Br. J. Pharmac. (1984), **83**, 007–014

Non-specific, time-dependent desensitization of the vas deferens and anococcygeus preparations of the rat to α_1-adrenoceptor antagonists and atropine

Igor Onnen

Laboratoire de Physiologie, Faculté de Médecine (Université Paris-Nord), Bobigny, France

1 Rat vas deferens preparations became desensitized to the α_1-adrenoceptor antagonist thymoxamine: after 6 h *in vitro*, the $t_{\frac{1}{4}}$ value (time to attain half the occupancy of receptors occupied at equilibrium) of the response to this drug was 1.50 fold greater in control strips (strips exposed to thymoxamine at 6 h) than in test strips (strips exposed to thymoxamine at 1 h).

2 The rate of action of the α_1-adrenoceptor antagonist AR-C 239 on the rat anococcygeus preparation was correlated with the rate of action of atropine. There was also a significant correlation between the $t_{\frac{1}{4}}$ ratios (1.37 and 1.30 for AR-C 239 and atropine respectively) observed in the control muscles at 6 h. The *in vitro* slowing is thus due to some change in the longitudinal muscle and not to a change in the receptors.

3 The *in vitro* slowing occured when either phenylephrine or methoxamine was the α_1-adrenoceptor agonist used.

4 The most likely mechanism of desensitization is a non-specific slowing of the access of drugs to receptors.

Introduction

Isolated tissues are known to develop desensitization to a variety of drugs. Desensitization is usually synonymous with tolerance (Paton, 1957; Huibobro-Toro & Foree, 1980) and is seen as decreased responsiveness to a drug resulting from a previous contact. Some cases of desensitization, such as the guinea-pig ileum's desensitization to atropine (Onnen & Olive, 1981) and antihistamine agents (Onnen & Olive, 1983), have the characteristics of a slowed (instead of decreased) responsiveness, resulting from the time spent by the preparation *in vitro* (as opposed to a previous contact with a drug).

The present experiments extended the study of *in vitro* desensitization to the interaction between the competitive antagonist, thymoxamine (Roquebert *et al.*, 1981) and the α_1-adrenoceptors of the rat vas deferens stimulated with phenylephrine. Comparison was made between the inhibition of phenylephrine by thymoxamine after 1 h (on test preparations) and after 6 h (on paired control strips, tissues, which were exposed to phenylephrine only in the first 6 h.

Additional experiments were carried out on the rat anococcygeus muscle, a tissue consisting almost entirely of longitudinal muscle (Gillespie, 1972) with the competitive α_1-adrenoceptor antagonist, AR-C 239 (2-[2-[4(*O*-methoxyphenyl)-piperazine-1-Yl]-ethyl]4, 4-dimethyl-1,3(2H-4H) isoquinolinedione; Mouillé *et al.*, 1980; Huchet, Andréjack & Schmitt, unpublished observations) and with atropine (Arunlakshana & Schild, 1959; Doggrell, 1981). Control muscles of these experiments received their first dose of agonist at the 5th h.

Methods

Variation in the affinity constants of thymoxamine on the vas deferens stimulated with phenylephrine

In each of 16 experiments, vasa deferentia were obtained from an adult Wistar rat (300–500 g) and their epididymal segments were immediately mounted in two separate organ baths containing 5 ml of Krebs-Henseleit solution (composition in mmol l^{-1}: NaCl 118, KCl 4.7, $CaCl_2$, $2H_2O$ 1.9, $MgSO_4$, $7H_2O$ 1.1, glucose 11, $NaHCO_3$ 24, NaH_2PO4 1.7) at 37°C, gassed by 5% CO_2 in 95% O_2 and containing cocaine (4 μmol l^{-1}) to block the

neuronal uptake of PE, and propranolol ($2.6\,\mu mol\,l^{-1}$) to block β-adrenoceptors. One strip served as the test strip, the other as the control. Contractions were measured isotonically.

The experiment started (zero time), and 40 min later phenylephrine (PE) was added cumulatively so that its concentration-response curve could be constructed. Thereafter, the EC_{50} value of PE was measured on each strip on sequential concentration-response curves in which PE was added to the baths every 3.5 min in concentrations of alternately 0.5, 1, and $2\,\mu mol\,l^{-1}$. The responses to PE were measured as the peaks of isotonic contractions, which reached 25–75% of the maximum response.

Affinity constants (Kbe, $t_{\frac{1}{2}}$ and 2-min Kb) of the test strip at the initial (0–2.5 h) period *K*be and $t_{\frac{1}{2}}$ were measured at 1 h. Once sensitivity to PE was stable, the test strip was exposed to thymoxamine $100\,nmol\,l^{-1}$ for 20–30 min. The progressive inhibition by thymoxamine of responses to PE was expressed as (1) dose-ratio DR (= ratio of equiactive concentrations of PE after and before thymoxamine) and (2) receptor occupancy o (= 100 (DR−1)/DR)) (Paton & Rang, 1965). The series of increasing inhibitions measured at times of exposure of 2 min (DR 2 min; o 2 min), 5 min (DR 5 min; o 5 min), etc..., and at equilibrium (DRe; oe) were used to calculate *K*be and $t_{\frac{1}{2}}$ (see below for definition and method of calculation). DRe was obtained in 5–20 min.

