Body temperature as a mouse pharmacodynamic response to bombesin receptor subtype-3 agonists and other potential obesity treatments

Joseph M. Metzger,1 Karen Gagen,1 Kate A. Raustad,1 Liming Yang,1 Amanda White,1 Sheng-Ping Wang,1 Stephanie Craw,1 Ping Liu,3 Thomas Lanza,3 Linus S. Lin,3 Ravi P. Nargund,3 Xiao-Ming Guan,2 Alison M. Strack,1 and Marc L. Reitman2

1Departments of Pharmacology, 2Diabetes and Obesity, and 3Medicinal Chemistry, Merck Research Laboratories, Rahway, New Jersey

Submitted 8 July 2010; accepted in final form 30 August 2010

Metzger JM, Gagen K, Raustad KA, Yang L, White A, Wang SP, Craw S, Liu P, Lanza T, Lin LS, Nargund RP, Guan XM, Strack AM, Reitman ML. Body temperature as a mouse pharmacodynamic response to bombesin receptor subtype-3 agonists and other potential obesity treatments. Am J Physiol Endocrinol Metab 299: E816–E824, 2010. First published August 31, 2010; doi:10.1152/ajpendo.00404.2010.—Treatment of rodents with a bombesin receptor subtype-3 (BRS-3) agonist reduces food intake and increases fasting metabolic rate, causing weight loss with continued treatment. In small mammals, core body temperature (Tb) is regulated in part by nutritional status, with a reduced Tb during fasting. We report that fed Brs3 knockout mice have a lower Tb, which is discordant with their nutritional status. Treatment of wild-type mice with a BRS-3 agonist increased Tb, more so when the baseline Tb was reduced such as by fasting or during the inactive phase of the light cycle. With repeated BRS-3 agonist dosing, the Tb increase attenuated despite continued weight loss efficacy. The increase in Tb was not prevented by inhibitors of prostaglandin E (PGE) production but was partially reduced by a β-adrenergic blocker. These results demonstrate that BRS-3 has a role in body temperature regulation, presumably secondary to its effect on energy metabolism, including effects on sympathetic tone. By making use of this phenomenon, the reversal of the fasting Tb reduction was developed into a sensitive single-dose pharmacodynamic assay for BRS-3 agonism and other antiobesity compounds acting by various mechanisms, including sibutramine, cannabinoid-1, and melanin-concentrating hormone-1 receptor blockers, and melanocortin, β-adrenergic, and cholecystokinin-1 receptor agonists. These drugs increased both the fasted Tb and the fasted, resting metabolic rates. The Tb assay is a robust, information-rich assay that is simpler and has a greater throughput than measuring metabolic rate and is a practical, effective tool for drug discovery.

MAMMALS ARE HOMEOTHERMS, regulating their core body temperature (Tb) within a narrow range (30). Tb impacts all facets of life, ranging from chemical reaction rates to defense against infection. Mammals typically live in environments below their thermoneutral range, so maintenance of Tb involves generating and conserving heat. Heat is generated as a byproduct of metabolic processes and via dedicated heat generation ("facultative thermogenesis") that occurs principally in brown adipose tissue, a specialized tissue whose only known function is efficient heat generation (5). Heat is conserved through a variety of mechanisms, including behavioral (nests, huddling, choice of warm environment), anatomic (fur, increased body size), and physiological (vasoconstriction, regulation of energy expenditure) mechanisms (11).

Small mammals maintain their Tb, despite their increased heat loss to the environment, by burning a significant fraction of their energy intake for warmth. For example, about one-third of the food intake is needed for heat generation by mice housed at 22°C. When food is not available, a controlled, graded reduction in Tb occurs, resulting in conservation of energy stores (9, 17, 29). In certain severe circumstances (severe food restriction and a cool and quiet environment), mice enter a torpid state, with a Tb reduction of 10 to –2°C above the ambient temperature, thus greatly reducing metabolic rate and the required energy supply (see, e.g., Ref. 8). These adaptations are a coordinated, regulated physiological response to the environment.

