Br. J. Pharmac. (1973), **49**, 445–456.

Some effects of histamine in the depolarized rat uterus

D. I. BLYTH*

Department of Biological Sciences, The Hatfield Polytechnic, Hatfield, Herts

Summary

1. The relaxant effect of histamine in the isolated rat uterus remained after the preparation was depolarized in a potassium Ringer.

2. The effect was abolished by the calcium-chelating agent, ethyleneglycol bis-aminoethyl ether-tetraacetic acid (EGTA).

3. Histamine caused relaxation during calcium-induced contractures in a depolarized uterus treated with EGTA, but did not produce relaxation during barium-induced contractures.

4. While responses of a normally polarized rat uterus to acetylcholine were inhibited by histamine, those of a depolarized uterus were enhanced by histamine. The inhibitory effects of isoprenaline and papaverine on acetylcholine responses were maintained in depolarized preparations.

5. Both the enhancing effect of histamine on responses to acetylcholine and the relaxation of calcium-induced contractures were abolished by burimamide, indicating that H_2-receptors mediate the effects of histamine in the depolarized tissue.

6. A reduction in the rate of exchange of calcium across the depolarized cell membrane was demonstrated with high concentrations of histamine.

7. The results are consistent with the hypothesis that in the rat uterus, the stimulation of H_2-receptors by histamine is accompanied by a reduction in calcium exchange across the membrane which may result in a decrease in the concentration of free intracellular calcium available to stimulate contraction of the myofilaments. Histamine may act by increasing the binding of calcium within the cell.

Introduction

The receptors for histamine in the rat uterus are similar to those involved in histamine-stimulated gastric secretion (Black, Duncan, Durant, Ganellin & Parsons, 1972). A knowledge of the immediate consequences of H_2-receptor stimulation in the rat uterus would not only help to explain how histamine exerts its relaxant effect on this tissue, but may provide information about the mechanisms involved in gastric secretion.

It has been shown (Blyth, 1972) that histamine keeps the resting membrane potential of the rat uterus at the normal level and suppresses spike discharge.

* Present address: Pharmacology Department, Glaxo Laboratories Ltd., Greenford, Middlesex.

The present study was undertaken to discover whether the relaxant effect of histamine remained after the membrane had been depolarized and, if so, whether the calcium ion was involved in the action.

Part of the work was based on the experiments of Schild (1966, 1967a, b) which showed that the relaxant effect of isoprenaline in a potassium-depolarized rat uterus was abolished by the addition of a calcium-chelating agent to the bathing fluid. Furthermore, while isoprenaline-induced relaxation could be obtained during a contracture to calcium, it did not occur when the uterus was stimulated with barium.

Results are presented in this paper which suggest that a similar situation exists with histamine and may be due to an effect of histamine on the rate of calcium exchange across the cell membrane.

Methods

Isolated-organ bath technique

The experiments were carried out on uteri from Wistar rats of about 160 g body weight. Only animals in natural oestrus were used and were chosen immediately prior to the experiment by examination of vaginal smears. The animal was killed by a blow on the head, the entire uterus removed, and one horn dissected free of fatty tissue in Tyrode solution at room temperature. The uterine horn was suspended in an isolated-organ bath filled with Tyrode solution to a calibrated level of 10 ml. The bathing fluids were continuously oxygenated. Tension was recorded isometrically with a Devices transducer type UF1 mounted on a rack and pinion to allow changes in tension to be made. The signal from the transducer was recorded on a Devices M2 pen recorder. The muscle preparation was placed under an initial tension of 1 gram.

Isotonic recording with a frontal-writing lever (magnification $\times 9$, loading on tissue 0·5–1·0 g) was sometimes used.

In some experiments, both horns from the same uterus were set up in parallel under identical conditions, one horn acting as a control for the other.

All experiments were carried out at temperatures within the range 20°–25° C.

Experiments were normally started by recording the spontaneous activity of the preparations in Tyrode solution. After a short time, the bathing fluid was changed for either KCl- or K_2SO_4-Ringer. These had the following compositions (mM): KCl-Ringer: KCl, 145 ; $KHCO_3$, 12 ; glucose, 6 ; K_2SO_4-Ringer ; K_2SO_4, 74 ; $KHCO^3$, 12 ; glucose, 6 ; sucrose, 59 (Schild, 1967b). Immersion of uteri in potassium Ringer always resulted in an immediate contraction followed by relaxation to the original baseline within 10 or 15 minutes.

