Brain Corticotropin-Releasing Factor (CRF) and Catecholamine Responses in Acutely Stressed Rats

SHUSO SUEMARU, KOZO HASHIMOTO AND ZENSUKE OTA

*Third Department of Internal Medicine, Okayama
University Medical School, Okayama*

Abstract

Ether-laparotomy stress produced a rapid increase in rat hypothalamic CRF concentration, followed by a rapid reduction and subsequent increase. Cold-restraint stress significantly reduced hypothalamic CRF concentration at 15 min after stress onset. Serum ACTH and corticosterone levels were significantly elevated at 15 min after the onset of both stresses. The CRF responses in the medulla oblongata were not similar to the hypothalamic CRF responses. Norepinephrine concentration in the hypothalamus was reduced, whereas dopamine concentration in the hypothalamus and medulla oblongata was significantly increased. Epinephrine concentrations in these tissues did not show any significant change throughout the stress period.

The observations lead to the following conclusions: hypothalamic CRF plays a major role in stimulating ACTH secretion under acute stress; the reduction in hypothalamic CRF is due to an excess release in the early phase of acute stress; hypothalamic CRF and medulla oblongata CRF are controlled by different mechanisms; norepinephrine in the hypothalamus may not be involved in stimulating hypothalamic CRF secretion in the early phase of acute stress; and catecholamines are regulated differently in the hypothalamus and medulla oblongata.

Since Vale *et al.* (1981) and Rivier *et al.* (1983) reported on sequence analysis and synthesis of 41-residue ovine and rat hypothalamic peptides (ovine and rat CRF), many investigators have used synthetic ovine and rat CRF. Some reports have shown the possible involvement of CRF, vasopressin and catecholamines as mediating factors in stress-induced ACTH secretion (Rivier *et al.*, 1983; Bruhn *et al.*, 1984; Labrie *et al.*, 1984; Axelrod and Reisine, 1984). Some investigators have reported that the CRF-induced ACTH release at the

pituitary is potentiated by catecholamines (Vale *et al.*, 1983; Labrie *et al.*, 1984; Murakami *et al.*, 1984). In addition, Fisher *et al.* (1982) reported that centrally administered CRF increased the sympathetic outflow to affect the cardiovascular function, and Valentino *et al.* (1983) reported on the relationship between CRF and noradrenergic neurons of the locus coeruleus in the medulla oblongata. But it is unclear whether catecholamines stimulate or inversely inhibit the release of hypothalamic CRF (Hillhouse *et al.*, 1975; Ganong, 1980; Fehm *et al.*, 1980; Tate *et al.*, 1983; Smythe *et al.*, 1983; Maickel and Martel, 1983), and little is

known about time course changes in brain CRF and catecholamine concentrations during stress and their interaction. Therefore, in the present study, we observed changes in CRF and catecholamine concentrations in the hypothalamus and medulla oblongata, the sympathetic nervous center, of rats after acute stress using rat CRF radioimmunoassay and ion-pairing reverse-phase high performance liquid chromatography (HPLC) with amperometric detection.

Materials and Methods

Animals

Male Wistar rats, weighing 250–300 g, were kept at a suitable temperature in a 12 hr light/dark cycle animal room (lights on 0700–1900 h) with food and water *ad libitum*. Before the day of experimentation, the animals were moved to a quiet laboratory to be allowed to acclimatize to the room conditions. The experiments were performed between 1300 h and 1500 h to avoid variations associated with the circadian rhythm as much as possible.

Stress

Two kinds of noxious stimuli were used. Ether-laparotomy stress: The animals were exposed to ether vapor for about 1 min in a jar, followed by laparotomy with intestinal traction. The abdominal incision was sutured. This surgical procedure was performed for less than 1 min as rapidly as possible and ether anesthesia was maintained during surgery. Cold-restraint stress: The animals were immobilized in a wire mesh (3×3 mm) made of stainless steel and left in a supine position in the cold room (6°C). The rats were decapitated at scheduled time intervals after the start of stress, i.e. in ether-laparotomy stress, at 0, 2.5, 15, 30, 60 and 120 min, and in cold-restraint stress, at 0, 15, 60 and 180 min. The reason why the same times were not chosen after the onset of the 2 stresses was that cold-restraint stress was considered to produce less rapid and more continuous responses than ether-laparotomy stress. Therefore, in cold-restraint stress, the observation at 2.5 min was omitted and the responses were observed until 180 min after the stress onset.

