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Effects of an Anti-β-Endorphin Serum on Tonic Immobility in Rabbits

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ALOISI, A. M., A. E. PANERAI AND G. CARLI. *Effects of an anti-β-endorphin serum on tonic immobility in rabbits.* PHARMACOL BIOCHEM BEHAV 51(4) 577-579, 1995.—The involvement of endogenous β-endorphin (β-EP) in tonic immobility (TI) was evaluated in rabbits following the intracerebroventricular (ICV) administration of a specific antibody. Rabbits were tested twice with a 1-week intersession interval. For each animal, results of Session 1 were used as the baseline. Six hours before the beginning of Session 2, a bilateral ICV injection of specific anti-β-EP serum or aspecific γ-globulin (vehicle) was performed. Results showed that pretreatment with anti-β-EP serum increased TI duration, whereas no change occurred in the vehicle group. In a parallel experiment we evaluated TI duration in the presence of formalin pain: TI increased in animals pretreated with aspecific γ-globulins and decreased in the animals pretreated with anti-β-EP serum. Results suggest that the β-EP system acts to limit TI duration, but that this effect is reversed by persistent pain.

β-Endorphin Animal hypnosis Pain Formalin pain Rabbit

TONIC immobility (TI), also called animal hypnosis, is a transitory immobility that can be induced experimentally in many animal species by short-lasting physical stimuli (5,10,11). In rabbits TI can be easily elicited by a brief period of restraint in the supine position. TI has been studied in our laboratory as an experimental model to investigate persistent pain and opiate mechanisms (4,6,7).

A morphine-like mechanism for TI has been suggested (4), mainly because of the similarities between this condition and the immobility produced by morphine or β-endorphin (β-EP) treatment (6,8,12,15,17,21). Like morphine, TI blocks the pain reactions to persistent nociceptive stimulation by eliciting cataplexia and high-voltage slow waves in the EEG (3). Indeed, in a condition of prolonged nociceptive stimulation, not only can TI still be produced, but its duration is actually increased (7). Naloxone counteracts the increase in TI duration normally following both morphine treatment (6,15,17,21) and persistent nociceptive stimulation (4,6); in the latter instance it is able to reduce TI duration below baseline levels (6). However, in the absence of nociceptive stimulation, naloxone alone does not reduce the duration of TI except at a high dose (15 mg/kg) (6,9).

Because morphine and naloxone provide only broad indications about the involvement of endogenous opiates, our experiment aimed to assess the role of endogenous β-EP in TI duration by injecting a β-EP specific antibody (13). In addition, the effect of the same antibody was tested in rabbits experiencing formalin pain (22), a condition known to be associated with the release of β-EP by several brain structures (16).

METHOD

Adult male rabbits (*Oryctolagus cuniculus*), weighing 2.5-3.0 kg, were used in this study. Each rabbit was anesthetized with sodium pentobarbital [35 mg/kg, intravenously (IV)] and two guide cannulae were bilaterally implanted in the lateral ventricles. The efficacy of the cannulae was assessed in vivo 2 days after surgery, by testing the drinking behavior of the animals after an intracerebroventricular (ICV) injection of 10 ng of the potent dipsogen angiotensin II and, postmortem, by a brain dissection performed following the injection of 10 μl of ink into the two ventricles. Testing took place in the afternoon (1400-1700 h) in a moderately lighted, sound-isolated recording chamber equipped with white noise and ventilation. Tonic immobility (TI) was elicited by restraining the rabbit for 30 s on its back (induction) in a U-shaped trough (11). When the induction procedure failed to elicit immobility or when the immobility lasted < 45 s, induction was repeated up to three

