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Behavioral Thermoregulation in the Study of Drugs Affecting Body Temperature

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COX, B., M. D. GREEN AND P. LOMAX. *Behavioral thermoregulation in the study of drugs affecting body temperature.* PHARMAC. BIOCHEM. BEHAV. 3(6) 1051–1054, 1975. – A method of measuring thermoregulatory behavior in the rat has been developed, which allows analysis of the mechanism of action of drugs which modify body temperature. The test measures the amount of time a rat will remain exposed to an infrared heat source before making an escape and this evidence has been used to divide drugs into those which act on the central thermostats and those which act on effector systems. A peripherally acting hypothermic drug (N-methyldiphenhydramine) increased the time of exposure to the heat lamp. Tri-iodothyronine increased body temperature and decreased exposure to the heat lamp. Intraventricular oxotremorine caused hypothermia but a decreased exposure to heat suggesting it acts to lower the set-point of the central thermostats. Both effects were blocked by atropine. The possibility that central cholinergic mechanisms in the hypothalamus have a function in determining the setting of the central thermostats is discussed.

Thermoregulatory behavior Rectal temperature Drug effects Oxotremorine Intraventricular injections Cholinergic mechanisms

ONE of the major problems in investigating the mechanism by which a drug modifies body temperature is the complexity of the system under study. If one accepts the set-point concept in physiological temperature regulation [8] then there are a number of ways in which drugs may act to alter body temperature. Drugs which lower body temperature may do so by lowering the set-point. In this situation effector systems will be modified so that a net heat loss occurs. Alternatively a drug may cause a lowering of body temperature by a direct action on the effector systems themselves without modifying set-point. The converse is true for drugs which increase body temperature. They may act either to raise the set-point or to activate directly heat gain effectors.

A number of workers have studied drug effects on animal thermoregulatory behavior in an attempt to elucidate the mechanism of action. In some cases the animal, usually a rat, is trained to press a bar either to obtain more heat [14] or to escape from heat [3]. Thus, if the body temperature rises but the animal continues to press for heat, then this suggests the drug is raising the set-point. In a similar situation in the escape test the animal would delay pressing the escape-bar.

Another test that has been used in thermoregulatory studies [15] involves placing a rat in a graded temperature chamber and noting the position the animal adopts within the gradient.

This paper describes a simple, but effective, method for measuring thermoregulatory behavior in the rat, which avoids some of the disadvantages of the earlier tests. This method has been used to analyse drug action on thermoregulatory mechanisms and to determine the way in which oxotremorine, a directly acting muscarinic agonist [11], produces hypothermia in the rat.

METHOD

Animals

All experiments were carried out on female Sprague Dawley rats with a weight range of 180 to 250 g.

Apparatus

Thermoregulatory behavior was assessed by measuring the amount of time the rat remained exposed to a source of radiant heat. The apparatus consisted of a rectangular chamber 80 × 8 cm with 35 cm high walls. A 250 W infrared heat lamp was placed 65 cm above the floor of the chamber and could be set in any position over the length of the chamber. Two 15 W cold fluorescent strip lights were also placed above the chamber to provide an even intensity of illumination. The behavioral experiments were carried out at an ambient temperature of 22–23°C.

Procedure

The experimental protocol was as follows: Rats were acclimatised to the chamber during two 30 min periods on the day prior to testing. After acclimatisation rats usually settled down at one or the other end of the chamber within 5 min of being introduced into the test situation. The infrared lamp was then positioned above the rat and switched on. The time that the rat remained under the lamp was noted (T_1). Escape from the heat source was taken as

the time at which the whole of the rat, including tail, moved outside the perimeter of the visible infrared beam. Immediately the rat escaped the lamp was switched off for an intertest period equal to the time of exposure to the lamp, but not less than 6 min. The test was then repeated (T_2). A third and final test T_3 was obtained using the same protocol. This provided 3 consecutive measurements of the rat's sensitivity to the heat load.

