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The Reversal Effects of Curcumin, an Herbal Remedy, on the Impairments Induced by VMAT-2 Inhibitor Tetrabenazine

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ABSTRACT

Substantial evidence has shown that dopamine (DA), particularly in the nucleus accumbens (NAc), is involved in behavioral activation and effort-related processes, such as overcoming work related response costs. Behavioral paradigms have been developed to assess effort-related choice behavior in rodents, including maze procedures and operant tasks. Interference with accumbens DA transmission through administration of the vesicular monoamine transportor-2 (VMAT-2) inhibitor tetrabenazine (TBZ) produces an alteration of response allocation in the concurrent FR5/chow choice procedure, biasing animals toward the lower effort alternative. The effects of TBZ are consistent with DA depletions and administration of DA D_1 or D_2 family antagonists. It has been suggested that these drug-induced shifts in effort-related choice behavior seen in rodents are analogous to symptoms such as psychomotor retardation, anergia, and fatigue, which can be observed in people with depression and other related disorders. Previous studies have shown the effects of TBZ on lever pressing and chow consumption can be attenuated through co-administration of the putative antidepressant MSX-3, an adenosine A_{2A} antagonist, the monoamine-oxidase (MAO)-B inhibitor, deprenyl, as well as the well-known antidepressant bupropion. Recently, clinical studies have shown antidepressant efficacy of curcumin, a MAO-A/B inhibitor, as an adjunct medication. Curcumin is a naturally occurring compound found in turmeric, a prominent ingredient of curry powder used in ethnic foods. Curcumin has proven successful in traditional rodent models of depression such as the tail suspension or forced swim test. The current study investigated the ability of curcumin to reverse the effects of TBZ on effort-related choice behavior. The effects of TBZ on lever pressing were partially reversed by ingestion of a high dose of curcumin two hours prior to testing. A major problem with curcumin is its poor bioavailability due to fast metabolism; therefore future studies should investigate ways to enhance the absorption of curcumin.

1 - Introduction

To survive, organisms must overcome environmental obstacles that separate them from motivationally significant stimuli. In order to overcome constraints, organisms need to exert effort through effort-related decision-making revolving around cost/benefit analyses (Salamone & Correa, 2002). There are numerous variables that influence these choices, with one of the most important being interactions based upon work requirements and reinforcement value (Salamone & Correa, 2002; Salamone et al., 2003, 2005, 2007). Substantial evidence has indicated that the mesolimbic dopamine (DA) pathway, particularly DA within the nucleus accumbens (NAc), is involved in effort-related processes, such as overcoming work-related response costs in instrumental behavior (Barbano & Cador, 2007; Phillips et al., 2007; Salamone et al., 2005, 2007). Interference with DA transmission can affect the relative allocation of behavior in animals responding on tasks that assess effort-based choice behavior; biasing animals towards the lower effort alternative (Floresco et al., 2008; Salamone et al., 2005, 2007).

Tests of effort-related choice behavior offer animals choices between a more highly valued reward that can be obtained by a high degree of effort vs. a low effort/low reward option. One procedure that is used to study the effects of dopaminergic manipulations on effort-related choice behavior is the concurrent FR5/chow feeding choice task. This operant choice task offers rats the option of lever pressing to obtain a more preferred food (BioServe high carbohydrate pellets), or approaching and consuming a concurrently available less preferred standard lab chow. Under baseline or control conditions, when the FR requirement is relatively low (i.e., FR1 or FR5), trained rats will receive most of their food from lever pressing and consume only a small quantity of the lab chow (Cousins et al., 1993; Nowend et al., 2001; Salamone et al., 1991, 1997). Interfering with DA neurotransmission through DA depletions and local or systemic administration of DA D_1 or D_2 family antagonists produce a reallocation of behavior, such that

