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Modelling the changes induced by chronic desipramine treatment on the factors governing the agonism at prejunctional α_2-adrenoceptors

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1 The adaptational changes induced after chronic desipramine treatment on functional responsiveness of α_2-adrenoceptor activation were investigated in prostatic portions of the rat vas deferens.

2 For this purpose, clonidine and xylazine were studied for their effects on twitch contractions elicited by electrical field stimulation of prostatic portions removed 48 h after the last injection to the animals of vehicle or desipramine (10 mg kg^{-1}, i.p.; 14 days). Operational model-fitting and the nested hyperbolic method were used to analyse the effects of irreversible receptor alkylation by N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ, 300 nM) on the α_2-adrenoceptor-mediated effects of clonidine, either in vehicle- or in desipramine-treated animals.

3 Treatment with desipramine decreased the potency (increased the EC_{50}) of clonidine and xylazine by about 12 and 9 fold respectively. However, the treatment did not modify the maximal effect (α) elicited by either agonist. The estimates of apparent affinity for clonidine did not depend on the method of calculation as the 'null' method and the 'operational' method gave similar answers. Estimates of τ values for both agonists revealed that chronic desipramine treatment resulted in significant decreases in the efficacy of agonists. However, desipramine treatment was not associated with significant changes in the affinity constant for clonidine while for xylazine, the operational model provided a higher estimate of K_A (lower affinity) after desipramine treatment.

4 The results indicate a large receptor reserve at prejunctional α_2-adrenoceptors which is modulated by chronic desipramine treatment.

5 The comparison of results obtained after chronic desipramine exposure with those by using EEDQ suggests that chronic desipramine treatment is not a useful experimental intervention for the purpose of estimating agonist affinities and efficacies.

Keywords: Prejunctional α_2-adrenoceptors; clonidine; xylazine; agonist affinities; agonist efficacies; desensitization; desipramine; operational model; rat vas deferens

Introduction

Prejunctional α_2-adrenoceptors are present on nerve endings in both the peripheral and central nervous system. These α_2-adrenoceptors (auto- or heteroreceptors) mediate a negative feedback mechanism modulating the Ca^{2+}-dependent stimulation-evoked release of neurotransmitter (Starke, 1981). On the basis that α_2-adrenoceptors exert an important physiological role in regulating neurotransmitter availability for a number of target cells, and that their expression levels at the plasma membrane may be regulated by a variety of physiological or pathological conditions, they are attractive targets for pharmacological exploitation.

Many studies in the central nervous system have described a reduction in physiological, biochemical and behavioural responses to clonidine (an agonist of α_2-adrenoceptors) following chronic treatment with antidepressant drugs (see Charney *et al.*, 1981). This effect has been attributed to a decrease in the number of presynaptic α_2-adrenoceptors. Indeed, a reduction in the number of α_2-adrenoceptors in the brain has been demonstrated following chronic administration of antidepressant drugs (Giralt & García-Sevilla, 1989). Likewise, it has been found that following chronic treatment with monoamine oxidase (MAO) inhibitors and tricyclic antidepressant drugs, the inhibitory action of clonidine in rat vas deferens (Finberg & Tal, 1985; García-Sevilla & Zubieta, 1986) and of xylazine in rat anococcygeus muscle (Vila *et al.*, 1990) on the contraction elicited by electrical field stimulation is reduced. The isometric twitch response evoked by electrical pulse stimulation in prostatic portions of rat vas deferens has proven to be a suitable response for the examination of the prejunctional activity of agonists at α_2-adrenoceptors (Blakely *et al.*, 1981; Badia & Sallés, 1989). In a previous study (Sallés *et al.*, 1994) the irreversible α_2-adrenoceptor antagonist N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) was used to estimate the affinity and efficacy of a series of clonidine derivatives for the α_2-adrenoceptors of the prostatic portion of rat vas deferens. According to the operational model of agonism (Black & Leff, 1983), agonist action can be described by four parameters: K_A, the agonist-receptor dissociation constant; τ, the operational efficacy; E_m, the maximal effect in a particular receptor system and n, the slope parameter for the function relating receptor occupancy to pharmacological effect. In theory, an experimental intervention could affect any of these parameters. Indeed, a number of publications have demonstrated how experimental interventions affect τ (Black *et al.*, 1985a; Barret *et al.*, 1986), both τ and E_m (Leff *et al.*, 1985) and both τ and n (Dougall & Leff, 1987). However, no such examples of interventions affecting α_2-adrenoceptor mediated responses are evident in the literature.

