Effects of Corticosterone in Drinking Water

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Abstract

Every day we experience some type of stress. High levels of stress are a common contributor to health problems. In our lab we have previously found that exposure to 10 days of unpredictable stress reduced motivation for a sucrose reward (Bond, Anderson, McWaters, & Matuszewich, 2015). When stress occurs, our bodies release the stress hormone cortisol (corticosterone in animals). The present study was designed to assess whether administering corticosterone through drinking water would elevate blood levels of corticosterone and therefore it can be used to accurately simulate chronic stress in rats. In this study rat’s plasma corticosterone levels were evaluated on day 5 and 10 after being administered corticosterone via drinking water for 10 days. The results demonstrated that corticosterone levels were actually lower in the blood of rats given corticosterone in their drinking water compared to rats given plain drinking water.
Effects of Corticosterone in Drinking Water

Stress effects everybody in some way on a daily basis. Some people experience stress more than others and some people are better able to deal with stress that comes about in their daily life. Stress as defined by Dr. Robert Sapolsky in his book *Why Zebras Don’t Get Ulcers* (2004) is “any unexpected experience in everyday life that creates a stress response within the body”. Stress is an important component to our survival; without it our species would have died out centuries ago and never would have evolved to be the Homo sapiens that we are today (Hadany et al., 2005). Stress is also what keeps us motivated, which has led us to our scientific discoveries and building of civilizations over the years.

**Adaptive Functioning of Stress**

Stress responses are controlled in part by the autonomic nervous system, which regulate automatic changes in bodily function (breathing, heart rate, etc.). The autonomic nervous system is split into two different divisions: sympathetic, which prepares the body for a strenuous physical reaction; and parasympathetic, which works to relax the body. The sympathetic nervous system is responsible for what we know as the “fight or flight” response. Humans have evolved from hunter gatherers and a typical stressor for them would be a predator. If there was a predator looking to eat you or your supply of food, you would quickly have to make the decision to run and hopefully save yourself or stay and try to fight off the predator: “fight or flight”. Our “fight or flight” response helps us pump blood faster, hold our digestion & other needs until a more appropriate time, helps us take in extra air and any other adaptions that might aid us in dealing with the stressor at that moment. The fight or flight response is a great system for a period when predators were the main stressor of the time, but the nature of stress has changed for
the modern society. People now tend to stress about non-active situations, such as bills, homework, social media. These stressors do not necessarily require the “fight or flight” response as responding to the stressor does not require an increase in blood flow or a reduction in digestion. Repeated exposure to 21st century stressors that don’t require increase blood flow to muscles can cause long term health problems—hypertension, insomnia, depression and/or anxiety to name a few.

Maladaptive Dysfunction of Stress

In the brain, the hypothalamus regulates behaviors associated with stress, including motivation, sexuality, aggression, hunger, and thirst (Lambert, 2018). The hypothalamus is found in the base of the brain, below the thalamus and above the pituitary gland. Neurons in the hypothalamus sends the chemical messenger, corticotropin releasing hormone (CRH), to the pituitary gland via the paraventricular nucleus (PVN). CRH binds to special proteins called CRH receptors in the pituitary gland that act to release another chemical messenger, adrenocorticotropic hormone (ACTH), which then travels to the adrenal cortex located on top of the kidneys (Takei, 2016). The zona fasciculata of the adrenal cortex then releases cortisol (or corticosterone in rats) to prepare the body for the “fight or flight” response (Takei, 2016). Cortisol is released into the bloodstream, where it then travels to many different areas of the body to help prepare the body for potential danger and increase body’s metabolism of glucose. Once a high level of circulating cortisol is reached, cortisol receptors in the hypothalamus and the pituitary gland inhibit the further release of hormones (e.g CRF or ACTH). This pathway is called the hypothalamic-pituitary-adrenal (HPA) axis and is regulated by the negative feedback system (Lambert, 2018). In a single stressful experience, the HPA is activated leading to
increases in CRF, ACTH, and cortisol, which then prevents further release of stress hormones and turns off the HPA axis. However, if this HPA axis is activated numerous times due to chronic stress the repeated increase in stress hormones can lead to the desensitization of the HPA axis. This means that the pituitary gland and hypothalamus have a hard time recognizing when to stop releasing hormones, and thus creates and an over-abundance of cortisol (Lambert, 2018).

While stress is a necessity for our survival & flourishing, chronic stress can have very negative health effects. Chronic stress results in the repeated release of stress hormones, causing heart rate to increase and the constriction of blood vessels resulting in your heart having to work harder. This constriction of blood vessels & high blood pressure over time can potentially lead to things such as heart disease, heart failure, stroke, aneurysms, vascular dementia, kidney damage, eyesight damage, trouble sleeping, depression, anxiety etc. All processes in your body that rely on good blood flow could potentially be affected by chronic stress.

In order to better understand the effects of high levels of chronic corticosterone that are associated with chronic stress, corticosterone can be directly administered. Experimentally, there are three routes of administration that are typically used when giving corticosterone to rats (Kott et al, 2016). These include a corticosterone solution via the rats drinking water, corticosterone injection, or the implantation of a time-release corticosterone pellet. The rational for using each type of manipulation differed as does the effect. For example, Previous research done by Kott and colleagues demonstrated that corticosterone in drinking water lowered neurogenesis levels in the hippocampus in rats compared to rats who had a corticosterone time-release pellet. Rats given corticosterone through drinking water and injection had lower levels of plasma corticosterone compared to rats that received a time-release pellet (Kott, et al, 2016). However, activation of corticosterone typically leads to the previously mentioned reduction in the
physiological responses of the HPA axis due to the negative feedback loop. Thus, while the
time-release corticosterone may be advantageous in providing a consistent level of
corticosterone, other research has shown that the hormones in the blood return to baseline levels
in as little as 3 days following pellet implantation (Gasparini et al., 2016). Moreover, the stress
hormone levels increase and decrease throughout a 24-hour period (Chan & Debano, 2010). We
can conclude from this research that the dosing with the time-release corticosterone does not
accurately simulate natural hormone release.

