Br. J. Pharmac. (1975), 53, 393-401

ACTION OF β-ADRENOCEPTOR ANTAGONISTS ON THE RESPONSE TO ISOPRENALINE IN THE OESTROGEN DOMINATED RABBIT UTERUS

BRITT-INGJERD NESHEIM
Institute of Pharmacology, University of Oslo, Blindern, Oslo 3, Norway

1 Log dose-response curves to isoprenaline from spontaneously contracting muscle strips from rabbit uterus have been obtained. The effects of the α-adrenoceptor antagonists phentolamine and phenoxybenzamine, the β-adrenoceptor antagonists propranolol and practolol, and (+)-propranolol on the log dose-response curves were studied.

2 Phentolamine 5.3×10^{-7} M had a stimulating effect on the muscle strips, moving the log dose-response curve of isoprenaline to the right. Phenoxybenzamine 2.9×10^{-5} M had no effect on the curves.

3 Propranolol 3.4×10^{-6} M had no effect on the curves from circular muscle strips, with either phentolamine 5.3×10^{-7} M or with phenoxybenzamine 2.9×10^{-5} M as α-blocker. The curves from the longitudinal muscle strips were shifted somewhat to the right, the same shift, however, being obtained with (+)-propranolol 3.4×10^{-6} M.

4 Practolol 3.8×10^{-6}, 3.8×10^{-5} and 3.8×10^{-4} M was without effect on the curves, either in the circular or in the longitudinal strips.

5 It is concluded that neither propranolol at 3.4×10^{-6} M nor practolol act as β-adrenoceptor antagonists in oestrogen dominated rabbit uterus. In all other tissues investigated, propranolol or practolol block the effect of isoprenaline. Whether the effect of isoprenaline in this tissue may be termed a β-effect, is then a question of definition. In addition there is no evidence for any extraneuronal uptake mechanisms for isoprenaline.

6 Variations in sensitivity to β-stimulation with the time of the year were observed, the sensitivity being greatest in the winter and lowest in the summer.

Introduction

The effects of stimulation of the α- and β-adrenoceptors in the rabbit myometrium are well known (for references, see Marshall, 1970). Stimulation of the β-adrenoceptors invariably causes inhibition of the spontaneous activity of the uterine smooth muscle. One of the procedures used for differentiating types of adrenoceptors is a quantitative determination of the capacity of an antagonist to reduce the sensitivity of the responding tissue to an agonist (Furchgott, 1967). Pharmacological testing of this type, involving different β-adrenoceptor antagonists, has again produced evidence for the existence of different types of β-adrenoceptors in different tissues (for review, see Furchgott, 1972). Previous *in vitro* experiments have shown (Nesheim, 1972) that the longitudinal and the circular muscle strips of the oestrogen-dominated rabbit uterus react differently to β-stimulation, the longitudinal muscle being much more sensitive to β-stimulation than the circular muscle. During these studies it was discovered that the inhibitory response to the β-adrenoceptor agonist isoprenaline could not be blocked to any great extent by the β-adrenoceptor antagonist propranolol.

The experiments presented here were undertaken in order to study in more detail the effect of β-adrenoceptor antagonists and, if possible, to decide to which group of β-adrenoceptors the ones of the rabbit myometrium belong.

Methods

Sixty-four rabbits, each weighing 2-4 kg, were used for the experiments. Ovariectomy was performed through incisions of about 2 cm in the flanks, after which a tablet of 25 mg diethylstilboestrol was implanted subcutaneously. One week later the animal was given a blow on the neck and thereafter bled to death by cutting the carotid arteries. The uterus was removed immediately and placed in modified Ringer solution as described previously (Nesheim, 1972). Nineteen rabbits were

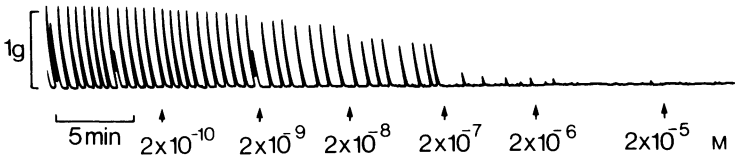

Figure 1 Tracing from an experiment on a longitudinal muscle strip from oestrogen dominated rabbit uterus. Reserpine-treated rabbit. Phenoxybenzamine present in the bath. To the left: spontaneous activity immediately before the addition of isoprenaline. At the arrows, isoprenaline was added to the bath.

given reserpine 1 mg/kg intraperitoneally or intravenously 24 h before they were killed.

