Br. J. Pharmac. (1984), **82**, 791–800

Contractile effects of cysteamine on the guinea-pig ileum

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1 Cysteamine (β-mercaptoethylamine HCl) (1.0–40.0 mM) induced a concentration-dependent increase in tonic and phasic contractions of segments of guinea-pig ileum *in vitro*. Myenteric plexus-longitudinal muscle (MPLM) preparations also responded with an increase in tonic contractions but phasic contractions were either greatly reduced or absent, indicating that these were a response of the circular muscle.

2 Atropine (5 μM) inhibited the cysteamine-induced contractions, whereas hexamethonium and guanethidine had no effect, suggesting that cysteamine was acting at least partly via a cholinergic mechanism involving muscarinic receptors.

3 Tetrodotoxin increased the phasic contractions of ileal segments, but had no effect on the tonic component.

4 Treatment of MPLM preparations with morphine (1 μM) resulted in a small reduction in responsiveness to cysteamine, and blocked electrically-induced contractions by at least 90%. Since morphine acts by inhibiting acetylcholine release via hyperpolarization of intrinsic neurones, a small but significant part of the cysteamine-induced contractions probably resulted from stimulation of acetylcholine release from intrinsic neurones.

5 Following a response to high cysteamine concentrations (> 15 mM) tissues were refractory to subsequent cysteamine administration. Cross-desensitization between cysteamine and acetylcholine also occurred, as short term (1–3 min) incubation of MPLM preparations with high concentrations of either compound (1–10 μM acetylcholine or 20 mM cysteamine) resulted in a reduced responsiveness to both.

6 A reduced sensitivity to acetylcholine or cysteamine was obtained following long-term (45 min) incubation with acetylcholine (1 μM). Removal of Na^+ from the incubation medium negated this effect. In contrast, the refractoriness to acetylcholine or cysteamine following long-term (45 min) incubation with cysteamine (20 mM) was accentuated in low Na^+ medium.

7 It is suggested that cysteamine induces a contraction of both the circular and longitudinal muscle of the guinea-pig ileum by stimulating the release of acetylcholine from intrinsic neurones, by an action at the level of the smooth muscle muscarinic receptor, and possibly by a non-cholinergic mechanism. However, the mechanisms by which acetylcholine and cysteamine induce tissue refractoriness probably differ.

Introduction

Cysteamine (β-mercaptoethylamine HCl) has been shown to have a number of effects on the gastrointestinal tract. It is a potent ulcerogen in rats (Selye & Szabo 1973; Groves *et al.*, 1974; Robert *et al.*, 1974; Fujii & Ishii 1975; Szabo *et al.*, 1979; Hernandez *et al.*, 1982) and this is accompanied by increased circulating levels of gastrin (Lichtenberger *et al.*, 1977b; Kirkegaard *et al.*, 1982) and increased gastric acid secretion (Groves *et al.*, 1974; Ishii *et al.*, 1976; Szabo *et al.*, 1977; 1979; Kirkegaard *et al.*, 1980; Hernandez *et al.*, 1982). Additional changes include reduced duodenal Brunner's gland secretion (Kirkegaard *et al.*, 1981; Poulsen *et al.*, 1981), increased gastric mucin release (Lamont *et al.*, 1983), delayed gastric emptying (Robert *et al.*, 1974; Lichtenberger *et al.*, 1977a; Poulsen *et al.*, 1982) and a reduction in both gastric and duodenal tissue somatostatin levels (Szabo & Reichlin, 1981; 1983).

Vagotomy has been demonstrated to inhibit cysteamine-induced gastric acid secretion (Ishii *et al.*,

1976; Szabo *et al.*, 1979; Kirkegaard *et al.*, 1980; Hernandez *et al.*, 1982) and to either inhibit (Lichtenberger *et al.*, 1977a) or augment (Poulsen *et al.*, 1982) the associated delay in gastric emptying, depending on the animal model used. This implied that at least some of the actions of cysteamine involved the autonomic nervous system, which was further supported by the observations that atropine administration blocked cysteamine-induced ulcer development and acid secretion (Fujii & Ishii 1975; Ishii *et al.*, 1976) and that chemical sympathectomy decreased the incidence of cysteamine-induced ulceration (Szabo *et al.*, 1979).

In view of the fact that cysteamine can influence gastric emptying it is possible that it has a more general action on gastro-intestinal smooth muscle. Therefore, in the present study the effects of cysteamine on the motility of the guinea-pig ileum have been investigated.

