Colchicine Lesions of Ventromedial Hypothalamus: Effects on Regulatory Thermogenesis in the Rat[1]

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PRESTON, E., J. TRIANDAFILLOU AND N. HAAS. *Colchicine lesions of ventromedial hypothalamus: Effects on regulatory thermogenesis in the rat.* PHARMACOL BIOCHEM BEHAV 32(1) 301–307, 1989.—Experiments were carried out to test whether the ventromedial hypothalamus (VMH) is the site of a pathway that stimulates thermoregulatory heat production in brown adipose tissue (BAT). Adult Sprague-Dawley rats received bilateral 50 nl microinjections of colchicine solution into the VMH (0.1, 0.32, 1.0 or 3.2 µg per side). Beginning a day later, hyperphagia developed consistently with 0.32 µg colchicine; and with higher doses there appeared the additional effect that for several days rats developed hypothermia when placed temporarily at 6°C. The degree of hypothermia was limited by activation of nonshivering thermogenesis (NST) in BAT, as evidenced by increased shivering after propranolol injection to block NST, and by increased GDP binding measured in IBAT mitochondria after cold exposure. The findings suggest that chemical lesioning to induce the VMH hyperphagia syndrome does not produce an obligatory impairment of thermoregulation against cold unless the dose of neurotoxin and lesion area extends beyond that which underlies the overeating response. Furthermore, when tolerance to cold is thus compromised, the effect is not readily explained in terms of simply disconnecting a proposed stimulatory pathway from the VMH to BAT.

Ventromedial hypothalamus   Brown adipose tissue   Thermogenesis   Colchicine   VMH lesions
Thermoregulation

STUDIES carried out primarily in the rat show that the heat production which occurs in brown adipose tissue during activation of its sympathetic nerves may serve two functions: defence of body temperature against cold (5) and dissipation of excess dietary calories during overnutrition (6). The ventromedial area of the hypothalamus (VMH) apparently plays a role in mediating the sympathetic outflow to BAT (2, 7–9, 11); but the supporting evidence has not clearly resolved whether the region mediates thermoregulatory or dietary adjustments of BAT thermogenesis, or both functions. For example, a role of the VMH in the thermoregulatory function of BAT is suggested by the finding that rises in IBAT temperature evoked by anterior hypothalamic cooling were attenuated by lidocaine microinjected into the VMH (9). Also, electrolytic VMH lesioning reduced the firing rate in sympathetic fibres innervating IBAT, and the normal increase in such firing evoked by exposure of the rat to cold (11). On the other hand, measurements of GDP binding to BAT mitochondria, the level of binding being a putative index of thermogenic activity, led to the conclusion that VMH lesions only disrupt the mechanism controlling diet-induced thermogenesis in BAT while that for thermoregulation remains functional (2,7).

Colchicine is a drug which binds tubulin and blocks axo-plasmic transport in neurons (3). Its microinjection into the VMH evokes the syndrome of hyperphagia and obesity commonly seen with other types of VMH lesions (1). In the present study we undertook to explore how such chemical lesions in the VMH might affect the thermogenic functions of BAT.

METHOD

Male Sprague-Dawley rats, 280–320 g, were housed singly at 24±0.5°C in hanging wire cages, with Purina rat chow and water freely available, and with room lighting on between 0600 and 1800 hr.