Collection of blood

Immediately after decapitation, blood was collected from the trunks into chilled glass tubes and centrifuged at 1,200 g at 4°C for 10 min. The serum was frozen and stored until ACTH and corticosterone assay.

Tissue extraction for CRF assay

The hypothalamus and medulla oblongata were removed from each rat immediately after decapitation. An obtained block of hypothalamic tissue included the median eminence, bounded by the optic chiasm and mammillary bodies rostrocaudally and by the anterior commissure dorsally. The medulla oblongata was dissected out as a block between the starting points of II and VIII cranial nerves. They were individually cut into two pieces at the midline, and the halves of the hypothalamus and medulla oblongata were put into iced plastic tubes, frozen and stored until extraction. The tissue samples were homogenized in a 2 ml solution composed of 80 % acetone and 20% 0.5 N HCl with an ultrasound sonicator. Ten microliters of each tissue homogenate was dried and stored for protein measurement by Lowry's method (Lowry *et al.*, 1951). The rest of the homogenate was centrifuged at 4,000 g at 4°C for 10 min, and the supernatant was transferred to another glass tube, and 3 ml of petroleum ether was added. Then they were mixed and centrifuged at 1,200 g at room temperature for 5 min, and the lower layer was transferred to another tube and dried at 45°C under a stream of nitrogen gas. The dried extracts were stored at –20°C until assay for CRF.

Radioimmunoassay of CRF

The radioimmunoassay of rat CRF was performed by the previously reported method (Hashimoto *et al.*, 1985). The rat CRF antiserum was developed in our laboratory. Synthetic rat CRF from Bachem Co. was used as tracer and standard. Iodination of rat CRF and subsequent RIA were carried out by the same procedure used for ovine CRF-RIA (Hashimoto *et al.*, 1983). The detection limit was 20 pg/tube.

Tissue extraction for catecholamine assay

The second halves of the hypothalamus and medulla oblongata were quickly put into plastic tubes with 1 ml of 0.2 M HClO₄, frozen and stored until extraction. After 50 ng/50 µl of 3, 4-dihydroxybenzylamine (DHBA) (Sigma) was ad-

ded to each tube as internal standard, the tissue samples were homogenized in the ultrasound sonicator. Ten microliters of tissue homogenate from each tube was stored for protein measurement. The rest of the tissue homogenate was centrifuged at 10,000 g at 4°C for 10 min, and 200 μ l of the supernatant was transferred to another conical tube containing 50 mg Al_2O_3 (Alumina) and 1.5 ml of Tris-HCl buffer (1.0 M Tris, 0.004% EDTA-2Na, pH 8.6). After mixing and centrifugation, the supernatant was aspirated off, and its precipitate was washed 3 times with distilled water. Catecholamine-absorbed alumina was placed into an MF-I filter kit (with RC58 Membrane filter 0.2 μ m, Bioanalytical Systems Inc. USA) and centrifuged at 1,200 g for 2 min to extract the catecholamines from alumina into

the receiving tube. Twenty-five or fifty microliters of extracted sample was injected into the HPLC system.

Determination of catecholamines

Norepinephrine, epinephrine and dopamine in the hypothalamus and medulla oblongata were determined by ion-pairing reverse-phase high performance liquid chromatography with amperometric detection. The chromatographic system was composed of: a pump, IRICA P-321; injector, IRICA U-350; column, Lichrosorb RP18 (5 μ m, 4 mm $\times$ 250 mm); amperometric detector, IRICA E-308; and recorder, HITACHI 056. The solvent consisted of 0.1 M KH_2PO_4 , 0.5 mM EDTA-2Na and 100 mg/l sodium 1-heptane sulfonate as 'ion-pairing' reagent. The flow rate

Fig. 1. Reverse-phase high performance liquid chromatography of standard catecholamines (left figure) and catecholamines in hypothalamic extract (right figure). 1, norepinephrine; 2, epinephrine; 3, DHBA; 4, dopamine.

was 1.5 ml/min, applied voltage was 0.75 V on the detector electrode and the strip chart speed was 5 mm/min. In these conditions, the intra-assay coefficient of variation was 9.2% and the interassay coefficient of variation was 12.8%. The assay sensitivities of norepinephrine, epinephrine and dopamine were 10 pg, 10 pg and 15 pg, respectively. Fig. 1 shows the chromatographic resolution of norepinephrine, epinephrine, dopamine and DHBA from the standard solution and extracted sample.

