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Effects of local anaesthetics on short-term desensitization of guinea-pig taenia caecum to histamine

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1 Short-term desensitization to histamine was induced by incubating guinea-pig taenia caecum with 10^{-4} M histamine for 30 min (desensitizing incubation) in normal Locke-Ringer solution or Ca-free Locke-Ringer solution containing 0.2 mM EGTA. This desensitization was measured as a reduction of the maximal contractile response.

2 The effects of the presence of local anaesthetics during the desensitizing incubation were examined. Results showed that tetracaine, procaine, procainamide, oxybuprocaine and lignocaine inhibited the desensitization, whereas dibucaine, benzocaine and mepivacaine did not.

3 The inhibitory effects of these drugs on the desensitization were not correlated with their lipid solubility nor with the potency of their known effects, such as membrane stabilization, Ca channel blockade, calmodulin antagonism, or inhibition of C-kinase.

4 It is concluded that the inhibitory effects of local anaesthetics on the desensitization are not due to their non-specific membrane-stabilizing effects *per se*, but to some other action.

Introduction

The mechanism of short-term desensitization to histamine is still unknown, although this phenomenon was first observed in 1935 by Barsoum & Gaddum Histamine-induced desensitization has been investigated by measuring histamine-mediated responses, such as cyclic GMP formation in mouse neuroblastoma cells (Taylor & Richelson, 1979), glycogen hydrolysis in mouse brain slices (Quach *et al.*, 1981), and contractile responses in guinea-pig ileum (Kenakin & Cook, 1980; Bielkiewicz & Cook, 1984) and guinea-pig taenia caecum (Uchida & Hirano, 1983; Uchida & Mita, 1985). We examined the mechanism of short-term desensitization of guinea-pig taenia caecum to histamine by blockade of various cellular responses. In such studies, test drugs should not significantly affect the contractile response of muscle to histamine. Most drugs that modify receptors, ion channels and intracellular signal transduction tend to affect the contractile response itself and their effects are difficult to remove by washing the tissue before it recovers from short-term desensitization. However, some local anaesthetics do not affect the contraction of muscle to histamine, and so we tested their effects on the desensitization. Local anaesthetics were expected to modify the extent of the histamine-induced desensitization, as they have various effects on lipids and proteins, and affect factors involving cell functions such as Ca channels (Spedding & Berg, 1985), calmodulin (Volpi *et al.*, 1981), and protein kinases (Mori *et al.*, 1980). In fact, some local anaesthetics increase the desensitization of nicotinic cholinoceptors (Heidmann & Changeux, 1978) and inhibit the desensitization of muscarinic cholinoceptors (Magaribuchi *et al.*, 1973; Higuchi *et al.*, 1983). So we tested their effects on short-term desensitization of guinea-pig taenia caecum to histamine. The relationship between the effects of these drugs on histamine-induced desensitization and the potencies of their various other actions described in other papers are discussed.

Methods

Guinea-pigs of either sex, weighing 250–400 g, were killed by a blow on the neck and were exsanguinated. Strips of taenia caecum were suspended in 10 ml organ baths bubbled with air at 30°C. The bathing solution was normal Locke-Ringer solution of the following composition (mM): NaCl 154, KCl 5.63, $CaCl_2$ 2.16, $MgCl_2$ 2.10, $NaHCO_3$ 5.95 and glucose 5.55. $CaCl_2$ was omitted and 0.2 mM EGTA was added to Ca-free solution. Cumulative contractile responses were recorded isotonically on a smoked drum using a lever with a

