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Is the Polar Bear *(Ursus maritimus)* a Hibernator?: Continued Studies on Opioids and Hibernation

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BRUCE, D. S., N. K. DARLING, K. J. SEELAND, P. R. OELTGEN, S. P. NILEKANI AND S. C. AMSTRUP. *Is the polar bear* (Ursus maritimus) *a hibernator?: Continued studies on opioids and hibernation.* PHARMACOL BIOCHEM BEHAV **35**(3) 705–711, 1990.—Polar bear behavior and biochemistry suggest they may have the ability to hibernate year-round, even though this species is not considered to be a true hibernator. This observation, plus the discovery of a hibernation-induction trigger (HIT) in the blood of black bears, prompted the examination of polar bear blood collected throughout the year for evidence of HIT, and to determine if it displayed opioid activity, as black bear blood does. A bioassay was conducted by injecting summer 13-lined ground squirrels with serum collected from polar bears at different seasons. One group of squirrels was previously implanted with osmotic pumps containing naloxone. The rest had pumps containing saline. Squirrels with saline pumps all hibernated significantly more than those with naloxone, except the group receiving blood from a November polar bear, observed to be highly active and hyperphagic. An in vitro study, using guinea pig ileum, showed that 400 nM morphine inhibited induced contractions and 100 nM naloxone reversed the inhibition. Ten mg of winter polar bear serum albumin fraction (to which HIT binds in ground squirrels and woodchucks) had a similar inhibiting effect, but naloxone, even at 4,000 nM, didn't reverse it. It is concluded that polar bear blood contains HIT, that it has an opioid effect, but may not itself be an opioid.

Hibernation Hibernation-induction trigger (HIT) Opioid Naloxone Polar bear *(Ursus maritimus)*
13-Lined ground squirrel *(Citellus tridecemlineatus)* Guinea pig ileum-myenteric plexus

HIBERNATION is a physiological state in an endotherm characterized by depressed body temperature (T_b), respiration, cardiovascular function and general metabolism. It is also a regulated state: hibernators avoid freezing and arouse spontaneously to euthermia and control levels of metabolism and system function (13). Well-known hibernators are found among rodents (e.g., ground squirrels, woodchucks, dormice), insectivores (e.g., European hedgehog), bats and carnivores (e.g., black bear).

Ever since Dawe *et al.* (7,8) showed that there is a chemical contained in the blood of hibernating woodchucks and 13-lined ground squirrels which upon injection could induce summer-active ground squirrels to enter hibernation, investigators have tried to explain the physiology and source of the inducer. This substance, still not isolated, has been dubbed "hibernation-induction trigger" (HIT), and has been employed as an inducer of summer hibernation in the 13-lined ground squirrel successfully (5–9, 26–30, 33, 34) as well as unsuccessfully (37).

It has been demonstrated that HIT is associated with the plasma albumin fraction (26). Other studies (3, 4, 21) suggest that hibernation is an opioid-dependent state. Plasma or its albumin fraction from the winter torpid black bear possesses an HIT substance and can effectively induce summer hibernation in the 13-lined ground squirrel (34); black bear HIT is ineffective at inducing summer hibernation in the presence of the opioid antagonist, naloxone (6). It has been shown that an opiate kappa agonist alone does not induce summer hibernation (27); in companion studies, neither mu nor kappa receptor agonists were effective at inducing summer hibernation, but the delta agonist DADLE (D-Ala^2-D-Leu^5-enkephalin) was (28,30). These investigators conclude that the delta opioid receptor and its endogenous ligand may be intimately involved in hibernation. Mu and kappa opioids actually antagonized HIT-induced hibernation in these studies, and may, therefore, play an important role in arousal from hibernation.

