sion of phospholipid vesicles, or to inactivation or loss of the transporter molecules during reconstitution, is unclear.

The high-affinity choline transporter is the sole supplier of choline for acetylcholine synthesis in the isolated terminal (*9*). In contrast, the low-affinity choline uptake process is found in virtually all cells and presumably subserves phosphorylcholine and subsequently phospholipid synthesis (*14*). Our results show that these two uptake systems can be reconstituted simultaneously into proteoliposomes and, in particular, that the high-affinity transporter retains kinetic and pharmacological properties that are similar to those seen in intact synaptosomes. This transport occurs in the absence of choline acetyltransferase activity, even though kinetic observations in intact terminals suggest that this enzyme is physically coupled to high-affinity uptake (*3, 9, 15*).

γ-Aminobutyrate and glutamate transport activities have also been described in membrane preparations derived from synaptosomes (*16*). However, these preparations resulted from the resealing of hyposmotically lysed terminals, in which we found no high-affinity, hemicholinium-3–sensitive, Na^+-dependent choline uptake. A liposome preparation derived from hyposmotically lysed synaptosomal membranes that retained choline uptake has been described (*17*); however, this uptake was not characterized pharmacologically or kinetically as high-affinity transport. For example, hemicholinium-3 only slightly inhibited choline uptake, and no dependence on external sodium or chloride ions was demonstrated.

The proteoliposomal preparation we have described appears to be appropriate for isolating and characterizing the high-affinity choline transporter, as well as for studying other neuronal transport mechanisms. We have found that the proteoliposomes take up serotonin at a low concentration in a sodium-dependent manner. In addition, we have shown in preliminary experiments that this preparation, when supplemented internally with acetylphosphate, coenzyme A, choline acetyltransferase, and phosphotransacetylase, releases newly synthesized acetylcholine when depolarized with K^+ in a calcium-dependent manner. The preparation may therefore also be useful for studying nonexocytotic release mechanisms.

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Endotoxin-Stimulated Opioid Peptide Secretion: Two Secretory Pools and Feedback Control in vivo

Abstract. *Small doses of endotoxin evoked a dramatic biphasic response of opioid peptide secretion into blood in sheep. The first phase began within minutes and coincided with a brief hypertensive response to endotoxin well before the appearance of fever or hypotension. The ratio of* β*-endorphin to* β*-lipotropin fell abruptly at the onset of the second phase of release, suggesting early depletion of a pool rich in* β*-endorphin and subsequent emergence of a pool rich in unprocessed precursor. The concentration of cerebrospinal fluid opioids increased tenfold during the second phase. Naloxone administration augmented endotoxin-induced opioid secretion in both early and late phases, suggesting a short-loop feedback regulation of stress-induced endorphin secretion.*

The pressor response to intravenously administered naloxone during endotoxin-induced hypotension in animals, and in some clinical cases of septic shock, suggests a role for endogenous opioids in mediating shock resulting from infection (*1*). Although pyrogens activate the pituitary-adrenal axis (*2*) and hence would be expected to cause release of β-endorphin (β-EP) (*3*), no direct evidence is available regarding the dynamics of opioid peptide secretion in relation to endotoxin-induced hyperthermia or hypotension. A primary role for endogenous opioids in producing vital sign responses to endotoxin might be indicated if peptide release precedes vital sign changes, or a secondary role if vital sign changes precede peptide release.

To characterize endorphin secretory dynamics in endotoxin fever, we used a model that permits rapid atraumatic sampling of blood simultaneously from the carotid artery, jugular vein, and sagittal sinus in alert, unanesthetized, partially restrained sheep (*4*). In addition, in 13 of 14 experiments, cerebrospinal fluid (CSF) was sampled from a lateral ventricle through an implanted cannula (*5*). All animals were tame, acclimated to the testing laboratory, and had free access to food and water at all times. Blood pressure was monitored through the carotid artery line, and temperature was recorded from a rectal thermocouple.

