MOLECULAR BIOLOGY OF STRESS

Organizers: Oren Zinder and Shlomo Breznitz

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Plenary Sessions

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Molecular Biology of Stress

Biochemical Basis for Integrated Pituitary and Autonomic Responses to Stress

Q 001 PRECLINICAL AND CLINICAL STUDIES WITH CORTICOTROPIN-RELEASING FACTOR: IMPLICATIONS FOR AFFECTIVE DISORDERS, Charles B. Nemeroff, Garth Bissette, Michael J. Owens, Pamela D. Butler and Clinton D. Kilts, Departments of Psychiatry and Pharmacology, Duke University Medical Center, Box 3859, Durham, N. C. 27710.

Since elucidation of the chemical identity of corticotropin-releasing factor (CRF) 7 years ago, considerable data have been provided which support an extra-endocrine role for CRF as a neurotransmitter in the CNS. In brief, CRF and high affinity CRF binding sites, putative receptors are distributed heterogenously throughout the mammalian CNS and the peptide is released from brain slices by $[K^+]$ in a Ca^{2+}-dependent manner. In addition the peptide, in certain brain regions, activates second messenger systems, i.e. adenylate cyclase and phosphoinositide hydrolysis. CRF also produces potent electrophysiological and behavioral effects in laboratory animals. We have tested two major hypotheses: (1) CRF acts both in its role as a hypophysiotropic hormone and as a CNS neurotransmitter to coordinate the organism's response to stress and (2) CRF is hypersecreted in patients with major depression, contributing both to the hypercortisolemia characteristic of this disorder and to many of the signs and symptoms of depression. To test the first hypothesis, we have measured the concentration of CRF in rats exposed to either acute or chronic stress (1) and observed marked alterations in CRF concentrations: a 50% decrease in the arcuate-median eminance area, consistent with increased CRF release and, a marked increase (↑100%) in the locus coeruleus, the origin of the A_6 NE-containing cells. The triazolobenzodiazepines, alprazolam and adinazolam, produce effects on CRF concentrations in the hypothalamus and LC that are opposite to the effects of stress. We have, in three studies, demonstrated increased concentrations of CRF in cerebrospinal fluid (CSF) in drug-free patients with major depression (2-4). Recently we have reported (5) a reduced number of CRF binding sites in the frontal cortex of suicide victims compared to controls; this finding is consistent with chronic CRF hypersecretion resulting in CRF receptor down regulation. This concateration of preclinical and clinical findings support the hypothesis that this peptide is involved in the pathogenesis of major depression. (Supported by NIMH MH-42088)

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Corticotropin Releasing Factor System

Q 002 BEHAVIORAL ACTIONS OF CORTICOTROPIN RELEASING FACTOR IN THE CENTRAL NERVOUS SYSTEM: George F. Koob. Research Institute of Scripps Clinic, 10666 N. Torrey Pines Road, BCR 1, La Jolla, CA 92037

Corticotropin-releasing factor (CRF) has been localized in the central nervous system in regions outside the hypothalamic pituitary adrenal axis, and has been hypothesized to have a role in the central nervous system in mediating behavioral responses to stress. Experiments in our laboratory have demonstrated that CRF has behavioral activating effects when injected intracerebroventricularly in rats. CRF dose-dependently increases activity in a familiar photocell cage environment. This activation persists after hypophysectomy, opiate receptor blockade, and low-dose dopamine receptor blockade, which suggests a unique mechanism of action. In aversive situations such as an open field test, CRF produces behavioral changes consistent with increased emotionality. In a conflict test CRF produces decreases in responding during the conflict component, and in a startle test CRF produces increases in startle amplitude. These effects are reversed by the benzodiazepine, chlordiazepoxide, and a CRF peptide antagonist. Results in our laboratory have also shown that the CRF antagonist, alpha helical CRF 9-41 injected by itself intracerebroventricularly can attenuate behavioral responses to stress. Alpha-helical CRF (1-25 μg ICV) can block stress-induced fighting in rats, and alpha helical CRF 9-41 can significantly block the acquisition of conditioned suppression. These results suggest that endogenous CRF systems in the central nervous system may have a role in mediating behavioral responses to stress. These data and the behavioral activating effects of CRF itself suggest that CRF may function as a fundamental brain activating system.

Other Neuropeptides in Stress-Mechanisms

Q 003 **PHARMACOLOGICALLY-INDUCED CHANGES IN SOMATOSTATIN BIOSYNTHESIS IN THE CENTRAL NERVOUS SYSTEM: STUDIES WITH CYSTEAMINE.** J.D.Fernstrom, R.P.S.Kwok, and J.L.Cameron. Departments of Psychiatry and Behavioral Neuroscience, and Center for Neuroscience, University of Pittsburgh, Pittsburgh PA 15213.

Members of the somatostatin (SRIF) family of peptides, particularly SRIF-14 and SRIF-28, are thought to function as neurohormones and probably neurotransmitters in the central nervous system (CNS). Because of these roles, it is important to understand how the production of these peptides is controlled, and as a corollary, whether and how drugs influence this process. For such studies, the quantitation of immunoreactive (IR) levels of these peptides is probably inadequate for determining the kinetic activity in the pathway. Accordingly, we have developed an in vivo method for estimating SRIF-14 and SRIF-28 synthesis rates, initially in hypothalamus. The method involves administering (^{35}S)cysteine into the third ventricle of urethane anesthetized rats, and removing hypothalami at intervals thereafter. The hypothalami are immediately homogenized, and extracts run on an HPLC to separate and quantitate labeled SRIF-14 and SRIF-28. Labeling of SRIF-14 and SRIF-28 is linear for 8 hr after (^{35}S)cysteine injection. Kinetic studies are confined to this timeperiod, since the linear period of labeling is most likely to provide valid information regarding synthesis rate. In practise, a single timepoint, 4 hr, is selected for study, since this is about midpoint in the linear accumulation period, and is associated with substantial numbers of counts in the SRIF-14 and SRIF-28 peaks. A similar method is also employed for estimating vasopressin (AVP) and oxytocin (OXT) synthesis rates. Both are cysteine-containing peptides. However, labeling of AVP and OXT plateaus 2 hr post label injection, and this timepoint is therefore used in AVP and OXT labeling studies. Few known neuropharmacologic agents appear to cause substantial alterations in the CNS levels of these cysteine-containing peptides; thus, we have initially focused on an uncommon drug, which produces profound (though temporary) changes in IR SRIF levels. This compound is the sulfhydryl agent cysteamine (CSH), and its administration to rats causes immediate and selective reductions in hypothalamic IR-SRIF levels. We have explored for CSH effects on SRIF synthesis, and find that the drug causes an immediate suppression of (^{35}S) labeling of SRIF-14 and SRIF-28, as well as of OXT and AVP. Unlike IR-SRIF, IR-AVP and OXT levels are unaffected by CSH. Though IR-SRIF levels are not restored for at least 3 days, labeling of all four cysteine-containing peptides is normal within 10-12 hr of CSH injection. These data, and other results to be presented, suggest that CSH may suppress SRIF, AVP and OXT syntheses, but only transiently, and that the initial reduction in IR-SRIF levels cannot be the result of synthesis inhibition. However, the rapid normalization of SRIF synthesis could be an important contributor to the restoration of SRIF levels in the days that follow drug injection.
[Supported by a grant from the NIH (NS20014)].

Q 004 MECHANISMS OF TRANSSYNAPTIC AND GLUCORTICOID REGULATION OF ADRENAL OPIATE PEPTIDES, Edmund F. La Gamma and Joseph D. DeCristofaro, Departments of Pediatrics and Neurobiology & Behavior, SUNY at Stony Brook, Stony Brook, NY 11794-8111.

Stimulus-secretion-synthesis coupling is long recognized as a critical determinant of neuroendocrine stress-responsiveness. Cold-stress is frequently employed as an experimental stimulus. During cold-stress the sympathoadrenal system is activated through increased transsynaptic impulses and glucocorticoid hormones are released. This stimulus causes adrenal catecholamine release and augments its biosynthesis. How is the co-localized opiate peptide system affected? To answer this question, rats were individually housed at 4°C. Cold stress decreased (or had no effect on) adrenal medullary opiate peptide levels. However, since certain severe stress-paradigms increase opiate levels (eg. insulin shock), we increased the intensity (severity) of cold-stress by wetting the animals twice per day. In contrast, this more severe stress now resulted in an increase in opiate levels to 195% of control. Since cold stress increases catecholamine release and biosynthesis by increasing transsynaptic impulse activity, we treated rats with cholinergic-nicotinic and cholinergic-muscarinic receptor agonists. Neither nicotine nor carbechol (muscarinic agonist) alone affected enkephalin levels. However, when adminstered simulataneously, there was a three fold rise in leu-enkephalin peptide and prohormone. Mechanisms of regulation were examined at the cellular level. Increased levels of the nicotinic receptor-linked second-messenger, cAMP, suppressed enkephalin biosynthesis, while the muscarinic receptor-linked second-messenger, cGMP, had no effect. Similar, to effects using combined receptor agonists, simultaneous administration of forskolin (which increases cAMP), and with elevated levels of cGMP a 3-fold rise in enkephalin peptide and prohormone levels was seen. These observations are consistent with our previous results showing that enkephalin levels (mRNA, prohormone, and peptide) are suppressed (ie. maintained at low levels) by nicotinic receptor activity and consequent membrane depolarization in a calcium ion dependent manner. Moreover, our data, using nuclear run-on assay, illustrates that depolarizing inhibitory influences result from effects at the genomic level. On the other hand, stress-related glucocorticoid hormones augment enkephalin pathways, consistent with an enhancer function. In summary, our observations suggest that co-localized catecholamine and opiate peptide neurotransmitters are differentially regulated. Altering the intensity of a stressful stimulus can either suppress or augment enkephalin biosynthesis. This may result from an interaction between various extracellular signals and their respective second-messenger pathways. Physiological effects on adaptive responses need to be determined.

Q 005 REGULATION OF NEUROPEPTIDES BY PEPTIDASE ACTION, Jeffrey F. McKelvy, Neuroscience Research Division, Abbott Laboratories, Abbott Park, IL 60064

As part of the response to a stressor, neural systems can adaptively alter the lifetimes of intercellular signals used in communication. In the case of neuropeptide signals, recent work suggests that the regulation of the activity of peptidase enzymes may contribute to the regulation of neuropeptide levels. The two types of peptidase activities which can be considered in this regard are degrading peptidases, those which break down biologically active peptides to inactive products, and processing peptidases, which liberate biologically active neuropeptides from biologically inert neuropeptide polyprotein precursors. The evidence for the regulation of these activities in a variety of systems will be reviewed, including that suggesting the possibility that processing peptidase activity could be regulated so as to alter the qualitative array of neuropeptides derived from a given neuropeptide polyprotein. In addition, recent evidence that peptide receptors may be liberated from the plasma membrane into the extracellular medium by membrane-bound peptidases as part of a receptor shuttling mechanism will be considered.

Molecular Mechanisms in Physical Stress

Q 006 BRAIN CRF AND CRF RECEPTORS IN DEVELOPMENT, Thomas R. Insel, Laboratory of Clinical Science, NIMH, Poolesville, MD 20837.