The polar bear apparently separated from the brown bear *(Ursus arctos)* group very recently. Polar bears appeared as a distinct form in the fossil record only during the late Pleistocene, about 100,000 years ago (16). Despite significant ecological and behavioral differences, and extended temporal separation of their stocks, some recent data suggest polar bears still share similar physiologies with black and brown bears. It has been suggested (22,24) that all three bear species share at least 4 physiological

stages: 1) hibernation, 2) walking hibernation, 3) normal activity, and 4) hyperphagia. Among the most significant differences between polar bears and other northern ursids is the absence of extended winter denning in all but pregnant female polar bears (1,2). Pregnant females must den in order to provide a suitable environment for neonates. Other sex and age classes, however, are known to fast and maintain low levels of activity for extended periods of time when stranded on land in summer (19). This occurs in the southern extreme of polar bear range near Hudson Bay. These investigators concluded that although some summer stranded polar bears used supplemental foods, feeding at that time of year did not seem to be necessary for survival or reproduction. Another study (31) found that weight losses of lactating female bears during this summer fasting period could be great, but that single bears (which would be metabolizing body stores only for themselves) showed relatively small losses. Nelson *et al.* (24) concluded that some blood values of alert, locomoting polar bears during this summer fasting period were physiologically similar to those of hibernating black bears, and suggested such animals were in a state of "walking hibernation."

Polar bears feed almost exclusively on a few species of ice-loving seals (2). Unlike Hudson Bay bears, those occurring in the Beaufort Sea and other higher latitude habitats, have access to sea ice and the seals it supports throughout the summer period. Whereas polar bears in Hudson Bay face a summer of fasting on land (19), polar bears in the Beaufort Sea and high arctic are hyperphagic in summer and autumn, and reach their peak weights by early winter (36). During the fall-through-spring period, when pregnant females occupy maternity dens, the remaining portions of the population remain active on the sea ice. Although food is theoretically available, most animals lose considerable weight during the dark winter months (Amstrup, unpublished data). If polar bears can facultatively invoke a metabolic state similar to hibernation, such action might be expected during this winter negative foraging period. Thus, using the terminology of Nelson *et al.* (24), polar bears wintering in the Beaufort Sea area should be either hibernators in dens or "walking hibernators" when not in dens. Conversely, bears that were actively foraging in summer and fall would not show metabolic signs of hibernation. Because Nelson *et al.* (24) examined many blood values that are known to change with variations in physiological states exclusive of hibernation (10), many questions about hibernation and "walking hibernation" in ursids remain.

The purposes of this study were to 1) examine blood collected from polar bears representing different stages in the annual life cycle for an HIT-like substance, by bioassay, injecting polar bear sera into summer-active ground squirrels, and 2) determine whether that substance has opioid properties similar to the HIT present in hibernating black bears (6,34).

METHOD

Polar Bear Capture and Blood Collection

Wild polar bears in the Beaufort Sea were captured by injection of immobilizing drugs administered by projectile syringes shot from hovering helicopters. Several authors have described capture procedures for polar bears in the pack ice environment (17, 18, 35). All of the polar bears from which sera were used in our experiments were immobilized with Telezol (A. H. Robbins Co.). Telezol is a mixture of a cyclohexamine CNS depressant, tiletamine hydrochloride, and the tranquilizer zolazepam hydrochloride. Telezol has strong analgesic and anesthetic properties and has been used effectively on polar bears since the mid-1980's (12). Blood was drawn from the femoral artery or vein into evacuated test tubes. At the end of each day in the field, sera were separated by centrifugation and frozen. Upon return from the field, sera were stored at $-80°C$.

We tested for indications of an HIT-like substance in sera from 4 polar bears captured as part of ongoing U.S. Fish and Wildlife Service studies:

1. Polar bear No. 1734 is an adult female whose activity had been monitored by the U.S. Fish and Wildlife Service for several years. Serum for use in these studies was taken from her when she was last captured on 15 April 1988. She was known to have occupied a maternity den through the period from 3 November 1987 to 10 April 1988. For these experiments we designated her as "FED," for "female polar bear emerging from a den." At the time of capture she was very lean from her long fast and the expense of lactation. She was immobilized with 1080 mg of Telezol.
2. Bear No. 6248 was a previously captured bear whose activity had also been monitored for several years. Unfortunately, she had shed her most recently attached radiocollar, and when captured (6 May) we were not certain whether she occupied a den during the winter of 1987–88. She showed no evidence of lactation, however, and was very fat for a bear in the spring season, suggesting she had either not fasted or had been very effective in conserving energy while doing so. Most likely, No. 6248 deferred a reproductive attempt this year. Because of her higher fat load, she required 1400 mg of Telezol to achieve immobilization. We designated No. 6248 as "NDF" for "nondenning female."
3. Male polar bear No. 6602 was unmarked prior to his capture on 18 April 1988. We have not observed male polar bears of any age to enter long-term dens in the Beaufort Sea region, and have no reason to feel this bear might have been in a den last winter. He was in extremely good condition at the time of capture, and was immobilized with 1400 mg of Telezol. For our experiments, this bear was designated "WM" for "winter male."
4. Finally, polar bear No. 6484 is a radiocollared female from which blood was taken on 20 November 1986. Activities of this bear had been monitored for some time prior to that capture. At the time her blood sample was drawn, No. 6484 was in excellent condition, weighing 200 kg. It took 2600 mg of Telezol to keep her effectively immobile during our marking and sampling procedures. Telezol is quite lipophillic, and bears captured in the autumn almost always require substantially more drug to make them tractable. We have designated No. 6484 as "FAF" for "fall-active female."

