DRUGS FROM EMASCULATED HORMONES: THE PRINCIPLES OF SYNTOPIC ANTAGONISM

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by

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In this lecture I want to give an outline of the early stages in the discovery of adrenaline β-receptor antagonists and of the histamine H_2-receptor antagonists. I will end with a brief personal view about future research.

Adrenaline P-receptor antagonists

The work that is the theme of this lecture began in the early summer of 1958 when I joined Imperial Chemical Industries' Pharmaceuticals Division. I had gone there to pursue a very clear project that had been developing in my mind for several years. The idea had clinical, therapeutic, physiological and pharmacological elements.

Clinically, angina pectoris was known to be precipitated by anxiety and emotion just as well as by exercise. Indeed, the initiation of pain by an injection of adrenaline had been used as a diagnostic test. Partial thyroidectomy had been found to relieve severe angina pectoris whether or not associated with hyperthyroidism. At that time, tachycardia seemed to me to be the connecting link in these disorders.

Therapeutically, nitroglycerine could quickly relieve an attack of angina. Nitroglycerine also produced facial flush and headache. The relief of angina was attributed to similar vasodilatation in the coronary arteries. However, the newer, synthetic, selective coronary vasodilators, such as dipyridamole, were clinically ineffective despite the enhanced coronary artery dilatation that they provided. Here was a question mark against the widely practiced industrial strategy of seeking better drugs to increase coronary blood flow for angina.

Physiologically, Smith and Lawson (1958) had found that hyperbaric oxygen, at two atmospheres pressure, reduced the incidence of ventricular fibrillation associated with occlusion of a coronary artery even although the oxygen carrying capacity of the blood had increased by a maximum of only 25%. Might not an equivalently small *decrease* in the myocardial demand for oxygen be just as effective? That was my question.

Myocardial oxygen consumption is determined by the work of the heart and is a function of arterial blood pressure and heart rate. Lowering blood

pressure by systemic vasodilatation might dangerously reduce the perfusion pressure and blood flow through disease-narrowed coronary arteries. Indeed, hypotension was known to be able to induce a heart attack. Heart rate, on the other hand, is largely determined by the cardiac autonomic nervous system. Heart rate would thus be reduced by cardiac sympathetic blockade. In addition, there was much discussion in those days about a postulated "anoxiating" action of adrenaline, proposing that the price of rapidly increasing cardiac power was a decrease in cardiac metabolic efficiency.

These clinical, therapeutic and physiological features of hearts coping with coronary artery disease all seemed to point to the potential advantage of annulling the actions of the sympathetic hormones, noradrenaline and adrenaline, on the heart.

Pharmacologically, the anti-adrenaline drugs were a well-recognised class in 1958. All of them showed a pattern of actions similar to those seen by Dale (1906) with the ergot alkaloids. Characteristically, they reversed the blood pressure rise produced by adrenaline to a fall in pressure, but they did not suppress the associated tachycardia. Konzett (1940) had shown that isoprenaline, the purely synthetic isopropyl derivative of noradrenaline, produced only the actions such as tachycardia, vasodilatation and bronchodilatation which the antiadrenaline drugs were not able to suppress. These were the actions of isoprenaline that Ahlquist (1948) could not explain on the basis of Cannon and Rosenblueth's (1939) prevailing hypothesis involving sympathins E and I. Ahlquist went on to propose that the widespread physiological effects of adrenaline were mediated by two classes of receptors, α and β. In this new classification, the antiadrenaline drugs of the day were a-receptor antagonists, and isoprenaline was a selective stimulant of β–receptors.

So I started at I.C.I. with a clear goal — I wanted to find a β-receptor antagonist. I expected this to reduce pulse rates at rest and during exercise and hoped that it would decrease the susceptibility of patients to angina pectoris. The unknown factor for me at that time was the significance of adrenaline's "anoxiating" activity.

John Stephenson was the medicinal chemist assigned to work with me. As no compounds were known to annul the actions of adrenaline on the heart, the programme had to be cold-started. The structure of isoprenaline (Fig. l), the selective Preceptor stimulant, was our only clue. We thought that if N-substitution of adrenaline with isopropyl produced a selective *agonist*, then perhaps substitution with a different, larger, group might produce a selective *antagonist*. We thought that symmetrical, doubled-up, analogues of isoprenaline and dibenzylethylamines, might be interesting targets.

We were making compounds and testing them, admittedly without success when, early in 1959, we read Powell and Slater's (1958) report about the properties of DCI, an analogue of isoprenaline in which the ring hydroxyl groups were replaced by chlorine atoms (Fig. 1). In trying to

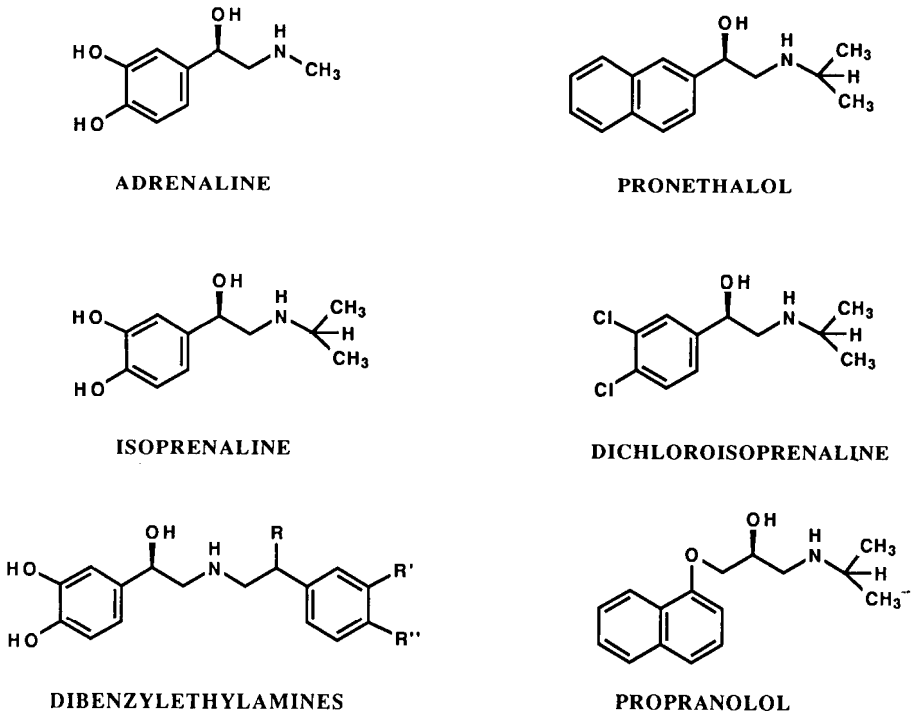


Figure 1. Chemical structures of adrenaline-related compounds.

exploit the bronchodilator properties of isoprenaline by making a long-acting variant, the Lilly group had discovered a compound that intrigued them by displaying — instead of isoprenaline's bronchodilator activity — the opposite property, namely antagonism. Soon afterwards, Moran and Perkins (1958) reported that DC1 could annul the inotropic effects of adrenaline on the heart and classified DC1 as a P-receptor antagonist. Stephenson immediately made some DC1 for us to test.

We had started our bioassays using the classical Langendorff preparation, the isolated, spontaneously beating guinea-pig heart. Isoprenaline is a powerful stimulant of both the rate and force of beating in this preparation, but we measured the amplitude of contractions which compounded both changes. In this system DC1 turned out to be as powerful a stimulant as isoprenaline and so was not at all what we were looking for. We had also developed a technique for simultaneously recording blood pressure and heart rate, in analogue form, in anaesthetised animals. Potential antagonists could now be given economically by slow intravenous infusion, and this allowed the effect on a wide range of systems to be monitored. Here, too, the powerful stimulant effects of DC1 on heart rate were clearly seen, although there was less hypotension due to vasodilatation than we had expected (Fig. 2). On the basis of these experiments, we decided that DC1 was not the lead we needed.

