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Different apparent modes of inhibition of α_{2A}-adrenoceptor by α_2-adrenoceptor antagonists

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Abstract

The inhibition of α_{2A}-adrenoceptor-mediated Ca^{2+} elevation by α_2-adrenoceptor antagonists was measured in HEL human erythroleukemia cells. The antagonists could be divided in two classes: those that displayed surmountable inhibition (right-shift of the agonist dose–response curve), and those that displayed different degrees of insurmountable inhibition (depression of the maximum signal and a possible right-shift of the agonist dose–response curve). The degree of surmountability of the inhibition correlated well with the measured antagonist dissociation rates, suggesting that the hypothesis of the antagonist dissociation rate governing the mode of inhibition of fast responses, holds true. HEL cells thus provide a useful model system for the investigation of physiological consequences of different dissociation rates. Also, the dissociation rates of antagonists not available in radiolabelled form can be predicted from the functional data. The data stresses the importance of measurement of kinetic parameters of the drug–receptor interaction in addition to the equilibrium binding constants. © 1997 Elsevier Science B.V.

Keywords: α_2-Adrenoceptor; Ca^{2+} signaling; Fura-2; Receptor kinetics; Receptor binding

1. Introduction

For G-protein coupled receptors the usual agonist–antagonist binding to the receptor site is reversible and mutually exclusive, i.e., competitive. Under equilibrium conditions, any antagonist at any concentration may thus be competed away from the receptor site by a sufficiently high agonist concentration. Yet it is known that many antagonists, which display competitive interaction in the binding studies, show an apparent insurmountable inhibition of agonist-induced signals (Rang, 1966; El-Fakahany et al., 1988; Kachur et al., 1988; Kenakin and Boselli, 1990; Patcheke, 1990; Minneman and Atkinson, 1991; Kukkonen and Åkerman, 1992; Vigne et al., 1993; Sakamoto et al., 1994). This has been suggested to be due to the non-equilibrium conditions present under response measurements, i.e., the slow dissociation of the antagonist from the receptor (Rang, 1966; Kenakin, 1993) and may thus be present in all the fast and/or rapidly declining responses mediated by receptors. Consequently it has been observed in the IP_3 or Ca^{2+} responses mediated by the endothelin ET_A receptors (Vigne et al., 1993; Sakamoto et al., 1994) and muscarinic receptors (El-Fakahany et al., 1988; Kukkonen and Åkerman, 1992).

The insurmountable effect of receptor antagonist has mainly been considered as an experimental artefact to be aware of when determining the antagonist K_d values. However, differences in the antagonist dissociation rates can be predicted to lead to pronounced differences in the in vivo effects. Furthermore, it is not known for many antagonists, whether the usual criteria for choice of antagonists for pharmacotherapy — which is based on the equilibrium affinity and selectivity — anyhow reflects the kinetic properties of the antagonists. We have therefore in the present study aimed to investigate both the dissociation rates of α_2-adrenoceptor antagonists and the functional

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consequence of these. As a model system we have used HEL human erythroleukemia cells which express α_{2A}-adrenoceptors (Michel et al., 1989). They are the only cell type where α_2-adrenoceptors have been shown to couple to relatively large calcium elevations (Michel et al., 1989). These cells were thus chosen as a suitable model for investigation of the possible insurmountable inhibition of a fast response by α_2-adrenoceptor antagonists. Because of the low expression level of α_{2A}-adrenoceptors in HEL cells, direct measurements of the antagonist dissociation rates were performed on Shionogi S115 mouse mammary tumor cells transfected with the human α_{2A}-adrenoceptor gene (Marjamäki et al., 1992).

2. Materials and methods

2.1. Cells

HEL cells were obtained from the American Type Culture Collection (Rockville, MD, USA). They were grown in RPMI-1640 medium supplemented with 100 U/ml penicillin (Nordvacc Media, Skärholmen, Sweden), 50 μg/ml streptomycin (Nordvacc Media) and 7.5% (v/v) fetal calf serum (Gibco, Paisley, UK) in 5% CO_2 at 37°C in a humidified incubator. α_{2A}-transfected Shionogi S115 cells (Marjamäki et al., 1992) were grown in Dulbecco's Modified Eagle Medium (Gibco) supplemented with 100 U/ml penicillin, 50 μg/ml streptomycin, 5% (v/v) fetal calf serum, 20 mM HEPES, 20 mM $NaHCO_3$, 1 mM Na^+-pyruvate and 10 nM testosterone in 5% CO_2 at 37°C in a humidified incubator.

