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Pharmacological analysis of the interaction between Bay K 8644 and 5-HT in rabbit aorta

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1 Bay K 8644 potentiated and augmented 5-hydroxytryptamine (5-HT)-induced contractions in the rabbit, isolated aorta preparation, as manifested in leftward shift and increase in the asymptote of 5-HT E/[A] (effect vs concentration) curves.

2 The operational model of agonism (Black & Leff, 1983) was used to analyse this interaction and the concomitant effects of irreversible receptor alkylation by phenoxybenzamine. The competitive effects of spiperone in the presence and absence of Bay K 8644 were also examined.

3 From these analyses it is concluded that Bay K 8644 elicits its potentiating effects by increasing the efficacy of 5-HT at the 5-HT_2 receptor with no alteration in affinity. This is consistent with the known effect of Bay K 8644 of causing an increase in the functional concentration of plasmalemmal calcium channels coupled to the 5-HT_2 receptors in this preparation.

4 The positively co-operative shape of the 5-HT E/[A] curves obtained in the aorta and the quantitative nature of their potentiation by Bay K 8644 indicated that the coupling of 5-HT_2 receptor occupancy to intracellular calcium concentration is linear and that the co-operativity resides in the subsequent relation between intracellular calcium and pharmacological effect.

5 Bay K 8644 may serve as a probe for differentiating between the types of calcium channels that transduce 5-HT receptor-mediated effects in different systems. Such information would be useful in the classification of agonist interactions with 5-HT receptors.

Introduction

5-Hydroxytryptamine (5-HT) induces contractions of rabbit isolated aortic rings. These contractions are subserved by a single class of 5-HT receptor, (Humphrey *et al.*, 1982; Black *et al.*, 1983) which has been designated 5-HT_2 on the basis of antagonist dissociation constant estimates and their correlation with corresponding estimates in central binding assays (Humphrey *et al.*, 1982). Moreover, the response appears to be dependent on extracellular calcium (Ratz & Flaim, 1984) with little, if any, dependence on intracellular calcium mobilisation. This is in contrast with the action of 5-HT on other vascular tissues (Ratz & Flaim, 1984; Van Neuten & Vanhoutte, 1981) and with the action of α-adrenoceptor agonists on the rabbit aorta (Karaki *et al.*, 1984; Cory *et al.*, 1984) which have been reported to be dependent on both intra- and extracellular calcium sources.

Thus, the action of 5-HT on rabbit aorta appears to obey a simple one-receptor: one-transducer scheme in which 5-HT_2 receptors subserve the opening of plasmalemmal calcium channels, ultimately resulting in muscle contraction. A previous analysis of the agonist action of 5-HT in rabbit aorta supports this view (Black *et al.*, 1985a). In that study, the effects of irreversible antagonism on 5-HT concentration-effect (E/[A]) curves were shown to accord quantitatively with the predictions of the operational model of agonism (Black & Leff, 1983) in which a one-receptor: one-transducer arrangement is implicitly assumed. Whereas the previous study involved experimental interventions at the receptor level, the present analysis of 5-HT agonism examines the effects of interventions at the transducer level.

Bay K 8644 is reported to promote transmembrane influx of extracellular calcium through specific calcium channels in both cardiac and vascular smooth muscle (Schramm *et al.*, 1983) possibly by acting as an allosteric effector of the channel to shift a pre-existing equilibrium between closed and open channels towards the open state, or by increasing channel open

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time (Triggle, 1984). Therefore, it was anticipated that Bay K 8644 would augment responses to 5-HT in rabbit aorta by increasing the efficiency of coupling between 5-HT_2 receptor occupancy and calcium influx. We have demonstrated such effects and analysed them using the operational model of agonism in order to define the nature of the potentiating effect observed. The results of the analysis are discussed (i) in terms of their implications for 5-HT_2 receptor:calcium channel coupling in this system and (ii) with respect to their contribution to the pharmacological classification of 5-HT receptor agonists.

Theory

The operational model of agonism (Black & Leff, 1983; Black *et al.*, 1985a) describes agonist action in a one-receptor : one-transducer system by the equation:

$$E = \frac{E_m \tau^n [A]^n}{(K_A + [A])^n + \tau^n [A]^n} \quad (1)$$

Where E is the pharmacological effect, of which E_m is the maximum possible value, [A] is the agonist concentration, K_A is the dissociation constant of the agonist, and τ and n respectively define the efficiency and sensitivity of transduction of agonist-receptor complexes, AR, into E. τ is defined in the model as the ratio of $[R_o]$, the total functional receptor concentration, over K_E, the value of [AR] for 0.5 E_m.