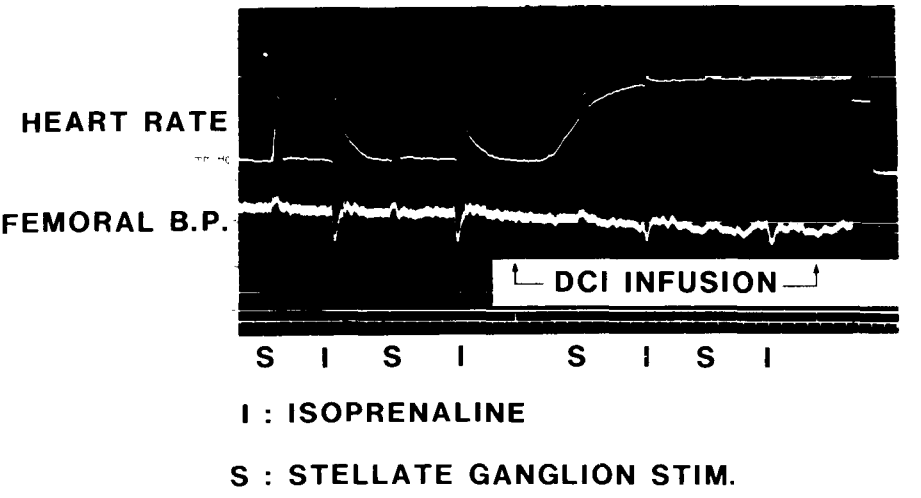
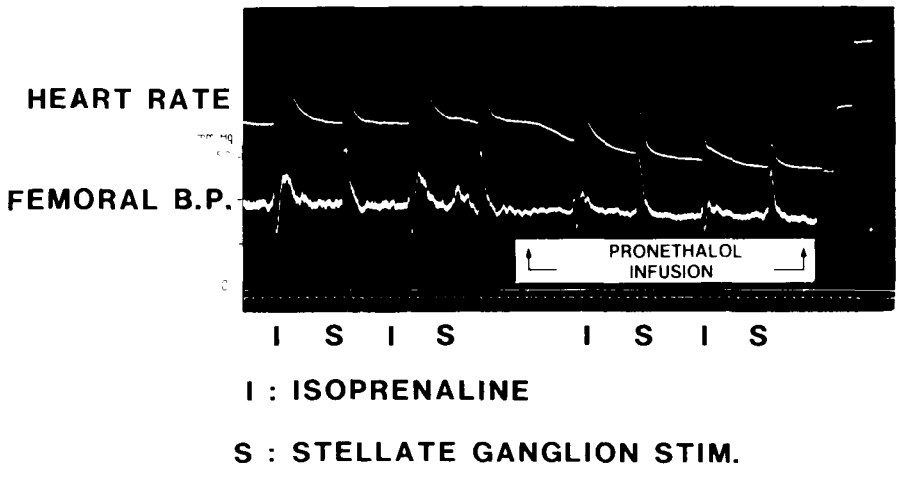


Figure 2. **Effects of DC1 (100μg/kg/min) (above) and pronethalol (l00μg/kg/min) (below) infusion upon heart rate and blood pressure responses to injections of isoprenaline (0.4μg/kg i.v.) and to sympathetic stimulation (square wave pulses, 10 ms duration, 2.5 volts, 15 pulses/s for 30s) in anaesthetised cats. Lower axis shows time markers in one minute intervals.**



As analytical pharmacologists, what we are allowed to see of a new molecule's properties is totally dependent on the techniques of bioassay we use. The prismatic qualities of an assay distort our view in obscure ways and degrees. Our only defence lies in restless improvement in technique and experimental design, in the hope that collimation of several techniques will improve the reliability of our vision. We would make the change self-consciously today, but then it was intuitive.

We developed a new in vitro assay based on guinea-pig cardiac papillary muscles as a way of measuring the contractile effects of isoprenaline independently of rate changes. Then, we reassessed many early compounds, including DCI. On the new preparation, DCI had no stimulant activities

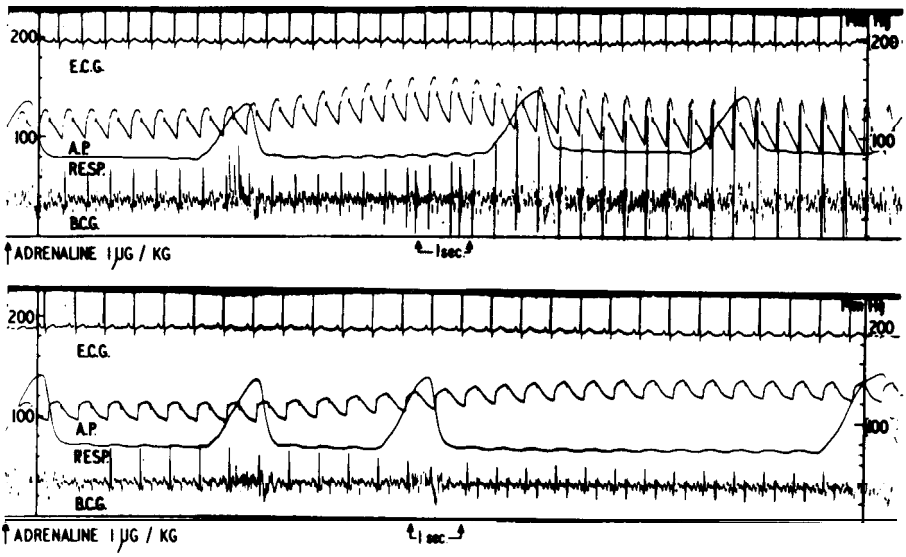


Figure 3. **Effects of 38,174 (pronethalol, 5mg/Kg i.v.) on cardiac and respiratory.. responses to adrenaline (1µg/Kg i.v.) in anaesthetised dogs. E.C.G.: electrocardiogram; A.P.: aortic blood pressure; RESP: respiration; B.C.G.: ballistocardiogram.**

itself but simply antagonised the effects of adrenaline and isoprenaline, although the stimulant activity on pacemaker tissue could be clearly seen in the atria1 preparation. We were astonished. Today, we classify DCI as a partial agonist. Ariens (1954) and R.P. Stephenson (1956) had introduced the concept of partial agonists a few years earlier but nothing in their writing, as far as I recall, alerted us to expect that the agonist activity of these compounds could be so tissue dependent.

I shall never forget John Stephenson's reaction to this discovery: "We'll make the naphthyl analogue of isoprenaline" (Fig. 1). He had realised immediately that while a fused benzene ring would have similar steric and electronic properties to the two chlorine atoms, there was also the possible advantage of extended π-bonding. Compound ICI 38 174 — nicknamed nethalide for a time, but finally christened pronethalol — was conceived in excitement and thrilled us at its birth. Pronethalol was an antagonist without any sign of agonist activity in both atrial and ventricular tissues. In anaesthetized animals, pronethalol reduced the resting heart rate and depressed the increments from isoprenaline or stimulation of cardiac sympathetic nerves (Fig. 2).

Having got over the first hurdle much more easily than I had dared to imagine, I was impatient to tackle the next one. How would someone, restricted by P-receptor blockade, cope with a surge of adrenaline or a burst of exercise? I had always imagined that the combination of Starling's "Law of the Heart" and the buffering capacity of the arterio-venous oxygen difference ought to be able to take up the slack of a reduction in cardiac output. We had developed the non-invasive technique of acceleration ballistocardiography to estimate the force of cardiac contractions in anaesthetised dogs (Fig. 3). Adrenaline increased heart rate, aortic blood pressure

and force of contractions. After pronethalol, basal heart rate and force were reduced and the effects of adrenaline were abolished. However, the vasodilator effects of adrenaline were also blocked, thus exposing the heart to a vasoconstrictor load mediated by the unblocked α-receptors. The heart was able to maintain its output and produced an enhanced rise in blood pressure. This was the experiment that convinced me that the new compound might be more than a laboratory curiosity. In fact, I did notice in these early experiments, that the cardiac ballistic action was reduced under load. I noticed also that the time taken from ventricular excitation to the opening of the aortic valves — that is from the R-wave to the upstroke of aortic pressure — was increased under load. These were tell-tale signs that the cardiac reserve was reduced, but I persuaded myself at the time that this was a reasonable price to pay for the possibility of increasing the work capacity of a heart with restricted coronary flow.