The 2-min *K*b value was measured on the effects of 2 min exposure to thymoxamine 100, 200 and $400\,nmol\,l^{-1}$ in the following way. After DRe had been obtained, thymoxamine was washed out; the test strip was then exposed to thymoxamine $200\,nmol\,l^{-1}$ for 2 min, and after washout of this second concentration of thymoxamine, to thymoxamine $400\,nmol\,l^{-1}$ for 20–30 min. The washout of antagonist was very effective (see Results).

To calculate the 2-min *K*b value, the effects of 2 min exposure to thymoxamine 100, 200 and $400\,nmol\,l^{-1}$ were plotted on a graph of log (DR−1) versus log concentrations of thymoxamine. The 2 min *K*b value, i.e. the concentration of thymoxamine producing a dose-ratio of two after 2 min exposure, was solved for y = log (2−1) = zero (Arunlakshana & Schild, 1959).

Affinity constants (Kbe, $t_{\frac{1}{2}}$ and 2-min Kb) of thymoxamine on the test and control strips at the (6–9 h) period At 6 h, the test strip for the second time and the control strip previously treated with PE only for the first time were exposed to thymoxamine $100\,nmol^{-1}$ for 20–30 min in order to measure *K*be and $t_{\frac{1}{2}}$ values. After washout of thymoxamine, the strips were exposed to thymoxamine $200\,nmol\,l^{-1}$ for 2 min and $400 nmol\,l^{-1}$ for 20–30 min (to calculate the 2-min *K*b value).

Variations in the affinity constants of the (phenylephrine-AR-C 239) and (carbachol-atropine) antagonisms on the anococcygeus muscle

In each of 18 experiments, anococcygeus muscles were taken from between the paravertebral muscles and the rectum of an adult Wistar rat. The test muscle received its first dose of PE at zero time, the control muscle not before the 5th h. Cocaine ($4\,\mu mol\,l^{-1}$) and propranolol ($2.6\,\mu mol\,l^{-1}$) were added to the Krebs-Henseleit solution only at the time the effects of PE and AR-C 239 were measured: 0–1.5 h for the test muscle and 5–7 h for the control muscle.

Affinity constants Kbe and $t_{\frac{1}{2}}$ values of AR-C239 and atropine on the test muscle at the initial (0–2.5 h) period At 1 h, once sensitivity to PE was stable, the test muscle was exposed to AR-C 239 $20\,nmol\,l^{-1}$ for 40–60 min (to measure *K*be and $t_{\frac{1}{2}}$ values). DRe was obtained at 20–30 min. Thereafter, at 2 h, the EC_{50} values of carbachol was measured and the test strip was exposed to atropine $6\,nmol\,l^{-1}$ for 20–40 min, to measure *K*be and $t_{\frac{1}{2}}$. Dre was obtained at 20–30 min.

Affinity constants Kbe and $t_{\frac{1}{2}}$ of AR-C239 and atropine on the control muscle at the (6–8 h) period At 5 h, the control muscle, hitherto merely kept in Krebs-Henseleit solution at 37°C and regularly washed, received its first dose of PE. The affinity constants of AR-C 239 and atropine were measured at 6 and 7.5 h respectively, as had been done on the test muscle at the initial (0–2.5 h) period.

Variation in the affinity constants of thymoxamine on the anococcygeus muscle stimulated with methoxamine

In each of 10 experiments, a test anococcygeus muscle received its first dose of methoxamine (MA) at zero time and was exposed to thymoxamine $100\,nmol\,l^{-1}$ for 30 min at 1 h, once sensitivity to MA was stable. Similarly, the control muscle received its first dose of MA at 5 h and was exposed to thymoxamine $100\,nmol\,l^{-1}$ for 30 min at 6 h. Propranolol ($0.5\,\mu mol\,l^{-1}$) was added to the Krebs-Henseleit solution at the time the effects of MA and thymoxamine were measured.

Definition and calculation of Kbe and $t_{\frac{1}{2}}$ values

*K*be is the concentration of antagonist producing at equilibrium a dose-ratio of two, i.e. a receptor occupancy (oe) of 50% :oe = 100 ((2−1)/2). The interaction between a competitive antagonist such as thymoxamine, AR-C 239 or atropine and its receptor corresponds to the equation:

log (DRe−1) = n log A −log *K*be (Arunlakshana & Schild, 1959).

where A is the concentration of antagonist (thymoxamine = 100 nmol l^{-1}; AR-C 239 = 20 nmol l^{-1}; atropine = 6 nmol l^{-1}) and n a constant which equals unity when the antagonist is competitive. If n equals unity, the equation becomes

$\log Kbe = \log A - \log (DRe - 1)$ or

$Kbe = A/(DRe - 1)$.