Operationally, τ determines the efficacy of an agonist in a system. Experimental manipulation of τ, by variation of either $[R_o]$ or K_E, allows estimation of agonist affinity (the reciprocal of K_A) and efficacy (the control value of τ) by fitting E/[A] data directly to Equation (1) (Black *et al.*, 1985a; Leff *et al.*, 1985). While $[R_o]$ manipulation can be achieved easily by use of irreversible antagonists, simple K_E manipulation may be difficult because a post-receptor intervention intended to alter this parameter may also cause changes in the other parameters which define the transducer relation, namely E_m and n. The effects of concomitant E_m changes have been fully discussed in a previous paper (Leff *et al.*, 1985). For the present, it suffices to say that such effects produce quantitatively different changes in E/[A] curve profile from those generated by simple K_E or $[R_o]$ changes alone. However, because $[R_o]$ and K_E changes produce congruent effects on E/[A] curves in theory, then, by comparing the effects of a post-receptor intervention with those of an irreversible antagonist, it is possible to deduce whether the former gives rise to a simple K_E change (Black *et al.*, 1985b; Leff *et al.*, 1985).

In the present study we apply this approach to analyse the effects of Bay K 8644 on 5-HT E/[A] curves in the presence and absence of irreversible receptor blockade by phenoxybenzamine (Pbz).

Methods

Rabbit thoracic aorta

Male NZW rabbits (2.0–2.5 kg) were killed by intravenous injection of pentobarbitone sodium (60 mg kg^{-1}). Isolated thoracic aortic ring segments were prepared according to the method of Stollak & Furchgott (1982) and suspended in 20 ml organ baths, thermostatically controlled at 37°C, containing modified Krebs solution, pH 7.4, of the following composition (mM): NaCl 118.41, $NaHCO_3$ 25.00, KCl 4.75, $KHPO_4$ 1.19, $MgSO_4$ 1.19, glucose 11.10, $CaCl_2$ 2.50, aerated continuously with 95% O_2 : 5% CO_2.

Tissue responses were measured as increases in isometric force using Grass FT03C strain gauges and displayed on Gould 2600S multichannel recorders.

Experimental protocol

The protocol adopted in these experiments was identical to that described previously (Black *et al.*, 1985a). Briefly, each tissue was subjected to a sighting dose of 10 μM 5-HT in order to establish tissue viability, then, after washing, a control 5-HT concentration-effect curve was performed. Following further washing a second curve was obtained. Where appropriate, Pbz was applied for 30 min in the period between first and second curves. Bay K 8644 and spiperone were administered for 30 min and 60 min respectively prior to construction of the second curve and both remained in the organ baths during those 5-HT additions. Both 5-HT curves were obtained cumulatively using 0.5 $\log_{10}$[M] increments.

Drugs and solutions

Drugs used were: pentobarbitone sodium (Sagatal, May and Baker), 5-HT creatinine sulphate complex (Sigma), benextramine tetrahydrochloride monohydrate (BHC) (Aldrich Chemical Company), phenoxybenzamine hydrochloride (Pbz) (Dibenyline, SK & F), spiperone (Janssen Pharmaceutica), Bay K 8644 (methyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)- pyridine- 5-carboxylate, prepared by Dr W.R. King, Wellcome Research Laboratories). 5-HT and BHC solutions were aqueous. Bay K 8644 and Pbz were prepared in ethanol and diluted in water. Spiperone was prepared in dimethylsulphoxide. At the concentrations used, ethanol and dimethylsulphoxide vehicles had no effect on tissue tone or on 5-HT E/[A] curves. The maximum volume of drug solution administered to any organ bath was 300 μl.