^{45}Ca efflux measurements

The technique used was that described by Feinstein (1963, 1966). Experiments were carried out at 21° C using both uterine horns in parallel. The preparations were mounted on glass rods, secured with cotton ligatures, and incubated in K_2SO_4-Ringer of the following composition (mM): K_2SO_4, 125 ; $KHCO_3$, 25 ; glucose, 11 (Feinstein, 1966), and containing 1 mM $CaCl_2$ and about 4 μCi/ml ^{45}Ca (as the chloride) for a period of 2 hours. This time is sufficient to allow for ^{45}Ca uptake

into the muscle cells. In fact, nearly all the exchangeable cellular calcium in rat uterus has been shown to equilibrate with external ^{45}Ca in 1 hour (van Breemen, Daniel & van Breemen, 1966).

The tissues were rinsed rapidly with non-radioactive, calcium-free K_2SO_4-Ringer to remove radioactive solution adhering to the surface of the tissue. Each horn was then passed through a series of 12 test-tubes, each containing 2 ml of calcium-free K_2SO_4 solution. The preparations remained in each tube for 10 minutes.

At the end of the 120 min period, the horns were passed through a further series of 12 tubes, each containing 2 ml of K_2SO_4 solution + 1 mM Ca, spending 5 min in each tube. The total period of washout was therefore 180 minutes.

When used, drugs were added to the tubes after 90 min of washout, i.e. 30 min prior to the addition of calcium. Only one horn was exposed to the drug, the other horn acting as a control.

Two aliquots of 0·5 ml were taken from each collection tube and placed in 10 ml of dioxan base scintillator solution made up as follows: 1,4-dioxan 1 litre; Cellusolve (2-ethoxy-ethanol) 200 ml; PPO (2,5-diphenyl-oxazole) 12 g; POPOP (1,4-di-(2(5-phenyloxazole))-benzene) 0·6 g and naphthalene 60 g.

At the end of the experiment, the uterine horns were removed from the glass rods, placed in vitreosil crucibles, and dry-ashed at 500° C in a muffle furnace overnight. The ashes from each horn were dissolved in 20 ml of scintillator solution and this volume divided into two for counting. The radioactivity in the resulting 100 samples was estimated in a Tracerlab Corumatic 100a liquid scintillation counter.

The relative ^{45}Ca efflux for a particular collection period was calculated as follows:

$$\text{Rate coefficient for } ^{45}Ca = \frac{\text{radioactivity appearing in washout solution during collection period}}{\text{average radioactivity in the muscle during the collection period} \times \text{duration of collection period}}$$

$$\text{Relatve } ^{45}Ca \text{ efflux per collection period} = \frac{\text{rate coefficient for } ^{45}Ca \text{ efflux during that collection period}}{\text{rate coefficient for } ^{45}Ca \text{ efflux during the 10 min period just prior to addition of Ca to the perfusion fluid (110–120 minutes).}}$$

The half-life of ^{45}Ca (160 days) is sufficiently long to make correction for decay unnecessary in these relatively short experiments.

The drugs used were: acetylcholine chloride (Koch-Light), burimamide (Smith, Kline and French), histamine acid phosphate (British Drug Houses), isoprenaline sulphate (Burroughs Wellcome), papaverine (Macfarlan Smith), propranolol (ICI) and tetracaine hydrochloride (Amethocaine, Hoechst Pharmaceuticals).

Results

The effect of histamine on acetylcholine-induced contractions of the depolarized rat uterus

When regular responses of the depolarized uterus were obtained to submaximal doses of acetylcholine in Ca-free K_2SO_4-Ringer, histamine, injected 1 min before

the next dose of acetylcholine, produced a fall in the level of resting tension but normally caused no inhibition of the response to acetylcholine. The tension of the muscle relaxed by histamine was not readjusted to 1 gram. Although a small inhibitory effect of histamine on acetylcholine contractions was seen in a few preparations, the most common observation was that histamine either had no effect at all on the response to acetylcholine or actually enhanced the response, the contraction appearing larger in the presence of histamine than in its absence. If the tissue was then returned to Tyrode solution and allowed to repolarize, histamine caused inhibition of the acetylcholine-induced contractions.

The apparent potentiation of the response to acetylcholine during exposure to histamine may be explained by the altered baseline. The lower initial tension and the increased length of the muscle may have allowed the dose of acetylcholine to cause a larger change in tension. Depolarization of the uterine cell membrane therefore appeared to remove the inhibitory effect exerted by histamine on acetylcholine-induced contractions in a normally-polarized preparation.

The effect of histamine on the decline in the response to acetylcholine in Ca-free K-Ringer

It has been reported that when an isolated rat uterus depolarized in a K-Ringer containing Ca, is stimulated with acetylcholine, responses to the drug can be obtained repeatedly over a period of several hours without deterioration (Evans, Schild & Thesleff, 1958). In Ca-free K-Ringer, however, it was found that the response of the uterus to acetylcholine declined progressively over a period of time (Edman & Schild, 1962). Similar decline occurs when contractions are induced in other depolarized tissues with adrenaline (Edman & Schild, 1963), histamine, 5-hydroxytryptamine and oxytocin (Schild, 1964).

