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ERRORS IN THE MEASUREMENT OF AGONIST POTENCY-RATIOS PRODUCED BY UPTAKE PROCESSES: A GENERAL MODEL APPLIED TO β-ADRENOCEPTOR AGONISTS

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1 The sensitization of guinea-pig atria and trachea to noradrenaline, isoprenaline, and salbutamol, produced by an inhibitor of neuronal (cocaine) and extraneuronal (metanephrine) uptake, was studied quantitatively. The data were compared to a theoretical model.

2 Cocaine produced near maximal sensitization to noradrenaline in guinea-pig atria (5 fold) at concentrations which produced only partial sensitization in guinea-pig trachea (4.7 fold sensitization of a maximum 11 fold). These results agreed with the model which predicts that there is a direct relationship between the amount of uptake inhibitor required to produce full sensitization and the magnitude of maximal sensitization demonstrable in the tissue. This makes extrapolation of uptake inhibition concentrations from tissue to tissue a potentially erroneous practice.

3 In normal trachea, salbutamol is 20 times more potent than noradrenaline but this difference is abolished (to 0.9 times) by cocaine (100 μM). This reduction of potency-ratio is due to the selective cocaine-induced sensitization of trachea to noradrenaline and raises a serious objection to the classification of salbutamol as a β_2 selective agonist.

4 Metanephrine produced very little sensitization of trachea to isoprenaline. Experiments with salbutamol showed metanephrine to be a simple competitive antagonist of β-adrenoceptors ($pK_b = 4.3$) and that this receptor antagonism masked sensitization to isoprenaline.

5 A theoretical model indicates that an inhibitor of agonist uptake requires a remarkable degree of selectivity for the uptake mechanism (i.e. K_b for receptors $10^4 \times K_I$ for uptake sites) to demonstrate tissue sensitization to the agonist. This analysis and the data with metanephrine indicate that a sinistral shift of the concentration-response curve is a poor indicator of the importance of uptake mechanisms in an isolated tissue.

6 An alternate method to determine the importance of agonist-uptake effects on concentration-response curves is described which utilizes agonist potency ratios. Agonist potency ratios in guinea-pig atria and trachea showed that the bronchoselectivity demonstrated by salbutamol (with respect to isoprenaline) is reduced from 54 to 7.8 by metanephrine reflecting the importance of extraneuronal uptake in trachea.

Introduction

Agonist potency ratios are one of the most powerful tools available for receptor classification. Determined by the relative position of concentration-response curves along the concentration axis, agonist potency ratios are parameters subject to the efficacy, equilibrium dissociation constant, and receptor-compartment concentration of the agonist. Apparent differences in agonist potencies, resulting from selective removal of agonists from the receptor compartment, reflect differences in the way organs respond to drugs and not differences in receptors. The minimization of these effects of removal mechanisms on agonist potencies must be achieved before agonist potency ratios can be considered valuable data for receptor classification (Furchgott, 1972). This paper describes quantitatively, the sensitization of guinea-pig atria and trachea to β-adrenoceptor agonists by inhibitors of agonist removal and compares the data to a theoretical model. The analysis provides general concepts that may be useful in experiments aimed at receptor-classification.

Methods

Guinea-pig atria

The hearts from male guinea-pigs (Hartley, 400 g), killed by cervical dislocation, were quickly excised

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and placed in cold oxygenated Krebs-Henseleit solution of the following composition (mM): Na^+ 143, K^+ 5.9, Mg^{2+} 1.2, Ca^{2+} 2.6, Cl^- 128, $H_2PO_4^-$ 24.9, SO_4^{2-} 1.2, HCO_3^- 25, D-glucose 10. The right atria were dissected free and mounted under 1 g tension in 17 ml organ baths filled with oxygenated solution at pH 7.2 and 34°C. The left atria were dissected free, the inner atrial wall removed, and the outer wall mounted under 1 g tension in tissue holders such that the outer surface rested on a single platinum punctate electrode milled flush with the surface (Blinks, 1965). Stimuli were delivered through the punctate electrode and an external platinum electrode in the bath fluid from a Grass S88 stimulator (1 Hz, 5 ms duration, threshold +30% voltage). Contractions of both right and left atria were transmitted via Grass FT 0.03 isometric transducers and displayed on a Beckman R-511A potentiometric chart recorder. Right atrial rate was processed from a Beckman 9857B rate meter and recorded as beats per minute and left atrial force, processed from a Beckman 9853A coupler, was recorded in g tension.

Guinea-pig trachea

The tracheae from male guinea-pigs (Hartley, 400 g) killed by cervical dislocation, were removed and placed in cold oxygenated physiological salt solution of the following composition (mM): Na^+ 144, K^+ 6, Mg^{2+} 1.2, Ca^{2+} 2.5, Cl^- 128, $H_2PO_4^-$ 1.0, HCO_3^- 25, SO_4^{2-} 1.2, D-glucose 11. The tracheae were trimmed of fatty tissue, cut transversely into rings comprising two tracheal ring sections, and the cartilage portion of the rings dissected away from the smooth muscle. The isolated tissue preparations used in this study were composed of a section of the smooth muscle from trachea connected to approx. 2 mm of cartilage at each end, in which notches had been cut to hold 5-0 silk thread. The tracheal smooth muscle segments were mounted under 1 g tension in 17 ml organ baths, filled with oxygenated solution at pH 7.2 and 37°C, such that contractions and relaxations could be detected by a grass FT. 0.03 isometric transducer and recorded, in g tension, on a Beckman R-511A potentiometric chart recorder (Beckman 9853A coupler). Phentolamine (3 μM) and disodium edetate (EDTA, 10 μM) were present in the bathing solution at all times to avoid complicating effects of α-adrenoceptor activation and catecholamine oxidation (Hughes, 1978), respectively, on β-adrenoceptor responses. Tracheae were contracted by the muscarinic agonist, bethanechol (10 μM), before relaxations to β-agonists were measured.