Radio Transmitter Implantation

Radio transmitters (Mini-Mitter Co., Sunriver, OR) were calibrated to determine the frequency at which they emitted signals at temperatures ranging from 5°C to 37°C and implanted into the abdominal cavity of all squirrels except controls. Transmitters were sutured to the inside of the rectus abdominus to prevent them from moving around the abdominal cavity. During surgery the incision was liberally swabbed with 10% povidone-iodine (Betadine). The rectus abdominus was then sutured with absorbable suture (Vicryl, size 4-0, size p-3 needles) and the incision was closed with wound clips. Following surgery each squirrel was given 0.25 cc of penicillin (Tech America, 300,000 units/ml) subcutaneously (SC) to insure against infection. Wound clips were removed 7–10 days later, according to the condition of the wound. Control squirrel temperatures were monitored with a thermistor-

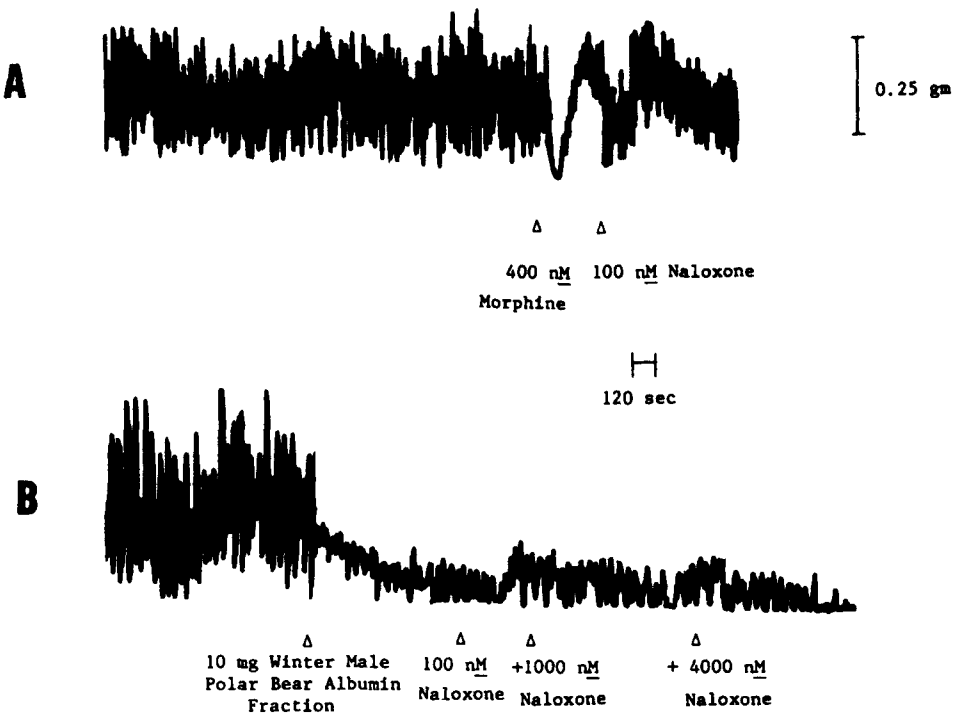

FIG. 1. (A) Effect of 400 nM morphine on induced contractility of the guinea pig myenteric plexus-longitudinal muscle preparation, and reversal of the inhibition by 100 nM naloxone. (B) Effect of 10 mg of polar bear albumin fraction on a similar prep, but failure of naloxone to antagonize the inhibition, even at 1000 and 4000 nM concentrations.

telethermometer (Bailey Instruments, Inc., model BAT-8).

