Biochemical Pharmacology, Vol. 39, No. 5, pp. 879–889, 1990.
Printed in Great Britain.

0006-2952/90 $3.00 + 0.00

HISTAMINE RELEASE FROM RAT MAST CELLS INDUCED BY METABOLIC ACTIVATION OF POLYUNSATURATED FATTY ACIDS INTO FREE RADICALS

EMANUELA MASINI,* BEATRICE PALMERANI,* FRANCESCO GAMBASSI,* ALESSANDRA PISTELLI,* ELIANA GIANNELLA,* BRUNELLA OCCUPATI,* MARIO CIUFFI,* TATIANA BANI SACCHI† and PIER FRANCESCO MANNAIONI‡

*Department of Preclinical and Clinical Pharmacology and †Department of Human Anatomy and Histology, Section of Histology, Florence University, School of Medicine, Florence, Italy

(*Received* 21 *February* 1989; *accepted* 6 *October* 1989)

Abstract—Polyunsaturated fatty acids (PUFA: arachidonic and linoleic acid) release histamine from isolated purified rat serosal mast cells only in the presence of oxidizing systems such as phenobarbital-induced rat liver microsomes, prostaglandin-H-synthetase (PHS) or soybean lipoxygenase. The release of mast cell histamine by activated PUFA has a long time-course and the electron microscopical features are consistent with an exocytotic secretion in the case of arachidonic acid and cell lysis in the case of linoleic acid. The phenomenon is associated with a significant increase in malonyldialdehyde (MDA) and conjugated diene generation, suggesting a relationship between histamine release and membrane lipid peroxidation. The secretion of histamine was inhibited by anti-free radical interventions such as D-mannitol, reduced glutathione and α-tocopherol. Some cyclooxygenase and lipoxygenase inhibitors, cimetidine and carnitine derivatives, are differentially active in the inhibition of mast cell histamine release by activated arachidonic acid. These results suggest that free radical derivatives of PUFA, generated by metabolic activation, trigger mast cell histamine release.

There is extensive evidence demonstrating that oxygen centered free radicals release mast cell histamine in various experimental conditions. Active species of oxygen generated in vitro by xanthine oxidase and hypoxanthine (XOD-HPX) release histamine from isolated rat mast cells without causing nonspecific cell lysis [1], and oxy-radicals produced in the oxidative burst of eosinophils and neutrophils [2–4] have been shown to initiate mast cell histamine secretion. Histamine release was also reported to occur when rat peritoneal mast cells are kept in hyperoxia, in a way which is linearly related to the oxygen tension [5].

A second model of histamine release by free radicals has recently been inferred from experiments showing that a host of xenobiotics such as paracetamol, cocaine and mitomycin C, all known to be activated into free radical species *in vivo* and *in vitro* [6–8], produce the release of histamine from rat serosal mast cells only in the presence of oxidizing systems (rat liver microsomes; prostaglandin-H-synthetase, PHS [9–11]).

The PHS catalysed oxidation of arachidonic acid may be regarded as a third source of endogenously generated free radicals. The radical nature of this reaction was suggested by the observation of an electron paramagnetic resonance signal following addition of substrate fatty acids to seminal vesicle microsomes [12]. Moreover, using the ESR spin-trapping technique, a free radical involved in the oxygenation of arachidonic acid by ram seminal vesicle microsomes has been identified as having the structure of a carbon-centered conjugated fatty acid free radical [13], and a free radical chain mechanism has been proposed to explain the conversion of arachidonic acid to the bicyclic endoperoxide PGG_2 [14]. Another intermediate of arachidonic acid oxidation, 15-hydroperoxy-eicosatetranoic acid (15-HPETE) has been more recently shown to decompose into carbon-centered radicals upon heme or ram seminal vesicle-catalysis [15]. De Groot *et al.* [16] have clearly demonstrated the involvement of a free radical in the oxidation of linoleic acid by soybean lipoxygenase using a water-soluble spin trap to detect the carbon-centered conjugated dienyl linoleic acid free radical, and an oxygen-centered free radical has been more recently identified in the lipoxygenase-linoleic acid system, using a different spin trap (α-4-pyridyl-1-oxide-*N*-*t*-butylnitrone) [17].

These observations prompted us to study whether the *in vitro* activation of polyunsaturated fatty acids (PUFA) by a variety of oxidizing systems could lead to mast cell histamine release, and to define the nature of the releasing process.

MATERIALS AND METHODS

Chemicals. Arachidonic acid (99% pure), linoleic acid (99% pure), prostaglandins (PGE_2; $PGF_{2\alpha}$; PGD_2; 6-keto-$PGF_{1\alpha}$; PGI_2), T_xB_2 and LTE_4 were purchased from Sigma Chemical Co. (Poole, U.K.). Non-steroidal anti-inflammatory drugs included: indometacin from Sigma; acetylsalicylic acid (ASA) from Bayer AG (Leverkusen, F.R.G.), nordihydroguaiaretic acid (NDGA) from Sigma; D-mannitol from Merck (Darmstadt, F.R.G.), superoxide dismutase (SOD) from Sigma; catalase from Wor-

‡ Send correspondence to: Prof. P. F. Mannaioni, Department of Preclinical and Clinical Pharmacology, Viale G.B. Morgagni 65, 50134 Florence, Italy.

Table 1. Mast cell histamine and lactate dehydrogenase (LDH) release induced by arachidonic acid in the presence of prostaglandin-H-synthetase (PHS)

Arachidonic acid* (M)	N	Prostaglandin-H-synthetase* 100 mUnits	500 mUnits	1000 mUnits	5000 mUnits
Histamine release (%)					
10^{-7}	6	4.7 ± 2.2	18.3 ± 1.4	25.4 ± 3.3	29.2 ± 1.9
10^{-6}	8	10.9 ± 1.5	22.7 ± 4.1	32.3 ± 4.7	36.2 ± 4.3
10^{-5}	8	12.0 ± 2.3	26.8 ± 5.8	35.3 ± 3.0	37.3 ± 5.9
10^{-4}	8	19.0 ± 2.1	29.7 ± 2.8	38.8 ± 5.1	44.8 ± 2.2
LDH release (%)					
10^{-7}	6	4.2 ± 2.1	6.2 ± 1.4	5.3 ± 2.3	4.2 ± 1.2
10^{-6}	6	5.7 ± 1.7	6.9 ± 1.3	5.8 ± 1.2	6.3 ± 2.4
10^{-5}	6	4.8 ± 2.1	5.8 ± 1.2	7.7 ± 1.8	7.4 ± 2.6
10^{-4}	8	6.3 ± 4.1	6.3 ± 1.3	6.8 ± 2.4	8.3 ± 2.4

* At the given concentrations both arachidonic acid and PHS, when incubated alone, produced only negligible histamine release which was subtracted from all the values.

