pK_I values of prazosin and idazoxan for receptors stimulated by neuronally released transmitter in the epididymal portion of rat isolated vas deferens

¹D. Mackay & ²M. Kengatharan

Department of Pharmacology, The Worsley Medical & Dental Building, University of Leeds, Leeds LS2 9JT

1 A new method has been used to measure pK_I values of prazosin and idazoxan against neuronally-released transmitter in the epididymal portion of the rat isolated vas deferens. The most reproducible results were obtained with a prolonged antagonist equilibration time (1 h).

2 Under these conditions the pK_I of prazosin was practically unaffected by addition of α , β -methylene-adenosine-5'-triphosphate (10 μ M) to desensitize purinoceptors. Addition of desmethylinipramine (DMI) (0.3 μ M) produced a small, but statistically non-significant, reduction.

3 The same method has been used to measure the pK_I of prazosin against exogenous noradrenaline. In the latter case addition of DMI (0.3 μ M) and corticosterone (30 μ M) together produced a statistically significant reduction in the apparent pK_I of prazosin.

4 The new method for estimating pK_I values shows that DMI itself acts either pseudo-irreversibly or non-competitively and may be reducing the apparent pK_I of prazosin.

5 The pK_I values obtained for prazosin and idazoxan against neuronally-released transmitter are in good agreement with those obtained by other workers for the actions of these drugs on α -adrenoceptors.

Keywords: Competitive antagonists; dissociation constants; affinity constants; pA₂ values; pK_I values; rat vas deferens; neurotransmitters; prazosin; idazoxan

Introduction

Dissociation constants, K_I, of antagonist-receptor complexes have been widely used to characterize receptors in isolated tissues. The classical method for estimating the pA₂ (or pK_I) of a competitive antagonist for its receptor is to measure, on a cell or isolated tissue, several agonist concentration-response curves, each curve at a fixed concentration of antagonist. Such results provide agonist dose-ratios (DR) for each concentration of antagonist [I]. The classical plot of log (DR – 1) against log [I] then provides a test for competitive antagonism and an estimate of the pA₂ (Arunlakshana & Schild, 1959). The classical method can only be used dependably if agonist and antagonist concentrations at the receptors are known. This usually limits the technique to exogenously applied agonist and often requires the presence of other drugs to block uptake or metabolism of agonist (or antagonist) by the tissue.

In the previous paper (Mackay, 1994) it was shown that if two or more antagonist concentration-response curves are measured, each at a fixed agonist concentration, then these curves can be analysed to give estimates of pK_I and of the agonist concentration-ratio(s). This method should be applicable if antagonism is competitive under equilibrium or pseudo-irreversible conditions, or non-competitive, provided that the agonist and antagonists all interact with the receptor on a 1:1 molecular basis. The present paper shows how this technique has been used to measure the pK_I values of prazosin and of idazoxan for receptors acted on by neurotransmitter released by field stimulation of the rat isolated vas deferens.

Theory

The theoretical basis of the technique, for measuring the dissociation constant of a competitive antagonist without

knowledge of agonist concentrations, has been set out and analysed in the preceding paper. Initially, the method was based on the general null equation for competitive antagonism under equilibrium conditions, namely

$$\frac{1 + [I]''/K_I}{1 + [I]'/K_I} = \frac{[A]''}{[A]'} \quad (1)$$

where concentration [A]' of agonist with [I]' of antagonist produce the same response, from an isolated cell or tissue, as that produced by [A]'' of agonist with [I]'' of antagonist. If each antagonist concentration-response curve is measured at a fixed agonist concentration then equation 1 can be rearranged to give

$$[I]'' = (m - 1)K_I + m [I]' \quad (2)$$

where the fixed agonist concentration-ratio is

$$m = [A]''/[A]' \quad (3)$$

As outlined in the previous paper, equation 2 can also be derived if the antagonist is competitive but acts under pseudo-irreversible conditions or if the antagonist acts non-competitively on the receptor. However, in these cases *m* is not the agonist concentration-ratio but is given by a more complex expression such as

$$m = \left\{ 1 + \frac{K_A}{[A]'} \right\} / \left\{ 1 + \frac{K_A}{[A]''} \right\} \quad (4)$$

where K_A is an apparent dissociation constant of the agonist-receptor complex.

