Suppressive Effects of Cholecystokinin and Bombesin on Growth Hormone and Prolactin Secretion in Urethane-Anesthetized Rats

TSUYOSHI KARASHIMA, TAIICHIRO OKAJIMA, KEN-ICHI KATO AND HIROSHI IBAYASHI

Third Department of Internal Medicine, Faculty of Medicine, Kyushu University, Fukuoka 812

Abstract

The effects of cholecystokinin octapeptide (CCK) and bombesin on rat plasma growth hormone (GH) and prolactin (PRL) levels were investigated with the animals under urethane anesthesia. Intraventricular administration of both CCK (0.3 μg) and bombesin (2 μg) completely suppressed the GH secretion induced by FK 33-824, chlorpromazine (CPZ) or prostaglandin E2 (PGE2). Both peptides also completely suppressed the PRL secretion induced by FK 33-824 or PGE2, and partially that induced by CPZ, but not that induced by domperidone. The intravenous administrations of CCK and bombesin had no or lesser potency in inhibiting the stimulated GH or PRL releases. These results indicate that the CCK and bombesin act much in the same manner to inhibit GH and PRL. These peptides may suppress the GH and PRL secretions via a hypothalamus-related action.

High concentrations of cholecystokinin (CCK)- and bombesin-like activities have been found in the hypothalamus (Innis et al., 1979; Brown et al., 1978). Gibbs et al. (1977) found that CCK and bombesin have some common biological actions. In fact, intraventricular administrations of both peptides decreased the appetite (Gibbs et al.; Morley, 1980) and produced a hypo-thermia (CCK: Katsuura et al., personal communication; bombesin: Brown et al., 1977). These peptides stimulate exocrine pancreatic secretions and produce contractions of the gall bladder (Williams, 1981). In addition, bombesin is a potent releaser of CCK in dogs (Miyata, 1978). As to the endocrinological aspect, several reports on CCK and bombesin were published but the results were inconsistent. We therefore further studied the effects of CCK and bombesin on GH and PRL release in order to compare the neuroendocrinological actions of these peptides.

Materials and Methods

Male Wistar rats weighing 250–300 g were kept in a light and temperature controlled room (12 hr dark-light cycle, light on at 8:00 a.m., 23 ± 2°C). Oriental Laboratory Chow and water were available ad libitum.

The rats were anesthetized with ketalar (160 mg/kg B.W.) and a heart catheter (right atrium) was implanted, according to the method of Steffens (1969). A cannula was implanted stereotaxically into the right lateral ventricle at a point...
Five to 7 days later, these rats were anesthetized with urethane (150 mg/100 g B.W.) between 10:00 and 12:00 a.m.. CCK octapeptide or bombesin dissolved in physiological saline was injected in a volume of 10 μl into the lateral ventricle. For intravenous administrations, each peptide dissolved in 0.3 ml saline was infused through the heart catheter. FK 33–824, chlorpromazine (CPZ), domperidone or prostaglandin E₂ (PGE₂) dissolved in 0.3 ml saline was infused through the heart catheter 10 min after the intraventricular injection of CCK or bombesin. Blood (1.0 ml) was withdrawn through the heart catheter before and 10 min, 20 min and 40 min after the injection of CCK and bombesin. An equal volume of saline was injected into the rats after each blood sampling, in order to minimize extracellular volume changes.

The following drugs were used: bombesin (Sigma), CCK octapeptide (Sigma), chlorpromazine (CPZ, Yoshitomi Pharm. Co.), Domperidone (Kyowa Hakko Co.), FK 33–824 (D-Ala²-MePhe⁴, met-(o)enkephalinol : Domme, Sandoz), prostaglandin E₂ (PGE₂, Ono Pharm. Co.).

Plasma growth hormone (GH) and prolactin (PRL) were determined by double antibody radioimmuno-assay using materials supplied by NIAMDD, NIH. The minimal sensitivities for GH and PRL were 1.5 ng/ml. The intra- and inter-assay coefficients of variation were less than 10%. Statistical analysis of data were performed using Student’s t-test.