Bombesin receptor subtype-3 (BRS-3) is a member of the bombesin receptor subfamily of G protein-coupled receptors (GPCRs), which also includes the neuromedin B and gastrin-releasing peptide receptors (19). BRS-3 is expressed chiefly in the brainstem, and several midbrain nuclei (12, 26, 31, 37). Despite its name and sequence similarity, BRS-3 has a low affinity for bombesin and its natural, endogenous ligand(s) is unknown (19). BRS-3 knockout (Brs3–/–) mice have a reduced metabolic rate and increased food intake and develop obesity and insulin resistance (22, 32). No alterations in Tb have been reported. The recent development of potent, selective, and bioavailable BRS-3 ligands (15, 25) has facilitated exploration of BRS-3 function. A BRS-3 antagonist increases food intake and body weight, whereas BRS-3 agonists increase metabolic rate and reduced food intake and body weight. BRS-3 agonist efficacy was maintained in Npy1r–/–, Agrp1r–/–, Mc4r–/–, Cnr1–/–, and Leprdb/db mice, suggesting that BRS-3 has a role in energy homeostasis that complements these regulatory pathways (12). BRS-3 agonists are a potential new approach to the treatment of obesity.

Given the reduction in metabolic rate in Brs3–/– mice, we decided to explore the role of BRS-3 in body temperature regulation. In the present study, using both genetic (Brs3–/–) mice and pharmacological tools (agonist ligands), we examined the interactions between BRS-3 and Tb. The Brs3–/– mice robustly increased the reduced Tb of the fasted state. We also explored the utility of Tb as a pharmacodynamic assay during drug development, comparing the effects of a panel of drugs on both Tb and metabolic rate. We conclude that drug effects on Tb can be a useful and efficient pharmacodynamic marker.
EXPERIMENTAL PROCEDURES

Compounds. Bag-1 [2-(4-[2-[5-(2,2-dimethylbutyl)-1H-imidazol-2-yl][ethyl]phenyl]pyridine; compound 9 in Ref. 25], Bag-4 [6-(4-tert-butylphenyl)sulfonyl]-7,8-dimethyl-2-(trifluoromethyl)-6,11-dihydro-5H-pyrido[2,3-b][1,5]benzodiazepine; P. Liu, T. Lanza, L. S. Lin, and R. P. Nargund, unpublished observations], MC4R agonist [(3S,4R)-3-[4-[2-(15-acetamidophenyl)-4-chlorophenyl]piperidin-1-yl]carbonyl]-1-tert-butyl-4-(2,4,6-trifluorophenyl)pyrrolidinium chloride; compound 2B in Ref. 13], and MCH1R antagonist 4-(benzyloxy)-1-[4-(2-pyrrolidin-1-ylethoxy)phenyl]pyridin-2(1H)-one (18) were synthesized at Merck Research Laboratories or purchased from Sigma-Aldrich (St. Louis, MO). 2(1H)-one (18) were synthesized at Merck Research Laboratories or provided by Dr. James Battey (22) and back-crossed at least eight generations onto a C57BL/6 background. Diet-induced obese (DIO) mice were fed a high-fat diet (Bio-Serv S2382, Frenchtown, NJ, or Research Diets 12492, New Brunswick, NJ) for at least 14 wk before study. Mice were housed in a temperature- and humidity-controlled environment with a 12:12-h light-dark cycle and with food and water available ad libitum. Food intake was measured as described (12). All animal procedures were performed in compliance with National Institutes of Health guidelines and approved by the Merck Research Laboratories Institutional Animal Care and Use Committee, Rahway, NJ.

