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Research Article

Iodine-131 Metaiodobenzylguanidine (MIBG) and NP-59 uptakes in the Rat Normal Tissues: Increase of Cholesterol uptake in Cold-Activated Brown Adipose Tissue

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Abstract

Objective Aim: NP-59 (I-131-6ß-iodomethyl-19-norcholesterol) and MIBG (I-131-metaiodobenzylguanidine) both are useful to detect tumors. There is a great need to understand their distributions in normal organs and tissues as well as ways to reduce normal uptakes in tumor imaging and/or treatment. Activated Brown Adipose tissue (BAT) has been promising for its role in optimal treatment of obesity and hypercholesterolemia. The study of cholesterol uptake in BAT may have a significant impact in managing hyperlipidemia and protecting from atherosclerosis.

Materials and Methods: Male Sprague-Dawley rats weighing 250—300 g were used in the study. All animals received 25 µCi of I-131-MIBG or NP-59 by the tail vein and were killed (N = 4 - 6 each) 3, 24 or 48 hours afterwards. For cold stressed animals were kept at 6 – 8 C in a cold room overnight prior to receiving labeled MIBG or NP-59. The animal continued to stay in cold room as compared to room temperature controls. To study the effect of norepinephrine-blocking agent, reserpine (4 mg/kg, IP) was given to rats 4 hours prior to I-131-MIBG infusion. Organs were immediately dissected. Radioactivity was counted and tissue concentrations were expressed as percent kilogram (body weight) dose per gram of tissue (% Kg dose/g).

Findings: Higher uptakes of I-131-MIBG were observed in organs rich in sympathetic innervation [BAT (brown adipose tissue), heart and spleen] as compared to liver and muscle. Reserpine reduced uptakes in BAT, heart, adrenals and spleen but not in liver in room temperature controls. BAT, but not in other organs, showed a paradoxical increase in reserpine-treated animal kept in cold room as compared to room temperature controls. NP-59 showed a near 6 folds increase in adrenals from 30 minutes to 48 hours and unchanged in cold stressed animals. The only tissue showed significant increases in NP-59 uptake under cold stress was BAT from 1.84 folds at 30 minutes to 1.44 folds at 48 hours ($p<0.05$ as compared to room temperature controls).

Conclusions: The uptake of MIBG is reduced in organs rich in sympathetic innervation under cold stress, but this reduction is partially blocked by reserpine. Findings may help to improve the efficacy of MIBG as a theragnostic agent. A noteworthy finding is the significant increase in NP-59, a cholesterol analog, in cold-activated BAT. This novel observation could serve as a valuable tool for studying the role of activated BAT in the overall cholesterol metabolism in humans. Furthermore, it presents a potent therapeutic avenue for mitigating hyperlipidemia and providing protection against atherosclerosis.

Key words: NP-59; MIBG; tumor imaging and treatment; brown adipose tissue activation; cholesterol; atherosclerosis

Introduction

Both radio-labelled MIBG and NP-59 are useful to detect tumors (1). It has been suggested that MIBG – an analog of guanethidine – shared the same uptake, storage and release mechanism as norepinephrine (NE) (2). The use of radiolabeled MIBG has been routinely incorporated into the diagnosis of neuroblastoma and other neuroendocrine tumors. With the recent FDA approval of high-dose I-131- MIBG for the treatment of advanced pheochromocytoma and its ongoing clinical trials to treat high-risk neuroblastoma, there is a great need to understand the mechanisms involved in MIBG disposition in normal organs and tissues. NP-59, I-131-6β-iodomethyl-19-norcholesterol, is a cholesterol analog. NP-59 is transported in circulation as cholesterol and bound to LDL (low density lipoproteins) that will bind to specific LDL receptors on adrenocortical cells (3). After binding to adrenocortical cells, the NP-59 is internalized and esterified without undertaking any further metabolism. Giving the importance of cholesterol, efforts to image its distribution and specifically its involvement in the adrenal glands has been continued (4). Recent development of fluorinated NP-59 has brought on a revival of cholesterol use imaging, PET in particular (5).

