Endogenous Opiates and Fasting

Gambert *et al.* (*1*) claim to demonstrate decreases in the concentration of hypothalamic β-endorphin but not pituitary β-endorphin, hypothalamic adrenocorticotropin (ACTH), or pituitary ACTH in rat brain after a 3-day fast. From this they conclude that hypothalamic β-endorphin "may serve as a mechanism for the down-regulation of feeding behavior, enhancing energy conservation during periods of food storage." The authors, therefore, appear to assume that the correlational relation between hypothalamic β-endorphin concentration and days of deprivation is somehow causally linked. Correlations, of course, are merely indicative of relations and do not in themselves suggest any causal interaction between the two variables. It may be, for example, that fasting simply alters locomotor activity, drinking behavior, or other factors that independently affect brain concentrations of endogenous opiates. Therefore, the actual shift in β-endorphin levels may or may not have anything to do with fasting per se. Moreover, Gambert *et al.* mistakenly cite a paper from our laboratory (*2*) in support of their contention that "concentrations of β-endorphin are increased in pituitaries from genetically obese mice and rats." Actually, far from suggesting a causal relation, our report demonstrates that elevated pituitary β-endorphin neither precedes nor is concomitant with weight gains of ob/ob mice. Thus, it is unlikely that pituitary concentrations of β-endorphin have any causal relation in the obesity syndrome of this mutant strain.

Further, we do not understand why the authors state "there was no change in immunoreactive ACTH in the pituitary in response to fasting, and, unlike the result of β-endorphin, there was no significant reduction in hypothalamic ACTH as a result of fasting up to 3 days." If a *t*-test (for independent means) is computed from the data provided in their table 2 (*1*) (hypothalamic ACTH: control subjects compared to 3-day fasted subjects), a *t*-value of 2.51 is obtained which, with d.f. = 30, is statistically reliable ($P < .025$). Similarly, a *t*-test computed on the pituitary data for these same two groups is also statistically significant [t (30) = 2.05, $P < .05$]. How then do the authors explain their conclusion that these differences are not statistically reliable? It may be, given the large number of *t*-tests they report, that the authors chose to employ a correction factor in the calculation of their post hoc tests. If this were the case the reader should have been so informed.

Even if the statistical tests were correctly done, they are inappropriate to the argument the authors wish to make. Because hypothalamic β-endorphin is significantly different before and after fasting, but hypothalamic ACTH, pituitary ACTH, and pituitary β-endorphin are not, the authors suggest that hypothalamic β-endorphin is affected by fasting differently from ACTH and pituitary β-endorphin. However, the appropriate way to test this conclusion is to directly compare, relative to control values, the percentage change in day-3 concentrations of hypothalamic β-endorphin (35.4 ± 14.8 percent) (mean ± standard error), with pituitary endorphin (77.5 ± 21.4 percent), hypothalamic ACTH (49.5 ± 20.4 percent), and pituitary ACTH (62.25 ± 19.1 percent). None of these differences is statistically significant (even without the use of a correction for multiple tests). Contrary to what Gambert *et al.* (*1*) assert, there is no reason to believe that the effects of fasting are not common to all the substances they measured.

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The questions raised by Ettenberg *et al.* are based on their incorrect interpretations of statements in our report (*1*):

1) We speculated but did not conclude that β-endorphin in the hypothalamus may affect feeding behavior; we concluded only that "hypothalamic β-endorphin is modified by acute starvation." From the design of the experiment, this is all we could logically conclude. The mechanism by which starvation affects this change in hypothalamic endorphins is unclear.

2) We did not misrepresent the report of Rossier *et al.* (*2*); we only related feeding and obesity to pituitary endorphin levels as part of the justification for the experiment. We did not say endorphins cause obesity, nor did we suggest that Rossier *et al.* said that.

3) The demonstration (*2*) of similar β-endorphin in ob/ob mice and their lean littermates until 4 months of age is based on data from 18 obese mice and 20 ?/+ controls spread over 5 ages. Our results (*3*) are at variance with their data and suggest that pituitary endorphin-like immunoreactivity is elevated in ob/ob mice at all ages and is higher in control animals immediately after weaning.

The evidence linking endorphins and feeding behavior is strong (*4*): (i) opioid peptides stimulate feeding in sated rats (*5*); (ii) opiate antagonists suppress food intake in otherwise normal but food-deprived rats (*6*), in genetically obese animals (*7*), in rats made obese by hypothalamic lesions (*8*), and in rats induced to eat by stress (tail pinch) (*9*); (iii) food deprivation in the rat results in analgesia, which is diminished by naloxone (*10*). We feel that our study adds evidence to support an endorphin-feeding relationship.

All groups of data in our report (*1*) were statistically analyzed first using an analysis of variance. Since we found no differences between the groups other than that of hypothalamic β-endorphin, further analysis by the *t*-test was reserved for this one group only. Ettenberg *et al.* only did *t*-testing within groups, choosing the most extreme values for comparison. Our statistical conclusions are further confirmed by Scheffe's test. We believe our data clearly show that fasting is associated with a decrease in hypothalamic β-endorphin.

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