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EFFECTS OF PLACEBO (SALINE) INJECTIONS ON CORE TEMPERATURE IN THE RAT

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Abstract

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1. Core temperature was telemetrically measured in 15 rats before (i.e., at baseline) and at 10-min intervals for 120 min following the injection of normal saline (1 ml/kg ip) or "no injection."
2. The sample exhibited a mean temperature increase of 0.60 ± 0.10°C (mean ± SEM) following injection.
3. This differed significantly from the mean increase of 0.13 ± 0.03°C following "no injection" ($p < 0.001$).
4. The injection of saline (1 ml/kg) affected a mean rise in core temperature of 0.55 ± 0.07°C ($p > 0.000001$) in 46 animals in a second experiment.
5. These data indicate that routine handling and a simple injection comprise significant and measurable stress which must be controlled in neuropharmacological studies employing a thermoregulation paradigm.

Keywords: Core temperature, hyperthermia, placebo, stress, telemetry

Abbreviations: intraperitoneally (ip), minute (min)

Introduction

Certain forms of stress produce hyperthermia in rats (Blasig et al, 1978; Briese and DeQuijada, 1970; Pechnick et al, 1984; Singer et al, 1986). The authors studied the effect of saline injection in adult, male Sprague-Dawley rats by measuring core temperature.

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Material and Methods

Experiment I

Fifteen adult, male Sprague-Dawley rats weighing 206 ± 3.2 g (mean ± SEM) were used in this experiment which was designed to determine the effect of simply handling these animals and injecting 1 ml/kg of normal saline.

Experimental Procedure

The core temperature of the 15 rats was measured at baseline (t = 0, i.e., prior to injecting saline). The thermic response to normal saline was then telemetrically measured every 10 min for 120 min. The 12 deviations between core temperature (prior to handling and the injection of saline) and each of the 10-min points after handling and injection were added and divided by 12. This yielded the mean thermic response for each individual animal. The process of serially measuring core temperature requires that an AM receiver be placed into the cage of each animal every 10 min. This is a mild disturbance which might in and of itself produce a stress induced change in mean core body temperature. The mean change in core temperature was therefore similarly measured in these animals for a 120-min period not preceded by the injection of saline.

Experiment II

Forty-six adult, male Sprague-Dawley rats weighing 250.1 ± 4.7 g (mean ± SEM) were used during this experiment which was designed to quantitate the magnitude of the thermic effect of simply handling the rat and injecting 1 ml/kg of saline.

Experimental Procedure

This experiment did not include a procedure for partialing out the effect of simply measuring core temperature serially. The effect of serial measurement, handling, and injection was the object of concern. The object of this experiment was to determine whether the thermic response exhibited by the animals differed from "0" or "no change relative to baseline. The mean thermic response of each rat was therefore paired with "0" for the purposes of data analysis.

Apparatus

Core temperature was measured using intraperitoneally implanted Model VM Mini-Mitters (Mini-Mitter Co., Sun River, OR). These telemetric thermosensors emit Hertzian waves detectable with an AM receiver at a rate directly proportional to temperature. The animals were allowed five days to recover from the surgical procedure prior to starting the study. The reliability and validity of this method is described elsewhere (Tocco-Bradley et al, 1985).

Statistical Analysis

Data were analyzed using the Wilcoxan sign Rank Test for matched pairs and Student's paired t-test. All measures of variance in the text refer to the standard error of the mean (SEM).

Table 1

Summary of Experimental Results

Experiment 1: (n = 15)	Thermic Response
[1] Handling and injection of saline (1 ml/kg ip)	+0.6 ± 0.1°C
[2] Multiple measurements of core temperature	+0.13 ± 0.03°C
Difference between [1] and [2] is significant at $p < 0.001$	
Experiment 2: (n = 46)	
[3] Handling and injection of saline (1 ml/kg ip)	+0.55 ± 0.07°C
Difference of [3] from baseline is significant ($p < 0.000001$)	

Legend: Core temperature was measured every 10 min for 120 min following the ip injection of saline in conditions 1 and 3. Temperature was simply measured every 10 min for 120 min in condition 2 (the animals were not touched). The telemetric measurement of core temperature requires the placement of an AM receiver into the cage of individually housed animals. This requires that the cages be opened and closed every 10 min for 120 min.