The early clinical studies seemed to confirm that judgement. Dornhorst and Robinson (1962) studied the interaction between pronethalol and isoprenaline in healthy volunteers. Isoprenaline, infused into the brachial artery, produced a large increase in forearm blood flow. However, when repeated after an intra-arterial infusion of pronethalol, the first route of administration into man, the vasodilator effect was abolished. Isoprenaline given by slow intravenous infusion increased heart rate, respiratory amplitude, arterial pulse pressures and forearm blood flow. The subjects in these studies often seemed to get a fear of impending doom and became visibly restless. After pronethalol, all of these effects of isoprenaline were suppressed (Fig. 4). By chance, an athlete and a loafer were the first pair to

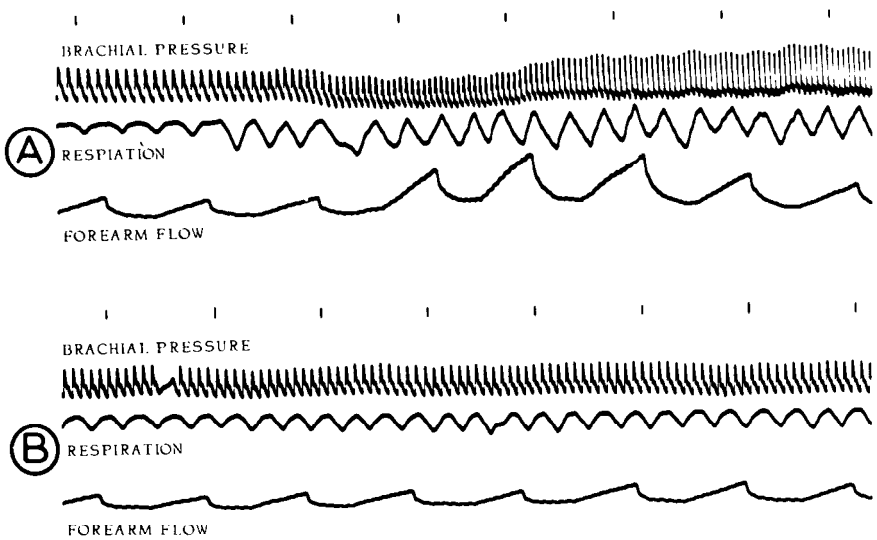


Figure 4. **Effects of intravenous infusion of isoprenaline (10μg/min), (A) before and (B) after intravenous infusion of pronethalol (110mg) upon arterial blood pressure, respiration and forearm blood flow in a healthy volunteer (reproduced by kind permission from the** Lancet**). Time intervals: 10 seconds.**

do maximal exercise after pronethalol. Compared to the control run, the athlete's heart rate at rest and exercise were little changed and his capacity to work was reduced. The loafer's heart rate was substantially reduced by pronethalol at rest and during exercise. He was less distressed by his lower heart rate. The potential benefits of β-adrenoceptor blockade for people with embarrassed hearts was also seen in the first patient with angina of effort. After pronethalol he was able to do more work before the onset of pain forced him to stop when his heart rate had eventually reached the same level as in the control run (Fig. 5).

Pronethalol always seemed to us to be a prototype drug, good enough to answer questions of principle, but not good enough to be marketable. So a large chemical group, directed by Crowther (Black et al. 1964), was assembled to try to find a more active, safer replacement for pronethalol. The discovery of ICI 45520, propranolol, a naphthyloxy propanolamine derivative, was the result (Fig. 1).

Our bioassays which had been developed as qualitative screens had now to be adapted for comparative quantitative bioassay. The isolated spontaneously-beating guinea-pig right atrial preparation proved to be excellent for

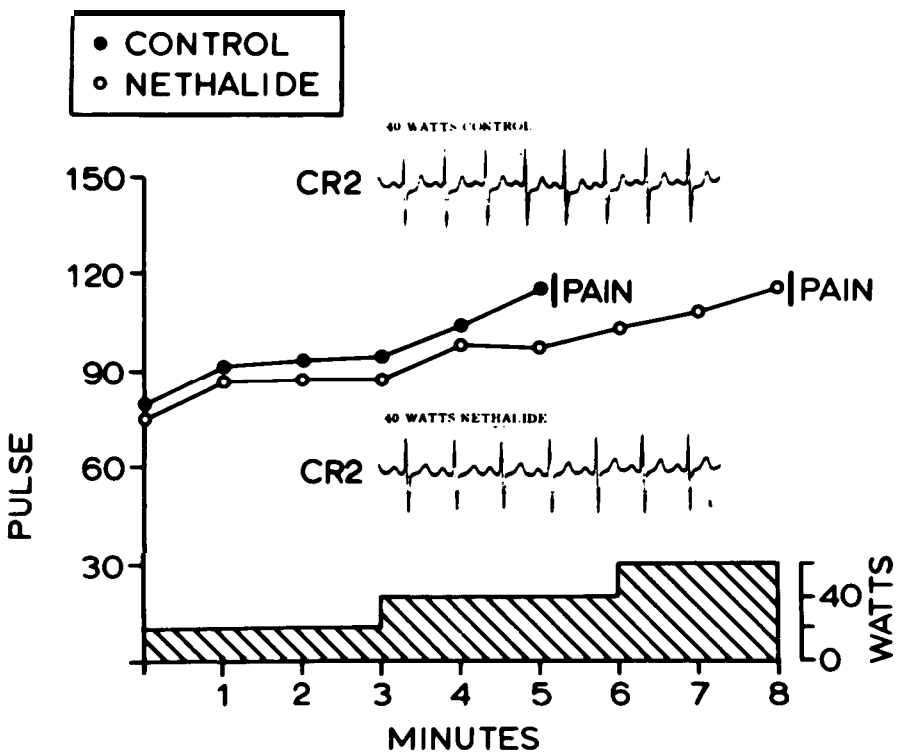


Figure 5. **Effect of nethalide (pronethalol, 250 mg p.o.) upon time taken and work exerted before the onset of chest pain in a patient with angina pectoris performing graded exercise on a bicycle. Inserts show the ECG before and after nethalide (reproduced by kind permission from the Lancet).**

these assays. The nature of the surmountable antagonism by propranolol was analysed by relating the rightward displacement of cumulatively derived dose-response curves to the concentration of antagonist. The linearity and slope of the Schild plot relating these variables indicated the likelihood that adrenaline and propranolol were competing for the same sites. This is the evidence that propranolol can be classified as a *syntopic* antagonist to the native hormones which activate β-adrenoceptors. Note here that I follow A. J. Clark (1933) in using the term 'hormone' very broadly: when one cell secretes a chemical to which another responds physiologically, I define that chemical as a hormone.

Histamine H_2-receptor antagonists

In 1964, I went to Smith, Kline & French Laboratories Ltd. to pursue another project that I had been thinking about for some time. Again, the idea had clinical, therapeutic, physiological and pharmacological implications.

The clinical problem was gastric and duodenal ulcers. I had thought a lot about the problem when I worked with Adam Smith (1953) on the effects of 5-hydroxytryptamine on gastric secretion. The immediate cause of ulceration was recognised to be hyperscretion of acid but the nature of the driving stimulus was unknown. The one clear fact was that patients with duodenal ulcers gave an exaggerated secretory response to histamine, the basis of a diagnostic test.

The therapeutic problem was that only surgical intervention, partial gastrectomy in those days, was recognized to be effective. The potential value of anticholinergic drugs, like atropine, was obscured by unacceptable side-effects. Antacids could be shown to promote ulcer healing, but only with clinically-unacceptable regimens.

The physiological problem was the relationship between gastrin and histamine, both of them powerful stimulants of acid secretion and both synthesized in the mucous membrane of the stomach. MacIntosh (1938) had proposed that histamine was the final stimulant of secretion when the vagus was stimulated and both Code (1965) and Kahlson (Rosengren and Kahlson, 1972) had extended that idea to gastrin as well, making histamine the final *common* chemostimulant. Mainstream thinking in gastroenterology, however, regarded gastrin as the *direct* hormone of secretion in its own right, thus, the question of the function of histamine in the stomach was unsettled (Grossman, 1974).

The pharmacological problem was the selective blocking properties of the antihistamines (Loew, 1947). The available antihistamines were a diverse group, chemically unrelated to histamine and reminiscent in this respect of the class of adrenaline α-receptor antagonists. They were powerful inhibitors of histamine-induced visceral muscle contractions but had no effect at all against histamine-induced acid secretion, uterine relaxation or cardiac stimulation. Other effects of histamine, such as vasodilatation were well

known to be insensitive to the antihistamines. The parallels with the spectrum of activity of the anti-adrenalines seemed obvious.

In 1964, I had no doubts that histamine had its "β-receptors" and that a new type of selective histamine antagonist could be found. Ambiguity about the physiological role of histamine in acid secretion left me unsure about the clinical value of such drugs. At the very least, however, I expected to answer the physiological question of the gastrin-histamine connection.

