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REGULATION OF RAT HYPOTHALAMIC CORTICOTROPIN-RELEASING HORMONE SECRETION IN VITRO: POTENTIAL CLINICAL IMPLICATIONS

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INTRODUCTION

Corticotropin-releasing hormone (CRH) is located in both the hypothalamus (1) and several extrahypothalamic brain regions (2). Different reports suggest that CRH not only stimulates pituitary adrenocorticotropin (ACTH) secretion (3), but also activates the sympathetic nervous system (4) and causes behavioral changes (5-10). Behavioral studies conducted primarily in rats have indicated that CRH administered intracerebroventricularly (icv) causes anxiety-like behaviors. Such behaviors are, in part, influenced by the animal's familiarity with the environment. In single-caged rats, tested in a familiar environment, CRH increases locomotion, rearing, and self-grooming (10) and decreases food intake (5,8). On the other hand, CRH administered to rats in a novel environment decreases locomotion, rearing, and food intake and increases self-grooming (10). In addition, icv CRH administration increases aggression and mounting behavior of male rats (10). In contrast, microinfusion of CRH into the arcuate hypothalamic nucleus and mesencephalic central gray area of estrogen-primed female rats causes decreased sexual receptivity (9). These effects of CRH are compatible with the concept that this peptide may be an important integrative factor that coordinates endocrine, autonomic, and behavioral responses to stress.

A voluminous literature regarding the regulation of hypothalamic CRH secretion has yielded conflicting and often unclear conclusions. For example, serotonin (5HT) has been reported to stimulate (12), inhibit (13) or have no effect (14) upon CRH secretion. Similarly, norepinephrine (NE) has been reported both to inhibit (15,16) and stimulate (17) the secretion of this peptide. On the other hand, certain other aspects of hypothalamic CRH regulation are poorly understood. These include: (1) the role of other neurotransmitters, such as epinephrine (E) and dopamine (DA); (2) the receptor subtypes mediating the effects of various neurotransmitters; (3) the interactions between the different neurotransmitter systems; (4) the evaluation of possible negative feedback control loops; and (5) the evaluation of interactions between products of the immune system and the hypothalamic CRH neuron. In experiments conducted in animals, factors such as use of relatively nonspecific pharmacologic probes, application of varying routes of drug administration (e.g. peripheral, icv, direct hypothalamic application), the confounding effects of anesthesia, and/or the use of indirect assessments of ACTH secretion (e.g. corticosterone, 11-OH corticosteroids, etc) may have contributed to the conflicting results on the regulation of CRH secretion. Furthermore, it should be noted that the oldest in vitro hypothalamic organ culture system employed to study CRH regulation had as end-point corticotropin-releasing bioactivity rather than CRH itself (12,14,16). Several hypothalamic or circulating

substances, however, are capable of releasing ACTH, such as vasopressin, oxytocin, E, 5HT, vasoactive intestinal peptide, peptide histidine isoleucine, cholecystokinin, and angiotensin II (18). In addition, the low sensitivity of these bioassays requiring either sample concentration and/or incubation of pooled hypothalami may have contributed to the conflicting results.

To circumvent the shortcomings of previous efforts to elucidate the regulation of hypothalamic CRH secretion, we developed an in vitro rat hypothalamic organ culture system which allowed us to measure immunoreactive CRH (IR-rCRH) secretion from single explanted rat hypothalami.

HYPOTHALAMIC ORGAN CULTURE

A more detailed account of the methods, including validation and quality control has been reported elsewhere (19). Briefly, adult male Sprague-Dawley rats were sacrificed by decapitation and the hypothalami sterilely excised. After an overnight preincubation in medium 199 (M199), single hypothalami were incubated in M199 according to the experimental protocols described below.

The experimental design consisted of serial passages of the hypothalamic explants in 6 different wells every 20 min, for a total period of 120 min. Basal IR-rCRH secretion was 36±2pg/hypothalamus/0.4 ml/20 min of incubation and remained constant for the length of the experiment. We employed three different experimental protocols. Protocol 1 was used when stimulation of IR-rCRH secretion was examined. For this purpose the hypothalami were incubated in plain medium in the first three wells (basal IR-rCRH concentration) and exposed to graded concentrations of the test substance in the next two wells (stimulated IR-rCRH concentration). Controls for protocol 1 were obtained by exposing the hypothalami to medium plus vehicle alone in the fourth and fifth well. Protocol 2 was used when inhibition of stimulation induced by a stimulatory neurotransmitter was examined. For this experimental protocol, the hypothalami were incubated in plain medium in the first two wells (basal IR-rCRH concentration). In the third, fourth, and fifth well the antagonist(s) was present, whereas in the fourth and fifth well the stimulant was added (stimulated IR-rCRH concentration). Finally, protocol 3 was used when inhibition of stimulation was examined. For this purpose, the inhibitor was added during the last hour of preincubation and throughout the experiment conducted as in protocol 1.

