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Inhibitory Patterns by CB 154 of Prolactin and Growth Hormone Responses to Partially Purified Bovine Hypothalamic Extracts (39879)

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Several ergot alkaloids, such as ergocryptine and ergocornine, are known to inhibit the release of prolactin (PRL) from the pituitary (1-4). It is thought that these ergot derivatives act mainly at the level of the pituitary rather than at the hypothalamic level (2, 5, 6), but little is known concerning their effects on the release of PRL stimulated by hypothalamic extracts. In the present study, we investigated the inhibitory pattern by 2-Br-α-ergocryptine (CB 154) of PRL and growth hormone (GH) responses to partially purified hypothalamic extracts of bovine origin (BHE). These BHE had both PRL-releasing (PRF) and GH-releasing (GRF) activities (7). The *in vitro* perifusion system of rat adenohypophysis previously described (7) was used.

Materials and methods. Male Wistar rats, weighing 180-220 g, were used as pituitary donors. The anterior pituitary was perifused using a multichannel perifusion apparatus which maximally can perifuse 20 incubation chambers simultaneously (7). In brief, one macroscopically intact pituitary was placed in an incubation chamber and perifused with Krebs-Ringer-bicarbonate-2 mg/ml glucose buffer (KRBG), pH 7.4, at a rate of 75 μl/min. The volume of KRBG in the incubation chamber was adjusted to 0.3 ml. Each effluent was collected every 10 min on a fraction collector specifically modified to collect 20 samples at the same time. Fractions No. 39-42 (BHE), following Sephadex G-25 gel filtration of bovine hypothalamic extracts (8, 9) which had both PRF and GRF activities (7), were dissolved in KRBG and infused for 20 min. CB 154 solution at a concentration of 600 μg/ml was obtained from Sandoz Pharmaceuticals and diluted to 1.0 μg/ml with KRBG.

Study 1. One pituitary was perifused with KRBG alone (control group) or KRBG containing 1 μg/ml of CB 154 (experimental group) for 260 min from the beginning of the study. BHE at a dose of 0.2 or 0.5 hypothalamic equivalents/1.5 ml was infused for 20 min starting at 190 min. Two pituitaries per group were used. A total of four pituitaries was perifused simultaneously.

Study 2. The perifusion was performed for 340 min. BHE at a dose of 0.2 hypothalamic equivalents/1.5 ml was administered for two 20-min intervals starting at 190 and 280 min. CB 154 was infused for 20 min, together with the initial BHE in the experimental group, and CB 154 was not administered in the control group. Eight pituitaries were used in the control group and six pituitaries were used in the experimental group. A total of 14 pituitaries was perifused simultaneously.

Study 3. The perifusion was performed for 340 min. BHE at a dose of 0.2 hypothalamic equivalents/1.5 ml was administered for 20 min starting at 280 min. CB 154 was administered for 20 min at 130-, 90-, or 40-min intervals prior to or together with BHE in the experimental group. In the control group CB 154 was not administered. Four pituitaries per group were used. A total of 20 pituitaries was perifused simultaneously.

The concentration of PRL and GH in each effluent was measured by double-antibody radioimmunoassay using NIAMDD kits (7). The results were expressed as nanograms per milligram of pituitary (amount of the hormone released from 1 mg of pituitary for 10 min) in terms of

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NIAMD-rat PRL-RP-1 and rat GH-I-2, for PRL and GH, respectively. The significance of the differences between groups was calculated by Student's t test or Duncan's new multiple range test.

Results. Study 1. Figures 1 and 2 show the patterns of PRL and GH responses to BHE with and without CB 154. The release of PRL was markedly stimulated during the perifusion with BHE at a dose of 0.2 hypothalamic equivalents/1.5 ml in the absence of CB 154 (Fig. 1A). The stimulated release of PRL was completely inhibited in the presence of 1.0 μg/ml of CB 154 (Fig. 1A). CB 154 did not affect the release of GH stimulated by this dose of BHE (Fig. 1B). When the dose of BHE was raised to 0.5 hypothalamic equivalents/1.5 ml, the inhibitory effect of CB 154 on PRL release was partially reduced (Fig. 2A). GH release stimulated by this dose of BHE was not suppressed in the presence of CB 154 (Fig. 2B).

Study 2. As shown in Fig. 3A, the release of PRL was clearly stimulated by both the initial and second BHE infusion. By administering CB 154 with the initial BHE the mean peak value of PRL levels stimulated by the initial BHE was significantly decreased from 2.99 ± 0.19 ng/mg of pituitary (mean ± standard error) (Fig. 3A) to 2.14 ± 0.20 ng/mg of pituitary (Fig. 3B) at 210 min ($P < 0.02$, Student's t test) and the mean peak value stimulated by the second BHE was also decreased from 2.08 ± 0.13 (Fig. 3A) to 0.80 ± 0.13 ng/mg of pituitary (Fig. 3B) at 300 min ($P < 0.01$, Student's t test).

