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Desensitization of histamine H_1 receptor-mediated inositol phosphate accumulation in guinea pig cerebral cortex slices

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1 Histamine stimulated the production of [^{3}H]-inositol phosphates in untreated (control) guinea-pig cerebral cortex slices with a best-fit EC_{50} of $17 \pm 4\,\mu M$, and a best-fit maximum response of $385 \pm 23\%$ over basal accumulation.

2 Histamine pretreatment desensitized guinea-pig cortex slices to a subsequent challenge with histamine, which was observed as a reduction in the best-fit maximum response to $182 \pm 32\%$ over basal accumulation.

3 The time-course for the histamine-induced production of [^{3}H]-inositol phosphates was approximately linear over 90 min of stimulation in both control and histamine pretreated slices. The rate of production in pretreated slices was significantly slowed compared to control, such that by 90 min of histamine stimulation the desensitized slices produced 2.8 times the basal [^{3}H]-inositol phosphate accumulation compared to 5.3 fold the basal [^{3}H]-inositol phosphate accumulation in the control slices.

4 Displacement of [^{3}H]-mepyramine binding to homogenates of guinea-pig cerebral cortex by mepyramine and histamine revealed that histamine pretreatment did not alter the apparent affinity of the H_1 receptor for histamine (control $K_d = 6.3 \pm 0.7\,\mu M$, desensitized $K_d = 7.9 \pm 1.6\,\mu M$) or mepyramine (control $K_d = 3.4 \pm 0.8$ nM, desensitized $K_d = 3.4 \pm 1.3$ nM), nor was there any reduction in the calculated maximum number of [^{3}H]-mepyramine binding sites (control $B_{max} = 192 \pm 31$ fmol mg^{-1} protein, desensitized $B_{max} = 220 \pm 50$ fmol mg^{-1} protein).

5 The histamine-mediated desensitization of response in guinea-pig slices was mediated by the H_1 receptor subtype, since the attentuated maximum histamine-stimulated [^{3}H]-inositol phosphate accumulation could not be prevented by inclusion of an H_2- (ranitidine) and an H_3- (thioperamide) receptor antagonist during the pretreatment period.

6 The desensitized histamine-stimulated [^{3}H]-inositol phosphate accumulation recovered to 90% of control levels over a period of 150 min after the removal of the conditioning dose of histamine, with a half-time of recovery of about 95 min.

Keywords: Histamine H_1 receptors; desensitization; [^{3}H]-inositol phosphates; guinea-pig cerebral cortex

Introduction

It is well known that many hormone- and neurotransmitter-induced signalling pathways are desensitized after persistent agonist stimulation. This ubiquitous regulatory mechanism is manifest as an attenuation of cellular responsiveness to continued or subsequent receptor activation and plays a pivotal role in regulating cellular homeostasis. In addition, *in vivo* receptor desensitization has clinical implications, being purported to have a role in the aetiology of several diseases (Brodde & Michel, 1989) and suggested to be responsible for the tolerance to certain drugs after long-term treatment (Brodde & Michel, 1989; Rickels *et al.*, 1983).

The molecular mechanisms underlying receptor desensitization appear multifaceted and can be divided into two phases: homologous desensitization (receptor-specific) which involves the selective loss of response to the stimulated receptor only, and heterologous desensitization (receptor non-specific) which in addition to a reduced responsiveness of the stimulated receptor, also involves an attenuation of responses induced by other receptor systems. The molecular basis for agonist-induced desensitization has been characterized in detail for the catecholamine-induced desensitization of the β-adrenoceptor system. Here, both phases of desensitization can be observed that appear to be mediated, at least in part, by protein phosphorylation (Huganir & Greengard, 1990). In contrast to the β-adrenoceptor-adenylate cyclase pathway, much less information is available on the mechanisms of agonist-induced desensitization of receptors coupled to phospholipase C (PLC). There is evidence that receptors coupled to PLC, such as muscarinic acetylcholine receptors, α_1-adrenoceptors, angiotensin II and histamine H_1 receptors, desensitize by both homologous and heterologous mechanisms and that modulation of the activity of protein kinase C can affect the development of desensitization (Hepler *et al.*, 1988; Mitsuhashi & Payan, 1988; Cowlen *et al.*, 1990), implicating a role for phosphorylation in this process (Huganir & Greengard, 1990).