One group (seven sheep) received intravenous bolus injections of endotoxin (*Escherichia coli* serotype 055:B5; 450 ng/kg) (*6*); a second group (three sheep) was given endotoxin plus naloxone (an intravenous bolus of 0.1 mg/kg injected with the endotoxin, then an infusion of 0.02 mg/kg per hour) (*7*); and a control group (four sheep) was given 0.9 percent saline only. In each experiment, lactated Ringer solution was infused into the jugular vein at a rate equal to the rate of blood withdrawal. Plasma and CSF samples were assayed for immunoreactive β-EP (iβ-EP) (*8*). The molecular weight profile of iβ-EP was obtained by G-50 gel chromatography of plasma extracts (*9*).

Figure 1A depicts vital sign changes in relation to plasma and CSF concentrations of iβ-EP in a representative animal treated with endotoxin. A composite diagram of results obtained in plasma of the seven sheep given endotoxin is also shown (Fig. 1B). Temperature in these sheep first rose to a level two standard deviations above baseline at a mean ± standard error (S.E.M.) of 30.0 ± 7.7 minutes after endotoxin administration. A brief period of hypertension always preceded hyperthermia, presumably reflecting vasoconstriction during the so-called "chill" phase long recognized as an immediate consequence of endotoxin administration (*10*). Hypotension followed the transient hypertension, after

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Table 1. Changes in vital signs and endorphin secretion after endotoxin administration. All values are means ± S.E.M. Peaks and troughs were identified as described in the text for each of the seven sheep treated with endotoxin only. Values for vital signs are differences from baseline values, and values for immunoreactive β-EP are obtained by dividing the value of plasma iβ-EP at a peak or a trough by the mean baseline value of iβ-EP in each animal. Values in parentheses indicate the times in minutes of the occurrence of peaks and troughs. The molar ratio of β-EP to β-LPH was determined in a subgroup of five sheep after correction for recoveries from extracts of plasma withdrawn from the jugular vein during early and late secretory phases of iβ-EP (see text).

Item	Measurement change		
	First peak	Trough	Second peak
Rectal temperature (°C)	0.91 ± 0.16 (67 ± 7)	0.42 ± 0.23 (112 ± 6)	1.56 ± 0.26 (222 ± 6)
Systolic pressure (mmHg)	12.4 ± 2.8 (18 ± 3)	−23.9 ± 3.4 (76 ± 8)	3.9 ± 8.5 (146 ± 10)
Diastolic pressure (mmHg)	10.2 ± 2.5 (18 ± 4)	−21.4 ± 4.1 (77 ± 9)	1.3 ± 5.7 (156 ± 10)
Immunoactive β-EP (relative to baseline)	12.3 ± 4.1 (38 ± 5)	3.8 ± 1.1 (70 ± 2)	22.0 ± 3.0 (151 ± 9)
Molar ratio of β-EP to β-LPH	1.42 ± 0.18		0.66 ± 0.11

which blood pressure returned to a level slightly above baseline. Levels of iβ-EP rose during the initial period of hypertension and, in the jugular vein, attained levels equal to 10.1 ± 4.4 times baseline, with the earliest increase two standard deviations above baseline detectable 16.0 ± 2.2 minutes after endotoxin administration. After a subsequent decline, the plasma levels of iβ-EP in the jugular vein rose to levels equal to 17.3 ± 3.3 times baseline. The four saline controls showed no significant changes in vital signs or levels of iβ-EP in plasma or CSF.

These patterns of response for vital signs and iβ-EP in animals given endotoxin alone were evident whether the data were analyzed by averaging values for all seven animals during serial 10-minute intervals (as above) or by identifying times and levels of peaks and troughs for each measurement in each animal and then averaging these times and levels independently (Table 1). Both forms of analysis are shown in Fig. 1B. Levels of iβ-EP in the CSF did not always rise in parallel with plasma levels during the first hour after endotoxin was administered. However, in the five sheep examined, an eventual increase in CSF iβ-EP to levels approximately ten times baseline appeared to parallel the second phase of elevated plasma iβ-EP.