If the responses measured at the higher agonist concentration, [A]'', can be fitted by the general logistic equation

$$r = \frac{\alpha - \delta}{1 + \left\{ \frac{[I]}{\gamma} \right\}^\beta} + \delta \quad (5)$$

¹ Author for correspondence.

² Present address: The William Harvey Research Institute, St. Bartholomew's Hospital Medical College, Charterhouse Square, London EC1M 6BQ.

(where r is the response, $[I]$ is the molar concentration of antagonist and α , β , γ and δ are adjustable parameters) then equations 2 and 5 can be combined to produce a general equation which can fit each member of a family of curves with common values of α , β , γ , δ and K_1 but with different values of m . The appropriate equation is

$$r = \frac{\alpha - \delta}{1 + \left\{ \frac{(m-1) K_1 + m [I]}{\gamma} \right\}^\beta} + \delta \quad (6)$$

which reduces to equation 5 for the control curve when $m = 1$. Equation 6 can be used to obtain estimates of pK_1 and m from two or more antagonist concentration-response curves whether the antagonism is competitive, pseudo-irreversible or non-competitive, provided that the agonist and antagonists all interact with the receptor on a 1:1 molecular basis.

The usefulness of equation 6 can be extended by deriving a null equation for two competitive antagonists, I and J , acting simultaneously against agonist A which acts on one type of receptor on the cell or tissue. When the antagonists are acting under equilibrium conditions the proportion of receptors occupied by the agonist would be

$$P_A = \frac{[A]/K_A}{\{1 + [A]/K_A + [I]/K_I + [J]/K_J\}} \quad (7)$$

From equation 7 a null equation can be derived relating those concentrations $[I]'$ and $[I]''$ of the first competitive antagonist which produce equal responses in the presence of fixed concentrations $[A]'$ and $[A]''$ of the agonist in the presence of a fixed concentration $[J]$ of the second competitive antagonist. The resulting equation is of the same form as equation 2 and therefore leads to equation 6 but with K_1 replaced by an apparent dissociation constant

$$K_{\text{Iapp}} = K_1 \{1 + [J]/K_J\} \quad (8)$$

If K_1 is already known then K_J can be calculated from K_{Iapp} .

Methods

Experimental techniques

Male Wistar rats (250–350 g) were killed by cervical dislocation and bled out. The vasa deferentia were removed and the epididymal portions placed in Krebs-Henseleit solution of the following composition (mM): Na^+ 143.3, K^+ 5.9, Ca^{2+} 2.6, Mg^{2+} 1.2, Cl^- 128.3, HCO_3^- 24.9, SO_4^{2-} 1.2, H_2PO_4^- 2.2, ascorbic acid 0.057 and glucose 10.0. The physiological saline was gassed with 95% O_2 /5% CO_2 .

Two different protocols were used in the experiments.

Protocol A

Each epididymal portion of vas deferens was cleared of excess fat and tissue, and mounted in an organ bath at 37°C. Contractions produced either by field stimulation via platinum electrodes positioned parallel to the tissue, or by addition of noradrenaline to the organ bath, were recorded on a Lectromed MX216 recorder via a Dynamometer UF1 isometric transducer. The resting tension on the tissue was 0.5 g.

After being set up in the organ bath, tissues were initially washed twice at 15 min intervals and then exposed to noradrenaline (1.25 μM and 12.5 μM) for 30 s periods, or stimulated with 30 s trains (alternatively at 12.5 and 25 Hz, pulse width 0.5 ms, supramaximal current (400 mA)) every 10 min for 30 min. After this period tissues were exposed to

noradrenaline (1 μM and 2 μM) or stimulated (12.5 Hz and 25 Hz) every 5 min for about 30 min by which time reproducible responses were obtained. During this time and thereafter, desmethylinipramine (0.3 μM) and propranolol (0.3 μM) were present in the bathing medium to block neuronal uptake of noradrenaline and effects on β -adrenoceptors. The effects of increasing concentrations of antagonist were then tested, each concentration being allowed to act for 30 min. When the responses were elicited by exogenous noradrenaline the antagonist was replaced after washout, either with the same or with a higher concentration as appropriate. When the responses were produced by field stimulation the organ bath was washed out and a higher antagonist concentration applied every 30 min. In some experiments corticosterone (30 μM) was also present to block extraneuronal uptake of noradrenaline.