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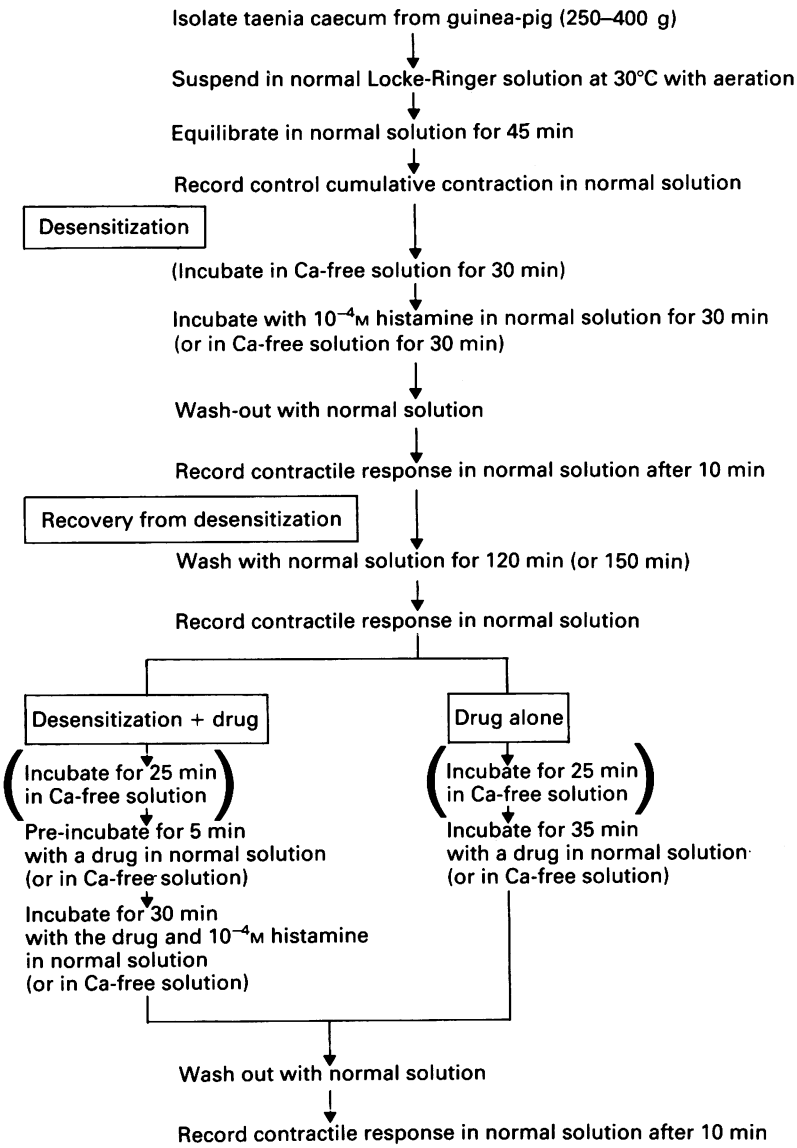

Figure 1 Procedure for measuring the mechanical response to histamine after desensitizing incubation in the normal solution or in Ca-free solution containing 0.2 mM EGTA. For conditions see Methods. Desensitization, the first desensitization; recovery from desensitization, recovery from the first desensitization; desensitization + drug, effects of drugs on the second desensitization; drug alone, effects of drugs alone without the second desensitizing incubation; the words in parentheses, apply to desensitization induced in Ca-free solution containing 0.2 mM EGTA.

load of about 0.5 g in normal Locke-Ringer solution. After the control responses had been recorded, the muscles were treated with 10^{-4} M histamine for 30 min (desensitizing incubation) in normal solution or Ca-free solution to induce desensitization as described previously (Uchida & Hirano, 1983). The muscles were then washed with normal solution and exactly 10 min after the end of the desensitizing incubation, their responses were recorded in normal solution as the first desensitization responses (desensitization). Subsequently muscles that had been desensitized in normal solution and in Ca-free solution were

incubated in normal solution without histamine for 120 min and 150 min, respectively. The responses were recorded in normal solution to confirm the complete recovery of the muscles from the first desensitization. Then a second desensitizing incubation was performed, under the same conditions as for the first desensitizing incubation, and 10 min later the contractile responses were recorded in normal solution as the second desensitization responses. For examination of the effects of drugs on the desensitization, the second desensitizing incubation was performed in the presence of drugs, which were added 5 min before the start of the second desensitizing incubation (desensitization + drug) and the second desensitization responses were compared with the first ones. Control muscles were not subjected to a second desensitization, but incubated with test drugs alone for the same period to examine the effects of the drugs (drug alone). Figure 1 shows the procedure used for measuring the mechanical response to histamine after desensitizing incubation in normal solution or Ca-free solution containing 0.2 mM EGTA. The drugs were used in concentration ranges that did not lower the maximal contractile response to 10^{-4} M histamine, since reduction of the maximal response was the criterion used to judge whether desensitization had occurred, and reduction of the maximal contraction by the drugs themselves would complicate interpretation of their effects on the desensitization. Contractile responses were expressed as a % of the maximal response of the control. Statistical significance was evaluated by Student's paired t test, and $P = 0.05$ was taken as the upper limit of significance.

Drugs used: tetracaine hydrochloride, procaine hydrochloride, procainamide hydrochloride and dibucaine hydrochloride (Sigma), benzocaine (Tokyo Chemical Industry). The following drugs were generous gifts; lignocaine hydrochloride (Fujisawa Pharmaceutical Co.), oxybuprocaine hydrochloride (Santen Pharmaceutical Co.) and mepivacaine hydrochloride (Yoshitomi Pharmaceutical Industries).

