Acetazolamide increases locomotion, exploratory behavior, and weight loss following social stress: A treatment for emotional eating?

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A B S T R A C T

Sympathomimetics are effective, centrally acting drugs that induce weight loss through their potent anorexic and locomotor properties. We reported that sympathomimetics antagonize catecholamine-dependent, alpha-2 adrenergic receptor-dependent signal transduction mediated by chloride/bicarbonate transport. We posit that other drugs that target cellular chloride/bicarbonate antiport would similarly demonstrate anorectic properties, induce locomotion, and diminish weight gain. Male and female inbred mice were housed in groups or stressed by prolonged social isolation. Mice consumed either normal chow or a high fat, high fructose corn syrup, (i.e. “Western”) diet. To inhibit chloride/bicarbonate transport, acetazolamide (ACT, 3 mM) was added to the drinking water. Rodents underwent evaluations of exploratory locomotion and learning with the object recognition test. Mice consuming a “Western” diet gain more weight compared to mice given a normal diet. When placed on a “Western” diet, stressed mice gained weight more rapidly than unstressed. The body weight of mice fed a normal diet with ACT was significantly reduced compared to control mice not given ACT (weight, g ± SEM), 23.7 ± 0.8 vs. 21.0 ± 0.5, p = 0.02. ACT did not reduce weight gain in animals chronically maintained on a “Western” diet. Compared to unstressed mice, living in social isolation reduced spontaneous exploratory locomotion time, an indicator of anxiety, in male mice (sec ± SEM) from 22.8 ± 3.5 to 12.2 ± 2.1 (p < 0.001), and in female mice, from 47 ± 5.7 to 19.6 ± 2.3 (p < 0.001). ACT had no effect on exploration time in unstressed mice, but ACT completely restored the diminished exploratory locomotion time found in stressed mice compared to unstressed mice. The ratio of time spent exploring new objects compared to familiar items (discrimination ratio [DR]) was reduced following social isolation in males from 2.6 ± 0.5 to 1.2 ± 0.2 (p < 0.05) and in females from 3.8 ± 0.6 to 1.5 ± 0.2 (p < 0.01). ACT normalized the DR ratio of the stressed mice. Decreased food consumption and greater locomotor activity induced by ACT may contribute to acute weight loss; this effect is diminished when rodents were maintained on an unhealthful Western diet. Inhibition of chloride/bicarbonate transport through agents such as acetazolamide could offer a safe, new approach to achieving weight loss.

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1. Introduction

There is a growing body of literature that in many, depression, social isolation, and stress can lead to inactivity, increased food consumption, and weight gain [1–10]. It has long been recognized that amphetamines and sympathomimetics (e.g. phenylpropanolamine and ephedrine) are effective, centrally acting drugs that induce weight loss through their potent effect to promote both anorexia and spontaneous locomotion [11,12]. However, because of their abuse potential, these agents are not first line therapy for treatment of weight gain brought on by emotional eating. Alpha-2 adrenergic receptors (α2AR) of catecholaminergic neurons of the paraventricular hypothalamus mediate satiety and spontaneous locomotion [13–21]. We reported that amphetamines and
sympathomimetic agents antagonize α2AR-dependent signal transduction mediated by chloride/bicarbonate co-transport [22,23]. We hypothesized that in addition to amphetamines and sympathomimetics, drugs that inhibit cellular chloride/bicarbonate antiporter would similarly induce anorexia, locomotion, and diminish weight gain with a greater safety profile — especially in disorders such as depression or social isolation where there is a decrease in activity and an increase in emotional eating. Specifically, we hypothesized that acetazolamide, a potent inhibitor of carbonic anhydrase, would reduce chloride/bicarbonate co-transport and decrease food intake, promote locomotion, and reduce weight gain from emotional eating.

2. Materials and METHODS

Male and female inbred mice, approximately twelve weeks of age, were housed socially in groups or they underwent the stress of living in social isolation or in solitary metabolic chambers for five days. Mice consumed either a normal healthful, rodent chow (10% kcals from fat; 72% kcals from carbohydrate; no high fructose corn syrup) or a “Western” diet rodent food (46% kcals from fat; 36% kcals from carbohydrate, sucrose [17.5%] and high fructose corn syrup [12.8%]). Water was provided ad libitum. To inhibit chloride/bicarbonate transport, a carbonic anhydrase inhibitor, acetazolamide (ACT, 3 mM) was added to the drinking water for some mice. For the Object Recognition Tests (ORT), in the morning, two identical, brown, 500 ml glass bottles were placed in a fixed spot in the testing enclosure. Mice were placed in the direct center of the testing area and given 300 s to explore their surroundings and the two identical objects in the testing area. After 300 s, the mouse was removed. During the evening session, one of the identical objects was replaced by a new, novel object (a volumetric-flask filled with blue-dyed water). The amount of time the mouse spends actively investigating the familiar and novel objects was recorded. Active investigation is considered sniffing, climbing, or brushing whiskers against an object. The data collected allowed for the analysis of novel versus familiar object investigation as well as total time spent exploring both objects. Rodents were weighed, and fat mass, lean mass, and body water was measured with magnetic resonance imaging (EchoMRI™). Food consumption and locomotor activity were measured using the Promethion™ Metabolic Measurement System.

