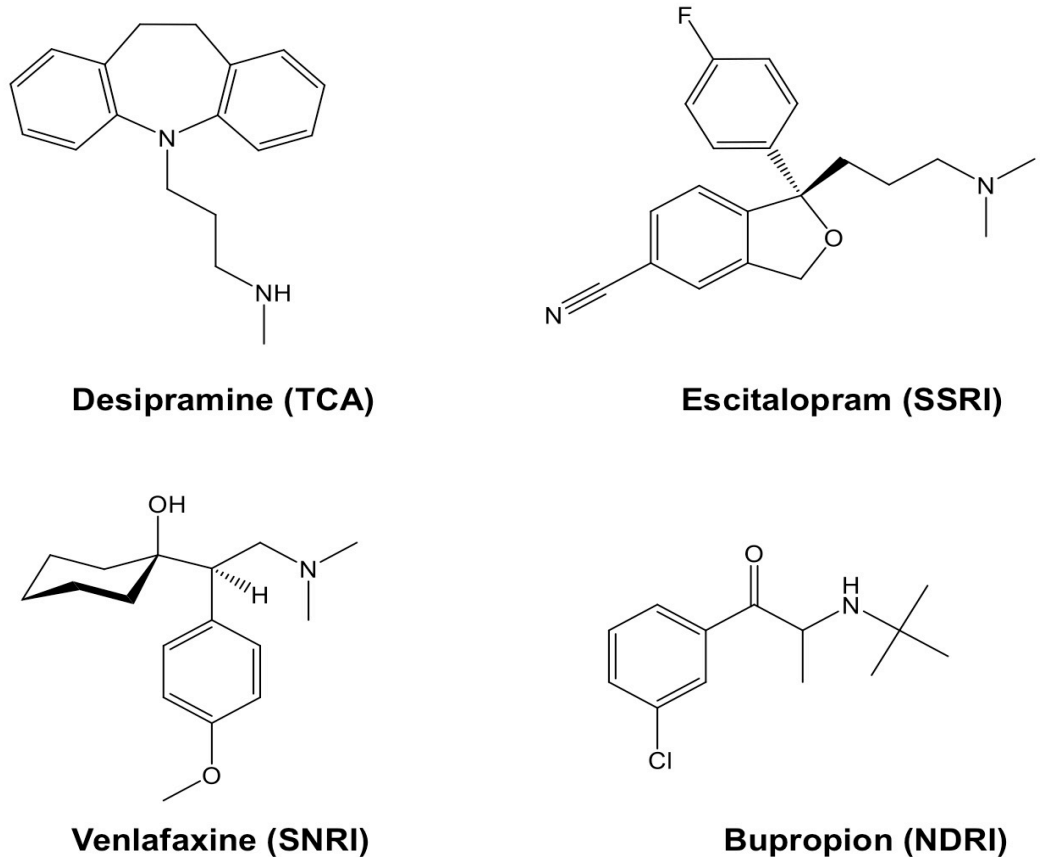


**Figure 1.** Overview of investigated antidepressants (desipramine (tricyclic antidepressant, TCA), escitalopram (selective serotonin reuptake inhibitor, SSRI), venlafaxine (serotonin-noradrenaline reuptake inhibitor, SNRI) and bupropion (norepinephrine–dopamine reuptake inhibitor, NDRI)).