The effect of histamine on the decline in the responses to acetylcholine was investigated. The experiments were carried out in Ca-free K_2SO_4-Ringer but no chelating agent was added to this solution. Both uterine horns were set up in parallel, one horn acting as a control for the other. Events in the control horn were timed to occur 10 s after those in the test horn. The preparations were stimulated with 2 μg/ml acetylcholine for 45 s every 3 min over a period of 1 hour. Both preparations were then returned to Tyrode solution for 1 h to repolarize. At the end of this time, the bathing fluid was changed for Ca-free K_2SO_4-Ringer again and the stimulation with acetylcholine restarted. After 13 min, the test horn was exposed to histamine and this exposure continued to the end of the 1 h period, stimulation with acetylcholine being continued throughout. The control horn, meanwhile, was stimulated with acetylcholine in K_2SO_4-Ringer as before. The procedure was then repeated for a third time.

On every change to K_2SO_4-Ringer, the preparations contracted, indicating that at least some repolarization had taken place while the muscle was in Tyrode solution. Each experiment lasted 5 hours. Six graphs were obtained from the data, three from each horn. Tension was recorded isometrically.

The response to acetylcholine was found, as reported, to decline progressively over each 1 h period, the decline having a half-time of about 20 minutes. The repolarization in Tyrode solution restored the response to almost its original level. The rates of decline of the acetylcholine responses were similar in horns from

the same uterus but became faster with time, the third curve falling off more steeply than the first.

During exposure to histamine, the resting tension of the preparation was reduced. The responses to acetylcholine, however, were enhanced so that histamine appeared to reverse the decline in the response. This potentiation of the responses to acetylcholine was shown in each of 12 preparations. On no occasion did histamine reduce the size of the acetylcholine response. Figure 1 shows a typical effect of an exposure to 50 μg/ml histamine on the decline of the acetylcholine response. In Figs. 1 to 3, the ordinates are the per cent response to 2 μg/ml acetylcholine in K_2SO_4-Ringer.

If one horn was treated with the H_2-histamine antagonist burimamide throughout, the enhancing effect of histamine on the acetylcholine responses was reduced or abolished, indicating that this effect of histamine was mediated via H_2-receptors. It can be seen from Fig. 2 that 5 μg/ml burimamide reduced the effect of 50 μg/ml

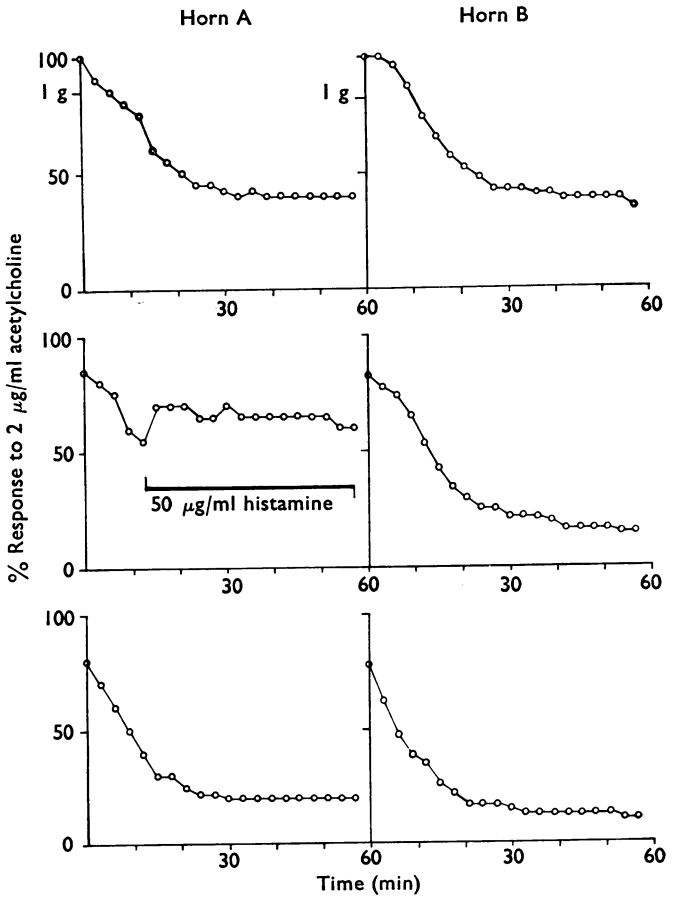

FIG. 1. The effect of an exposure to 50 μg/ml histamine on the decline in the response to acetylcholine (2 μg/ml) of rat uterus preparations depolarized in calcium-free K_2SO_4-Ringer at 23° C. The contact time for acetylcholine was 45 seconds. The tissues were allowed to repolarize in Tyrode solution for 1 h between each of the three periods of stimulation. Horns A and B were from the same uterus. Horn B acted as the control. The ordinates are the per cent tension, recorded isometrically, produced regularly by 2 μg/ml acetylcholine in (drug-free) K_2SO_4-Ringer.