Measurement of serum ACTH and corticosterone

Serum ACTH was measured by radioimmunoassay using the ACTH radioimmunoassay kit (CIS). The serum was used for ACTH assay as no significant difference was observed in the ACTH values between serum and plasma. Serum corticosterone was measured by fluorescence spectrophotometry. Samples were extracted by CHCl_3 , and the corticosterone fluorescence in the sulfuric acid reagent was measured by a fluorescence spectrophotometer (Guillemin *et al.*, 1959).

Statistical analysis

Statistical analysis was conducted by Duncan's new multiple range test.

Results

CRF, ACTH and corticosterone after ether-laparotomy stress

Hypothalamic immunoreactive CRF (IR-CRF) concentration showed a remarkable increase (more than two-fold) at 2.5 min after the onset of ether stress, but was significantly reduced (less than half) at 15 min after stress compared to 0 time level, followed by the recovery to 0 time level at 30 min. On the other hand, the IR-CRF concentration in the medulla oblongata did not change in the early period of stress, significantly decreased at 60 min and recovered to 0 time level at 120 min (Fig. 2). Corresponding to hypothalamic IR-CRF, serum ACTH level was greatly increased to 240.4 ± 38.4 (mean $\pm$ SEM) pg/ml 2.5 min after stress onset, proceeding to 342.6 ± 62.3 pg/ml and to 272.7 ± 45.2 pg/ml at 15 and 120 min, respectively. The serum corticosterone level was significantly elevated 15 min after stress onset. The levels were

Fig. 2. Brain IR-CRF concentration after ether-laparotomy stress. Each column and horizontal line represent mean and SEM of 5 or 6 determinations.
* $p < 0.05$ vs 0 time;
** $p < 0.01$ vs 0 time.

Fig. 3. Serum ACTH and corticosterone levels after ether-laparotomy stress. Each column and horizontal line represent mean and SEM of 5 or 6 determinations. * $p < 0.01$ vs 0 time.

Fig. 4. Brain catecholamine concentration after ether-laparotomy stress. Open columns indicate norepinephrine (NE); dark columns, epinephrine (E); shadow columns, dopamine (D). Each column and horizontal line represent mean and SEM of 5 or 6 determinations. * $p < 0.05$ vs 0 time; ** $p < 0.01$ vs 0 time.

4.6 ± 2.9 , 44.3 ± 3.1 , 56.9 ± 4.3 , 49.0 ± 2.9 and 52.4 ± 4.3 $\mu\text{g/dl}$, at 0, 15, 30, 60 and 120 min, respectively (Fig. 3).

Catecholamines after ether-laparotomy stress

Norepinephrine concentration was significantly reduced and dopamine increased in the hypothalamus 120 min after stress onset. But epinephrine did not show a significant change throughout the period of stress. On the other hand, the dopamine concentration

in the medulla oblongata was significantly increased at 15 and 30 min. Norepinephrine and epinephrine concentrations in the medulla oblongata did not show a significant change (Fig. 4).

CRF, ACTH and corticosterone after cold-restraint stress

As in ether-laparotomy stress, the hypothalamic IR-CRF concentration was significantly reduced to less than half of the 0

time level at 15 min after stress onset, and subsequently showed a gradual and significant increase, exceeding the 0 time level at 180 min. On the other hand, the IR-CRF concentration in the medulla oblongata showed a significant reduction at 180 min (Fig. 5). In contrast to the reduction in the hypothalamic IR-CRF concentration, the serum ACTH level was markedly elevated to 594.3 ± 92.6 pg/ml 15 min after the begin-

ning of stress, and remained at a high level until 180 min (535.8 ± 76.4 pg/ml). The serum corticosterone level was significantly elevated 15 min after stress onset. The levels were 8.2 ± 3.1 , 30.6 ± 1.6 , 38.5 ± 2.5 and 40.4 ± 2.5 μ g/dl at 0, 15, 60 and 180 min, respectively (Fig. 6).

Catecholamines after cold-restraint stress

In the hypothalamus, the norepinephrine

Fig. 5. Brain IR-CRF concentration after cold-restraint stress. Each column and horizontal line represent mean and SEM of 5 or 6 determinations. * $p < 0.05$ vs 0 time.

Fig. 6. Serum ACTH and corticosterone levels after cold-restraint stress. Each column and horizontal line represent mean and SEM of 5 or 6 determinations. * $p < 0.01$ vs 0 time.

