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# SHORT COMMUNICATIONS

## Quantitative relationship between histamine and $^{35}S$ release from isolated rat mast cells due to the beta-adrenoceptor blocking drug Kö 1124

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Isolated rat mast cells release histamine after exposure to different pharmacological stimuli [1]. Beta-adrenoceptor blocking drugs possess a high membrane affinity resulting in different nonspecific activities [2–4]. We have found that at least two groups of these drugs exist concerning the effect on isolated rat mast cells. Most of beta-adrenoceptor blocking drugs do not release histamine *in vitro*. On the other hand time-dependent release of histamine occurred with beta-adrenoceptor blocking drug Kö 1124 (1–3'-butylphenoxy-2-hydroxy-3-isopropylamino propane·HCl Boehringer Ingelheim) without corresponding release of granules [5]. In this study we investigated the mechanism of Kö 1124 and the relationship between histamine release and degranulation induced by this drug of isolated rat mast cells.

*Materials and methods.* Male albino Wistar rats (300–350 g) were used in all experiments. Mast cells were isolated from pleural and peritoneal washes of decapitated rats in buffered salt solution (130 mmol·$1^{-1}$ NaCl adjusted to pH 6 with Sorensen phosphate buffer). After differential centrifugation in 30 and 40% Ficoll (Pharmacia) mast cells were washed twice in buffered salt solution (154 mmol·$^{-1}$ NaCl, 2.7 mmol·$1^{-1}$ KCl, 0.9 mmol·$1^{-1}$ $CaCl_2$ with 10% v/v Sörensen phosphate buffer $Na_2HPO_4$ + $KH_2PO_4$ mmol·$1^{-1}$ pH = 7.0) with 0.5 mg/ml of human serum albumin (Imuna Š. Michal'any) [6]. Mast cells were pooled, stained with toluidine blue, counted in Bürker chamber and finally diluted to get 250,000 cells per 1 ml of sample. In each experiment 2 ml samples were used. After incubation at different temperatures samples were cooled to 0° and centrifuged at 450 *g* for 10 min at 4°. The supernatant was decanted and sediment resuspended in 1 ml of 0.02 mol·$1^{-1}$ HCl, swirled and left for 10 min at room temperature. One ml of 154 mmol·$1^{-1}$ NaCl was added and tubes were heated for 6 min at 70° to release all remaining histamine. The histamine was determined fluorometrically both in supernatant and sediment [7] omitting the extraction procedure [8]. Released histamine in supernatant was calculated as the percentage from the total histamine content in the sample.

For labelling the mast cell granules with $^{35}S$ rats were injected 14 to 16 days before mast cell isolation with $Na_2[^{35}S]O_4$ (Rotop DDR) subcutaneously (2.5 mCi = $9.25 \cdot 10^7$ Bq per rat −9). For $^{35}S$ activity determination aliquots from supernatant and sediment were taken. For scintillation counting the samples were prepared [10] by use of 10 ml commercial scintillation fluid (SLS 31 Spolana Neratovice). The $^{35}S$ activity was determined on Packard–Tri-Carb liquid scintillation counter 3390. The percentage of $^{35}S$ activity (dpm) released in the supernatant was calculated from the total activity in the sample.

All determinations were made in duplicate. Each value is the mean from four to six experiments ± S.E.M. Results were evaluated by Student's *t*-test.

*Results.* The effect of different incubation temperatures, pH and calcium ions on histamine release induced by Kö 1124 is summarized in Table 1. The control release of histamine at pH 8 was 12.5 per cent, at pH 9 16.5 per cent. At 45° the control histamine release was 7.4 per cent.

In the presence of Kö 1124 the release of histamine increased from 1.24 at $10^{-5}$ mol·$1^{-1}$ to 2.95 at $10^{-3}$ mol·$^{-1}$. Figure 2 demonstrates the effect of cocaine and some of histamine by Kö 1124 was below 20 per cent, at pH 7 it increased to 60 per cent and at pH 8 to 87 per cent. Kö 1124 released 67 per cent of histamine in calcium free solution and 61 per cent at the calcium concentration used in buffered salt solution (1 mmol·$1^{-1}$). Increasing the calcium concentration to 10 mmol·$1^{-1}$ resulted in the inhibition of histamine release to 1.4 per cent.

