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Suppression of Guinea Pig Ileum Induced Contractility by Plasma Albumin of Hibernators

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BRUCE, D. S., D. L. AMBLER, T. M. HENSCHEL, P. R. OELTGEN, S. P. NILEKANI AND S. C. AMSTRUP. *Suppression of guinea pig ileum induced contractility by plasma albumin of hibernators.* PHARMACOL BIOCHEM BEHAV **43**(1) 199–203, 1992.—Previous studies suggest that hibernation may be regulated by internal opioids and that the putative "hibernation induction trigger" (HIT) may itself be an opioid. This study examined the effect of plasma albumin (known to bind HIT) on induced contractility of the guinea pig ileum muscle strip. Morphine (400 nM) depressed contractility and 100 nM naloxone restored it. Ten milligrams of lyophilized plasma albumin fractions from hibernating ground squirrels, woodchucks, black bears, and polar bears produced similar inhibition, with partial reversal by naloxone. Five hundredths mg of D-Ala^2-D-Leu^5-enkephalin (DADLE) also inhibited contractility and naloxone reversed it. Conclusions are that hibernating individuals of these species contain an HIT substance that is opioid in nature and summer animals do not; an endogenous opioid similar to leu-enkephalin may be the HIT compound or give rise to it.

Hibernation Woodchuck Ground squirrel Black bear Polar bear Guinea pig ileum
Hibernation induction trigger

PLASMA from hibernating bears, ground squirrels, bats, and woodchucks will cause summer-active 13-lined ground squirrels to hibernate (4–7,19). Studies have also shown that this "hibernation induction trigger" (HIT) is found in the albumin fraction of the plasma (15), is a protein (13), and has profound effects on the blood components of hibernators (14,20).

The mechanism by which HIT induces hibernation is unknown. It has been postulated that the mechanism entails an endogenous opioid (1,9). For example, in a previous study it was demonstrated that infusion of naloxone (an opioid antagonist) via an osmotic minipump blocked HIT-induced hibernation in summer-active ground squirrels (2). Woodchuck HIT even has opioid-like effects in primates, causing wretching and decreases in metabolism, renal function, heart rate, and core body temperature (16). HIT has also been shown to depress electrically induced contraction of the guinea pig ileum myenteric plexus-longitudinal muscle preparation (GPI), as does morphine (2,3,17).

It has been suggested that HIT may trigger hibernation by releasing endogenous opioids (2,3). Studies have also shown that neither mu- nor kappa-agonists were effective at inducing summer hibernation, but the delta-agonist, D-Ala^2-D-Leu^5-enkephalin (DADLE) was (11,17). Mu- and kappa-agonists actually antagonized HIT-induced hibernation in these studies and may therefore play an important role in arousal from hibernation. DADLE has also been shown to suppress induced contractility in the mouse vas deferens that is reversed by naloxone (21).

Since the albumin fraction of plasma binds the putative HIT substance and is not species specific, we designed an experiment to demonstrate HIT presence in plasma albumin of four hibernating species. If such plasma albumin contains an opioid/HIT compound, it should have an inhibitory effect on induced contractility of the GPI; contractility should be restored by naloxone. DADLE, a known agonist of opioid delta-receptors that has been shown to induce hibernation in summer-active ground squirrels as effectively as plasma albumin fractions from hibernating woodchucks (17), was also

