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Cyclic AMP and Cyclic GMP Response to Stress in Brain and Pituitary: Stress Elevates Pituitary Cyclic AMP[1,2]

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KANT, G. J., J. L. MEYERHOFF, B. N. BUNNELL AND R. H. LENOX. *Cyclic AMP and cyclic GMP response to stress in brain and pituitary: Stress elevates pituitary cyclic AMP.* PHARMAC. BIOCHEM. BEHAV. 17(5) 1067–1072, 1982.—Male rats were exposed to six stressors (saline injection, cold, forced running, Formalin injection, immobilization, electric footshock) for 15, 30, or 60 min. Following sacrifice by microwave irradiation, cyclic AMP and cyclic GMP levels were measured in pituitary, pineal and 8 regions of rat brain. All stressors except saline increased plasma corticosterone, plasma prolactin and pituitary cyclic AMP levels compared to control animals. The magnitude of the pituitary cyclic AMP response was highly correlated with the intensity of the stress as determined by the levels of plasma prolactin. Electric footshock increased pituitary cyclic AMP levels over 10 fold and plasma prolactin over 60 fold. Cyclic AMP levels in other brain regions were not altered. Cerebellar cyclic GMP was increased only by stressors that involved increased motor activity.

Pituitary Stress Cyclic AMP Cyclic GMP Cerebellum Prolactin Corticosterone Growth hormone

ACUTE stress activates a multitude of neuroendocrine and neurochemical responses [33]. Stress releases hormones including β-endorphin, prolactin, and adrenocorticotropic hormone (ACTH) from the pituitary, while growth hormone secretion in rats is suppressed [10, 16, 21, 23, 39, 45]. Centrally, the turnover of norepinephrine (NE), dopamine (DA) and serotonin (5-HT) is increased in some brain regions following stress [2, 6, 7, 38, 42, 48, 50, 51].

Adenosine 3′,5′-monophosphate (cyclic AMP) and guanosine 3′,5′-monophosphate (cyclic GMP) function as second messengers in the CNS as well as the periphery, mediating the effects of neurotransmitters and hormones at some receptors [3, 20, 46]. In the pituitary gland, cyclic AMP appears to be involved in the release and/or synthesis of pituitary hormones. Incubation of pituitaries in vitro with cyclic AMP analogues increases the release of hormones into the medium [24, 43, 44]. Moreover, neurotransmitters and releasing factors have been reported to increase pituitary cyclic AMP levels in vitro [4, 13, 26, 41, 53]. In previous studies we have found that pituitary cyclic AMP levels in vivo are responsive to dopaminergic, cholinergic, and adrenergic agonists [17, 18, 28, 35].

In order to determine accurate in vivo levels of cyclic AMP free of postmortem artifact, we sacrificed the rats in our studies using a high power microwave system. This sacrifice technique required brief (30 sec) immobilization of the rat in a plastic applicator tube which, by itself, was without effect upon cyclic nucleotide levels (unpublished data). When we investigated the effect of longer periods of immobilization on cyclic AMP and cyclic GMP levels in various regions of the brain, we found that immobilization for 15 minutes increased levels of cyclic AMP in the pituitary but did not affect levels of cyclic AMP in any other brain region [34]. Although other investigators have suggested that stress elevates cerebellar cyclic GMP [8,9], we found that cerebellar cyclic GMP levels were decreased following 15 min of immobilization.

We hypothesized that other stressors in addition to immobilization might increase pituitary cyclic AMP in vivo, possibly via central release of neurotransmitters or releasing

[1]In conducting the research described in this report, the investigator(s) adhered to the *Guide for the Care and Use of Laboratory Animals*, as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council.

[2]This material has been reviewed by the Walter Reed Army Institute of Research, and there is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

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factors into the portal circulation. In addition, cyclic nucleotide levels in other brain regions might be responsive to increased neurotransmitter turnover after different stressors.

METHOD

Animals

Male rats (Wistar-derived from the Walter Reed Colony) were handled for two weeks prior to the experiment to minimize non-specific stress effects. Animals were individually housed and food and water were freely available. All experiments were performed between 0830 and 1230 to minimize circadian effects.