Histamine is an established neurotransmitter in the mammalian CNS, where it is found in discrete pathways (Pollard & Schwartz, 1987). It interacts with three pharmacologically distinct receptors subtypes in the CNS, H_1, H_2 and H_3, to regulate a plethora of physiological actions, such as arousal state, locomotor activity, brain energy metabolism, neuroendocrine, autonomic and vestibular functions, feeding, drinking, sexual behaviour and analgesia (Wada *et al.*, 1991). Much work in the brain has focused on the histamine H_1 receptor which is coupled to inositol phospholipid hydrolysis, and thus to the mobilization of intracellular calcium (Hill, 1990). The responses induced by H_1 receptors can be desensitized after prolonged agonist stimulation in both a homologous (Nakahata & Harden, 1987; Dillon-Carter & Chuang, 1989; Cowlen *et al.*, 1990) and heterologous (Brown *et al.*, 1986; McDonough *et al.*, 1988) manner. Previous studies have shown that the histamine-induced hydrolysis of membrane inositol phospholipids (Nakahata & Harden, 1987; Cowlen *et al.*, 1990) is desensitized by agonist pretreatment, as well as the intracellular pathways distal to calcium mobilization, such as histamine-stimulated guanosine 3′:5′-cyclic monophosphate (cyclic GMP) formation (Taylor & Richelson, 1979), -glycogen

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hydrolysis (Quach et al., 1981) and -calcium fluxes (Brown et al., 1986). However, the H_1 agonist-induced desensitization of histamine-stimulated inositol 1,4,5-trisphosphate (IP_3) production has only been characterized in cultured cells and not in more complex CNS tissue preparations, such as brain slices, which contain a heterogeneous populations of communicating cells. In this paper we describe the desensitization of H_1 receptor-mediated inositol phosphate production in guinea-pig cerebral cortex slices.

Methods

Accumulation and extraction of [^{3}H]-inositol phosphates

Guinea-pig cerebral cortices (male, Dunkin Hartley strain, 350–500 *g*) were cross-chopped into 350 × 350 μm slices (McIlwain tissue chopper), dispersed and washed 3 times in Krebs-Henseleit medium (composition in mM: NaCl 116, KCl 4.7, $MgSO_4$ 1.1, KH_2PO_4 1.2, $NaHCO_3$ 25, $CaCl_2$ 2.5, D-glucose 11). The slices were then incubated at 37°C in continuously gassed (95% O_2:5% CO_2) Krebs-Henseleit medium for 60 min with 4 changes of medium. A proportion of the slices were preincubated in histamine (100 μM) containing Krebs-Henseleit medium and the remainder preincubated in Krebs-Henseleit medium alone for 30 min at 37°C with continuous gassing (95% O_2:5% CO_2). In experiments to determine the receptor subtype mediating the desensitization effect, ranitidine (100 μM, H_2 antagonist) and thioperamide (1 μM, H_3 antagonist) were included in both the control and histamine pretreatment mediums. The slices were washed thoroughly (4 × 50 ml Krebs-Henseleit medium) and transferred to an Eppendorf Multipipette tip and allowed to settle under gravity. Aliquots of the slices (50 μl, approx 1.5 mg protein) were added to 190 μl of Krebs-Henseleit medium containing 0.33 μM *myo*-[2-^{3}H]-inositol (1 μCi per incubation) and 10 mM LiCl, in insert vials. The vials were gassed (95% O_2:5% CO_2), capped and incubated for 30 min in a shaking water bath (37°C) before the addition of 10 μl solution of Krebs-Henseleit medium or histamine (10^{-6}–10^{-3} M). After incubation for a further 45 min, the reaction was terminated by addition of 250 μl of an ice-cold solution of 10% perchloric acid containing EDTA (1 mM) and phytic acid (1 mg ml^{-1}). Tubes were then allowed to stand on ice for 20 min before addition of 400 μl of freshly prepared trioctylamine/1,1,2 trichlorotrifluoroethane mixture (1:1 v:v) to extract the inositol phosphates (Sharpes & McCarl, 1982). The samples were then vortex mixed for 15 s and centrifuged at 1500 *g* for 3 min. The upper aqueous phase (350 μl) was transferred to new insert vials and neutralized with 3 ml of HEPES (100 mM, pH 7.5), and each sample was then applied to an AG1-X8 (formate form, 100–200 mesh, Biorad) anion-exchange column. [^{3}H]-inositol and [^{3}H]-glycerophosphoinositol were eluted with 10 ml water and 10 ml 60 mM ammonium formate/5 mM sodium tetraborate, respectively, and [^{3}H]-inositol mono-, bis- and tris-phosphates ([^{3}H]-IP_n) were collectively eluted with 10 ml 800 mM ammonium formate/100 mM formic acid. Quicksafe scintillation fluid (10 ml, Zinsser Analytic) was added to each fraction and tritium determined by scintillation counting.