Protocol B

Each epididymal portion of vas deferens was cleared of fat, desheathed, and mounted in an organ bath at 37°C. Contractions of the vas deferens were produced either by field stimulation via platinum electrodes positioned parallel to the tissue, or by addition of noradrenaline to the organ bath. In this protocol the contractile responses were recorded on a Lectromed MX216 recorder via a Gould-Statham isometric transducer, and a damped output ($t_{1/2} = 1.5$ s) was fed from the MX216 to a Chessel flat-bed recorder to produce more easily measurable traces. The resting tension on the tissue was 0.5 g.

After being set up in the organ bath the tissues were stimulated with 30 s trains (alternately at 5 and 10 Hz, pulse width 0.5 ms, supramaximal current (400 mA)) every 5 min for 30 min then every 10 min for 60 min. During this time the tissue was washed by overflow every 15 min. Any additional drugs required to be present during the experiment were added at this stage and replaced after completion of each subsequent wash. When noradrenaline concentration-response curves were being measured the responses were produced by bolus injections of the agonist. The contact time of the agonist was that required to obtain plateau or peak responses (at least 25 s). After draining and refilling the organ bath 3 times, a rest period of 7 min was allowed before adding the next bolus injection of noradrenaline. After measurement of the first agonist concentration-response curve a rest period of 1 h was allowed before the above procedure was repeated. After the second curve, which was usually very similar to the first, a chosen concentration of antagonist was added to the organ bath and allowed to act for 1 h before the next agonist concentration-response curve was obtained. When antagonist was also present in the organ bath it was replaced each time the agonist was washed out. The above procedure was repeated until the effect of a suitable number of concentrations of antagonist had been tested. When the effect of the antagonist was being measured on responses to various frequencies of nerve stimulation (range 10 Hz to 1 Hz) the procedure was essentially the same as that described above except that the organ bath was washed out and antagonist or other drugs added only after the completion of each frequency-response curve.

Materials and solutions

Prazosin hydrochloride, desmethylinipramine hydrochloride (DMI), corticosterone, α, β -methylene-adenosine 5'-triphosphate (α, β -Me ATP) and (–)-noradrenaline bitartrate, were all supplied by Sigma Chemical Co. ($\pm$)-Propranolol hydrochloride was a gift from I.C.I. All inorganic salts were of A.R. grade.

Solutions of noradrenaline were prepared daily in ascorbic acid solution (100 μM) and stored on ice. Prazosin hydrochloride was dissolved in either methanol or ethanol, dilutions being made with ethanol. Solutions of corticosterone were

also prepared in ethanol. The volumes of alcoholic solutions added to the organ bath were kept low, so that the alcohols themselves had no detectable effect on tissue responses.

Analysis of results

The general curve-fitting programme ALLFIT was modified, as described in the appendix of the preceding paper, to enable equation 6 to be fitted to sets of data, each set consisting of two or more antagonist concentration-response curves measured at constant agonist concentrations on a single sample of tissue. In some experiments these constant agonist concentrations were produced by applying exogenous agonist to the tissue. In others, field stimulation was applied at different frequencies, for sufficiently long periods of time to produce either steady or peak responses. These responses were assumed to correspond to steady, though unknown, interstitial concentrations of neurotransmitter.

Each experiment gave an estimate of pK_i and of the agonist concentration-ratio(s). Unless otherwise stated values are given as mean $\pm$ s.e.mean based on n results. Depending on the experimental design, tests of significance between groups were based either on the Kruskal-Wallis non-parametric test followed by distribution-free multiple comparisons, or on Student's paired t tests (Dunn, 1964; Snedecor & Cochran, 1967).

Results

Protocol A

Preliminary experiments showed that when this protocol was followed the tissue was capable of giving reproducible responses for about 4 h. Typical results obtained with exogenous noradrenaline and field stimulation are shown in Figure 1a and b respectively. Using prazosin as an antagonist of exogenous noradrenaline, values of pK_i and of the agonist concentration-ratio, m , were measured in the presence of propranolol and DMI, with and without corticosterone. Analogous experiments were carried out using field stimulation instead of exogenous noradrenaline. In the latter case the effects of α, β -Me-ATP on pK_i and m were also tested. These results are summarised in Table 1.

