Br. J. Pharmac. (1978), **63**, 665–669

THE EFFECTS OF UPTAKE$_1$ ON α-ADRENOCEPTOR ANTAGONIST POTENCY IN DOG SAPHENOUS VEIN

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1 The effects of α-adrenoceptor antagonists on contractile responses to noradrenaline and methoxamine were examined in isolated saphenous vein strips of the dog.

2 Phentolamine, labetalol and thymoxamine antagonized responses to methoxamine to a greater extent than responses to noradrenaline.

3 Cocaine (3.0×10^{-5} mol/l) increased the potency of noradrenaline by about eight fold, but had little or no effect on the potency of methoxamine.

4 In the presence of cocaine (3.0×10^{-5} mol/l) the potency of the antagonists against noradrenaline was increased such that the pA_2 values were then similar to those obtained against methoxamine.

5 It is concluded that the lower potency of phentolamine, labetalol and thymoxamine as antagonists of noradrenaline than of methoxamine is due to the presence of an avid saturable uptake$_1$ process in the saphenous vein.

Introduction

As part of a study to characterize and determine the distribution of α-adrenoceptors and 5-hydroxytryptamine-receptors in vascular smooth muscle, the interaction between noradrenaline and a number of α-adrenoceptor antagonists was investigated in the isolated saphenous vein of the dog. It was noticed that the potencies of the α-adrenoceptor antagonists in the saphenous vein were unusually low when compared to their potencies against noradrenaline in other vascular preparations. The present study was undertaken in order to determine the reason for this anomaly.

Methods

Lateral saphenous veins were removed from dogs of either sex, anaesthetized with thiopentone (25 mg/kg i.v.) and barbitone (300 mg/kg i.p.). The vessels were cut spirally into strips and either used at once or stored overnight in a modified Krebs solution (Apperley, Humphrey & Levy, 1976) at 4 to 6°C and used the following day. Four strips, obtained from a single vessel, were mounted in 20 ml organ baths at 37°C. The strips were approximately 1 to 3 cm long at an applied resting tension of about 0.5 g. Isometric tension was measured with a Statham Microscale Accessory (model UL5) attached to a transducing cell (model UC3).

Agonist and antagonist activities were quantified as described previously for the rabbit aorta (Apperley *et al.*, 1976). In each experiment cumulative concentration-effect curves to an agonist were obtained in 4 strips. The agonist was then washed from the baths and graded concentrations of an antagonist were added to 3 of the strips whilst the fourth acted as a control. After 30 min, cumulative concentration-effect curves to the agonist were redetermined on all 4 strips. The degree of antagonism was quantified as the dose-ratio, that is, the ratio of the EC_{50} values in the presence and absence of the antagonist. Correction was made for any spontaneous change in sensitivity by dividing the dose-ratios obtained in the strips exposed to antagonist by the dose-ratio obtained in the control strip. The sensitivity of the control strips to noradrenaline decreased during the experiments by about two fold. However, when the agonist was methoxamine or noradrenaline in the presence of cocaine, the decrease was usually two or three fold but was sometimes up to six fold. With all the agonists there was sometimes a slight reduction of the maximum response in the second control concentration-effect curve. The results were plotted graphically in the form of a Schild plot (Arunlakshana & Schild, 1959) and the slope of the regression and a pA_2 value estimated from each experiment.

Drugs

The following drugs were used: (−)-noradrenaline bitartrate, mol. wt. 337.3 (Koch-Light); methoxamine hydrochloride, mol. wt. 247.7 (Burroughs Wellcome);

phentolamine methanesulphonate, mol. wt. 377.5 (Ciba); labetalol hydrochloride, mol. wt. 364.9 (AH 5158, Allen & Hanburys Research); thymoxamine hydrochloride, mol. wt. 315.8 (Warner) and cocaine hydrochloride, mol. wt. 339.8 (May & Baker). Methoxamine, phentolamine, labetalol, thymoxamine and cocaine were dissolved in isotonic saline (0.9% w/v NaCl solution) and noradrenaline in isotonic saline containing ascorbic acid (0.2 mg/ml).

Results

Agonists

Noradrenaline (1.0×10^{-7} to 1.0×10^{-4} mol/l) and methoxamine (1.0×10^{-7} to 1.0×10^{-4} mol/l) produced concentration-dependent contractions with maximal responses of 3.56 ± 0.24 g and 3.94 ± 0.33 g (mean ± s.e., $n_1 = n_2 = 24$) respectively at 1.0×10^{-4} mol/l. The EC_{50} values for noradrenaline (1.9×10^{-6} mol/l) and methoxamine (2.3×10^{-6} mol/l) were similar.