Inhibition of [^{3}H]-mepyramine binding

Guinea-pig cerebral cortex slices were prepared and pretreated as described above. The histamine preincubated and control slices were then washed thorougly with ice-cold Krebs-Henseleit medium (2 × 50 ml) followed by ice-cold *N*-Tris-(hydroxymethyl)-methyl-2-amino-ethanesulphonic acid (TES-KOH) buffer (10 mM, pH 7.5, 2 × 50 ml), prior to homogenization (Teflon-glass homogenizer, 3 passes). Measurement of the binding of [^{3}H]-mepyramine to the cerebral cortex homogenates (385 ± 39 μg protein, $n = 8$) was performed immediately. [^{3}H]-mepyramine (1 nM) binding and inhibition by various concentrations of competing ligand (mepyramine: 1×10^{-12}–1×10^{-7} M and histamine 1×10^{-8}–1×10^{-2} M) was allowed to come to equilibrium at 30°C for 60 min before the reaction was terminated by rapid filtration (Brandel 24-place cell harvester) through GF/B filters (presoaked in 0.3% polyethylenimine), essentially as described by Treherne & Young (1988). The buffer used was 10 mM TES-KOH (pH 7.5) and the non-specific binding component was defined by use of 1 μM temelastine in each experiment. For each experiment the concentration of [^{3}H]-mepyramine was determined by scintillation spectrometry and the protein concentration determined according to Lowry *et al.* (1951).

Analysis of data

The histamine-induced concentration-response curves for [^{3}H]-inositol phosphate accumulation in cerebral cortex slices were expressed as a percentage increase above basal values (unstimulated) in each experiment and fitted to a Hill equation (logistic equation) according to Crawford *et al.* (1990). Each point was weighted according to the reciprocal of the variance associated with it. The errors associated with the best-fit parameters are appreciable with this approach, since the number of points on each curve is limited by the need to obtain curves for both histamine-pretreated and control slices within the same experiment. However, fitting the curves in this way does have the advantage of giving unbiased estimates of EC_{50} and maximum response.

To obtain a best-fit value of the IC_{50} ± estimated s.e.mean for curves of mepyramine and histamine inhibition of [^{3}H]-mepyramine binding to cerebral cortex homogenates, the curves were fitted as above to the equation:

$$\%\text{ of the uninhibited binding of [}^3\text{H]-mepyramine} = \frac{100-\text{NSB}}{((A/IC_{50})^n + 1)} + \text{NSB}$$

with IC_{50}, n and NSB as variables. A is the concentration of inhibitor, IC_{50} the inhibitor concentration giving 50% inhibition of specific binding, n the Hill coefficient and NSB non-specific binding. Analysis of statistical significance of differences between observations was performed by the Student's *t* test (unpaired data) using MultiStat statistics package implemented on a Macintosh LCII computer.

Chemicals

[^{3}H]-*myo*-inositol (specific activity 15–18 Ci mmol^{-1}) was obtained from New England Nuclear and histamine dihydrochloride and mepyramine maleate were purchased from Sigma. Ranitidine was a gift from Glaxo and Thioperamide was purchased from Cookson Chemicals. All other compounds were analytical grade and purchased mainly from BDH and Sigma

Results

Effect of histamine pretreatment on histamine-stimulated [^{3}H]-inositol phosphate accumulation in guinea-pig cerebral cortex slices

The concentration-response curve for histamine-stimulated [^{3}H]-inositol phosphate ([^{3}H]IP_n) accumulation in guinea pig cerebral cortex slices in the presence of 10 mM Li^+ is shown in Figure 1. Histamine stimulated the production of [^{3}H]-inositol phosphates in untreated (control) guinea-pig cerebral cortex slices with a best-fit EC_{50} of 17 ± 4 μM (mean ± s.e.mean, 6 experiments), with a 1 mM histamine response of 385 ± 24% over basal accumulation. The histamine response is mediated by the H_1 receptor subtype, since mepyramine (1 μM), a selective H_1 antagonist, complete-

ly abolished (99.6% inhibition, mean of 2 experiments) the response to 100 μM histamine, whereas the H_2 receptor antagonist, ranitidine (100 μM, 6% inhibition, mean of 2 experiments), and a selective H_3 receptor antagonist, thioperamide (1 μM, 7% inhibition, mean of 2 experiments), were ineffective. The antagonist results and the EC_{50} value for the histamine-stimulated [^{3}H]-IP_n accummulation in untreated slices of 17 μM are in accordance with previous reports (Daum *et al.*, 1983; 1984).

Preincubation of the cerebral cortex slices for 30 min with 100 μM histamine resulted in a subsequent concentration-response curve for histamine with an EC_{50} of 23 ± 19 μM (4 experiments); this value is not significantly different from the EC_{50} value from control slices. The best-fit maximum histamine-stimulated [^{3}H]-IP_n production in histamine-pretreated slices was 182 ± 32% (4 experiments) over basal accumulation (Figure 1). Significant differences were obtained at the two highest histamine concentrations tested, 10^{-4} M ($P<0.002$, Student's t test) and 10^{-3} M ($P<0.009$), between the response induced in control compared to pretreated brain slices. In several experiments we observed in both control and desensitized slices that histamine concentrations of above 10^{-3} M induce an [^{3}H]-IP_n accumulation less than that obtained with 10^{-3} M histamine.