To carry out bilateral chemical or sham lesioning of the VMH, each rat was anesthetized with sodium pentobarbital (60 mg/kg IP) and its head mounted in a Kopf stereotaxic apparatus. The atlas coordinates (12) for microinjection were as follows: AP=0, Depth=9.5 mm below dura; Lateral=0.7 mm (except lateral=1.0 mm in specified cases). Through a burr hole in the skull, a drug-loaded injection cannula was lowered to the VMH site. The cannula consisted of a 33-ga hypodermic tube lying within, and tip extending 1.5 mm beyond, an outer 26-ga supporting tube. A 0.5-µl capacity Hamilton syringe was used to infuse 50 nl of isotonic injectate over 15 sec. This contained 0, 0.1, 0.32, 1.0 or 3.2 µg
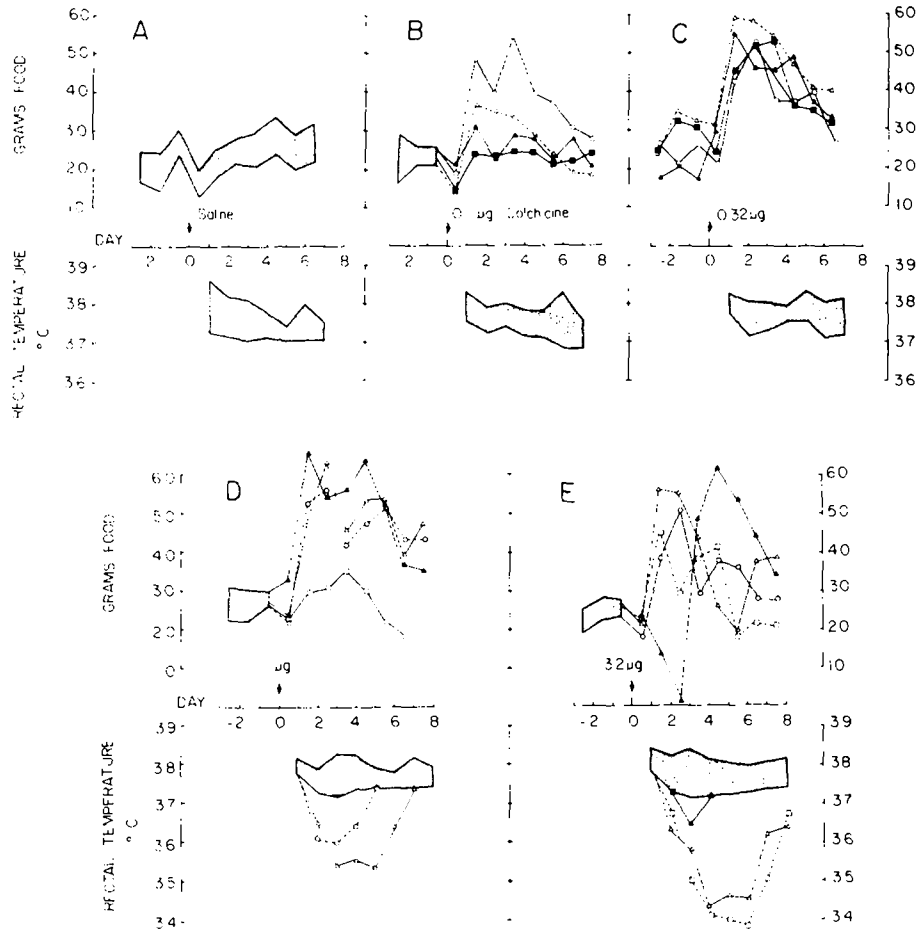
FIG. 1. Effect of intrahypothalamic microinjection of colchicine on food intake and body temperature response to cold exposure. Each panel summarizes data from 4 rats which received bilateral microinjection into the VMH area of saline (panel A) or colchicine solution (0.1–3.2 μg per side, panels B–E) on day 0. Upper half of panels: each broken line plot shows food intake for individual rats except when intakes were similar (all values enclosed within the shaded regions). Lower half of panels: rectal temperature was measured immediately before and after daily exposure of the rat to cold for 1.5 hr, beginning on day 1. Shaded regions enclose all these measurements except in 5 animals in which temperatures were considered below normal after cold exposure; these postexposure values are plotted separately (D,E).

colchicine (Sigma Chemical Co.) dissolved in NaCl solution. The injection was repeated on the opposite side of the hypothalamus and then the skin wound was closed and the rat allowed to recover overnight before experimental use. When tests were completed, histological assessment of injection sites was carried out. Each rat was anesthetized, the brain vasculature perfused with formalin, and coronal brain slices (20 μm) were taken through the hypothalamic region using a microtome/cryostat. These were mounted on glass slides, stained with cresyl violet, and examined by light microscopy. Injection sites were located by examination of successive sections to follow the track of injury left by the injection cannula to its termination, as revealed by the more densely staining granular material present along the course of cannula penetration.

In one group of rats, denervation of IBAT was carried out under pentobarbital anesthesia. This involved making a transverse skin incision just anterior to the IBAT, separating the anterior portion of the IBAT from the muscles of the

scapulae, gently raising the tissue to expose the five intercostal nerve bundles entering the pads on each side, and severing the bundles with fine scissors. The incision was closed with silk suture and, after recovery from anesthesia, the rat was returned to its cage.