As shown previously (Black *et al.*, 1985a; Leff *et al.*, 1990) elucidation of undefined interventions depends partly on a comparison of their effects with those of a well-characterized

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irreversible antagonist at the receptor in question. As mentioned above, EEDQ has been shown to be an irreversible α_2-adrenoceptor ligand useful for characterization of α_2-adrenoceptor-mediated agonism (Sallés *et al.*, 1994). The objective of this study was to compare the use of EEDQ with the effect of chronic desipramine treatment on responses to the full agonist, clonidine and the partial agonist, xylazine in the rat vas deferens preparation. Such a comparison should indicate if the latter intervention is a useful means of obtaining reliable agonist affinity and efficacy estimates.

Methods

Animals and chronic desipramine treatment

Male Wistar rats (200–225 g) with free access to food and water, and maintained on a 12 h light/dark schedule were injected i.p. daily for 14 days either with saline or desipramine 10 mg kg^{-1}. Animals were killed by decapitation 48 h after the last injection. Both vasa deferentia were removed, carefully cleaned and bisected into prostatic and epididymal portions and used for functional studies.

Rat isolated field-stimulated prostatic portions

The prostatic portion of the rat vas deferens was placed between platinum electrodes in 20 ml organ baths and incubated at 32°C with Krebs bicarbonate solution of the following composition (in mM): NaCl 112.0, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.1, $MgSO_4$ 1.2, $NaHCO_3$ 25.0 and glucose 11.1. The solution was maintained at 32 ± 0.5 °C and gassed with 95% O_2, 5% CO_2. Organ responses were recorded by means of an isometric PIODEN (UF-1) transducer attached to an OmniScribe pen recorder.

Experimental protocols

General At the beginning of each experiment, a force of 0.5 g wt was applied to each tissue. This was followed by a 45 min stabilization period before electrical stimulation was started. At the end of the equilibration period the tension was re-instated. Contractions of the tissue were elicited by field stimulation at supramaximal voltage, with square wave electrical pulses (3 ms duration, 0.1 Hz, 20-40 V) delivered via a Grass S44 stimulator, conditions known to produce neurogenic responses, the stimuli being about 15% greater than that required to elicit a maximum contraction. Reproducible responses to supramaximal electrical stimulation were established after about 25 min. The tissues were rinsed every 15 min during these periods by replacing the organ bath contents with fresh buffer, prior to the addition of drugs.

Irreversible receptor inactivation

Agonist-concentration-effect (E/[A]) curves were constructed by cumulative additions of clonidine or xylazine at 0.5 $\log_{10}$ unit increments as previously described (Sallés *et al.*, 1994). Responses were measured as the height of the twitch contractions in the presence of agonist and are expressed as percentage inhibition of the height of the basal twitch contractions (in g wt of tension) induced by electrical stimulation (Sallés *et al.*, 1994). Only a single curve was generated in each tissue. A multiple curve design could not be used because it was difficult to remove clonidine despite repeated washing. Furthermore, a gradual loss of size of twitch responses was observed. The following protocol was therefore adopted. Experiments were performed on paired prostatic portions of vas deferens from a single rat. One portion of the pair was treated with a single concentration of the irreversible antagonist, while the other was treated with the vehicle used to dissolve the alkylating agent.

Prostatic portions of vas deferens from rats chronically treated with saline or desipramine were incubated with EEDQ (300 nM) for 15 min, after which excess inhibitor was removed by several changes of the organ bath Krebs solution over the following 30 min as described elsewhere (Sallés *et al.*, 1994). Ten minutes later, agonist concentration-response curves were obtained as described above.

Analysis of data

Pragmatic logistic curve fitting Each individual set of E/[A] curve data, recorded as fractional inhibitions of twitch were fitted to a logistic function of the form:

$$E = \frac{\alpha \times [A]^m}{[EC_{50}]^m + [A]^m} \quad (1)$$

in which E and [A] are the pharmacological effect and the concentration of agonist, respectively; α, $[EC_{50}]$ and m are the asymptote, location and slope parameters, respectively. Location parameters were actually estimated as negative logarithms (pEC_{50}).

