

10.7

ROLE OF TNF IN THE FEVER MECHANISM INDUCED BY INTRAVENOUS PERFLUOROCARBON EMULSION IN RATS. J.D. Bradley, S. Otto, D. Smith, G. Neshund, and S.F. Flaim. Alliance Pharmaceutical Corp., San Diego, CA 92121.

Intravenous (i.v.) infusion of perfluorochemical emulsions (PFC) produces transient, delayed fevers in animals and humans. Our objective was to determine whether increases in plasma TNF levels correlated with the fever profile following PFC infusion. Rats were chronically fitted with intraperitoneal body temperature telemeters and a jugular vein catheter and allowed to recover overnight. Animals were then infused with saline (3 mL/kg, n=4), lipopolysaccharide (0.1 µg LPS/rat, n=4), or a 90% w/v PFC emulsion (3 mL/kg, n=4) and core body temperature was monitored by telemetry for 12 hrs. A fourth group of animals was pretreated with dexamethasone (Dex, 0.2 mg/kg) at both 12 and 2 hrs prior to PFC infusion (n=4). Rats were unrestrained with free access to food and water. Blood was taken at baseline and 1, 2, 3, and 6 hrs post infusion to determine serum TNF levels by ELISA. The table shows TNF levels (pg/mL) and mean area under the curve (AUC) of the change in body temperature. Data are Means ± SEM.

GROUP	AUC	Baseline	1 hr	2 hrs	3 hrs	6 hrs
Saline	13.7	0 ± 0	0 ± 0	3.8 ± 1.4	1.4 ± 0.9	0.3 ± 0.3
LPS	15.4	1.4 ± 1.4	3179 ± 799	235 ± 65	25 ± 7	3.5 ± 3.5
PFC	27.9	0 ± 0	110 ± 36	197 ± 60	162 ± 37	35 ± 9
Dex + PFC	10.9	1.2 ± 1.2	0 ± 0	1.3 ± 0.9	0.6 ± 0.6	0 ± 0

There were no significant changes in TNF or body temperature in saline control animals. After LPS, TNF levels increased sharply by 1 hr. Despite high TNF levels, this dose of LPS did not induce fever (while higher LPS doses induce fevers in this model). A moderate, but sustained increase in TNF was observed after PFC. The sustained increase in TNF is presumably related to the gradual removal of PFC from the circulation by macrophages. After PFC, rats exhibited a transient fever which peaked at ~1.25°C above baseline at 5 hrs. Dex ablated both the increase in TNF and the fever following PFC. These data suggest that serum TNF levels do not always correlate with fever. However, the timecourse or duration of TNF release may play a role in the production of fever.

10.9

L-NAME, A NITRIC OXIDE SYNTHASE INHIBITOR, DOES NOT ALTER THE FEBRILE RESPONSE TO LPS. N.C. Long and S.A. Shore. Physiology Program, Harvard Sch. of Public Health, Boston, MA 02115.

Previous studies have shown that injection of antiserum against tumor necrosis factor (TNF) enhances the febrile response to lipopolysaccharide (LPS), suggesting that this cytokine may act as an endogenous cryogen, limiting the magnitude of fever (Long et al., *Am. J. Physiol.*, R332, 1990). One potential mechanism for this response is that TNF moderates fever by inducing the release of nitric oxide (NO), a potent vasodilator, which could lead to increased heat loss. NO could also act centrally to alter the febrile response. To test the hypothesis that NO is involved in moderating fever, we compared the febrile response of rats that had been treated with the NO inhibitor L-NAME, to that seen in rats treated with its inactive enantiomer, D-NAME. L-NAME and D-NAME were administered continuously in the rats' drinking water (70 mg/100 ml) starting 8 days before the study began. The L-NAME-treated rats had a mean blood pressure of 151 mmHg, while the D-NAME animals had a mean blood pressure of 127 mmHg (p=0.007), confirming the efficacy of the L-NAME treatment. Body temperature (Tb) of the rats was monitored by implanted biotelemetry devices. We found no difference in the baseline Tb of the rats (L-NAME: 36.4±0.2°C vs D-NAME: 36.3±0.3°C; p=0.71). The injection of 10 µg/kg of LPS (i.p.) into the L-NAME-treated rats induced a mean change in Tb of 1.11±0.18°C between 2 and 8 h post-injection. This did not differ from the fever seen in the D-NAME rats during the same time interval (1.09±0.12, p=0.92). These data do not support the hypothesis that the vasodilatory effect of NO mediates the cryogenic effect of TNF during LPS fever. It is possible, however, that NO acts centrally, and that the L-NAME did not reach the region of the brain responsible for this response. Supported by HL19170.

10.8

HIV-1 glycoprotein120 ALTERS RAT SLEEP. MR Opp, TK Hughes, Jr., and EM Smith. Depts of Psych & Behav Sci and Microbiol. Univ of Texas Med Branch, Galveston, TX 77555

Excessive daytime fatigue and sleepiness are prominent and persistent symptoms associated with HIV infection. Concentrations of cytokines increase during the course of HIV infection, and some cytokines, particularly IL-1, are somnogenic. gp120, the major envelope protein of HIV-1, has many neurological actions and induces IL-1 secretion in brain. As such, gp120-induced IL-1 secretion within the CNS is one potential mechanism whereby sleep may be altered during HIV infection. Alternatively, gp120 may exert direct actions on "sleep centers" within the CNS.

