



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



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ABSTRACT

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 v. 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 unhealthy 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 ($\alpha 2AR$) of catecholaminergic neurons of the paraventricular hypothalamus mediate satiety and spontaneous locomotion [13–21]. We reported that amphetamines and

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sympathomimetic agents antagonize α_2 AR-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 antiport 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 [17.5%]). 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 water was increased in the ACT treated mice (Fig. 1). There was less fat mass in mice consuming a *healthful diet* with ACT compared to a healthful diet only (all values expressed as weight in grams \pm SEM) of 3.61 ± 0.26 vs. 4.68 ± 0.34 , $p = 0.048$; however, there were no significant differences in lean mass (weight in grams \pm SEM, 16.67 ± 0.28 vs 17.69 ± 0.37 , $p = \text{n.s.}$) or lean-to-fat mass ratio (4.76 ± 0.28 vs. 4.10 ± 0.31 , $p = \text{n.s.}$). In addition to no differences in body weight in mice consuming a *western diet* with or without ACT, there were no differences seen in fat mass (weight in grams \pm SEM, 11.25 ± 1.00 vs. 10.36 ± 1.37 , $p = \text{n.s.}$), lean mass (weight in grams \pm SEM, 18.96 ± 0.34 vs. 18.09 ± 0.74 , $p = \text{n.s.}$), lean-to-fat

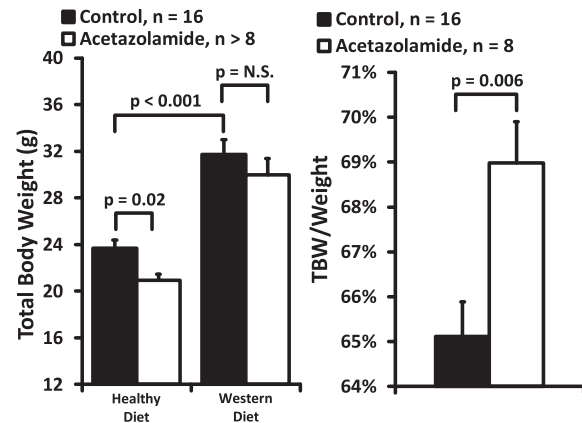


Fig. 1. Total body weight and total body water in group housed mice fed a healthy, control diet or a high fat, high corn syrup “Western” diet with and without acetazolamide (ACT). (Left) Group housed, female mice fed a standard, nutritious, control rodent chow over four months gained less weight compared 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 \pm SEM) 23.7 ± 0.75 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 \pm SEM) from $65.11 \pm 0.77\%$ vs $68.98 \pm 0.92\%$.

mass ratio (1.94 ± 0.22 vs. 2.15 ± 0.25 , $p = \text{n.s.}$), or total body water content (in percent \pm SEM) $52.14\% \pm 1.69\%$ vs $53.18\% \pm 1.57\%$, $p = \text{n.s.}$).

Compared to mice living in group housing, the Western diet increased the rate of weight gain to a greater degree in mice stressed by habitation 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 \pm SEM, 0.50 ± 0.01 vs. 0.50 ± 0.01 , $p = \text{n.s.}$), average VO_2 consumption (mL/min \pm SEM, 1.73 ± 0.02 vs. 1.73 ± 0.03 , $p = \text{n.s.}$), or VCO_2 production (mL/min \pm SEM, 1.34 ± 0.03 vs. 1.35 ± 0.02 , $p = \text{n.s.}$) 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 potentially 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 grouped 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 grouped housed mice, but it completely reversed the reduction in discrimination ratio induced