Operational model-fitting E/[A] data obtained experimentally were fitted to the operational model of agonism in order to estimate agonist efficacies and affinities (Black & Leff, 1983; Black *et al.*, 1985b):

$$E = \frac{E_m \times \tau^n \times [A]^n}{(K_A + [A])^n + \tau^n \times [A]^n} \quad (2)$$

in which E_m is the maximum possible effect; K_A is the dissociation constant of the agonist from the receptor (this was estimated as the negative logarithm, that is, pK_A); τ is the ratio $[R_o]/K_E$, where $[R_o]$ is the total, functional receptor concentration and K_E defines the value of occupancy, [AR], for half E_m; n is the slope parameter for the assumed logistic relation linking [AR] to effect, E. Operationally, τ defines the efficacy of an agonist in a system.

A combination of two analytical methods was employed, the receptor inactivation method for the full agonist clonidine and the comparative method for the partial agonist, xylazine (Leff *et al.*, 1990). In the case of clonidine, two experimental situations, absence or presence of desipramine treatment, were considered and, for each of these cases, two different conditions, no exposure or exposure to the irreversible antagonist EEDQ, were applied. Resulting clonidine E/[A] curve data were fitted simultaneously to equation 2 to obtain estimates of E_m, n, K_A and τ. First, the four parameters were allowed to be different between vehicle and desipramine-treated animals. The hypothesis that desipramine intervention could alter some of these parameters is addressed and tested below. When EEDQ was introduced within the same experimental situation, τ was allowed to vary but with common E_m, n and K_A. In the case of xylazine, the partial agonist, only vehicle and desipramine-treated rats were considered and no irreversible antagonist was added. Vehicle and desipramine-treated xylazine E/[A] curve data were fitted to equation 2 to estimate τ and K_A for each experimental situation. In this fitting procedure, E_m and n values were fixed at the estimates obtained from the corresponding clonidine analysis and kept fixed. This procedure was carried out for vehicle and desipramine-treated animals.

In data analysis, the results of logistic curve fitting will be considered in terms of the operational model. In order to do this, Black & Leff (1983) defined the logistic curve parameters in terms of their operational model counterparts:

$$\alpha = \frac{E_m \times \tau^n}{1 + \tau^n} \quad (3)$$

$$EC_{50} = \frac{K_A}{(2+\tau^n)^{1/n} - 1} \quad (4)$$

$$m = \frac{n \times (2+\tau^n) \times ((2+\tau^n)^{1/n} - 1)}{(2+\tau^n)^{1/n} \times (1+\tau^n)} \quad (5)$$

Therefore, model equations (3), (4) and (5) establish the predicted behaviour of the asymptote, location and slope curve parameters with variations in the estimated operational model parameters, E_m, τ, n and K_A.

Null approach Clonidine E/[A] curve data were also analysed by the nested hyperbolic null method (James *et al.*, 1989). This method which is analytically simpler than the classical method of Furchgott (1966) involves fitting the control E/[A] curve data to equation 1 whilst simultaneously fitting the post-inactivation E/[A] curve data to the following equation:

$$E = \frac{\alpha}{\left(\frac{EC_{50}}{q \times K_A \times [A']} \times (K_A + [A'] \times (1-q))\right)^m + 1} \quad (6)$$

where q represents the fractional receptor concentration remaining following inactivation.

Statistics

Experimental points and results from pragmatic logistic curve fitting are expressed as mean ± s.e.mean. The statistical significance of the observed differences was assessed by the Student's two tailed *t* test. In all cases, significance was set at a *P* value less than 0.05. The number of separate preparations used in each experimental protocol is indicated as '*n*' in the legend to the Figures and Tables.

Experimental data were directly fitted to the mathematical models described above with the 'AR' programme (derivative-free, nonlinear, regression analysis) within the BMDP statistical software package (Dixon, 1990), implemented on a Vax 6610 computer. It is assumed that estimates of K_A, EC_{50} and τ are log-normally distributed; therefore, each of these is expressed as a logarithmic value. α, m, E_m and n are assumed to be approximately normally distributed on the natural scale (Fleming *et al.*, 1972; Leff *et al.*, 1990).