Muscle strips, 10-15 mm long and 2 mm wide, were cut from the uterus in the longitudinal and the circular direction. The strips were transferred to organ baths, as described by Nesheim (1972). Muscle tension was recorded isometrically. All strips were allowed to equilibrate in the bath for at least one hour. Adrenoceptor antagonists were added immediately after the strips were mounted.

The β-adrenoceptor agonist used was isoprenaline, at concentrations of 2×10^{-11} M-2×10^{-5} M. The dose-response curves were obtained by cumulative addition of isoprenaline, always starting with the lowest concentration and multiplying it by ten with each addition of the drug. The time interval between each addition of drug was six minutes. Each muscle strip was used only once.

The muscle strips had spontaneous activity. Isoprenaline inhibited this activity (Figure 1). The degree of inhibition of the spontaneous activity caused by the β-adrenoceptor agonist was calculated by the following procedure: the height of each contraction during one 5 min interval just before any drug had been added was measured and these heights were added together. Then the heights of each of the contractions in a 5 min interval after addition of each concentration of the drug were added together. The first minute after the addition of the drug was ignored in order to allow the drug to produce its effect. The difference between the sums obtained before any drug had been added and after addition of each concentration of the drug, expressed as percentage of the sum obtained before the addition of the drug, was used as a measure of the inhibition.

As stated previously (Nesheim, 1972), the responses to a certain concentration of isoprenaline varied a great deal, both within the same rabbit and between different rabbits. The variations in response between different muscle strips from one rabbit were smaller than the variations between the different rabbits. Therefore, the median of the responses from the individual muscle strips taken from each rabbit was used in the statistical calculations. The median of these medians is plotted in the figures.

The log dose-response curves were obtained by fitting the logistic function $y = Y \cdot x^n/(x^n + k^n)$ to the experimentally determined points by a non-linear least squares method (Parker & Waud, 1971). In this equation Y = maximum response, x = drug concentration giving response y, $k = ED_{50}$, n = a power determining the slope of the curve. During the fitting procedure, all three parameters Y, k and n could be varied, or any one or two of them could be kept constant.

The experiments were done in five series. In each series the effect of one of the antagonists was compared to controls in such a way that approximately the same number of muscle strips were used with antagonist and as controls from each animal. Each experimental series consisted of 10-12 rabbits, and each rabbit yielded 30-40 muscle strips.

To estimate the effect of the β-adrenoceptor antagonists each rabbit was considered separately. For each concentration of isoprenaline the median response of all muscle strips was taken. The median log dose-response curves obtained in this way, with and without β-adrenoceptor antagonist, were compared. In order to do this the two curves were fitted simultaneously with the same n and Y. Thus for each rabbit median curves with and without β-adrenoceptor antagonist were obtained, which gave the best fit to the experimentally determined points, given that the only effect of the drug was to change ED_{50}. The ratio of ED_{50} with and without antagonist was calculated for each rabbit. Three parameters characterizing the distributions of these ratios within each experimental series are shown in Table 2. These parameters are the median (x) and the upper and lower 90% confidence limits. When the value 1.0 is included between the upper and lower confidence limits, this means that the antagonist had no statistically significant effect.

As it could not safely be assumed that the distributions of the ED_{50} ratios were symmetrical, the method described by Noether (1971) was used to find the confidence limits. The statistical