Studies with several neuropeptides have suggested that activation at critical phases during development may have lasting effects on receptor sensitivity. In a series of studies we have investigated the ontogeny of CRF receptors in brain and we have looked for long-term effects of CRF administration during development in the rat.

Using homogenate binding and *in vitro* receptor autoradiographic techniques (De Souza et al., 1985; 1986), ^{125}I-tyr-O-rCRF specific binding was noted as early as E17, reached a peak at P8, then decreased to adult levels by P21. The distribution of binding shifted from striatum to cortex in the first postnatal week. In spite of the marked density of receptors early in development, CRF potency for generating cAMP was maximal at P14, suggesting that many of the early striatal binding sites were not linked to cyclase.

To evaluate whether CRF has behavioral effects in development, we administered the peptide to rat pups during a 2-minute isolation test. Acute central administration of CRF to rat pups (age 8 days) was associated with decreased ultrasonic calls during social isolation. Acute peripheral administration was associated with increased plasma corticosterone levels but no change in ultrasonic calls during social isolation.

Chronic administration of CRF (10 μg/day SQ) from days 1-7 postnatal resulted in increased resting levels of cort and earlier eye opening in infancy. Adults treated with CRF as pups showed increased open-field exploration, and in preliminary studies, differences in CRF receptor number in pituitary (but not brain).

These studies suggest that CRF and its receptor are functional in the first week of postnatal life and that this peptide may have "organizational" as well as "activational" effects during development. Similar effects have been previously reported with early administration of other neuropeptides as well as sex steroids. These new observations with CRF might provide a model by which stress during development could have long-term consequences on the regulation of the stress response.

Q 007 NEUROENDOCRINE STRESS RESPONSES DURING FETAL AND NEONATAL LIFE. James F. Padbury, Alma Martinez, Siang Thio, Elizabeth Burnell. Perinatal Research Laboratories, Harbor-UCLA Medical Center, Torrance, CA 90509.

Catecholamines (CA) and opiate peptides (OP) are co-stored throughout the sympathoadrenal system (SAS). In sympathetic ganglia, sympathetic neurons and the adrenal medulla, the predominant OP are enkephalins (ENK). ENK are stored as free penta-peptides (methionine enkephalin and leucine enkephalin) and as larger molecular weight forms. OP, including the ENK, have been suggested as neuromodulators of SAS activity but there is little in vivo data on the interaction of these neuroendocrine systems or on co-secretion of ENK peptides and CA. To determine the role of OP as modulators of CA release, we determined the effect of Naloxone (NLX) on CA responses to hypoxia in chronically catheterized fetal sheep (n=11) from 122 to 142 days gestation. Dose response data were generated by random assignment of animals to NLX at 4 dosages (0.1, 0.5, 1.0 and 2.0 mg/kg). Fetuses were made hypoxic (Pa02 13 mmHg) by giving 12-14% 02 to the ewe. Hypoxia increased both Norepinephrine (NE) and Epinephrine (E) in all sheep. CA response to hypoxia was augmented by NLX only at the 0.1 mg/kg dose. Plasma E levels by 20 minutes of hypoxia during the 0.1 mg/kg NLX dose (geometric mean 5366 pg/ml) was greater than with hypoxia alone (997 pg/ml), $p<0.05$ ANOVA. Increased blood pressure and initial reflex bradycardia during hypoxia were observed in all animals. Peak systolic blood pressure with 0.1 mg/kg NLX (114±4 mmHg) was greater than hypoxia alone (102±10 mmHg) but not significantly ($0.05<p<.1$). To determine both the extent of co-secretion of ENK and CA in response to physiologic stimuli and which species of ENK peptides are released, we studied the effect of the same hypoxia model on fetal plasma CA, free ENK and total ENK. Total ENK was defined as ENK immunoreactivity observed after selective proteolytic cleavage of the larger molecular weight forms. Free ENK were measured by radioimmunoassay (RIA). Total ENK was measured by RIA after sequential enzymatic digestion of plasma with trypsin and carboxypeptidase B. The baseline free ENK (M±SEM) level did not change during hypoxia (228±72 pg/ml). Total ENK increased by an average of 300% (range 78% to 804%) from baseline (7331± 2600 pg/ml). Hypoxia increased both plasma E and NE ($p<.05$). There was a significant positive correlation between total ENK and E during hypoxia ($r=0.763$, $p<0.001$) but not NE. Conclusions: 1) The fetal plasma E response to hypoxia was augmented by NLX, 2) The dose dependent effect of NLX suggests mediation by mu opiate receptors, 3) Both plasma CA and a high molecular weight form of ENK are increased during hypoxia, 4) There is a significant correlation between total ENK and E responses. Speculation: ENK are co-stored and co-secreted in adrenergic but not noradrenergic cells and exert a paracrine limit on E release. Post-translational processing of ENK occurs largely after secretion.

Q 008 ENZYMATIC AND MORPHOLOGICAL ADAPTATION TO PHYSICAL EXERCISE IN YOUNG, MIDDLE AGED AND OLD MICE: A MODEL FOR PHYSICAL STRESS, A.Z. Reznick*, E. Steinhagen-Thiessen**, M. Silbermann*, and D. Gershon***, *The Rappaport Institute for Research in the Medical Sciences, Technion, Haifa; **Medical Clinic, Eppendorf University Hospital of Hamburg, W. Germany and ***Dept. of Biology, Technion, Haifa, Israel.

Old age has been shown to be involved in reduced enzyme activity. Enzmyes involved in energy providing systems, such as creatine phospho -kinase (CPK) and aldolase (Ald) as well as superoxide dismutase (SOD) a protective enzyme against damaging active oxygen species, were reported to undergo significant change in senescent animals. The response of muscular tissues to stress of physical exercise via adjust -ment of enzyme levels was studied in CFW-1 and C57BL/6 mice ranging from young to old age. Physical exercise was enforced with the aid of a motor-driven rotating wheel for either 5 or 10 weeks. Cardiac and hindleg muscles were obtained weekly and were assayed for CPK, Ald and SOD. Control trained animals showed a 15-40% decline in enzyme specific activity as a function of age. Muscles of young animals showed an increase of 20-50% in the activity of the above enzymes after the training program. By contrast, muscles of 27-month-old animals undergoing a similar exercise regimen exhibited a 10-40% decline in enzyme activity. These findings were followed by morpho- logical changes indicative of muscular atrophy and degeneration. The latter were manifested by a marked proliferation of fibroblasts, the accumulation of an atypical adipose tissue and an increased number of macrophages. By and large, middle-aged animals responded in a similar fashion to the young ones. Hence, old animals did not appear to be able to cope with physical stress as did young animals. When mice were trained for longer periods of time (12-14 months) beginning at either young or middle-age, the levels of the above enzymes were elevated in a fashion similar to that obtained in young mice trained for short and/or long periods of time. These results indicate that if training is started prior to a critical physiological threshold, it may encompass beneficial effects on enzyme activity and thereby mitigate the aging effect upon the activity of CPK, Ald and SOD.

Q 009 CATECHOLAMINES AND STRESS IN THE NEWBORN, Theodore A. Slotkin and Frederic J. Seidler, Department of Pharmacology, Duke Univ. Med. Ctr., Durham, NC 27710. Catecholamines released into the fetal and neonatal circulation play key roles in the adaptation of the organism to extrauterine life and in subsequent control of tissue development. Circulating catecholamine levels rise toward the end of gestation, culminating in a surge at parturition in which levels may exceed 50 to 200 nmols per liter. In comparison, severe stress in an adult rarely achieves one-tenth this amount. The surge is derived primarily from the adrenal medulla and the release of catecholamines is triggered, in part, by the hypoxia associated with birth. The immature adrenal medulla possesses an unique catecholamine secretory mechanism which does not require centrally-derived neuronal stimulation of the tissue; in contrast, adrenomedullary catecholamine release in adulthood requires an intact nerve supply. The catecholamines released during hypoxia play a vital role in permitting the neonate to survive stress: adrenalectomy or pharmacologic blockade of adrenergic receptors result in complete loss of the ability to withstand low oxygen conditions. The primary actions appear to be on respiratory and cardiovascular function; surfactant release is evoked by activation of β-adrenergic receptors, and cardiac conduction is maintained in neonatal hypoxia through stimulation of α-receptors, which exist in high concentration in the neonatal heart. Both the non-neurogenic secretory capability of the neonatal adrenal and the unusual response patterns disappear concurrently with the development of innervation of the tissues. Accordingly, factors which alter the development of innervation (drugs, maternal stress, malnutrition, endocrine status) compromise the ability of the organism to withstand hypoxia, conditions which may play a role in Sudden Infant Death Syndrome.

Catecholamines derived from sympathetic neurons are important for development of peripheral tissues such as heart, kidney and lung. Low tonic activity early in neonatal life provides a positive trophic influence on cell acquisition in sympathetic target tissues. In addition, a second period of unusual sensitivity to catecholamines occurs well into the postnatal period, associated with transient sympathetic hyperactivity. At this time, catecholamines are programming the future development of receptors, end-organ sensitivity to neuronal stimulation and differentiation of sympathetically-innervated tissues. This second phase of catecholaminergic activity also is associated with hypersensitivity of the organism to stress. Again, perturbations in the development of innervation and/or onset of CNS regulation of sympathetic activity can result in permanent anomalies of structure and function of the nerve pathways themselves and of the tissues they innervate. Such alterations may be responsible for subsequent hypo- or hyperreactivity to stress.

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Psychological Instigation of Stress

Q 010 INFORMATION INDUCED STRESS IN HUMANS, Shlomo Breznitz, R.D.Wolfe Centre for Study of Psychological Stress, University of Haifa, Haifa, 31999 Israel. In a series of laboratory and field experiments, subjects were exposed to painful and/or exhausting tasks. Information about the anticipated duration of the tasks was controlled. The main experimental variations consisted of four types: No Information, Full Information, Encouraging Information followed by Disappointment, and Discouraging Information followed by Encouragement. Endurance, as well as autonomic and hormonal indicators were significantly affected by information.

Q 011 AFFECT STATE-DEPENDENT ACCESS TO ARCHIVAL MEMORY, Herbert Weingartner, George Washington Univ., Washington, DC.

Changes in affective states are associated with alterations in various psychobiologically distinct types of cognitive operations. Some of these cognitive changes are expressed as alterations in information processing, storage and retrieval processes that reqire cognitive capacity and effort. Affective states, like many psychoactive drugs, also serve as discriminative stimuli, which serve as a context biasing the types of recent and biographical events that are likely to be retrieved from memory. This, in turn, determines how we percieve ongoing events and how those events are encoded and organized in memory.

Adrenergic Mechanisms in Response to Stress

Q 012 ADRENERGIC RESPONSES FOLLOWING RECOGNITION OF STRESS, Irwin J. Kopin, NINCDS, National Institutes of Health, Bethesda, Maryland, 20892.

