CAFFEINE STIMULATES β-ENDORPHIN RELEASE IN BLOOD
BUT NOT IN CEREBROSPINAL FLUID

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Summary

Plasma β-endorphin and prolactin profiles were obtained from groups of unstressed, adult male rats. The infusion of caffeine (20 mg/kg) via a chronic, indwelling intra-atrial cannula results in a prompt and sustained (2-2.5 h) rise in plasma β-endorphin levels. The infusion of the opiate antagonist naloxone causes a modest (40%) decrease in plasma β-endorphin and blunts the elevation in plasma β-endorphin following caffeine administration. In contrast, plasma prolactin levels were unchanged following caffeine administration and were decreased by treatment with naloxone. Caffeine treatment did not effect CSF β-endorphin levels or the release of β-endorphin from hemipituitaries incubated in vitro.

Caffeine is a widely consumed compound that has powerful central nervous system stimulant properties. Among its many other actions this methylxanthine derivative stimulates cardiovascular function, relaxes smooth muscle and influences blood vessel caliber (1). Caffeine has achieved popularity as a component of numerous analgesic preparations. It has already been suggested that methylxanthines may relieve vascular headaches by acting as a cerebral vasoconstrictor or by decreasing cerebrospinal fluid (CSF) pressure (2,3), but other mechanisms are also plausible.

Although there is considerable controversy regarding the precise role of endogenous opioid peptides in modulating pain perception there is little question that several of the endorphins can elicit analgesia. β-endorphin is a particularly potent analgesic agent; its administration into CSF or brain sites causes long-lasting analgesia in man (4) and in rats (5). Electrical stimulation of the periaqueductal gray (PAG) area of patients with pain produces analgesia which is reversible with naloxone (6). Further, stimulation of the PAG for pain relief is accompanied by increased CSF β-endorphin levels (7). These and other studies point to a possible physiological role for endorphins, and particularly β-endorphin, in the regulation of pain sensitivity. Since caffeine is so widely consumed in foods, beverages and analgesic preparations, we began investigation of the effects of this methylxanthine on blood and CSF β-endorphin levels.

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Materials and Methods

Animals: Male Sprague-Dawley rats (300-350 g; Charles River Laboratories, Wilmington, MA) were housed 4 per cage and given ad libitum access to water and food (Rodent Laboratory Chow, Ralston Purina Co.). The animals were exposed to light from 0600 to 1800 hr daily; the temperature was maintained at 22°C. Animals were implanted with chronic right atrial silastic cannulae under pentobarbital anesthesia (8,9). While under anesthesia some animals also received chronic intracisternal cannulae for subsequent collection of multiple CSF samples (10). For this procedure cannulae were constructed from a 23 mm length of 21 G hypodermic tubing and were fitted with a stylet of 26 G hypodermic tubing. A 25 cm piece of polyethylene tubing (PE 20, dead space 0.025 ml) was connected to the cannula for CSF sampling. After recovering for 1 week, rats were housed in isolation chambers (9) for experimentation. In these studies, each rat served as its own control, with at least 2 days between experiments.

Experimental Protocols: In blood studies hormone secretory patterns were obtained from groups of 6 rats; blood samples were drawn every 15 min between 1215 and 1545 hr. Naloxone (Narcan HC1, a gift of Endo Laboratories, Garden City, NY; 10 mg/kg) or saline (0.2 ml) was injected via the atrial cannulae after the first blood sample (1215 hr); caffeine (caffeine anhydrous, Sigma Chemical Co., St. Louis, MO; 20 mg/kg) or saline were injected similarly at 1245 hr. The order of treatments was randomized. All blood samples (0.5 ml) were immediately centrifuged; plasma was frozen until assayed. Red cells were resuspended in normal saline and returned to the rat when the next blood sample was taken. In another set of experiments 6 rats had hourly CSF samples (0.11 ml) withdrawn between 1200 and 1600 hr. Caffeine (20 mg/kg) or saline (0.2 ml) was injected via a chronic right atrial cannula at 1230 hr. CSF samples were quickly frozen until assayed.