The values are expressed as means ± SE of the reported number of experiments performed in duplicate.

Table 2. Mast cell histamine and lactate dehydrogenase (LDH) release induced by arachidonic acid in the presence of S10 mix liver microsomes from phenobarbital treated rats

Arachidonic acid* (M)	N	S10 Mix liver microsomes* 200 μL	400 μL	600 μL
Histamine release (%)				
10^{-6}	6	18.9 ± 2.7	32.3 ± 5.3	44.8 ± 3.6
10^{-5}	6	28.7 ± 3.6	41.3 ± 3.1	48.7 ± 5.3
10^{-4}	6	32.8 ± 2.4	43.7 ± 4.8	49.6 ± 6.2
LDH release (%)				
10^{-6}	6	6.3 ± 1.1	6.9 ± 2.7	4.3 ± 2.6
10^{-5}	6	7.1 ± 2.6	8.3 ± 1.8	8.4 ± 1.9
10^{-4}	6	8.3 ± 3.1	7.8 ± 2.6	7.1 ± 2.3

* At the given concentrations, both arachidonic acid and S10 mix liver microsomes, when incubated alone, produced only negligible histamine release which was subtracted from all the values.

The values are expressed as means ± SE of the reported number of experiments performed in duplicate.

thington Biochem; DL-α-tocopherol and reduced glutathione (GSH) from Sigma; L-acetyl carnitine and L-propionyl carnitine were kindly provided by Sigma Tau. Prostaglandin-H-synthetase (PHS) purified from calf seminal vesicles was purchased from Miles and soybean lipoxygenase from Sigma. The S-10 mix liver homogenate fractions were prepared from the liver of phenobarbital treated rats (75 mg/kg per day intraperitoneally for 6 days) according to Garner *et al.* [18]. The protein content of the enzymatic preparation was adjusted 7.5 mg/mL and the activity was 0.15 units/g protein. One unit of the enzymatic activity was defined as the enzyme that catalyses the formation of 1 μmol PGE_2 per min.

Analysis of arachidonic acid metabolism. The incubation of arachidonic acid (10^{-6} M) with PHS (1000 mUnits) was carried out at 37°, gas phase air, in a medium of the following composition: 145 mM NaCl; 0.9 mM $CaCl_2$; 2.4 mM KCl; 0.45 mM $MgCl_2$; 0.1% glucose; 0.1% human serum albumin (Behringwerke, Marburg/Lahn, F.R.G.) adjusted to pH 7.4 with 1% Sörensen phosphate buffer. After 30 min incubation the reaction was stopped by chilling the tubes and one end product of arachidonic acid metabolism, 6-keto-$PGF_{1\alpha}$, was evaluated by radioimmunoassay techniques according to Coker *et al.* [19].

Isolation of mast cells. Male Wistar albino rats weighing between 200 and 400 g were used for the experiments. Rats were anaesthetized in an atmosphere of ether and air and then killed by decapitation. Saline (8 mL; NaCl 154 mM) adjusted to pH 6 with 10% Sörensen phosphate buffer were injected into the peritoneal cavity of each rat and 2 mL of the same solution were injected into the pleural cavity. Peritoneal and pleural washings contained about 5% mast cells. Isolation of mast cells from other cells was achieved by density gradient centrifugation in Ficoll, as previously described [20] or by using a Beckman elutriation system (Rotor JE-6, chamber

Table 3. Mast cell histamine and lactate dehydrogenase (LDH) release induced by arachidonic acid in the presence of soybean lipoxygenase

Arachidonic acid* (M)	N	Soybean lipoxygenase* 100 mUnits	500 mUnits	1000 mUnits
Histamine release (%)				
10^{-7}	4	7.3 ± 1.4	9.7 ± 1.8	12.3 ± 2.6
10^{-6}	4	8.2 ± 3.6	10.2 ± 4.3	14.3 ± 0.7
10^{-5}	4	7.0 ± 2.1	10.9 ± 2.1	16.2 ± 1.8
10^{-4}	4	6.2 ± 0.8	9.8 ± 2.9	18.4 ± 7.2
LDH release (%)				
10^{-7}	4	2.3 ± 1.4	3.8 ± 2.1	3.1 ± 0.8
10^{-6}	4	3.2 ± 0.9	4.6 ± 1.8	4.7 ± 0.9
10^{-5}	4	2.4 ± 0.8	5.2 ± 1.9	5.4 ± 2.1
10^{-4}	4	4.3 ± 1.6	4.9 ± 0.7	5.0 ± 1.8

* At the given concentrations, both arachidonic acid and soybean lipoxygenase, when incubated alone, produced only negligible histamine release which was subtracted from all the values.

The values are expressed as mean ± SE of the reported number of experiments performed in triplicate.

size 4.5 mL) according to Glick *et al.* [21]. Evan's buffer was used as elutriation fluid consisting of NaCl 138 mM; KCl 2.7 mM; glucose 6.5 nM; Tris–HCl 2.5 nM and 0.1% bovine serum albumin. The elutriation was carried out at 5° at the rotor speed of 2400 ± 10 rpm for 1 hr and at a flow rate of 15 mL/min. Several fractions were collected by successive increments in flow rate, according to Beaven *et al.* [22].

A final yield of 90–95% pure mast cells was achieved. Unless otherwise indicated the incubation medium in which the mast cells were resuspended had the same composition as that used to evaluate the metabolism of arachidonic acid.

Incubation. Mast cell incubations were conducted in 10 mL test tubes at 37° in a metabolic shaker for 30 min, unless otherwise stated; the gas phase was air. The final composition of the incubations was 5×10^4 mast cells and 2 mL of the incubation medium containing various concentrations of PUFA (arachidonic acid and linoleic acid) alone or in the presence of different amounts of the oxidizing systems (PHS, soybean lipoxygenase and S10 mix liver microsomes), as indicated. The reaction was stopped by chilling the tubes in an ice-water bath. Cells were then separated from the medium by centrifugation (400 *g* for 5 min) at 4° and histamine and lactate dehydrogenase were measured in the supernatants and in the pellets.

Histamine assay. Histamine was measured fluorimetrically using the method of Shore *et al.* [23] as modified by Kremzner and Wilson [24]. In the supernatants, *o*-phthaldialdehyde was added directly to the sample after alkalinization. The same procedure was used for the cells, after extraction with HCl (0.1 M) using the method of Bergendorff and Uvnäs [25]. The authenticity of histamine in control and treated cells was demonstrated either by recording the excitation and fluorescence spectra or through bioassay according to a two by two design [26]. Histamine release (supernatant histamine) was expressed as a percentage of the total present in the cells plus supernatant. Spontaneous histamine release ranged between 1 and 8% and was subtracted.