Fig. 7. Brain catecholamine concentration after cold-restraint stress. Open columns indicate norepinephrine (NE); dark columns, epinephrine (E); shadow columns, dopamine (D). Each column and horizontal line represent mean and SEM of 5 or 6 determinations. * $p < 0.05$ vs 0 time.

level was significantly reduced at 60 and 180 min, but the dopamine level was slightly, but not significantly increased at 180 min. The epinephrine level did not show a significant change. On the other hand, the catecholamines in the medulla oblongata did not change throughout the period of stress (Fig. 7).

Discussion

Data concerning the changes in CRF concentrations in the rat hypothalamus during stress are controversial. Hypothalamic CRF levels were increased (Hiroshige *et al.*, 1969; Vernikos-Danellis, 1965) or not increased (Sirett and Purves, 1973; Yasuda and Greer, 1976) after acute stress. Such a difference may be due to the use of different bioassay systems. Bioassayable

CRF in the hypothalamus is composed of many substances with ACTH releasing activity (vasopressin, angiotensin, norepinephrine, epinephrine, etc.) as well as the authentic CRF.

Therefore, in the present study, CRF was measured using rat CRF radioimmunoassay. In ether-laparotomy stress, the hypothalamic IR-CRF concentration showed a rapid and remarkable increase 2.5 min after stress onset, followed by a rapid reduction at 15 min. A similar reduction was also observed at 15 min in cold-restraint stress. This rapid change under stress is fairly consistent with the data of Hiroshige *et al.* (1971), using CRF bioassay, in which the hypothalamic CRF content in the adult rat increased 2 min after the start of ether-laparotomy stress, followed by a reduction at 20 min. Serum ACTH and corticosterone concentrations reached peak levels at 15

and 30 min, respectively. These prompt changes in IR-CRF, ACTH and corticosterone concentrations suggest that hypothalamic IR-CRF plays a major role in stimulating ACTH secretion in stress. And moreover, these prompt changes in IR-CRF levels may indicate a rapid excess synthesis or processing of CRF and a subsequent excess release in the hypothalamus, since the change in the hypothalamic CRF concentration is a net balance between CRF synthesis and release. One hour after the start of stress, both the synthesis and the release of CRF seemed to be equally accelerated. Three hours after cold-restraint onset, the synthesis of CRF might exceed its release. In this study, the IR-CRF concentration was measured in the whole hypothalamus as it contained CRF neurons which secreted CRF into portal vessels, although the IR-CRF concentration was different in the median eminence from that in the rest of the hypothalamic tissue.

The IR-CRF level in the medulla oblongata did not show a similar change to that in the hypothalamus. This suggests that hypothalamic CRF and medulla oblongata CRF are controlled by different mechanisms.

Many investigators have reported on the relationship between brain catecholamines and stress-induced ACTH secretion. Some have argued that norepinephrine is an excitatory hypothalamic monoamine which releases ACTH under stress, as its content decreases and turnover increases in stress (Smythe *et al.*, 1983). However, Ganong (1977, 1980) reported that norepinephrine or its agonists acted as an inhibitor in stress-induced ACTH secretion. Some investigators have reported a reduction in the epinephrine concentration in the hypothalamus and pons-medulla in stress (Kvetnansky *et al.*, 1978; Roth *et al.*, 1982; Sudo, 1983) and others have reported an increased dopamine turnover in caudate, putamen and amygdala (Heffner *et al.*, 1984) or decreased

dopamine turnover in the medial palisade zone of the median eminence (Fuxe *et al.*, 1983).

In the present investigation, the hypothalamic level of norepinephrine was reduced 120 min after the start of ether-laparotomy stress, and reduced 60 and 180 min after cold-restraint onset. The dopamine concentration in the hypothalamus was increased 120 min after ether-laparotomy stress onset and slightly, but not significantly increased 180 min after cold-restraint onset. But epinephrine in the hypothalamus did not show any significant change throughout the period of stress. It may be difficult to discuss the turnover rate of catecholamines from our results, as changes in tissue content may be reflected in synthesis, storage, release or inactivation. However, in view of other reports (Palkovits *et al.*, 1975; Smythe *et al.*, 1983; Fuxe *et al.*, 1983; Sudo, 1983), our results may indicate that the turnover rate of norepinephrine is increased but that of dopamine is decreased in the hypothalamus after acute stress. But norepinephrine exhibited a delayed response to stress compared with CRF in the hypothalamus. Therefore, norepinephrine does not seem to be involved in stimulating hypothalamic CRF secretion and the subsequent release of ACTH from the anterior pituitary in the early phase of stress. Palkovits *et al.* (1975) reported that acute stress (formalin, immobilization and cold) reduced both the norepinephrine and dopamine concentrations in the arcuate nucleus without affecting the median eminence concentrations, considering that utilization (release or axonal transport) exceeds synthesis of both norepinephrine and dopamine following stress, therefore catecholamines, particularly in the medial basal hypothalamus, appear to be involved in the hypothalamic regulation of releasing and releasing-inhibiting hormones. As our study did not detect catecholamine alterations in individual hypothalamic nuclei, our data were not able to be compared with theirs.