Stressors

Six putative stressors (cold, forced running, saline injection (IP), Formalin injection (SC), immobilization and electric footshock) were tested at each of three time points; 15, 30 and 60 min duration. The six stress paradigms were performed as follows: Animals subjected to cold were first sprayed with tap water to wet their fur and then returned to their home cage which was placed in a 4°C chamber. Forced running was applied by placing the rats in a motorized running wheel (diameter=38 cm) at 5 rpm. After a single saline (IP) or Formalin (SC) injection, animals were returned to their home cage until sacrificed. Animals were immobilized in the 5.7 cm diameter plastic cylinder used as the applicator in the microwave inactivation system used for sacrifice. Electric footshock was delivered in a shock chamber on a variable time schedule with a shock duration of 5 sec and an average intershock interval of 30 sec. Six animals were sacrificed for each condition at each of three time points. In addition, 12 control rats were removed from their home cages and sacrificed immediately.

Assay Procedures

All animals were sacrificed by high-power microwave irradiation for 5 sec [5, 27, 30, 36]. Following microwave irradiation, the rats were decapitated and trunk blood was collected and the plasma stored for subsequent hormone assays. The heads were cooled on dry ice and the following brain regions were dissected: pituitary, pineal, cerebellum, hypothalamus, septal region, frontal cortex, striatum, ventral striatum (n. accumbens plus olfactory tubercle), olfactory bulb and interpeduncular region. The tissue pieces were weighed and sonicated in 50 mM sodium acetate buffer pH 6.2. The sonicates were centrifuged at 25,000 × g for 15 minutes and the supernatants were stored at −70°C until assayed. Cyclic AMP and cyclic GMP were determined using antibodies developed and characterized in our laboratories [29, 31, 49]. For measurement of the cyclic nucleotides in the smaller brain regions, a modification of the method described by Harper and Brooker [14] was employed. Highly specific antisera were used at usual final dilutions of 1:400,000 for cyclic AMP and 1:20,000 for cyclic GMP. The data was analyzed by computer using a nonlinear four parameter logistic model weighted for nonuniformity of variance [47].

Prolactin and growth hormone were determined by radioimmunoassay using materials provided by the NIAMD and the results are expressed in terms of ng/ml of the RP-1 standards. Rat plasma samples were assayed for corticosterone by radioimmunoassay using an antibody produced in our laboratory in rabbit against corticosterone-21-hemisuccinate: BSA [29].

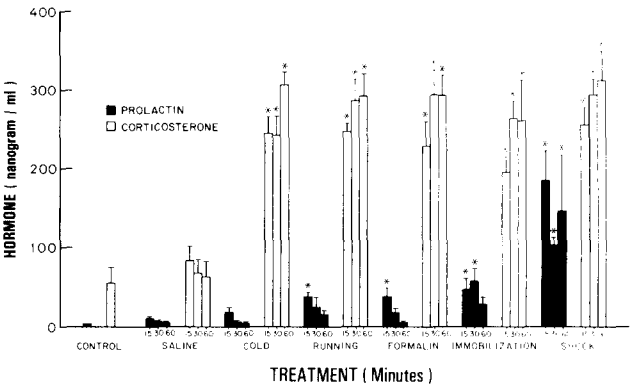

FIG. 1. Hormone levels in rats following stress. Controls were sacrificed immediately upon removal from home cage. Stressors are described in the text. Vertical bars are Mean±SEM, N=6 rats. Data were analyzed by one way analysis of variance, $*p<0.05$.

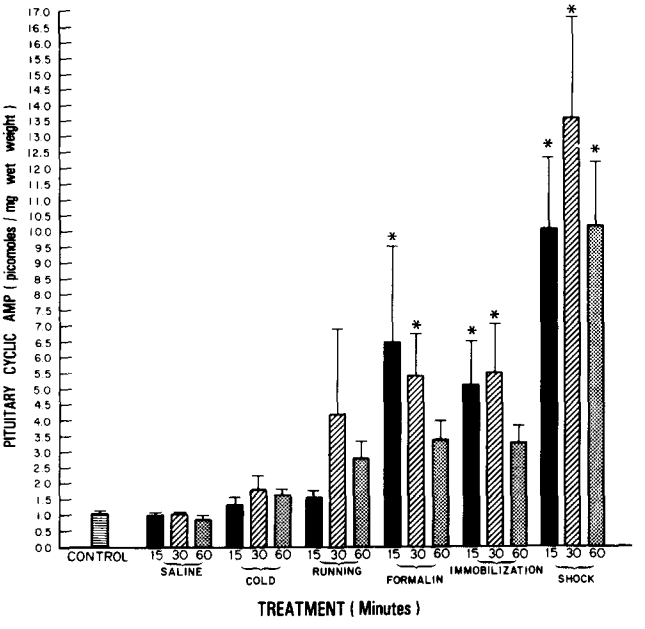

FIG. 2. Pituitary cyclic AMP levels in rats following stress. See Fig. 1 legend for details.