Analysis of 5-HT E/[A] data

Response data were recorded in grams force from the second 5-HT curve obtained in each tissue. Individual sets of curve data were fitted to a logistic function of the form:

$$E = \frac{\alpha\,[A]^m}{[A_{50}]^m + [A]^m} \qquad (2)$$

In which α, $[A_{50}]$ and m are the asymptote, location and slope parameters, respectively. Location parameters were actually estimated as logarithms and they are quoted as $p[A_{50}]$ values, that is, $-\log_{10}[A_{50}]$. For the analysis of competitive interactions, this fitting procedure also performed one-way analyses of variance comparing computed slope and asymptote parameters between and within treatment groups. Further analysis of competitive antagonism was performed by fitting computed $\log_{10}[A_{50}]$ values to the following linear form of the Schild equation (Trist & Leff, 1985):

$$\log_{10}[A_{50}] = \log_{10}[A_{50}^c] + \log_{10}(1 + [B]^n/K_B) \qquad (3)$$

In which $[A_{50}^c]$ is a control $[A_{50}]$ value, [B] is the concentration of antagonist, K_B is its dissociation constant and n is its order of reaction with the receptor (unity for simple competition). If n was not significantly different from unity it was constrained to this value in order to estimate pK_B ($-\log_{10}K_B$).

E/[A] curve data were also analysed in terms of the operational model of agonism by fitting to Equation (1), in which agonist affinity was estimated as pK_A.

Finally, the protocol adopted allowed a paired anlaysis to be performed comparing control and Pbz-treatment curves. This served as a check for consistency between the present data set and that obtained previously (Black *et al.*, 1985a) as regards estimates of 5-HT affinity and efficacy.

All the fitting procedures used were unweighted iterative least squares minimization computer programmes. They were locally written with the exception of BMDP Module AR (BMDP Statistical Software, 1981) which was the programme used to fit data to the model of agonism.

All parameter estimates are given as mean values with standard errors and the number of degrees of freedom associated with estimates are given in parentheses.

Results

Effect of Bay K8644 on 5-HT E/[A] curves

In a preliminary experiment (data not shown) treatment of tissues with Bay K 8644 in the concentration range 3 nM to 300 nM caused dose-dependent leftward-shifts of 5-HT E/[A] curves accompanied by increases in asymptote. Replication of the 300 nM treatment gave the results illustrated in Figure 1, where Bay K 8644 caused a 0.75 $\log_{10}$ unit potentiation of 5-HT effects, the control curve $p[A_{50}]$ value being 6.62 ± 0.05 (9) and the treatment value being 7.37 ± 0.06 (5). The asymptote was increased by 2.08 g from a control value of $3.91\,g \pm 0.21\,g$ (5) to a treatment value of $5.99\,g \pm 0.52\,g$ (9). Both the potentiation and augmentation were significant at the $P < 0.05$ level. Occasionally, administration of Bay K 8644 rendered tissues spontaneously contractile, transient responses of 2 min duration occurring at an irregular frequency. These effects were superimposed on the 5-HT responses and often rendered them unmeasurable. Such data were not included in the analysis. In two of the ten replicate treatments used for analysis, Bay K 8644 caused an increase in force amounting to 0.5 g. In the other cases no effect was seen and on average the effect was negligible.

Antagonism by spiperone of 5-HT in the presence and absence of Bay K8644

Spiperone is a reliable competitive antagonist for the detection and definition of 5-HT_2 receptors in phar-

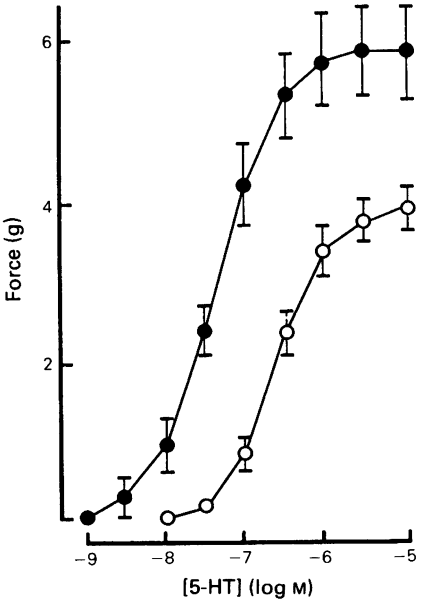

Figure 1 Effect of Bay K 8644 on 5-HT E/[A] curves: 6 replicate control (○) and ten replicate Bay K 8644 (300 nM) – treatment (●) curves were obtained. The diagram shows averaged data in g force together with s.e. bars.

macological assays (Humphrey *et al.*, 1982; Cohen *et al.*, 1983; Maayani *et al.*, 1984). Figure 2 illustrates the results of an analysis of spiperone effects in the presence and absence of 300 nM Bay K 8644. Standard errors on E/[A] curve asymptotes were in the order of 0.4 g in the absence of Bay K 8644 and 0.7 g in the presence of Bay K 8644. Analysis of asymptotes and slopes indicated no deviation from parallelism. Analysis of the curve displacements produced by spiperone using Equation (3) accorded with simple competition, giving pK_B estimates of 9.18 and 9.39 in the presence and absence of Bay K 8644 respectively. These values were not significantly different.

