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Hypothermia Induced by Δ^9-Tetrahydrocannabinol in Rats With Electrolytic Lesions of Preoptic Region[1]

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SCHMELING, W. T. AND M. J. HOSKO. *Hypothermia induced by Δ^9-tetrahydrocannabinol in rats with electrolytic lesions of the preoptic region.* PHARMAC. BIOCHEM. BEHAV. 5(1) 79–83, 1976. – The preoptic region (POR) is a primary central site for thermoregulation. Bilateral lesions of POR disrupt thermoregulation, and in rats, produce a characteristic syndrome including hyperthermia. Δ^9-Tetrahydrocannabinol (Δ^9-THC), a potent hypothermic agent, appears to mediate this effect via some central mechanism. The studies reported here suggest that Δ^9-THC induces hypothermia at a site other than POR. Male Sprague-Dawley rats were divided into 2 groups, one with subsequently confirmed bilateral POR lesions and a sham operated group. The lesioned animals developed hyperthermia (+2.1° ± 0.1°C, $p<0.01$) within 2 hr after surgery when compared to the sham operated controls. Δ^9-THC was administered intraperitoneally (5 and 10 mg/kg). Rectal temperature was recorded at 30 min intervals for 2.5 hr. Both lesioned and nonlesioned rats exhibited hypothermia within 30 min of Δ^9-THC administration. The hypothermic response to 5 and 10 mg/kg Δ^9-THC in the lesioned animals was significantly greater ($p<0.05$) and showed a trend toward longer duration than the hypothermia induced in the sham operated controls. These data demonstrate that Δ^9-THC is able to induce a hypothermic response in rats whose body temperatures were elevated by POR ablation. Although Δ^9-THC does not appear to act primarily at POR to induce hypothermia, it is evident that an intact POR plays a role in modifying the duration and magnitude of Δ^9-THC induced hypothermia.

Hypothermia Δ^9-Tetrahydrocannabinol (THC) Lesions Preoptic Region (POR)

Δ^9-TETRAHYDROCANNABINOL (Δ^9-THC), the major pharmacologically active constituent of marihuana, has been shown to be a potent hypothermic agent [1,15]. The magnitude of induced hypothermia is dependent on dose, route of administration, and prevailing ambient temperature [1, 5, 12, 15, 16, 41]. The site of action of Δ^9-THC for induction of hypothermia has been postulated to be in the central nervous system [1, 8, 11, 12]. The preoptic region (POR) of the anterior hypothalamus has been demonstrated to be a major central site of thermoregulation [3, 4, 18, 27]. Bilateral lesions of POR disrupt normal thermoregulation [38] and in rats produce a syndrome which is characterized by hyperactivity and hyperthermia. The purpose of the studies reported here was to determine whether systemically administered Δ^9-THC would induce hypothermia in rats with acute bilateral electrolytic lesions of POR.

METHOD

Surgical Procedure

Male Sprague-Dawley rats (150–350 g) were used in these studies. Ambient temperature was maintained at 24°C ± 1°C. Humidity was not regulated. Rectal temperature was determined by means of a thermister probe inserted 5 cm into the rectum. Animals were anesthetized with fluothane and the head was stereotaxically oriented by means of a headclamp which does not require ear bar placement [23], thereby eliminating the possibility of corda-tympani damage which could alter normal thermoregulatory mechanisms [39]. Using bregma as the reference point and moving to coordinates 2.0 mm anterior and 1.0 mm lateral [34], the skull was burred open bilaterally. Epoxy insulated, 23 ga., stainless steel electrodes were stereotaxically lowered to a depth 8.0 mm below the surface of the cortex. POR was lesioned bilaterally by passing a 4.0 mA DC cathodal current for a duration of 15 sec between each electrode, and a 20 ga. needle under the skin of the neck. In sham operated control rats, electrodes were lowered 4.0 mm below the cortex; no current was passed. The skull defect was covered with gelfoam to control minor bleeding, and the incision sutured. The righting reflex could be elicited within 2–5 min, and all animals were moving about freely within 15 min after fluothan administration was terminated.

