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AM 6545: A Novel Peripheral CB1 Antagonist

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Abstract:

Obesity and other related metabolic disorders are a common problem in the United States. Consequently, several drug therapies have been developed in an attempt to address this problem. Many older appetite suppressants, such as amphetamines, were dangerous and potentially addictive. For the last few years, the endocannabinoid system was investigated as a potential target for appetite suppression. Unfortunately, early cannabinoid CB1 antagonists came with an unacceptable side effect profile of their own, which is largely due to central actions of these drugs. In an attempt to reduce the side effect profile, researchers are investigating peripherally acting cannabinoid antagonists, which do not penetrate the blood brain barrier. This study investigated AM 6545, a novel peripheral cannabinoid antagonist, for its effects on food reinforced instrumental behavior. In the end, the results indicated that AM6545 produced a dose-related suppression of lever pressing for food reinforcement.

Introduction:

Obesity and excessive weight gain have become major health problems in the United States and other parts of the developed world. Currently it is estimated that approximately 66% of adults in the US are overweight. Excess weight is associated with a number of health risks including reduced life span, diabetes, and cardiovascular disease, and these problems place an enormous strain upon health care systems (Yanovski and Yanovski, 2002; Moore 2008). The recognition of this growing health problem has stimulated interest in understanding the complex behavioral, neural and hormonal systems that regulate food intake. In the last few years, the endocannabinoid system has attracted considerable attention (Salamone et al. 2007). Drugs that block CB1 cannabinoid receptor transmission can reduce food intake and food-reinforced behavior in animals (Salamone et al. 2007; Sink et al. 2008a, 2008b). CB1 antagonists such as rimonabant are being studied in human clinical trials for their possible appetite suppressant and weight loss effects (Pi-Sunyer et al., 2006). However, these drugs also can produce aversive effects such as nausea (McLaughlin et al. 2005; Sink et al. 2008a; Pi-Sunyer et al. 2006; Salamone et al. 2007), as well as psychological side effects such as depression and anxiety in some people (Pi-Sunyer et al. 2006). These aversive effects would complicate the clinical profile of CB1 antagonists as possible treatments for obesity.

A new research direction has focused on creating CB1 antagonists that do not penetrate the blood brain barrier (Kunos G et al. 2008). The CB1 receptors responsible for nausea are located on the brainstem (Salamone et al. 2007). However, peripheral CB1 receptors are also present on adipocytes and have a role in energy regulation (De Kloet AD and Woods SC, 2009). Additionally, evidence suggests that CB1 inverse agonists behave differently from CB1 antagonists. CB1 antagonists suppress feeding, while a CB1 inverse agonist suppresses feeding

with an induction of nausea (Sink et al. 2008a). An ideal appetite suppressant would therefore be a CB1 antagonist as opposed to a CB1 inverse agonist to avoid inducing nausea. Furthermore, it should not cross the blood brain barrier in order to avoid anxiogenesis, or the creation of anxiety. To that end, A. Makriyannis's research team created AM 6545, a peripherally acting CB1 antagonist. This compound was a novel drug that had not been tested before. This experiment sought to characterize the behavioral effects of AM 6545 on appetite suppression. Operant conditioning was the paradigm used to measure food related motivational behaviors. Previous research has suggested that cannabinoid antagonists not only suppress appetite, they also suppress food related motivational behaviors (Sink et al. 2008b). Additionally, peripherally, the endocannabinoid system is important for energy regulation (Pavon et al. 2008). Thus, in view of research indicating that the endocannabinoid system in the periphery is involved in energy regulation, it presents a target for pharmacotherapy. The present experiment sought to study the effects of different doses of AM6545 on food reinforced lever pressing in order to better understand the dose required to observe an effect.

In summary, CB1 antagonists are being studied for their potential to reduce appetite and lead to weight loss for patients that take them. However, the endocannabinoid system also is involved in other functions such as pain, nausea and anxiety. Early appetite suppressants that acted on the endocannabinoid system were inverse agonists such as Rimonabant, which is nonselective and led to unpleasant side effects such as nausea and anxiety. Attempts are being made to reduce the side effect profile of this class of medication by seeking cannabinoid neutral antagonists. One promising new development is the synthesis of peripherally acting cannabinoid antagonists such as AM 6545. AM 6545 is expected to lead to a reduction in lever pressing due to its suspected appetite suppression effects.