### Results

The effects of intraventricular injection (ivt) of CCK (1 ng to 300 ng) and bombesin (10 ng to 10 μg) on the basal levels of GH and PRL were observed (Table 1). Both CCK and bombesin significantly lowered the basal GH levels, dose-dependently. Neither peptide, however, changed the basal PRL levels with the same dose ranges given. The intravenous administrations of CCK (1 μg/100 g B.W.) and bombesin (10 μg/100 g B.W.) did not significantly lower the basal GH and PRL levels.

To evaluate the inhibitory effects on the GH and PRL releases, CCK or bombesin was given intraventricularly 10 min before an intravenous administration of substances which would stimulate GH and PRL. Fig. 1 shows that an intravenous administration of FK 33–824 (10 μg/100 g B.W.), a Met-enkephalin analogue, increased the plasma GH (at 10 min, P<0.05; at 20 and 40 min, P<0.01) and PRL levels (at 10, 20 and 40 min, P<0.01). Intraventricular administration of CCK (300 ng) or bombesin (0.5 and 2.0 μg) completely suppressed the FK 33–824-induced GH and PRL releases (Fig. 1). An intravenous injection of CPZ (10 ng/100 g B.W.), a monoamine antagonist, significantly increased the plasma GH (at 10 min, P<0.05; at 20 and 40 min, P<0.01)

### Table 1. Effect of intraventricularly injected CCK and bombesin on plasma rat GH

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of rats</th>
<th>Plasma GH (ng/ml)</th>
<th>% decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>(12)</td>
<td>12.2± 1.0</td>
<td>12.2± 0.7</td>
</tr>
<tr>
<td>CCK-8</td>
<td>(6)</td>
<td>15.2± 1.2</td>
<td>14.8± 0.5</td>
</tr>
<tr>
<td>0.03</td>
<td>(6)</td>
<td>13.2± 0.7</td>
<td>12.4± 0.8</td>
</tr>
<tr>
<td>0.1</td>
<td>(6)</td>
<td>12.5± 1.4</td>
<td>10.2± 0.8</td>
</tr>
<tr>
<td>0.3</td>
<td>(6)</td>
<td>17.2± 1.4</td>
<td>10.6± 1.6*</td>
</tr>
<tr>
<td>Bombesin</td>
<td>(6)</td>
<td>19.8± 0.7</td>
<td>18.8± 0.6</td>
</tr>
<tr>
<td>1</td>
<td>(6)</td>
<td>18.2± 1.5</td>
<td>17.5± 0.4</td>
</tr>
<tr>
<td>3</td>
<td>(6)</td>
<td>17.8± 0.6</td>
<td>14.2± 1.2*</td>
</tr>
<tr>
<td>10</td>
<td>(6)</td>
<td>18.5± 1.5</td>
<td>15.3± 0.4*</td>
</tr>
</tbody>
</table>

Values given are the mean±S.E. * P<0.05 vs respective control (=0 min)
and PRL (at 10, 20 and 40 min, P<0.01) (Fig. 2). CCK (0.3 μg; ivt) and bombesin (2 μg; ivt) completely suppressed the CPZ-induced GH and partially suppressed the CPZ-induced PRL release (Fig. 2).

Domperidone, a peripheral dopamine antagonist, increased the plasma PRL (at 10, 20 and 40 min, P<0.01) but decreased the GH (at 10 min, 24.7±5.2, S.E. to 11.4±2.7, S.E.: P<0.05 at 20 and 40 min, not significant). An intraventricular administration of CCK (0.3 μg) or bombesin (2 μg) did not inhibit the domperidone-induced PRL release (Fig. 3).