Osmotic Minipump Implantation

Alzet osmotic pumps were implanted SC interscapularly in each squirrel. We used model 2ML4 which has a mean pumping rate at 37°C of 2.58 ± 0.14 μl/hr, and a mean fill volume of 2158 ± 70 μl; the pump should administer solution for approximately 35 days. After an incision was made in the back, a tunnel was formed by spreading a hemostat SC. The pump was then inserted into the tunnel delivery-port end first. As before, the incision was liberally swabbed with 10% povidone-iodine (Betadine). All incisions were closed with absorbable suture. Thirty-one of the squirrels received pumps filled with saline. The other eight received pumps with naloxone delivered at a rate of 1 mg/kg body weight per hour. Seven days were allowed for recovery before intrasaphenous injections were made.

Intrasaphenous Injection

Whole frozen serum samples from four polar bears (see above) were thawed at 7°C before use. Serum samples, syringes and needles were kept on ice throughout the injection period. In late May, all ground squirrels were injected intrasaphenously with polar bear serum or with saline (Tyrode's solution) as follows. Group 1 (N = 6), with saline in the pumps, received 0.2 cc serum from a winter male polar bear (WM). Group 2 (N = 7) had saline pumps and were injected with serum from a nondenning female (NDF). Group 3 (N = 8) had saline pumps and received serum from a winter female bear emerging from the den (FED). Group 4 (N = 8) had naloxone in the pumps and received serum from the same bear as group 3: female emerging from the den (FED-N). The animals in group 5 were controls. All 10 had saline pumps. Half received fall active female serum (FAF). The rest were injected with saline (SAL).

Observation of Injected Ground Squirrels

Following injection all squirrels were placed into separate cages in a cold room at an ambient temperature (T_a) of 7 ± 2°C. Each squirrel was given ample bedding material and had unlimited access to food (Wayne Rodent Blox) and water. Squirrels were monitored at the same time each day for 28 days. Readings included core body temperature and respiratory rate. Criteria for hibernation were T_b less than 4°C above T_a (7°C) and respiratory rate less than 10/min (euthermic control rate = 120/min). Squirrels with readings above these were classified as being hypothermic, but not yet in deep hibernation. Most hypothermic animals had T_b's within a few degrees of euthermia (36–37°C), or were in the process of entering, or arousing from, a bout of hibernation.

Preparation of Guinea Pig Ileum Myenteric Plexus-Longitudinal Muscle Strip

The guinea pig ileum myenteric plexus-longitudinal muscle strip (GPIl) is a well-known model demonstrating opioid depression of electrically induced contraction (11). The GPIl was excised from male Hartley guinea pigs weighing 300–400 g, and a 5 cm segment removed from a section 15 cm from the ileo-cecal junction. The longitudinal muscle was removed from the ileal segment (32), and suspended in a muscle chamber (volume 25 cc) containing Krebs bicarbonate buffer [composition (mM): NaCl