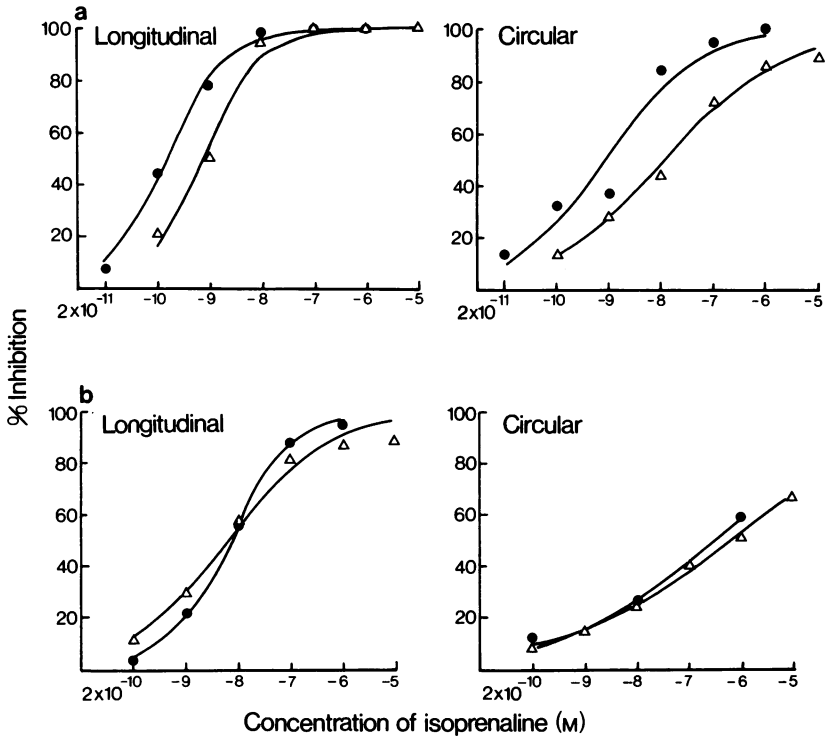

Figure 2 Log dose-response curves to isoprenaline in circular and longitudinal muscle strips from rabbit uterus. Abscissae: concentration of isoprenaline (M). Ordinates: % inhibition of spontaneous activity. (a) With phentolamine 5.3×10^{-7} M added to the bath (△); without phentolamine (●). Median results from 12 rabbits. (b) With phenoxybenzamine 2.9×10^{-5} M added to the bath (△); without phenoxybenzamine (●). Reserpine-treated rabbits. Median results from 6 rabbits.

method used for determination of the significance of the difference between the experimental log dose-response curves was a one-sided Wilcoxon–van Elteren test (van Elteren, 1960), and for determination of the significance of the difference between any two points on the curves Wilcoxon two sample test was used.

The drugs used were: isoprenaline sulphate, phentolamine (Regitin), phenoxybenzamine, (±)-propranolol (Inderal), practolol (Eraldin), (+)-propranolol and reserpine (Serpasil).

Results

Isoprenaline had some α-activity in high concentrations. When no α-adrenoceptor antagonist was present in the bath, the stimulation caused by isoprenaline 2×10^{-5} M was similar to that of isoprenaline 2×10^{-4} M when the α-adrenoceptor antagonist was present. Lower concentrations were used to study the β-activity.

Effect of phentolamine

Figure 2a shows the effect of the presence of phentolamine 5.3×10^{-7} M in the organ bath on the log dose-response curves to isoprenaline. The muscle strips were less sensitive to isoprenaline in the presence of phentolamine ($\alpha < 0.001$). Phentolamine might have been expected to leave the curves unchanged or to have shifted them in the opposite direction if isoprenaline in the concentrations used had had any α-activity and phentolamine had blocked this activity. Phentolamine therefore must have stimulating properties on the rabbit myometrium.

When all three parameters Y, k and n were allowed to vary during the curve fitting, Y ended up close to 100%. During the fitting procedure of the curves plotted in Figure 2a, Y was kept constant at 100%. The same procedure was adopted for all subsequent curves where Y ended up close to 100% (Figures 2-4).

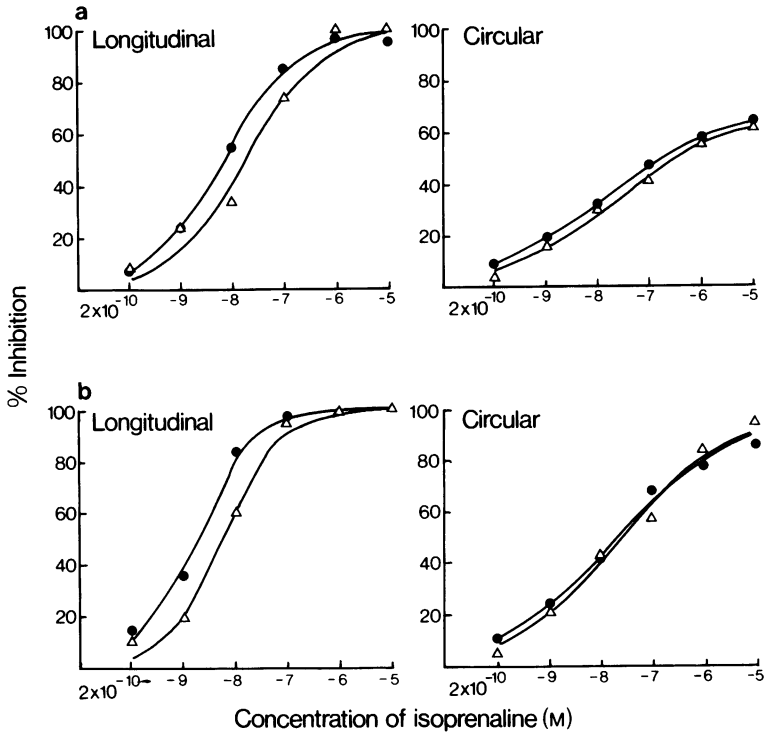

Figure 3 Abscissae and ordinates as in Figure 2. Phentolamine 5.3×10^{-7} M was always present in the organ bath. (a) Controls (•), with (±)-propranolol 3.4×10^{-6} M (△). Median results from 12 rabbits. (b) Controls (•), with (+)-propranolol 3.4×10^{-6} M (△). Median results from 10 rabbits.