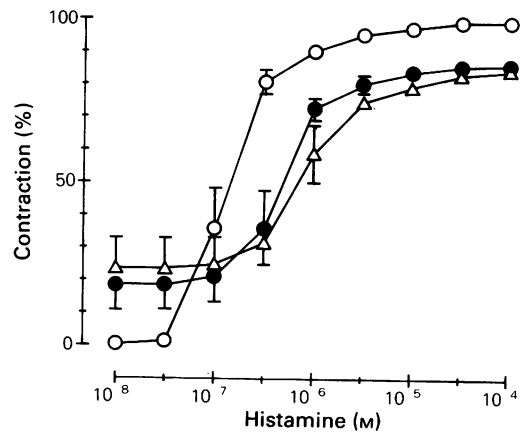

Figure 2 Repeated desensitization induced in the presence of external Ca ion. The contractile responses of guinea-pig taenia caecum to histamine were recorded isotonically on a smoked drum before (○) and 10 min after (●) the first desensitizing treatment with 10^{-4} M histamine for 30 min in normal Locke-Ringer solution. When the muscles had completely recovered from desensitization by subsequent incubation in normal solution without histamine for about 120 min, a second desensitizing treatment was performed and the contractile responses were recorded 10 min later (△). Contractile responses are expressed as percentages of the maximal contractile response of the control. The initial base line was taken as 0% and subsequent base lines are expressed as percentages of the maximal contraction of the control: the base lines are identical to the values with 10^{-8} M histamine, which did not cause contraction in any case. Each point represents the mean of 4 experiments and vertical lines indicate s.e.

Results

Desensitization to histamine induced in the presence of external Ca ion

Reappearance of histamine-induced desensitization
After desensitization in normal solution, the base line of the dose-response curve of guinea-pig taenia caecum was higher, and the maximal response (to 10^{-4} M histamine) was lower than that of the control (Figure 2). The EC_{50} value, defined as the concentration eliciting 50% of the maximal contraction of the control, was $0.16 \pm 0.03\,\mu$M for the control, $0.51 \pm 0.12\,\mu$M after the first desensitization and $0.95 \pm 0.27\,\mu$M after the second desensitization. Thus the EC_{50} value was increased more than 3 fold by desensitization, and there was no significant difference between the EC_{50} values after the first and second desensitizations. The base line (basal tone) of guinea-pig taenia caecum varied independently of desensitization, but no change was observed throughout the experiment in the maximal contraction of control tissues that had not been subjected to desensitizing incubation. Moreover, the extent of reduction in the maximal response to 10^{-4} M histamine after desensitizing incubation with histamine was constant in different preparations, and a higher concentration of histamine (10^{-3} M) did not elicit additional contraction of desensitized tissue. Thus 10^{-4} M histamine evoked the maximal response in all preparations. The EC_{50} value was affected to such an extent by the response to lower concentrations of histamine, which was altered by base line changes, that the EC_{50} was a much less reliable indicator of desensitization than reduction in

Table 1 Effects of local anaesthetics on desensitization to histamine induced in the presence of external Ca ion

Drug	*Conc.* (M)	*Maximal contraction* (% of control) *Desensitization*	*Desensitization + drug*	*Basal tone* (% of control) *Desensitization*	*Desensitization + drug*	n
None		86.8 ± 1.5	85.0 ± 1.8†	18.5 ± 7.4	23.7 ± 9.3†	4
Tetracaine	3×10^{-5}	81.1 ± 0.9	86.0 ± 1.1*	41.4 ± 7.3	13.5 ± 8.3**	4
	10^{-4}	86.5 ± 1.9	91.6 ± 2.4**	−0.9 ± 7.3	−5.4 ± 5.4	4
Procaine	3×10^{-4}	88.3 ± 1.5	94.2 ± 1.7**	18.0 ± 10.2	−2.3 ± 2.4	4
Procainamide	10^{-3}	84.2 ± 1.8	94.0 ± 0.5**	22.7 ± 4.4	3.4 ± 2.5*	6
Oxybuprocaine	3×10^{-5}	84.3 ± 2.8	91.6 ± 1.3*	15.6 ± 1.5	−2.6 ± 1.4**	4
Lignocaine	10^{-4}	84.0 ± 2.7	90.2 ± 1.8**	22.9 ± 4.1	5.6 ± 4.9*	5
Dibucaine	10^{-5}	87.5 ± 0.9	88.5 ± 2.2	42.8 ± 8.8	19.5 ± 7.9***	4
	3×10^{-5}	85.1 ± 1.2	87.0 ± 2.2	9.9 ± 2.5	0.1 ± 0.9*	4
Benzocaine	3×10^{-4}	80.2 ± 2.7	83.1 ± 1.2	27.4 ± 10.1	22.8 ± 5.7	4
	10^{-3}	81.2	83.1	24.8	59.4	2
Mepivacaine	10^{-4}	84.9 ± 2.8	83.2 ± 2.7	16.2 ± 1.8	3.4 ± 4.7*	4