Lactate dehydrogenase determination. Release of cytoplasmic LDH was assayed by measuring spectrophotometrically the LDH catalysed reduction of pyruvate to lactate in the presence of NADH, as described by Bergmeyer and Bernt [27].

Determination of lipid peroxidation. The determination of lipid peroxidation in mast cell homogenate was based on the reaction of malonyldialdehyde (MDA), the end product of lipid peroxidation, with 2-thiobarbituric acid (TBA) to form a pink-colored substance [28] and on the measurement of conjugated dienes [29]. The reaction mixture for MDA contained 0.2 mL of sample, 0.2 mL of 2 mM chlortetramethoxypropane, 0.2 mL of 8.1% sodium dodecylsulfate, 1.5 mL of 20% acetic acid and 1.5 mL of 0.8% aqueous solution of TBA. The mixture was made up to 4 mL with distilled water and heated to 95° for 30 min. After cooling, 1 mL of the mixture of *n*-butanol and pyridine (15:1, v/v) was added. The mixture was shaken vigorously, centrifuged at 4000 rpm for 10 min, and the absorbance of the organic phase (upper layer) was measured at 532 nm [30].

For determination of conjugated dienes, lipids were extracted from 0.4 mL of samples with 1 mL of 95% ethanol. After 1 mL of a 60% glacial acetic acid and 40% chloroform solution plus 0.2 mg acetyl thioglycolate per mL and 0.08 mL of a saturated KI solution were added to the samples. After 15 min the mixture was titrated with standard iodine and diene absorbance was measured at 234 nm [29].

Morphology of isolated mast cells. Isolated mast cells were incubated for 30 min at 37° in the medium previously described containing various concentrations of PUFA, alone or in the presence of the specific oxidizing system (PHS, soybean lipoxygenase). The cells were then collected by centrifugation and fixed by the addition of a double volume of cold glutaraldehyde (3% in 0.1 M Na–

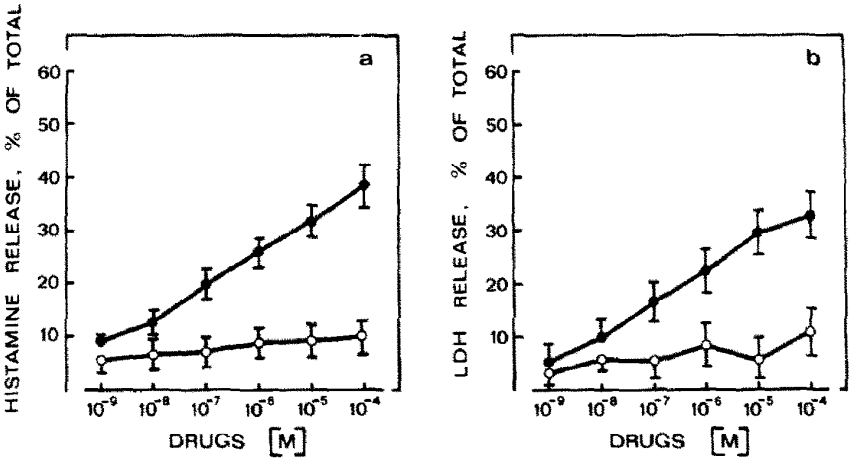

Fig. 1. The release of histamine (a) and of lactate dehydrogenase (LDH) (b) from rat mast cells by linoleic acid alone (○—○) and in the presence of PHS (500 mUnits; ●—●). The values are expressed as means ± SE of six experiments in duplicate.

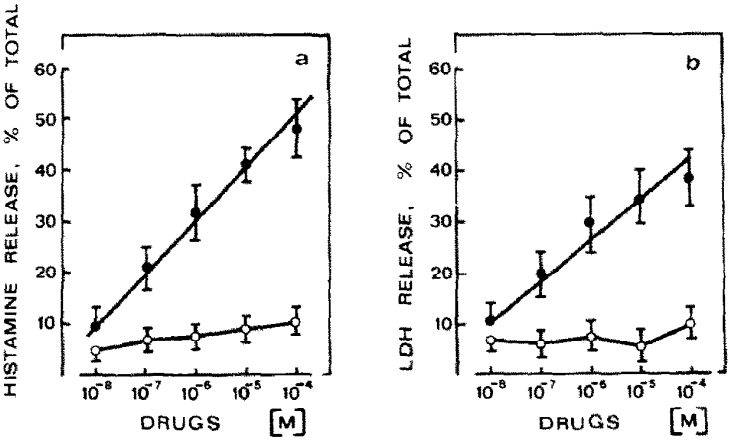

Fig. 2. The release of histamine (a) and of lactate dehydrogenase (LDH) (b) from rat mast cells by linoleic acid alone (○—○) and in the presence of soybean lipoxygenase (500 mUnits; ●—●). The values are expressed as means ± SE of eight experiments in duplicate.

cacodylate buffer, pH 7.3, 0–4° for 15 min; room temperature for 60 min). Post fixation was in 1% osmium tetroxide in 0.1 M Na–cacodylate buffer at room temperature, according to the procedure described by Lawson *et al.* [31].

Statistical analysis. Significant differences in the per cent histamine and LDH release among different experimental groups were determined by the paired *t*-test.

RESULTS

Interaction between the prostanoid generating system and mast cell histamine release

The incubation of given concentrations of arachidonic acid (10^{-6} M) with PHS (1000 mUnits) resulted in the appearance of measurable amounts of 6-keto-$PgF_{1\alpha}$ (2.5 ng/mL ± 0.3; mean values of six experiments).

Among the final products stemming from arachidonic acid metabolism either through the cyclooxygenase or lipoxygenase pathway, none of them was capable of eliciting mast cell histamine release when incubated with isolated purified mast cells (data not shown). Arachidonic acid, up to the concentration of 10^{-4} M, and PHS, up to the concentration of 5000 mUnits, failed to evoke any significant histamine release when they were incubated separately with rat mast cells (Table 1).