Effect of phenoxybenzamine

Since phenoxybenzamine can release endogenous catecholamines in some tissues (Furchgott, 1972) the experiments were done on reserpine-treated rabbits. Figure 2b shows the effect on the log dose-response curves to isoprenaline of phenoxybenzamine 2.9×10^{-5} M. The curves are fairly similar. There is no statistically significant difference between any two points on the curves when tested with a Wilcoxon two sample test.

Effect of (±)-propranolol with phentolamine as α-blocker

Three concentrations of (±)-propranolol were used: 2.0×10^{-6}, 2.7×10^{-6} and 3.4×10^{-6} M. The two lowest concentrations had little effect on the log dose-response curves, and complete series with these concentrations were therefore not carried out. Figure 3a shows log dose-response curves to isoprenaline with and without (±)-propranolol 3.4×10^{-6} M present in the bath. Phentolamine 5.3×10^{-7} M was used to block the α-adrenoceptors.

The curves from the circular strips were very similar and were not statistically significantly different. The curve from the longitudinal strips was shifted somewhat (statistically significant) to the right, the median ratio between the ED_{50} with and without (±)-propranolol being 2.4 (Table 2).

Effect of (+)-propranolol

Figure 3b shows log dose-response curves to isoprenaline with and without (+)-propranolol 3.4×10^{-6} M present in the bath. Phentolamine 5.3×10^{-7} M was used for blocking the α-adrenoceptors.

As for (±)-propranolol, the curves from the circular strips were almost identical. (+)-Propranolol shifted the curve from the longitudinal strips to the right (statistically significant), the median ratio between the ED_{50} with and without (+)-propranolol being 8.0 (Table 2).

Effect of practolol

Practolol in the concentrations 3.8×10^{-6}, 3.8×10^{-5} and 3.8×10^{-4} M was used. Phentolamine 5.3×10^{-7} M was used for blocking the α-adrenoceptors. Tables 1 and 2 show that practolol did not move the curves significantly to the right, and larger concentrations of practolol did not give any increase in blocking effect.

Effect of (±)-propranolol with phenoxybenzamine as α-blocker

The rabbits were pretreated with reserpine. Figure 4 shows log dose-response curves to isoprenaline with and without (±)-propranolol 3.4×10^{-6} M. Phenoxybenzamine 2.9×10^{-5} M was always present in the organ bath. The curves from the circular strips were almost identical, while in the longitudinal strips propranolol shifted the log dose-response curve to the right (statistically significant), the median ratio between the ED_{50} with and without (±)-propranolol being 7.0.

Variations in sensitivity with time of the year

In Figure 5 the log dose-response curves to isoprenaline from all the control curves from the experimental series described above are shown, with reference to the time of the year. For the sake of clarity only the curves from the circular strips are shown; the longitudinal strips showed exactly the same behaviour. In addition to the results from experiments already described one curve obtained from results with reserpine-treated rabbits is shown in Figure 5.

Table 1 Values of n, ED_{50} and Y obtained by fitting the experimental log dose-response curves to the logistic function $y = Y \cdot x^n/(n^n + k^n)$ (see text).

