REPORTS

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

Warren E. Lux, Jr.[1], Giora Feuerstein[1] and Alan I. Faden [1,2]

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

Abstract Soybean lipoxygenase, 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 lipoxygenase-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 D_4 (LTD_4), 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 that caused by lipoxygenase, 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 D_4 (LTD_4) (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 LTD_4 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 lipoxygenase is an enzyme which catalyzes the formation of 15-hydroperoxy eicosatetraenoic acid (15-HPETE) from arachidonic acid (13). Like LTD_4 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 lipoxygenase 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 lipoxygenase 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 lipoxygenase (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 lipoxygenase (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 lipoxygenase (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 endotoxemia (1, 2, 6), hypovolemia (3, 6) or spinal injury (4, 5, 7). In shock caused by LTD_4 (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 lipoxygenase results in a similar kind of TRH-responsive, naloxone-resistant shock. Whether this shock results from

1 Neurobiology Research Unit, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814

2 Address correspondence to: Alan I. Faden, M. D., Neurobiology Research Unit, USUHS, 4301 Jones Bridge Road, Bethesda, MD 20814

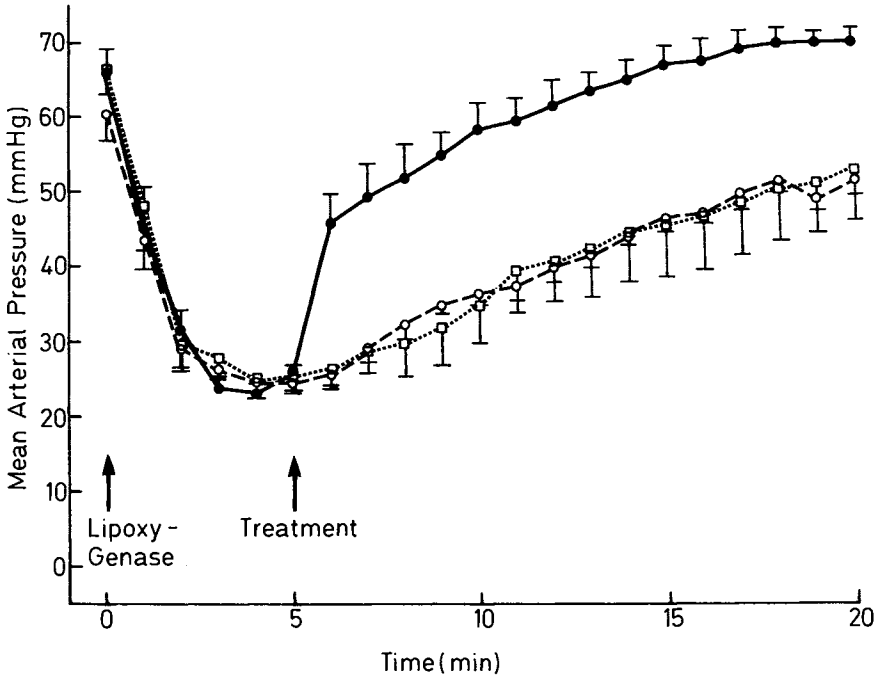

Fig. 1 Effect of TRH (2 mg/kg IV) (●——●) and naloxone (2 mg/kg IV) (□····□) on the hypotension produced by soybean lipoxygenase (150 mg/kg IV) in unanesthetized guinea pigs. Controls are shown by ○——○. The hypotension is promptly reversed by TRH whereas naloxone is without effect. Results are the mean ± SEM for six animals in each group.

in vivo formation of the vasoactive lipid 15-HPETE is not certain, but the fact that ovalbumin produced no cardiovascular change suggests that lipoxygenase-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 endorphin effects. Whether the beneficial actions of TRH in naloxone-sensitive shock models are similarly independent of endogenous opioid systems remains to be determined.