Metabolic rate measurement. To measure resting metabolic rate with minimal contributions from physical activity and facultative thermogenesis, assays were performed at thermoneutrality using the time points with minimal physical activity. Vehicle controls were studied simultaneously with the drug-treated mice. Standard assay conditions are described; individual experiments varied slightly. DIO C57BL/6 mice were implanted intraperitoneally with radiotelemetry probes (E-Mitter, Mini Mitter, Bend, OR) at least 7 days before the study and were conditioned to dosing with vehicle for 5–7 days. Three days prior to study, mice were acclimatized to individual housing in the metabolic chambers of an Oxymax System (Columbus Instruments, Columbus, OH) equipped with a photobeam system to measure motor activity. At 1400 on the day prior to study, mice were shifted to thermoneutrality (29–30°C). At 1700, the 12-h dark period started, and food was removed (access to water continued). At 0800 the next morning, the mice were dosed and measurement of metabolic rate was continued. Changes in O2 and CO2 were recorded every 17 min (70 s settle and 40 s measure). To quantify the effect of drug treatment on resting metabolic rate, the mean metabolic rate from 1 to 6 h after dosing was expressed as a percentage of baseline (the 3-h period prior to dosing). The 1-h period immediately following dosing was excluded due to reequilibration of the chambers and activity related to dosing. Readings taken during periods with >50 beam breaks per 17 min or >5 beam breaks per 110-s measurement period were considered nonresting and excluded.

Body temperature measurement. To minimize Tb variability, such as that caused by physical activity, care was taken to keep the vivarium undisturbed with no or minimal entry of personnel into the room during measurement. Vehicle controls were studied simultaneously with the drug-treated mice, and all experiments were performed at thermoneutrality (22°C). Standard assay conditions are described; individual experiments varied slightly. DIO C57BL/6 mice were implanted intraperitoneally with radiotelemetry probes (E-Mitter, Mini Mitter, Bend, OR) at least 7 days before the study and were conditioned to oral dosing. On the day prior to dosing, 1 h before the dark period, mice were transferred to clean cages with access to water but without access to food. The following morning (3 h into light phase), vehicle or drug was administered and the response measured for the next 6 h. Core temperature and physical activity were recorded at 5-min intervals using VitalView software (Mini Mitter). The ΔT1–2h is calculated as the mean Tb from 1–2 h after dosing minus the mean Tb from 2.5–0.5 h prior to dosing. The h immediately following dosing was excluded due to activity related to dosing.

Mouse rectal temperature in conscious animals was measured with a thermocouple thermometer (model BAT-10R) using a RET-3 probe (both from World Precision Instruments, Sarasota, FL). The tip of the probe was coated with KY jelly and inserted to the length of the probe (~2 cm). Temperature was recorded once the value settled for >5 s.

Statistics. One-way ANOVA with repeated measures followed by the Dunnett’s post hoc test or two-way ANOVA with repeated measures followed by Bonferroni’s posttest was used for comparing multiple treatments vs. the control group. Student’s t-test (paired or unpaired, as appropriate) was used when two groups were compared. Statistical analyses used two-tailed tests using P < 0.05 as statistical significance.

RESULTS

BRS-3 agonists increase body temperature. Mice lacking leptin (lepob/ob) have a reduced Tb (16) and thus might provide a sensitive model for testing BRS-3’s role in Tb regulation. Treatment of fasted lepob/ob mice with the BRS-3 agonist Bag-1 increased rectal temperature by 4°C for at least 8 h (Fig. 1A). The Bag-1 Tb effect was similar in magnitude to that of the β3-adrenergic agonist CL-316243 (4, 35), used as the positive control.