Results from the behavioral test were presented in two ways. (a) The mean duration of stay under the heat lamp was calculated for each of the three test sessions (T_1, T_2 and T_3). (b) For each individual rat the mean exposure to the lamp was obtained for the whole of the test ($T_1 + T_2 + T_3/3$) and this value then used to calculate an average time for the whole group (grand mean) as shown in Table 1.

Rectal temperatures were measured using a thermistor probe inserted to a depth of 6 cm. When a time course of drug effect was measured the rats were held in restraining cages for the duration of the experiment, but when temperature measurements were made during the behavioral experiment rats were only restrained for a sufficient time to allow the temperature to be recorded (usually less than 1 min).

Drugs for injection were prepared in sterile NaCl (0.9 percent) solution and injected either IP (dose volume 1 ml/kg) or intracerebroventricularly (dose volume 1 μl). All concentrations were expressed in terms of the free base.

Since we were using N-methyldiphenhydramine iodide in a parallel study in our laboratories the compound was used as a peripherally acting hypothermic agent. The N-methyl derivative was prepared from diphenhydramine HCl by precipitating the base with NaOH, shaking and extracting with ether and reacting with CH_3I at 0°C for 3 hr. After recrystallisation a 40 percent yield of pure white crystals (M. P. 188–189°C) was obtained which gave a single spot on TLC.

Appropriate saline controls were used in all the studies. For intracerebroventricular (i.vent.) injections cannula guides were implanted under pentobarbital anaesthesia (45 mg/kg IP) and at least 7 days allowed to elapse before experimentation. Histological verification of the injection site was routinely carried out.

RESULTS

Reproducibility of the Test

Table 1 shows the mean duration of stay under the heat lamp for 2 groups of control rats (A and B) for each of the 3 individual test sessions T_1, T_2 and T_3. In both cases the longest duration of stay under the lamp occurred in the first test session T_1. There was a consistent pattern of behavior from the two groups which were not significantly different from each other in any of the sessions.

Table 1 also shows the mean duration of exposure to the lamp for control groups computed from the average for each of the 3 individual tests. There was no significant difference between the 2 control groups with respect to time of exposure to the heat lamp.

Rectal temperature measurements for each group are also shown in Table 1. During a 90 min pretest period the mean rectal temperature of the two control groups did not vary by more than 0.1°C. Immediately after removal from the behavioral experiment their rectal temperatures had increased by approximately 1°C.

Drug Responses

The effect of injection of oxotremorine (4 μg i. vent.) on the rectal temperature of restrained rats is shown in Fig. 1. This dose produced a maximum fall in rectal temperature of 3.4 °C which occurred during the first 45 min of the test. The average times that the behavioral studies with oxotremorine were carried out is also shown on this figure.

When oxotremorine (4 μg i.vent.) was injected 10 min prior to behavioral testing there was a significant decrease in the mean exposure time of rats to the heat lamp when compared with saline pretreated controls (Table 2). That oxotremorine had produced a fall in body temperature during the period of the behavioral test was shown by the rectal temperature measurement which was 1.0°C lower than saline controls. Atropine (4 mg/kg IP) produced no significant change in either the rectal temperature or the mean exposure time of rats to the radiant heat source when injected 1 hr before the test. After atropine pretreatment the effect of oxotremorine was antagonised. Both the mean rectal temperature and the mean exposure to the heat source were not significantly different from controls.

The effect of a 1 hr pretreatment with N-methyldiphenhydramine is also shown in Table 2. This drug caused both a significant decrease in rectal temperature and a significant increase in the duration of exposure to the heat lamp. Tri-iodothyronine (5 μg IP) caused a significant increase in mean rectal temperature of rats which was accompanied by a significant decrease in the time of exposure to the heat lamp.

