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STATE-DEPENDENT VARIATION IN THE INHIBITORY EFFECT OF [D-Ala^2, D-Leu^5]-ENKEPHALIN ON HIPPOCAMPAL SEROTONIN RELEASE IN GROUND SQUIRRELS

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SUMMARY

Accumulated evidence has suggested that increased endogenous opioid activities may facilitate the onset of hibernation either directly or possibly through modulation of other neurotransmitter systems. The seasonal change of [D-Ala^2, D-Leu^5]-enkephalin (DADLE), a δ receptor agonist, in modulating K^+ (35 mM)-induced [^{3}H]-5-hydroxytryptamine (5-HT) release from the hippocampal and hypothalamic slices of euthermic and hibernating Richardsons' ground squirrels was therefore investigated. DADLE (0.1 - 10 μM) had no effect on 5-HT release in the hypothalamic slices but elicited a dose-related inhibition on [^{3}H]-5-HT release from the hippocampal slices of the euthermic ground squirrel. The inhibitory effect of DADLE was completely reversed by naloxone (10 μM), but not by tetrodotoxin (1 μM). In contrast, DADLE failed to alter the K^+-induced 5-HT release from the hippocampal slices of the hibernating ground squirrel. This state-dependent reduction in responsiveness to an opioid is consistent with the hypothesis that enhanced endogenous opioid activity in the hibernating phase could lead to down regulation of the opioid receptors and minimize its inhibition on hippocampal serotonergic activity. A high 5-HT activity would inhibit midbrain reticular activating system indirectly through non-serotonergic fibers, which in turn facilitate the onset or maintenance of hibernation.

Hibernation is a complex process which involves not only adaptational changes in peripheral tissues, but also extensive seasonal adjustments of central nervous system (CNS) thermoregulatory functions. Among the numerous neurotransmitters and neuromodulators within the CNS (for review see 1 and 2), the endogenous opioid has been implicated to be one of the likely candidates involved in the regulation of hibernation. For instance, increased overall brain levels of met- and leu-enkephalins (3) and increased met-enkephalin immunoreactivities have been observed in specific hypothalamic areas and the lateral septum during hibernation (4). Administration of opioid receptor antagonists, such as naloxone or naltrexone, to hibernating animals either reduces the incidence (5) and duration of hibernation (6), or

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initiates a premature termination of hibernation (7). Further, seasonal variations in thermoregulatory (8) and feeding responses (9) to exogenous opioids have also been reported which are correlated with the endogenous shift in physiological state during the annual hibernation cycle.

In rodents, a certain degree of overlap in the distribution of opioid receptors and serotonergic terminals in hippocampus has been demonstrated (10,11,12). It has been shown recently that the release of hippocampal 5-hydroxytryptamine (5-HT) can be modulated by various opioids (13). Based on the current model of neuronal regulation of body temperature (T_b) in sleep and hibernation (14), inhibition of the brain-stem reticular activating system by the septal-hippocampal complex is a fundamental determinant in initiating and maintaining T_b depression. Since the physiological exemplification of both opioid and 5-HT activities is primarily inhibitory (15,16,17), and these neurotransmitters have been implicated in the regulation of hibernation (for review see 1), it thus raises the possibility that these two neurotransmitters within the hippocampus may work in concert to initiate and maintain hibernation. If so, a state-dependent variation would be expected to reflect the seasonal difference in thermoregulation. To test this, the opioid receptor-modulated [^{3}H]-5-HT release in hippocampal and hypothalamic slices was investigated in a hibernator, the Richardson's ground squirrel.

Methods

Mature Richardson's ground squirrels (Spermophilus richardsonii) of both sexes were used in this study. They were live-trapped in the suburb of Edmonton, Alberta, and kept individually at an ambient temperature of 22°C under 12L:12D photoperiod with ad lib food (rat chow supplemented with sunflower seeds) and water. The pre-hibernation phase was characterized by a rapid weight gain followed by a weight plateau and anorexia. These animals were then placed in the cold (5°C) and dark without food in a walk-in environmental chamber and were examined daily for exhibition of hibernation by using sawdust technique. Hibernating state ground squirrels were animals having completed at least two hibernation bouts and still hibernating (T_b = 5-7 °C) by the time of sacrifice. The non-hibernating phase was evident when the animals showed no significant weekly weight change for at least two months before used for the experiment (T_b = 37-38 °C).