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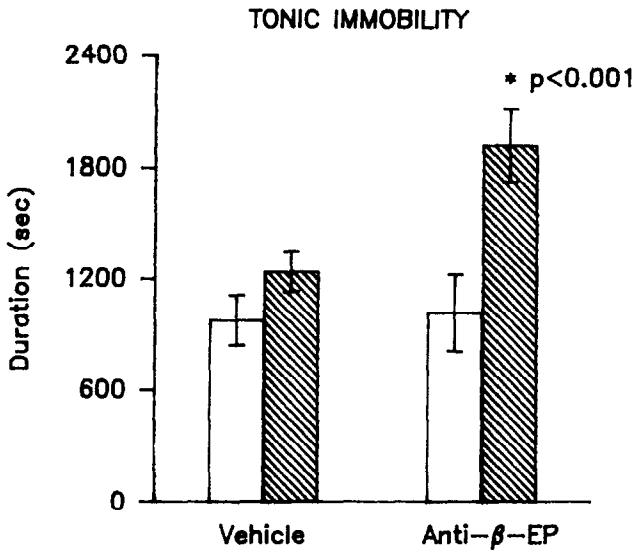

FIG. 1. TI duration (mean ± SEM) recorded before (Session 1, open bars) and after (Session 2, hatched bars) an ICV injection of vehicle (n = 8) or anti-β-EP serum (n = 8). *$p < 0.05$, LSD test.

times. The onset of TI was manifested by the disappearance of righting reflexes. The attempt by the animal to recover normal posture was considered the end of TI.

All animals were studied twice with a 1-week interval between the two sessions. Because of the high variability in TI duration among the rabbits, Session 1 was carried out to determine the individual baseline results and the effects of the anti-β-EP serum were assessed in Session 2. In each of the two sessions, TI was evaluated on the basis of five induction trials, at 2-min intertrial intervals. For each session, the parameters taken into account were: total amount of the time spent in TI (s) and, because each of the five trials could require up to three inductions, the number of inductions performed. Six hours before the beginning of Session 2, each animal was removed from its home cage and bilaterally injected with 10 μl of either γ-globulins extracted from normal serum (vehicle, n = 8) or anti-β-EP serum (anti-β-EP, n = 8), both redissolved in sterile water. After the ICV injections, the animals were left undisturbed in their home cages; this time lag was chosen on the basis of a previous study (13). The anti-β-EP serum is directed against the C-terminal portion of β-EP molecule; it displays 100% cross-reactivity with human β-lipotropin, camel β-EP 1-27 and camel β-EP 1-26, acetyl c-β-EP 1-31, 1-27, and 1-26, and 0.1% cross-reactivity with Met-enkephalin, but does not recognize ACTH, α-MSH, dynorphin, and Leu-enkephalin.

In a parallel experiment the same methodology as in the first experiment was used to evaluate the effects of the anti-β-EP serum in rabbits experiencing formalin pain. After Session 1, because in some of these animals TI duration was very low (mean ± SEM: 518 ± 195) (see Fig. 2), and because from previous observation (6) a large decrease could be expected after one of the treatments (i.e., anti-β-EP), these animals were assigned to the group in which an increase was expected (i.e., vehicle). In addition to the ICV injection of aspecific γ-globulin in the low TI duration rabbits (n = 5, vehicle/formalin) or anti-β-EP serum in the high TI duration subjects (n = 5, anti-β-EP/formalin), 5 min before the beginning of Session 2, all animals were injected subcutaneously (SC) with formalin (0.8 ml, 20%) in the dorsal part of the left hind paw. Attention was paid to the ethical guidelines for investigation of experimental pain in conscious animals (23).

Data were analyzed by two-way analysis of variance (ANOVA) involving the factors Treatment (vehicle, anti-β-EP) and Session (Sessions 1 and 2, repeated). Posthoc analysis was performed by the least significant difference (LSD) test. The data of the formalin-treated groups were analyzed by the paired t-test.

RESULTS

Two-way ANOVA applied to TI duration values revealed a significant effect for the main factors Treatment [$F(1, 14) = 4.29$, $p < 0.05$] and Session [$F(1, 14) = 13.14$, $p < 0.003$], whereas only a marginally significant effect was found in the interaction Treatment × Session [$F(1, 14) = 3.97$, $p = 0.06$] (Fig. 1). Posthoc comparison showed that these results were due to a significant increase present in the anti-β-EP group in Session 2 with respect to Session 1 ($p < 0.001$), whereas no significant difference was found in the vehicle group between the two sessions. The number of inductions was not modified by treatment.