2.2. Media

The TES buffered medium (TBM) was composed of 137 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 10 mM glucose, 1.2 mM $MgCl_2$, 4.2 mM $NaHCO_3$, 0.44 mM KH_2PO_4 and 20 mM TES (2-[(2-hydroxy-1,1-bis[hydroxymethyl]ethyl)amino] ethane sulfonic acid), pH adjusted to 7.4 with NaOH.

2.3. Chemicals

(−)-adrenaline, oxymetazoline (3-[(4,5-dihydro-1H-imidazol-2-yl-)methyl]-6-[1,1-dimethylethyl]-2,4-dimethylphenol HCl) and yohimbine (17α-hydroxyyohimban-16α-carboxylic acid methyl ester HCl) were purchased from Sigma (St. Louis, MO, USA) and guanfacine (*N*-[aminoiminomethyl]-2,6-dichlorobenzenacetamide) from Sandoz (Basel, Switzerland). Atipamezole (4-[5]-2,3[dimethylbenzyl]imidazole HCl), detomidine ([±]-4-[5]-2,3-[dimethylbenzyl]imidazole HCl) and *l*-medetomidine ([−]-4-[5]-[1-(2,3-dimethylphenyl)ethyl]-1H-imidazole) were from Orion, Orion Pharma (Turku, Finland). Rauwolscine (17α-hydroxy-20α-yohimban-16β-carboxylic acid methyl ester HCl), RX821002 (2-[2-(2-methoxy-1,4-benzodioxanyl)]imidazoline HCl) and UK14,304 (5-bromo-*N*-[4,5-dihydro-1H-imidazol-2-yl]-6-quinoxalinamine) were from Research Biochemicals International (Natick, MA, USA) and fura-2 acetoxymethyl ester from Molecular Probes (Eugene, OR, USA). Digitonin was from Merck (Darmstadt, Germany). MK-912 (L657,743; [2*S*,12b*S*]1′,3′-dimethylspiro-[1,3,4,5′6,6′,7,12b-octahydro-2H-benzo(b)furo(2,3-a)-quinazoline]-2,4′-pyrimidin-2′-one) was a kind gift from Dr. Douglas J. Pettibone (Department of New Lead Pharmacology, Merck, Sharp and Dohme Research Laboratories, West Point, PA, USA). [^{3}H]idazoxan (44.0 Ci/mmol) and [^{3}H]RX821002 (52.0 Ci/mmol) were purchased from Amersham (Amersham, UK) and [^{3}H]MK-912 (81.0 Ci/mmol), [^{3}H]rauwolscine (74.0 Ci/mmol) and [^{3}H]yohimbine (82.5 Ci/mmol) from DuPont NEN (Boston, MA, USA).

2.4. Dissociation kinetics

The time course of dissociation of radiolabelled antagonists from α_{2A}-adrenoceptors at 37°C was determined using washed homogenate from mouse Shionogi-115 cells stably transfected with the human α_{2A}-adrenoceptor subtype (Marjamäki et al., 1992). The cells were homogenized in 50 mM Tris, 5 mM EDTA buffer (pH 7.5) using Potter-Elvehjelm homogenizer at 4°C and the homogenate was centrifuged at $48\,000 \times g$ for 30 min. The resulting pellet was resuspended, centrifuged at $48\,000 \times g$ for 30 min and the final pellet suspended in 50 mM KH_2PO_4, pH 7.5, divided into aliquots and stored frozen. Homogenates at a protein concentration of 2 mg/ml were first incubated in 50 mM KH_2PO_4, pH 7.5 for 30 min with radioligand (0.5–2 nM), whereafter the dissociation was initiated by the addition of 100 μM oxymetazoline and stopped by a rapid filtration of the samples through glass fibre filters at timed intervals. Filters were washed 3 times with 5 ml ice-cold incubation buffer, dried and counted for radioactivity in a scintillation counter. Each time point was determined three of four times in triplicate. The non-specific binding was estimated in parallel samples which received 100 μM oxymetazoline prior to the addition of the radioligand.