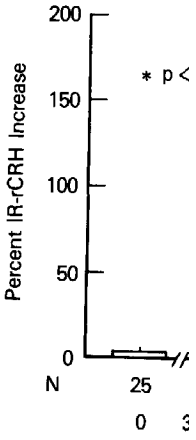
The concentration of CRH in the medium was measured directly by RIA, using a specific anti-rCRH rabbit antiserum developed in our laboratory (20).

EXCITATORY NEUROTRANSMITTERS

Acetylcholine

Acetylcholine (ACh) has been proposed by several investigators as an excitatory neurotransmitter of the hypothalamic-pituitary-adrenal (HPA) axis (12,16). The locus of ACh action on the HPA axis has been proposed to be within the central nervous system (CNS). Concordantly, it has been shown that ACh does not affect pituitary ACTH secretion in vitro (21). We examined the hypothesis that ACh stimulates the HPA axis by inducing hypothalamic CRH secretion (22).

ACh induced IR-rCRH secretion in a dose-dependent fashion, at concentrations ranging from 3.3×10^{-10} to 10^{-5} M (Fig 1). The maximal stimulatory effect of ACh on IR-rCRH secretion was antagonized by the simultaneous presence of atropine and hexamethonium, a muscarinic and a nicotinic receptor antagonist, respectively. Further evidence for the cholinergic regulation of the CRH neuron was provided by the findings that both carbachol, a muscarinic receptor agonist, and nicotine, a nicotinic receptor agonist, stimulated IR-rCRH secretion in a dose-dependent fashion, although, their maximal stimulatory effects were lower than that produced by ACh. Carbachol- and nicotine-induced IR-rCRH secretion were antagonized by atropine or hexamethonium, respectively. ACh stimulated IR-rCRH secretion in the presence of ritanserin, a serotonin$_2$ receptor antagonist and phentolamine, an α-adrenergic antagonist suggesting



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responsivity to stress (26).

Serotonin

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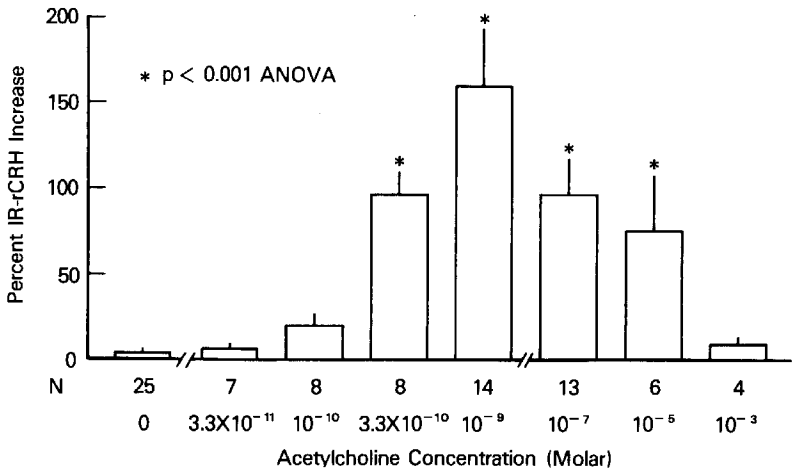


Fig. 1. IR-rCRH secretion by single explanted hypothalami (mean±SE) exposed for 40 min to graded concentrations of acetylcholine, as indicated. Results are expressed as percent increase above baseline. Statistical analysis was done employing one-way analysis of variance (ANOVA) followed by the Ryan-Einot-Gabriel-Welsch multiple range test (REGWQ). Logarithmic transformation of the data was applied to correct for heteroscedasticity, detect by the Bartlett test. Acetylcholine induced a dose-dependent increase of IR-rCRH ($p<0.001$). N=number of hypothalami tested for each dose level (from reference 22).

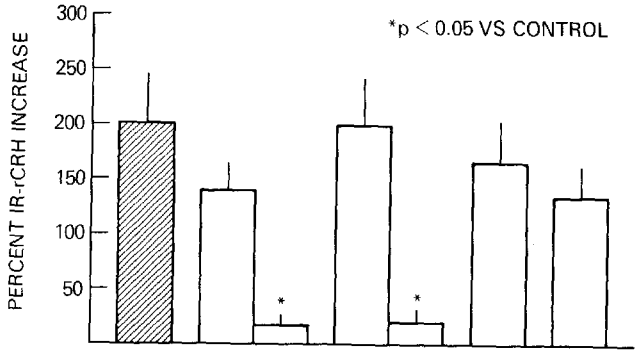
that the cholinergic stimulation of CRH secretion is not mediated by serotonergic or α-adrenergic interneurons.

