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Circannual Variations in Bear Plasma Albumin and Its Opioid-Like Effects on Guinea Pig Ileum

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BRUCE, D. S., E. C. BAILEY, D. P. SETRAN, M. S. TRAMELL, D. JACOBSON, P. R. OELTGEN, N. D. HORTON AND E. C. HELLGREN. *Circannual variations in bear plasma albumin and its opioid-like effects on guinea pig ileum.* PHARMACOL BIOCHEM BEHAV 53(4) 885–889, 1996.—Previous studies suggest that hibernation is controlled by an opioid system. In this study we examined the effect of plasma albumin fractions drawn from black bears at timed intervals while in hibernation or during the awake state in fall and winter, on induced contractility of the guinea pig ileum. Four hundred nM morphine produced typical suppression of contractility and 400 or 1000 nM naloxone (an opiate antagonist) restored it. Twenty mg of lyophilized albumin fraction from the winter hibernating bear caused similar suppression, the effect being greater than that of either summer bear or winter-active bear plasma albumin. Naloxone reversed the suppression in all cases. Strong suppression of contractility was also demonstrated with 2.5 nM [D-Pen^{2,5}]-enkephalin (DPDPE), a δ agonist, but only minor suppression with 2.5 nM dynorphin A, a κ agonist. Results support the opioid nature of the albumin-bound hibernation-induction trigger substance, that it binds to the δ opiate receptor, and that δ agonist opioid production may increase during the hibernation season.

Hibernation Hibernation-induction trigger Black bear Opioid Guinea pig ileum Naloxone DPDPE Dynorphin A

DAWE and Spurrier (6) demonstrated the ability of blood from a hibernating animal to induce hibernation in summer-active 13-lined ground squirrels, suggesting the existence of a blood-borne substance that triggers the onset of mammalian hibernation. This hibernation induction trigger or HIT has been shown to be bound to the albumin fraction of the plasma (13), to be a small protein (14), and to be present in woodchucks (8,13), two species of bats (2), the 13-lined ground squirrel (6,7), the black bear (3,18), and the polar bear (4,5).

Although HIT appears to be ubiquitous in hibernators, the mechanism of its action is unknown. There is strong evidence that hibernation may be regulated through endogenous opioids (1,11). Summer hibernation in HIT-injected ground squirrels is antagonized by simultaneous infusion of naloxone, a potent opiate antagonist (3). In in vitro studies, HIT suppresses electrically stimulated contractions of the guinea pig ileum myenteric plexus-longitudinal muscle strip in a manner similar to morphine; contraction is restored with naloxone (3–5,15). Of the three opiate receptors (mu, kappa, and delta), the delta receptor seems to bind to HIT during induction of summer hibernation (15,16), while mu and kappa receptor ligands antagonize HIT-induced summer hibernation in 13-lined ground squirrels, and may be involved, rather, in arousal from hibernation.

In this study, we examined the effects of plasma albumin fractions from the black bear (*Ursus americanus*) on induced contractility of the guinea pig ileum (GPI). Blood samples were collected monthly during the hibernating season from October to March; summer samples were collected in July and August. One bear entered hibernation in November and remained in hibernation for the duration of the study. The other bear remained active throughout the winter hibernation

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season, moving about daily and eating during the same period. Our hypothesis was that bears in hibernation should have higher concentrations of albumin-bound HIT than summer-active or winter-active bears.

The objectives of this study were therefore to: 1) examine black bear plasma samples for opioid activity using the GPI assay and naloxone, and 2) record seasonal fluctuations of HIT (opioid) levels throughout the winter hibernation season in an active and a hibernating black bear.

METHODS

Experimental Animals

Two black bears (*Ursus americanus*) were captured in the wild and monitored in captivity over a 7-month period. One bear (bear 2) entered hibernation after 2 months in captivity (as determined by denning behavior and cessation of feeding), and remained in hibernation for the duration of the investigation. The other bear (bear 1) remained active during the entire period, feeding daily. Blood samples were drawn monthly from each bear from October to March. The blood was centrifuged in the cold, the plasma harvested and stored at −25 to −75°C until thawed for albumin fraction isolation. Plasma collected from three summer active bears was pooled and similarly prepared for the GPI assay. Complete details on housing and handling these bears are found elsewhere (10).