We next looked at the effect of BRS-3 agonists in DIO C57BL/6N mice by using continuous temperature monitoring. When Bag-1 was dosed at the onset of the dark (active) phase, it caused a slight increase in Tb (the largest 1-h increase was 0.45°C from 6 to 7 h post-dosing, with a 0.08°C mean increase from 1 to 5 h post-dose), which did not exceed the daily maximum Tb (Fig. 1B, top). When Bag-1 was given early in the light (resting) phase, a larger increase was observed (0.66°C mean increase from 1 to 5 h post-dose; Fig. 1B, middle). As expected, fasted mice decreased their Tb (Fig. 1B, bottom). When Bag-1 was dosed 18 h into the fast, an increase in Tb was seen (1.05°C mean increase from 1 to 5 h post-dose; Fig. 1B, bottom). During fasting, Tb is more variable and the increase induced by Bag-1 similarly showed more variability. We previously observed that the increase in metabolic rate caused by Bag-1 was not due to an increase in physical activity (12). Similarly, physical activity changes did not explain the increase in Tb (not shown). In summary, the data demonstrate that BRS-3 agonist treatment increased Tb, particularly when Tb was reduced by fasting or during the light phase, but the Tb increase typically did not exceed the daily maximum. The largest magnitude effect observed was the reversal of the low Tb of the fasted state.

We next characterized the thermal biology of Brs3−/− mice by using continuous Tb monitoring. Brs3−/− mice showed normal fed day-night rhythms, but with Tb reduced compared with wild-type (WT) mice by 0.49°C during the dark phase (37.54 ± 0.07°C in WT vs. 36.86 ± 0.16°C in KO, P = 0.01) and 0.72°C during the light phase (36.42 ± 0.05°C in WT vs. 35.70 ± 0.12°C in KO, P < 0.001; Fig. 2A). The Brs3−/− mice showed an exaggerated decrease in Tb with fasting, being reduced by 1.13°C during the latter half of the dark phase (35.96 ± 0.11°C in WT vs. 34.83 ± 0.24°C in KO, P < 0.001). The stress-induced increase in Tb during handling for dosing was intact in the Brs3−/− mice. Brs3−/− mice showed no significant increase in Tb in response to Bag-1 (Fig. 2B), demonstrating that the Tb increase was an on-target, BRS-3 mechanism-based effect.
The dose response for Bag-1 was multiphasic (Fig. 3 A). Vehicle dosing causes a 1.4°C Tb increase attributed to handling stress. At low doses (3 and 10 mg/kg), Bag-1 caused a slight prolongation of the Tb increase. A higher dose (30 mg/kg) increased the duration without appreciably changing the onset timing or magnitude of the maximum Tb. The highest Bag-1 dose (100 mg) yielded a qualitatively different response. The initial increase was followed by a dip at 1 h and then an increase to a plateau that lasted until the end of the light phase. The triphasic response with high doses was observed reproducibly with multiple BRS-3 agonists of diverse structure (not shown).

Continuous Tb monitoring is a straightforward quantification method with high information content. We explored its use for routine compound screening during drug development. We chose the mean Tb from the 2 h before dosing as the baseline and used 60–120 min after dosing to calculate ΔTb.2h, a robust, sensitive measure of response. Omitting 0–60 min after dosing removed most of the temperature increase and variability due to handling. The ED50 of ΔTb.2h for Bag-1 is 9.1 ± 3.2 mg/kg (Fig. 3B). Using the mean temperature change over longer times, such as from 60 to 360 min, reduces the assay sensitivity, but is useful if a less sensitive assay is desired.

BRS-3 agonist-induced increase in temperature attenuates with repeated dosing. Continued agonist stimulation of most receptors attenuates their biological response (24). Repeated BRS-3 stimulation did not attenuate the response in cell-based signaling assays or in electrophysiological studies in arcuate nucleus slices, and weight loss is maintained with continued dosing whereas the reduction in food intake is transient (12). We investigated whether the increase in Tb attenuates over 12 days by using different dosing regimens of the BRS-3 agonist Bag-4 (Fig. 4). When Bag-4 was dosed once every 3 days (Q3D), there was a robust increase in Tb following each dose. When dosed every other day (Q2D), Tb increased after each dose, but the magnitude was slightly less. With daily dosing (QD) there was less response on the second day, with further attenuation thereafter.