Application of the Kruskal-Wallis test suggested the presence of significant differences between the groups of results shown in Table 1 ($P < 0.05$). Distribution-free multi-

Figure 1 (a) Antagonist concentration-response curves for prazosin acting against 2 μ M (■) and 1 μ M (▲) noradrenaline on the epididymal portion of the rat isolated vas deferens at 37°C. Each concentration of antagonist was allowed to act on the tissue for 30 min. Other drugs present were propranolol (0.3 μ M), desmethylinipramine (0.3 μ M) and corticosterone (30 μ M). The pK_i of prazosin estimated from these curves was 8.14. (b) Antagonist concentration-response curves for prazosin acting against neurotransmitter released by field stimulation of the epididymal portion of the rat isolated vas deferens at 37°C. The stimulation frequencies were 25 Hz (■) and 12.5 Hz (▲). Each concentration of antagonist was allowed to act on the tissue for 30 min. Other drugs present were propranolol (0.3 μ M), desmethylinipramine (0.3 μ M) and corticosterone (30 μ M). The pK_i estimated from these curves was 8.61.

Table 1 Estimates of pK_i from antagonist concentration-response curves for prazosin at 37°C, with a 30 min contact time*

	Stimulus	pK_i	m_2	No. of results
(a)	Exogenous noradrenaline	8.02 ± 0.02	$2.05 \pm 0.11^{**}$	5
(b)	Exogenous† noradrenaline	8.17 ± 0.09	$1.83 \pm 0.08^{**}$	5
(c)	Field stimulation	8.51 ± 0.07	4.41 ± 0.50	6
(d)	Field† stimulation	8.52 ± 0.32	4.91 ± 1.83	4
(e)	Field†† stimulation	8.24 ± 0.12	2.77 ± 0.50	4

*The experiments were on the epididymal segment of rat isolated vas deferens. Desmethylinipramine (0.3 μ M) and propranolol (0.3 μ M) were always present. Values are given as mean $\pm$ s.e.mean. **With exogenous noradrenaline the applied agonist concentration-ratio was 2.0. †Corticosterone (30 μ M) was also present during these experiments. †† α, β methylene-adenosine 5'-triphosphate (10 μ M) was present during these experiments.

ple comparisons indicated no significant difference between individual groups but a significant difference when the results obtained with exogenous noradrenaline were compared with those obtained using electrical stimulation ($P < 0.05$).

Protocol B

Preliminary experiments showed that when this protocol was followed the tissue was capable of giving reproducible responses for about 6 h. Typical results obtained with exogenous noradrenaline and field stimulation are presented as antagonist concentration-response curves in Figures 2a and b respectively. Using prazosin as an antagonist of exogenous noradrenaline, values of pK_i and of the agonist concentration-ratios, m , were measured in the presence of propranolol, with and without DMI plus corticosterone. Analogous experiments were carried out to test the effect of various combinations of propranolol, DMI, α, β -Me-ATP and idazoxan on estimates of pK_i using field stimulation instead of exogenous noradrenaline. These results are summarised in

Figure 2 (a) Antagonist concentration-response curves for prazosin acting against 9.8 (●), 3.1 (■), 0.98 (▲) and 0.31 μM (▼) noradrenaline on the epididymal portion of the rat isolated vas deferens at 37°C. Each concentration of antagonist was allowed to act on the tissue for 1 h. Propranolol (0.3 μM) was also present. The pK_i of prazosin estimated from these curves was 9.08. (b) Antagonist concentration-response curves for prazosin acting against neurotransmitter released by field stimulation of the epididymal portion of the rat isolated vas deferens at 37°C. The stimulation frequencies were 10 (●), 6.67 (■), 3.33 (▲) and 2.50 Hz (▼). Each concentration of antagonist was allowed to act on the tissue for 1 h. Propranolol (0.3 μM) was also present. The pK_i estimated from these curves was 9.04.

Table 2. Application of paired t tests gave $P < 0.01$, $P > 0.5$, $P > 0.1$ and $P < 0.01$ respectively for the pairs in groups (a), (b), (c) and (d) in Table 2.

Table 3 summarises estimates of pK_i and m for prazosin against exogenous noradrenaline, in the presence of various combinations of propranolol, DMI and corticosterone using protocols A and B.