Irreversible antagonism by phenoxybenzamine of 5-HT in the presence and absence of Bay K8644

Pbz irreversibly inhibits 5-HT-induced responses in rabbit aorta (Stollak & Furchgott, 1982; Black *et al.*, 1985a). We have confirmed its reliable use as an agent for quantifying 5-HT affinity and efficacy by showing that concomitant application of the reversible competitive antagonist, methysergide, protects against receptor alkylation. This demonstrates that Pbz is competitive but irreversible at the 5-HT_2 receptor.

Figure 3 shows the effect of Pbz (0.1 μM and 0.3 μM, each for 30 min) on 5-HT E/[A] curves in the presence and absence of Bay K 8644. The curves obtained in the absence of Bay K 8644 (Figure 3b) confirm previous data (Stollak & Furchgott, 1982; Black *et al.*, 1985a) by undergoing asymptote depression with little rightward-shift. In contrast, the curves obtained in the presence of Bay K 8644 (Figure 3a) undergo a parallel rightward-shift (at 0.1 μM Pbz) before subsequent depression (at 0.3 μM Pbz) but even then, only to the position of the control 5-HT curve (shown by the

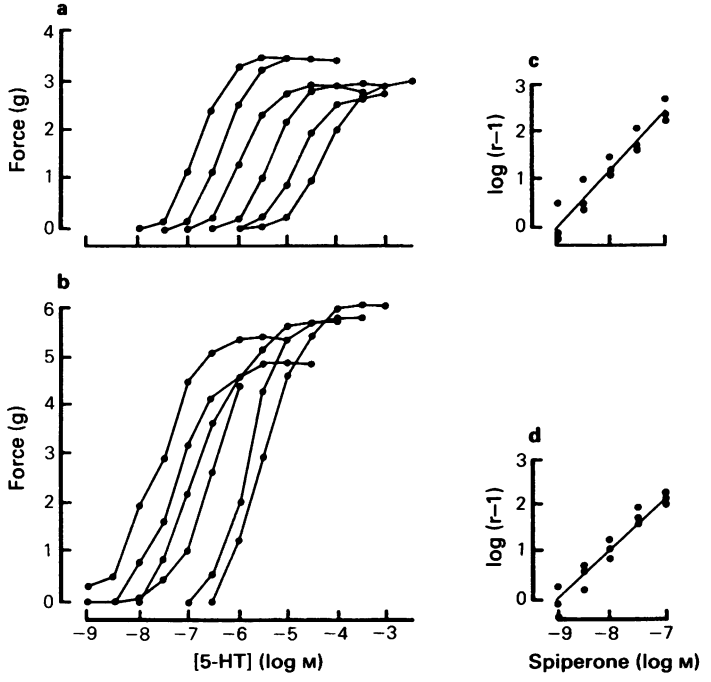

Figure 2 Antagonism of 5-HT by spiperone in the presence and absence of Bay K 8644: (a) and (b) show the effects of spiperone on 5-HT E/[A] curves in the absence and presence of Bay K 8644 (300 nM) respectively. In each case, analyses of variance on slope and asymptote parameters revealed no significant differences; (c) and (d) illustrate the concentration ratios (r) produced by spiperone in the absence and presence of Bay K 8644 respectively. The data are shown in Schild plot form for display purposes only. The lines drawn through the data were obtained by fitting Equation (3) directly to $\log_{10}[A_{50}]$ values.

In the absence of Bay K 8644 the estimated value of n, equivalent to the Schild plot slope was 1.13 ± 0.10 (s.e., 15 d.f.) which was not significantly different from unity; the pK_B estimated with n constrained to unity was 9.39 ± 0.15 (s.e., 16 d.f.).

In the presence of Bay K 8644, n was estimated to be 1.05 ± 0.07 (s.e., 15 d.f.) which again was not significantly different from unity. With n constrained to unity the estimated pK_B values was 9.18 ± 0.10 (s.e., 16 d.f.)