Animals were terminated by decapitation and brains were rapidly removed. Either hippocampus or hypothalamus was dissected out and sliced to a thickness of 0.3 mm using a McIlwain tissue chopper. The slices were incubated for 30 min at 37°C in 1 ml oxygenated Krebs' medium (pH 7.4) containing ascorbic acid (0.2 mM), pargyline (1 mM) and 0.1 μM [^{3}H]-5HT creatinine sulfate (specific activity 9.3 Ci/mmol, Amersham). After labelling, aliquots of 100 μl of tissue suspension were transferred to each of four superfusion chambers (about 80 mg of wet tissue per chamber) and superfused with Krebs' medium with a flowrate of 1 ml/min at 37°C. Slices within each chamber were stimulated twice at 46 min (S_1) and 76 min (S_2) after the onset of superfusion by exposure to a medium containing 35 mM KCl for 6 min. The S_1 was used as self-control and the drugs were added to the superfusion medium immediately after S_1 and remained present throughout the rest of the experiment.

Samples of the superfusate were collected at 2 min intervals 30 min after the onset of superfusion. At the end of the experiment, the slices were solubilized with 1.0 ml 1 N NaOH and the radioactivity in the slices and superfusate were determined by liquid scintillation spectrometry. The amount of ^{3}H released in a 2-min sample was expressed as fraction of total tissue ^{3}H content within the same chamber at the onset of the respective collection period. The percentage of radioactivity released above the basal level by the two pulses of K^+ was expressed as the ratio of S_2/S_1 for both the control and drug-treated slices. In order to quantify the effects of drugs on the stimulation-evoked outflow, the S_2/S_1 ratios of the drug-treated slices were compared with the ratios calculated under the respective control conditions. Statistical analyses were performed by using the two-tailed Student t-test. Significance was set at $p<0.05$ unless

otherwise stated.

The following chemicals were used: [D-Ala², D-Leu⁵]-enkephalin (DADLE), naloxone HCl and tetrodotoxin (TTX) were purchased from Sigma (St. Louis). Working solutions were dilutions using Kreb's solution of stock (1 mM), which were prepared with distilled water and stored at -10°C.

Results

Since an increase in brain enkephalin level has been reported in the hibernating animal (3,4), DADLE, a more stable and selective enkephalin analog was selected for the present study. The 5-HT outflow elicited in the hypothalamic control slices during the first period of stimulation (S_1) was 8.17±0.55% and the ratio of S_2/S_1 was 0.74±0.03 (n=7), these values were about the same as those observed in the hippocampal control slices (S_1 was 8.37±0.59% and S_2/S_1 was 0.72±0.04, n=7, respectively)(Fig. 1). In experimental slices, addition of DADLE (0.1 - 10 μM), immediately after S_1, inhibited the K⁺-evoked release of [³H]-5HT from the hippocampal slices in a dose-related manner (Fig. 1). The optimal suppression (about 26%) was observed in the presence of 10 μM DADLE. In contrast, inclusion of similar concentrations of DADLE in the perfusion medium failed to affect 5-HT release from the hypothalamic slices of the euthermic ground squirrels (Fig. 1). Only about 12% reduction in 5-HT outflow was observed even in the presence of the highest concentration of DADLE (10 μM) used in the present study.

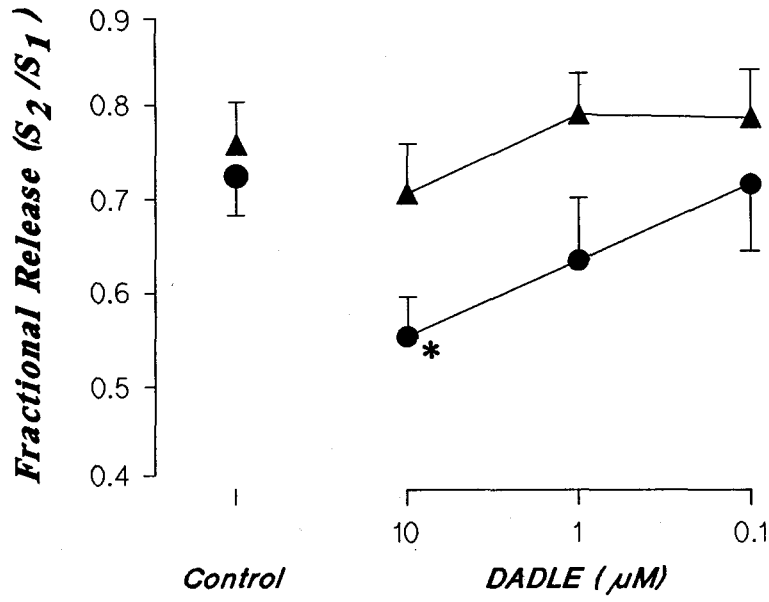

FIG 1

Effect of DADLE on K⁺-evoked [³H]-5HT release from either hippocampal (●) or hypothalamic (▲) slices of the euthermic Richardsons' ground squirrels. Each point represents the mean±s.e.m from seven experiments.