Two experiments were also carried out to test the action of increasing concentrations of DMI against exogenous noradrenaline in the presence of propranolol (0.3 μM). For DMI these experiments gave $pK_i = 6.12 \pm 0.29$, $m_2 = 1.70 \pm 0.19$, $m_3 = 3.33 \pm 0.73$ and $m_4 = 7.07 \pm 2.27$. The applied agonist concentration-ratios, to be compared with the above estimates of m , were 3.16, 10.0 and 31.6 respectively.

Discussion

The theoretical basis of the new method for measuring pK_i values of antagonists was set out and tested in the previous paper. How well the modified version of ALLFIT can fit equation 2 to real antagonist concentration-response curves, obtained with either exogenous noradrenaline or nerve stimulation, can be judged by examining Figures 1a and b, 2a and b. An important assumption made when applying this method of analysis is that the agonist and antagonist are acting on only one type of receptor. For this reason propranolol was always present in these experiments to block any actions of noradrenaline on β -adrenoceptors. Electrical stimulation of the rat vas deferens is known to cause concurrent release of noradrenaline and adenosine-5'-triphosphate (McGrath, 1978; Brown *et al.*, 1983; Sneddon & Burnstock, 1984) which act independently on their postsynaptic receptors to produce contraction of the smooth muscle (Ventura & Pennefather, 1991). The P_2 -purinoceptor agonist α, β -MeATP blocks the effects of ATP by desensitizing these receptors (Sneddon & Burnstock, 1983; Stjarne & Astrand, 1985). In the experiments described here, α, β -MeATP produced a transient contraction after which addition of more α, β -MeATP failed to produce any response. The presence of α, β -MeATP caused the response to field stimulation to be reduced by up to 50%. Nevertheless, with protocol B, the presence of α, β -MeATP caused no significant change in the pK_i obtained for prazosin, as shown in Table 2. A similar result was obtained with protocol A (Table 1). The reason for this finding is not entirely clear though it may be due to some cancellation of error. In particular it should be emphasised that the new method for estimating pK_i depends on relative positions of antagonist concentration-response curves, and is not sensitive to some kinds of curve distortion.

Table 2 Estimates of pK_i from antagonist concentration-response curves for prazosin at 37°C, with a 1 h contact time*

Paired vasa deferentia					
		First vas deferens		Second vas deferens	
	Stimulus	pK_i	Other drugs present	pK_i	Other drugs present
(a)	Exogenous noradrenaline	9.12 ± 0.05 ($n = 4$)	Propranolol	8.69 ± 0.07 ($n = 4$)	propranolol DMI, cortico- sterone
(b)	Field stimulation	8.86 ± 0.06 ($n = 4$)	Propranolol DMI	8.81 ± 0.16 ($n = 4$)	propranolol DMI, α, β -Me-ATP
(c)	Field stimulation	8.81 ± 0.12 ($n = 9$)	Propranolol	8.60 ± 0.09 ($n = 9$)	propranolol, DMI
(d)	Field stimulation	8.94 ± 0.11 ($n = 3$)	Propranolol α, β -Me-ATP	7.70 ± 0.20 ($n = 3$)	propranolol, idazoxan, α, β -Me-ATP

*The experiments were on the epididymal segment of rat isolated vas deferens. Drug concentrations were propranolol 0.3 μM , DMI (desmethylinipramine) 0.3 μM , corticosterone 30 μM , α, β -Me-ATP (α, β methylene-adenosine 5'-triphosphate) 10 μM and idazoxan 10 μM . Values are given as mean $\pm$ s.e.mean based on n results.

Table 3 Estimates of pK_i for prazosin at 37°C, and noradrenaline concentration-ratios, m , from antagonist concentration-response curves*