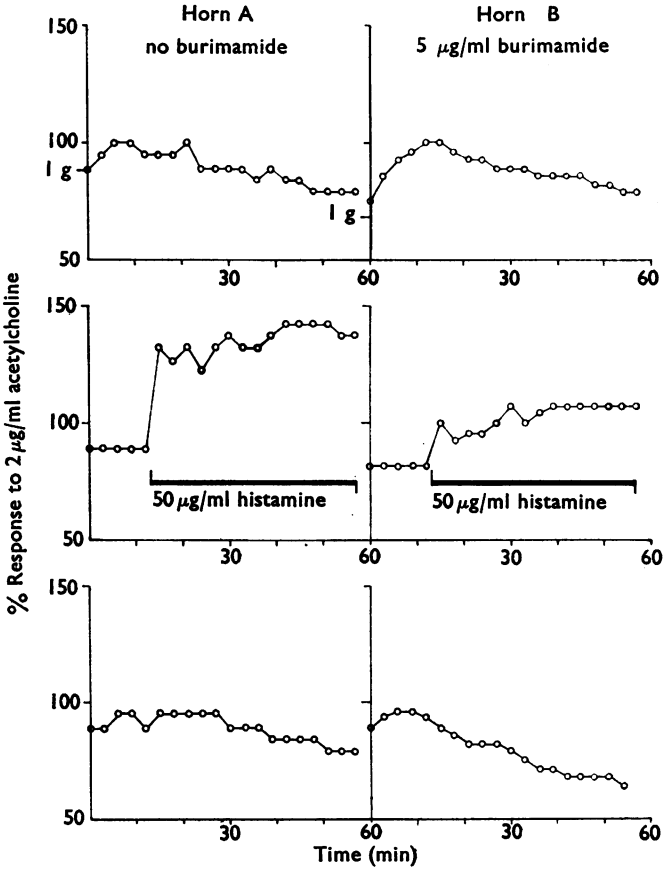

FIG. 2. The reduction in the effect of 50 μg/ml histamine on the decline in the acetylcholine response, by 5 μg/ml burimamide, to which horn B was exposed throughout. The preparations were depolarized in K_2SO_4-Ringer at 22° C. Ordinates as in Figure 1. In this experiment, the responses of horn A to 2 μg/ml acetylcholine were increased beyond the original level during exposure to histamine.

histamine while having no effect itself on the responses to acetylcholine. The effect of the same concentration of histamine could be blocked completely by increasing the concentration of burimamide to 10 μg/ml.

Unlike histamine, isoprenaline produced its normal inhibition of acetylcholine responses. This was demonstrated by an experiment in which one horn of a uterus was exposed to 50 μg/ml histamine and the other horn to 10 ng/ml isoprenaline. While both drugs caused a fall in resting tension, histamine enhanced the responses to acetylcholine and isoprenaline inhibited them (Figure 3).

When papaverine was similarly compared with histamine, it too produced the normal inhibition of acetylcholine responses while histamine enhanced them (Figure 3).

The augmentation of the acetylcholine responses seen with histamine in depolarizing conditions, therefore, appears not to be a property of smooth muscle relaxants in general, but to be peculiar to histamine.