Time-course of histamine-stimulated [^{3}H]-inositol phosphate accumulation in guinea-pig cerebral cortex slices

The rate of histamine-stimulated (100 μM) and basal (unstimulated) accumulation of [^{3}H]-IP_n in untreated and histamine-pretreated guinea-pig cerebral cortex slices is shown in Figure 2. The accumulation of [^{3}H]-IP_n ([^{3}H]-inositol mono-, bis- and trisphosphates) in non-pretreated slices of guinea-pig cerebral cortex rises at a near linear rate, reaching 5.3 fold over basal by 90 min of histamine stimulation. In histamine-pretreated slices the time course of accumulation of [^{3}H]-IP_n also rises at a near linear rate, but the rate of accumulation of [^{3}H]-IP_n is significantly reduced ($P<0.03$, Student's t test) and by 90 min of stimulation the response is only 2.8 times basal levels. The basal levels for both untreated and pretreated slices steadily increase at approximately the same rate over the 90 min stimulation period (Figure 2). Note that there is a slight increase in the basal accumulation of [^{3}H]-IP_n in pretreated compared to control slices, but this difference is insignificant relative to the attenuation of histamine-stimulated response in treated compared to control tissue.

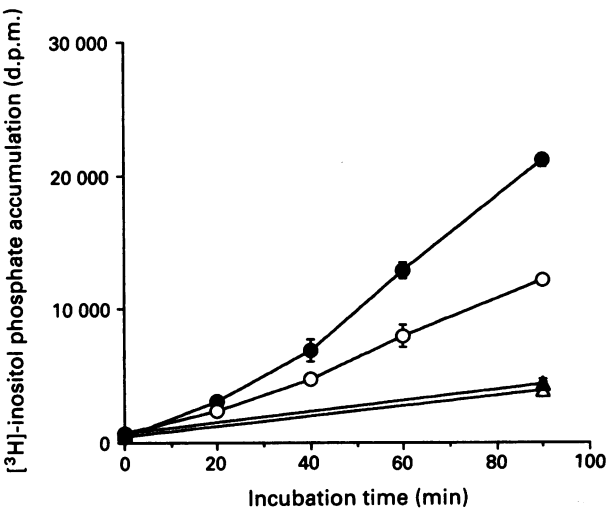

Figure 2 Time course of histamine-induced (100 μM) and basal accumulation of [^{3}H]-inositol mono-, bis- and tris phosphates ([^{3}H]-IP_n) in guinea-pig cerebral cortex slices. Cerebral cortex slices were prepared, preincubated, prelabelled with [^{3}H]-*myo*-inositol, and stimulated with histamine (10^{-4} M) or Krebs-Henseleit medium (basal) in the presence of LiCl (10 mM), and the [^{3}H]-IP_n extracted and measured as described under Methods. The graph is expressed as mean d.p.m. values ± s.e.mean of triplicate determinations from a representative experiment. Very similar results were obtained on a further two occasions. At all time points the histamine-treated slices were significantly lower (to at least $P<0.03$) than the corresponding control slices. At 90 min incubation the basal [^{3}H]-IP_n accumulation was not significantly different under the two conditions. The lines represent histamine-induced response in control (●) and histamine-pretreated (○) slices and basal (Krebs-Henseleit medium) response in control (▲) and histamine-pretreated (△) slices.