The quantitative relationship between histamine release and degranulation of mast cells induced with Kö 1124 is demonstrated in Fig. 1. Although the histamine and granule release were both stimulated by Kö 1124 release curves did not run in parallels. With a concentration of $10^{-3}$ mol·$1^{-1}$ the release of histamine was 98 per cent but the release of $^{35}S$-labelled granules was only 29.2 per cent. The ratio of per cent histamine release to per cent $^{35}S$ release increased from 1.24 at $10^{-5}$ mol·$1^{-1}$ to 2.95 at $10^{-3}$ mol·$^{-1}$. Figure 2 demonstrates the effect of cocaine and some metabolic and enzymatic inhibitors on histamine and $^{35}S$-labelled granules release by Kö 1124. Mast cells were first preincubated with inhibitors for 5 min at 37° then incubation was continued with Kö 1124 for 5 min. Except iodoacetic acid (IAA—Calbiochem) metabolic inhibitors

Table 1. The effect of temperature, pH and calcium ions on histamine release from rat mast cells after Kö 1124 ($5 \cdot 10^{-4}$ mol·$1^{-1}$)*

| Temperature (°C) | Per cent release | pH | Per cent release | $CaCl_2$ (mmol·$1^{-1}$) | Per cent release |
|---|---|---|---|---|---|
| 0 | 3.5 ± 1.2 | 4 | 18.0 ± 3.4 | 0 | 66.8 ± 5.8 |
| 5 | 7.5 ± 2.4 | 5 | 11.4 ± 0.7 | 1 | 60.9 ± 4.3 |
| 15 | 9.2 ± 2.1 | 6 | 10.9 ± 1.4 | 3 | 17.6 ± 3.6 |
| 22 | 20.1 ± 5.1 | 7 | 61.4 ± 8.8 | 5 | 18.4 ± 3.2 |
| 37 | 69.2 ± 0.9 | 8 | 86.6 ± 0.2 | 10 | 1.4 ± 0.8 |
| 45 | 85.8 ± 2.8 | 9 | 83.1 ± 0.5 | | |
| 48 | 86.1 ± 1.7 | | | | |

* Control values subtracted (see results). Each value is the mean from 5 experiments ± S.E.M.

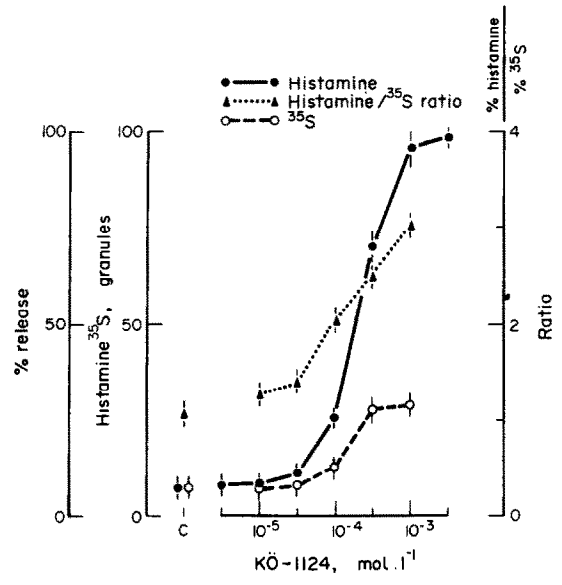


Fig. 1. The quantitative relationship between dose-dependent histamine release and $^{35}$S-labelled granule release from isolated rat mast cells induced with Kö 1124 after 5 min at 37°. Each value is the mean from 5 experiments ± S.E.M. ●—histamine release; ○—$^{35}$S release; ▲—histamine to $^{35}$S ratio.

and cocaine (Spofa) alone did not release more than 10 per cent of histamine. Cocaine and 2,4-dinitrophenol(2,4-DNP—Lachema) decreased the release of histamine induced by Kö 1124 significantly while n-ethylmaleimide(n-EM—Koch Light), iodoacetic acid and ouabain(Merck) were uneffective.