Effects of simultaneous incubation of PUFA with oxidizing systems on mast cell histamine release

Table 1 shows the effects on mast cell histamine release of combining a variety of concentrations of arachidonic acid with PHS. It is apparent that a substantial amount of histamine is released at any given concentration of arachidonic acid when in the presence of a suitable amount of PHS. The release

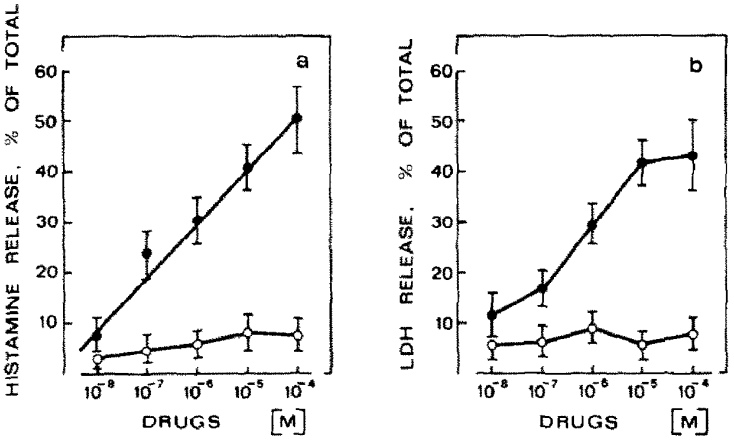

Fig. 3. The release of histamine (a) and of lactate dehydrogenase (LDH) (b) from rat mast cells by linoleic acid alone (○—○) and in the presence of S10 mix liver microsomes from phenobarbital treated rats (400 μL; ●—●). The values are expressed as means ± SE of six experiments in duplicate.

of histamine increased as a function of both the concentrations of arachidonic acid and PHS. However, the increase in the release of histamine was not linear, and almost reached saturation at high concentrations of both arachidonic acid and PHS. Similar results were obtained when mast cells were incubated with arachidonic acid in the presence of S10 mix liver microsome fractions obtained from phenobarbital treated rats (Table 2). A lower but still significant release of histamine was obtained by the incubation of arachidonic acid with soybean lipoxygenase (Table 3). It is worth noting that the release of histamine was not concomitant with any significant appearance of LDH in the supernatants (Tables 1–3).

Linoleic acid behaves differently from arachidonic acid. In fact, when incubated alone with rat mast cells, linoleic acid (10^{-8}–10^{-4} M) fails to evoke any significant release of histamine (Figs 1–3). However, when incubated with a fixed concentration of PHS, linoleic acid elicits a concentration-dependent histamine release (Fig. 1). Linoleic acid was also activated to release histamine by the more specific oxidizing system, soybean lipoxygenase (Fig. 2) and by phenobarbital-induced rat liver microsomes (Fig. 3). Unlike arachidonic acid, the release of histamine was linear with the concentrations of linoleic acid, and in any case was coupled with a progressive increase of LDH release in the supernatants.

Definition of mast cell histamine release induced by activated PUFA

The time-course of mast cell histamine release induced by activated PUFA is shown in Fig. 4. The releasing process evoked by arachidonic acid and linoleic acid in the presence of PHS increases almost linearly in the first 15 min of incubation and reaches the plateau between 15 and 20 min; at that time the release is definitely maximal.

A relationship was found between the release of histamine by activated PUFA and lipid peroxidation as measured by the generation of MDA and conjugated dienes (Fig. 5). In duplicates from the same

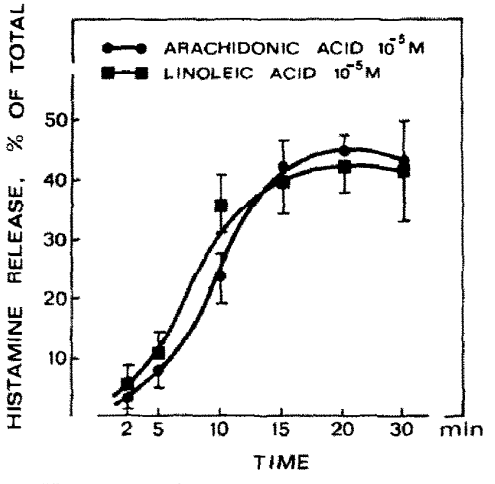

Fig. 4. Time-course of mast cell histamine release induced by PUFA in the presence of PHS (500 mUnits). The values are expressed as means ± SE of six experiments in duplicate.

inoculum, both arachidonic acid and linoleic acid release a parallel amount of histamine and MDA from rat mast cells, in the presence of PHS. However, activated linoleic acid is associated with higher levels of lipid peroxidation in comparison with activated arachidonic acid (Fig. 5b and c).

The electron microscopic analysis of the effect of the various treatments on rat mast cells is reported in Fig. 6. Cell morphology was unaltered by incubation with arachidonic acid or linoleic acid alone (Fig. 6a and c). The features of mast cells exposed to arachidonic acid in the presence of PHS were consistent with a process of sequential exocytosis (Fig. 6b), while the features of mast cells exposed to activated linoleic acid (Fig. 6d and e) were consistent with a process of cytotoxic cell disruption.

Effect of drugs on mast cell histamine release induced by activated PUFA

We have previously reported that some free radical

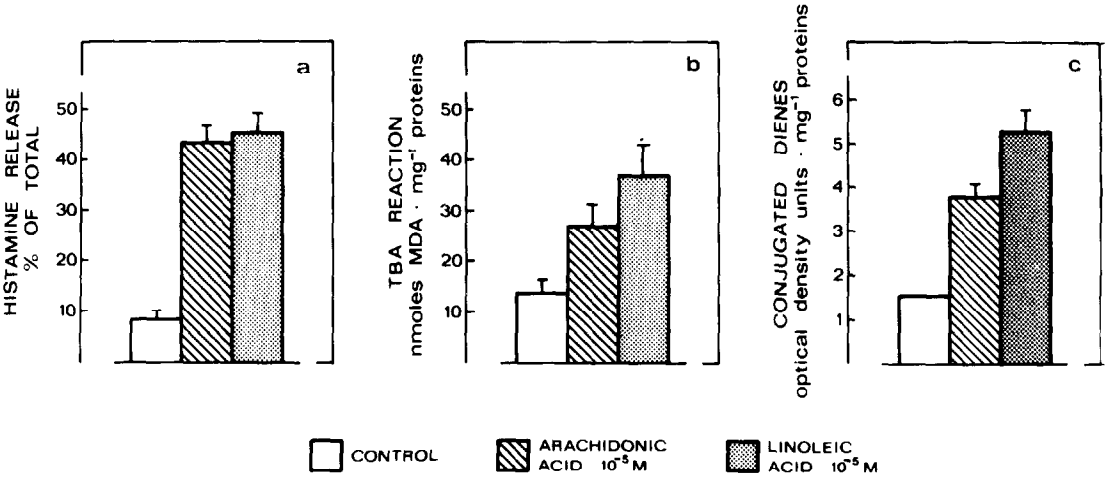

Fig. 5. Correlation between histamine release (a), MDA production (b) and conjugated dienes (c) in isolated rat serosal mast cells induced by PUFA in the presence of PHS (500 mUnits). The values are expressed as mean ± SE of 10 experiments in duplicate.

scavengers abated the release of histamine induced by the incubation of arachidonic acid with PHS [11]. This is also true for linoleic acid (Table 4). Free radical scavengers (D-mannitol, GSH and α-tocopherol) were capable of significantly reducing the release of histamine induced by linoleic acid in the presence of soybean lipoxygenase. It is worth noting that, at the concentrations used, these drugs did not modify the release of histamine induced by compound 48/80 in rat serosal mast cells.