lever-pressing is significantly decreased and consumption of the lab chow is increased (Nunes et al., 2010; Salamone et al., 2002; Sink et al., 2008; Worden et al., 2009). A similar behavioral profile is produced after administration of the reversible vesicular monoamine transporter-2 (VMAT-2) inhibitor tetrabenazine (TBZ). By inhibiting VMAT-2, TBZ blocks vesicular storage and depletes monoamines, with its greatest impact being upon striatal DA (Pettibone et al., 1984). Additionally, TBZ has been shown to affect DA signal transduction in a manner that is consistent with reduced ventral striatal DA D_1 and D_2 receptor neurotransmission (Nunes et al., 2013). The concurrent FR5/chow choice task has been extensively validated to demonstrate that the effects of interference of NAc DA transmission are not due to changes in appetite, food intake, or preference (Salamone et al., 1991), and do resemble effects of reinforced evaluation by prefeeding (Nunes et al., 2013; Salamone et al., 1991) or appetite suppressant drugs (Salamone et al., 2002; Sink et al., 2008).

It has been suggested that tasks measuring effort-based decision-making could be useful to model the effort-related motivational symptoms such as fatigue, psychomotor slowing, and anergia seen in depression and other related disorders (Salamone & Correa, 2012). It has been reported that these symptoms are among the most common in psychiatric medicine but are the most difficult to treat as they are resistant to antidepressant treatment (Fava et al., 2013; Stahl, 2002). Therefore, these paradigms may be beneficial for developing adjunct medication to treat these motivational dysfunctions. Tests of effort-related decision-making have been developed in humans (Treadway et al., 2011), and have shown that individuals with major depressive disorder show reduced selection of higher effort alternatives (Treadway et al., 2012). Reversal studies (e.g., co-administration of another compound) are used as pre-clinical assessment of potential treatments. Co-administration of the adenosine A_{2A} antagonist, MSX-3, was able to attenuate the shifts in behavior induced by TBZ (Nunes et al., 2013). Additionally, the widely used

antidepressant drug bupropion, a catecholamine uptake blocker, and L-deprenyl, a monoamine oxidase-B (MAO-B) inhibitor, attenuate the effort-related shifts induced by TBZ (Nunes et al., 2013; Randall et al., submitted).

Curcumin is a naturally occurring compound found in turmeric powder, that has been shown to possess action as an inhibitor of both MAO-A and MAO-B enzymes (Kulkarni et al., 2005). By inhibiting the activity of monoamine oxidase, curcumin prevents the breakdown of monoamines and thereby increases their availability. Levels of both serotonin (5-HT) and DA were increased following administration of curcumin, and levels of norepinephrine (NE) were not altered (Kulkarni et al., 2008). There is a dose-dependent effect of curcumin on the MAO-B enzyme. Curcumin's action on this enzyme is achieved at high doses as compared to inhibition of the MAO-A enzyme which is achieved a low doses (i.e., 5 mg/kg vs. 40 mg/kg; Kulkarni et al., 2008). The antidepressant profile of curcumin has been explored in animal models of behavioral despair, which are employed as antidepressant screening paradigms. Co-administration of curcumin with DA depleting agents, such as reserpine, decreased immobility time (Kulkarni et al., 2008). Moreover, curcumin further enhanced the anti-immobility effect of the nonselective MAO inhibitor, tranylcypromine, as well as selegiline, a MAO-B inhibitor, in the mouse forced swim test (Kulkarni et al., 2008). Together these studies confirm the MAO inhibiting activity of curcumin. Recently, curcumin has been shown to be effective in human-clinical trials as an adjunct medication for depression (Bergman et al., 2013).

