Pharmacology Biochemistry and Behavior, Vol. 58, No. 3, pp. 621–625, 1997

0091-3057/97 $17.00 + .00

ELSEVIER

PII S0091-3057(97)00063-4

Hibernation-Induction Trigger. I. Opioid-like Effects of Prairie Dog Plasma Albumin on Induced Contractility of Guinea Pig Ileum

D. S. BRUCE,*[1] E. C. BAILEY,* S. K. CRANE,* P. R. OELTGEN,† N. D. HORTON† AND H. J. HARLOW‡

*Department of Biology, Wheaton College, Wheaton, IL 60187
†Department of Pathology, University of Kentucky College of Medicine and Pathology Service, Lexington Veterans Administration Medical Center, Lexington, KY 40536
‡Department of Zoology and Physiology, University of Wyoming, Laramie, WY 82070

Received 11 December 1995; Revised 17 December 1996; Accepted 17 December 1996

BRUCE D. S., E. C. BAILEY, S. K. CRANE, P. R. OELTGEN, N. D. HORTON AND H. J. HARLOW. *Hibernation-induction trigger. I. Opioid-like effects of prairie dog plasma albumin on induced contractility of guinea pig ileum.* PHARMACOL BIOCHEM BEHAV **58**(3) 621–625, 1997.—Studies have shown that plasma albumin fractions (PAFs) from hibernating mammals can inhibit induced contractility of the guinea pig ileum similarly to morphine. This study examined PAFs from two species of prairie dogs, one that undergoes natural seasonal hibernation (white-tailed, WT) and one that does not but can be induced to hibernate (black-tailed, BT). Dose–response curves of lyophilized PAF yielded IC_{50} values (mg) of 20.23 for summer WT, 15.53 for hibernating WT, 15.45 for summer BT, and 13.16 for winter-active BT. Winter samples from both species have IC_{50}s lower than samples from summer animals, indicating greater potency of winter PAFs in suppressing guinea pig ileum contractility and therefore the presence of more opioid ligands in winter prairie dog plasma. Studies to elucidate receptor selectivity of PAF continue. © 1997 Elsevier Science Inc.

Hibernation Prairie dog plasma Guinea pig ileum HIT Opioid

THE induction of hibernation in summer-active ground squirrels (*Citellus tridecemlineatus*) via infusion of plasma or plasma albumin from hibernating ground squirrels, bats, black bears or woodchucks indicates the presence of a circulating substance responsible for the onset of hibernation (7–9). This hibernation induction trigger (HIT), is present in plasma albumin fractions (PAFs) (12), and may exert its effects preferentially at delta (δ) opioid receptor sites, according to preliminary studies (13,14). The opioid-like activity of HIT can be reversed by the opioid antagonist naloxone both in vitro and in vivo (3,4,6,14). Previous investigations have indicated that the level of HIT changes seasonally in mammalian hibernators (2,3,6). In the present study, the winter and summer levels of HIT in PAFs were assayed in two prairie dog species: the white-tailed prairie dog (*Cynomys leucurus*), a natural hibernator, and the black-tailed prairie dog (*C. ludovicianus*), which does not hibernate in the wild. However, the black-tailed prairie dog is physiologically capable of hibernation, and can be induced to do so in fall or winter by the withholding of food and water and placement in a cold, dark room. PAFs obtained from both winter and summer prairie dog blood samples were examined for their effects on induced contractility of the guinea pig ileum–myenteric plexus preparation, a highly sensitive model demonstrating opioid depression of electrically induced contraction (10).

MATERIALS AND METHODS

Experimental Animals

Black-tailed and white-tailed prairie dogs, approximately 970–980 g body weight, were captured in the wild and main-

[1]To whom all reprint requests should be sent. E-mail: david.s.bruce@wheaton.edu

tained in captivity for the duration of the investigation. Blood samples were obtained via cardiac puncture; active animals were first anesthetized with Ketamine HCl, 60 mg/kg body weight. Hibernating prairie dogs received no anesthesia. Blood was drawn from 9 white-tailed (WT 1–9) and 10 black-tailed prairie dogs (BT 1–10); prairie dogs BT 1–5 and WT 1–5 had blood drawn during the winter months, and prairie dogs BT 6–10 and WT 6–9 had blood drawn during the summer months.

