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Interaction between GABAergic neurotransmission and rat hypothalamic corticotropin-releasing hormone secretion in vitro*

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Corticotropin-releasing hormone (CRH) has been considered a major coordinator of the overall physical and behavioral response to stress. Moreover, prolonged hypersecretion of CRH has been implicated in the pathogenesis of disorders characterized by anxiety and/or depression. Drugs acting through the γ-aminobutyric acid/benzodiazepine (GABA/BZD) receptor system have anxiolytic and/or antidepressant properties whereas benzodiazepine inverse agonists cause anxiety and stimulate the pituitary–adrenal axis in vivo. To examine the involvement of the GABA/BZD system in the regulation of hypothalamic CRH secretion, we studied the effects of various agonists and antagonists of $GABA_A$ and $GABA_B$ receptors using a sensitive rat hypothalamic organ culture with radioimmunoassayable CRH (IR-rCRH) as endpoint. The $GABA_A$ and $GABA_B$ receptor agonist GABA inhibited serotonin (5-HT)-induced IR-rCRH secretion from 10^{-9} to 10^{-6} M, but failed to do so at 10^{-5} M. The $GABA_A$ receptor agonist muscimol was a weak inhibitor of 5-HT-induced IR-rCRH secretion, being effective only at the concentration of 10^{-6} M. In contrast, the specific $GABA_B$ receptor agonist baclofen was able to inhibit 5-HT-induced IR-rCRH secretion from 10^{-7} to 10^{-5} M. The rank of potency was thus, GABA >> baclofen > muscimol. Bicuculline, a $GABA_A$ receptor antagonist, partially reversed the inhibitory effects of GABA. Diazepam, a classic benzodiazepine which interacts with the benzodiazepine-site of the $GABA_A$ receptor complex, inhibited 5-HT-induced IR-rCRH secretion from 3.3×10^{-9} to 10^{-5} M, an effect that could be reversed by the BZD inactive ligand Ro15-1788. The diazepam inverse agonist γ-carboline-3-carboxylic acid methylester was a potent stimulator of IR-rCRH secretion and its effect was antagonized only by high concentrations of diazepam but not by Ro15-1788. These results suggest that the GABA/BZD system is involved in the regulation of CRH secretion. It appears that both $GABA_A$ and $GABA_B$ receptors mediate the suppressive effects of GABA upon 5-HT-induced CRH secretion. We speculate that drugs acting through the GABA/BZD system may exert, at least in part, their anxiolytic and/or antidepressant effects via suppression of central CRH secretion.

INTRODUCTION

Corticotropin-releasing hormone (CRH) plays a role in coordinating endocrine, physiological and behavioral responses to stressful stimuli[29,51]. This 41 amino acid neuropeptide is a principal stimulus of the hypothalamic–pituitary–adrenal (HPA) axis[51] and produces a variety of effects resembling stress responses, including increases in heart rate, blood pressure, and oxygen consumption, as well as elevated plasma level of catecholamines, vasopressin (AVP) and glucose[11]. Intracerebroventricular (i.c.v.) administration of CRH produces a dose-dependent behavioral activation, characterized by increased locomotion and combativeness[9,48]. Additional behavioral effects at higher concentrations can be considered anxiogenic (e.g. enhancing responses to acoustic stimuli)[10,49]. In concert with these activating effects,

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vegetative functions such as feeding and sexual behavior are inhibited by centrally administered CRH[9,39,47].

In contrast to the activating effects of CRH, γ-aminobutyric acid (GABA) or benzodiazepines (BZDs) appear to suppress HPA axis function[1,24,25,27,30,51] and to decrease physiological responses to stress, such as hypertension[5] and gastric ulceration[18]. Moreover, activation of the GABA/BZD neurotransmitter system also exerts behavioral effects in opposition to those of CRH by decreasing arousal and inducing effects which can be characterized as anxiolytic[10].

GABAergic effects are mediated by primarily two types of receptors within the central nervous system (CNS), the $GABA_A$ and the $GABA_B$ receptors[7]. The $GABA_A$ receptor is a macromolecular complex with separate binding sites for GABA, BZDs and barbiturate-like substances. The GABA binding site of the $GABA_A$ receptor is identified by the binding of the antagonist bicuculline, whereas muscimol is the classic pharmacologic agonist for this site. Activation of the $GABA_A$ site results in the opening of the chloride ionophore linked to this receptor, leading to an influx of chloride ions and hyperpolarization of the cell, and hence, inhibition of cell firing. An additional binding site on the $GABA_A$ macromolecular complex is that for BZDs, whose action enhances the binding of GABA to the $GABA_A$ receptor GABA binding site, functionally increasing the frequency with which GABA-operated chloride channels open[7]. Binding of β-carboline-3-carboxylic acid methylester (β-CCM) to a region of the BZD binding site of the $GABA_A$ receptor, known as the inverse agonist site, produces effects antithetical to those of BZDs, including intense anxiety and seizures[15].

Differently from $GABA_A$ receptor, the GABA site of the $GABA_B$ receptor shows no affinity for bicuculline and only weak affinity for muscimol which acts as an antagonist. (−)-b-*p*-chlorophenyl GABA (baclofen) is a potent pharmacologic agonist of $GABA_B$ receptor. Moreover, the $GABA_B$ receptor is not linked to BZDs, nor is it linked to the chloride ionophore. Activation of the $GABA_B$ receptors decreases the influx of extracellular calcium ions[7]. GABA may also interact with a third receptor, the so-called 'peripheral type' BZD receptor, also recently described in the CNS. In contrast to the robust binding of GABA to the $GABA_A$ and $GABA_B$ receptors, it appears that GABA has only a weak affinity for this receptor[32,43].