Although we cannot rule out a direct effect of systemically administered cholinergic agonists on the adrenal cortex itself (23), our data support the hypothesis that the HPA axis activation mediated by ACh occurs via stimulation of CRH secretion. Although the physiologic role of the stimulatory cholinergic pathway is unknown, there is evidence to suggest that it may play a role in the regulation of the HPA axis during stress. In this regard, it has been shown that central implantation of atropine inhibits stress-induced activation of the HPA axis in rats (24,25) and that the septo-hippocampal cholinergic system, which undergoes rapid activation during acute stress, may play an important role in both endocrine (HPA axis activation) and emotional responsivity to stress (26).

Serotonin

Serotonin has been implicated in the regulation of the HPA axis. In this regard, administration of 5HT precursors, releasers, reuptake inhibitors or receptor agonists increase plasma ACTH, β-endorphin (β-EP) and corticosterone levels in laboratory animals (27,28). Furthermore, it has been reported that various stressors associated with HPA axis activation also enhance brain 5HT turnover (29). A number of experiments have been performed using pituitary cells or hypothalamic explants to determine the site(s) of 5HT action on the HPA axis. Whereas, it seems clear that 5HT is capable of stimulating pituitary ACTH in vitro (30), the experiments examining hypothalamic explant release of corticotropin-releasing bioactivity have been less conclusive and 5HT has been reported to stimulate (2), inhibit (13) or to have no effect (14) upon CRH secretion.

In our hands, 5HT stimulated IR-rCRH secretion in a dose-dependent fashion with peak of activity at the 10^{-9} M concentration (19). We attempted also to examine which 5HT receptor subtypes were involved in this response and the possible interactions between 5HT and other neurotransmitter systems. Serotonin-induced IR-rCRH secretion was antagonized by both ketanserin and ritanserin, which are specific $5HT_2$ receptor antagonists, but was not affected by the blockade of cholinergic (atropine plus hexamethonium) or α-adrenergic (phentolamine) receptors (Fig. 2).



N	17	7	15	7	8	8	15
SEROTONIN (Log M)	−9	−9	−9	−9	−9	−9	−9
KETANSERIN (Log M)	—	−9	−7	—	—	—	—
RITANSERIN (Log M)	—	—	—	−9	−7	—	—
ATROPINE (Log M)	—	—	—	—	—	−7	—
HEXAMETHONIUM (Log M)	—	—	—	—	—	−7	—
PHENTOLAMINE (Log M)	—	—	—	—	—	—	−7

Fig. 2. IR-rCRH secretion by single explanted hypothalami exposed to 10^{-9}M serotonin (shaded bar) or serotonin plus different concentrations of serotonergic, cholinergic and α-adrenergic antagonists. None of these antagonists had any effect on basal IR-rCRH secretion. Results (mean±SE) are expressed as percent increase above baseline. *p<0.05 vs serotonin alone (ANOVA followed by REGWQ test), N=number of hypothalami tested (from reference 19).

Our data suggest that 5HT stimulates hypothalamic CRH secretion primarily via a $5HT_2$ receptor type. This effect does not appear to be mediated by cholinergic or α-adrenergic interneurons, as previously suggested (12). Serotonin appears capable of stimulating the HPA axis not only by releasing hypothalamic CRH and pituitary ACTH, but also by releasing hypothalamic arginine vasopressin (31). In spite of these redundant effects of 5HT on the HPA axis, the physiologic role of this neurotransmitter is unknown. Poorly understood are also its effects on circadian or stress-induced HPA axis activation.

Norepinephrine

The concomitant activation of the CRH and the locus coeruleus-norepinephrine (LC-NE) systems constitutes one of the principal adaptations during either physical or emotional stress (32-34). Hence, an understanding of potential interactions between these two systems may be essential to the study of stress mechanisms. Recent data suggest that CRH significantly increases the locus coeruleus firing rate. However, the effect of NE upon CRH secretion is a matter of considerable debate. Many studies, utilizing indirect assessment of CRH secretion and in the context of widely divergent experimental designs, have failed to convincingly demonstrate whether NE is inhibitory or excitatory to the hypothalamic CRH neuron (15-17). To evaluate the effect of NE

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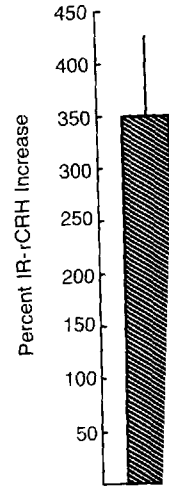

N	10
Norepinephrine (10^{-9} M)	+
Phentolamine (log M)	−
Propanolol (log M)	−
Prazosin (log M)	−
Yohimbine (log M)	−

Fig. 3. IR-rCRH secretion by
norepinephrine (shaded bar) or
norepinephrinergic antagonists. N
rCRH secretion. Results (mean±
*p<0.05 vs norepinephrine alone.
were employed for the statistical
reference 35).

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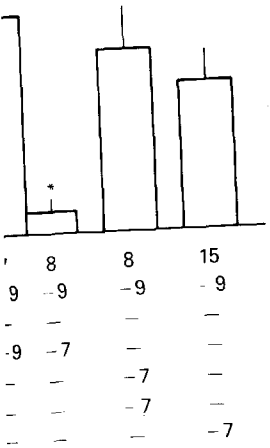

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on hypothalamic CRH secretion we tested the direct effects of a multiplicity of catecholamine agonists and antagonists upon CRH secretion in vitro (35).