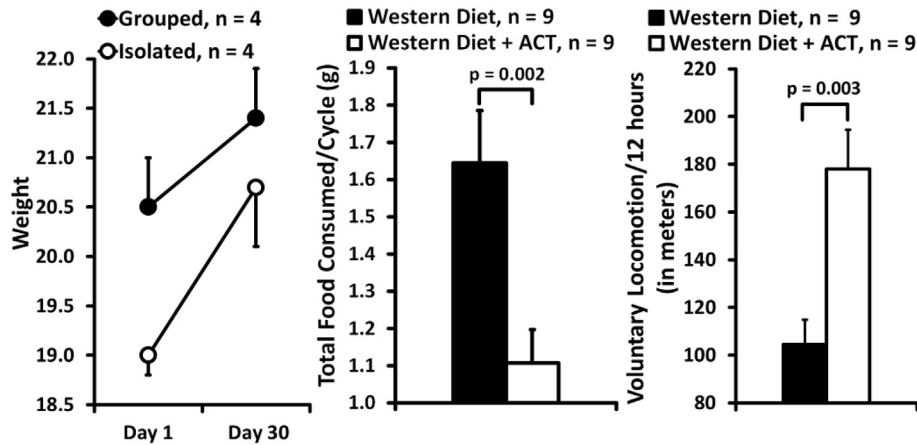


Fig. 2. Effect of social isolation stress on weight gain, appetite, and locomotion in animals fed a high fat, high fructose corn syrup “Western” diet. (Left) When placed in the stress of social isolation and while on a high fat, high fructose corn syrup, compared to group housing, female mice gain weight at a significantly greater rate. We observed nearly a 300% greater rate in weight gain in socially isolated mice over one month when compared to group housed mice fed a high fat, high fructose corn syrup “Western” diet. (Middle) ACT decreased food consumption (all values expressed in grams \pm SEM/12 h) fell from 1.64 ± 0.14 to 1.11 ± 0.09 in mice undergoing the stress of social isolation but fed a nutritious diet. (Right) In contrast, exploratory locomotion (all values expressed in meters \pm SEM/12 h) increased from 104.85 ± 10.27 to 178.07 ± 16.34 .

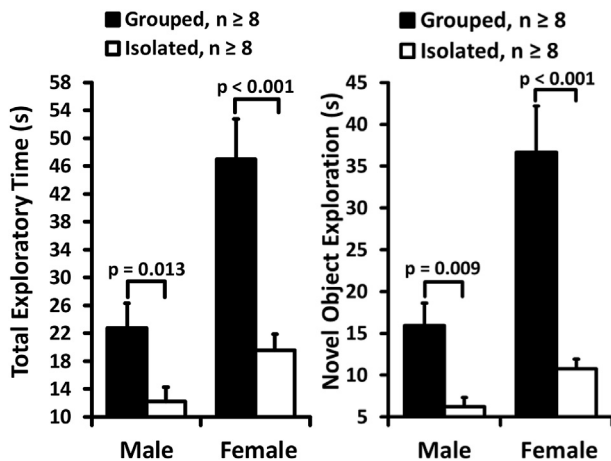


Fig. 3. Effect of social isolation stress in male and female mice on total exploratory locomotion time and time spent exploring new objects over 5 min of exposure. (Left) Compared to male mice, female mice express more spontaneous, exploratory behavior. However, social isolation stress diminished the time spent exploring new territory among both male and female mice. Stress induced by social isolation reduced the time male mice (all values expressed in seconds \pm SEM) spent exploring new territory from 22.8 ± 3.5 to 12.2 ± 2.1 , and among females from 47 ± 5.7 to 19.6 ± 2.3 . (Right) Similarly, the time mice spent exploring new objects was decreased in male from 15.9 ± 2.7 to 6.23 ± 1.1 , and in female mice from 36.7 ± 5.6 to 10.8 ± 1.2 .

by the stress of social isolation (Figs. 6 and 7).