Gel chromatography of extracts of plasma samples taken before and after endotoxin administration confirmed that increases in iβ-EP were of the magnitude indicated by assay of unextracted plasma and were due to increases in both β-EP and β-lipotropin (β-LPH). However, in each of the five sheep so examined, the molar ratio of β-EP to β-LPH was invariably higher in the first phase of secretion of iβ-EP (mean ratio ± S.E.M., 1.416 ± 0.178) than in the second (0.664 ± 0.113, $P < .001$). To distinguish whether the altered molecular weight profile of iβ-EP in early as opposed to late phases of secretion arose simply because of differences in the metabolic clearance rates of β-EP and β-LPH, we determined this ratio in serial plasma samples drawn over a 3-hour interval after endotoxin administration in two of these five sheep. In contrast to the continuous decline in molar ratio that might be expected solely on the basis of different clearances, this ratio fell abruptly within 1 hour after endotoxin administration in both animals and then remained constant, suggesting early depletion of a "fast-turnover" pool rich in β-EP, and subsequent unmasking of a "slow-turnover" pool in which β-LPH predominated. Although the source of plasma iβ-EP is presumed to be the pituitary, a gradient from the carotid artery to the jugular vein was not uniformly present. Concentrations of iβ-EP in the sagittal sinus rarely exceeded those in the carotid artery, as might be expected for retrograde flow of peptide from pituitary to brain or for efflux of peptide directly from brain to periphery (*4*). Indeed, these studies cannot exclude nonpituitary contributions to plasma iβ-EP, for example, white blood cells (*11*).

Naloxone given with endotoxin produced hyperthermic effects no different from those seen with endotoxin alone (*12*), nor did naloxone consistently block endotoxin-induced hypotension (Fig. 1C). However, animals given naloxone plus endotoxin had a somewhat greater initial rise in blood pressure than the group given endotoxin only [systolic, 26.7 ± 9.2 (S.E.M.) mmHg ($P < .05$); diastolic, 16.4 ± 6.1 ($P < .1$)]. Secretion of iβ-EP into plasma in both early and late phases was much greater in animals given naloxone plus endotoxin than in those given endotoxin only [ratio to baseline value ≥ 54 ± 19.4 (S.E.M.) in the first phase ($P < .05$); ≥ 65 ± 8.3 in the second phase ($P < .001$)]. This rise frequently exceeded the upper limits of estimation in our assay, even though plasma samples from all experimental groups were assayed together. Levels of CSF iβ-EP on the average increased as much in animals given naloxone plus endotoxin as in those given endotoxin only. However, the highest level of CSF iβ-EP in these studies was seen in an animal treated with naloxone plus endotoxin and occurred just before it had the only febrile convulsion witnessed in these studies; the convulsion was followed by a further increase in CSF iβ-EP (Fig. 1C).