Assays: Antiserum for human β-endorphin (βh-endorphin) was generated in a New Zealand white rabbit using methods previously described (11). RIA was performed using a 1:7,000 or a 1:10,000 final dilution of antiserum (Batch No. 2A-IV) and approximately 5,000 cpm 125I-labeled β-endorphin in a final volume of 0.5 ml (buffer was 0.04 M sodium phosphate, pH 7.5, containing 0.01 M EDTA, 0.05 M NaCl, 0.01% sodium azide, and 0.5% BSA). Assay tubes containing standards (1 to 500 pg camel β-endorphin (βc-endorphin) plus 0.05 ml hypox plasma in plasma assay) or samples (0.05 ml plasma or CSF) were incubated with the antibody at 4°C for 24 hr before the addition of label. Two days later appropriately titered normal rabbit serum (0.15 ml) and goat anti-rabbit γ globulin (0.1 ml) were added. After an additional 24 hr incubation the assay tubes were centrifuged and the radioactivity in the pellet was determined. Plasma prolactin levels were determined by RIA using materials provided by the NIH Rat Pituitary Program.

Gel permeation chromatography: Pooled rat sera (10 ml) were extracted with talc (Ormont Drug and Chemical Co., Englewood, NJ) as described by Wardlaw and Frantz (12). The serum extract was dissolved in 2 ml 0.1 M acetic acid/0.1% BSA and was added to a 0.9 x 45 cm Sephadex G-50 (fine column). The column was eluted with acetic acid/BSA at 4°C; 1 ml fractions were collected. Fractions were lyophilized, reconstituted in 1 ml RIA buffer, and assayed for β-endorphin. Pooled rat CSF (10 ml) was lyophilized, and the resulting material was subjected to chromatography and RIA as described above. Labeled βh-endorphin and βh-LPH were used to standardize the column.
In vitro pituitary release of \( \beta \)-endorphin: Rat hemi-pituitaries were incubated in vitro (13) to assess \( \beta \)-endorphin release. Eight hemi-pituitaries (dissected from 300-350 g rats) were incubated in 16 mm tissue culture wells with 2 ml Medium 199 (Difco Laboratories, Detroit, MI). The pituitaries were preincubated for 30 min, the medium was changed, and incubation was continued for 60 min with various concentrations of caffeine. \( \beta \)-endorphin was measured in 0.002 ml of medium.

Data analysis: Mean plasma \( \beta \)-endorphin and prolactin levels were calculated for individual rats between 1245-1545 hr. The resulting data were analyzed statistically using one-way analysis of variance and the Neuman-Keuls test (14). \( \beta \)-endorphin release in vitro was compared using a two-tailed t-test.

Results

The displacement of labeled \( \beta_h \)-endorphin by varying amounts of \( \beta \)-endorphin and other peptides is shown in Fig. 1. Compared to \( \beta_h \)-endorphin, the peptides \( \beta \)-endorphin, \( \beta_h \)-LPH, and \( \beta \)-LPH have 56, 24, and 17% crossreactivity by weight, respectively, when assessed by 50% displacement of tracer binding to the antibody. Other peptides including \( \alpha \)-endorphin, \( \gamma \)-endorphin and methionine enkephalin have no crossreactivity at concentrations up to 5 ng/tube.

At 1:10,000 final antibody concentration the sensitivity of the RIA, defined as the concentration of \( \beta \)-endorphin which results in binding of labeled \( \beta_h \)-endorphin 2 SD below mean binding in absence of \( \beta \)-endorphin, is 2.6 ± 0.4 pg/tube (mean of 5 assays). At this antibody concentration 50% displacement of tracer binding to antibody occurs with 19.8 ± 1.2 pg/tube of \( \beta_h \)-endorphin (mean of 5 assays).

Recovery of labeled \( \beta_h \)-endorphin and \( \beta \)-LPH added to rat sera and extracted with talc was routinely 60-70%. Chromatography of extracted rat sera over a Sephadex G-50 column shows the majority (90%) of \( \beta \)-endorphin immunoreactivity comigrates with \( \beta \)-endorphin, whereas a smaller peak (10%) is associated with \( \beta \)-LPH (Fig. 2).

Chromatography of pooled rat CSF over Sephadex G-50 results in a different profile of immunoreactivity (Fig. 3): 69% of the \( \beta \)-endorphin immunoreactivity migrates with \( \beta \)-endorphin, 24% with \( \beta \)-LPH, and about 5% in the void volume.
FIG. 2
Profile of β-endorphin immunoreactivity from rat sera. Ten ml pooled rat sera was extracted with talc and chromatographed over a 45 cm Sephadex G-50 (fine) column. About 90% of the immunoreactivity migrates in fractions corresponding to the molecular weight of β-endorphin.

FIG. 3
Profile of β-endorphin immunoreactivity from rat CSF. Ten ml pooled rat CSF was lyophilized and chromatographed over a 45 cm Sephadex G-50 (fine) column. Over 69% of the immunoreactivity was eluted in fractions corresponding to the molecular weight of β-endorphin.

