

FREE RADICALS AND HISTAMINE

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Our interest in the theme of free radicals and histamine was stimulated by the observation that the incubation of xanthine oxidase and hypoxanthine with rat peritoneal mast cells resulted in the release of histamine [1]. We followed this pathway and were encouraged to proceed by the demonstration that rat serosal mast cells coincubated with human neutrophils activated by the chemotactic peptide (fMLP) underwent massive granular exocytosis and histamine release [2]. Subsequent evidence has been provided for the release of histamine by oxy-radicals, such as in hyperoxic exposure, antracycline cardiomyopathy, and oleic acid-induced pulmonary vessels injury [see 3, for a review]. We also demonstrated that in the coincubation model of rat mast cells with activated human neutrophils, the production of superoxide from human neutrophils paralleled the release of histamine from rat mast cells [3]. Besides the oxy-radicals, it was generally accepted that free radical metabolites can be generated from almost any aromatic xenobiotic by the intervention of specific isoforms of hepatic microsomal cytochrome P-450. In further experiments, we selected paracetamol, cocaine and mitomycin C, compounds relevant to human pathology and all known to be activated by carbon-centered or nitrogen-centered free radicals. To test the release of histamine by their oxidative metabolites, we developed a bioassay in which isolated purified rat mast cells were incubated with a variety of concentrations of the drugs under study, alone or in the presence of S-10 liver homogenate fraction of rats treated with microsomal enzyme inducers (Aroclor 1254, PCB; phenobarbital). Under these circumstances, paracetamol, cocaine and mitomycin C release histamine from mast cells only in the presence of liver microsomes. The process was exocytotic in nature (except in the lysis induced by mitomycin C) and the free radical nature of the event was suggested by the concomitant generation of markers of membrane lipid peroxidation (malonyldialdehyde; conjugated dienes) and by the abatement of the release of histamine by free radicals scavengers [3, 4]. In the same experimental model, drugs of abuse (morphine) or drugs used in the treatment of heroin addiction (methadone) release histamine in a free radical driven manner [5]. Histamine release by free radicals is implicated in the pathophysiology of inflammation. Using the ESR spin trapping technique, a carbon-centered free radical intermediate was demonstrated in the prostaglandin-synthetase (PHS) oxidation of arachidonic acid, identified as the bicyclic endoperoxide PGG-2 [6]. Using rat serosal mast cells as detector of histamine release, we have shown that arachidonic acid evokes the release of histamine only after metabolic activation in the presence of PHS isolated from calf seminal vesicles [3, 7]. Two basic steps in inflammation, such as the prostanoid cascade and the release of histamine could be linked: arachidonic acid released by phospholipase A₂ not only initiates the synthesis of prostaglandins but also the release of mast cell histamine via the PHS-generated free radical intermediates.

The concept that reperfusion of ischemic heart causes a paradoxical extension of the ischemic damage (reperfusion injury) is widely accepted. The implication of free radicals in the pathophysiology of reperfusion injury starts from the direct measurement of free radical generation following reperfusion of ischemic myocardium [3]. During ischemia, xanthine-dehydrogenases are converted to xanthine-oxidases and the breakdown of ATP leads to xanthine, thus producing the xanthine-xanthine-oxidase system, capable of generating superoxide anions when the oxygen tension increases in the reperfusion phase. In turn, superoxide would release histamine from resident cardiac mast cells, causing arrhythmias, ventricular fibrillation and amplifying cardiac damage. In an *in vitro/ex vivo* model of focal ischaemia/reperfusion (I/R) in isolated guinea pig hearts, we have shown that in the reperfusion phase, cardiac mast cells lose granule metachromasia, histamine and lactate dehydrogenases (LDH) are released and severe arrhythmias are induced. These effects were abated by a free radical scavenger N-t-butyl-phenyl-nitron (BPN) and by allopurinol [3].

The gaseous transmitter nitric oxide (NO) has been shown to produce beneficial hemodynamic effects [8]. In an *in vitro/ex vivo* model of focal I/R of guinea pigs hearts, we have shown that perfusion of the heart with NO donors (SNP; SIN-1) or generators (GTN) reduces the release of histamine and LDH, the loss of mast cells metachromasia and the severity of arrhythmias. These effects were potentiated by superoxide dismutase (SOD) [9]. In an *in vivo* model of focal I/R of rat heart, a selective SOD mimetic M40403, exerts a protective effect against I/R injury [10]. These results suggest that NO, SOD and a SOD mimetic produce myocardial salvage in I/R, supporting a key role of superoxide in the reperfusion injury, in which the release of histamine by superoxide is a significant event. In the same model, pretreatment with hemin increases the expression and activity of both heme-oxygenase-1 and inducible NO-synthase, suggesting that the gaseous transmitter carbon monoxide (CO) cooperates with NO in abating the release of histamine by free radicals in I/R [11]. Should these experimental data be translated into the clinical setting, the release of histamine by free radicals produced by drug metabolism might be relevant in the pathophysiology of adverse drug reactions. The release of histamine by free radicals produced by PHS within the prostanoid cascade and the release of histamine by free radicals produced in I/R, may be relevant in the pathophysiology of inflammation and of cardiac reperfusion injury. In this context, the endogenously generated NO and CO could act as an antioxidant defense, down-regulating the release of histamine by free radicals.

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