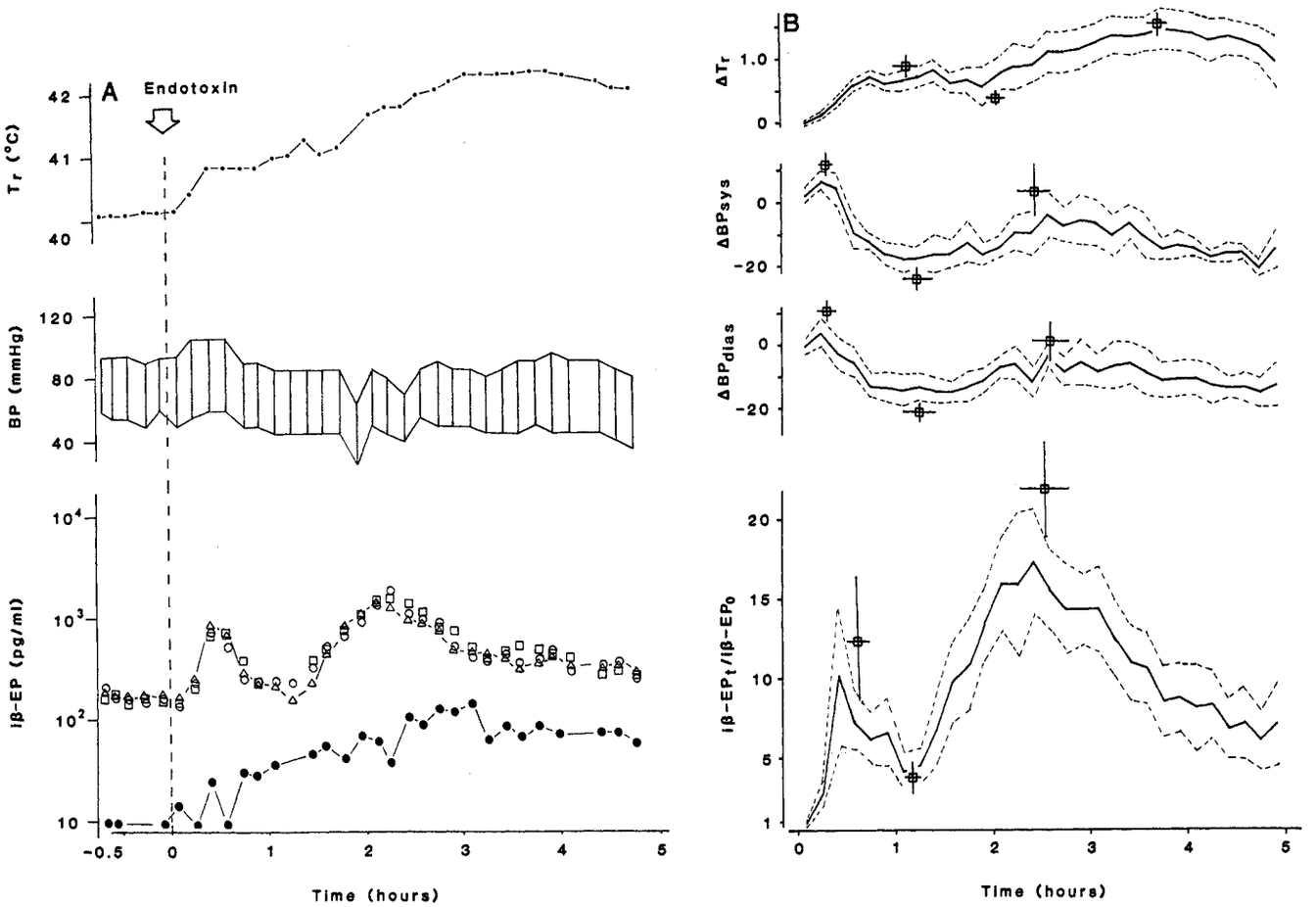
These studies permit several conclusions.

1) The results demonstrate by direct measurement a biphasic rise in plasma iβ-EP in response to endotoxin and hence extend earlier observations of a biphasic release of plasma corticosteroids in endotoxin fever (*2, 13*). The first phase of iβ-EP secretion may be terminated by rapidly rising plasma levels of corticosteroids; further study will be necessary to test this hypothesis.

2) Our studies indicate that the initial release of both β-EP and β-LPH into the periphery occurs before, and hence not in response to, endotoxin-induced hyperthermia or hypotension, even though the latter can independently provoke release of adrenocorticotropic hormone (ACTH) (*14*) and, presumably, iβ-EP (*3*). Our studies cannot exclude hypertension as a stimulus to iβ-EP release, but studies of ACTH (*14*) make this unlikely. Rather, the transient hypertension may be a result of iβ-EP release (*15*).

