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HISTAMINE H_3-RECEPTOR ACTIVATION AUGMENTS VOLTAGE-DEPENDENT Ca^{2+} CURRENT VIA GTP HYDROLYSIS IN RABBIT SAPHENOUS ARTERY

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SUMMARY

1. Actions of histamine on the voltage-dependent $Ba^{2+}(Ca^{2+})$ currents (I_{Ba}, I_{Ca}) were investigated using the whole-cell patch-clamp technique on dispersed smooth muscle cells from the rabbit saphenous artery.

2. Histamine (half-maximal dose, EC_{50} = 530 nM) augmented the I_{Ba} evoked by a brief depolarizing pulse (100 ms duration; to +10 mV from a holding potential of −80 mV) in a concentration-dependent manner. The maximum augmentation was obtained with 30 μM-histamine (1·29 times control). This augmentation of I_{Ba} was inhibited by the H_3-antagonist, thioperamide (K_i = 30 nM, slope of the Schild plot = 1·0), but not by H_1- or H_2-antagonists (mepyramine or diphenhydramine, or cimetidine, respectively).

3. An H_3-agonist, $R\alpha$-methylhistamine (EC_{50} = 93 nM), also augmented I_{Ba} in a concentration-dependent manner at a holding potential of −80 mV and the maximum augmentation (1·25 times control) was obtained with 10 μM. This augmentation was also inhibited by thioperamide, but not by the above H_1- and H_2-antagonists.

4. Intracellularly applied 500 μM-guanosine 5′-triphosphate (GTP) enhanced, but 1 mM-guanosine 5′-*O*-(2-thiodiphosphate) (GDPβS) abolished, the histamine-induced augmentation of I_{Ba}. When one of the non-hydrolysable GTP analogues, guanosine 5′-*O*-(3-thiotriphosphate) (GTPγS; > 5 μM), guanylyl-imidodiphosphate (GMP-PNP; 200 μM) or guanylyl (β,γ-methylene)-diphosphonate (GMP-PCP; 1 mM) was intracellularly applied, the I_{Ba} amplitude evoked without the application of histamine was not affected, but the excitatory effect of histamine on I_{Ba} was reversed to an inhibition. Pre-treatment with pertussis toxin (PTX: 300 ng/ml and 3 μg/ml) did not modify the histamine-induced responses in the absence or presence of GTPγS.

5. 4β-Phorbol 12,13-dibutylate (PDBu) increased the amplitude of I_{Ba}. However, this action of PDBu was not enhanced by the application of GTP (500 μM) in the pipette, but additional application of histamine further increased the amplitude of I_{Ba}. Pre-treatment with a potent non-selective protein kinase inhibitor, 1-(5-isoquinolinesulphonyl)-2-methylpiperazine dihydrochloride (H-7; 100 μM), did not

modify the histamine-induced current augmentation or inhibition observed in the presence or absence of intracellular GTPγS.

6. These results indicate that (i) the histamine H_3-receptor is present on the smooth muscle membrane of the rabbit saphenous artery, (ii) H_3-receptor stimulation augments I_{Ba} via activation of a PTX-insensitive GTP-binding protein (G-protein) directly or via unknown mechanisms that do not involve the actions of protein kinase C or cyclic nucleotide-activated protein kinases, (iii) GTP hydrolysis is required for the action of the G-protein in augmenting I_{Ba}, whereas stimulation of G-protein in the presence of non-hydrolysable GTP analogues inhibits I_{Ba}.

7. Effects similar to those observed on the actions of histamine by intracellularly applied guanine nucleotides were also seen on the noradrenaline- or angiotensin II-induced I_{Ba} augmentation. Therefore, G-protein-mediated GTP hydrolysis may be a common mechanism for the receptor-mediated augmentation of the voltage-dependent Ca^{2+} channel in smooth muscle cells of the rabbit saphenous artery.

INTRODUCTION

In vascular smooth muscle cells, activation of the voltage-dependent Ca^{2+} channel and of receptor-operated second-messenger synthesis – especially synthesis of inositol 1,4,5-trisphosphate ($InsP_3$) through hydrolysis of phosphatidyl inositol 4,5-bisphosphate by phospholipase C – are thought to be essential factors for the generation of contraction that is due to an increase in the concentration of cytosolic Ca^{2+}. Stimulation of agonist receptors also activates the receptor-operated cation channel through activation of G-protein. For example, in the rabbit ear artery, ATP produces a receptor-operated inward current (mainly carried by Na^+ but also by Ca^{2+}) through activation of the purinergic receptor (Benham & Tsien, 1987; Benham, 1989) and thus triggers the voltage-dependent Ca^{2+} channel. In addition, ATP can positively or negatively modify the voltage-dependent Ca^{2+} channel evoked in the rabbit portal vein (Xiong, Kutamura & Kuriyama, 1991). In smooth muscle cells of the rabbit mesenteric artery, noradrenaline (10 mM) depolarized the membrane via activation of the α_1-adrenoceptor-operated cation channel and thus produced a Ca^{2+}-induced action potential. In addition, noradrenaline causes the synthesis of $InsP_3$ (Hashimoto, Hirata, Itoh, Kanmura & Kuriyama, 1986). This means that activation of the purinergic receptor or of the α_1-adrenoceptor triggers the receptor-operated cation channel and subsequently activates the voltage-dependent Ca^{2+} channel by depolarizing the membrane. On the other hand, acceleration or inhibition of the voltage-dependent Ca^{2+} channel can occur following stimulation of the purinergic receptor or α_1-adrenoceptor with or without activation of the receptor-operated cation channel (Droogmans, Declerck & Casteels, 1987; Nelson, Standen, Brayden & Worley, 1988). A relationship between agonist-induced activation of the voltage-dependent Ca^{2+} channel and G-protein has been suggested in vascular smooth muscle cells (Droogmans *et al.* 1987; Benham & Tsien, 1988; Loirand, Pacaud, Mironneau & Mironneau, 1990). However, the cellular mechanism involved in agonist-induced activation of the voltage-dependent Ca^{2+} channel is still controversial inasmuch as some investigators suppose the involvement of protein kinase C (Loirand *et al.* 1990) and others of cyclic AMP (Fukumitsu, Hayashi, Tokuno & Tomita, 1990).

In vascular smooth muscle tissues, three subtypes of the histamine receptor have

been reported, viz. histamine-1 (H_1), histamine-2 (H_2) and histamine-3 (H_3) receptors. H_1-receptor activation synthesizes $InsP_3$ and diacylglycerol (DG), the H_2-receptor synthesizes cyclic adenosine 5′-monophosphate (cyclic AMP) in smooth muscle cells (Chand & Eyre, 1975; Hill, 1990) and the H_3-receptor is thought to be distributed on nerve terminals and to negatively regulate neurotransmitter release (Arrang, Garbarg, Lancelot, Lecomte, Pollard, Robba, Schunack & Schwartz, 1987; Ishikawa & Sperelakis, 1987; Schwartz, Arrang, Garbarg & Pollard, 1990). A possible distribution of the H_3-receptor on smooth muscle cells has also been reported (Ea-Kim & Oudart, 1988).

In the present experiments, we set out to clarify the action of histamine on the voltage-dependent Ca^{2+} channel in dispersed smooth muscle cells of the rabbit saphenous artery, using the whole-cell patch-clamp procedure. The results obtained using biophysical procedures indicate that the H_3-receptor is distributed on these smooth muscle cells. Activation of the H_3-receptor (i) augments the voltage-dependent Ba^{2+} current (I_{Ba}) via GTP hydrolysis mediated by a pertussis toxin (PTX)-insensitive G-protein, but (ii) inhibits the I_{Ba} via activation of a PTX-insensitive G-protein in the presence of non-hydrolysable GTP analogues.

METHODS

Male albino rabbits (1·7–2·0 kg) were anaesthetized with sodium pentobarbitone (40 mg/kg I.V.; Pitman-Moore Inc., Washington Cross, NJ, USA) and exsanguinated. The saphenous artery was isolated in physiological salt solution (PSS) and the tunica adventitia and surrounding connective tissue carefully removed, as far as possible, with fine scissors and forceps under a binocular microscope. The endothelium was also removed by gently rubbing with a small cotton ball. The procedure for cell dispersion was similar to that previously described (Momose & Gomi, 1980; Terada, Nakao, Okabe, Kitamura & Kuriyama, 1987). In brief, small segments of the tissue were rinsed with Ca^{2+}-free PSS, then incubated in warmed Ca^{2+}-free PSS containing 0·25% collagenase (Wako Pure Chemicals, Osaka, Japan), 0·1% bovine serum albumin (essentially fatty acid free; Sigma Chemical Co., St Louis, MO, USA), and 0·1% trypsin inhibitor (type II-S, Sigma) at 36 °C for 40 min. After collagenase treatment, tissues were transferred to fresh Ca^{2+}-free PSS, and single cells were dispersed by gentle agitation with a glass pipette. Dispersed cells were collected by mild centrifugation (600 r.p.m. for 1 min) after removal of pieces of undigested tissue with a fine nylon mesh (100 × 100 μM). Dispersed cells were re-suspended in fresh 0·5 mM-Ca^{2+}/0·5 mM-Mg^{2+} solution, stored in ice-cold water and used within 6 h. Experiments were performed at room temperature (20–25 °C).

