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TECHNICAL NOTE: EFFECT OF HANDLING ON STRESS-INDUCED HYPERTHERMIA IN ADULT RABBITS

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Abstract: Animals perceive humans as a threat, therefore handling could cause stress reactions and fear in rabbits. Stress induced hyperthermia (SIH) is a physiological response produced when a stressful situation takes place and is supposed to occur in rabbits. In this work, the SIH response is assessed when different groups of rabbits (young males, nulliparous does and multiparous does) are handled. A benzodiazepine (Midazolam®) was administered to half of the animals to assess handling response also under drug effects. Rectal temperature was not increased by handling in male rabbits and multiparous does, whereas nulliparous females showed presented SIH after 30 min of handling. In addition, Midazolam® has been revealed as useful for pharmacological validation of SIH after 25 min from injection.

Key Words: rabbit, stress-induced hyperthermia, benzodiazepine, handling, behaviour.

INTRODUCTION

Handling of rabbits may cause fear, since domestic animals can develop fear of human beings (Rushen *et al.*, 1999). Thus, rabbits may perceive human presence and handling practices as a threat, triggering physiological responses.

Bouwknecht *et al.* (2007) explained that when an animal is exposed to a stressor, the "flight or fight" response takes place, while heart rate and body temperature increase. This rise in temperature is known as stress induced hyperthermia (SIH) and it is part of an individual's response to stressful situations that occurs both before and during exposure to stress-inducing stimuli (Olivier *et al.*, 2003). The SIH response is present in any mammal that has been tested, including humans, pigs, mice, rabbits or rats (Vinkers *et al.*, 2008) and it has been reported when animals are subjected to stressors such as handling (Moe and Bakken, 1997).

Benzodiazepines are the most frequently used psychotropic agents in drug treatment for anxiety disorders (Schmitt and Hiemke, 1998). In this case, behavioural and physiological reactions related to fear response may change when individuals are treated with benzodiazepines.

Physiological reactions such as SIH can be pharmacologically validated and it is thus important to consider that administration of a drug involves handling of animals as well as an injection, so could induce SIH response by itself (Vinkers *et al.*, 2009). It is therefore of interest to study the effects of handling and injection stress in SIH when validating a behavioural test to ensure that physiological responses correspond to any studied stimuli.

The aim of this work was to assess whether handling and injection produces SIH in different groups of adult rabbits.

MATERIALS AND METHODS

A total of 56 rabbits from synthetic line A of the Universidad Politécnica de Valencia (Baselga, 2002) were used in this study. The experiment was carried out in an experimental house located in Segorbe (Castellón, Spain). Rabbits were grouped in 3 categories: 16 multiparous does (MD) in the second week of their third gestation and 6 mo of age

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(average weight 4.17±0.35 kg); 16 young male rabbits (MR) 3.5 mo of age (average weight 3.65±0.34 kg); 24 young nulliparous does (ND) 3.5 mo of age (average weight 3.65±0.24 kg).

Animals were individually housed in commercial cages as of 2 mo of age. Cage dimensions were 40×90×35 cm (width×length×height).

Rabbits from each group were randomly assigned to control (CON) or Midazolam® (MDZ) group (n=8 in MD and MR; n=12 in ND). Rabbits were gently removed from their home cages and those in the MDZ group received an intramuscular injection of 0.5 mg/kg of MDZ (15 mg/3 mL, Midazolam®, NORMON Laboratory, Spain, while rabbits in CON group received the same dose of saline solution by intramuscular injection.

Prior to injection, basal rectal temperature (Tb) was measured in all the animals with a digital thermometer inserted approximately 1.5 cm into the animal's rectum. Animals were then returned to the cage and over the following 60 min rectal temperature was carefully measured every 5 min (Ti) to assess the possible effect of handling in SIH. This test was designated accumulated temperature test (ATT).

This procedure was revised and accepted by the Animal Welfare and Ethics Committee of the Instituto Valenciano de Investigaciones Agrarias (IVIA, Valencia).

Statistical analysis

Changes in rectal temperature were assessed using a repeated measures procedure with time as the within subject factor and the animal as experimental unit. For each type of animal (MR, MD or ND), treatment group (control or MDZ) and time (0, 5, 10,...60) were included as explanatory factors. Possible interactions were also considered. Analyses were performed by Mixed procedure and Tukey-Kramer adjustments were used for comparisons. All the statistical analyses were carried out using SAS (SAS, 2009) and the significance level was set as 0.05.

RESULTS AND DISCUSSION

Regarding time effect, which implies handling effect, ND showed an increase in rectal temperature during the ATT both in CON and in MDZ groups ($P<0.0001$). In this type of animals, differences between Tb and Ti were significant from minute 30 in control group and from minute 35 in MDZ group (Figure 1). However, MR and MD behaved in a different way, as shown in Figure 1. In CON groups, time had no effect on rectal temperatures, so there was no handling-induced hyperthermia. On the contrary, the effect of time was statistically significant in MR treated with Midazolam® ($P<0.0001$) which showed a decrease in rectal temperature of 0.82 °C from the beginning to the end of the test. MD in MDZ group also showed a decrease in rectal temperature during the test (0.29 °C), but it was not statistically significant ($P=0.1099$). In this case, time effect in fact corresponds with the Midazolam® effect, which acts by reducing body temperature and is confirmed when assessing the effect of the interaction time-treatment ($P=0.0007$). Thus, in MR and MD differences in rectal temperature were found between CON and MDZ groups ($P<0.0001$ both in MR and in MD) and began to statistically differ from minute 25 until the end of the test. These results allow us to establish that Midazolam® reduced rectal temperature, although the drug effect needed 25 min to fully develop.