3) Our finding that the second phase of iβ-EP secretion occurs during the recovery from endotoxin-induced hypotension excludes falling blood pressure as the trigger for this second phase.

4) The presence of a distinct shift in the molecular weight profile of iβ-EP between the first and second phases of secretion provides evidence in vivo for two secretory pools of iβ-EP; this is consistent with recent findings in vitro (*16*) and analogous to findings for other pituitary hormones (*17*). Moreover, rec-



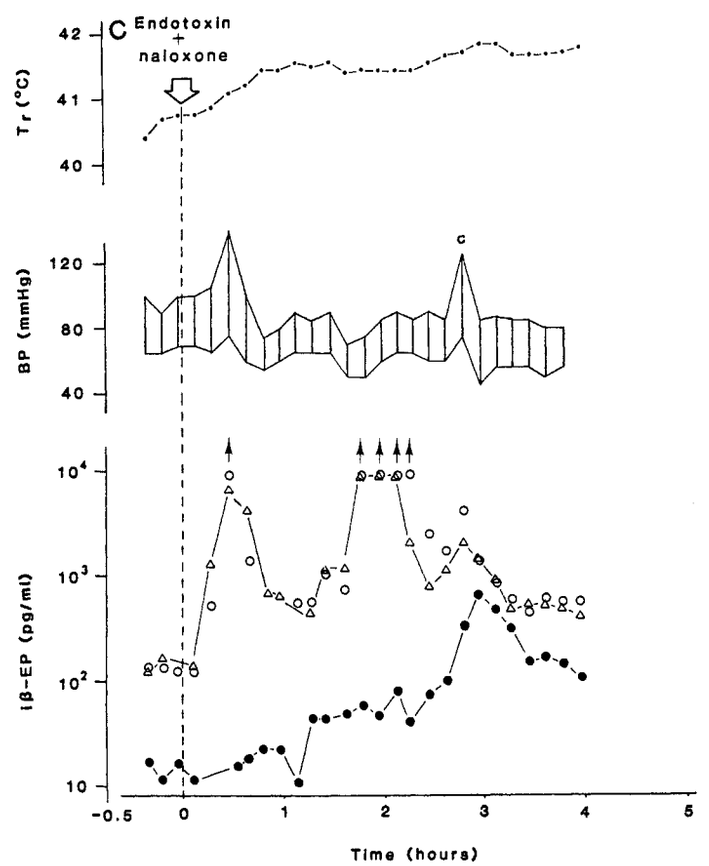


Fig. 1. (A) Rectal temperature (T_r), blood pressure (*BP*), and immunoreactive β-endorphin (iβ-EP) in plasma and CSF in a representative sheep given endotoxin. Immunoreactive β-endorphin in the (○) jugular vein, (△) carotid artery, and (□) sagittal sinus were assayed with 50 μl of plasma per assay tube, using standards to which plasma was added. Immunoreactive β-endorphin in the (●) CSF was measured with 100 μl of CSF per assay tube, using a curve derived from plasma-free standards. Estimation limits of iβ-EP averaged 60 pg/ml for plasma and 30 pg/ml for CSF. A log scale is used for iβ-EP. (B) Mean (solid line) ± S.E.M. (dotted line) of differences of vital signs from baseline values and ratios of iβ-EP to baseline values in seven sheep treated with endotoxin only. Crossbars show means ± S.E.M. obtained by identifying each peak and trough in individual animals and then averaging their times and levels independently. (C) Responses [same scales as in (A)] in an animal given endotoxin plus naloxone; *C* on blood pressure graph indicates convulsion.

ognition that changes in the molecular weight profile of iβ-EP occur during stimulated secretion could help to clarify conflicting findings on the molar ratio of β-EP to β-LPH (*18*); the range of reported values might reflect differences in time elapsed from the onset of stimulated secretion to collection of plasma specimens.