The bioassay systems were easily selected. For in vitro assays, guinea-pig ileal muscle was the classical system for studying antihistamines such as mepyramine. Guinea-pig atrial tissue looked like a good assay for mepyramine-refractory histamine responses. The assay for acid secretion was harder to choose. No in vitro assays were available at that time. We chose the Ghosh and Schild (1958) method of lumen perfusion of the stomach of anaesthetized rats, but the method worked reliably only after it had been substantially modified by Parsons (1969), my new colleague.

The chemical programme, from the start, concentrated on making analogues and derivatives of histamine. As the whole project was conceived by

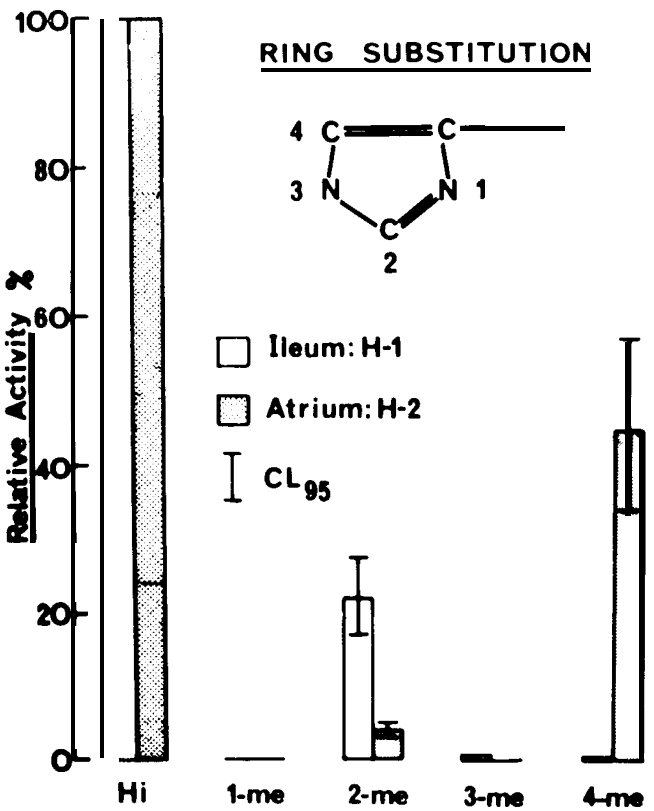


Figure 6. **Selectivity of histamine and several methyl-substituted analogues in the guinea-pig ileum (histamine H_1 receptor assay) and right atrium (histamine H_2 receptor assay). Activity values, relative to histamine were calculated from parallel line assays. Error bars show 95% confidence limits.**

analogy with the adrenaline P-receptor story, I was particularly anxious to concentrate on varying the imidazole ring end of histamine. Some of the early ring-substituted compounds turned out to be very important (Black et al. 1972) (Fig. 6). Methyl substitution on either of the ring nitrogens produced inactive compounds. Methyl substitution on the 2-position was found to be less active than histamine itself, but nevertheless showed a clear preference for the ileal assay. However, 4-methylhistamine was exciting for

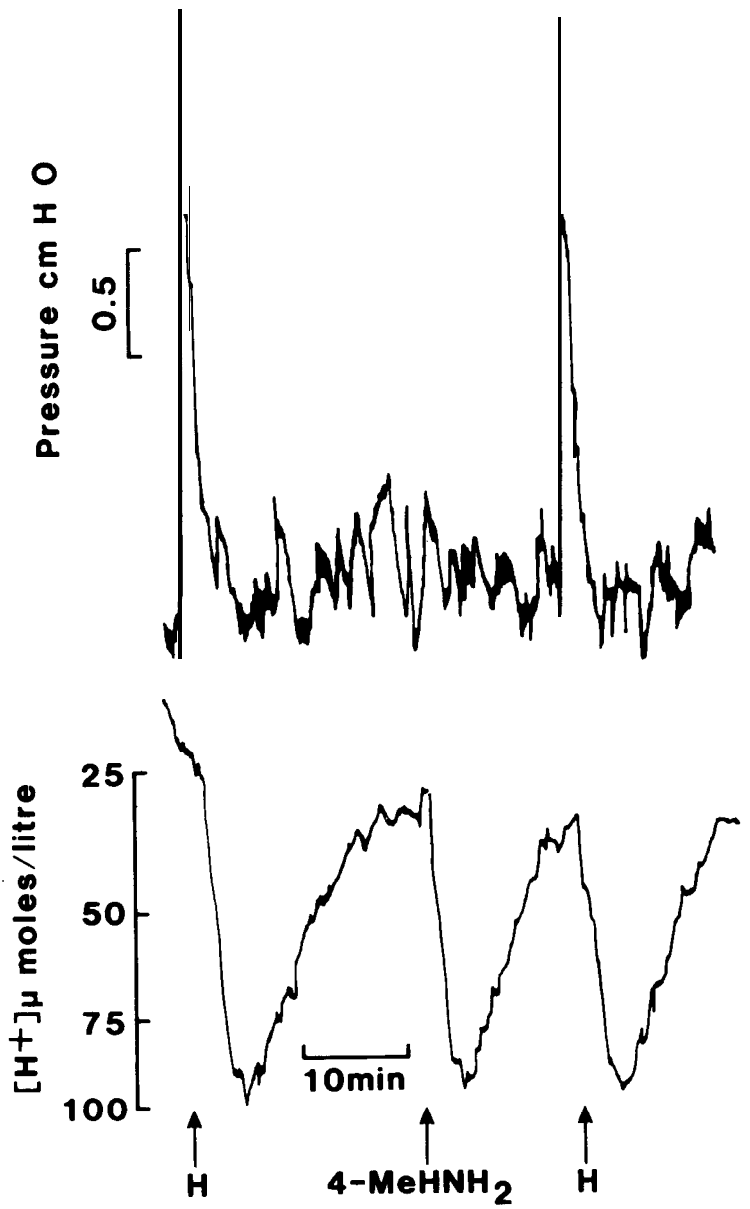


Figure 7. **Effects of i.v. bolus injections of histamine (H, 2ug/kg) and 4-methyl histamine (4MeHNH$_2$, 5ug/kg) upon secretion of gastric acid and stomach wall contraction in an anaesthetised rat.**

me. Nearly half as active as histamine on the atrial assay, 4-methylhistamine was practically inactive on the ileal assay. We were able to confirm this selectivity in vivo. In the anaesthetized rat preparation, an injection of histamine produced a fast, spasmodic contraction of the stomach wall followed by a phasic burst of acid secretion (Fig. 7); 4-methylhistamine produced an equivalent output of acid without any muscle contractions. Thus, 4-methylhistamine was a selective agonist analogous to isoprenaline at adrenergic receptors. This result was the compelling clue which kept us going through several lean years of negative screening.

This observation assumed even greater importance when we compared 4-methylhistamine and 2-methylhistamine with the 1,2,4-triazole analogue of histamine. Using a number of additional assays, both in vitro and in vivo, we found that the triazole analogue was clearly non-selective, whereas 2-methylhistamine was selective for the mepryamine-sensitive responses and 4-methylhistamine was selective for the refractory responses (Fig. 8). When Ash and Schild (1966) proposed that the mepyramine-sensitive histamine receptors should be classified as H_1, we used this pattern of bioassay results to argue for the homogeneity of a non-H_1 class of histamine receptor.