The responses of an organism to stressors which are perceived as threats to the preservation of conditions essential for life are mediated by a complex hierarchal network of neuroendocrine systems. The initial manifestations of such stress responses are usually mediated by the sympathoadrenal medullary system and include stimulus-specific responses targetted to correct or compensate for the disturbance.

Sympathoadrenal medullary activity is the efferent limb of many homeostatic reflexive responses, but these reflexes are modulated or overridden by afferents from higher brain regions to alter bodily functions in anticipation of physiological requirements or with emotional state. Failure of homeostasis or severe stress elicit more generalized responses which include physiological changes which do not enhance homeostasis and may indeed be harmful.

Because catecholamines from the adrenal are released directly into the circulation, plasma epinephrine levels reflect adrenal medullary activity. Although activation of sympathetic nerve terminals results in release of norepinephrine (NE), inactivation of NE is mainly by reuptake into the neurone and differences in regional patterns of responses, blood flow changes, and anatomical factors influence how much of the released NE reaches the systemic circulation. Thus plasma NE levels may not reflect changes in sympathetic neuronal activity. Furthermore, alterations in receptors and the mechanisms they activate may influence responses Alternative methods for assessing sympathetic neuronal responses from NE metabolites and by examining receptors are being studied.

Adrenal Gland-Target Tissue of Stress

Q 013 ASCORBIC ACID REGULATES BIOSYNTHESIS OF THE STRESS HORMONE NOREPINEPHRINE IN SITU, Mark Levine, William Hartzell, Kuldeep Dhariwal, Philip Washko, Digestive Diseases Branch, NIH, Bethesda MD. 20892. Ascorbic acid regulates dopamine beta-monooxygenase activity and therefore synthesis of the product norepinephrine in chromaffin tissue from bovine adrenal medulla. However, the mechanism in situ is more complex than for the isolated enzyme dopamine beta-monooxygenase. In chromaffin cells, the enzyme is localized within chromaffin granules. By contrast, ascorbic acid in chromaffin cells is found both inside chromaffin granules and in cytosol in millimolar concentration. Furthermore, ascorbic acid does not enter isolated granules, and enters granules in cells very slowly. We have therefore tested the roles of intragranular and extragranular ascorbic acid as regulators of norepinephrine biosynthesis in isolated bovine chromaffin granules. When the substrate dopamine required Mg-ATP for uptake into granules, extragranular ascorbic acid increased intragranular norepinephrine formation with a Km of 240 uM. Under these conditions there was a relatively low concentration of intragranular dopamine. Therefore, we also used conditions where the amount of intragranular dopamine available to the enzyme was much higher. Under these conditions, dopamine uptake occurred by diffusion, independent of Mg-ATP. The Km of norepinephrine formation for ascorbic acid was 370 uM. However, even though dopamine uptake was Mg-ATP independent, norepinephrine biosynthesis still specifically required Mg-ATP as well as ascorbic acid. The Km of norepinephrine formation for Mg-ATP was 130 uM. Mg-ATP did not have a direct action on dopamine beta-monooxygenase to increase norepinephrine biosynthesis, nor did Mg-ATP increase biosynthesis by maintenance of a membrane potential. The specific mechanism by which extragranular Mg-ATP and ascorbic acid synergistically increased intragranular norepinephrine formation is not yet fully understood. In addition to extragranular ascorbic acid, we demonstrated that intragranular ascorbic acid was required for norepinephrine biosynthesis. All of these data indicate that norepinephrine biosynthesis in situ may be regulated concurrently by two distinct intracellular pools of ascorbic acid, and by Mg-ATP. These findings have direct implications for predicting how much ascorbic acid is required in cells for maximal norepinephrine synthesis.

Q 014 ENDOGENOUS NEUROPEPTIDES MAINTAIN ADRENAL CATECHOLAMINE OUTPUT DURING STRESS, Bruce G. Livett, Zhou Xinfu, Zeinab Khalil and Philip D. Marley. Department of Biochemistry, University of Melbourne, Parkville, Victoria, 3052, Australia.
A classical autonomic response to stress is the increased output of catecholamines from the adrenal gland. This is brought about by an increased autonomic outflow in the splanchnic nerves increasing cholinergic activation of the adrenal medulla. In species such as the rat and cow, this is principally or exclusively a nicotinic response. Numerous studies in-vitro with perfused bovine adrenals and isolated bovine chromaffin cells have shown that this nicotinic response exhibits desensitization particularly to high concentrations of acetylcholine (ACh). Given the marked sensitivity of the nicotinic response to agonist-induced desensitization in-vitro, it is surprising that the adrenal maintains a high output of catecholamines following sustained electrical stimulation in-vitro and in response to stress in-vivo. A possible mechanism for this maintained response is suggested from studies in-vitro : chromaffin cells incubated first in ACh or nicotine at concentrations that greatly desensitized the response to a subsequent EC_{50} concentration of agonist, showed no desensitization at all if substance P (SP,1-10uM) was also present in the first incubation (1). It was subsequently shown in-vivo that if SP was depleted from the splanchnic nerve of the rat by neonatal capsaicin treatment, the adrenal could not maintain its neurogenic output of catecholamines in response to stress (2,3). Three stessors have been studied : insulin (i.v.), cold and histamine (i.v.) With all three, the neurogenic component of adrenal catecholamine release (2,3) was abolished in the capsaicin pre-treated animals. The recently discovered mammalian tachykinins neurokinin A and neurokinin B are some 100 fold less active than SP at inhibiting the nicotinic response and are approximately 30 fold less potent than SP at protecting against nicotinic desensitization. It is of interest however that all three tachykinins tested were more active at protecting against nicotinic desensitization than at inhibiting the nicotinic response. This suggests that they control catecholamine secretion by the novel action of modulating nicotinic receptor desensitization. We conclude that SP-containing (capsaicin-sensitive) components of the splanchnic nerves in the rat are essential for maintaining the output of adrenal catecholamines under conditions of stress.

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Q 015 GLUCOCORTICOIDS AND STRESS. Allan Munck and Paul M. Guyre, Department of Physiology, Dartmouth Medical School, Hanover, NH 03756.

Increased glucocorticoid secretion is a well-known component of stress responses. The resulting elevated levels of glucocorticoids are generally thought to be necessary to protect the organism against stress. How glucocorticoids protect has remained a mystery, however. A common view has been that they stimulate the body's defense mechanisms. While some defense mechanisms may indeed require basal or maintenance levels of glucocorticoids in order to function, evidence accumulated over many decades indicates that high glucocorticoid levels usually suppress defense mechanisms. The antiinflammatory actions of glucocorticoids, discovered forty years ago, were a dramatic manifestation of such suppression. These actions were then classified as pharmacological, partly because they appeared to lack a physiological basis.

An alternative view of the physiological role of glucocorticoids in stress is that they protect not against the source of stress itself but against the organism's own defense mechanisms, preventing them from overshooting and themselves causing damage. That view not only provides a physiological basis for antiinflammatory and related immunosuppressive actions, but is also consistent with other well-established roles of glucocorticoids, such as that of counterhormones to insulin in regulation of blood glucose levels.

At the cellular level many of the suppressive actions of glucocorticoids appear to be effected through inhibition of the activity or production of intercellular mediators, including eicosanoids and cytokines such as IFN-γ, IL-1, IL-2 that regulate immune and inflammatory responses, and other hormones and neuropeptides such as ADH, CRH, ACTH,β-endorphin and insulin. Regulation by glucocorticoids of some these mediators has been shown to be via inhibition of transcription of their mRNAs, and of others via induction of inhibitors of their synthesis or activity. Through networks of such intercellular mediators the effects of glucocorticoids can be propagated to cells and systems that are not primary targets of the hormones.

Psychoimmunology

Q 017 CELLULAR AND MOLECULAR CONSEQUENCES OF PSYCHOLOGICAL STRESS, Ronald Glaser, L. David Tomei, Susan Kennedy, and Janice Kiecolt-Glaser, The Ohio State University Medical Center and Comprehensive Cancer Center, Columbus, Ohio 43210.

Data from a number of laboratories suggest that various psychological stressors may modulate the immune response in humans. Although various stressors are thought to affect the development and course of infectious and malignant disease, presumably through the impact on the immune response, research with human subjects is still relatively limited. There is, however, good evidence that major life changes can have adverse immunological consequences. For example, one of the earlier human studies showed that bereaved spouses had a poorer proliferative response to mitogen stimulation two to six weeks after the spouse's death than non-bereaved controls. Data from our laboratory have documented an association between academic stress and a variety of immunological parameters, including re-activation of latent Epstein-Barr virus, decrements in the percentages of cell populations of lymphocytes including helper T-lymphocytes and natural killer (NK) cells, as well as a decrease in NK cell lysis. We have also demonstrated the down-regulation of lymphokine production as measured by the ability of concanavalin A stimulated peripheral blood lymphocytes (PBLs) to synthesize γ interferon. We have expanded our studies to examine other cell functions that might be affected by psychological stress. We have demonstrated that psychological stress can have an impact at the molecular level, as demonstrated in decrements of DNA repair in lymphocytes obtained from psychiatric patients, as well as the synthesis of an enzyme associated with DNA repair (O6-methyl transferase) in rats. There is very good evidence in the literature that faulty repair of damaged DNA leads to an increased incidence of cancer, and even small deviations from normal DNA repair levels may have important longer term consequences for cancer. We now have preliminary data that suggests that programmed cell suicide (apoptosis) may also be affected by psychological stress. These studies suggest that psychological stress could lead to progressive accumulation of errors within the genome of a variety of cell types, particularly of the immune system, thereby reducing immune competence and leading to increased risk of environmental associated cancer as well as infectious diseases.

P 018 IMMUNOLOGICAL CONSEQUENCES OF STRESS, Nicholas R.S. Hall, Maureen P. O'Grady, Robert C. Steiner, Adrian J. Dunn, George Clark and Allan L. Goldstein. Department of Biochemistry, George Washington University, Washington DC 20037.

The impact of stress upon the immune system has been evaluated using both rodent and human models. In the animal studies, we have used exposure to viral infection in neonatal and adult animals as a stress inducing protocol. Use of this model is correlated with elevated corticosterone production and a reduction in hypothalamic levels of norepinephrine that are almost indistinguishable from those changes that have been reported following exposure of animals to electric foot shock or restraint stress. Correlated with these changes are reductions in a number of immune system measures including mitogen responsiveness as well as thymus weight. Pre- or neonatal exposure of rodents to viral infection or to immune system products produced in response to the viral antigen have been found to result in changes in immune system measures that are more pronounced in females than in males when the animals attain adulthood. In a separate line of inquiry, we have found that a partially purified extract of bovine thymic tissue - thymosin fraction 5 - is able to ameliorate certain measures of stress induced immunosuppression.

Using a human model system, we have found that measures of health and immunity are impaired in individuals who exhibit osteologic evidence of early exposure to stress-inducing events. Vertebral canal diameter has previously been found to be correlated with age of death. This permanent biological marker is adversely affected by some of the same hormonal changes that have been implicated in impairing the immune system. In a recently completed survey, a number of immunologic measures have been correlated with this early marker of stress-exposure. In summary, exposure to viral infection represents an event that parallels more traditional models of stress in both its neuroendocrine and immunologic consequences. Furthermore, exposure to such a stress during early development can have measurable consequences upon the adult immune system.

