divided in the same manner into 3 groups of 6 animals each: infected, heat-exposed and control. All animals were then injected i.p. with 1 ml of a 10% suspension of washed sheep erythrocytes, while those of the infected group were additionally injected with a sublethal dose of live *Salmonella enteritidis* (0.0002 mg bacterial dry weight)[8]. 6 h later, the animals of the heat-exposed group were placed at an ambient temperature of 37 °C, while the thermodes of the hypothalamus-cooled group were perfused with water the temperature of which was regularly adjusted to induce a change in body temperature similar to that elicited by *S. enteritidis*[8]. Both experimental treatments, heat exposure and cooling of the hypothalamus, were discontinued after 5 days, and the animals were followed for another 11 days. Throughout the experiments, body temperature was measured every 8 h, and blood samples were taken at various time intervals. All methods, including the determination of the titre of antibodies against sheep erythrocytes, have been described in detail elsewhere[6].

As there were no significant differences between the control animals with and without thermodes in any of the parameters studied in this work (see also Banet et al.[6]), these animals were pooled into a single control group. Furthermore, because 4 of the heat-exposed animals died during their exposure, though their rectal temperature never increased above 40 °C, the results of this group are not included in this report. 2 other animals, one each from the infected and hypothalamus-cooled groups, also died and their results were excluded. The cause of their death is not known, but it was noted that in both animals body temperature increased to nearly 41 °C on the 3rd experimental day.

*Results.* The figure summarizes the results. During the 1st 5 experimental days, the animals of the hypothalamus-cooled and infected groups maintained higher core temperatures (mean ± SE: 39.7 ± 0.1 and 39.5 ± 0.2 °C, respectively) than those of the control group (37.8 ± 0.2 °C; $p < 0.001$, t-test). The animals of the hypothalamus-cooled group tended to have a lower titre of antibodies than those of the infected group but the highest titres achieved were not significantly different. The highest titres of both groups of experimental animals were, however, significantly higher than those of the control ones ($p < 0.005$).

*Discussion.* Intermittent cooling of the hypothalamus stimulates the humoral immune response[6]. Since antibody titre increased after continuous and prolonged cooling of the hypothalamus, the present results show that the production of antibodies is not impaired when a high body temperature is sustained. Endogenous pyrogen has also been shown to increase the titre of antibodies[5], and this effect appears to be similar to that induced by intermittent cooling of the hypothalamus[6]. Since cooling the hypothalamus simulates the febrile response[7], it is likely that the main effect of pyrogen on antibody titre is due to its febrile rather than to any other of its actions.

Infection with *S. enteritidis* also increased the titre of antibodies. The responses of a host to a pathogen are, of course, too complex to allow a simplistic interpretation of this increase in antibody titre. However, since the effect of infection on antibody titre was similar to that of cooling the hypothalamus, it is possible that a large part of the former effect may be attributable to the febrile action of the endogenous pyrogen induced by the pathogen. These experiments thus support the view that a sustained febrile response stimulates the humoral immune response, but present no evidence as to whether this effect is mediated by the increase in body temperature or by other nonthermal aspects of the febrile response[6,8].

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## Histamine binding to $H_2$ receptors stimulates phospholipid methylation in mast cells

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*Summary.* Rat peritoneal mast cells incubated in vitro in the presence of L-[methyl-$^3$H] methionine and exposed to histamine undergo a rapid but transient increase in phospholipid methylation. By using specific $H_1$- and $H_2$-receptor antagonists, and histamine analogues differing in their $H_2$-receptor agonist potency, it has been demonstrated that this metabolic event is dependent on histamine binding to $H_2$-receptors.

There is substantial evidence that histamine release from basophils and mast cells is modulated by histamine itself through a $H_2$-mediated effect which is apparently coupled to the activation of adenylate cyclase[2,3]. The mechanism(s) responsible for this coupling remain to be determined. Hirata et al.[4] have recently reported that binding of the $\beta$-adrenergic receptor to its agonist, L-isoproterenol, stimulates phospholipid methylation and translocation which, in turn, increases membrane fluidity and coupling of the $\beta$-adrenergic receptor with adenylate cyclase in rat reticulocyte ghosts. Since in mast cells stimulation of both $H_2$ and $\beta$-adrenergic receptors results in adenylate cyclase activation[3], we have investigated whether binding of histamine to the $H_2$-receptors also increase phospholipid methylation in these cells.