A very large number of compounds were made, predominantly ring substitutions and fused ring heterocycles, all of them inactive. I vividly remember wondering suddenly if the strategy was all wrong. Perhaps we should have spent more time exploring the role of the side-chain amino

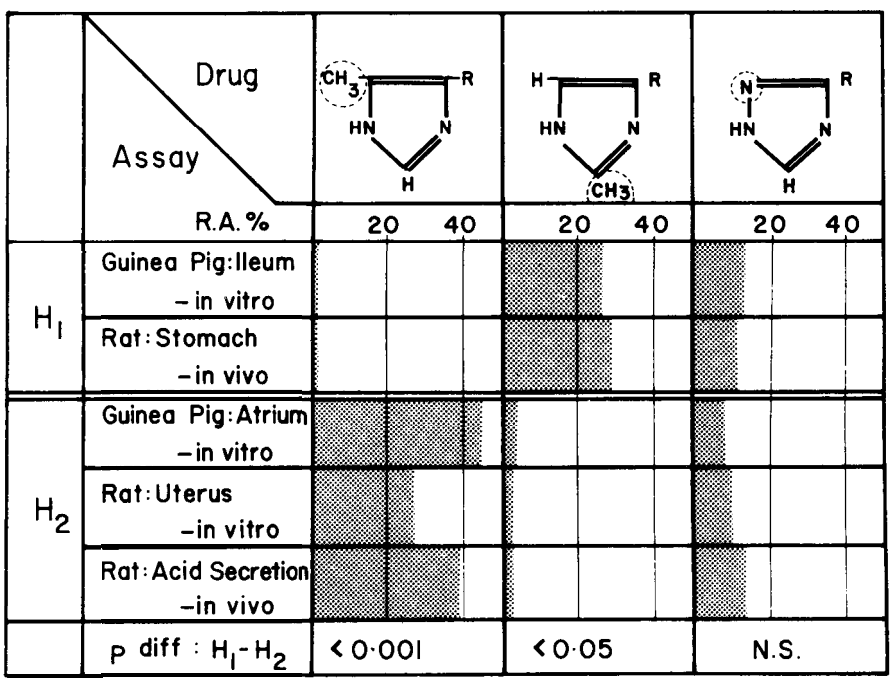


Figure 8. **Selectivity of 2-methylhistamine, 4-methylhistamine and 1,2,4-triazole analogue of histamine in several in vitro and in vivo, H_1- and H_2- histamine receptor assays. R.A. %: relative activity to histamine was calculated from parallel line assays.**

group. On this suggestion, Parsons quickly scanned through the earlier compounds looking for examples of side-chain variations. He came up with N^{α}-guanylhistamine (Fig. 11). This compound was one of the earliest we had tested and had proved to be quite a potent agonist when injected, like histamine, intravenously. However, over the years we had changed the design of the screening assay. A continuous intravenous infusion of histamine was used to produce a stable background of near-maximal acid secretion. A new compound could now be quickly screened by giving a succession of increasing doses intravenously. Even antagonism of very short duration would be detectable. In the new experimental design, the guanidino- analogue of histamine now exhibited a small degree of inhibition, about 5% reduction. There it was – a partial agonist! Guanylhistamine on histamine receptors was the analogue of DC1 on β-adrenoceptors. However, unlike DCI, the efficacy of histamine had been reduced by modifying the side-. chain rather than the ring system.

This was the lead that Ganellin and his colleagues in chemistry had been waiting for. One of the early analogues simply increased the length of the side chain from ethyl to propyl. In the rat stomach assay this compound showed good antagonism of histamine without much agonist activity of its own. However, in other species, particularly cat and dog, the compound was nearly a full agonist. Similarly, in the isolated guinea-pig atrial preparation, although much less potent than histamine, the compound achieved a maximum response about 80% of the histamine maximum. The true nature of this partial agonism could be seen by repeating the dose-response curve in the presence of a nearly maximal concentration of histamine. Only

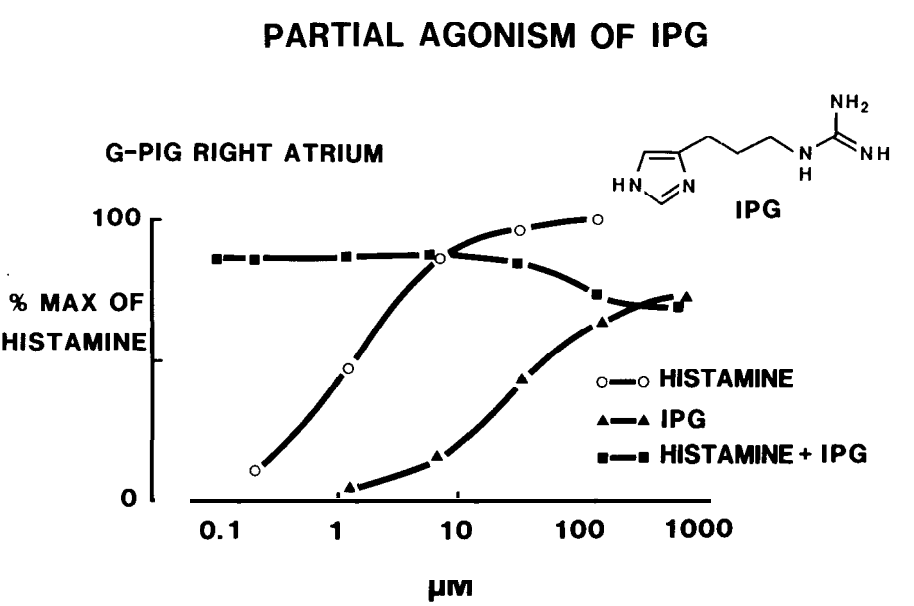


Figure 9. **Effects of cumulative additions of histamine, imidazole propylguanidine (IPG) and IPG in the presence of 10μM histamine on the pacemaker frequency of the isolated guinea-pig right atrium. Error bars have been removed for clarity (n=7).**

antagonism was now seen with the maximum inhibition, about 20%, being equal to the agonist maximum (Fig. 9).

Lengthening the side-chain to four carbon atoms and replacing the strongly basic guanidino group by the neutral methyl thiourea group produced burimamide, the first antagonist of moderate activity which had low enough efficacy to avoid being an agonist in any of our assays (Fig. 11). Burimamide, having relatively low potency and poor oral bioavailability, was clearly only a prototype. Ganellin saw the way forward. The non-basic, electron-releasing side-chain in burimamide, compared to the basic, electron-withdrawing, side-chain in histamine, raised the pK_a of the ring and favoured the opposite tautomer. Inserting the electro-negative thioether linkage in place of a methylene and introducing the 4-methyl group to favour H_2-receptor selectivity produced metiamide, which was much more potent and better absorbed than burimamide (Fig. 11). Toxicity associated with thiourea was then eliminated by replacing the thiourea sulphur with a cyano-imino group to produce cimetidine.

On the atrial assay in vitro, burimamide produced surmountable antagonism,, shown by rightward parallel displacement of the histamine dose-' response curves. When analysed by the Schild method, burimamide behaved like a syntopic antagonist to histamine. The estimated dissociation constants (K_B) were found to be independent of the potency of the titrating agonist and also independent of the tissue (atrium or uterus) used for the assay, substantially confirming the syntopic classification (Fig. 10). The high value on the ileum, an H_1 system, disclosed the compound's selectivity. As Ash and Schild (1966) had proposed the notation H_1, we proposed that burimamide should be classed as an H_2-receptor antagonist (Black et al. 1972).

BURIMAMIDE K_B (μM)

	ATRIUM	UTERUS	ILEUM
HISTAMINE	7.8	6.6	288
4-Me HISTAMINE	7.2		
2-Me HISTAMINE	6.9		

Figure 10. **Equilibrium dissociation constants (K_B) for burimamide in guinea-pig right atrium and rat uterus (H_2-histamine receptor assays) and in guinea-pig ileum (H_1-histamine receptor assay) against histamine and its analogues.**

The analytical capability to distinguish an antagonist which acts at the same site as the native hormone from one which does not act syntopically — that is a functional antagonist — seems to me to be important in drug research for two reasons. For a defined homogeneous population of receptors, widely disseminated across tissues, the properties of the syntopic antagonist can be generalised. As the mechanism of action of the functional antagonist is unknown, however, its properties have to be identified on a tissue-by-tissue basis. Again, the analytical power of a syntopic antagonist — that is its ability to prove hormonal involvement in physiological processes — is likely to be greater than for a functional antagonist. Of course, a compound that is a syntopic antagonist at one receptor system can also be a functional antagonist at a different receptor system. However, a possible combination of syntopic and functional properties would be expected to vary between different molecules, and the confusion can be eliminated by building up a class of syntopic antagonists that are chemically distinct but pharmacologically homogeneous. Syntopic antagonists are the best tools that analytical pharmacologists possess.