2.5. Ca^{2+} measurement

The fluorescent Ca^{2+}-indicator fura-2 was used to monitor changes in intracellular Ca^{2+} (Grynkiewicz et al., 1985). The cells were spun down, resuspended in TBM and loaded with 4 μg/ml fura-2 acetoxymethyl ester for 20 min at 37°C. Thereafter the cells were spun down and resuspended in TBM containing only 100 μM $CaCl_2$, and kept at room temperature. The measurement of intracellular free calcium was carried out as follows: About 10^6 cells were spun down, resuspended in 350 μl of normal TBM at 37°C and placed in a stirred quartz microcuvette in a thermostated cellholder within a fluorescence spectro-

photometer. Fluorescence was monitored either with a Hitachi F-4000 or a Hitachi F-2000 fluorescence spectrophotometer at the wavelengths 340 nm (excitation), 505 nm (emission). The antagonists were added 5 min prior to the addition of UK14,304 (or noradrenaline). When the predicted association rates (k_{on}) were calculated for each ligand (k_{on} = dissociation rate/K_d), this time appeared to be sufficient to obtain at least 80% of the equilibrium occupancy for all the used antagonist concentrations. The experiments were calibrated using 60 μg/ml digitonin, which gives the maximum value of fluorescence (F_{max}) and 10 mM EGTA, which gives the minimum value of fluorescence (F_{min}). The free Ca^{2+}-concentration was calculated from the fluorescence (F) using the equation

$$[Ca^{2+}] = (F - F_{min})/(F_{max} - F) \times 224 \text{ nM}$$

in which the extracellular fura-2 fluorescence is subtracted from the F values.

2.6. Data analysis

The equation for linear mixed inhibition (Cornish-Bowden, 1974) describes inhibition of an enzyme with an inhibitor binding both to the substrate site and an allosteric site with different affinities under steady-state conditions. When transformed into a form applicable to receptors it has been shown to be useful in the analysis of the apparent insurmountable inhibition (Kukkonen and Åkerman, 1992)

$$\Delta[Ca^{2+}] = \frac{[A] \times \Delta[Ca^{2+}]_{max}}{[A] \times (1 + ([I]/K_i')) + EC_{50} \times (1 + ([I]/K_i))}$$

$\Delta[Ca^{2+}]$ is the $[Ca^{2+}]_{basal}$ subtracted from $[Ca^{2+}]$; $\Delta[Ca^{2+}]_{max}$ is the maximum Ca^{2+} increase; [A] and [I] the concentrations of the agonist and the antagonist, respectively; EC_{50} the [A] producing half-maximal stimulation; K_i and K_i' the competitive (surmountable) and the non-competitive (insurmountable) inhibition constants, respectively. When applied to the analysis of the apparent insurmountable inhibition it gives direct measures for both the right-shift of the dose–response curve (K_i) and the depression of the maximum signal (K_i').

The non-linear least square curve fitting was performed using SigmaPlot for Windows (Jandel Scientific, Corte Madera, CA) or GraphPad Prism (GraphPad Software, San Diego, CA). Mean ± S.E.M. is given unless specifically indicated. n indicates number of independent experiments performed on separate batches of cells.

3. Results

The dissociation kinetics of [^{3}H]MK-912, [^{3}H]rauwolscine, [^{3}H]RX821002 and [^{3}H]yohimbine exhibited a clear first order reaction. Dissociation of the [^{3}H]idazoxan was too fast to be measured at room temperature; at 4°C a time dependent decrease in binding could be seen although the dissociation was still too fast for precise calculation of the off-rate constant (k_{off}). The ligands can thus be put in order according to their dissociation rates: [^{3}H]idazoxan ≫ [^{3}H]RX821002 > [^{3}H]yohimbine > [^{3}H]rauwolscine > [^{3}H]MK-912 (Table 1).