Concentration-response curves

All concentration-response curves were obtained in a cumulative manner (Van Rossum, 1963) at increments of 0.5 log units. Atrial and tracheal tissues were equilibrated in the organ baths for 1 h, with changes of bath solution every 15 min, before the start of experiments. Measurements of agonist potencies were obtained from complete concentration-response curves in all experiments. Concentration-response curves before and after incubation of tissues with cocaine or metanephrine did not deviate from parallelism; therefore, sensitization, denoted by x, to agonists was measured by the shift to the left of the curves and expressed as a multiple decrease in the concentration of agonist producing half the maximal response. Comparison of the experimental data with the model made accurate determinations of potency ratios mandatory, therefore frequent parallel control tissues were monitored to detect spontaneous changes in tissue sensitivity to agonists. Test tissues were not corrected for spontaneous changes in tissue sensitivity of control tissues but rather the results were used only from those experiments in which the control tissues showed less than 20% deviation from the initial concentration-response curves throughout the experiment.

Measurement of receptor antagonism

Compliance to simple competitive antagonism was tested by comparison of equiactive dose-ratios of agonists (denoted by dr), obtained in the presence of antagonist (B), to the Schild equation for simple competitive antagonism (Arunlakshana & Schild, 1959):

$$\log(dr - 1) = n \log[B] - \log K_B \qquad (1)$$

Statistical calculations

All agonist potencies are expressed as the geometric mean of the negative log molar concentrations producing half the maximal response to the agonist. Comparisons of Schild regressions for metanephrine on atria and trachea were carried out according to the analysis of covariance of regression lines (Snedecor & Cochran, 1967). This statistical comparison yields variance ratios (F) to determine whether or not calculated regression lines are different in slope and elevation respectively.

Drugs

Drugs used in these experiments were hydrochlorides of (−)-isoprenaline (Sigma Chemical Co.), (−)-noradrenaline (Sigma Chemical Co.), cocaine (Mallinckrodt Co.) and (±)-metanephrine (Sigma Chemical Co.), and EDTA (ethylendiamine-tetra-acetic acid disodium salt, Matheson Coleman and Bell Co.). I wish to thank CIBA Pharmaceutical Co. for the gift of phen-

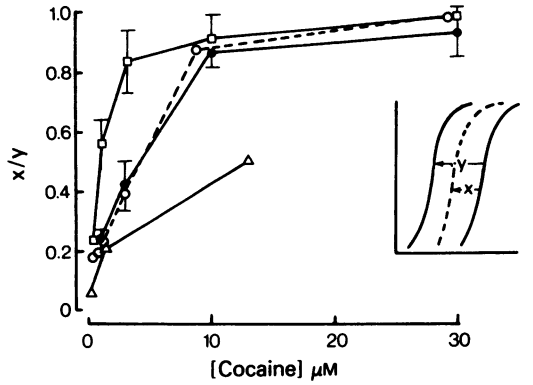

Figure 1 Sensitization of guinea-pig tissues to noradrenaline as a function of cocaine concentration. Ordinates; sensitization (x) expressed as a fraction of the maximal sensitization (y) obtainable in a particular tissue. Abscissae: molar concentration of cocaine. Inset: Illustration of the measurement of x and y from noradrenaline concentration-response curves (ordinates: fraction of maximal responses to noradrenaline, abscissae; log molar concentrations of noradrenaline). (□) Guinea-pig paced left atria, y = 5, n = 20; (●) guinea-pig trachea, y = 11, n = 15; (○) guinea-pig right atria, data recalculated from Trendelenburg, 1968, y = 13; (△) guinea-pig trachea, data recalculated from Foster, 1967, y = 36. Bars represent s.e. means.

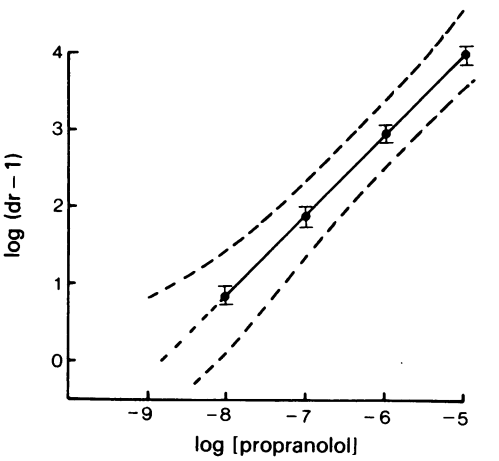

Figure 2 Schild regression for propranolol in guinea-pig trachea with salbutamol as the agonist. Ordinates; equiactive concentration-ratios of salbutamol minus one (log scale). Abscissae: log molar concentrations of propranolol. Heavy broken line shows extrapolation of the regression to the pK_B. Curved broken lines represent 95% confidence limits for the slope of the regression. Bars represent s.e. means; n = 12.

tolamine hydrochloride, Schering Corp. for the gift of salbutamol, and A.B. Hässle for the gift of terbutaline.