No correction (Furchgott, 1967) was made for spontaneous (i.e. not due to the antagonists) changes in sensitivity of the tissue to agonists because these changes were so small, viz 3–15% per h, as to be negligible over a 10–30 min period, which was the range of times requested to attain DR e.

$t_{\frac{1}{2}}$ is the time to attain one-half of the equilibrium receptor occupancy. If oe and ot are the occupancies at equilibrium (oe) and at any given time of the exposure to an antagonist (ot), the (oe − ot) values represent the % of receptors which remain to be occupied before reaching equilibrium, in other words the % of receptors still available for the antagonist (Paton & Rang, 1965). When plotted on a log scale against time, the (oe − ot) values form two successive straight lines. The first line, formed between 0, 2 and 5 min declines very rapidly with a slope K which is converted into $t_{\frac{1}{2}} = 0.693/K$; the second line, after 5 min, declines slowly. In some cases, $t_{\frac{1}{2}}$ could not be calculated between 0, 2 and 5 min because it exceeded by at least a conventional 20% the $t_{\frac{1}{2}}$ value calculated between 0 and 2 min, thus indicating non-linearity between 0, 2 and 5 min. In such cases (4, 6 and 5 experiments of Tables 1, 2 and 3 respectively), $t_{\frac{1}{2}}$ was calculated between 0 and 2 min.

Statistical analysis

Affinity constants measured at 6–9 h were compared to the values of the test strips at 0–2.5 h by means of the Wilcoxon paired *t* test. Desensitization was revealed by increased 2-min *K*b or $t_{\frac{1}{2}}$ values and was expressed in each experiment as $t_{\frac{1}{2}}$ ratio, i.e. the ratio of $t_{\frac{1}{2}}$ values at 6 h (control or test strip) and at 1 h (test strip). The values were expressed as means ± s.e.mean. *P* values < 0.05 were considered to be significant. The concentrations of drugs refer to the base.

Solutions were made up just before use.

Results

Variation in the affinity constants of thymoxamine on the vas deferens stimulated with phenylephrine

In the (0–2.5 h) period, the *K*be value for thymoxamine was 33 nmol l^{-1} (Table 1) and the 2-min *K*b value 61 nmol l^{-1}.

The (PE-thymoxamine) antagonism developed more slowly at 6 h in both test and control strips (Figure 1, Table 1). Firstly, although the *K*be values remained unchanged, the $t_{\frac{1}{2}}$ values were greater than at the initial (0–2.5 h) periods (test strips $P = 0.01$, $n = 16$; control strips $P < 0.01$, $n = 16$); the $t_{\frac{1}{2}}$ ratios of test and control strips were correlated ($r = 0.5$, $n = 16$, $P = 0.05$). The 2-min *K*b values were greater (test strips $P = 0.01$; control strips $P < 0.01$, $n = 16$) than in the initial (0–2.5 h) periods.

Variations in the affinity constants for the (phenylephrine–AR-C239) and (carbachol–atropine) antagonisms on the anococcygeus muscle

Anococcygeus muscles were much more sensitive to PE (mean EC_{50} = 60 nmol l^{-1}, Table 2) than vasa deferentia (mean EC_{50} = 1.2 μmol l^{-1}, Table 1).

In the (0–2.5 h) period, the *K*be value of AR-C239 was 0.80 nmol l^{-1} (Table 2), which shows that this drug is much more potent than thymoxamine (*K*be = 30 nmol l^{-1}, Table 1). The *K*be value of atropine was 0.54 nmol l^{-1} (Table 2), a value already reported in this tissue (Doggrell, 1981). The $t_{\frac{1}{2}}$ values

Table 1 Variation in the *K*be, $t_{\frac{1}{2}}$ and 2-min *K*b values for thymoxamine, and in the EC_{50} value and maximum response to phenylephrine (PE) on the vas deferens (16 experiments)

	Test strips		*Control strips*
	0–2.5 h	6–9 h	6–9 h
*K*be value (nmol)	33 ± 19	30 ± 4	30 ± 4
$t_{\frac{1}{2}}$ value (s)	56 ± 13	76 ± 17	80 ± 15
$t_{\frac{1}{2}}$ ratio	unity	1.38 ± 0.17	1.50 ± 0.23
2-min *K*b value (nmol)	61 ± 10	92 ± 15	91 ± 16
EC_{50} value of PE (μmol l^{-1})	1.20 ± 0.22	1.70 ± 0.44	2.10 ± 0.52*
Max. response to PE (cm)	0.58 ± 0.1	0.48 ± 0.90	0.47 ± 0.90**

*K*be is 100 nmol l^{-1} (DRe − 1). Affinity constants for thymoxamine were obtained from data of Figure 1.

*1.62 ± 0.34 μmol at 1 h.