PGE$_2$ (5 μg/100 g B.W., iv) increased the plasma GH and PRL (GH: at 10 min, P<0.01, at 20 min, P<0.05, PRL: at 10 and 20 min, P<0.01) (Fig. 4). CCK (0.3 μg, ivt) or bombesin (2 μg, ivt) completely suppressed the PGE$_2$-induced GH and PRL.

---

Fig. 1. The effects of cholecystokinin (CCK) or bombesin on the FK 33-824-induced GH or PRL release. Vertical bars indicate the mean±S.E. values of 6 rats. The degree of significance of the difference between control (= saline ivt+FK 33-824 iv) and CCK- or bombesintreated rats is shown as *: P<0.05 and **: P<0.01. upper panel: GH, lower panel: PRL

a and b ●: saline ivt+FK 33-824 10 μg/100 g B.W. iv
a ○: CCK 30 ng ivt+FK 33-824 iv
a ■: CCK 300 ng ivt+FK 33-824 iv
b ○: bombesin 0.5 μg ivt+FK 33-824 iv
b ■: bombesin 2.0 μg ivt+FK 33-824 iv
releases (Fig. 4).

The inhibitory effects of the intravenously injected CCK and bombesin on GH and PRL release were also observed (Fig. 5). CCK (1 μg/100 g B.W.) or bombesin (10 μg/100 g B.W.) given before the intravenous injection of FK 33–824 did not change the FK 33–824-induced GH release. Bombesin (10 μg) suppressed the FK 33–824-induced PRL release, while 1 μg of bombesin and CCK did not.

---

**Fig. 2.** The effects of cholecystokinin (CCK) (a) or bombesin (b) on the CPZ-induced GH and PRL release. Vertical bars indicate the mean ± S.E. values of 6 rats. The degree of significance of the difference between control (= saline ivt+FK 33–824 iv) and CCK- or bombesin-treated rats is shown as *: P<0.05, **: P<0.01. upper panel: GH, lower panel: PRL

a and b ○: saline ivt+CPZ 500 μg/100 g B.W., iv
a ○: CCK 300 μg ivt+CPZ iv
b ○: bombesin 2 μg ivt CPZ+ iv
Discussion

The effects of CCK and bombesin on GH release

Concerning the effect of bombesin, Rivier et al. (1978) reported that it increases the GH level in the urethane-anesthetized, estrogen-primed male rat. However, we found that bombesin inhibited the basal GH level, though evaluation of the drop in the basal GH level encounters a problem in urethane-anesthetized rats because of their low basal level (Kato et al., 1973). Abe et al. (1981) reported that in the urethane-anesthetized male rat, bombesin suppresses the PGE$_2$ (iv) or beta-endorphin (ivt)-induced GH release, corresponding to the results of our observations. Concerning the effect of CCK, Vijayan et al. (1979) reported that CCK (ivt) increases the GH release in conscious rat. It has not been reported, however, that CCK has an inhibitory action on GH release. The cause of these discrepancies cannot be explained, though they may be partly due to different experimental conditions.

Neither CCK nor bombesin is reported to inhibit the GH secretions in in vitro experiments (Westendorf and Schoubrunn, 1982: Malarkey et al., 1981). In this study the inhibitory effects of the two peptides on the GH release were more potent when administered intraventricularly than intravenously. These results suggest central sites of actions of these peptides.

Abe et al. (1981a) reported that bombesin appears to stimulate the secretion of somatostatin from the hypothalamus into hypophysial portal blood, thereby inhibiting GH release from the anterior pituitary in

Fig. 3. The effects of cholecystokinin (CCK) or bombesin on the domperidone-induced PRL release. Vertical bars indicate the mean ±S.E. values of 6 rats. The degree of significance of the difference between control and CCK or bombesin treated rats is shown as *: P<0.05 and **: P<0.01.