A variety of cyclooxygenase and lipoxygenase inhibitors were studied for their effects on mast cell histamine release induced by activated arachidonic and linoleic acid (Fig. 7). At concentrations of the same magnitude as those found in pharmacological treatments *in vivo* (10^{-6}–10^{-5} M), acetylsalicylic acid was more active than nordihydroguaiaretic acid.

It is worth noting that acetylsalicylic acid, at the same concentrations capable of blocking the release of histamine by activated arachidonic acid, did not modify the histamine release from rat serosal mast cells induced by graded doses of compound 48/80 (data not shown).

In previous experiments we have demonstrated a protective effect on histamine release and reperfusion arrhythmias afforded by carnitine derivatives and by cimetidine [32]. Figures 8 and 9 show that two carnitine esters inhibit the release of histamine by PHS-activated arachidonic acid and that cimetidine, but not ranitidine, reduces the release of histamine under the same circumstances, although at high concentrations. None of these drugs afford any protection toward the release of histamine by compound 48/80 in rat serosal mast cells (data not shown).

DISCUSSION

The present experiments confirm and extend our previous reports on the release of mast cell histamine by free radicals, generated *in vitro* during the metabolic activation of xenobiotics or in the ischemia-reperfusion model [11]. Many circumstantial evidences also suggest that the release of histamine is an indirect phenomenon, driven by a free radical mechanism. In fact, arachidonic acid and linoleic acid fail to produce the release of histamine in any significant amount when incubated alone with rat serosal mast cells, but acquire histamine releasing properties in the presence of specific oxidizing systems. There is direct proof [13, 17, 33–35] that arachidonic acid and linoleic acid generate free radicals when activated *in vitro* by specific oxidase (PHS; cytochrome P-450; soybean lipoxygenase).

Moreover, membrane phospholipid attack by free radicals leads to lipid peroxidation which can be evaluated in biological samples by a variety of methods [36], such as the measurement of thiobarbituric (TBA) reactive species and conjugated dienes [28, 29]. The present results have shown a linear relationship between the release of histamine by activated PUFA and the appearance of lipid peroxidation products, thus suggesting that lipid peroxidation is a consensual event to histamine release. However, more pronounced lipid peroxidation seems to take place in the case of activated linoleic acid.

It is therefore conceivable that free radicals, formed during the metabolic activation of PUFA, would produce both the release of histamine and the peroxidation of membrane phospholipids. We are aware that prostaglandin endoperoxides or their derivatives account for significant proportions of TBA-reactive substances found in serum [37] and that auto-oxidation of PUFA is a suggested mechanism for the formation of TBA-reactive material from prostaglandin-like endoperoxides [38]. However, a linear relationship between the release of histamine and the production of TBA-reactive compounds was also found when mast cells were challenged with different free radical generating systems,

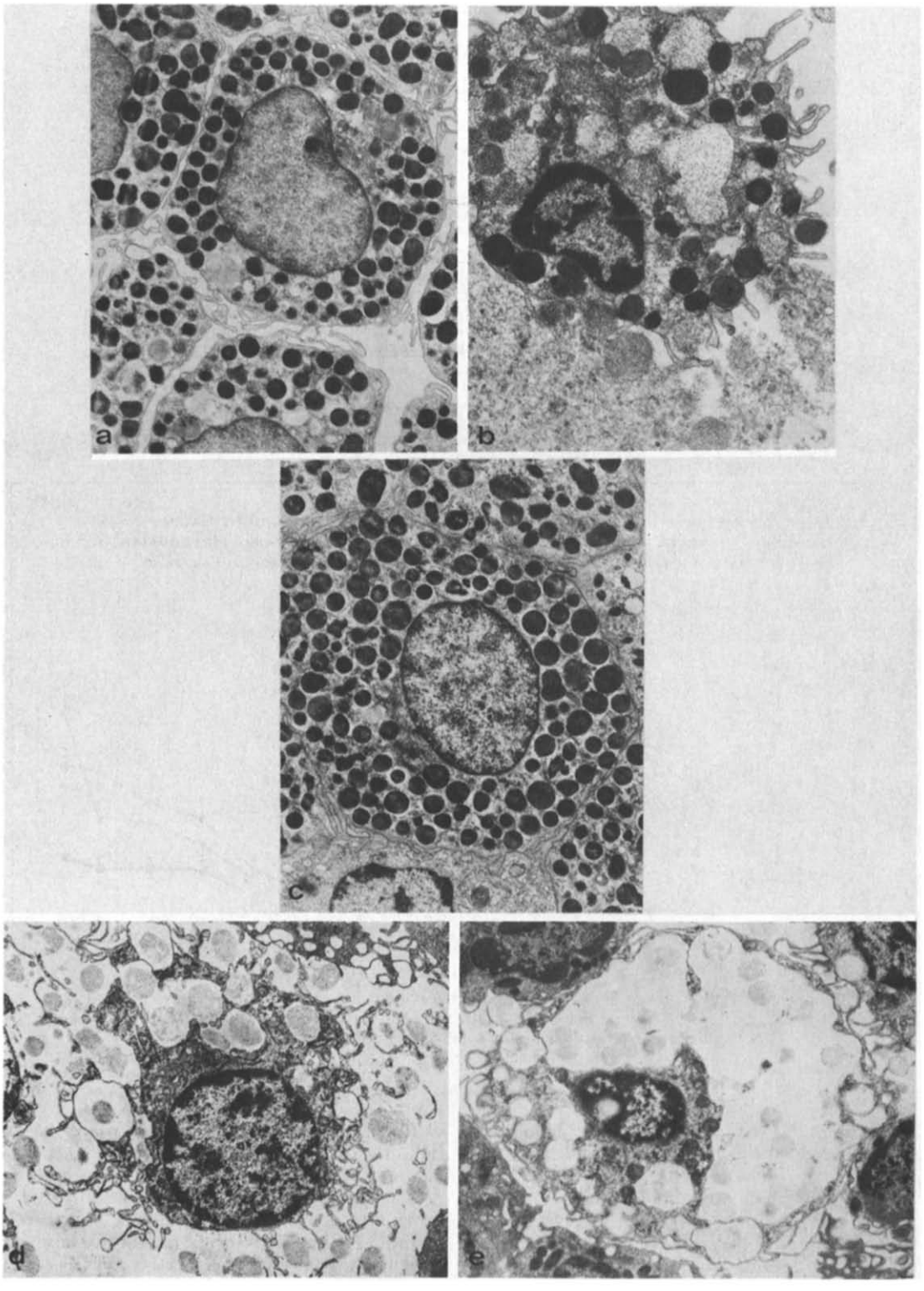

Fig. 6. Electron micrographs of isolated rat mast cells incubated in Tyrode's buffer, pH 7.4 with arachidonic acid alone (a; 10^{-6} M × 6000) or supplemented with PHS 500 mUnits (b; ×10,000); with linoleic acid alone (c; 10^{-6} M × 6000) or supplemented with PHS 500 mUnits (d; ×10,000) or soybean lipoxygenase 500 mUnits (e; ×7500).