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 α 2AR signal

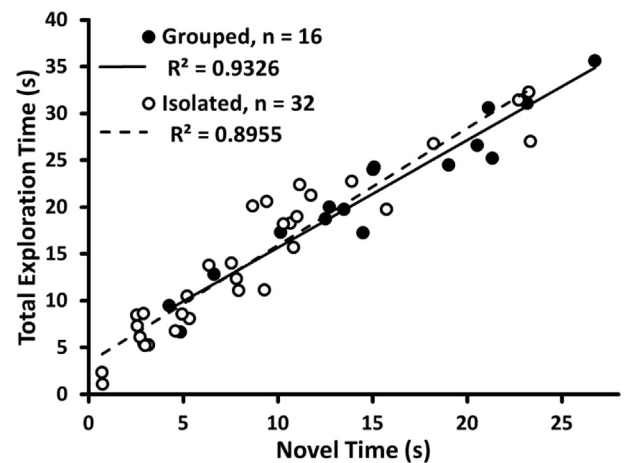


Fig. 4. The time spent exploring new objects correlated with the total time male and female mice spent exploring new territory. The time group housed mice, or mice stressed by social isolation, spent exploring new, “novel” objects was highly correlated with the time the mice spent exploring new “territory.” The linear correlations among these two cohorts were virtually superimposable ($y = 1.1(x) + 4.2$ and $y = 1.2(x) + 3.4$).

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 α 2AR agonists such as clonidine inhibit lipolysis, and acetazolamide-dependent inhibition of α 2ARs 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,

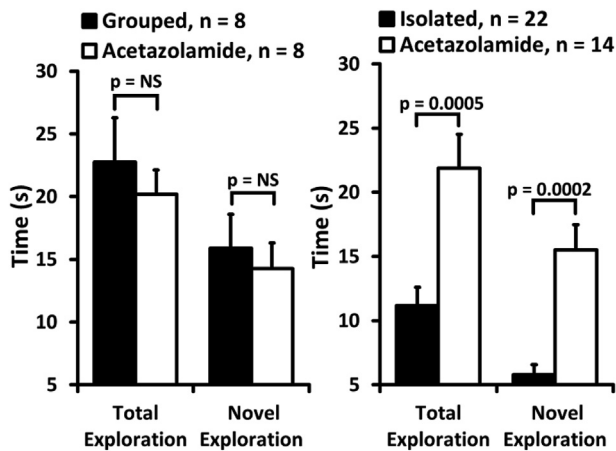


Fig. 5. Acetazolamide (ACT) did not increase exploratory locomotion time or the investigation of novel objects in group housed mice, but ACT increased both exploratory locomotion and novel exploration in mice subjected to the stress of social isolation. (Left) In unstressed male mice, reared in socially grouped housing, ACT had no effect on the total exploratory locomotion time, 22.8 ± 3.5 vs. ACT 20.2 ± 1.9 , $p = n.s.$, or on time spent exploring novel objects, 15.9 ± 2.7 vs. 14.0 ± 2.5 . (Right) However, in male and female mice stressed by social isolation, ACT restored total exploratory locomotion time, 11.2 ± 1.4 vs. 21.9 ± 2.7 , and also increased time spent exploring new objects, 5.8 ± 0.8 vs. 15.5 ± 1.9 .