Recording of the membrane currents

Recordings of the macroscopic currents were made in ways similar to those described by Hamill, Marty, Neher, Sakmann & Sigworth (1981). One drop of the cell suspension was placed in a small chamber (0·2 ml in volume) on the stage of a differential interference inverted microscope (TMD-Diaphoto: Nikon Co., Tokyo, Japan). Patch electrodes (outer diameter 2·0–3·5 μM; 3–5 MΩ) were prepared using an electrode puller and heat polisher (PP-83 & MF-83; Narishige Sci. Inst. Lab., Tokyo, Japan) and manipulated using three-dimensional oil-driven manipulators (MO-102; Narishige). A high-resistance seal (> 10 GΩ) was obtained by application of negative pressure to the patch electrode (10–30 cmH_2O). For the recording of the voltage-dependent barium current, the pipette was filled with high-Cs^+ solution with or without the guanine nucleotides indicated in the text, and 10 mM-Ba^{2+} solution was superfused in the bath.

Membrane currents were monitored on a high-gain digital oscilloscope and a conventional thermo-writing pen recorder (VC-10 & RJG-4124; Nihon Kohden, Tokyo, Japan) through a patch-clamp amplifier (CEZ-2300; Nihon Kohden), and stored on magnetic tape using a video-cassette recorder via a PCM data-recording system (NV-21; National Co., Tokyo & PCM-501E; Sony Co., Tokyo, Japan).

Capacitative and leak currents were subtracted using the 'P/n' (n = 2–4) method described by

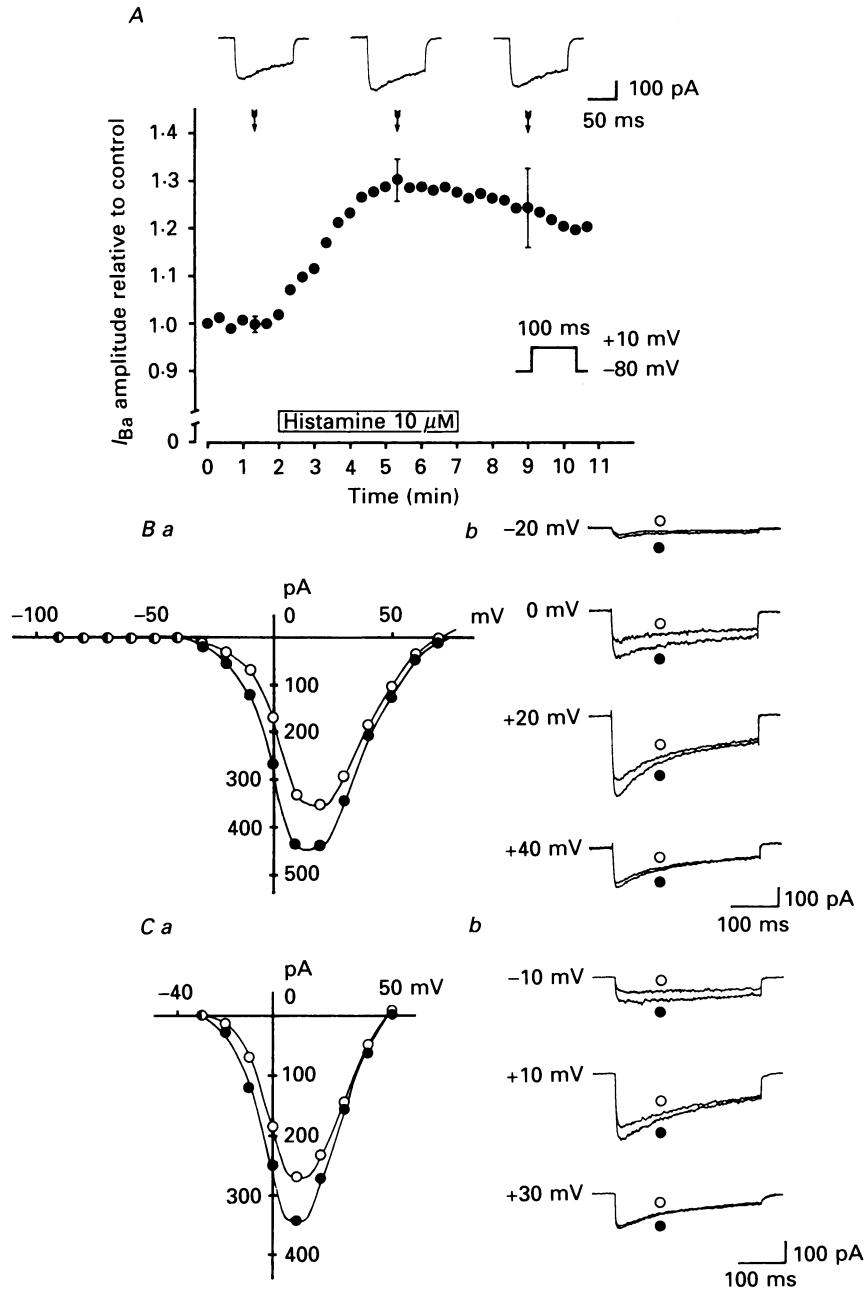

Fig. 1. Effects of histamine on I_{Ba} recorded from smooth muscle cells of rabbit saphenous artery. *A*, time course of 10 μM-histamine-induced changes in the amplitude of I_{Ba}. I_{Ba} was elicited by a depolarizing potential to +10 mV from the holding potential of −80 mV every 20 s. The I_{Ba} amplitude evoked before histamine application was normalized as 1·0 (control). Each point shows the mean value of six experiments, and vertical bar shows the value of S.D. Inset traces were taken at the times indicated by arrows. *Ba* and *Ca*, current–voltage relationships for I_{Ba} before (control; ○) and after (●) application of histamine (30 μM) at a holding potential of −100 mV (*B*) or −40 mV (*C*) using a pulse duration of 300 ms. The peak amplitude of I_{Ba} has been plotted. Measurements of I_{Ba}

Almers & Palade (1981) for the measurement of macroscopic currents on a digital oscilloscope (4094B; Nicolet Inst. Corp., Madison, WI, USA) and a hard copy was obtained using an X–Y plotter (7440A, Hewlett–Packard Co., San Diego, CA, USA).

Solutions and drugs

The ionic composition of the PSS was: NaCl, 134 mM; KCl, 6 mM; $CaCl_2$, 2·5 mM, and glucose, 12 mM. The Ca^{2+}-omitted solution was made be replacement of $CaCl_2$ with an equimolar amount of NaCl. The 0·5 mM-Ca^{2+}/0·5 mM-Mg^{2+} solution was made by addition of 0·5 mM-$CaCl_2$ and 0·5 mM-$MgCl_2$ in the Ca^{2+}-omitted solution. The 10 mM-Ba^{2+} solution contained $BaCl_2$ (10 mM) with TEA-Cl (135 mM) and glucose (10 mM). The high-Cs^+ pipette solution had the following ionic composition: CsCl, 145 mM; $MgCl_2$, 5 mM; Na_2ATP (adenosine triphosphate), 5 mM, and EGTA, (ethyleneglycol-bis(β-aminoethylether)-N,N,N',N'-tetraacetic acid), 4 mM. The pH of the solutions was adjusted to 7·25–7·30 using 10 mM-HEPES (N-2-hydroxyethylpiperazine-N'-2-ethansulphonic acid) titrated with Tris (tris(hydroxymethyl)aminomethane). The chamber was perfused with the appropriate solution at a constant rate (1 ml/min) throughout the experiments. Complete exchange of the solution was accomplished within 1 min.

Drugs used in the external solution were histamine (Sigma), $R\alpha$-methylhistamine (Eisai Co., Ltd, Tokyo, Japan), mepyramine, diphenhydramine, cimetidine, noradrenaline, angiotensin II (Sigma) and thioperamide (Eisai). Guanine nucleotides added to the high-Cs^+ internal solution were GTP, guanosine 5′-diphosphate (GDP), guanosine 5′-monophosphate (GMP), GDPβS, GTPγS, GMP-PNP and GMP-PCP (Böehringer-Mannheim, Germany). All drugs were dissolved in high concentration in distilled water and diluted by more than 100 times before bath or internal application.