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Experimental Protocol

Two hr after completing surgery (lesioning of POR or sham operations), rectal temperature was taken and the animals were given intraperitoneal Δ^9-THC in a 5 or 10 mg/kg dose. Control animals received vehicle intraperitoneally in a volume equal to that used to administer the 10 mg/kg Δ^9-THC dose. Rectal temperature was recorded at 30 min intervals for a period of at least 2.5 hr. A separate group of 4 rats with POR lesions were kept for 24 hr postsurgery. These animals received 5 mg/kg Δ^9-THC, intravenously at that time. Of the 21 lesioned animals, 6 were used as vehicle controls, 7 received 5 mg/kg Δ^9-THC, and 8 received 10 mg/kg Δ^9-THC. Eight of the 22 sham lesioned rats were used as vehicle controls, 6 received 5 mg/kg Δ^9-THC, and 8 received 10 mg/kg Δ^9-THC.

Δ^9-THC Preparation

Δ^9-THC was obtained from NIMH in 5 ml sealed vials containing 1 g of Δ^9-THC in 95% ethanol. The Δ^9-THC for every experiment was freshly prepared from stock solution. One hundred microliters of the 20% stock were rubbed up in 100 μl of Tween 80. The suspension was diluted to 4 ml with sterile water. The vehicle control was prepared in the same manner, with 95% ethanol being substituted for the Δ^9-THC stock solution.

Verification of Lesion Sites

At the end of each set of experiments the rats were sacrificed with an overdose of pentobarbital. The brains of lesioned animals were removed and placed in 10% Formalin-saline containing 0.5% Na ferrocyanide to fix the tissue and to develop Prussian blue marks at the lesion sites. After a few days in the fixing solution, the brains were blocked, frozen sectioned, and lesion sites were localized using the technique described by Hosko [24].

Figure 1 shows the distribution of lesions made in the course of this study. Markers indicate the relative center of each lesion. From this point, the lesion extended approximately 1.5–1.75 mm in dorsoventral direction with a diameter of approximately 1.5 mm. Due to the short time period between lesion and sacrifice, minimal phagocytosis was evident in the areas of lesion. In all cases the lesion largely obliterated POR with minimal encroachment on adjacent structures.

Statistical Analysis

Statistical analysis of data was by Student's t-test for paired or group comparisons. Significance was set at $p<0.05$.

RESULTS

The characteristic hyperthermia induced in rats by POR lesions is shown in Fig. 2. Mean deep rectal temperature (T_{Re}) recorded 2 hr postoperatively, are significantly ($p<0.01$) elevated over both the preoperative T_{Re} and the T_{Re} of sham operated rats. The hyperthermia began almost immediately after surgery, rose quickly in the first 15 min and was generally at or near peak elevation within 90 min. The 2 hr postoperative T_{Re} of sham operated rats was lower than the preoperative T_{Re} for this group, a common sequel to anesthesia and surgery.

The hyperthermia we observed in our lesioned animals correlates well with reports by other investigations [2, 20,

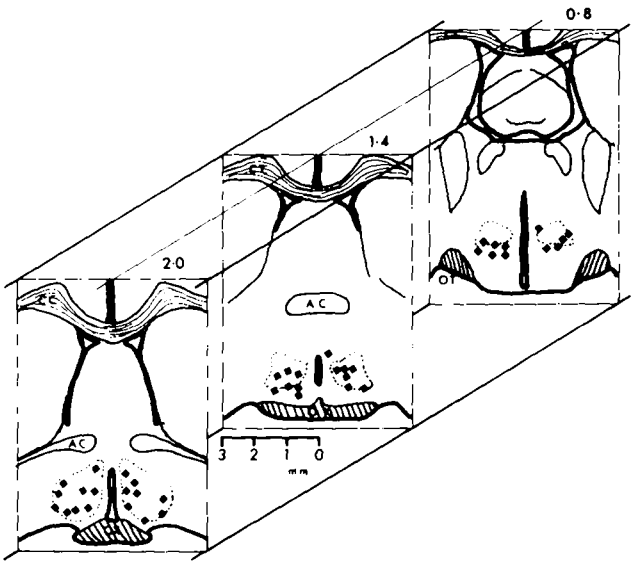

FIG. 1. Distribution of electrolytic lesions in anterior hypothalamus. The markers indicate the center of each lesion site. Coordinates are from the Atlas of Pellegrino and Cushman, 1967.