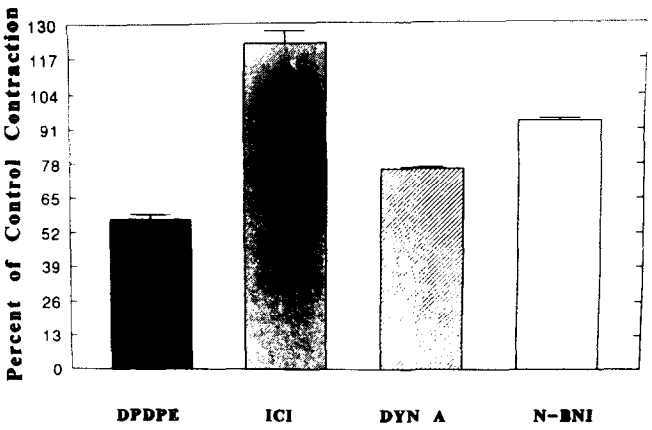

FIG. 2. Mean (± SEM) suppression of GPI-induced contractility by DPDPE and dynorphin A, and recoveries with ICI-174,864 and nor-BNI.

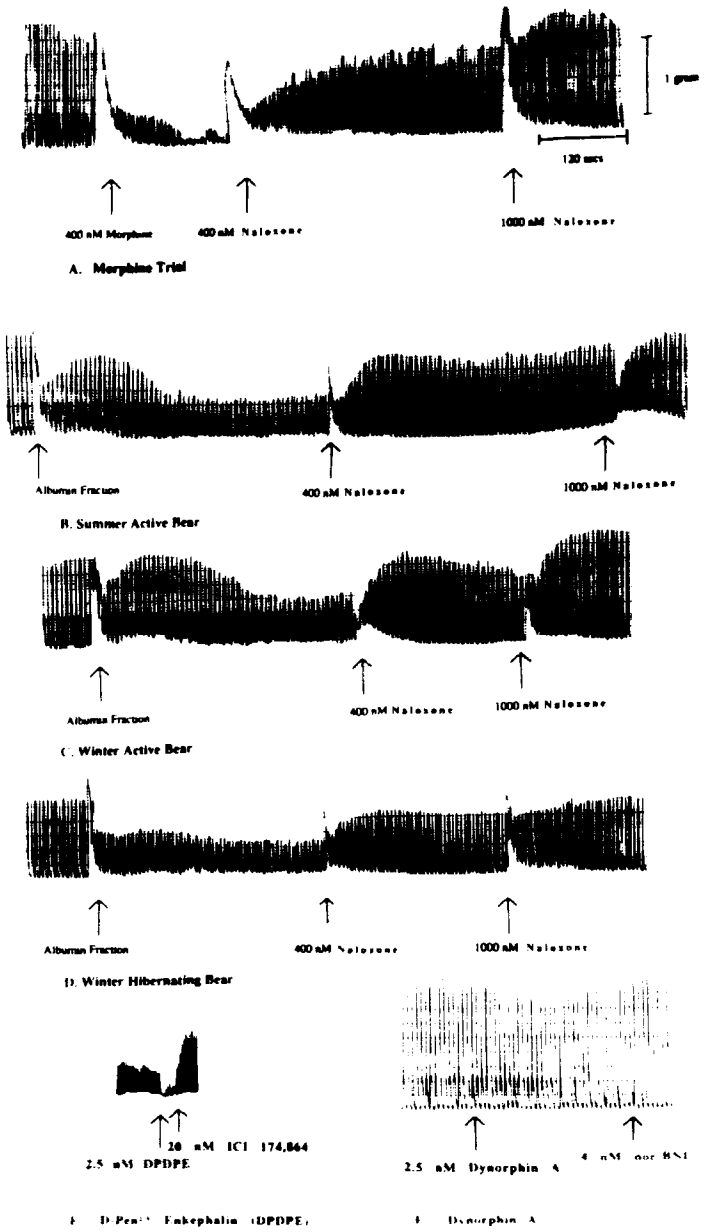

FIG. 1. Representative GPI contraction recordings.

