Changes of Endogenous Morphine and Codeine Contents in the Fasting Rat

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Accepted for publication January 15, 1991

ABSTRACT
The alteration of endogenous opiate alkaloids during fasting state was investigated in rats. The concentrations of morphine and codeine in the cortex, midbrain, pons plus medulla, cerebellum, adrenal gland and pancreas were measured using radioimmunoassay for the opiates following high pressure liquid chromatography. The morphine and codeine contents of fasting rats showed maximum elevated levels in cortex, pons plus medulla and pancreas after 2 days of fasting, but after 1 day in midbrain. The opiate content of the cerebellum showed a tendency for a continuous increase during the 4 days. Adrenal glands of fasting rats had elevated levels at days 3 and 4, although there were great fluctuations within the groups.

Although morphine is well known as a major component of Papaver somniferum, this compound was also found in several species of animals. Thus, morphine was identified in the skin of toads (Oka et al., 1985), rats and rabbits (Oka et al., 1985), the brain of the rat (Donnerer et al., 1986), beef (Goldstein et al., 1985) and sheep (Kodaira et al., 1989) and in other organs, including the adrenal gland (Goldstein et al., 1985; Donnerer et al., 1986).

The existence and formation of endogenous morphine and codeine in mammalian tissues (Donnerer et al., 1987) have led to the question of their function. Opioid substances have been suggested to be involved in the feeding behavior of monkeys (Nakano et al., 1986) and to decrease feeding in food-deprived rats following either intracerebroventricular or subcutaneous administration (Fernandez-Tome et al., 1988; Leshem, 1988).

Kaye et al. (1982) found higher levels of opioid activity, as determined by radioreceptor assay, in the cerebrospinal fluid of patients with anorexia nervosa who were severely underweight. Germer and Sharp (1982) failed to find any difference in beta endorphin immunoreactivity in the cerebrospinal fluid of control subjects and anorexia nervosa subjects. In this study, data are presented that show that the endogenous levels of morphine and codeine are elevated in various brain regions, in the adrenal gland and the pancreas of the rat during a 4-day starvation period.

Methods
Sprague-Dawley rats weighing 140 to 180 g were used. The rat chow did not contain morphine or codeine, as measured by HPLC and RIA. The rats were fasted up to 4 days with access to water. The brain, pancreas and adrenal gland were removed at various time intervals during the fast. Brain regions were dissected by the method of Glowinski and Iversen (1966). The brain regions, adrenal glands and pancreas were pooled from three rats for each determination of morphine and codeine. Tissues were homogenized with a Polytron in 10 volumes of 0.01 N HCl and the concentration was brought to 10% with the addition of concentrated HCl. The homogenate was heated to 100°C for 30 min and then centrifuged for 15 min at 5000 x g. The supernatant was extracted into 10% butanol in chloroform at pH 10 and then back-extracted into 0.01 N HCl, as described by Cardinal et al. (1967). After a preparaturation on Sep-Pak C18, column, morphine and codeine were eluted from the cartridge with 7 ml of 0.1 M pyridine/acetic acid (pH 6.2) containing 2.5% n-propanol. The eluate was evaporated to dryness, dissolved in 1 mM HCl and injected on to a RP18-HPLC column. The samples were eluted with a linear 0 to 25% v/v gradient of 0.1 M pyridine/acetic acid (pH 6.2) followed by a gradient of n-propanol/0.1 M pyridine/acetic acid at a flow rate of 1.5 ml/min. The eluted fractions were monitored with an RIA that measures both morphine and codeine (Donnerer et al., 1986), using [3H]dihydromorphine.

Statistics. All values reported are means ± S.E. The effects of starvation on morphine and codeine content were compared to the appropriate controls and tested for significance using a Student’s t test. Differences were declared statistically significant when P ≤ .05.

Results
Morphine and codeine showed no change in the cortex after a 1-day fast, but both opiate alkaloids rose to a maximum level after day 2 of fasting (about 700% increase). After 3 days, both morphine and codeine were still elevated and by day 4 the

ABBREVIATIONS: HPLC, high pressure liquid chromatography; RIA, radioimmunoassay.
Fig. 1. Morphine and codeine content in the cerebral cortex of rat during the 4-day fast. In the cerebral cortex of the feeding rat, the morphine and codeine contents were 0.43 ± 0.09 pmol/g tissue and 0.63 ± 0.08 pmol/g tissue, respectively, and these values were represented as 100%; morphine: •; codeine: ○. One experiment contained the cerebral cortex of three rats. The results are percent of mean ± S.E. of four experiments. Asterisks represent significant difference from day 0, P < .05.

concentrations of both alkaloids were back to control values (fig. 1). A different pattern is seen in the midbrain during the 4-day starvation period (fig. 2). Morphine content rose after day 1 (about 260%) and returned to control level by day 2 and remained at basal value. Codeine exhibited a tendency to become elevated by day 2; however, the rise is not statistically significant. Figure 3 shows the effects of the 4-day fast on the levels of the opiate alkaloids in the cerebellum. Although the codeine content rose during the 4-day fast period, the changes are not statistically significant. The morphine content was elevated by day 3 and by day 4 had returned to control value. The morphine and codeine contents rose about 150% in the pons medulla brain region after day 1 and by day 2 was about 250% greater than control. Both alkaloids returned to normal values by day 3. Day 4 results indicated that both morphine and codeine were increasing again (fig. 4).

The two opiate alkaloids were assayed in two peripheral tissues. The concentrations of morphine and codeine in the pancreas increased about 100% at day 1 and almost 200% by day 2, when peak values were attained. On days 3 and 4 the alkaloids declined to control levels (fig. 5). Figure 6 indicates that in the adrenal gland both opiate alkaloids, morphine and codeine, only changed after day 3 of the fast and remained elevated on day 4. There was a doubling of the levels in the adrenal gland.