In the light of the apparently antithetical effects of CRH and the GABA/BZD system on various physiological and behavioral parameters, the present study was undertaken to investigate whether the GABA/BZD system is directly involved in the regulation of hypothalamic CRH secretion. To accomplish this we evaluated the effects of GABA and a variety of GABA/BZD agonists and antagonists on hypothalamic CRH secretion in vitro, using a sensitive hypothalamic organ culture system which employed one rat hypothalamic explant per incubation chamber and radioimmunoassayable CRH (IR-rCRH) as endpoint.

MATERIALS AND METHODS

Materials

Serotonin hydrochloride (5-HT), GABA, bicuculline methobromide, muscimol and diazepam were purchased from Sigma (St. Louis, MO). β-CCM was purchased from Research Biochemicals Inc. (Natick, MA). Baclofen and Ro15-1788 were gifts from Ciba Geigy (Summit, NJ) and Hoffman-La Roche (Nutley, NJ), respectively.

Hypothalamic organ culture

Male Sprague–Dawley rats (175–225 g, Taconic Farm, Silver Spring, MD) were sacrificed by decapitation and the hypothalami were rapidly removed with fine-point-curved scissors using a sterile technique, from the optic chiasm anteriorly, to the mammillary bodies posteriorly, and along the hypothalamic sulci laterally. The depth of the fragments was about 3 mm. Immediately after the explantation, the hypothalami (one hypothalamus per well, 48-multiwell plates, Costar, Cambridge, MA) were incubated in 1.0 ml of medium 199 with modified Earle's salt (Gibco, Grand Island, NY) containing 0.1% BSA (fraction V, Sigma, St. Louis, MO) and 20 μM bacitracin (Aldrich, Milwaukee, WI), in a water-jacketed incubator at 37 °C, under 5% CO_2 atmosphere. The experiments (see Experimental protocols below) were performed after an overnight (18 h) preincubation. Detailed biological and histological studies on freshly explanted and overnight preincubated hy-

pothalami are given elsewhere[12]. In brief, hypothalami preincubated overnight exhibited IR-rCRH content and histologic appearance, studied by both light and electron microscopy, similar to freshly explanted tissues. Some afferent nerve demyelination was observed presumably as a result of denervation. The paraventricular nucleus (PVN) cells of hypothalami preincubated overnight excluded Trypan blue.

Following each experiment (see Experimental protocols below), we indirectly confirmed tissue viability by exposing each hypothalamus to a depolarizing concentration of potassium chloride (60 mM) for 20 min. Explants that failed to respond to KCl with an increase of IR-rCRH of at least 90% (approximately 10 intra-assay coefficients of variation) above baseline were excluded from analysis. Lack of response to KCl was observed on approximately 20% of the hypothalami employed.

Experimental protocols

An incubation volume of 0.4 ml was used for all experiments. To determine the effects of 5-HT and β-CCM on hypothalamic IR-rCRH secretion the hypothalami were incubated serially in 6 wells for 20 min per well, for a total period of 120 min. During this time the IR-rCRH secretion in plain medium remained constant. In particular, the hypothalami were incubated in plain medium in the first 3 wells (unstimulated IR-rCRH concentration), exposed to 10^{-9} M 5-HT or graded concentrations of β-CCM in the next two wells (stimulated IR-rCRH concentration), and exposed to 60 mM KCl in the sixth well. The hypothalami were transferred from one well to another using 3 × 3 mm nylon mesh grid (Small Parts, Miami, FL).

To evaluate the effects of GABA, muscimol, baclofen, bicuculline, diazepam and Ro15-1788 on 5-HT-or β-CCM-induced IR-rCRH secretion, graded concentration of the above-mentioned pharmacologic agents were added during the last hour of preincubation and throughout the experiment performed as indicated above. Finally, bicuculline and Ro15-1788 were added simultaneously to GABA or diazepam, respectively, to examine whether the effect of GABA could be reversed by a $GABA_A$ antagonist, and whether the inhibitory effect of diazepam upon 5-HT-induced IR-rCRH secretion could be antagonized by Ro15-1788.

CRH radioimmunoassay

The concentration of rCRH in the medium was measured directly, without prior extraction, by RIA, using specific anti-rCRH serum (TS-3) developed in our laboratory[43]. Rat CRH, labeled by the chloramine-T method[26] with ^{125}I on the histidine-residue, was stored at 4 °C for up to 8 weeks. An appropriate aliquot was passed through a 0.9 × 58 cm column of Sephadex G-50 fine (Pharmacia, Piscataway, NJ), before use. Aliquots of 100 μl of rCRH standard solution or media samples were incubated for 48 h at 4 °C with TS-3 antiserum (final dilution 1:21,000) diluted in assay buffer. After 48 h, 100 μl of [^{125}I]rCRH (3000–3200 cpm) were added and left to incubate for 24 h. Separation of bound from free labelled rCRH was achieved by incubating each tube for 12–16 h at 4 °C with 50 μl goat anti-rabbit serum diluted 1:5 with assay buffer containing 1% BSA. The tubes were centrifuged at 2000 *g* for 20 min, the supernatants were aspirated and the precipitates were counted in a gammacounter. All standards were measured in triplicate and the media samples in duplicate. Total binding of labelled rCRH by TS-3 antiserum was 32 ± 1.9% (mean ± S.E.M.) and non-specific binding was 2.1 ± 0.3%. Sensitivity (ED_{90}) of the assay was 2.0 ± 0.1 pg/assay tube (20 pg/ml of media). Parallelism was obtained with the incubation medium. The intra-assay coefficients of variation were 8.1 and 14.7%, respectively. TS-3 antiserum showed no significant (<0.01%) cross-reactivity with the following peptides: LH-RH, GH-RH, TRH, somatostatin, substance P, porcine vasoactive intestinal peptide (VIP), neuropeptide Y, hACTH (1–39), β-endorphin, α-MSH, β-MSH, dynorphin (1–17), AVP, and oxytocin (OT).