Materials and Methods:

Subjects:

A total of eight four month old male Sprague Dawley rats were obtained from Harlan, Indiana. They were kept on a 12 hour light dark cycle; the lights were on at 8 am, and turned off at 8 pm. They were drug naïve, and had not received any previous training nor taken part in any previous studies. The animals were handled and weighed for three days after arrival. Following that, they were food deprived to 85% of their free feeding body weight, and were singly housed in plastic cages (20 cm X 22 cm X 42 cm). They had continuous access to water whenever they were in their plastic home cages, or not performing in an experiment. This experiment was approved by the University of Connecticut's Institutional Animal Care and Use Committee (IACUC).

Materials:

The operant chamber (Med Associates, Georgia, VT; ENV-001) had one active lever. The food dispenser was a Med Associates ENV-203 pellet dispenser stocked with 45 mg precision dustless rodent purified diet pellet (Bioserve, Frenchtown, NJ). The operant chamber was linked through Med Associates interfaces (DIG 721 – outputs, DIG 711 – inputs, and 700 – decoder) to a computer running the Med Associates program (Med PC). This computerized system was able to record the number of lever presses, or the number of responses committed by the animal. The drug utilized was a novel CB1 inverse agonist, AM 6545, that does not penetrate the blood brain barrier. This drug was supplied by A. Makriyannis from Northeastern University.

Behavioral Procedure:

After the rats were received, they were allowed to habituate to the vivarium for one week. They were then weighed to determine the free feeding body weight, after which they were food deprived to 85% of body weight for the initial lever press training. Rats also received tail markings in order to provide identification, and were housed two to a cage. Next, they received a few days of magazine training. Lights were turned off at the beginning of every session as a cue for the rats to begin responding. Magazine training consisted of a continuous reinforcement schedule with a pellet dispensed every thirty seconds. This was intended to teach the rats where their food was dispensed into the chamber, and to learn to associate the pellet dispenser sound with food delivery. Magazine training lasted for a 30 minute interval each day. All the animals were handled and weighed every day following IACUC guidelines.

Following magazine training, the rats were then put on continuous reinforcement training for a couple of weeks. The primary difference between magazine training and continuous reinforcement was the lack of automatic pellet dispensation. This ensured that the rats would not expect pellets to be given to them. Continuous reinforcement training lasted for 30 minutes per day. As soon as the subjects reached a suitable baseline (around 300 lever presses per 30 minute session), the difficulty of the reinforcement schedule was increased. A switch was made to Fixed Ratio 5 (FR5) reinforcement. With this schedule, the animal needs to press the lever five times in order to be reinforced. The animals continued training on the FR5 schedule until they reached a baseline (average of 1500 lever presses). Training consisted of five days of operant conditioning (the weekdays) followed by two off days (the weekend). FR5 training lasted 30 minutes per day. Rats were provided supplemental food over the weekend and whenever they

approached their 85% weight level. Once the rats reached the behavioral criteria, they began drug testing.

Drug Treatment Procedure:

An initial pilot study was performed to determine the appropriate doses to use. For the drug experiment five different IP injection treatment conditions were used: vehicle, 2.0 mg/kg, 4.0 mg/kg, 8.0 mg/kg and 16.0 mg/kg. The vehicle for this drug was 10/10/80 mixture of TWEEN, DMSO, and saline. The experiment used a within-groups design, with each rat receiving all IP drug treatments in a randomly varied order (one treatment per week, with none of the treatment sequences repeated across different animals in the same experiment). Baseline (i.e., non-drug) sessions were conducted four additional days per week. An additional week of drug testing was built into the schedule to account for equipment malfunctions. The additional week was utilized because of a feeder jam on one of the previous testing days.

