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Uptake and washout of I-123-MIBG in neuronal and non-neuronal sites in rat hearts: Relationship to renal clearance

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We investigated the uptake and washout of I-123-metaiodobenzylguanidine (MIBG) in neuronal (both intra- and extravesicular) and non-neuronal sites in the heart and its relationship to renal clearance. Acute renal failure was induced in rats by ligating the renal vessels, and the findings were compared with those of sham-operated rats. Each group consisted of control, reserpine-treated and 6-hydroxydopamine (6-OHDA)-treated subgroups. Rats were sacrificed at 10 minutes and 4 hours after injection of MIBG. MIBG activity was calculated in specimens of heart, spleen, lung and blood. At 10 minutes, no significant difference in MIBG uptake in the heart was observed among the subgroups or between sham-operated and renal failure rats despite a significantly higher blood MIBG activity in the latter. At 4 hours, however, the hearts of both reserpine-treated and 6-OHDA-treated rats showed significantly lower MIBG uptake than control rats. Furthermore, the hearts of renal failure rats showed higher MIBG uptake in the control and reserpine-treated rats than in the corresponding subgroups in sham-operated rats. Intra and extravesicular neuronal uptake of MIBG in the heart were estimated using control, reserpine-treated and 6-OHDA-treated rats. Vesicular uptake values were similar in both the sham-operated group (0.51% ID/g) and the renal failure group (0.44% ID/g). But extravesicular neuronal uptake values were quite different in the renal failure group (0.86% ID/g) and the sham-operated group (0.19% ID/g). In conclusion, uptake to and washout from extravesicular neuronal sites may depend on the concentration of MIBG in the blood or the state of renal clearance, but vesicular uptake may be independent of these factors.

Key words: MIBG, heart, renal clearance, reserpine, 6-OHDA

INTRODUCTION

RADIOIODINATED META-IODOBENZYLGUANIDINE (MIBG), an analog of guanethidine, which blocks sympathetic neurons, has been used extensively to visualize human pheochromocytoma,[1] neuroblastoma,[2] and sympathetic innervation of the heart.[3] MIBG is thought to share the mechanism of uptake and storage with norepinephrine (NE).[4,5] Once inside the neuron, MIBG accumulates both inside and outside the vesicles,[6] and uptake to the vesicles is saturable, ATP and Mg^{++} dependent,[7] and more stable than extravesicular uptake.[8] There may be additional non-specific uptake of MIBG in non-neuronal tissues, which may release MIBG more quickly than neuronal uptake.[5,9,10]

Uptake of NE into the storage vesicles is Mg^{++} and ATP dependent, but at high plasma concentrations, NE may enter the vesicles by passive diffusion.[11] Reserpine is known to block both the active and passive uptake of NE and guanethidine into the storage vesicles,[11,12] and at high doses, reserpine can completely deplete the vesicular NE storage and block further uptake for an extended period.[10,13,14] Gasnier et al.[7] showed, in an experiment with bovine chromaffin granules, that transport of MIBG through membrane was also saturable and the uptake was similarly blocked by reserpine.

High doses of 6-hydroxydopamine (6-OHDA), a neurotoxic agent, which is actively transported to the sympathetic nerve endings, is thought to destroy the nerve endings, especially in the heart.[15–17] Because by this chemical sympathectomy, both intra- and extravesicular uptake of MIBG can be blocked,[18] MIBG would be assumed to accumulate only in non-neuronal tissues.

Most of the radioactivity excreted through the kidneys

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after injection of MIBG is in an intact form, especially in the first few hours.[19,20] In humans, 60–70% of MIBG is excreted through kidneys within 24 hours and a significant fraction of injected MIBG is filtered through the kidneys in the first few minutes.[21,22] Impairment of renal function or complete blocking of renal excretion may increase the activity of MIBG in the blood, and may thereby increase the uptake of MIBG in various sympathetic innervated organs and decrease the release of MIBG from those organs.[23]

Previously we reported[24] rapid clearance of MIBG from the hearts of patients with cardiomyopathy but patients on hemodialysis showed slow clearance from the heart. The purpose of this study is firstly, to investigate the effect of renal clearance of MIBG on its uptake into and washout from the heart, and secondly, to evaluate which compartment, neuronal or non-neuronal, is more liable to the renal clearance. For this purpose, we ligated the renal vessels of rats and treated them with either reserpine or 6-OHDA, and the activity of MIBG in organs and blood was determined at early and delayed times.