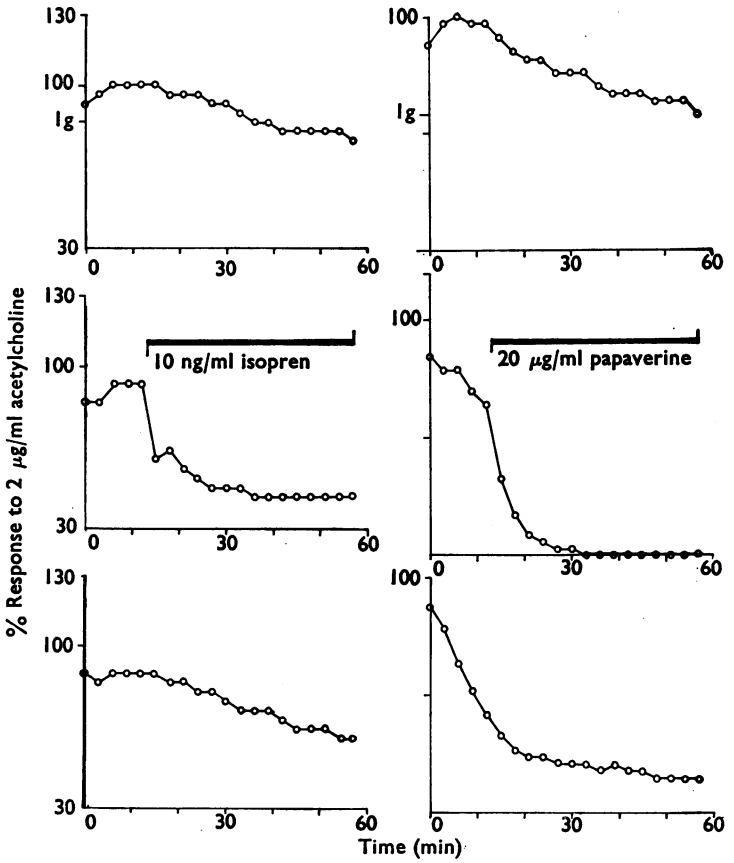

FIG. 3. The effects of 10 ng/ml isoprenaline (left panel) and 20 μg/ml papaverine (right panel) on the decline of acetylcholine responses in rat uterus preparations depolarized in K_2SO_4-Ringer. The drugs were tested on uterine horns from different animals. Both isoprenaline and papaverine produced reductions in the size of the responses to acetylcholine. Ordinates as in Figure 1.

The effect of histamine on Ca-induced increases in tension in the depolarized rat uterus

The inhibition of Ca-induced contractions by histamine in a depolarized rat uterus was small, particularly when compared with the effect of isoprenaline. Histamine was found to produce little or no shift in the log concentration-response curve to Ca. The relaxation caused by histamine during Ca-induced increases in tension in the depolarized uterus cannot therefore be a result of competitive antagonism of the Ca ion by histamine.

The experimental procedure was based precisely on that described by Schild 1967a, b). The preparations were set up in parallel and were depolarized in KCl-Ringer. Activity was recorded isometrically.

The relaxing action of histamine on resting tension was found to be abolished by the addition of 1 mM ethyleneglycol bis-aminoethyl ether-tetraacetic acid (EGTA) to the KCl-Ringer, suggesting that histamine, like isoprenaline, requires the presence of Ca in order to exert its effect.

Two horns of the same uterus, depolarized in KCl-Ringer, were exposed to 1 mM EGTA until they no longer responded to histamine or isoprenaline. The time interval between similar events in the two horns was 10 seconds. Ca (2 mM) was then added to one horn and Ba (2 mM) to the other. Both uterine horns contracted and shortening was allowed to proceed isotonically by constant adjustments to the levels of the transducers. Hence although the muscles had shortened, they remained under the original tension. The effects of histamine and isoprenaline were then tested on each horn. The drugs were each left in the bath for 2 minutes. The preparations were then allowed to repolarize in Tyrode solution for 15–30 min, were depolarized again in KCl-Ringer and the procedures repeated in a cross-over arrangement, the first horn now being stimulated with Ba instead of Ca, and the second horn with Ca instead of Ba.

On every occasion, histamine caused relaxation of the Ca contracture but had no effect on the Ba contracture. The results from a typical experiment of this type are illustrated in Figure 4. These results suggest that not only is Ca necessary for the relaxant effect of histamine, but that Ba cannot substitute for Ca in this role. It also follows that histamine cannot be exerting its effect directly on the contractile elements since this would result in a relaxation of the Ba contracture as well as the Ca contracture. Some interference by histamine with the function of Ca is indicated.

While the β-adrenoceptor antagonist, propranolol, blocked the effect of isoprenaline but not that of histamine, burimamide blocked the effect of histamine but not that of isoprenaline (Figure 5).

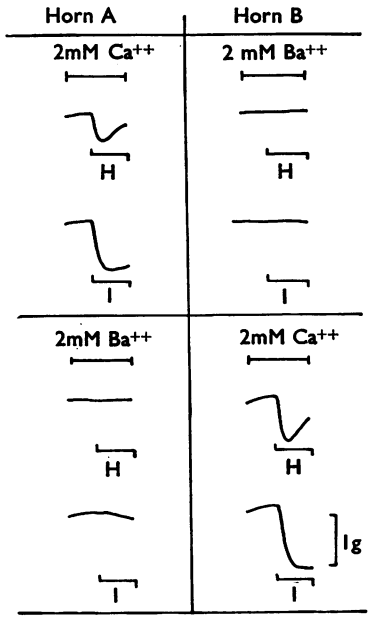

FIG. 4. The effects of 100 μg/ml histamine (H) and 10 ng/ml isoprenaline (I) on contractures induced by 2 mM Ca and 2 mM Ba in two horns of a rat uterus depolarized in KCl-Ringer containing 1 mM EGTA at 20° C. The histamine and isoprenaline were each left in the bath for 2 minutes. Both drugs produced relaxation during the Ca contractures but had no effect on the Ba-contractures.