* Significantly different from hippocampal control, $p<0.05$

Because of the lack of inhibitory effect of DADLE on the hypothalamic slices, further studies on the pharmacological profile were carried out only on the hippocampal slices and the results are shown in Fig. 2. Inclusion of naloxone (10 μM), an opioid receptor antagonist, in the perfusion medium simultaneously with DADLE (10 μM) significantly attenuated the inhibitory effect of DADLE on K^+-evoked 5-HT release from the hippocampal slices. However, addition of TTX (1 μM), a compound which is known to inhibit the propagation of action potentials by blocking voltage-dependent sodium channel (18), in the medium did not affect the inhibitory effect of DADLE (Fig. 2). Either addition of TTX (1 μM) or naloxone (10 μM) alone in the perfusion medium did not affect the K^+-evoked release of [^{3}H]-5HT (Fig. 2).

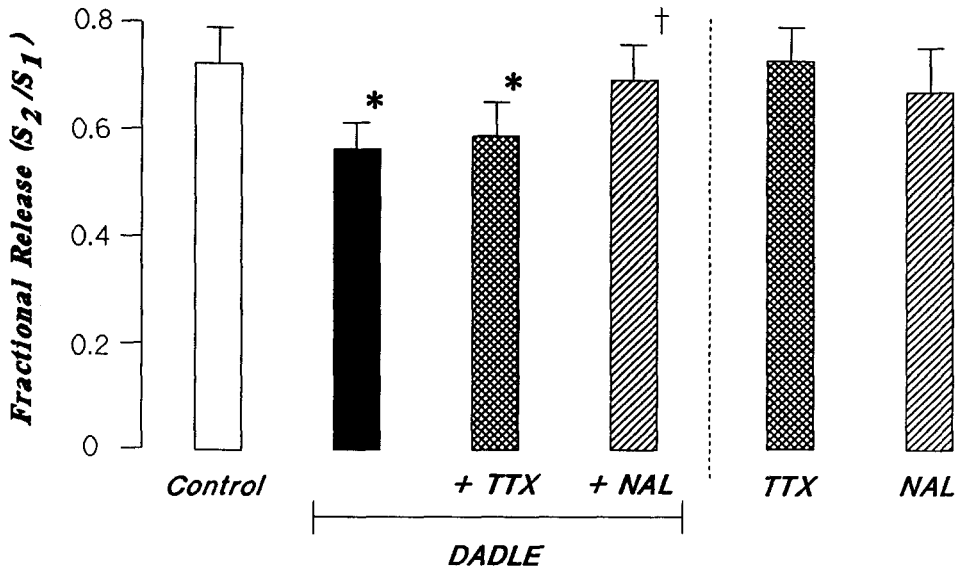

FIG 2

Effects of naloxone (NAL)(10 μM) and tetrodotoxin (TTX)(1 μM) on DADLE (10 μM) inhibitory effect on K^+-evoked [^{3}H]-5HT release from the hippocampal slices of the Richardsons' ground squirrel. Each bar represent the mean value of seven experiments. Vertical bar indicates the S.E.M.

* Significantly different from control, $p<0.05$;

† Significantly different from DADLE control, $p<0.05$.

To evaluate the possible state-dependent change in responsiveness to opioid, the effect of DADLE on 5-HT release from the hippocampal slices of the hibernating ground squirrel was compared with that from the euthermic animal and the results are summarized in Fig. 3. The pattern of 5-HT release in the hippocampal control of the hibernating animal (S_1 was 8.49±0.61% and S_2/S_1 was 0.80±0.09, n=5, respectively) was approximately the same as that observed in the euthermic hippocampal slices. In contrast to the dose-related inhibitory effect observed in the euthermic hippocampal slices, the same concentrations of DADLE (0.1 - 10 μM) did not affect the K^+-evoked 5-HT release (Fig. 3).