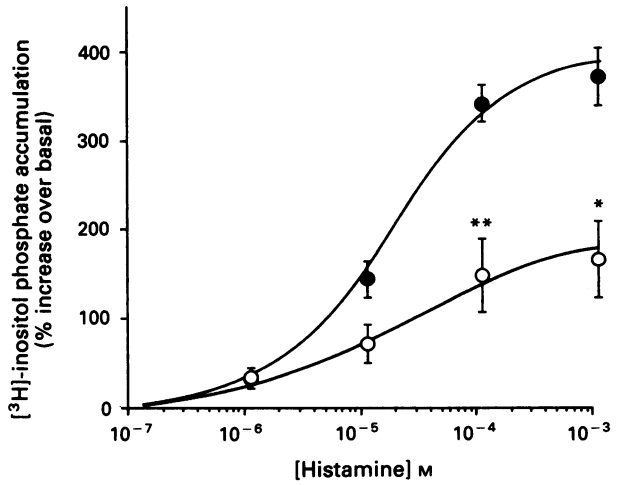

Figure 1 Concentration-response curve for histamine-induced [^{3}H]-inositol mono-, bis- and tris-phosphates ([^{3}H]-IP_n) formation in control (●) and desensitized (○) guinea-pig cerebral cortex slices. Cerebral cortex slices were prepared, preincubated, prelabelled with [^{3}H]-*myo*-inositol, and stimulated with histamine (10^{-6} M–10^{-3} M) in the presence of LiCl (10 mM), and the accumulated [^{3}H]-IP_n extracted and measured as described under Methods. In order to allow for variations in cellular response between experiments, values were standardized against [^{3}H]-IP_n accumulation in cells without histamine stimulation (basal). Each point is expressed as % increase over basal and is the mean ± s.e.mean of 6 (control) and 4 (histamine-pretreated) independent experiments.

Significantly different from control at $*P<0.009$ and $**P<0.002$, respectively. The basal [^{3}H]-IP_n accumulation ranged from 1455–2051 d.p.m. in control slices and 2307–3023 d.p.m. in desensitized slices. Note that these values are not corrected for variations in protein concentrations (i.e. number of brain slices per incubation) between control and histamine-pretreated preparations.

Inhibition of [^{3}H]-mepyramine binding to untreated (control) and desensitized guinea-pig cortex homogenates by mepyramine and histamine

The inhibition of [^{3}H]-mepyramine binding to untreated (control) and desensitized guinea-pig cortex homogenates by mepyramine and histamine is shown in Figure 3. The displacement of [^{3}H]-mepyramine (1 nM) binding to untreated and desensitized guinea-pig cortex homogenates by unlabelled mepyramine occurs with an apparent equilibrium dissociation constant, K_d, of 3.4 ± 0.8 nM (mean ± s.e.mean, 4 experiments) and 3.4 ± 1.3 nM, respecticely. However, both mepyramine inhibition curves had Hill coefficients less than unity (0.44 ± 0.04 and 0.58 ± 0.1, respectively) which may be a consequence of the experimental protocol adopted, although the reason for the low slope is not clear. The calculated maximum number of binding sites, B_{max}, for [^{3}H]-mepyramine was 192 ± 31 fmol mg^{-1} protein (untreated) and 220 ± 50 fmol mg^{-1} protein (desensitized). Histamine displacement of [^{3}H]-mepyramine binding to untreated and desensitized guinea-pig cortex homogenates reveals apparent

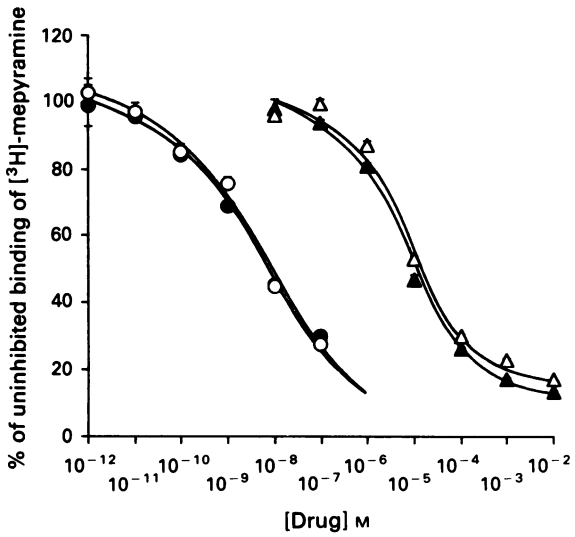

Figure 3 Inhibition of [^{3}H]-mepyramine binding to guinea-pig cortex homogenates by mepyramine and histamine. Guinea-pig cortex slices were prepared and pretreated with histamine (100 μM, 30 min) or untreated (control), and homogenized as described under Methods. Results are expressed as % of the total binding of [^{3}H]-mepyramine and are means ± s.e.mean of triplicate determinations from 4 experiments. The lines represent control homogenates displaced by mepyramine (●) and histamine (▲) and histamine pretreated homogenates displaced by mepyramine (○) and histamine (△).

