Quantitation of the Alpha and Beta Adrenoceptors and Inhibitory Responses in the Rabbit Intestine.

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INTRODUCTION

Ahlquist in 1948 introduced the concept of alpha and beta adrenergic receptors by using agonists (noradrenaline, adrenaline, isoprenaline and the alpha-methyl derivatives of noradrenaline and adrenaline) to identify and classify these adrenoceptors in the vascular beds, heart uterus and intestines of Cats, dogs rats and rabbits. Receptors may also be classified by observing the modification of agonists responses by selective antagonists. Thus the use of alpha adrenoceptor blockers (for example phentolamine) and later of beta adrenoceptor blockers such as propranolol have further confirmed the original concept and experiments of Ahlquist. The inhibitory responses of the small intestine was assigned to alpha receptor stimulation (Ahlquist 1948), but Furchgott (1959) proposed a third adrenergic receptor as being responsible in an attempt to resolve the apparent anomally of alpha receptor stimulation producing smooth muscle relaxation. Ahlquist and Levy (1959) and Furchgott (1960) showed that the small intestine of dog and rabbit contain both alpha and beta receptor, stimulation of which produce relaxation. This was as a result of the discovery of beta adrenoceptor antagonist dichloroisoprenaline (DCI) by Powell and Slater (1958).

Experimental observations have suggested that the alpha receptors are located on cell membranes of several tissues and on nerve cells associated with smooth muscles and muscle fibres, while the beta adrenoceptors are associated with metabolic activity and cyclic AMP within the cell (Brody and Diamond 1967, Haylett & Jenkinson 1972) Intestinal smooth muscle relaxes very rapidly following alpha receptor stimulation as compared with beta adrenoceptor stimulation (Van Rossum and Mujic 1965; Brody and Diamond 1966).

The work reported here is an attempt to quantify the contribution or importance of each of the two adrenoceptors to the inhibitory responses observed. We have also used the receptors and their antagonists to assess the alpha and beta adrenoceptor stimulant potency of a number of the commonly used sympathomimetic amines.

METHODS

Isolated segments 2cm length of the ileum of a freshly killed rabbit of either sex was set up in tyrode solution aerated with air at a temperature of 37 + 0.5oC. The contents of the isolated ileum was washed out with tyrode solution using a pipette. Pendular movements were recorded on a Kymograph with an isotonic frontal writing lever with a load of 2 gramme and a magnification of 6. The load was applied to prevent the development of a high level background tone in this tissue (Lum, Kermani and Heilman, 1966). Under these conditions, drugs caused an inhibition of pendular movement with little or no effect on the baseline.

A constant volume of 20ml was maintained in the organ bath; drugs were added either by replacement with tyrode containing the required drug concentrations (antagonists) or by adding volumes of the agonist drug not exceeding 1ml directly to the bath to reach the 20ml level. The antagonists were allowed a drug-tissue contact time of at least 15 minutes for equilibrium to develop between antagonist and receptors; the drug-tissue contact time for agonists was 60 seconds and this was observed to be enough for maximal effect. Three changes of the bath fluid with fresh tyrode were sufficient to restore the tissue to baseline after resting for 2 minutes.

TABLE I

Relative Potency of Agonists in Causing Inhibition in The Rabbit Intestine By Stimulating Either Alpha or Beta Receptor Using Adrenaline Without Any Antagonist As Standard.

Drugs	Log dose at 50%	Log dose Ratio	Dose Ratio	Relative Potency
Adrenaline	0.95	0	1	1
Noradrenaline in Propranolol	1.15	0.70	1.585	0.63
Phenylephrine in Propranolol	1.75	0.80	6.310	0.16
Isoprenaline in Phentolamine	2.4	1.45	28.18	0.038
Salbutamol in Phentolamine	3.15	2.20	158.5	0.0062

Dose-response relationships: Several and graded doses of each agonist were used to find the dose-response relationship before and after each adrenoceptor blocker; similar dose-response relationship were found in the presence of graded increasing concentrations of antagonists. It was thus possible to calculate the potency of agonist drugs in (a) causing inhibition of the intestinal pendular movements (b) stimulating specific receptors, in the presence of high concentrations

of either propranolol or phentolamine (10-4M). pA2 Values were found according to the method of Arunlakshana and Schild (1959).

