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

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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, thermoregulation; fasting; drug discovery)

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 specific brain regions, including the hypothalamus, caudal 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 knockouts (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 Npy−/−Agrp−/−, 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-(2,2-dimethylbutyl)-1H-imidazo-2-yl][ethyl]phenyl]pyridine; compound 9 in Ref. 25], Bag-4 [6-[(4-[(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-(1S)-(acetylamino)propyl]-4-chlorophenyl)piperidin-1-yl]carbonyl]-1-[(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(H)-one (18) were synthesized at Merck Research Laboratories or Banyu Pharmaceutical, as described. Sibutramine, AM251, and CL-316243 were purchased from Sigma-Aldrich (St. Louis, MO).

Mice. Male C57BL/6N mice were purchased from Taconic (Germantown, NY), and B6-V-Lep ob/J (40)

Metabolic rate measurement. To measure resting metabolic rate with minimum 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. Male mice were individually housed and 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 photocell 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 within the same thermocouple temperature (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 ΔT<sub>b</sub> is calculated as the mean Tb from 1–2 h after dosing minus the mean Tb from 2.5–5 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 (lep<sup>ob/ob</sup>) have a reduced Tb (16) and thus might provide a sensitive model for testing BRS-3’s role in Tb regulation. Treatment of fasted lep<sup>ob/ob</sup> 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 β<sub>3</sub>-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 T<sub>b</sub>. 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 T<sub>b</sub> (Fig. 1B, top). 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 T<sub>b</sub> (Fig. 1B, bottom). When Bag-1 was dosed 18 h into the fast, an increase in T<sub>b</sub> was seen (1.05°C mean increase from 1 to 5 h post-dose; Fig. 1B, bottom). During fasting, T<sub>b</sub> 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 T<sub>b</sub> (not shown). In summary, the data demonstrate that BRS-3 agonist treatment increased T<sub>b</sub>, particularly when T<sub>b</sub> was reduced by fasting or during the light phase, but the T<sub>b</sub> increase typically did not exceed the daily maximum. The largest magnitude effect observed was the reversal of the low T<sub>b</sub> of the fasted state.

We next characterized the thermal biology of Brs<sup>3</sup><sup>−/−</sup> mice by use of continuous T<sub>b</sub> monitoring. Brs<sup>3</sup><sup>−/−</sup> mice showed normal fed day-night rhythms, but with T<sub>b</sub> 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 Brs<sup>3</sup><sup>−/−</sup> mice showed an exaggerated decrease in T<sub>b</sub> 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 T<sub>b</sub> during handling for dosing was intact in the Brs<sup>3</sup><sup>−/−</sup> mice. Brs<sup>3</sup><sup>−/−</sup> mice showed no significant increase in T<sub>b</sub> in response to Bag-1 (Fig. 2B), demonstrating that the T<sub>b</sub> increase was an on-target, BRS-3 mechanism-based effect.
The dose response for Bag-1 was multiphasic (Fig. 3A). Vehicle dosing causes an ∼1.4°C $T_b$ increase attributed to handling stress. At low doses (3 and 10 mg/kg), Bag-1 caused a slight prolongation of the $T_b$ increase. A higher dose (30 mg/kg) increased the duration without appreciably changing the onset timing or magnitude of the maximum $T_b$. 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 $T_b$ 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 $T_b$ from the 2 h before dosing as the baseline and used 60–120 min after dosing to calculate $\Delta T_{1-2h}$, a robust, sensitive measure of response. Omitting 0–60 min after dosing removed most of the temperature increase and variability due to handling. The $ED_{50}$ of $\Delta T_{1-2h}$ 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 was maintained with continued dosing whereas the reduction in food intake is transient (12). We investigated whether the increase in $T_b$ 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 $T_b$ following each dose. When dosed every other day (Q2D), $T_b$ 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, $\Delta T_{1-5h}$ (Fig. 5A). The initial $\Delta T_{1-5h}$ value was 0.82°C. It is notable that with each regimen, each time drug was dosed, the $\Delta T_{1-5h}$ was positive, while on each non-drug-dosing day it was negative. The $\Delta T_{1-5h}$ values for Bag-4 and vehicle days were each...
averaged over days 4 through 12. The mean drug day $\Delta T_{1-5h}$ 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 QD) (means $\pm$ SE). The mean vehicle day $\Delta T_{1-5h}$ values were 0.18 $\pm$ 0.12°C and 0.25 $\pm$ 0.10°C for Q3D and Q2D, respectively. Taken together, these data suggest that attenuation of the increase in $T_h$ is minor with Q3D dosing, intermediate with the Q2D regimen, and nearly complete with daily dosing. This experiment also suggests that there is a rebound, with reduced $T_h$ on the days with vehicle dosing.