This problem of the resolving power of a receptor antagonist was seen from the beginning with metiamide. The histamine-induced acid secretion in the rat assay, having reached a plateau, was promptly inhibited when metiamide was given intravenously. Metiamide was found to be equally effective at inhibiting pentagastrin-induced secretion but much less effective against carbachol-induced secretion. Failure to inhibit cholinergically-stimulated secretion showed that metiamide was not a non-specific inhibitor of acid secretion. The ability of metiamide to inhibit the effects of gastrin

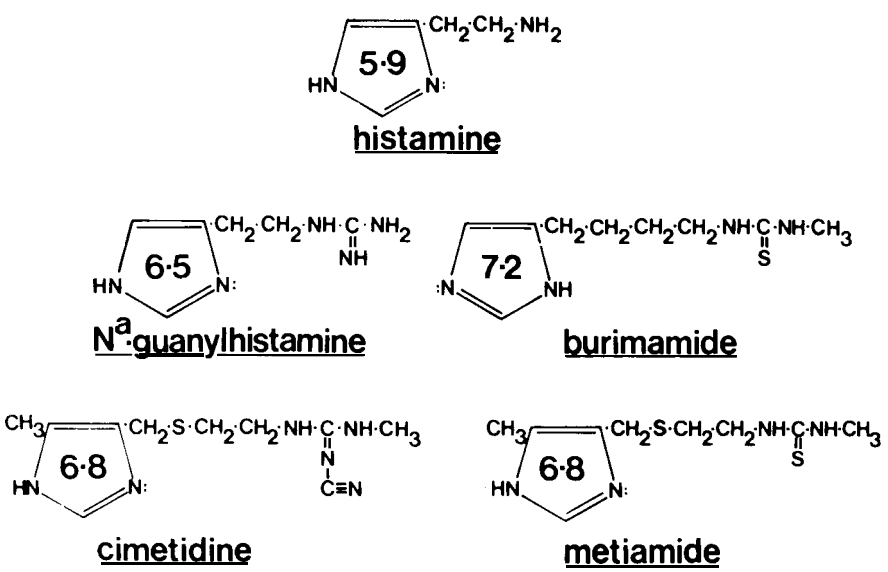


Figure 11. **Chemical structures of histamine-related imidazoles. Inset numbers: pK$_a$ values.**

pointed to potential clinical utility, but it was not at all clear that this result might contribute to a resolution of the gastrin-histamine controversy.

The problem became clear when the interactions between metiamide and the various stimulants were studied quantitatively in dogs with Heidenhain pouches (Black, 1973). Metiamide displaced the histamine dose-response curves in parallel to the right, as would be expected for a surmountable, syntopic antagonist (Fig. 12). Carbachol's steep dose-response curves were relatively refractory to inhibition. However, the flatter dose-response curves to pentagastrin were depressed downwards as well as being displaced to the right. At that time I did not know what to expect if H_P-receptor blockade inhibited gastrin only because histamine was the final common chemostimulant. I could not rule out the possibility that these drugs were functional antagonists of gastrin. Subsequently, when other workers had confirmed the different patterns of inhibition in other species, and with different compounds, an unspecific inhibitory action seemed unlikely. The pattern also became understandable when I was able to model indirect competitive antagonism and applied the model to tyramine, a well-characterised indirectly acting agonist, to show that it was inhibited insurmountably by propranolol, just like the gastrin-metiamide interaction (Black et al. 1980).

When we took the H_2-receptor antagonists into human volunteers, there were no surprises in the patterns of secretory inhibition. However, we did get a surprise, right at the start, with burimamide. We followed the standard clinical practice of giving the volunteers mepyramine before giving them histamine intravenously. Even so, the subjects showed marked skin and conjunctival vasodilatation. The surprise was that treatment with burimamide completely blocked this vasodilatation. In the laboratory, burimamide

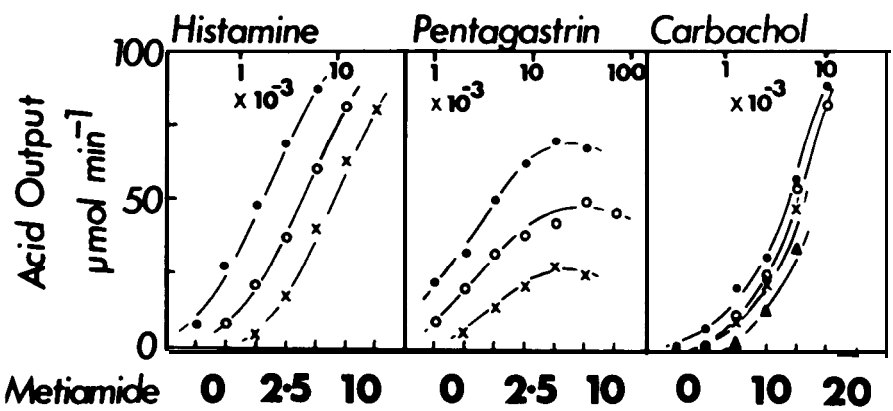


Figure 12. **Effects of i.v. infusion of metiamide against histamine-, pentagastrin- and carbachol-induced gastric acid secretion in dogs with Heidenhain pouches. Doses shown are μmol/kg/min for the agonists (upper abscissae); μmol/kg/h for metiamide (closed circles 0, open circles 2.5, crosses 10, triangles 20). Reproduced by kind permission from: International Symposium of H_2receptor blockade, 1973, Eds. Wood and Simpkins, Publ. SK&F Laboratories.**

alone had had no effect on histamine-induced vasodilatation. As both H_1- and H_2-receptors were involved, both antagonists were needed. This finding thus explained the results of Folkow et al. (1942) of 30 years earlier.

Hormone-receptor antagonists in the future

The histamine project was started by analogy with my experience of the adrenaline project. In retrospect, I think they have some features in common which helped them to succeed. Both started from well-recognised clinical problems at a time when they could be illuminated by specific hypothetical modelling at the laboratory level. The laboratory modelling defined the chemical starting points and the types of bioassay. The clinical problem defined how the newly classified drug should be tested in volunteers and patients. If the intimate coupling of clinical experience and pharmacological modelling has the effect of helping to eliminate wishful thinking in drug research, then the limiting step in the future will be the development and improvement of these models.

Models in analytical pharmacology are not meant to be descriptions, pathetic descriptions, of nature; they are designed to be accurate descriptions of our pathetic thinking about nature. They are meant to expose assumptions, define expectations and help us to devise new tests.

Traditionally, pharmacological modelling of hormone-receptor systems has been based on the application of the Law of Mass Action to reversible interactions. Therefore, they are all chemical, molar models characterised by thermodynamic parameters. The discovery, often in a homologous series of compounds, that not all agonists could produce the same maximum response (now defined as partial agonists) led to models which had both binding, or affinity, parameters *and* efficacy, or response-generating, parameters. In both of the studies that I have sketched for you today, chemical modification of a native hormone produced, first of all, selective agonists, then quite separate chemical changes produced partial agonists and finally pure antagonists. The assumption is that partial agonists and antagonists are associated with a relative loss of efficacy — emasculated hormones.

The discovery of partial agonists was, in both reports, crucial to the development of syntopic antagonists. Yet I very nearly, and could quite easily, have failed to discover them. The choice of tissue for the assay was vital. So, why is the expression of efficacy so tissue dependent? How can we try to choose tissues which are most likely to allow us to detect partial agonists?

To illuminate these questions, Leff and I developed an operational model of agonism which defined three mutually-connected surfaces such that knowing the shape of the function on any two allowed us, syllogistically, to deduce the necessary shape of the function on the third space (Black and Leff, 1983) (Fig. 13a). On the right-hand side of the graphical display of the model is the measured function that relates agonist concentration, on a logarithmic scale (log A), to the tissue effect which it produces. The pharmacological assumption is that the agonist initiates an effect by binding to a