This effect was quantified as the difference between the Bag-4- and vehicle-treated groups at 1–5 h after dosing, ΔTb.1–5h (Fig. 5A). The initial ΔTb.1–5h value was 0.82°C. It is notable that with each regimen, each time drug was dosed, the ΔTb.1–5h was positive, while on each non-drug-dosing day it was negative. The ΔTb.1–5h values for Bag-4 and vehicle days were each

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Fig. 1. Bag-1 increases body temperature (Tb) in mice. A: leptin-deficient (lepob/ob) mice were fasted overnight and dosed with either Bag-1 (100 mg/kg po, red), CL-316243 (0.01 mg/kg sc, blue), or vehicle (po, black) in the morning. Rectal temperatures were measured immediately before dosing (arrow) and every 2 h thereafter. Temperature changes at all times post-dosing Bag-1 and CL-316243 are different from vehicle, P < 0.05. Baseline temperatures were 29.1 ± 0.3, 31.0 ± 0.0, and 30.9 ± 0.6°C in Bag-1, CL-316243, and vehicle groups, respectively (means ± SE; n = 8/group). B: Tb was monitored continuously using implanted E-mitters (see EXPERIMENTAL PROCEDURES). C57BL/6 mice were treated with Bag-1 (100 mg/kg po, red) or vehicle (po, black). Dosing is indicated by an arrow, dark phase by black bars, and fasting by hatched bar. Data are means ± SE; n = 5/group.

The dose response for Bag-1 was multiphasic (Fig. 3A). Vehicle dosing causes an ~1.4°C Tb increase attributed to handling stress. At low doses (3 and 10 mg/kg), Bag-1 caused a slight prolongation of the Tb increase. A higher dose (30 mg/kg) increased the duration without appreciably changing the onset timing or magnitude of the maximum Tb. The highest Bag-1 dose (100 mg) yielded a qualitatively different response. The initial increase was followed by a dip at ~1 h and then an increase to a plateau that lasted until the end of the light phase. The triphasic response with high doses was observed reproducibly with multiple BRS-3 agonists of diverse structure (not shown).

Continuous Tb monitoring is a straightforward quantification method with high information content. We explored its use for

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Fig. 2. Lack of effect of Bag-1 on Tb in Brs3−/− mice. A: diet-induced obese (DIO) male Brs3−/− (49 g, solid line) and wild-type (WT; 39 g, dotted line) C57BL/6 mice were treated with Bag-1 (100 mg/kg po, red) or vehicle (po, black). Dosing is indicated by arrows, dark phase by black bars, and fasting by hatched bar. Data are means ± SE; n = 8/group.

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averaged over days 4 through 12. The mean drug day \( T_{1–5h} \) values were 0.21 \( \pm \) 0.12°C for QD, 0.46 \( \pm \) 0.02°C for Q2D (\( P < 0.001 \) vs QD), and 0.71 \( \pm \) 0.12°C for Q3D (\( P < 0.001 \) vs. vehicle), and QD (\( P < 0.001 \) vs. vehicle) groups, respectively. Thus, despite the near-complete attenuation of the agonist-induced increase in \( T_b \), the QD group showed the most weight loss, suggesting that mechanisms underlying the weight loss remain sensitive to the drug.

Role of BRS-3 in thermal biology. An increase in \( T_b \) could be caused by fever or non-fever-induced hyperthermia. Fever is a regulated rise in \( T_b \) and is typically induced by cytokines that increase local PGE and RANKL production (14, 28). We examined the effect of pharmacological agents that inhibit PGE production on Bag-1-induced increases in \( T_b \). Acetaminophen, given using a dose regimen that significantly reduced lipopolysaccharide-induced fever (not shown), had no significant effect on the \( T_b \) rise caused by Bag-1 (\( \Delta T_{1–2h} \) 2.03 \( \pm \) 0.30 vs. 1.87 \( \pm \) 0.05°C, without vs. with acetaminophen; Fig. 6, top). Similarly, indomethacin (10 mg/kg po) did not prevent the increase in \( T_b \) caused by 10 or 30 mg/kg Bag-1 administered 30 min later (not shown). These data indicate that some BRS-3 agonist effect is independent of PGE.