For conditions see text. n = number of experiments. Desensitization, the first desensitization in the absence of drugs; desensitization + drugs, the second desensitization in the presence of drugs. Basal tone is expressed as a percentage of the maximal contraction of the control. †Value under 'desensitization + drug' is that for the second desensitization without any drug-treatment. The significance of differences between values for 'desensitization' and 'desensitization + drug' was determined by Student's paired t test. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

the maximal contraction. Therefore, reduction of the maximal response to 10^{-4} M histamine was used as a reliable indication that desensitization had occurred. The maximal responses in the first and second desensitizations were 86.8 ± 1.5 and 85.0 ± 1.8% of that of the control, respectively, indicating that the desensitization was reproducible in the muscle preparations. The shape of the dose-response curves of muscle that had been incubated in normal solution for about 120 min after the first desensitization was the same as that of the control with a maximal response of 100.2 ± 1.5% of the control value (data not shown). Thus, in these conditions recovery from the first desensitization was complete, and the effects of drugs on the desensitization could be examined by comparing the second desensitization induced in the presence of the drugs (desensitization + drug) with the first desensitization (desensitization) of the same muscle as shown in the figures and tables. This method was considered more reliable than comparing the responses of different muscles with and without drugs.

Effects of local anaesthetics on histamine-induced desensitization Table 1 shows the effects of local anaesthetics on the desensitization. The maximal contractile response was not altered by treatment with any of these local anaesthetics alone (drug alone). So changes of the maximal response after desensitization in the presence of drugs could be regarded as their effects on the desensitization. When added alone, dibucaine, procaine, and oxybuprocaine lowered the sensitivity of the muscle to lower concentrations of histamine, but tetracaine, procainamide, lignocaine and mepivacaine did not affect the contractile response to any concentration of histamine. Benzocaine tended to increase the sensitivity to histamine.

When desensitizing incubation was carried out in the presence of tetracaine, procaine, oxybuprocaine, lignocaine or procainamide, the reduction of the maximal response induced by desensitization became significantly less and the maximal response approached that of the control. That is, these drugs inhibited the induction of desensitization. However, dibucaine, benzocaine and mepivacaine did not inhibit the induction of desensitization. Typical examples of the dose-response curves obtained are shown in Figures 3, 4 and 5 for tetracaine, dibucaine and benzocaine respectively. Benzocaine did not affect the shape of the dose-response curve on desensitization. Tetracaine and dibucaine, similar to all the other local anaesthetics tested, (except benzocaine) inhibited the rise of the base line on desensitization (Table 1). However, these two drugs had different effects on the reduction of the maximal contractile response on desensitization.

Desensitization to histamine induced in the absence of external Ca ion

Reappearance of histamine-induced desensitization Previously we found that histamine-induced desensitization of guinea-pig taenia caecum occurred even in Ca-free solution containing 0.2 mM EGTA. On desensitization in the absence of external Ca ion (Figure 6), the base line was much higher than that in the presence of external Ca ion, because it was increased by simply changing the bathing solution from Ca-free solution to normal solution to measure

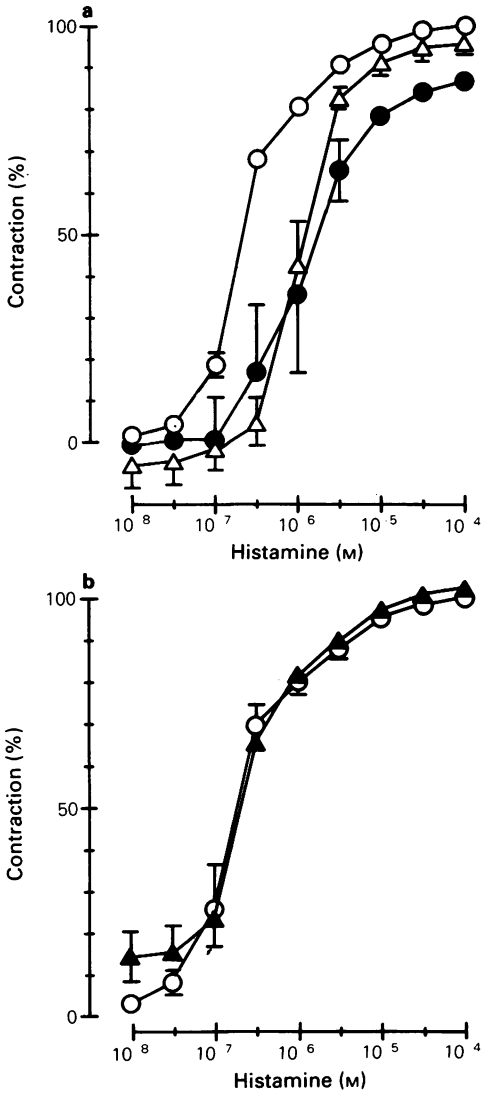

Figure 3 Effect of 10^{-4} M tetracaine on desensitization induced in the presence of external Ca ion. (a) The contractile responses of guinea-pig taenia caecum to histamine were recorded isotonically on a smoked drum before (○) and 10 min after (●) the first desensitizing treatment with 10^{-4} M histamine for 30 min in normal Locke-Ringer solution. After muscles had completely recovered from desensitization by subsequent incubation for about 120 min in normal solution without histamine, the second desensitizing treatment was performed in the presence of 10^{-4} M tetracaine and the contractile responses were recorded 10 min later (△). (b) The contractile responses were recorded before (○) and 10 min after treatment with 10^{-4} M tetracaine alone (▲). Contractile responses are expressed as a % of the maximal contractile response of the control. Each point represents the mean of 4 experiments and vertical lines indicate s.e. The base line was as for Figure 2.