During the foregoing surgical procedures and recovery from anesthesia, rectal temperature was continuously monitored using a YSI (Yellow Springs Instr. Co.) type 402 thermistor, inserted to a depth of 7–8 cm, and telethermometer. Temperature was maintained between 37–38°C using a voltage-regulated electrical heating pad. For single measurements of temperature during experiments, the rat was placed within a standard plastic housing box (lwh: 43×21×15 cm), with wire top removed and containing animal bedding, and the thermistor was inserted while the rat was gently restrained by holding the base of its tail.

In a first series of experiments, the tolerance of VMH-lesioned rats to cold exposure was tested each morning on a daily basis. The rat was removed from its housing, placed in

the plastic rat box, and rectal temperature was taken. The wire top was put in position and the animal was then placed for a 1.5-hr period in a room controlled at 6±0.5°C. At the end of this period the rat's temperature was measured again, and the animal then removed from the cold and returned to its normal housing.

In a second series of experiments, lesioned rats did not undergo exposure to cold until 3 days had elapsed after microinjection. On the third day, the rat was removed from its cage, and lightly anesthetized with inhalation of 2% halothane. Two stainless steel needle electrodes (Grass Instrument Co.) were inserted beneath the skin of each flank and held in place with a single 4-0 silk suture. The recording leads along with that of a thermistor probe in the rectum and a ground contact (paste electrode) were taped securely to the base of the rat's tail. The rat was allowed to awaken and then given a 40-min recovery period before undergoing a 1-hour period of cold exposure. Temperature and electromyogram signals were recorded using a Beckman R611 polygraph.

In a third series of experiments, changes in the thermogenic state of IBAT were assessed biochemically 4 days after colchicine or sham lesioning, or denervation of IBAT, by measuring specific binding of GDP to isolated BAT mitochondria. At 1600 hr on day 3 some rats were placed in a 6°C room and remained there with food and water for 16 hr overnight while others remained at 24°C. Between 0800–0900 hr on day 4, rats were removed individually from their environmental room, rectal temperature was measured and the animal decapitated. The IBAT pads were rapidly excised and immediately chilled in ice-cold medium containing 0.25 M sucrose, 0.2 mM EDTA and 1 mM HEPES at pH 7.2 (15). The tissue was then placed on a moist, chilled surface while dissection was carried out to remove adhering white fat, muscle and connective tissue. After weighing, the IBAT was homogenized and mitochondria were isolated by centrifugation, following procedures described elsewhere (15). The specific binding of ^{3}H-GDP (New England Nuclear NET47, 5 Ci/mmol) to mitochondria was measured by the method of Nicholls (10) as modified by others (4). Total IBAT protein and mitochondria protein were estimated by a modification (13) of the Lowry method.

The data were analyzed statistically by analysis of variance, and if this revealed significant differences between mean values, Duncan's new multiple range test was applied. Such differences are termed significant when $p < 0.05$; for simplicity, indications of p values are limited to $p < 0.05$ or $p < 0.01$.

RESULTS

Figure 1 summarizes daily measurements of food intake before and after microinjection of various colchicine doses or saline into the VMH of 20 rats (n=4 per dose). Beginning the day after treatment, rats that received intrahypothalamic colchicine showed evidence of pronounced hyperphagia. This appeared consistently in the group microinjected with the dose of 0.32 μg (Fig. 1C) in which the amount of chow consumed daily by individual rats exceeded their average 3-day prelesion intake by the following amounts (grams, mean±SEM, on days 2 through 7 respectively): 24.8±4.8, 26.4±1.2, 21.5±3.1, 16.6±4.9, 12.4±2.6 and 7.25±3.0. These increments were significantly greater on days 2, 3, 4 ($p < 0.01$) and 6 ($p < 0.05$) compared to corresponding increments in saline injected rats (Fig. 1A) which were as follows (days 2 through 7, respectively): −0.7±2.0, 2.5±1.3, 1.0±2.2, 6.1±1.5, 2.6±1.4, and 4.3±1.7.

One of 4 rats lesioned with 0.1 μg colchicine (Fig. 1B) and 3 of 4 at 1 μg (Fig. 1D) also exhibited marked increases in food intake. Changes in food intake in rats lesioned with 3.2 μg colchicine (Fig. 1E) appeared to be more variable than those seen at 1 or 0.32 μg colchicine, and one animal exhibited temporary aphagia.