Previous studies that have investigated the antidepressant-like effects have administered curcumin orally via gavage feeding (Kulkarni et al., 2008; Xu et al., 2005). Gavage is the introduction of a solution into the stomach by means of inserting a small-diameter tube into the esophagus, and delivering the drug directly into the stomach by means of a syringe. Although highly effective, this method can cause esophageal injury as well as restraint-associated distress,

particularly with repeated use (Brown et al., 2000). Stress induced alterations include changes in gastric secretion and mobility, changes in heart rate and increases in plasma glucocorticoids (Kent et al., 1986; Brown et al., 2000). Moreover, this stress-induced response can last up to one hour after (Bonnichsen et al., 2005). A prior study reported a 32% mortality rate attributed to granulomatous inflammation caused by gavaging that ultimately lead to asphyxia (Germann & Ockert, 1994).

The aim of the current study is to investigate the effects of ingested curcumin pellets to attenuate TBZ induced shifts in behavior in rodents responding on the concurrent FR5/chow choice procedure. Self-administration of curcumin pellets by animals will reduce adverse effects and distress due to gavaging and thereby provides an alternative to gavage. In addition, blood assays and tissue harvesting were conducted to determine rate of absorption and metabolism of curcumin.

2. Materials and Methods

2. 1 Animals

Adult male Sprague-Dawley rats (Harlan-Sprague Dawley, Indianapolis, IN, USA) were pair housed in a colony maintained at 23° C with 12-h light/dark cycles (lights on at 7:00h). Rats (n=7) weighed approximately 300-350 grams at the beginning of the study, and were initially food restricted to 85% of their free-feeding body weight for operant training. Rats were fed supplemental chow to maintain the food restriction throughout the study, with *ad libitum* water, and were allowed modest weight gain throughout experiments. Animal protocols were approved by the University of Connecticut institutional animal care and use committee, and followed NIH guidelines.

2.2 - Pharmacological Agents and Dose Selection

Tetrabenazine (9,10-dimethoxy-3-(2-methylpropyl)-1,3,4,6,7, 11b hexahydrobenzo[a]quinolizin-2-one), the VMAT-2 inhibitor, was purchased from Tocris Bioscience (Bristol, UK). Tetrabenazine was dissolved in a vehicle solution of 0.9% saline (80%) and DMSO (20%). 1N HCl /mL volume was then added to adjust the pH and get the drug completely into solution. The final pH of the tetrabenazine solution was 3.5. The saline with 20% DMSO vehicle solution was administered as the vehicle control. The 0.75 mg/kg dose of tetrabenazine which was used for the FR5/chow choice task was based on extensive pilot work done in our laboratory. All injections were administered intraperitoneal (IP).

Curcumin pellets were formulated by grinding 28 mg of BioServe (Frenchtown, NJ, USA) and 7 mg of curcumin together, to give a total weight of 35 mg per pellet. Curcumin pellets were pressed fresh weekly and were stored in a desiccator. All pellets were made by the University of Connecticut, School of Pharmacy. The dosages of curcumin were adjusted by pellet number. Animals needed to consume at least half of the pellets to be considered successful in achieving the desired dose.

2.3 Behavioral Procedures

Concurrent FR5/chow-choice procedure: Behavioral sessions were conducted in operant conditioning chambers (28x23x23 cm^3, Med Associates, Georgia, VT, USA) during the light period. Rats (n = 9) were initially trained to lever press on a continuous reinforcement schedule (30 minute sessions, during 5 days/week) to obtain 45mg pellets, (Bioserve, Frenchtown, NJ, USA), and then were shifted to the FR5 schedule (30 minute sessions, 5 days/week) and trained for several additional weeks until reaching a predetermined baseline number of lever presses (i.e., consistent responding • 1,200 lever presses). Animals needed to consistently reach baseline criteria for the course of approximately one week before being introduced to the concurrent FR5/chow-feeding choice procedure. In this task, weighed amounts of laboratory chow