5) The enhancement of iβ-EP release during naloxone administration is evidence of "short-loop" feedback—previously observed only in unstressed subjects (*19*)—modulating stress-induced secretion of endogenous opioids. This finding implies that the amelioration of endotoxic shock by naloxone (*1*) occurs despite acute augmentation of already high circulating levels of β-EP and β-LPH.

6) The demonstration of late increases in CSF iβ-EP after endotoxin administration suggests that physiological stimuli, such as fever, that provoke an outpouring of iβ-EP into the periphery may occur in concert with alterations of central opioid peptide metabolism. Though not in itself evidence either for direct secretion of iβ-EP from the brain into the CSF or "leakage" of iβ-EP into the CSF through the blood-brain barrier, this last finding supports the hypothesis that certain behavioral correlates of fever may be manifestations of altered central metabolism of peptides related to iβ-EP (*20*).

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Significance of Tissue *myo*-Inositol Concentrations in Metabolic Regulation in Nerve

Abstract. *Approximately 25 percent of resting energy utilization in isolated nerve endoneurium is inhibited by medium containing defatted albumin and selectively restored by arachidonic acid but is unaffected by indomethacin or nordihydroguaiaretic acid. The same component of energy utilization is inhibited by small decreases in endoneurial* myo-*inositol, which decrease incorporation of carbon-14–labeled arachidonic acid into phosphatidylinositol. The fraction of the resting oxygen uptake inhibited by ouabain is decreased 40 to 50 percent by a reduced tissue* myo-*inositol concentration or by defatted albumin. Metabolic regulation by rapid, basal phosphatidylinositol turnover is dependent on the maintenance of normal tissue* myo-*inositol concentrations.*

The significance of the high concentrations of *myo*-inositol (MI) normally maintained in most mammalian tissues (*1*) is unknown. This question has much broader import, but is central to a hypothesis that a decrease in nerve MI is a critical factor in the pathogenesis of diabetic neuropathy (*2*). *myo*-Inositol is directly incorporated into phosphatidylinositol (PI) by CDPdiacylglycerol—inositol 3-phosphatidyltransferase (PI synthetase) in the terminal step of PI synthesis (*3*). Studies with ^{32}P-labeled inorganic phosphate and labeled MI indicate that most tissues contain pools of membrane PI that undergo a rapid cycle of partial degradation and resynthesis (PI turnover) (*3*); this occurs in peripheral nerve axons (*4*). The cycle is initiated by a phospholipase C cleavage of PI and ultimately is completed by the PI synthetase reaction (*3*). The predominant species of PI in tissues is 1-stearyl-2-arachidonoyl (*3, 5*), and free arachidonic acid (AA) may also be released and reincorporated during PI turnover (*3, 6*). Rapid PI turnover in specific pools serves as a mechanism for metabolic regulation (*3, 6*).

Recently, interest in PI turnover has centered on its role in regulating free AA levels and prostanoid metabolism (*6*) and its relationship to the regulation of cytoplasmic free Ca^{2+} (*3, 7*); a role in regulating Na^+,K^+-adenosinetriphosphatase (ATPase) in some tissues is also postulated (*3*). The regulatory functions ascribed to rapid PI turnover in specific pools requires the largely unnoted assumption that the resynthesis of PI in these pools is normally independent of the MI concentration, although most PI synthetases have a relatively high Michaelis constant (K_m) for MI—for instance, 1.5 m*M* for guinea pig brain PI synthetase (*8*). We found evidence that in an endoneurium preparation approximately 25 percent of resting energy utilization, including a component of Na^+,K^+-ATPase activity, is regulated through PI turnover by mechanisms that do not appear to involve prostanoid metabolism. The activity of this fraction of energy utilization is acutely and reversibly inhibited by depletion of free AA or by small decreases in tissue MI, which decrease [1-^{14}C]AA incorporation into PI approximately 40 percent. Regulation of this fraction ap-

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