Wellcome Film Project

Histamine Receptors and Peptic Ulcer Disease
The Scientific Basis of Medicine

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Black-and-white
Duration: 00:37:17:15

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<**Opening titles**>

<**Professor J Black to camera**>

A new class of drugs, histamine H_2-receptor antagonists have been developed recently and their effects on gastric secretion in health and disease are again raising old questions about the role of histamine in gastric function. Professor Wyllie will summarise what we know about the effects in man of metiamide and cimetidine, which are the best known examples of these new drugs. And I will give a synopsis for the evidence of classification and a note of a few of their properties. Much of the work I will refer to, and wish to acknowledge, was done by a group at the SK&F Research Institute, particularly by Duncan and Ganellin, associated with Brimblecombe and Durant, Hesselbo, Parsons and the late Victor Smart.

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Before presenting the evidence for their pharmacological classification, I would like to give this problem some background.

The development of the first antihistamines was begun by Bovet and his colleagues in 1936. Ten years later, Lowe reviewed the pharmacological properties of the early compounds and subsequent drugs, typified by mepyramine and tripelennamine, have been found to fall into a similar pattern.

<**Black over table listing properties of antihistamine drugs**>

Here then is a sum of their properties. 1) Histamine contracts smooth muscle in many organs including bronchi, blood vessels, gut and uterus. Quite small doses of antihistamines can block these effects. 2) Histamine stimulates acid secretion from the mammalian stomach, stimulates cardiac pacemakers and inhibits the rat uterus. However, even massive doses of histamine antagonists fail to suppress these actions of histamine. 3) Other histamine responses, notably the fall in blood pressure and the skin weal and flare, could only be partially suppressed by even very large doses of antihistamines.

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Now, pharmacologists had met this phenomenon before; namely the phenomenon that drugs which otherwise seemed to be specific hormone antagonists simply failed to block all of the action of that hormone. Atropine didn't block the actions of acetylcholine at the neuromuscular junction. The ergot alkaloid or phenoxybenzamine could not block the actions of adrenaline or noradrenaline on the heart. A resolution of this kind of problem was proposed by Ahlquist when he established a pattern among the tissue responses to noradrenaline and its conjoiners, and saw how two populations of noradrenaline receptors, which he called alpha and beta, could explain his results.

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Phenoxybenzamine and related drugs were therefore classified as alpha-receptor antagonists and this paved the way for the discovery of selective beta-receptor antagonists.

<**Black over tables showing patterns of different hormone responses to various antihistamines**>

Look now at the pattern of noradrenaline responses more clearly: α-receptor mediated responses are usually very fast twitches of visceral muscle which can show rapid fade, whereas beta-receptor mediated responses, be they excitatory or inhibitory, usually take several minutes to reach a well-maintained plateau. The alpha-receptors can be selectively stimulated by agonists such as methoxamine, and isoprenaline was a selective agonist of beta-receptors. The alpha-receptor antagonists were chemically heterogeneous and had low specificity; the new β-receptor antagonists were all found to be conjoiners of isoprenaline and produced relatively specific blockade.

Compare what was known about histamine responses with this pattern, particularly look at the comparison between histamine responses which are sensitive, like guinea pig ileum, or refractory like guinea pig atrium to blockade bar mepyramine. Ileal muscle responds to histamine by a fast twitch which rapidly fades. Atrial muscle, exposed to histamine, gradually increases its frequency of beating and reaches a well-maintained plateau several minutes later. Similar slow but well-maintained responses were shown by a rat uterus and rat gastric secretion when exposed to pulses of histamine.

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This table shows the contrast. Further, pyridyl ethylamine had been found to be a selective agonist of mepyramine-sensitive responses and N alpha-methylated derivatives of histamine had been claimed to be especially active stimulants of gastric secretion. Like the alpha-receptor antagonists, the known antihistamines were

heterogeneous in a chemical sense and had relatively low specificity. For example, many showed anticholinergic and anti-adrenaline activity.

<**Black to camera**>

Now, by analogy with noradrenaline responses, this pattern suggested that the histamine receptors were probably not homogenous either, and that selective antagonists of histamine receptors, subserving its stimulant effect on gastric secretion and other tissues, ought to be discoverable. The clear message of this pattern was to make close analogues of histamine and then test them as inhibitors of gastric secretion.

Following the analogy of beta blockers which had the common isoprenaline side chain and varied aromatic ring systems, all the early chemical efforts were directed to making substitutions in the imidazole ring of histamine. However, no antagonists were found. Nevertheless, some of the simpler methylated histamines showed unusual agonist activity.