Analysis:

The number of lever presses for each animal was recorded each day. Additionally, averages for each day were calculated along with standard deviations and standard errors of the mean. This was done to track the variability in the data. Three different measures were collected during this study. The first was the number of lever presses as a function of drug dose. The second graph was the percentage of previous day lever presses as a function of drug dose. The advantage to performing this comparison is that the animals varied in their baseline lever pressing from week to week. The final comparison made was percentage of previous two days lever pressing as a function of dose. This comparison was made to ensure that the rat's previous

day's lever pressing was not anomalous. Ultimately, the variability in lever pressing for each rat in between weeks did not affect the shape of the relationship. As a result statistical analysis was only performed on the lever pressing as a function of dose. A univariate General Linear model ANOVA was performed to find whether or not the mean lever presses across doses was significant. A Tukey *post-hoc* test was performed on the data to determine which groups differed significantly from one another. No statistical analysis was performed on the other data set, however in the future it may be worth investigating.

Results:

Figure 1 shows the number of lever presses across different drug treatments. ANOVA revealed that there was a significant overall effect of treatment condition ($F = 10.638$, $p < 0.05$). As seen in Figure 1, it can be seen that as the dosage of drug increased, the number of lever presses decreased. Figures 2 and 3 illustrate a similar phenomenon. Figure 2 expresses the lever pressing data as a percentage of the previous baseline day. As seen in figure 2, this measure of lever pressing also showed a dose-related decline with increasing dose. Several treatment groups differed significantly from one another. Both the 8.0 and 16.0 mg/kg groups significantly differed from vehicle ($p < 0.001$). The mean lever presses recorded at the 2.0 mg/kg dose also was significantly different from the mean lever presses on both the 8.0 mg/kg ($p = 0.027$) and 16.0 mg/kg ($p = 0.002$) doses. Finally the mean lever presses on 8.0 mg/kg of drug was significantly different from the mean lever presses on 16 m/kg of the drug ($p = 0.05$).

In summary, AM 6545 leads to a suppression in lever pressing in a linear dose dependent manner. The 2.0 mg/kg and 4.0 mg/kg doses did not suppress lever pressing. The 8.0 mg/kg and 16.0 mg/kg doses were significantly different from the vehicle dose. Anecdotally, rats on the

highest dose had a tendency to lie on their backs in their home cage, and to move less than rats on the lower doses or vehicle. Furthermore, drug treated rats were less active and less responsive to auditory stimuli.

Discussion:

AM 6545 does reduce lever pressing in a dose dependent manner. However, the reason for the suppression in lever pressing is not entirely clear cut. This piece of evidence on its own is not enough to suggest that AM 6545 is an appetite suppressant in of itself. There are several possible causes of this phenomenon. Firstly, it is possible that AM 6545 causes a reduction in motor activities that are necessary for lever pressing. Secondly, it is possible that AM 6545 induces nausea. Thirdly it is possible that AM 6545 causes a reduction in the tendency to work for food. Finally, and ideally, AM 6545 would cause a reduction in appetite while not inducing nausea or a reduction in locomotion. Fortunately, different procedures exist to elucidate the cause of the lever suppression. While this experiment provides a starting point for further research on this drug, additional follow-up studies will be required to more fully elucidate its character.

If AM 6545 causes ataxia or a suppression in locomotion, several procedures could be used to verify or discount this case. One method of ascertaining what effect AM 6545 has on locomotion would be to use stabilimeters, which are small activity cages. Stabilimeters could track the movement of the rat for the duration of the session. Alternately, a large open field chamber could be used to measure locomotion. Previous research has suggested that cannabinoid antagonists do not affect locomotor activity. However, more information about a

drug profile is always better, so as a result, testing its locomotor activity would be a good idea. In addition, the roto-rod test could be used to assess ataxia.

Another possibility is that AM 6545 does not affect appetite, but does assess the tendency of the animal to work for food by pressing the lever. A food choice paradigm (Sink et al. 2008b) that gives concurrent access to both lever pressing for food and laboratory chow could be used to address this issue. AM 6545 should not affect the tendency to work for food, as this effect is generally seen as related to brain function, and this drug is not supposed to cross the blood brain barrier.

Conditioned gaping has been long used as a paradigm for measuring nausea in rats (Salamone et al. 2007; Sink et al. 2008a). Ideally, AM 6545 would not make rats nauseous, as one of the chief complaints against Rimonabant was its tendency to induce nausea (Pi-Sunyer et al. 2006). This point is important to the future development of this drug. If it does induce a large amount of nausea, then it would be a setback to this line of research.