The operational model of agonism (Black & Leff, 1983) provides an explicit description of E/[A] curves, allowing the evaluation of supposed changes in curves profiles after an experimental intervention. In the present study the experimental intervention is desipramine treatment. Thus, as established above, the pharmacological system comprised two experimental situations, prostatic portions from rats chronically treated with saline or desipramine. For clonidine, two conditions were included in each situation, namely, absence or presence of the irreversible antagonist, EEDQ. The effect of desipramine treatment was assessed by determining the goodness of fit of a series of models, with selected constraints, with respect to a reference model. Test and reference models both contain vehicle and desipramine-treated rats and control and EEDQ inactivation. In the reference model the four parameters E_m, n, K_A and τ for desipramine treatment were allowed to be different from corresponding values for vehicle. In the test models, one or more of these parameters are kept common for saline and desipramine treatment. Typically, both in reference and test models two different values for τ accounting for the absence or presence of EEDQ in each situation were always allowed. In the case of xylazine no irreversible antagonist was added, and E_m and n for vehicle and desipramine-treated animals were assumed from clonidine analysis and kept fixed. Comparisons between desipramine and vehicle were made by constraining K_A or τ. This procedure follows the rationale fully discussed in a previous paper (Leff & Giles, 1992) in which the similarity or otherwise of affinity and the efficacy in two different tissues for a series of agonists was analyzed. Briefly, the goodness of fit of a model under the imposition of various constraints is assessed by means of an *F*-test (Ratkowsky, 1983). This approach is statistically optimal, because experimental errors are not systematically distorted (De Lean *et al.*, 1978). The *F* value was calculated as follows:

$$F = \frac{\frac{SS_2 - SS_1}{df_2 - df_1}}{\frac{SS_1}{df_1}} \quad (7)$$

where SS_1 is the residual sum of squares, df_1 the number of degrees of freedom of the reference model, SS_2 and df_2 the corresponding ones for an alternative model in which desipramine and vehicle systems are constrained to share common E_m, n, K_A and/or τ. A significant impairment in goodness of fit, ($P<0.05$) when parameters are shared, indicates that the parameters of the two sets of E/[A] curves were experimentally different; in other words, the analysis that permitted one or more of the parameters to be shared without a significant increase in the residual variance was taken to be the best fit.

Drugs

Drugs used and their sources were: desipramine hydrochloride and EEDQ (N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline) (Sigma), clonidine hydrochloride (Boehringer Ingelheim), xylazine hydrochloride (Bayer, A.G.). EEDQ was dissolved initially in absolute ethanol and stored as 30 mM solution at −22°C. Further dilutions were made daily in propylene glycol and distilled water (final v/v/v ratio 1:1:2). At final concentrations to which tissues were exposed, the vehicle did not influence tissue responsiveness. Desipramine hydrochloride was dissolved in 0.9% w/v NaCl.

Results

Effect of chronic desipramine treatment on functional prejunctional α_2-adrenoceptors of the prostatic portion of rat vas deferens

The results of these studies are presented in Figure 1 and Table 1. There was no significant change in strength of contractile response of the prostatic portion to electrical field stimulation following chronic treatment with desipramine 48 h after 14 daily doses of the drug. The average twitch sizes (expressed in g wt) were: saline-treated rats 1.12 ± 0.06 ($n=16$) and desipramine-treated rats 0.98 ± 0.05 ($n=16$). However, after chronic treatment with desipramine the E/[A] curves for clonidine and xylazine were shifted to the right (Figure 1). Desipramine treatment reduced the potency (increased the EC_{50}) of clonidine and xylazine by about 12 fold ($P<0.001$) and 9 fold ($P<0.001$) respectively (Table 1) without affecting the maximum responses (α).

The effects of EEDQ (300 nM for 15 min) on clonidine E/[A] curves were examined in prostatic portions of saline- and desipramine-treated rats. The average data are illustrated in Figure 2. Irrespective of the treatment received by the animals, although the curves obtained were in keeping with the expectation of the effects of irreversible antagonism, they were equally resistant to asymptote depression (Table 1). Quantitative evaluation of the effects of EEDQ irrespective of the treatment received by the animals was carried out using the nested hyperbolic method and the operational model (see below).

Quantitative analysis of agonism

Nested hyperbolic method All the E/[A] curves depicted in Figure 2 including both experimental data sets were analysed

simultaneously, but independently to obtain the respective K_A and q values for clonidine. The estimated pK_A values for clonidine were 7.00 ± 0.08 and 7.17 ± 0.11 for vehicle- and desipramine-treated rats, respectively. Furthermore, the statistical analysis show no significant difference resulting from desipramine pretreatment. However, the estimated value for q × 100 (the theoretical fraction of functional receptors remaining after EEDQ inactivation) was consistently lower in the naive than in the desipramine-treated group, being 1.1% and 16.7% for vehicle- and desipramine-treated rats, respectively.