Originally developed as a structural analog of norepinephrine for adrenal cortex imaging, MIBG is taken up into tumor cells by the norepinephrine transporter (NET), which is highly expressed in neuroendocrine tumors (6). We demonstrated marked accumulation of MIBG in normal tissues including Brown adipose tissue (BAT) in rats. The high accumulation of radioactive MIBG in the normal tissues presents some problems. The uptake into normal tissues may interfere with radioactive labelled MIBG tumor imaging with an increase of false positive rate (8). Furthermore, uptake of I-131-MIBG into normal tissues can compete with tumor uptake, leading to reduce its therapeutic efficacy. Thus, manipulations to reduce the physiological uptake of MIBG may improve its therapeutic efficacy.

There has been resurgence in interest in BAT following radiological and histological identification of metabolically active BAT in adult humans (7, 8). Imaging enables BAT to be studied non-invasively and therefore imaging studies have contributed a significant amount to what is known about BAT function in humans.

The disappearance of MIBG from BAT was found to be slower than that from heart or spleen. In this report, we examine the effect of reserpine, which selectively blocks the NE into storage vesicles of adrenergic nerve, on MIBG uptake in BAT, both at room temperature or in cold. BAT is the only organ known to play a main physiological role for both cold-induced (non-shivering) and diet-induced thermogenesis (9, 10). BAT differs from white fat by its rich content of sympathetic fibers directly innervating the adipocytes (11). BAT activation is also known to reduce hypercholesterolemia and protects from atherosclerosis development (12). In the present study, we made a novel observation that cold stress significant enhance BAT uptake of NP-59 that may have physiological and clinical significance in our effort to prevent atherosclerosis. NP-59 may serve as a tool to study the role of activation of BAT in the amelioration of atherogenesis,

Methods And Materials

Animal Preparations

Male Sprague-Dawley rats weighing 250-300 g were used in the study. sixteen rats were equally divided into two groups (weight matched); one group was kept at room temperature and another at 6 - 8 C in a cold room overnight prior to MIBG injections and throughout the experiment. All animals received 25 **μ**Ci of I-131-MIBG by the tail vein and were killed (N=4 each) 3 and 24 hours afterwards. The experiment was repeated with 24 rats by adding another group sacrificed 48 hours afterwards. Similar experiments were carried out in 44 rats, cold exposure vs. room temperature control at 0.5, 3, 24 and 48 hours, 6 animals in each group (except 4 animals at 3 hours), treated with 25 **μ**Ci of I-131-NP-59 (19-iodocholesterol, also as I-131-iodocholesterol).

Another 12 rats were used for the study of the effect of reserpine. Reserpine (4 m1/kg, IP) was given to 4 rats 4 hours prior to I-131-MIBG administration to selectively block the norepinephrine and guanethidine into storage vesicles of the adrenergic nerve (13). Control rats (N=8) received saline injections. All animals received 25 **μ**Ci of I-131-MIBG by the tail vein and were killed at 3 and 24 hours afterwards. Rats were kept at room temperature or at 6—8 C in a cold room overnight prior to MIBG or NP-59 injections and throughout the experiment. Preliminary experiments showed that reserpine - treated rats could not survive 24 hours cold exposure; hence, only 3 hours cold data was presented in the reserpine—treated group.
Animal care was performed by following the procedures outlined by the Guide for the Care and Use of Laboratory Animals (14). The study was approved by the Subcommittee on Research Animal Care of the Show Chwan Memorial Hospital, Taipei Veterans General Hospital, and VA-UC Irvine Medical Center. All animals tolerated the procedures, consumed food and water, and moved freely in their cage without apparent distress.