It may be necessary to measure the turnover rate of catecholamines in individual hypothalamic nuclei and median eminence to confirm the interaction between CRF and catecholamines on the stress-induced ACTH release.

In the medulla oblongata, dopamine but not norepinephrine and epinephrine increased 15 and 30 min after ether-laparotomy stress, and the catecholamine concentration did not show a change throughout the period of cold-restraint stress. The results suggest that catecholamines in the hypothalamus and medulla oblongata are controlled by different mechanisms.

Acknowledgements

The authors wish to thank Dr. T. Hattori for advice and help in the catecholamine determinations, Dr. K. Murakami, Dr. Y. Kawada, Miss R. Akiyama and Mr. M. Aoi for technical assistance.

References

- Axelrod, J. and T. D. Reisine (1984). Stress hormones: their interaction and regulation. *Science* **224**, 452-459.
- Bruhn, T. O., P. M. Plotsky and W. Vale (1984). Effect of paraventricular lesions on corticotropin-releasing factor (CRF)-like immunoreactivity in the stalk median eminence: studies on the adrenocorticotropin response to ether stress and exogenous CRF. *Endocrinology* **114**, 57-62.
- Debreceeni, L. and G. Hartman (1983). Effect of stress and cholinergic stimulation on ACTH release in alpha-methyl-DOPA pretreated rats. *Acta physiol. Hung.* **61**, 99-103.
- Fehm, H. L., K. H. Voigt, R. E. Lang and E. F. Pfeiffer (1980). Effects of neurotransmitters on the release of corticotropin releasing hormone (CRH) by rat hypothalamic tissue *in vitro*. *Exp. Brain Res.* **39**, 229-234.
- Fisher, L. A., J. Rivier, C. Rivier, J. Spiess, W. Vale and M. R. Brown (1982). Corticotropin-releasing factor (CRF): central effects on mean arterial pressure and heart rate in rats. *Endocrinology* **110**, 2222-2224.
- Fuxe, K., K. Andersson, P. Eneroth, R. A. Siegel and L. F. Agnati (1983). Immobilization stress-induced changes in discrete hypothalamic catecholamine levels and turnover, their modulation by nicotine and relationship to neuroendocrine function. *Acta physiol. Scand.* **117**, 421-426.
- Ganong, W. F. (1977). Neurotransmitters involved in ACTH secretion: catecholamines. *Ann. NY Acad. Sci.* **297**, 509-516.
- Ganong, W. F. (1980). Neurotransmitters and pituitary function: regulation of ACTH secretion. *Fed. Proc.* **39**, 2923-2930.
- Guillemin, R., G. W. Clayton, H. S. Lipscomb and J. D. Smith (1959). Fluorometric measurement of rat plasma and adrenal corticosterone concentration. *J. Lab. Clin. Med.* **53**, 830-832.
- Hashimoto, K., K. Murakami, N. Ono, J. Kageyama, Y. Aoki, J. Takahara and Z. Ota (1983). A specific radioimmunoassay for corticotropin releasing factor (CRF) using synthetic ovine CRF. *Life Sci.* **32**, 1001-1007.
- Hashimoto, K., T. Hattori, K. Murakami, S. Suemaru, Y. Kawada, J. Kageyama and Z. Ota (1985). Reduction in brain corticotropin-releasing factor (CRF) in spontaneously hypertensive rats. *Life Sci.* **36**, 643-647.
- Heffner, T. G., G. Vosmer and L. Seiden (1984). Increased transport of 3, 4-dihydroxyphenyl acetic acid from brain during performance of operant behavior in the rat *Brain Res.* **293**, 85-91.
- Hillhouse, E. W., J. Burden and M. T. Jones (1975). The effect of various putative neurotransmitters on the release of corticotropin releasing hormone from the hypothalamus of the rat *in vitro*. I. The effect of acetylcholine and noradrenaline. *Neuroendocrinology* **17**, 1-11.
- Hiroshige, T., T. Saito, R. Ohta and S. Itoh (1969). Increase of corticotropin-releasing activity in the rat hypothalamus following noxious stimuli. *Jap. J. Physiol.* **19**, 866-875.
- Hiroshige, T., Sato and K. Abe (1971). Dynamic changes in the hypothalamic content of corticotropin-releasing factor following noxious stimuli: delayed response in early neonates in comparison with biphasic response in adult rats. *Endocrinology* **89**, 1287-1294.
- Kvetnansky, R., I. L. Kopin and J. M. Saavedra (1978). Changes in epinephrine in individual hypothalamic nuclei after immobilization stress.