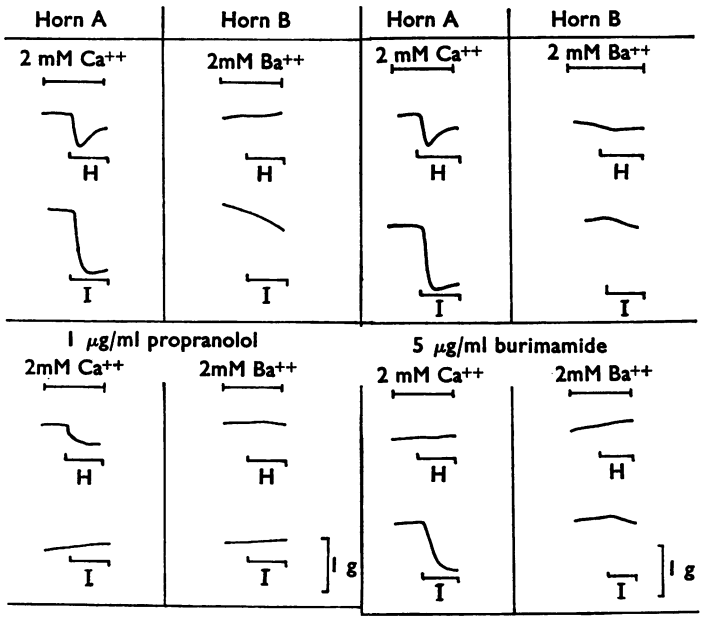

FIG. 5. Comparison of the effects of the β-adrenoceptor antagonist, propranolol with that of the H_2-receptor antagonist, burimamide. The left panel shows that propranolol (1 μg/ml) blocked the relaxant action of 10 ng/ml isoprenaline (I) on Ca-induced contracture in a rat uterus depolarized in KCl-Ringer containing 1 mM EGTA, at 22° C. The action of 100 μg/ml histamine (H) was unaffected. The right panel shows that burimamide (5 μg/ml) blocked the relaxant action of 40 μg/ml histamine (H) on Ca-induced contracture but not the action of 10 ng/ml isoprenaline (I).

Papaverine and nitrite (as KNO_2), two non-specific smooth muscle relaxants, were also tested on Ca and Ba contractures. Papaverine caused relaxation of both Ca and Ba contractures in a KCl-depolarized uterus, the effect with Ba being much less than that with Ca. Nitrite also produced relaxation in both Ca and Ba contractures, its effect being approximately the same in each case. Figure 6 demonstrates the differences between the effects of histamine, papaverine and nitrite in preparations from the same animal. It would seem that Ba, at least to some extent, can substitute for Ca in the relaxant actions of papaverine and nitrite but not in the action of histamine.

The effect of histamine on calcium exchange in the depolarized rat uterus

The addition of 1 mM Ca to the perfusing solution produced a marked increase in the rate of efflux of ^{45}Ca from the uterine horns as described by Feinstein (1966) and van Breemen *et al.* (1966).

In a double control experiment, in which neither horn was exposed to a drug, the rates of ^{45}Ca efflux from the two horns were the same.

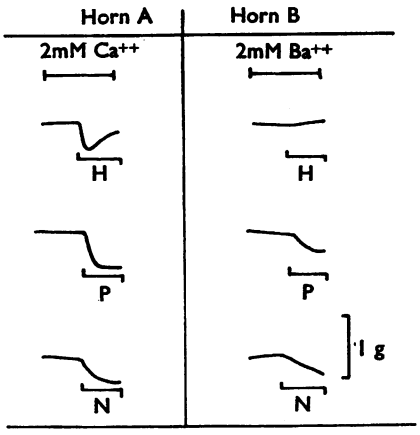

FIG. 6. A demonstration of the differences between the effects of 100 μg/ml histamine (H), 20 μg/ml papaverine (P) and 50 mM nitrite (N) on Ca- and Ba-induced contractures in a rat uterus depolarized in KCl-Ringer containing 1 mM EGTA, at 22° C. While histamine relaxed only the Ca contracture, papaverine and nitrite relaxed both the Ca and Ba contractures.