The release of $^{35}$S-labelled granules was in the presence of inhibitors at control values. As is evident from Fig. 2

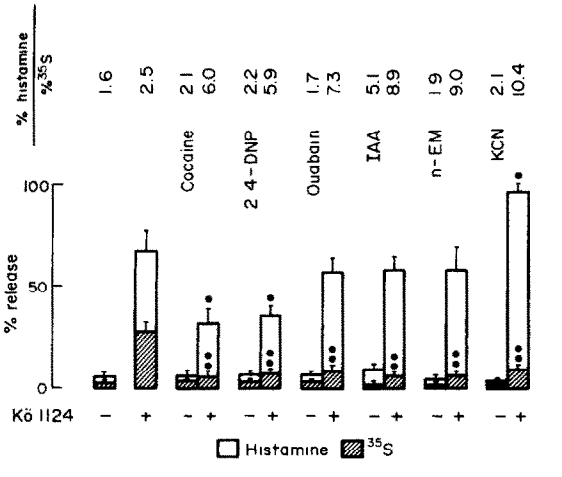


Fig. 2. The effect of different inhibitors (concentrations in parenthesis in mol·l$^{-1}$) on histamine and $^{35}$S release from isolated rat mast cells induced with Kö 1124 ($5 \cdot 10^{-4}$). Cocaine ($3 \cdot 10^{-3}$), n-ethylmaleimide (n-EM: $10^{-4}$), iodoacetic acid (IAA: $10^{-3}$), potassium cyanide (KCN: $3 \cdot 10^{-3}$), 2,4-dinitrophenol (2,4-DNP: $10^{-3}$), ouabain ($3 \cdot 10^{-4}$). The sign + or − indicates the presence or absence of Kö 1124 in particular samples. Each value is the mean from 5 experiments ± S.E.M. ● $P \leq 0.05$; ●● $P \leq 0.01$.

all of these drugs reduced significantly ($P \leq 0.01$) the amount of $^{35}$S release induced with Kö 1124. The ratio of per cent histamine release to per cent $^{35}$S release indicates that in the presence of cocaine 6 times more histamine than $^{35}$S-labelled granules was released whereas with KCN (potassium cyanide—Lachema) the release of histamine was 10 times higher.

*Discussion.* The release of histamine from isolated rat mast cells induced with beta-adrenoceptor blocking drug Kö 1124 was dose-dependent. Inhibition of this release by cold was similar to that described for both selective and nonselective histamine release [11, 12]. The highest release of histamine at pH 8 may be related to the increased partitioning of beta-adrenoceptor blocking drugs to hydrophobic medium at such pH [4]. The release of histamine with Kö 1124 occurred in calcium free solution and was inhibited with increased extracellular calcium. At this high concentration extracellular calcium ions might depress membrane permeability, decrease ionization at the membrane and tend to dehydrate cell surfaces [13]. The release of histamine and $^{35}$S-labelled granules induced by Kö 1124 was not parallel. The higher the concentration of Kö 1124 the more histamine relative to $^{35}$S was released.

As the degranulation requires energy, cocaine and other inhibitors probably blocked the release of $^{35}$S labelled granules stimulated by Kö 1124 by interfering with the triggering mechanism dependent on energy supply. The decrease in the histamine release by cocaine and 2,4-DNP remains unexplained. The release of histamine without corresponding degranulation could be a result of intracellular exchange of Kö 1124 for histamine as it has been shown for beta-adrenoceptor blocking drug VULM 111 [14].

Our results indicate that the beta-adrenoceptor blocking drug Kö 1124 release histamine and $^{35}$S-labelled granules from isolated rat mast cells probably as a result of high membrane and/or granule affinity [15]. The release of histamine was dose, temperature, pH and calcium dependent. The release of $^{35}$S labelled granules was significantly lower as compared with the amount of released histamine. It was inhibited with cocaine, 2,4-dinitrophenol, ouabain, iodoacetic acid, n-ethylmaleimide and KCN. The release of histamine was diminished only with cocaine and 2,4-dinitrophenol.

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# Stereochemistry in the oxidative metabolism of styrene by hepatic microsomes