Results

Sensitization to noradrenaline produced by cocaine

Cocaine produced transient positive inotropic and chronotropic responses in atria but no lasting change in basal responses in either atrial or tracheal tissues. Concentrations of cocaine of 1 μM to 100 μM produced a concentration-dependent sensitization of both tissues to noradrenaline. Sensitizations (x) to noradrenaline were measured from complete concentration-response curves and were expressed as a fraction of the observed maximal sensitization for that tissue (denoted by y). The relationship of x/y to concentrations of cocaine for the three tissue preparations is shown in Figure 1. Also included in Figure 1, for comparison, are data recalculated from results of two other authors, a study of cocaine-induced sensitization to noradrenaline on guinea-pig atria (chronotropic responses) by Trendelenburg (1968) and sensitizations of guinea-pig trachea by Foster (1967). Figure 1 shows that different tissues, given the same concentration of cocaine, do not sensitize to the same extent and that the greater the magnitude of maximal effect of cocaine (y), the more cocaine is required to reach full sensitization.

Since blockade of agonist removal, (in this case most probably, neuronal uptake) produced variable sensitization of concentration-response curves to noradrenaline, it followed that this could in turn alter the potency-ratios of noradrenaline and an agonist not taken up by the tissue. To test this hypothesis salbutamol was used as a β-adrenoceptor agonist which is not a substrate of any removal mechanism in these tissues.

Salbutamol as an agonist in trachea

Cocaine produced no sensitization of trachea to salbutamol at concentrations up to 100 μM. At higher concentrations variable effects were observed from sensitization (two to four fold) to depression of maximal response (to 50% of control values). Propranolol produced a simple competitive inhibition of salbutamol in guinea-pig trachea as shown by the linear Schild regression with slope not significantly different from unity (1.1, 95% limits 1.0 to 1.2), see Figure 2. These results indicated that, in the concentration range used in these experiments, salbutamol was not removed at any appreciable rate from the receptor compartment.

Potency ratios of β-adrenoceptor agonists in trachea

Potency ratios (pr) of noradrenaline and salbutamol,

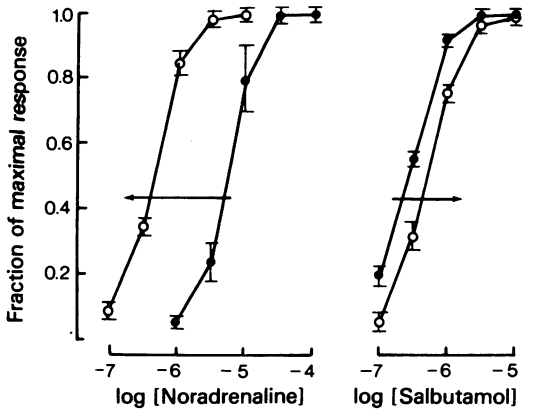

Figure 3 Effect of cocaine (100 μM) on concentration-response curves to noradrenaline and salbutamol in guinea-pig trachea. Ordinates; relaxation of trachea as a fraction of maximal relaxation to the agonist. Abscissae; molar concentrations of agonist (log scale). Responses in the absence (●) and presence (○) of cocaine 100 μM. Concentration-response curves for each agonist obtained in the same tissue, $n = 6$. Bars represent s.e. means. Cocaine produced a shift to the left of the concentration-response curve to noradrenaline and a slight shift to the right for salbutamol.

measured as concentrations of agonist producing half the maximal response, were obtained in the absence and presence of cocaine (1 μM to 100 μM). Figure 3 illustrates the selective sensitization of the trachea to noradrenaline produced by cocaine in the same tracheal preparation. In trachea not pretreated with cocaine, salbutamol is 20 times more potent than noradrenaline while in the presence of 100 μM cocaine, salbutamol is only 0.9 times as potent. No appreciable change in the potency-ratios of salbutamol and terbutaline, another β-adrenoceptor agonist which is not a substrate for neuronal uptake, was produced by cocaine.

The potency-ratio profiles for isoprenaline, noradrenaline and salbutamol in the absence and presence of cocaine are given in Table 1. In normal trachea the potency ratio profiles of isoprenaline, noradrenaline, and salbutamol are in accordance with what has been accepted for a β_2-adrenoceptor organ (Levy & Apperley, 1978). However, there is a striking change in the relative potencies of salbutamol and noradrenaline after cocaine which presents the paradox that salbutamol, an ostensibly β_2 selective agonist, is equipotent with noradrenaline, an ostensibly β_1 selective agonist.

These results show that the selective removal of noradrenaline from the receptor compartment could apparently endue salbutamol with selectivity for the trachea. This has implications for the bronchoselectivity of salbutamol as calculated by the potency-ratio method in trachea and atria. Therefore, the previous analysis was applied to the extraneuronal uptake of isoprenaline in guinea-pig atria and trachea and the calculation of bronchoselectivity for salbutamol. The inhibitor of extraneuronal uptake chosen was metanephrine.

Sensitization of trachea to isoprenaline by metanephrine

Metanephrine (10 μM to 1 mM) had no effect on tracheal tone but did slightly sensitize the tissues to isoprenaline at concentrations of 10 μM to 100 μM, see Figure 4. However, 1 mM metanephrine produced a mean shift to the right of the isoprenaline concentration-response curve of 0.4 ± 0.15 log units. This suggested the possibility that metanephrine antagonizes the β-adrenoceptor at high concentrations and that this antagonism is masked by a concomitant sensitization resulting from blockade of extraneuronal uptake. Therefore the antagonism of salbutamol, not a substrate for uptake in this tissue, by metanephrine was investigated.