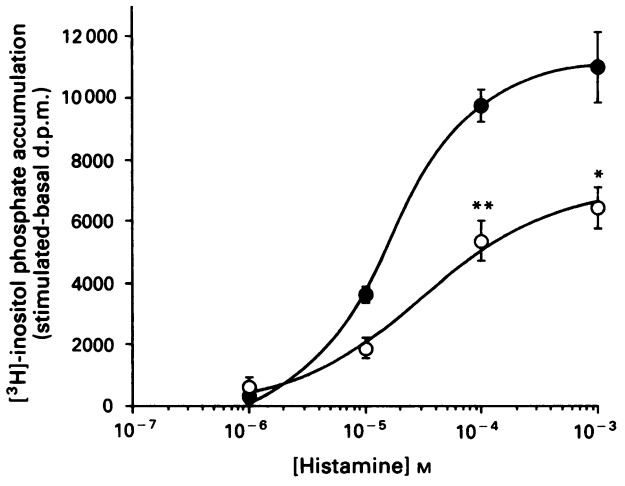

Figure 4 Effect of histamine H_2 and H_3 receptor antagonists on the development of histamine-mediated desensitization of [^{3}H]-inositol mono-, bis and tris phosphates ([^{3}H]-IP$_n$) accumulation in guinea-pig cerebral cortex slices. Cortical slices were prepared according to the Methods section and then pretreated with histamine (100 μM) in the presence of ranitidine (100 μM) and thioperamide (1 μM) (○, histamine + inhibitors) or pretreated with ranitidine (100 μM) and thioperamide (1 μM) alone (●, control + inhibitors). After extensive washing the slices were rechallenged with histamine (10^{-6}–10^{-3} M) for 45 min before the extraction and measurement of [^{3}H]-IP$_n$ accumulation as described under Methods. Each point is given as the stimulated – basal [^{3}H]-IP$_n$ accumulation in d.p.m. values (mean ± s.e.mean of triplicate determinations) from a representative experiment. The same experiment was repeated on two other occasions with similar results. Significantly different from control- + inhibitors at $^*P<0.007$ and $^{**}P<0.004$, respectively.

K_d values of 6.3 ± 0.7 μM (mean ± s.e.mean, 4 experiments; Hill coefficient 0.64 ± 0.03) and 7.9 ± 1.6 μM (Hill coefficient 0.65 ± 0.06), respectively. In all cases the parameters determined for the untreated guinea-pig cortex homogenates were not significantly different from those obtained in histamine-pretreated homogenates.

Effects of H_2 and H_3 receptor antagonists on histamine-induced desensitization

The histamine concentration-response curve for the accumulation of [^{3}H]-IP$_n$ in guinea-pig cortex slices pretreated with histamine (30 min) in the presence of ranitidine (100 μM) and thioperamide (1 μM) (histamine + inhibitors) and pretreated with ranitidine and thioperamide alone (control- + inhibitors) is shown in Figure 4. The EC_{50} for histamine of 18 ± 1 μM (mean ± s.e.mean, 3 experiments) derived from the control + inhibitors slices is not significantly different (Student's t test) from that obtained in non-pretreated (control) slices. The EC_{50} value for histamine in the histamine- + inhibitors pretreated brain slices of 30 ± 16 is also not significantly different from the EC_{50} value derived from slices pretreated with histamine alone (Figure 1), nor is it significantly different from the control + inhibitors EC_{50} value. The response to both 1 mM and 0.1 mM histamine in guinea-pig cerebral cortex slices pretreated with histamine (100 μM) in the presence of ranitidine and thioperamide were significantly ($P<0.007$ and $P<0.004$, respectively, Student's t test) attenuated compared to control + inhibitors brain slices (Figure 4), consistent with the involvement of histamine H_1 receptors in the desensitization process.

Recovery from histamine-induced desensitization of [^{3}H]-inositol phosphate accumulation in guinea-pig cerebral cortex slices

Histamine-stimulated [^{3}H]-IP$_n$ accumulation was measured in untreated and histamine-pretreated slices at various times

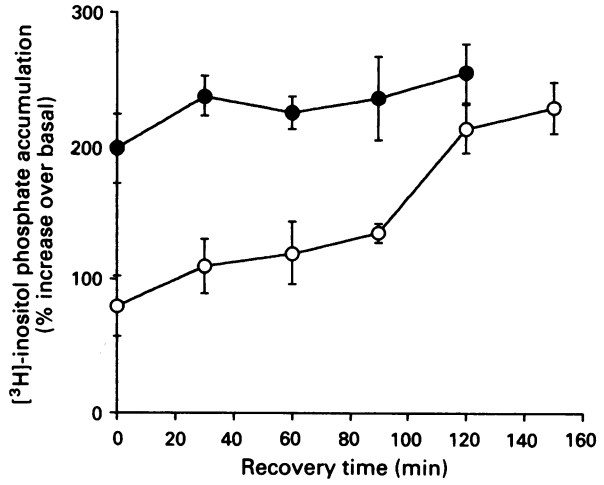

Figure 5 Time-course for recovery from desensitization of [^{3}H]-inositol mono-, bis- and tris-phosphates ([^{3}H]-IP$_n$) accumulation in guinea-pig cortex slices. Cerebral cortex slices were prepared, histamine preincubated, and prelabelled with [^{3}H]-*myo*-inositol as described under Methods. Slices from both control (●) and histamine-pretreated (○) preparations were allowed to recover for between 0 and 150 min (in Krebs-Henseleit medium at 37°C continuously gassed with 95% O_2:5%CO_2) before being stimulated with histamine (10^{-4} M) for 45 min and the [^{3}H]-IP$_n$ extracted and measured as described under Methods. Values at each time-point were calculated as a % increase over basal [^{3}H]-IP$_n$ accumulation and are the mean ± s.e.mean of three independent experiments. The histamine-stimulated [^{3}H]-IP$_n$ accumulation was significantly reduced in the desensitized slices (○) compared to controls at 0, 30, 60 and 90 min of recovery at $P<0.03$, 0.007, 0.01 and 0.03, respectively (Student's t test).

