Inflammatory Mediators

Edited by
G.A. Higgs
T.J. Williams

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INFLAMMATORY MEDIATORS

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Preface

The visual manifestations of inflammation were first correlated with changes in blood vessels in the 18th century and in the following century the cellular components were first observed. The contribution of the present century was the chemical mediator. This may seem a dubious contribution to any individual who is first faced with the cacophony of inflammatory mediators, with their disparate sources, functions and structures. To others, inflammatory mediators, with all their diversity and complexity, remain a constant source of fascination.

This book, based on a symposium held at the Royal College of Surgeons in August 1984, gives an insight into the current views on inflammatory mechanisms. We have asked experts to review the latest findings in their particular area of interest. We have deliberately requested contributions from individuals with a wide interest so that this book covers vasoactive amines, prostaglandins, leukotrienes, platelet activating factor, fibrin-derived peptides, complement-derived peptides, neuropeptides and cytokines. With this wide approach this book will prove useful to clinicians, established research workers and will also provide an introduction to those beginning their careers in research.

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Gerry Higgs and Tim Williams
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1
Inflammatory Mediators: Where are they Going?

G. Majno

It was most appropriate that a Symposium on Inflammatory Mediators should take place in London, where the first inflammatory mediator - histamine - was born.

It was actually a rebirth: the chemical compound histamine saw the light in 1907, in Germany, as a derivative of histidine. Its first link with biology was not inflammation but, more modestly, putrefaction (1). Within four years G. Barger and Sir Henry Dale described its effects on smooth muscle, in 1918 Sir Henry Dale and A.H. Richards found that it increased vascular permeability; and finally, in 1924, Sir Thomas Lewis and R.T. Grant postulated that a histamine-like substance was liberated in injured skin (2). The curtain was raised.

In this short introduction I can scarcely review the history, philosophy and pharmacology of the inflammatory mediators. I will just inflict upon you some thoughts of a pathologist who has flirted with histamine (and with a few of its rivals) for a quarter of a century.

My first thought concerns the enormous number of inflammatory mediators that are known today, and the consequences of this expansion. It has been said that medical knowledge doubles every 10 years. With this assumption, if we start with one mediator in the early twenties, we end up with 64 in 1980. This is just about right, because in 1984 we must have at least 100 mediators that are so named (including about 50 lymphokines). The true number is probably much greater.

I do not envy the reviewers of the year 2000. But let us not pretend that the situation is well in hand today. The concept of "inflammatory mediator" may sound reasonably limited, but in fact the field has become so specialised that no single individual known to me masters it all (and if I am insulting someone here, I will be delighted to apologise). The leukotriene people are totally removed from 5-HT, the immunologists speak essentially to each other, the free radical people are in a world of their own, few of the above have ever heard of

Figure 1: An example of vascular labelling by an inflammatory mediator. Rat cremaster muscle; 5 minutes after local injection of 5-HT, a suspension of carbon black was injected i.v. One hour later all the venules are blackened, i.e. "labelled" (carbon particles seep through endothelial gaps, and are trapped against the intact basement membrane). Scale: 100 mμ (from Majno et al., 1961, ref. 3).

vascular labelling, and so on. We have even run out of human names: some of the new ones can scarcely be memorised, let alone pronounced, like HPETE or AGEPC. Working conditions in the field of inflammation today remind me of those that must have prevailed in the tower of Babel.

As the number of mediators has grown, so has the number of targets. In the beginning the only target was the microcirculation: two effects were known (for histamine, and then for histamine-like mediators): (a) changes in arteriolar smooth muscle, leading to arteriolar dilatation or constriction; and (b) some mysterious change in the endothelium causing it to leak; the dogma was that mediator(s) induced an increase in "capillary permeability" (1) and there are many physiologists who still believe it. It took another 40 years or so to work out what was really going on. That was one of my most exciting scientific adventures, in collaboration with Dr. Palade and Dr. Schoefl (3). It turned out that after the injection of histamine, 5-HT or bradykinin, the capillaries remained intact; the leakage came from the venules, through gaps between endothelial cells. Seven more years, and we found that the gaps were caused by endothelial contraction (4) later confirmed in vivo by Joris et al. (5). This was intellectually pleasing, because it showed that the two apparently unrelated effects of histamine-type mediators (changes in muscular tone, and changes in permeability) could now be explained by a single cellular response: contraction.