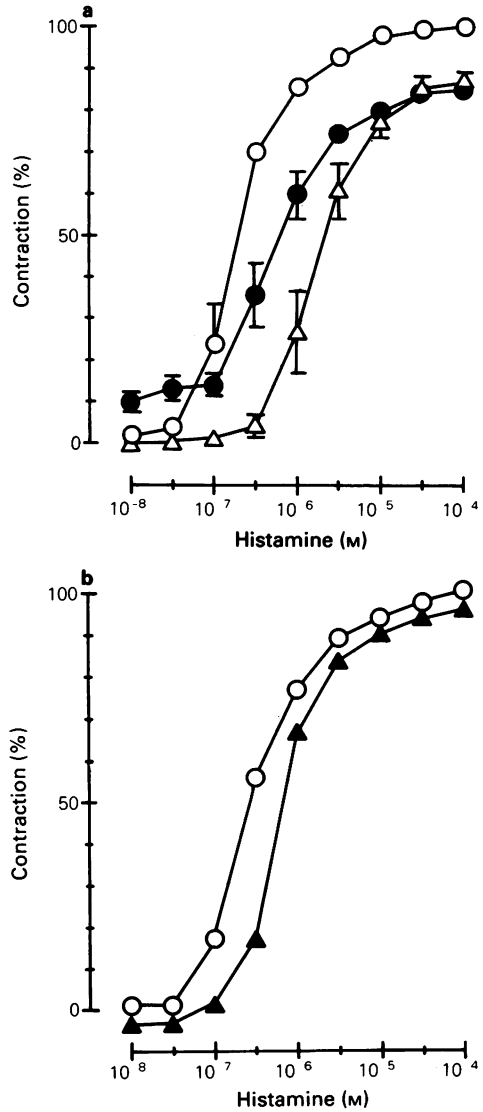

Figure 4 Effect of 3×10^{-5} M dibucaine on histamine-induced desensitization in the presence of external Ca ion. Conditions were as for Figure 3. (a) Contractile responses to histamine were recorded before (○) and after (●) the first desensitizing treatment, and after the second desensitizing treatment in the presence of 3×10^{-5} M dibucaine (△). In (b) contractile responses were recorded before (○) and after (▲) treatment with 3×10^{-5} M dibucaine alone. Each point represents the mean of 4 experiments and vertical lines indicate s.e.