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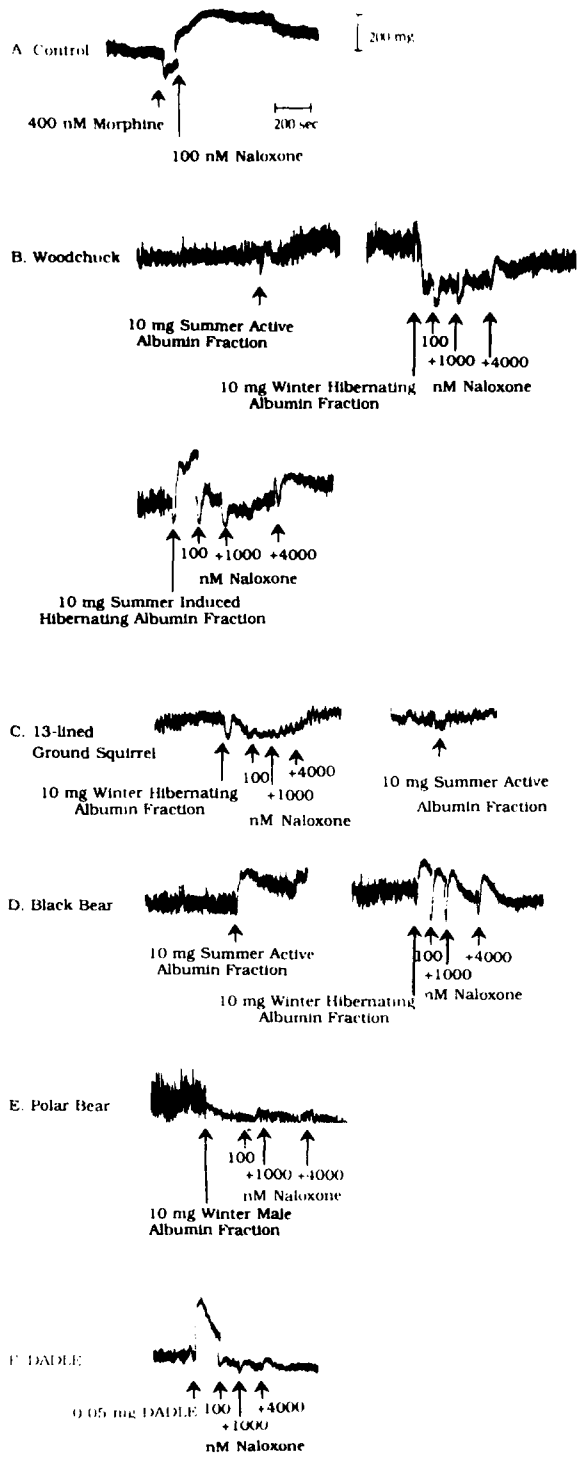

FIG. 1. (A) Effect of 400 nM morphine on induced contractility of the GPI preparation and reversal of inhibition by 100 nM naloxone. (B–E) Effects of albumin fractions from summer or winter animals of four species on the same preparation and partial reversal by naloxone. (F) Effects of DADLE on the ileal preparation and partial reversal by naloxone.

examined to see if it could inhibit GPI contractility. The objectives of the study, therefore, were to:

1. observe effects of plasma albumin fractions from 13-lined ground squirrels, woodchucks, black bears, and polar bears in winter hibernation or the summer-active state on induced contractility of the GPI;
2. record the effects of DADLE on GPI contractility; and
3. determine whether naloxone antagonizes the effects of HIT or DADLE on the GPI.

METHOD

Animals

Thirteen-lined ground squirrels (*Citellus tridecemlineatus*) and woodchucks (*Marmota monax*) were live-trapped in the summer and placed (in individual cages) into a cold room hibernaculum in late fall. Blood samples were drawn by cardiac puncture from woodchucks when animals were in deep torpor as indicated by posture, responsiveness, and respiratory rate. The black bear blood sample was collected in March in western Pennsylvania while the bear was still in its den, nearing the end of winter torpor. Both summer-active and hibernating samples were obtained from ground squirrels by decapitation and from a restrained summer black bear's femoral vein. The polar bear sample was taken from a male (#6602) in late winter on the Beaufort Sea in Alaska. Details of this animal's behavior and capture are found elsewhere (3).

As Amstrup reported [cited in (3)], the only polar bears to exhibit extended winter denning are pregnant females. During the fall–spring period, when pregnant females occupy maternity dens, the remaining portions of the population remain active on the sea ice. Food is theoretically available, but most polar bears lose significant weight during the dark winter months. If polar bears can facultatively invoke a metabolic state similar to hibernation, it would be expected during this winter negative foraging period. To use Nelson et al.'s terminology (10), polar bears wintering in the Beaufort Sea area should be either hibernators in dens or "walking hibernators" when not in dens. The polar bear in this study was captured on April 18 and immobilized with 1400 mg Telezol (A.H. Robbins Co., Richmond, VA).

All whole-blood samples from ground squirrels, woodchucks, and black bears were centrifuged in the cold (7°C) to separate plasma from packed cells. Polar bear blood was allowed to clot in the field and then centrifuged to harvest the serum. Yields were frozen and stored at −75°C until thawed for albumin fraction preparation.

Albumin Fraction Preparation

Affinity chromatography procedures were employed to isolate albumin from whole ground squirrel, woodchuck, and black bear plasma and polar bear serum. Details are found elsewhere (15). The resulting albumin fraction was lyophilized and 10 mg lyophilisate dissolved in Krebs solution just before being added to the muscle bath.