MATERIALS AND METHODS

Radiopharmaceuticals, chemicals, and animals: I-123-MIBG was purchased from Daiichi Radioisotopes Co., Ltd., Tokyo, Japan. The specific activity was 1.1–3.7 GBq/mg of MIBG. A 0.925 MBq of it (approximately 0.84 μg of MIBG, calculated from the lowest specific activity) was administered to each rat through the tail vein. The reserpine solution for injection was purchased from Daiichi Pharmaceuticals Co., Ltd., Tokyo, Japan. 6-OHDA hydrobromide (95% chemically pure) was purchased from Aldrich Chemical Co., USA. Male Sprague-Dawley rats, weighing 200–250 g, were purchased from SLC Co., Ltd., Shizuoka, Japan. Each rat was anesthetized by intraperitoneal injection of pentobarbital sodium (40–50 mg/kg), after being submerged in an ether-filled jar.

Method of blocking MIBG uptake into sympathetic vesicles: Reserpine was used to block the vesicular accumulation of MIBG. Reserpine, 4 mg/ kg body weight was selected for intraperitoneal administration according to the method of Nakajo et al.[8] This dose was sufficient to induce the maximal pharmacological effects.[25] The drug was administered to the rats intraperitoneally 4 hours before MIBG injection because it was shown that maximal depletion of endogenous NE from rat hearts occurs 4 hours after administration.[25]

Method of chemical sympathectomy of myocardial innervation: According to Kawa et al.,[16] 150 mg/kg body weight of 6-OHDA was injected intraperitoneally into rats 2 weeks before MIBG administration. A negligible amount of endogenous NE was found in the rat hearts 2 weeks after intraperitoneal injection of that dose.[16]

Method of inducing acute renal failure and sham-operated models: Acute renal failure was induced in half of the rats. After anesthesia, the abdomen was opened by a midline incision. Both kidneys were carefully exposed and the renal vessels were tied near the renal hilus with cotton thread. Bleeding from the incision line was checked as far as possible. After ligating the renal vessels, the abdomen was closed with 4–5 sutures. Sham surgery was performed in the remaining half of the rats by making the same incision, then closing the abdomen. For both 10-minute and 4-hour uptake, MIBG was injected soon (within 1–2 minutes) after closing the abdomen in all renal failure and sham-operated rats.

Tissue distribution study: There were three subgroups (conditions) of rats (control, reserpine-treated, and 6-OHDA-treated) in each model (sham-operated and renal failure). Rats were killed by rapid thoracotomy at 10 minutes for early and 4 hours for delayed data after injection of MIBG. Just before killing, blood samples were collected from the abdominal aorta by removing the abdominal sutures. The total heart, excluding large vessels, part of the spleen and the lung on both sides were taken and thoroughly washed with normal saline and blotted. The chambers of the heart were cut open to clear all clotted blood. All samples were weighed and counted in an auto well gamma counter (Aloka, Japan) and expressed as a percentage of the injected dose per gram of tissue (% ID/g).

Data analysis: All statistical data were expressed as the mean and one standard deviation (sd). Multi-point comparisons were made by two-way analysis of variance (ANOVA) and two-point comparisons were made by Student's t-test. If ANOVA showed significant differences between groups or among conditions, then two-point Student's t-test was carried out.

RESULTS

Figure 1 shows MIBG uptake into the heart and the significance of differences among the groups and conditions. The uptake at 10 minutes was higher than that at 4 hours in all groups and conditions. There were statistically no significant differences in uptake at 10 minutes among control, reserpine-treated and 6-OHDA-treated rats in the respective groups or between corresponding subgroups of sham-operated and renal failure rats. At 4 hours, however, there were significant differences in uptake among control, reserpine-treated, and 6-OHDA-treated as well as between corresponding subgroups of sham-operated and renal failure rats but not in 6-OHDA-treated rats. In 6-OHDA-treated rats, the uptake was similar in the sham-operated and renal failure groups. The differences between the mean uptake value in the control and that in the reserpine-treated rats at 4 hours were 0.51% ID/g (1.16 minus 0.65) for sham-operated and 0.44% ID/g (1.72 minus 1.28) for renal failure rats. The differences