(Laboratory Diet, 5P00 Prolab RHM 3000, Purina Mills, St. Louis, MO, USA; typically 20-25 grams, four-five large pieces) were concurrently available in the chamber during the 30 min FR5 session. Rats also received feeding study training and were trained to consume 10 Bioserve pellets on baseline days 2 hours prior to running. Before inclusion in the experiment, all animals were exposed to 10 (highest dose, 160 mg/kg) curcumin to insure consumption. Animals were trained on the procedure until approximately the entire dosage (i.e., 8/10 pellets) was consumed. On experimental testing days, when TBZ was administered, the rats were administered 80 mg/kg and 160 mg/kg of the curcumin pellet formulation 2 hours prior to running. At the end of the FR5/chow choice session, rats were immediately removed from the chambers, lever pressing totals were recorded, and amount of chow consumed was determined by weighing the remaining food and spillage. Rats were trained until reaching and maintaining stable levels of baseline lever pressing and chow intake. Once animals achieved baseline rates experimental testing began.

2.4 Experimental Procedures

Animals received a vehicle injection one week prior to beginning testing in order to habituate them to being injected. Additionally, animals received curcumin pellets one week prior to testing in order to assure accurate consumption. All experiments used a within-group design in which each rat received all drug or vehicle treatments in the experiment in a randomly varied order (one treatment per week). Baseline training sessions (i.e., non-drug) were conducted 4 days per week.

2.4.1 Experiment 1: Ability of MAO-inhibitor curcumin to reverse the effects of TBZ.

Rats were trained before drug testing as described above. Rats (n=7) were given 15 minutes to consume pellets administered 2 hours prior to testing. Animals received the following treatments; vehicle plus 10 Bioserve pellets (VEH/BIO), tetrabenazine plus 10 Bioserve pellets (TBZ/BIO), tetrabenazine plus 80 mg/kg curcumin (TBZ/80), and tetrabenazine plus 160 mg/kg

curcumin (TBZ/160). Animals who were over 400 grams, received one extra pellet. Remnants of the pellets were collected and weighed to determine the total dose of curcumin administered. Next, rats received an injection of either 0.75 mg/kg TBZ or vehicle 90 minutes prior to testing.

2.4.2 Experiment 2: Assays of tissue levels of curcumin to determine rate of absorption

Rats were randomly assigned to consume either 80 or 160 mg/kg curcumin 2 hours prior to live decapitations. Animals were placed into CO_2 for approximately 2 minutes and were quickly removed. Cardiac punctures were performed to acquire at least 5 cc's of blood. Afterwards, the liver, brain, and small intestine were removed for analysis.

2.5 Data Analysis

In experiment 1, total number of lever presses and gram quantity of chow intake from the 30 min session were analyzed using repeated measures ANOVA. A computerized statistical program (SPSS 21.0 for Windows) was used to perform all analyses. When there was a significant ANOVA, non-orthogonal planned comparisons using the overall error term were used to assess the differences between each treatment and the control condition. The number of comparisons was restricted to the number of treatments minus one (Keppel, 1991). For the effect size calculations partial eta-squared was used (^{2}). In experiment 2, a bivariate correlation was run between dose of curcumin administered and curcumin tissue concentration. Pearson's correlation coefficient (r) was used to determine the linear dependence between the two variables.

3. Results

Experiment 1: Ability of curcumin to reverse the effects of TBZ.

The MAO A/B inhibitor, curcumin, produced a partial reversal of the effects of TBZ in animals tested on the FR5/chow feeding choice test (Figure 1). Repeated measures ANOVA showed that there was an overall significant effect of drug treatment on lever pressing [$F(3,18)=10.17$, $p<0.001$]. Planned comparisons revealed that TBZ produced a significant

reduction in lever pressing compared to vehicle control conditions ($p<0.05$). Co-administration of 160 mg/kg curcumin with TBZ significantly increased lever pressing compared to TBZ plus vehicle (planned comparisons, $p<0.05$). The drug treatment effect was marked by a moderately high effect size (^{2} = 0.629). The overall treatment effect for chow consumption was also statistically significant [$F(3,18)=12.121$, $p<0.001$]. Chow intake was significantly increased by TBZ relative to the vehicle-vehicle condition (planned comparisons, $p<0.05$). Chow intake was significantly reduced at the 160 mg/kg dose of curcumin (planned comparisons, $p<0.05$). The effect size of drug treatment on chow consumption was moderately high (^{2} = 0.669). Thus, co-administration of curcumin (160 mg/kg) with TBZ significantly increase lever pressing and decreased chow consumption compared to TBZ-vehicle treated animals.