The ATT allows the assessment of changes in rectal temperature related to consecutive manipulation of animals. Our results indicate that the injection and manipulation of the animals themselves do not induce a rise in body temperature in male rabbits and multiparous does, as no differences in rectal temperature were recorded during the test. However, this assertion cannot be extrapolated to nulliparous does, since rectal temperature in these animals is significantly higher than initial temperature from minute 30 in control group. The nulliparous does show a higher degree of response to handling and this could mean that they may be more sensitive to stress related to manipulation than multiparous does and young males. It is known that human contact reduces fear responses toward humans (Csatadi *et al.*, 2007), although many studies related to this aspect are performed when the animals are very young (Csatadi *et al.*, 2007; Verga *et al.*, 2007; Verwer *et al.*, 2009). Nevertheless, handling programmes can also reduce fear reactions (Podberscek *et al.*, 1991), so previous experience with handling could be a plausible explanation for the differences found between nulliparous does (unhandled animals) and multiparous does (handled animals due to inseminations, palpations or weanings, among others). However, this hypothesis does not fit with the differences

found with male rabbits, as these animals were not previously handled.

In this sense, gender differences in the response to stress have been recorded in rodents and females show higher activity of the hypothalamic-pituitary-adrenal (HPA) axis than males (Handa *et al.* 1994). It is possible that rabbits also show this type of variations in HPA activity depending on gender, and this would be the reason why nulliparous showed a higher reaction to handling than males. However, following this hypothesis, multiparous does should also have shown an increase in rectal temperature, but similar temperatures to those recorded in males were registered. A possible explanation could be related to the pregnancy of multiparous does when the test was carried out, as pregnancy is apparently associated with an attenuated response of the sympathoadrenal and HPA systems, at least in humans (Kajantie and Phillips, 2006). Thus, pregnancy could have dampened the gender-related effect and this could explain why response to stress is similar in multiparous does and male rabbits and different in nulliparous does. To the best of our knowledge, no studies concerning gender and pregnancy effects on stress have been performed in rabbits, so these assumptions cannot be confirmed and further research is needed.

We also applied Midazolam® treatment to reduce any hyperthermia produced by a potentially stressful situation such as manipulation. When assessing the effect of Midazolam® on rectal temperature, differences between CON and MDZ group were only found in male rabbits and multiparous does. Midazolam® in these cases caused hypothermia in treated male rabbits and a non significant decrease of temperature in treated multiparous does, while body temperature in control animals remained nearly constant. These differences are significant as of minute 25, so we can assume a full effect of the drug from this moment on. The differences in the hypothermia caused by Midazolam® could be due to a stronger effect of the drug on males. Nevertheless, this hypothesis requires further research, since no literature related to gender differences in sensitivity to benzodiazepines in rabbits was found. Again, nulliparous behaviour was opposite, since no differences were found between control and treatment group. Following the preceding assumptions, young females seem to perceive manipulation as a stressful event and this could be the reason why temperature increases during the test both in control and in MDZ groups.

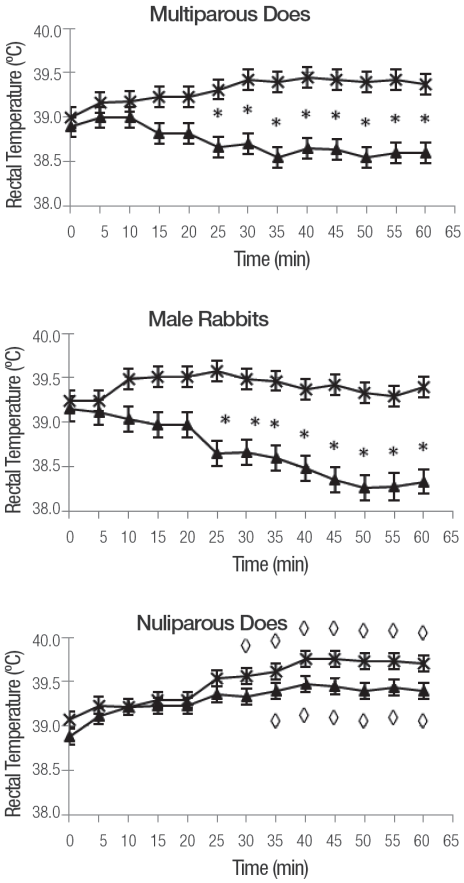

Figure 1: Mean±standard error of rectal temperature from initial time to minute 60 in non injected with Midazolam® (control) and injected with Midazolam® (treatment) male rabbits, multiparous does and nulliparous does. *: Significant differences between treatment and control ($P<0.05$). ◊: Significant differences between initial temperature and temperature measured every 5 min, ($P<0.05$). —✕— Control, —▲— Treatment.

CONCLUSIONS

This study has demonstrated that rectal temperature can be used to physiologically validate fear or stress tests in male rabbits and multiparous does, since manipulation does not cause hyperthermia itself and temperature changes may be attributed to stressors in the study. In nulliparous does, reactivity to manipulation seems to be higher and other physiological factors should be studied to perform a more accurate

validation. This work also implies that Midazolam® treatment, applied half an hour before a stressful event, can be used to annul fear or stress response in young male rabbits and multiparous does. This effect is complete 25 min after injection of the drug.

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