Tissue Distribution Studies

Animals were anesthetized with Nembutal (l ml/kg, IP) and killed by exsanguination via cardiac puncture. Organs were immediately dissected and blotted to remove any blood. After weighing the organs, radioactivity was counted in an automated gamma counter with corrections made for the back-ground. Tissue concentrations were expressed as percent kilogram (body weight) dose per gram of tissue (% Kg dose/g) by simultaneous counting duplicates of 0.01% injected dose to normalize the difference in rat weight.

Materials

I-131-MIBG was purchased from University of Michigan (Ann Arbor, MI) and had specific activity of at least 1.8 mCi/mg. I-131 - NP-59 was purchased from University of Michigan and PDRadiopharma Inc., Tokyo, Japan. Crystallized, > 99.0% (HPLC) reserpine was obtained commercially from Sigma~Aldrich Chemical (St. Louis, MO).

Statistical analysis

For statistical analysis, the student's t-test was used. P-values < 0.05 were considered to be significant.

Results

The time course of tissue uptakes of MIBG in cold - stressed rats was compared with those of room temperature control (Fig. 1). At 3 hours following administration of I-131-MIBG, higher uptakes were observed in BAT, heart, and spleen (0.626 ± 0.064, 0.460 ± 0.054, and 0.210 ± 0.038 10^{-}

[3]% kg dose/g ± S.E, N=4, respectively) (Table 1), organs with rich(0.050 ± 0.004% kg dose/g). sympathetic innervation, than in liver (0.072± 0.007% kg dose/g) and muscle

Organ/Time post- Infusion	3 h	24 h	48 h
Serum: Room Temp.	0.059±0.005	0.010±0.002	0.002±0.002
Cold	0.046±0.010	0.008±0.002	0.002±0.002
Adrenal: Room Temp.	0.626±0.064	0.336±0.008	0.298±0.022
Cold	0.510±0.028	0.334±0.052	0.174±0.008
BAT: Room Temp.	0.662±0.068	0.186±0.006	0.044±0.004
Cold	0.350**±0.058	0.034**±0.002	0.010**±0.004
Heart: Room Temp.	0.460±0.054	0.023±0.001	0.012±0.005
Cold	0.229**±0.017	0.013**±0.001	0.006**±0.001
Liver: Room Temp.	0.072±0.007	0.009±0.001	0.003±0.001
Cold	0.064±0.004	0.008±0.001	0.003±0.001
Kidney: Room Temp.	0.093±0.016	0.013±0.001	0.003±0.001
Cold	0.063±0.007	0.008±0.002	0.110±0.003
Spleen: Room Temp.	0.210±0.038	0.022±0.001	0.006±0.001
Cold	0.196±0.029	0.016**±0.001	0.005±0.002
Muscle: Room Temp.	0.050±0.004	0.010±0.001	0.006±0.001
Cold	0.041±0.004	0.007±0.001	0.002±0.001

+Tissue concentration is expressed as 10^{-3} % kg per g ± SE (n = 4);

* p<0.05; ** p<0.01 vs. room temperature controls.

Table 1: MIBG Distribution in normal rat tissues: Room Temperature vs. Cold Exposure

The accumulation of MIBG in BAT exceeds that in the heart at 3 h, P=0.01. In addition, the disappearance of MIBG from BAT was found to be slower than that from the heart at 24 h (0.186±0.006 and 0.023±0.001% Kg dose/g, respectively, P < 0.05). Comparing to room temperature control, cold exposure significantly decreased tissue uptakes of MIBG in BAT and heart (47.2% and 50.2%, respectively at 3 hours; and 81.7% and 43.5%, respectively, at 24 hours), but not in the spleen or adrenal glands, at 3 hours (Table 1). The results were reproducible in the separate cold stress study except adrenal that showed inconsistent cold effect at 24 hours (Figure. 1).