Analysis of data

Results, expressed as mean ± S.E.M. percent IR-rCRH increase above unstimulated secretion throughout the study, were calculated applying the following formula:

$$\Delta CRH\,(\%) = \frac{(\text{Stimulated IR-rCRH concentration} - 1)}{\text{Unstimulated IR-rCRH concentration}} \times 100$$

Statistical evaluation was done using one-way analysis of variance (ANOVA) followed by the Duncan

multiple range test (DUN). Control data were pooled as indicated in the legend to the figures. Logarithmic transformation of the data, applied to correct for heteroscedasticity (detected by the Bartlett test), was used before ANOVA and DUN analyses. Significance was accepted at a P-value less than 0.05. Mean ± S.E.M. concentrations of GABA, muscimol, baclofen and diazepam producing 50% inhibition (ID_{50}) of 5-HT-induced IR-rCRH secretion were calculated using the 4 parameters logistic equation. A computer program, 'ALLFIT', developed by DeLean et al., was employed for the computations[20].

RESULTS

Immunoreactive-rCRH secretion from hypothalami incubated in plain medium was 36 ± 2 pg/0.4 ml/20 min of incubation. Serotonin 10^{-9} M induced 250 ± 52% (n = 22) IR-rCRH increase above baseline ($P <$ 0.005 vs unstimulated release). The effects of GABA, muscimol, baclofen and diazepam on 5-HT-induced IR-rCRH secretion are shown in Fig. 1. GABA (Fig. 1A) inhibited 5-HT-induced IR-rCRH secretion from 10^{-10} to 10^{-6} M, but not at the concentration of 10^{-5} M ($P < 0.05$ vs 5-HT alone, DUN). Muscimol (Fig. 1B) inhibited 5-HT-induced IR-rCRH secretion only at 10^{-6} M concentration (27 ± 16 vs 250 ± 52% increase above baseline, $P < 0.05$, DUN), and failed to inhibit this parameter at 10^{-5} M. Baclofen (Fig. 1C) inhibited 5-HT induced IR-rCRH secretion from 10^{-7} to 10^{-5} M concentrations ($P < 0.05$ vs 5-HT alone, DUN). Diazepam inhibited 5-HT induced IR-

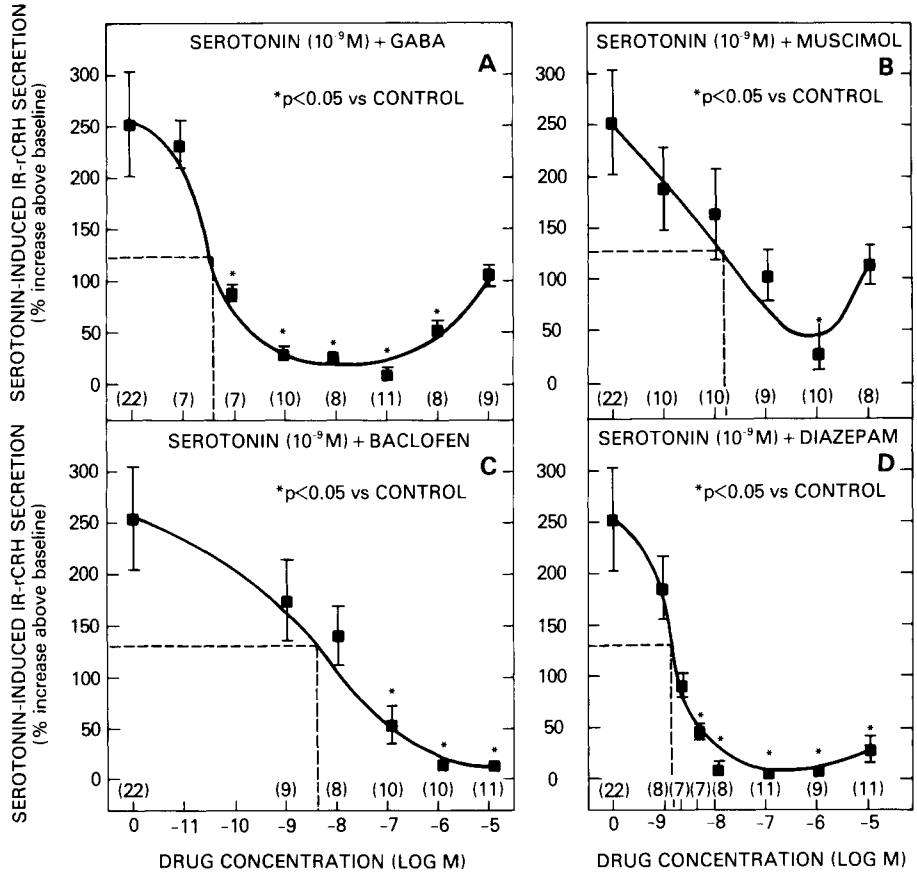

Fig. 1. IR-rCRH secretion by single explanted rat hypothalami exposed to 10^{-9} M serotonin and serotonin plus graded concentrations of GABA (A), serotonin plus graded concentrations of muscimol (B), serotonin plus graded concentrations of baclofen (C) and serotonin plus graded concentrations of diazepam (D). Results (mean ± S.E.M.) are expressed as percent increase above baseline. $^{*}P <$ 0.05 vs serotonin alone, Duncan test. The number of hypothalami tested per each dose level is indicated in parentheses. Dotted lines intercept curves at ED_{50} level. Control data were pooled from different experiments.