The basal intracellular $[Ca^{2+}]$ ($[Ca^{2+}]_i$) in HEL cells was 97 ± 7 nM (n = 25). As previously reported (Michel et al., 1989), α_2-adrenoceptor stimulation led to an elevation of $[Ca^{2+}]_i$ (Fig. 1). UK14,304 had an EC_{50} of 44.2 ± 5.4 nM and $\Delta[Ca^{2+}]_{max}$ of 298 ± 11 nM (n = 25). In

Table 1
The binding off-rates (k_{off}) and the inhibition displayed by the α_2-adrenoceptor antagonists and weak agonists on the UK14,304-induced Ca^{2+} elevation

Class	Compound	K_i (nM)	K_i' (nM)	K_i'/K_i	K_d (nM) [a]	k_{off} (min^{-1})	$t_{1/2}$ (min)
Surmountable antagonist	idazoxan	36.4 ± 14.7	∞	∞	19.2 ± 5.0	fast [b]	—
	l-medetomidine	52.3 ± 16.9	∞	∞	34.3 ± 12.6	—	—
Insurmountable antagonist	atipamezole	2.76 ± 0.55	170 ± 43	62.8 ± 13.2	1.16 ± 0.51	11.6 [c], 14.1 ± 3.0 [d]	0.0598, 0.0543 ± 0.0124
	RX 821002	6.39 ± 1.19	24.4 ± 3.7	4.19 ± 0.65	0.97 ± 0.32	0.890 ± 0.020	0.780 ± 0.018
	yohimbine	5.76 ± 0.43	12.0 ± 4.7	2.16 ± 0.98	7.48 ± 3.95	0.381 ± 0.015	1.82 ± 0.07
	rauwolscine	9.82 ± 2.55	14.6 ± 3.2	1.88 ± 0.80	4.75 ± 1.28	0.257 ± 0.021	2.73 ± 0.22
	MK-912	1.74 ± 0.41	2.37 ± 0.26	1.78 ± 0.67	1.23 ± 0.03	0.184 ± 0.002	3.77 ± 0.04
Weak agonist	oxymetazoline	15.3 ± 1.4	∞	∞	—	—	—
	detomidine	22.3 ± 3.4	∞	∞	—	—	—
	guanfacine	58.0 ± 9.0	∞	∞	—	—	—

When the maximum signal obtained in the presence of the antagonist was not significantly different from the maximum signal obtained in the absence of the antagonist, the K_i' was fixed to infinity. The agonist data (oxymetazoline, detomidine, guanfacine) was treated in a similar way as the slight apparent insurmountable inhibition observed was most probably an artefact. Number of batches of cells is 2–5.

[a] The values are means ± S.E.M. of 2–8 previous reports with the α_{2A}-adrenoceptors either heterologously expressed in Chinese hamster ovary cells (Uhlén et al., 1994), fibroblasts (Jansson et al., 1994b), S115 cells (Marjamäki et al., 1993; Jansson et al., 1994a; Halme et al., 1995) or Sf9 cells (Jansson et al., 1995) or endogenously expressed in human platelets (Galitzky et al., 1990; Gerhardt et al., 1990; Gobbi et al., 1990; Sjöholm et al., 1992; Renouard et al., 1994).

[b] See Section 3 for details.

[c] Calculated from Fig. 1 in Halme et al. (1995).

[d] Calculated from Fig. 2 by extrapolation.

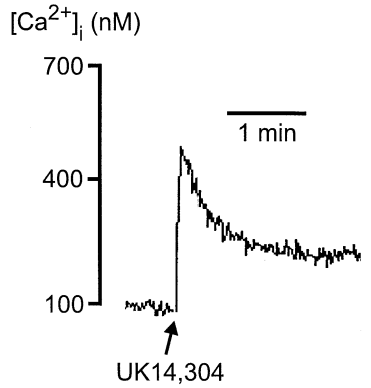

Fig. 1. A typical Ca^{2+} response to 10 μM UK14,304.