Norepinephrine stimulated IR-rCRH secretion in a dose-dependent fashion with peak of effect in the monomolar range. The effect of NE was antagonized by the mixed α antagonist phentolamine, by the α_1 antagonist prazosin and by the α_2 antagonist yohimbine, but not by the β-blocker L-propanolol (Fig 3).

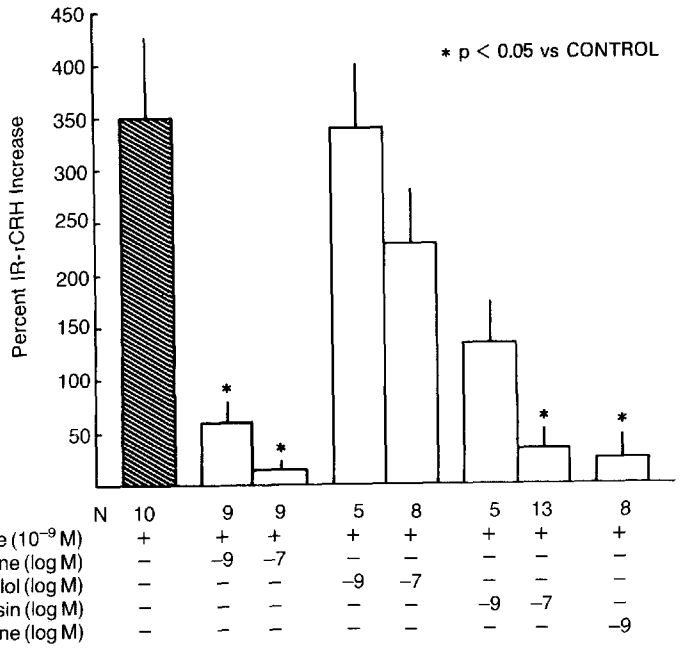

Fig. 3. IR-rCRH secretion by single explanted hypothalami exposed to 10^{-9}M norepinephrine (shaded bar) or norepinephrine plus different concentrations of norepinephrinergic antagonists. None of these antagonists had any effect on basal IR-rCRH secretion. Results (mean±SE) are expressed as percent increase above baseline. *p<0.05 vs norepinephrine alone. ANOVA followed by the Duncan multiple range test were employed for the statistical evaluation. N=number of hypothalami tested (from reference 35).

In agreement with these data were the findings that the α_1 agonist phenylephrine and the α_2 agonist clonidine both stimulated IR-rCRH secretion in a dose-dependent fashion. On the other hand, while the β agonist isoproterenol caused a weak, non dose-dependent increase in IR-rCRH secretion, this effect could not be antagonized by L-propanolol. Despite pretreatment with serotonin and ACh receptor antagonists the effect of NE on IR-rCRH secretion was undiminished, suggesting that NE-induced CRH secretion is not mediated by either neurotransmitter. On the other hand, pretreatment with GABA significantly attenuated NE-induced IR-rCRH secretion.

The present data support the hypothesis that NE is excitatory rather than inhibitory upon CRH secretion when it acts directly at a hypothalamic locus. The stimulatory NE effect on hypothalamic CRH secretion may be of interest in light of recent data showing that CRH administration to awake, unrestrained rats markedly increases the LC firing rate (36). These data, taken together, suggest that hypothalamic CRH neurons and NE neurons in regions such as the LC may participate in a mutually reinforcing positive feedback loop. This observation may be pertinent to stressful situations and psychiatric conditions characterized by activation of both the LC-NE and the CRH systems (37).

Epinephrine and Dopamine

Epinephrine has been reported to inhibit the central component of the HPA axis in vivo (38), whereas dopamine (DA) has been reported to have no effect upon corticotropin-releasing bioactivity in vitro (14). We found that E stimulates IR-rCRH secretion in a dose-dependent fashion. Peak of effect, however, was observed at about 100 times higher concentrations than NE. The stimulatory effect of E could be antagonized by equimolar concentrations of phentolamine, but not by propanolol suggesting that this effect is mediated through α adrenergic receptors.

Dopamine had a weak stimulatory effect on IR-rCRH secretion. This effect was antagonized by the specific D_1 receptor antagonist SCH23390, but not by phentolamine. These data suggest that DA effect on hypothalamic CRH secretion is receptor-mediated and not due to in vitro conversion of this neurotransmitter into NE or E (35).