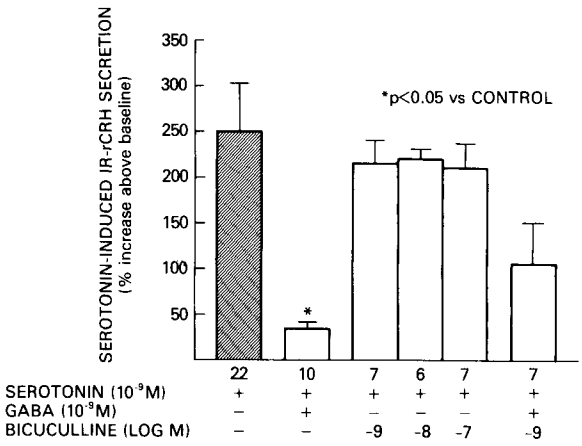

Fig. 2. IR-rCRH secretion by single explanted rat hypothalami exposed to 10^{-9} M serotonin (shaded bar), serotonin plus GABA, serotonin plus bicuculline alone or serotonin plus GABA and bicuculline. Results (mean ± S.E.M.) are expressed as percent increase above baseline. n = number of hypothalami tested for each dose level. Control data were pooled from different experiments.

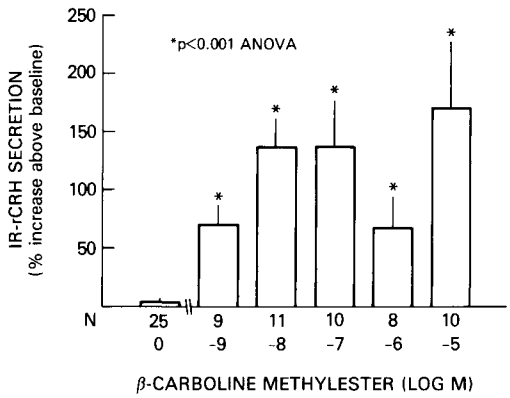

Fig. 4. IR-rCRH secretion by single explanted rat hypothalami exposed to graded concentrations of β-carboline methylester (β-CCM). Results (mean ± S.E.M.) are expressed as percent increase above baseline. *$P < 0.001$, ANOVA. n = number of hypothalami tested for each dose level.

rCRH secretion in a dose-dependent fashion from 3.3×10^{-9} to 10^{-5} M concentration ($P < 0.05$ vs 5-HT alone, DUN), but not at 10^{-9} M (182 ± 34 vs 250 ± 52% increase above baseline). Mean ± (S.E.M.) concentrations of GABA, muscimol, baclofen and diazepam capable of inhibiting 5-HT-induced IR-rCRH secretion by 50% were $5.5 \pm 1.1 \times 10^{-11}$, $1.0 \pm 0.4 \times 10^{-8}$, $5.6 \pm 1.9 \times 10^{-9}$ and $1.9 \pm 0.2 \times 10^{-9}$ M, respectively.

The inhibitory effect of 10^{-9} M GABA upon 5-HT-induced IR-rCRH secretion was partially reversed by equimolar concentrations of bicuculline. On the other hand, bicuculline alone (from 10^{-9} to 10^{-7} M concentrations) did not have any effect on 5-HT-induced IR-rCRH secretion (Fig. 2). The inhibitory effect of 3.3×10^{-9} M diazepam upon 5-HT-induced IR-rCRH secretion could be antagonized by the BZD inactive ligand Ro15-1788 used at 10^{-8}, but not

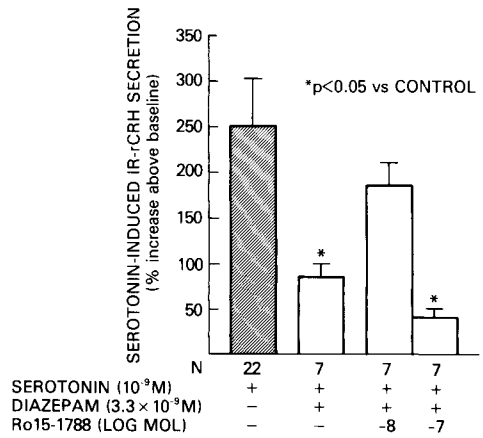

Fig. 3. IR-rCRH secretion by single explanted rat hypothalami exposed to 10^{-9} M (shaded bar), serotonin plus diazepam or serotonin plus diazepam plus Ro15-1788. Results (mean ± S.E.M.) are expressed as percent increase above baseline. *$P < 0.05$ vs 5-HT alone, Duncan test. n = number of hypothalami tested for each dose level. Control data were pooled from different experiments.