Statistics

An overall analysis of variance was performed for each region for each nucleotide. In Figs. 2 and 3, all stress groups were included in the analysis. For Tables 1, 2, and 3 only the displayed data were included. If analysis of variance revealed a significant F ratio, then post hoc *t*-tests between control and individual stress groups were performed.

RESULTS

As judged by the plasma corticosterone response, all

TABLE 1

CYCLIC AMP LEVELS IN PITUITARY, PINEAL AND BRAIN FOLLOWING FOOTSHOCK

Region	Cyclic AMP (pmole/mg wet weight) Control	Shock-15	Shock-30	Shock-60
Pituitary	1.06 ± 0.09	10.1 ± 2.3*	13.6 ± 3.2*	10.2 ± 2.1*
Hypothalamus	1.04 ± 0.06	1.10 ± 0.10	1.20 ± 0.09	1.04 ± 0.06
Interp. n.	1.16 ± 0.15	1.36 ± 0.13	1.43 ± 0.17	1.29 ± 0.21
Frontal ctx.	1.28 ± 0.14	1.25 ± 0.09	1.22 ± 0.06	1.41 ± 0.24
Ven. striatum	0.646 ± 0.046	0.704 ± 0.056	0.767 ± 0.050	0.638 ± 0.053
Striatum	0.458 ± 0.033	0.523 ± 0.095	0.466 ± 0.036	0.425 ± 0.024
Septal region	0.568 ± 0.044	0.594 ± 0.057	0.589 ± 0.038	0.647 ± 0.091
Olfactory bulb	1.10 ± 0.04	1.07 ± 0.10	0.838 ± 0.146	0.909 ± 0.048
Cerebellum	0.827 ± 0.031	0.798 ± 0.037	0.806 ± 0.047	0.672 ± 0.046*
Pineal (pmole/pineal)	2.40 ± 0.31	3.05 ± 0.41	3.97 ± 0.52	2.61 ± 0.40

Values represent Mean±SEM. N=6. *Differs significantly from control, $p<0.05$.

TABLE 2

CYCLIC GMP LEVELS IN PITUITARY, PINEAL AND BRAIN FOLLOWING FOOTSHOCK

Region	Cyclic GMP (pmole/mg wet weight) Control	Shock-15	Shock-30	Shock-60
Pituitary	0.077 ± 0.007	0.091 ± 0.017	0.084 ± 0.009	0.081 ± 0.014
Hypothalamus	0.066 ± 0.005	0.106 ± 0.011*	0.113 ± 0.007*	0.115 ± 0.012*
Interp. n.	0.075 ± 0.005	0.146 ± 0.024*	0.144 ± 0.016*	0.171 ± 0.043*
Frontal ctx.	0.090 ± 0.010	0.091 ± 0.008	0.085 ± 0.008	0.088 ± 0.009
Ven. striatum	0.100 ± 0.006	0.115 ± 0.007	0.097 ± 0.010	0.101 ± 0.005
Striatum	0.065 ± 0.003	0.093 ± 0.017	0.063 ± 0.006	0.073 ± 0.004
Septal region	0.089 ± 0.005	0.126 ± 0.013*	0.114 ± 0.008*	0.098 ± 0.008
Olfactory bulb	0.079 ± 0.004	0.089 ± 0.006	0.095 ± 0.018	0.081 ± 0.009
Cerebellum	1.18 ± 0.46	3.34 ± 0.49*	2.47 ± 0.32*	2.50 ± 0.33*
Pineal (pmole/pineal)	0.295 ± 0.051	1.41 ± 0.42*	0.724 ± 0.194	0.651 ± 0.106