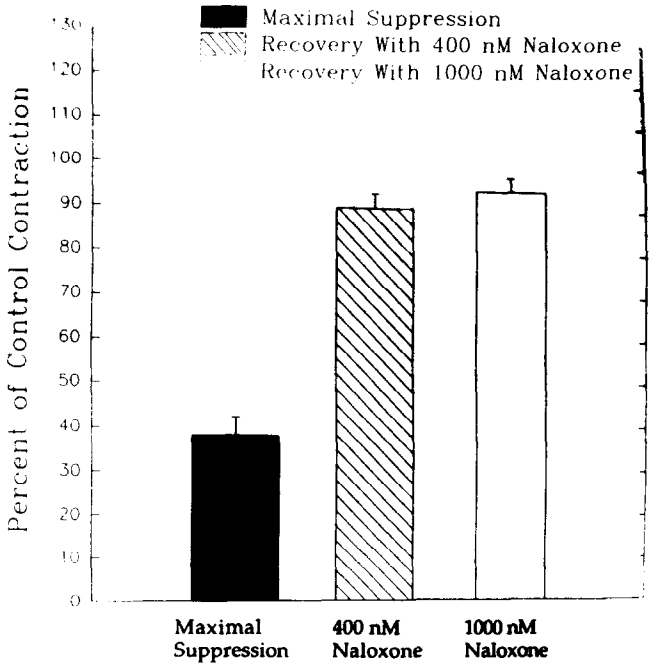

FIG. 3. Average suppression of induced contractility of guinea pig ileum (GPI) by summer-active bear plasma albumin fraction.

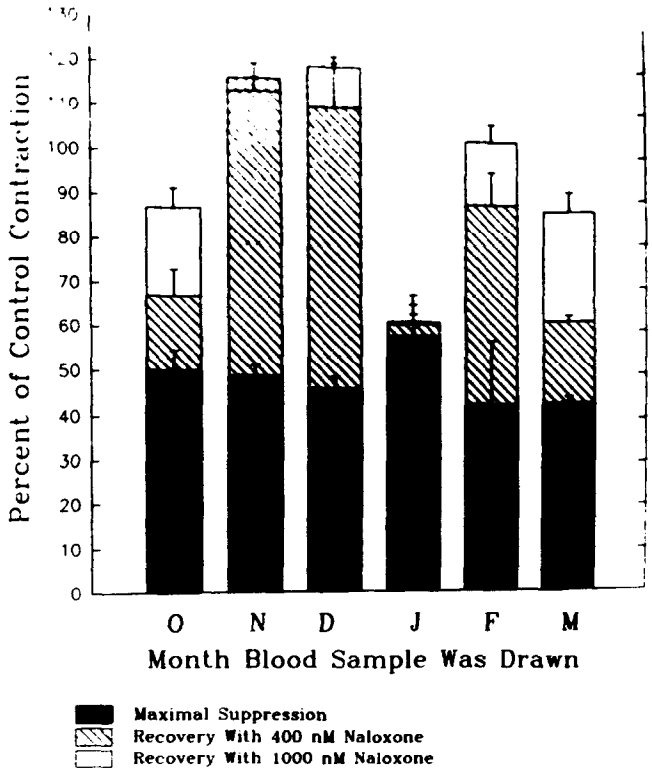

FIG. 4. Average suppression of induced contractility of guinea pig ileum (GPI) by winter-active bear (bear 1) plasma albumin fraction.

Albumin Fraction Preparation

Affinity chromatography with Affi-Gel Blue (Bio-Rad Industries, Richmond, CA) as the matrix was used to obtain a highly homogeneous albumin fraction. In our protocol, a 15.5 × 1 cm affinity chromatography column was packed with a 10 ml bead volume. The resulting albumin fraction was lyophilized, and 20 mg of lyophilisate dissolved in Krebs solution before being added to the muscle chamber.

Guinea Pig Ileum (GPI) Isolation and Assay

Male Hartley guinea pigs weighing 350–600 g were sacrificed by cervical dislocation, and a 1.5 cm segment of ileum longitudinal muscle with attached myenteric plexus, 15 cm proximal to the ileo-cecal junction, was isolated and prepared by the method of Rang (17). The GPI was suspended in a 10 cc muscle chamber containing Krebs bicarbonate buffer solution with 70 mM hexamethonium bromide and 0.125 mM mepyramine maleate (9), which was continually aerated with 95% O_2, 5% CO_2 and maintained at 37°C.