By use of the affinity constant values aforementioned, the receptor occupancy was calculated for each concentration of clonidine and plotted as fractional receptor occupancy against the fractional responses (see legend Figure 3). Note that the occupancy-effect relationship of naive and desipramine-treated tissues are nonlinear and similar in shape. However, as shown, clonidine induces the maximal effect in vehicle-treated rats when only 25% of the receptors were occupied, but in desipramine-treated rats, 100% of the receptors were required to elicit the maximal response. The plot of the responses for clonidine against the theoretical percentage of receptor occupancy resulted in hyperbolic curves (Figure 3) that were fitted by using the equation $E = (E_m[AR]/[R_o])/(K_E/[R_o] + [AR]/[R_o])$, where K_E represents the value of [AR] for half E_m (maximal response), E the response, [AR] the receptor occupancy and $[R_o]$ the total functional receptor concentration. Nonlinear regression analysis of these hyperbolic curves resulted in $K_E/[R_o]$ values as follows: $K_E/[R_o] = 1.44\%$ in vehicle-treated rats, and $K_E/[R_o] = 21.1\%$ in desipramine-treated rats.

Operational model The data for the E/[A] curves for clonidine with and without partial alkylation of α_2-adrenoceptors with EEDQ (300 nM) obtained in prostatic portions of vas deferens

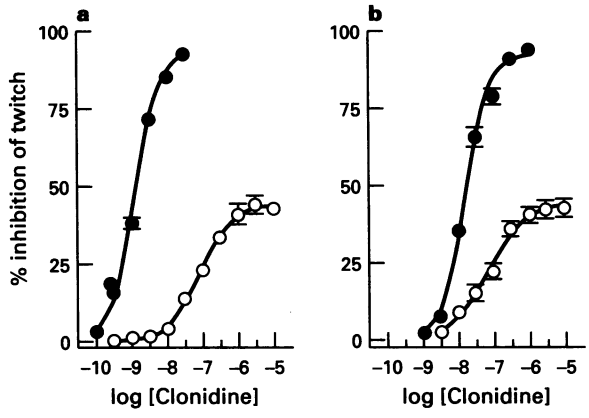

Figure 2 Effect of EEDQ pretreatment on clonidine-evoked inhibition of twitch responses in the prostatic portion of the rat vas deferens from vehicle- (a), and desipramine-treated animals (b). Average clonidine E/[A] curves obtained following a 15 min exposure to vehicle (●) or 0.3 μM EEDQ (○) are depicted. The lines drawn through the data are the results of pragmatic logistic fitting (Eq.(1), see Methods section). Values are mean with s.e. mean ($n = 6$).

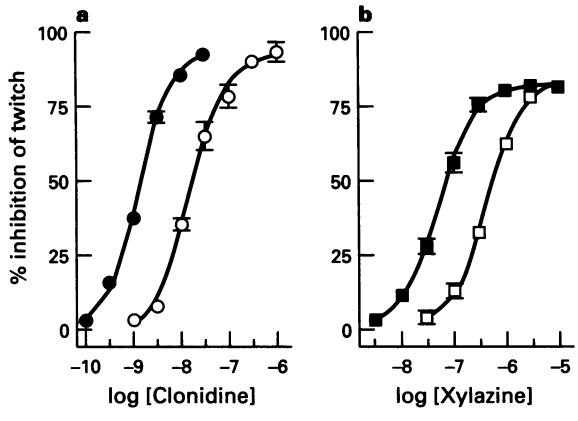

Figure 1 Concentration-response curves for the inhibition of twitch contractions of prostatic portions of the vas deferens by clonidine (a) or xylazine (b) in rats chronically treated with saline (● for clonidine, ■ for xylazine) or desipramine (○ for clonidine, □ for xylazine). Values are mean with s.e. of at least 6 experiments. The lines fitted through the data are calculated according to Eq. (1) in the text.

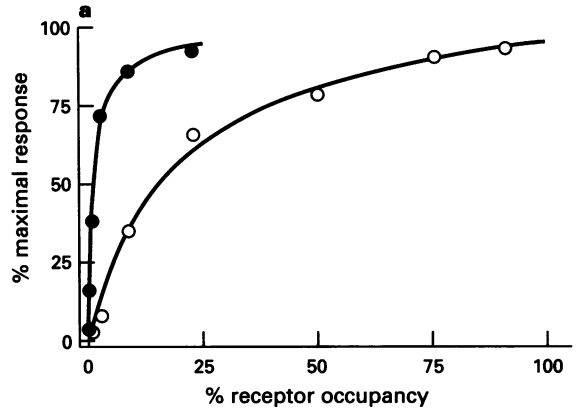

Figure 3 Plot of percentage receptor occupancy versus response for clonidine in vehicle - (●) or desipramine -treated (○) animals. The percentage occupancy of the receptor was calculated from the equation $[AR]/[R_o] = [A]/(K_A + [A])$ where $[AR]/[R_o]$ is the fractional receptor occupancy, K_A is the dissociation constant and [A] is the concentration of the agonist clonidine.