Metanephrine as an antagonist of β-adrenoceptors in trachea

Metanephrine produced a concentration-dependent

Table 1 Potency ratios for β-adrenoceptor agonists in guinea-pig trachea

	Normal trachea			*Cocaine* (100 μM)		
Agonists†:	*Iso*	*> Sal >*	*NA*	*Iso*	*> NA =*	*Sal*
$-\log(EC_{50})$	7.9 (7.6 to 8.2)	6.7 (6.4 to 7.0)	5.4 (5.0 to 5.8)	7.8 (7.5 to 8.1)	6.54 (6.2 to 6.7)	6.5 (6.2 to 6.8)
n	15	25	25	4	6	6
Potency-ratios	1	15.8	316	1	18	20

†Iso = isoprenaline; Sal = salbutamol; NA = noradrenaline.
$-\log(EC_{50})$ = negative log of the concentration of agonist required to produce half the maximal response.
Values in parentheses represent 95% confidence limits. Potency-ratios for salbutamol and noradrenaline were always determined in the same tracheal preparation.

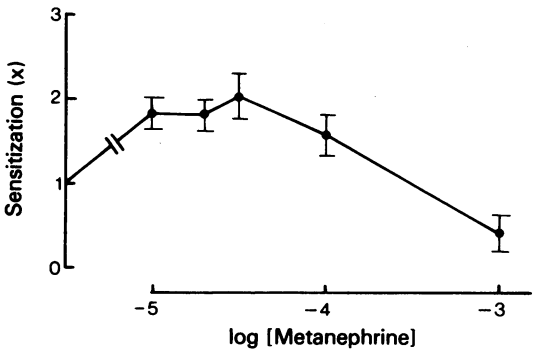

Figure 4 Maximal sensitization of isoprenaline concentration-response curves as a function of concentrations of metanephrine. Ordinates; sensitization expressed as the antilog of the shift to the left of the concentration of isoprenaline required for half the maximal response along the log scale of the concentration axis. Abscissae; molar concentration of metanephrine (log scale). Values greater than unity represent sensitization, less than unity, receptor antagonism. Bars represent s.e. means, $n = 14$.

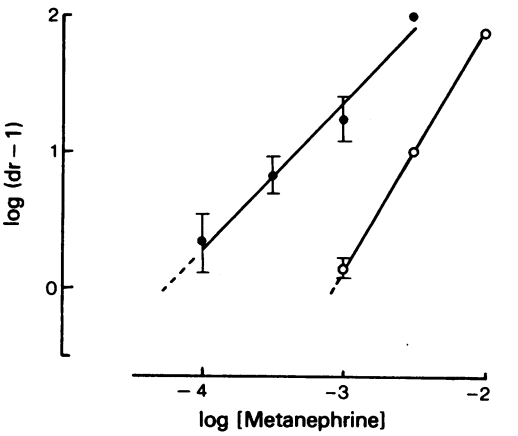

Figure 5 Schild regressions for metanephrine in guinea-pig trachea, salbutamol and isoprenaline as the agonists. Ordinates; equiactive concentration ratios of agonist minus one (log scale). Abscissae; log molar concentration of metanephrine. (●) Salbutamol as the agonist, $n = 12$; (○) isoprenaline as the agonist, $n = 8$. Bars represent s.e. means.

simple competitive antagonism of salbutamol. The Schild regression, shown in Figure 5, is linear, has a slope not significantly different from unity (1.0, 95% limits 0.8 to 1.3) and indicates that the pK_B (minus log dissociation constant) of metanephrine for β-adrenoceptors is 4.3 (95% limits 3.0 to 5.6). Concentrations of metanephrine of 1 mM to 10 mM antagonized isoprenaline responses as well but the Schild regression suggested something more than only simply competitive antagonism (slope = 1.4, 95% limits 0.9 to 1.9), most likely a cancellation of observed antagonism by concomitant sensitization to isoprenaline.

The antagonism of salbutamol and isoprenaline by metanephrine was also measured in guinea-pig isolated right atria. In this tissue extraneuronal uptake of isoprenaline, although demonstrable by biochemical techniques (Bönisch & Trendelenburg, 1974) is of insufficient activity to produce alteration in receptor compartment concentrations of isoprenaline (Wöppel & Trendelenburg, 1973; Kenakin & Black, 1978). In accordance with these data, metanephrine produced only receptor antagonism of isoprenaline and salbutamol and with these agonists, pK_B values for metanephrine of 4.0 and 4.5 were obtained with isoprenaline and salbutamol as the agonists respectively. An analysis of covariance indicated that the Schild regressions for metanephrine in atria were not significantly different from that in trachea (isoprenaline/atria vs salbutamol/trachea, slope, $F = 0.2$, d.f. = 1, 13; elevation, $F = 9$, d.f. = 1, 14; salbutamol/atria vs salbutamol/trachea, slope, $F = 0.1$, d.f. = 1, 16; elevation, $F = 1.5$, d.f. 1, 17). This indicates that metanephrine antagonism of receptors in atria was indistinguishable from that in trachea.