Values represent Mean±SEM. N=6. *Differs significantly from control, $p<0.05$.

tested conditions except saline injection appeared to be stressful to the rats. As shown in Fig. 1, corticosterone levels were increased 5-fold after all tested stressors except saline injection. Prolactin responded rapidly to the stressors. There was an increase in plasma prolactin even after saline injection and shock caused a 60-fold increase in prolactin levels. Plasma growth hormone levels generally decreased with stress: Control 253±131 ng/ml; saline (60 min) 84±27 ng/ml; cold (60 min) 15±3 ng/ml; running (60 min) 13±2 ng/ml; Formalin (60 min) 18±7; immobilization (60 min) 15±4 ng/ml; shock (60 min) 16±4 ng/ml.

All stressors except saline increased pituitary cyclic AMP compared to controls as shown in Fig. 2; however, the increases after cold and running were not statistically significant. There were no significant effects of any stressor on cyclic AMP levels in any other region examined, with the exception of a decrease in cerebellar cyclic AMP in the 60 min shock group. Levels of cyclic AMP in all brain regions examined following shock are shown in Table 1.

The increases in cerebellar cyclic GMP (Fig. 3) seen after certain stressors appear to be related to the change in motor activity produced by some stressors and not related to the stress itself. Stressors such as cold, running, and footshock increased cerebellar cyclic GMP, while Formalin and immobilization did not. In fact, immobilization, which obviously decreases motor activity, resulted in decreased cyclic GMP levels in the cerebellum. Similarly, cyclic GMP levels in some other brain regions were increased after shock (Table 2) but not after immobilization (Table 3).

DISCUSSION

The results of these studies indicate that pituitary cyclic AMP is responsive to stress. The elevation in pituitary cyclic AMP appeared to be proportional to the apparent severity of the stressor as judged by plasma prolactin response, with the most severe stressors having the greatest effect. Cyclic AMP mediates the actions of many neurotransmitters and hormones at particular sites. Possibly, hormones released by stress feedback on the pituitary and initiate the cyclic AMP

TABLE 3

CYCLIC GMP LEVELS IN PITUITARY, PINEAL, AND BRAIN FOLLOWING IMMOBILIZATION

Region	Cyclic GMP (pmole/mg wet weight) Control	Immob.-15	Immob.-30	Immob.-60
Pituitary	0.077 ± 0.007	0.098 ± 0.031	0.065 ± 0.011	0.057 ± 0.004
Hypothalamus	0.066 ± 0.005	0.073 ± 0.006	0.066 ± 0.005	0.076 ± 0.012
Interp. n.	0.075 ± 0.005	0.072 ± 0.008	0.071 ± 0.012	0.085 ± 0.015
Frontal ctx.	0.090 ± 0.010	0.079 ± 0.006	0.088 ± 0.009	0.080 ± 0.012
Ven. striatum	0.100 ± 0.006	0.099 ± 0.014	0.099 ± 0.014	0.081 ± 0.005
Striatum	0.065 ± 0.003	0.058 ± 0.004	0.073 ± 0.014	0.063 ± 0.010
Septal region	0.089 ± 0.005	0.101 ± 0.007	0.100 ± 0.014	0.095 ± 0.007
Olfactory bulb	0.079 ± 0.004	0.066 ± 0.006	0.078 ± 0.009	0.082 ± 0.009
Cerebellum	1.18 ± 0.46	0.521 ± 0.087	0.365 ± 0.044	0.454 ± 0.110
Pineal (pmole/pineal)	0.295 ± 0.051	0.180 ± 0.036	0.180 ± 0.036	0.209 ± 0.048

Values represent Mean±SEM. N=6.