Experiment 2: Assays of tissue levels of curcumin to determine rate of absorption

The bioavailability of curcumin despite the dose administered produced similar blood serum concentrations (Figure 2). A bivariate correlation between dose consumed and concentration revealed that 80 and 160 mg/kg produced nearly identical levels. There was a very strong correlation for both 80 mg/kg ($r=0.918$) and 160 mg/kg ($r=0.907$).

4. Discussion

TBZ is currently used to treat Huntington's disease, and is known to produce depressive side effects, including motivational symptoms, in some patients (Frank et al., 2009; 2010). By inhibiting VMAT-2, TBZ affects monoamine storage, but studies indicate that the greatest effects are on striatal DA (Pettibone et al., 1984; Nunes et al., 2013). Similar to the effects produced by interfering with NAc DA, TBZ produced a shift in choice behavior in rats responding on the concurrent FR5/chow choice task. Compared to control animals, TBZ-treated animals have decreased lever pressing and increased consumption of the lab chow. Animals administered TBZ expend minimal effort when an alterative food source is available at lower

cost (i.e., the freely available chow). Although TBZ-treated rats show significant reductions in food-reinforced lever pressing, they remained directed towards the acquisition and consumption of food. Similar to the effects produced by administration of DA D_1 or D_2 family antagonists, the effects induced by TBZ substantially differed from pre-feeding to reduce food motivation (Salamone et al., 1991), and appetite suppressant drugs such as amphetamine (Cousins et al., 1994) and CB_1 antagonists and inverse agonists (Sink et al., 2008), all of which failed to increase chow consumption at doses that suppressed lever pressing.

The TBZ induced shifts in behavior can be attenuated by co-administration of the adenosine A_{2A} antagonist, MSX-3, the catecholamine uptake inhibitor bupropion, and the MAO-B inhibitor deprenyl (Nunes et al., 2013; Randall et al., submitted). The present study investigated the MAO-A and B inhibitor, curcumin, to reverse the behavioral effects of TBZ. Co-administration of 160mg/kg curcumin two hours prior testing can successfully attenuate TBZ induced effects. Previously, curcumin has been shown to produce antidepressant-like effects in rodent tasks such as the forced swim test (Xu et al., 2005) and the tail suspension task (Xu et al., 2005). Curcumin produces effects in these paradigms that are comparable to well-known antidepressants such as fluoxetine, desipramine, and imipramine (Lucki, 1997). The ability of curcumin to attenuate the shift in behavior is similar to the MAO-B inhibitor l-deprenyl (Randall et al., submitted). Moreover, in antidepressant screening paradigms curcumin has been combined with subthreshold doses of antidepressants, and has further attenuated immobility time (Sanmukhani et al., 2011). In addition, a recent human clinical trial has paired curcumin as an adjunct medication to treatment with fluoxetine (Prozac) for depression (Sanmukhani et al., 2013). Patients with add-on curcumin plus fluoxetine had a better ranking on the Hamilton Depression Rating Scale compared to those without curcumin (Sanmukhani et al., 2013). Taken together, these studies suggest that curcumin could further potentiate current antidepressants.

