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Modulation of Endogenous Opiate Production: Effect of Fasting

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SUMMARY: The endogenous opiate alkaloid content in tissues from fed, 24 h and 48 h fasted rats was determined. Plasma morphine and codeine concentrations did not change in response to fasting. Morphine levels in the spleen increased 3-fold after 24 h of fasting and were lower than fed rats by 48 h of fasting; no change was detected in spleen codeine levels. Brain morphine levels were elevated 5-fold after 24 h of fasting and were two-fold higher than those of fed rats after 48 h of fasting. Brain codeine levels did not change with fasting. These results indicate that opiate alkaloids are endogenously produced in rodent tissues, particularly in the spleen, liver, and adrenals. The synthesis of morphine, in the spleen and brain, is maximally stimulated after 24 h of fasting, without alterations in tissue codeine synthesis. These suggest differential regulation of the endogenous synthetic pathways of morphine and codeine in response to the stress of fasting.

The presence of non-peptide opiates in mammalian tissues has been reported several times during the last decade. Gintzler et al. (1) identified a non-peptide morphine-like compound that cross-reacted with morphine-specific antibodies in the mouse brain, bound to opiate receptors and had kinetics similar to those of morphine and naloxone. Later, Spector et al (2) and Weitz et al (3) demonstrated that this substance, when purified and analyzed by HPLC, was identical to morphine by immunological, pharmacological and physical-chemical criteria (4). These findings were followed by the demonstration that the final steps of the biosynthetic pathway to morphine in mammals were similar to those in the poppy plant (5,6,7,8). It has been postulated that the production of these alkaloids, similar to those of the opioid peptides, is elevated under situations of stress (9), as for example following food restriction (10). We recently presented evidence for an important immunomodulatory role of these endogenous alkaloids during periods of stress (11). All of these finding lead to hypothesize that the production rates of these alkaloids must be altered during periods of complete fast, and speculated that their elevations could potentially participate in the behavioral and metabolic responses observed during food restriction.

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MATERIALS AND METHODS: Animal preparation. Male Sprague-Dawley rats (275-325 g BW, Charles River, Wilmington, MA) were housed in a controlled environment, exposed to a 12:12 h light-dark cycle and fed standard rat chow (Purina) for one week before being randomly assigned to the fed (control) or fasted groups. Rats were sacrificed by decapitation at 24 and 48 h after initiating the fasting period. Time-matched fed controls were sacrificed after 24 and 48 h. Since values for control rats did not vary over the duration of the experiment, all values were combined. Trunk blood and selected tissue samples of spleen, liver, adrenals, thymus, kidney and brain were freeze-clamped (-80°C) until analysis of morphine and codeine.

Opiate analysis. Analysis was carried out using a combined HPLC/RIA technique (12) which is a modification of two previous published methods (13,14). Ethylmorphine (Sigma, St. louis, MO.) was used as an internal standard. Separation of morphine, codeine and ethylmorphine was achieved by a 50 min isocratic HPLC run. The flow rate was 1.0 ml/min, and 50 one-minute fractions were collected, dried at 50°C overnight and re-suspended in PBS for detection of immunoreactivity by a modified commercially available kit (Morphine Abuscreen RIA; Roche Diagnostics, Branchburg, NJ). The intra-assay coefficient of variation was 8% and the inter-assay coefficient of variation was less than 20%.

RESULTS: Body weight. Fasting resulted in 3% and 5% weight loss after 24 and 48 h, respectively. Liver glycogen decreased significantly from 29±4 mg/g in the fed rats to 6±1 mg/g and 7±1 mg/g after 24h and 48 h of fasting, respectively.

Opiate levels. Plasma morphine levels of the fed animals averaged 57±34 fmol/ml (Fig. 1) and did not increase after 24 h (90±37 fmol/ml) 48 h (41±17 fmol/ml) of fasting. Of the tissues analyzed, liver had the highest content of morphine in the fed rats and averaged 1905±632 fmol/g. Fasting for 2 days did not result in any changes in liver morphine or codeine contents. Morphine content of the adrenals did not show a significant increase in response to fasting and averaged 551±221 fmol/g (Fig. 1).