**0.60 ± 0.1 cm at zero time.

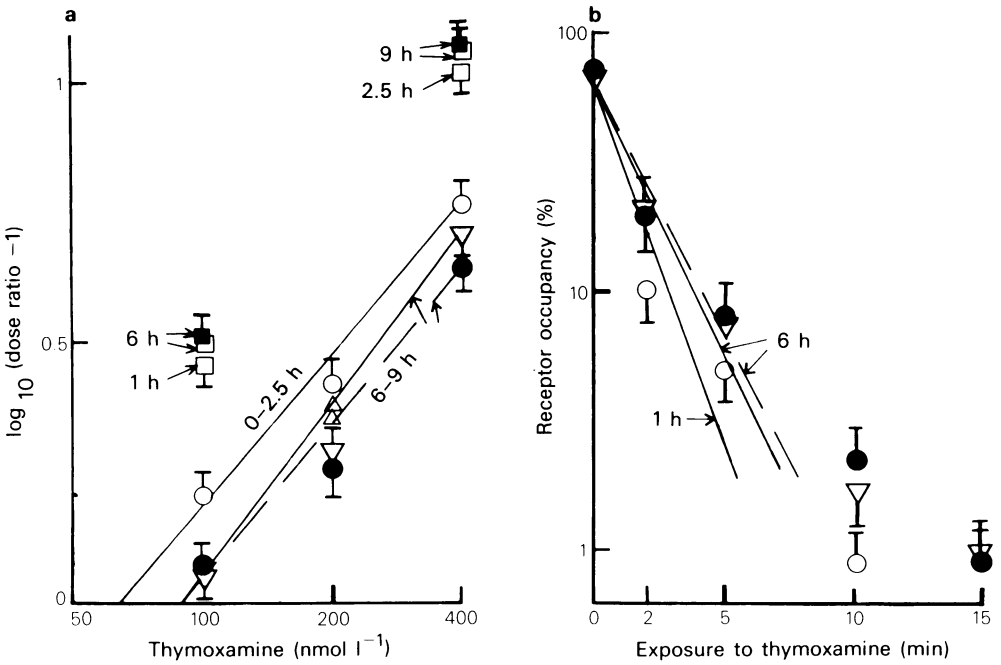

Figure 1 Variation in the effects of thymoxamine on the rat vas deferens stimulated with phenylephrine. (a) (1) Equilibrium effects (DRe) of 100 nmol l^{-1} thymoxamine (□ = test strips at 1 h and at 6 h; ■ = control strips at 6 h). *K*be values 100 nmol l^{-1}/(DRe−1) are given in Table 1. (2) Concentration-effect curves (2-min exposure) of the test strips at 0-2.5 h (○) and at 6–9 h (▽) and of the control strips at 6–9 h (●). The 2-min *K*b values (intercepts with y = zero) are given in Table 1. The mean slope ± s.e.mean (the slope was in each experiment $\Delta y/\Delta x$) was 1.2 ± 0.1 ($n = 16$) at the (0–2.5 h) period. (3) In addition are shown (a) the effects of 2 min exposure to 200 nmol on the test strips at 4 h and at 5 h (Δ: the smaller effect was at 5 h) and (b) the equilibrium effects of 400 nmol thymoxamine (□ = test strips at 2.5 h and at 9 h; ■ = control strips at 9 h); the slope between the equilibrium effects of 100 and 400 nmol l^{-1} were 0.9 ± 0.1 at 1 h (test strips) and 1.05 ± 0.1 at 6 h (control strips). (b) Graphs plotting the % (at a log scale) of α_1-adrenoceptors available for thymoxamine against time (○ = control strips at 1 h; ▽ = test strips at 6 h; ● = control strips at 6 h) by 100 nmol thymoxamine. Mean $t_{\frac{1}{4}}$ values are given in Table 1. $t_{\frac{1}{4}}$ was measured in each experiment, at 1 h on the test strip and at 6 h on both the test and control strips, and was expressed as 0.693/K where K was the slope of the graph between 0, 2 and 5 min (in 4 experiments between 0 and 2 min).

Table 2 Variations in the *K*be and $t_{\frac{1}{4}}$ values for AR-C239 and atropine and in the EC_{50} values and maximum responses to phenylephrine and carbachol on the rat anococcygeus muscle (18 experiments)

	AR-C239		*Atropine*	
	Test muscles (1st h)	*Control muscles* (6th h)	*Test muscles* (2nd h)	*Control muscles* 7th h)
*K*be value (nmol)	0.78 ± 0.12	0.70 ± 0.14	0.55 ± 0.07	0.55 ± 0.05
$t_{\frac{1}{4}}$ value (s)	73 ± 13	93 ± 12	60 ± 10	74 ± 13
$t_{\frac{1}{4}}$ ratio	unity	1.37 ± 0.20	unity	1.30 ± 0.25
EC_{50} value of agonist (μmol)	0.06 ± 0.01	0.09 ± 0.015	0.15 ± 0.04	0.28 ± 0.09
Max. response to agonist (cm)	0.80 ± 0.12	0.73 ± 0.10	0.78 ± 0.10*	0.73 ± 0.10**

*K*be for AR-C239 is 20 nmol l^{-1}/DRe−1). *K*be for atropine is 6 nmol l^{-1}/(DRe−1).
*measured at 3 h.
**measured at 8 h.