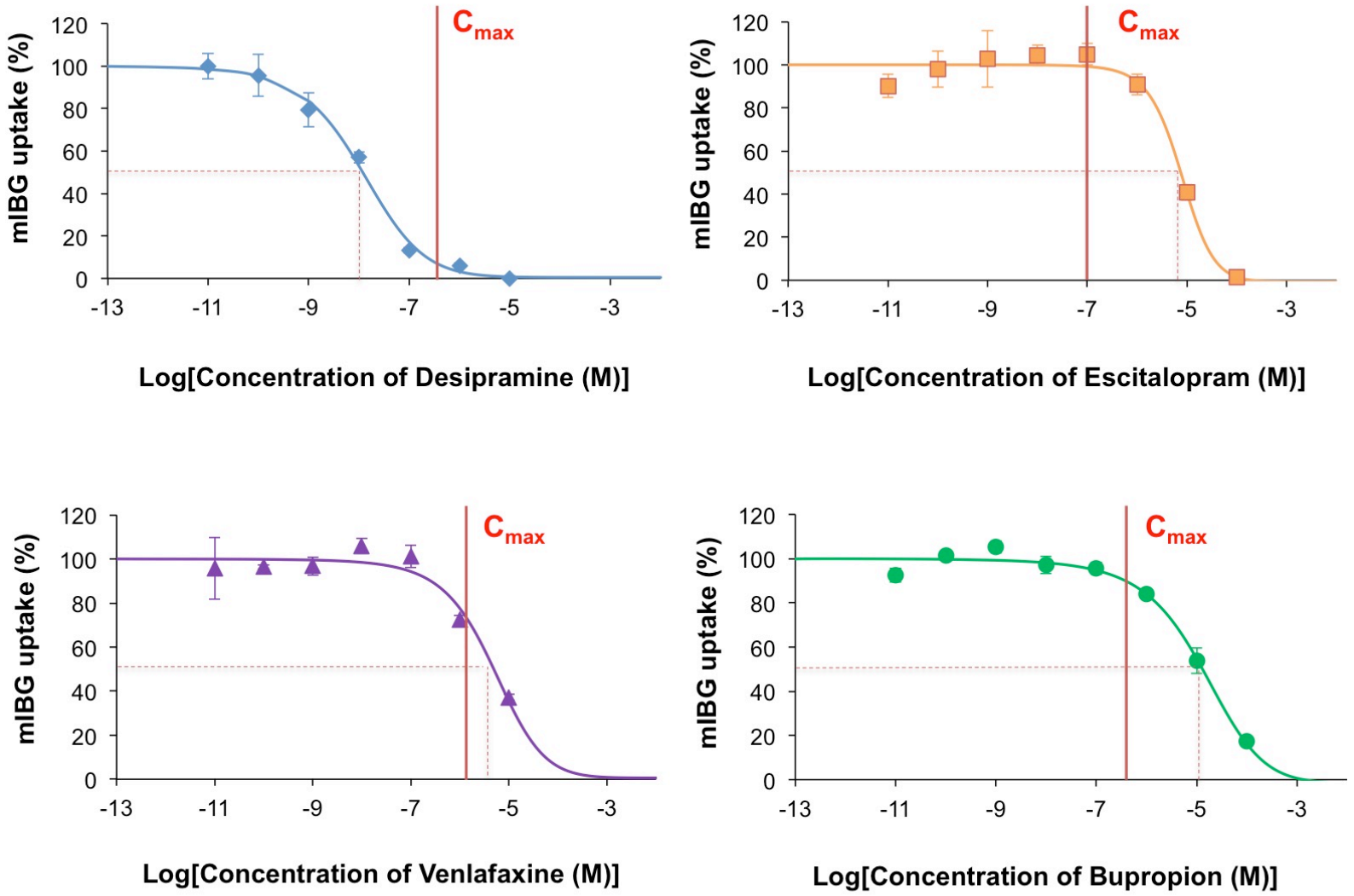


**Figure 2.** Dose-response curves obtained from *in-vitro* $^{131}$I-metaiodobenzylguanidine (mIBG) uptake assays using increasing concentrations of different antidepressants. Reported maximum concentration ($C_{max}$) as derived by clinical studies (*21*) are displayed on each graph. Dotted lines indicate respective $IC_{50}$ values.

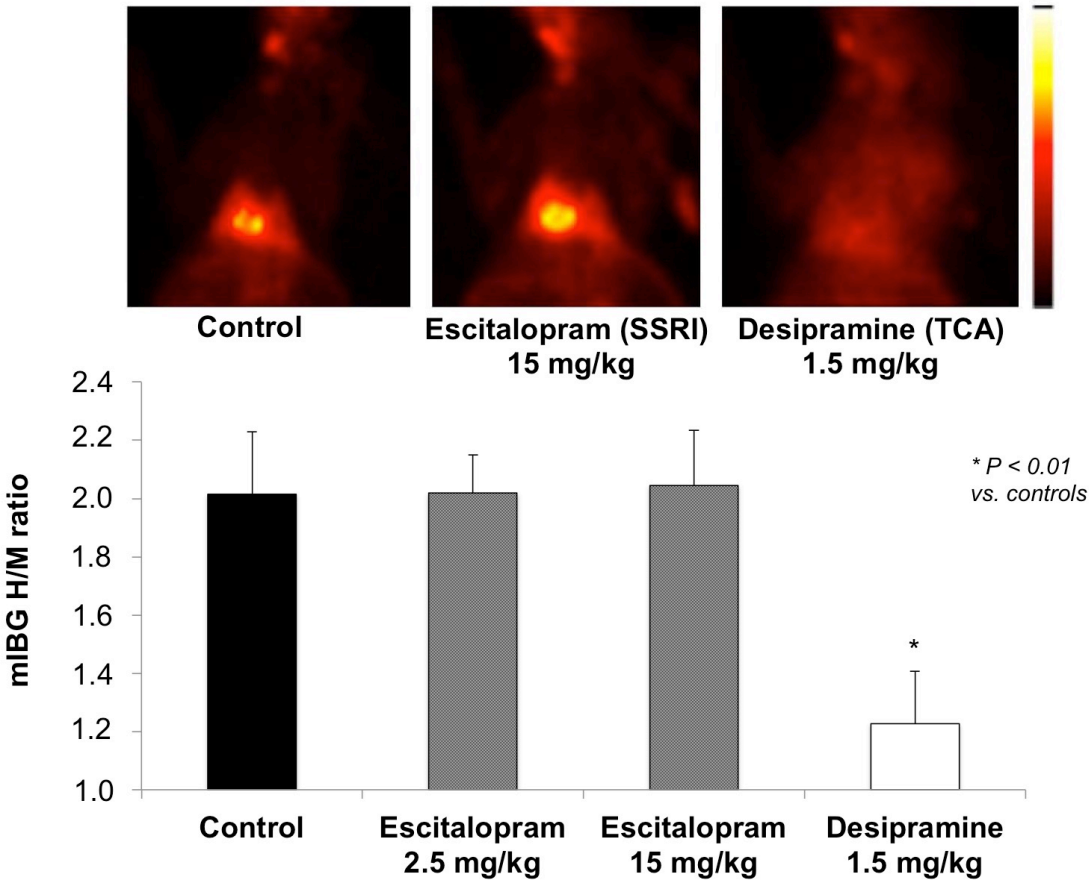


**Figure 3.** Results of the *in-vivo* $^{123}$I-metaiodobenzylguanidine (mIBG) studies after dose-increasing pretreatment with the selective serotonin reuptake inhibitor (SSRI) escitalopram compared to desipramine. In an *in-vivo* rabbit model, heart-to-mediastinum ratios (H/M ratio) decreased significantly after blockade of uptake-1 with desipramine 1.5mg/kg (*p<0.01). There is no reduction of H/M ratio after pretreatment with increasing doses of escitalopram (2.5mg/kg and 15mg/kg, respectively).