Methods

Guinea-pigs (200–350 g) of either sex were killed either by a blow on the neck or by a short exposure to an atmosphere of CO_2. No differences in responses were observed between animals killed by the two methods. The abdomen was opened by a mid-line incision and the small intestine removed. The terminal 10 cm of the ileum was discarded and segments 1.5–2 cm in length cut from the remaining ileum. A modified Krebs-bicarbonate buffer, gassed with 95% O_2 plus 5% CO_2 to achieve pH 7.4, was used to gently flush the lumen of the segment and for subsequent incubations unless otherwise indicated. Myenteric plexus-longitudinal muscle (MPLM) preparations were separated from ileal segments by the method of Paton & Zar (1968). The composition of the Krebs solution (mM) was: NaCl 120, KCl 4.4, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.5, $NaHCO_3$ 25, glucose 8.8 and choline chloride 0.01. In some experiments the physiological salt solution of Joiner (1973) was used and the composition of this (mM) was: NaCl 125, KCl 2.7, $CaCl_2$ 1.8, glucose 11, Tris (hydroxymethyl aminoethane) (THAM) 23.8 and choline chloride 0.01 adjusted to pH 7.4 with HCl. Low Na^+ solutions were identical to the above except that NaCl was replaced by its osmotic equivalent of THAM chloride. Segments of MPLM preparations were transferred to 10 ml tissue baths containing Krebs or THAM buffer continuously gassed with 95% O_2 plus 5% CO_2 and maintained at 37°C. One end of the tissue was attached via a thread to the bottom of the bath and the other end was suspended

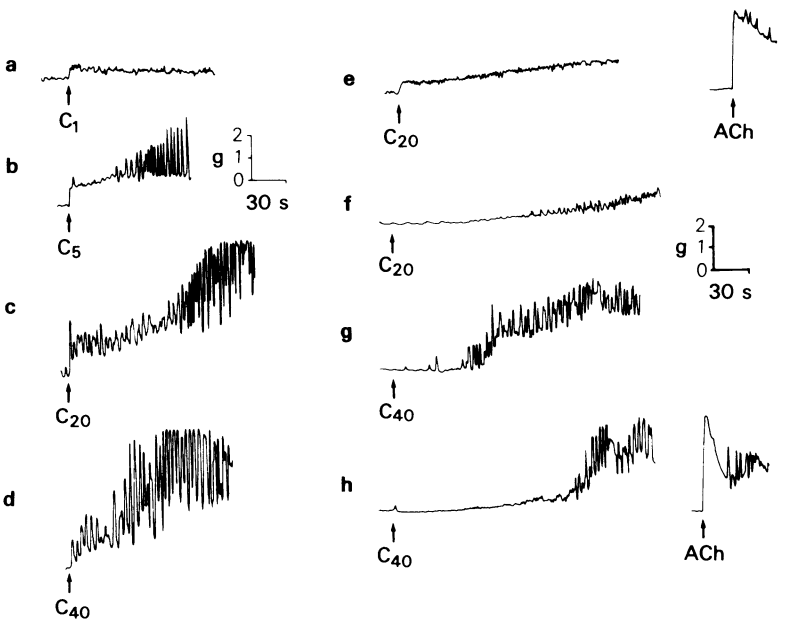

Figure 1 The effect of increasing concentrations of cysteamine on contraction of guinea-pig ileal segments. Cysteamine was added to the bath to give final concentrations of (a) 1 mM (C_1), (b) 5 mM (C_5), (c) 20 mM (C_{20}) and (d) 40 mM (C_{40}). Following stimulation with 20 mM cysteamine (c) the preparation was washed once and after the tension had returned to the baseline was again stimulated with 20 mM cysteamine (e). After a further single wash the response to 20 mM cysteamine was again measured (f). (g) and (h) Show the responses to sequential additions of 40 mM cysteamine, according to the above protocol, following the stimulation with 40 mM cysteamine as in (d).

from a Statham G10B force transducer. The segments were given a basal tension of 1 g and MPLMs 0.5 g. Isometric muscular contractions were recorded on a Gilson Polygraph. Preparations were allowed to equilibrate for at least 1 h before the addition of drugs, during which time the solution was changed approximately every 15 min. Where applicable field stimulation was performed via two parallel platinum electrodes held in a lucite chamber using supramaximal voltage, of 0.5 ms duration and a frequency of 0.3 Hz.

In the majority of experiments, two adjacent preparations from the same guinea-pig were mounted in separate chambers. As explained in the results section there was reduced tissue responsiveness when a second cysteamine dose was administered within 2 min of obtaining a cysteamine induced contraction. Therefore, unless otherwise stated, tissues were washed with a minimum of 20 ml of Krebs buffer and allowed to equilibrate for at least 5 min between additions.