- Brain Res.* 155, 387–390
- Labrie, F., V. Giguere, L. Prolx and G. Lefevre (1984). Interactions between CRF, epinephrine, vasopressin and glucocorticoids in the control of ACTH secretion. *J. Steroid Biochem.* 20, 153–160.
- Lowry, O. H., N. J. Rosenbrough, A. L. Farr and R. J. Randall (1951). Protein measurement with Folin phenol reagent. *J. Biol. Chem.* 193, 265–275.
- Maickel, R. P. and R. R. Martel (1983). Brain biogenic amines and pituitary-adrenocortical function in the rat. *Pharmacol. Biochem. Behav.* 19, 321–325.
- Murakami, K., K. Hashimoto and Z. Ota (1984). Effect of angiotensin II, catecholamines and glucocorticoid on corticotropin-releasing factor (CRF)-induced ACTH release in pituitary cell culture. *Acta Med. Okayama* 38, 349–355.
- Palkovits, M., R. M. Kobayashi, J. S. Kizer, D. M. Jacobowitz and I. L. Kopin (1975). Effects of stress on catecholamines and tyrosine hydroxylase activity of individual hypothalamic nuclei. *Neuroendocrinology* 18, 144–153.
- Rivier, C. and W. Vale (1983). Roles of corticotropin-releasing factor, catecholamines and vasopressin in modulating stress-induced ACTH release in the adult rat. *Nature* 305, 325–327.
- Rivier, J., J. Spiess and W. Vale (1983). Characterization of rat hypothalamic corticotropin-releasing factor. *Proc. Natl. Acad. Sci. USA* 80, 4851–4855.
- Roth, K. A., I. M. Mefford and J. D. Barchas (1982). Epinephrine, norepinephrine, dopamine and serotonin: differential effects of acute and chronic stress on regional brain amines. *Brain Res.* 239, 417–424.
- Sirett, N. and H. D. Purves (1973). The assay of corticotropin-releasing factor in ACTH primed 'grafted' rats. In: *Brain-Pituitary-Adrenal Interrelationship* (A. Brodich and E. S. Redgate ed.), Karger, Basel. pp. 79–98.
- Smythe, G. A., J. E. Bradshaw and R. F. Vining (1983). Hypothalamic monoamine control of stress-induced adrenocorticotrophic release in the rat. *Endocrinology* 113, 1062–1071.
- Sudo, A. (1983). Time course of the changes of catecholamine levels in rat brain during swimming stress. *Brain Res.* 276, 372–374.
- Tate, P. W., D. C. Newell, E. E. Cook, D. R. Martinson and T. C. Hagen (1983). Lack of effect of serotonin and norepinephrine on CRF release from hypothalamus *in vitro*. *Horm. Metabol. Res.* 15, 342–346.
- Vale, W., J. Spiess, C. Rivier and J. Rivier (1981). Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and β -endorphin. *Science* 213, 1394–1397.
- Vale, W., J. Vaughan, M. Smith, G. Yamamoto, J. Rivier and C. Rivier (1983). Effects of synthetic ovine corticotropin-releasing factor, glucocorticoids, catecholamines, neurohypophysial peptides and other substances on cultured corticotrophic cells. *Endocrinology* 113, 1121–1131.
- Valentino, R. J., S. L. Foote and G. Aston-Jones (1983). Corticotropin-releasing factor activates noradrenergic neurons of the locus coeruleus. *Brain Res.* 270, 363–367.
- Vernikos-Danellis, J. (1965). Effect of stress, adrenalectomy, hypophysectomy and hydrocortisone on the corticotropin-releasing activity of the rat median eminence. *Endocrinology* 76, 122–126.
- Yasuda, N. and M. A. Greer (1976). Rat hypothalamic corticotropin-releasing factor (CRF) content remains constant despite marked acute or chronic changes in ACTH secretion. *Neuroendocrinology* 22, 48–56.