As shown in Fig. 2, the paired t-test applied to TI duration in the formalin-injected groups revealed a significant difference between the two sessions in both the vehicle/formalin and anti-β-EP/formalin groups (n = 5, $t = 9.5$, $p < 0.005$, and $t = 6.3$, $p < 0.01$, respectively). This was due to the increase in TI duration in the animals belonging to the vehicle/formalin group and to the decrease recorded in the anti-β-EP/formalin group (Fig. 2).

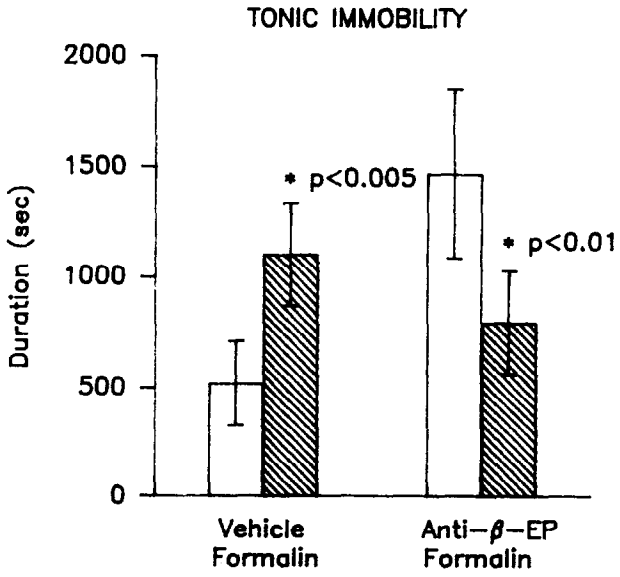

FIG. 2. TI duration (mean ± SEM) recorded before (Session 1, open bars) and after (Session 2, hatched bars) an ICV injection of vehicle (n = 5) or anti-β-EP serum (n = 5) in formalin-injected animals. *$p < 0.05$, paired t-test.

DISCUSSION

The increase in TI duration following pretreatment with anti-β-endorphin (β-EP) serum suggests the presence of a central β-EP system that stimulates the arousal mechanisms involved in restoring normal posture and vigilance. The present results are in line with sporadic observations in newborn chickens, in which the same effect was described after pretreatment with very low doses (0.1 and 0.5 mg/kg) of naloxone (14,15), and contradict the original hypothesis that the β-EP system would delay TI termination (7).

Further evidence of involvement of the β-EP system in the modulation of arousal mechanisms comes from experiments in which an increase in the locomotor/exploratory activities were found after an ICV injection of a low dose of β-EP (18) or the induction in the animal of low-intensity, short-lasting pain (1); this latter effect was completely counteracted by an ICV injection of a specific anti-β-EP serum (2).

In previous experiments we showed that TI duration is increased by formalin pain and that this increase is abolished by naloxone (6). It was then suggested that following formalin injection, there is a release of opiates that in turn potentiate TI. In the present experiment we confirmed the potentiation induced by pain and identified β-EP as being responsible for this effect.

It is interesting that treatment by the anti-β-EP serum as well as by naloxone (6) not only counteracts the increase in TI duration induced by pain, but produces a clear decrease in TI duration. Further studies will be necessary to demonstrate whether this effect depends on the disinhibition of the nociceptive system through blockage of the β-EP system, and/or upon the modifications of neuronal excitability and of the activity of opioid receptors described to occur in a condition of persistent pain (19,20).

In conclusion, our results indicate that the β-EP system exerts an opposite effect on the duration of TI according to the absence or presence of pain. This confirms that β-EP is implicated in several integrated complex responses and that its effects depend on environmental conditions.

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