Cysteamine, acetylchloline chloride, tetrodotoxin, morphine sulphate, choline chloride and atropine sulphate were obtained from Sigma Chemical Co., St. Louis, MO. Hexamethonium bromide was from K and K Labs, Inc., Plainview, N.Y. Guanethidine sulphate was a generous gift from CIBA Pharmaceuticals, Mississauga, Ontario. Drugs were administered to the bath in the smallest possible volume, not exceeding 100 μl, in a concentration calculated to give the desired final concentration. Cysteamine was initially dissolved in a small volume of distilled water and the pH adjusted to 7.0 with 2 M NaOH before the final dilution. All other chemicals were of reagent grade or higher purity. Statistical analysis was performed using Students t test for paired or unpaired samples where applicable; P values <0.05 were considered to be significant.

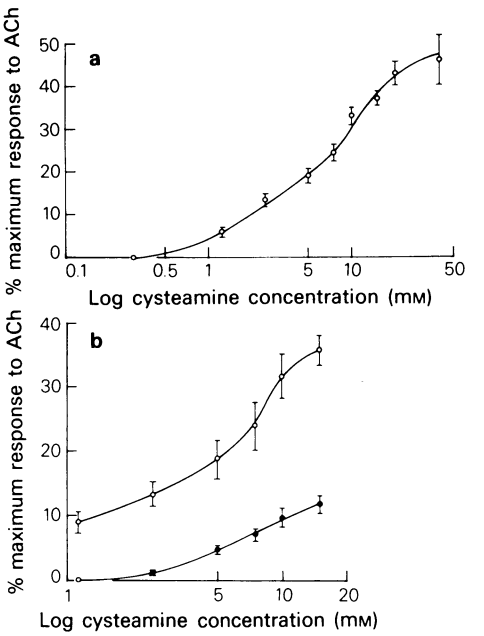

Figure 2 Concentration-response curves of the guinea-pig ileum to cysteamine and the effect of atropine on cysteamine-induced contractions. (a) Contraction is expressed as the mean ± s.e.mean (vertical line) of the maximum tension generated within the first 30 s of contact with cysteamine as a % of the maximal response to acetylcholine (100 μM). Over the range 1.25–15 mM $n=17$, and for 20 mM and 40 mM $n=6$. (b) Concentration-response curve to cysteamine before and in the presence of atropine (5 μM). Each point represents the mean ± s.e.mean (vertical line), $n=6$. (○) Control response; (●) response in presence of atropine (5 μM).

Results

(a) *Effect of cysteamine*

Cysteamine (1.0–40.0 mM) caused a concentration-dependent increase in both tonic and phasic contraction of the ileal segments (Figure 1 a–d). The response consisted of rapid rise to a peak within 30 s followed either by a plateau or, at higher concentrations of cysteamine (>2.5 mM: Figure 1 b–d), a further rise in tension accompanied by large increases in rhythmical contractions. This latter response equalled, or exceeded, that obtained with 100 μM acetylcholine.

When the ileum was treated with a high concentration of cysteamine (>15 mM) subsequent responses were reduced in size and delayed in time of onset. Following stimulation with 20 mM cysteamine (Figure 1c), after washing the tissue, the tension returned to basal values. A second addition of 20 mM cysteamine now induced a much smaller contractile response (Figure 1e), whereas the response to a maximal acetylcholine stimulus (100 μM) was identical to that obtained before the addition of cysteamine. After a further single wash, a 20 mM cysteamine produced a response only after a considerable period of delay (Figure 1f) and the rhythmic contractions were of a reduced intensity. A similar reduction in response and delay in time of onset of contraction also occurred following 40 mM cysteamine (Figure 1g and h).

Due to this reduction in responsiveness, subsequent studies were performed with cysteamine in contact with the tissue for periods not exceeding 2 min, except in the experiments described in Results section (f). Following washing and a further 5 min