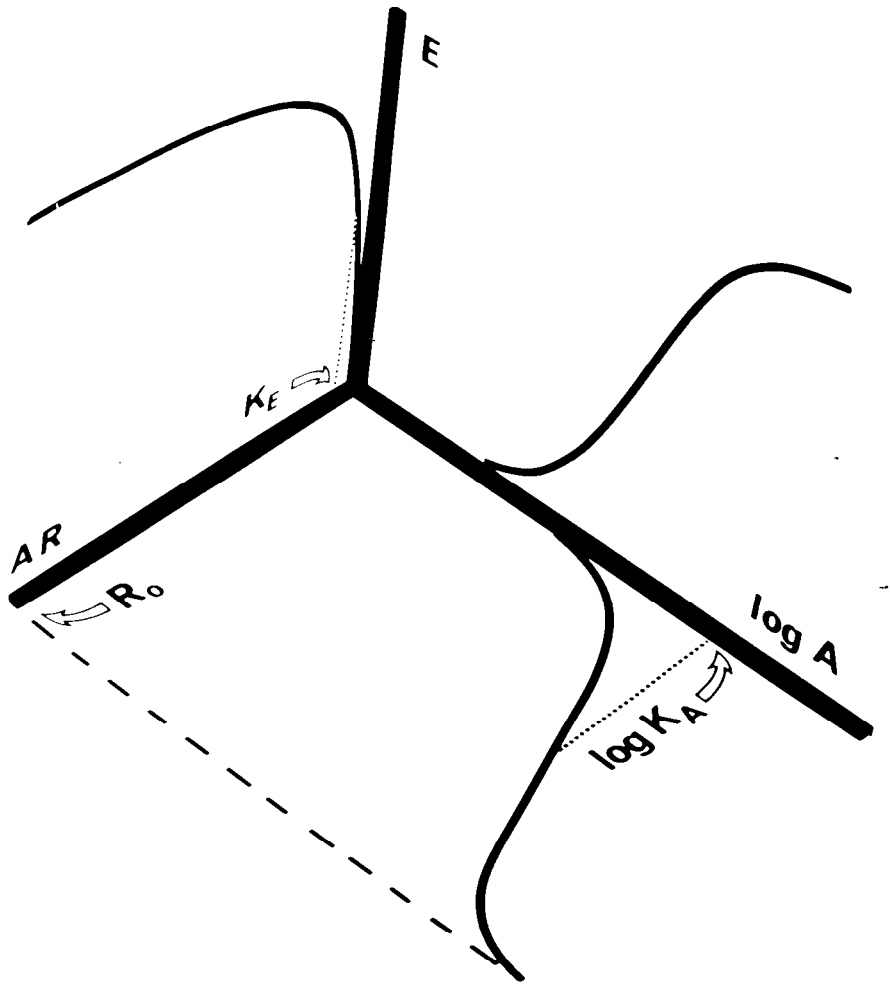


Figure 13a. **Three-dimensional display of the Operational Model of Agonism (Black and Leff, 1983). E: pharmacological effect, log [A]: logarithmic concentration of agonist A, log K_A: log equilibrium agonist dissociation constant, [R_o]: operational receptor concentration, [AR]: concentration of receptors occupied by A, K_E: concentration of AR required for half-maximal tissue response. The three planes of the figure represent pharmacological effect (right panel), binding or affinity (base panel) and efficacy or transduction (left panel).**

receptor (R); then the bound receptor (AR) activates a messenger system that produces the effect. Therefore, the base of the display shows the assumed relationship between agonist concentration and the concentration of bound receptors — the *affinity* relation. Then, the left-hand panel shows the deduced relation between bound receptor concentration and effect — the *efficacy* relation. The behaviour of this model is critically determined by the ratio of R_o, the total receptor concentration, to K_E, the concentration of bound receptor needed to produce a half-maximal effect. For example, when R_o is equal to K_E the agonist can only produce a half-maximal response, thus defining a 50% partial agonist (Fig. 13b).

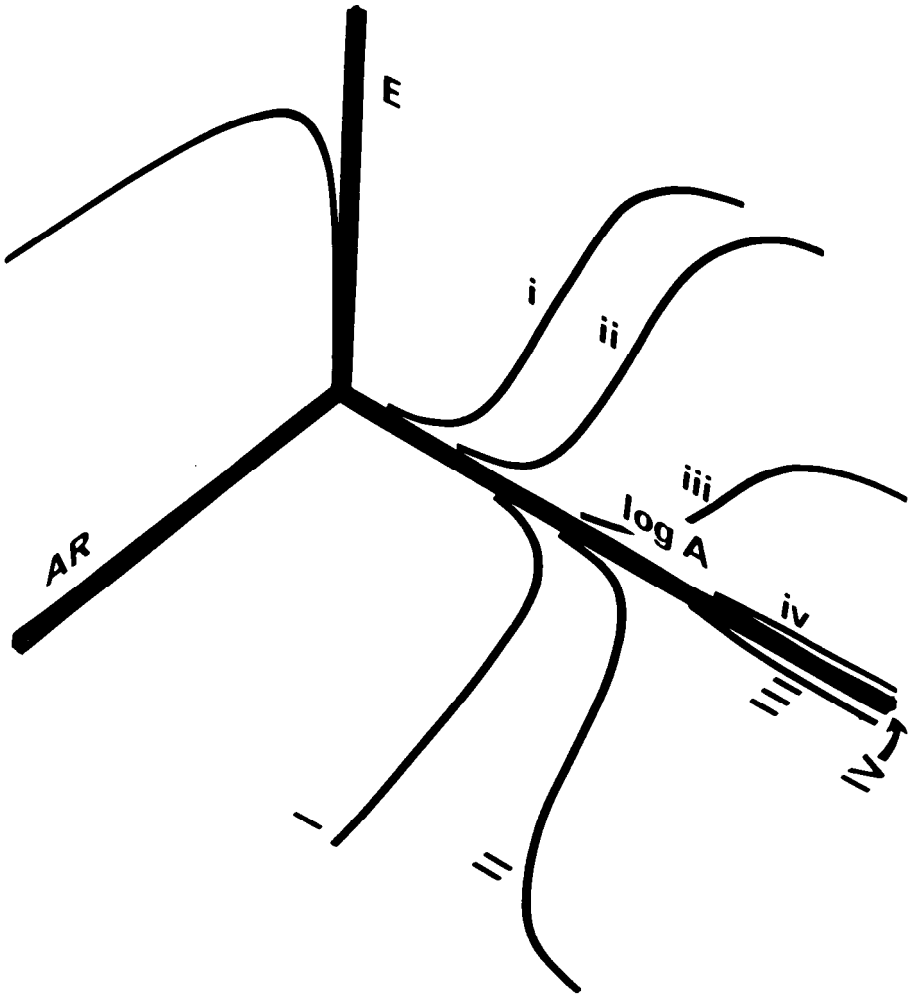


Figure 13b. **Predictions of the influence of changes in [R_o] upon pharmacological effect using the Operational Model of Agonism. Curves I to IV show R_o concentration decreasing successively by ten-fold giving rise to effect curves i to iv, respectively.**

One of the first uses of the model was to fit simultaneously all of the data which Kenakin and Beek (1980) had got from comparing isoprenaline and prenalterol, a partial agonist at β-receptors, on six different tissues (Fig. 13c). The model of agonism allowed all of the data to be fitted by the theoretical curves shown when only one parameter, total receptor density Ro, was allowed to vary. The concentration of receptors is now known to vary between tissues, so that this seems to me to be an attractive way of accounting for the tissue dependence of a partial agonist's efficacy. Superimposing all the dose-response curves clearly demonstrates that the tissues most sensitive to isoprenaline support the greatest maximum responses to prenalterol and vice versa (Fig. 13d). Practically, therefore, the best way to avoid missing a partial agonist is to measure the potency of the native hormone or full agonist on as many tissues as possible and select assays

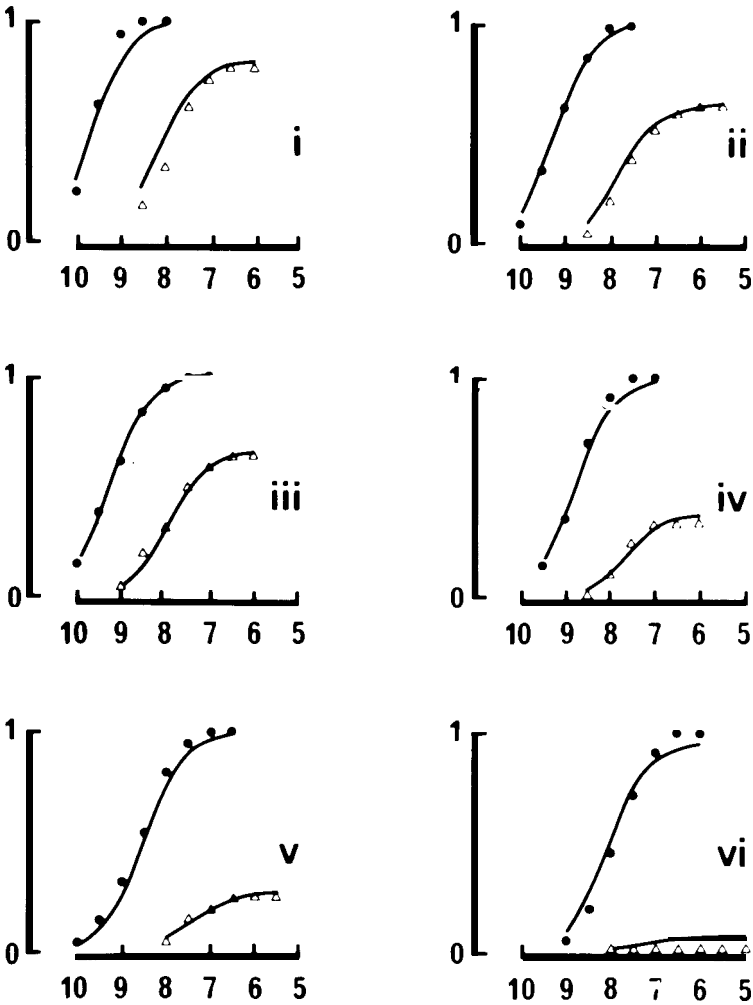


Figure 13c. **Behaviour of isoprenaline (circles) and prenalterol (triangles) in (i) guinea-pig tracheal muscle, (ii) cat left atria, (iii) rat left atria, (iv) cat papillary, (v) guinea-pig left atria and (vi) guinea-pig extensor digitorum longus muscle; (Kenakin and Reek, 1980). The data has been regressed to the Operational Model of Agonism allowing only [R_o] to vary between tissues. Abscissa: log molar agonist concentration. Ordinate: Fractional response to isoprenaline.**

expressing both high and low efficacy. This seems to be a robust test, relatively insensitive to the mechanisms underlying the differences in sensitivity.