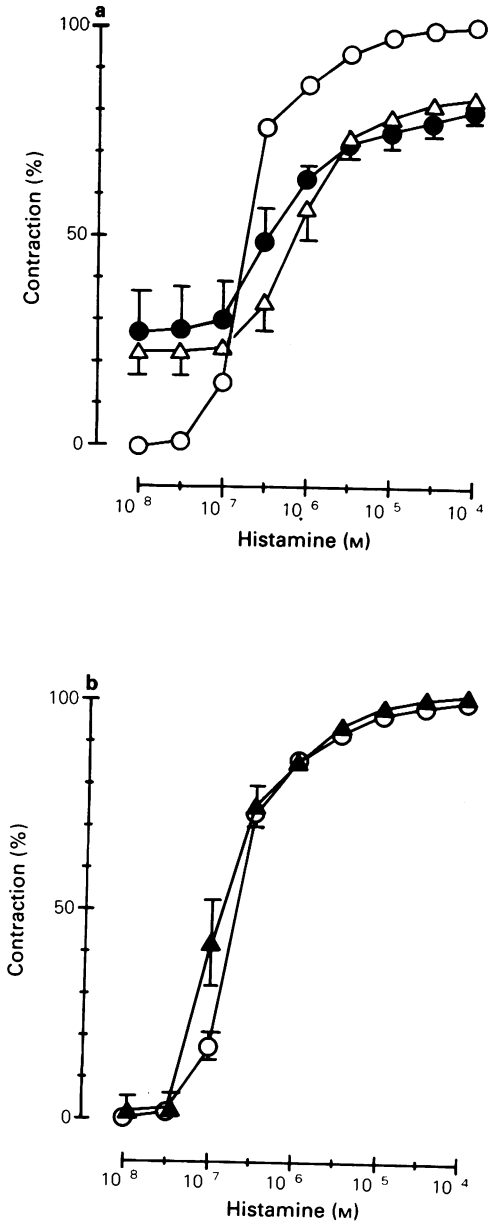

Figure 5 Effect of 3×10^{-4} M benzocaine on histamine-induced desensitization in the presence of external Ca ion. Conditions were as for Figure 3. (a) Contractile responses were recorded before (○) and after (●) the first desensitizing treatment, and after the second desensitizing treatment in the presence of 3×10^{-4} M benzocaine (△). In (b) contractile responses were recorded before (○) and after (▲) treatment with 3×10^{-4} M benzocaine alone. Each point represents the mean of 4 experiments and vertical lines indicate s.e.

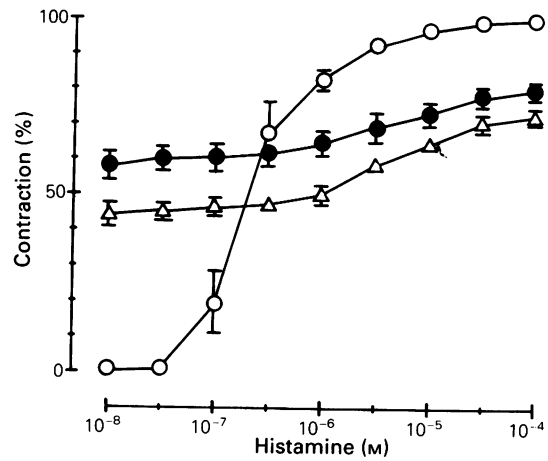

Figure 6 Repeated desensitization induced in the absence of external Ca ion. Conditions were as for Figure 2 except that Ca-free Locke-Ringer solution containing 0.2 mM EGTA was used and muscles were allowed to recover from desensitization for about 150 min in normal solution without histamine, before the second desensitization. Responses were recorded before (○) and 10 min after (●) the first desensitizing treatment, and 10 min after the second desensitizing treatment (△). Each point represents the mean of 4 experiments and vertical lines indicate s.e.

the contractile response (Uchida & Hirano, 1983). The base line on the second desensitization induced in Ca-free solution was lower than that on the first desensitization (Figure 6). However, the maximal responses on the first and second desensitizations were 79.9 ± 2.7 and $72.7 \pm 2.3\%$ of the control, respectively, indicating that desensitization induced in Ca-free solution was also reproducible, but that the extent of the second desensitization was slightly greater than that of the first ($P < 0.05$). The effects of drugs on the desensitization induced in Ca-free solution were examined by a similar protocol to that in normal solution.

Effects of tetracaine and dibucaine on histamine-induced desensitization Tetracaine inhibited the induction of desensitization (Figure 7 and Table 2), but dibucaine did not (Figure 8 and Table 2) as in normal solution. That is, the effects of the anaesthetics tested on the desensitization were independent of external Ca ion, just as induction of desensitization was independent of external Ca ion.