In mice, sympathetic nervous system stimulation is a major regulator of thermogenesis by brown adipose tissue (1, 5). When propranolol, a nonselective \( \beta \)-adrenergic antagonist, was coadministered with Bag-1, a modest reduction of \( T_b \) was observed, which trended toward statistical significance (\( \Delta T_{1–2h} \) 2.03 \( \pm \) 0.30 vs. 1.40 \( \pm \) 0.12°C, without vs. with propranolol, \( P = 0.08 \); Fig. 6, bottom).

\( T_b \) and metabolic rate effects with other antiobesity treatments. To place the BRS-3 data in context, we explored the generality of the reversal of the fasting-induced decrease in \( T_b \) by testing various potential antiobesity compounds acting by different mechanisms. The compounds tested were AM251, a selective cannabinoid receptor-1 inverse agonist (36); 4-(benzyloxy)-1-[4-(2-pyrrolidin-1-ylethoxy)phenyl]pyridin-2(1H)-one, a selective melanin-concentrating hormone receptor-1 antagonist (18); melanotan II (MTII), a nonselective melanocortin agonist (33); SR-146131, a selective cholecystokinin receptor-1 agonist (3); compound 2B, a selective melanocortin receptor-4
agonist (13); sibutramine, a serotonin and norepinephrine re-uptake inhibitor (27); and CL-316243, a selective β3-adrenergic receptor agonist (4). Relatively high drug doses were used to ensure that the drug effects lasted for the duration of the assays. All eight compounds increased Tb under the conditions of this assay (Fig. 7A). Some showed a delay, and others exhibited a triphasic profile, having a reduction in Tb before the sustained increase. As observed with BRS-3 agonists, the response depended on dose, which affected both the duration and shape of the initial response (data not shown). A number of the compounds produced Tb increases of similar magnitude as BRS-3 agonists.

We previously reported that Bag-1 increases fasting, resting metabolic rate (12). The panel of antiobesity compounds was next tested for effects on resting metabolic rate in independent experiments but using the same time interval (1–6 h after dosing) and doses as the Tb assay. Except for sibutramine, all of the compounds increased fasting, resting metabolic rate (Fig. 7B). Unlike the other agents, sibutramine caused a large increase in locomotor activity, which increased the total metabolic rate [10 and data not shown]. Therefore, data with significant locomotor activity were specifically excluded when the resting metabolic rate was measured. This exclusion of many data points made calculation of the resting metabolic rate for sibutramine more uncertain.

In Fig. 8, we compared the drug effects on fasting resting metabolic rate and fasting inactive-phase Tb. Although there was not a significant correlation between the changes in Tb and metabolic rate, it is notable that all eight drugs increased both Tb and metabolic rate. The lack of correlation may be due to an intrinsic lack of quantitative coupling between these metrics across therapeutic mechanisms. Alternatively, the degree of correlation may have been underestimated,
since 1) T_b and metabolic rate were measured in separate studies and in different cohorts of mice, and 2) both assays are exquisitely sensitive to environmental conditions (noise, light cycle, vibration due to construction, room temperature, etc.). Taken together, the data demonstrate that antiobesity compounds reverse the reduced T_b of fasted mice, and while T_b is not strictly a surrogate measure of an effect on fasted, resting metabolic rate, the T_b assay, once characterized for a given mechanism, should be useful in obesity drug discovery.
DISCUSSION