Intrinsic activities of the agonists were calculated by comparing maximum responses of the agonists with that of adrenaline. That is, Intrinsic Activity=Magnitude of maximal response to agonist/Magnitude of maximal response to adrenaline.

TABLE 2

Relative Potency of Agonists in Stimulating Alpha Receptors Using Noradrenaline as Standard.

Agonists	Log dose at 50%	Log dose Ratio	Dose Ratio	Relative Potency
Noradrenaline	1.95	0	1	1
Adrenaline	1.68	0.27	1.862	1.57
Phenylephrine	2.1	0.15	1.413	0.71
Isoprenaline	2.8	0.85	7.079	0.14
Salbutamol	3.45	1.50	31.62	0.032

TABLE 3

Relative Potencies of Agonists in Stimulating Beta Receptors Using Isoprenaline as Standard

Agonists	Log dose at 50%	Log dose Ratio	Dose Ratio	Relative Potency
Isoprenaline	2.5	0	1	1
Adrenaline	2.15	0.35	2.24	1.45
Noradrenaline	2.77	0.27	1.862	0.54
Salbutamol 3.6	3.6	1.1	12.59	0.079
Phenylephrine	4.3	1.8	63.10	0.0159

Three concentrations (2 x 10–7M, 10–5M, and 10–4M) of the antagonists Propranolol and phentolamine were chosen after several experiments had shown that doses below 2 x 10–7M had very little or no effect while doses above 10–4M showed some intrinsic activity of the antagonist. Propranolol reduced the tone of the tissue above 10–4M while phentolamine produced increased tone. These effects prevented the responses to agonist drugs from being graded.

Affinity Constant (Kaff): This was found by using each agonist in presence of specific antagonist (for example noradrenaline in Phentolamine) except Adrenaline for which both antagonists wereneeded to find dose response relationships. The dose at 50% maximum response was found for each, and affinity constant was calculated from the relationship where:
Kaff = 1/A(50)
A(50) = dose producing 50% maximum response
Kaff = Affinity constant.

RESULTS

General observation:

A difference was observed in the character of the inhibitory responses of the rabbit ileum to alpha and beta receptor stimulants. The response to alpha receptor stimulation as shown by adrenaline in the presence of propranolol was rapid in onset and recovery began while drug was still in contact with the tissue, that is, before wash out. In contrast, the response to beta adrenoceptor stimulation of the intestine as demonstrated by adrenaline in the presence of phentolamine was relatively slow in onset and the inhibitory response was maintained throughout the drug tissue contact time.

1ng of adrenaline, noradrenaline, phenylephrine and isoprenaline produced some inhibitory response of the spontaneous pendular contractions of the isolated rabbit ileum while it took up to 64ng of salbutamol to produce any effect. High doses of phentolamine (10–8M) blocked the inhibitory effect of noradrenaline, adrenaline and phenylephrine considerably but did not have much effect on isoprenaline and salbutamol. High doses of Propranolol (10–8M) had considerable effect on salbutamol and isoprenaline but did not significantly affect noradrenaline and phenylephrine. Both phentolamine and propranolol had pronounced effects on the inhibitory response adrenaline.

TABLE 4

Affinity Constants of Agonists For their Respective Receptors

Agonists	Receptor	Affinity Constant x 107 Utrelmol
Noradrenaline	alpha	1.196
Phenylephrine	alpha	0.2951
Isoprenaline	beta	0.8401
Salbutamol	beta	0.0159
Adrenaline	alpha	1.454
Adrenaline	beta	1.296

Potency: The potency of agonists in causing inhibition was compared with adrenaline as standard.
The order of potency was Adrenaline 1.0, Noradrenaline 0.63, Phenylephrine 0.16, Isoprenaline 0.038, Salbutamol 0.0062 (Table 1).