Overeating in colchicine rats was associated with more rapid weight gain, e.g., body weight in the 0.32 μg colchicine rats (Fig. 1C) increased by 43.6±8.7 (SEM) grams over the 7-day period postlesion compared to 17.2±3.1 ($p < 0.05$) for saline controls (Fig. 1A).

Starting the day after treatment, these same rats were exposed daily to 6°C for 1.5 hr. Rectal temperature measurements taken at the beginning and end of cold exposure are summarized immediately below the food intakes in each panel (Fig. 1). Four out of eight rats that had been lesioned with 1 or 3.2 μg colchicine exhibited relatively low rectal temperatures after daily cold exposure (Fig. 1D, E). This response, termed here as cold intolerance, lasted several days and then began to diminish. A relatively low body temperature was evident in a fifth rat for 1 day (Fig. 1E), but this may have been related to the temporary aphagia of this rat at the time. Cold intolerance was not evident in rats lesioned with 0.1 or 0.32 μg colchicine.

Examination of coronal brain sections revealed injection cannula tracks in 18 of the 20 rats. These terminated within or close to the ventromedial hypothalamic nucleus (Fig. 2). The 4 rats which exhibited cold intolerance had been injected in sites very close to or overlapping with those in rats that only showed hyperphagia. However, 3 of 4 rats showing cold intolerance had been microinjected using the lateral coordinate of 0.7 mm rather than 1.0 mm, and 0.7 mm was utilized for further study of the phenomenon.

In the second group of experiments, control and lesioned rats were prepared on the third day after microinjection for chronic recording of body temperature and EMG to determine whether thermoregulation of lesioned rats in the cold would be impeded by propranolol. It was reasoned that if VMH lesions impair the neuronal mechanism which activates NST in BAT, other nonadrenergically-mediated defences would predominate, and responsiveness to beta adrenergic blockade would be diminished in colchicine rats. Figure 3 shows typical examples of data obtained in 3 rats during a one-hour period of cold exposure, midway through which propranolol was injected. The control rat (Fig. 3A) and the rat lesioned with 0.32 μg colchicine (B) both maintained their body temperature during the first 30 min of cold exposure, while the animal lesioned with 1 μg showed a spontaneous fall in body temperature (C). After injection of propranolol, all 3 rats experienced a fall in body temperature. The sample EMG records taken after propranolol injection show increased bursts of muscle electrical activity in all three cases. This presumably reflects the increase in shivering thermogenesis required to compensate for adrenergic blockade and loss of NST. Possible differences in lead position and recording efficiency would account for differences in EMG amplitude between animals. However, qualitatively, it appeared that rats lesioned with 1 μg colchicine exhibited less EMG activity before propranolol, even though the animals were hypothermic.