Pertussis toxin (PTX; List Biol. Lab. Inc., Campbell, CA, USA) was dissolved in phosphate buffer, pH 7·1, at a concentration of 300 μg/ml. It was used to incubate cells for 4 h at 37 °C, or for intracellular application. In the intracellular application method, 300 ng/ml or 3 μg/ml PTX and 5 mM-dithiothreitol (DTT; Böehringer-Mannheim) were incubated at 37 °C for 20 min with the high-Cs^+ solution and then 1 mM-nicotinamide adenine dinucleotide (NAD; Böehringer-Mannheim) was added. 4β-Phorbol 12,13-dibutyrate (PDBu; Sigma) was first dissolved in dimethylsulphoxide (DMSO; Katayama Chemicals, Osaka, Japan) at a concentration of 10 mM and then diluted to a final concentration of 300 nM (0·003% DMSO). Control injections of DMSO alone were without effect. 1-(5-Isoquinolinesulphonyl)-2-methylpiperazine dihydrochloride (H-7; Seikagaku Kogyo Co., Ltd, Tokyo, Japan) was dissolved in distilled water and used at a final concentration of 100 μM to incubate cells for more than 10 min at room temperature (25 °C) before current recording.

Statistics

Values were expressed as mean ± standard deviation (S.D.), and statistical significance estimated by Student's t test except where the use of a paired test is indicated in the text. P values less than 0·05 were considered to be significant.

RESULTS

Although a voltage-dependent Ca^{2+} current (I_{Ca}) of small amplitude could be recorded from the freshly dispersed smooth muscle cells of the rabbit saphenous artery using PSS as the external solution, an external solution containing 10 mM-Ba^{2+} instead of Ca^{2+} was used throughout the following experiments since this provided an inward current (I_{Ba}) of greater amplitude.

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amplitudes were made 5–7 min after application of histamine. In *Bb* and *Cb*, traces evoked by depolarizing pulses of various amplitudes in the presence or absence of histamine are superimposed. The holding potential was −100 mV in *Bb* and −40 mV in *Cb* and the pulse duration 300 ms in each case.

The effects of histamine receptor agonists and antagonists on I_{Ba} in the rabbit saphenous artery

Histamine (10 μM) slowly augmented the I_{Ba} evoked by repetitive depolarizing pulses to +10 mV (20 s interval: holding potential −80 mV) (Fig. 1*A*) and the

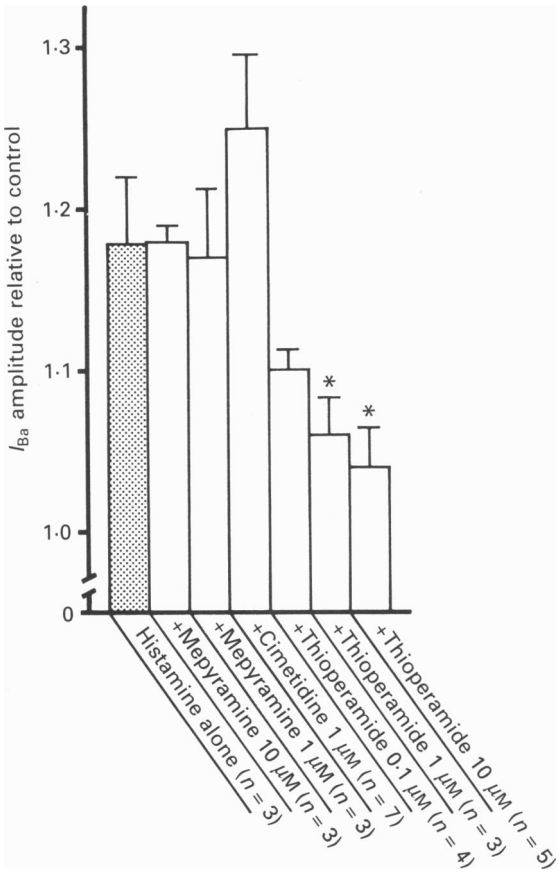

Fig. 2. Effects of H_1-, H_2- and H_3-histamine receptor antagonists on 1 μM-histamine-induced I_{Ba} augmentation. I_{Ba} was obtained using a depolarizing potential to +10 mV from the holding potential of −80 mV every 20 s. Smooth muscle cells were continuously exposed to solution containing the given antagonist starting 5 min before application of histamine. The I_{Ba} amplitude evoked before application of histamine was normalized as 1·0 (control). Each column shows the mean ± S.D. value. No antagonist itself modified the I_{Ba} amplitude. * indicates $P < 0{\cdot}05$ (compared with the response to histamine alone).

augmentation gradually declined after the histamine was removed. Figure 1*B* shows the effect of 30 μM-histamine on the current–voltage relationship with the holding potential at −100 mV. In 10 mM-Ba^{2+} solution, application of a depolarizing pulse (to levels more positive than −30 mV) evoked an inward current, the maximum amplitude being evoked by depolarizing pulses to between +10 and +20 mV (○). Application of histamine (30 μM) increased the I_{Ba} evoked by each depolarizing pulse (in the range −30 to +20 mV; ●). As the voltage steps which showed the most

prominent effects of histamine were the same as those that indicate activation of the so-called T-type calcium channel (Nilius, Hess, Lansman & Tsien, 1985), the effect of histamine was examined using a holding potential of −40 mV (Fig. 1*C*). With this holding potential, histamine (30 μM) also augmented I_{Ba}, but the effect was greater with depolarizing pulses to levels more negative than +20 mV. Furthermore, the shape of the current traces was not affected by histamine (Fig. 1*Bb* and *Cb*). These results indicate that histamine was activating mainly the so-called L-type calcium channel.

The effects of known histamine receptor antagonists on the histamine-induced I_{Ba} augmentation were examined (Fig. 2). The holding potential was placed at −80 mV and depolarizing pulses to +10 mV applied every 20 s. The amplitude of I_{Ba} thus evoked was normalized as 1·0 (control). Histamine (1 μM) augmented the I_{Ba} amplitude to 1·18 ± 0·04 times control. Pre-treatment with one of the H_1-antagonists, mepyramine (1 and 10 μM) or diphenhydramine (10 μM), had no effect on the histamine (1 μM)-induced I_{Ba} augmentation (1 μM-mepyramine, 1·18 ± 0·01 times control; 10 μM, 1·17 ± 0·04 times control; 10 μM-diphenhydramine, 1·20 ± 0·04 times control). Cimetidine (1 μM), an H_2-antagonist, did not affect the I_{Ba} augmentation, either (1·25 ± 0·04 times control). On the other hand, thioperamide ($\gtrsim$ 1 μM), an H_3-antagonist, induced a significant inhibition of the 1 μM-histamine-induced I_{Ba} augmentation in a concentration-dependent manner (with 1 μM, 1·06 ± 0·02 times control; with 10 μM, 1·04 ± 0·02 times control).

The effects of the H_3-receptor agonist, *R*α-methylhistamine, on I_{Ba} were then examined. Figure 3*Aa* shows the current–voltage relationship in the presence or absence of 10 μM-*R*α-methylhistamine. *R*α-Methylhistamine induced augmentation of the I_{Ba} evoked by depolarizing pulses (to levels between −40 and +20 mV), at a holding potential of −100 mV. Figure 3*Ab* shows current traces evoked by depolarizing pulses with the holding potential of −100 mV in the presence or absence of 10 μM-*R*α-methylhistamine and indicates that the shape of the traces was not affected by *R*α-methylhistamine. Figure 3*B* shows the effect of pre-treatment with 1 or 10 μM-thioperamide on the *R*α-methylhistamine-induced I_{Ba} augmentation. As it did in the case of the histamine-induced I_{Ba} augmentation, thioperamide induced an inhibition of the action of *R*α-methylhistamine on I_{Ba} in a concentration-dependent manner (1 μM-*R*α-methylhistamine alone, 1·21 ± 0·04 times control; with 1 μM-thioperamide, 1·09 ± 0·03 times control; with 10 μM-thioperamide, 1·04 ± 0·03 times control).