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The incubation of styrene, one of the principal precursors in the industrial production of polymer materials, with liver microsomes in the presence of NADPH leads to the formation of phenyloxirane as an intermediate, which is rapidly metabolized further into phenylethanediol [1–6]. The first step of this metabolic pathway, epoxide formation, is catalyzed by liver monooxygenases, and the formed chemically reactive epoxide, phenyloxirane, has been noticed as a toxic metabolite since it was reported to be a mutagen in the *Salmonella typhimurium* TA strains used in the Ames testing system [5, 7–11], and suggested to be a skin tumorigen in mice [12]. It has a reactivity to bind covalently to rat liver macromolecules, especially to protein, *in vivo* and *in vitro* [5, 13]. The second step is hydrolysis of the epoxide formed, which is catalyzed by microsomal epoxide hydrolase. In this step the toxic epoxide can be hydrolyzed to the chemically and toxicologically less active glycol, phenylethanediol. Phenyloxirane is one of the most frequently used substrates for the assay of microsomal epoxide hydrolase activity [14–16]. Except the previous demonstrations that the enzymatic phenylethanediol formation from racemic phenyloxirane was initiated by the introduction of a hydroxy group specifically to the 3-position of the oxirane ring [17] and also that rabbit liver microsomal epoxide hydrolase had almost no preference for either *R*- and *S*-phenyloxirane, producing phenylethanediol of much less than 1% optical purity from racemic phenyloxirane [17]. It is of interest that rats given styrene excreted optically active mandelic acid in urine [18]. This is suggestive of the stereo-selective formation of phenylethanediol from styrene in the animal body since the acid proved to be yielded directly from the diol [18].

As to cyclic system, a number of stereochemical studies have been carried out on hepatic microsomal epoxidation and epoxide hydrolysis, e.g. epoxidation of benzo[*a*]pyrene [19, 20], naphthalene [17], cholesterol [21], pregnenolone [22], and 1,3,5(10),16-estratetraene [23], and hydrolysis of benzo[*a*]pyrene 4,5-, 7,8-, and 9,10-epoxides [20], benzo[*a*]pyrene 7,8-diol-9,10-epoxides [19, 20], naphthalene oxide [17], cholesterol 5,6-epoxides [21], pregnenolone 5,6-epoxides [22], 2,3-epoxysteroids [24], estratetraenol epoxides [23], cyclohexene oxide [17], and 4-*tert*-butyl-1,2-epoxycyclohexanes [25]. However, nothing is known of stereochemistry, including absolute configurations of oxygenated carbons, in the microsomal epoxidation of acyclic olefins especially regarding the chirality formation from an achiral molecule and in their hydrolysis especially regarding the chiral selectivity in enantiomeric epoxides although stereoselectivity in hepatic microsomal hydrolysis has been partially shown with a few epoxides such as stilbene oxides [26], mono-substituted stilbene oxides [27], and 9,10-epoxystearates [28]. It is of importance to investigate the optical selectivity in the microsomal oxidative metabolism of the vinyl side chain of sytrene as a model substrate in these respects. In connection with this, previous workers provided tentative evidence that there was little difference between rates of hepatic microsomal hydrolysis of the *R*- and *S*-enantiomers in racemic phenyloxirane [17].

A promising approach to this problem could be to separate and determine *R*-, *S*-, and racemic phenyloxiranes since it appears from the results of the previous investigations that the epoxides yielded from the hydrocarbon by hepatic microsomes in the presence of NADPH are completely converted during incubations at intervals longer than 30 min into phenylethanediol [5, 6] without inversion of the absolute configurations at 2-position of the oxiranes [17]. For approaching to the problem, we synthesized *R*- and *S*-phenyloxiranes and *R*- and *S*-phenylethanediols with purities all higher than 98 per cent and established an HPLC (high performance liquid chromatography) method for the resolution of the enantiomers of phenylethanediol as their diastereoisomeric di-*R*-(+)-MTPA (α-methoxy-α-trifluoromethylphenylacetic acid) esters. In the present communication, we wish to report that there exists a remarkable difference in the rates of microsomal hydrolysis of phenyloxirane enantiomers and also that hepatic microsomal epoxidation of the sytrene vinyl group proceeds with low stereoselectivity to yield *S*-phenyloxirane at slightly higher rate than the *R*-isomer. Finally, we will also provide evidence for the equivalent mutagenicity of *R*- and *S*-phenyloxiranes toward *Salmonella typhimurium* TA strains.

*R*- and *S*-phenyloxiranes used in the present investigation were synthesized as follows: *R*-(−)- and *S*-(+)-phenylethanediols derived from *R*-(−)- and *S*-(+)-mandelic acids (Tokyo Chemical Industry Co. Ltd., Tokyo) with enantiomeric purities more than 98 per cent, respectively, by the reduction with lithium aluminium hydride in the standard manner [29], were reacted with a 0.9 molar ratio each of *p*-toluene-sulfonyl (tosyl) chloride in dry pyridine at 0° for 15 hr and at room temperature for 1 hr. Resulting *R*- and *S*-phenyltosyloxymethylcarbinols (m.p. 70° each) were