Albumin Fraction Preparation

Affinity chromatography with Affi-Gel Blue (Bio-Rad Industries, Richmond, CA) as the matrix was used to obtain a highly homogeneous PAF. A 15.5- × 1-cm affinity chromatography column was packed with 10 ml bead volume equilibrated with 0.02 M Na-phosphate buffer, pH 6.8. The albumin fraction was eluted with a 0.02 M Na-phosphate and 1.4 M NaCl buffer, pH 5.7, after which it was lyophilized and stored at −75° C.

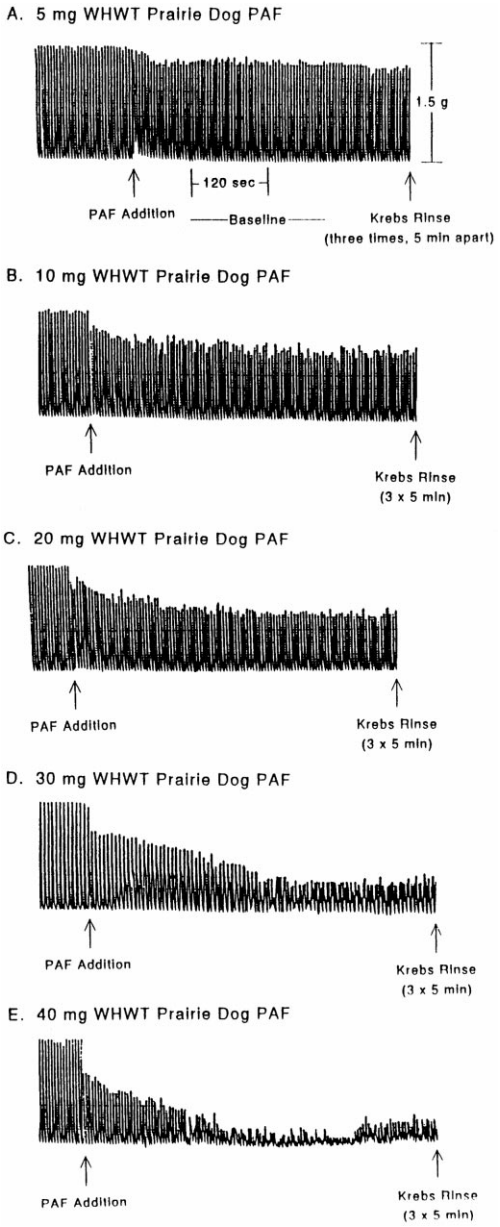

FIG. 1. Representative GPI contractions.

Guinea Pig Ileum (GPI) Isolation and Assay

Male Hartley guinea pigs weighing 400–600 g were killed by cervical dislocation. A 1.5-cm segment of ileal longitudinal muscle with attached myenteric plexus was removed 15 cm proximal to the ileocecal junction and prepared by the method of Rang (16). The GPI was suspended in a 10-cc muscle chamber containing Krebs bicarbonate buffer with 70 μM mepyramine maleate and continually aerated with 95% O_2– 5% CO_2 at 37°C.

Contractions of the GPI 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 transducer (Grass Instrument Co., West Warwick, RI) on a Gould 3400 thermal trace recorder (Gould, Inc., Cleveland, OH). A resting tension of approximately 250 mg was maintained on the GPI at all times. After isolation, the GPI was allowed to equilibrate for 90 min, with Krebs buffer rinses every 15 min.

The experimental procedure for the dose–response curves was as follows. During dose–response studies, the opioid sensitivity of the GPI was first corroborated with two successive additions of 400 nM morphine (Sigma Biochemical Co., St. Louis, MO) followed by 100 nM naloxone. All 19 prairie dog albumin samples were then assayed three times at 30 mg for their ability to inhibit the GPI contraction and for subsequent restoration with naloxone; in each case, naloxone at 100 nM was sufficient to restore GPI contractions to control levels. After the initial assay, two typical samples from each of the four groups of prairie dogs [summer-active white-tail (SAWT); winter-hibernating white-tail (WHWT); summer-active black-tail (SABT); and winter-active black-tail (WABT)] were chosen for dose-response curve determination. Lyophilized prairie dog PAF was reconstituted in 11 ml of Krebs buffer and applied to the GPI in progressively increasing amounts, with levels of 5, 10, 20 and 40 mg albumin; sufficient PAF was available from SAWT to allow runs at 50 mg albumin. In each case, runs for a given group (e.g., WHWT) were made on ileal segments from two guinea pigs, alternating between segments from different regions of the ileum from a given guinea pig.