The potency of agonists in stimulating specific receptors was calculated. For alpha adrenoceptors, noradrenaline was used as standard and all agonists were observed in the presence of high concentrations of propranolol 10–5M. The values found were: Adrenaline 1.57, Noradrenaline 1.0 Phenylephrine 0.71 Isoprenaline 0.14 Salbutamol 0.032 (Table 2). For beta adrenoceptors, Isoprenaline was used as standard and all agonists were observed in the presence of high concentration of phentolamine 10–5M. The order of

potency was Adrenaline 1.45, Isoprenaline 1.0, Noradrenaline 0.54 Salbutamol 0.079, Phenylephrine 0.0159 (Table 3).

From tables 2 and 3, it is apparent that adrenaline stimulated alpha and beta receptors about equally but more of alpha effect in this tissue. It is observed that alpha adrenoceptors stimulation produced greater inhibition. The relative potencies were calculated by finding the reciprocal of the displacement of each agonist from the standard.

TABLE 5

Data for PA2

A	B	B–A (X)	X –1	Log X–1	–Log Molar Conc from	pA2 Values from graphs
Salbutamol in propranolol 2.45	2.895 3.15 3.4	3.229 5.012 8.913	2.229 4.012 7.913	0.3592 0.6033 0.8984	6.6990 5.0000 4.0000	8.9
Salbutamol in phentolamine 2.52	2.98 3.35 3.85	3.052 6.761 18.98	2.052 5.761 17.98	0.3118 0.7605 1.2114	6.6990 5.0000 4.000	7.6
Isoprenaline in phentolamine 1.65	1.93 2.26 2.45	2.788 4.078 6.310	1.788 3.078 5.310	0.2516 0.4871 0.7604	6.6990 5.0000 4,0000	8.0
Isoprenaline in propranolol 1.65	2.2 2.4 2.8	3.548 5.623 6.761	2.548 4.623 5.761	0.4048 0.6649 0.7604	6.6990 5.000 4.000	9.1
Phenylephrine in propranolol 1.05	1.35 1.65 1.95	2.153 3.981 7.943	1.183 2.981 6.943	0.2463 0.4781 3.1798	6.6990 5.000 4.000	7.4
Phenylephrine in phentolamine 1.1	2.62 3.35 2.25	33.11 177.8 1514	32.11 176.8 1513	1.5515 2.3475 3.1798	6.6990 5.000 4.000	9.2
Noradrenaline in propranolol 1.35	1.88 2.13 2.25	3.388 6.026 7.943	2.388 5.026 6.943	0.3692 0.7012 0.9156	6.6990 5.000 4.00	8.1
Noradrenaline in phentolamine 1.35	2.13 2.8 3.06	6.026 32.48 60.12	5.026 31.48 59.21	0.7012 1.4487 1.7913	6.6990 5.000 4.000	9.1
Adrenaline in propranolol 0.8	1.22 1.42 1.6	3.245 4.169 6.310	2.245 3.169 5.31	0.3651 0.5019 0.7251	6.6990 5.0000 4.000	9.2
Adrenaline in phentolamine 0.8	1.1 1.3 1.7	2.665 3.762 7.943	1.665 2.762 6.943	0.2238 0.4740 0.8416	6.6990 5.0000 4.000	9.2

Key: A = 50% max response to agonist alone
B = 50% max. response to agonist in presence of antagonist
B – A* The displacement i.e. dose ratio (antilog).

Intrinsic Activity: As described under methods was calculated. Adrenaline which produced maximum inhibition was taken as 100% and the effect of the others were compared with it.
Noradrenaline = 100/100 = 1.0
Phenylephrine = 100/100 = 1.0
Isoprenaline = 100/100 = 1.0.
Salbutamol = 99.46/100 = 0.99.
Thus the intrinsic activities of the agonists used were 1 except Salbutamol which was approximately 1. One may therefore conclude that they are all full agonists.

Affinity Constant: This was calculated for each receptor and the values are shown in Table 4.
pA2 Values were also calculated and shown on Table 5. The slope in each case is about 1 showing that the antagonists were acting competitively.