The two pK_B values for spiperone obtained in the presence and absence of Bay K 8644 were not significantly different.

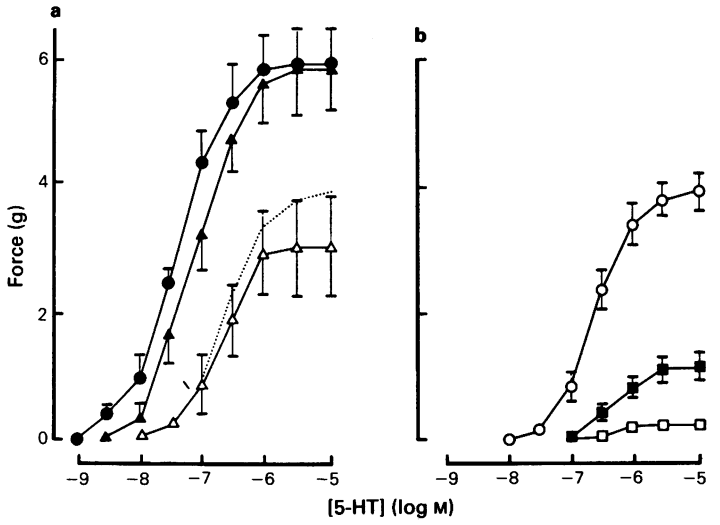

Figure 3 Effect of phenoxybenzamine (Pbz) on 5-HT E/[A] curves in the presence and absence of Bay K 8644: 5-HT E/[A] curves were obtained in the presence (a) and absence (b) of Bay K 8644 following 30 min exposure to 0.1 μM (▲,■) or 0.3 μM (△,□) Pbz. Each point is the mean of 5–10 replicates. Vertical bars are standard errors. The dotted line in (a) represents the 5-HT control curve.

dotted curve). Therefore Bay K 8644 and Pbz acted in simple opposition.

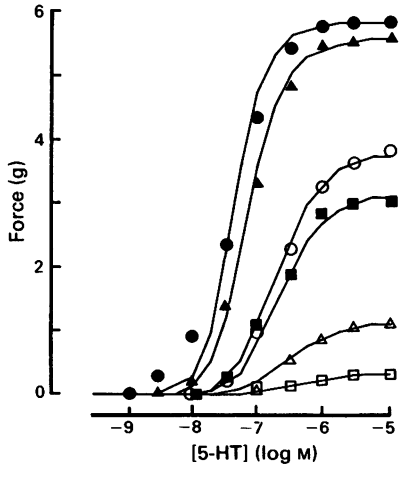

Figure 4 Operational model-fitting of the 5-HT-Bay K 8644 phenoxybenzamine (Pbz) interaction: the average 5-HT curve data shown in Figure 3a and b were fitted simultaneously to the operational model of agonism (Equation (1)). Only τ varied between curves, single estimates of E_m, n and K_A being made for the whole family. Closed symbols represent data obtained in the presence of Bay K 8644, open symbols represent data obtained in its absence.

Model-fitting of data

The data obtained with each combination of Bay K 8644 and Pbz were fitted simultaneously to the operational model of agonism, Equation (1), assuming common values of E_m, K_A and n for all the curves but with a different value of τ corresponding to each condition. Figure 4 shows the average data and the model-fitted lines. The parameter values corresponding to these lines were as follows: $E_m = 6.12$ g; $n = 2.30$; $pK_A = 6.84$; τ_1 (Bay K 8644 alone) $= 4.28$; τ_2 (Bay K 8644 + 0.1 μM Pbz) $= 2.91$; τ_3 (control) $= 1.26$; τ_4 (Bay K 8644 + 0.3 μM Pbz) $= 1.05$; τ_5 (0.1 μM Pbz) $= 0.53$; τ_6 (0.3 μM Pbz) $= 0.27$. Evidently, the family of curves were fitted acceptably well, assuming that only operational efficacy varied between them.

In addition, the data shown in Figures 3a and b were each fitted alone giving pK_A estimates of 6.99 and 6.70 respectively.

Discussion

The objective of this study was to define, operationally, the nature of the interaction between Bay K 8644 and 5-HT in the rabbit aorta. The methods used are, in principle, applicable to the characterization of any initially unspecified intervention which modifies E/[A] curve profiles.