(0–150 min) after the removal of histamine (pretreated) and the washing procedure to assess the recovery of the histamine response (Figure 5). The histamine response was relatively constant at 230 ± 9% (mean ± s.e.mean, 5 experiments) increase over basal throughout 150 min of the experiment in the untreated (control) slices. In the desensitized slices the histamine-induced response was only 80 ± 23% over basal immediately after the removal of histamine ($t = 0$), before recovery of the response occurred over the following 150 min. The histamine-induced response in the desensitized slices was significantly lower (Student's test) than the corresponding control slices at 0 ($P < 0.03$), 30 ($P < 0.007$), 60 ($P < 0.01$) and 90 ($P < 0.03$) min of recovery. After removal of histamine the desensitized tissue had recovered to 84% of the control response by 120 min and to 90% by 150 min and was not significantly different from the histamine response measured in the control slices (at 120 min). The half-time for the recovery of the histamine-mediated [^{3}H]-IP_n accumulation was estimated at 95 min.

Discussion

The results presented here show clearly that histamine H_1 receptor-mediated [^{3}H]-IP_n accumulation in guinea-pig cerebral cortex slices can be desensitized by a prior acute treatment of the cells with histamine. The desensitization effect is mediated by histamine acting on H_1 and not H_2 or H_3 receptors and is manifest as a reduction in the maximum histamine-stimulated IP_n accumulation and an attenuated time-course for [^{3}H]-IP_n production. This desensitization effect is essentially the same as that observed for histamine H_1 receptor-induced [^{3}H]-IP_n accumulation in HeLa cells (Bristow & Young, 1991; Bristow & Zamani, 1993) and the P_{2Y} purinoceptor-coupled phosphoinositidase C system in Turkey erythrocytes (Martin & Harden, 1989). The slowed time course for [^{3}H]-IP_n production is a very subtle way of desensitizing the system to agonist stimulation, but the effect of this reduced rate of [^{3}H]-IP_n formation on the intracellular calcium mobilization and the ultimate cellular function is unknown and awaits further investigation. The desensitization of the response does not appear to involve a reduction in the affinity of the H_1 receptor for histamine or the antagonist mepyramine, nor does it seem to be the result of a reduction in the number of H_1 receptors in the cerebral cortex slice preparation. Characterization of the histamine-mediated desensitization phenomenon shows that the effect is reversible with an almost full recovery of the response after removal of the desensitizing stimulus.

Histamine pretreatment of rat cerebellar granule cells (Dillon-Carter & Chuang, 1989), N1E-115 mouse neuroblastoma cells (Taylor & Richelson, 1979), HeLa cells (Bristow & Zamani, 1993), BC3H-1 muscle cells (Brown *et al.*, 1986), 1321N1 human astrocytoma cells (McDonough *et al.*, 1988), DDT_1 MF-2 cells (Cowlen *et al.*, 1990), guinea-pig ileum (Donaldson & Hill, 1986), rabbit aorta (Lurie *et al.*, 1985), guinea-pig jejunum (Leurs *et al.*, 1990) and also of mouse cerebral cortex slices (Quach *et al.*, 1981) has been shown to attenuate histamine-induced intracellular responses. We are able to extend this list to include the H_1 receptor-mediated [^{3}H]-IP_n production in guinea-pig cerebral cortex slices. The type of desensitization mechanism shown in the previous studies varies, with both homologous and heterologous forms of H_1 receptor desensitization being reported. Homologous desensitization is thought to involve an alteration at the level of the receptor molecules, perhaps by phosphorylation, although no evidence has been presented to date to support this contention for H_1 receptors. Some evidence does support the role of PKC in regulating H_1 receptor responsiveness (Mitsuhashi & Payan, 1988; Murray *et al.*, 1989; Cowlen *et al.*, 1990) and acute histamine stimulation of cells does appear to cause protein phosphorylation (Levin & Santel, 1991; Raymond *et al.*, 1991). However, the PKC-mediated attenuation of H_1 receptor responses may be a non-selective desensitization mechanism, since in HeLa cells the agonist-mediated desensitization of histamine H_1 receptors appears to occur independently of PKC activation (Smit *et al.*, 1992; Zamani & Bristow, unpublished observations).