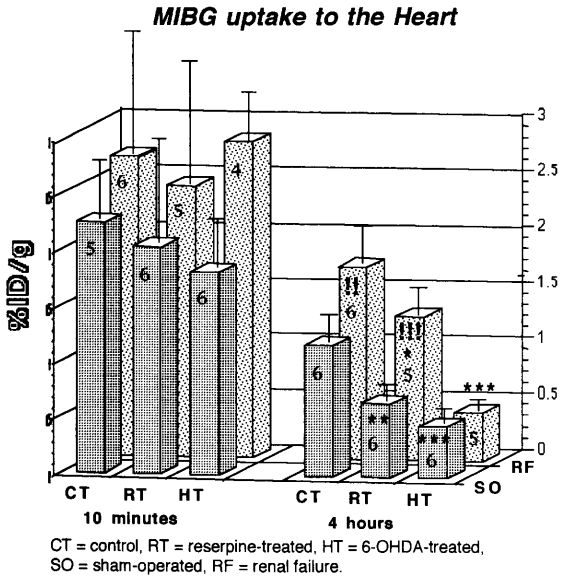

Fig. 1 MIBG uptake to the heart (mean ± 1 sd) at 10 minutes and 4 hours after injection and significant differences among groups and conditions. The numbers on the column represent numbers of samples. * = comparison among control, reserpine-treated and 6-OHDA-treated rats in respective groups. ! = comparison between corresponding subgroups of sham-operated and renal failure rats. */! = $p < 0.05$, **/!! = $p < 0.01$, ***/!!! = $p < 0.001$

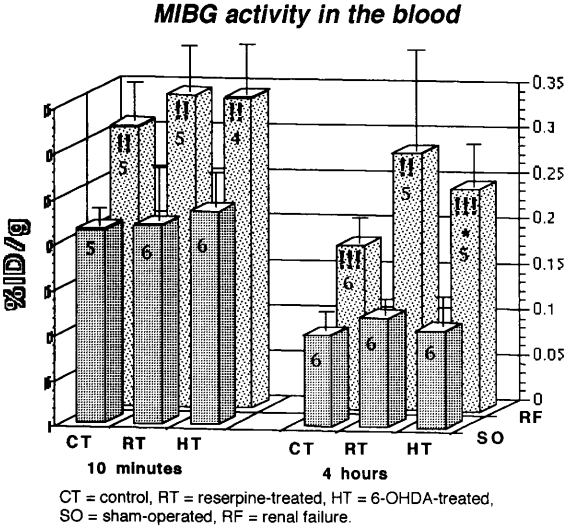

Fig. 2 MIBG activity (mean ± 1 sd) in the blood at 10 minutes and 4 hours and significant differences among groups and conditions. Note, significantly higher blood MIBG activity in the renal failure group. The numbers on the column represent numbers of samples. * = comparison among control, reserpine-treated and 6-OHDA-treated rats in respective groups. ! = comparison between corresponding subgroups of sham-operated and renal failure rats. */! = $p < 0.05$, **/!! = $p < 0.01$, ***/!!! = $p < 0.001$

between the mean uptake value in the control and that in the 6-OHDA-treated rats at 4 hours were 0.70% ID/g (1.16 minus 0.46) for sham-operated and 1.30% ID/g

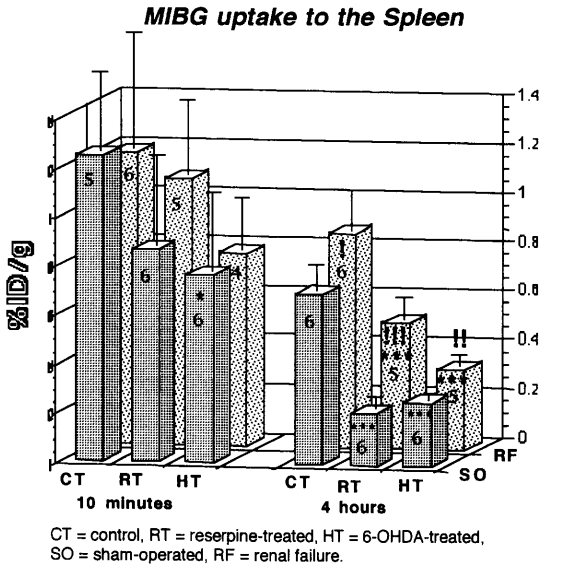

Fig. 3 MIBG uptake (mean ± 1 sd) to the spleen at 10 minute and 4 hours and significant differences among groups and conditions. Note, the uptake pattern was similar to that in the heart (Fig. 1) except for that in 6-OHDA-treated rats. The numbers on the column represent numbers of samples. * = comparison among control, reserpine-treated and 6-OHDA-treated rats in respective groups. ! = comparison between corresponding subgroups of sham-operated and renal failure rats. */! = $p < 0.05$, **/!! = $p < 0.01$, ***/!!! = $p < 0.001$