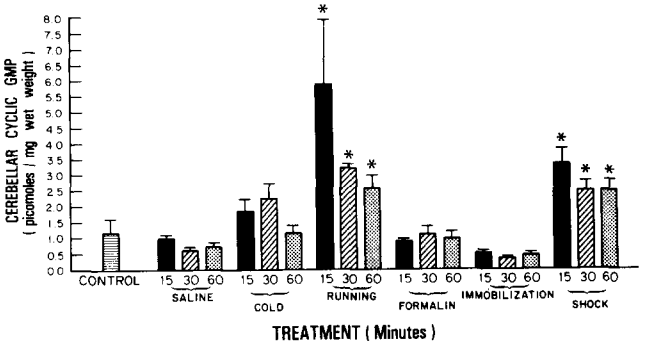

FIG. 3. Cerebellar cyclic GMP levels in rats following stress. See Fig. 1 legend for details.

response. The elevated cyclic AMP might serve to regulate further hormone release or as a signal for hormonal synthesis. Alternatively, centrally derived transmitters or releasing hormones might act directly to elevate pituitary cyclic AMP in a selected cell subtype. The cyclic AMP could then alter the release or synthesis of pituitary hormones [25] and link stress-activated central circuits with pituitary hormonal output.

Biogenic amines have been implicated in the control of various pituitary hormones including growth hormone, prolactin, ACTH and the gonadotropins [1, 32, 40]. We have found that pituitary cyclic AMP levels increase in vivo after administration of several neurotransmitter agonists including apomorphine, isoproterenol, oxotremorine and nicotine [17, 18, 28, 35]. Increased central turnover of NE, DA, acetylcholine or other transmitters caused by stress could directly or indirectly impinge upon pituitary cyclic AMP.

Since some catecholamine receptors in the CNS are thought to be linked to adenylate cyclase [15,19] and, since catecholamine turnover is increased by stress, we also examined the cyclic AMP response to stressors in regions of the brain containing NE and DA terminals. Although both the mesolimbic DA pathway [11,52] and the cells of the locus coeruleus are activated by stress [22], none of the respective projection areas had increased levels of cyclic AMP (Table 1).

Cerebellar cyclic GMP has been reported to be a biochemical marker for stress. Early studies reported that cyclic GMP was elevated by cold, forced swimming and exposure to a hot plate [8,9]. The lack of a cyclic GMP increase following immobilization prompted us to investigate the role of motor activity in the cerebellar cyclic GMP elevations reported by others. We found that cerebellar cyclic GMP increased with voluntary motor activity [34,37]. In the study reported here with a variety of stressors, it is also clear that activity rather than stress is responsible for elevated cyclic GMP levels in cerebellum and other brain areas.

As expected, stress increases plasma prolactin and corticosterone and decreased growth hormone. This has been demonstrated previously by our laboratory and others [23,29]. In this experiment where different stressors were examined in the same study, a difference in response to stress by prolactin and corticosterone can be seen. All stressors maximally elevated corticosterone within 15 minutes. In contrast, prolactin response appears proportional to the severity of the stressor. Although plasma prolactin is known to increase following various stressors in rats and humans [12], the function of the prolactin response to stress is not known.

In summary, we have shown that pituitary cyclic AMP increases after acute stress. The amplitude of the increase seem to be highly correlated with the intensity of the stress response as measured by prolactin release. In contrast, cerebellar cyclic GMP which has been postulated to be a sensitive marker for stress appears to be responsive only to the increased motor activity evoked by some stressors. The mechanism and function of the pituitary cyclic AMP response is not clear at present, but the possibility that cyclic AMP may link centrally-mediated release of neuroactive compounds with pituitary hormonal output remains an attractive hypothesis.