Figure 4*A* shows the relationship between the concentration of histamine or *R*α-methylhistamine and the I_{Ba} augmentation they induced (expressed relative to the maximum effect produced by each drug). Depolarizing pulses to +10 mV from the holding potential of −80 mV were applied every 20 s to obtain a stable I_{Ba} control response. The maximum increase in this response was calculated to be 1·29 ± 0·02 times control for histamine (30 μM) and 1·25 ± 0·03 times control for *R*α-methylhistamine (3 μM). The mean value for the maximum increase induced by each drug was normalized as 1·0 in each case, and the relative values plotted against the concentration of drug (see Fig. 4*A*). The best fit of the results to the theoretical curves was obtained using the following values: an ED_{50} value of 530 nM for histamine and 93 nM for *R*α-methylhistamine and a Hill coefficient of 1·65 for each

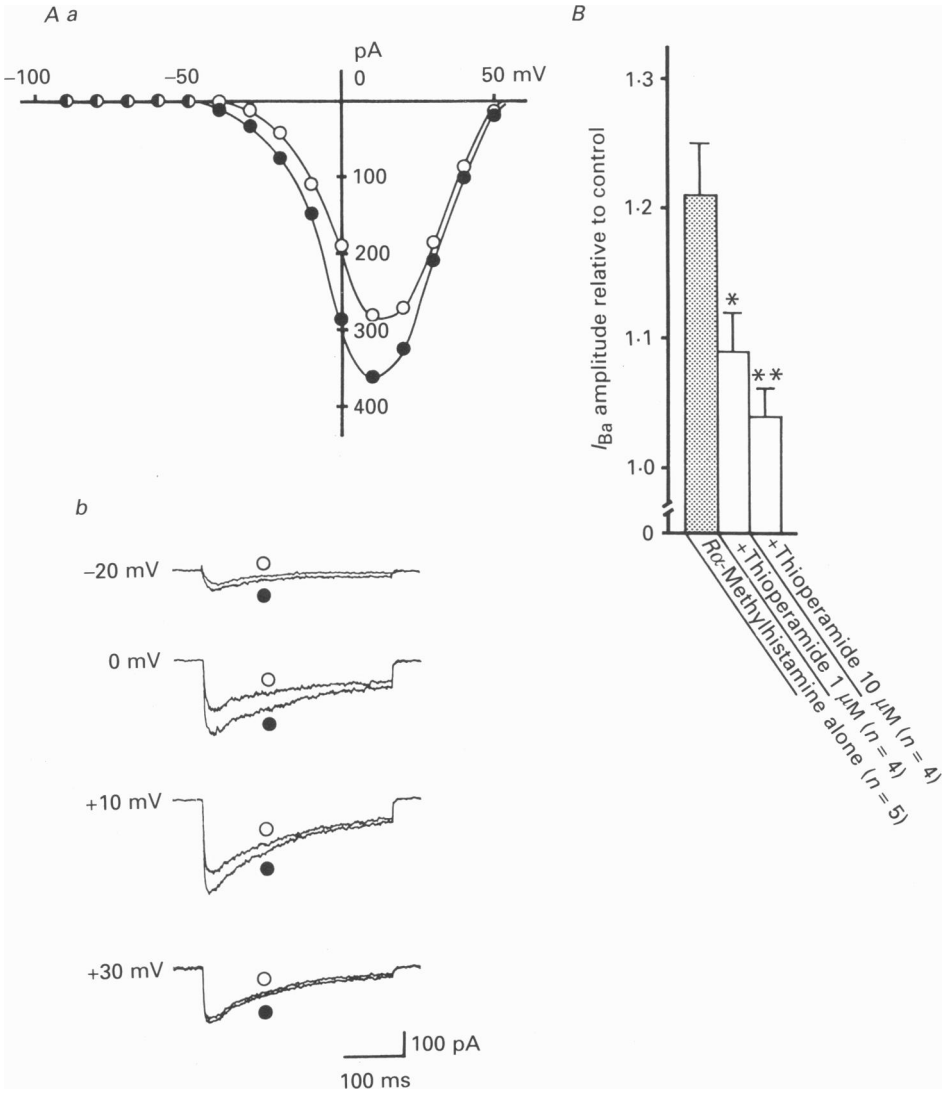

Fig. 3. Current–voltage relationships for I_{Ba} obtained before (control, ○) and after application of *R*α-methylhistamine (10 μM, ●; *A*,) and effects of thioperamide on the 1 μM-*R*α-methylhistamine-induced I_{Ba} augmentation (*B*). In *A*, the holding potential was −100 mV and the pulse duration 300 ms. The peak amplitude of I_{Ba} has been plotted. The amplitude of I_{Ba} in the presence of histamine was measured 5–7 min after application of *R*α-methylhistamine. In *A b*, traces evoked by depolarizing pulses of various amplitudes before and after application of *R*α-methylhistamine are superimposed. In *B*, cells were continuously exposed to a solution containing 1 or 10 μM-thioperamide starting 5 min before application of 1 μM-*R*α-methylhistamine. I_{Ba} was elicited by a depolarizing potential to +10 mV from a holding potential of −80 mV. The amplitude of I_{Ba} evoked before application of *R*α-methylhistamine was normalized as 1·0 (control). Thioperamide itself did not modify the amplitude of I_{Ba}. * indicates $P < 0{\cdot}05$ and ** indicates $P < 0{\cdot}01$ (both compared with the response to *R*α-methylhistamine alone).

drug. Figure 4*B* shows a Schild plot of the inhibitory effects of thioperamide on the histamine-induced I_{Ba} augmentation. The pA_2 ($-\log K_d$) value for thioperamide was $-7{\cdot}5$ (inhibition constant, $K_i = 30$ nM) and the slope was 1·0. These results indicate that the histamine-induced I_{Ba} augmentation occurred through activation of the histamine H_3-receptor, and not of the H_1- or H_2-receptor.

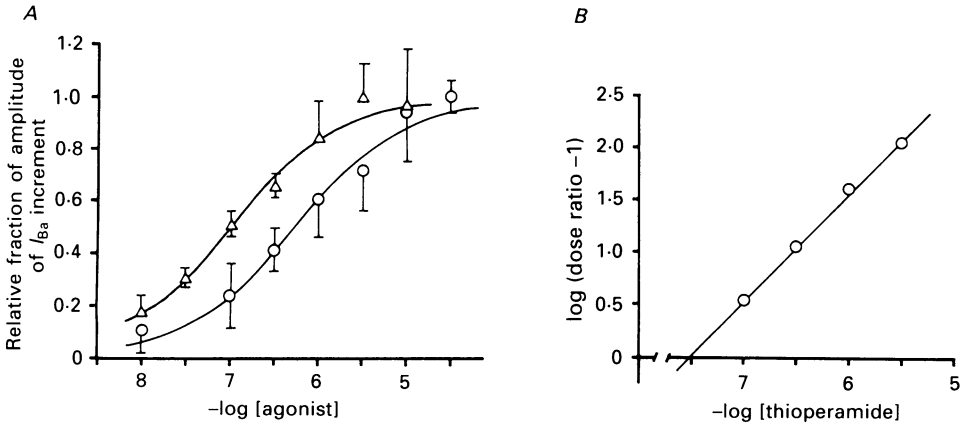

Fig. 4. *A*, concentration–response relationships for effect of histamine (○; mean ± S.D., $n = 3$–9) and *R*α-methylhistamine (△; mean ± S.D., $n = 3$–5) on I_{Ba}. I_{Ba} was obtained using a depolarizing potential to +10 mV from a holding potential of −80 mV. The vertical axis indicates the change in the amplitude of I_{Ba}, expressed relative to the mean maximum response induced by histamine (30 μM) and *R*α-methylhistamine (3 μM) (each of which was normalized as 1·0). Continuous lines were drawn using the equation $Y = 1/[1+(B/X)^n]$, where $B = 10^{-6{\cdot}28}$ and $10^{-7{\cdot}02}$ for histamine and *R*α-methylhistamine, respectively, and $n = 1{\cdot}65$ for both drugs. *B*, Schild plot of thioperamide action on histamine-induced I_{Ba} augmentation. Individual points are mean values ($n = 4$). The pA_2 value is $-7{\cdot}53$ and the slope is 1·02.

The effects of guanine nucleotides on the histamine-induced change in I_{Ba}

To study further the mechanism of the histamine-induced augmentation of I_{Ba}, GTP and its analogues were intracellularly applied. The amplitude of I_{Ba} evoked before application of histamine was normalized as 1·0 (control). This control amplitude, which was elicited by a depolarizing pulse to +10 mV from the holding potential of −80 mV, was not modified by any of the guanine nucleotides used (in the absence of guanine nucleotides, 276·9 ± 85·8 pA, $n = 8$; with 500 μM-GTP, 232·5 ± 97·3 pA, $n = 6$; with 200 μM-GTPγS, 304 ± 120·2 pA, $n = 6$; and with 1 mM-GDPβS, 199·4 ± 108·2, $n = 5$). However, as shown in Fig. 5*A a* and *A b*, when 500 μM-GTP was intracellularly applied, histamine (10 μM) increased I_{Ba} to a much larger extent than in the absence of GTP (in the absence of guanine nucleotides, 1·29 ± 005 times control, $n = 6$; with 500 μM-GTP, 1·88 ± 0·22 times control, $n = 5$; $P < 0{\cdot}05$). The amplitude of the I_{Ba} gradually declined after removal of the histamine. By contrast, when 200 μM-GTPγS was added to the pipette solution, 10 μM-histamine reduced the amplitude of I_{Ba} (Fig. 5*A a* and *A b*; 0·56 ± 0·09 times control, $n = 6$, which is significantly different from the effect of histamine in the absence of guanine nucleotides, $P < 0{\cdot}01$). On the other hand, 1 mM-GDPβS merely prevented the