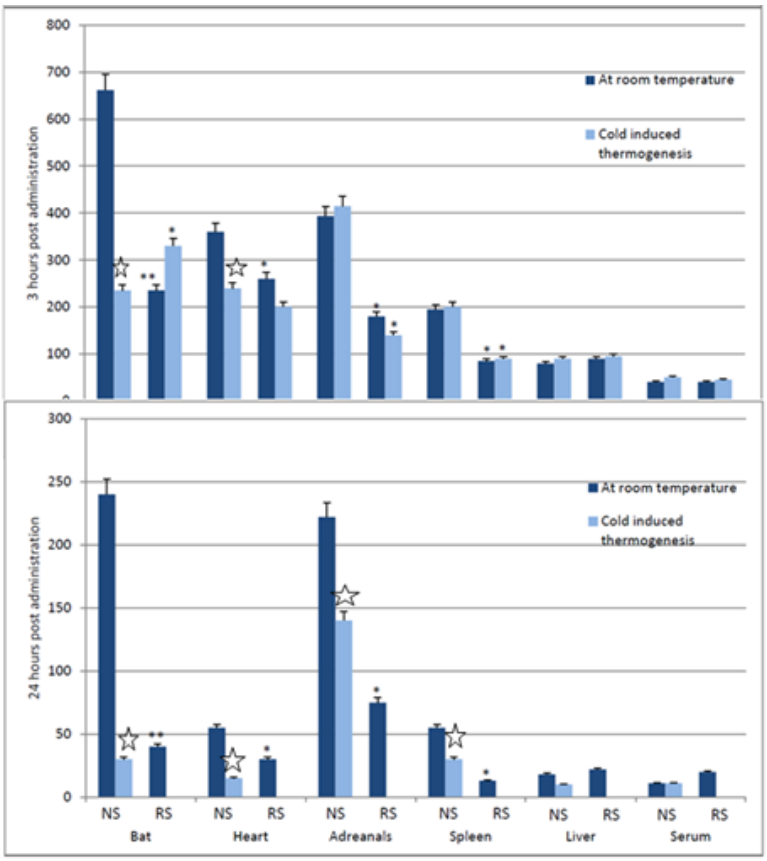

Figure 1: MIBG Distribution in normal rat tissues: Room Temperature vs. Cold Exposure; Saline-treated vs. Reserpine Treatment:

Tissue Concentration is expressed as 10^{-3}% kg dose/g + SE.
NS: normal saline treated controls; RS: reserpine treated.
* p < 0.05 cf. NS control; ** p <0.01.
P < 0.01 cold exposure cf. room temperature controls.

The effect of reserpine on tissue uptakes of I-131-MIBG in cold-stressed or room temperature control rats is summarized in Fig. 1. In room temperature, reserpine significantly decreased MIBG accumulation in BAT (68.5%), heart (25.2%), adrenals (56.0%), and spleen (60.8%), but not in the liver (0%), as compared to saline-treated control. Percentages (10^{-3} kg dose/g tissue) of accumulation was also higher in BAT (0.463% and 0.198%, respectively) than that in the spleen (0.115% and 0.040%, respectively) or heart (0.089% and 0.026%, respectively) at 3 and 24 hours in room temperature control. It is interesting to observe that BAT from reserpine treated rats 3 hours after cold exposure showed higher MIBG uptakes than those from sa1ine-treated control rats (0.226% ±0.014% and 0.345 ±0.029%, respectively, mean ± S.E., p<0.0l). No such paradoxical increases in other tissues were observed.

In Table 2, the tissue NP-59 uptakes in normal rat kept in room temperature is compared with cold stressed animals at 30 minutes, 3, 24 and 48 hours. The highest uptake (expressed as 10^{-3}% kg dose/g) is observed in adrenal gland at 30 minutes (3.555+0.217), 3 (6.475+1.001), 24 (15.870+0.361) and 48 (20.220+1.580) hours. Cold stress did not alter the uptakes, at 48 hours (20.620+1.090). The next high uptake is observed in liver at 30 minutes (1.402+0.048) and gradually reduced to 11.2% at 48 hours (0.157+0.011). The uptake ratio significantly improved in adrenal gland to liver from 30 min (2.5-fold) to 48 hours (131- fold). The uptake is not significantly altered in adrenal, heart, liver, kidney, spleen or muscle. However, surprisingly, significant more uptakes of NP-59 is noted in cold-stressed BAT, 1.84 folds at 30 minutes, 1.64 folds at 3, 1.39 folds at 24 and 1.44 folds at 48 hours (p<0.05) (Table 2).