The mechanism of action of TRH in naloxone-resistant shock, moreover, remains unclear. In the LTD_4 model, plasma catecholamines rise during the hypotensive phase, and this sympatho-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 sympatho-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 LTD_4 (9, 10) or PAF (in review).

The site of action of TRH, on the other hand, is somewhat more clearly defined and appears to be within the central nervous system (CNS). Intracerebroventricular (ICV) administration of TRH has been shown to produce a pressor effect in normal animals (15); it has also been shown to reverse LTD_4 hypotension at doses which are ineffective when given systemically (10). In addition, preliminary observations in PAF-induced shock suggest a comparable response to ICV TRH in that model as well (in review). Although the precise CNS sites responsible for the cardiovascular actions of TRH are not known, this peptide has been localized within a number of brain cardio-regulatory centers, including the anterior hypothalamus, nucleus tractus solitarius and nucleus ambiguus (16, 17). Moreover, a particularly potent pressor effect has been demonstrated by discrete injections of TRH into the pre-optic region of the anterior hypothalamus of the rat (18).

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 pathophysiologic role of endorphins in different shock states may vary. In lipoxygenase-induced shock, as in LTD_4, PAF and anaphylactic shock, endorphins appear to play a less important pathophysiologic 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 09 212. 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

1. Holaday, J. W., Faden, A. I. (1978) Nature 275, 450–451.
2. Faden, A. I., Holaday, J. W. (1980) J. Pharmacol. Exp. Ther. 212, 441–447.
3. Faden, A. I., Holaday, J. W. (1979) Science 205, 317–318.
4. Faden, A. I., Jacobs, T. P., Mougey, E., Holaday, J. W. (1981) Ann. Neurol. 10, 326–332.
5. Holaday, J. W., Faden, A. I. (1980) Brain Res. 189, 295-299.
6. Holaday, J. W., D'Amato, R. J., Faden, A. I. (1981) Science 213, 216–218.
7. Faden, A. I., Jacobs, T. P., Holaday, J. W. (1981) N. Engl. J. Med. 305, 1063–1067.
8. Lux, W. E. Jr., Feuerstein, G., Faden A. I. (1983) Prostaglandins Leukotrienes Med. 10, 301–308.
9. Lux, W. E. Jr., Feuerstein, G., Smith, G. P., Faden, A. I. (1983) in Leukotrienes and Other Lipoxygenase Products (Piper, P. J., ed.), pp. 338–342, John Wiley & Sons, Chichester.

10. Lux, W. E. Jr., Feuerstein, G., Faden, A. I. (1983) Nature 302, 822–824.
11. Lux, W. E. Jr., Feuerstein, G., Snyder, F., Faden, A. I. (1983) Circ. Shock 10, 262.
12. Lux, W. E. Jr., Feuerstein, G., Faden, A. I. (1983) Eur. J. Pharmacol. 90, 301–302.
13. Aharony, D., Smith, J. B., Smith, E. F., III, Lefer, A. M. (1981) Prostaglandins Med. 7, 527–535.
14. Smith, M., Gunther, R., Zaiss, C., Flynn, J., Demling, R. (1981) Circ. Shock 8, 647–656.
15. Horita, A., Carino, M. A., Weitzman, R. E. (1979) in Catecholamines: Basic and Clinical Frontiers, Vol. 2 (Usdin, E., Kopin, I. J., Barchas, J., eds.), pp. 1140–1142, Pergamon Press, New York.
16. Cummings, S., Elde, R., Ells, J., Lindall, A. (1982) Soc. Neurosci. Abstr. 8, 110.
17. Lechan, R. M., Jackson, I. M. D. (1982) Soc. Neurosci. Abstr. 8, 111.
18. Feuerstein, G., Hassen, A. H., Faden, A. I. (1983) Peptides 4, 617–620.

Importance of Structural Free Space to the Solvent Power of Water

Reinhard Hüttenrauch[1, 2], Petra Zielke[1], and Sabine Fricke[1]

Received: August 28, 1983; accepted: November 17, 1983.