(1.72 minus 0.42) for renal failure rats, and the differences between the mean uptake value for reserpine-treated and that for 6-OHDA-treated rats were 0.19% ID/g (0.65 minus 0.46) for sham-operated and 0.86% ID/g (1.28 minus 0.42) for renal failure rats.

Figure 2 shows the activity of MIBG in the blood and the significance of differences among groups and conditions. No difference was observed among control, reserpine-treated and 6-OHDA-treated rats either at 10 minutes or at 4 hours in the respective groups, but when the corresponding subgroups of sham-operated and renal failure groups were compared, significantly higher blood MIBG activity was observed under all conditions in renal failure rats at both 10 minutes and 4 hours.

Figure 3 shows MIBG uptake in the spleen and the significance of differences among groups and conditions. At 10 minutes, uptake patterns were similar to those in the heart. At 4 hours, however, disparities were observed especially in the 6-OHDA-treated rats, where significant differences were observed between the sham-operated and renal failure groups.

Figure 4 shows MIBG activity in the lungs and the significance of differences among groups and conditions. At 10 minutes, lung uptake in reserpine-treated rats was significantly higher than that in the control and 6-OHDA-treated subgroups in sham-operated rats, but at 4 hours reserpine-treated rats showed higher uptake in both groups. At 4 hours, renal failure rats showed significantly higher

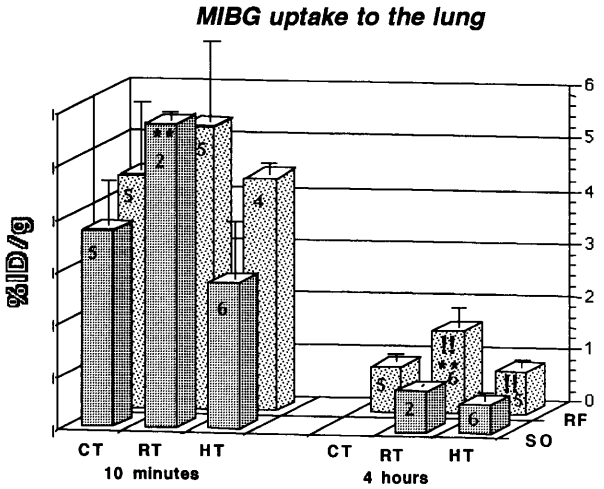

Fig. 4 MIBG uptake (mean ± 1 sd) to the lung and significant differences among groups and conditions. Note, at 10 minutes, the absolute uptake in the lung was higher than those in the heart (Fig. 1) and spleen (Fig. 3) but at 4 hours uptake values were less than those of heart and spleen in control rats, indicating rapid washout from loosely bound non-neuronal sites. * = comparison among control, reserpine-treated and 6-OHDA-treated rats in respective groups. ! = comparison between corresponding subgroups of sham-operated and renal failure rats. **/!! = $p < 0.01$.

Table 1 Washout rate from the heart, spleen and lungs in all groups and conditions

	Control	Reserpine-treated	6-OHDA-treated
Sham-operated:			
Heart	47.9	68.0	74.7
Spleen	44.4	75.9	65.7
Lungs	NA	86.4	80.6
Renal failure:			
Heart	35.7	47.1	85.0
Spleen	26.7	53.0	58.1
Lungs	80.1	70.5	81.4

[Washout rate was calculated as: (10 min uptake − 4 hours uptake)/10 min uptake × 100. Uptake represents mean uptake values], NA = not available

lung activity.

Table 1 shows the washout rate of MIBG, calculated from the means of early and delayed % ID/g uptake, in the heart, spleen and lungs. A rather high washout rate was observed in the lungs in all the conditions. Washout from the heart and the spleen in renal failure rats was slower than in sham-operated rats.