Organ/Time post- Infusion	0.5 h	3 h	24 h	48 h
Serum: Room Temp.	0.310±0.016	0.197±0.010	0.094±0.026	0.057±0.008
Cold	0.284±0.034	0.230±0.004	0.076±0.016	0.052±0.012
Adrenal: Room Temp.	3.555±0.217	6.475±1.001	15.870±0.361	20.220±1.580
Cold	2.632±0.109	6.735±0.535	13.700±1.220	20.620±1.090
BAT: Room Temp.	0.096±0.010	0.143±0.013	0.182±0.008	0.175±0.014
Cold	0.177*±0.022	0.234*±0.014	0.254*±0.010	0.252*±0.010
Heart: Room Temp.	0.076±0.002	0.123±0.014	0.130±0.007	0.105±0.007
Cold	0.086±0.007	0.115±0.005	0.131±0.002	0.097±0.002
Liver: Room Temp.	1.402±0.048	0.893±0.086	0.276±0.025	0.157±0.011
Cold	1.208±0.083	0.754±0.004	0.277±0.005	0.166±0.003
Kidney: Room Temp.	0,110±0.002	0.141±0.011	0.130±0.011	0.124±0.008
Cold	0.098±0.010	0.140±0.006	0.124±0.003	0.110±0.003
Spleen: Room Temp.	0.540±0.013	0.711±0.07	0.411±0.016	0.244±0.013
Cold	0.445±0.085	0.703±0.033	0.371±0.020	0.242±0.011
Muscle: Room Temp.	0.019±0.003	0.025±0.011	0.026±0.002	0.025±0.002
Cold	0.014±0.001	0.024±0.002	0.024±0.001	0.023±0.001

Table 2: NP-59 Distribution in normal rat tissues: Room Temperature vs. Cold Exposure;

+Tissue concentration is expressed as 10^{-3}% kg dose/g ± SE (n = 4 – 6);

* p<0.05 vs. room temperature controls.

Discussion

The present data demonstrate that tissue concentration of I-131-MIBG, in room temperature (RT) control rats, is higher in BAT than in other tissues including heart, spleen and adrenals. The reserpine blocking studies show that MIBG was 65.5-85.4% (Fig. 1) of the total uptake-accumulated intra-vesicular in BAT, which was also higher than that in heart and spleen.

The reduced MIBG in BAT in cold-exposed control rats may be due to the higher level of circulating catecholamines in cold-stressed rats, competing with I-131-MIBG for adrenergic neurons. Prior study by Baba et. al. showed an increase of I-131-MIBG uptake at 20 to 60 minutes [15]; without showing a time course of MIBG uptake beyond one hour. They postulated that initial increase of uptake within one hour of MIBG infusion was due to increase blood flow in the activated BAT. Their postulation of mechanism cannot explain the reduced uptake MIBG at 3 and 24 hours in BAT from cold stressed animals. It is even less clear, however, in regard to the mechanism concerning increased MIBG accumulation in BAT in reserpinized cold-stressed rats. Nevertheless, the mechanism may include the possibility of the discharge of MIBG in cold-stressed animals.

In normal, lean rodents, it is established that thermogenesis in BAT is an important component of energy expenditure in the maintenance of leanness in hyperphagic cold-acclimated animals [9]. Currently there is no direct evidence that, in human beings, thermogenesis in BAT acts as an energy buffer. However, indirect evidence indicates that human beings may have similar control mechanisms. The high accumulation of MIBG in BAT provides a means for imaging and assessing the total amount of "functional" BAT in humans with strong correlation to ^{18}F-FDG uptake in PET/CT, as a marker of metabolic activity [16].