Curves from Figure	*Muscle and treatment*	*n*	*ED_{50}*	*Y*
2a	Longitudinal, without α-blocker	0.8	3.2×10^{-10}	
	Longitudinal, with phentolamine	0.8	1.5×10^{-9}	
	Circular, without α-blocker	0.5	1.9×10^{-9}	
	Circular, with phentolamine	0.4	2.5×10^{-8}	
2b	Longitudinal, without α-blocker	0.7	1.4×10^{-8}	
	Longitudinal, with phenoxybenzamine	0.5	1.2×10^{-8}	
	Circular, without α-blocker	0.3	6.5×10^{-7}	
	Circular, with phenoxybenzamine	0.3	1.3×10^{-6}	
3a	Longitudinal, control	0.6	1.4×10^{-8}	
	Longitudinal, with (±)-propranolol	0.6	3.5×10^{-8}	
	Circular, control	0.4	2.8×10^{-8}	68%
	Circular, with (±)-propranolol	0.4	4.2×10^{-8}	66%
3b	Longitudinal, control	0.8	3.2×10^{-9}	
	Longitudinal, with (+)-propranolol	0.8	1.1×10^{-8}	
	Circular, control	0.4	4.6×10^{-8}	
	Circular, with (+)-propranolol	0.4	5.6×10^{-8}	
	Longitudinal, control	0.8	1.5×10^{-9}	
	Longitudinal, with practolol 3.8×10^{-6} M	0.8	2.2×10^{-9}	
	Circular, control	0.4	2.5×10^{-8}	
	Circular, with practolol 3.8×10^{-6} M	0.4	5.4×10^{-8}	
	Longitudinal, control	0.8	2.6×10^{-9}	
	Longitudinal, with practolol 3.8×10^{-5} M	0.8	3.8×10^{-9}	
	Longitudinal, with practolol 3.8×10^{-4} M	0.7	1.2×10^{-8}	
	Circular control	0.4	6.6×10^{-8}	
	Circular, with practolol 3.8×10^{-5} M	0.4	9.7×10^{-8}	
	Circular, with practolol 3.8×10^{-4} M	0.3	1.8×10^{-7}	
4	Longitudinal, control	0.4	2.2×10^{-8}	
	Longitudinal, with (±)-propranolol	0.4	8.8×10^{-8}	
	Circular, control	0.3	8.7×10^{-7}	
	Circular, with (±)-propranolol	0.3	1.0×10^{-6}	

Where Y is not given in the table, it was kept constant at 100%.

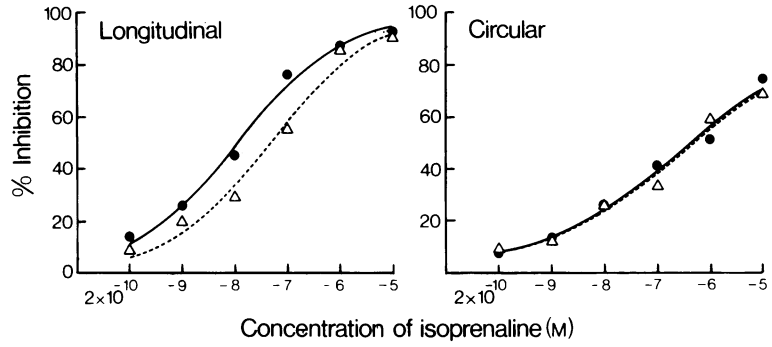

Figure 4 Abscissae and ordinates as in Figure 2. Phenoxybenzamine 2.9 x 10^{-5} M was always present in the organ bath. Controls (•), with (±)-propranolol 3.4 x 10^{-6} M (△). Reserpine-treated rabbits. Median results from 10 rabbits.

The muscle strips were most sensitive to inhibition of the spontaneous activity with isoprenaline during winter time, less sensitive in the spring and least sensitive during the summer. The differences between the curves are statistically significant ($\alpha = 0.05$ for the difference between the curves December-February 1972-73/ February-May 1973, $\alpha < 0.0001$ for the difference between the curves February-May 1973/March-June 1971).

Table 2 Median ratio of equiactive concentrations (X) of isoprenaline in presence and absence of a β-antagonist.

Muscle	*β-antagonist (M)*	*α-antagonist (M)*	*X*	*90% confidence limits*	*Number of animals*
Circular	(±)-propranolol $3.4 \cdot 10^{-6}$	phentolamine $5.3 \cdot 10^{-7}$	1.0	0.1-1.3	10
Circular	(±)-propranolol $3.4 \cdot 10^{-6}$	phenoxybenzamine $2.9 \cdot 10^{-5}$	1.1	0.8-3.0	10
Circular	(+)-propranolol $3.4 \cdot 10^{-6}$	phentolamine $5.3 \cdot 10^{-7}$	0.7	0.3-3.3	10
Longitudinal	(±)-propranolol $3.4 \cdot 10^{-6}$	phentolamine $5.3 \cdot 10^{-7}$	2.4	1.4-4.8	11
Longitudinal	(±)-propranolol $3.4 \cdot 10^{-6}$	phenoxybenzamine $2.9 \cdot 10^{-5}$	7.0	3.3-10.1	10
Longitudinal	(+)-propranolol $3.4 \cdot 10^{-6}$	phentolamine $5.3 \cdot 10^{-7}$	8.0	2.7-11.2	10
Circular	practolol $3.8 \cdot 10^{-6}$	phentolamine $5.3 \cdot 10^{-7}$	1.2	0.6-7.0	12
Circular	practolol $3.8 \cdot 10^{-5}$	phentolamine $5.3 \cdot 10^{-7}$	1.7	0.1-2.8	10
Circular	practolol $3.8 \cdot 10^{-4}$	phentolamine $5.3 \cdot 10^{-7}$	2.1	0.5-8.1	10
Longitudinal	practolol $3.8 \cdot 10^{-6}$	phentolamine $5.3 \cdot 10^{-7}$	3.4	2.5-4.0	12
Longitudinal	practolol $3.8 \cdot 10^{-5}$	phentolamine $5.3 \cdot 10^{-7}$	2.1	0.4-5.6	9
Longitudinal	practolol $3.8 \cdot 10^{-4}$	phentolamine $5.3 \cdot 10^{-7}$	0.8	0.3-1.2	9