TABLE 1

COMPARISON OF TIME OF EXPOSURE TO HEAT LAMP AND RECTAL TEMPERATURES OF 2 GROUPS OF CONTROL RATS

Group	No. of Animals	Exposure to Heat Lamp (min ± SEM)				Rectal Temperature (°C ± SEM)	
		T_1	T_2	T_3	Grand Mean	a	b
A	6	9.7 ± 1.2	4.2 ± 1.1	3.6 ± 0.9	5.9 ± 0.7	37.7 ± 0.2	38.6 ± 0.3
B	6	8.0 ± 1.3	3.7 ± 0.4	5.7 ± 0.9	5.8 ± 0.6	37.7 ± 0.1	38.7 ± 0.1

T_1, T_2, T_3 = individual consecutive test sessions a = immediately before test b = immediately after test

TABLE 2

EFFECT OF DRUGS ON THE TIME OF EXPOSURE TO THE HEAT LAMP AND RECTAL TEMPERATURE OF RATS

Drug (dose)	No. of Animals	Mean Exposure to Heat Lamp (min ± SEM)	Mean Rectal* Temperature (°C ± SEM)
Saline (1 ml/kg IP & 1 μl i.vent)	6	5.8 ± 0.96	37.7 ± 0.17
Oxotremorine (4 μg i.vent)	8	2.4 ± 0.18†	36.7 ± 0.04†
Atropine (4 mg/kg IP)	6	5.3 ± 0.79	37.6 ± 0.28
Oxotremorine (4 μg i.vent) + Atropine (4 mg/kg IP)	6	5.8 ± 0.48‡	37.9 ± 0.38‡
N-methyldiphenhydramine (25 mg/kg IP)	4	9.8 ± 1.23†	35.8 ± 0.12†
Tri-iodothyronine (5 μg IP)	6	3.0 ± 0.50†	38.5 ± 0.33

*Measured immediately before commencing behavioral test
†Significantly different from saline controls, ‡significantly different from oxotremorine ($p<0.05$ Mann Whitney U test)

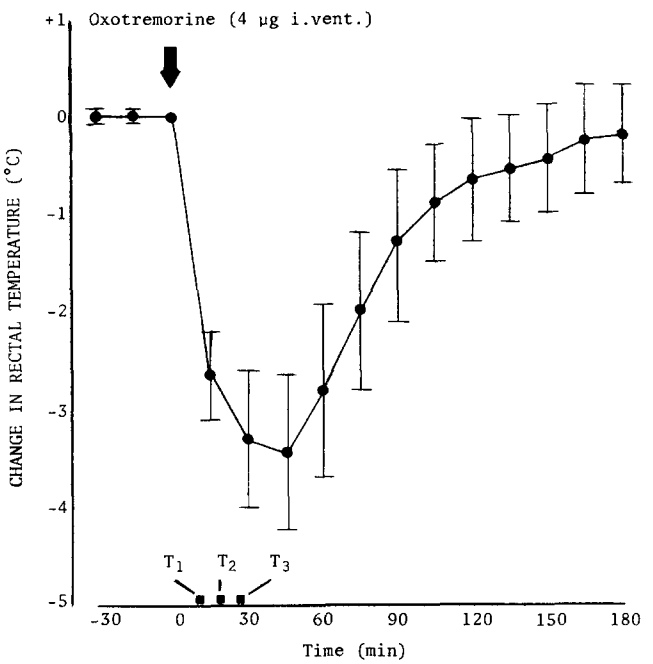

FIG. 1. Time course of oxotremorine (4 μg i. vent.) on rat rectal temperature. Each point is the mean change ± SEM from 6 observations. T_1, T_2 and T_3 represent points on the time scale where behavioral tests were performed.

DISCUSSION

That the behavioral test reported in this paper is capable of giving consistent results in control rats is well demonstrated by the comparisons made in Table 1. However it is necessary to establish that the response measured is indicative of thermoregulatory events and not other factors.