*Brs3\(^{-/}\) mice have a reduced metabolic rate and develop mild obesity, but they had not been noted to have a reduced T\(_{\text{b}}\) (32). In this report, we present observations on the role of BRS-3 in thermal biology. The T\(_{\text{b}}\) in the *Brs3\(^{-/}\) mouse is reduced, but day-night rhythmicity is maintained. Similarly intact is the stress-induced increase in T\(_{\text{b}}\) that occurs with handling. When *Brs3\(^{-/}\) mice are fasted, their T\(_{\text{b}}\) falls further. We hypothesize that these changes in *Brs3\(^{-/}\) T\(_{\text{b}}\) reflect an incorrect signal that energy stores are insufficient, rather than BRS-3 having a direct intrinsic effect on the control of body temperature; presumably the underlying central thermoregulatory mechanisms remain undisrupted in the *Brs3\(^{-/}\) mice. These T\(_{\text{b}}\) changes are qualitatively similar to those seen in leptin-deficient (*lep\(^{ ob/ob}\)*) (16) and lipostatic (8) mice and polygenic (NZO) (20) and monogenic (7) obese mouse models.

BRS-3 agonist treatment of fasted WT mice increases their T\(_{\text{b}}\), with smaller effects in fed mice. The ability of a BRS-3 agonist to increase T\(_{\text{b}}\) in *lep\(^ { ob/ob}\) mice (this report) and to reduce food intake in *lep\(^ { ob/ob}\) mice (12) is consistent with BRS-3 functioning downstream of, or in parallel to, leptin signaling. Possibly, an endogenous BRS-3 agonist ligand helps signal the “energy replete” state to the BRS-3-expressing cells (and/or a low BRS-3 tone could signal low energy stores).

To understand the effect of a perturbation (such as a drug) on energy homeostasis, it is important to understand where its primary action occurs. If the primary driver is a peripheral increase in energy expenditure, an increase in food intake partially compensates, with the net result being some weight loss. Conversely, if the primary action restricts energy intake, a reduction in metabolic rate partially compensates but there still is weight loss. The situation is different if the effect is mediated by an alteration affecting central regulation. If the brain is signaled that the body has too little energy available, there is both a reduction in metabolic rate and an increase in food intake, as seen with BRS-3 antagonist treatment and in *Brs3\(^{-/}\) mice. Conversely, when the brain senses plentiful energy stores, food intake is reduced and metabolic rate is higher than expected, as observed with BRS-3 agonist treatment. Measuring T\(_{\text{b}}\) under appropriate conditions provides a window into the central assessment of energy status.

It is important to note that T\(_{\text{b}}\) information complements other physiological measurements such as indirect calorimetry, which directly measures metabolic rate and allows determination of fuel source. Similarly, food intake measurements can provide meal pattern and behavior information in addition to the quantity consumed. Thus, the T\(_{\text{b}}\) assay is best used first in addition to other physiological measurements to characterize the system and can then be applied in a focused manner, such as for compound screening.

Are the T\(_{\text{b}}\) effects of BRS-3 solely secondary effects of its role in fuel homeostasis and regulation of metabolic rate, or does BRS-3 also have primary effects on T\(_{\text{b}}\)? BRS-3 mRNA and binding activity (receptors) are located in brain regions that control both T\(_{\text{b}}\) and metabolic rate, such as the medial preoptic area, diagonal band, and paraventricular, dorsomedial, and arcuate hypothalamic nuclei (12). In addition, as shown in Fig. 8, the increases in T\(_{\text{b}}\) and metabolic rate induced by a BRS-3 agonist were similar to effects observed with other antiobesity treatments. Thus, the hypothesis that the T\(_{\text{b}}\) increase with a BRS-3 agonist is the result of increased heat production (i.e., non-fever-induced hyperthermia) rather than a programmed increase (i.e., fever) is consistent with the available data, including the lack of inhibition by acetaminophen and indomethacin. However, a primary, fever-like effect on T\(_{\text{b}}\) cannot be definitively ruled out without further experiments.

The weight loss effect of chronic treatment with BRS-3 agonists is moderate (12), less than that seen with mechanisms such as inhibition of type 1 cannabinoid receptor (CB1R) (6). However, the weight loss with BRS-3 agonists appears to be sensitive to the energy status of the animal, with greater weight loss in DIO than in lean mice, and possibly preservation of weight once a certain threshold is reached.