Q 019 STRESS AND IMMUNOLOGY, Marvin Stein, M.D., Mount Sinai School of Medicine, New York, New York 10029.

Considerable evidence demonstrating a relationship between stress and immune function is accumulating, and a complex chain of psychologic and biologic processes are involved. This presentation will review experimental and clinical studies concerned with the influence of stress effects on immunity as well as central nervous system and endocrine processes which may be involved. Attention will be directed to the issues of specificity and homeostasis.

Model Systems and Methods

Q 020 CONTRIBUTION OF THE MOTHER TO INFANT IMMUNITY IN NONHUMAN PRIMATES, Christopher L. Coe, William B. Ershler, Christine M. Erickson, Ralph M. Albrecht and Gabriele Lubach, Departments of Psychology, Medicine and Veterinary Science, University of Wisconsin, Madison, WI 53706.

Our research on nonhuman primates has shown that infancy may be a particularly sensitive period for evaluating the impact of stress on the immune system. At this point in the life span, social interactions with the mother play a essential role in ensuring normal development and in buffering the infant against environmental perturbations. The importance of the mother becomes immediately evident following disruption of the mother-infant relationship or through study of abnormal rearing environments. We will describe a wide range of immunological alterations which occur in primate infants following separation from the mother. Changes in humoral and cellular immunity have been observed (e.g., antibody responses, hemolytic complement activity, macrophage chemiluminescence and superoxide production, delayed hypersensitivity, and lymphocyte proliferation responses). The shift in macrophage activity may be particularly important because it persists for more than one month after the period of psychoendocrine activation. By varying psychological aspects of the separation environment, we have also been able to evaluate how different levels of psychoendocrine activation selectively affect immune responses. Other studies have focused on the immunological consequences of nursery rearing and early weaning of infants from the mother. When monkeys are examined at one year of age, it is possible to show significant effects of the prior rearing environment on lymphocyte proliferation responses. We will also describe how prenatal events relating to the transplacental transfer of maternal antibody can have a prolonged effect on the immunological development of the infant. Collectively, the studies indicate that stressful experiences in infancy can have long-lasting effects on immune responses and, further, that the immunobiology of the mother-infant relationship provides a unique model for stress research.

Q 021 EFFECTS OF ANXIETY ON REGIONAL CEREBRAL BLOOD FLOW AND METABOLISM: RELATIONSHIPS WITH COGNITIVE PERFORMANCE, Ruben C. Gur, The Brain Behavior Laboratory, Departments of Psychiatry and Neurology, University of Pennsylvania, Philadelphia, Pennsylvania 19104.

The effect of anxiety on human performance is likely mediated by its effect on regional brain activity, yet there is limited availability of data on how anxiety affects brain function and how levels of brain activity may influence performance on cognitive tasks. A series of studies will be reported in which these relationships have been examined using neuroimaging techniques for quantitation of regional cerebral blood flow and metabolism. A curvilinear "inverted-U" relationship was found between anxiety and cortical activity. This relationship could be demonstrated in subjects performing cognitive tasks, and it corresponded to the relationship between anxiety and task performance. These findings may suggest a neural mechanism underlying the operation of the so-called Yerkes-Dodson postulate of a curvilinear relationship between "strength of stimulus" and "rapidity of habit-formation". It is proposed that if there is an adaptive role to this reduction in cortical activity, it would be in response to shifting control of behavior to subcortical regions more important in fight-or-flight situations.

Q 022 PSYCHOBIOLOGY OF MOTHER-INFANT RELATIONSHIPS, Seymour Levine and Sandra G. Wiener, Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, Stanford, CA 94305.

A series of studies will be presented which have examined the physiological and behavioral responses of nonhuman primates, squirrel monkeys and rhesus macaques, following disruptions of mother-infant relationships. These studies have demonstrated that reliable increases in circulating levels of plasma cortisol and of increases in the monoamine metabolite of norepinephrine, MHPG, found in CSF, occur following separation of the infant from its mother. The presence of familiar conspecifics during the time of separation reduces both physiological responses compared to those elicited by total isolation. Visual access to the mother during separation also ameliorates the biological responses measured. However, when separated in the presence of unfamiliar conspecifics, the physiological responses are exaggerated when compared to animals which are totally isolated. The behavior expressed by the infant during separation, particularly separation-induced vocalizations, are not concordant with these physiological indices of affect. The rate of vocalization produced when the infant has visual access to the mother was higher than when the infant was totally isolated. However, when allowed access to familiar conspecifics, the rate of vocalization was lower than during total isolation, with no vocalization produced while the separated infant was in the unfamiliar social group. The curvilinear relationship between vocalization and the physiological indices of arousal has led to a revision of the traditional concept that separation-induced infant vocalization is reflective of distress. Instead, these data support a hypothesis that vocalizations may serve as a coping response that reduces the physiological indices of arousal. However, social interaction with familiar conspecifics may involve a non-vocal coping response (e.g., proximity contact to other monkeys) which reduces both the behavioral and physiological responses to maternal separation.

Q 023 MECHANISMS OF GASTRIC AND DUODENAL EROSION IN THE RAT. Herbert Weiner, Dept. of Psychiatry & Biobehavioral Science, University of California, Los Angeles, CA 90024.

Various stressors (cold restraint, electric shock, etc) applied to rats increase gastric contractility and are associated with gastric erosions. Intracisternal (IC) thyrotropin releasing hormone (TRH) increases contractility, gastric acid secretion and the incidence of erosion formation. Corticotropin releasing hormone (CRH) is released by stress, and acting centrally produces autonomic and endocrine changes. We have studied the role of IC CRH on gastric contractility in anesthetized rats (N=6). Contractility was measured by extraluminal force transducers sutured to the gastric corpus. Frequency, amplitude of contractions and motility index were analyzed by computer. Baseline measures, after recovery from surgery were obtained in 24 hr fasted rats. Contractility was stimulated by IV carbachol (100 mg/kg/hr) or IC injection of the TRH analog, RX 77368. Contractility thus induced was inhibited by IV atropine (1 mg/kg). IC CRH (30-1000 ng)produced a dose-dependent suppression of RX 77368 ($p < 0.05$) but had no effect on that induced by IV carbachol; saline IC had none either. IV CRH was 1/10th as potent in suppressing contractions produced by IC RX 77368. Diminution of gastric contractions after IC CRH (1 mg) occurred within 5 minutes of administration, and lasted for at least 60 minutes. These data show that IC CRH injection acts centrally to inhibit gastric contractions stimulated centrally (i.e. by IC RX 77368) but not peripherally (i.e. by carbachol), and by inference reduces the risk of erosion formation induced by some stressors.

Neuropeptides

Q 100 PROPRANOLOL ANTAGONIZES THE ENHANCED CONDITIONED FEAR PRODUCED BY CRF. Belinda J. Cole and George F. Koob. Scripps Clinic and Research Foundation. La Jolla. CA 92037.

Both corticotropin releasing factor (CRF) and activation of the norepinephrine (NE) containing nucleus locus coeruleus (LC) induce behaviors normally exhibited in threatening situations. Furthermore, CRF has been shown to activate NE neurones in the LC. The purpose of the present series of experiments was to investigate the role of NE in the enhanced fear (as measured in the conditioned emotional response paradigm) and behavioral activation (as measured by locomotor activity in photocell cages) induced by CRF, using the beta-adrenoceptor antagonist propranolol. d,l, Propranolol itself was found not to affect performance of a conditioned emotional response (CER), but it did antagonize the enhanced conditioned fear produced by CRF. This antagonism was shown to be specific to l, and not d, propranolol, suggesting that it does not result from non-specific effects. In contrast, propranolol was shown to potentiate the locomotor hyperactivity induced by CRF. These results suggest that activation of beta-adrenoceptors may be an important mechanism in the 'anxiogenic' effects of CRF. In addition, they suggest that the neurochemical mechanisms that underlie the 'anxiogenic' and the 'activating' behavioral effects of CRF are neuropharmacologically distinct.

Q 101 EFFECT OF ENDOGENEOUS OPIOIDS ON THYMIC ENDOCRINE FUNCTION, M. Dardenne, W. Savino,M.Cl. Gagnerault and J.F. Bach. INSERM U 25 - Hôpital Necker - Paris - France.

Cumulative data now strongly demonstrate that several neuropeptides including endogenous opioids can have immunomodulatory functions. Most of the studies so far carried out focused on the action of these substances directly on lymphocytes. We decided to investigate whether or not thymic epithelial cells (TEC) - the major component of the thymic microenvironment - could also be modulated by endogeneous opioids.

We approached this problem by subjecting primary cultures of human TEC lines to several opioids (α - β - or γ - endorphins, (as well as Met- or Leu-enkephalins) applied in concentrations ranging from 10^{-6} to 10^{-9} M. On the following days we measured the levels of thymulin (a chemically-defined thymic hormone known to stimulate some steps of T-cell differentiation) in the culture supernatants, as well as the numbers of thymulin containing cells, evaluated by immunofluorescence with an anti-thymulin monoclonal antibody.

After treatment of TEC cultures with β-endorphin or Leu-enkephalin we detected a significant increase in the level of thymulin in the culture media, paralleled by an augmentation in the percentage of thymulin containing cells. In addition, the intensity of this stimulatory effect was correlated with the dose applied to the cells. Preincubation of the opioids with the specific antibodies abrogated the opioid-induced stimulatory effect on TEC. Importantly, no effect on thymulin production was observed with the other opioids used, whatever the dose. These results suggest that, at least in vitro, β-endorphin and Leu-enkephalin stimulate the hormonal function of the thymic epithelium . These findings suggest the concept that the modulatory role of endogenous opioids on the immune system is not restricted to lymphocytes but can also take place at the level of cells belonging to T-cell differentiating microenvironments.

Q 102 PERIPHERAL STIMULATORY FACTORS ARE INVOLVED IN ACTH RELEASE DURING STRESS IN RATS. D.Jezova, R. Kvetnansky, F.J.H. Tilders * , G.B. Makara**

Ins. Exp. Endocrinology, CPS SAS, Bratislava, Czechoslovakia, *Dpt. Pharmacol., Free Univ., Amsterdam, The Netherlands and **Inst. Exp . Medicine, Hungarian Acad. Sci., Budapest, Hungary

The ACTH levels in plasma increase during stress even if the dominant regulatory pathways via CRF and AVP have been interrupted. The mechanisms involved in this small residual response of ACTH during immobilization stress in rats were investigated. Possible stimulation of ACTH release by circulating catecholamines was excluded. The immunoneutralization of CRF using both a polyclonal or a monoclonal antibody significantly, though not completely inhibited ACTH response to stress in rats with knife lesions of the medial basal hypothalamus. These results indicate that peripheral CRF activity and other yet unidentified peripheral factors contribute to the rise in plasma ACTH during stress.

Q 103 REGULATION OF T CELLS BY DENDRITIC CELLS. David R Katz, Angela Brennan and Philip King, Pathology Department, University College & Middlesex School of Medicine, LONDON W1P 8AA, United Kingdom.