Results

Experiment I: Thirteen (13) animals exhibited an increase in core temperature relative to the condition of "no injection" following saline administration, and two demonstrated no change between these conditions ($p = 0.00006$, Wilcoxan Sign Rank Test for matched pairs). The mean thermic response to saline injection was 0.60 ± 0.10°C

compared to mean change in core temperature of 0.13 ± 0.03°C following "no injection" ($p > 0.001$, $t = 4.15$, $df = 14$) (Table 1).

Experiment II: Forty three (43) of the 46 animals exhibited a mean increase in core temperature, two exhibited no change and one a slight fall ($p < 0.000001$ Wilcoxan Sign Rank Test). The mean thermic response relative to baseline, 0.55 ± 0.07, was also highly significant ($p < 0.000001$, $t = 7.84$, $df = 45$).

Discussion

Interpretations of Data

The injection of normal saline produced increases in core temperature in 13 of 15 animals ($p = 0.00006$) and significant difference in mean thermic response between phases of 0.47 ± 0.11°C ($p < 0.001$) in Experiment I. Forty-three (43) of 45 animals exhibited a rise in core temperature in Experiment II ($p < 0.000001$), and the sample displayed a mean increase in core temperature of 0.55 ± 0.07°C ($p < 0.000001$). Overall, handling and the injection of saline produced increases in core temperature in 56 of 61 animals, no change in four, and a minimal decrease in one.

These data suggest that routine handling and injection are biologically significant events. Stress can raise a rat's temperature (Blasig et al., 1978; Briese and DeQuijada, 1970; Pechnick et al, 1984; Singer et al, 1986). The increase in core temperature measured in this experiment is most apt to be a nonspecific response to the stress of handling and injection. These data have important implications. Experiments sensitive to the effects of stress should be designed to control for the nonspecific effects of routine handling and injection. This particularly applies to studies using temperature as an endpoint. The use of an ABA or repeated measures design is one means of controlling for this factor. This strategy (in principle) allows for a mathematical cancellation of nonspecific intra-individual effects between phases of a study. This strategy is illustrated in several recent studies in which a thermoregulation strategy was employed (Dilsaver and Davidson, 1985; Dilsaver and Alessi, 1987; Dilsaver et al, 1986, 1987, 1988).

Implications of Data

It is also possible to control the effect of routine handling and injection by using each animal as its own saline control. This strategy involves measuring the thermic response to saline and contrasting this with the thermic response to an agonist. For example, the authors first measured the thermic response to saline (1 ml/kg) and then measured the thermic response to nicotine (Dilsaver and Davidson, 1987). This provides a more accurate estimate of nicotine's capacity to produce hypothermia than does the administration of nicotine alone. Indeed, a drug producing no change in core temperature could be exerting a significant hypothermic effect given that the usual response to handling and injection is a significant hyperthermic response.

Advantages of Telemetry

Other investigators have also reported that various stressors raise the body temperature of animals. This study presents two advantages. First, we used telemetry to measure core temperature. A rectal probe, for example, requires immediate handling or restraint. Restraint stress alters core temperature. Secondly, we measured temperature at multiple points over a long period of time. Thus, placebo (saline) injections have a persistent effect on core temperature.

Conclusions

Simple manipulations associated with studies requiring the use of the common laboratory rat can be stressful. The effect of merely measuring core temperature telemetrically had a modest effect. This study demonstrates that merely handling the rat and injecting saline can produce a robust hyperthermic response. Experimental interventions producing dramatic changes in the thermic responsiveness of the rat (please see Dilsaver and Alessi, 1988, for examples) render the effects of handling and injecting negligible. However, the results of studies in which the magnitude of the effect of an agent or of a manipulation altering temperature could be shaped by these minor stresses.

Singer et al (1986) reported that the stress of placing a rat in an open field results in a hyperthermic response which is blocked by salicylate. It is conceivable that the hyperthermic response produced by other stressors such as manual manipulation and the injection of

saline can also be blocked by salicylate. Whether this is the case is now being studied in our laboratory.

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