Salbutamol/isoprenaline potency ratios

As was observed with salbutamol/noradrenaline potency ratios and cocaine, metanephrine (100 μM) produced an 8 fold change in the potency ratio of salbutamol and isoprenaline in the guinea-pig trachea. The changes in potency ratio, as a function of concentrations of metanephrine, is shown in Figure 6. A maximal sensitization of 8.0 would have been observed for isoprenaline in the presence of 100 μM metanephrine had the receptor blocking property of this uptake inhibitor not produced an opposing effect. As shown in Figure 6, the potency ratios for salbutamol and isoprenaline in atria did not change with increasing metanephrine concentrations. Therefore, the relative activity of these agonists was selectively altered by extraneuronal uptake in trachea, making comparison of potencies between atria and trachea subject to extraneuronal uptake of isoprenaline. This in turn would introduce errors into the determination of the bronchoselectivity of salbutamol.

Bronchoselectivity of salbutamol

The potency of salbutamol, relative to isoprenaline, is greater in guinea-pig trachea than in guinea-pig atria. If this is a result of agonist activities on β-adrenoceptors only, it would be grounds for the statement that salbutamol demonstrates receptor bronchoselectivity.

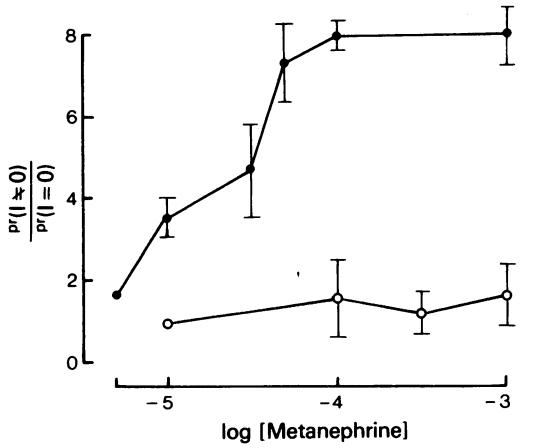

Figure 6 Potency-ratios of isoprenaline and salbutamol produced by various concentrations of metanephrine. Ordinates; isoprenaline/salbutamol potency ratios (pr) measured in the presence of various concentrations of metanephrine divided by the pr in the absence of metanephrine. Abscissae; molar concentrations of metanephrine (log scale). Data obtained from guinea-pig trachea (●), $n = 22$, and guinea-pig atria (○), $n = 8$. Bars represent s.e. means.

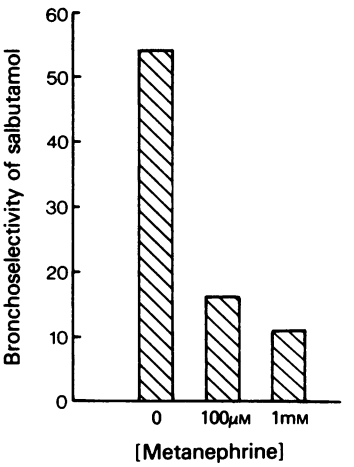

Figure 7 Histogram showing the bronchoselectivity of salbutamol in guinea-pig tissues in the absence and presence of metanephrine. Histograms show bronchoselectivity of salbutamol calculated by the ratio of the pr of this agonist and isoprenaline in guinea-pig trachea and atria. In the absence of metanephrine, salbutamol is 54 times more potent, with respect to isoprenaline, on trachea than atria. Metanephrine (100 μM) reduced this bronchoselectivity to 7.8. A higher metanephrine concentration (1 mM) had little further effect (bronchoselectivity = 8.5).

However, if part of the bronchoselectivity of salbutamol is due to a selective diminution, by extraneuronal uptake, of isoprenaline potency in guinea-pig trachea, then the true measure of bronchoselectivity is the ratio of the potency ratios of the agonists in the two tissues after inhibition of extraneuronal uptake. Figure 7 shows the observed bronchoselectivity of salbutamol as a function of metanephrine concentration. Whereas salbutamol appears to be 54 times more potent in trachea than in atria (relative to isoprenaline) in the absence of metanephrine, blockade of extraneuronal uptake by this drug reduces this bronchoselectivity to 7.8.

Like cocaine, metanephrine altered potency ratios of agonists but unlike cocaine, it did not produce a sizable sensitization of isoprenaline responses to account for the change in potency ratios. The sensitization is masked by the receptor blocking activity of metanephrine.

A model of sensitization in isolated tissues

Mathematical models describing the effects of agonist removal mechanisms on concentration-response curves have been presented elsewhere (Langer & Trendelenburg, 1969; Waud, 1969; Brimijoin, Pluchino & Trendelenburg, 1970; Furchgott, 1972). Working within the framework of existing models the three main experimental findings described in this paper can be predicted mathematically.

(a) The dependence of sensitization on the concentration of uptake inhibitor [I] is intimately related to y, the maximal sensitization obtainable (this effect was shown experimentally in Figure 1).

Sensitization of tissues (denoted by x) can be calculated by the following equation derived in the appendix):

$$x = \frac{y(1 + [I]/K_I + [A]_b/K_{AR})}{y + [I]/K_I + [A]_b/K_{AR}} \qquad (2)$$

where y is the maximal sensitization, [I] the concentration of uptake inhibitor, $[A]_b$ the concentration of agonist in the receptor compartment, K_I the equilibrium dissociation constants of the uptake inhibitor of the site of uptake and the K_{AR} the equilibrium-dissociation constant of the agonist for the site of removal.

Figure 8 illustrates the calculated sensitization (expressed as x/y) as a function of $[I]/K_I$ where it can be seen that the greater the maximal sensitization observed in a given system, the more inhibitor of removal is required to achieve full effect. This agrees with the observations for cocaine in guinea-pig tissues (Figure 1).