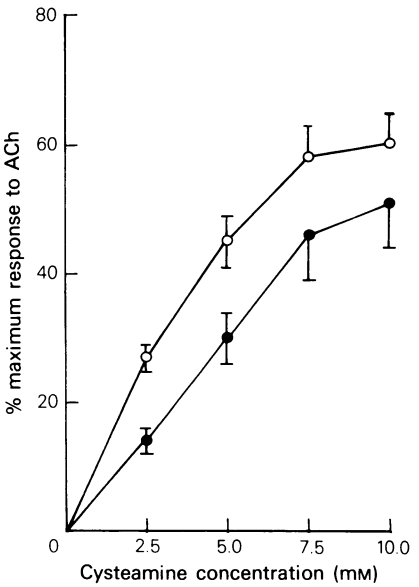

Figure 3 Effect of morphine on responses of myenteric plexus-longitudinal muscle (MPLM) preparations to cysteamine. MPLM preparations were equilibrated in Krebs solution and responses to cysteamine measured. After washing out the cysteamine, and a further 3 min equilibration, electrical stimulation (supramaximal voltage, 0.5 ms, 0.3 Hz) was performed for 30 s and on cessation of this stimulation the response to cysteamine again tested. Electrical stimulation was now repeated, using the same stimulus parameters, morphine was added and when maximal inhibition occurred the electrical stimulation was stopped and responses to cysteamine measured. Responses shown are mean ± s.e.mean (vertical line) post-electrical stimulation (○; $n = 8$) and post-electrical stimulation plus 1 μM morphine (●); $n = 8$).

equilibration period, repetitive doses resulted in almost identical responses. Unless otherwise indicated, the data are therefore represented either as the peak response obtained within the first 30 s of administering cysteamine, expressed as a percentage of the maximal response obtained with acetylcholine in that preparation, or in g tension. Under these conditions the log concentration-response curve to cysteamine was sigmoidal (Figure 2a) with half maximal contraction at a concentration of 7 mM. With MPLM preparations similar concentration-response curves were obtained but the secondary rhythmical contractions were either greatly reduced or completely absent ($n = 6$; results not shown).

(b) *Effect of atropine on cysteamine-induced contractions*

Administration of atropine (5 μM) before stimulating guinea-pig ileal segments with cysteamine inhibited both the initial rise in tension and the sustained phasic contractions. The response to acetylcholine (100 μM) was abolished by this concentration of atropine ($n = 10$) but responses to cysteamine or acetylcholine returned following periods of extensive

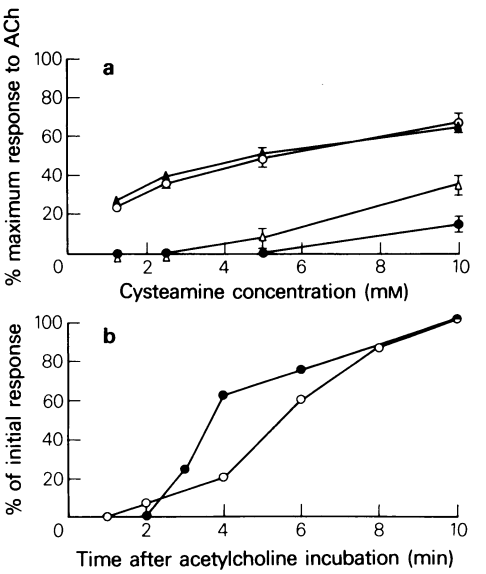

Figure 4 Effect of short term exposure to acetylcholine on subsequent tissue responses to cysteamine (a) and recovery of cysteamine responsiveness following acetylcholine treatment (b). (a) Longitudinal muscle-myenteric plexus strips were initially tested for cysteamine responses over the concentration range 1.25–10.0 mM (○). The time of incubation with cysteamine was 1 min and a 3 min recovery period was allowed between doses. Tissues were then exposed for 1 min to acetylcholine at a concentration of 10 mM (●), 1.0 mM (△) or 0.1 mM (▲). After washing out the acetylcholine, responsiveness to cysteamine was immediately tested according to the above protocol. Results are presented as the mean ± s.e.mean (vertical line) of at least six experiments. (b) Longitudinal muscle-myenteric plexus strips were incubated with acetylcholine (10 μM) for 1 min. After washing the strip once, cysteamine 2.5 mM (○) or 5.0 mM (●) was added at one of the time periods indicated in min. The strips were then re-washed. Following a 3 min equilibration period, 10 μM acetylcholine was again added and the above protocol repeated for a different time interval. Data are presented as the mean of two experiments at each concentration of cysteamine. Repetitive stimulations by acetylcholine under the experimental conditions induced responses which varied by not more than 5%.

washing ($n = 20$). The concentration-response curves to cysteamine in the presence and absence of atropine were parallel over the range 1–7.5 mM (Figure 2b), but above this concentration the response curves diverged. The degree of inhibition by atropine of responses to cysteamine 7.5 mM, 10.0 mM and 15 mM were 71%, 69% and 67%, respectively, whereas at lower cysteamine concentrations (1.25 mM and 2.5 mM) blockade was almost complete: 100% and 93%, respectively.