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REFERENCES

1. Advis, J. P., J. W. Simkins, J. Bennett and J. Meites. Serotonergic control of prolactin release in male rats. *Life Sci.* **24:** 359–366, 1979.
2. Bliss, E. L., J. Ailon and J. Zwanziger. Metabolism of norepinephrine, serotonin and dopamine in rat brain with stress. *J. Pharmac. exp. Ther.* **164:** 122–134, 1968.
3. Bloom, F. E. The role of cyclic nucleotides in central synaptic function. In: *Advances in Biochemical Psychopharmacology*, vol. 15, edited by E. Costa, E. Giacobini and R. Paoletti. New York: Raven Press, 1976, pp. 273–282.
4. Borgeat, P., G. Chavancy, A. Dupont, F. Labrie, A. Arimura and A. V. Schally. Stimulation of adenosine 3,5,-cyclic monophosphate accumulation in anterior pituitary gland *in vitro* by synthetic luteinizing hormone-releasing hormone. *Proc. natn. Acad. Sci. U.S.A.* **69:** 2677–2681, 1972.
5. Brown, P. V., R. H. Lenox and J. L. Meyerhoff. Microwave enzyme inactivation system: electronic control to reduce dose variability. *IEEE Trans. biomed. Engng* **25:** 205–208, 1978.
6. Cassens, G., M. Roffman, A. Kurac, P. J. Orsulak and J. J. Schildkraut. Alterations in brain norepinephrine metabolism induced by environmental stimuli previously paired with inescapable shock. *Science* **209:** 1138–1140, 1980.
7. Corrodi, H., K. Fuxe and T. Hökfelt. The effect of immobilization stress on the activity of central monoamine neurons. *Life Sci.* **7:** 107–112, 1968.
8. Dinnendahl, V. and S. W. Gumulka. Stress induced alterations of cyclic nucleotide levels in brain: effects of centrally acting drugs. *Psychopharmacology* **52:** 243–249, 1977.
9. Dinnendahl, V. Effects of stress on mouse brain. Cyclic nucleotide levels *in vivo*. *Brain Res.* **100:** 716–719, 1975.
10. Euker, J. S., J. Meites and G. D. Riegle. Effects of acute stress on serum LH and prolactin in intact, castrate and dexamethasone-treated male rats. *Endocrinology* **96:** 85–92, 1975.
11. Fadda, F., A. Argiolas, M. R. Melis, A. H. Tissari, P. L. Onali and G. L. Gessa. Stress-induced increase in 3,4-dihydroxyphenylacetic acid (DOPAC) levels in the cerebral cortex and in n. accumbens: reversal by diazepam. *Life Sci.* **23:** 2219–2224, 1978.
12. Frantz, A. G. The regulation of prolactin secretion in humans. In: *Frontiers in Neuroendocrinology*, vol. 3, edited by W. F. Ganong and L. Martini. London: Oxford University Press, 1978, pp. 337–374.
13. Gourdji, D., D. Bataille, N. Vauclin, D. Grouselle, G. Rosselin and A. Tixier-Vidal. Vasoactive intestinal peptide (VIP) stimulates prolactin (PRL) release and cAMP production in a rat pituitary cell line (GH3/B6). Additive effects of VIP and TRH on PRL release. *FEBS Lett.* **104:** 165–168, 1979.
14. Harper, J. F. and G. Brooker. Femtomole sensitive radioimmunoassay for cyclic AMP and cyclic GMP after 2'0 acetylation by acetic anhydride in aqueous solution. *J. cycl. Nucleotide Res.* **1:** 207–218, 1975.
15. Iversen, L. L. Catecholamine-sensitive adenylate cyclases in nervous tissues. *J. Neurochem.* **29:** 5–12, 1977.
16. Jobin, M., L. Ferland and F. Labrie. Effect of pharmacological blockade of ACTH and TSH secretion on the acute stimulation of prolactin release by exposure to cold and ether stress. *Endocrinology* **99:** 146–151, 1976.
17. Kant, G. J., J. L. Meyerhoff and R. H. Lenox. *In vivo* effects of apomorphine and 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone (RO20-1724) on cyclic nucleotides in rat brain and pituitary. *Biochem. Pharmac.* **29:** 369–373, 1980.
18. Kant, G. J., V. E. Bates, R. H. Lenox and J. L. Meyerhoff. Increases in cyclic AMP levels in rat brain regions *in vivo* following isoproterenol. *Biochem. Pharmac.* **30:** 3377–3380, 1981.