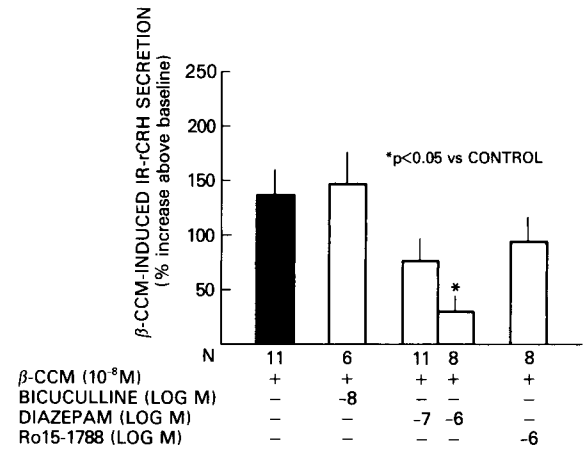

Fig. 5. IR-rCRH secretion by single explanted rat hypothalami exposed to 10^{-8} M β-CCM (closed bar), β-CCM plus bicuculline, β-CCM plus diazepam or β-CCM plus Ro15-1788. *$P < 0.05$ vs β-CCM alone, Duncan test. n = number of hypothalami tested for each dose level.

at the concentration of 10^{-7} M (Fig. 3).

β-Carboline methylester stimulated IR-rCRH secretion at all concentrations used, from 10^{-9} to 10^{-5} M ($P < 0.05$ vs concentration zero, DUN; Fig. 4). Bicuculline (10^{-8} M) or Ro15-1788 (10^{-6} M) did not have any effect on IR-rCRH secretion induced by 10^{-8} M β-CCM. In contrast, 10^{-6} M diazepam was able to inhibit β-CCM-induced IR-rCRH secretion ($P < 0.05$ vs β-CCM alone, DUN; Fig. 5).

DISCUSSION

The results of the present paper agree with many studies which have indicated that both GABA and BZDs inhibit HPA axis function in vivo. In this regard, GABA administered i.c.v. to rats suppresses surgical stress-induced activation of the HPA axis[30], and it appears to restrain the activation of this axis by ether[31]. In addition, both plasma corticosterone[2] and the hypothalamic GABA system[14] show circadian fluctuations which correlate negatively with each other. The above effects of GABA on the HPA axis appear to be exerted mainly at a central level[25]. Subcutaneous, but not i.c.v., injection of γ-aminohydroxybutyric acid, a GABA derivative which does not cross the blood–brain barrier in humans, does not have any effect on HPA axis function[50]. Furthermore, Plotsky et al. have recently reported that GABA injected i.c.v. in rats caused inhibition of immunoreactive CRH concentration in the hypophysial portal blood system[37]. The presence of $GABA_A$ receptors[19] in the hypothalamus and the presence of a dense network of glutamate decarboxylase (GABA synthesizing enzyme)-positive nerve fibers evenly distributed throughout the hypothalamus[53] provide further evidence for a hypothalamic site of action. It should be noted, however, that a possible pituitary site for GABA action on the HPA axis has also been reported[3].

Benzodiazepines cause a dose-dependent, time-limited suppression of cortisol levels in normal volunteers and in depressed patients[1,24,25,51]. The suppression occurs at doses that do not cause significant signs of sedation. In animal experiments BZDs seem to suppress corticosterone levels during stress-induced HPA axis activation, and in rats this effect is counteracted by the imidazo-benzodiazepine Ro15-1788 (ref. 6), a relatively specific and potent BZD receptor ligand[38] with presumably little biological activity of its own. (Recently, some anticonvulsant[40] and proconvulsant[16] effects of Ro15-1788 were reported). In a similar vein, intravenous infusion of the inverse benzodiazepine receptor agonist β-carboline-3-carboxylic acid ethylester in rhesus monkeys was shown to cause signs of anxiety accompanied by a pronounced rise of plasma ACTH and cortisol. Both effects could be prevented by diazepam or Ro15-1788 pretreatment[17,34]. In the present study, Ro15-1788 was able to antagonize the inhibitory effects of diazepam on 5-HT-induced IR-rCRH secretion only when employed at the concentration of 10^{-8} M. Surprisingly, this effect was lost at 10^{-7} M and Ro15-1788 at higher concentrations was unable to antagonize the stimulatory effects of β-CCM on IR-rCRH secretion. A possible explanation is that at high concentrations Ro15-1788 exerts agonist or antagonist effects of its own[16,40]. This hypothesis is supported by two sets of data. First, Ro15-1788 inhibited 5-HT-induced IR-rCRH secretion when employed at concentrations greater than 10^{-7} M, suggesting that it may have some benzodiazepine agonist effect. Second, Ro15-1788 at 10^{-9} M antagonized the inhibitory effect of the triazolo-benzodiazepine alprazolam on 5-HT-induced IR-rCRH secretion (Kalogeras and Calogero, unpublished observations). Other explanations for the inability of Ro15-1788 to inhibit β-CCM-induced IR-rCRH secretion may be its lower binding affinity for the receptor[8,33]. Less likely, the effect of β-CCM on IR-rCRH secretion might not be mediated by the benzodiazepine receptor.

The mixed $GABA_A$ and $GABA_B$ receptor agonist GABA, the $GABA_A$ agonist muscimol and the $GABA_A$ agonist baclofen were all able to inhibit 5-HT-induced IR-rCRH secretion in vitro. Baclofen was twice as effective as muscimol. This difference may reflect poor penetration of muscimol into the cultured tissue. Surprisingly, GABA lost its inhibitory activity at 10^{-5} M concentration. We have no definitive explanation for this phenomenon, but it could reflect the capacity of the $GABA_A$ receptor to rapidly undergo desensitization upon exposure to pharmacologic concentrations of $GABA_A$ agonists[13,44]. In contrast to the $GABA_A$ receptor, inhibition of 5-HT-induced CRH secretion by $GABA_B$ activation showed no loss of efficacy when baclofen was used at concentrations as high at 10^{-5} M, though the lowest

maximal inhibitory concentration was lower than that required for $GABA_A$-mediated inhibition.