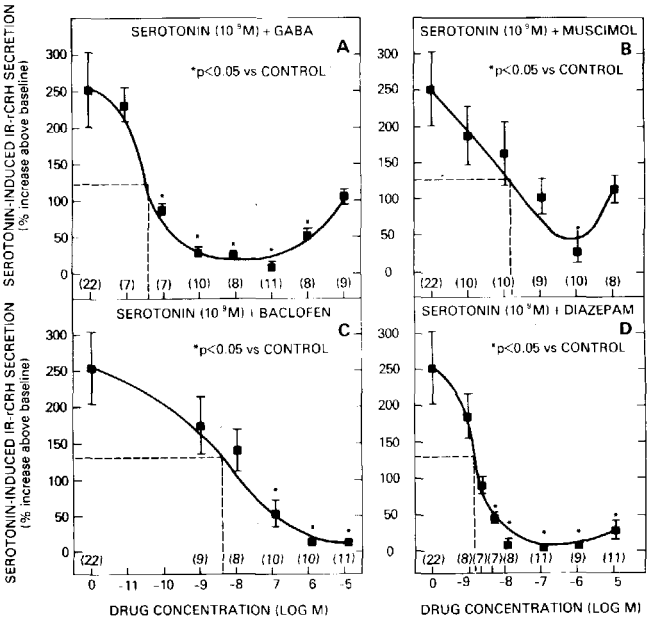

Fig. 4. IR-rCRH secretion by single explanted hypothalami exposed to 10^{-9}M serotonin serotonin plus graded concentrations of GABA (Panel A), serotonin plus graded concentrations of mascimol (Panel B), serotonin plus graded concentrations of baclofen (Panel C), or serotonin plus graded concentrations of diazepam (Panel D). Results (mean±SE) are expressed as percent increase above baseline. *p<0.05 vs serotonin alone (ANOVA followed by Duncan test). Dotted lines intercept curves at ED_{50} level. ED_{50}s were calculated employing the four parameter logistic equation. A computer program, "ALLFIT", developed by DeLean et al., was employed for the computations (43). The number of hypothalami tested for each dose level is indicated in parentheses (from reference 44).

INHIBITORY NEUROTRANSMITTERS

Gamma-Aminobutyric Acid

In contrast to the activating effect of icv administration of CRH on the stress system, GABA and benzodiazepine (BZD) agonists seem capable of not only decreasing excessive responses to stress, such as hypertension (39) and gastric ulceration (40), but

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EGULATORY NEGATIV

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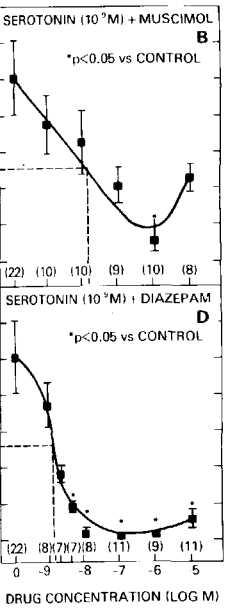

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lso of suppressing the activity of the HPA axis (41). Moreover, activation of the GABA/BZD neurotransmitter system also exerts behavioral effects in opposition to those of CRH by decreasing arousal and inducing effects which can be characterized as anxiolytic (42). In the light of the apparently antithetical effects of CRH and the GABA/BZD system on various physiological and behavioral parameters, we sought to evaluate the effects of the GABA/BZD system on 5HT-induced CRH secretion (43).

GABA inhibited 5HT-induced IR-rCRH secretion from 10^{-10} to 10^{-6} M concentrations (Fig 4, panel A). Muscimol, a $GABA_A$ receptor agonist, inhibited 5HT-induced IR-rCRH secretion only at 10^{-6} M (Fig 4, panel B). Baclofen, a $GABA_B$ receptor agonist was more potent than muscimol, but not than GABA, in suppressing 5HT-induced IR-rCRH secretion (Fig 4, panel C). Diazepam, a classic benzodiazepine which interacts with the BZD site of the $GABA_A$ receptor complex, inhibited 5HT-induced IR-rCRH secretion from 3.3×10^{-9} to 10^{-5} M (Fig 4, panel D).

These results suggest that the GABA/BZD system is involved in the regulation of CRH secretion. It appears that both $GABA_A$ and $GABA_B$ receptor types mediate the suppressive effect of GABA upon 5HT-induced CRH secretion. It is not entirely unexpected that activation of the principal inhibitory system in brain (45) would diminish the responsivity to stimulatory inputs of the CRH neuron which is postulated to be a mediator of arousal and of the stress response (5-10). In this regard, it is tempting to postulate that some of the anxiolytic effect of $GABA_A$ and $GABA_B$ receptor agonists may be exerted via inhibition of the CRH neuron.