3. Results

We found that group housed, unstressed mice fed a high fat, high fructose corn syrup (“Western”) diet gained more weight over three months than unstressed rodents fed a nutritious rodent chow. ACT more effectively reduced the weight gain over three months in the rats fed the healthful chow compared to the mice fed the Western diet and living in group housing for three months (Fig. 1). There was less weight gain in rodents fed ACT with the healthy diet. This reduction in weight gain by ACT during a healthful diet was not due to the diuretic effect of ACT; total body weight and total body water in group housed mice fed a healthy, control diet or a high fat, high corn syrup “Western” diet with and without acetazolamide (ACT). (Left) Group housed, female mice fed a standard, nutritious, control rodent chow over four months gained less weight compared to animals similarly fed but who were also given acetazolamide (ACT, 3 mM) in their drinking water. The control diet mice achieved a weight of (all values expressed in grams ± SEM) 23.7 ± 0.73 compared to mice fed the standard diet with ACT, 20.95 ± 0.52. When mice were changed to a high fat, high fructose corn syrup diet, their weight increased significantly, but ACT had no effect on this chronic weight gain in group housed mice. (Right) The lower weight gains in animals fed the standard diet and given ACT was not due to the diuretic effect of this drug, as in these animals, total body water (TBW) actually increased (values expressed as percentage body water per gram body weight ± SEM) from 65.11 ± 0.77% vs 68.98 ± 0.92%.

Compared to mice living in group housing, the Western diet increased the rate of weight gain to a greater degree in mice stressed by habituation in social isolation by 300%. Significantly, ACT acutely inhibited food consumption and increased voluntary locomotion in mice acutely stressed for five days by social isolation while being fed a Western diet (Fig. 2). Although there were differences in food consumption and activity, there was no change in total energy expenditure (kcal/hour ± SEM, 0.50 ± 0.01 vs. 0.50 ± 0.01, p = n.s.), average VO2 consumption (mL/min ±SEM, 1.73 ± 0.02 vs. 1.73 ± 0.03, p = n.s.), or VCO2 production (mL/min ±SEM, 1.34 ± 0.03 vs. 1.35 ± 0.02, p = n.s.) between Western diet fed rodents in the presence or absence of ACT. There was a strong effect of sex on the effect of the voluntary locomotion behavior of mice. Female mice demonstrated significantly greater exploratory behavior and learning as demonstrated by the novel object exploration tests. However, in mice of both sexes, the stress of social isolation significantly reduced exploratory behavior and novel recognition times (Fig. 3) as compared to mice in group housing. Novel exploration time is dependent upon the willingness of a rodent to explore surroundings and remember objects they have previously been exposed. The time rodents spent exploring new objects correlated significantly with the total time rodents spent exploring new territory. However, the stress of social isolation did not affect the correlation between exploration time and novel exploration (Fig. 4).

ACT potently reversed the suppression of total exploration time and novel exploration in the mice that were stressed by social isolation to the level observed in the group housed, unstressed mice (Fig. 5). Similarly, reductions in the discrimination ratio, a reflection of memory needed for learning, induced by the stress of social isolation was sensitive to ACT. Specifically, ACT had no effect on the discrimination ratio of group housed mice, but it completely reversed the reduction in discrimination ratio induced.
4. Discussion

Acute stress, such as that induced by social isolation, increases food consumption and decreases spontaneous, voluntary locomotor activity in both laboratory rodents and humans [24–26]. These behaviors can lead to weight gain which contributes to the development of the metabolic syndrome and, eventually, increased cardiovascular morbidity in men and women. It has been shown that stress induces “emotional eating,” i.e. the increased consumption of less nutritious food that results in weight gain. We sought to identify pharmacologic antagonists that could modify the behavior outcomes of social stress that often results in emotional eating and unhealthy weight gain. We have shown that many of the effects of the stress hormone, epinephrine, through α2AR signal transduction are mediated by carbonic anhydrase-dependent chloride/bicarbonate exchange [22,23].