Another useful product of those experiments was the phenomenon of vascular labelling: a few drops of India ink in the blood stream of an experimental animal can demonstrate, simply, cheaply, and strikingly, all the leaking vessels (Fig. 1). This is how we could establish that practically all the leakage occurred in the venules.

Back to the number of targets: until the 1930's the microcirculation attracted all the attention. In the late 30's, Valy Menkin showed that also the accumulation of leukocytes could be explained in terms of mediators, i.e. of endogenous substances capable of attracting white blood cells out of vessels (6). His "leukotaxine" was probably a mixture of polypeptides and can no longer be identified with any specific mediator, but the point was made: white blood cells could also be a target for mediators.

Today, any cell is a fair target for mediators, including mast cells, fibroblasts, epithelia, smooth muscle, not to mention the endothelium and all the white blood cells. It is now known that histamine can send messages even to the lymphocytes (7) and that lymphocytes can issue orders to almost any cell. Long gone are the easy times when, in order to ascertain whether any given substance was a mediator, it was enough to inject it, and check whether it made either vessels leak or white blood cells emigrate.

As the number of cell targets has increased, the number of possible responses has increased even more dramatically, because every

cell, when prodded, will express its own reaction in terms of its own particular receptors and its own particular metabolism. The functional responses may be extremely subtle: a cell can be induced to express or reveal a new antigen on its surface, without any visible change (8); or the target cell may simply become "activated". We have learned that all inflammatory cells can exist in these two conditions -resting and activated - and this may ultimately become true for all cells. Activated cells then produce new mediators of their own. The inflammatory soup is becoming thicker and thicker.

The situation is further complicated by the fact that many of the known mediators have opposite effects, or effects that become reversed as a function of concentration; and then some mediators last only seconds or less, so that they can only be presumed to exist. I will venture to conclude that nobody today has, or could have, a clear picture of the mediator interactions in a focus of inflammation.

Why should there by so many mediators? A tentative answer could be that inflammation is essential for survival, thus nature has made sure that it will unfailingly be triggered, by creating a tremendous excess of messages. But there may be another reason.

If it is true that the mediators can "say" almost anything, are we really dealing with inflammatory mediators, or with a chemical language of broader significance? The expression "inflammatory mediators" may be in part a misnomer. Cells must communicate, and they must do so almost exclusively by chemical messages. Perhaps most of the substances that we now call inflammatory mediators really represent the basic language of cells, which speak it not only when they are angry and inflamed (or turned malignant), but also when they quietly communicate in their daily business. To this end, it is most appropriate that an alphabet for that language (arachidonic acid) be placed on the surface,in the cell membrane, where it is ready to be "spoken out".

Capillaries - the Ivory Tower

Having spent much of my time emphasising the number and variety of the inflammatory mediators, of their targets, and of their effects, I would like to point out an extraordinary contradiction: as far as I know, despite all this pharmacologic arsenal, not a single mediator does anything to the capillaries. It is not a marvel of nature that these tiny vessels should be so carefully spared? The world may be all inflamed around them, and yet the evidence shows that they only participate passively, by increasing or decreasing filtration according to pressure and flow, and of course by leaking if they are directly injured. But unless they are physically damaged they will simply carry on their duty with utter disregard of the fuss all around.