REGULATORY NEGATIVE "FEEDBACK"INFLUENCES

The activity of the HPA axis is regulated not only by circadian and stress-related excitatory inputs or by inhibitory neural modulation, but also by various negative feedback control loops (46). Negative feedback is exerted by glucocorticoids at the pituitary corticotroph cell, the hypothalamic CRH-secreting neuron and possibly at sites of the limbic system such as the amygdala and the hippocampus (46). ACTH has been proposed to exert negative feedback effects on basal secretion of hypothalamic CRH (47,48) and on its own as well secretion (49). With regard to CRH, it has been suggested that this peptide exerts no ultrashort loop negative feedback under resting conditions but may stimulate its own secretion in stress states (50). Direct evidence for a positive or a negative ultrashort CRH feedback loops would, however, be necessary to clarify this issue. We examined the effects of ovine CRH (oCRH), ACTH, β-EP, α-melanocyte-stimulating hormone (α-MSH), corticotropin-like intermediate lobe peptide (CLIP), ovine β-lipotropin (ovine β-LPH) and dexamethasone (DEX) on basal and neurotransmitter-stimulated CRH secretion (51).

Ovine CRH and DEX inhibited unstimulated IR-rCRH secretion with ED_{50}s at the 10^{-8} M range. ACTH had no detectable suppressive effect at 10^{-8} M. Ovine CRH, ACTH and DEX inhibited 5HT-, ACh- and NE-induced IR-rCRH secretion in a dose-dependent fashion (Fig. 5). β-EP, α-MSH and CLIP also inhibited 5HT-induced IR-rCRH secretion. Of these latter peptides, the strongest inhibitor was β-EP and the weakest CLIP. Ovine β-LPH had only a weak inhibitory effect on 5HT-induced IR-rCRH secretion. Generally, the concentrations required for 50% suppression of neurotransmitter-stimulated IR-rCRH secretion were significantly lower than those required for a similar suppression of unstimulated IR-rCRH secretion.

Our data suggest the presence of multiple regulatory negative feedback loops operating at the level of the CRH-producing neuron. These include an ultrashort CRH-mediated loop, a short POMC gene-derived peptide loop and a long glucocorticoid-mediated negative feedback loop. The source of ACTH and other fragments of the POMC gene capable to exert negative feedback effects on hypothalamic CRH secretion in vivo has not been definitively elucidated. Several lines of evidence suggest that the most likely source is the arcuate nucleus of the hypothalamus where POMC content is high (52). ACTH-containing cells in the arcuate nucleus receive afferent input from CRH neurons located in the paraventricular nucleus (PVN) (9) and send projections to the PVN (53). In addition, direct application of CRH onto the arcuate nucleus of the rat results in the release of both ACTH and β-EP (54).

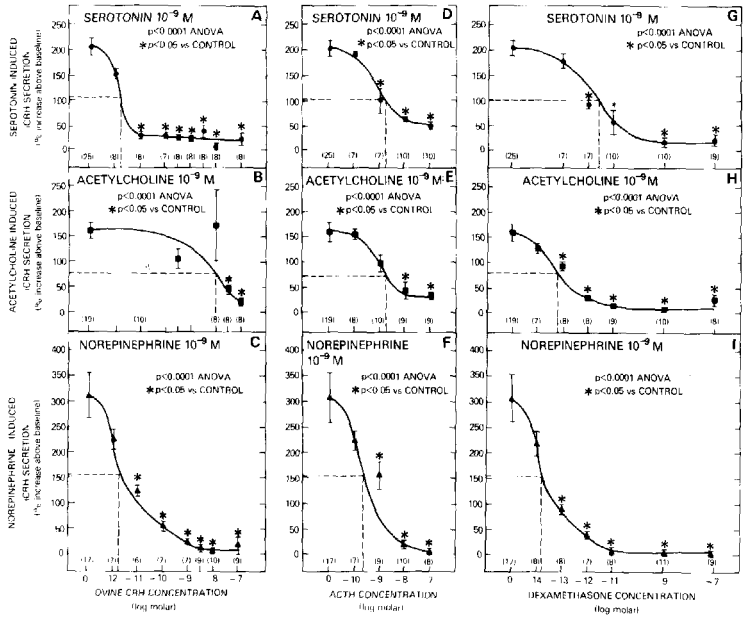

Fig. 5. Effects of graded concentrations of oCRH (left panels), ACTH (central panels) and dexamethasone (right panels) on iCRH secretion by single explanted rat hypothalami stimulated by serotonin (upper panels), acetylcholine (middle panels) or norepinephrine (lower panels). Results (mean±SE) are expressed as percent increase above baseline. *p<0.05 vs control (ANOVA followed by Duncan test). Dotted lines intercept curves at ED_{50} level. The number of hypothalami tested at each dose level is indicated in perentheses (from reference 51).

POTENTIAL EXTRAHYPOTHALAMIC INFLUENCES

Activation of the HPA axis is observed during the immune response. Several mediators of immune phenomena, such as inflammation, allergy and anaphylaxis have been shown capable of activating this axis. These include interleukin-1 (IL-1), interleukin-2 (IL-2), thymosins, and lipids (55-60).

A number of studies have been performed to clarify the site of action of IL-1 within the HPA axis. In the rat, IL-1-induced ACTH release was completely abolished by immunoneutralization with CRH antiserum (55,58). Accordingly, IL-1 caused no ACTH secretion by cultured rat anterior pituitary cells (55,58). We have shown that IL-1 stimulated IR-rCRH secretion by cultured rat hypothalami in a dose dependent-fashion down to the concentration of 10^{-15} M (58).