GPI Preparation

The GPI is a well-known model demonstrating opioid depression of electrically induced contraction (8). Male or female Hartley guinea pigs averaging 460 g were killed by cervical dislocation and the small intestine isolated. A 3-cm long section of ileum was excised 15 cm proximal to the ileocecal junction. The longitudinal muscle with attached myenteric

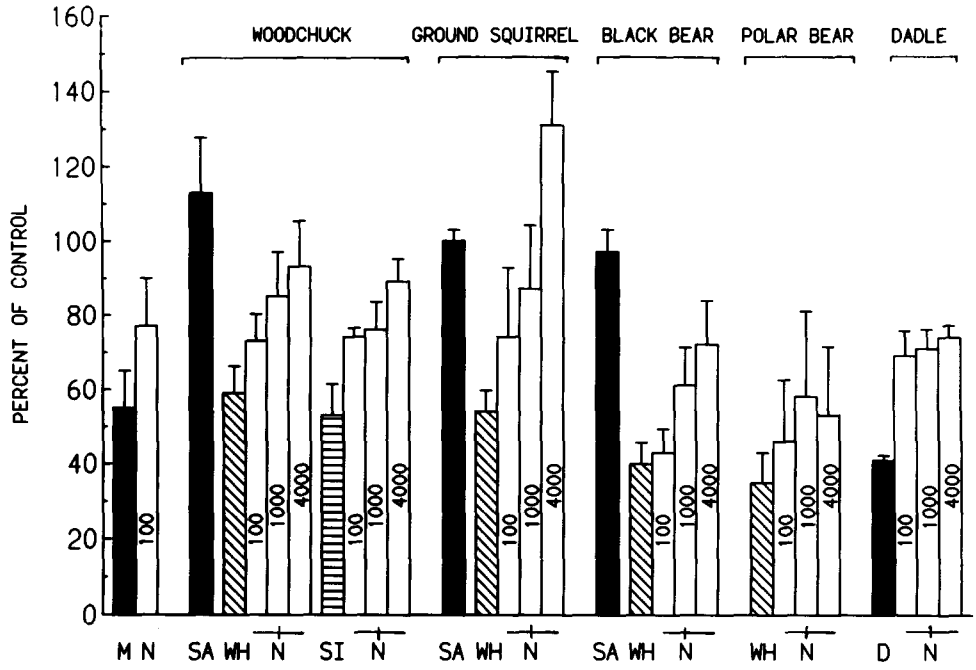

FIG. 2. Suppression of induced muscle contractility of the GPI by morphine and plasma or serum albumin fractions from four species of hibernating mammals, and DADLE, expressed as percentage of control contractility. M, L-morphine (400 nM); N, naloxone (nM); SA, summer-active fraction; WH, winter-hibernating fraction; SI, summer-induced hibernating fraction; D, DADLE.

plexus was removed by the method of Rang (18) and suspended in a muscle chamber containing Krebs bicarbonate buffer solution with 70 μM hexamethonium bromide and 0.125 μM mepyramine maleate (8). A temperature-controlled (37°C) Phipps and Bird (Richmond, VA) isolated organ bath enclosed the muscle chamber, which was aerated by bubbled 95% O_2:5% CO_2. Field stimulation through two platinum electrodes from a Grass (Quincy, MA) SD-9 stimulator (80V, 0.1 Hz, 0.25-ms pulse duration) induced contractions of the GPI strip. Contractions were recorded using a Grass FT-03 force transducer with a Gould (Cleveland, OH) 3400 thermal trace recorder. During a 90-min equilibration period, the GPI was rinsed with Krebs solution at 10-min intervals; a resting tension of 500 mg was maintained for all recordings.

TABLE 1

SUPPRESSION OF CONTRACTILITY IN GPI BY MORPHINE, PLASMA ALBUMIN FRACTIONS, OR DADLE AND RESTORATION BY NALOXONE

	Mean % of Control (± SEM)	Recovery With Naloxone (% of Control ± SEM)		
		100 nM	1,000 nM	4,000 nM
400 nM morphine	55 (10.1)	77 (13.0)	–	–
Woodchuck				
Summer active	113 (14.6)	–	–	–
Winter hibernating	59 (7.2)	73 (7.2)	85 (12.0)	93 (12.3)
Summer-induced hibernating	53 (8.4)	74 (2.5)	76 (7.5)	89 (6.1)
13-Lined ground squirrel				
Summer active	100 (3.0)	–	–	–
Winter hibernating	54 (5.6)	74 (18.7)	87 (17.1)	131 (14.3)
Black bear				
Summer active	97 (6.0)	–	–	–
Winter hibernating	40 (5.7)	43 (6.3)	61 (10.2)	72 (11.8)
Polar bear*				
Winter hibernating	35 (8.0)	46 (16.5)	58 (23.0)	53 (18.5)
DADLE	41 (1.3)	69 (6.7)	71 (5.2)	74 (3.2)

*No summer fraction.