Previous studies have administered the dopamine norepinephrine reuptake inhibitor, bupropion, with TBZ in operant task (Nunes et al., 2013; Randall et al., submitted) and maze paradigms (Yohn et al., submitted). Bupropion, like l-deprenyl and curcumin, also produced a partial reversal of TBZ induced deficits in choice behavior (Nunes et al., 2013; Randall et al., submitted; Yohn et al., submitted). Future studies should investigate the ability of subthreshold doses of bupropion plus curcumin to attenuate effort-related shifts in behavior caused by TBZ.

Various animal models (Shankar et al., 1980) or human clinical studies (Lao et al., 2006) have proved that curcumin is extremely safe even at high doses. The pharmacological safety of curcumin makes it a potential compound for the treatment of depression, however, a major issue with curcumin is that is has very poor bioavailability. Curcumin has low intrinsic activity, high rate metabolism and poor absorption (Anand et al., 2007). Previous studies have indicated that the route of administration plays a role in achievable serum levels and tissue distribution (Anand et al., 2007). For instance a study conducted by Ravindranath & Chandrasekhara (1981) revealed less than 3% of curcumin was found in the liver, small intestine, and stomach tissues at the highest dose of curcumin. Moreover, the percentage of curcumin absorbed remained constant regardless of dose, indicating that administration of more curcumin does not result in higher absorption (Ravindranath & Chandrasekhara, 1981). Similarly, the blood serum results from the current study are consistent with the literature. The concentration of curcumin in blood serum is independent of dosage. For example, both the 80 and 160 mg/kg dose of curcumin displayed roughly the same total concentration of curcumin. Additionally, previous studies have shown that curcumin has extremely low serum levels (Anand et al., 2007). Similar to rodents, oral administration of 2 grams curcumin daily in humans produced undetectable serum levels (Cheng et al., 2001). Together, these studies with the current results suggest there is a dose-dependent limitation to bioavailability. The addition of an excipient to curcumin has been shown to improve

the bioavailability (Anand et al., 2007; Kulkarni et al., 2008; Shoba et al., 1998). Therefore, future studies should investigate the potential of curcumin paired with an adjuvant.

Neusilin has been proposed as a possible bioavailability enhancer that may be paired with curcumin (Williams et al., 2014; Yan et al., 2011). Using curcumin prepared with phytosomes has also been shown to improve the uptake into the blood (Marczylo et al., 2007), and that the peak of curcumin in blood is 15 minutes after oral gavage administration. Other studies have used piperine, another natural compound found primarily in hot jalapeno peppers, to increase the bioavailability of oral curcumin dosing by 154% (Kulkarni et al., 2008; Shoba et al., 1998). Piperine's combined effects with curcumin have also been studied examining the anti-depressant like effect on chronic-stress induced behavior (Bhutani et al., 2009). Prior studies have demonstrated that piperine enhances the oral bioavailability of curcumin in both rats and humans at doses that were devoid of adverse side effects (Shoba et al., 1997). Piperine has been shown to inhibit the metabolism of curcumin and enhance its bioavailability.

Additionally, during collection it was noted that, majority of the curcumin pellets were still located within the stomach and small intestine. Taking this into consideration, future studies should extend the lead-time of curcumin. Prior studies have found that curcumin remains active in the system 90 minutes to three hours after administration (Anand et al., 2007). Moreover, previous studies have used IP or oral injections of curcumin, which have faster absorption rates (Pan et al., 1999). Prior studies have shown that negligible amounts of curcumin have been found in blood plasma of rats after oral administration of 1 g/kg of curcumin indicating that curcumin was poorly absorbed from the gut (Wahlstrom & Blennow, 1978). Since very little curcumin is absorbed from the gut and small intestine, future studies should investigate rate of absorption of curcumin with an excipient.

As mentioned before, curcumin has already been employed in studies testing its effects as an enhancer of antidepressant medication (Kulkarni et al., 2008; Sanmukhani et al., 2013). With the results from this study, we provide further evidence that it can be used for depression, schizophrenia, and other related disorders that have motivational symptoms. People with neurological disorders, especially those related to inflammation, may consider using curcumin as a natural herbal remedy, by suggesting a change of diet involving an increased intake of curry foods. This adds to the growing field of complementary and alternative medicine, and may be able to help those who cannot take the normally prescribed medications due to either medical or personal reasons.