A macrophage-derived cytokine, tumor necrosis factor-α (TNF-α) (Bernardini et al. unpublished information) and IL-2 (58) both stimulated hypothalamic IR-rCRH secretion in vitro. Neither IL-1 nor IL-2 had any effect on pituitary ACTH secretion in vitro. To examine whether the effects of IL-1 on CRH secretion were prostanoid-mediated, we employed the cyclo- and lipooxygenase inhibitors indomethacin (INDO) and eicosatetraynoic acid (ETYA) and the lipooxygenase inhibitor nordihydroguaiaretic acid (NDGA). Both INDO and ETYA inhibited IL-1-induced IR-rCRH secretion, whereas NDGA had no effect. These data suggest that cyclooxygenase products of arachidonic acid, such as prostaglandins (PG) may mediate the IL-1 effects on hypothalamic CRH secretion (61). IL-2 effect on IR-rCRH secretion were not affected by arachidonic acid metabolites. Accordingly, cyclooxygenase metabolites of arachidonic acid, such as $PGF_{2\alpha}$ and thromboxane (TX) B_2, as well as the TXA_2 receptor agonist U-46,619

imulated hypothalamic IR-rCRH secr
echanism involved in the CRH respon
esponse to this cytokine.

We have also shown that platele
latelet-aggregating properties which
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We have shown that epidermal gr
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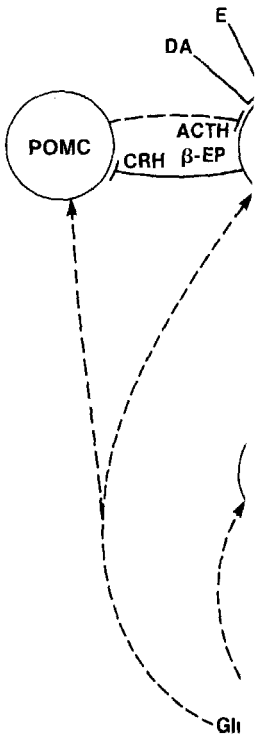
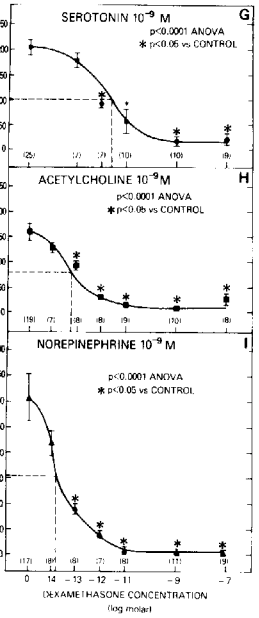


Fig. 6. Schematic representation
adrenal axis and of the functional
neuron and other brain regions,
proopiomelanocortin (POMC) gene-d
system in the hindbrain. CRH act
coerulus (LC) and the central sym

hypothalamic POMC gene-derived pe
be regulated by central stimulatory i
(ACh), norepinephrine (NE), epineph
inputs mediated by the GABA/benz
negative feedback loops. These in
hypothalamic POMC gene-derived
negative feedback loop.



ft panels), ACTH (central panels)
retion by single explanted rat
acetylcholine (middle panels) or
re expressed as percent increase
d by Duncan test). Dotted lines
pthalami tested at each dose level

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the immune response. Several
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not affected by arachidonic acid
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XA_2 receptor agonist U-46,619

timulated hypothalamic IR-rCRH secretion in vitro (62). It is interesting that the mechanism involved in the CRH response to IL-1 is similar to that shown for the fever esponse to this cytokine.

We have also shown that platelet activating factor (PAF), a glycerolipid with platelet-aggregating properties which is released during the inflammatory response, timulates ACTH secretion in the rat in vivo and hypothalamic IR-rCRH secretion in vitro (60,63).

We have shown that epidermal growth factor (EGF), a polypeptide mitogen that participates in wound healing, stimulates the HPA axis in primates in a dose-dependent fashion (64). To define whether the locus of stimulation was the hypothalamus and/or the pituitary gland we examined the capacity of mouse EGF to directly stimulate