DISCUSSION

The rapidity of onset of inhibition by alpha receptor agonists confirms the fact that alpha receptors are believed to be located on the cell membrane whereas beta receptors are intracellular, (Brody and Diamond 1967) or may lead to a chain of intracellular events responsible for the mechanical response. Inhibition evoked by alpha receptors is the result of hyperpolarization of cell membranes consequent upon a primary increase in potassium ion (K+) permeability, whereas that for beta receptor is due to intracellular action (Jenkinson & Morton 1967).

The inhibitory actions of both alpha and beta receptor stimulants were blocked by the alpha and beta adrenoceptor antagonists used, phentolamine and propranolol.

Using a combination of propranolol and phentolamine, more block was obtained on the stimulant effect of Adrenaline. This shows or confirms that the two receptors are present in the intestine. The fact that both alpha and beta receptor blockers produce block of the adrenergic receptor agonists also shows that both alpha and beta receptors are present in the rabbit intestine. Agonists like noradrenaline and phenylephrine which are more potent on alpha receptor were inhibited to some extent by propranolol. Similarly, Isoprenaline was antagonised to some extent by phentolamine. These observations further show the presence of both receptors in thetissue as well as the double activity of the agonists.

The potency of agonists in causing inhibition was compared (Table 1). It showed that adrenaline is the most active. This is further confirmed by the activity ratio of each agonist in causing inhibition by stimulating each specific receptor (Table 2 and 3).

The result shows that the stimulation of alpha receptors caused more inhibition than the stimulation of beta receptors. Quantitatively, one could say that taking Noradrenaline as standard for alpha receptor stimulant effect and comparing it with isoprenaline, their activities was 0.63: 0.038, that is, 17:1. Therefore noradrenaline is seveteen times more effective in causing inhibition in rabbit intestine through alpha receptor stimulation than Isoprenaline through beta receptor stimulation. This infers that alpha receptors are much more potent than beta receptors in causing the relaxation of the rabbit intestine's pendular movement.

Salbutamol which is more specific for beta 2 receptor found in the intestine was not used in this comparison because there is a controversy on whether it is beta 2 or beta 1 receptor that is found in the intestine. Schild (1973) classified the beta receptor in the rabbit intestine as beta 1. However according to other workers Dunlop & Shanks (1968) it was beta 2, since its action in stimulating the intestine beta adrenoceptor to produce inhibition, is different from the response of the heart to beta adrenoceptor stimulation, which is excitation, but similar to that on the bronchioles. Using the pA2 values (Table 5), as an index it is observed that for salbutamol is slightly lower than that for isoprenaline, using propranolol. The difference is not significant: 9.1 for isoprenaline and 8.9 for salbutamol so they are probably acting on the same receptors but with different efficacies.

From the pA2 values, it can also be seen that there are two distinct receptors alpha and beta, with the agonists each being more specific for one agonist except adrenaline which acts on both receptors about equally.

The potency of each agonist in stimulating each of the two receptors was worked out. It was found that in each case, adrenaline was the most potent, and it followed the pattern observed for the inhibitory responses.

Phenylephrine has very little beta receptor stimulant effect while salbutamol has very little alpha stimulant effect. Noradrenaline is about half as potent as isoprenaline in stimulating beta receptors in the intestine. Adrenaline was almost equipotent in stimulating both alpha and beta receptors. This can also be seen from the affinity constants of agonists for receptors, (Table 4). Adrenaline has maximal affinity for both receptors. Its affinity for alpha receptor was found to be greater than that for the beta receptor. It therefore can be conclusively said that the inhibitory effect elicited in the rabbit intestine is caused more by the alpha receptor than by beta receptor.

If the potencies of phenylephrine and salbutamol is causing inhibition in the intestine are compared, the ratio is 0.16; 0.0062 that is, 26:1 (Table 1). This also shows that inhibition due to the beta adrenoceptor.

The mean of the potency ratios for noradrenaline/Isoprenaline and Phenylephrine/Salbutamol was found to be 21.5:1, that is, alpha adrenoreceptor stimulation is 21.5 times more potent than beta adreno-

receptor stimulation in causing inhibition in the rab bit intestine.

SUMMARY

The pendular movement of the isolated rabbit intestine was inhibited to different degrees by sympathomimetic amines-adrenaline, noradrenaline, phenylephrine, isoprenaline and salbutamol in this order.