Table 1 Parameter estimates obtained by pragmatic logistic fitting of agonist E/[A] curves performed in prostatic portions of saline- and desipramine-treated rats

	pEC_{50}	α	m
Saline-treated rats			
Clonidine	8.89 ± 0.04	0.95 ± 0.02	1.21 ± 0.10
Clonidine + EEDQ	7.09 ± 0.04	0.45 ± 0.01	0.93 ± 0.06
Xylazine	7.32 ± 0.02	0.82 ± 0.01	1.19 ± 0.05
Desipramine-treated rats			
Clonidine	7.81 ± 0.05***	0.92 ± 0.03	1.24 ± 0.15
Clonidine + EEDQ	7.17 ± 0.08	0.44 ± 0.02	0.80 ± 0.10
Xylazine	6.36 ± 0.04***	0.84 ± 0.03	1.14 ± 0.09

Tissues were removed 48 h after last of 14 daily injections of desipramine (10 mg kg^{-1}) or saline. Mean values are given with s.e.mean. ***$P < 0.001$ for difference from control value (t test).

from animals chronically treated with saline or desipramine were fitted simultaneously using the operational model of agonism (equation 2). This provided estimates of E_m, n, pK_A and τ for clonidine in both experimental conditions. Results are summarized in Table 2. These estimates were then tested for their similarity between treatments by imposing a number of selected constraints on the model parameters and analysing the effect of these constraints on the residual sum-of-squares associated with model-fitting. The results of these analyses are described in full in Table 3. In brief, it was found that: (i) a significant worsening of fit was obtained when the E_m value was assumed to be the same irrespective of the treatment; (ii) a significant worsening of fit occurred when the efficacy (τ) value for clonidine was assumed to be the same after both treatments, although no worsening occurred when the n and pK_A values for clonidine were assumed to be the same irrespective of the treatment. Therefore, chronic treatment with desipramine is associated with significant changes in efficacy (τ) for clonidine and E_m of the system.

As explained in the Methods section (see also Leff *et al.*, 1990; Sallés *et al.*, 1994), the comparative method was employed to establish the affinity and efficacy of xylazine. In terms of the operational model, clonidine was used to provide estimates of E_m and n before proceeding with the comparative analysis. Concentration-effect curve data for xylazine from both treatments were then fitted simultaneously using the operational model of agonism (equation 2). This provided estimates of affinity (pK_A) and efficacy (τ) for xylazine after both treatments (Table 2). In this case, the aforementioned statistical analysis found that: (i) a significant worsening of fit was obtained when the affinity (pK_A) of xylazine was assumed to be the same irrespective of the treatment; (ii) a significant worsening of fit occurred when the efficacy (τ) value for xylazine was assumed to be the same irrespective of the treatment (Table 3). Therefore, it can be concluded that chronic treatment with desipramine is associated with a significant decrease either in efficacy (τ) and in affinity (pK_A) for xylazine.

Discussion

The aim of this study was to quantify the changes induced by chronic desipramine treatment on responses to α_2-adrenoceptor agonist in the rat vas deferens and to compare them with the changes induced by the irreversible antagonist EEDQ. For this purpose, the nested hyperbolic method and the operational model were used, which offer the advantage of directly fitting the experimental data (James *et al.*, 1989; Leff *et al.*, 1990). The present study, therefore extends previous work on the determination of agonist affinities and efficacies at functional presynaptic α_2-adrenoceptors (Sallés *et al.*, 1994).

The application of the above methods requires that various conditions are fulfilled. On the basis of previous results (Sallés *et al.*, 1994) it was assumed that the agonists analysed, clonidine and xylazine, interacted with a homogeneous population of receptors belonging to the α_2-adrenoceptor subtype. Another issue of concern is the possible interference of postjunctional α_1-adrenoceptors. Thus, although the twitch response is suitable for examining the prejunctional activity of α_2-adrenoceptor agonists and antagonists, α_1-adrenoceptor agonist drugs act postjunctionally to produce intermittent contractions of the vas deferens, and to potentiate the twitch response (MacDonald & McGrath, 1980). Our previous data (Badia & Sallés, 1989; Sallés *et al.*, 1994) showed that the imidazolidine like drugs, St-587 and St-591, behave as partial agonists at prejunctional α_2-adrenoceptors only after complete blockade of α_1-adrenoceptors. Hence, it is important to choose an α_2-adrenoceptor agonist with little or no postjunctional effect in this tissue. In our and others studies (Hyland & Docherty, 1985) with prostatic portions of rat vas deferens, xylazine has proven to be the most suitable α_2-adrenoceptor partial agonist.