Observation of the rats during the test showed that they did not respond to stimuli such as noise, vibrations or the switching on of the heat lamp. That the escape behavior was related to the heat from the lamp, and not the light, was suggested by the relatively long duration of stay under the heat source. In contrast when a white light source was used some rats moved away from the light within a few seconds, a finding particularly evident in rats whose pupils were dilated with atropine. Thus the red light heat source would seem to be preferable to a white light as used by some workers [15]. The height of the lamp above the test chamber was such that it did not increase the surface temperature of objects inside the chamber to more than 38°C. Therefore it is unlikely that the heat lamp was producing a nociceptive stimulus.

There were a number of positive indications that the behavior was linked to a thermoregulatory mechanism. Soon after exposure to the lamp rats adopted the sprawled languid posture regarded as typical for a heat stressed animal [4]. As exposure continued the rats displayed grooming or saliva spreading activity, which is regarded as a vestigial heat loss response [7]. Finally rats escaped from the heat source by moving to the other end of the chamber and at this time obvious vasodilation of tail blood vessels

could be seen. All the above observations support the contention that the measured response is a function of thermoregulatory behavior.

This test has the advantage over bar pressing experiments mentioned in the introduction in that it only requires of the animal an ambulatory response, there is no need to pretrain the rat and the rat uses innate, rather than conditioned, thermoregulatory behavior. The test relies in part on a reproducible behavior trait in the rat, which after acclimatisation, exhibits an apparent preference for a selected position within the test chamber. This positional preference appeared stronger than minor changes in the environmental conditions and thus made the gradual thermal gradient type of experiment [15] impractical. The rat's choice of position within the gradient appeared to be determined by its positional preference and to be independent of small differences in the temperature conditions.

In control rats T_1 was always longer than T_2 and T_3. This behavior did not seem to be related to the learning of an avoidance response, as rats showed the same pattern when tested on consecutive days. Rather, it seemed related to the thermoregulatory events. The rats allowed their rectal temperatures to increase by approximately 1°C during the test and presumably the largest part of this heating up process took place during T_1. By using the overall mean for the 3 consecutive test sessions a reproducible measure of thermoregulatory behavior was obtained.

Perhaps the best evidence that the behavioral response was linked to thermoregulatory events came from the drug studies. N-methyldiphenhydramine, a quaternary derivative of the antihistamine diphenhydramine, produced a fall in rectal temperature. Like other quaternary derivatives [5, 9, 10] this drug will not penetrate the central nervous system. Therefore the lowering of rectal temperature must be a peripheral effect, with a heat loss occurring in spite of a normal set-point. It would be predicted that this drug should increase the time of exposure to the heat lamp, as the animal adjusts its behavior to correct the fall in core temperature. This indeed was shown to be the case. A similar argument can be applied to the results with tri-iodothyronine. This drug raised rectal temperature as a consequence of increased metabolic activity [6] and again the set-point should be unchanged. Rats spent less time under the heat lamp after tri-iodothyronine pretreatment as would be predicted from its mechanism of action.

The role of cholinergic systems in thermoregulation has been the subject of some controversy. Some workers [1, 2, 13] reported an increase in body temperature after central injection of cholinergic drugs whilst others [11] reported a decrease. Crawshaw [4] suggested that the response obtained depended on the degree of restraint in the animal and that motor activity could mask a hypothermic response. In our experiments there was a fall in rectal temperature after intraventricular injection of the cholinomimetic agent oxotremorine even though the rats were unrestrained. Furthermore these rats also spent less time under the heat lamp. This result, a lowering of rectal temperature yet less exposure to heat, suggests that oxotremorine is acting to produce a downward setting of the central thermostats. Blockade of oxotremorine's effect with atropine indicate that the response is mediated by muscarinic receptors. A previous report [4] noted that acetylcholine injection into the preoptic area of the anterior hypothalamus caused rats to adopt a posture similar to that in heat stressed animals, and that this occurred immediately following the injection. This observation is consistent with a lowered set point in the presence of a normal body temperature.

Thus, taken as a whole, these results support the idea of a physiological role for cholinergic systems in thermoregulation [12] with a cholinergic link in the anterior hypothalamus being involved in the setting of a central thermostat.

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