order to evaluate whether there was any receptor reserve with respect to the Ca^{2+} elevation by UK14,304, the method of Stickle and Barber (1989) was applied. They have used a slowly dissociating β-adrenoceptor antagonist, propranolol, to inactivate a part of β-adrenoceptor population. Propranolol had a dissociation half-time of 150 s; during the 1 min exposure of a propranolol-equilibrated receptor population to agonist, there would be a 25% dissociation from the receptors. We used a similar approach to inactivate the receptors with MK-912. This compound had a dissociation half-time of 226 s (Table 1); during the time under which the maximum response had to be obtained (<10 s), only 5% dissociation would be reached. Therefore, under these conditions, the inhibition would be practically irreversible. When there is no receptor reserve for a given response, receptor removal (inactivation) causes a depression of the maximum response with little or no change in the EC_{50} value (Kenakin, 1993). As MK-912 caused a depression of the maximum signal without any change in the EC_{50} value (Table 1), there cannot be considered to be any receptor reserve for the UK14,304 mediated Ca^{2+} elevation. Furthermore, adrenaline caused a $92 \pm 23\%$ ($n = 2$) higher Ca^{2+} elevation in HEL cells than UK14,304, showing that UK14,304 is a partial agonist with respect to this response. The absence of receptor reserve enabled us to investigate other antagonists. UK14,304 induced signal was inhibited both by α_2-adrenoceptor antagonists and weak agonists. The antagonists could be separated into two different groups. Idazoxan behaved apparently surmountably, i.e., shifted the UK14,304 dose–response curve to the right without depression of the maximum signal (Fig. 2A; Table 1). *l*-medetomidine did not cause any Ca^{2+} elevation of its own and behaved also as a surmountable antagonist against UK14,304 (Fig. 2A; Table 1). The other antagonists atipamezole, rauwolscine, RX821002 and yohimbine both shifted the UK14,304 dose–response curve to the right and depressed the maximum signal in different degrees (Fig. 2B; Table 1). Detomidine, guanfacine and oxymetazoline also depressed the maximum signal to some degree (~ 20%) and shifted the UK14,304 dose–response curve to the right: however, they also caused some Ca^{2+} elevation by themselves and after saturation of this, any higher concentration of the antagonists only produced a further right-shift without no further depression of the maximum signal (Fig. 2C). Thus, this minor depression of the maximum signal is most likely caused by desensitization or Ca^{2+} store depletion.

The insurmountable effect of rauwolscine, yohimbine, RX821002, MK-912 and atipamezole is not likely to be a result of any non-specific interaction with the signal transduction pathway as they did not affect the thapsigargin

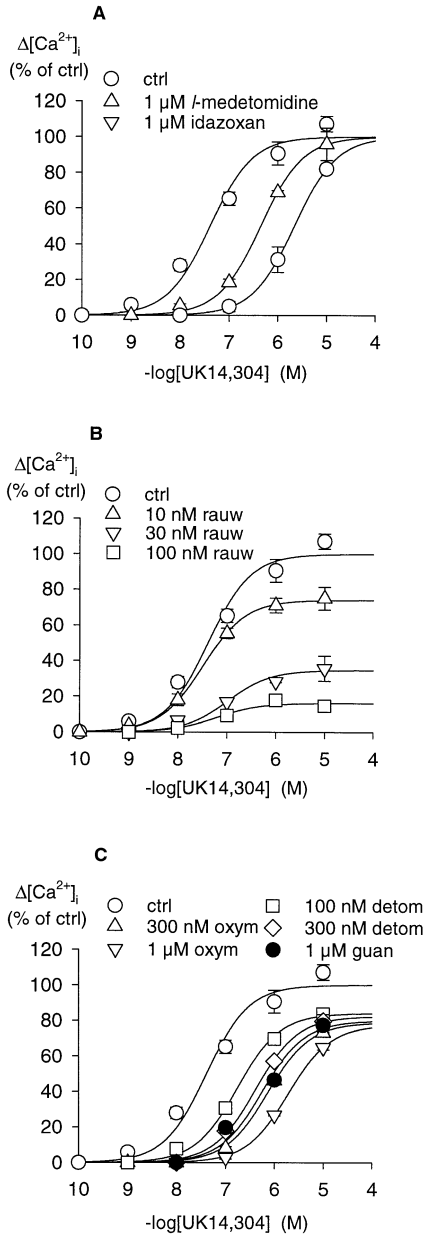

Fig. 2. The effect of antagonist or weak agonist preincubation (5 min) on UK14,304-induced Ca^{2+} elevation. In (A) the surmountable behavior represented by *l*-medetomidine and idazoxan, in (B) the insurmountable behavior represented by rauwolscine (rauw), and in (C) the weak agonists oxymetaxoline (oxym), detomidine (detom) and guanfacine (guan).