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Appendices

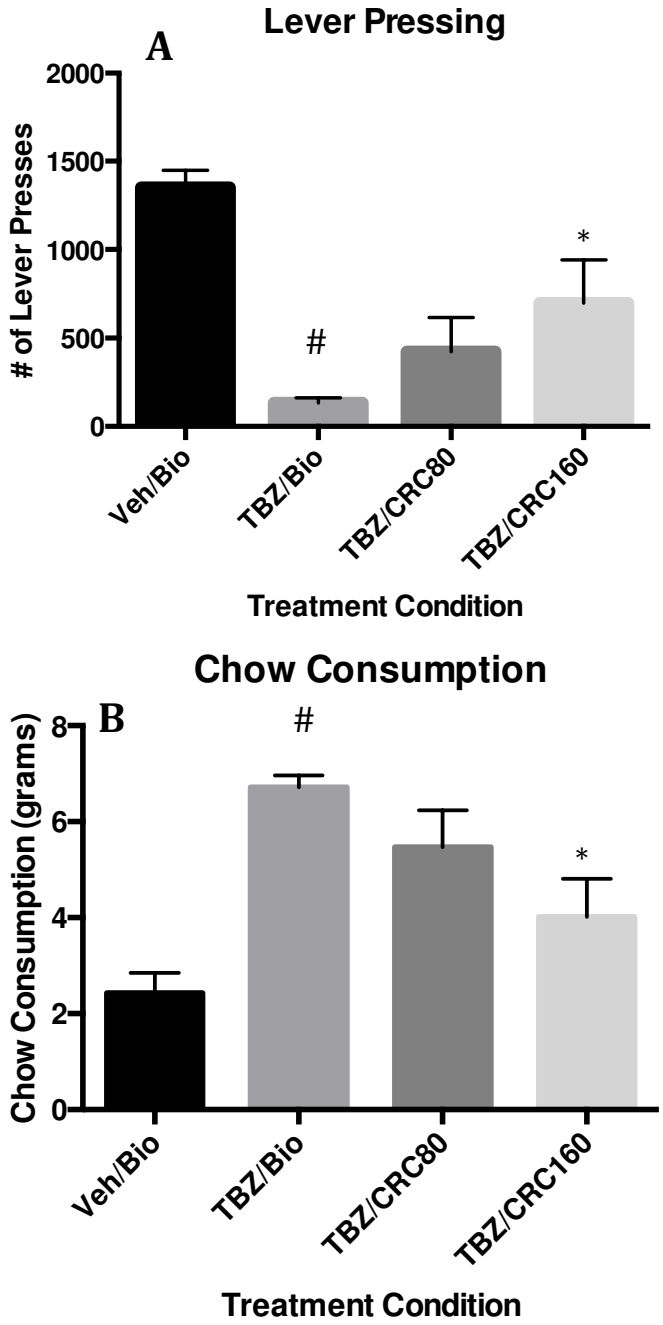

Figure 1: The effects of the MAO-A/B inhibitor curcumin on TBZ-induced changes in rats performing on the concurrent FR5/chow choice task. Rats received IP injections of vehicle or 0.75 mg/kg 90 minutes prior to testing. In addition, rats were administered curcumin two hours prior to testing. (A) Mean (±SEM) number of lever presses (FR5 schedule) during the 30 minute session. (B) Mean (±SEM) gram quantity of chow intake. TBZ significantly decreased lever pressing and increased chow consumption relative to vehicle (# $p<0.05$). Administration of 160 curcumin to TBZ-treated rats 2 hours prior to testing significantly increased lever pressing and decreased chow consumption relative to treatment with TBZ alone (* $p<0.05$).