Table 1 summarizes the average rectal temperature data obtained in the three groups of rats. The phenomenon of cold intolerance was only present in the rats lesioned with the higher dose of colchicine (1 μg), in which rectal temperature had fallen significantly during the first 30 min of cold exposure. These rats also exhibited a greater sensitivity to pro-
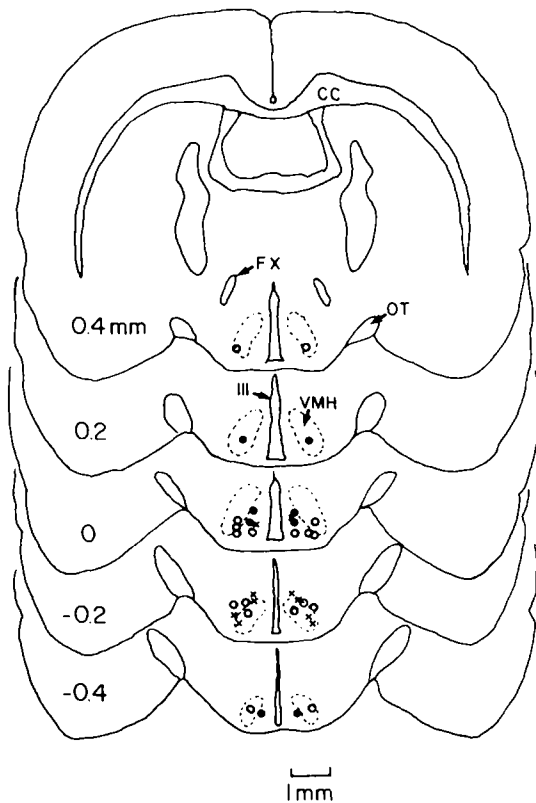
FIG. 2. Diagrammatic representation of coronal sections through the brain, showing location of hypothalamic microinjection sites in rats that had been utilized for the experiments summarized in Fig. 1. Each bilateral pair of symbols are from a single rat. Filled circles: these rats were microinjected with 1 or 3.2 μg colchicine and exhibited hyperphagia, and several days of cold intolerance. Open circles: hyperphagia only. Crosses: neither response occurred. Anterior-posterior (AP) positions shown on the left are with reference to bregma. Abbreviations: FX—fornix; OT—optic tract; VMH—ventromedial hypothalamic nucleus; CC—corpus callosum; III—third ventricle.
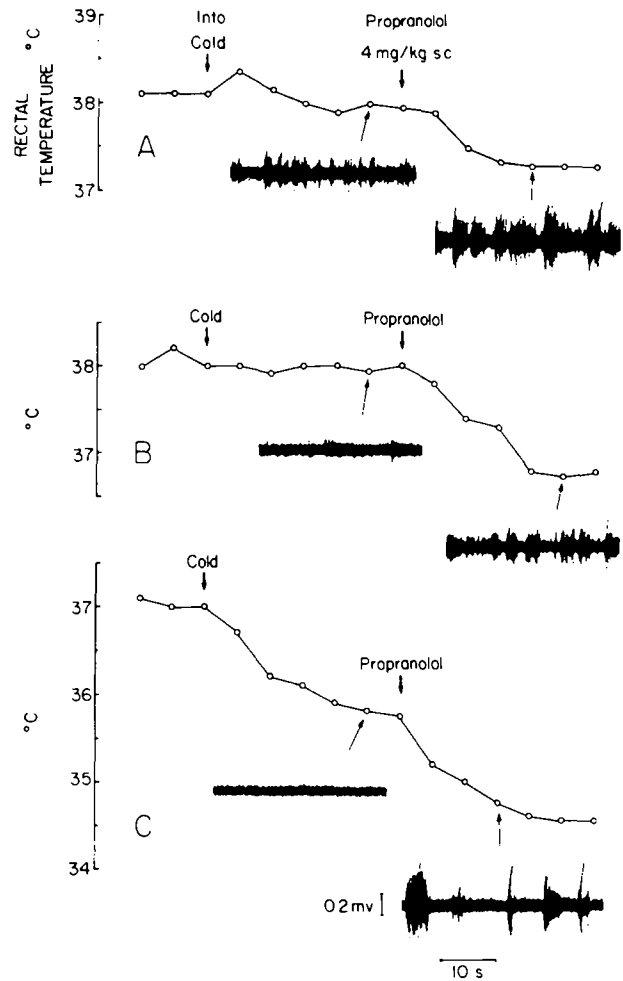

FIG. 3. Effect of cold exposure and propranolol injection on rectal temperature and EMG activity recorded in 3 rats. The line graph in each panel shows rectal temperature level (plotted at 5-min intervals) over a 10-min period immediately preceding placement of the rat in the cold, and thereafter during 60 min of continuous cold exposure. Propranolol was injected midway through the cold exposure period. The time and millivolt scale shown in the lowermost panel (C) applies to all of the EMG recordings, sampled at the points indicated by the arrows.

pranolol injection, indicated by the larger decline in body temperature during the 30 min following injection.

Table 2 gives data from experiments to determine whether VMH colchicine lesioning would alter the effect of cold exposure of the rat on IBAT properties measured afterwards in vitro. Four colchicine-lesioned rats (0.32 μg) placed in the cold for 16 hr overnight had a GDP binding elevated to 3.5 times the mean value obtained in lesioned rats that were not cold exposed (A-4 vs. A-3). The same experiment in nonlesioned rats showed that cold exposure caused GDP binding to be elevated to 3.9 times the normal value at 24° (A-2 vs. A-1). The colchicine rats maintained at 24°C failed to exhibit dietary activation of IBAT and increased GDP binding associated with hyperphagia, but rather a possible suppressing influence was indicated (A-3 vs. A-1). The colchicine animals (A-3) had a cumulative 4-day food intake which averaged 61±6 (SE) % higher than the mean intake of controls (p<0.01). At either 6 or 24°C, there was no significant differences in rectal temperature of lesioned rats vs. controls just prior to sacrifice on day 4.