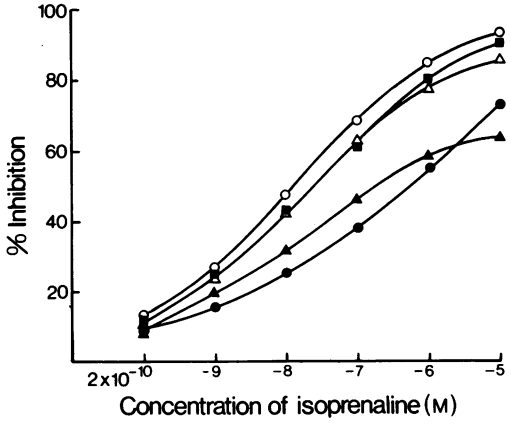

Figure 5 Variations in sensitivity with time of the year. Log dose-response curve to isoprenaline in circular muscle strips from rabbit uterus. December-February 1972-73 (○), February-May 1973 (■), January-March 1972 (rabbits were reserpine-treated) (△), March-June 1971 (▲), May-August, 1973 (reserpine-treated) (●). Phentolamine 5.3 x 10^{-7} M was present in the bath, except for (●), where phenoxybenzamine 2.9 x 10^{-5} M was used.

Discussion

This investigation has confirmed the difference between the circular and longitudinal muscle strips from the rabbit uterus in the reaction to stimulation of the β-adrenoceptors; even though the sensitivity of the strips varied with the time of the year, the longitudinal strips were always more sensitive than the circular ones (Nesheim, 1972).

In addition, it has been shown that phentolamine, but not phenoxybenzamine, reduces the sensitivity of muscle strips from the rabbit uterus to inhibition with isoprenaline. Phentolamine has been shown to have stimulating properties on muscle strips from rat uterus (Levy & Tozzi, 1963). It has never been shown to have any β-blocking activities. It is therefore reasonable to assume that the reduction in sensitivity to β-adrenoceptor stimulation by phentolamine seen in the experiments reported here was due to an unspecific stimulation of the muscle strips, although the spontaneous activity seemed to be of the same magnitude regardless of whether phentolamine was added to the bath or not.

Depending on the K_B (or pA_2) obtained with the different β-adrenoceptor antagonists in different tissues and on the relative potencies of agonists, there is evidence for multiple subgroups of β-adrenoceptors (for review and references, see Furchgott, 1972). The curves from the circular uterine muscle strips are not shifted at all with propranolol 3.4 x 10^{-6} M, and the shift that is obtained in the longitudinal strips is mimicked by the same concentration of (+)-propranolol. Both (±)- and (+)-propranolol are membrane stabilizers (Langslet, 1970). (+)-Propranolol has little, if any, β-blocking effects (Howe & Shanks, 1966). It therefore seems reasonable to assume that the shift in the log dose-response curve from the longitudinal strips was due to this unspecific membrane stabilizing effect of propranolol. It must be concluded that propranolol under these experimental conditions has no measurable β-blocking effect on the rabbit uterus. This fact places the β-adrenoceptors in the rabbit uterus in a group entirely different from those reported from other smooth muscle.

Practolol has less membrane stabilizing activity than propranolol (Hellenbrecht, Lemmer, Wiethold & Grobecker, 1973). It was therefore assumed that although practolol is a relatively selective cardiac β-adrenoceptor antagonist, its concentration might be high enough to confirm whether any β-blocking activity might be achieved at all in the rabbit uterus under these experimental conditions. The log dose-response curves were not moved significantly to the right even with these high concentrations of practolol, and larger concentrations of practolol did not have any greater effect than lower concentrations. Thus, no β-blocking ability of practolol could be demonstrated in this preparation.