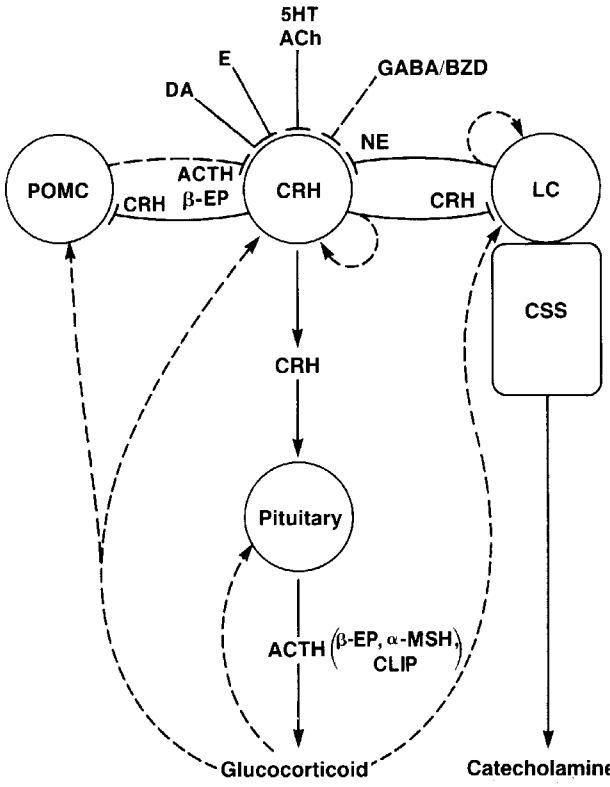

Fig. 6. Schematic representation of the regulation of the hypothalamic-pituitary-adrenal axis and of the functional interrelationships between the hypothalamic CRH neuron and other brain regions, such as the hypothalamic region producing proopiomelanocortin (POMC) gene-derived peptides and the centers of the sympathetic system in the hindbrain. CRH activates the pituitary-adrenocortical axis, the locus coerulus (LC) and the central sympethetic system (CSS) and stimulates secretion of hypothalamic POMC gene-derived peptides. The activity of the CRH neuron appears to be regulated by central stimulatory inputs mediated by serotonin (5HT), acetylcholine (ACh), norepinephrine (NE), epinephrine (E) and dopamine (DA), by central inhibitory inputs mediated by the GABA/benzodiazepine (GABA/BZD) system and by multiple negative feedback loops. These include an ultrashort CRH-mediated loop, a short hypothalamic POMC gene-derived peptide loop and a long glucocorticoid-mediated negative feedback loop.

hypothalamic IR-rCRH or pituitary ACTH secretion in vitro. Mouse EGF stimulated IR-rCRH secretion in a dose-dependent fashion, but failed to cause pituitary ACTH secretion. These data suggest that EGF, and/or one of its naturally occurring analogs, may participate in the physiological activation of the HPA axis at times during which the concentrations of these factors are raised in the systemic circulation and/or locally in the hypothalamus. Such states may include trauma, surgery, and possibly emotional stress.

SUMMARY AND CONCLUSION

In summary, 5HT, ACh, NE, E and DA appear to stimulate hypothalamic CRH secretion whereas activation of the GABA/BZD system seems to decrease the responsivity of the CRH neuron to stimulatory neurotransmitters (Fig. 6). Hypothalamic CRH released from the hypothalamic neuron not only activates the HPA axis, but also stimulates the locus coeruleus-norepinephrine system (LC) and the central sympathetic system (CSS). CRH also induces secretion of hypothalamic POMC gene-derived peptides, such as ACTH, β-EP, α-MSH and CLIP. These peptides as well as CRH itself, decrease the responsivity of the CRH neuron to stimulatory inputs. In addition, glucocorticoids restrain the activity of both the CRH neuron and the locus coeruleus and may also inhibit the secretion of POMC gene-derived peptides by the POMC neurons of the arcuate nucleus.

Hypothalamic CRH secretion is regulated also by a number of mediators of the immune response, such as IL-1, IL-2, TNF-α and $PGF_{2\alpha}$, PAF and EGF. Although the physiologic significance of this regulation is largely unknown, it is tempting to speculate that cytokines and mediators of inflammation released in vivo may activate the HPA axis to trigger a glucocorticoid-mediated counter-regulatory mechanism to restrain the immune system (Fig. 7).

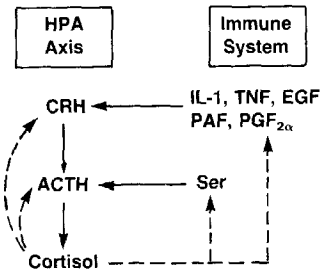

Fig. 7. Schematic representation of the interactions between the HPA axis and the immune system. Continuous lines represent stimulatory inputs and interrupted lines represent inhibitory inputs.

In conclusion, our in vitro hypothalamic organ culture system allowed us to examine the regulation of CRH secretion in a direct and specific manner. Some of our observations may help with better understanding of the role played by CRH in the complex symptomatology of stress. In making extrapolations and interpretations from the in vitro data, however, we should try to keep in mind the words of Claude Bernard, "... If we break up a living organism by isolating its different parts it is only

or the sake of ease in analysis and by no
ndeed when we wish to ascribe to a
ignificance we must always refer it to th
n relation to the effects in the whole".

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for the sake of ease in analysis and by no means in order to consider them separately. Indeed when we wish to ascribe to a physiological quality its value and true significance we must always refer it to this whole and draw our final conclusions only in relation to the effects in the whole".

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