Effect of Thyrotropin-Releasing Hormone on Lipoxyngease-Induced Hypotension in the Unanesthetized Guinea Pig

Warren E. Lux, Jr.¹, Giora Feuerstein¹ and Alan I. Faden ¹,²

Received: July 24, 1983; accepted: October 22, 1983.

Abstract  Soybean lipoxyngease, an enzyme which catalyzes the formation of the vasoactive lipid 15-hydroperoxy eicosatetraenoic acid (15-HPETE) from arachidonic acid, was administered to unanesthetized guinea pigs previously prepared with indwelling vascular cannulae for continuous cardiovascular monitoring. Administration of this enzyme (150 mg/kg IV) resulted in profound hypotension in this model, but no cardiovascular change was observed after administration of equal weight or equimolar amounts of another protein (ovalbumin). The lipoxyngease-induced hypotension, moreover, was promptly reversed by the peptide thyrotropin-releasing hormone (TRH) (2 mg/kg IV) but not by the opiate receptor antagonist naloxone (2 mg/kg IV). This TRH-naloxone dissociation was comparable to that previously observed in hypotension produced by leukotriene D₄ (LTD₄), platelet-activating factor (PAF), or antigen-induced anaphylaxis in the same species. Thus, although its properties as a "physiologic" opiate antagonist led to the early trials of TRH in endotoxic, hypovolemic and spinal shock, it is now apparent that TRH reverses several other forms of experimental shock, including those caused by lipoxyngease, through non-endorphin-related mechanism.

A role for endogenous opioids (endorphins) in the pathophysiology of some forms of circulatory shock has been suggested by the demonstration that the opiate receptor antagonist naloxone improves cardiovascular function and survival in endotoxic (1, 2), hypovolemic (3) and spinal (4, 5) shock models. A similar therapeutic effect of thyrotropin-releasing hormone (TRH) in these same models has been attributed to its "physiologic" anti-opiate properties (6, 7). However, recent studies from this laboratory have demonstrated that TRH, but not naloxone, reverses hypotension caused by the administration of either leukotriene D₂ (LTD₂) (8, 9, 10) or platelet-activating factor (PAF) (11) to unanesthetized guinea pigs. Moreover, we have shown that this TRH-naloxone dissociation also applies to experimental anaphylactic shock (12), a condition in which a number of vasoactive substances, including LTD₂ and/or PAF, may play a pathophysiologic role. Such findings suggest a non-endorphin-related mechanism of action for TRH in these models and raise the possibility that such a mechanism applies to other shock models as well.

Soybean lipoxyngease is an enzyme which catalyzes the formation of 15-hydroperoxy eicosatetraenoic acid (15-HPETE) from arachidonic acid (13). Like LTD₂ and PAF, 15-HPETE is a lipid compound which has been shown to contract vascular and non-vascular smooth muscle in vitro (13). In addition, administration of soybean lipoxyngease to unanesthetized sheep causes changes in the pulmonary microcirculation which are thought to be secondary to in vivo 15-HPETE generation (14). We therefore studied the systemic cardiovascular effects of soybean lipoxyngease administration in our unanesthetized guinea pig model and examined the therapeutic effects of TRH and naloxone.

Materials and Methods

Male Hartley strain guinea pigs weighing 500–600 g had indwelling femoral arterial and venous cannulae surgically implanted by a method described previously (8, 9). Studies were performed 24 or more hours after cannulation at which time the animals were fully awake and freely moving in their home cages. At the time of study, blood pressure was continuously measured from the arterial cannula and recorded on a physiograph (Narco MK-IV). Soybean lipoxyngease (Sigma, Type I, 150 mg/kg IV) was administered to 18 animals randomly divided into three groups. The experimental groups received either TRH (Beckman, 2 mg/kg IV, n=6) or naloxone (Endo, 2 mg/kg IV, n=6). The control group (n=6) received equal volume physiologic saline. The doses of TRH and naloxone were selected on the basis of previously demonstrated efficacy in other shock models (2, 3, 4, 6, 7).

Results

Soybean lipoxyngease (150 mg/kg IV) produced profound hypotension to approximately 25 mmHg in this model, and a depressor effect was still apparent at 20 min. This hypotension was reversed by TRH, whereas naloxone had no effect (Fig. 1).

The difference in mean arterial pressure between the TRH group and either the naloxone group or the saline control group was significant throughout the period of study following pharmacologic intervention (repeated measurement ANOVA; F = 23.25, p < 0.001, n=6 for TRH vs. naloxone; F = 81.92, p < 0.001, n=6 for TRH vs. controls). Animals given a protein other than lipoxyngease (IV ovalbumin in either equal weight or equimolar amounts) exhibited no cardiovascular change (data not shown).

Discussion

Both TRH and naloxone have previously been shown to improve shock caused by endoxemia (1, 2, 6), hypovolemia (3, 6) or spinal injury (4, 5, 7). In shock caused by LTD₂ (8, 9, 10), PAF (11) or anaphylaxis (12), however, TRH is effective, whereas naloxone is not. The current study demonstrates that the administration of soybean lipoxyngease results in a similar kind of TRH-responsive, naloxone-resistant shock. Whether this shock results from
in vivo formation of the vasoactive lipid 15-HPETE is not certain, but the fact that ovalbumin produced no cardiovascular change suggests that lipoygenase-induced hypotension is unlikely to be a non-specific phenomenon secondary to a large protein load. Although it has previously been postulated that TRH's mechanism of action in shock involves "physiologic" opiate antagonism (6, 7), the TRH-naloxone dissociation in the current study, as well as that in the several prior ones, indicates that the therapeutic effect of TRH in these models is unlikely to be mediated by alteration of endogenous opioid effects. Whether the beneficial actions of TRH in naloxone-sensitive shock models are similarly independent of endogenous opioid system remains to be determined.

The mechanism of action of TRH in naloxone-resistant shock, moreover, remains unclear. In the LTD4 model, plasma catecholamines rise during the hypotensive phase, and this sympathetic-adrenomedullary activation is enhanced by both TRH (10) and indomethacin (in review). Only TRH, however, improves the blood pressure (9, 10). Furthermore, at pharmacologic doses, the pressor effect of TRH in normal animals has been shown to be independent of associated catecholamine release (15). Taken together, these data suggest that sympathetic-adrenomedullary mechanisms are unlikely to account for all of the non-endorphin-related therapeutic effects of TRH. Neither do parasympathetic mechanisms appear to be critical since N-methylatropine does not improve blood pressure in shock produced by either LTD4 (9, 10) or PAF (in review).

Regardless of the mechanism or site of action of TRH, however, the existence of naloxone-resistant shock models, such as the current one, also implies that the pathophysiology of endorphins in different shock states may vary. In lipoygenase-induced shock, as in LTD4, PAF and anaphylactic shock, endorphins appear to play a less important pathophysiological role than in shock due to endotoxemia, hypovolemia or spinal injury. Thus, the ability of TRH to alter shock through non-endorphin-related mechanisms adds to its therapeutic potential and provides the rationale for the ongoing evaluation of this peptide in the pharmacology of experimental shock of diverse etiologies.

Acknowledgements
The authors are grateful for the technical assistance of G. P. Smith, the statistical support of F. A. Meeks and the assistance of E. M. Bell and J. C. Mosely in preparing the manuscript.

This research was supported by the Uniformed Services University of the Health Sciences Protocol No. R 09212. The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the view of the Department of Defense or the Uniformed Services University of the Health Sciences. The experiments reported herein were conducted according to the principles set forth in the Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, National Research Council (DHEW Publication [NIH] 78-23, 1978).

References