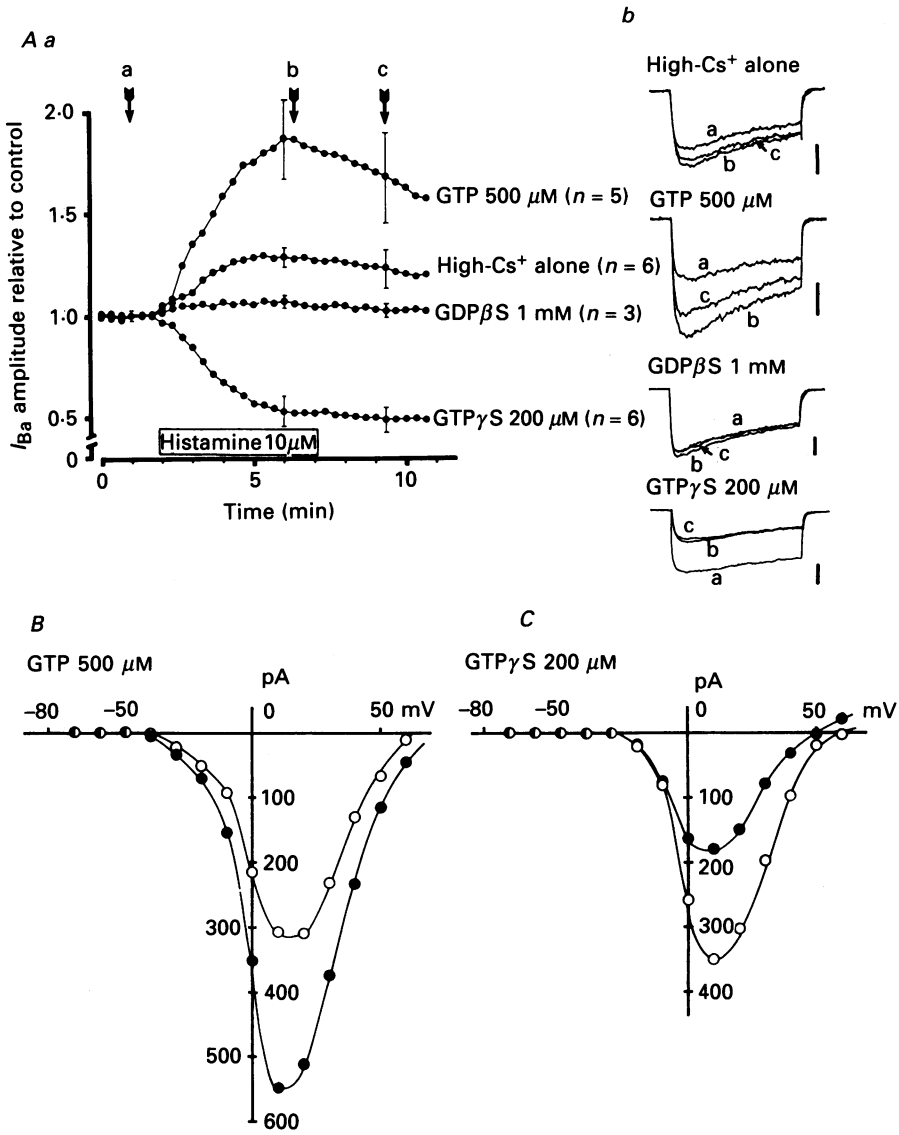

Fig. 5. Effects of guanine nucleotides on histamine-induced I_{Ba} augmentation. *A a*, time course of 10 μM-histamine-induced changes in the amplitude of I_{Ba} following internal application of guanine nucleotides. I_{Ba} was elicited by a depolarizing potential to +10 mV from a holding potential of −80 mV every 20 s, and the amplitude of I_{Ba} evoked before application of histamine was normalized as 1·0 (control). Ba^{2+} solution (10 mM) was perfused in the bath and one of the following guanine nucleotides was added to the high-Cs^+ pipette solution; 500 μM-GTP, 200 μM-GTPγS or 1 mM-GDPβS. The pipette solution in the absence of guanine nucleotides is described as 'high-Cs^+ alone' in the figure. Each point indicates the mean (±S.D.) of three to six experiments. *A b*, typical traces recorded before (a) and during (b) application of, and after removal (c) of, histamine (10 μM, recorded at the times indicated by arrows in *A a*). Each vertical bar represents 100 pA and the pulse duration was 100 ms. *B* and *C*, current–voltage relationships for I_{Ba} before (control, ○) and after application of 10 μM-histamine (●) with 500 μM-GTP (*B*) or 200 μM-

enhancing action on I_{Ba} induced by 10 μM-histamine (Fig. 5*A a* and *A b*; 1·05±0·02 times control, $n = 3$, which again is significantly different from the effect of histamine alone, $P < 0·05$). Figure 5*B* and *C* shows the effect of histamine (10 μM) on the current–voltage relationships observed with the presence in the pipette of either 500 μM-GTP (Fig. 5*B*) or 200 μM-GTPγS (Fig. 5*C*). The current–voltage relationships observed in the presence of GTP (500 μM) with histamine showed little change in voltage dependence after the application of 10 μM histamine alone (Fig. 5*B*; compare with Fig. 1*B*), i.e. in the presence of GTP the relation curve did not shift along the voltage axis. On the other hand, inhibition of the amplitude of I_{Ba} by histamine in the presence of internal GTPγS (200 μM) occurred in a voltage-dependent manner. The latter inhibition was most obvious at voltages more positive than +10 mV (Fig. 5*C*).

Figure 6 shows the effects of intracellular application of GTP analogues and other nucleotides on the histamine (10 μM)-induced modification of I_{Ba}. I_{Ba} was elicited by a depolarizing potential to +10 mV from the holding potential of −80 mV every 20 s, and the I_{Ba} amplitude evoked before application of histamine was normalized as 1·0 (control). The values obtained from smooth muscle cells to which various nucleotides had been added intracellularly were compared statistically with the value obtained from cells in the absence of guanine nucleotides (the latter being 1·28±0·06 times control, $n = 9$). On the other hand, GDP, GMP, ITP or UTP (500 μM) had no action on the histamine-induced I_{Ba} augmentation (with 500 μM-GDP, 1·49±0·06 times control, $n = 4$; with 500 μM-GNP, 1·31±0·06 times control, $n = 4$; with 500 μM-ITP, 1·30±0·10 times control, $n = 5$; with 500 μM-UTP, 1·31±0·08 times control, $n = 5$; for all these values, $P > 0·05$ when they are compared with the value in the absence of guanine nucleotides). In these studies, 5 mM-ATP was present in the pipette solution throughout the experiments. To remove any possible influence of ATP on the histamine-induced augmentation, the ATP in the pipette solution was replaced by 5 mM-GTP. Under these conditions, the control I_{Ba} amplitude evoked by a depolarizing pulse (to +10 mV from the holding potential of −80 mV) was marginally reduced (5 mM-ATP, 277·0±85·8 pA, $n = 8$; 5 mM-GTP, 167·5±108·3 pA, $n = 4$; $P > 0·05$, no significant difference), but histamine (10 μM) enhanced the amplitude of I_{Ba} to the same extent as when 5 mM-ATP and 500 μM-GTP were present (1·70±0·18 times control, $n = 4$, $P < 0·05$ when this is compared with the value in the absence of guanine nucleotides, but $P > 0·05$ when it is compared with the value obtained with 5 mM-ATP and 500 μM-GTP, which was 1·88±0·22 times control, as reported above).

To evaluate the mechanisms involved in the different actions of GTP and GTPγS on the histamine-induced I_{Ba} modification, various concentrations of GTPγS were applied intracellularly. As before, the I_{Ba} amplitude evoked before application of histamine was normalized as 1·0 (control). Low concentrations of GTPγS ($\leqslant 1$ μM) had no action on the histamine-induced I_{Ba} augmentation (with 0·2 μM-GTPγS, 1·20±0·10 times control, $n = 4$; with 1 μM, 1·15±0·17 times control, $n = 3$; $P > 0·05$

GTPγS (*C*) in the pipette. The holding potential was −80 mV and the pulse duration was 300 ms. The peak amplitude of I_{Ba} has been plotted. I_{Ba} amplitudes were measured 5–7 min after application of histamine.

when either value is compared with that in the absence of guanine nucleotides). With higher concentrations of intracellular GTPγS ($\geqslant 5$ μM), the effect of 10 μM-histamine was reversed, i.e. it significantly inhibited the amplitude of I_{Ba} (with 5 μM-GTPγS, $0{\cdot}70 \pm 0{\cdot}08$ times control, $n = 3$; with 30 μM, $0{\cdot}64 \pm 0{\cdot}09$ time control, $n = 5$; with