The final follow-up study that is needed is a feeding study. The rats should be allowed free access to food during a session. At the end of the session, the food consumed by the rats could be measured. Previous research has shown that Cannabinoid antagonists produce a type of feeding suppression distinct from other drugs (Sink et al. 2008a, 2008b). This study is the most essential one, because it would address all of the possible drug effects in a comprehensive manner.

If AM 6545 were to be an effective and useful appetite suppressant drug, it should not affect locomotion or cause nausea. Nausea was one of the reasons people ceased taking Rimonabant during clinical trials. The final piece of the puzzle is whether or not AM 6545 actually crosses the blood brain barrier. This investigation is beyond the scope of this

experiment. Hopefully, future immunological data will be able to help clarify the biochemical characteristics of AM 6545. If for instance AM 6545 was in fact a CB1 inverse agonist, then future investigations involving this compound would be less desirable. Alternately, it may better help explain why AM 6545 produces the behavioral changes it does, such as the possible nausea. In short, this experiment is reliant that AM 6545 behaves biochemically the way it is supposed to.

In conclusion, there is reason to be optimistic about the future of appetite suppressants. Peripherally acting CB1 antagonists have the potential to avoid some of the psychological side effects of systemic CB1 antagonists. More research on this compound is needed, but hopefully it will allow for a new generation of treatment options for obesity. More research is needed on the characteristics of AM 6545, but based off of what has been observed, it is a very interesting compound worthy of future study.

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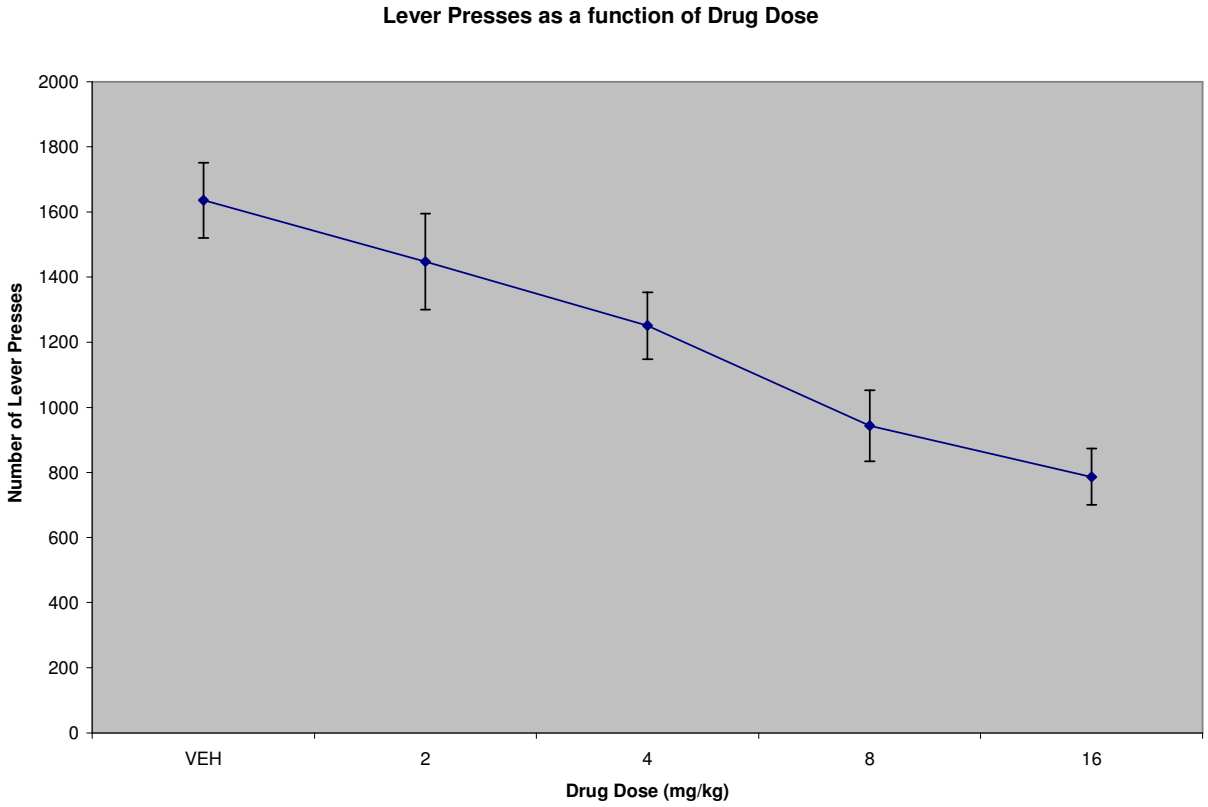