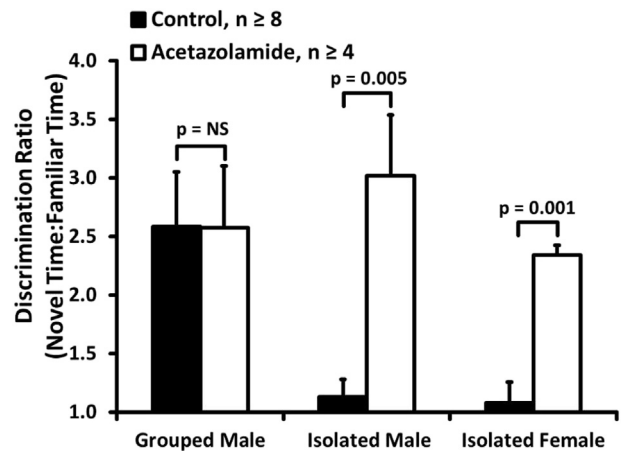


Fig. 7. Acetazolamide (ACT) increased the discrimination ratio (DR), i.e. relative time mice spent exploring new objects in new territories, only in mice stressed by social isolation. There was no difference in the DR among grouped male rodents who were not given ACT and those who had consumed ACT; the values for the DR were identical (values expressed in arbitrary units ± SEM) 2.6 ± 0.5 . However, among mice stressed with social isolation, ACT normalized their DR to that of the group housed mice as seen in males, 1.2 ± 0.2 vs. 2.8 ± 0.5 , and females, 1.08 ± 0.18 vs. 2.34 ± 0.09 .

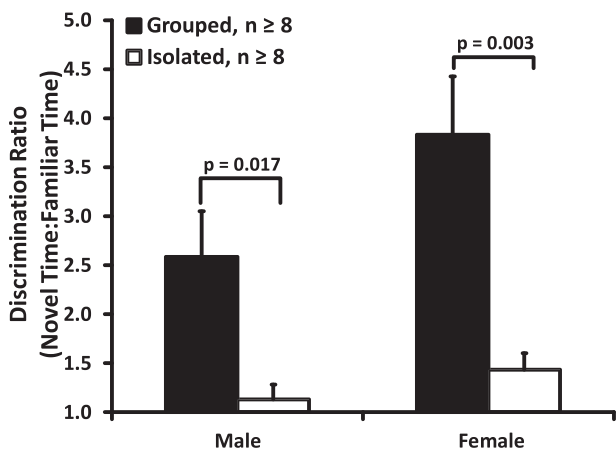


Fig. 6. The stress of social isolation diminished the discrimination ratio (DR) in both male and female mice. Grouped male mice spent more time exploring novel objects relative to known objects, i.e. the DR, than their counterparts stressed by social isolated (values expressed in arbitrary units ± SEM), 2.6 ± 0.5 vs. 1.2 ± 0.2 . A similar decrement in DR was found among the isolated female mice, 3.8 ± 0.6 vs. 1.5 ± 0.2 .

has been found to effectively reduce weight in humans although the mechanism of action is unknown [30,31].

Activation of the $\alpha 2AR$ increases food intake in rodents [15]. Amphetamines and sympathomimetics (e.g. phenylpropranolamine and ephedrine) are effective, centrally acting drugs that induce weight loss through their potent anorectic and locomotor properties [11,12]. These effects of the amphetamine could be due to the inhibition by these sympathomimetics of central $\alpha 2ARs$ in the paraventricular hypothalamus (PVH) that modify satiety and locomotion [13–18]. As noted above, we have previously shown that $\alpha 2AR$ 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 anorectic 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 anorectic action of amphetamine. It is inferred that post-synaptic β -adrenergic receptors are also necessary for the feeding response. Clonidine, an $\alpha 2AR$ agonist, induces feeding behavior in rodents, and this observation gives further evidence that it is specifically the $\alpha 2ARs$ 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 $\alpha 2AR$ antagonist yohimbine has been shown to reduce food intake [17]. More recently, a genetic polymorphism of the $\alpha 2AR$ 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 $\alpha 2ARs$, most likely in the PVH, 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 $\alpha 2AR$ activation, most likely in the PVH, also contribute to weight loss by initiating, or by facilitating, spontaneous movement. Clonidine has been shown to induce hypoactivity, and dexmedetomidine, another potent $\alpha 2AR$

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 $\alpha 2AR$. Although amphetamine may have other actions that result in weight loss, it appears that the anorexic and hyperactivity effect are mediated through $\alpha 2ARs$ 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.; 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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Ryan Woodman conducted this work as a clinician scientist trainee in the Division of Endocrinology in the Department of Medicine at the University of Missouri School of Medicine. Jeffrey Student and 2LT Coleman Miller, USAR, were medical student biomedical research trainees at the University of Missouri School of Medicine.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.metop.2020.100023>.

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