We report that the carbonic anhydrase inhibitor acetazolamide diminishes food intake in socially stressed animals. The effect of acetazolamide on weight loss was not due to the effect of the carbonic anhydrase inhibitor on taste perception that could make food less palatable. In a study in which sucrose was added to acetazolamide in the drinking water and food intake was held constant, acetazolamide still induced nearly 10% reduction in body weight [27,28]. Acetazolamide could have an effect on basal metabolic rate as the formation of triglycerides stores requires the presence of carbonic anhydrase-dependent bicarbonate to form fat depots, but it should be kept in mind that α2AR agonists such as clonidine inhibit lipolysis, and acetazolamide-dependent inhibition of α2ARs can result in decreased fat mass [29]. However, it is shown from our data that acetazolamide also increases spontaneous locomotion associated with exploratory behavior which will expend calories and cause weight loss. It is noteworthy that another carbonic anhydrase inhibitor, topiramate, used in the treatment of seizures,
has been found to effectively reduce weight in humans although the mechanism of action is unknown [30,31].

Activation of the 2AR increases food intake in rodents [15]. Amphetamines and sympathomimetics (e.g. phencyclidine/propranololamine and ephedrine) are effective, centrally acting drugs that induce weight loss through their potent anorexic and locomotor properties [11,12]. These effects of the amphetamine could be due to the inhibition by these sympathomimetics of central 2ARs in the paraventricular hypothalamus (PVH) that modify satiety and locomotion [13–18]. As noted above, we have previously shown that 2AR signal transduction can be inhibited by blocking carbonic anhydrase-dependent chloride/bicarbonate transport with acetazolamide in platelets and vascular smooth muscle [22,23]. In this current report, we show that acetazolamide demonstrates potent anorectic properties, increases locomotion, and diminishes weight gain during exposure to social stress similar to the action of amphetamines. This effect of acetazolamide on weight was abolished in mice fed a Western diet for >12 weeks. It is possible that the high-fructose corn syrup based Western diet may alter signaling of appetite hormones and feeding behaviors independent of the carbonic anhydrase-dependent chloride/bicarbonate transport. Whether this effect is sugar concentration dependent or related to diet duration would need further exploration.

Electrical stimulation of medial forebrain noradrenergic fibers releases catecholamines in the hypothalamus which increase the rewarding value of food, and stimulated animals can be induced to eat non-preferred foods or to feed after satiety. On the other hand, damage to the lateral hypothalamus produces a deficit in noradrenergic function and reduces the rewarding value of food [13]. Administration of α-methyl-tyrosine, an inhibitor of catecholamine synthesis, into the hypothalamus inhibits the anorexic effect of amphetamine [14]. Implicit in this observation is that pre-synaptic catecholamines are necessary for amphetamine-induced anorexia. Furthermore, administration of the β-adrenergic receptor antagonist, propranolol, inhibited the anorexic action of amphetamine. It is inferred that post-synaptic β-adrenergic receptors are also necessary for the feeding response. Clonidine, an 2AR agonist, induces feeding behavior in rodents, and this observation gives further evidence that it is specifically the 2ARs that mediate catecholaminergic feeding behavior in the ventral hypothalamus [15]. Because amphetamine antagonizes clonidine binding, it is not surprising that amphetamine inhibits the effect of clonidine on feeding behavior [16]. Similarly, central administration of the specific 2AR antagonist yohimbine has been shown to reduce food intake [17]. More recently, a genetic polymorphism of the 2AR gene on chromosome 10 in humans (rs1800544) has been associated with increased caloric consumption and predilection for sweet food products and weight gain in patients using atypical antipsychotics [18,19]. Clearly, stimulation of 2ARs, most likely in the Pbh, enhances feeding behavior in satiated laboratory rodents.

In addition to inducing anorexia, amphetamines and sympathomimetic drugs increase spontaneous locomotion and the caloric expenditure rises. Similarly, inhibition of 2AR activation, most likely in the Pbh, also contribute to weight loss by initiating, or by facilitating, spontaneous movement. Clonidine has been shown to induce hypoactivity, and dexmedetomidine, another potent 2AR
agonist, has also been shown to reduce spontaneous locomotion [10,11]. The effect of dexmedetomidine on hypoactivity is abrogated in mice with targeted disruption of the gene encoding the α2AR. Although amphetamine may have other actions that result in weight loss, it appears that the anorexic and hyperactivity effect are mediated through α2ARs in a carbonic anhydrase dependent manner.

At the very least, it is suggested from our findings that inhibition of carbonic anhydrase-dependent chloride flux in the PVH may offer a salutary countermeasure against emotional eating. It remains to be determined as to how acetazolamide-dependent inhibition of chloride transport increases locomotion and diminished nutrient consumption.

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Ethics statement
All experiments were approved by our institutional committee for the use of laboratory animals in research.

Author contribution statement
All authors contributed to this work. R.W. jointly conceived the study and planned experiments with W.L. with input from J.S., T.M., and C.M. performed the experiments with R.W. All authors discussed the results and implications and commented on the manuscript at all stages.

Declaration of competing interest
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Appendix A. Supplementary data
Supplementary data related to this article can be found at https://doi.org/10.1016/j.metabol.2020.100023.

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