In further experiments, rats that had been previously lesioned with 1 μg colchicine and exposed to cold overnight had a lower mean temperature and GDP binding than controls similarly exposed (Table 2, B-2 vs. B-1). Mean GDP binding in colchicine rats was, nevertheless, substantially elevated (2.9 times) relative to the value obtained in another group of rats (Experiment C) which underwent the same protocol, but instead of microinjection on day 0, had undergone denervation of the IBAT. This difference also indicated the importance of neuronal rather than humoral factors in GDP binding response to cold exposure.

Measurements of IBAT mass throughout these experiments showed that IBAT pads were usually heavier in colchicine-lesioned rats compared to controls, and in some instances, IBAT protein was higher.

TABLE 1

EFFECT OF 60-MIN COLD EXPOSURE AND PROPRANOLOL INJECTION ON RECTAL TEMPERATURE OF RATS WITH COLCHICINE LESIONS OF THE VENTROMEDIAL HYPOTHALAMUS

Colchicine Dose μg	Rectal Temperature °C			Rectal Temperature Decrease °C Between	
	0 min	30 min	60 min	0–30 min	30–60 min
0 (control)	38.2 ± 0.2	38.2 ± 0.2	37.3 ± 0.1¶	0.1 ± 0.1	0.9 ± 0.2¶
0.32	37.7 ± 0.1†	37.4 ± 0.2†	36.2 ± 0.3†¶	0.3 ± 0.1	1.2 ± 0.1¶
1.0	37.5 ± 0.2*	36.5 ± 0.3*§#	34.8 ± 0.4*‡¶	0.9 ± 0.1*‡	1.7 ± 0.2*§¶

Values are mean ± SEM. Rats were tested 3 days after microinjection (n=6 rats per treatment group). Each rat was placed in a 6°C room for 60 min, beginning at time 0 min. Propranolol, 4 mg/kg SC was injected immediately after the rectal temperature measurement at 30 min. Statistical comparisons between colchicine dosage groups: *($p<0.01$) or †($p<0.05$) for colchicine groups vs. corresponding control value. ‡($p<0.01$) or §($p<0.05$) comparing 1 μg vs. 0.32 μg dose. Within-treatment groups: ¶($p<0.01$) or #($p<0.05$) comparing temperature values at 30 min vs. 0 min, at 60 min vs. both 30 and 0 min, and comparing decreases, 30–60 min vs. 0–30 min.

TABLE 2

MEASUREMENTS OF IBAT PROPERTIES AND RECTAL TEMPERATURE IN RATS SUBJECTED TO COLCHICINE LESIONING OF THE VMH OR DENERVATION OF IBAT

Experiment Group	Treatment		IBAT			Rectal Temperature °C
	Colchicine μg	Exposure °C	Mass (mg)	Protein (mg)	GDP binding (pmol/mg)	
A-1	0	24	397 ± 46	21.2 ± 1.9	82 ± 7	37.7 ± 0.3
2	0	6	280 ± 20	23.2 ± 1.3	323 ± 31*	37.7 ± 0.3
3	0.32	24	704 ± 75‡	23.2 ± 2.5	55 ± 6	37.8 ± 0.2
4	0.32	6	470 ± 20*§	30.1 ± 1.4†§	193 ± 21*‡	37.3 ± 0.4
B-1	0	6	360 ± 34	35.8 ± 5.1	292 ± 33	37.8 ± 0.1
2	1.0	6	872 ± 134*	43.6 ± 2.6	200 ± 22†	36.5 ± 0.3*
C IBAT Denervation		6	406 ± 30*	27.3 ± 2.6†§	70 ± 16†‡	37.4 ± 0.1†

Values are mean ± SEM for IBAT properties and rectal temperature of rats sacrificed on the morning of day 4, after the following treatments. On day 0, groups A-1–A-4 and B-1, B-2 received bilateral VMH microinjections of colchicine or vehicle (0 μg); in group C, IBAT was denervated. All groups except A-1, A-3 had spent 16 hr overnight at 6°C immediately prior to sacrifice on day 4. IBAT properties shown are total brown fat mass and protein and pmol GDP bound per mg mitochondrial protein. n=4 rats per treatment group except for group C (n=3).

Symbols for following data comparisons: *($p\leq0.01$) or †($p\leq0.05$) for A-2 vs. A-1, A-4 vs. A-3, B-2 vs. B-1, C vs. B-2. ‡($p\leq0.01$) or §($p\leq0.05$) for A-3 vs. A-1, A-4 vs. A-2, C vs. B-1.