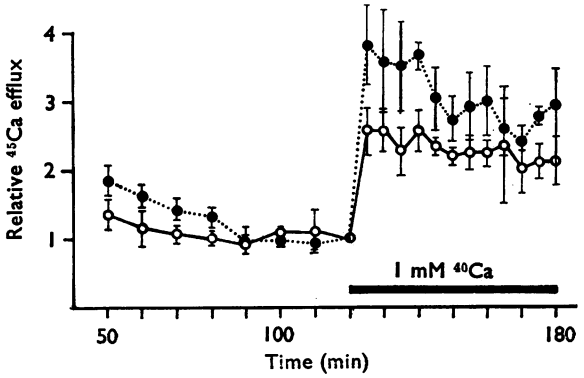

FIG. 7. Relative ^{45}Ca efflux from rat uterus. Means and standard errors from 4 experiments in which one horn (open circles) was exposed to 1 mg/ml (3·3 mM) histamine from 90 min onwards. The control horn (closed circles, broken line) was not exposed to the drug. A reduction in the rate of calcium exchange occurred in the horn exposed to histamine. All preparations were depolarized in K_2SO_4-Ringer at 21° C.

The rate of efflux remained unchanged during experiments in which one horn was exposed to a concentration of histamine of 50 μg/ml. This concentration of histamine would normally be sufficient to cause relaxation in a depolarized uterus.

A reduction in the rate of ^{45}Ca efflux was seen, however, when concentrations of 1 mg/ml (3·3 mM) histamine were used. Figure 7 shows the means and standard errors of data from four experiments in which one horn was exposed to 1 mg/ml histamine from 90 min onwards. This reduction in calcium exchange by histamine was as great as that produced by the equivalent molar concentration of tetracaine.

Discussion

The relaxant action of histamine on the isolated rat uterus has been shown to remain after the membrane is depolarized in high-potassium solution. It therefore

seems likely that the effect of histamine on the membrane (Blyth, 1972) is a result of an action which is independent of membrane polarization, although 'stabilization' of the membrane, that is suppression of action potential generation or conduction by non-specific inhibition of changes in membrane potential (Shanes, 1958), would alone explain the relaxant action in a normally-polarized uterus, since under physiological conditions, spike generation is necessary to maintain tension or initiate a contraction (Kuriyama, 1961).

Since the effects of histamine in both enhancing responses to acetylcholine and relaxing contractures to calcium are antagonized by burimamide, H_2-receptors (Black *et al.*, 1972) may be assumed to mediate the effects of histamine in a depolarized uterus as they do under normal conditions. In this respect, histamine resembles several other drugs whose effects remain in depolarized tissues and are mediated by the same receptors under both polarizing and depolarizing conditions (Schild, 1964).

Histamine cannot be said to competitively antagonize calcium in the rat uterus, but its action does seem to involve an interference at some stage with the normal function or movement of calcium. The effect of histamine was abolished by a calcium-chelating agent and restored by the addition of small quantities of calcium. The addition of barium, however, did not restore the effect. The possibility that histamine has a direct effect on the contractile elements is not supported by the results. Such an action would mean that histamine might be expected to relax contractures induced by barium in addition to those induced by calcium.

If the decline in the response of the uterus to acetylcholine is assumed to be due either directly (Edman & Schild, 1962; Feinstein, Paimre & Lee, 1968) or indirectly (Isozima & Bozler, 1963) to a progressive loss of intra-cellular calcium, the enhancing effect of histamine on these responses may be due to an action which prevents the loss of calcium from the cell. Since acetylcholine may cause a contraction by releasing bound calcium (Edman & Schild, 1962), histamine may exert its effect by increasing the amount of calcium bound within the cell or at the cell membrane. The effect of acetylcholine in mobilizing calcium from this store would then be improved.

The inhibitory effect of high concentrations of histamine on calcium exchange in the depolarized uterus supports this hypothesis. The difficulties of demonstrating the effects of drugs on calcium metabolism have been discussed by Lüllman (1970), who points out that only a small fraction of the cellular calcium may be involved in the action of a drug and that a change in this particular fraction may be masked by a much greater amount of calcium which does not contribute to the response. This situation appears to exist in the present study since, although low concentrations of histamine had no visible effect on calcium exchange, high concentrations (1 mg/ml) of the drug did cause a reduction in the process.

A decreased influx of calcium may be a result of increased binding of calcium at the membrane and would account for the antagonism of calcium-induced contractures, the 'stabilization' of the membrane potential, and the suppression of spikes, which, in the rat uterus, are thought to be due to the entry of calcium ions (Abe, 1968). The resulting fall in the concentration of free intracellular calcium would bring about relaxation.