(c) *Effect of hexamethonium, guanethidine and tetrodotoxin on cysteamine-induced contractions*

In five experiments (MPLM preparation) application of hexamethonium (100 μM) before cysteamine (1.25–10.0 mM) had no effect on the tonic or phasic contractions, whereas administration of guanethidine (5 μM) reduced the resting tension of the ileum but had no effect on cysteamine-(1.25–10.0 mM) induced contractions ($n = 6$; data not shown).

Addition of tetrodotoxin (4.1 μM) before cysteamine had no effect on the overall increase in tension developed by either the ileal segments or MPLM preparations. The amplitude of rhythmic contractions was, however, increased in tetrodotoxin-treated segments. The concentration of tetrodotoxin used completely blocked contractions of the ileum induced by field stimulation in all experiments ($n = 6$).

(d) *Effect of morphine on cysteamine-induced contractions of myenteric plexus longitudinal muscle preparations*

Following incubation with morphine (1 μM), responses to cysteamine (2.5 and 5.0 mM) were significantly reduced (Figure 3) by 45 ± 9% and 34 ± 8%, respectively ($P < 0.01$). With this concentration of morphine, contractions induced by electrical stimulation were reduced by at least 90%. Cysteamine responses measured pre- and post-electrical stimulation alone did not differ significantly.

(e) *Effect of acetylcholine on cysteamine-induced contractions*

Exposure of longitudinal muscle-myenteric plexus preparations to acetylcholine induced a refractoriness to subsequent additions of cysteamine. Following a 1 min incubation with 10 μM acetylcholine, there was a complete densensitization to all cysteamine concentrations below 10 mM, and the response to the latter was reduced by 80 ± 6% (Figure 4a). Treatment with 1 μM acetylcholine induced a total refractorines to 1.25 mM and 2.5 mM cysteamine whereas contractions induced by 5 mM and 10 mM cysteamine were reduced by 84 ± 10% and 46 ± 5%, respectively. The reductions in responsiveness following acetylcholine (10 μM and 1 μM) were significant at all concentrations of cysteamine ($P < 0.01$). Lower concentrations of acetylcholine

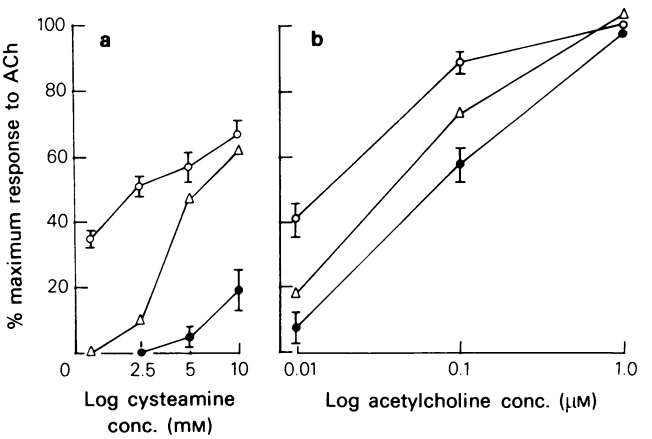

Figure 5 Responses of myenteric plexus-longitudinal muscle (MPLM) preparations before and after short-term exposure to acetylcholine or cysteamine. MPLM preparations were equilibrated in THAM buffer and responses to cysteamine (a) or acetylcholine (b) were measured. Tissues were then incubated for 1 min in either 1 μM acetylcholine or 20 mM cysteamine, washed once and responses again measured. (●) Responses after pre-incubation in acetylcholine; (△) responses after pre-incubation in cysteamine; (○) pre-incubation control responses. Data are presented as the mean ± s.e.mean (vertical line) of 5–8 experiments on different preparations.