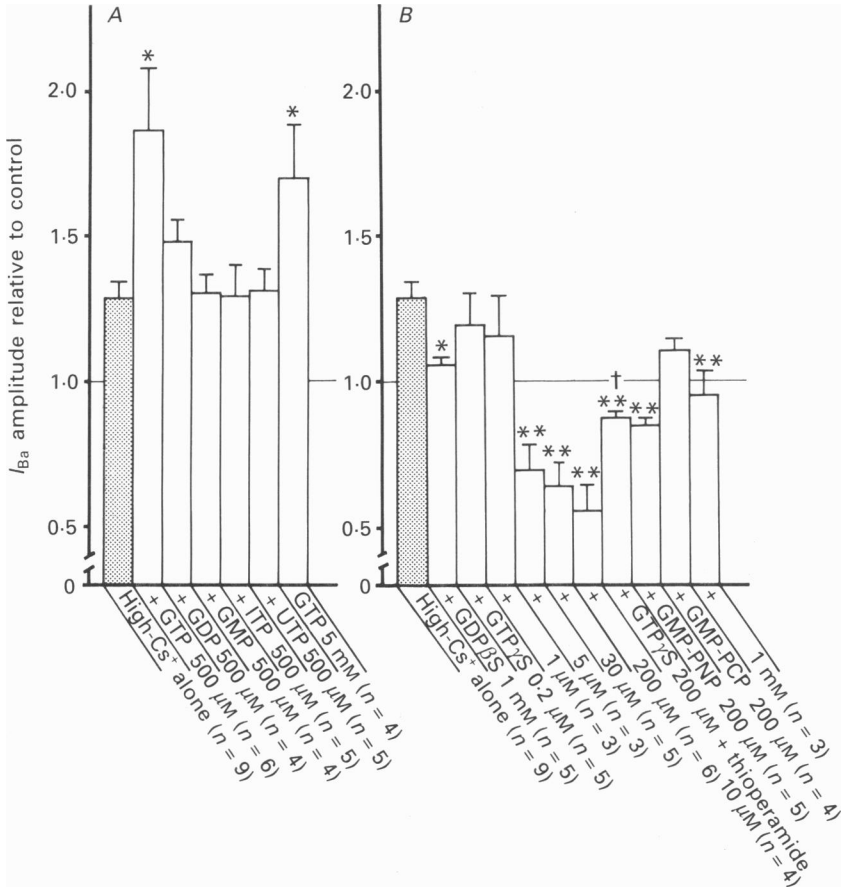

Fig. 6. Effects of the presence of various hydrolysable guanine nucleotides and related nucleotides (*A*) and GTPβS or non-hydrolysable GTP analogues (*B*) in the pipette solution on the action of histamine (10 μM) on I_{Ba}. The pipette solution in the absence of guanine nucleotides is described as 'high-Cs^+ alone' in the figure, and the indicated concentration of nucleotide was added to it, except for '5 mM-GTP' solution where the ATP of the high Cs^+ solution was completely replaced by GTP. The external solution was 10 mM-Ba^{2+} solution. I_{Ba} was elicited by a depolarizing potential to +10 mV from a holding potential of −80 mV every 20 s. The amplitude of I_{Ba} evoked before application of histamine was normalized as 1·0 (control). Each column shows mean ± S.D. * indicates $P < 0{\cdot}05$ and ** indicates $P < 0{\cdot}01$ when compared with the corresponding value in the absence of guanine nucleotides (high-Cs^+ alone in the pipette). † indicates that the values obtained in the presence of 200 μM-GTPγS and 200 μM-GTPγS with 10 μM-thioperamide also show a significant difference ($P < 0{\cdot}05$).

200 μM, $0{\cdot}56 \pm 0{\cdot}09$ times control, $n = 6$; $P < 0{\cdot}01$, when any of these is compared with that obtained in the absence of guanine nucleotides). The inhibitory action of 10 μM-histamine on I_{Ba} in the presence of 30 μM-GTPγS was not antagonized by a

higher concentration of EGTA (50 mM) in the pipette solution (0·59±0·09 times control, $n = 5$; $P > 0·05$, when this is compared with 30 μM-GTPγS and 4 mM-EGTA in the pipette), whereas the inhibitory action in the presence of 200 μM-GTPγS was partially antagonized by pre-treatment with 10 μM-thioperamide in the bath (0·86±0·01 times control, $n = 4$; $P < 0·05$, when this is compared with the value obtained without thioperamide in the presence of 200 μM-GTPγS). Intracellular GMP-PNP (200 μM) and GMP-PCP (1 mM), which are non-hydrolysable GTP analogues, also caused significant inhibition of I_{Ba} amplitude by 10 μM-histamine (with GMP-PNP, 0·84±0·02 times control, $n = 5$; with 200 μM-GMP-PCP, 1·10±0·03 times control, $n = 4$; and with 1 mM-GMP-PCP, 0·95±0·09 times control, $n = 3$; $P < 0·01$ when values of GMP-PNP and 1 mM-GMP-PCP are compared with that obtained in the absence of guanine nucleotides).

The effects of pertussis toxin, phorbol ester and protein kinase inhibitor on the histamine-induced augmentation and inhibition of I_{Ba} amplitude in rabbit saphenous artery

First, the effect of pertussis toxin (PTX) on the histamine-induced I_{Ba} augmentation and inhibition were examined. In the present experiment, smooth muscle cells were pre-incubated for 4 h at 37 °C in the presence or absence of 300 ng/ml PTX, and the effects of histamine on the amplitude of I_{Ba} compared in PTX-treated and untreated cells. A depolarizing potential to +10 mV from the holding potential of −80 mV was applied every 20 s, and the I_{Ba} amplitude thus elicited before application of histamine normalized as 1·0 (control). Histamine (10 μM) enhanced the I_{Ba} amplitude in the PTX-treated cells, and there was no significant difference between the size of the I_{Ba} increment whether the cells were PTX-treated or untreated (PTX-untreated cells, 1·28±0·06 times control, $n = 3$; PTX-treated cells, 1·21±0·05 times control, $n = 3$; $P > 0·05$). Insensitivity to PTX (300 ng/nl) was also observed on intracellular application of PTX with 5 mM-DTT and 1 mM-NAD (in the absence of PTX but with 5 mM-DTT and 1 mM-NAD, 1·35±0·13 times control, $n = 4$; with 300 ng/ml PTX, 1·26±0·10 times control, $n = 8$; $P > 0·05$). An even higher concentration of PTX (3 μg/ml) also had no effect on the I_{Ba} augmentation (1·22±0·08 times control, $n = 7$; $P > 0·05$, when this is compared with the value in the absence of PTX).

Pre-incubation with 300 ng/ml PTX for 4 h at 37 °C did not prevent the histamine-induced inhibition of I_{Ba} amplitude seen in the presence of intracellular GTPγS (200 μM) (PTX-untreated cells, 0·56±0·09 times control, $n = 6$; PTX-treated cells, 0·65±0·06 times control, $n = 6$; $P > 0.05$). Furthermore, when a solution containing 30 μM-GTPγS, 300 ng/ml (or 3 μg/ml) PTX, 5 mM-DTT and 1 mM-NAD was applied to the cells intracellularly, the inhibition of I_{Ba} amplitude produced by histamine (10 μM) was of the same extent as that recorded without PTX (when PTX was omitted from the above solution, 0·64±0·09 times control, $n = 5$; with 300 ng/ml PTX, 0·64±0·08 times control, $n = 5$; and with 3 μg/ml PTX, 0·69±0·06, $n = 6$; $P > 0·05$ when these values are compared). These results indicate that the G-protein related to the histamine-induced I_{Ba} augmentation is PTX insensitive.

The effects of 4β-phorbol 12,13-dibutyrate (PDBu) on I_{Ba} and 1-(5-isoquinoline-sulphonyl)-2-methylpiperazine dihydrochloride (H-7) on the histamine-induced I_{Ba} augmentation were investigated to examine the possible relationship between the