This explanation of the action of histamine in the rat uterus is almost certainly a simplification of the processes actually taking place when the drug comes into contact with a rat uterine cell. One or more intermediate steps may occur before histamine causes a reduction in calcium exchange. Indeed, the reduction in calcium exchange may merely accompany some other action of histamine and may not be its principal mechanism in causing relaxation.

The likelihood of the immediate consequences of H_2-receptor stimulation in the rat uterus being the same as those at other H_2 sites is questionable, since a decreased calcium exchange and membrane 'stabilization' in gastric mucosa or guinea-pig atria (both H_2 sites) might be expected to oppose those actions normally exerted by histamine on these tissues, i.e. stimulation of acid secretion and increased cardiac contractility, respectively. However, it is possible that the relaxant action of histamine on some vascular smooth muscle, to which H_2-receptor stimulation may contribute (Black *et al.*, 1972), involves processes similar to those taking place in the rat uterus.

I am very grateful to Dr. J. W. Black for the generous gift of burimamide and to Hoechst Pharmaceuticals for the gift of Amethocaine.

REFERENCES

ABE, Y. (1968). The effect of sodium and calcium on the action potential of pregnant rat myometrium. *J. Physiol., Lond.*, **200**, 1–2*P*.

BLACK, J. W., DUNCAN, A. M., DURANT, C. J., GANELLIN, C. R. & PARSONS, E. M. (1972). Definition and antagonism of histamine H_2-receptors. *Nature, Lond.*, **236**, 385–390.

BLYTH, D. I. (1972). The effect of histamine on the electrical activity of rat uterus. *Br. J. Pharmac.*, **45**, 126–128.

BREEMEN, VAN C., DANIEL, E. E. & BREEMEN, VAN D. (1966). Calcium distribution and exchange in the rat uterus. *J. gen. Physiol.*, **49**, 1265–1298.

EDMAN, K. A. P. & SCHILD, H. O. (1962). The need for calcium in the contractile responses induced by acetylcholine and potassium in the rat uterus. *J. Physiol., Lond.*, **161**, 424–441.

EDMAN, K. A. P. & SCHILD, H. O. (1963). Calcium and the stimulant and inhibitory effects of adrenaline in depolarized smooth muscle. *J. Physiol., Lond.*, **169**, 404–411.

EVANS, D. H. L., SCHILD, H. O. & THESLEFF, S. (1958). Effects of drugs on depolarized plain muscle. *J. Physiol., Lond.*, **143**, 474–485.

FEINSTEIN, M. B. (1963). Inhibition of caffeine rigor and radioactive calcium movement by local anesthetics in frog sartorius muscle. *J. gen. Physiol.*, **47**, 151–172.

FEINSTEIN, M. B. (1966). Inhibition of contraction and calcium exchangeability in rat uterus by local anesthetics. *J. Pharmac. exp. Ther.*, **152**, 516–524.

KURIYAMA, H. (1961). In: *Recent studies on the electrophysiology of the uterus.* Ciba Foundation Study Group No. 9. pp. 51–71. London, Churchill.

FEINSTEIN, M. B., PAIMRE, M. & LEE, M. (1968). Effects of local anesthetics on excitation-coupling mechanisms. *Trans. N.Y. Acad. Sci.*, **30**, 1073–1081.

ISOZIMA, C. & BOZLER, E. (1963). Role of calcium in initiation of contraction in smooth muscle. *Amer. J. Physiol.*, **205**, 681–685.

LÜLLMAN, H. (1970). Calcium fluxes and calcium distribution in smooth muscle. In: *Smooth Muscle.* pp. 151–165. Ed. Bülbring, E., Brading, A. F., Jones, A. W. & Tomita, T. London, Arnold.

SCHILD, H. O. (1964). Calcium and the effects of drugs on depolarized smooth muscle. In: *Pharmacology of Smooth Muscle*, Proc. 2nd. International Pharmacological Meeting, **6**, 95–104, Ed. Bülbring, E. Pergamon, N.Y.

SCHILD, H. O. (1966). Calcium and the relaxant effect of isoproterenol in the depolarized rat uterus. *Pharm. Rev.*, **18**, 495–501.

SCHILD, H. O. (1967a). Need for calcium in isoprenaline-induced relaxation of the depolarized rat uterus. *Nature, Lond.*, **215**, 650–651.

SCHILD, H. O. (1967b). The action of isoprenaline in the depolarized rat uterus. *Br. J. Pharmac. Chemother.*, **31**, 578–592.

SHANES, A. M. (1958). Electrochemical aspects of physiological and pharmacological action in excitable cells. *Pharm. Rev.*, **10**, 59–273.

(*Received February 5*, 1973)