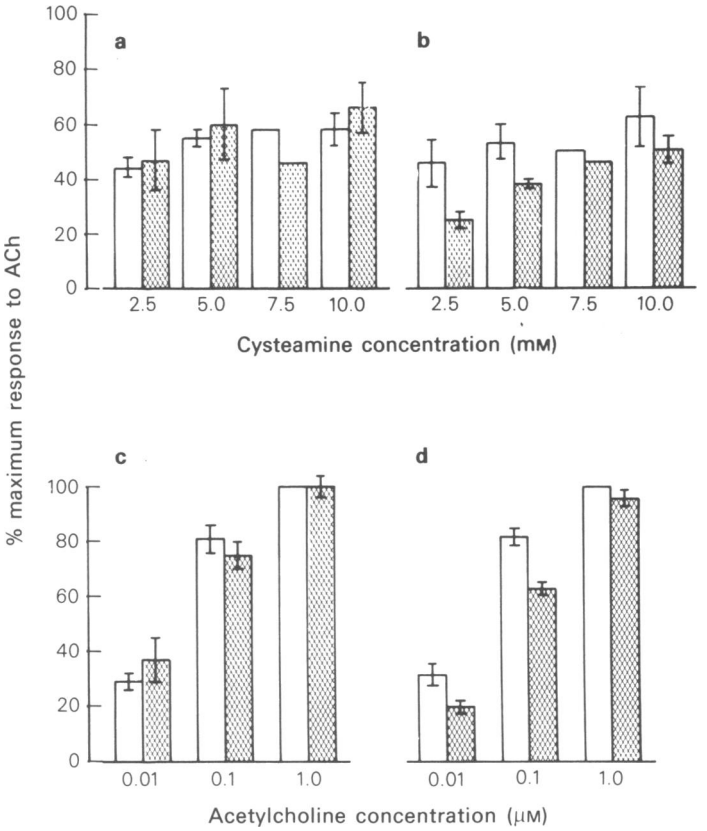

Figure 6 Responses of myenteric plexus-longitudinal muscle (MPLM) preparations before and after long-term exposure to acetylcholine in normal or low Na^+ medium. The responses of two MPLM preparations to cysteamine (a) and (b) or acetylcholine (c) and (d) were initially measured (open columns). One preparation was then incubated for 45 min in 1 μM acetylcholine in normal THAM buffer (b) and (d) and the second preparation under the same conditions but with low Na^+ buffer (a) and (c). Following a wash, and 15 min incubation in normal THAM buffer, tissue responsiveness was again tested (hatched columns). Data are presented as mean ± s.e.mean (vertical bar) of five experiments except for 7.5 mM cysteamine ($n = 2$). Responses after pre-incubation in acetycholine were significantly different ($P < 0.01$) from controls only for 2.5 and 5.0 mM cysteamine and 0.01 and 0.1 mM acetylcholine in normal Na^+ medium (b) and (d).

(<0.10 μMM) had no effect on subsequent cysteamine-induced contractions (Figure 4a). This refractoriness to cysteamine was reversible: reaching pretreatment responses after 10 min of incubation in acetylcholine free buffer (Figure 4b).

(f) *Effect of a reduction of sodium ion on desensitization induced by cysteamine and acetylcholine*

Since there is evidence that the loss of tissue sensitivity following long-term exposure to high doses of acetylcholine can be reversed by incubation in a medium containing low concentrations of Na^+ (Joiner, 1973), studies were performed to ascertain whether this applied to the cross-desensitization between acetylcholine and cysteamine. The THAM buffer system of Joiner (1973) (see Methods) was utilized in these experiments. Responses of MPLM preparations to acetylcholine and cysteamine (Figure 5) were similar to those obtained in Krebs-bicarbonate solution. Following exposure of the tissues for 1 min to 1 μM acetylcholine subsequent responses to all doses of cysteamine and acetylcholine (0.01 and 0.1 μM) were significantly reduced ($P < 0.01$). Treatment of the tissues with 20 mM cysteamine for 1 min also induced a refractoriness to both acetylcholine (0.01 and 0.1 μM) and cysteamine (1.25 and 2.5 mM) but to a lesser degree than the pretreatment with 1 μM acetylcholine ($P < 0.01$).