Previous studies which examine dendritic cell (DC) regulation of T cell function have emphasised the role of class II MHC determinants on the surface of these cells, and that DC do not process antigen via a conventional phagolysosome pathway, but the precise mechanism of DC function has not been clarified.

We have been investigating the cellular mechanism of DC function using DC isolated from human tonsillar tissue. These cells have typical morphology and phenotype and are potent inducers in both syngeneic and mitogen responses. Using periodate-pulsed T cells in an oxidative mitogenesis assay, we have shown that both anti-class I and anti-class II MHC antibodies inhibit the response. A panel of monoclonal antibodies against CD4, CD11a and CD11c determinants inhibit the response. Antibodies against CD11b and CD14 are not inhibitory. Neither anti-IL-1α nor anti-IL-1β have an inhibitory effect, despite the fact that both antisera bind to the surface of the DC. Exogenous recombinant IL-1 will augment the response, but the same concentration has no effect on the periodate pulsed cells alone.

Taken in conjunction with our previous study, which showed that DC express receptors for the monocyte product dihydroxycholecalciferol (1,25DHCC) (the active metabolite of vitamin D3) our results add further support to the hypothesis (i) that synergy between DC and macrophages is due to DC binding of macrophage derived mediators rather than to uptake of processed fragments of antigen; and (ii) that the differential role of the these two types of presenting cell is a function of whether or not an associated inflammatory mediator is required in the particular immune induction process.

Q 104 NEUROPHARMACOLOGY OF NEONATAL RAT VOCALIZATIONS, P. Kehoe, Trinity College, Hartford, CT 06040. Mammalian infant separation from parents and siblings evokes a quantifiable emotional response in rats, ultrasonic vocalizations. Individual isolation of the rat pup seems to be a severe biosocial stress as judged by these ultrasounds which are a potential stimulus for maternal retrieval. The neuropharmacology of these cries reveal interesting and complex data. Opiates and opiate antagonists modulate neonatal rat's vocalizations and response to nociception. Morphine decreases vocalizations and produces analgesia while naltrexone increases crying and reverses isolation-induced analgesia. Neonatal cries and analgesia are both obviously influenced by opioids, suggesting that the level of distress calls in isolated pups is reduced by an endogenous opioid peptide release. Moreover, cocaine, a dopamine stimulant, in doses from 2.5- 20 mg/kg causes a significant reduction in crying of the isolated pup while producing hyperactivity. This quieting or hyperactive effect of cocaine is not reversed with naltrexone (.5-2mg/kg). In contrast to the calming effects of morphine and cocaine (drugs which promote positive associations in pups), the noradrenergic alpha-2 receptor agonist, clonidine, produces a dose-dependent high level of calling and extreme hyperactivity in pups up to 15 days of age, while Day 18 pups were quieted by the same doses. More importantly, these ultrasonic vocalizations were emitted in the nest situation as well as isolation, suggesting a noradrenergic mechanism for initiation of crying.

Q 105 INTERACTION OF CHRONIC STRESS WITH SALINE LOAD ON CIRCULATING NEUROPEPTIDE Y AND CATECHOLAMINES. EVIDENCE FOR A DIFFERENTIAL LONG-TERM REGULATION, Pierre Mormède, Vincent Castagné, Roger Corder and Rolf Gaillard, INRA-INSERM U259, Bordeaux, France (PM, VC) and Clinique Médicale, Hôpital Cantonal Universitaire, Geneva, Switzerland (RC and RG).

Neuropeptide tyrosine (NPY) is a vasoconstrictor peptide present in noradrenergic nerves and coreleased with noradrenaline in acute stress situations (Castagné et al, Regul Pep 19: 55, 1987). The present experiments were designed to evaluate the effect of chronic stress and saline overload, two treatments known to induce hypertension, on NPY and catecholamine plasma levels. Male rats were unilaterally nephrectomized and received ad libitum saline (0.9%) as drinking water. Controls received tap water. One half of the animals were restrained in a plastic mesh for 1 hour every day at the beginning of the dark phase of the cycle. After 12 days of these treatments, rats were equipped with an indwelling jugular catheter and 24 hr later blood was collected in undisturbed animals , after gentle handling and after a 20-min electric shock session. Although both chronic stress and saline load reduced NPY levels (by 29 and 22% respectively), their association increased plasma NPY by 29%. This was true in basal and acute stress conditions. A completely different picture was found for noradrenaline plasma levels: they were increased by chronic stress in basal condition (+58%) and in response to shock (+45%) but saline load had no effect.

These results show that chronic stress and saline load interact to increase circulating levels of neuropeptide Y which may play a role in the genesis of hypertension. Furthermore, the long-term influence of environmental factors on NPY and CA appears to be completely different, although they are colocalized in the same neurons.

Q 106 CRF LEVELS IN HYPOPHYSIAL PORTAL BLOOD OF CONSCIOUS, UNRESTRAINED CASTRATED RAMS

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Hypophysial portal and peripheral blood were obtained from conscious, unrestrained castrated rams to measure simultaneously the secretion of CRF, LH-RH, ACTH, Cortisol and LH. The cannulas for portal blood collection were surgically implanted through a transnasal transphenoidal approach 5 days before the collection. Portal and peripheral blood were collected simultaneously at 10- min intervals for 8-9 h. Peripheral ACTH and Cortisol levels were within the basal range and increased sharply after an hemorrhagic stress (acute 15% blood volume depletion). CRF was detectable in all portal samples, displayed small pulsatile variations under basal conditions and showed a large increase after hemorrhage. The pulsatile portal LH-RH and peripheral LH secretion were unaltered after hemorrhagic stress.
In conclusion, these data provide the first direct information on CRF secretion in portal blood of conscious, unrestrained animals. They indicate that CRF-induced release by hemorrhage has no effect on LH-RH and LH secretion.

Q 107 EFFECTS OF STRESS INTENSITY AND MODALITY ON ADRENAL CATECHOLAMINE METABOLISM AND HEMODYNAMIC VARIABLES: ROLE OF OPIOID RECEPTORS, H.M. Rhee and D. Hendrix, Dept of Pharmacology, Oral Roberts University School of Medicine, Tulsa, OK 74137,
Previous study indicates that the biosynthesis of adrenal catecholamines is effected primarily by neuronal and humoral factors. Denervation of splanchnic nerve reduced the activity of catecholamine synthesizing enzymes. Stressful stimuli or reserpine treatment also attenuated the activity of adrenal catecholamine synthesis. The main purpose of this paper was to test 1) the effect of several types of stress on adrenal catecholamine levels and cardiovascular parameters; 2) the effect of typical opioid receptor agonists and/or antagonists on adrenal level of methionine enkephalin. Young male Sprague-Dawley rats were anesthetized for the chronic cannulation of carotid artery and jugular vein. After 3 days recovery period from the surgery the rats were subjected to either immobilization, heat stress or swimming exercise for various durations. The heat stress under red light (35°C) for 5 min, three times with 5 min interval increased arterial blood pressure from 109.5+/- 2.5 (N=11) to 152.3 +/- 5.6 (N=11) mmHg. Similar alterations in mean and diastolic pressures and heart rate were also noted after the specific stress. Immobilization of the rats in small chambers for 60 min increased significantly blood pressure (109.5 +/- 2.5 vs. 125.9 +/- 2.3 mmHg). The identical immobilization stress 10 min after naloxone (2 mg/kg, I.P.) elevated significantly ($p < 0.02$) blood pressure (125.9 +/- 2.3 vs. 133.9, DF=20). Although treatment of opioid receptor agonists such as DAGO or DADLE produced variable results depending on stresses, the data suggest that opioid receptors in the central and/or in periphery modulate cardiovascular and adrenergic performance in normal as well as stressful conditions.

Q 108 ACTH RESPONSE TO STIMULATION WITH VASOPRESSIN AND CRF GIVEN CONCURRENTLY TO NORMAL SUBJECTS. Alan G. Robinson, Michelle M. Roberts and David B. Jarrett, Departments of Medicine and Psychiatry, University of Pittsburgh.
The diurnal rhythm of ACTH and cortisol is well established. Two hypothalamic secretagogues for ACTH which might be responsible for the the diurnal rhythm are vasopressin and CRF. As the greatest release of ACTH in response to vasopressin, is when the vasopressin acts synergistically with CRF, we postulated that the ACTH release in response to intravenously administered vasopressin would depend in large measure on endogenous levels of CRF. Thus, we interpeted the greater release of ACTH when vasopressin was administered in the morning (compared to the evening) as due to a higher level of endogenous CRF in the morning. If the exaggerated release of ACTH in the morning were due to endogenous CRF, we then hypothesized that if AVP and CRF were given together there would be no diurnal difference in response. We, therefore, developed a protocol in which both vasopressin and CRF were administered together using stepwise graded increases in dosages similar to that we reported for vasopressin alone. The doses administered were 0.14 AVP and 0.01 CRF; 0.43 AVP and 0.05 CRF; 1.4 AVP and 0.1 CRF; and, 4.3 AVP and 0.5 CRF (mU/kg and ug/kg, respectively). Each dose pair was administered 20 minutes apart with blood samples obtained every 5 minutes. With this protocol, the release of ACTH at 7 a.m., was greater than at 11 p.m. in 4 of 5 subjects tested. Thus, we found a diurnal variation in response to vasopressin and CRF given together which was similar to that found with vasopressin alone. The data are consistent with the conclusion that some factor(s) other than CRF and/or vasopressin are important in modulating the diurnal release of ACTH and the response of ACTH to administered vasopressin and CRF.

Q 109 GLUCOCORTICOID REGULATION OF CRF RNA LEVELS, Rosalie M. Uht and Jeffrey F. McKelvy, SUNY at Stony Brook, Stony Brook, NY 11794.

Data from Northern analyses and in situ hybridization studies indicate that levels of CRF RNA are increased after adrenalectomy (ADX) and that this increase is attenuated by the administration of glucocorticoids (Jignami et al., Endocrinology 117:1314, 1985; Young et al., Neurosci. Lett.s 70:198, 1986; Kovacs et al, Neuroendo. 46:365, 1987). To evaluate this increase over time, we are measuring hypothalamic CRF RNA levels by Northern analyses at 1, 3, 5, and 7 days after ADX in the rat. Four experimental groups are being studied at each point: normals, sham ADX + placebo, ADX + placebo and ADX + corticosterone (CORT). At sacrifice, hypothalami are frozen and sera are collected for CORT levels. Hypothalamic RNAs are isolated by a guanidine thiocyanate/CsCl method, separated by electrophoresis and electroblotted onto nylon membranes. The blotted RNAs are hybridized to a rat CRF cDNA (gift of Robert Thompson) which is ^{32}P-labeled by random priming. We are currently evaluating methods of quantifying the CRF RNA levels. Our preliminary data suggests that CRF RNA is elevated in the rat hypothalamus by 7 days after ADX and that this increase is attenuated by CORT.