Partial agonists, as empirical facts, have been recognised for many years. Pharmacological modelling of partial agonism and the related concept of efficacy has, however, developed more slowly. Fundamental problems about the nature of efficacy, either as a molar, thermodynamic, concept or as a molecular problem in wave mechanics have still to be tackled. However, there seems no doubt about the pragmatic utility in drug research of

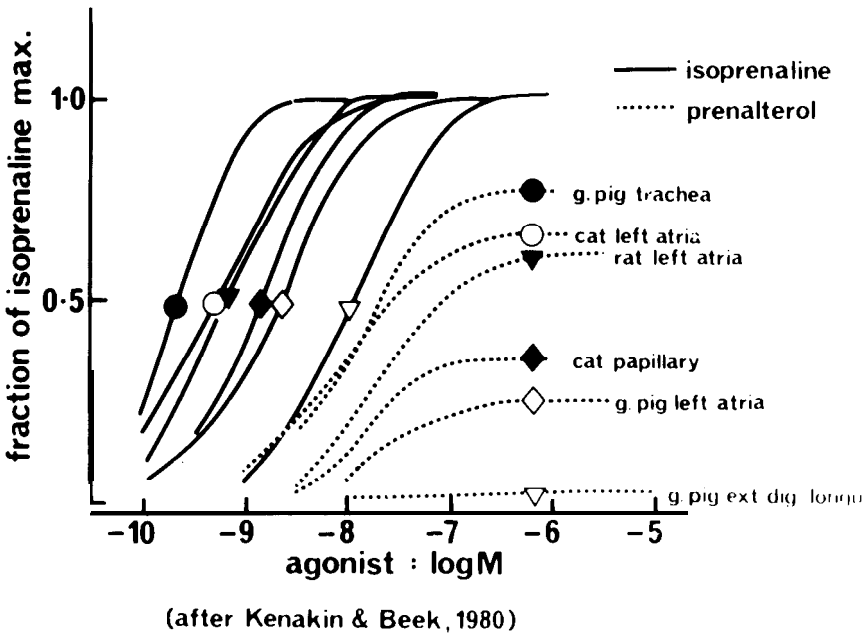


Figure 13d. **Representation of the model-simulated curves from Fig. 13c superimposed on a single pair of axes.**

distinguishing potency changes from efficacy changes in the bioassay of hormone analogues. The discovery of a partial agonist is the vital clue in developing useful syntopic antagonists.

We have gone on to use the model to study the effects of the slope of dose-response curves (Black et al. 1985a), functional antagonism (Leff et al. 1985), indirect competitive antagonism (Black et al. 1985b), dual receptor systems and receptor distribution when there is a relatively high concentration of transducer molecules.

The dose-response relation can, of course, be broken down into any two necessarily-connected steps. Thus, the gastrin dose-response curve of acid secretion can be broken down into the gastrin/histamine-released relation followed by the histamine-released/acid secretion response relation. Two of the relations are known from measurement and the third, the gastrin/histamine-released relation can be deduced. Using this model, we were able to make a correct estimate of the K_B of an H_2-receptor antagonist from the family of unsurmountable curves produced by its interaction with pentagastrin, an important piece of evidence linking local histamine release to the physiological action of gastrin. (Black et al. 1985b.)

The approach I have outlined so far has regarded hormones and their conjugate receptors as simple, linear, "command-control" systems. The approach has undoubtedly had some success. Nevertheless, I think if we want to continue to try to develop new drugs by mimicking and manipulating physiological chemical control systems, our ideas will have to become more sophisticated. There is plenty of evidence now that hormone recep-

tors and their dependent messengers are not insulated from each other. Mutually-enhancing interactions between any two receptor-messenger systems can occur at many different points leading to different kinds of physiological advantage.

When one hormone can interact with two allosterically-linked receptors on the same cell, the continuous gearing can change the relatively-flat concentration-response curve characteristic of the Mass-Action Law behaviour of a one-receptor system into steep curves. This greatly reduces the change in concentration needed to sweep the cellular response through its full range. This could be an advantage for fast-responding cells. When there are two hormones and two receptors, mutual potentiation can lead to threshold changes, pulsing signals and, more importantly, by making the activity of one hormone depend on the other, the convergence changes the type of behaviour from obligatory responses to conditional responses, like nerve cells, based on summation.

The rich possibilities of hormonal convergence plus inter-receptor amplification are now being discovered in the area of neuroendocrine secretion. While co-existence of multiple hormones in a single nerve ending does not necessitate co-transmission, there seems as yet no need to doubt it. Hökfelt, in a recent review (Hökfelt et al 1987), pointed out that the distribution of these hormones was not random. For example, neurones classified as 5-HT-, noradrenaline- or dopamine-transmitting had each got different groups of peptides co-existing in their terminals. Neurobiologists have plenty of ideas about the significance of the very large and rapidly growing number of pharmacologically active substances which have been identified in nervous tissue. However, as an outsider looking in on all their excitement, I sense that my colleagues have problems with the Principle of Parsimony. Neurotransmission involving discrete, microscopic events is unlikely to generate problems with chemical crosstalk in the brain at large. So, is there not now an embarrassing number of potential neurotransmitters?

On the other hand, biologists concerned with brain development probably do need an abundance of specific cell markers. Sperry's Chemoaffinity Hypothesis (1963), one of the earliest attempts to account for the details of pattern development in the embryonic brain, required that cells have individual chemical identification markers almost down to the level of single cells. Edelman's modulation hypothesis (1984) — how the composition and density of nerve cell adhesion molecules can be locally regulated by the cells themselves — is chemically much more economical. These molecules subserving cell-to-cell interactions can provide a framework for guiding neurite growth cones. Diffusible growth factors, such as the specific Nerve Growth Factor, can provide a general engine for neural growth into a supporting network. The question that intrigues me, however, is whether the framework and the engine are enough to account for the exquisite fine-tuning of synaptic connections which occurs during brain maturation and for the control of synaptic plasticity now known to be a feature of the mature brain.

I like the idea that these synaptic connections are determined chemotactically. An effective chemotactic address might then involve the co-operative signalling of two or more chemicals. The possibilities for chemotactic signatures are factorial. If an effective signal involved just three chemicals, then 100 hormones could provide over a hundred thousand different signatures in any one compartment.

As our ideas about hormone-receptor systems become progressively more complicated in terms of multiplex pathways, hierarchy due to cellular conjunctions and biochemical cascades, the reductionist methods of molecular biology would seem to offer modern drug research a way out: simplify the systems by receptor isolation and expression. Molecular biology undoubtedly holds out the promise of the most direct and productive route ever to screening chemicals as hormone receptor reagents. However, once classified at the molecular level, a new reagent will have to be evaluated at the level of tissue complexity to confirm its classification and define its selectivity. These tissue bioassays, such as I've discussed today, may seem old-fashioned but, properly designed, they are arguably the best methods we have for making reliable predictions about clinical outcomes. They have served us well but they need to be continually improved, both technically and in their related operational models, to match our changing ideas. Molecular biology will continue to provide drug research with extraordinary analytical methods and lend a richer texture to our imagination.

These reflections suggest that there will be both great opportunities, and potential dangers, for the development of specific hormone receptor reagents in the future. The limiting factors, however, are likely to be the verisimilitude of our models and the complexity of our bioassays. Analytical pharmacology has got an important and exciting future

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