More prolonged (45 min) incubation in normal

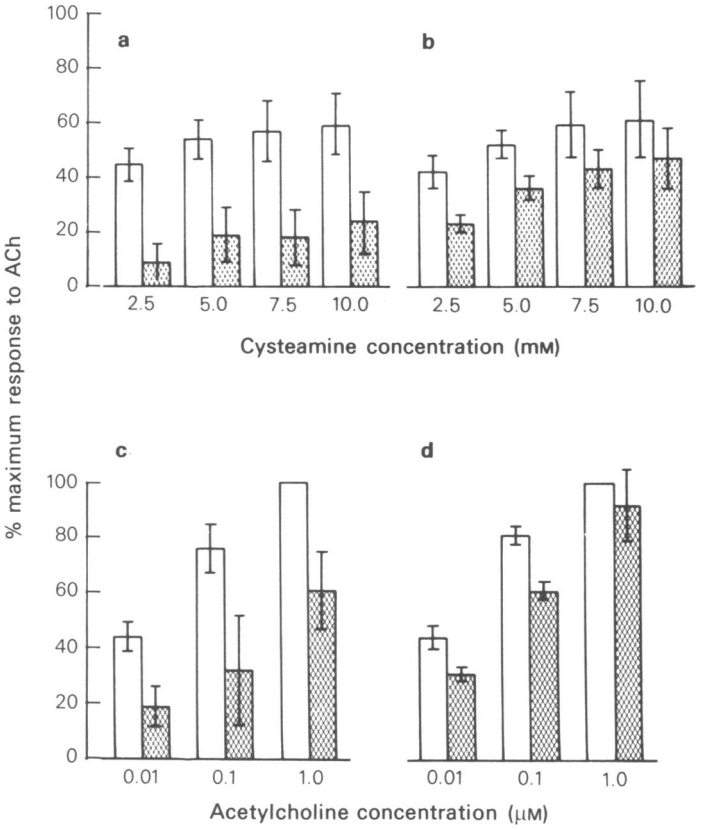

Figure 7 Responses of myenteric plexus-longitudinal muscle (MPLM) preparations before and after long-term exposure to cysteamine in normal or low Na^+ medium. The responses of two MPLM preparations to cysteamine (a) and (b) or acetylcholine (c) and (d) were initially measured (open columns). One preparation was then incubated for 45 min in 20 mM cysteamine in normal THAM buffer (b) and (d) and the second preparation under the same conditions but with low Na^+ buffer (a) and (c). Following a wash and 15 min incubation in normal THAM buffer, tissue responsiveness was again tested (hatched columns).Data are presented as mean ± s.e.mean (vertical bar) of five experiments. Responses after pre-incubation in cysteamine were significantly different from controls for all responses, except those to 7.5 and 10 mM cysteamine (b) and 1.0 μM acetylcholine (d) in normal Na^+ medium.

Na^+ medium with 1 μM acetylcholine (Figure 6 b and d) produced a less marked reduction in responsiveness to cysteamine and acetylcholine than short term exposure. Responses to 2.5 and 5.0 mM cysteamine were reduced by 41 and 28%, respectively and those to 10 nM and 100 nM acetylcholine by 35 and 23%, respectively. Following incubation with acetylcholine in low Na^+ medium (Figure 6 a and c), there was no significant reduction in responsiveness to acetylcholine or cysteamine at any dose level. Tissues incubated in THAM buffer containing normal or low Na^+ concentrations in the absence of acetylcholine did not differ in their subsequent responsiveness to acetylcholine (10 μM) ($n=3$).

Such a reversal of reduced responsiveness with low Na^+ did not occur in tissues incubated for 45 min with 20 mM cysteamine (Figure 7). In contrast, the degree of refractoriness to both cysteamine (Figure 7a and b) and acetylcholine (Figure 7c and d) was increased for all concentrations ($P<0.01$).

Discussion

Cysteamine exerts a wide range of actions on the gastro-intestinal tract some or all of which may be related to its ulcerogenic properties. The only studies on the effect of cysteamine on motility have suggested that delayed gastric emptying resulted from a relaxation of the stomach and blockade of gastric peristalsis (Poulsen *et al.*, 1982). The doses of cys-

teamine administered orally or subcutaneously, in studies on its ulcerogenic effects have varied between 10 and 100 mg kg^{-1} body weight (Selye & Szabo, 1973; Groves *et al.*, 1974; Robert *et al.*, 1974; Fujii & Ishii, 1975; Szabo *et al.*, 1979; Hernandez *et al.*, 1982). Similar doses have been utilized for investigations on other gastro-intestinal functions. If one assumes a complete distribution in total body water, then circulating levels would range between 1.5 and 15 mM. Following oral administration, local concentrations could be higher. In the present study concentrations of cysteamine within this range have been demonstrated to cause concentration-dependent increases in both tonic and phasic contractions of the guinea-pig ileum. These actions were both rapid in time of onset and reversible upon removal of the drug, providing that tissues were only subjected to a short period of contact. The secondary, phasic contractions were largely absent in longitudinal muscle-myenteric plexus preparations, suggesting that they resulted from actions on the circular muscle.

Attempts to elucidate the site of action of cysteamine suggest that it may act at more than one site. Since the responses to cysteamine were partially inhibited by atropine, but not by hexamethonium, tetrodotoxin or guanethidine, it seems probable that part of cysteamine's action is exerted either via stimulation of post-ganglionic, cholinergic neurones or by binding to, or interacting with the smooth muscle muscarinic receptor. However, higher concentrations of cysteamine (>2.5 mM) were still stimulatory in the presence of an atropine concentration capable of totally blocking maximal acetylcholine-induced concentrations. A non-muscarinic component also, therefore, probably exists.