DISCUSSION

To date, both *in vitro* and *in vivo* studies have been performed to determine the uptake mechanisms, storage sites and release of MIBG by using both animal models and cell cultures.[4,5,26,27] Two mechanisms of uptake into the sympathetic neuron are postulated. One is uptake 1, which is sodium and energy dependent, temperature sensitive, high affinity, and low capacity uptake, and the other is simple diffusion, which is sodium independent, temperature sensitive, low affinity and high capacity uptake. Once inside the neuron, MIBG accumulates both inside and outside the vesicles and uptake to the vesicles is saturable, ATP and Mg^{++} dependent.[7] Furthermore, another uptake site has been postulated, that is uptake to non-neuronal tissue.[9,10] According to an experiment with isolated amine-storing vesicles from the hypothalamus of the pig, reserpine inhibits both the active and passive transport of NE into those vesicles.[28] Passive transport which occurs in the presence of high amounts of NE is inhibited by reserpine at 37°C, though it does not influence the diffusion of amine at 0°C.[11,28] There is evidence that the effects of reserpine on adrenergic nerve storage particles are virtually permanent and that recovery from the effects of drugs occurs only at newly synthesized storage particles.[29] Chang and Fearn[30] found no fluorescent structures, and noradrenaline and adrenaline were almost completely depleted in ventricular tissue from the rats treated with 2.5 mg/kg reserpine intraperitoneally for 2 successive days and killed on the 3rd day. Gasnier et al.[7] showed, in an experiment with bovine chromaffin granules, that transport of MIBG through membrane was saturable and the uptake was blocked by reserpine. They also showed that there was only baseline uptake after reserpine treatment. We therefore, assume that MIBG may accumulate in both extra-vesicular sites (cytosol) inside the neuron and non-neuronal sites after reserpine treatment. Since non-neuronal uptake of MIBG was released quickly,[8,31,32] MIBG accumulated in the heart in reserpine-treated rats 4–6 hours after injection may mostly be uptake to the extravesicular site.

The neurotoxic drug 6-OHDA induces sympathectomy similar to cardiac denervation[18] when administered at high doses by peripheral routes. Kawa et al.[16] found a negligible amount of endogenous NE in the heart 2 weeks after intraperitoneal injection of 150 mg/kg 6-OHDA in rats. Priola et al.[18] found that tyramin was completely ineffective in producing positive sympathetic responses both in cardiac denervated and 6-OHDA treated animals and suggested that elimination of releasable catecholamines from the heart had been achieved either by complete cardiac denervation or chemical sympathectomy. It therefore can be concluded that after large doses of 6-OHDA, there would be no sympathetic nerve in the heart and radioactivity in the heart in 6-OHDA-treated rats would represent uptake by non-neuronal sites.

Our experiment was an *in-vivo* study; that is, the temperature was the body temperature of rats, so there might have been no chance of diffusion into the vesicles in reserpine-treated rat hearts. The difference between the

mean uptake value for the control and that for reserpine-treated rats at 4 hours may reflect the uptake to the vesicles. This value in the sham-operated group was similar to that in the renal failure group, 0.51% ID/g and 0.44% ID/g respectively, indicating that vesicular uptake was not related to blood MIBG activity. The difference between the mean uptake value in the control and that in 6-OHDA-treated rats at 4 hours may reflect uptake by the neurons (both intra- and extravesicular). Furthermore, the difference between the mean uptake value in reserpine-treated rats and that in 6-OHDA-treated rats would represent extravesicular neuronal uptake. Both these pools were larger in the renal failure group than in the sham-operated rats, 1.30% ID/g vs. 0.70% ID/g and 0.86% ID/g vs. 0.19% ID/g, respectively. These findings indicate that extravesicular neuronal uptake was related to blood MIBG activity.

There were no statistically significant differences in MIBG uptake in the heart at 10 minutes among the control, reserpine-treated and 6-OHDA-treated or between sham-operated and renal failure rats. At 4 hours, however, both reserpine-treated and 6-OHDA-treated rats showed significantly lower uptake than the control in both sham-operated and renal failure rats. Moreover, there were also significant differences between reserpine-treated and 6-OHDA-treated rats. Higher uptake in both control and reserpine-treated rats in the renal failure group may be due to increased uptake to the extravesicular pool inside the neurons. In 6-OHDA-treated rats, there was no difference in uptake between the sham-operated and renal failure groups. If the differences in mean uptake values between reserpine-treated and 6-OHDA-treated rats at 4 hours represent extravesicular neuronal uptake, then impairment of renal clearance would increase the uptake to the extravesicular neuronal sites because a significant difference was observed between the sham-operated and renal failure groups (0.19% ID/g vs. 0.86% ID/g).