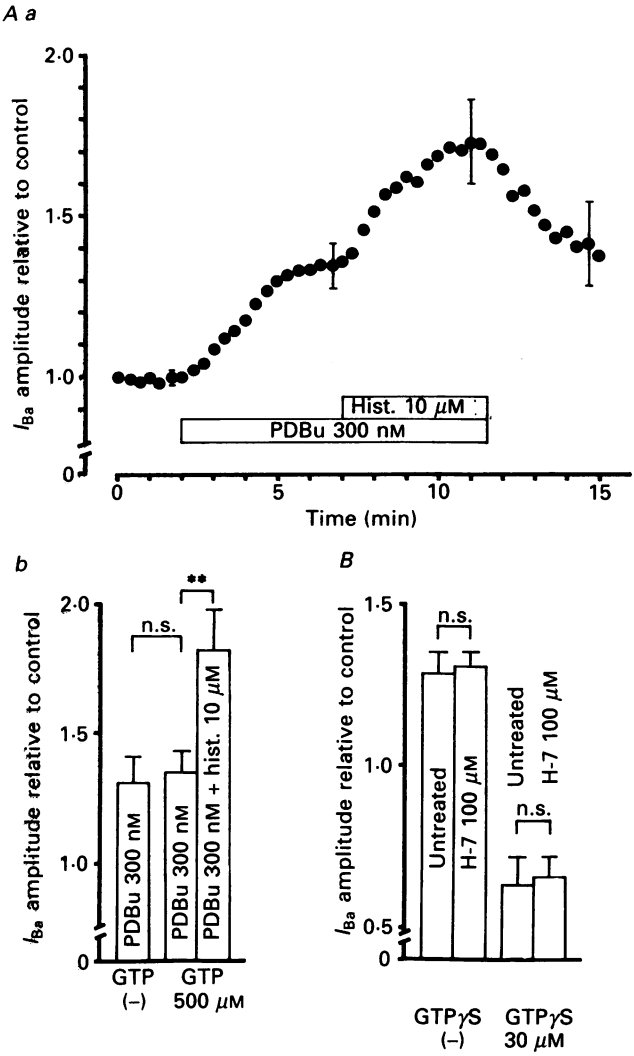

Fig. 7. *A*, effect of the protein kinase C stimulator, PDBu, on I_{Ba} and the effect of histamine (Hist.) on the response. *A a* shows the time-dependent effect of PDBu (300 nM) and of subsequent addition of histamine (10 μM) on I_{Ba} with 500 μM-GTP in the pipette. I_{Ba} was elicited by a depolarizing potential to +10 mV from a holding potential of −80 mV every 20 s. The amplitude of I_{Ba} evoked before application of PDBu was normalized as 1·0. Each point indicates mean ±S.D. of four experiments. *A b*, the effect of PDBu on I_{Ba} in the presence or absence of 500 μM-GTP in the pipette solution and of histamine (10 μM). *B*, the effect of the protein kinase inhibitor, H-7 (100 μM), on the histamine-induced I_{Ba} augmentation or inhibition in the presence or absence of 30 μM-GTPγS. Experimental procedure is the same as Fig. 6 and H-7 was applied as indicated in Methods. ** indicates $P < 0{\cdot}01$. n.s. indicates no significant difference ($P > 0{\cdot}05$).

actions of histamine and protein kinase C. I_{Ba} was elicited by a depolarizing potential to +10 mV from the holding potential of −80 mV every 20 s, and the I_{Ba} amplitude evoked before application of PDBu was normalized as 1·0 (control). PDBu (300 nM)

increased the I_{Ba} amplitude to 1·31 ± 0·10 times control ($n = 5$; Fig. 7*A b*). With GTP (500 μM) in the pipette, PDBu (300 nM) evoked no greater increase in amplitude (1·35 ± 0·07 times control, $n = 4$; $P > 0·05$ when this is compared with the values for PDBu without GTP). Additional application of histamine (10 μM) increased the I_{Ba} amplitude markedly (1·73 ± 0·14 times control, $n = 4$; comparison of this value with the value for PDBu alone in the presence of GTP gave a value of $P < 0·01$ using Student's paired *t* test). Figure 7*A a* shows the time course of the effect of PDBu and additional application of histamine on I_{Ba} with 500 μM-GTP in the pipette solution. Figure 7*B* shows the effect of 100 μM-H-7, an inhibitor of protein kinase C and also of cyclic nucleotide-dependent protein kinases, on the histamine (10 μM)-induced augmentation and inhibition of I_{Ba} in the absence or presence of GTPγS. I_{Ba} was elicited by a depolarizing potential to +10 mV from the holding potential of −80 mV every 20 s, and the I_{Ba} amplitude evoked before application of histamine was normalized as 1·0 (control). Pre-treatment of the cells for 10 min with 100 μM-H-7 induced no effect on either the histamine-induced augmentation or inhibition of I_{Ba} amplitude (in the absence of GTPγS, 1·30 ± 0·04 times control, $n = 5$; $P > 0·05$, when this is compared with the value for cells untreated with H-7 and not containing GTPγS; with 30 μM-GTPγS, 0·66 ± 0·06 times control, $n = 5$; $P > 0·05$, when this is compared with the value for H-7-untreated, GTPγS-containing cells). These results indicate that the protein kinase C system participates in neither the augmentation nor the inhibition of I_{Ba} induced by histamine.

Effects of other excitatory agonists on the amplitude of I_{Ba}

The effect of noradrenaline and angiotensin II on I_{Ba} amplitude were studied with or without guanine nucleotides in the pipette solution and compared with the actions of histamine. I_{Ba} was evoked by depolarizing pulses to +10 mV from the holding potential of −80 mV every 20 s. Both noradrenaline (100 μM) and angiotensin II (100 nM) enhanced the I_{Ba} amplitude (noradrenaline, 1·23 ± 0·04 times control, $n = 5$; angiotensin II, 1·18 ± 0·01 times control, $n = 3$). With 500 μM-GTP in the pipette solution, the augmentation of I_{Ba} induced by both noradrenaline and angiotensin II was much greater than in the absence of GTP (noradrenaline, 1·47 ± 0·06 times control, $n = 3$; $P < 0·01$, when this is compared with the value in the absence of GTP; angiotensin II, 1·47 ± 0·11 times control, $n = 3$; $P < 0·05$, when this is compared with the value in the absence of GTP). On the other hand, when GTPγS (200 μM) was added in the pipette, both noradrenaline and angiotensin II inhibited the I_{Ba} amplitude (noradrenaline, 0·61 ± 0·08 times control, $n = 5$; $P < 0·01$, when this is compared with the value in the absence of GTP; angiotensin II, 0·55 ± 0·08 times control, $n = 3$; $P < 0·01$, when this is compared with the value in the absence of GTP). These actions of GTP and GTPγS were similar to those observed on the response to histamine. Figure 8 shows current traces of I_{Ba} evoked by depolarizing potentials of 100 ms duration to +10 mV from the holding potential of −80 mV before and after application of noradrenaline (100 μM) and angiotensin II (100 nM) with guanine nucleotides (500 μM-GTP or 200 μM-GTPγS) present in, or absent from, the pipette solution.

DISCUSSION

Identification of the histamine receptor subtype on the smooth muscle cell membrane of the rabbit saphenous artery

It is a well-known fact that histamine H_1- and H_2-receptors are present on vascular smooth muscle membranes: the former is coupled with phospholipase C to synthesize

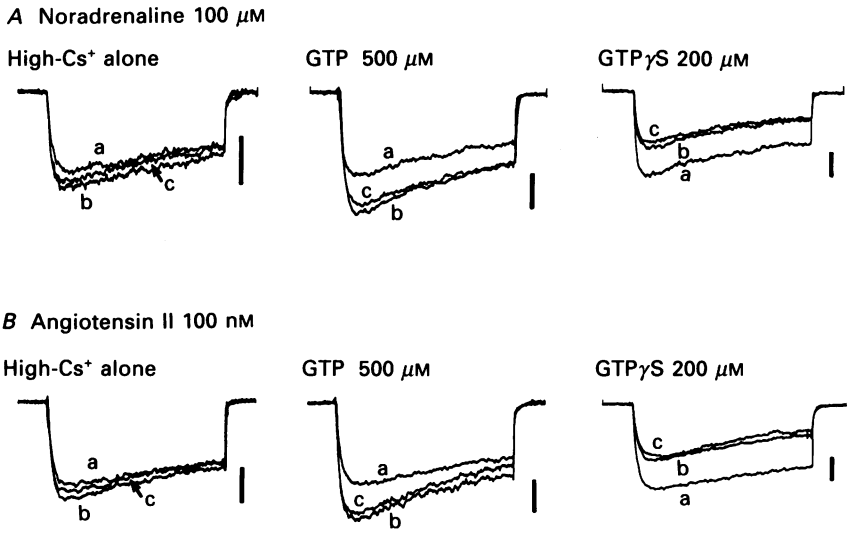

Fig. 8. Effects of GTP and GTPγS on noradrenaline- and angiotensin II-induced I_{Ba} augmentation in smooth muscle cells of rabbit saphenous artery. Ba^{2+} solution (10 mM) was perfused in the bath. High-Cs^+ internal solution (high-Cs^+ alone) and GTP (500 μM)- or GTPγS (200 μM)-added high Cs^+ solution were used in the pipette. I_{Ba} was elicited by a depolarizing potential to +10 mV from the holding potential of −80 mV. Each vertical bar represents 100 pA and the pulse duration is 100 ms. Traces recorded before (a) and during (b) application of, and after removal of (c), noradrenaline (*A*, 100 μM) and angiotensin II (*B*, 100 nM) are superimposed.

inositol 1,4,5-trisphosphate ($InsP_3$) and diacylglycerol (DG), and the latter synthesizes cyclic adenosine 5′-monophosphate (cyclic AMP) (Chand & Eyre, 1975; Edvinsson, Gross & Mohamed, 1983; Ottosson, Jansen & Edvinson, 1988; Hill, 1990). On the other hand, the H_3-receptor is thought, in general, to be present on nerve terminals and to regulate either the release of neurotransmitters (Arrang *et al.* 1987; Ishikawa & Sperelakis, 1987; Schwartz *et al.* 1990) or histamine synthesis (Arrang, Garbarg & Schwartz, 1983; Arrang *et al.* 1987; Schwartz *et al.* 1990) through unknown cellular mechanisms (North, 1989; Arrang, Roy, Morgat, Shunack & Schwartz, 1990). In peripheral nerve terminals in the guinea-pig mesenteric artery, it has been reported that histamine and *N*α-methylhistamine both inhibit the amplitude of excitatory junction potentials without any change in the membrane properties of the arterial muscle cells (Ishikawa & Sperelakis, 1987). This would suggest that the H_3-receptor is distributed on nerve terminals but not on vascular smooth muscle cells. However, Ea-Kim & Oudart (1988) have reported the possible

existence of the H_3-receptor on the smooth muscle cell membrane, though their evidence was inconclusive because they used tissues stimulated by high K^+ in which nerve terminals would also be depolarized. The present experiments provide the first clear evidence of the presence of the H_3-receptor on smooth muscle membranes, in this case in the rabbit saphenous artery.