Table 4. Effect of free radical scavengers on mast cell histamine release induced by linoleic acid (10^{-5} M) in the presence of soybean lipoxygenase (500 mUnits)

		Histamine release (%)*	Inhibition of histamine release (%)	N
Linoleic acid plus soybean lipoxygenase	10^{-5} M 500 mUnits	44.3 ± 7.3	—	6
SOD	50 mcg	36.2 ± 3.5	—	4
Catalase	50 mcg	47.8 ± 4.2	—	4
D-Mannitol	10^{-3} M	35.6 ± 4.8	—	6
D-Mannitol	10^{-2} M	18.4 ± 2.7†	58.4	6
GSH	10^{-4} M	36.8 ± 5.6	—	4
GSH	10^{-3} M	19.7 ± 7.2†	55.5	4
α-Tocopherol	10^{-4} M	20.4 ± 3.2†	53.5	6

The values are expressed as means ± SE of the reported number of experiments performed in duplicate.

* The cells were incubated for 30 min at 37° in a buffered salt solution pH 7.4 with the drugs and the releasing system.

† $P < 0.005$.

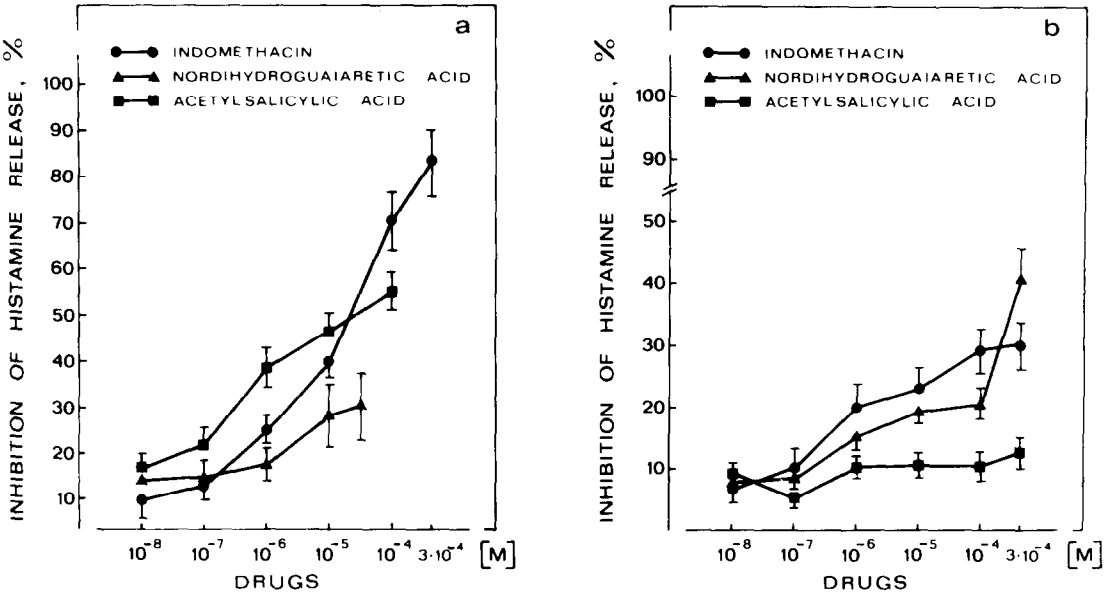

Fig. 7. The effect of prostaglandin synthesis inhibitors on mast cell histamine release induced by arachidonic acid (10^{-6} M: a) and linoleic acid (10^{-6} M: b) in the presence of PHS (500 mUnits). The values are expressed as means ± SE of five experiments in triplicate.

thus supporting the hypothesis that lipid peroxidation products originate from free radical-driven membrane peroxidation, rather than from the autooxidation of arachidonic or linoleic acid [39].

A further indirect proof of the involvement of free radicals in the release of histamine by activated PUFA comes from the experiments carried out with free radical scavengers. The preferential inhibition of the release of histamine by activated PUFA induced by some free radical scavengers (GSH; α-tocopherol; D-mannitol) and the lack of effects by others (superoxide-dismutase, SOD; catalase) is in keeping with our previous results using other sources of free radicals [10, 11]. The differential sensitivity of the histamine-releasing process toward the free radical scavengers allows one to speculate about the nature of the radical(s) tentatively involved. A free radical chain mechanism has been proposed to explain the conversion of arachidonic acid to PGG and to 5-HEPETE [14]; free radicals are also generated during the reduction of PGG to PGH_2 and in the oxygenation of linoleic acid by soybean lipoxygenase [17]. To these purported promoters of mast cell histamine release it should be worth adding the hydroxyl radical, since the hydroxyl radical scavenger, D-mannitol, is the most active compound capable of abating the mast cell histamine release induced by activated PUFA. It is also worth noting that a

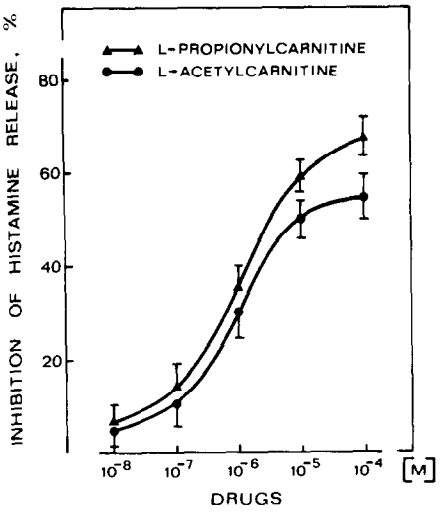

Fig. 8. Effect of L-carnitine derivatives on histamine release induced by arachidonic acid (10^{-6} M) in the presence of PHS (1000 mUnits). The values are expressed as means ± SE of five experiments in triplicate.

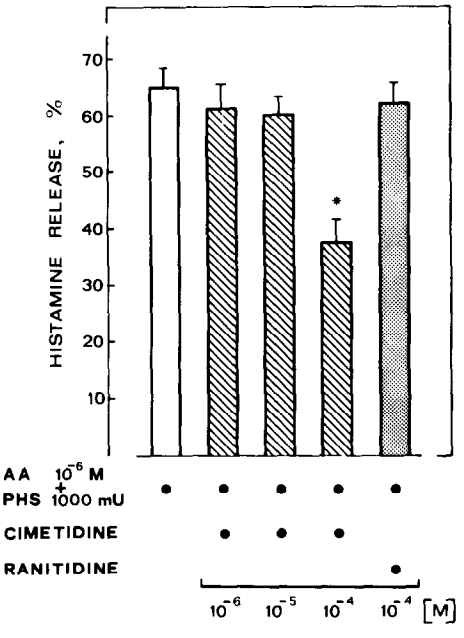

Fig. 9. Effect of cimetidine and ranitidine on histamine release induced by arachidonic acid (10^{-6} M) in the presence of PHS (1000 mUnits). The values are expressed as means ± SE of five experiments in triplicate. * $P < 0.001$.

transient formation of superoxide radical, a precursor of hydroxyl radical, has been reported to be involved in PUFA-induced brain swelling [40]. However, we are aware that the evidence of a free radical-driven release of histamine is only circumstantial, and must await the unequivocal demonstration of free radical production by adequate techniques.