Q 110 CHARACTERIZATION OF HUMAN HISTAMINE H1 RECEPTORS BY MEANS OF A NEW SPECIFIC LIGAND :[^{125}I] IODOBOLPYRAMINE, Françoise Villemain, Lucienne Chatenoud and Jean-François Bach, INSERM U25 - Hôpital Necker - 161, rue de Sèvres 75015 Paris - France.

Histamine is an important immunomodulator and neuromediator. Concerning immune reactions, histamine has been shown to play an important role not only in immediate hypersensitivity reactions but also in various lymphocyte cooperation mechanisms that mediate delayed type hypersensitivity reactions. [^{125}I] Iodobolpyramine is a recently described radioiodinated ligand specific for histamine H1 receptors shown to be a highly sensitive probe to detect histamine H1 receptors in guinea-pig brain. The original molecule that is SK&F 94461, an aminopentyl analogue of mepyramine, acts as a H1 receptors antagonist. Using [^{125}I] Iodobolpyramine on intact peripheral human T lymphocytes, a single specific binding site for this ligand has been identified. This binding is reversible (d-chlorpheniramine at 10^{-6}M molecular concentration has been used as the cold competitor), saturates at 0.5-0.6 nM and the binding equilibrium is achieved after 30 min of incubation at 27°C. [^{125}I] Iodobolpyramine binds with high affinity to the intact human T lymphocytes (Kd = 0.12-0.28nM). By scatchard analysis 2335 ± 1500 specific binding sites/cell have been observed. In competition experiments using a panel of various H1 receptor antagonists (mepyramine, SK&F 94461, d-and l-chlorpheniramine, triprolidine, doxepine) and histamine itself (as an agonist), IC50 values consistent with the expected rank order of potency for interactions with already described H1-receptors have been found. In preliminary experiments the characterization of H1 receptors on the various T cell subpopulations indicates that H1 receptors are present on CD8+ suppressor/cytotoxic T cells in significantly higher proportions (approximately a ten-fold increase) as compared to CD4+ helper/inducer T cells. These results provide evidence for : 1) the role of histamine as an immunomodulator through its direct action on H1 type receptors, 2) quantitative difference in the number of H1 binding sites among functionally distinct human T cell subsets.

Q 111 THE PERIPHERAL BENZODIAZEPINE RECEPTOR AS A REGULATORY SUBUNIT OF THE O2-PRODUCING MOLECULAR COMPLEX IN MACROPHAGES, F.Zavala and B. Descamps-Latscha,INSERM U 25,CNRS UA 122,Hôpital Necker,Paris,France.

We previously demonstrated (F. Zavala and M. Lenfant, ANN.NY.Acad.Sci.1987, 496,24Ø) that :1) macrophages express a high number of benzodiazepine (BZD) receptors specific for peripheral and mixed type molecules; 2) nanomolar concentrations of BZD modulate in vitro the arachidonic acid (AA) induced oxidative burst of macrophages,an effect reversed by the peripheral BZD antagonist PK 11195; 3)in vivo administration of low doses of BZD (1mg/kg) in mice markedly enhance the humoral response to sheep red blood cells.
Using the murine macrophage hybridoma cell line,2C11.12.4, the present study shows that, following the induction of the oxidative responsiveness by a 48h in vitro treatment with LPS or gamma IFN, the affinity of the BZD receptors is consistently decreased on both intact cells and membrane preparations.This specific BZD binding is inhibited by the lipoxygenase inhibitor NDGA (IC5Ø=5µM).Lastly,the capacity of AA to stimulate the O2- production of 2C11.12.4 membranes in the presence of the physiological substrate NADPH is inhibited by BZD.
Altogether these data suggest that the peripheral BZD receptor could be a regulatory subunit of the O2- producing molecular complex in macrophages and a target for the immunomodulating effect of both mixed and peripheral types of BZD.

Stress Psychology and Physiology

Q 200 ALARM CHEMOSIGNALS AND IMMUNE SUPRESSION, R. Cocke and D. Thiessen. Department of Psychology, Univ. of Texas, Austin, Tx. 78712

Alarm chemosignals from stressed rodents are avoided by conspecifics and are attractive to predators. When the alarm odors are presented to conspecifics they produce physiological changes such as increased body temperature, alterations in CNS neurotransmitter levels and opioid analgesia. We recently have found evidence that the presentation of these odors also affect the immune response. The mitogenic stimulation of B- lymphocytes is suppressed in animals which have been exposed to stress odors compared to animals exposed to control odors. Based on these observations, it appears that the aviodance of odors from stressed conspecifics might be advantagous for disease resistance as well as predator avoidance.

Q 201 SHOCK INDUCED IMMUNOSUPPRESSION: NALTREXONE SENSITIVE AND INSENSITIVE PARAMETERS. J.E. Cunnick, D.T. Lysle, A. Armfield and B.S. Rabin. University of Pittsburgh, Pittsburgh, PA 15217.

Stressors can cause a naltrexone reversible suppression of NK activity in rat spleens. We used our previously reported paradigm in which a signaled-footshock suppresses the mitogenic response of whole-blood and spleen lymphocytes. Male Lewis rats were injected with naltrexone or PBS prior to administering the shock. The rats received 16 signaled footshocks over a 64 min session. All experimental and control subjects were assayed for whole-spleen NK activity and whole-blood and spleen responsiveness to non-specific T-cell mitogens. Our results demonstrate a naltrexone-reversible suppression of NK activity. However, stress induced suppression of mitogen responsiveness was not reversible with naltrexone.

Research by others has shown that anxiogenic drugs can cause immunosuppression. Our research has indicated that there may be a psychological component producing some of the immunosuppression induced by a signaled footshock. Therefore we administered diazepam or PBS to experimental and control rats prior to a session of signaled footshock. Our results show that diazepam is unable to reverse the immunosuppression of NK activity or the suppressed mitogenic responsiveness of T cells.

These results indicate that there are different mechanisms inducing immunosuppression of NK cells and T cells in these shock stressed rats.

Q 202 CLASSICAL CONDITIONING OF A SECONDARY IMMUNE RESPONSE, Laura Czajkowski[1*], Ken Hill[1], Carl Cheney[+], John Rose[1*], [1]Salt Lake City VAMC, [*]University of Utah, [+]Utah State University.

The effects of classical conditioning on a secondary immune response to BSA were examined over a 42 day period. Lewis rats were immunized with BSA on day 0, and received antigen boost on day 14. Conditioning occurred on day 14 and consisted of a single pairing of a novel flavor, saccharine, with cyclophosphamide (50 mg/kg) to induce immune suppression. Test treatments consisted of re-exposure to the conditioned stimulus (flavor only). Antibody production was assessed using the Enzyme Linked Immunoassay (ELISA) with an evaluation of both antibody titer and affinity. Taste aversion was demonstrated on days 21 and 28 (Tests 1 and 2) in the treatment group. Decreased titer and affinity were noted in the treatment groups on days 38 and 42. The results demonstrated a conditioned immunosuppression consistant with previous studies. The findings extended the current literature by demonstrating the effect on both antibody titer and affinity in a secondary immune response. Conditioned immunosuppression was only demonstrated after cessation of the taste aversion response. A further conclusion of this research was the effective use of the ELISA to quantify the conditioned immunosuppression.

Q 203 LONG-TERM EFFECTS OF INESCAPABLE STRESS ON DAILY RUNNING ACTIVITY AND ANTAGONISM BY DESIPRAMINE, Paul H. Desan, Lee H. Silbert, and Steven F. Maier, Stanford University, Stanford, CA, 94305, and University of Colorado, Boulder, CO, 80309.

The behavioral consequences of exposure to stressors such as inescapable shock are usually transitory if testing is conducted in an environment different from that in which the stressor was administered. The behaviors tested have generally been motivated by discrete stimuli in the environment (e.g., activity in reaction to shock) or have been part of homeostatic regulatory mechanisms (e.g., eating). Here we investigated the effects of inescapable shock on a behavior that is not so tightly tied to motivating and reinforcing conditions, daily activity in a familiar home cage/running wheel environment. Rats lived in the wheel environment for 44 - 85 days before treatment. Inescapable shock produced only a transient reduction of water intake and body weight, but daily running was depressed for 14 - 42 days (the maximum period studied), depending on the conditions. This long-term effect on activity occurred despite the fact that shock was administered in an environment very different from the animal's home running wheel environment. The activity reduction was reversed by desipramine in a dose dependent fashion. Indeed, the activity of inescapably shocked animals treated with the optimum dose of desipramine exceeded that of control animals undergoing neither stress or drug treatment. The maximum effect of desipramine required 7 days of treatment. Desipramine did not affect the activity of control subjects.

Q 204 **A PROBLEM-SOLVING PROGRAM FOR ADOLESCENT STRESS MANAGEMENT,** **Marianne Frauenknecht and David R. Black, Purdue University, West Lafayette, IN 47907**

New approaches to alleviate detrimental effects of excessive stress in adolescents are sorely needed although programs of this type are currently scarce. The direct and indirect consequences of stress on adolescents are evidenced by the disporportionate increase in morbidity and mortality among teenagers. The negative effects of stress are compounded because many adolescents are ill-equipped to manage excessive stress due to lack of experience, or poorly developed or nonexistent coping skills. Research has shown that problem solving is a coping skill that is deficient among certain adolescents groups such as those who are behaviorally and emotionally disturbed, chemically dependent, or suicidal. Problem-solving training during adolescence is needed to provide skills for stress reduction during a critical period of development. Problem-solving programs can be applied at all levels of prevention to adolescents with identified problems, high-risk groups, or asymptomatic individuals. As a general coping strategy for stress, problem solving can be used to incorporate the selective use of other stress management techniques and to manage various stressors in different situations. Problem solving can also be used as a stress management skill to identify the most salient problem, choose effective options, and initiate and maintain a selected course of action. Rationale is offered for a problem-solving program designed for adolescents in the high school setting, the advantages of a problem-solving program for stress management, a model of problem solving for adolescent stress management, and selected program materials.

Q 205 RELATIONSHIPS AMONG PSYCHOLOGICAL AND HORMONAL VARIABLES ASSOCIATED WITH A WRITTEN EXAMINATION. G.A. Hudgens, S.E. Slager, R.T. Chatterton, Jr., L.G. Keith, R.W. Rebar, and F.A. DeLeon-Jones, U.S. Army Human Engineering Laboratory, Aberdeen, MD, Department of Obstetrics and Gynecology, Northwestern University, Chicago, IL, and West Side VA Hospital, Chicago, IL.

As part of a program to identify profiles of psychological and physiological responses associated with kinds and levels of stressors, male medical students (N=26) taking a final written examination were recruited for study. The Multiple Affect Adjective Checklist (MAACL) and a Subjective Stress Scale (SSS) were administered within 15 min before and within 15 min after the examination. Serum hormone levels were measured in 4 blood samples taken before and 4 samples after the exam. The students returned for evaluation under control conditions. A group of non-students was also evaluated under the control conditions. Before the exam the anxiety subscale (Anx) of the MAACL and the SSS were both significantly elevated over the self-control values ($P<0.005$) and the non-student controls. Anx was correlated with prolactin levels ($r=-0.43$, $P<0.025$) and with luteinizing hormone (LH) levels ($r=-0.48$, $P<0.012$) measured before the exam and with scores on the exam ($r=-0.40$, $P<0.045$). The pre-exam SSS was related to LH measured before the exam ($r=-0.47$, $P<0.015$) but not to the score on the exam. Cortisol was elevated pre-exam relative to non-students, but not to the self-control period and did not relate to the psychological scales. After the exam the depression ($P<0.035$) and hostility ($P<0.01$) subscores (MAACL), and the SSS ($P<0.005$) were increased relative to the the self-control period. These were not related to hormone levels or performance on the exam. We conclude that cortisol is chronically elevated in these students and is not related to this particular event. The anxiety level appears to be a primary variable that is reflected by levels of specific hormones, including LH & prolactin, and significantly relates to subsequent depression, hostility, and to performance on the exam.