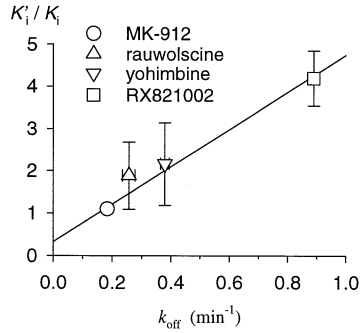

Fig. 3. Correlation of the dissociation rate constant (k_{off}) and the insurmountability as expressed by K_i'/K_i. The parameters for the linear regression ($y = A \times x + B$) are the following: $A = 4.418 \pm 0.445$ min, $B = 0.3265 \pm 0.1464$.

(100 nM) or thrombin (0.3 U/ml) induced Ca^{2+} elevation (data not shown). Also, the behavior and the calculated values of K_i and K_i' for rauwolscine and *l*-medetomidine were independent of the agonist used (UK14,304 or adrenaline, data not shown).

We have previously used the equation for linear mixed inhibition (Cornish-Bowden, 1974) for the analysis of insurmountable inhibition (Kukkonen and Åkerman, 1992). This equation fails to mechanistically follow the current situation, as it describes a completely different phenomenon, namely the mixed competitive–non-competitive inhibition at steady state. However, it is the best of the known methods to date as it gives comparable measures describing both the right-shift of the dose–response curve (K_i) and the depression of the maximum signal (K_i'). $K_i \approx K_i'$ indicates a solely insurmountable inhibition (MK-912) while $K_i < K_i'$ indicates an almost fully surmountable inhibition. The K_i'/K_i-ratio should thus be a measure of the extent of the surmountability. If the hypothesis that the dissociation rate of the antagonist determines the degree of surmountability (Rang, 1966; Kenakin, 1993) holds true, then a direct correlation between the dissociation rate and the K_i'/K_i-ratio is expected. In accordance with this, a linear relationship between k_{off} and K_i'/K_i was observed for those antagonists, whose k_{off} could be determined (Fig. 3). It becomes clear from the study of Halme et al. (1995) that the dissociation of [^{3}H]atipamezole is too fast to be reliably measured even at 25°C. However, the first rapid dissociation in that study can be measured and the 'lowest possible' value for k_{off} obtained. This can be recalculated to apply to 37°C assuming a similar temperature dependence as for [^{3}H]rauwolscine and for [^{3}H]RX821002. The value, 11.6 min^{-1} is close to the value (14.1 ± 3.0 min^{-1}) obtained by extrapolation of the regression line in Fig. 3 (Table 1).

4. Discussion

The results of this study show that different α_2-adrenoceptor antagonists display different modes of inhibition of the α_2-adrenoceptor agonist mediated Ca^{2+} elevations. The inhibition ranges from an almost totally insurmountable inhibition, i.e., depression of the maximum signal without change in EC_{50}, to a slight depression of the maximum signal with a marked increase in EC_{50}, and finally to a completely surmountable inhibition, i.e., increase in EC_{50} without depression of the maximum signal.

The phenomenon of insurmountability with competitive antagonists has previously been suggested to be due to the slow dissociation of the antagonist — which has been pre-equilibrated with the receptor population — i.e., a pseudo-irreversibility of the receptor–antagonist interaction (Rang, 1966; Kenakin, 1993). In the present study we have aimed to evaluate the rightness of this hypothesis by examining a variety of antagonists of similar equilibrium constants. For the study of the effect of antagonist dissociation rate on response inhibition, it is of paramount importance first to evaluate the possibility of the presence of a receptor reserve with respect to a given response in the test system, i.e., more receptors than are needed to produce a maximum response. The approach of Stickle and Barber (1989) suggested that there was no receptor reserve present with respect to the UK14,304-induced Ca^{2+} elevation in HEL cells.