The only explanation I can offer is that capillaries represent the critical segment of the circulation, where the vital exchanges between blood and tissues take place. All the rest is plumbing. This particular

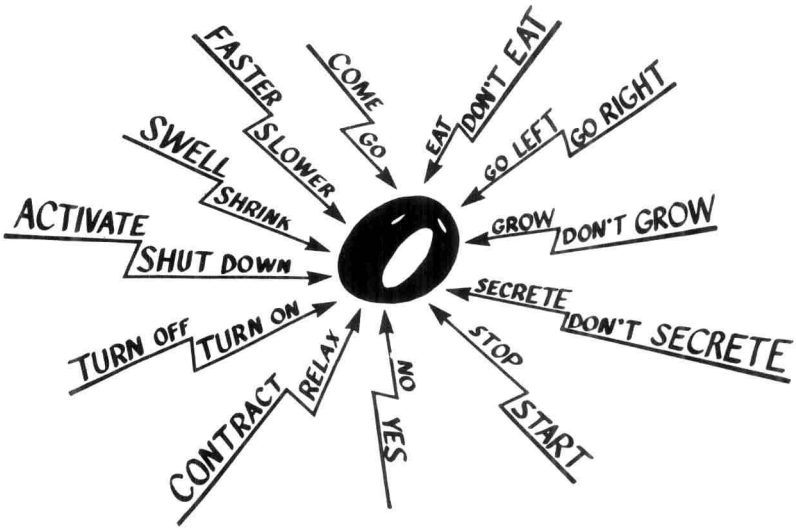

Figure 2: Dilemmas of a cell caught in a focus of inflammation.

part of the system must be spared at all cost: life in the tissues must go on, even if battles are raging in the connective tissue spaces.

To translate this loose talk into pharmacological terms, it could be that the capillaries do not respond because they have not been endowed with the required receptors. It may well turn out that all the permeability-increasing mediators known today have receptors only in the venules. For histamine, this has been elegantly shown in the laboratory of N. and M. Simionescu (9) using a complex of histamine and ferritin that could be visualised under the electron microscope.

Incidentally, if it is true that all the leakage-inducing mediators affect the venules, how do they induce them to leak? Is it always by endothelial contraction? Surprisingly, very little is known along these lines. The only "new" mediator that has been tested as regards its capacity to induce endothelial contraction is leukotriene E_4: it does (10). Conceivably, other mechanisms of endothelial leakage could exist, such as the unzipping of endothelial cells. This area of mediator biology needs further study.

To close, I would like you to put yourselves, for just a moment, in the place of a cell somewhere in the tissue spaces, suspended in the inflammatory soup, and prodded from all sides by mediators. Imagine the cacophony: hundreds of orders, with conflicting instructions, all shouted at the same time (Fig. 2).

I wish someone could tell us how each individual cell, immersed in all this noise, knows how to pick out the significant messages, and act accordingly.

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2
The Role of Histamine in Inflammation

J.C. Foreman

The study of inflammation has traditionally been an area for the pathologist and, more recently, the immunologist. However, the pharmacologist has, I believe, a major role to play, especially in the assessment of putative mediators of inflammation.

Although originally designed to examine the role of putative neurotransmitters, Dale's criteria can usefully be applied to mediators of inflammation and this is an area where pharmacologists have a contribution to make in the study of inflammation. A modification of Dale's criteria, which should be fulfilled if a substance is to be considered a mediator of inflammation, can be stated as follows.

1. The inflammatory response should be mimicked by application of the putative mediator in appropriate concentrations to the relevant tissue both in vitro and in vivo.

2. The mediator should be released from the tissue during an inflammatory reaction both in vivo and in vitro.

3. Enzymes for the production of the mediator should be present in the tissue, and their activity should demonstrably increase when there is increased turnover of the mediator during the inflammatory reaction.

4. Since inflammatory reactions do not persist unmodified indefinitely, there should be a mechanism for terminating the action of the mediator: such a mechanism may include catabolism, uptake of the mediator or desensitisation of the tissues to its action.

5. Predictable effects should arise from the pharmacological modification of synthesis, storage, release, metabolism or action of the putative mediator both in vitro and in vivo.

6. Clinical conditions where there is excess or deficiency of the mediator should have the predictable effects on the inflammatory reaction.