The release of histamine by activated PUFA has two distinct features. In fact, the release of histamine induced by linoleic acid in the presence of a variety of oxidizing systems is associated with a concomitant release of LDH and with an electron-microscopic pattern of cell lysis. Arachidonic acid releases histamine without any leakage of LDH and with an electron-microscopic pattern of sequential exocytosis. This implies that arachidonic acid and linoleic acid produce different reactive species, capable of causing a perturbation of plasma lipid membranes leading to cytotoxic release or to an active exocytotic secretion of histamine. Both these processes are consistent with a role for free radicals in cell pathophysiology. Destruction of pancreatic β-cells by alloxan and dialuric acid [41, 42], erythrocyte hemolysis by naphtoquinonesulfate [43], bacteriolysis by streptonigrin [44] are examples of cell disruption related to membrane lipid peroxidation due to the generation of free radicals.

The possibility that the metabolic activation of PUFA could lead to free radical species capable of triggering the sequential exocytosis of mast cell granules is even more interesting. When mast cells are challenged *in vitro* with some free radical generating systems, the release of histamine was coupled with a net increase of free cytosolic calcium [45], while no substantial breakdown of polyphosphoinositides was observed [39, 45]. Calcium ionophore A 23187 behaves in a similar fashion by promoting the concomitant secretion of mast cell histamine and an increase in cytosolic calcium without affecting the hydrolysis of inositol phospholipids [39, 45]. The similarity between calcium ionophore and free radical generating systems in the biochemical events linked with mast cell histamine release suggests that free radicals behave as endogenously formed calcium ionophores, capable of triggering the sequential exocytosis of histamine containing granules. However, Ca^{2+} ionophores activate the hydrolysis of inositol phospholipids in rat basophil leukemia cells [46] and phosphatidylinositol turnover was slightly increased in antigen sensitized rat peritoneal mast cells stimulated with antigen or with concanavalin A [47]. The discrepancy within these results could be accounted for by the different mechanisms of histamine release in different cell populations (basophil leukemia cells versus rat mast cells), or in the same cells challenged with different stimuli (antigen; concanavalin A; ionophore A 23187).

From this perspective, pharmaceutical compounds which allegedly react with free radicals are worthy of a thorough analysis: cimetidine and carnitine abate the release of histamine by activated arachidonic acid, probably by trapping the generated free radical(s). In fact, none of the drugs which in the present experiments inhibit the release of histamine by activated arachidonic acid, was shown capable of influencing the release of histamine induced by a classical secretagogue, such as compound 48/80 or calcium ionophore.

Acknowledgement—These investigations were supported by a grant from MPI 40% and by a grant of CNR Rome, National Pharmacological group.