The 2-methylhistamine and the 4-methylhistamine derivatives were particularly interesting. Their activity as agonists expressed as a percentage of histamine's activity was estimated on a number of tissues – some on isolated tissues in vitro and some in whole animals, some on guinea pig tissues and some on rat tissues. Nevertheless, a clear pattern emerged.

<**Black over table showing patterns of methylhistamine**>

1) Using mepyramine-sensitive tissues, namely guinea pig ileum contraction in vitro or rat stomach contraction in vivo, 4-methlyhistamine was found to have very little histamine-like activity, whereas 2-methylhistamine had about a fifth of the agonist activity of histamine. 2) Using mepyramine refractory responses, the frequency of beating of guinea pig atria in vitro, the inhibition of rat uterus in vitro or the stimulation of rat gastric secretion in vivo, 4-methylhistamine was found to have between 30 and 40% of the activity of histamine as an agonist, whereas 2-methylhistamine had now a

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very low order of activity. 3) Other agonists, such as the 1,2,4-triazole analogue of histamine did not show this selective agonist activity.

<**Black to camera, then over illustration showing chemical properties of various antihistamines**>

Remember that Ahlquist had used a similar pattern to establish his dual receptor hypothesis and you will realise that these results were very encouraging indeed. Fortunately, antagonists were eventually found following the tantalisingly tenuous lead that the guanyl derivative of histamine showed. In addition to its histamine-like agonist activity, guanyl histamine also was found to have some weak antagonist activity which could just be seen when it was injected into rats during a plateau of maximum histamine-stimulated acid secretion.

Here is this lead compound, the guanyl analogue of histamine, and subsequent modification of this molecule led, by lengthening of the side chain and by replacing the guanidine with thiourea, led to burimamide. Later, modifications produced metiamide in which a sulphur is introduced into the side chain and a methyl group into the imidazole ring and the compound now under active development, cimetidine, replaces the sulphur of thiourea with a cyanoamino group.

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<**Black to camera, alternating with series of graphs showing properties of different drugs**>

From the point of view of the subsequent analysis, metiamide and cimetidine differ little in pharmacological activity but show important differences in toxicity. Let's look now at some of the properties of these drugs.

The main screening test dealt with continuous sub maximal histamine-stimulated gastric acid secretion in anaesthetised rats. When an active drug, such as metiamide, was injected during a plateau of secretion, prompt inhibition of the

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secretion was produced. Here is a typical kind of record and the plateau is inhibited by the intravenous injection of metiamide. Now, you can imagine how exciting it was to find that burimamide and metiamide not only inhibited this effect of histamine on acid secretion but also inhibited the effects of histamine on increasing atrial frequency and on inhibiting the uterus of the rat.

On the other hand, mepyramine-sensitive contractions of visceral muscle, such as the contraction of guinea ileum in vitro, were not antagonised by these drugs – the pattern seemed to be complete. But was it? A plateau of acid secretion in the rat preparation could also be produced by continuous intravenous infusion of either pentagastrin or carbachol. Metiamide was found able to inhibit pentagastrin-stimulated secretion as easily as it had inhibited histamine. This result has been confirmed in many laboratories and in many species.

However, metiamide failed to inhibit carbachol-stimulated secretion; although this result has by no means been confirmed in other species or in other laboratories, nevertheless, in the original experiments, the result indicated that metiamide was not simply depressing the secretory capacity of gastric glands. Did the inhibition of pentagastrin by metiamide mean, then, that this drug was a non-specific inhibitor of secretion?

Well, there was a hypothesis, originated by MacIntosh and developed by Carlson and his colleagues, which was illuminating on this point. In their hypothesis, feeding acting directly on the antrum, or indirectly via the vagus, is seen to release the hormone gastrin; gastrin is believed not to stimulate acid secretion directly, but indirectly by releasing histamine from special histamine-secreting cells located in the vicinity of the parietal cells. Note that in this hypothesis, a direct action of acetylcholine on the parietal cells is allowed for.

Recent results with isolated cat gastric mucous membrane, secreting in vitro, are in apparent conformity with this model. Here is metiamide, inhibiting histamine and inhibiting pentagastrin to the same extent; whereas secretion stimulated by acetylcholine is untouched.

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However, there is also much evidence against this two-cell hypothesis. To decide whether the anti-gastrin actions of these antagonists is due to blockade of histamine receptors or due to some other action, more demanding criteria for the classification is needed. Fortunately, pharmacological tests can help. We need to know if metiamide is acting on the same side as histamine, that is, if it is a simple competitive antagonist and, if so, we want to estimate a characteristic equilibrium constant for this interaction. Then if the same dissociation constant is estimated on two different tissues, the same type of receptors can be assumed to be involved.