Discussion

Histamine-induced short-term desensitization in

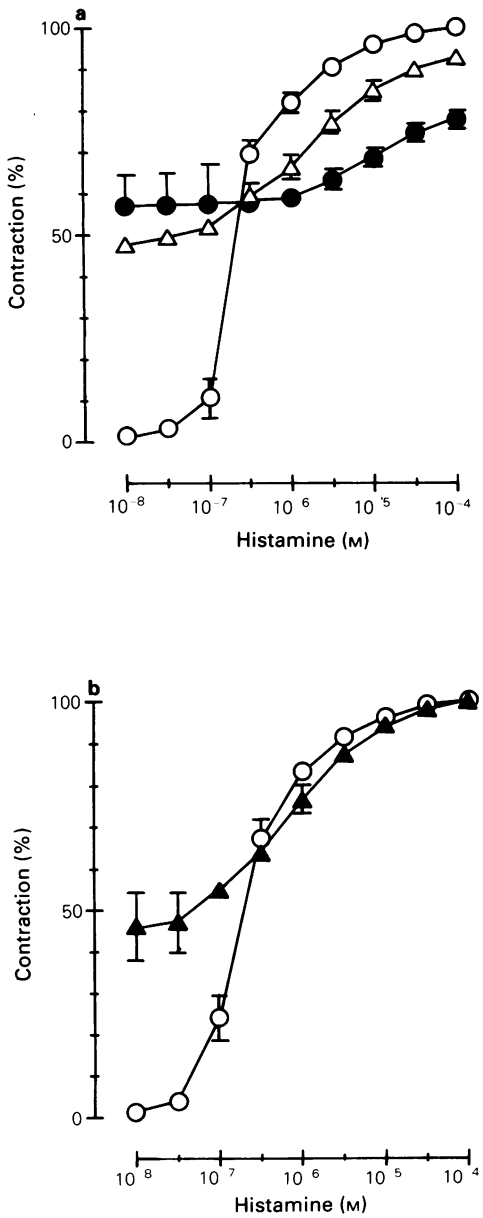

Figure 7 Effect of 10^{-4} M tetracaine on histamine-induced desensitization in the absence of external Ca ion. Conditions were as for Figure 6, except that 10^{-4} M tetracaine was added during desensitization. (a) Contractile responses were recorded before (○) and after (●) the first desensitizing treatment, and after the second desensitizing treatment in the presence of tetracaine (△). In (b) responses were recorded before (○) and after (▲) treatment with (10^{-4} M) tetracaine alone. Each point represents the mean of 4 experiments and vertical lines indicate s.e.

guinea-pig taenia caecum is characterized by a reduction of the maximal contraction. However, changes in the base line may confuse the apparent dose-response relationships after treatments. Since indomethacin, a cyclo-oxygenase inhibitor, suppressed the increase in the base line after desensitizing incubation with histamine, prostaglandins may influence the basal tone of the muscle (data not shown). Local anaesthetics such as dibucaine, tetracaine and procaine have been found to inhibit prostaglandin formation *in vitro* (Kunze *et al.*, 1974), so this effect may contribute to their inhibitory effects on the increase in base line. However, both the maximal contraction of the non-desensitized tissue and reduction of the maximal response by desensitization are not affected by indomethacin (unpublished observations). Thus prostaglandins do not appear to be involved in the desensitization. Other factors may also influence the base line. For instance, it was raised after Ca-free treatment, probably by the sudden change of the bathing solution from Ca-free to normal solution, but the maximal contraction of the non-desensitized tissue and its reduction by desensitization were not affected (Uchida & Hirano, 1983). Therefore, in this work we monitored desensitization as a change in the maximal response irrespective of the change in base line.

Our results showed that not all local anaesthetics inhibit the induction of short-term desensitization to histamine. Moreover, the inhibitory effects of these drugs were not correlated to their membrane-stabilizing potencies. The most potent anaesthetics, dibucaine and tetracaine, had different effects on histamine-induced desensitization, and lignocaine and mepivacaine, which have very similar structural formulae and local anaesthetic potencies, also did not have the same effects: tetracaine and lignocaine inhibited the desensitization, whereas dibucaine and mepivacaine did not.

If this desensitization occurred as a result of a reaction inside the cells after receptor-stimulation during the desensitizing treatment, it would be expected that: (1) the desensitization should occur even in Ca-free solution containing 0.2 mM EGTA, where the concentration of internal Ca ion would be so low that the muscle could not contract; (2) it should be inhibited by tetracaine not by dibucaine; and (3) these drugs would become incorporated into the cell membrane to reach and inhibit the factors inside the cells involved in desensitization. However, the lipid solubility of dibucaine is more than that of tetracaine.

Another possibility is that the effects of different drugs is due to differences in their specificities for sites that are desensitized or factors that are involved in the desensitization. Ca ion, which is needed for muscle contraction, enters the cells via Ca channels, so lowering of Ca channel function could result in the desensitized state. Calmodulin is important in signal

Table 2 Effects of tetracaine and dibucaine on desensitization induced in the absence of external Ca ion

Drug	Conc. (M)	*Maximal contraction* (% of control) *Desensitization*	*Desensitization + drug*	*Basal tone* (% of control) *Desensitization*	*Desensitization + drug*	n
None		79.9 ± 2.7	72.7 ± 2.3*,†	58.1 ± 4.1	44.1 ± 3.2***,†	4
Tetracaine	10^{-4}	78.0 ± 2.2	92.9 ± 1.6**	56.9 ± 1.8	47.9 ± 6.6	4
Dibucaine	3×10^{-5}	79.2 ± 3.0	81.2 ± 1.0	54.8 ± 3.4	13.5 ± 14.8*	4

For conditions see text. n = number of experiments. Desensitization, the first desensitization in the absence of drugs; desensitization + drug, the second desensitization in the presence of drugs. Basal tone is expressed as a percentage of the maximal contraction of the control. †Value under 'desensitization + drug' is the second desensitization without any drug-treatment. The significance of differences between values for 'desensitization' and 'desensitization + drug' was determined by Student's paired t test. *$P<0.05$; **$P<0.01$; ***$P<0.001$.

transduction of Ca ion, so inactivation of calmodulin could also lead to the desensitized state. Since most local anaesthetics inhibit Ca channels (Spedding & Berg, 1985) and calmodulin (Volpi *et al.*, 1981), their inhibitory effects during desensitization might protect Ca channels and calmodulin from being desensitized. But although dibucaine has stronger inhibitory effects than tetracaine on Ca channels and calmodulin, it did not inhibit desensitization, whereas tetracaine did. So the inhibitory effects of the drugs on the desensitization are unlikely to be due to their actions on Ca channels or calmodulin.