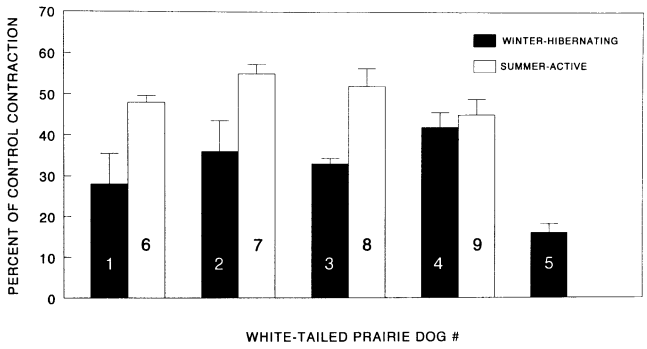

FIG. 2. Average inhibition of induced GPI contractility by 30 mg plasma albumin fractions from winter-hibernating and summer-active white-tailed prairie dogs.

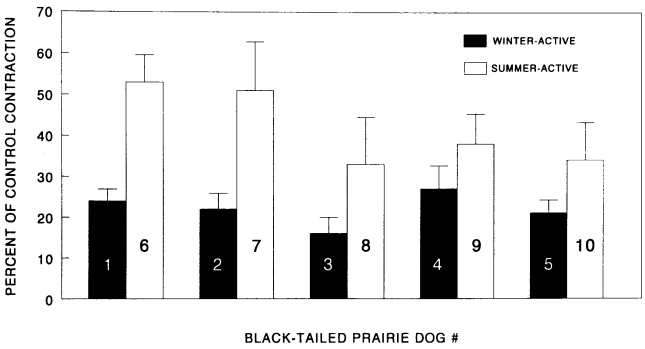

FIG. 3. Average inhibition of induced GPI contractility by 30 mg plasma albumin fractions for winter-active and summer-active black-tailed prairie dogs.

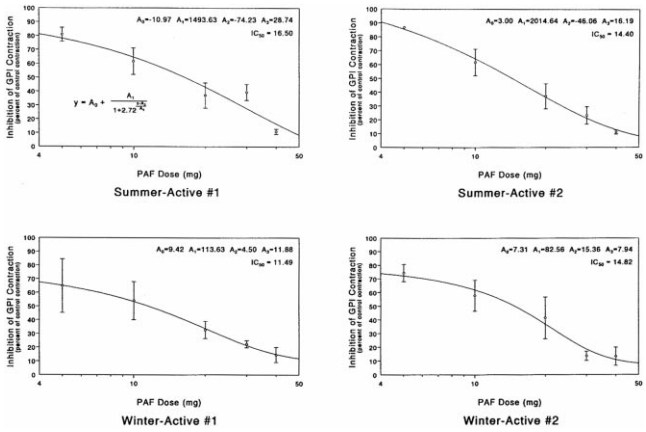

FIG. 5. Black-tailed prairie dog dose-response curves.

For a single dose–response curve, the GPI was rinsed twice with Krebs buffer during a 10-min period between PAF additions to restore contractions to control levels before applying the next, higher dose of albumin. After completing one dose series for a given sample, the GPI was allowed another 15 min, with Krebs rinses every 5 minutes, to re-establish baseline contraction. In this manner, any change in control contraction could be accounted for in the dose–response curve. Three replicates of each dose series were conducted, and the inhibition of contraction for each albumin dose was recorded as a percentage of control contraction. For a given group of prairie dogs, calculated IC_{50} values from each animal (e.g., WABT prairie dogs 1 and 2) were averaged and the standard error of the mean (SEM) determined.