GABA was effective in inhibiting 5-HT-induced CRH secretion at concentrations that are about one thousandth of those required for GABA to produce different effects[3,13]. Some endocrine effects of GABA and GABAergic agonists and antagonists, however, have been reported at concentrations as low as 10^{-11} M[37,54]. Development of hypothalamic hypersensitivity probably mediated by an increase of receptor number and/or increased receptor affinity to both agonists and antagonists might explain why GABA was effective in inhibiting IR-rCRH secretion at these low concentrations. The development of hypersensitivity after abolition of regulatory influences from higher centers seems to be a general characteristic of the nervous system, expressed clearly in systems associated with motor function (e.g. development of spasticity) or regulation of blood pressure (hypersensitivity of blood vessel tone following interruption of autonomic system regulatory input)[21]. An alternative and/or additional explanation for the hypersensitivity of our 18 h-preincubated explants may be the demyelination of afferent axons that takes place following explantation ('denervation') during the in vitro preincubation[12]. An analogous phenomenon is the decreased seizure threshold of patients with demyelinating disease.

CRH function and GABAergic neurotransmission appear to play antithetical effects on various physiological and behavioral parameters. In contrast to the behavioral activation induced by i.c.v. administration of CRH, activation of either the $GABA_A$ or $GABA_B$ receptor systems increases seizure threshold, decreases arousal, and exerts anxiolytic effects[1,5,18,24,25,28,46,51], while antagonism of the $GABA_A$ system with agents such as β-CCM or bicuculline produces intense anxiety and promotes seizures[17,34,35]. Furthermore, alterations of the CRH system and of the endogenous GABAergic neurotransmission have been implicated in a variety of illnesses, including major depressive disorders and panic-anxiety disorder. In depression, levels of GABA in the plasma and in the cerebrospinal fluid (CSF) are reduced[22,36], compatible with the clinical observation that depressed patients classically show dysphoric hyperarousal and sustained anxiety. The same patients have a hyperactive HPA axis with high-normal or frankly elevated concentrations of CSF CRH[23]. Patients with panic-anxiety disorder show clear evidence for CRH hypertension[41], and the intermittent and explosive attacks associated with the illness can be treated and largely prevented by the administration of GABA/BZD agonists[45].

It is not entirely unexpected that activation of the principal inhibitory system in the brain[4] would exert a profound inhibition of the CRH neuron, which is postulated to be a mediator of arousal and of the stress response[9,10,48,49]. Moreover, in the light of the putative role of CRH in major depression[23] and panic disorder[41], it is tempting to speculate that the anxiolytic and the antistress effects of drugs acting through the GABA/BZD system are, at least in part, mediated by inhibition of the CRH system.

REFERENCES

1 Adam, K., Oswald, F. and Shapiro, C., Effects of loprazolam and of triazolam on sleep and overnight urinary cortisol, *Psychopharmacology*, 82 (1984) 389–394.

2 Ahlers, I., Smajda, B. and Ahlersova, E., Circadian rhythm of plasma and adrenal corticosterone in rats: effect of restricted feeding schedules, *Endocrinol. Exp.*, 14 (1980) 183–190.

3 Anderson, R.A. and Mitchell, R., Effects of γ-aminobutyric acid receptor agonists on the secretion of growth hormone, luteinizing hormone, adrenocorticotropin hormone and thyroid-stimulating hormone from the rat pituitary gland in vitro, *J. Endocrinol.*, 108 (1986) 1–8.

4 Baxter, C.F., The nature of γ-aminobutyric acid. In A. Lajtha (Ed.), *Handbook of Neurochemistry, Vol. 2*, Plenum, New York, 1970, pp. 289–353.

5 Benson, H., Herd, J.A., Morse, W.H. and Kelleher, R.T., Hypotensive effects of chlordiazepine, amobarbital and chlorpromazine on behaviorally elevated blood pressure in squirrel monkey, *J. Pharmacol. Exp. Ther.*, 173 (1970) 399–406.

6 Bizzi, A., Ricci, M.R., Veneroni, E., Amato, M. and Garattini, S., Benzodiazepine receptor antagonists reverse the effect of diazepam on plasma corticosterone in stressed rats, *J. Pharm. Pharmacol.*, 36 (1984) 134–135.

7 Bowery, N.G., Hill, D.R., Hudson, A.L., Price, G.W., Turnbull, W.J. and Wilkin, G.P., Heterogeneity of mammalian GABA receptors. In N.G. Bowery (Ed.), *Actions and Interactions of GABA and Benzodiazepine*, Raven, NY, 1984, pp. 81–108.

8 Braestrup, C. and Nielsen, M., GABA reduces binding of ^{3}H-methyl β-carboline-3-carboxylate to brain benzodiazepine receptors, *Nature (Lond.)*, 294 (1981) 472–474.

9 Britton, D.R., Koob, G.F., Rivier, J. and Vale, W., Intraventricular corticotropin-releasing factor enhances behavioral effects of novelty, *Life Sci.*, 31 (1982) 363–367.

10 Britton, W.T., Morgan, J., Rivier, J., Vale, W. and Koob, G.F., Chlordiazepoxide attenuates response suppression induced by corticotropin-releasing factor in the conflict test, *Psychopharmacology*, 86 (1985) 170–174.

11 Brown, M.R., Fisher, L.A., Rivier, J., Spiess, J., Rivier, C. and Vale, W., Corticotropin-releasing factor effects on the sympathetic nervous system and oxygen consumption, *Life Sci.*, 30 (1982) 207–210.