A decreased function of the receptors by some conformational modification, such as phosphorylation, may also contribute to the desensitization. In fact, receptor phosphorylation has been suggested to be one cause of desensitization (Stadel *et al.*, 1983). However, tetracaine and dibucaine do not affect cyclic AMP-dependent protein kinase or cyclic GMP-dependent protein kinase, and the inhibitory effect of dibucaine on protein kinase C is stronger than that of tetracaine (Mori *et al.*, 1980). So the inhibitory effects of the drugs on the desensitization do not seem to be due to actions on these protein kinases, although other protein kinases or some other unknown factor(s) on which tetracaine has a more selective effect than dibucaine may modify receptor function.

Another possibility is that the receptors themselves are changed on sustained agonist-binding. On desensitization in normal solution, the maximal response induced by 10^{-4} M histamine was not significantly affected by the presence of local anaesthetics. Thus the binding of histamine to its receptor may be unchanged by the presence of the drugs. However, some other sites on the receptor may be changed during the desensitizing incubation and this change might decrease signal transduction to the effector system. Possibly some local anaesthetics interact with such sites without causing changes in their structure. As the effects of local anaesthetics were not correlated with their membrane-stabilizing potencies, these drugs may have specific sites of binding for their effects. Therefore, we considered the structure activity relationship of the local anaesthetics in inhibiting desensitization. Of the synthetic local anaesthetics tested, para-aminobenzoates, i.e. procaine, tetracaine, and oxybuprocaine, were effective. The analogue of procaine, procainamide, was also effective, but benzocaine, which is smaller because it has no alcoholic amino group, was not effective. Of the amide-type local anaesthetics tested, lignocaine, which is similar in size to procaine, was effective, but mepivacaine, which is more bulky than lignocaine because of its methylpiperidine ring, was not effective. Dibucaine, which is more bulky, was ineffective in spite of its strong membrane-stabilizing action.

Whatever the mechanism involved, our results show that the main inhibitory effects of these drugs are not caused by the usual membrane-stabilizing actions or hydrophobic interactions, although these may contribute to the effects. The inhibitory actions of effective local anaesthetics on the induction of desensitization are probably due to specific actions on unknown desensitizing factors. For elucidating the mechanisms involved in the early stage of induction of desensitization it is important to determine the system blocked by tetracaine and lignocaine, but not by dibucaine or mepivacaine.

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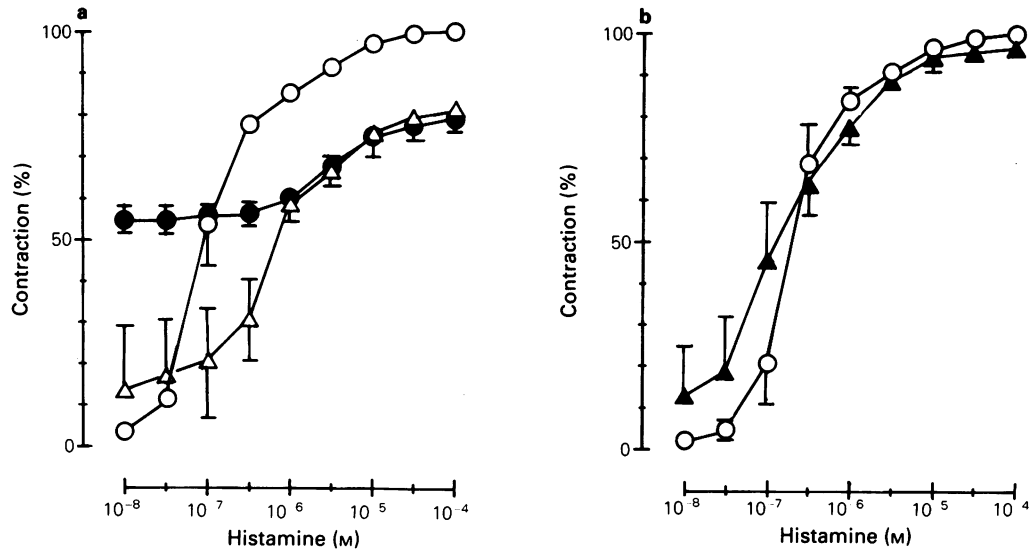

Figure 8 Effect of 3×10^{-5} M dibucaine on histamine-induced desensitization in the absence of Ca ion. Conditions were as for Figure 7. (a) Contractile responses were recorded before (○) and after (●) the first desensitizing treatment; and after the second desensitizing treatment in the presence of dibucaine (△). In (b) responses were recorded before (○) and after (▲) treatment with 3×10^{-5} M dibucaine alone. Each point represents the mean of 4 experiments and vertical lines indicate s.e.

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