In the present experiments, histamine and *R*α-methylhistamine augmented the amplitude of I_{Ba} with EC_{50} values of 530 and 93 nM, respectively. In the central nervous system, the ratio of the EC_{50} values for histamine and the selective H_3-agonist has been reported to be between 8 and 15·5 (Arrang *et al.* 1987). *R*α-Methylhistamine also has an agonistic action on the H_1-receptor when higher concentrations are used ($> 10\ \mu$M; Arrang *et al.* 1987), and the ratio of EC_{50} values for histamine and *R*α-methylhistamine (= 5·7) obtained in the present experiments was rather smaller than those reported for nerve cells. However, thioperamide, but not mepyramine or diphenhydramine, prevented the I_{Ba} augmentation induced by either histamine or *R*α-methylhistamine. Thus, the H_3-receptor is responsible for the augmentation of I_{Ba} in the rabbit saphenous artery, and not the H_1-receptor. The K_i value for thioperamide in respect of responses induced by H_3-receptor activation has been reported to be 2·0–31 nM in the central nervous system, and it was 30 nM in the present experiments. These results clearly indicate that stimulation of the H_3-receptor but not of H_1- or H_2-receptors augments I_{Ba} in smooth muscle cell of the rabbit saphenous artery. The physiological importance of the H_3-receptor in arterial cells is not yet known, but it may lead to contraction in the rabbit saphenous artery by a positive feedback mechanism in co-operation with activation of the H_1-receptor, which has been postulated to induce contraction of arterial smooth muscle (Hagen & Paegelow, 1979; Ottosson *et al.* 1988; Hill, 1990).

Mechanisms underlying the augmentation and inhibition of I_{Ba} induced by histamine

Although a possible contribution of guanine nucleotides to the actions of the H_3-receptor have been suggested in rat brain membranes (Arrang *et al.* 1990), no cellular mechanism related to the H_3-receptor has yet been demonstrated (North, 1989; Arrang *et al.* 1990). ! y contrast with the effects of guanine nucleotides on neurone (Dolphin & Scott, 1989) and the cardiac muscle (Shuba, Hesslinger, Trautwein, McDonald & Pelzer, 1990), these substances themselves did not modify the amplitude of I_{Ba} in the smooth muscle cells of the rabbit saphenous artery. With a GTP (500 μM)-containing pipette solution, histamine enhanced I_{Ba} to $1{\cdot}87 \pm 0{\cdot}22$ times control, whereas GDP (500 μM) and GMP (500 μM) did not enhance the action of histamine. On the other hand, when GDPβS (1 mM) was in the pipette solution, histamine produced no effect on I_{Ba} (only $1{\cdot}05 \pm 0{\cdot}02$ times control) suggesting that at least a certain kind of G-protein, which is PTX insensitive, is required for this histamine-induced I_{Ba} augmentation. Benham & Tsien (1988) reported that the noradrenaline-induced increase of arterial I_{Ba} was enhanced when GTP (200 μM) was present in the pipette. In the present experiments, we found that in the smooth muscle cells of the rabbit saphenous artery, angiotensin II as well as noradrenaline augmented the I_{Ba} amplitude and addition of GTP in the pipette further enhanced these effects. These observations may indicate the presence of a common pathway through which these agonists activate the voltage-dependent Ca^{2+} channels. One

possible candidate for involvement in the mechanism of I_{Ba} activation is the protein kinase C system, because PDBu was able to mimic the excitatory responses in the rabbit saphenous artery (and also in the rat portal vein; Loirand *et al.* 1990). On the other hand, PDBu produced an augmentation of I_{Ba} which was no greater when GTP was in the pipette solution, but the additional application of histamine increased the I_{Ba} amplitude quite markedly. These observations indicate that PDBu and histamine may act on I_{Ba} via different paths. Moreover, 100 μM-H-7, a potent inhibitor of protein kinases, including protein kinase C (Kuwano & Hidaka, 1984), had no effect on the histamine-induced I_{Ba} augmentation. Furthermore, the histamine-induced augmentation of I_{Ba} was not due to activation of the H_1-receptor, which synthesizes diaclyglycerol and activates the protein kinase C system. These observations indicate that protein kinase C is not involved in the histamine-induced I_{Ba} augmentation in the rabbit saphenous artery.

Recently, Fukumitsu *et al.* (1990) reported that the voltage-dependent Ca^{2+} channel in the pig coronary artery was activated by β-adrenoceptor stimulation through a cyclic AMP-dependent process, because forskolin mimicked the isoprenaline-induced response. This mechanism may not operate in the rabbit saphenous artery, because (i) the stimulation of the H_2-receptor activates adenylate cyclase and synthesizes cyclic AMP (Soll & Wollin, 1979; Chew, Hersey, Sachs & Berglindh, 1980; Hill, 1990), but an H_2-antagonist did not prevent the histamine-induced augmentation of I_{Ba}, (ii) 100 μM-H-7, which has a K_i value of 3·0 μM for cyclic AMP-dependent protein kinase (Hidaka, Inagaki, Kawamoto & Sasaki, 1984), produced no effect on the histamine-induced augmentation of I_{Ba}, and (iii) cyclic AMP in the pipette did not augment the amplitude of I_{Ba} (M. Oike, K. Kitamura & H. Kuriyama, unpublished observations).

In our experiments, when GTPγS (> 5 μM) or other non-hydrolysable GTP analogues (GMP-PNP or GMP-PCP) were in the pipette, the actions of histamine, noradrenaline and angiotensin II were reversed to inhibition. These actions of the non-hydrolysable GTP analogues were not due to a mechanism involving Ca^{2+}-induced inactivation of the Ca^{2+} current, because a high concentration of EGTA (50 mM) in the pipette did not prevent the reduction of I_{Ba} amplitude by these agonists, and, moreover, 10 mM-Ba^{2+} instead of Ca^{2+} was used throughout the experiments. The histamine-induced inhibition of I_{Ba} in the presence of GTPγS was blocked by 10 μM-thioperamide, suggesting that this inhibition is also mediated by the H_3-receptor. Loirand *et al.* (1990) reported that in the rat portal vein, GTPγS (10–100 μM) in the pipette augmented the amplitude of I_{Ba} even in the absence of noradrenaline, and that noradrenaline showed little effect on I_{Ba} when it was already increased by GTPγS. However, neither GTPγS (< 200 μM) nor the other non-hydrolysable GTP analogues by themselves modified the amplitude of I_{Ba} in the rabbit saphenous artery. Therefore, in this preparation, G-protein may be coupled tightly with histamine receptors – and with other receptors, too – and activation of these receptors may be essential to activate G-protein. Thus, GTP hydrolysis mediated by a PTX-insensitive G-protein is the most likely mechanism for the voltage-dependent Ca^{2+} channel stimulation by histamine, and presumably by the other two agonists in the rabbit saphenous artery. This conclusion is reinforced by the observations that (i) I_{Ba} was markedly augmented by histamine even when the

ATP (5 mM) in the pipette was completely replaced by GTP (5 mM), (ii) low concentrations of GTPγS never produced augmentation of I_{Ba}, while GTP always augmented it, and (iii) neither UTP (500 μM) nor ITP (500 μM) modified the histamine-induced augmentation. Recently, Tooze, Weiss & Huttner (1990) reported that GTPγS reduced the formation of the secretory vesicles of Golgi, which had been known to be mediated by a G-protein system, and that GTP prevented this action of GTPγS. Thus, they concluded that GTP hydrolysis plays an essential role in the regulation of vesicle formation.

In conclusion, in smooth muscle cells of the rabbit saphenous artery, histamine stimulates the H_3-receptor and accelerates the voltage-dependent Ca^{2+} channel. Acceleration of the voltage-dependent Ca^{2+} channel induced by the H_3-receptor requires GTP hydrolysis. Noradrenaline- and angiotensin II-induced Ca^{2+} channel augmentation may also depend on the hydrolysis of GTP. Thus, G-protein-mediated GTP hydrolysis would play a key role in modulating the voltage-dependent Ca^{2+} channel in the rabbit saphenous artery. However, it is still unclear whether hydrolysis of GTP activates the voltage-dependent Ca^{2+} channel directly or through some subsequent second messenger system the nature of which is unknown but which does not involve protein kinase C and cyclic nucleotide-dependent kinases.

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