Antagonists

Phentolamine (1.0×10^{-8} to 1.0×10^{-6} mol/l), labetalol (1.0×10^{-7} to 1.0×10^{-5} mol/l) and thymoxamine (1.0×10^{-7} to 1.0×10^{-5} mol/l) were investigated as antagonists of methoxamine and noradrenaline (Table 1). Each antagonist produced concentration-dependent antagonism of responses to methoxamine, displacing the agonist concentration-effect curves to the right in a parallel manner (see Figure 1).

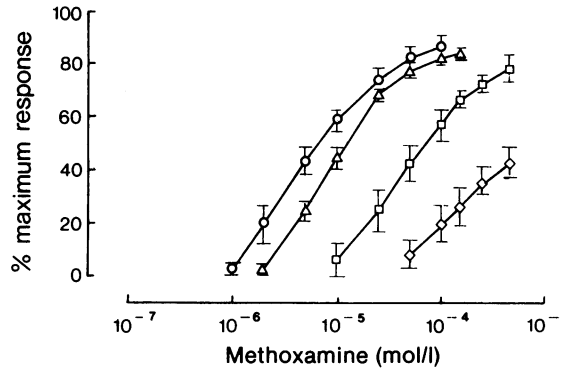

Figure 1 Dog isolated saphenous vein. Mean concentration-effect curves for methoxamine in the presence of labetalol 1.0×10^{-7} mol/l (△), 1.0×10^{-6} mol/l (□) and 1.0×10^{-5} mol/l (◇). Only the second control curve (○) is shown for clarity (see text). Each point is the mean of 4 determinations and the vertical bars represent s.e. means. Note the potent antagonism of methoxamine by labetalol.

Table 1 The interaction of phentolamine, labetalol and thymoxamine with α-adrenoceptor agonists in the dog saphenous vein

		Agonist		
Antagonist		*Noradrenaline*	*Noradrenaline (and cocaine 3×10^{-5} mol/l)*	*Methoxamine*
Phentolamine	pA_2	7.00 (6.69–7.31)	7.69* (7.24–8.14)	7.90* (7.66–8.14)
	Slope	1.01 (0.70–1.32)	0.91 (0.78–1.04)	1.00 (0.80–1.20)
Labetalol	pA_2	6.25 (5.22–7.28)	7.05 (6.63–7.47)	7.06 (6.87–7.25)
	Slope	0.54† (0.13–0.95)	0.86 (0.67–1.05)	0.95 (0.77–1.13)
Thymoxamine	pA_2	5.37 (4.07–6.67)	6.88* (6.50–7.26)	7.11* (6.80–7.42)
	Slope	0.50† (0.05–0.95)	0.96 (0.74–1.18)	0.83 (0.66–1.00)

Each value is the mean of 4 to 7 observations (95% confidence limits)
* Significantly different from pA_2 value against noradrenaline ($P < 0.05$); † significantly different from unity.

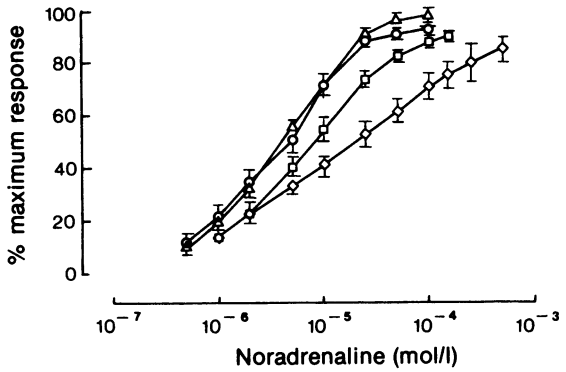

Figure 2 Dog isolated saphenous vein. Mean concentration-effect curves for noradrenaline in the presence of labetalol 1.0×10^{-7} mol/l (△), 1.0×10^{-6} mol/l (□) and 1.0×10^{-5} mol/l (◇). Only the second control curve (○) is shown for clarity (see text). Each point is the mean of 7 determinations and the vertical bars represent s.e. means. Note the weak antagonism of noradrenaline by labetalol (compare Figures 1 and 3).

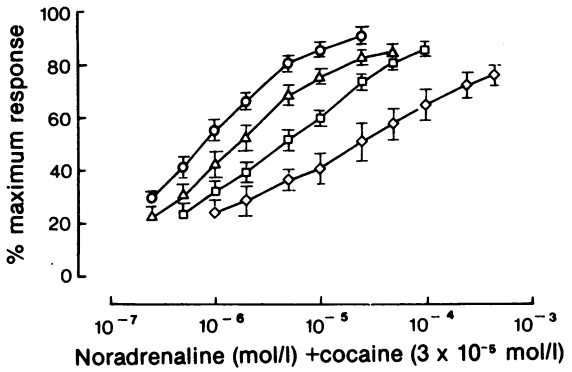

Figure 3 Dog isolated saphenous vein. The effect of cocaine (3.0×10^{-5} mol/l) on mean concentration-effect curves for noradrenaline in the presence of labetalol 1.0×10^{-7} mol/l (△), 1.0×10^{-6} mol/l (□) and 1.0×10^{-5} mol/l (◇). Only the second control curve (○) is shown for clarity (see text). Each point is the mean of 6 determinations and the vertical bars represent s.e. mean. Note the increased potency of noradrenaline and the more potent antagonistic action of labetalol (compare Figure 2).