*Material and methods.* Purification of mast cells. Male Sprague-Dawley rats (200-250 g) were anesthetized with ether, exsanguinated by cutting the carotid arteries and laparatomized; peritoneal cells were harvested in a medium (MCM buffer) containing 150 mM NaCl, 3.7 mM KCl, 3.0 mM $Na_2HPO_4$, 3.5 mM $KH_2PO_4$, 0.9 mM $CaCl_2$, 5.6 mM dextran, 0.1% (w/v) bovine serum albumin (BSA), 0.1% gelatin and 10 units $ml^{-1}$ heparin (pH 6.8). After purification by a BSA density gradient centrifugation method[5], mast cells of 90% or greater purity were obtained. Cell concentration was determined by a standard microhae-

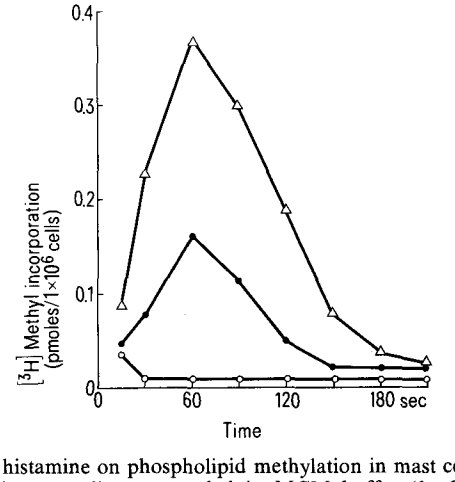
matocrit procedure and cell viability was checked by trypan blue exclusion.

Determination of phospholipid methylation. Purified mast cells were resuspended in 10 ml of MCM buffer containing 0.2 mCi of L-[methyl-$^3$H]methionine (1.66 µM final concentration) and 0.1% BSA at a final concentration of $1 \times 10^6$ cells ml$^{-1}$. After 30-min preincubation at 37 °C, 1 ml aliquots of the cell suspension were transferred to a test tube and the reaction was started by the addition of histamine or histamine analogues at the concentrations indicated. In some experiments, preincubated mast cells were kept at 37 °C for an additional period of 5 min in the presence of increasing amounts of $H_1$ or $H_2$ antagonists and then exposed to histamine (5 µM final concentration). At different times after stimulation, 200 µl samples were pipetted into tubes containing 2 ml of chloroform/methanol/2 M HCl (6:3:1) for phospholipid extraction. After vortex mixing, the aqueous phase was removed and the chloroform phase was washed twice with 1.5 ml of 0.1 M KCl in 50% methanol. Then 1 ml of the chloroform phase was removed, dried at room temperature, dissolved in 5 ml of Triton X-100/toluene-base scintillator, and assayed for radioactivity. Identification of methylated phospholipids was carried out by TLC as described by Hirata et al.[4].

Statistical analysis. Differences from control values were analyzed by Student's t-test. Comparisons were of independent mean values.

Reagents. L-[methyl-$^3$H]methionine ($^3$H-meth) (12 Ci mmole$^{-1}$) was purchased from Amersham Radiochemicals; histamine analogues were kindly supplied by Smith Kline and French Laboratories; all other reagents were obtained from Sigma Chemical Co.

*Results.* When mast cells prepared from rat peritoneal fluids were incubated with histamine, the incorporation of the [$^3$H]methyl groups from $^3$H-meth into cellular phospholipid increased considerably in the 1st 30–60 sec in a dose-response fashion and then gradually decreased to the control level (fig.). Chromatographic analysis of methylated phospholipids on silica gel G plates revealed that the formation of mono and dimethyl phosphatidylethanolamine and phosphatidylcholine markedly increased upon stimulation with histamine. In an attempt to determine the exact receptor specificity involved in this metabolic event, the effect of specific $H_1$- and $H_2$-receptor antagonists was tested. In these experiments the antagonists were preincubated with mast cell suspensions before histamine pulsing. As shown in table 1, histamine-induced phospholipid methylation was inhibited by the $H_2$-receptor antagonists burimamide and cimetidine but not by the $H_1$-receptor antagonists mepyramine and diphenhydramine. Additional evidence for linking histamine-induced phospholipid methylation to $H_2$-receptor binding was obtained by the use of histamine analogues. Histamine, 4-methyl and 3-methylhistamine were incubated with mast cell suspensions at 2