The pharmacological tests for competition and the estimation of equilibrium constants are based on the chemical interpretation of those response curves. If the interaction between pools of agonist molecules and corresponding receptors is occurring reversibly on the basis of the mass action laws, then the fraction of receptors theoretically occupied by agonist molecules, at any concentration, would be described by a familiar symmetrical sigmoid log-concentration fractional occupancy curve. However, non-specific binding, penetration into other compartments, inactivation and non-linear relations between receptor occupancy and response mean that measured dose-response curves cannot be interpreted in chemical terms.

However, if a simple competitive antagonist interferes with agonist receptor occupation and alters none of those factors which transmute a theoretical concentration occupancy curve into a real dose-response curve, then those dose-response curves will be displaced to the same extent as the theoretical curves. Here is the displacement of the measured dose-response curves, and here is the displacement of the underlying theoretical concentration occupancy curves. This displacement is referred to as the dose ratio.

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<**Black to camera, then over series of graphs and tables charting dose ratio quantities, very occasionally to camera in between**>

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Now, this assumption provides the pharmacological tests for simple competitive antagonism, and these tests are three. 1) That there should be parallel dextral displacement of log dose-response curves. 2) That the relationship described by the Schilt equation – namely, the plot of log dose ratio minus 1 against the logarithm of the antagonist concentration, should be linear and should have a slope of 1 and 3; this relationship should be independent of the affinity of the agonist being used for its receptors.

What did we find then? Metiamide was found to produce parallel dextral displacement of histamine dose-response curves on isolated guinea pig atria. And the relationship between the extent of the displacement of these curves and the concentration of antagonists is shown in these Schilt plots. Note that the logarithm of the molar concentration of metiamide is plotted against the displacement of the curves as estimated by the logarithm of the dose-ratio minus 1. Each point, on this curve, is a single estimate of dose-ratio, the overall relationship is linear over a 1000-fold range of antagonist concentration, and the slope is not significantly different from 1.

At the point where the dose-ratio is 2 and the log DR minus 1 equals nothing, we can make an estimate of the dissociation constant of the metiamide histamine receptor complex, and here it is, the dissociation constant being approximately 1 micromolar. This concentration can be conveniently expressed in logarithmic mode and given the symbol pA_2, as suggested by Schilt, and on analogy with pH. Now, we can use the pA_2s for metiamide and examine other agonists on other tissues.

This next table shows that pA_2 measurements with metiamide are independent of the relative activity of the agonists used. Note also that tolazoline, an imidazoline derivative, more usually classified as a noradrenaline α-receptor antagonist, also competes with metiamide for histamine receptors in the heart. However, this table shows the more important information – that the pA_2 is independent of the tissue used, that is, the receptors subserving these quite different responses appear to be homogeneous.

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Previously, Ashe and Schilt had made similar measurements using mepyramine and the mepyramine-sensitive tissues in the trachea and in the gut. This table shows the pA_2 values Schilt and his colleagues found. The pA_2 on guinea pig ileum was found to be fairly independent of the relative activity of three different histamine-like agonists. But more important, the pA_2 was found to be independent of the tissue which it was measured on, namely guinea pig ileum, guinea pig trachea or human trachea. On this basis, Ashe and Schilt proposed that these receptors were homogeneous and suggested the notation H_1- receptor to describe them.

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<**White over table listing histamine receptors, then to camera**>

By extension, with this work we have suggested that the homogeneous receptors subserving acid secretion, cardiac stimulation and uterine relaxation should be classified as H_2-receptors. Note in this table some additions from the early one I showed. Leukocytes are now shown, leukocytes both as far as basophils and as lymphocytes are concerned, are shown containing H_2-receptors. And neurones are shown here along with weals and hypotension as containing or involving both H_2- and H_1-receptors.

Now, in all our studies so far, in animals, truly the only pharmacological effects we see, when doses of these drugs are given, are changes in evoked gastric secretion. We're learning then, I think, two things about these drugs. One is that by their water solubility they probably penetrate very little into the brain and, hence, don't involve histamine receptors there. And secondly, perhaps the histamine receptors in other tissues, in leukocytes or on peripheral nerves normally play only a sub-dominant physical role.

Anyway, after extensive toxicity tests, these drugs were then taken into human study and Professor Wyllie will now describe what was found.