Study 3. The results are summarized in Fig. 4. When the pituitary was perifused

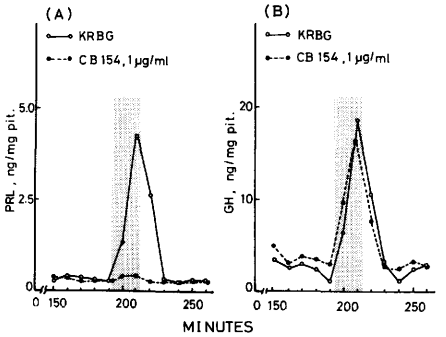

FIG. 1. The effect of CB 154 on PRL and GH release stimulated by partially purified bovine hypothalamic extracts (BHE). BHE at a dose of 0.2 hypothalamic equivalents/1.5 ml was infused for 20 min in the presence and absence of CB 154. Shaded area indicates duration of BHE infusion. A representative pattern of duplicate experiments, which were performed simultaneously, is shown. The response of PRL and that of GH are shown in (A) and (B), respectively. pit. = pituitary.

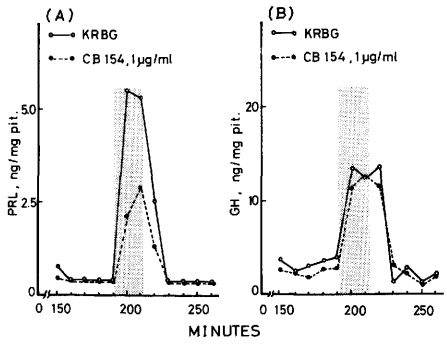

FIG. 2. The effect of CB 154 on PRL and GH release stimulated by BHE. BHE at a dose of 0.5 hypothalamic equivalents/1.5 ml was infused for 20 min in the presence and absence of CB 154. Shaded area indicates duration of BHE infusion. A representative pattern of duplicate experiments, which were performed simultaneously, is shown. The response of PRL and that of GH are shown in (A) and (B), respectively. pit. = pituitary.

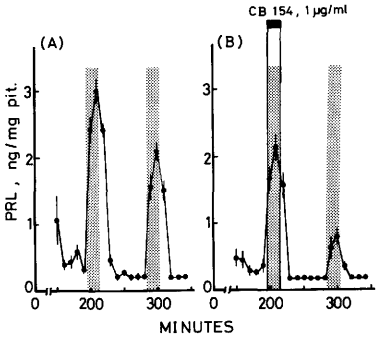

FIG. 3. The effect of a pulsatile administration of CB 154 on PRL release stimulated by BHE. In the control group (A), BHE at a dose of 0.2 hypothalamic equivalents/1.5 ml was infused for two 20-min intervals starting at 190 and 280 min. Shaded area indicates duration of BHE infusion. In the experimental group (B), CB 154, indicated by a black bar, was administered for 20 min together with the first administration of BHE. Eight pituitaries were used for the control group and six pituitaries for the experimental group. A total of 14 pituitaries was perifused simultaneously. Each closed circle and vertical bar gives the mean ± standard error of eight (A) or six observations (B). pit. = pituitary.

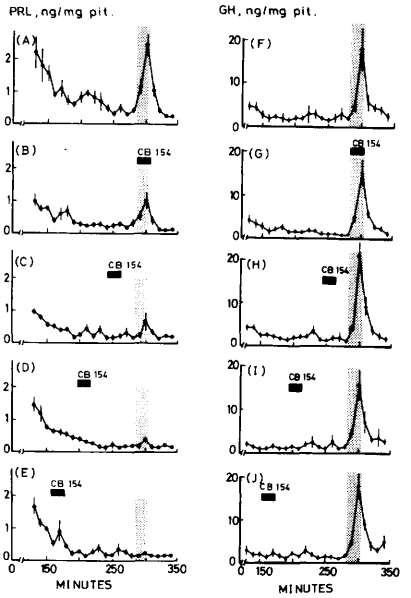

FIG. 4. The effect of the interval between the administrations of CB 154 and BHE on PRL and GH release stimulated by BHE. BHE at a dose of 0.2 hypothalamic equivalents/1.5 ml was administered for 20 min starting at 280 min. Shaded area indicates duration of BHE infusion. CB 154, indicated by a black bar, was administered for 20 min at 130-min (E,J), 90-min (D,I), and 40-min (C,H) intervals prior to and together with (B,G) a 20-min infusion of BHE. Four pituitaries per group were used. A total of 20 pituitaries was perifused simultaneously. (A) through (E) represent the patterns of PRL response, and (F) through (J) represent the patterns of GH response. Each closed circle and vertical bar gives the mean ± standard error of four observations. pit. = pituitary.