Effect of histamine on phospholipid methylation in mast cells. Rat peritoneal mast cells resuspended in MCM buffer ($1 \times 10^6$ cells ml$^{-1}$) were preincubated for 30 min at 37 °C in the presence of 0.2 mCi of $^3$H-meth. 1 ml aliquots of the cell suspension were then transferred to a test tube and the reaction was started by the addition of 0.1 ml buffer without (○) and with 1 µM (●) or 5 µM (△) (final concentration) histamine. During the preincubation, 0.1–0.2 pmoles of [$^3$H]methyl was incorporated. This value was subtracted from the observed value for each point. The data shown are typical of 3 separate experiments.

Table 1. Effect of $H_1$- and $H_2$-antagonists on histamine-induced phospholipid methylation in mast cells

| Cells exposed to | Final concentration (µM) | Histamine-induced phospholipid methylation (pmoles/$1 \times 10^6$ cells) |
|---|---|---|
| Buffer alone | | 0.45 ± 0.06 |
| Buffer + mepyramine | 0.05 | 0.47 ± 0.07 |
| Buffer + mepyramine | 0.50 | 0.41 ± 0.08 |
| Buffer + mepyramine | 5.00 | 0.36 ± 0.03 |
| Buffer + diphenhydramine | 0.05 | 0.42 ± 0.04 |
| Buffer + diphenhydramine | 0.50 | 0.48 ± 0.04 |
| Buffer + diphenhydramine | 5.00 | 0.40 ± 0.05 |
| Buffer + burimamide | 0.05 | 0.31 ± 0.05 |
| Buffer + burimamide | 0.50 | 0.22 ± 0.07* |
| Buffer + burimamide | 5.00 | 0.12 ± 0.06* |
| Buffer + cimetidine | 0.05 | 0.18 ± 0.05* |
| Buffer + cimetidine | 0.50 | 0.08 ± 0.02* |
| Buffer + cimetidine | 5.00 | 0.02 ± 0.01* |

Rat peritoneal mast cells in MCM buffer ($1 \times 10^6$ cells ml$^{-1}$) were preincubated at 37 °C for 30 min in the presence of 0.2 mCi of $^3$H-meth. 1-ml aliquots of the cell suspension were transferred to a test tube, kept at 37 °C and exposed for 5 min to increasing amount of the indicated histamine antagonists. The reaction was started by the addition of 5 µM (final concentration) histamine and stopped 60 sec later. Phospholipid methylation was measured as described in the text. The value for [$^3$H] methyl incorporated during the preincubation was subtracted from the experimental values. Results are expressed as means ± SEM (3 experiments). *Differences from control value (buffer alone) significant at $p < 0.05$.

Table 2. Effect of histamine and histamine analogues on phospholipid methylation in mast cells

| Substances | Final concentration (µM) | Increase in phospholipid methylation (pmoles/$1 \times 10^6$ cells) |
|---|---|---|
| Histamine | 1 | 0.25 ± 0.05 |
| Histamine | 10 | 0.48 ± 0.10 |
| 4-Methylhistamine | 1 | 0.10 ± 0.02 |
| 4-Methylhistamine | 10 | 0.22 ± 0.06 |
| 3-Methylhistamine | 1 | 0.00 |
| 3-Methylhistamine | 10 | 0.04 ± 0.02 |

Rat peritoneal mast cells in MCM buffer ($1 \times 10^6$ cells ml$^{-1}$) were preincubated at 37 °C for 30 min in the presence of 0.2 mCi of $^3$H-meth. 1 ml aliquots of the cell suspension were then transferred to a test tube. The reaction was started by the addition of histamine or histamine analogues at the indicated concentrations and stopped after 60-sec incubation at 37 °C. Results are expressed as means ± SEM (3 experiments).

different final concentrations (1 or 10 μM) and their stimulatory effect on phospholipid methylation was tested. As shown in table 2, the order of stimulatory potency was histamine > 4-methylhistamine > 3-methylhistamine. This is in accord with the known $H_2$-receptor agonist potency order of these analogues[6], and suggests the involvement of an $H_2$-receptor in the methylation of mastocyte phospholipid by histamine.