Recording Technique

Once the GPI strip stabilized, trials using 400 nM morphine, 10-mg albumin fractions dissolved in 30 ml Krebs solution, and 0.05 mg DADLE in 30 ml Krebs solution were performed. During experiments in which depressed contractility was observed, naloxone at 100, 1,000, and 4,000 nM concentrations was applied successively to see if contractility could be restored.

RESULTS

Figure 1A shows the inhibitory effect of 400 nM L-morphine (as morphine sulfate, Sigma Chemical Co., St. Louis, MO) on induced GPI contractility and its restoration with 100 nM naloxone. (This and other panels show representative results from three to five trials with each substance.) Figures 1B–1D show the effects of summer and winter plasma albumin fractions from woodchucks, ground squirrels, and black bears on GPI contractility. The effect observed is a decrease in contraction amplitude of individual contractions (in several cases, a tonic increase occurs initially when the albumin fraction is added). Figure 1E is a recording of the effect of serum albumin fraction from a winter polar bear on the GPI prep, and Figure 1F shows the effect of DADLE on the same prep. It is noted that summer fractions from the various species had little or no effect on induced contractility; winter-hibernating fractions all suppressed contractility, as did plasma albumin fraction from a woodchuck induced to hibernate in the summer by injection of hibernating woodchuck plasma from the previous winter (panel B). Naloxone partially reverses such contractility inhibition (panels B–E), based partly upon dose. DADLE also inhibits contractility (panel F) and can be partially antagonized by naloxone. Figure 2 and Table 1 demonstrate suppression of contractility by the several hibernating fractions and DADLE [as a percentage of control contractility (i.e., the period of contraction before a substance was added)], as well as restoration by naloxone (as % of control).

DISCUSSION

As displayed in Fig. 1, 10-mg albumin fractions from summer-active animals of each species had no suppressing effect on contractility; summer woodchuck albumin fraction actually increased contractility about 40% above control. This suggests that plasma from summer-active woodchucks does not contain an HIT substance but actually contains a substance that opposes such activity and enhances contractility of the guinea pig ileal strip. Oeltgen et al. (11,17) have shown that the delta-opioid agonist, DADLE, effectively induced summer hibernation in ground squirrels but mu- and kappa-opioid agonists antagonized HIT-induced hibernation. It could be speculated that the effect of the summer woodchuck plasma in the present study was an expression of the presence of kappa-agonists. Oeltgen et al. (12), in fact, demonstrated that dynorphin A (a kappa-opioid from brain) antagonizes HIT-induced hibernation in summer-active ground squirrels. By contrast, fractions from winter-hibernating animals produced contractility suppression from 27% (polar bear) to 65% of control (woodchuck) (Fig. 2, Table 1). Naloxone antagonized the suppression in all cases, especially at 4000 nM, although it did not return contractility to control levels.

These results support the hypothesis that mammals in winter hibernation possess an HIT substance in their blood that has an effect on the GPI model similar to morphine, suppressing induced contractility. That suppression is at least partially reversible by the opiate antagonist naloxone. The HIT substance is lacking in summer animals of each species (no polar bear summer serum was available) since no suppression of contractility is seen when summer albumin fractions are employed. DADLE, a ligand that specifically binds the delta-opiate receptor, similarly suppresses GPI contractility. That inhibition can also be partially reversed by naloxone.

CONCLUSIONS

We conclude from this study that winter members of these species possess an HIT substance in their plasma albumin: woodchuck [*M. monax*], 13-lined ground squirrel [*C. tridecemlineatus*], black bear [*Ursus americanus*], and polar bear [*Ursus maritimus*]. Summer animals of the first three species do not (no information was gathered on summer polar bear serum). The HIT compound appears to be opioid in nature, bind to the delta-opiate receptor, and therefore to be similar to leu-enkephalin.

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