Discussion

It is well known that endogenous opioids can modulate the release of various neurotransmitters and their effects depended on the brain site studied and the type of opioid receptor subtypes

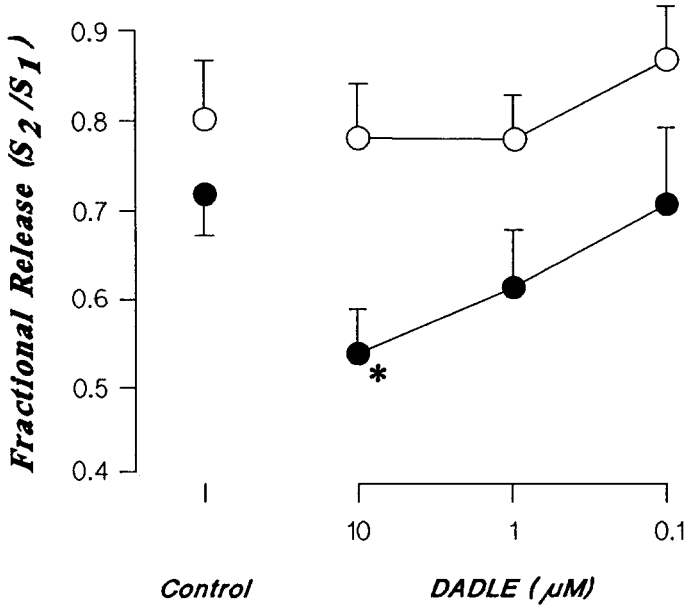

FIG 3
Effect of DADLE on K^+-evoked [^{3}H]-5HT release from hippocampal slices of either euthermic (●) or hibernating (○) Richardsons' ground squirrels. Each point represents the mean±s.e.m from 5-7 experiments.
* Significantly different from corresponding hippocampal control, $p<0.05$

activated (16). In the present study, we have demonstrated that DADLE caused a dose-related suppression of K^+-evoked [^{3}H]-5-HT release from the hippocampal slices of the non-hibernating Richardsons' ground squirrel. However, the same concentrations of DADLE did not affect the stimulated release of 5-HT from the hypothalamic slices. Even though a different δ receptor agonist was used in our study, the concentration of DADLE (10 μM) causing a significant inhibition of 5-HT release was comparable to the concentration of [D-Pen^2, D-Pen^5]enkephalinused in rats for the same purpose (13). Pretreating the hippocampal slices with naloxone (10 μM) completely attenuated the inhibitory effect of DADLE, indicating that DADLE elicited its effect via the opioid receptors. To further evaluate whether DADLE inhibits 5-HT release directly via receptors that are located on 5-HT neuronal terminals, or indirectly via an interneuron, TTX, a compound that blocks axonal conduction by preventing Na^+ influx (17), was used. Inclusion of TTX (1 μM) in the perfusion medium did not affect the inhibitory effect of DADLE, suggesting that DADLE elicits its effect directly via opioid receptors on the serotonergic terminals.

The most interesting finding of the present study is the lack of responsiveness of the hippocampal slices from the hibernating ground squirrel to the same concentration range of DADLE. As the pattern of K^+-evoked 5-HT release is about the same in both hibernating and euthermic animals, it is unlikely that the failure of DADLE to affect the 5-HT outflow in the hibernating animals is due to general depression. Rather, the lack of responsiveness may indicate an endogenous change in the hippocampal opioid system during the hibernating phase. To date, there has been much evidence indicating an increase in central opioid activity during the hibernating phase (see Introduction). It is quite possible that the reduced

responsiveness to DADLE observed during the hibernating phase may be due to a reduction in the opioid receptor efficacy resulting from an increase in endogenous opioid activity during this part of the annual cycle. This suggestion is supported by the finding that an overall hippocampal opioid receptor binding efficacy is decreased in the hibernating ground squirrel (19). However, since μ-and k-opioid receptors have also been shown to exist within the hippocampus (11,12), further studies are required to examine whether any state-dependent changes in the responsiveness of 5-HT overflow also occur after the activation of these two opioid receptor subtypes.