RESULTS

Figure 1 shows a series of GPI contraction recordings used in construction of a dose–response curve for PAF from hibernating white-tailed prairie dog 1. Note the decrease in contractility with each increment of PAF. The results of the initial 30-mg assay for each of the white-tailed and black-tailed prairie dog PAF samples are shown in Figs. 2 and 3, respectively. These results demonstrate that PAF from winter animals of both species is more potent as a GPI contractility suppressor than PAF from summer animals (i.e., the IC_{50} value for winter PAF is lower than the summer value). Figure 4 is a composite of the dose–response curves for the four white-tailed prairie dogs studied (two SAWT and two WHWT), and Fig. 5 shows curves for the black-tailed prairie dogs (two SABT and two WABT).

The IC_{50}s (mg, with S.E.M.) for all prairie dogs studied are shown in Table 1. Mean IC_{50}s are 20.23 ± 0.02 for SAWT; 15.53 ± 2.14 for WHWT, 15.45 ± 1.05 for SABT; and 13.16 ± 1.67 for WABT. In both WT and BT prairie dogs, there is greater suppression of contractility at every dose by the winter PAF. Table 2 displays the results of unpaired *t*-tests comparing contractility suppression at 5-, 10-, 20-, 30- and 40-mg PAFs between SAWT and WHWT, between SABT and WABT, between SAWT and SABT and between WHWT and WABT groups. No significant differences are demonstrated for suppressions at any PAF doses from WHWT vs. WABT, whereas many differences for other comparisons are significant (1).

DISCUSSION

The PAFs from white-tailed and black-tailed prairie dogs, a hibernating and normally nonhibernating species, respectively, were assayed on the GPI to quantify the level of PAF

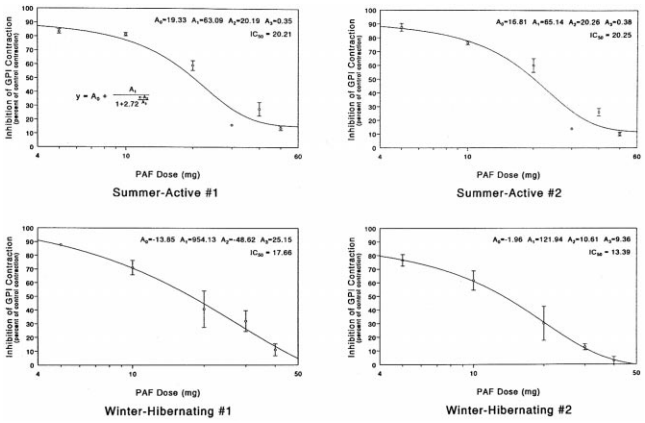

FIG. 4. White-tailed prairie dog dose-response curves.

TABLE 1

IC_{50} STATISTICS FOR ALL PRAIRIE DOGS STUDIED

	Animal IC_{50}	Treatment IC_{50}*
White-tailed prairie dog		
Summer-active 1	20.21	SAWT = 20.23 ± 0.02
Summer-active 2	20.25	
Winter-hibernating 1	17.66	WHWT = 15.53 ± 2.14
Winter-hibernating 2	13.39	
Black-tailed prairie dog		
Summer-active 1	16.50	SABT = 15.45 ± 1.05
Summer-active 2	14.40	
Winter-active 1	11.49	WABT = 13.16 ± 1.67
Winter-active 2	14.82	

*Mean ± SEM.

TABLE 2

RESULTS OF UNPAIRED *t*-TESTS COMPARING GPI CONTRACTILITY AT VARIOUS PRAIRIE DOG PAF DOSAGES

Dose (mg)	SAWT vs. WHWT	SABT vs. WABT	SAWT vs. SABT	WHWT vs. WABT
5	0.365	0.177	0.636	0.238
10	0.024*	0.402	0.017**	0.287
20	0.022*	0.723	0.014**	0.883
30	0.160	0.031*	0.006***	0.357
40	0.0004****	0.508	0.0002****	0.179

*Significant at $p \leq 0.05$.
**Significant at $p \leq 0.02$.
***Significant at $p \leq 0.01$.
****Significant at $p \leq 0.001$.