Q 206 EFFECT OF PROLACTIN (PRL) ON HUMAN NK ACTIVITY. Lina Matera, Alessandra Cesano, Fabrizio Veglia and Giampiero Muccioli, University of Turin, 10126 Turin, Italy.

Stressfull stimuli have been reported to enhance tumor growth and this effect has been suggested to be mediated, in part, by impairment of the lytic function of Natural Killer (NK) cells. The molecular bases of this phenomenon are believed to rest on the increased production of glucocorticoids, which have an inhibitory effect on NK activity, by the adrenal cortex, in response to signals from the hypothalamus, via the pituitary. Here we have focussed on the effect of the pituitary hormone prolactin (PRL), which is known to increase during stress, on NK cell function in vitro. Peripheral blood lymphocytes (PBL) containing 10% NK cells and NK-purified cell fractions were tested for NK activity against the cell line K562. PRL had no effect on the NK activity, mediated by unseparated PBL, when used at physiological doses (20 ng/ml), but produced a 30 and 40% reduction ($p<0.05$ and <0.02) at the concentrations of 100 and 200 ng/ml respectively. By contrast, the K562 cytotoxicity of purified NK cells was strongly enhanced (30% increase, $p < 0,02$) by physiological doses of PRL and only minimally affected at the highest PRL concentration (200 ng/ml). These results indicate that PRL modulates the activity of NK cells through both NK-directed and accessory cell-mediated mechanisms, the outcome of this influence being a severe depression of NK function.

Q 207 CHRONIC STRESS CAN INTERFERE WITH LOW-DOSE ANTIGEN PRIMING, Jan A. Moynihan, Todd Schachtman, Nicholas Cohen, and Robert Ader, University of Rochester Medical Center, Depts. of Psychiatry and of Microbiology and Immunology, Rochester, NY 14642.

The effects of chronic stress on the in vivo secondary antibody response to the soluble protein antigen keyhole limpet hemocyanin (KLH) was investigated. Male C3H/HeJ mice, 6-8 weeks of age, received daily footshock (60 signalled 10 sec footshocks, 0.6 ma) for 7 days before and 7 days after they were intraperitoneally injected with I ug KLH (without adjuvant). In this strain of mice, 1 ug KLH does not elicit readily detectable (ELISA) IgM and IgG serum antibodies. However, it does prime the animals; that is, good secondary IgM and IgG responses occur following a reexposure to 1 ug KLH three weeks after priming.

There was a statistically significant reduction in the secondary IgM and IgG anti-KLH titers in footshocked animals (relative to apparatus-control animals) that received a secondary immunization with 1 ug KLH. This reduction did not occur in animals that received a secondary immunization with 5ug KLH. Thus, chronic stress can interfere with the antigen-priming efficacy of a low concentration of antigen.

This paradigm is novel and of potential clinical import, because low antigen concentrations may be more reflective of naturally-occurring situations than are responses to the bolus antigen concentrations used in most systems.

We are currently varying shock protocols to determine minimal stress levels required to alter primary and secondary responses, and evaluating the relationship of plasma corticosterone levels to antibody titers.

Q 208 ACCUMULATION OF INTERLEUKIN-2 mRNA AND ITS SUPPRESSION BY DEXAMETHASONE OCCUR BY POSTTRANSCRIPTIONAL REGULATION IN A MOUSE T CELL LINE, Barbara A. Sorg and Raymond Reeves, Washington State University, Pullman, WA 99164. We examined the effects of the glucocorticoid, dexamethasone (dex), on the production of interleukin-2 (IL-2) and its mRNA in the cell line, LBRM-33.4A2. Dex (10nM) inhibited production of IL-2 by 76% with a corresponding decrease in IL-2 mRNA (69%) in Concanavalin A-stimulated cells. Accumulation of IL-2 mRNA following stimulation required protein synthesis, and its inhibition by dex was dose-dependent, while β-tubulin mRNA levels were unaffected by dex. The increase in IL-2 mRNA following stimulation was not accompanied by a change in the transcriptional rate, as determined by nuclear run-off. Treatment with dex also did not alter the transcriptional level of the IL-2 gene. Previous observations have shown that A-U-rich sequences are common in the 3'-noncoding regions of mRNAs for several lymphokines and oncogenes, and may confer instability upon these mRNAs. Several of these are also suppressed by glucocorticoids. Consistent with this, we found that the protooncogene c-myc mRNA, which has a less extensive A-U region than does IL-2 mRNA, is inhibited by dex at a posttranscriptional level to values between those for IL-2 mRNA and controls (33%). Thus, the observation of dex-induced suppression, which may be mediated by the A-U regions, has implications for understanding suppression by glucocorticoids of a large set of immune response genes. Supported by NIH AI-07025 and USDA 85-CRCR-1-1730.

Q 209 INHIBITION OF RAT MAMMARY TUMORS BY STRESS: RELATION TO LYMPHOCYTE RESPONSIVENESS TO MITOGENS AND CORTICOSTERONE LEVEL, J. Ross Stevenson, Hamid Noorbakhsh, Elton Chu, Benjamin H. Newberry, and Brent C. Bruot, Kent State University, Kent, OH 44242
Female Sprague Dawley rats injected with the carcinogen dimethylbenzanthracene developed fewer mammary tumors if they were subjected to 33 days of 14 hr daily restraint stress, as previously reported (Newberry et al., Psychosom. Med., 38: 155. 1976). Splenic lymphocytes taken from these rats displayed the same responsiveness to the mitogens Con A and LPS and thymic lymphocytes the same responsiveness to Con A as ones from control rats. The same result was obtained when rats not injected with the carcinogen were used. Male Lewis rats were subjected for up to 40 days to a daily footshock regime similar to that previously found to inhibit mammary tumor development (Newberry et al., Psychosom. Med., 34: 295. 1972). When splenic lymphocytes from these rats were stimulated with Con A or LPS one day after the last shock session, depressed responsiveness was seen, though corticosterone had returned to baseline. However, when the same tests were made on the day of the last shock session, responsiveness was increased in spite of greatly increased plasma corticosterone level.

Q 210 CORTICOTROPIN-RELEASING FACTOR (CRF) AND PHYSIOLOGICAL STRESSORS ALTER ACTIVITY OF RAT NORADRENERGIC LOCUS COERULEUS NEURONS (LC) IN A SIMILAR MANNER, Rita J. Valentino, George Washington University, Washington, D.C. 20037.
The hypothesis that stress activates LC neurons by releasing CRF was tested by comparing the effects of CRF and certain stressors on LC discharge. CRF (1.0 and 3.0 ug, i.c.v.) increased spontaneous discharge rates of LC neurons recorded in both anesthetized and unanesthetized rats, although LC cells of unanesthetized rats were more sensitive to CRF. Additionally, CRF disrupted the response of LC cells to repeated presentation of phasic sensory stimuli. This response is characterized by increased discharge occurring 10-20 msec after the stimulus followed by a longer period of relative inhibition. In anesthetized rats presented with footshock and unanesthetized rats presented with tones, CRF disrupted the sensory response by increasing baseline discharge and decreasing or not altering the excitatory component of the response. Two stimuli that elicit CRF release, hemmorhage and nitroprusside infusion, produced identical increases in LC spontaneous discharge rates (25% above baseline rates). The similar effects of CRF and the physiologic stressors on LC neuronal disharge are consistent with the hypothesis that certain stressors activate LC neurons by releasing CRF. During this activation the LC may be less responsive to phasic sensory stimuli. Because the LC projects to widely diverse brain regions, activation of LC by CRF during stress may be a mechanism by which CRF effects may be amplified throughout the CNS, ultimately resulting in an integrated response to stressful stimuli.

Q 211 OPIATE ACTION IN THE PERIAQUEDUCTAL GRAY MATTER CAUSES SUPPRESSION OF IMMUNE FUNCTION. R.J. Weber and A. Pert. Laboratory of Neuroscience, NIDDK and Biological Psychiatry Branch, NIMH, Bethesda, MD 20892.
Numerous observations indicate that opiates can affect immune function (Weber and Pert, Central and Peripheral Endorphins, Muller and Genazzini (ed.), 1984). Intracerebroventricular (ICV) administration of low doses of morphine suppressed NK cell activity, suggesting that this effect was mediated through the CNS (Shavit, et al, J. Immunol., 135:834, 1985). In order to localize the CNS effects of morphine on immune function, rats were implanted with bilateral cannulae guides aimed for various brain structures. One week following surgery the animals received bilateral injections of morphine (5 ug in 1 ul saline) in each structure. Three hours following administration of morphine the rats were sacrificed, spleens removed, and NK cell activity measured. Injections of morphine into the anterior hypothalamus, arcuate nucleus, medial amygdala, medial thalamus and dorsal hippocampus had no significant effect on NK cell activity when compared to uninjected controls. Injection of morphine into the periaqueductal gray (PAG), however, produced a significant suppression of NK cell activity, when compared to saline injected animals. The suppression of NK cell activity was blocked by prior peripheral administration of naltrexone. These findings suggest that the central actions of opiates on NK cell function are mediated through opiate receptors in the PAG.

Q 212 INFLUENCE OF PSYCHOSOCIAL STRESS ON MOUSE MAMMARY TUMOR GROWTH, Joanne Weinberg, Gail D. Bellward and Joanne T. Emerman, University of British Columbia, Vancouver, B.C., Canada.

We have been examining the effects of psychosocial stress on mammary tumor growth rate in mice, as mediated by endocrine and immune variables. Male mice were injected (s.c.) with cells from the androgen-responsive Shionogi mouse mammary carcinoma, or were sham injected, and assigned to groups: those raised alone were placed in groups (IG) or remained alone (II); those raised in groups remained in groups (GG) or were isolated (GI). Animals were also subjected to acute daily stress (placement in novel environment, 15 min/day). After 23-24 days, animals were sacrificed. Tumor growth was increased in II and GI, and decreased in IG, compared to GG control animals. In tumor bearing animals, plasma corticosterone levels did not differ among groups; however, adrenal corticosterone content was increased in the IG condition. Testosterone levels were generally decreased in tumor bearing animals. There were no differences in androgen or glucocorticord receptor affinity or capacity in tumor cytosols. In all tumor-bearing animals, spleen weights were increased and both lymphocyte proliferation and antibody titre were decreased; however some differential effects of group were observed. These data indicate marked effects of housing condition and novelty stress on tumor growth and immune responsiveness.