The net result of the reduced responsiveness to DADLE is a reduction of the inhibitory effect on serotonergic activity, this may lead to an increase of 5-HT release under neural stimulation from hippocampal slices of the hibernating animal. This finding tends to corroborate with the previous report that an increase of 5-HT turnover is observed in the hippocampus of the hibernating red-cheeked ground squirrel (20). Because of the similarity between the hibernation stage and the non-rapid eye movement sleep (21) and the endogenous serotonergic system has been suggested to play an important role in the regulation of sleep-wakefulness (22,23), alteration of central 5-HT metabolism has also been implicated in hibernation. The general consensus is that increased brain serotonergic activity facilitates entry into hibernation since the 5-HT turnover is higher during hibernation (20,24). Furthermore, injection of parachlorophenylalanine (25) or lesioning of the raphe nucleus (25,26) has been shown to prevent the onset of hibernation. Taken together, it may be speculated that the down-regulation of hippocampal opioid receptors resulted by an increase in endogenous opioid activity heightens serotonergic activity, which in turn indirectly elicits an inhibition on the midbrain reticular activating system through non-serotonergic fibers descending from the hippocampus and thus facilitates the initiation of sleep and entry into hibernation. Further studies are required to provide additional evidence in support of this scheme.

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References

1. L.C.H. WANG, Advances Comparative and Environmental Physiology. Vol. 2, R. Gilles (ed.), 1-46, Springer-Verlag, Berlin (1988)
2. L. JANSKY, J. Therm. Biol. 15: 329-347 (1990)
3. L.I. KRAMAROVA, S.H. KOLAEVA, R.Y. YUKHANANOV, AND V.V. ROZHANETS, Comp. Biochem. Physiol. 74C: 31-33 (1983)
4. T.F. LEE, F. NURNBERGER, M.L. JOURDAN AND L.C.H. WANG, Thermoregulation: Research and clinical application, eds P. Lomax and E. Schonbaum (eds.), 200-203, Karger, Basel (1989)
5. W. KROMER, Experientia 36: 581-582 (1980)
6. A.L. BECKMAN AND C. LLADOS-ECKMAN, C. Brain Res. 328: 201-205 (1985)
7. D.L. MARGULES, B. GOLDMAN AND A. FINCK, Brain Res. Bull. 4: 721-724 (1979)
8. L.C.H. WANG, T.F. LEE AND M.L. JOURDAN, Pharmacol. Biochem. Behav. 26: 565-571 (1987)
9. S.E. NIZIELSKI, A.S. LEVINE, J.E. MORLEY, K.A. HALL AND B.A. GOSNELL, Physiol. Behav. 37: 5-9 (1986)
10. A. PARENT, L. DESCARRIES AND A. BEAUDET, Neurosci. 6: 115-138 (1981)
11. N.A. SHARIF AND J. HUGHES, Peptides 10: 499-522 (1989)
12. K. STENGAARD-PEDERSEN, Prog. Histochem. Cytochem. 20 (No. 3): 47-67 (1989)
13. F. PASSARELLI AND T. COSTA, J. Pharmacol. Expt. Ther. 248: 299-305 (1989)
14. H.C. HELLER, Ann. Rev. Physiol. 41: 305-321 (1979)

15. M.F. CHESSELET, Neuroscience 12: 347-375 (1984)
16. P. ILLES, Rev. Physiol. Biochem. Pharmacol. 112, 139-233 (1989)
17. M. SEGAL, Brain Res. 94: 115-131 (1975)
18. C.Y. KAO, Pahramcol. Rev. 18: 997-1049 (1966)
19. A.L. BECKMAN, Living in The Cold, H.C. Heller, X.J. Musacchia and L.C.H. Wang (eds.), 225-234, Elsevier, New York (1986)
20. N.K. POPOVA AND N.N. VOITENKO, Pharmacol. Biochem. Behav. 14: 773-777 (1981)
21. H.C. HELLER, J. WALKER, G. FLORANT, S.F. GLOTZBACH AND R.J. BERGER, Strategies in Cold: Natural Torpidity and Thermogenesis, L.C.H. Wang and J. Hudson (eds.), 225-266, Academic Press, New York (1978)
22. M. JOUVET, Exp. Brain Res. Suppl. 8: 81-94 (1984)
23. W.P. KOELLA, Experientia 40: 309-338 (1984)
24. R. NOVOTNA, L. JANSKY AND Z. DRAHOTA, Gen. Pharmacol. 6: 23-26 (1975)
25. D.C. SPAFFORD AND E.T. PENGELLEY, Comp. Biochem. Physiol. 38A: 239-250 (1971)
26. B. CANGUILHEIM, J.L. MIRO, E. KEMPF AND P. SCHMITT, Am. J. Physiol. 251: R755-761 (1986)