The use of radiolabeled MIBG has been routinely incorporated into the diagnosis of neuroblastoma and other neuroendocrine tumors [1]. With the recent FDA approval of high-dose I-131-MIBG for the treatment of advanced pheochromocytoma and its ongoing clinical trials to treat high-risk neuroblastoma, there is a great need to understand the molecular mechanisms involved in MIBG disposition in normal organs and tissues. Originally developed as a structural analog of norepinephrine for adrenal cortex imaging, MIBG is taken up into tumor cells by the norepinephrine

transporter (NET), which is highly expressed in neuroendocrine tumors [5].

Whole-body scintigraphy shows that besides NET-mediated uptake into tumor lesions, MIBG also has extensive uptake and accumulation in several normal tissues, including the liver, heart, intestines, and adrenal glands as demonstrated in the present and other studies [7, 8]. The high accumulation of radioactive MIBG in the normal tissues presents several complications. Firstly, uptake into normal tissues may interfere with I-123-MIBG tumor imaging, leading to an increased rate of false negatives [7]. Secondly, uptake of I-131-MIBG into normal tissues can compete with tumor uptake, resulting a reduction of antitumor efficacy. Thirdly, the high uptake and accumulation in normal tissues are associated with several radiation-induced toxicities [8]. Therefore, an understanding of how MIBG is transported in normal tissues is essential for developing strategies to enhance MIBG efficacy while minimizing toxicity and maximizing therapeutic efficacy. In the present study, cold stress reduced uptake in adrenal gland and heart which may help to reduce normal tissue toxicity.

Unfortunately, cold stress does not reduce the hepatic uptake. Although the liver is not a site for MIBG elimination, hepatic accumulation accounts for approximately 30% of the injected dose [7]. Bayer et al., [16] proposed to improve tumor selective uptake of MIBG by reducing Organic-cation-transporter-3 (Oct3)-mediated tissue uptake. They showed that MIBG uptake was decreased in small intestine and kidney in mice treated with corticosteroids. MIBG is almost exclusively eliminated by the kidney, with more than 90% of the administered dose excreted unchanged in the urine [8].

Most recently, researchers have found nerve pathways that supply BAT, a type of tissue that releases chemical energy from fat metabolism as heat -- a finding that could pave the way toward using it to treat obesity and related metabolic conditions [11].

In the present study, we made novel observation of a cholesterol analog, NP-59 (I-131-6β-iodomethyl-19-norcholesterol) uptake is significantly increased in BAT from cold stressed rats (Table 2). To our knowledge this is the first study showing a physiologic condition, there is a direct increase of cholesterol uptake into activated BAT. BAT activation is known to reduce hypercholesterolemia and protects from atherosclerosis development (12). The mechanism may involve the enhancement of the selective uptake of fatty acids from triglyceride-rich lipoproteins into activated BAT; subsequently accelerating the hepatic clearance of the cholesterol-enriched remnants (18). Findings support the notion that high metabolic activity of activated BAT exerts its theroprotective properties via increased systemic cholesterol flux through the lipoprotein compartment [19, 20]. The uptake of I-131-NP-59 or fluorinated NP-59 as a tool to study the role of activated BAT may be used when the anti-aging gene Sirtuin 1 is activated in brown adipose tissue. Sirutuin 1 is the heat shock gene and in BAT is responsible for circulation and metabolism of fat [21, 22]. Sirtuin 1 measurements may be important to activated BAT and the use of NP-59 to explore the treatment of atherogenesis.

Conclusions:

Our novel observation that cold stress significant enhance BAT uptake of cholesterol that may have physiological and clinical significance. I-131-NP-59 or fluorinated NP-59 may serve as a tool to study the role of activated BAT as a potential therapeutic avenue to ameliorate hyperlipidemia and protect from atherosclerosis.

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