The effect of dose interval on food intake was also monitored (Fig. 5B). With daily dosing, there was an initial reduction followed by an overshoot at days 4-5. With Q3D and Q2D dosing, in general there was a slight reduction on drug days, with a tendency toward increased food intake on the vehicle days. The body weights are shown in Fig. 5C. At 12 days, the %weight changes were +3.2 $\pm$ 0.6, +3.4 $\pm$ 0.6, and +0.7 $\pm$ 1.2 and -3.2 $\pm$ 1.4 for the vehicle, Q3D, and Q2D ($P = 0.05$ vs. vehicle), and QD ($P < 0.001$ vs. vehicle) groups, respectively. Thus, despite the near-complete attenuation of the agonist-induced increase in $T_h$, 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 $T_h$ could be caused by fever or non-fever-induced hyperthermia. Fever is a regulated rise in $T_h$ 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 $T_h$. Acetaminophen, given using a dose regimen that significantly reduced lipopolysaccharide-induced fever (not shown), had no significant effect on the $T_h$ 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 $T_h$ 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 $T_h$ 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).

$T_h$ 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 $T_h$ by testing various potential antiobesity compounds acting by different mechanisms. The compounds tested were AM251, a selective cannabinoid receptor-1 inverse agonist (36); 4-(benzylxoy)-1-[4-(2-pyrrolidin-1-ylethoxy)phenyl]pyridin-2(1H)-one, a selective melanin-concentrating hormone receptor-1 agonist (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-adrener-
gic 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 com-
ounds 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, sibutra-
mine 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 calcu-
lation 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<sup>−/−</sup> mice have a reduced metabolic rate and develop mild obesity, but they had not been noted to have a reduced Tb (32). In this report, we present observations on the role of BRS-3 in thermal biology. The Tb in the Brs3<sup>−/−</sup> mouse is reduced, but day-night rhythmicity is maintained. Similarly intact is the stress-induced increase in Tb that occurs with handling. When Brs3<sup>−/−</sup> mice are fasted, their Tb falls further. We hypothesize that these changes in Brs3<sup>−/−</sup> Tb 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<sup>−/−</sup> mice. These Tb changes are qualitatively similar to those seen in leptin-deficient (lep<sup>ob/ob</sup>) (16) and lipoatrophic (8) mice and polygenic (NZO) (20) and monogenic (7) obese mouse models.

BRS-3 agonist treatment of fasted WT mice increases their Tb, with smaller effects in fed mice. The ability of a BRS-3 agonist to increase Tb in lep<sup>ob/ob</sup> mice (this report) and to reduce food intake in lep<sup>ob/ob</sup> 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<sup>−/−</sup> 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 Tb under appropriate conditions provides a window into the central assessment of energy status.

It is important to note that Tb 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 Tb 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 Tb 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 Tb? BRS-3 mRNA and binding activity (receptors) are located in brain regions that control both Tb 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 Tb and metabolic rate induced by a BRS-3 agonist were similar to effects observed with other antiobesity treatments. Thus, the hypothesis that the Tb 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 Tb 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 more 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 Tb increase and food intake decrease caused by BRS-3 agonists largely attenuate with continued dosing. This suggests that tachyphylaxis for Tb and food intake occurs downstream of the initial receptor signaling, anywhere from that initial neuron to multiple synapses downstream. Alternatively, the body weight and Tb/food intake effects could be mediated by divergent signaling pathways, with only the Tb/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 ΔT<sub>b</sub> metric (quantifying reversal of the fasting-induced reduction in Tb) has proved useful as a pharmacodynamic screening assay during BRS-3 agonist discovery. The lack of the Tb response to multiple BRS-3 agonists in Brs3<sup>−/−</sup> 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 \( T_b \) 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 \( T_b \) (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 \( T_b \) 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 \( T_b \) assay as a measure for energy homeostasis effects. Similarly, the central integration and regulation of energy status are disrupted, for example with divergent \( T_b \) and metabolic rate responses, \( T_b \) would be misleading. Thus, an understanding of the biology should be established before relying on \( T_b \) as the pharmacodynamic assay.

What is the applicability of the mouse \( T_b \) changes and \( \Delta T_{1-2h} \) assay to larger homeotherms, such as humans? Regulated changes, sometimes large, in \( T_b \) are characteristic of energy homeostasis in small homeotherms. Larger mammals, such as rhesus monkeys (23) and humans (2, 21), have much smaller reductions in \( T_b \) in response to starvation. Although unlikely, it remains to be tested whether the relatively large \( T_b \) increases with BRS-3 agonists observed in mice will be observed in larger mammals.

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DISCLOSURES

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

REFERENCES


23. Lane MA, Baer DJ, Rumpler WV, Weindruch R, Roth GS. Calorie restriction lowers body temperature in rhesus monkeys, consistent with a postulated anti-aging mechanism in rodents. Proc Natl Acad Sci USA 93: 4159–4164, 1996