19. Kebabian, J. W., G. L. Petzold and P. Greengard. Dopamine-sensitive adenylate cyclase in caudate nucleus of rat brain and its similarity to the "dopamine receptor." *Proc. natn. Acad. Sci. U.S.A.* **69:** 2145–2149, 1972.
20. Kebabian, J. W., F. E. Bloom, A. L. Steiner and P. Greengard. Neurotransmitters increase cyclic nucleotides in postganglionic neurons: immunocytochemical demonstration. *Science* **190:** 157–159, 1975.
21. Keim, K. L. and E. B. Sigg. Physiological and biochemical concomitants of restraint stress in rats. *Pharmac. Biochem. Behav.* **4:** 289–297, 1976.
22. Korf, J., G. J. Aghajanian and R. M. Roth. Increased turnover of norepinephrine in the rat cerebral cortex during stress: role of the locus coeruleus. *Neuropharmacology* **12:** 933–938, 1973.
23. Krulich, L., E. Hefco, P. Illner and C. B. Read. The effects of acute stress on the secretion of LH, FSH, prolactin, and GH in the normal male rat, with comments on their statistical evaluation. *Neuroendocrinology* **16:** 293–311, 1974.
24. Labrie, F., G. Pelletier, P. Borgeat, J. Drouin, F. Ferland and A. Belanger. Mode of action of hypothalamic regulatory hormones in the adenohypophysis. In: *Frontiers in Neuroendocrinology*, vol. 4, edited by L. Martinin and W. F. Ganong. New York: Raven Press, 1976, pp. 63–93.
25. Labrie, F., G. Pelletier, A. Lemay, S. Lemaire, G. Poirer, N. Barden, G. Beraud, R. Boucher, M. Gautheir and A. Delean. Hypophysiotropic hormones, cyclic AMP and anterior pituitary protein synthesis and release. In: *Hormones and Brain Function*, edited by K. Lissak. New York: Plenum Press, 1973, pp. 157–182.
26. Labrie, F., R. Veilleux, G. Lefevre, D. H. Coy, J. Sveiras-Diaz and A. Schally. Corticotropin releasing factor stimulates accumulation of adenosine 3', 5'-monophosphate in rat pituitary corticotrophs. *Science* **216:** 1007–1008, 1982.
27. Lenox, R. H., P. V. Brown and J. L. Meyerhoff. Microwave inactivation—a technique with promise and pitfalls. *TINS* **2:** 106–109, 1979.
28. Lenox, R. H., G. J. Kant and J. L. Meyerhoff. Regional sensitivity of cyclic AMP and cyclic GMP in rat brain to central cholinergic stimulation. *Life Sci.* **26:** 2201–2209, 1980.
29. Lenox, R. H., G. J. Kant, G. R. Sessions, L. L. Pennington, E. H. Mougey and J. L. Meyerhoff. Specific hormonal and neurochemical responses to different stressors. *Neuroendocrinology* **30:** 300–308, 1980.
30. Lenox, R. H., O. P. Gandhi, J. L. Meyerhoff and H. M. Grove. A microwave applicator for *in vivo* rapid inactivation of enzymes in the central nervous system. *IEEE Trans. microwave Theory Technol.* **24:** 58–61, 1976.
31. Lenox, R. H., J. L. Meyerhoff, O. P. Gandhi and H. L. Wray. Regional levels of cyclic AMP in rat brain: pitfalls of microwave inactivation. *J. cycl. Nucleotide Res.* **3:** 367–379, 1977.
32. Martin, J. B., P. Brazeau, G. S. Tannenbaum, J. O. Willoughby, J. Epelbaum, L. C. Terry and D. Durand. Neuroendocrine organization of growth hormone secretion. In: *The Hypothalamus*. New York: Raven Press, 1978, pp. 329–357.
33. Mason, J. W., J. T. Maher, L. H. Hartley, E. H. Mougey, M. J. Perlow and L. G. Jones. Selectivity of corticosteroid and catecholamine responses to various natural stimuli. In: *Psychopathology of Human Adaption*, edited by G. Serban. New York: Plenum Publishing Corp., 1976, pp. 147–171.
34. Meyerhoff, J. L., G. J. Kant, G. R. Sessions, E. H. Mougey, L. L. Pennington and R. H. Lenox. Brain and pituitary cyclic nucleotide response to stress. In: *Perspectives in Behavioral Medicine*, vol. 2, edited by R. B. Williams. New York: Academic Press, in press.

