Endogenous Cholecystokinin (CCK) Exerts a Tonic Inhibitory Action on Rat Pituitary-Adrenocortical Axis, Acting through the CCK-B Receptor Subtype

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ABSTRACT

Cholecystokinin (CCK) is a regulatory peptide widely distributed in the body tissues and organs, that acts through two subtypes of receptors, called CCKA and CCKB, which are predominantly located in the periphery and the central nervous system, respectively. We have investigated the role of endogenous CCK in the functional regulation of the pituitary-adrenocortical axis in both normal and ether-stressed rats. To this task selective antagonists of CCKA and CCKB receptors (CCKA-α and CCKB-α) were administered (a single subcutaneous injection of 20 nmol/kg), and their effects on hormonal plasma concentrations were examined at various times after the injection. CCKB-α did not alter ACTH plasma concentration at 60 and 120 min in intact rats, but it raised corticosterone blood level. Conversely, CCKB-α increased ACTH plasma concentration at 60 and 120 min in ether-stressed rats, without changing the concentration of corticosterone. CCKB-α evoked a marked rise in the level of circulating ACTH in both group of rats at 15 min. CCKA-α was ineffective in both normal and stressed animals. These findings allow us to conclude that (i) endogenous CCK, acting through the CCKB receptor subtype, exerts a tonic inhibitory action on rat pituitary-adrenocortical function; and (ii) the mechanism of this action of CCK mainly involves the decrease in the pituitary ACTH release.

Cholecystokinin (CCK) is a regulatory peptide involved in the regulation of satiety and stress and anxiety disorders, which is widely distributed in the central nervous system and peripheral organs and tissues. CCK exerts its biological effects through two receptor subtypes, named CCKA and CCKB, which belong to the G protein-coupled receptor superfamily and are predominantly located in the periphery and the central nervous system, respectively (for review, see 2).

The presence of CCK and its receptors has been demonstrated in both the central and the peripheral branches of the mammalian hypothalamo-pituitary-adrenal (HPA) axis (for review, see 4, 6), and, accordingly, evidence is available that the systemic administration of CCK activates HPA in the rat, mainly via the CCKA receptor subtype (8, 11).

Investigations dealing with the possible regulatory role played by endogenous CCK on HPA axis function are lacking. It, therefore, seemed worthwhile to explore this topic by studying the effects of selective antagonists of CCK receptors on the HPA axis of normal and stressed
rats.

MATERIALS AND METHODS
Adult (2-month old) female Wistar rats were kept under a 12:12 h light-dark cycle (illumination onset at 8:00 a.m.) at 23°C, and maintained on a standard diet and tap water ad libitum. Animals were given daily subcutaneous (s.c.) injections of 0.2 ml 0.9% NaCl for 7 days to dampen the response of their HPA axis to handling stress. Groups of rats were stressed by ether inhalation for 10 min, as detailed previously (10).

Normal and stressed rats were divided into three groups, two of which were given a s.c. injection of 20 nmol/kg of the selective CCK-A- and CCK-B-receptor antagonists (CCKA-a and CCKB-a) PD140548 and PD135158, respectively (5, 7), which were purchased from Research Biochemical International (Natick, U.S.A.). This dose of the antagonists was previously found to be effective in inhibiting CCK receptors (8). The third group received a s.c. injection of the saline vehicle. The rats were decapitated at time 0 (just before the injection), 15, 60 and 120 min after the injection (8 rats for each time point). The plasma levels of corticosterone were not measured 15 min after the treatment. All the injections were made at 9:00 a.m. The trunk blood was collected in the presence of EDTA (1 mg/mL), and plasma was separated and stored at −30°C.

ACTH was extracted from plasma and purified (1), and its concentration measured by RIA, using a commercial kit (ACTH Double Antibody) purchased from Diagnostic Products Corporation (Los Angeles, U.S.A.). Corticosterone was extracted from plasma and purified (12), and its concentration assayed by RIA, as previously detailed (10). Intra- and interassay variation coefficients were: ACTH 6% and 9%; and corticosterone 7% and 9%, respectively.

Data were expressed as means (SEM, and their statistical comparison was done by ANOVA, followed by the Multiple Range Test of Duncan.

RESULTS AND DISCUSSION
Neither CCKA-a nor CCKB-a affected ACTH plasma level at 60 and 120 min in normal rats (Fig. 1). In contrast, CCKB-a, but not CCKA-a, significantly raised corticosterone blood concentration (Fig. 2). As expected, ether stress marked-

![Fig. 1: Effect of CCK-receptor antagonists on ACTH plasma concentration in normal and ether-stressed rats. Data are means (SEM) (n=8). *P<0.01 from baseline (0-time); ^P<0.01 from the respective control value.](image1)

![Fig. 2: Effect of CCK-receptor antagonists on corticosterone plasma concentration in normal and ether-stressed rats. Data are means (SEM) (n=8). *P<0.01 from baseline (0-time); ^P<0.01 from the respective control value.](image2)
ly increased both ACTH and corticosterone plasma concentrations at both 60 and 120 min. CCKB-a, but not CCKA-a, evoked a further significant rise in ACTH blood level (Fig. 1), without affecting that of corticosterone (Fig. 2). CCKB-a, but not CCKA-a, markedly enhanced the level of circulating ACTH at 15 min in both normal and stressed rats (Fig. 3).

Taken together, the present findings indicate that the acute blockade of CCKB receptors evokes in normal rats a transient stimulatory effect on ACTH release at 15 min, which results in a significant rise in corticosterone secretion detectable at 60 and 120 min. This suggests that endogenous CCK, acting through the central CCKB subtype of receptors, exerts a tonic suppressive action on the central branch of the rat HPA axis. Hence, the physiological relevance of the previously reported CCKA receptor-mediated stimulatory effect of exogenous CCK on rat HPA axis (8) remains very doubtful. We have previously found that endogenous CCK exerts a CCKA receptor-mediated tonic inhibition of corticosterone secretion from regenerating rat adrenals (8), coupled with a stimulatory effect on the proliferation of their cells (9). Our present results may indicate that these last effects of endogenous CCK are independent of any action on the ACTH release: they could be mediated by CCKA receptors located on adrenals and coupled with the cascades activating cell proliferation. The inhibition of corticosterone production may be consequent to this primary effect of CCK, inasmuch as it is well known that an inverse correlation occurs between adrenocortical cell proliferative and secretory activities (for review, see 13).

Available literature data indicate that endogenous CCK plays a role in the response to various stress stimuli. In the rat, stresses affect both CCK levels and CCKB-receptor binding in different brain regions, among which the hypothalamus (for review, see 3). Ether stress, as applied in the present study, is perhaps the most potent factor activating HPA axis. In spite of this, the acute blockade of CCKB receptors significantly enhances ether-evoked pituitary ACTH release, thereby suggesting an important role of endogenous CCK in dampening exceedingly high responses of the HPA axis to stress. CCKB-a does not significantly magnify corticosterone response to stress. This observation suggests that in the rat ether stress evokes a maximal stimulation of corticosteroid release, the glucocorticoid secretory capacity of the adrenal cortex being the limiting factor of the response of the HPA axis to stresses.

REFERENCES