Discussion

It has been reported that in feeding disorders the alpha adrenergic function in the medial hypothalamus (Leibowitz, 1978; 1980; Jhanwar-Umiyal et al., 1982) and possibly other neurochemical systems (Ebert et al., 1984; Leibowitz and Hor, 1982) are implicated. This paper presents data indicating that the endogenous content of the opiate alkaloids, morphine and codeine, may also be a factor as they are elevated in various brain regions of the rat during food deprivation. Because our dissections of brain regions involved large areas, we probably diluted out those changes which occur in discrete brain regions, and consequently, the changes in concentrations we report are minimum changes. However, it is apparent from this study that different areas of the brain exhibit different rates of elevation of both opiate alkaloids.

The cortical content increased after the first day of food deprivation and peaked by 48 hr. Although one cannot evaluate mood changes in the rat, it is known that mood changes occur in patients with anorexia nervosa (Crisp, 1980). Although the mood changes observed in patients have been postulated to be adrenergic or serotonergic in origin, in view of our findings, morphine should also be considered.

The midbrain also showed marked elevation in morphine content. The brainstem contains the center that regulates body temperature, and thermoregulation is critical for the survival of the organism. The thermoregulatory center can be influenced by various neurotransmitters and hormones. Feldberg and Meyers (1965) showed that monoamine can regulate body temperature; Meyers (1981) and Meyers and Walter (1977) reported...
Fig. 3. Morphine and codeine content in the cerebellum of rats during the 4-day fast. In the cerebellum of the feeding rat, the morphine and codeine contents were $0.37 \pm 0.07$ pmol/g tissue and $0.48 \pm 0.08$ pmol/g tissue, respectively, and these values were represented as 100%; morphine: $\bullet$; codeine: $\circ$. One experiment contained the cerebellum of three rats. The results are percent of mean $\pm$ S.E. of four experiments. Asterisks represent significant difference from day 0, $P < .05$.

Fig. 4. Morphine and codeine content in the pons plus medulla of rat during the 4-day fast. In the pons plus medulla of the feeding rat, the morphine and codeine contents were $0.59 \pm 0.10$ pmol/g tissue and $0.47 \pm 0.08$ pmol/g tissue, respectively, and these values were represented as 100%; morphine: $\bullet$; codeine: $\circ$. One experiment contained the pons plus medulla of three rats. The results are percent of mean $\pm$ S.E. of three experiments. Asterisks represent significant differences from day 0, $P < .05$.

Fig. 5. Morphine and codeine content in the pancreas of rat during the 4-day fast. In the pancreas of the feeding rat, the morphine and codeine contents were $0.12 \pm 0.05$ pmol/g tissue and $0.11 \pm 0.06$ pmol/g tissue, respectively, and these values were represented as 100%; morphine: $\bullet$; codeine: $\circ$. One experiment contained the pancreas of three rats. The results are percent of mean $\pm$ S.E. of four experiments. Asterisks represent significant differences from day 0, $P < .05$.

Fig. 6. Morphine and codeine content in the adrenal gland of rat during the 4-day fast. In the adrenal gland of the feeding rat, the morphine and codeine contents were $4.21 \pm 0.09$ pmol/g tissue and $6.21 \pm 1.0$ pmol/g tissue, respectively, and these values were represented as 100%; morphine: $\bullet$; codeine: $\circ$. One experiment contained the adrenal gland of three rats. The results are percent of mean $\pm$ S.E. of four to six experiments. Asterisks represent significant differences from day 0, $P < .05$. 
on the role of serotonin in thermoregulation. Prostaglandin E also exerts an influence on thermoregulation (Veale et al., 1977). The systemic administration of morphine can evoke a change in body temperature (Goodman and Gilman, 1985). Thus, the question has to be raised whether the elevated content of endogenous morphine seen during the 4-day fast may be to effect the thermoregulatory center to elicit hypothermia in order to decrease the rate of metabolism during starvation. The respiratory center has been described to be located in the reticular formation of the medulla. One of the pharmacological actions of the opiate alkaloids is to depress respiration. During starvation it is reasonable to assume that in order to conserve energy the oxidative processes are reduced.

Although we did not investigate any neuroendocrine disturbances in these animals during the 4-day fast period, it is well documented that anorectic patients have neuroendocrine disturbances (Vigursky, 1977; Fisher et al., 1982; Doerr et al., 1980; Casper and Frohman, 1982). Morphine is known to have an effect on many endocrine systems and whether endogenous morphine contributes to neuroendocrine dysfunction during feeding disorders awaits further studies.

Administered morphine produces hyperglycemia (Feldberg and Gupta, 1974) through an effect on the posterior hypothalamus, leading to sympathetic splanchnic outflow to the adrenal medulla (Bonsin, 1971). Our data indicate that in the pancreas, endogenous content of morphine and codeine increased during starvation. The elevated opiate alkaloids might serve two functions: 1) to modulate the release of insulin from the beta cells so that during starvation more glucose becomes available to the brain and 2) to modify the hyperphagic action of insulin. It has been reported (Moore et al., 1981; Mills and Medlicott, 1984) in studies with anorexia nervosa patients, that the narcotic antagonist naloxone led to a depression of plasmic nonesterified fatty acids and beta hydroxybutyrate, which would indicate an inhibition of lipolysis. This inhibition was associated with weight gain in these same patients. Our data would suggest that the role of these endogenous opiate alkaloids be studied both in regard to lipid metabolism and their actions in modulating eating disorders.

References


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