Spleen morphine content increased significantly from an average of 1209±364 fmol/g in the fed rats to 4428±1589 fmol/g after 24 h of fasting; these levels returned to lower than fed

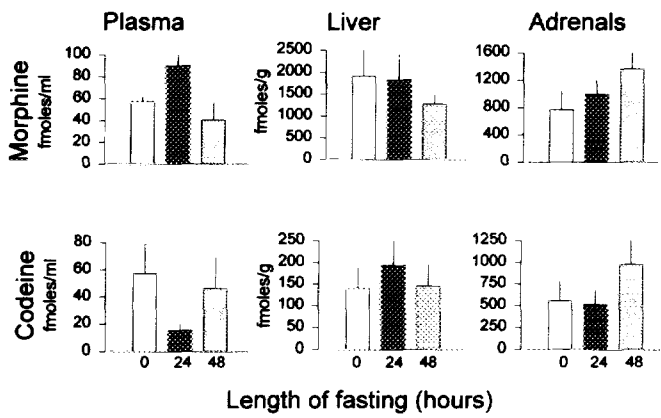

Figure 1. Endogenous morphine (top panels) and codeine (bottom panels) levels in plasma, liver and adrenals of fed, 24 h fasted and 48 h fasted rats. N=7-9 per group.

values after 48 h of fasting. Spleen codeine content did not increase with fasting (Fig. 2). Brain morphine levels showed a significant increase from 329±93 fmol/g in the fed rats to 1736±693 fmol/g after 24 h of fasting; the levels were still elevated after 48 h of fasting but were not statistically different from levels of fed rats. Brain codeine levels did not show any significant increase in response to fasting (Fig. 2).

Thymus morphine content averaged 381±100 fmol/g in the fed rats and increased to 507±107 fmol/g after 24 h of fasting (Fig. 3). Thereafter, levels remained elevated and averaged 506±63 fmol/g. Thymus codeine content was not detectable in the fed rats; was 70±37 fmol/g in the 24 h fasted rats and 36±36 fmol/g in the 48 h fasted rats. Kidney had the lowest levels of morphine among the tissues analyzed and averaged 61±16 fmol/g. The content of morphine in the kidney did not show any significant change as a result of fasting and averaged 57±12 fmol/g and 63±10 fmol/g in the 24 and 48 h fasted rats, respectively. Codeine levels were 21±4 fmol/g in the fed animals and did not differ significantly from those of 24 h and 48 h fasted rats.

DISCUSSION

The present findings indicate that fasting is associated with significant tissue-specific alterations in morphine levels without any noticeable change in tissue codeine. These changes were not accompanied by any significant alterations in plasma levels of either opiate, suggesting that their tissue-specific production rates and/or binding are selectively altered with caloric deprivation.

Previous studies from our laboratory have demonstrated a clear and direct correlation between intravenous or intracerebroventricular morphine administration and the degree of

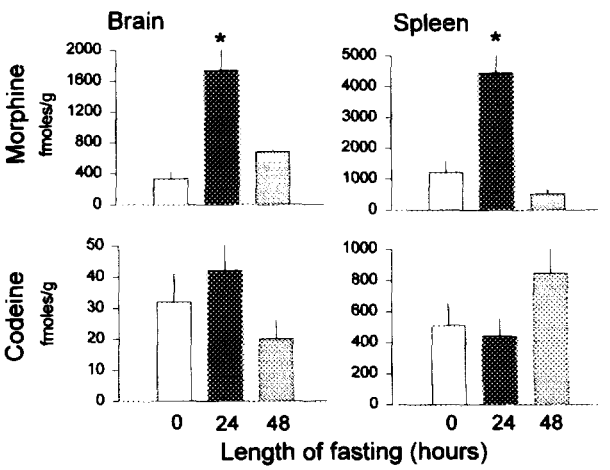

Figure 2. Endogenous morphine (top panels) and codeine (bottom panels) levels in brain and spleen of fed, 24 h fasted and 48 h fasted rats. N=7-9 per group, *p<0.05 compared to fed animals.