In the case of Bay K 8644 some kind of augmenta-

tion or potentiation of 5-HT curves was anticipated on the basis of its reported effects on calcium channels (Schramm *et al.*, 1983). However, consistency between observation and qualitative expectation does not necessarily mean that the experimental results can be attributed entirely, or even in part, to effects on calcium channels. Furthermore, even if the post-receptor nature of the interaction was assumed, an attempt to analyse the data quantitatively was necessary in order to distinguish between the different kinds of post-receptor interactions that may result (Leff *et al.*, 1985). The operational model of agonism (Black & Leff, 1983) served as the basis for such an analysis. Alterations at the receptor level can influence either $[R_o]$ or K_A (competitive antagonism would be a K_A effect here). Alterations at the transducer level can effect K_E, E_m or n. The curve shape alterations caused by $[R_o]$, K_E, E_m and n changes have been described in previous papers (Black & Leff, 1983; Black *et al.*, 1985a,b).

If the leftward-shift of 5-HT E/[A] curves by Bay K 8644 had been the consequence of an increase in the affinity of the agonist for the 5-HT_2 receptor, for example, by an allosteric interaction with the receptor, then different estimates of agonist affinity may have been expected in the presence and absence of Bay K 8644. In fact, the pK_A estimate for 5-HT in the presence of Bay K 8644 was slightly lower (6.70) than that observed in the absence of Bay K 8644 (6.99) although we consider these differences as meaningless. Therefore, there is no evidence that Bay K 8644 modifies agonist affinity. Also, the pK_B estimated for spiperone was unaltered by Bay K 8644 which is consistent with a receptor-independent action of the latter.

The logical alternative to explain the potentiating effect of Bay K 8644 is an increase in efficacy. In a previous, model-fitting analysis of 5-HT agonism in the rabbit aorta preparation (Black *et al.*, 1985a), 5-HT was estimated to produce only 85% of the maximum possible effect, E_m. The present analysis indicated that the asymptotic effect of 5-HT was 65% of E_m. However, in other respects the two analyses gave quantitatively similar results. The pK_A estimated in the previous analysis was 6.95; here it was 6.99. The dissimilarity between the two analyses can be explained by a difference in the average tissue receptor concentration between the two groups of animals used. Importantly, both analyses indicate that 5-HT operates as a partial agonist in this sytem. Therefore, an increase in efficacy, that is τ in the model, either by increasing $[R_o]$ or by decreasing K_E, would be anticipated to potentiate and augment 5-HT effects. In either case, irreversible antagonism should simply reverse the changes in E/[A] curve shape by decreasing τ. Figure 3a shows that Pbz had such an effect on the Bay K 8644-induced potentiation and augmentation, the 0.3 μM Pbz treatment restoring the 5-HT curves approximately to the control position. The results indicated that Bay K 8644 alone modified τ to the extent that 5-HT then behaved as a full agonist, since 0.1 μM Pbz appeared to cause parallel shift of 5-HT curves in the presence of Bay K 8644. In contrast, in the absence of Bay K 8644 (see Figure 3b), the same concentration of Pbz caused marked depression of the curves.

Qualitatively, these data are consistent with an increase in τ by Bay K 8644. Indeed the whole family of curves in the presence and absence of Bay K 8644 and Pbz could be fitted on the basis of changing τ. However, without a quantitative analysis of the data shown in Figure 3 it would not be possible to deduce whether both τ and E_m were affected by Bay K 8644, or simply τ. A previous analysis (Leff *et al.*, 1985) showed that concomitant E_m and τ changes can affect E/[A] curve profiles in a qualitatively similar way to simple τ variation. However, if data corresponding to the former case are analysed as if only τ were varying, an erronously low estimate of K_A is obtained. Conversely, if K_A is known or independently estimable, the experimental intervention under consideration can be tested by comparing the K_A estimate produced by the intervention with the known one. Three estimates of pK_A have already been quoted from the present and previous analyses: 6.99, 6.95 and 6.70. The model-fitted lines through the family of curves shown in Figure 4 correspond to a pK_A of 6.84. From the similarity between these estimates we conclude that the effects of Bay K 8644 and Pbz, alone and in combination, simply involve alterations in τ.