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<**Professor J Wyllie to camera**>

Professor Black has described to you how histamine H_2- receptors were discovered and how specific antagonists were invented, but he has not answered the all-important question whether such experiments provide a scientific basis for a new medicine.

I hope to show you that they do, by referring to two things. First, gastric secretion. I shall show you, by reference to experiments in man, how the results from animals were duplicated, speaking about gastric secretion stimulated by histamine, pentagastrin and insulin. And secondly, I shall talk about the effects of H_2 blockade in patients with duodenal ulcer.

Let us first consider histamine-stimulated gastric acid secretion in man.

<**Wyllie over graph showing histamine-stimulated gastric acid secretion in man, then back to camera**>

This graph shows gastric acid secretion here, in millimoles per 15 minutes, first under basal conditions and then during a continuous infusion of histamine at 40 micrograms per kilogram per hour. This produced secretion at a maximal rate, with a steady plateau level, there. These are the results from 5 subjects. Now, in exactly similar experiments in 9 subjects, an infusion of the H_2-antagonist, metiamide, was superimposed on part of the histamine infusion – these are the metiamide levels there, you can see that inhibition of gastric acid secretion occurred right down to very low levels.

We conclude from this that in man, as in animals, H_2-receptor blockade suppresses histamine-stimulated gastric acid secretion. We may say that's very interesting but would it be of any practical importance? Pathological states in which excessive stimulation by histamine on the main lesion are not well defined.

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Well, one case of systemic mastocytosis has been described with hyper-histaminaemia and duodenal ulceration, and the important point, H_2 blockade suppressed the levels of gastric secretion. Furthermore, Lorenz has measured elevated plasma histamine levels in a variety of circumstances including during operations, and he has speculated that following major trauma, raised histamine blood levels are responsible for the troublesome upper gastrointestinal haemorrhage which sometimes occurs. It is therefore interesting that preliminary reports indicate that bleeding under these circumstances can be controlled by H_2 blockade.

Now my next point: gastrin-stimulated gastric acid secretion. Whether or not we believe that histamine is indicated in pathological or physiological stimulation of gastric secretion in man, we cannot deny that gastrin is important. So we would like a way of inhibiting gastrin-stimulated secretion.

<**Wyllie over graph detailing methods of inhibiting gastrin-stimulated secretion, then to camera**>

It was most gratifying to find that H_2 blockade did just that. Here, I show acid secretion, first basal, then stimulated with histamine and then inhibited with metiamide. This is, in fact, a compression of the results in the last slide. And over here the results of exactly similar experiments in which the stimulus was pentagastrin – you see basal secretion, stimulated secretion and then inhibited secretion.

An identical inhibition occurred with pentagastrin as occurred with histamine. And from this we may conclude that gastric secretion in man is blocked by H_2-receptor antagonists. The first hint that this might be of practical importance was found from the following experiment.

<**Wyllie over graph showing importance of previous results, then to camera**>

The patient was a young man with Zollinger-Ellison syndrome due to G-cell hypoplasia, as described by Pierce. You can see that his basal output was about 20 millimoles per hour, which is very high, and that further stimulation occurred under

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pentagastrin infusion, up to about 60 millimoles per hour. When an infusion of metiamide was given (here are the blood levels of metiamide), acid secretion was secreted, right down to a very low level.

He was, in fact, the first patient ever to have a prolonged treatment with a H_2-receptor antagonist. The pain of his ulceration was dramatically relieved and within 3 weeks the ulcers had partially healed. We now know that cimetidine is effective in all, or nearly all, cases of Zollinger-Ellison syndrome. It relieves symptoms, it reduces the abnormally raised gastric acid secretion, it stops the diarrhoea and it heals the ulcers. Moreover, if drug treatment is maintained, then the effects of H_2 blockade seem to continue indefinitely.

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<**Wyllie to camera, then over graph showing results on H_2 blockade and vagal stimulation**>

So much for a rather rare pathological state. But we have long believed that human gastric secretion depends partly on gastrin and partly on vagal stimulation. Does H_2 blockade inhibit vagally stimulated secretion? Yes, it does. The best demonstration of this is the following experiment by Carter in Edinburgh in which volunteers have received an insulin infusion which produced hypoglycaemia which resulted in an efferent discharge down the vagus nerves and so, in this secretion of gastric acid, we've a fairly well-maintained plateau in the latter part of the experiment.

When, in exactly similar experiments in the same volunteers, an infusion of cimetidine was given, as shown here, gastric acid secretion started as before and then rapidly died away to almost zero.