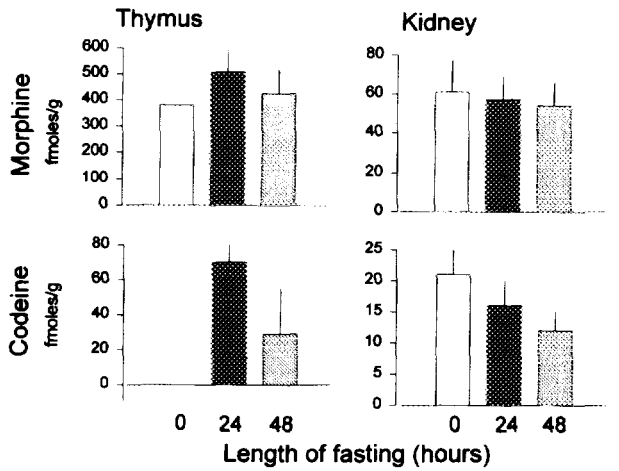

Figure 3. Endogenous morphine (top panels) and codeine (bottom panels) levels in thymus and kidney of fed, 24 h fasted and 48 h fasted rats. N=7-9 per group.

hyperglycemia (15,16). As a result we attributed the metabolic effects of morphine to direct central nervous system (CNS) stimulation (16). Although the location of the receptors involved in coordinating the metabolic responses are not known, we presented evidence for an associated activation of the endogenous endorphinergic, the pituitary-adrenal and the sympathetic nervous systems (17,18). While the doses of morphine used previously would have resulted in much higher CNS concentrations than those seen in the present study, it is still likely that the amounts of CNS endogenous alkaloids detected as a result of fasting could have played a role in the modulation of metabolic responses during caloric deprivation. It is also possible that the endogenous alkaloids play different, yet important roles in the reinforcing mechanisms associated with fasting. A role of the opiate system in the CNS in the etiology of anorexia nervosa (19) has been suggested. Animal studies have demonstrated that morphine administration into the CNS (at doses of 120, 10 and 5 μg) produces significant suppression of food and water intake (20) and has direct effects on lateral and ventromedial hypothalamic neurons (21) which directly influence motivation and cessation of eating (22). Additionally, recent studies have demonstrated that chronic food restriction is associated with region-specific elevations in prodynorphin-derived peptide content and in μ-receptor binding in the brain (23). Thus, it is possible to speculate that the μ-receptor binding is the direct result of increased endogenous morphine production and release into the CNS in response in fasting.

It is interesting to note that of the tissues analyzed, the liver had the highest concentration of morphine but no alteration in liver morphine or codeine was detected as a result of fasting. Thus, it is possible to speculate that the previously described (5) liver opiate synthetic pathways were not enhanced after 48 h of fast. The elevated levels of endogenous morphine in the spleen

and thymus suggest the possibility of a specific immunomodulatory role of these alkaloids during stress. It has been well documented that exogenous administration of opiates have marked suppressive effects on the immune system (24,25,26). In addition, the synthesis of endogenous opioid peptides by cells of the immune system has been also reported (27). We have also presented additional evidence showing that the endogenous morphine produced in the endolymph of the mussel, *Mytilus edilus*, suppressed the IL-1 induced chemotaxis and chemokinesis of monocytes (10). Taken together, these findings suggest the possibility of the splenocytes synthesizing morphine which could potentially modulate immune function in a paracrine or autocrine fashion.

In conclusion, the results from the present study, clearly indicate that fasting modulates tissue levels of morphine, particularly in neural and immune tissues such as brain and spleen. Unlike morphine, codeine levels did not change with fasting. The potential biologic role of these opiates is quite likely tissue and consequently function-specific. Thus, it is possible to speculate that while in the brain the role of morphine might be related to behavioral and metabolic effects of fasting, in the spleen the rise in opiates might be closely related to nutrient-dependent modulation of the immune function.

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