opioid activity in two closely related congeneric species. Eight dose–response curves were constructed: two for WABT, two for WHWT, and two summer-active dose–response curves for both species. In both species, the winter PAF sample had greater activity than the corresponding summer albumin sample, with IC_{50} values of 15.53 mg vs. 20.23 for white-tailed prairie dogs and 13.16 vs. 15.45 mg for black-tailed prairie dogs. Furthermore, both SABT and WABT PAF samples were more potent than the corresponding white-tailed samples. If hibernation is controlled only by an inducing ligand, the black-tailed prairie dog, normally a nonhibernator in nature, should have little or no opioid activity in either summer or winter PAFs. Results of this study—that all black-tailed prairie dog PAF samples and even SAWT PAF samples contained significant opioid activity, as determined by GPI bioassay—could suggest the mediation of (an) opioid(s) other than the HIT substance. As studies by Oeltgen et al. (13,15) have shown, μ– and κ-agonists antagonize HIT-induced hibernation. The additional opioid that could be present in the circulation of hibernators during the summer and throughout the year in facultative hibernators such as the black-tailed prairie dog might have significant μ– or κ–receptor activity. Thus, hibernation may be regulated by a multiopioid system. Alternatively or additionally, the effects seen could be due to seasonal changes in receptor populations in the animals' central nervous systems.

We did compare across species and detect a significant difference between SAWT and SABT at four of the five PAF dosages used (Table 2). In comparing WHWT and WABT, no significant differences are detected. For both comparisons, no physiological conclusions can be drawn, because we do not yet know the composition of HIT contained in plasma albumin, and it is almost certain that the protein composition of albumin from the white-tailed prairie dog is different from that of the black-tailed prairie dog. Therefore, until the HIT compound is characterized chemically, no direct comparisons between species can be made.

The GPI longitudinal muscle–myenteric plexus model is a sensitive screen to demonstrate opioid activity, but because it contains all three opioid receptor types (μ, κ and δ), it does not have high specific receptor selectivity. Future research in hibernation induction must examine the validity of a possible multiopioid, multireceptor hibernation control system. The use of opioid assays that will identify receptor-selective opioids is the logical first step in such an investigation; a variety of such assays is currently available. The hamster vas deferens (HVD) and mouse vas deferens (MVD) are tissues selectively rich in the δ opioid receptor (11,17), making them ideal assays to determine whether a specific δ receptor opioid is present in winter-hibernating PAF and absent from summer PAF and winter-active PAF samples. Albumin fractions could also be assayed with κ-specific and μ-specific opioid assays to determine the levels of each class of opioid on a seasonal basis and in relation to the physiologic state of the experimental animal. The GPI assay has demonstrated that opioid activity is present in albumin from hibernating species and in at least one species, the black-tailed prairie dog, which is not a natural hibernator. The HVD, MVD and related assays could provide resolution of PAF opioid activity into κ–, μ–, and δ–receptor-specific components. Information concerning receptor selectivity of timed albumin samples would be a significant step toward characterization of the proposed HIT, and perhaps one or several hibernation arousal triggers. Investigations designed to provide such information are currently underway. We describe studies with the mouse vas deferens in another report (5).

CONCLUSIONS

From these results, we conclude that PAFs from the white-tailed prairie dog, a hibernating species, and the black-tailed prairie dog, which normally does not hibernate, manifest significant opioid activity. In both species, winter albumin was more potent than summer albumin at inhibiting induced contractility of the GPI. However, all summer albumin samples contained significant opioid activity, as demonstrated by the GPI opioid assay. These data do not suggest a facile on/off mechanism regulating the hibernating state; rather, they are consistent with the existence of a multiopioid system regulating the entry into, and arousal from, hibernation. Such a dynamic system is supported by studies of Oeltgen et al. (13,15) and Bruce et al. (3); there is also the possibility that opioid receptor populations may change seasonally. Future research concerning the induction and control of hibernation must utilize receptor-specific opioid assays to resolve seasonal opioid activity in PAF samples into opioid-specific components.

ACKNOWLEDGEMENTS

This study was supported in part by the Wheaton College Summer Student Research Program, U.S. Army Medical Research & Development Command Contract DAMD 17-87-C-7070, and the Great Lakes Cluster of the Pew Foundation. We thank Alan S. Krober for help with statistical analysis and data presentation.

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