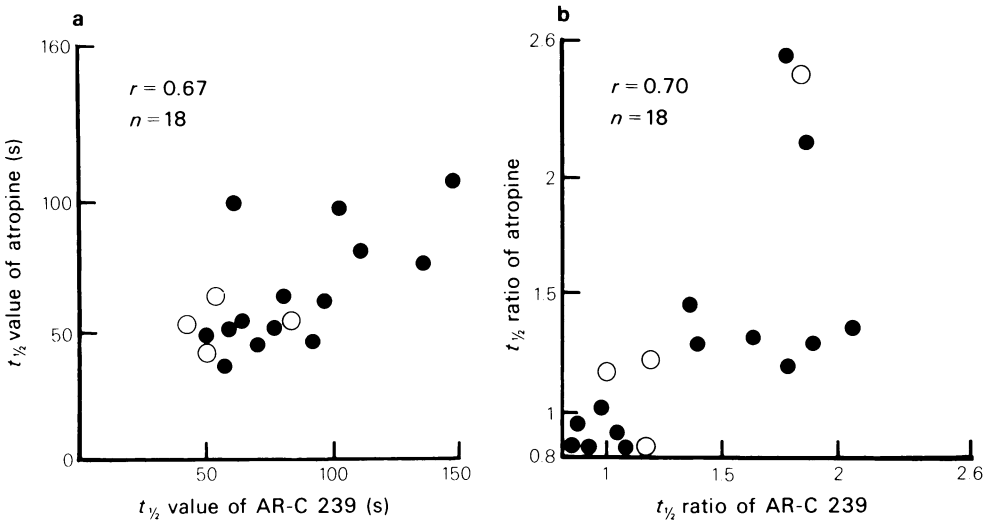

Figure 2 Correlation between the rates of action of AR-C239 and atropine and between the slowing of these rates of action on the anococcygeus muscle. (a) $t_{\frac{1}{2}}$ values of AR-C239 and atropine at the initial (0–2.5 h) period. Mean $t_{\frac{1}{2}}$ values are given in Table 2. (b) $t_{\frac{1}{2}}$ ratios of AR-C239 and atropine. The $t_{\frac{1}{2}}$ ratio of an antagonist was in each experiment the ratio of $t_{\frac{1}{2}}$ values of the control strip (measured at 6–8 h) and of the test strip (measured at 0–2.5 h). Mean $t_{\frac{1}{2}}$ ratios are given in Table 2. In (a) and (b), open symbols (○) represent experiments where the α_1-adrenoceptor agonist was methoxamine.

of AR-C239 and atropine showed a significant ($P=0.01$, $n=18$) correlation (Figure 2a): in other words, a muscle which rapidly responds to AR-C239 is expected to respond rapidly to atropine.

The rate of development of antagonism in the (6–8 h) period is shown in Table 2: $t_{\frac{1}{2}}$ values were greater than in the (0–2.5h) period (AR-C239: $P=0.01$, $n=18$; atropine: $P=0.05$, $n=18$), while *K*be values remained unchanged. In most cases, the slowing of the response of the tissue to AR-C239 (Figure 2b) was associated with a comparable slowing of the response to atropine ($P=0.001$, $r=0.70$, $n=18$), indicating that the phenomenon did not depend upon the drug *per se*, but was more a characteristic of the preparation.

Histological studies were performed in four experiments where the $t_{\frac{1}{2}}$ ratios of AR-C239 were 0.84, 1.5, 1.8 and 1.9. These studies showed that each test and control muscle was a bundle of muscle cells which was surrounded by serous cells on half of its surface only.

Variation in the affinity constants of thymoxamine on the anococcygeus muscle stimulated with methoxamine

At 1 h, the *K*be value (33 nmol l^{-1}, Table 3) was similar to that of the vas deferens (Table 1) and the $t_{\frac{1}{2}}$ value (27 s, $n=10$) was smaller than in the vas deferens (56 s, Table 1).

Table 3 Variation in the *K*be and $t_{\frac{1}{2}}$ values for thymoxamine and in the EC_{50} value and maximum response to methoxamine on the rat anococcygeus muscle (10 experiments)

	Test muscles (1st h)	*Control muscles* (6th h)
*K*be value (nmol)	33 ± 6	29 ± 5
$t_{\frac{1}{2}}$ value (s)	27 ± 7	37 ± 9
$t_{\frac{1}{2}}$ ratio	unity	1.47 ± 0.35
EC_{50} value of MA (μmol l^{-1})	0.47 ± 0.2	0.62 ± 0.32
Max. response to MA (cm)	0.93 ± 0.15	0.74 ± 0.20

*K*be is 100 nmol l^{-1}/(DRe − 1).