While it is not possible to attribute the τ change unequivocally to an effect solely on K_E—in principle Bay K 8644 could affect $[R_o]$—this alteration seems the most likely in the light of the reported action of Bay K 8644 on calcium channels (Schramm *et al.*, 1983). Accepting a K_E change as the explanation for the potentiating effect of Bay K 8644 allows some deductions to be made about the coupling of 5-HT_2 receptor occupancy by 5-HT to calcium influx in this system. A shift of calcium channels from a closed to open state, or a prolongation of open time by Bay K 8644 (Triggle, 1984), would have the operational effect of increasing functional open channel density. The transducer function in the model of agonism can be considered as a sequence of two saturable processes: a $[Ca]_i/[AR]$ function and an $E/[Ca]_i$ function, $[Ca]_i$ signifying the intracellular calcium concentration. The logical consequence of increasing calcium channel density is an increase in the asymptote of the $[Ca]_i/[AR]$ function. However, if this relation were saturating in the range of E production, then a concomitant K_E and E_m change would be predicted (Leff *et al.*, 1985). But, if the relation were effectively linear in the range of E production, then the increase in channel density would

cause a simple K_E reduction. For the present data this argument implies that receptor occupancy is coupled linearly to $[Ca]_i$ elevation. A similar conclusion can be drawn for other calcium channel coupled receptor systems. For example, Gião T. Rico (1971a,b) showed for both the muscarinic receptor and the histamine (H_1) receptor in the guinea-pig isolated ileum preparation that extracellular calcium depletion mimicked irreversible receptor antagonism.

Interestingly, such systems appear to operate in accordance with the traditional receptor-stimulus model of agonist action (Stephenson, 1956; Furchgott, 1966; Kenakin & Beek, 1980) which, as Black & Leff (1983) have shown, is one possible formulation of the operational model of agonism. Stimulus S, in the traditional model, can be considered to be $[Ca]_i$ if the $[Ca]_i/[AR]$ relation is linear, the proportionality constant between [AR] and $[Ca]_i$ being equivalent to 'efficacy' in the traditional model.

The linear coupling between 5-HT_2 receptor occupancy and calcium channel opening does not necessarily imply a direct physical linkage between receptor and channel; a cascade of sequential relations each operating in a linear fashion will produce an overall linear coupling. What is apparent, however, is that both occupied receptors and Bay K 8644 operate on the same calcium channel pool, directly or indirectly. This result is not consistent with the claim of Yamamoto *et al.* (1984) that Bay K 8644 is able to differentiate between potential and receptor operated calcium channels in rabbit aorta. Potassium-induced depolarization of rabbit aorta smooth muscle cell membranes was reported to be necessary for Bay K 8644 to elicit its so-called calcium agonism (Schramm *et al.*, 1983). In the present study, experiments were not conducted under depolarized conditions which presumably explains the insignificant intrinsic effects of Bay K 8644 obtained. The simplest explanation for these findings is that the same pool of calcium channels, opened by 5-HT_2 receptor-mediated agonism or depolarization, are stabilised in their open state by Bay K 8644, the number of open channels being negligible under basal conditions.

Another implication of linear coupling between [AR] and $[Ca]_i$ is that the shape of the overall transducer, E/[AR], function is determined by the E/$[Ca]_i$ relation. The results of model-fitting in the present and the previous (Black *et al.*, 1985a) studies indicate that the E/[AR] relation is steep compared with a rectangular hyperbola, the value of n being estimated at 2.3 here. This implies that the relation between $[Ca]_i$ and E is positively co-operative. This conclusion is supported by direct studies of the relation between intracellular calcium and contraction of rabbit aortic rings using ^{45}Ca (Van Breemen, 1977) which suggested that 'two or more calcium ions are necessary to activate a single functional myosin group'.

The present study has shown that Bay K 8644 increases 5-HT_2 receptor-mediated agonist efficacy in the rabbit aorta. 5-HT, which behaves as a partial agonist under control conditions, produced effectively full agonism under the influence of Bay K 8644, τ being raised by a factor of about 3.5 according to model-fitting. Efficacy is essentially related to the transduction process. Quantification of agonist affinity and efficacy makes a valuable contribution to pharmacological classification studies and is critical in the design of agonist drugs (for review see Kenakin, 1984). Assuming that the increase in efficacy by Bay K 8644 is due to a specific interaction with the calcium channel coupled to the 5-HT_2 receptor, then this compound may be considered to be a probe for 5-HT transduction processes, possibly serving to detect differences in the type of calcium channels linked to 5-HT receptors. Such information would contribute to the classification of 5-HT mimetics and the receptors and transducers subserving their actions.

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