<**Wyllie to camera**>

Perhaps this means that the vagus nerve stimulates secretion in man in this type of experiment mainly by releasing gastrin. The implication of these experiments with

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histamine, gastrin and insulin hypoglycaemia is that food-stimulated secretion in man should be inhibited by H_2 blockade. And this is, indeed, the case as has been shown by Fortran and Walsh and by Misiewicz, Pounder, Milton, Thomson and their colleagues. For simplicity I have spoken only about gastric acid secretion but I should add that pepsin secretion is also inhibited.

And now we come to the really critical question. Taking patients with duodenal ulceration who, we know, have got elevated gastric acid and pepsin secretion above the normal values – shall we be able to cure them by H_2 blockade? The patient simply wants to have his pain relieved. The surgeon or physician cannot see the pain and may have difficulty in assessing it but he can see the ulcer by passing a fibre optic endoscope into the duodenum. And so we have two questions: what happens to ulcer pain and what happens to the duodenal ulcer itself when H_2 blockade is instituted?

<**Wyllie over graph showing the results of a trial of metiamide to treat duodenal ulcer, then a table showing the rates of ulcer healing in a similar trial** >

And this next slide shows the effect of metiamide in the first double-blind trial of this type; there have been many similar ones since with similar results. Patients were randomly allocated, either to have a placebo or to have one of two doses of metiamide. They kept records which showed how frequently they had pain and the slide simply shows that taking dummy tablets for a month produced no change in the incidence of pain, whereas taking either dose of metiamide produced a progressive diminution in the frequency of symptoms. And you will notice that the improvement was gradual. This is a feature of the response to H_2 blockade – the patients usually have to take the drug for several days before observing any benefit; thereafter improvement is rapidly progressive.

Relief of pain was associated with ulcer healing and on the next slide I show the ulcer healing rate in the first 104 patients treated with H_2 blockade in double-blind

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trials against a placebo. Healing was assessed endoscopically and unless the ulcer healed completely it was regarded as not having healed.

<**Wyllie to camera**>

Superiority of the treatment group over the placebo group is significant beyond any reasonable doubt so we can say the question I began with is answered; Professor Black's scientific experiments have resulted in a new medicine. But this is not the end of the story.

Many new problems arise, for example, you may say what about the patient whose ulcers did not heal in those trials? Perhaps they received an inadequate dose of drug? I should explain that the doses of H_2-receptor antagonists, selected for use in these trials, were based on experiments just like the ones I have shown you just now. One made estimates of the blood level of H_2-receptor antagonists to produce at least 50% inhibition of maximally stimulated acid secretion. And then one studied blood levels following oral administration of the drugs.

<**Wyllie over tables showing effects of cimetidine on ulcer healing rate, to camera in between**>

And the next slide shows a typical result from a starved subject receiving cimetidine; blood concentration of cimetidine rose rapidly to a peak at about 1 hour and then declined approximately exponentially with a half time of about 110 minutes. From such considerations one estimated that about 1 gram a day of cimetidine, given in 4 divided doses, would probably give satisfactory suppression of secretion. Indeed, extensive studies by Pounder, Misiewicz and his colleagues have confirmed that these estimates were, indeed, reasonable.

And yet not all the ulcers heal in 4 to 6 weeks, this next slide shows the ulcer healing rate, here, and plus or minus 95% confidence limits for nearly 200 patients who took cimetidine and for 94 patients who were on a zero dose, that is the placebo. You can

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see that increasing the cimetidine dose from about 1 gram a day do about 2 grams a day produced little, if any, improvement in the results.

Many possible explanations for this come to mind. One possibility that has been discussed is that there is individual variation, perhaps related to the original size of the ulcer. Another is that 4 to 6 weeks treatment may simply not be enough for some patients. Northfield has shown that an increase in the healing rate occurs at least up to 12 weeks of treatment. Moreover, in some cases failure of treatment was undoubtedly due to failure of the patient to take the prescribed medicine.

After short courses of treatment, say 4 to 6 weeks, if the drug is stopped many patients soon relapse. But evidence is accumulating that this can be prevented by prescribing a single dose, a low dose, of the drug to be taken at night. No doubt we still have a lot to learn about the best way to prescribe H_2-receptor antagonists, and there have been other problems.

Metiamide seems to have caused a few cases of reversible granulocytopenia and for this reason it has been superseded by cimetidine which, fortunately, seems to be remarkably non-toxic.

In conclusion, histamine H_2-receptor blockade with cimetidine enables chronic duodenal ulcers to heal and affords complete relief of symptoms in the great majority of patients. Perhaps there are unforeseen difficulties ahead, but I think that cimetidine is destined to play an important role in the treatment of duodenal ulceration.

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