A slowing of the effects of thymoxamine occurred at 6 h in that there was a 1.47 fold increase in $t_{\frac{1}{2}}$ value, with no change in *K*be value.

Discussion

The present study shows that after 6 h *in vitro* the α_1-adrenoceptor agonist-antagonist reaction was slowed in its onset ($t_{\frac{1}{2}}$ and 2-min *K*b values were increased) but kept the same equilibrium state (*K*be values remained unchanged). This *in vitro* slowing has four main characteristics. Firstly, it seems to be a general effect since it occurs with at least four types of receptors in two animal species, the 2-min *K*b ratio (ratio of 2-min *K*b values at 6–9 h and at 0–2.5 h) being 1.52 in the rat vas deferens (Table 1), 1.4 in the muscarinic (Onnen & Olive, 1981) and 1.7 in the histamine H_1 (Onnen & Olive, 1983) receptors of the guinea-pig ileum, and 1.4 in the 5-HT receptors of the rat uterus (Onnen, unpublished results). Secondly, the *in vitro* slowing is not specific for one drug or receptor type. Thirdly: the slowing is due to some change within the longitudinal muscle since it is exerted in the vas deferens, a complex four-layer structure (serous layer, longitudinal and circular muscles, and mucous layer), as well as in the anococcygeus muscle, a simple structure consisting almost entirely of longitudinal muscle (Gillespie, 1972). Fourthly: the desensitization to antagonists is entirely due to effects in the 5–6 h of *in vitro* life, and is not due to previous contact with agonists.

Two properties of the anococcygeus preparation were apparent in this study: the tissue was much more sensitive to PE and responded more rapidly to thymoxamine than did the vas deferens. The sensitivity to PE suggests that anoccygeus muscle either has a high density of α_1-adrenoceptors (the greater the sensitivity to an agonist, the greater the density of receptors: Land *et al.*, 1977) or is very sensitive to cocaine (the concentration of cocaine required to block 50% of noradrenaline neuronal uptake is 0.92 μmol in this tissue (Doggrell & Woodruff, 1977) instead of 2.8 μmol in the vas deferens (Drew *et al.*, 1978)). The great sensitivity to PE might also be due to the absence of extraneuronal uptake (Gibson & Pollock, 1973). The suggestion of a high density of α_1-adrenoceptors seems to be valid in that 5 of the 10 anococcygeus muscles in Table 3 were taken from the same animals that provided vasa deferentia for separate experiments (Onnen, unpublished result). In these experiments, the EC_{50} value of MA, an agonist not influenced by cocaine (Leighton, 1982), was much smaller ($P = 0.02$, $n = 5$) in the anococcygeus muscle (0.47 ± 0.22 μmol l^{-1}) than in the paired vasa deferentia (3 ± 1.3 μmol l^{-1}). On the other hand, in these 5 experiments, $t_{\frac{1}{2}}$ values of thymoxamine were not much smaller in anococcygeus muscles (27 ± 5 s at 1 h; 39 ± 8 s at 6 h) than in the paired vasa deferentia (33 ± 5 s at 1 h; 45 ± 6 s at 6 h): this result refutes the possibility that thymoxamine acts much faster on the anococcygeus muscle than on the vas deferens.

The mechanism of desensitization of the tissues to α_1-adrenoceptor antagonists and atropine is non-specific. After 6 h *in vitro* there is a change in one of the factors that influence the rate of the reaction between an antagonist and its specific receptors. One factor is rate of the access of the antagonist from the bath fluid to the receptors; a second factor is the rate of binding between an antagonist and its specific receptors, whilst a third factor is the number of acceptors, i.e. sites which are not specific for a given antagonist: the greater the number of acceptors, the slower the effect and the greater the residual effect of the antagonist after washout (Salazar *et al.*, 1976).

In the first suggested mechanism, the number of acceptors might increase after 6 h *in vitro*, resulting in a slower effect and in a greater residual effect of the antagonist. This mechanism is unlikely, because putative acceptors for antagonists were blocked or unavailable in that (a) the β-adrenoceptors of the rat vas deferens would be occupied by propranolol, and (b) β-receptor mediated effects are not important in the rat anococcygeus muscle (Gillespie, 1972). Furthermore, thymoxamine (Leighton *et al.*, 1979) and AR-C 239 (Huchet, Andréjak and Schmitt, unpublished observation) share with MA and PE (Drew, 1977; Leighton *et al.*, 1979) a 100–1000 fold greater activity on α_1-adrenoceptors than on α_2-adrenoceptors; whereas, carbachol (Doggrell, 1981), thymoxamine at 400 nmol l^{-1} (Drew *et al.*, 1978) and MA (Leighton, 1982) have no affinity for neuronal uptake sites. The drugs used in the study, carbachol, (McGrath & Olverman, 1977; Burnstock *et al.*, 1978) MA (Doggrell & Woodruff, 1978) and presumably thymoxamine do not release noradrenaline or acetylcholine. The second piece of evidence against an 'acceptor' mechanism of desensitization is that the residual effect of thymoxamine was not greater at 6 h (1.20 ± 0.15, $n = 16$) than at 1 h (1.22 ± 0.15). The residual effect of thymoxamine was in each experiment of Table 1 the ratio of EC_{50} values of PE after the end of washout and just before the exposure to thymoxamine 100 nmol l^{-1}.