The experiments utilizing morphine were designed to investigate the possible existence of a presynaptic action of cysteamine on post-ganglionic cholinergic neurones. Morphine exerts its inhibitory action on electrically-induced contractions of the guinea-pig ileum by inhibiting acetylcholine release (Paton, 1957; Schauman, 1957) via hyperpolarization of intrinsic neurones (Szerb, 1982; North, 1982). In the present study, cysteamine-induced contractions were reduced by a maximum of 45% whereas electrically-induced contractions were totally inhibited. There was no significant inhibition of contractions induced by high concentrations of cysteamine (>5 mM). It seems, therefore, that a small but significant fraction of cysteamine's action can be explained by a stimulation of acetylcholine release from post-ganglionic intrinsic neurones, although this proposal needs to be substantiated by direct measurements of release. Inhibition of cysteamine-induced contraction by atropine was profound, with total inhibition at lower cysteamine concentrations (1.25 mM) but only 67–71% at concentrations above 5.0 mM. This greater degree of inhibition by atropine suggests that cysteamine also exerts direct effects on the smooth muscle muscarinic receptor. This possibility is difficult to rationalize on a structural basis. It has generally been demonstrated that the minimal structural requirement for a cholinoceptor agonist is a quarternary ammonium group, and that there is a critical distance between this moiety and an ester group (Horn, 1975). Recently, the importance of guanine nucleotide regulation of muscarinic receptor activity has become apparent (Roeske *et al.*, 1983; Uchida *et al.*, 1983). It is therefore possible that cysteamine is acting on the nucleotide binding site leading either to a direct stimulation of subsequent events resulting in contraction, or to a sensitization of the tissue to acetylcholine released by a presynaptic action of cysteamine. The latter seems unlikely in view of this compound's desensitizing action on the ileum.

The finding that exposure of tissues to cysteamine induced a marked resistance to subsequent doses led to a comparison of this 'desensitization' phenomenon with that induced by acetylcholine. Desensitization by acetylcholine has generally been considered to be of a 'non-specific' nature (Morgenstern & Bluth, 1976; Hurwitz & McGuffee, 1979) and studies by Joiner (1973) and Paton & Rothschild (1965) raised the possibility that changes in sodium ion distribution may be involved. Cross-desensitization between cysteamine and acetylcholine occurred following either short or long incubations with high concentrations of either compound. In agreement with Joiner (1973), incubation in a low Na^+ medium ablated the acetylcholine-induced desensitization to subsequent acetylcholine treatment. The reduced responsiveness to cysteamine following acetylcholine was similarly reversed. However, in contrast to these results, cysteamine-induced desensitization was not reversed by a low Na^+ medium, but a potentiation of this effect was obtained. The explanation for this difference is not readily apparent. The majority of data supports an interaction with the smooth muscle muscarinic receptor but the mode of cysteamine-induced desensitization may be through a different mechanism from that demonstrated for acetylcholine.

Different pathways may operate in the action of cysteamine on gastrin and acid secretion and gastric emptying. Stimulation of gastrin release has been shown to be mediated via a mechanism which is sensitive to β-adrenoceptor blockade (Kirkegaard *et al.*, 1982), whereas the gastric acid stimulating action of cysteamine is blocked by hexamethonium (Ishii *et al.*, 1976), suggesting either a preganglionic or ganglionic site of action. This would be compatible with the loss of cysteamine action on acid secretion (Ishii *et al.*, 1976) or gastric emptying (Lichtenberger *et al.*, 1977a) following vagotomy, although the latter effect has not always been observed (Poulsen *et al.*,

1982). An explanation for the pronounced relaxation of the stomach and blockade of gastric peristalsis following *in vivo* cysteamine treatment may be proposed from this present study. Either a depletion of acetylcholine stores or a desensitization of muscle to endogenous acetylcholine could result in both phenomena.

In more general terms, it is possible that the effects of cysteamine reflect an action of sulphur-containing substances. A number of thiol compounds have been used to potentiate the smooth muscle actions of substances such as bradykinin and histamine (Cirstea, 1965; Hall & Bonta, 1974; Watson & Iversen, 1982) and the potentiating effect of cysteine has been attributed to a facilitation of acetylcholine release (Potter & Walaszek, 1972). Cysteine and methionine also induce contraction of the guinea-pig ileum (Lewis *et al.*, 1972; Bakich & McIntosh, unpublished), although the mode of action is again unknown. A common stimulatory mechanism for the sulphur-containing amino acids and their respective amines may exist.

This work was supported by grants from the British Columbia Health Care Research Foundation and the Medical Research Council of Canada. Dr Y. N. Kwok is a Postdoctoral Fellow of the Canadian Medical Research Council. We would also like to thank Mary Forsyth for preparation of the manuscript and Kurt Henze for the art work. Please address correspondence to C. M.

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(*Received October 13, 1983.*
Revised January 30, 1984.)