- a and b ○: saline ivt+ domperidone 10 µg/100 g B.W. iv
- a ○: CCK 300 ng ivt + domperidone iv
- b ○: bombesin 2 µg ivt + domperidone iv
urethane-anesthetized rat, by measuring the plasma levels of immunoreactive somatostatin in hypophysial portal blood. In this study, the inhibitory effects of the two peptides were observed in the GH releases induced by various substances, i.e. FK 33–824, CPZ and PGE₂, which act by various mechanisms. Beta-endorphin enhances rat GH secretion, probably via GH releasing factor (GRF), without changing the somatostatin level in hypophysial portal blood (Abe et al., 1981b). CPZ increases the GH release in urethane-anesthetized rat by antiodopaminergic rather antinoradrenergic action (Weiner and Ganog, 1981). As domperidone, which does not cross the blood brain barrier, did not increase the plasma GH, the CPZ-induced GH release is thought to...
be due to central antidopaminergic action, though it cannot be decided whether dopamine stimulates a GRF release or/and inhibits a somatostatin release in anesthetized rat. On the other hand, PGE₂ is thought to act directly on the pituitary in the rat (Lee, 1981). Therefore, these findings suggested that CCK and bombesin may increase somatostatin from the hypothalamus, thus suppressing the GH release induced by GRF or PGE₂ on a pituitary level.

The effects of CCK and bombesin on PRL release

Neither CCK nor bombesin is reported to release the PRL in in vitro experiments (Westendorf and Schoubrunn, 1982; Malarkey et al., 1981). In this study, the in-

---

Fig. 5. The effects of intravenous infusion of cholecystokinin (CCK) or bombesin on the FK 33-824-induced GH and PRL releases. Vertical bars indicate the mean±S.E. values for 6 rats. The degree of significance of the difference between control and CCK or bombesin treated rats is shown as **: P<0.01.

a and b ○: FK 33-824 10 μg/100 B.W., iv
a ○: CCK 1 μg/100 g B. W., iv + FK 33-824, iv
b₁ ○: bombesin 10 μg/100 g B.W., iv + FK 33-824, iv
b₂ ○: bombesin 1.0 μg/100 g B.W., iv + FK 33-824, iv
■: bombesin 10 μg/100 g B.W., iv + FK 33-824, iv
hibitory effects of intravenous injections of two peptides on the PRL release were not observed. These results suggest central sites of actions of these peptides. Concerning the effect of CCK, Vijayan et al. (1979) reported that CCK (ivt) increases the PRL release in conscious rat. Concerning the effects of bombesin in in vitro experiment, Rivier et al. (1978) reported that it increases the PRL level in the estrogen-primed male rat. However, Matsushita et al. (1983) reported that gastrin-releasing peptide (GRP), which has a C-terminal decapeptide fragment almost identical to bombesin, inhibited rat PRL release induced by FK 33–824 and domperidone (1 μg/100 g/B.W., iv) but not by domperidone (10 μg/100 g/B.W., iv), and this corresponded to our findings on CCK and bombesin. The cause of these discrepancies cannot be explained, though they may be partly due to different experimental conditions. Matsushita et al. (1983) suggest that GRP inhibits PRL secretion by acting through the brain to stimulate the dopaminergic mechanism via bombesin receptor. This hypothesis may be applied on our observation, because CCK and bombesin inhibited the PRL release induced by various stimuli, such as FK 33–824, PGE₂ and CPZ. When either CCK or bombesin stimulates dopamine release at the hypothalamus, it is possible that a moderate increase in PRL release induced by FK 33–824 or PGE₂ could be blocked by both peptides. However, if pituitary dopamine receptors were occupied by domperidone, the dopamine centrally increased by these peptides could exert no effect to lower PRL release at the pituitary level. CPZ, which is a central and peripheral dopamine antagonist, may increase the PRL releases at the level of both the hypothalamus and the pituitary. In the present series of experiments, there was a difference between domperidone and CPZ in the inhibitory responses, despite similar increases in PRL induced by these anti-dopaminergic agents. This fact may be explained by the theory that a central but not a peripheral antidopaminergic action is blocked by the central dopamine-releasing actions of these peptides.

References