In the second possible mechanism of desensitisation, receptors might have changed their functional state after 6 h *in vitro*, resulting in a slower binding with antagonists. Changes in receptors are suggested by the decreased sensitivity to PE after 6 h (Table 1). However this mechanism of desensitization is unlikely for two reasons. First, desensitization to thymoxamine occurred in control vasa deferentia (Table 1) even though they retained the same sensitivity to PE between 1 h and 6 h: this lack of variation of sensitivi-

ty to an agonist suggests a stable functional state of the receptors and cannot be explained by the existence of spare receptors as there is no spare α_1-adrenoceptor reserve in the rat vas deferens (Moran *et al.*, 1969). Furthermore, the rate of access of drugs to receptors and not the rate of interaction, is the rate-limiting step for the interaction between an antagonist and receptors. For example, atropine is known to act about ten fold faster when it is placed in close contact with the receptors by microionotophoresis as opposed to simple addition to the bath (Bolton, 1977). If the interaction between drugs and receptors was rate-limiting, one would expect from the work of Roberts & Stephenson, (1976) that the 'offset' $t_{\frac{1}{4}}$ values for thymoxamine 100 and 400 nmol l^{-1} would be equivalent, and that the ratio of 'offset' and 'onset' $t_{\frac{1}{4}}$ values for thymoxamine 100 nmol l^{-1} would equal DRe. Neither prediction was fulfilled. The offset $t_{\frac{1}{4}}$ value for thymoxamine 400 nmol l^{-1} was 950 ± 180 s which was greater than that for thymoxamine 100 nmol l^{-1} (650 ± 180 s) ($P = 0.05$, $n = 7$). The ratio of 'offset' (610 ± 150 s) and 'onset' (56 ± 13 s) $t_{\frac{1}{4}}$ values was 12 ± 4, which was significantly greater than DRe (4 ± 1) ($P = 0.01$, $n = 7$).

Since none of the mechanisms discussed above is satisfactory, the most likely explanation of desensitization is a slowed access of drugs to receptors. The rate of action of AR-C 239 was correlated with that of atropine, as was the time-dependent slowings of these rates of action. These correlations are highly consistent with, and strongly support the assumption, that access is rate-limiting. The existence of these correlations suggests a common pathway in the accessibility of drugs to receptors, which decreases after several hours *in vitro*, thus explaining the unspecific nature of desensitization. During the access stage, AR-C 239 and atropine follow a pathway which leads each of these agents from the bath fluid to its specific receptors through the intercellular spaces of the tissue. The pathway is to a very great extent common to AR-C 239 and atropine since the receptors for these agents, i.e. the end of the pathway, are situated, like receptors for neurotransmitters (Goldstein *et al.*, 1974) within or at the outer surface of the smooth muscle cell membranes. Under these conditions, the common pathway may be more or less accessible to drugs at 1 h, explaining that some tissues respond rapidly to these drugs and other tissues do not. Some alteration, the exact nature of which remains to be determined, occurs after several hours *in vitro* and reduces the accessibility of drugs to receptors, thus explaining the non-specific slowing in the responses to these drugs.

The histological work was done by J.L. Kemeni and Mme B. Quenette. Thymoxamine was supplied by Laboratoire Dedieu, AR-C 239 by Laboratoire Karl Thomae and methoxamine by Laboratoire Wellcome. I am indebted to Dr Georges Roux for his help in translating the text.

References

ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmac. Chemother.* **14,** 48–58.

BOLTON, T. B. (1977). Rate of offset of slow-acting muscarinic antagonists is fast. *Nature,* **270,** 354–356.

BURNSTOCK, G., COCKS, T. & CROWE, R. (1978). Evidence for purinergic innervation of the anococcygeus muscle. *Br. J. Pharmac.*, **64,** 13–20.

DOGGRELL, S.A. (1981). Effect of antimuscarinic agents on the contractile response to cholinomimetics in the rat anococcygeus muscle. *Br. J. Pharmac.*, **73,** 829–835.

DOGGRELL, S.A. & WOODRUFF, G.N. (1977). Effects of antidepressant drugs on noradrenaline accumulation and contractile responses in the rat anococcygeus muscle. *Br. J. Pharmac.*, **59,** 403–409.

DOGGRELL, S.A. & WOODRUFF, G.N. (1978). Effects of drugs on the accumulation and spontaneous release of noradrenaline in the rat anococcygeus muscle. *Br. J. Pharmac.*, **63,** 465–470.