As a direct relationship between the binding and response could be established, the other antagonists were tested. The results obtained support the previously presented hypothesis: within a certain range of dissociation rates a direct correlation of the dissociation rate and surmountability of the antagonist effect could be seen. Therefore, it seems logical and likely that the differences in the insurmountability of the antagonist interaction are caused by the differences in the dissociation rates. At the extremes the situation would change somewhat: when the dissociation rate would be low enough the inhibition should become solely insurmountable. This is exemplified by MK-912, which is essentially insurmountable, due to its essentially completely irreversible effect, and even if the dissociation rate of some antagonist was lower no further increase in insurmountability would be observed in this system. Similarly, when dissociation is 'fast enough', inhibition should become surmountable. Even though a direct correlation of the dissociation rates and surmountability (as expressed by the quantity K_i'/K_i) can be observed, the binding kinetics to homogenates cannot be used to predict antagonistic behavior on the level of a cellular response without also taking into consideration the kinetics and the mechanism, e.g., amplification of the agonist mediated response. Also, many antagonists dissociate too fast to enable a reliable measurement of the dissociation rate. This correlation can, however, be used the other way round, to predict dissociation rates of the ligands that are not available in radiolabelled form. Unfortunately, all the antagonists tested are either relatively slow (MK-912, rauwolscine, RX821002 and yohimbine) or too fast (atipamezole, idazoxan), limiting the k_{off} range of the

'standard curve', where interpolation can be used, to 0.2–0.9 min^{-1}. Results obtained by extrapolation should always be treated with some caution; however, extrapolation to the measured K_i'/K_i ratio for atipamezole gives a k_{off} value that is very close to the value suggested by dissociation rate measurements at 25°C (Halme et al., 1995). Certainly, the results suggest that this approach can be used in a semiquantitative manner if not necessarily in a quantitative manner. Semiquantitatively the antagonists can be put in the order idazoxan/*l*-medetomidine > atipamezole ≫ RX821002 > yohimbine > rauwolscine > MK-912. The order of idazoxan and *l*-medetomidine cannot be resolved.

Any insurmountability in antagonist interaction can theoretically be avoided if the response is measured under equilibrium conditions (El-Fakahany et al., 1988). However, many responses are too rapidly inactivating for such measurements. Thus the observed insurmountability has several important consequences. It can cause an error in the antagonist affinity measurement in functional studies. This should, however, be easily detectable as irregularities in Schild plots. Equilibrium binding affinities and IC_{50} values measured in vitro are not likely to represent the true in vivo potency of these antagonists since most of the responses — even those not displaying rapid desensitization — are likely to be rapid in vivo as rapid removal and inactivation systems for agonists, especially for neurotransmitters, often exist. Thus, the in vivo behavior of several antagonists may be governed by the kinetics of their dissociation from the receptor. The features described here can also explain pharmacokinetic differences of ligands. Insurmountability may lead to cumulative effects, compartmentalization and increase factual elimination times. A high degree of surmountability again will lead to reduced potency of the antagonist in situations with excessive receptor activity. A marked difference between different antagonists may be seen especially in situations where the transmitter release has increased as a result of chronic antagonist treatment. Therefore we suggest, that as the dissociation rates do not seem to be in any relationship to the equilibrium binding affinities (Fig. 4), also the kinetic properties of the receptor antagonists — especially the dissociation rate constants — should be investigated when the selectivities for receptor subtypes are evaluated.

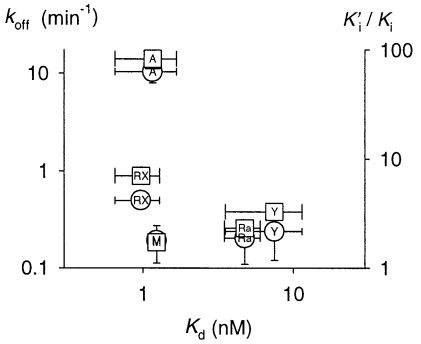

Fig. 4. Correlation of k_{off} and K_d (○) and k_{off} and K_i'/K_i (□). The ligands are the following: atipamezole (A), RX821002 (RX), MK-912 (M), rauwolscine (Ra) and yohimbine (Y).

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