with BHE alone, the mean peak value of PRL levels observed at 300 min was 2.43 ± 0.34 ng/mg of pituitary (A). A concomitant administration of CB 154 and BHE (B) caused a partial but significant inhibition of PRL release (1.03 ± 0.25 ng/mg of pituitary) as compared with BHE alone ($P < 0.05$, Student's t test). In the groups pretreated with CB 154 (C through E), the mean peak values of PRL levels observed at 300 min were 0.67 ± 0.26 (C), 0.41 ± 0.09 (D), and 0.25 ± 0.04 ng/mg of pituitary (E). Application of Duncan's new multiple range test revealed that the value of (D) was significantly smaller than that of (B) ($P < 0.05$) and the value of (E) was also significantly smaller than that of (B) ($P < 0.05$). These results indicate that the longer the interval between the treatments of CB 154 and BHE, the stronger is the suppressive effect of CB 154 on PRL release. The basal secretion of PRL was not clearly modified by a pulse of CB 154 (C,D, and E). CB 154 did not affect the release of GH stimulated by BHE whenever the drug was given (G through J).

Discussion. Although the ergot alkaloids have been demonstrated to be effective in inhibiting PRL secretion in animals and humans, the mechanism of the inhibitory activity is not well understood. The alkaloids are generally considered to act mainly at the level of the pituitary on the basis of the findings that they are effective in the *in vitro* studies (1, 2, 6) and in hypophysectomized rats with the transplanted pituitaries beneath the kidney capsules (3, 10). However, one report indicates that the drugs act rather on the hypothalamus (5). Taking another view, Gautvik *et al.* (11) demonstrated that the mode of the inhibitory action of CB 154 was different from that of colchicine (12).

It was clearly demonstrated in the present study that CB 154 selectively suppressed only the release of PRL stimulated by BHE. This provides evidence that CB 154 manifests its inhibitory effect of PRL release at the level of the pituitary by canceling PRF activity in BHE.

Our present results shown in Fig. 3 suggest that CB 154 could affect the later PRL response to BHE despite the fact that this drug had already been washed out. This was confirmed by the results obtained in Study 3 that, once the pituitary was exposed to a pulse of CB 154, the inhibitory action lasted even after being washed out. This effect became more marked as the interval between the administrations of CB 154 and BHE was prolonged (Fig. 4). There is a possibility that CB 154 plays an inhibitory role in PRL release by competing against PRF at the receptor sites of lactotrophs, since the suppressive effect is dependent on the dose of BHE (Figs. 1 and 2). However, considering the findings obtained in the present study, it is more likely to assume that this drug might reduce the affinity of PRF binding to the receptor sites of the pituitary in a different manner. It is not suggested that the inhib-

itory action of CB 154 is ascribed to cellular destruction (13).

It was shown in the *in vitro* static method using rat hemipituitaries that thyrotropin-releasing hormone (TRH) did not have the activity to stimulate the release of PRL (9, 14). However, recent studies indicated that PRL release was stimulated by TRH in dispersed cell culture of rat pituitary (15). PRF activity in BHE used in our study is not attributed to TRH (9). Moreover, TRH did not consistently stimulate PRL secretion in the present perifusion system (unpublished data). Thus, it is not clear whether or not ergot alkaloids can suppress TRH-mediated PRL release in normal rat pituitary *in vitro*. However, the alkaloids have been shown to possess the activity to inhibit TRH-induced PRL release in rat pituitary tumor cells *in vitro* (11) and in man *in vivo* (16). On the other hand, basal secretion of PRL in this study was not affected by a pulse of CB 154. This lack of effect might be ascribed to a relatively low level of basal PRL release.

There are reports showing that CB 154 can affect the release of GH in some patients with acromegaly (17). In the assay system used in the present study we could not observe any influence of CB 154 on GH release from normal rat pituitary. This suggests that CB 154 can act on GH release under some pathological conditions.

Summary. The effect of CB 154 on PRL and GH release stimulated by BHE with PRF and GRF activities was investigated in perifused rat pituitaries *in vitro*. The perifusion with 1.0 μg/ml of CB 154 for 260 min completely suppressed PRL release stimulated by BHE at a dose of 0.2 hypothalamic equivalents/1.5 ml starting at 190 min for 20 min. When the dose of BHE was raised to 0.5 hypothalamic equivalents/1.5 ml, the inhibitory effect was partially reduced. In the next study, BHE was administered twice for 20-min duration at a 70-min interval. CB 154 was administered together with the initial BHE. CB 154 inhibited PRL release not only with the initial BHE but also with the second BHE. When CB 154 was administered prior to BHE at different times for a 20-min interval, it was noticed that the longer the elapsed time between CB 154 and BHE, the stronger was the suppressive effect of CB 154 on PRL release. The basal secretion of PRL was not modified by a pulse of CB 154. Also, CB 154 did not affect BHE-mediated GH release. These results suggest that CB 154 manifests, at least in part, its inhibitory effect on PRL release at the level of the pituitary by canceling PRF activity.

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