DISCUSSION

A main finding of this study is that the effects of colchicine lesioning of the VMH on food intake and thermoregulation in the cold could be dissociated on the basis of the dose that had been microinjected into a limited hypothalamic area. Although both low and high doses caused hyperphagia, the larger doses (1 or 3.2 μg) had the added effect of impairing thermoregulation so that at 6°C the rats became hypothermic. Use of the more medial injection coordinate (0.7 mm lateral, as opposed to 1.0 mm) appeared more reliably associated with this cold intolerance; however, there

was little firm basis to distinguish between injection sites evoking one or both responses. Fifty nanolitres of injectate, as a spherical volume, would have a diameter of 0.457 mm, which is substantive relative to the region through which injection sites were distributed (Fig. 2). Since the actual physical distribution of this injectate volume and the diffusion of the drug itself are unknown, it is quite possible that addition of cold intolerance to the hyperphagic response required a more widespread lesion through the ventromedial nucleus, or that it was exclusively related to effects in a nearby region. There was also evidence, e.g., Fig. 1D, that

the cold intolerance could disappear while hyperphagia persisted, further suggesting a distinction between the neuronal elements involved.

For several reasons, it is difficult to ascribe the cold intolerance to a functional disconnection between the VMH and the sympathetic innervation that activates thermogenesis in brown fat, as has been suggested to occur with VMH lesions (9,11). First of all, rats rendered cold intolerant by colchicine still exhibited elevated GDP binding after overnight cold exposure, showing that the tissue had undergone thermogenic activation. Secondly, the injection of propranolol during the hypothermic response in rats lesioned with 1 μg colchicine caused a subsequent decline in body temperature over 30 min which exceeded that seen in controls (Table 1). If the sole defect in VMH-lesioned rats is the functional disconnection of sympathetic innervation of BAT, one might have expected a smaller temperature drop after propranolol in colchicine rats, since the animals would be preferentially utilizing shivering thermogenesis, which is not adrenergically mediated. The heightened sensitivity to propranolol thus invites speculation that the colchicine rat that is cold intolerant suffers a thermoregulatory impairment more complex than simple interruption of an efferent pathway to BAT. For example, both the spontaneous fall in core temperature in the cold, and large declines after propranolol could reflect an impairment in error signalling in the thermoregulatory mechanism due to changes in the thresholds for activation of its neuronal components. Thus, core temperature in these animals exposed to cold may have to deviate to a threshold well below the normal setpoint temperature before the defences of NST and shivering thermogenesis are recruited.

The observation of differential effects of colchicine microinjection dosage on appetite vs. cold tolerance may help explain how divergent views have arisen concerning importance of the VMH for thermoregulatory modulation of BAT metabolism. The arrival at opposite conclusions may have been indirectly governed by whether or not the lesioning method had affected neuronal elements additional to, or independent of those associated with the VMH-hyperphagia syndrome. Our findings with lower colchicine dosage, presumably involving more restricted chemical lesioning, are in keeping with those reported in several earlier studies. On the basis of biochemical assessment of BAT activation, e.g., increased GDP binding in response to cold but not overnutrition, it was concluded that thermal (7), electrolytic (14), and

knife-cut (2) lesioning of he VMH blocks dietary but spares thermoregulatory activation of BAT. Our correlation of higher VMH colchicine dosage with cold intolerance points to the possibility that more widespread impairment of the VMH was the determining factor in those earlier studies which concluded that VMH lesions functionally disconnect the thermoregulatory pathway to BAT. More specifically, it was shown that microinjection of lidocaine prevented the rise in IBAT temperature normally evoked by localized cooling of the anterior hypothalamus (9) and that electrolytic lesioning blocked the increase in sympathetic nerve activity to IBAT normally evoked by skin cooling (11). It should be qualified here that the cold intolerance we observed after colchicine lesioning was more readily explained in terms of altered thresholds for activation of thermoregulatory pathways to BAT rather than complete functional disconnection. However, it is still possible that the latter could occur with more extensive lesioning.