12 Calogero, A.E., Bernardini, R., Margioris, A.N., Bagdy, G., Gallucci, W.T., Munson, P.J., Tamarkin, L., Tomai, T.P., Brady, L., Gold, P.W. and Chrousos, G.P., Effects of serotonergic agonists and antagonists on corticotropin-releasing hormone secretion by explanted rat hypothalami, submitted.

13 Cash, D.J. and Subbarao, K., Gamma-aminobutyric acid (GABA)-mediated transmembrane chloride flux with membrane vesicles from rat brain measured by quench flow technique: kinetic homogeneity of ion flux an receptor desensitization, *Life Sci.*, 41 (1987) 437–445.

14 Cattabeni, F., Maggi, A., Monduzzi, M., De Angelis, L. and Racagni, G., Circadian fluctuations of rat hypothalamus, *J. Neurochem.*, 31 (1978) 565–567.

15 Corda, M.G., Blaker, W.D., Mendelson, W.B., Guidotti, A. and Costa, E., β-Carbolines enhance shock-induced suppression of drinking in rate, *Proc. Natl. Acad. Sci. (U.S.A.)*, 80 (1983) 2072–2076.

16 Corda, M.G., Costa, E. and Guidotti, A., Specific proconvulsant action of an imidazobenzodiazepine (Ro15-1788) on isoniazid convulsions, *Neuropharmacology*, 21 (1982) 91–94.

17 Crawley, J.N., Ninan, P.T., Pickar, D., Chrousos, G.P., Linnoila, M., Skolnick, P. and Paul, S.M., Neuropharmacological antagonism of the β-carboline-induced 'anxiety' response in rhesus monkeys, *J. Neurosci.*, 5 (1985) 477–485.

18 Dasgupta, S. and Mukherjee, B., Effect of chlordiazepoxide on stomach ulcers in rabbits induced by stress, *Nature (Lond.)*, 215 (1967) 1183.

19 DaVanzo, J.P., Chamberlain, J. and McConnaughey, M.M., Influence of environment on GABA receptors in muricidal rats, *Pharmacol. Biochem. Behav.*, 25 (1986) 95–98.

20 DeLean, A., Munson, P.J. and Rodbard, D., Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay, and physiological dose–response curves, *Am. J. Physiol.*, 235 (1978) E97–E102.

21 Ganong, W.F., *Review of Medical Physiology*, 12th edn., Lange Medical Publication, Los Altos, CA, 1985, 654 pp.

22 Gold, B.I., Bowers, M.B., Roth, R.H. and Sweeney, D.W., GABA levels in cerebrospinal fluid of patients with psychiatric disorders, *Am. J. Psychiatry*, 137 (1980) 362–364.

23 Gold, P.W., Loriaux, D.L., Roy, A., Kling, M.A., Calabrese, J.R., Hellner, C.H., Nieman, L.K., Post, R.M., Pickar, D., Gallucci, W., Avgerinos, P., Paul, S., Oldfield, E.H., Cutler, G.B. and Chrousos, G.P., Responses to corticotropin-releasing hormone in the hypercortisolism of depression and Cushing disease: pathophysiologic and diagnostic implications, *N. Engl. J. Med.*, 314 (1986) 1329–1335.

24 Gram, L.F., Christensen, L., Kristensen, C.B. and Kragh-Sorensen, P., Suppression of plasma cortisol after oral administration of oxazepam in man, *Br. J. Clin. Pharmacol.*, 17 (1984) 176–178.

25 Gram, L.F. and Christensen, P., Benzodiazepine suppression of cortisol secretion: a measure of anxiolytic activity, *Pharmacopsychiatry*, 19 (1986) 19–22.

26 Greenwood, F.C., Hunter, W.N. and Glover, J.S., The preparation of ^{131}I-labelled human growth hormone of high specific radioactivity, *Biochem. J.*, 89 (1962) 114–123.

27 Jones, M.T., Gillham, B., Altaher, A.R.H., Nicholson, S.A., Campbell, E.A., Watts, S.M. and Thody, A., Clinical and experimental studies on the role of GABA in the regulation of ACTH secretion: a review, *Psychoneuroendocrinology*, 9 (1984) 107–123.

28 Kalin, N.H., Shelton, S.E. and Barksdale, C.H., Separation distress in infant rhesus monkeys: effects of diazepam and Ro15-1788, *Brain Research*, 408 (1987) 192–198.

29 Koob, G.F., Stress, corticotropin-releasing factor, and behavior. In R.B. Williams (Ed.), *Perspectives on Behavioral Medicine, Vol. 2: Neuroendocrine Control and Behavior*, Academic, New York, 1985, pp. 39–52.

30 Makara, G.B. and Stark, E., Effect of gamma-aminobutyric acid (GABA) and GABA antagonist drugs on ACTH release, *Neuroendocrinology*, 16 (1974) 178–190.

31 Manev, H. and Pericic, D., Hypothalamic GABA system an plasma corticosterone in ether stressed rats, *Pharmacol. Biochem. Behav.*, 18 (1983) 847–850.

32 Marangos, P.J., Patel, J., Boulenger, J.P. and Clark-Rosenberg, R., Characterization of peripheral-type benzodiazepine binding sites in brain using [^{3}H]Ro 5-4864, *Mol. Pharmacol.*, 22 (1982) 26–32.

33 Mohel, H. and Richards, J.G., Agonist and antagonist benzodiazepine receptor interact in vitro, *Nature (Lond.)*, 294 (1981) 763–765.