Our results show that chronic desipramine treatment was followed by a significant reduction in agonist potencies with no depression of the maximum responses. In agreement with our results, previously it has been shown that treatment of rats with MAO inhibitors and/or tricyclic antidepressant drugs

Table 2 Unconstrained operational model-fitting of clonidine and xylazine E/[A] curves of saline and desipramine-treated animals in absence and presence of EEDQ (300 nM)

Agonist	E_m	*n*	$log\ \tau_1$	$log\ \tau_2$	pK_A
Clonidine					
Control	95.42 ± 1.54	1.21 ± 0.06	1.90 ± 0.10	−0.06 ± 0.03	7.00 ± 0.08
Desipramine	109.48 ± 5.13	1.28 ± 0.12	0.60 ± 0.09	−0.16 ± 0.04	7.17 ± 0.11
Xylazine					
Control	–	–	0.72 ± 0.04	–	6.57 ± 0.06
Desipramine	–	–	0.40 ± 0.03	–	5.78 ± 0.06

τ_1 and τ_2 denote respectively τ values in the absence and the presence of EEDQ (300 nM)

Table 3 Goodness-of-fit analysis of operational model fitting of clonidine and xylazine E/[A] curves after desipramine treatment and EEDQ receptor inactivation

Agonist	*Constraint*	*Residual sum of squares*	*Degrees of freedom*	F-*ratio*	P-*value*
Clonidine	None	4001.42	182	–	–
	pK_A	4043.37	183	1.91	0.17
	τ	6096.29	184	47.64	<0.0001
	E_m	4224.32	183	10.14	0.0017
	n	4007.53	183	0.28	0.60
Xylazine	None	4813.66	128	–	–
	pK_A	6939.87	129	56.53	<0.0001
	τ	6117.14	129	34.66	<0.0001

Models sharing one parameter for control and antidepressant treatment are compared to a model with no constraints

over a similar period leads to a reduction in the responsiveness of prejunctional α_2-adrenoceptors of whole vas deferens (Finberg & Tal, 1986; García-Sevilla & Zubieta, 1986). One hypothesis which has been proposed to explain these findings, suggests that chronic administration of antidepressant drugs elevates the synaptic levels of noradrenaline, and finally leads to a down-regulation of presynaptic α_2-adrenoceptors (Cohen *et al.*, 1980). In fact, a reduction of α_2-adrenoceptor density in the central nervous system, as measured by ligand binding techniques, has been repeatedly demonstrated (see Meana *et al.*, 1992 and references therein). However, subsensitive functional responses to receptor activation during chronic antidepressant drug treatment may also involve desensitization of signal transduction pathways distal to the receptor (for review see Manji, 1992). The changes in agonist potency observed in this study could be potentially explained by either of the above mechanisms.

Previous studies have shown a nonlinear relationship between α_2-adrenoceptor occupancy and inhibition of adenylate cyclase activity in human platelets (Lenox *et al.*, 1985), and inhibition of [^{3}H]-noradrenaline release from both rat heart (Fuder *et al.*, 1986) and cerebral cortex (Adler *et al.*, 1987). Our previous data showed that there is a reserve for prejunctional α_2-adrenoceptors on the postganglionic neurones in the prostatic portion of the rat vas deferens (Sallés *et al.*, 1994). Analysis of the present data by the null approach (nested hyperbolic method), and the subsequent plotting of the hyperbolic occupancy-response relationship (see Figure 3) demonstrate that there is a receptor reserve for clonidine which is modulated by desipramine pretreatment. Thus, the $K_E/[R_o]$ value for clonidine was increased after chronic desipramine treatment ($K_E/[R_o]$ control = 1.4% versus $K_E/[R_o]$ desipramine = 21.1%) resulting in a more linear occupancy-effect relationship under the latter condition.