Another important aspect of the relationship between VMH and brown fat concerns the mediation of diet-induced thermogenesis. Following other types of VMH lesioning, BAT activation and increased GDP binding fails to occur in response to overnutrition. On the contrary, lowering of GDP binding was reported (2, 7, 8, 14). Colchicine-lesioned rats exhibited similar responses in this respect (Table 2, A3 vs. A1). The lowering of GDP binding associated with hyperphagia in electrolytically-lesioned rats could be prevented by restricting food intake (14), a finding we have confirmed also for colchicine rats (unpublished data). The observations in this study and those of others (2, 7, 14) suggest that the mechanism for thermoregulatory modulation of BAT is still functional in the VMH rat. One might therefore speculate that the diminishing of BAT activity associated with VMH hyperphagia indicates a thermoregulatory downward modulation of the tissue, unmasked by VMH damage and loss of the dietary mechanism that is believed to normally increase BAT metabolism during overnutrition. Such a thermoregulatory lessening of BAT thermogenesis could be a counter-response to the greater heat production of food processing during the period of hyperphagia.

REFERENCES

1. Avrith, D.; Mogenson, G. J. Reversible hyperphagia and obesity following intracerebral microinjection of colchicine into the ventromedial hypothalamus of the rat. Brain Res. 153:99–107; 1978.
2. Coscina, D. V.; Chambers, J. W.; Park, I.; Hogan, S.; Himms-Hagen, J. Impaired diet-induced thermogenesis in brown adipose tissue from rats made obese with parasagittal hypothalamic knife-cuts. Brain Res. Bull. 14:585–593; 1985.
3. Dasheiff, R. M.; Ramirez, L. F. The effects of colchicine in mammalian brain from rodents to rhesus monkeys. Brain Res. Rev. 10:47–67; 1985.
4. Desautels, M.; Zaror-Behrens, G.; Himms-Hagen, J. Increased purine nucleotide binding, altered polypeptide composition, and thermogenesis in brown adipose tissue mitochondria of cold acclimated rats. Can. J. Biochem. 56:378–383; 1978.

5. Foster, D. O.; Frydman, M. L. The distribution of cold-induced thermogenesis in conscious warm or cold acclimated rats reevaluated from changes in tissue blood flow: the dominant role of brown adipose tissue in the replacement of shivering by non-shivering thermogenesis. Can. J. Physiol. Pharmacol. 57:257–270; 1979.
6. Himms-Hagen, J.; Triandafillou, J.; Gwilliam, C. Brown adipose tissue of cafeteria-fed rats. Am. J. Physiol. 241:E116–E120; 1981.
7. Hogan, S.; Coscina, D. V.; Himms-Hagen, J. Brown adipose tissue of rats with obesity-inducing ventromedial hypothalamus lesions. Am. J. Physiol. 243:E338–E344; 1982.
8. Hogan, S.; Himms-Hagen, J.; Coscina, D. V. Lack of diet-induced thermogenesis in brown adipose tissue of obese medial hypothalamic-lesioned rats. Physiol. Behav. 35:287–294; 1984.

9. Imai-Matsumura, K.; Matsumura, K.; Nakayama, T. Involvement of ventromedial hypothalamus in brown adipose tissue thermogenesis induced by preoptic cooling in rats. Jpn. J. Physiol. 34:939–943; 1984.
10. Nicholls, D. G. Hamster brown adipose tissue mitochondria. Eur. J. Biochem. 65:581–585; 1975.
11. Nijima, A.; Rohner-JeanRenaud, F.; JeanRenaud, B. Role of ventromedial hypothalamus on sympathetic efferents of brown adipose tissue. Am. J. Physiol. 247:RF650–RF654; 1984.
12. Pellegrino, L. J.; Pellegrino, A. S.; Cushman, A. J. A stereotaxic atlas of the rat brain. 2nd. ed. New York: Plenum Press; 1979.

13. Schachterle, G. R.; Pollack, R. L. A simplified method for the quantitative assay of small amounts of protein in biological material. Anal. Biochem. 51:654–655; 1973.
14. Seydoux, J.; Ricquier, D.; Rohner-JeanRenaud, F. F.; Assimacopoulos-Jeannet, F.; Giacobino, J. P.; JeanRenaud, B.; Birardier, L. Decreased guanine nucleotide binding and reduced equivalent production by brown adipose tissue in hypothalamic obesity. FEBS Lett. 146(1):161–164; 1982.
15. Slinde, E.; Pedersen, J. I.; Flatmark, T. Sedimentation coefficient and buoyant density of brown adipose tissue. Anal. Biochem. 65:581–585; 1975.