There is some evidence for an active extraneuronal uptake mechanism for isoprenaline in guinea-pig trachea (Foster, 1967, 1969), this uptake mechanism being inhibited by phenoxybenzamine. Furchgott (1972) has proposed that β-adrenoceptor agonists and antagonists might compete for such uptake mechanisms; in this way an erroneously high K_B would be produced. When phenoxybenzamine was used as a combined α-blocker and blocker of eventual uptake mechanisms in these experiments, the log dose-response curve to isoprenaline did not differ from the control curve without phenoxybenzamine, and the β-blocking effect of propranolol was no greater than when phentolamine was used for blocking the α-receptors. Thus no evidence has been produced for any uptake mechanisms for isoprenaline in the rabbit uterus.

The question may be raised whether the relaxation caused by isoprenaline in the spontaneously contracting rabbit uterus may be called a β-effect at all. The answer depends on how a β-effect is defined: if the definition includes the statement that the effect can be blocked by known β-adrenoceptor antagonists, the relaxation caused by isoprenaline in this preparation cannot

be termed a β-effect. In all instances where an effect has been thought to be mediated through β-adrenoceptors, the effect has been paralleled by a rise in cyclic adenosine 3′,5′-monophosphate. This is also the case in this preparation (Nesheim, Osnes & Øye, 1975). In this respect, the relaxation of the rabbit myometrium caused by isoprenaline is similar to β-effects in all other tissues.

Propranolol is a potent, nonselective β-adrenoceptor antagonist (Bristow, Sherrod & Green, 1970; Wasserman & Levy, 1972). It has been shown to antagonize the effects of isoprenaline in isolated uterus from the rat (Diamond & Brody, 1966; Wasserman & Levy, 1972), the mouse (Magaribuchi & Osa, 1971), and in man both in the pregnant and non-pregnant state (Stander & Barden, 1966; Sullivan & Marshall, 1970; Andersson, Ingemarsson & Persson, 1973; Lossius & Nesheim, unpublished results). The lack of β-blocking activity in the rabbit uterus described here, was therefore most surprising. Willems & de Schaepdryver (1966) reported an inhibition of the response of the oestradiol dominated rabbit uterus to isoprenaline by propranolol *in vivo*. They recorded the response from the longitudinal muscles only. In their experiments propranolol completely blocked the blood pressure response to isoprenaline, while the response from the uterus was diminished (not quantitated in the paper). It might well be that this diminished response was the same as that seen in the longitudinal muscle strips in these experiments, caused by the membrane stabilizing effect of propranolol.

Practolol has no β-blocking abilities in the rat (Wasserman & Levy, 1972) or the cat (Levy, 1973) uterus. It was therefore not unexpected that practolol was also without antagonistic action in the rabbit uterus.

Variations with the time of the year of the sensitivity of the rabbit uterus to β-stimulation might be related to anovulation which has been observed from October to March, although the tendency is not absolute (Asdell, 1964). This is in very good agreement with the experience in this laboratory. However, this difference in sensitivity has no relevance to the other conclusions drawn from these experiments, since the log dose-response curves that are compared have always been obtained simultaneously from the same rabbits. It stresses the importance of making experiments at the same time of the year and, if possible, on the same animals when effects of adrenergic stimulation on smooth muscle from the reproductive tract are compared.

I would like to thank Dr Lars Walløe for helping me with the curve fitting and with the statistical methods. I am grateful to Professor Edith Bülbring for having given valuable comments on the manuscript. Financial support was provided by Norsk Medisinaldepot.

References

ANDERSSON, K.-E., INGEMARSSON, I. & PERSSON, C.G.A. (1973). Relaxing effects of β-receptor stimulators in isolated, gravid human myometrium. *Life Sciences*, **13**, 335-344.

ASDELL, S.A. (1964). *Patterns of Mammalian Reproduction*, p. 195. Ithaca, New York: Comstock Publishing Associates, Cornell University Press.

BRISTOW, M., SHERROD, T.R. & GREEN, R.D. (1970). Analysis of beta receptor drug interactions in isolated rabbit atrium, aorta, stomach and trachea. *J. Pharmac. exp. Ther.*, **171**, 52-61.

DIAMOND, J. & BRODY, T.M. (1966). Effect of catecholamines on smooth muscle motility and phosphorylase activity. *J. Pharmac. exp. Ther.*, **152**, 202-211.

ELTEREN, P.H. van (1960). On the combination of independent two sample test of Wilcoxon. *Bull. Inst. int. Statist.*, **37**, 351-361.