Figure 1: This figure shows the relationship between drug dose and number of lever presses. 8 mg/kg was the minimum dose necessary to produce a suppression in lever pressing compared to the vehicle.

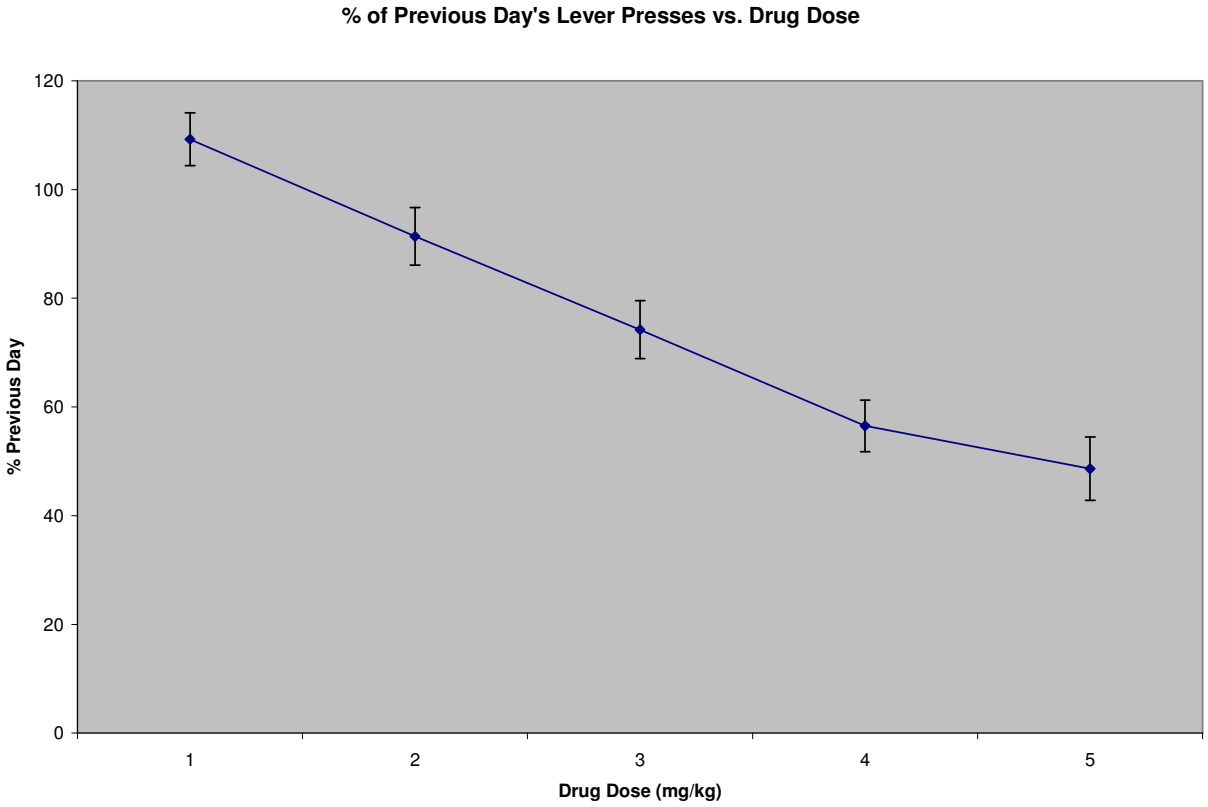

Figure 2: This figure shows the relationship between the percentage of previous day's lever presses as a function of drug dose. It is included because the baseline lever pressing varied from week to week.