35. Meyenhoff, J. L., G. J. Kant, R. H. Lenox, L. L. Pennington and D. R. Collins. Effects of muscarinic and nicotinic agonists and antagonists on brain regional cyclic nucleotides. *Soc. Neurosci. Abstr.* **6:** 534, 1980.
36. Meyerhoff, J. L., R. H. Lenox, P. V. Brown and O. M. Gandhi. The inactivation of rodent brain enzymes *in vivo* using high-intensity microwave irradiation. *Proc. IEEE* **68:** 155–159, 1980.
37. Meyerhoff, J. L., R. H. Lenox, G. J. Kant, G. R. Sessions, E. H. Mougey and L. L. Pennington. The effect of locomotor activity on cerebellar levels of cGMP. *Life Sci.* **24:** 1125–1130, 1979.
38. Morgan, W. W., P. K. Rudeen, K. A. Pfeil. Effect of immobilization stress on serotonin content and turnover in regions of the rat brain. *Life Sci.* **17:** 143–150, 1975.
39. Mueller, G. P. Beta-endorphin immunoreactivity in rat plasma: variations in response to different physical stimuli. *Life Sci.* **29:** 1669–1674, 1981.
40. Nansel, D. D., G. A. Gudelsky, M. J. Reymond, W. B. Neaves and J. C. Porter. A possible role for lysosomes in the inhibitory action of dopamine on prolactin release. *Endocrinology* **108:** 896–902, 1981.
41. Naor, Z., G. Snyder, C. P. Fawcett and S. M. McCann. Pituitary cyclic nucleotides and thyrotropin-releasing hormone action: the relationship of adenosine 3,5-monophosphate and guanosine 3,5-monophosphate to the release of thyrotropin and prolactin. *Endocrinology* **106:** 1304–1310, 1980.
42. Palkovits, M., R. M. Kobayashi, J. S. Kizer, D. M. Jacobowitz and I. J. Kopin. Effects of stress on catecholamines and tyrosine hydroxylase activity of individual hypothalamic nuclei. *Neuroendocrinology* **18:** 144–153, 1975.
43. Peake, G. T. The role of cyclic nucleotides in the secretion of pituitary growth hormone. In: *Frontiers of Neuroendocrinology*, vol. 3, edited by W. F. Ganong and L. Martini. London: Oxford University Press, 1973, pp. 173–208.
44. Pelletier, G., A. Lemay, G. Beraud and F. Labrie. Ultrastructural changes accompanying the stimulatory effect of N6-monobutyrl adenosine 3,5-monophosphate on the release of growth hormone (GH), prolactin (PRL) and adrenocorticotropic hormone (ACTH) in rat anterior pituitary gland *in vivo*. *Endocrinology* **91:** 1355–1370, 1972.
45. Rice, R. W. and V. Critchlow. Extrahypothalamic control of stress-induced inhibition of growth hormone secretion in the rat. *Endocrinology* **99:** 970–976, 1976.
46. Robison, G. A., R. W. Butcher and E. W. Sutherland. *Cyclic AMP*. New York: Academic Press, 1971.
47. Rodbard, D., R. H. Lenox, H. L. Wray and D. Ramseth. Statistical characterization of the random error in the radioimmunoassay dose-response variable. *Clin. Chem.* **22:** 350–358, 1976.
48. Saavedra, J. M., R. Kvetnansky and I. J. Kopin. Adrenaline, noradrenaline and dopamine levels in specific brain stem areas of acutely immobilized rats. *Brain Res.* **160:** 271–280, 1979.
49. Steiner, A. L., A. W. Parker and D. M. Kipnis. Radioimmunoassay for cyclic nucleotides. *J. biol. Chem.* **247:** 1106–1113, 1972.
50. Thierry, A. M., G. Blanc and J. Glowinski. Effects of stress on the disposition of catecholamines localized in various intraneuronal storage forms in the brain stem of the rat. *J. Neurochem.* **18:** 449–461, 1971.
51. Thierry, A. M., F. Javoy, J. Glowinski and S. Kety. Effects of stress on the metabolism of norepinephrine, dopamine, and serotonin in the central nervous system of the rat. I: Modifications of norepinephrine turnover. *J. Pharmac. exp. Ther.* **163:** 163–171, 1968.
52. Tissari, A. H., A. Argiolas, F. Fadda, G. Serra and G. L. Gessa. Footshock stress accelerates non-striatal dopamine synthesis without activating tyrosine hydroxylase. *Naunyn-Schmiedeberg's Arch. Pharmac.* **308:** 155–157, 1979.
53. Zor, U., T. Kaneko, H. P. G. Schneider, S. M. McCann, I. P. Lowe, G. Bloom and B. Borland. Stimulation of anterior pituitary adenyl cyclase activity and adenosine 3,5-cyclic phosphate by hypothalamic extract and prostaglandin E1. *Proc. natn. Acad. Sci. U.S.A.* **63:** 918–925, 1969.