REFERENCES

1. Ohmori H, Komoriya K, Azuma A, Kurozomi S and

Oto Y, Xanthine oxidase-induced histamine release from isolated rat peritoneal cells: involvement of hydrogen peroxide. *Biochem Pharmacol* **28**: 333–334, 1979.
2. Henderson WR, Chi EY and Klebanoff SS, Eosinophil peroxidase-induced mast cells secretion. *J Exp Med* **152**: 265–279, 1980.
3. Stendhal O, Molin L and Lindroth M, Granulocyte-mediate release of histamine from mast cells. *Int Arch Allergy Appl Immunol* **70**: 277–284, 1983.
4. Fantozzi R, Brunelleschi S, Rubino A, Tarli S, Masini E and Mannaioni PF, FMLP-activated neutrophils evoke histamine release from mast cells. *Agents Actions* **18**: 155–158, 1986.
5. Damas J, Lagneaux D and Le Comte J, Liberation d'histamine par le mastocyte du rat en hyperoxie. *C R Soc Biol* **175**: 266–268, 1981.
6. West PR, Harman LS, Josephy PD and Mason RP, Acetaminophen: enzymatic formation of a transient phenoxyl free radical. *Biochem Pharmacol* **33**: 2933–2936, 1984.
7. Evans MA, Microsomal activation of *N*-hydroxy-norcocaine to a reactive nitroxide. *Toxicologist* **1**: 1–6, 1981.
8. Komiyama T, Kikuchi T and Sugiura Y, Generation of hydroxyl radical by anticancer quinone drugs, carbazil-quinone, mitomycin C, alacinomycin A and adriamy-cin, in the presence of NADPH-cytochrome P-450 reductase. *Biochem Pharmacol* **31**: 3651–3656, 1982.
9. Masini E, Lodovici M, Fantozzi R, Brunelleschi S, Conti A and Mannaioni PF, Histamine release by free radicals: paracetamol-induced histamine release from rat peritoneal mast cells after in vitro activation by monooxygenase. *Agents Actions* **18**: 85–88, 1986.
10. Mannaioni PF, Giannella E, Palmerani B, Pistelli A, Gambassi F, Bani Sacchi T, Bianchi S and Masini E, Free radicals as endogenous histamine releasers. *Agents Actions* **23**: 129–142, 1988.
11. Mannaioni PF and Masini E, The release of histamine by free radicals. *Free Radical Biol Med* **5**: 177–197, 1988.
12. Nugteren DH and Van Dorp DA, The participation of molecular oxygen in the biosynthesis of prostaglandins. *Biochim Biophys Acta* **88**: 654–656, 1966.
13. Mason RP, Kalyanaraman B, Tainer BE and Eling TE, A carbon-centered free radical intermediate in the prostaglandin synthetase oxidation of arachidonic acid. *J Biol Chem* **255**: 5019–5022, 1980.
14. Siedlik PH and Marnett LJ, Oxidizing radical gen-eration by prostaglandin-H-synthase. *Methods Enzymol* **105**: 412–416, 1984.
15. Schreiber J, Mason RP and Eling TE, Carbon-centered free radical intermediates in the hematin- and ram seminal vesicle-catalyzed decomposition of fatty acid hydroperoxides. *Arch Biochem Biophys* **251**: 17–24, 1986.
16. De Groot JJMC, Garssen GJ, Vliegenthart JFG and Boldingh J, The detection of linoleic acid radicals in the anaerobis reaction of lipoxygenase. *Biochim Biophys Acta* **326**: 279–284, 1973.
17. Connor HD, Fischer V and Mason RP, A search for oxygen-centered free radicals in the lipoxygenase/lino-leic acid system. *Biochem Biophys Res Commun* **141**: 614–621, 1986.
18. Garner RC, Miller EC and Miller JA, Liver microsomal metabolism of aflatoxin B to a reactive derivate toxic to *Salmonella typhimurium*-Ta_{1530}. *Cancer Res* **32**: 2058–2066, 1972.
19. Coker SJ, Clarke B and Zeitlin IJ, Radioimmunoassay techniques for the determination of the local release of prostaglandins and thromboxanes. *J Pharmacol Methods* **7**: 207–217, 1982.
20. Blandina P, Fantozzi R, Mannaioni PF and Masini E, Characteristics of histamine release evoked by ace-tylcholine in isolated rat mast cells. *J Physiol* **301**: 281–293, 1980.
21. Glick D, Von Redlich D, Juhos ET and McEwen CR, Separation of mast cells by centrifugal elutriation. *Exp Cell Res* **65**: 23–26, 1971.
22. Beaven MA, Aiken DL, Woldemussie E and Soll AH, Changes in histamine synthetic activity, histamine con-tent and responsiveness to compound 48/80 with matu-ration of rat peritoneal mast cells. *J Pharmacol Exp Ther* **224**: 620–626, 1983.
23. Shore PA, Burkhalter A and Cohn VR, A method for the fluorimetric assay of histamine in tissues. *J Pharmacol Exp Ther* **127**: 182–186, 1959.
24. Kremzner LT and Wilson IB, A procedure for the determination of histamine. *Biochim Biophys Acta* **50**: 364–367, 1961.
25. Bergendorff A and Uvnäs B, Storage of 5-hydroxy-triptamine in rat mast cells. Evidence for an ionic binding to carboxyl groups in a granule heparin-protein complex. *Acta Physiol Scand* **84**: 320–331, 1972.
26. Schild HO, A method for conducting a biological assay on a preparation giving repeated graded responses illus-trated by estimation of histamine. *J Physiol* **101**: 115–130, 1942.
27. Bergmeyer HU and Bernt E, Lactate dehydrogenase UV-Assay with pyruvate and NADH. In: *Methods for Enzymatic Analysis* (Ed. Bergmeyer HU), Vol. 2, pp. 574–579. Verlag Chemie, Academic Press, New York, 1974.
28. Dahle LK, Hill EG and Holman RT, The thiobarbituric acid reaction and the autoxidation of polyunsaturated fatty acid methyl esters. *Arch Biochem* **98**: 253–261, 1962.
29. Pryor WA and Castle L, Chemical methods for the detection of lipid hydroperoxides. *Methods Enzymol* **105**: 293–298, 1984.
30. Ohkawa H, Onishi N and Yagi K, Assay for lipid peroxides in animal tissues by thiobarbituric acid reac-tion. *Analyt Biochem* **85**: 351–358, 1979.
31. Lawson D, Raff MC, Gomperts BD, Fewtrell C and Gilula NB, Molecular events during membrane fusion. A study of exocytosis in rat peritoneal mast cells. *J Cell Biol* **72**: 242–259, 1977.
32. Masini E, Gambassi F, Giannella E, Palmerani B, Pistelli A, Carlomagno L and Mannaioni PF, Ischemia-reperfusion injury and histamine release in isolated guinea-pig heart: the role of free radicals. *Agents Actions* **27**: 154–157, 1989.
33. Chance B, Sies H and Boveris A, Hydroperoxide metabolism in mammalian organs. *Physiol Rev* **59**: 527–605, 1979.
34. Hawco F, Hulett L and O'Brien PJ, Hydroxyl radical formation during P_{450} function and benzopyrene hydroxylation. In: *Microsomes, Drug Oxidations and Chemical Carcinogenesis* (Ed. Estabrook RW), p. 419. Academic Press, New York, 1980.
35. Capdevila J, Parkhill L, Chacos N, Okita R, Master BSS and Estabrook RW, The oxidative metabolism of arachidonic acid by purified cytochromes P_{450}. *Biochem Biophys Res Commun* **101**: 1357–1363, 1981.
36. Smith CV and Anderson RE, Methods for deter-mination of lipid peroxidation in biological samples. *J Free Radical Biol Med* **3**: 341–344, 1987.
37. Shimizu T, Kondo K and Hayaishi O, Role of prosta-glandin endoperoxides in the serum thiobarbituric acid reaction. *Arch Biochem Biophys* **206**: 271–276, 1981.
38. Pryor WA, Stanley JP and Blair E, Autoxidation of polyunsaturated fatty acids: II. A suggested mechanism for the formation of TBA-reactive materials from prostaglandin-like endoperoxide. *Lipids* **11**: 370–379, 1976.

39. Masini E, Giannella E, Pistelli A, Palmerani B, Gambassi F, Occupati B and Mannaioni PF, Histamine release by free radicals: the relationship with the signal transduction system. *Agents Actions* **27**: 72–79, 1989.
40. Chan PH and Fishman RA, Transient formation of superoxide radicals in polyunsaturated fatty acid-inducing brain swelling. *J Neurochem* **35**: 1004–1007, 1980.
41. Cohen G and Keikkila RE, The generation of hydrogen peroxide, superoxide radical and hydroxyl radical by 6-hydroxy-dopamine, dialuric acid and related cytotoxic agents. *J Biol Chem* **249**: 2447–2451, 1974.
42. Keikkila RE and Cohen G, Cytotoxic aspects of the interaction of ascorbic acid with alloxan and 6-hydroxy-dopamine. *Ann NY Acad Sci* **258**: 221–225, 1975.
43. Goldberg B and Stern A, Superoxide anions as mediator of drug-induced oxidative hemolysis. *J Biol Chem* **251**: 6468–6471, 1976.
44. Misra HP and Fridovich I, The univalent reduction of oxygen by reduced flavins and quinones. *J Biol Chem* **247**: 188–191, 1972.
45. Masini E, Giannella E, Palmerani B, Pistelli A, Gambassi F and Mannaioni PF, Prostaglandin-H-synthetase activates xenobiotics into free radicals: histamine release and biochemical characteristics. *Int Arch Allergy Appl Immunol* **88**: 134–135, 1989.
46. Lo TN, Saul W and Beaven MA, The actions of Ca^{2+} ionophores on rat basophilic (2H3) cells are dependent on cellular ATP and hydrolysis of inositol phospholipids. *J Biol Chem* **262**: 4141–4145, 1987.
47. Cockcroft S and Gomperts BD, Evidence for a role of phosphatidylinositol turnover in stimulus-secretion coupling. *Biochem J* **178**: 681–687, 1979.