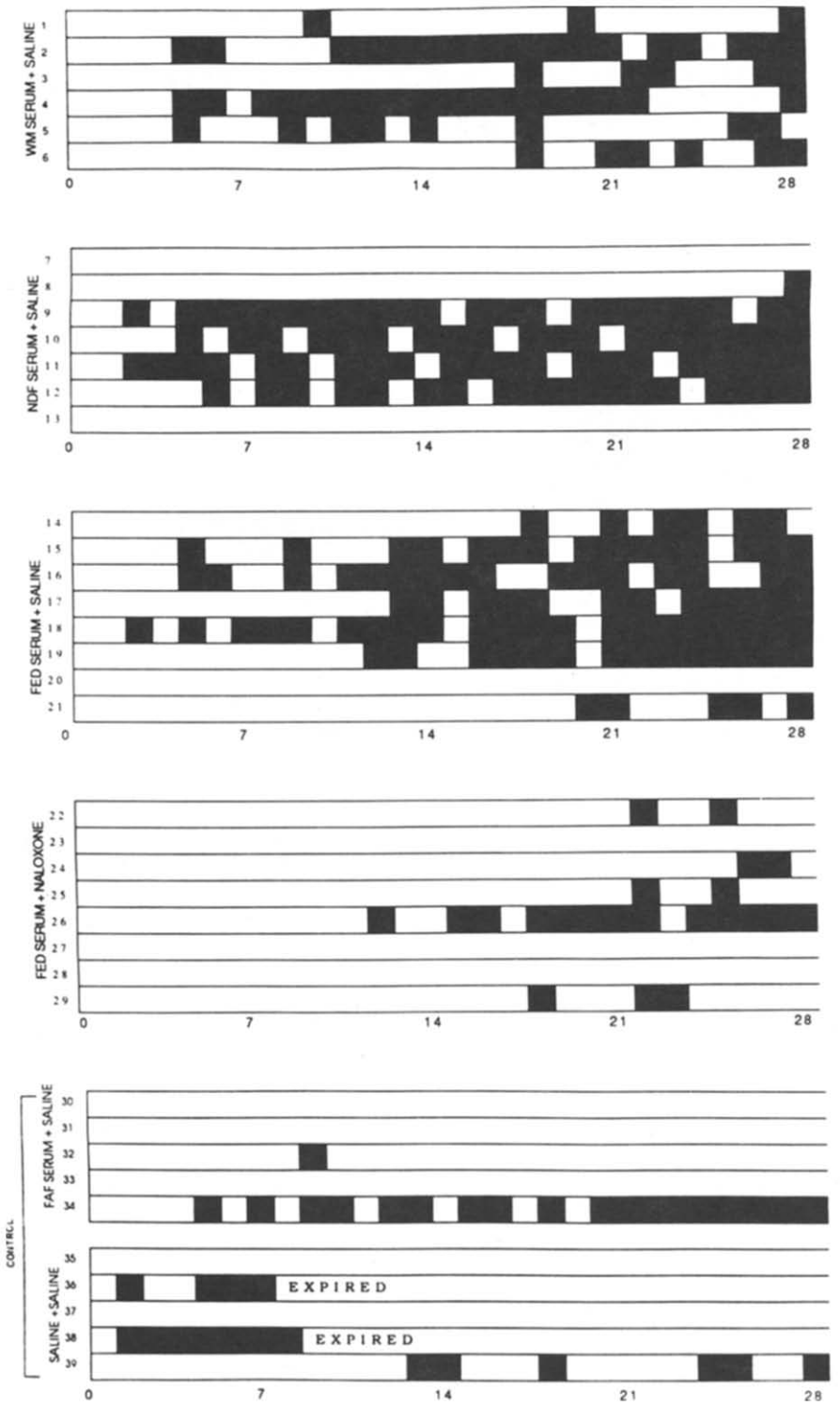

FIG. 2. Patterns of summer hibernation in all injected ground squirrels. Black bars indicate days in hibernation. Legend: WM = winter male polar bear serum (+ saline in the osmotic minipump); NDF = serum from a nondenning winter female polar bear (+ saline in the pump); FED = serum from a female polar bear emerging from a den (+ saline in the pump); FED + Naloxone = serum from the same bear, but with naloxone in the pump; FAF = serum from a late fall active female polar bear (+ saline in the pump); Saline + Saline = Tyrode's solution injected + Tyrode's solution in the pump. The FAF/Sal groups were controls.

TABLE 1

FREQUENCY AND DURATION OF SUMMER HIBERNATION BOUTS IN GROUND SQUIRRELS INJECTED WITH POLAR BEAR SERUM OR SALINE

Group	Bout Frequency	*p* Value	Bout Duration (Days)	*p* Value
(1) Winter Male (WM)	4.00 ± 0.52*	<0.01† <0.05‡	2.38 ± 0.59*	n.s.† <0.05‡
(2) Nondenning Female (NDF)	3.43 ± 1.11	n.s.† n.s.‡	3.38 ± 0.46	<0.05† <0.05‡
(3) Female Emerging from Den (FED)	4.00 ± 0.73	<0.05† <0.05‡	2.78 ± 0.34	n.s.† <0.05‡
(4) Female Emerging from Den (Naloxone) (FED-N)	1.38 ± 0.49	— n.s.‡	1.38 ± 0.44	— n.s.‡
(5) Fall Active Female/or Saline (FAF/ SAL) (Control)	1.50 ± 0.73	n.s.† —	1.80 ± 0.52	n.s.† —

*Mean (± SEM) number of hibernation bouts and mean bout duration during the 28-day experiment.