Simultaneous operational model analysis of E/[A] curves for clonidine (with and without prior exposure to EEDQ 300 nM) shows that vehicle and desipramine data sets can be fitted together to a model sharing pK_A and n (see Table 3). The affinity constant values were in close agreement with the values obtained when using the nested hyperbolic method. The similarity between estimates seems to make complex statistical analysis inappropriate. For each experimental condition, two τ values, τ_1 and τ_2, corresponding to the control and EEDQ-treatment curves, were estimated (see Table 2). The ratio τ_2/τ_1 provided by operational model fitting reflects the reduction in $[R_o]$ due to EEDQ-treatment, and it is equivalent to q in the method of Furchgott (1966), both corresponding to the fractional concentration of receptors remaining after irreversible antagonism. The average value of τ_2/τ_1 was 0.011 compared with an average of q of 0.011 in vehicle-treated animals, while in desipramine-treated animals, these values were 0.175 and 0.167, respectively. Once again a similarity between the two methods of estimation is evident. However, a paradox is seen from the fraction of receptors remaining unblocked (i.e. q values) which indicate that approximately 99% of the receptors are blocked in naive prostatic portions, but approximately 84% of the receptors are blocked in desipramine-treated tissues. A possible explanation for this discrepancy is discussed below.

To use the operational model of agonism to interpret the changes in clonidine and xylazine E/[A] curves caused by desipramine treatment, the definitions of α, m and EC_{50} in terms of the model parameters, K_A, τ, n and E_m (Black & Leff, 1983; see Methods section) were used. In the case of α, according to the operational model, the asymptote depends on τ, E_m and n (equation 3). For instance, using the estimates of τ, E_m and n for clonidine given in Table 2 for the unconstrained fit in control and desipramine-treated animals, it can be observed that the asymptote values predicted by eq. (3) ($\alpha_{control} = 0.95$, $\alpha_{treatment} = 0.93$) were in close agreement with the values obtained by pragmatic analysis (Table 1). Thus, although decreases in τ in principle can lead to decreases in α, in this case these effects were minimal due to the increase in E_m. Therefore, it is logical to deduce, that the increase in E_m is responsible for the lack of effects of the loss of efficacy of agonists on the asymptote values for clonidine and xylazine. Certainly, constraining E_m to a single value for all the clonidine E/[A] data resulted in a drastic loss of goodness-of-fit of the operational model (see Table 3). Moreover, an increase in the E_m value could help to reinterpret the xylazine affinity decrease after desipramine treatment and the aforementioned paradox that q values are higher in desipramine treated tissues than in normal ones. That is, the statistically different xylazine pK_A values under control and desipramine-treated situations could be the result of using, wrongly, the clonidine E/[A] curve to estimate E_m (and n). Similarly, an increase in E_m would result in an overestimate of τ_2 (and of τ_1) leading to different q estimates.

The lack of effects on m of clonidine and xylazine E/[A] curves accompanying chronic desipramine treatment could in theory have been the result of the concomitant lack of effects on n. Thus for clonidine, according to the operational model definition on m (eq. 5) the factor by which τ changed (20 fold) is not sufficient to produce flattening of curves. Assuming that the value of n is 1.28 (see Table 2) the value of m, for a τ value of 3.95 is predicted by eq. 5 to be 1.17 which is close to the experimentally obtained value (1.24).

The increase in EC_{50} values, that is, the rightward shift of agonist E/[A] curves accompanying chronic desipramine treatment, according to the eq. 4, must depend on the changes produced on pK_A, τ and n. Summarizing the analysis shown in Table 3, significant changes in location of clonidine and xylazine E/[A] curves after desipramine treatment were the result of consistent changes in τ's for both agonists, although judged by the goodness of fit, clonidine E/[A] curves, in contrast to xylazine, failed to disclose evidence of changes in agonist affinity.

The foregoing discussion relies on the assumption that either the null method or the operational model is an appropriate method for the estimation of *in vitro* dissociation constants under restricted conditions. Indeed, the correspondence between estimates of dissociation constants for clonidine produced by the two methods was very good. The operational definition of τ as $[R_o]/K_E$ makes evident that any intervention leading to a decrease in $[R_o]$ (irreversible antagonist) or an increase in K_E (post-receptor action) may be used to estimate agonist dissociation constants. However, a post-receptor intervention may affect other parameters of the transducer system, namely E_m and n. Indeed, it has been shown (Leff *et al.*, 1985) that a concomitant variation of E_m and K_E does not allow reliable K_A estimations. In the present study, the statistical analysis showed a worsening of fit when E_m or τ for vehicle and desipramine-treated tissues were constrained to share a common value. These findings indicate that the use of chronic desipramine treatment is not a suitable experimental procedure for the estimation of agonist affinities and efficacies at prejunctional α_2-adrenoceptors.

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