The effects of GPCR agonists can be attenuated in multiple ways. Examples include inhibitory phosphorylation of the GPCR, receptor internalization, and reduction of downstream signaling (24). BRS-3 does not appear to be acutely downregulated at the receptor level (T. M. Kelly, unpublished observation) and both weight loss (12) and the increase in fasting, resting metabolic rate (X.-M. Guan and J. M. Metzger unpublished observation) induced by BRS-3 agonists are maintained with continued dosing. Yet, interestingly, the T\(_{\text{b}}\) increase and food intake decrease caused by BRS-3 agonists largely attenuate with continued dosing. This suggests that tachyphylaxis for T\(_{\text{b}}\) and food intake occurs downstream of the initial receptor signaling, anywhere from that initial neuron to multiple synapses downstream. Alternatively, the body weight and T\(_{\text{b}}\)/food intake effects could be mediated by divergent signaling pathways, with only the T\(_{\text{b}}\)/food intake pathway(s) undergoing tachyphylaxis. Additional detailed pharmacological studies with BRS-3 agonists in vitro and in vivo are needed to explore this hypothesis.

The \(\Delta T_{1-2h}\) metric (quantifying reversal of the fasting-induced reduction in T\(_{\text{b}}\)) has proved useful as a pharmacodynamic screening assay during BRS-3 agonist discovery. The lack of the T\(_{\text{b}}\) response to multiple BRS-3 agonists in *Brs3\(^{-/}\) mice demonstrates that the assay indeed measures a property of
BRS-3 agonism. One advantage of the assay is its large dynamic range with greater sensitivity at times shortly after dosing. This assay has a high information content due to the continuous monitoring, allowing recognition of spurious data and requiring as few as four mice per group. In addition, the assay has a relatively high throughput, which we estimate to be tenfold greater than measuring metabolic rate via indirect calorimetry. The applicability of the Tb assay to a wide array of antiobesity drugs covering the majority of central antiobesity mechanisms suggests wide utility of the assay, well beyond the drug discovery process.

Some compounds caused a triphasic response in Tb (increase, decrease, increase) before returning to baseline. BRS-3 does this slightly at high doses, as do both melanocortin compounds (34). The physiology underlying these observations, and whether they have a common neurophysiological basis, is not currently known.

Despite its great potential as a pharmacodynamic measure of the central nervous system-derived integrated energy status of the mouse, the Tb assay could be misleading in certain situations. For example, if a compound has a primary effect on thermal regulation (i.e., causes fever), this would interfere with using the Tb assay as a measure for energy homeostasis effects. Similarly, if the central integration and regulation of energy status are disrupted, for example with divergent Tb and metabolic rate responses, Tb would be misleading. Thus, an understanding of the biology should be established before relying on Tb as the pharmacodynamic assay.

What is the applicability of the mouse Tb changes and ΔT1–2h assay to larger homeotherms, such as humans? Regulated changes, sometimes large, in Tb are characteristic of energy homeostasis in small homeotherms. Larger mammals, such as rhesus monkeys (23) and humans (2, 21), have much smaller reductions in Tb in response to starvation. Although unlikely, it remains to be tested whether the relatively large Tb increases with BRS-3 agonists observed in mice will be observed in larger mammals.

ACKNOWLEDGMENTS

We thank James Battey (National Institutes of Health) for the Brs3 knockout mice and Doug MacNeal and Cai Li for comments on the manuscript. Correspondence information for J. M. Metzger: Merck Research Laboratories, P.O. Box 2000, 148011-135, 126 E. Lincoln Ave., Rahway, NJ 07065 (E-mail: joseph.metzger@merck.com).

DISCLOSURES

The authors are current or former employees of Merck & Co., Inc., and own stock and/or have stock options in the company.

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