(b) Any receptor-blocking property of an inhibitor of agonist uptake will completely mask sensitization (see Figure 4) and in fact, a remarkable degree of

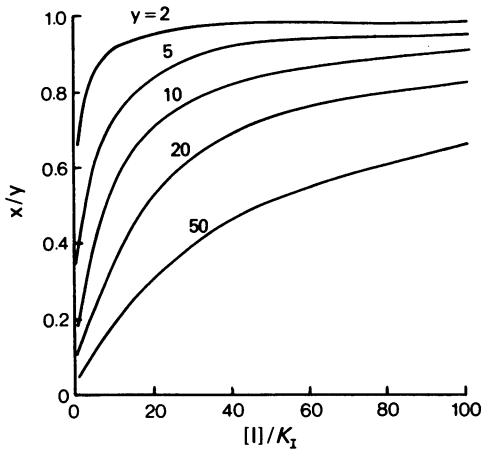

Figure 8 Sensitization of concentration-response curves as a function of uptake inhibitor concentration. Ordinates; sensitizations of concentration-response curves, at half maximal responses (x), expressed as a fraction of the maximally observed sensitization, y. Abscissae; molar concentrations of uptake inhibitor as a fraction of the equilibrium dissociation constant of the inhibitor for the site of removal. Numbers next to the lines represent the value of y, the maximal sensitization. Note how as y increases, greater concentrations of [I] are required to produce comparable fractional sensitization.

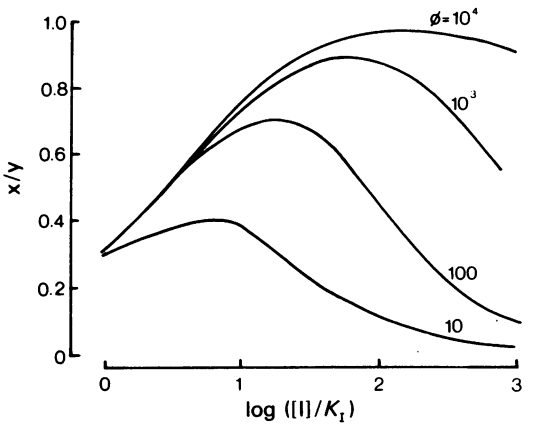

Figure 9 Sensitization produced by inhibitors of agonist removal possessing receptor blocking properties. Ordinates; sensitization expressed as a fraction of the true maximal sensitization (calculated for y = 5). Abscissae; concentrations of uptake inhibitor as a fraction of K_I (log scale). Numbers to the left of the lines indicate ϕ (K_B/K_I). Note how the maximal sensitization is not approached unless $\phi > 10^4$.

selectivity for the uptake site may be required to demonstrate sensitization.

The following equation (derived from the model) was used to calculate sensitization (x) to an agonist produced by an inhibition of agonist uptake with blocking activity at the agonist receptors (measurable equilibrium dissociation constant for the receptor, K_{BI}):

$$x = \frac{y(1 + [I]/K_I + [A]_b/K_{AR})}{(y + [I]/K_I + [A]_b/K_{AR})(1 + [I]/K_I \cdot 1/\phi} \quad (3)$$

where $\phi = K_{BI}/K_I$.

Considering a theoretical system where y = 5, Figure 9 shows sensitizations (calculated from equation 3), expressed as a fraction of the maximal sensitization, as a function of I/K_I. It can be seen that unless $\phi > 10^4$, the maximal sensitization will never be observed. This makes the observed tissue sensitization after uptake removal a poor index for the importance of uptake systems on concentration-response curves. In the case of metanephrine where ϕ = 10 to 20, very little sensitization would be predicted (Figure 9), and in fact very little was observed (Figure 4).

(c) The potenty-ratios of two agonists taken up by a tissue at different rates depends not only upon $[I]/K_I$ but also upon y, the maximal sensitization obtainable for each agonist. The following equation was derived from the model:

$$\frac{pr_{(observed)}}{pr_{(true)}} = \frac{y'' + [I]/K_I}{y + [I]/K_I} \quad (4)$$

Thus, the observed potency ratio will be in error by a factor determined by differences (reflected by y''/y) in extra-receptor uptake of the agonists in the receptor compartment. As the removal mechanism becomes inhibited, $(y'' + [I]/K_I)/(y + [I]/K_I)$ approaches unity and $pr_{(observed)} = pr_{(true)}$, i.e. the observed potency ratio reflects the true potency ratio in the receptor compartment. Figure 10 shows the calculated changes in potency ratios of two agonists in four different tissues, one not taken up by the tissues (y'' = 1, like salbutamol) and one taken up to different maximal sensitizations (y = 5 to 50, like noradrenaline). The larger the difference between y'' and y, the more uptake inhibitor is required to visualize the true potency ratio. The dependence of the potency-ratios of noradrenaline and salbutamol on metanephrine concentration was shown experimentally in Figure 6.