*Discussion.* The results of the present investigation indicate that exposure to histamine analogues causes a transient increase of methylated phospholipids in mast cells. The same metabolic event has been reported to occur in rat erythrocyte ghosts following β-adrenoreceptor activation[4], in human monocytes stimulated by chemotactic factors[7], in murine T lymphocytes exposed to mitogenic lectins[8], and in rat mastocytes stimulated by antibody raised against IgE receptors[9]. It seems, therefore, that methylation of phospholipids is an essential step in the recognition and transduction of regulatory signals by eukaryotic cells. It can be supposed that the insertion of methyl groups into the hydrocarbon chains of the fatty acid residues of phospholipids may favor signal transduction by loosening the packing of the lipid molecules in the bilayer thereby increasing membrane fluidity and permeability. Support for this view comes from the finding that phospholipid methylation and demethylation are apparently coupled in rat erythrocytes[10] and in *Dictyostelium discoideum*[11] to the flux of calcium ions across the membrane. The stimulatory effect of histamine on adenylate cyclase activity could thus be generated by localized changes in lipid structure or by changes in binding of $Ca^{2+}$ to the regulatory unit of adenylate cyclase system. Unfortunately, our attempts to demonstrate changes in viscosity or $Ca^{2+}$ binding associated with phospholipid methylation in membranes isolated from mast cells were unsuccessfull, due to the low yield of material available for study, and no final conclusion could be reached.

1 We are grateful to Smith Kline and French Laboratories for the generous gift of histamine analogues.

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## Orally administered melatonin stimulates the 3α/β-hydroxysteroid oxidoreductase but not the 5α-reductase in the ventral prostate and seminal vesicles of pinealectomized rats[1]

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*Summary.* Pinealectomized rats were treated orally with melatonin (MEL) for 14 days. Prostates and seminal vesicles were investigated for the activity of the Δ 4-3-ketosteroid-5α-oxidoreductase (5α-R) and of the 3α/β-hydroxysteroid-oxidoreductase (3α-HSO). The activity of the 3a-HSO was significantly stimulated ($p < 0.01$, Friedman test) when compared to controls. The activity of the 5α-R did not change significantly.

Melatonin (MEL) is primarily considered to be an antigonadotropic hormone[2] which exerts its action by a blockade of the hypothalamic LHRH secretion[3] and by stimulatory or inhibitory influences on the secretion of prolactin[4]. However, some results have reinforced the opinion that it may also have a direct influence on the metabolism of steroids in peripheral organs such as the ovary[5], testes[6,7] and adrenals[8]. Recently we have demonstrated that MEL affects the activity of the reduced NADP: 3α-hydroxysteroid-oxidoreductase (E.C. No. 1.1.1.50) in human hyperplastic prostatic tissue when added in vitro[9].

The aim of this study is to demonstrate whether or not MEL is capable of causing enzymatic changes in the rat prostate and seminal vesicles if administered orally for 14 days to animals in which the endogenous MEL rhythm has been interrupted by pinealectomy and constant illumination.

*Material and methods.* Adult male Wistar rats weighing 320–370 g were used in this study. Water and laboratory chow were available ad libitum. The intestinal absorption of 125 μCi $^3$H-MEL (NEN) plus 1 mg unlabeled MEL (Sigma) was investigated in 4 rats. Blood samples of 20 μl were taken from the retroorbital vein plexus 30, 45, 60, 90, 120, 150, 210, 270 and 720 min after force-feeding and measured for total radioactivity content. The presence of $^3$H-MEL and its metabolites was determined 12 h after force-feeding by TLC in the system chloroform: methanol = 9:1.

16 rats were pinealectomized using a technique similar to that suggested by Kuszak and Rodin[10]. Eight of them received 2 mg MEL (Sigma) in 1 ml of triolein (Sigma) per day orally for 14 days via a bulb headed feeding tube fitted to a syringe (group A) while the remaining 8 rats (group B) and 8 sham-operated animals (group C) were treated with only 1 ml of triolein. All rats were further maintained under constant light in order to suppress the pineal gland in the sham-operated controls and to lower extrapineal MEL secretion.

All hormone and control treatments started 1 day after pinealectomy or after sham-operation and were carried out at 19.00 h. On the 14th day of treatment the rats were bilaterally orchidectomized at 19.00 h and sacrificed 24–27 h later[11]. After exsanguination the ventral lobes of the prostates were removed, dissected free of connective