34 Paul, S.M., Ninan, P., Insel, T. and Skolnick, P., Benzodiazepine receptor-mediated experimental 'anxiety' in rhesus monkey after infusion of 3-carboethoxy-β-carboline (β-CCE). In L.F. Gram, E. Usdin, S.G. Dahl, P.K. Sorensen, F. Sjoqvist and P.L. Morselli (Eds.), *Clinical Pharmacology in Psychiatry: Bridging the Experimental Therapeutic Gap*, Macmillan, London, 1982, pp. 395–401.

35 Petty, F. and Sherman, A.D., GABAergic modulation of learned helplessness, *Pharmacol. Biochem. Behav.*, 15 (1981) 567–570.

36 Petty, F. and Schlesser, M.A., Plasma GABA in affective illness, *J. Affect Dis.*, 3 (1981) 339–343.

37 Plotsky, P.M., Otto, S. and Sutton, S., Neurotransmitter modulation of corticotropin releasing factor secretion into the hypophysial-portal circulation, *Life Sci.*, 41 (1987) 1311–1317.

38 Polc, P., Laurent, J.P., Scherschlicht, R. and Haefely, W., Electrophysiological studies on the specific benzodiazepine antagonist Ro15-1788, *Naunyn-Schmiedeberg's Arch. Pharmacology*, 316 (1981) 317–325.

39 Rivier, C. and Vale, W., Influence of corticotropin-releasing factor on reproductive functions in rat, *Endocrinology*, 114 (1984) 914–921.

40 Robertson, H.A. and Riives, M.L., A benzodiazepine antagonist is an anticonvulsant in an animal model for limbic epilepsy, *Brain Research*, 270 (1983) 380–382.

41 Roy-Byrne, P.P., Uhde, T.W., Post, M.A., Gallucci, W., Chrousos, G.P. and Gold, P.W., The CRH-stimulation test in patients with panic disorder, *Am. J. Psychiatry*, 143 (1985) 896–899.

42 Schoemaker, H., Boles, R.G., Horst, W.D. and Yamamura, H.I., Specific high-affinity binding sites for [^{3}H]Ro 5-4864 in rat brain and kidney, *J. Pharmacol. Exp. Ther.*, 225 (1983) 61–69.

43 Schuermeyer, T.H., Avgerinos, P., Gold, P.W., Gallucci, W.T., Tomai, T.P., Cutler, G.B., Loriaux, D.L. and Chrousos, G.P., Human corticotropin-releasing factor in man: pharmacokinetic properties and dose–response of plasma adrenocorticotropin and cortisol secretion, *J. Clin. Endocrinol. Metab.*, 59 (1984) 1103–1108.

44 Schwartz, R.D., Suzdak, P.D. and Paul, S.M., Gamma-aminobutyric acid (GABA) and barbiturate-mediated 36 Cl^- uptake in rat brain synaptoneurosomes: evidence for rapid desensitization of the GABA receptor-coupled chloride channel. *Mol. Pharmacol.*, 30 (1986) 419–426.

45 Sheehan, D.I., Coleman, J.H., Greenblatt, D.J., Jones, K.J., Levine, P.H., Orsulak, P.J., Peterson, M., Schildkraut, J.J., Uzogara, E. and Watkins, D., Some biochemical correlates of panic attacks with agoraphobia and their response to a new treatment, *J. Clin. Psychopharmacol.*, 4 (1984) 66–75.

46 Sherman, A.D. and Petty, F., Neurochemical basis of the action of antidepressant on learned helplessness, *Behav. Neurol. Biol.*, 30 (1980) 119–134.

47 Sirinathsinghji, D.J.S., Rees, L.H., Rivier, J. and Vale, W., Corticotropin-releasing factor is a potent inhibitor of sexual receptivity in the female rat, *Nature (Lond.)*, 305 (1983) 232–234.

48 Sutton, R.E., Koob, G.F., Le Moal, M., Rivier, J. and Vale, W., Corticotropin-releasing factor produces behavioral activation in rats, *Nature (Lond.)*, 291 (1982) 331–333.

49 Swerdlow, N.R., Geyer, M.A. and Vale, W., Corticotropin-releasing factor potentiates acoustic startle in rat: blockade by chlordiazepoxide, *Psychopharmacology*, 88 (1986) 147–152.

50 Takahara, J., Yumoki, S., Yakushiji, W., Yamauchi, J., Hosogi, H. and Ofuji, T., Stimulatory effect of gamma-aminobutyric acid (GABA) on growth hormone, prolactin and cortisol release in man, *Horm. Metab. Res.*, 12 (1980) 31–34.

51 Tormey, W.P., Dolphin, C. and Darragh, A.S., The effect of diazepam on sleep, and the nocturnal release of growth hormone, prolactin, ACTH and cortisol, *Br. J. Clin. Pharmacol.*, 8 (1979) 90–92.

52 Vale, W., Spiess, J., Rivier, C. and Rivier, J., Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and β-endorphin, *Science*, 213 (1981) 1394–1397.

53 Vincent, S.R., Hokfelt, T. and Wu, J.Y., GABA neuron system in hypothalamus and the pituitary gland: immunohistochemical demonstration using antibodies against glutamate decarboxylase, *Neuroendocrinology*, 34 (1982) 117–125.

54 Willoughby, J.O., Jervois, P.M., Manadue, M.F. and Blessing W.W., Activation of GABA receptors in the hypothalamus stimulates secretion of growth hormone and prolactin, *Brain Research*, 374 (1986) 119–125.