The above findings may be explained in the following ways: (a) the initial uptake of MIBG in the rat heart soon after injection depends on the initial concentration of MIBG in the blood and is not related to the presence or absence of sympathetic neurons, this has also been suggested by others.[9,33] This initial uptake of MIBG into the heart may be assumed to represent MIBG loosely bound to the non-neuronal tissue, probably interstitial cells or myocardial cells. Thereafter, this loosely bound MIBG would either be released into the blood or taken up specifically by the neurons (if any). (b) Because sympathetic neurons were intact both in the control and reserpine-treated rats, the higher uptake of MIBG into the heart at 4 hours in the renal failure group may be due to higher blood MIBG activity (Fig. 2) which caused either increased uptake or prevented release of MIBG from the extravesicular sites. Because the kidneys play an important role in the early clearance of intact MIBG from the plasma,[19–22] the absence of renal clearance causes an increase in the level of plasma MIBG[23] and consequently may cause increased extravesicular neuronal uptake by both uptake-1 and simple diffusion mechanisms both in control and reserpine-treated rats in the renal failure group. (c) There was no effect of plasma MIBG activity on vesicular uptake or on release from the denervated hearts, because the same uptake values were observed in 6-OHDA-treated rats at 4 hours in both groups.

Similar uptake values in the 6-OHDA-treated subgroup for sham-operated and renal failure rats may be explained in either of 2 ways: (1) there would be uptake to a fixed non-neuronal compartment which is independent of blood MIBG activity or renal clearance, or (2) there may be incomplete degradation of sympathetic neurons and/or appearance of regenerated neurons in the sham-operated rats, but the latter was the less likely because all rats were treated in the same way, and, denervation in the heart was felt to be more complete than that in other organs (spleen, vas differences, etc.), almost like selective cardiac denervation.[17,18] Further clarification of this is needed.

In the same way, activity in the spleen could be explained, although certain disparities at 4 hours may be due to incomplete destruction of the sympathetic neurons,[16,17] but lung tissue has negligible sympathetic innervation and no definite change in MIBG activity was expected in reserpine-treated or 6-OHDA-treated compared to control rats. The uptake at 10 minutes may therefore be due to MIBG loosely bound to non-neuronal sites, which may be related to the initial concentration of MIBG in the blood. Higher MIBG activity in reserpine-treated rats may be due to the reserpine effect that caused hypotension and bradycardia[34] which has also been suggested by other investigators,[8] and the higher activity in the renal failure group at 4 hours may be due to activity in the extracellular fluid. Despite the high absolute uptake, most of which was loosely bound, in the lung at 10 minutes, there was lower activity than that in control hearts at 4 hours (Fig. 1), indicating rapid release from non-neuronal binding sites.

Washout from the lungs as well as from the 6-OHDA-treated rat hearts in both sham-operated and renal failure groups was faster and similar, indicating that MIBG uptake into the non-neuronal sites is loosely bound and release from these sites would not depend on the state of renal clearance. We also found rapid clearance of MIBG from the hearts with dilated cardiomyopathy where the normal architecture is replaced by fibrotic tissues.[24] On the other hand a impairment of renal clearance would decelerate the release of MIBG from the organs with intact sympathetic nerves, which is in agreement with our last report,[24] where patients on hemodialysis for renal failure without any cardiac complication showed slow clearance of MIBG. These important findings may help in interpreting MIBG study in man, especially in renal failure and cardiomyopathic patients.

CONCLUSION

The initial uptake soon after injection of MIBG represents mostly uptake into the non-neuronal tissues and thereafter loosely bound MIBG washes away and finally specific neuronal uptake remains or occurs. The uptake to and washout from extravesicular neuronal sites may be dependent on both the plasma concentration of MIBG and the state of renal clearance, but vesicular uptake may be independent of these factors. Washout from the non-neuronal sites may not depend on the state of renal clearance.

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