Rats were surgically prepared with instrumentation to allow determination of sleep/wake activity. The rats were injected ICV with one of two doses of recombinant gp120 (ABT, Inc., Cambridge, MA: 100- or 500 ng) or with pyrogen-free saline (vehicle). Rats responded with increases in the amount of time spent in non-rapid-eye-movements sleep (NREMS) relative to values obtained after vehicle injection, regardless of the dose of gp120 injected. This period of enhanced NREMS lasted 4 - 8 h depending on dose, and was followed by a reduction in sleep relative to values obtained after vehicle administration. We speculate this period of reduced sleep may be due to gp120- and/or IL-1-induced elevations in endogenous cytokine inhibitors, possibly the IL-1 receptor antagonist, IL-10, or corticotropin-releasing hormone. These substances all reduce sleep in experimental animals.

10.10

COLOCALIZATION OF FOS-LIKE IMMUNOREACTIVITY AND NITRIC OXIDE SYNTHASE ACTIVITY FOLLOWING IMMUNOLOGICAL STIMULATION. J.K. Elmquist, T.E. Scammell, C.D. Jacobson, and C.B. Saper. Dept. of Neurology, Harvard Medical School/Beth Israel Hospital, Boston, MA 02115, and Dept. of Veterinary Anatomy, Iowa State University, Ames, IA 50011.

Nitric oxide (NO) is a biologically active molecule implicated in numerous physiological functions including participation in the acute phase response (APR). Recent studies suggest that NO is involved in regulating neuroendocrine function following immune system activation including alteration of ACTH, vasopressin, and oxytocin secretion. Additionally, inhibition of NO production following LPS challenge has detrimental effects (including increased mortality rates) indicating a regulatory role of NO after immunological stimulation. Previous experiments have demonstrated that systemic administration of lipopolysaccharide (LPS) mimics various aspects of the APR. We recently demonstrated that i.p. administration of LPS induces the expression of Fos-like immunoreactivity (Fos-IR) in nuclear groups of the rat brain thought to be involved in regulation of autonomic homeostasis. In the present study, we have used i.v. LPS as a model of sepsis and have mapped the resultant Fos-IR. In addition, we have examined subsets of cells in the brain that contain both Fos-IR and NADPH-diaphorase staining (NO synthase activity) following LPS challenge. Cells that contained both NO staining and Fos-IR were observed in the periventricular, supraoptic, and paraventricular hypothalamic nuclei, and in the medial preoptic area. Double staining was also seen in circumventricular organs including the organum vasculosum of the lamina terminalis (OVLT) and in the subfornical organ. The results of this study provide anatomical evidence for the role of NO in regulating neuroendocrine responses following immune activation. These studies further suggest that neuronal NO is involved in coordination of the complex physiological responses of the APR.

METHODOLOGICAL ISSUES IN CYTOKINE MEASUREMENT

11.1

PRO-IL-1β IS RELEASED FROM MONOCYTES IN VITRO IN A FORM THAT IS RESISTANT TO PROCESSING BY IL-1β CONVERTING ENZYME. Mark D. Wewers* and Heidi A. Pone. The Ohio State University, Columbus, OH. 43210

The processing and release of 31 kDa proIL-1β to the mature 17 kDa form of IL-1β is still poorly understood. In this context, we and others have noted that a 31 kDa form of IL-1β is released from mononuclear phagocytes in response to endotoxin stimulation in vitro (*J. Immunol.* 149:3052, 1992). Since the site of processing of the 31 kDa proIL-1β is not known, we hypothesized that the released proIL-1β may represent IL-1β in a pre-processing phase or IL-1β that has been modified to prevent processing. To study released proIL-1β, we measured supernatant proIL-1β from endotoxin stimulated monocytes by immunoprecipitation of ³⁵S-methionine labeled protein, by Western blots, and by our recently developed enzyme linked immunoassay (ELISA) specific for proIL-1β (*J. Immunol. Meth.* 165:269, 1993). Although supernatant proIL-1β represented 20-40% of the total released IL-1β as measured by SDS-PAGE with densitometry, this proIL-1β was not detected by our proIL-1β specific ELISA. The ELISA's inability to detect proIL-1β was not due to inadequate sensitivity or subsequent degradation in the ELISA. Importantly, the 31 kDa protein was confirmed to be proIL-1β since its immunoprecipitation was specifically blocked by the immunogenic peptide used to generate the proIL-1β specific antibody. Finally, since supernatant proIL-1β can be immunoprecipitated, we asked whether affinity purified supernatant proIL-1β can be processed to mature IL-1β when incubated with recombinant IL-1β converting enzyme (ICE) (Merck). In two separate purifications, immunoprecipitated cytosolic proIL-1β was processed by ICE but identically purified supernatant proIL-1β was not. These findings imply that proIL-1β can be released from monocytes in a unique form that may reveal important clues to monocyte regulation of proIL-1β processing and release.

11.2

DEVELOPMENT OF A HUMAN INTERLEUKIN-1 BETA PRECURSOR ELISA. Leslie S. Casey, Lisa Esposito, Cynthia Alley, Beverly Lytwyn, and Richard S. Dondero. Cistrion Biotechnology, Inc., Pine Brook, NJ 07058.

A highly-sensitive, rapid, and specific ELISA was developed for measurement of the 33kD human interleukin-1β (IL-1β) precursor protein. The monoclonal antibody-based ELISA reproducibly detects as little as 10pg/ml of precursor. Proteolytic cleavage of IL-1β precursor by IL-1 converting enzyme (ICE) or other enzymes leads to release of the active, 17.5kD form of IL-1β, which is known to be a key mediator of inflammation in normal immunity and in disease. Cleavage of purified IL-1β precursor protein *in vitro* resulted in markedly reduced signals in the IL-1β precursor ELISA, with concomitant increased signals in assays measuring the active form. Because of the previous lack of availability of a sensitive, specific assay, study of regulated expression of the IL-1β precursor protein has lagged behind that of the active form. Development of this new ELISA for the human IL-1β precursor protein provides an important tool for this unexplored area of cytokine research.