Contractions were induced by field stimulation via two platinum coil electrodes (stimulus parameters: 80 V, 120 mA, 0.1 Hz, 0.1 ms pulse duration), and recorded with a Grass FT03 force transducer (Grass Instrument Co., West Warwick, RI) on a Gould 3400 thermal trace recorder (Gould, Inc., Cleveland, OH). A resting tension of 250 mg was applied to the contracting GPI. The GPI was allowed to equilibrate for 90 min with Krebs solution rinses every 15 min.

Twenty milligrams of lyophilized bear plasma albumin was dissolved in 11 ml of Krebs buffer and immediately injected into the muscle chamber. If suppression of contractility occurred, naloxone at 400 nM and 1000 nM concentrations was applied in succession to determine if contractility could be restored. At least three albumin samples from each collection date for each bear were applied. Between runs, the GPI was rinsed with Krebs buffer and allowed to reequilibrate to control levels prior to injection of the next albumin sample.

RESULTS

Figure 1 shows representative GPI contraction records. In panel A is the typical suppression of induced contractions by 400 nM morphine and recovery with 400 and 1000 nM naloxone. Panel B shows the effect of adding albumin fraction from summer active black bears and naloxone reversal. Panel C is a typical recording following addition of fraction from the winter active bear, and D shows a record from the winter hibernating bear's albumin. Panels E and F are typical recordings of the effects of 2.5 nM [D-Pen^{2,5}]-enkephalin (DPDPE), a potent δ agonist, and 2.5 nM dynorphin A, a selective κ agonist, and recoveries with 20 nM ICI-174,864 (a selective δ antagonist) or 4 nM nor-binaltorphimine (nor-BNI), a κ antagonist. Figure 2 shows the average suppressions produced by DPDPE and dynorphin. Figure 3 shows the average suppression of contractility by summer-active bear albumin fractions, and Fig. 4 demonstrates average suppression by fractions from the winter-active bear blood samples. Figure 5 shows average suppression by albumin fractions from the winter hibernating bear, and Fig. 6 summarizes suppression of GPI contractility by winter-active and winter-hibernating black bear blood samples. Table 1 shows the results of a two-way analysis of variance (bear, month) with multiple replications. Variation due to bear (hibernating, nonhibernating) as well as due to bear-month interaction were statistically significant

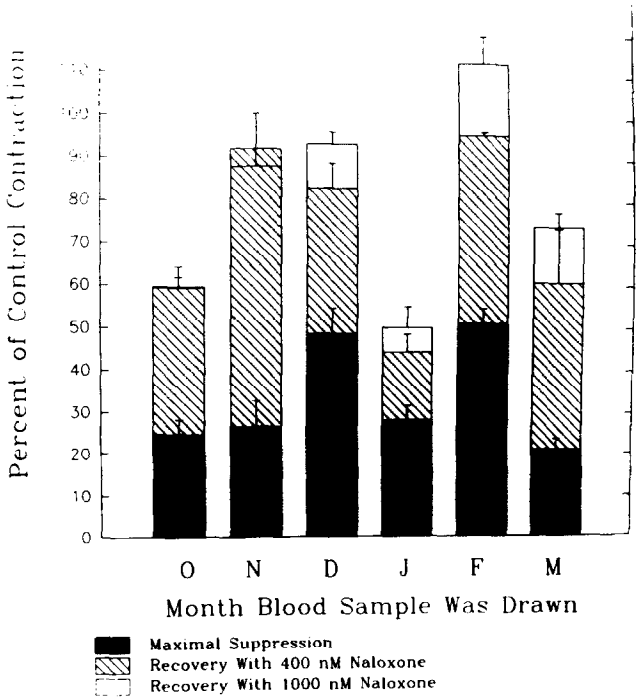

FIG. 5. Average suppression of induced contractility of GPI by hibernating bear (bear 2) plasma albumin fraction.