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Adrenergic Mechanisms; Adrenal Gland

Q 300 INVOLVEMENT OF NOREINEPHRINE IN THE EFFECT OF RESTRAINT BUT NOT CRF ON EXPLORATORY BEHAVIOR.

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Both stress and CRF activate brain noradrenergic systems and norepinephrine (NE) has been postulated to participate in the regulation of both behavioral and emotional responding in stress and anxiety. We have investigated the involvement of noradrenergic systems in mediating the restraint- and CRF-induced decrease in exploratory behavior as tested in the multicompartment chamber (Arnsten & Segal, 1979).

The α_2-antagonist, idazoxan (1.0 mg/kg), which increases NE release and locus coeruleus firing decreased the mean time per contact with the environmental stimuli. In contrast, clonidine, an α_2-agonist increased the mean time per contact with the stimuli. A similar effect was observed in animals tested 3 days after an injection with DSP-4, which selectively depletes brain NE. Both clonidine and DSP-4 antagonized the effect of restraint on stimulus interaction and when combined blocked the effect of restraint. The α_1-antagonist, prazosin, also increased the mean time per contact. In contrast to the results obtained in stressed animals, neither clonidine nor DSP-4 diminished the ability of ICV CRF to decrease stimulus contact-times.

These results suggest an involvement of noradrenergic systems in the restraint-induced, but not the CRF-induced, decrease in investigatory behavior. The action of NE in stress may be mediated through a post-synaptic α_1-receptor.

Q 301 INDIVIDUAL DIFFERENCES IN THE ADRENOCORTICAL STRESS RESPONSE AND AGING, S.R. Bodnoff, D.H. Aitken, V. Viau, Ch. Van Berkel, R.M. Sapolsky and M.J. Meaney. Douglas Hospital Research Centre, Dept. of Psychiatry, McGill University, Montreal, Canada and Dept. of Biological Sciences, Stanford University, Stanford,CA.

Animals handled during the first three weeks of life show increased glucocorticoid receptor binding capacity in hippocampus, a critical brain structure in the control of pituitary-adrenal activity. As adults, handled animals exhibit increased sensitivity to the inhibitory effects of both corticosterone and dexamethasone on ACTH secretion, as well as increased glucocorticoid secretion during and following stress. By 14-16 months of age, non-handled animals show increased corticoid secretion under both basal and stressed conditions, hippocampal cell loss, and profound spatial memory deficits. Handled animals show no age-related increases in basal corticosterone, only moderate corticoid hypersecretion following stress, no hippocampal cell loss, and spatial memory skills that are indistinguishable from those of young, adult animals. These data show that a subtle environmental manipulation can modify the adrenocortical response to stress and alter the aging process in certain CNS systems.

Q 302 DEMONSTRATION OF DIRECT PROJECTIONS FROM THE AMYGDALA TO THE PARAVENTRICULAR HYPOTHALAMIC NUCLEUS: POSSIBLE ROLE IN STRESS-INDUCED ACTH RELEASE, Thackery S. Gray and Michael Carney, Loyola University, Maywood, IL 60153

Studies have demonstrated that the amygdala, specifically the central amygdaloid nucleus (Ce), is important for the expression of ACTH and corticosterone responses in rat models of stress. Presumably, the amygdala can affect the neuroendocrine system through direct and/or indirect connections to the paraventricular nucleus of the hypothalamus (PVN). To date, there are no studies that have definitively demonstrated a direct projection from the Ce to the PVN. In the present study, the highly-sensitive anterograde tracer phaseolis vulgaris leucoagglutinin lectin (PHA-L) was iontophoretically injected into the Ce of rats via standard sterotaxic technique. Injections of the tracer localized within the medial part of the Ce produced axonal labeling that extended into the PVN. The medial and lateral parvocellular subdivisions of the PVN contained numerous labeled axons and presumed terminals. Axonal labeling was sparsely distributed within all other subdivisions of the PVN including magnocellular regions. Axonal labeling was also observed within the lateral hypothalamus, central grey, parabrachial nucleus, locus coeruleus, ventrolateral medulla and dorsal vagal complex. Thus, there is a direct projection from the medial Ce to the regions of the PVN that can affect the release of ACTH from the anterior pituitary. In addition, the same population of PVN projecting neurons within the medial Ce also innervates autonomic regions of the brainstem. Through these pathways the medial Ce is capable of simultaneously affecting neuroendocrine hypothalamic and autonomic brainstem areas in response to stressful or fear producing stimuli.

Q 303 NUTRITIONAL INFLUENCES ON CHROMAFFIN CELL DEVELOPMENT, C. Lau[1], A.M. Cameron[1], J. Bartolome[2], J.M. Bell[2] and T.A. Slotkin[2], [1]Northrop Services & US EPA, Research Triangle Park, NC, and [2]Duke University Medical Center, Durham, NC.

The developing brain is generally protected from growth retardation associated with nutritional deprivation. To investigate whether such protective mechanisms extend to the peripheral sympathetic nervous system, maturation of the chromaffin cells of the adrenal medulla and development of splanchnic innervation were evaluated in rats whose nutritional status had been altered during the neonatal period by increasing (16-17 pups/litter) or decreasing (5-6 pups/litter) the litter size from the standard one of 11-12 pups per litter. Ontogeny of adrenal catecholamine (CA) stores and activities of CA-biosynthetic enzymes tyrosine hydroxylase (TH) and phenylethanolamine N-methyltransferase (PNMT) were monitored, along with activity of choline acetyltransferase (ChAT), a marker enzyme for the preganglionic neurons innervating the chromaffin cells. Neonatal nutritional deprivation induced by large litter size slowed body weight gain and retarded development of the chromaffin cells as CA stores, TH and PNMT activities were subnormal throughout the study. The effects on the adrenal persisted despite apparent recovery of body weight deficits post-weaning. These developmental alterations did not appear to be associated with non-specific overcrowding stress, as plasma corticosterone levels were not elevated in the large litter group. Neonatal nutritional enrichment promoted body weight gain but failed to enhance development of adrenal CA or TH, although the PNMT activity was slightly but significantly elevated. Unlike effects on the chromaffin cells, altered neonatal nutritional status had little or no effect on the development of cholinergic innervation: ontogeny of ChAT activity did not differ among the three litter groups. These results suggest that maturation of chromaffin cells is dependent upon nutritional status, and that ontogenetic gains are close to optimum at normal nutritional status. (Supported by EPA 68-02-4450, EPA CR813769 and USPHS HD-09713).

Q 304 ESCAPABLE AND INESCAPABLE STRESS: EFFECTS ON BRAIN AND ADRENAL NEUROTRANSMITTER BIOSYNTHETIC ENZYME mRNA, Pascale Montpied, Robert C. Drugan, Sandra L. Cottingham, Jacqueline N. Crawley, and Steven M. Paul, Clinical Neuroscience Branch, NIMH, Bethesda, MD 20892.

The lack of control over an environmental stressor (e.g. inescapable tail shock in rats) results in a behavioral syndrome referred to as "learned helplessness"; characterized by an impairment in learning, reductions in aggression and social dominance, decreased food intake, decreased immune functions, and gastric ulcer formation. Approximately 50% of Sprague-Dawley rats develop this syndrome following inescapable tail shock, whereas the other 50% do not (coping animals). The propensity of animals to develop learned helplessness is a stable (i.e. trait) characteristic, suggesting that genetic factors are important in determining the organism's response to stress. Several neurotransmitter systems have been implicated in the development of learned helplessness, including those for norepinephrine and GABA. Consequently, we have examined the rate-limiting enzymes for catecholamine (tyrosine hydroxylase, TH) and GABA (glutamic acid decarboxylase, GAD) synthesis in tissues of rats exposed to escapable or inescapable shock. Further, to assess whether changes in the expression of the TH or GAD gene following inescapable stress are associated with learned helplessness, the levels of TH and GAD mRNA have been quantified in brain and adrenal by Northern analysis. These data and their implications will be presented.

Q 305 RAPID INCREASE IN GLYCEROL PHOSPHATE DEHYDROGENASE POLY(A)+RNA AS A CORTICOID-DEPENDENT STRESS RESPONSE IN THE ADULT RAT BRAIN, Nancy R. Nichols, Jeffrey N. Masters and Caleb E. Finch, USC, Los Angeles, CA 90089-0191.

The hypothalamic-pituitary-adrenal (HPA) axis is activated in response to stress and corticosteroids are elevated in serum. The hippocampus is both a glucocorticoid target with a high abundance of corticoid receptors and a site of feedback inhibition on HPA-axis. Recently, we have identified two relatively abundant RNA species in rat hippocampus coding for 35 and 20 kd polypeptides, that respond to acute corticosterone (CORT) treatment and vibratory stress. In order to identify these species, we have isolated CORT-responsive clones from a hippocampal cDNA library in λgt10 by differential screening using cDNA probes prepared from adrenalectomized (ADX) or CORT treated rats (10 mg/d for 3 d). Different RNAs recognized by 3 clones increase 3- to 10-fold (CORT/ADX) when analyzed by RNA blot hybridization. One of these hybidizes to a RNA of 2.9 kb, shows a pattern corresponding to white matter, oligodendrocyte distribution by in situ hybridization, and has a DNA sequence homologous to the mouse glycerol phosphate dehydrogenase (GPDH) gene. This effect is rapid, within 2 h CORT, mediated by the type II glucocorticoid receptor and responds in a dose-dependent manner to increasing CORT (0.2 to 10 mg/rat). We have seen a similar response in intact rats after 2 h vibratory stress (3- to 4-fold) and no response in stressed ADX rats. Therefore, GPDH gene activity represents a glucocorticoid-dependent stress response. The brain enzyme is frequently cited for its role in phospholipid synthesis during myelination and may be important in neuronal membrane stability during stress.

Q 306 ONTOGENY OF THE GLUCOCORTICOID RECEPTOR: AN IMMUNOCYTOCHEMICAL STUDY P. Rosenfeld,* J.A.M. Van Eekelen,+ S. Levine,* H.M. Westphal,++ and E.R. De Kloet.+
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+Rudolf Magnus Institute for Pharmacology, Medical Faculty, University of Utrecht.
++Institute for Molecular Biology and Cancer Research, Marburg, F.R.G.

The ontogeny of the Type 2 glucocorticoid receptor (GR) in the rat brain was examined using a monoclonal antibody raised against the rat liver GR. This antibody recognizes the DNA binding form of the receptor.

GRir was high perinatally, decreased until around day 12, and thereafter increased, achieving adult levels by day 20 in most brain areas. In some regions, such as the hippocampal CA3-4 cell fields and the suprachiasmatic nucleus of the hypothalamus, GRir was only present early in ontogeny. GRir localization showed a distinctive developmental trend towards greater compactness within the cell fields and a greater restriction of GRir to the cell fields with exclusion of the adjoining areas. Adrenalectomy reduced overall staining, which was increased by administration of the selective glucocorticoid agonist RU 28362.

Our results suggest the glucocorticoid receptor system has a high degree of molecular and cellular plasticity; such plasticity may provide a basis for understanding the role of glucocorticoids during development.