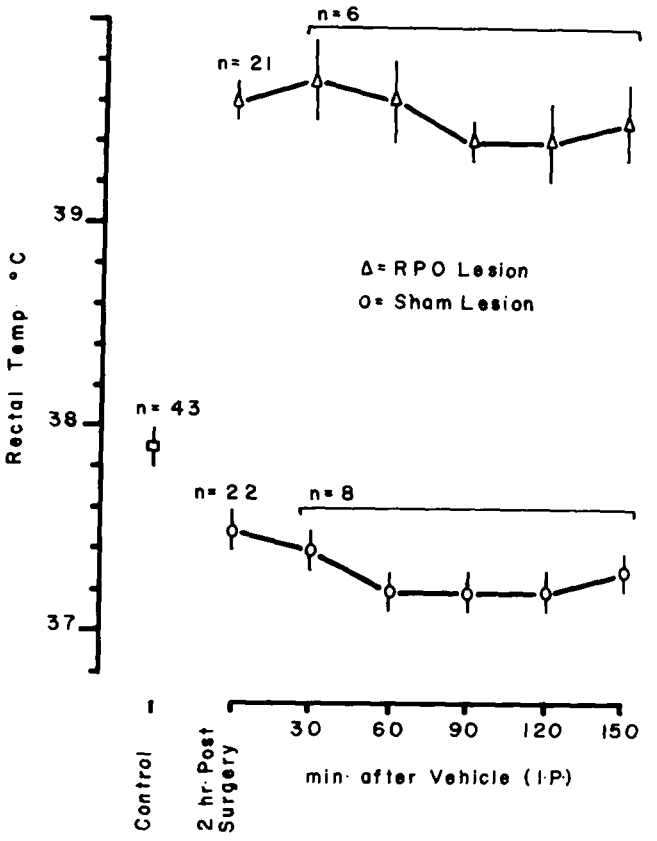

FIG. 2. Effect of anterior hypothalamic lesions, sham lesions and vehicle on deep rectal temperature in the rat.