The mean plasma β-endorphin profiles from groups of unstressed, free-running male rats is presented in Fig. 4. Plasma β-endorphin levels in control animals given 2 iv saline injections vary between 200 and 500 pg/ml.

FIG. 4
Plasma β-endorphin (βE) profiles from groups of rats given infusions of saline (closed circles), naloxone (open circles), caffeine (closed triangles), or naloxone plus caffeine (open triangles). Naloxone (10 mg/kg) or saline were infused after the first blood sample; caffeine (20 mg/kg) or saline were infused after the third blood sample. Caffeine doubled mean plasma βE levels compared to saline controls between 1:00 and 3:45 PM. Naloxone decreased βE levels by 40% and partially blocked the βE response to caffeine infusion.
over the 3.5 hr sampling period. One hr after the injection of naloxone plasma β-endorphin levels had fallen to under 200 pg/ml and remained between 100-200 pg/ml for about 2 hrs. The iv infusion of caffeine (20 mg/kg) after the third blood sample results in a rapid (15 min) elevation in plasma β-endorphin levels to over 650 pg/ml; β-endorphin levels remained elevated in most animals for about 2 hrs after injection. In animals pretreated with naloxone, plasma β-endorphin levels also increased after caffeine injection, but fell to control levels within 30-60 min. Mean plasma β-endorphin levels calculated between 1245 and 1545 hr also reflect this trend (Table 1): naloxone treatment causes a 40% decrease in plasma β-endorphin; caffeine administration more than doubles β-endorphin values; and naloxone plus caffeine treatment results in β-endorphin values similar to saline-treated rats.

Mean plasma prolactin levels in control (saline-injected) rats vary from about 5-18 ng/ml over the 3.5 h sampling period (Fig. 5). Naloxone injection causes an immediate fall in prolactin to 2-4 ng/ml; prolactin levels remain suppressed for about 2.5 hrs. Caffeine injection causes a small surge in prolactin to about 24 ng/ml but hormone levels quickly (15 min) fall to

![Graph of plasma prolactin levels](image)

### FIG. 5

Plasma prolactin profiles from groups of rats given iv infusions of saline (closed circles), naloxone (open circles), caffeine (closed triangles), or naloxone plus caffeine (open triangles). Naloxone (10 mg/kg) or saline were infused after the first blood sample; caffeine (20 mg/kg) or saline were infused after the third blood sample. Caffeine did not affect mean plasma prolactin levels compared to saline controls between 1:00 and 3:45 PM. Naloxone decreased plasma prolactin levels in both saline and caffeine-treated rats.

### TABLE 1

<table>
<thead>
<tr>
<th>Treatments</th>
<th>βE (pg/ml)#</th>
<th>PRL (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>288.0 ± 42.0</td>
<td>9.4 ± 1.5</td>
</tr>
<tr>
<td>Saline</td>
<td>590.3 ± 67.5</td>
<td>10.4 ± 2.8</td>
</tr>
<tr>
<td>Naloxone</td>
<td>173.3 ± 24.2</td>
<td>4.6 ± 0.9</td>
</tr>
<tr>
<td>Naloxone</td>
<td>290.1 ± 50.2</td>
<td>4.1 ± 1.1</td>
</tr>
</tbody>
</table>

Hormonal secretory patterns were obtained from groups of 6 adult male rats bearing chronic right atrial cannula; blood samples were drawn every 15 min between 1215 and 1545 h. Naloxone (10 mg/kg) or saline (0.2 cc) were injected via the cannula after the first blood sample (1215 h); caffeine (20 mg/kg) or saline similarly were injected at 1245 h. βE and PRL values are average levels calculated between 1300-1545 h, mean ± SEM. P<0.05 vs. saline control.
control levels. Naloxone pretreatment results in suppressed plasma prolactin levels which are unaffected by caffeine administration. Mean plasma prolactin levels also reflect this trend (Table 1); caffeine administration causes an insignificant rise in prolactin whereas both groups receiving naloxone have decreased prolactin.

Caffeine incubated directly with rat pituitaries in vitro did not affect α-endorphin release (Table 2).