DREW, G.M. (1977). Pharmacological characterization of the presynaptic adrenoceptor in the rat vas deferens. *Eur. J. Pharmac.*, **42,** 123–130.

DREW, G.M., HILDITCH, A. & LEVY, G.P. (1978). Effect of labetalol on the uptake of [^{3}H]-noradrenaline into the isolated vas deferens of the rat. *Br. J. Pharmac.*, **63,** 471–474.

FURCHGOTT, R.F. (1967). Techniques for studying antagonism and potentiation of sympathomimetic drugs on isolated tissues. In *Pharmacological Techniques in Drug Evaluation,* vol 2. ed. Siegler, P.E. & Moyer, J.H. pp. 256–266. Chicago: Yearbook Medical Publishers.

GIBSON. A., & POLLOCK, D. (1973). The effects of drugs on the sensitivity of the rat anococcygeus muscle to agonists. *Br. J. Pharmac.*, **49,** 506–513.

GILLESPIE, J.S. (1972). The rat anococcygeus muscle and its response to nerve stimulation and to some drugs. *Br. J. Pharmac.*, **45,** 404–416.

GOLDSTEIN, A., ARONOW, L. & KALMAN, M.S. (1974). *Principles of Drug Action.*, p.179. New York: J.E. Wiley.

HUIDOBRO- TORO, J.P. & FOREE, B. (1980) Dual agonist-antagonist effects of 5-hydroxytryptamine (5 HT) in the guinea pig ileum: evidence for a selective receptor desensitization effect. *Eur. J. Pharmac.*, **61,** 335–345.

LAND, B.R., PODLESKI, T.R., SALPETER, E.E. & SALPETER. M.M. (1977). Acetylcholine receptor distribution mechanism in myotubes culture correlated to acetylcholine sensitivity. *J. Physiol.*, **269,** 155–177.

LEIGHTON. H.J. (1982). Quantitative assessment of the pre- and postsynaptic alpha adrenoceptor antagonist potency of amitriptyline. *J. Pharmac. exp. Ther.*, **220,** 299–304.

LEIGHTON, J., BUTZ, K.R. & PARMETER L.L. (1979). Ef-

fects of α adrenergic agonists and antagonists on neurotransmission in the rat anococcygeus muscle. *Eur. J. Pharmac.*, **58,** 27–38.

McGRATH, J.C. & OLVERMAN, H.J. (1977). Release of [^{3}H]-noradrenaline by field stimulation and by drugs from the anococcygeus muscle. *Br. J. Pharmac.*, **60,** 305–306 P.

MORAN, J.F., TRIGGLE, C.C. & TRIGGLE, D.J. (1969). The mechanism of action of 2-halogenoethylamines at the adrenergic α-receptor and a further investigation of the "spare receptor" hypothesis. *J. Pharm. Pharmac.*, **21,** 38–46.

MOUILLÉ, P., HUCHET, A.M, CHELLY, J., LUCET, B., DOURSOUT, M.F. & SCHMITT, H. (1980). Pharmacological properties of AR-C 239.2-[2-(4(0-methoxyphenyl)-piperazine-1-4)-ethyl] 4, 4-dimethyl- 1, 3(2 H-4 H) isoquinolinedione, a new α_1-adrenoceptor blocking drug. *J. cardiovasc. Pharmac.*, **2,** 175–191.

ONNEN, I. & OLIVE. G. (1981). Desensitization to the anticholinergic effects of atropine on isolated guinea pig ileum. *J. Pharmac. Meth.*, **5,** 249–253.

ONNEN, I. & OLIVE, G. (1983). Desensitization of the isolated guinea pig ileum to antihistaminic agents. *Eur. J. Pharmac.*, **90,** 185–191.

PATON, W.D.M. (1957). The action of morphine and related substances on contraction and on acetylcholine output of coaxially stimulated guinea pig ileum. *Br. J. Pharmac.*, **12,** 119–128.

PATON, W.D.M. & RANG, H.P. (1965). The uptake of atropine and related drugs by intestinal smooth muscle of guinea-pig in relation to acetylcholine receptors. *Proc. R. Soc. B.* **163,** 1–44.

ROBERTS, F. & STEPHENSON, R.P. (1976). The kinetics of competitive antagonists on guinea-pig ileum. *Br. J. Pharmac.*, **58,** 57–70.

ROQUEBERT, J., DEMICHEL, P., MALEK, A. & CANELLAS, J. (1981). Etude comparative de l'activité alphabloquante pré et post-synaptique du Thymoxamine et de ses métabolites sur le canal déférent de rat. *J. Pharmac. (Paris)*, **12,** 15–25.

SALAZAR, M. SHIMADA, K. & PATIL, P.N. (1976). Iris pigmentation and mydriasis. *J. Pharmac. exp. Ther.*, **197,** 79–88.

(Received August 8, 1983.
Revised January 9, 1984.)