Here we show that H_1 receptor desensitization in guinea-pig cerebral cortex involves a reduction in the maximum histamine-induced response, an effect that has also been found in NIE-115 neuroblastoma cells (Taylor & Richelson, 1979), BC3H-1 muscle cells (Brown *et al.*, 1986), DDT_1 MF-2 smooth muscle cell line (Cowlen *et al.*, 1990) and HeLa cells (Bristow & Zamani, 1993). We observe about a 60% desensitization of the 10^{-3} M histamine-stimulated [^{3}H]-IP_n production, which is of similar magnitude to the degree of desensitization of response observed in mouse brain slices (Quach *et al.*, 1981), NIE-115 cells (Taylor & Richelson, 1979) and DDT_1 MF-2 smooth muscle cells (Cowlen *et al.*, 1990). However, due to the delay inherent in our experimental procedure, [^{3}H]-IP_n accumulation was measured 70 min after the removal of the desensitizing stimulus, and considering our findings that the desensitization process is reversible (Figure 5), the actual maximum extent of desensitization of this system may be greater than the earliest measurable value of 60% inhibition.

Our study of guinea-pig cortex slices and homogenates do not show any significant alteration in the EC_{50} values for histamine-induced [^{3}H]-IP_n accumulation, the maximum number of binding sites (B_{max}) for [^{3}H]-mepyramine, nor was there any change in the apparent affinity of the H_1 receptor for histamine and mepyramine. However, histamine inhibition of [^{3}H]-mepyramine binding may not be a sensitive measure of receptor-effector coupling, since GTP, GPPNHP or GTPγS produce only a small, or no, shift in the histamine inhibition curves in membranes from guinea-pig whole brain (Chang & Snyder, 1980), guinea-pig cerebellum (Bristow & Young, unpublished observations) or HeLa cells (Arias-Montaño & Young, unpublished observations). The [^{3}H]-mepyramine binding evidence suggests that the desensitization process in guinea-pig cortex does not involve an alteration in H_1 receptor affinity for agonists or antagonists. Thus, the attenuation of the response is likely to occur at sites distal to the initial agonist binding interaction and may involve a change in the activity of the transduction pathway.

The lack of a reduction in the B_{max} for [^{3}H]-mepyramine binding to histamine-pretreated cerebral cortex homogenates does not exclude the possibility that the reduction in response is due to a loss of functional H_1 receptors from the cell surface. In fact our results are in contrast to the situation reported in mouse cerebral cortex, where a small (18%) reduction in [^{3}H]-mepyramine binding sites was observed after a similar desensitization protocol to that used here (Quach *et al.*, 1981). We believe that our findings of an unchanged B_{max} are more consistent with the mechanism involved in acute (< 2 h stimulation) agonist desensitization of other receptor systems, which do not appear to involve receptor down-regulation and degradation (Huganir & Greengard, 1990). Furthermore, a result indicating a reduction in the B_{max} of H_1 receptors using [^{3}H]-mepyramine should be viewed with caution, since the physiochemical nature of this ligand make it freely accessible to both cell-surface and intracellular compartments, and thus it is unlikely to detect H_1 receptor down-regulation. A reduction in [^{3}H]-mepyramine binding sites would only be observed if desensitization involved the concomitant degradation of H_1 receptors, and the report of Quach *et al.* (1981) clearly shows that this is not the case.

It is clear from previous research that histamine-induced H_1 receptor desensitization can occur by different processes that seem to be dependent on the tissue preparation. For instance, homologous desensitization occuring at the level of the H_1 receptor has been shown to occur in the DDT_1 MF-2 smooth muscle cell line (Cowlen *et al.*, 1990) and mouse cerebral cortex slices (Quach *et al.*, 1981), whereas the

heterologous type of receptor desensitization involving the H_1 receptor and the attenuation of other receptor-mediated events after prolonged histamine pretreatment has also been reported in HeLa cells (Bristow & Zamani, 1993), BC3H-1 smooth muscle cells (Brown *et al.*, 1986) and 1321N1 human astrocytoma cells (McDonough *et al.*, 1988). In guinea-pig cerebral cortex slices the homologous or heterologous nature of the histamine H_1 receptor desensitization is unresolved. Preliminary studies on the effect of histamine pretreatment on either carbachol- (muscarinic acetylcholine receptor agonist) or noradrenaline (α_1-adrenoceptor agonist) induced $[^3H]$-IP_n accumulation in guinea-pig cortex slices have to date failed to provide consistent answers (Bristow and Banford, unpublished observation) and the mechanism of histamine-induced desensitization must remain a target for future studies.

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