Hypothesis

The current project investigated the effects of administering corticosterone through the
effects of administering corticosterone through the drinking water on plasma corticosterone
levels. Administering corticosterone via drinking water can be a good administration method of
corticosterone due to its less invasive nature than other options such as a surgical implant that
slowly releases corticosterone into the rat’s system (Gasparini et al., 2016) The goal of the
present study is to determine if corticosterone administered through drinking water could
accurately mimic effects of chronic stress. We hypothesize that the synthetic corticosterone
administered in drinking water to male and female rats would increase plasma corticosterone
levels. However, it is possible that due to the negative feedback process, exogenous
corticosterone would have the reverse effect and lower corticosterone levels. This research is
important in determining whether corticosterone administered through drinking water is effective
in altering plasma corticosterone levels similar to the effects chronic stress.
Method

Subjects

Out of 24 adult male and female Sprague-Dawley rats, half received corticosterone in drinking water for 10 consecutive days. Six adult male and six adult female rats were given corticosterone in their water. All rats were handled and weighed on a daily basis and all procedures were approved by NIU’s Animal Care and Use Committee.

Procedure

Corticosterone (Sigma-Aldrich, C2505) was dissolved in 100% molecular grade ethanol at a 1 mg/ml concentration. The diluted corticosterone was then diluted further with tap water to a 1% ethanol solution with a concentration of 75 µg/mL corticosterone. The remaining 12 rats (control) received 1% ethanol water for the ten days, matched to the rats receiving the corticosterone administration. Ten days was selected based on our laboratory’s previous research that found that ten days of chronic stress altered motivation (Bond et al, 2015).

On days five and ten of the ten day treatment, blood samples were extracted through the tails of the rats. Each rat was placed in a plastic restraining tube with the tail facing out. For female rats, a small towel was placed in the top of the restrainer to reduce the movement of the rat. A heating pad was placed under the restrainer with a towel between the heating pad and the restrainer to promote the flow of blood. The rat’s tail was warmed by submerging in warm water for two minutes. Once a sampling site of the lateral tail vein at approximately midpoint on the length of the tail was identified the tail was extended and a 3 mm needle was inserted into the lateral tail vein. After collecting approximately 20µl of blood, the needle was removed from the tail and gauze were applied with gentle pressure to stop any additional bleeding. The rat was
returned to its home cage where they were monitored for approximately 15 minutes to make certain the bleeding had totally ceased.

**Plasma Corticosterone Assay**

The blood samples were assayed for Corticosterone using an ELISA blood kit. First, the Assay buffer reagent was prepared by diluting 10 ml of the supplied concentrate with 90 ml of distilled water. The corticosterone standard solution was diluted with standard diluent. The concentration of corticosterone standard ranged from 20,000, 4,000, 800, 160 and 32 pg/ml respectively. Standard ELISA Assay procedure guidelines were followed. First, 100µl of standard diluent was pipetted into the appropriate wells. Next, 50µl of blue Conjugate and 50µl of yellow Antibody was pipetted into each well. Then the plate incubated at room temperature on a plate shaker for two hours at ~500 rpm. Then the contents of the wells were emptied and washed by adding 400µl of wash solution to every well for a total of three washes. After the final wash, 5µl of the blue Conjugate with the pNpp Substrate solution was added to every well. The plates were then incubated at room temperature for one hour without shaking. Finally, 50µl of Stop Solution to every well and the plate was read at 405 nm.

**Results**

We appear to be approaching a significant difference between rats who drank corticosterone in their water compared to rats that were given vehicle water to drink (F(1, 4) = 6.41, p = .065). There was no evidence for a significant effect of between males and females (F(1, 4) = 2.62, p = .181). There was no significant effect of which day rats were given corticosterone (F(1, 4) = 0, p = .989). Being that we have a small sample size for males and females it is possible we would find an effect of sex with a larger sample size based on results shown in Figure 1.
Discussion

Conclusions

From the preliminary results, we can conclude that administering corticosterone in the drinking water of male and female rats reduced corticosterone levels found in the blood compared to rats that were given regular drinking water. This demonstrates to us that administration of synthetic stress hormones like corticosterone through drinking water may not be a valid way to examine the effects of stress hormones in rats. We can also conclude that while female levels of corticosterone in rats appeared higher at a base level than male rats; it also appeared higher in females for the group of rats that received regular drinking water. The findings do not support our hypothesis that plasma corticosterone levels would be higher in rats given corticosterone via drinking water.

Limitations

Administration times of corticosterone and drinking water occurred during the day time when our researchers were typically available to be in the lab and rats are nocturnal creatures so they are typically more active, meaning they do their eating and drinking in the evening. The results do not support our hypothesis that corticosterone would be lower than baseline levels if given through the rats’ drinking water. This may be due to our small sample size. Future studies with larger sample sizes could easily predict an effect of sex based on the direction of our statistics.

Future Implications

Future studies should explore the other two routes of administration previously mentioned (injection, and time-release pellet). It could also be beneficial if this study could take place primarily in evening hours so that researchers can be doing the administration and blood
sampling while the rats are in their natural awakened states similar to how humans are awake to respond to their chronic stress. Future studies might also benefit from a larger sample size to see if there truly is an effect of sex.
References


Figure 1. Males v. females and cort water v. water on day 5 and day 10