†Compared to Group 4 (FED-N) by Student's unpaired *t*-test. n.s. = no significant difference.

‡Compared to Group 5 (FAF-SAL) by Student's unpaired *t*-test. n.s. = no significant difference.

118, KCl 4.75, $CaCl_2$ 2.54, KH_2PO_4 1.19, $MgSO_4$ 0.12, $NaHCO_3$ 25, glucose 11 (15). To this was added 70 μM hexamethonium bromide and 0.125 μM mepyramine maleate (11)]. The muscle chamber containing the GPII was inside a Phipps & Bird isolated organ bath maintained at 37°C, and aerated by bubbled 95% O_2–5% CO_2. Contractions were induced by field stimulation through two platinum electrodes, one formed into a ring around the GPII and the other placed parallel to the muscle strip. A Narco F-60 force transducer and Narco Physiograph recorded contractions induced by a Grass SD-5 stimulator with these stimulus parameters: 80 volts, 0.1 Hz, and 0.25 msec pulse duration. Resting tension was 1 g. The GPII was allowed to equilibrate for 60–90 min, with fresh Krebs solution added every 10 min.

Albumin Fraction Preparation

Affinity chromatography procedures were employed at 4°C to absorb albumin selectively from whole polar bear serum. Details are found elsewhere (26). The result is lyophilized albumin fraction. Ten mg of the fraction containing albumin was dissolved in Krebs solution just before addition to the muscle bath.

RESULTS

Morphine (as morphine sulfate, 400 nM) (Sigma Chemical Co.) has a typically inhibiting effect on electrically induced contraction of the GPII (Fig. 1A). One hundred nM naloxone reverses the inhibition. Ten mg of winter male polar bear serum albumin fraction similarly inhibits GPII contraction (Fig. 1B), but naloxone fails to reverse the inhibition, even at 1,000 or 4,000 nM concentrations. Results were similar in 4 experiments.

The patterns of summer hibernation of all ground squirrels can be seen in Fig. 2. Day zero represents the eighth day after osmotic pump implantation, and one day after intrasaphenous injection of serum or saline. Pumps should empty by day 28. Saline-pump squirrels injected with polar bear serum from a winter male (WM), a nondenning winter female (NDF), or a winter female emerging from a den (FED) hibernated significantly more than the squirrels with naloxone pumps that had been injected with serum from the female bear emerging from the den (FED-N), or control squirrels injected with serum from a fall active female polar bear or with saline (FAF-SAL), as shown in Table 1.

Examining the average percentage of days in hibernation for all experimental groups (Fig. 3), it is apparent that those which hibernated least were the FED-N and FAF-SAL squirrels. The first demonstrates that naloxone effectively inhibits induction of summer hibernation by serum from a female polar bear emerging from a den. Serum from the same bear is very effective at such induction if the squirrels' pumps contain saline (FED). Results from the FAF-SAL group suggest that serum from fall active polar bears, or Tyrode's solution, are ineffective inducers of summer hibernation.

DISCUSSION

In previous studies (6,34) it was shown that blood from winter torpid black bears *(Ursus americanus)* induced summer hibernation in 13-lined ground squirrels. Results of the present study show that blood from polar bears *(Ursus maritimus)* is equally effective in this regard. Summer hibernation in the test species was successfully induced after intrasaphenous injection of 0.2 cc of serum from a nondenning winter male, a nondenning winter female, and a female just emerging from a winter den. Serum from the latter bear was ineffective at inducing summer hibernation if