Protocol*	Other drugs† present	$\log_{10}K_i$	m_2	m_3	m_4	No. of results
A	Propranolol,	8.03 ± 0.02	$2.07 \pm 0.14^{**}$	—	—	5
	DMI	8.01 ± 0.08	2.00 fixed**	—	—	
A	Propranolol, DMI,	7.99 ± 0.08	$1.88 \pm 0.10^{**}$	—	—	5
	corticosterone	8.06 ± 0.10	2.00 fixed**	—	—	
B	Propranolol	9.12 ± 0.05	$3.25 \pm 0.25^{\dagger\dagger}$	$11.3 \pm 1.6^{\dagger\dagger}$	$40.7 \pm 10.0^{\dagger\dagger}$	4
		9.07 ± 0.08	3.16 fixed††	10.0 fixed††	31.6 fixed††	
B	Propranolol, DMI,	8.69 ± 0.07	$2.69 \pm 0.05^{\dagger\dagger}$	$9.2 \pm 0.7^{\dagger\dagger}$	$30.6 \pm 8.0^{\dagger\dagger}$	4
	corticosterone	8.69 ± 0.05	3.16 fixed††	10.0 fixed††	31.6 fixed††	

*The experiments were on the epididymal segment of rat isolated vas deferens. The results were obtained using two protocols with different contact times for antagonist, 30 min for protocol A and 1 h for protocol B. Values are given as mean $\pm$ s.e.mean. †Drug concentrations were propranolol 0.3 μ M, DMI (desmethylinipramine) 0.3 μ M and corticosterone 30 μ M. **The applied noradrenaline concentration-ratio was 2.0. ††The applied noradrenaline concentration ratios were 3.16, 10.0 and 31.6.

This is clear from the fact that this method can be used for competitive, pseudo-irreversible or non-competitive antagonism.

It was suggested in the preceding paper that the new method should permit measurement of pK_i values even when uptake or metabolism of the agonist is not blocked, provided that these processes remain constant during the experiment. In principle these comments should apply whether the agonist is applied exogenously or released endogenously. Statistical analysis of the pK_i estimates obtained with protocol A (see Table 1) detected no significant differences between individual groups. This suggests that neither corticosterone nor α , β -MeATP significantly affected the estimation of pK_i by the new method. However, further statistical analysis showed that the values of pK_i obtained with exogenous noradrenaline were significantly lower than those obtained using electrical stimulation. Since the latter effect was not found with protocol B, the lower pK_i values obtained with exogenous noradrenaline using protocol A are presumably due to some poorly controlled factor in these particular experiments. The main conclusion from examination of Table 1 is that use of protocol A, with a 30 min contact time, produced estimates of the pK_i for prazosin against exogenous noradrenaline which were lower than those measured against neurally released transmitter, and both sets of values obtained with protocol A were lower than those obtained with protocol B (see Table 2).

Use of paired instead of unpaired experiments might be expected to enable detection of smaller treatment effects. Statistical analysis of the paired sets of results shown in Table 2, which were obtained using protocol B with a 1 h contact time for the antagonist, suggests that the pK_i -value for prazosin against exogenous noradrenaline was significantly reduced by the combined presence of DMI and corticosterone (group (a), $P < 0.01$). Using protocol B, DMI without corticosterone seemed to produce a small reduction in the pK_i of prazosin which however is not statistically significant (group (c), $0.2 > P > 0.1$). As will be seen from the results section, DMI itself appeared to act as a non-competitive or pseudo-irreversible antagonist against exogenous noradrenaline since the values of m differed greatly from the applied agonist concentration ratios. The estimated pK_i of 6.12 for DMI can be compared with values of 6.3 to 6.7 reported by McCulloch & Story (1972). A pK_i

of 6.8 was obtained by U'Pritchard *et al.* (1978) for DMI as a competitor at [3 H]-WB-4101 ('noradrenergic') binding sites in rat brain. If the effect of DMI on the rat isolated vas deferens can be considered 'competitive' at low concentrations then 0.3 μ M DMI might be expected to reduce the apparent pK_i of prazosin by 0.15 to 0.40 log units.

The presence of idazoxan (10 μ M) produced a highly significant reduction in the apparent pK_i of prazosin against released transmitter (Table 2, group (d), $P < 0.01$). Assuming the true pK_i value of prazosin to be 8.81 (group (c), Table 2) the pK_i of idazoxan against released transmitter can be calculated from equation 8 to be 6.07. This is close to the value of 6.10 obtained for idazoxan acting as an antagonist of α_1 -adrenoceptors on rat anococcygeus muscle (Doxey *et al.*, 1983).