Abstract: The contribution of structural free space to the solvent power of water was examined by a systematic modification of the geometric factor. Gaps and holes, available to foreign molecule occupation, are thought to be filled at low concentrations (max. 1 %) of aliphatic alcohols. The effect upon solvency reached approximately 10 %, which suggests that spatial parameters affect solvent power. The results demonstrate the importance of solvent purity in the dissolution process.

According to the structural model of Eyring (1, 2) liquids represent highly disordered solids containing numerous vacancies and holes (Fig. 1). Under normal conditions each liquid exhibits approximately 3 % empty volume, while at the critical point the fraction of holes reaches 50 %. The vacancies are able to move at random; their formation requires about 10^{-20} J work each. In general, the holes of the perforated structure occupy the size of one or several liquid molecules. However, in this form the hole theory and the hole defects model are only applicable to non-polar liquids with low interaction energy of about 10^{-21} J (3). Definite numerical data are available for the free volume (hole volume) of several liquids (4, 5).

As far as polar liquids, such as water, are concerned, an "Orientation Defect Model" has been developed (6) on the basis of misorientation of hydrogen atoms. Accordingly, the properties of water could be described as a "Two-State-H-Bond Breaking Equilibrium" between OH-groups with and without hydrogen bondings (in short: Two-State Model). The orientation defects (Bjerrum-Defects) probably cause cluster limitations and cluster interfaces. At the border of ordered zones, however, structural holes may be formed even in the case of polar liquids (Fig. 1) (7). Thus, some authors (8) suggest that water also consists of a perforated structure characterized by holes.

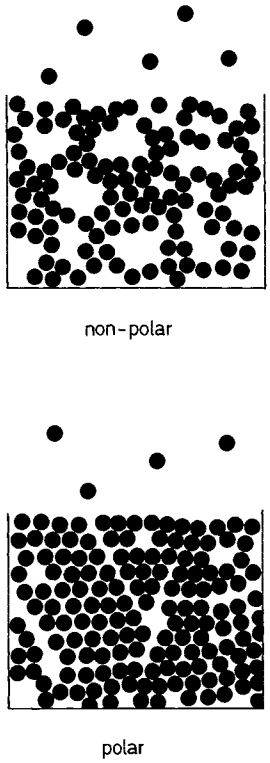

Fig. 1 Scheme of hole defects in liquid structure.

In addition to the free space caused by disordering, the principle of ordering contributes considerably to the empty volume in solvent structure. The tetrahedral short-range order of water implies such a poor space economy that only 12 % of the volume is occupied by matter. The anomalies of water could be attributed to this low degree of space filling that arises from its highly ordered structure (9). Hence, there are different structural features forming non-occupied places and regions.

We examined the potential importance of structural free space to the solvency of water as a provocative new concept.

Materials and Methods

The water system was modified with the addition of low aliphatic alcohols of relatively small molecular volumes: methanol, ethanol, *n*-propanol, 1-butanol, 2-butanol, and 2-methyl-2-propanol. As a test substance for the determination of solvent power the new lipophilic steroid dienogest (a peroral gestagen, 17α-cyanomethyl-17β-hydroxy-estra-4,9(10)-dien-3-on; $C_{20}H_{25}NO_2$; MW 311.4) was chosen.

The dissolution kinetics were investigated with a closed flow system using the paddle-principle. The dissolved drug concentration was measured spectrophotometrically at 315 nm.

Results

With the use of low aliphatic alcohols as solvency-blocking agents, we found that the saturation concentration of dienogest in water as a function of alcohol content showed extreme values in each case (Fig. 2).

Remarkably, small amounts of the alcohols influenced the solubility of the steroid negatively, although they repre-

[1] Department of Research and Development, VEB Jenapharm, Otto-Schott-Straße 13, DDR-6900 Jena.
[2] Correspondence.