Discussion

The quantitative analysis of cocaine-induced sensitization of guinea-pig trachea and atria to noradrenaline shows that concentrations used in pharmacological experiments to block uptake processes completely

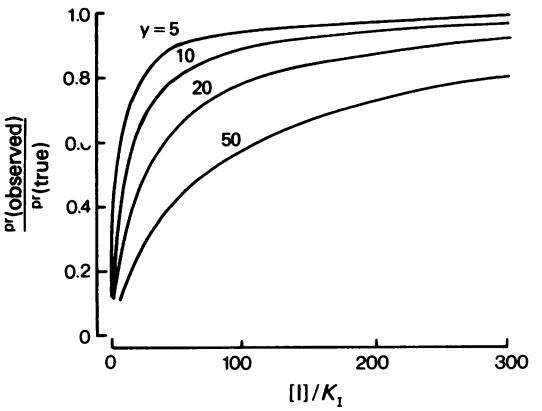

Figure 10 Theoretically predicted changes in the potency-ratios of two agonists, one removed and one not removed, by an active mechanism in the receptor compartment, as a function of uptake inhibition. Ordinates; potency ratios obtained after partial inhibition of agonist removal as a fraction of the true potency ratio. Abscissae; concentrations of uptake inhibitor as a fraction of the equilibrium dissociation constant of the inhibitor for the site of removal (K_I). Numbers next to the line represent values of y for the agonist removed by the uptake mechanism. Note how a greater concentration of uptake inhibitor is required to normalize the observed pr to the true pr when the agonist taken up is more severely modulated by the removal mechanism (larger y).

may not be extrapolated from tissue to tissue. The reason for this lies in the factors governing the maximal effects of uptake on concentration-response curves (y) in different tissues. The rate of diffusion of agonist into the receptor compartment, the relative geometry of the receptors and sites of agonist removal (for example, the neuro-muscular interval, Trendelenburg, 1972; Guimaraes & Paiva, 1977), the volume/surface ratio of isolated tissue preparations (Ebner & Waud, 1978), and the concentrations of agonist utilized to elicit response compared to the K_m for the removal process ($[A]_a/K_m$ ratio) all can affect the observed maximal effects of agonist removal on concentration-response curves. Some of these factors may not even be uniform within a sample of a given isolated tissue. Therefore, it is probably not correct to assume that for every tissue there is a constant value of y. For example, the maximal effect of extraneuronal uptake on isoprenaline responses of guinea-pig trachea appears to be quite variable (y = 5, O'Donnell & Wanstall, 1976; y = 6, Geddes, Jones, Dvorsky & Lefcoe, 1974; y = 8.5, this paper; y = 30, Foster, 1967). The fact that the tissue preparations could have been contracted to different extents therefore modifying $[A]_a/K_m$ ratios, and that methods of preparation, age, and size of the animals can affect volume/surface ratios and diffusion characteristics of isoprenaline into the tissue, all suggest that this variation in estimates of y can be accounted for and perhaps expected. Therefore, although a given concentration of uptake inhibitor produces a given inhibition of the uptake process, the above mentioned processes, which can vary from tissue to tissue, determine the magnitude of effect of the remaining fraction of active removal of agonist.

From the studies of Lands and co-workers (Lands, Arnold, McAuliff, Luduena & Brown, 1967a; Lands, Luduena & Buzzo, 1967b) noradrenaline has been classified as a β_1 selective agonist and the guinea-pig trachea a predominantly β_2 selective organ. The subsequent finding that salbutamol is more potent than noradrenaline in guinea-pig trachea has led to the assumption that salbutamol is a β_2 selective agonist. The reduction of the pr of noradrenaline and salbutamol by cocaine suggests that the higher potency of salbutamol relative to noradrenaline is an artifact of neuronal uptake. While it can be argued that noradrenaline and salbutamol act on a mixed β_1 and β_2 receptor population in guinea-pig trachea (Furchgott, Wakade, Sorace & Stollak, 1975; Furchgott & Wakade, 1975), this argument removes the definitive criterion on which salbutamol was originally classified as β_2 selective, namely that the guinea-pig trachea is a solely β_2 receptor containing organ. Thus salbutamol may appear to be more active than noradrenaline in trachea by virtue of the fact that it is not a substrate for neuronal uptake. This is important for receptor classification in other organs where salbutamol is used to classify beta-adrenoceptors.

The fact that salbutamol was 54 times more active in trachea than atria relative to isoprenaline indicates a functional bronchoselectivity. Since this observed bronchoselectivity was reduced to 7.8 fold by metanephrine, a large portion of the bronchoselectivity was due to the proportionately greater effect of extraneuronal uptake on isoprenaline concentration-response curves in trachea and to the fact that salbutamol is not a substrate for extraneuronal uptake.

The effects of metanephrine on isoprenaline concentration-response curves in trachea illustrate an important point, namely that uptake inhibitor-induced sensitization of tissues is a poor criterion on which to evaluate the importance of removal mechanisms on concentration-response curves. Metanephrine, shown to be a potent inhibitor of extraneuronal uptake of catecholamines in rat heart (Burgen & Iversen, 1965), failed to produce a sizable sensitization of concentration-response curves to isoprenaline in guinea-pig trachea, previously shown to possess an active metanephrine-sensitive uptake mechanism for isoprenaline (Foster, 1969). The competitive inhibition of salbutamol by metanephrine showed that the sensitization to isoprenaline was being masked by receptor-antag-

onism. The method of agonist potency ratios eliminates this problem since the receptor antagonism effects cancel. The calculated effect of extraneuronal uptake by this method (y = 8) agrees approximately with the apparent loss of bronchoselectivity of salbutamol (54 to 7.8). The theoretical analysis of the receptor effects of uptake inhibitors indicates that uptake inhibitors must possess a remarkable degree of selectivity to produce sensitization of the concentration-response curve. Thus for metanephrine pK_I is 5.3 to 5.6 and pK_B for this agent on β-adrenoceptors is 4.3 yielding a calculated selectivity for uptake sites of $K_B/K_I = \phi = 10$ to 20. This is well below the theoretical limit of $\phi \geq 10^4$ required to show complete sensitization of concentration-response curves.