FOSTER, R.W. (1967). The potentiation of the responses to noradrenaline and isoprenaline of the guinea-pig isolated tracheal chain preparation by desipramine, cocaine, phentolamine, phenoxybenzamine, guanethidine, metanephrine and cooling. *Br. J. Pharmac. Chemother.*, **31**, 466-482.

FOSTER, R.W. (1969). An uptake of radioactivity from (±)-^{3}H-isoprenaline and its inhibition by drugs which potentiate the responses to (−)-^{3}H-isoprenaline in the guinea-pig isolated trachea. *Br. J. Pharmac.*, **35**, 418-427.

FURCHGOTT, R.F. (1967). The pharmacological differentiation of adrenergic receptors. *Ann. NY. Acad. Sci.*, **139**, 553-570.

FURCHGOTT, R.F. (1972). The classification of adrenoceptors (adrenergic receptors). An evaluation from the standpoint of receptor theory. In: *Handbook of Experimental Pharmacology*, Vol. 33, ed. Blaschko, H. & Muscholl, E., pp. 283-335. Berlin, Heidelberg, New York: Springer-Verlag.

HELLENBRECHT, D., LEMMER, B., WIETHOLD, G. & GROBECKER, H., (1973). Measurements of hydrophobicity, surface activity, local anesthesia, and myocardial conduction velocity as quantitative parameters of the non-specific membrane affinity of nine β-adrenergic blocking agents. *Naunyn-Schmiedeberg's Arch. Pharmac.*, **277**, 211-226.

HOWE, R. & SHANKS, R.G. (1966). Optical isomers of propranolol. *Nature, Lond.*, **210**, 1336-1338.

LANGSLET, A. (1970). Membrane stabilization and cardiac effects of d,l-propranolol, d-propranolol and chlorpromazine. *Eur. J. Pharmac.*, **13**, 6-14.

LEVY, B. (1973). Practolol blockade of cardiac but not vascular or uterine beta-adrenergic receptors in the anesthetized cat. *Arch. int. Pharmacodyn.*, **204**, 143-146.

LEVY, B. & TOZZI, S. (1963). The adrenergic receptive mechanisms of the rat uterus. *J. Pharmac. exp. Ther.*, **142**, 178-184.

MARGARIBUCHI, T. & OSA, T. (1971). Effects of catecholamines on electrical and mechanical activity of the pregnant mouse myometrium. *Jap. J. Physiol.*, **21**, 627-643.

MARSHALL, J.M. (1970). Adrenergic innervation of the female reproductive tract: Anatomy, physiology and pharmacology. *Ergebn. Physiol.*, **62**, 6-67.

NESHEIM, B.-I. (1972). Effect of noradrenaline and isoprenaline on the circular and longitudinal muscle of the oestrogen dominated rabbit uterus. *Acta pharmac. tox.*, **31**, 296-304.

NESHEIM, B.-I., OSNES, J.-B. & ØYE, I. (1975). Role of cyclic adenosine 3',5'-monophosphate in the isoprenaline-induced relaxation of the oestrogen dominated rabbit uterus. *Br. J. Pharmac.*, **53**, 403-407.

NOETHER, G. (1971). *Introduction to Statistics. A fresh approach.* p. 107. Boston: Houghton Mifflin Company.

PARKER, R.B. & WAUD, D.R. (1971). Pharmacological estimation of drug-receptor dissociation constants. Statistical evaluation. I. Agonists. *J. Pharmac. exp. Ther.*, **177**, 1-12.

STANDER, R.W. & BARDEN, T.B. (1966). Adrenergic receptor activity of catecholamines in human gestational myometrium. *Obst. Gyn.*, **28**, 767-774.

SULLIVAN, S.F. & MARSHALL, J.M. (1970). Quantitative evaluation of exogenous amines on the contractility of human myometrium in vitro. *Amer. J. Obstet. Gynec.*, **107**, 139-148.

WASSERMAN, M.A. & LEVY, B. (1972). Selective beta adrenergic receptor blockade in the rat. *J. Pharmac. exp. Ther.*, **182**, 256-263.

WAUD, D.R. & PARKER, R.B. (1971). Pharmacological estimation of drug-receptor dissociation constants. Statistical evaluation. II. Competitive antagonists. *J. Pharmac. exp. Ther.*, **177**, 13-24.

WILLEMS, J.L. & SCHAEPDRYVER, A.F. de (1966). Adrenergic receptors in the oestradiol and allyl-oestrenol dominated rabbit uterus. *Archs. int. Pharmac. Ther.*, **161**, 269-274.

(Received June 4, 1974)
(Revised August 23, 1974)