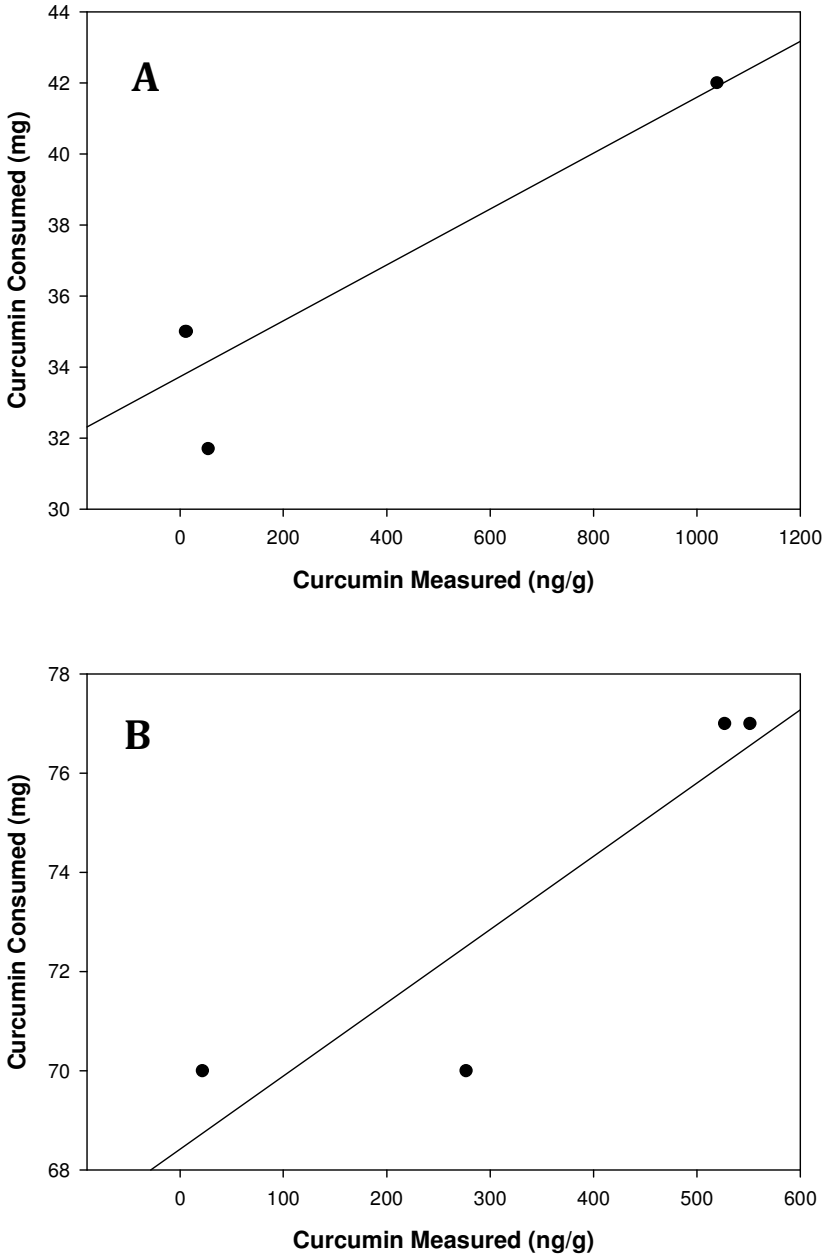

Figure 2: Blood serum concentrations of curcumin to assess rate of absorption. Rats received either 80 or 160 mg/kg curcumin two hours prior to collection. (A) Linear trend with a strong positive correlation of the amount of curcumin consumed and serum levels in the 80 mg/kg dose (r=0.918). (B) Positive correlation of the effects of 160 mg/kg curcumin on amount detectable in blood serum (r=0.907). Despite the dose and amount consumed, both doses have similar serum concentration levels.