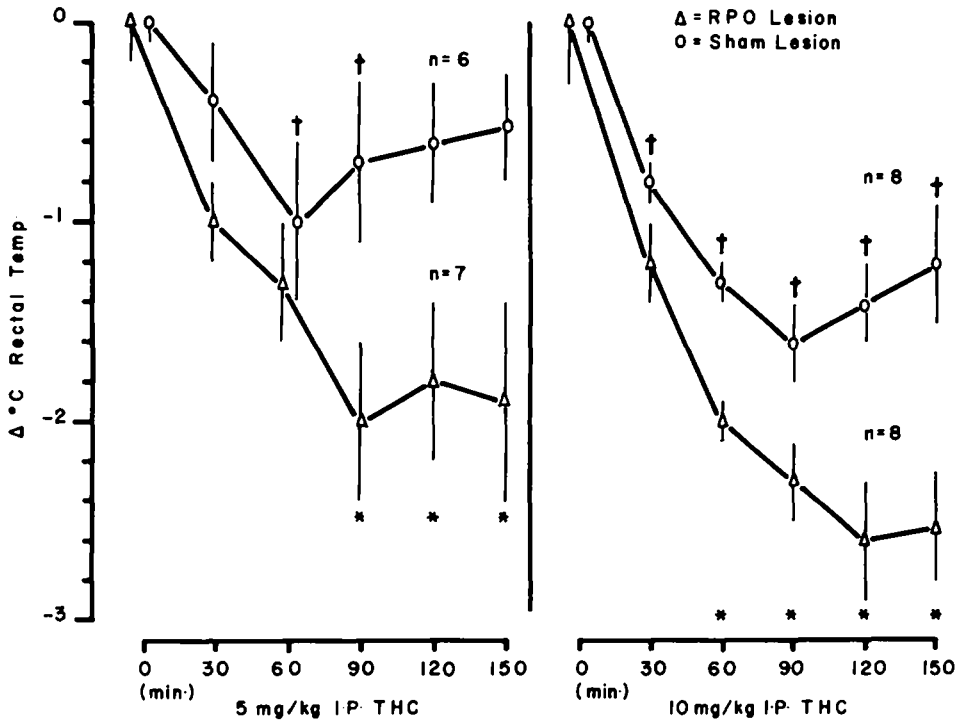

FIG. 3. Effect of intraperitoneal Δ^9-THC in doses of 5 and 10 mg/kg on rectal temperature of anterior hypothalamic lesioned and sham lesioned rats. Δ^9-THC was administered 2 hr after surgery. At this time, the rectal temperature of the lesioned rats was 39.6°C and that of the sham operated rats 37.5°C. Dagger, significantly different ($p<0.05$) from sham lesioned vehicle controls (see Fig. 2). Asterisk, significantly greater ($p<0.05$) hypothermia than that produced by THC in sham lesioned rats.

25, 30, 35]. Hyperactivity and hypersensitivity were evident in every lesioned animal. After recovery from anesthesia, the rats were in continuous motion and an exaggerated startle response could be elicited by small movements or sounds. A few of the lesioned animals exhibited varying degrees of hyperphagia (4 animals) and/or polydipsia (3 animals) – 3 of these animals exhibited both increased eating and drinking. The fourth did not appear to increase water intake.

Seven rats died within 1 hr after completion of surgery. Following initial recovery, the rats slowly became comatose, frequency and depth of respiration decreased, a pink froth exuded from the mouth and nose and death resulted. This syndrome of pulmonary edema which is associated with midline anterior hypothalamic lesions has been previously described [30,33]. None of the rats utilized in the study reported here had any symptoms of pulmonary edema.

Within 15 minutes of Δ^9-THC administration (5 and 10 mg/kg dose), the spontaneous activity of the rats was markedly depressed. Within 30 min of administration, the animals exhibited no spontaneous motor activity. However, an exaggerated startle response could be elicited by tapping the cage.

Δ^9-THC produced depression of T_{Re} in both lesioned and sham operated rats (Fig. 3). The magnitude and time course of T_{Re} depression by Δ^9-THC in the sham operated animals are similar to those reported by other investigators using intact rats [5, 29, 37].

Depression of T_{Re} was significantly greater in lesioned animals at dose levels of 5 and 10 mg/kg Δ^9-THC. It is evident from the curves in Fig. 3 that the peak hypothermic response in sham operated animals occurred at 60–90 min. The T_{Re} in these animals was shifting toward control levels by 150 min. In POR lesioned animals, T_{Re} depression induced by Δ^9-THC continued for 90–120 min and then tended to plateau at the level of maximal depression for at least 30 min. These data indicate that the hypothermic effect of Δ^9-THC was augmented, and the duration of effect was prolonged in the lesioned, as compared to the sham operated rats. Administration of vehicle did not significantly alter postsurgery T_{Re} in either lesioned of sham operated animals (Fig. 2).

DISCUSSION

Various investigations have found marked differences in the magnitude of response when Δ^9-THC was administered by different routes [12,37]. Ho *et al.* [22] has demonstrated that after intraperitoneal administration of tritiated Δ^9-THC to rats, a major portion of the drug remained in the abdominal cavity. Liu [26] however disagrees with the physiological importance of these findings. Since a number of investigators have utilized intraperitoneal administration of Δ^9-THC to evoke thermoregulatory responses in the rat [29,37], this route was chosen as the major route of administration for the studies reported here.

Since POR is involved in the regulation of food and water intake as well as in thermoregulation we felt that decrements of homeostatis other than those involving temperature regulation could occur if the postlesion experi-

mental period was extended over a long period of time. As a consequence, we administered Δ^9-THC 2 hr postsurgery. To verify that the hyperthermic response was not transitory under the conditions of our series, a small group of animals was followed for 24 hr postsurgery. In this group, the hyperthermia remained essentially undiminished (Fig. 4), demonstrating that the lesions induced a reasonably long term alteration in thermoregulatory patterns. The hypothermic response evoked by administration of 5 mg/kg IV Δ^9-THC to these animals further supports our finding that the lesion did not block the hypothermic effect of Δ^9-THC.