It is worth emphasising that for those experiments in which agonist was applied exogenously, the values of the agonist concentration-ratios estimated by this new method of analysis are generally in good agreement with the ratios actually applied to the tissue (see Table 3). This agreement, which was found whether the contact time for the antagonist was 30 min or 1 h, suggests that in these experiments either prazosin was acting competitively or there was appreciable spare receptor capacity for the agonist. Since prazosin seems to equilibrate slowly with the receptors the latter explanation may be the correct one.

The values obtained with protocol B are in good agreement with the value of 8.97 reported by Minneman *et al.* (1982) and 9.26 reported by Aboud *et al.* (1993), who used 45 min and 60 min contact times respectively. Lower pK_i values were obtained with protocol A, probably because that involved less drastic removal of connective tissue and a shorter equilibration time for the antagonist.

The results discussed above provide general support for the new method for measuring pK_i values of antagonists from antagonist concentration-response curves. They also confirm the potential of the new method for use in situations where the concentration of agonist is controllable but unknown. In the present series of experiments with a 1 h equilibration time for antagonist, most estimates of the pK_i of prazosin against released transmitter were in the range 8.7 to 9.1, while that of idazoxan was close to 6.1. These estimates are consistent with values obtained by other workers for interaction of these antagonists with α_1 -adrenoceptors.

References

- ABOUD, R., SHAFII, M. & DOCHERTY, J.R. (1993). Investigation of the subtypes of α_1 -adrenoceptor mediating contractions of rat aorta, vas deferens and spleen. *Br. J. Pharmacol.*, **109**, 80–87.
- ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmacol. Chemother.*, **14**, 48–57.

- BROWN, D.A., DOCHERTY, J.R., FRENCH, A.M., MACDONALD, A., MCGRATH, J.C. & SCOTT, N.C. (1983). Separation of adrenergic and non-adrenergic contractions to field stimulation in the rat vas deferens. *Br. J. Pharmacol.*, **79**, 379–393.
- DOXEY, J.C., ROACH, A.G. & SMITH, C.F.C. (1983). Studies on RX781094: a selective, potent and specific antagonist of α_2 -adrenoceptors. *Br. J. Pharmacol.*, **78**, 489–505.
- DUNN, O.J. (1964). Multiple comparisons using rank sums. *Technometrics*, **6**, 241–252.
- MACKAY, D. (1994). A new method for estimating dissociation constants of competitive and non-competitive antagonists with no prior knowledge of agonist concentrations. *Br. J. Pharmacol.*, **111**, 219–226.
- MCCULLOCH, M.W. & STORY, D.F. (1972). Antagonism of noradrenaline and histamine by despiramine in the isolated artery of the rabbit ear. *Br. J. Pharmacol.*, **46**, 140–150.
- MCGRATH, J.C. (1978). Adrenergic and 'non-adrenergic' components in the contractile response of the vas deferens to a single indirect stimulus. *J. Physiol.*, **283**, 23–39.
- MINNEMAN, K.P., FOX, A.W. & ABEL, P.W. (1982). Occupancy of α_1 -adrenergic receptors and contraction of rat vas deferens. *Mol. Pharmacol.*, **23**, 359–368.
- SNEDDON, P. & BURNSTOCK, G. (1984). Inhibition of excitatory junction potentials in guinea-pig vas deferens by α , β -methylene-ATP. Further evidence for ATP and noradrenaline as co-transmitters. *Eur. J. Pharmacol.*, **100**, 85–90.
- SNEDECOR, G.W. & COCHRAN, W.G. (1967). *Statistical Methods*, 6th edition. Ames: Iowa State University Press.
- STJARNE, L. & ASTRAN, D.P. (1985). Relative pre- and postjunctional roles of noradrenaline and adenosine 5'-triphosphates as neurotransmitters of the sympathetic nerves of guinea-pig and mouse vas deferens. *Neurosci.*, **14**, 929–946.
- UPRICHARD, D.C., GREENBERG, D.A., SHEEHAN, P.P. & SYNDER, S.H. (1978). Tricyclic antidepressants: Therapeutic properties and affinity for α -noradrenergic receptor binding sites in the brain. *Science*, **199**, 197–198.
- VENTURA, S. & PENNEFATHER, J.N. (1991). Sympathetic co-transmission to the cauda epididymis of the rat: characterisation of postjunctional adrenoceptors and purinoceptors. *Br. J. Pharmacol.*, **102**, 540–544.

(Received August 9, 1993
Accepted October 5, 1993)