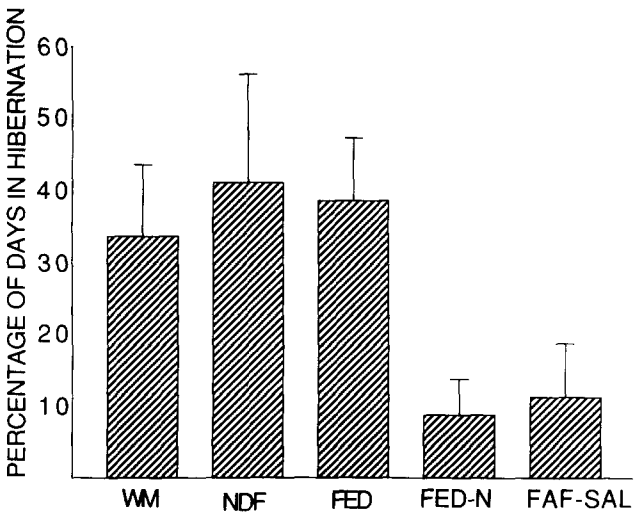

FIG. 3. Percentage of days in summer hibernation for ground squirrels injected with polar bear serum or saline. See Fig. 2 legend.

naloxone-filled osmotic pumps were previously implanted in the squirrels. Saline alone or serum from a late fall active female polar bear were also ineffective inducers (Table 1, Figs. 2 and 3). Two squirrels in this group (Nos. 36 and 38) became deeply hypothermic and died only 7 and 8 days into the experiment.

Since the polar bear developed from the brown/black bear line (16), it is not surprising that they share a system for controlling metabolism and thermoregulation. Results of the present study strongly suggest that polar bears in winter or spring possess an HIT substance. Induction capacity of that substance can be effectively blocked in vivo by the opiate antagonist, naloxone, suggesting that it could be an opioid compound. It has been shown that such a compound in the black bear might not itself be an opioid (6), because naloxone did not reverse the inhibition of guinea pig ileum contraction caused by black bear HIT. In the present study, similar results were obtained with polar bear serum albumin fraction. This suggests that there is a polar bear HIT, that it has an in vitro opioid effect (i.e., depresses GPII electrically induced contraction), but may not itself be an opioid since naloxone doesn't block its action on the GPII (see Fig. 1). This leads us to speculate that polar bear HIT, perhaps like that of the black bear (6), may either be a precursor to, or a releaser of, endogenous opiate.

Serum from a female polar bear in November (FAF), when the bear is highly active and feeding heavily, is ineffective as an inducer of summer hibernation in the ground squirrel. This could mean that there is very little endogenous HIT being produced and circulating in the wild polar bear in late fall. During the winter denning period or even if not denning, and in spring and summer when displaying the "walking hibernation" state described earlier (22), HIT levels may be increased, thereby triggering the metabolic changes characteristic of hibernation.

It has been stated (15) that because hibernation may be a case of convergent evolution (20), no hibernation factor should be expected to be universal in promoting hibernation. While this may be true, it does seem that an HIT substance from the following species can effectively induce summer hibernation in the 13-lined ground squirrel *(Citellus tridecemlineatus): C. tridecemlineatus* (7–9, 33); *Marmota monax* (woodchuck) (8,9); *Myotis lucifugus* (little brown bat) and *Eptesicus fuscus* (big brown bat) (5); *Ursus americanus* (black bear) (6,34); and now *Ursus maritimus* (polar bear). Results of the present study further substantiate that there exists a common HIT, shared by species across several mammalian orders, now including the Carnivora. Although it may ultimately be shown that there are at least subtle molecular structural differences in HIT from one species to another, the fact that the 13-lined ground squirrel's thermoregulatory and metabolic machinery responds to all in a similar manner points to a molecule similar in structure and biochemistry across species lines. It is likely an opioid or a releaser of one, and binds to the delta opioid receptor, according to recent evidence (28,30). The recently demonstrated summer hibernation-inducing effect of melatonin (29) is intriguing, since its main source, the pineal gland, is implicated in the regulation of circadian and circannual rhythmicity. It may, therefore, explain the relationships between seasonal changes in day length and the natural hibernation-arousal cycle. At present it is not yet clear what HIT is. As Oeltgen *et al.* (29) emphasize, melatonin could release endogenous delta opioid, or delta opioid could release melatonin. The result is induction of hibernation. Which occurs, or if perhaps other possibilities exist, awaits further investigation. It is clear from the present study that polar bears *do* produce an HIT substance, and that its effects are antagonized by naloxone in vivo.

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