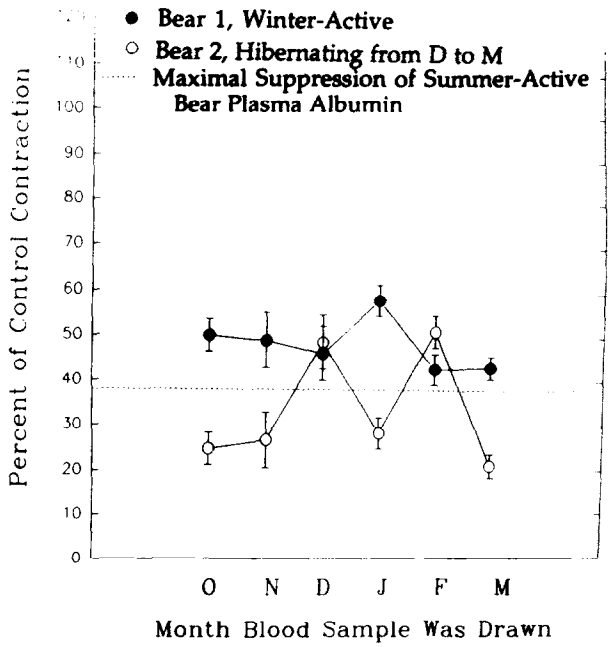

FIG. 6. Average suppression of induced contractility of GPI by plasma albumin samples from a winter-active black bear (bear 1) and a winter-hibernating black bear (bear 2).

(p = <0.01 or <0.02, respectively), whereas variation due to month the sample was collected was not significant.

DISCUSSION

It has previously been demonstrated that plasma albumin from torpid black bears is an effective inducer of summer hibernation in 13-lined ground squirrels (18), and that plasma from torpid black bears and denning polar bears suppresses GPI contractility similarly to morphine (4,5). In the present study, a series of plasma albumin samples from two black bears, one that hibernated and a second that remained active throughout the winter, as well as samples from three summer bears, were assayed for opioid activity.

Suppression by bear albumin of the electrically stimulated contractions of the GPI in a manner similar to morphine indicate the presence of the hibernation induction trigger, HIT. Summer bear albumin inhibited GPI an average of 40% of control contractility (Fig. 3). Albumin from the winter bear that remained active and feeding caused mean suppression of 46 to 60% of control, depending on the sample month (Fig. 4). Plasma albumin from the hibernating bear caused mean suppression of GPI contractility to between 22 and 50% of control, depending on month of draw. Even before the bear was considered to be in hibernation (e.g., still feeding lightly), its plasma albumin was effective at suppressing GPI induced contractility (in October and November; Fig. 5). This could be

TABLE I

DIFFERENCES IN MEAN GPI CONTRACTILITY SUPPRESSION BETWEEN WINTER-ACTIVE AND WINTER-HIBERNATING BLACK BEAR PLASMA ALBUMIN SAMPLES (TWO-WAY ANOVA, WITH REPLICATIONS)

Source	*df*	*F*-Statistic	*p*
Month	5	0.50	0.77
Error	15		
Bear	1	20.82	0.02*
Error	3		
Month/Bear	5	4.51	0.01*

*Significant.

an expression of "walking hibernation" (12): a bear can be alert and locomoting, but possess a biochemistry similar to that of a bear in hibernation, as shown by blood values of BUN, ketones, etc. Plasma albumin samples are effective at suppressing GPI-induced contractility, based on bear (winter hibernating vs. winter active) ($p < 0.02$) and bear-month interaction ($p < 0.01$), employing two-way ANOVA (Table 1). Furthermore, there is a strong suggestion that the albumin-bound HIT compound is opioid-like in nature, binding to the δ opiate receptor, since suppression is similar to that of 2.5 nM DPDPE, and can be similarly reversed with the δ antagonist, ICI-174,864. Dynorphin A, a κ receptor agonist, is much less effective at suppressing contraction (Fig. 2).

These results express the opioid nature of the regulatory system operating during hibernation. As has been demonstrated (15,16), it is the delta agonist that is effective at inducing summer hibernation; mu and kappa agonists antagonize induction of summer hibernation. In the present study, it could be speculated that mu and kappa receptor agonist ligands are produced in greater amounts during the summer or during periods in winter when the bear does not hibernate, but continues to remain active and feeding.

Blood samples drawn during these times are not as effective at inhibiting GPI induced contractility, as shown in this study. During hibernation, or just preceding it, delta agonist opioid production may increase, as evidenced by the greater suppression of GPI contractility.

It appears that this regulation is a very dynamic one, with perhaps all three opioid receptor agonists being produced year round, but at critically different levels, depending upon season. It remains to be seen how such levels are regulated, what the precise molecular structure of the HIT compound is, and how endogenous and exogenous cues "flip the hibernation switch" on and off.

ACKNOWLEDGEMENTS

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