In conclusion, it has been shown that agonist uptake processes in isolated tissues can produce artifacts that can be erroneously attributed to receptor differences. The theoretical model, although undoubtedly an oversimplification, is derived from existing models which have been already proven useful in describing receptor kinetics (Langer & Trendelenburg, 1969; Furchgott, 1972) and assists in describing these experimental findings in general terms applicable to all isolated tissue systems.

I wish to thank Dr J. W. Black for useful comments and interesting discussion.

Appendix

(a) *Calculated sensitization*

The rate of entry of a drug into the receptor compartment has been described by a resistance term, referred to as the transfer rate constant (k_t), introduced by Furchgott (1972) and will be used in these calculations. Assuming that a steady state is attained in the tissue bath whereby the rate of entry of drug into the receptor compartment equals the rate of removal of drug and that removal can be adequately described by Michaelis-Menten kinetics, Furchgott (1972) derived an equation relating the concentration of drug in the receptor compartment ($[A]_b$) to the concentration in the external solution ($[A]_a$):

$$\frac{[A]_b}{[A]_a} = \frac{1 + [A]_b/K_{AR}}{1 + [A]_b/K_{AR} + R_{max}/k_t \cdot K_{AR}} \quad (5)$$

where K_{AR} is the equilibrium dissociation constant of the agonist for the site of removal and R_{max} is the maximal rate of removal. When removal of the agonist from the receptor compartment is completely inhibited, then $[A]_a = [A]_b$ and the maximal sensitization (y) is given by (Furchgott, 1972):

$$y = \frac{R_{max}}{k_t \cdot K_{AR}} \cdot \frac{1}{(1 + [A]_b/K_{AR})} + 1 \quad (6)$$

Assuming competitive inhibition of the removal process by an inhibitor I possessing an equilibrium dissociation constant for the site of removal denoted by K_I, equation 5 can be rewritten:

$$\frac{[A]_b}{[A]_a} = \frac{1 + [A]_b/K_{AR} + [I]/K_I}{1 + [A]_b/K_{AR} + [I]/K_I + R_{max}/k_t \cdot K_{AR}} \quad (7)$$

With equation 7, the expected sensitization (x) can be calculated for any multiple of $[I]/K_I$ by setting $x = [A]_a/[A]'_a$ where $[A]'_a$ is the external solution concentration of drug in the presence of I, equiactive to $[A]_a$ in the absence of I:

$$x = \frac{y(1 + [I]/K_I + [A]_b/K_{AR})}{y + [I]/K_I + [A]_b/K_{AR}} \quad (8)$$

(b) *Calculated sensitization-uptake inhibitor also blocks receptors*

The preceding analysis has been confined to theoretically very selective inhibitors of agonist removal. Considering that the agonist recognizes two separate sites, namely the receptor and the site of removal from the receptor compartment, it should not be assumed that the inhibitor of agonist removal would have no affinity for the receptor at higher concentrations. If the drug has agonist activity this can be observed experimentally but if receptor antagonism results from inhibitor-receptor interaction this may be more difficult to detect. Thus, depending on the relative affinities of the inhibitor for the two sites, concomitant sensitization and receptor antagonism could result. The net effect would be that the full sensitization of the tissue to the agonist may never be observed. The following calculations will be confined to inhibitors of uptake which also antagonize the receptors for the agonist competitively since this effect can be predicted by the Schild equation. Other actions such as toxicity to the tissue would produce qualitatively identical effects on the observed sensitization to agonists but of an unpredictable magnitude. Considering competitive antagonism of receptors by I, an empirical constant which serves to illustrate this effect can be defined as the ratio of the dissociation constants of the inhibitor for the drug receptor (denoted K_{BI}) and the site of agonist removal (K_I). This factor will be defined as K_{BI}/K_I and denoted by ϕ.

Incorporating the Schild equation (equation 1) into equation 8 the observed sensitization (x) is given by:

$$x = \frac{y(1 + [I]/K_I + [A]_b/K_{AR})}{(y + [I]/K_I + [A]_b/K_{AR})(1 + [I]/K_I \cdot 1/\phi)} \quad (9)$$

(c) Calculated changes in potency-ratios

Considering two agonists, A and A″ such that the tissue demonstrates a maximal sensitization to these agonists of magnitude y and y″ respectively then the ratio of the equiactive concentrations in the external bath can be calculated from equation 7 and rearranged to:

$$\frac{[A]''_a}{[A]_a} = \frac{[A]''_b}{[A]_b} \times \frac{(1 + [A]''_b/K''_{AR} + [I]/K_I + R''_{max}/k''_t \cdot K''_{AR})(1 + [A]_b/K_{AR} + [I]/K_I)}{(1 + [A]_b/K_{AR} + [I]/K_I + R_{max}/k_t \cdot K_{AR})(1 + [A]''_b/K''_{AR} + [I]/K_I)} \quad (10)$$

Equiactive concentrations in the receptor compartment would represent the true potency ratio ($[A]''_b/[A]_b = pr_{(true)}$) while $[A]''_a/[A]_a$ would reflect the potency ratio in the external solution observed by experimentally ($pr_{(observed)}$). Therefore from equations 6 and 10 and assuming $[A]_b \ll K_{AR}$ and $[A]''_b \ll K''_{AR}$ for simplicity:

$$\frac{pr_{(observed)}}{pr_{(true)}} = \frac{y'' + [I]/K_I}{y + [I]/K_I} \quad (11)$$

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