**TABLE 2**

<table>
<thead>
<tr>
<th>Conc. Caffeine (µg/ml)</th>
<th>Medium α-Endorphin Level (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>15.8 ± 2.3</td>
</tr>
<tr>
<td>10</td>
<td>13.2 ± 3.7</td>
</tr>
<tr>
<td>100</td>
<td>13.5 ± 1.0</td>
</tr>
<tr>
<td>1000</td>
<td>16.5 ± 2.7</td>
</tr>
</tbody>
</table>

α-endorphin release into medium by hemipituitaries incubated with caffeine at the indicated concentrations for 60 min. Caffeine did not modify α-endorphin release into medium at any concentration tested.

Hourly CSF α-endorphin levels in 6 rats are shown in Fig. 6. Caffeine injection did not modify CSF α-endorphin levels at any time after its administration (Table 3).

**FIG. 6**

CSF αE levels in individual rats on different days given iv infusions of either saline (closed circles) or 20 mg/kg of caffeine (open circles). Drugs were administered via a chronic venous cannula 30 min after the first CSF sample. Caffeine infusion did not significantly affect CSF αE levels at any time point after its administration.

**TABLE 3**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cerebrospinal Fluid α-Endorphin Level (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 hour</td>
</tr>
<tr>
<td>Saline</td>
<td>136.5 ± 26.0</td>
</tr>
<tr>
<td>Caffeine</td>
<td>147.9 ± 17.0</td>
</tr>
</tbody>
</table>

CSF α-endorphin level in groups of 6 rats given i.v. infusions of saline or caffeine (20 mg/kg). Drugs were administered 30 min. after the first CSF sample.
Discussion

In this study the administration of caffeine to rats results in a prompt and sustained rise in plasma β-endorphin levels. The mechanism for caffeine's effect on blood β-endorphin has not been entirely clarified, but would seem to occur by stimulation of pituitary β-endorphin secretion. As pituitary β-endorphin is released by stress (15), one might propose that caffeine's stimulation of β-endorphin is simply the result of stress. However, prolactin, which is also released by stress (16), was only slightly affected by caffeine in our study and was not affected in the study of Spindel et al (17). Thus stress alone is unlikely to be the explanation for the rise in plasma β-endorphin observed after caffeine administration.

Caffeine's actions on the hypothalamus are likely responsible for the increase in pituitary β-endorphin secretion. Caffeine has been shown to inhibit the enzyme phosphodiesterase (18) and to directly stimulate adenosine receptors (19). Other studies indicate that caffeine increases the brain levels of two putative neurotransmitters, serotonin and norepinephrine (20,21). Ultimately, caffeine's actions on neurons in the central nervous system may affect the release of hypothalamic releasing factors which regulate anterior pituitary function. Caffeine suppresses the release of two other pituitary hormones, GH and TSH (17). The effect of caffeine on β-endorphin release appears indirect since addition to pituitaries in vitro was without effect.

In this study, pretreatment with the opiate antagonist naloxone blocks β-endorphin release after caffeine. This finding suggests that caffeine enhances pituitary β-endorphin release by a mechanism involving the stimulation of opiate receptors in the brain. Agents that interact with opiate receptors have already been shown to modify pituitary β-endorphin release. Morphine treatment in the rat (22) and in the dog (23) is associated with increased β-endorphin release. In the dog, the i.v. infusion of naloxone enhances β-endorphin release (23), whereas in normal rats naloxone has no effect on basal (23,24) or stress-induced β-endorphin secretion (24). These various effects of naloxone and morphine are attributed to actions on brain rather than pituitary sites, as neither of these agents has any effect on pituitary β-endorphin release in vitro (23). In the present study, naloxone decreased basal β-endorphin release by 40% and prolactin by over 50%. The effect of naloxone on prolactin release has already been demonstrated (24,25). The decrease in β-endorphin release following naloxone is a new finding, and is the opposite response to that reported in the dog (23). Whether stress contributes to the stimulation of β-endorphin release by naloxone in the dog was not determined. Species difference may account for these divergent effects.

Although caffeine elevates blood β-endorphin levels it has no effect on CSF β-endorphin (Table 3). This finding indicates that there is a dissociation of blood and CSF β-endorphin responses in the rat. There also does not appear to be any transfer from the blood to the CSF compartments after elevation of circulating levels of β-endorphin.

The dose of caffeine used in this study is 20 mg/kg, an amount that when given i.p. is shown to elevate serum caffeine levels to 15 to 18 μg/ml (17). Humans drinking 2 to 3 cups of brewed coffee (250 mg or more of caffeine) can achieve blood caffeine levels in excess of 15 μg/ml (26,27). Whether caffeine stimulates β-endorphin release in humans is unknown, although studies are presently underway to address this question.
Acknowledgments

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References