The receptors involved are classified as alpha and beta adrenergic receptors since the inhibition of pendular movement was antagonised by phentolamine and propranolol which are alpha and beta adrenergic receptor antagonists respectively. Both adrenoceptor blockers when combined antagonized more the effect of adrenaline than each blocker when used alone.

Taking adrenaline as a unit standard, the potency of each agonist in inhibiting the pendular movements was found to be; adrenaline, 1; Noradrenaline, 0.63; Phenylephine, 0.16; Isoprenaline, 0.038; Salbutamol, 0.0062.

It was found that alpha adrenoreceptor stimulation caused more of the inhibition in the rabbit intestine than the beta adrenoreceptor.

REFERENCES

1. Ahlquist, R.P. (1948) A study of the adrenotropic receptors Amer. J. Physiol. 153, 586-600.
2. Ahlquist, R.P. & Levy, B. (1959) Adrenergic receptive mechanism of Canine ileum. J. Pharmac. exp. Therap. 127, 146-149.
3. Arulakshana, O. & Schild, H.O. (1959) Some quantitative uses of drug antagonists. Brit. J. Pharmac. Chemother. 14, 48-58.
4. Black, J.W., Duncan, W.A.M. & Shanks, R.G. (1965). Comparison of some properties of pronothalol and propranolol. Brit. J. Pharmac. Chemother. 25, 577-591.
5. Blinks, J.R. (1967) Evaluation of the cardiac effect of Several beta adrenergic blocking agents Ann. N.Y. Acad. Sci. 139 673-685.
6. Brody, T.M. & Diamond, J. (1967) Blockade of the biochemical correlates of contraction and relaxation in uterine and intestinal smooth muscle Ann. N.Y. Acad. Sci. 139, 772-780.
7. Dunlop, D. & Shanks, R.G. (1968) Selective blockade of adrenoceptive beta receptors in the heart. Brit. J. Pharmac. Chemother. 32, 201-218
8. Furchgott, R.F. (1959) The receptors of epinephrine and nerepinephrine (adrenergic receptors) Pharmac. Review 11; 429-441.
9. Furchgott, R.F. (1960) Receptors for Sympathomimetic amines. Ciba Foundation Symposium on Adrenergic Mechanisms. Edited by G.E.W. Welstanhelme & M.O'Conner PP. 246-252. Little Brown & Co. Beston. 1960.
10. Haylett, D.G. & Jenkinson, D.H. (1972) Effects of Noradrenaline on Potassium efflux, membrane potential and electrolyte levels in tissue slices prepared from guinea-pig liver. J. Physiol. London 225, 721-750
11. Jenkinson, D.H. & Morton, I.K.M. (1967) Adrenergic blocking drugs as tools in the study of the actions of Catecholamines on the Smooth muscle membrane. Ann. N.Y. Acad. Sci. 139 762-771
12. Lands, A.M. Arnold, A., McAuliff, J.P., Luduena F.P. & Brown, Jun. T.G. (1967) Differentiation of Receptor Systems activated by Sympathomimetic amines. Nature, London 214, 597-598.
13. Lum, B.K.B., Kermani, M.H., & Heilman, R.D. (1966) Intestinal relaxation produced by sympathomimetic amines in the isolated rabbit jejunum: selective inhibition by adrenergic blocking agents and by cold storage. J. Pharmac Exp. Therap. 154, 463-471.
14. Levy. B. & Ahlquist, R.P. (1961) Analysis of adrenergic blocking activity. J. Pharmac. exp. Therap. 133, 202-210.
15. Powell, C.E. & Slater, I.H. (1958) Blocking of inhibitory adrenergic receptors by a dichloro analog of isoproterenol. J. Pharmac exp. Therap. 122, 480-488.
16. Rossum, J.M. Van & Mujic, M. (1965) Classification of Sympathomimetic drugs on the rabbit intestine. Archs. int. Pharmacedyn. Therap. 155.
17. Schild H.O. (1949). Pax and competitive drug antagonism Brit. J. Pharmac. Chemother. 4, 277-280.