The role of the anterior hypothalamus in thermoregulation is well documented. Temperature sensitive neurons in POR have been found in virtually all vertebrates [6, 14, 40]. Evidence from lesion studies [17,38], from regional heating and cooling experiments [14,19], from intrahypothalamic and intraventricular injection of a variety of pharmacological agents [9, 28, 31] have documented the functional role of the anterior hypothalamus in the maintenance of normal body temperature. The hyperthermia which results following POR lesions in the rat appears to involve disruption of thermoregulatory mechanisms concerned with "heat loss" [4]. Although the fine control of temperature regulation is impaired in lesioned animals, a degree of behavioral and physiological thermoregulation remains under the control of thermosensitive areas located in posterior hypothalamus [21], mesencephalon [32], cutaneous areas [3] and spinal cord [7,36].

The hypothermia induced by Δ^9-THC in POR lesioned rats could be explained as follows: release of lower thermoregulatory centers from tonic interaction with POR results in increased sensitivity of the residual thermoregulatory system to stress loading with decreased accuracy of compensatory mechanisms. In these terms, in the intact animal, POR attenuates the magnitude and duration of the hypothermic response induced by Δ^9-THC.

Our findings suggest that the thermoregulatory response to Δ^9-THC does not originate at anterior hypothalamus since Δ^9-THC induced hypothermia is augmented and prolonged in POR lesioned animals. Our data however does indicate that an intact POR plays a significant role in modifying the duration and magnitude of Δ^9-THC induced hypothermia.

Evidence for a central hypothermic action of Δ^9-THC is based on intracerebroventricular injection studies with the drug and its metabolites by various investigators [1, 8, 11, 12] who reported increased potency with administration by this route. Fuxe and Ungerstedt [10] and Glowinski *et al.* [13] have demonstrated that intraventricular action of many drugs is largely restricted to structures lying proximal to the ventricle.

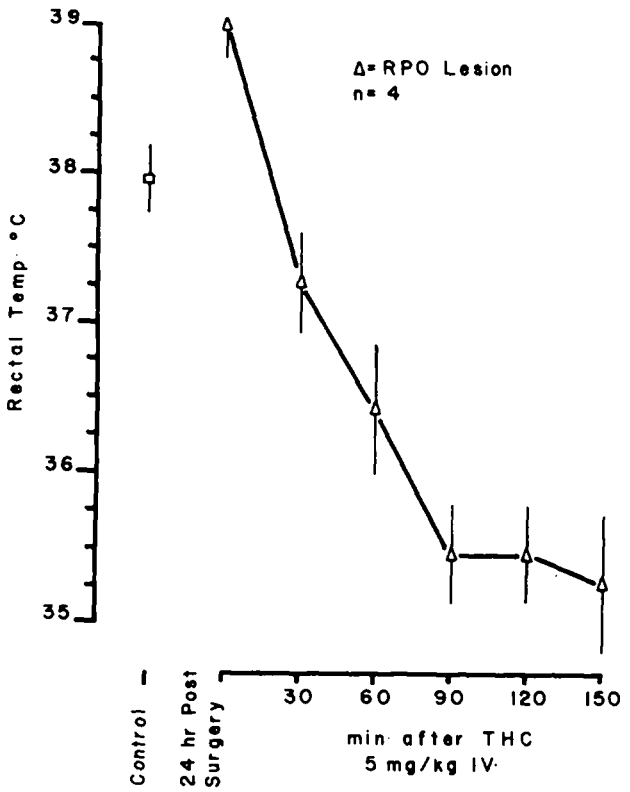

FIG. 4. Effect of intravenous Δ^9-THC (5 mg/kg) on rectal temperature of anterior hypothalamic lesioned rats. Δ^9-THC was administered 24 hr after surgery. Note that significant postlesion hyperthermia persisted for at least 24 hr.

It could be reasonably postulated that a likely site for the central localization of the hypothermic action of Δ^9-THC is at posterior hypothalamic or mesencephalic thermosensitive areas which lie in juxtaposition to the ventricular wall.

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