The slopes of the Schild plots were not significantly different from unity which suggest that the antagonism was competitive in each case (Arunlakshana & Schild, 1959).

When noradrenaline was the agonist, the potency of each antagonist was lower than that against methoxamine. With phentolamine there was parallel displacement of the noradrenaline concentration-effect curves and the slope of the Schild plot was close to unity, but the pA_2 was significantly lower than that against methoxamine. Labetalol produced parallel displacement of the noradrenaline concentration-effect curve at the lower concentrations of antagonist but at concentrations of 1.0×10^{-5} mol/l and above, the slope of the noradrenaline curve became shallower (Figure 2). The pA_2 for labetalol against noradrenaline, although lower, was not significantly different from that against methoxamine but the slope of the Schild plot was less than unity. Thymoxamine also displaced the noradrenaline concentration-effect curve in a parellel manner at low concentrations of antagonist. However, with thymoxamine 10^{-5} mol/l the noradrenaline curve was biphasic, that is, the curve was parallel to the control curve over the lower portion (up to about 50% of the maximum) then plateaued between about 10^{-5} and 10^{-4} mol/l before becoming parallel to the control curve again. In this case only, the EC_{40} value was used for calculation of the dose-ratio. The pA_2 for thymoxamine against noradrenaline was lower than that against methoxamine and the slope of the Schild plot was less than unity (Table 1).

Effect of cocaine

Cocaine (1.0×10^{-6} to 5.0×10^{-5} mol/l) markedly potentiated the responses to noradrenaline. At 3.0×10^{-5} mol/l the noradrenaline concentration-effect curve was displaced to the left by a factor of 7.7 (3.6–16.7), the mean (95% confidence limits) of 4 observations. In contrast, cocaine (3.0×10^{-5} mol/l) did not potentiate responses to methoxamine (methoxamine dose-ratio 1.1 [0.8–1.5], mean [95% confidence limits] of 6 observations).

When the antagonist potencies of phentolamine, labetalol (Figure 3) and thymoxamine were re-determined against noradrenaline in the presence of cocaine (3.0×10^{-5} mol/l) the noradrenaline concentration-effect curves were displaced to the right in a parallel manner and the pA_2 values for all three antagonists were similar to those obtained against methoxamine. In addition, the slope of the Schild plot for each antagonist was not significantly different from unity (Table 1).

Discussion

The pA_2 values for phentolamine, labetalol and thymoxamine against noradrenaline were about one whole unit lower in the dog saphenous vein than is commonly found in other vascular preparations (Birmingham & Szolcsanyi, 1965; Furchgott, 1967; Gulati, Parikh & Umar, 1968; Patil, Fudge & Jacobowitz, 1972; Kennedy & Levy, 1974). This might be

thought to support the suggestion that there are subclasses of α-adrenoceptors (Sheys & Green, 1972; Janis & Triggle, 1973). However, a more plausible explanation is that the adrenergic neuronal uptake process (uptake$_1$) interfered with the estimates of antagonist potency.

In the saphenous vein, cocaine markedly potentiated responses to noradrenaline, presumably as a result of its uptake$_1$ blocking action (Iversen, 1965). Such potentiation is unlikely to result from a non-specific sensitizing action (Kalsner, 1975), as responses to methoxamine were not affected. In the presence of cocaine, the pA_2 values for the antagonists against noradrenaline were then close to those expected on 'classical' α-adrenoceptors. These findings are consistent with those obtained in some other tissues (see Furchgott, 1972 for references) where adrenoceptor antagonists are apparently less potent against noradrenaline than against agonists which are not inactivated by uptake$_1$. This phenomenon is thought to occur because of the presence of the saturable uptake$_1$ mechanism (Langer & Trendelenburg, 1969). At low concentrations the uptake of noradrenaline is directly proportional to the organ bath concentration and so the concentration at the receptors is reduced by a constant proportion. However, as the bath concentration of noradrenaline is increased, the capacity of the uptake$_1$ system approaches saturation and the increase in concentration at the receptors is disproportionately greater than the increase in bath concentration. Once saturation is complete there should be a direct relationship between the increase in concentration of noradrenaline at the receptors and the increase in bath concentration. Normally the concentrations of noradrenaline required to obtain a concentration-effect curve are too low to saturate the uptake$_1$ system. However, in the presence of a competitive antagonist, higher concentrations of noradrenaline are required to produce a response and saturation begins to occur. There will then be a disproportionate increase in the concentration of noradrenaline at the receptors and hence a smaller shift in the concentration-effect curve. Furchgott (1972) has derived equations that predict the effects of a saturable uptake system on the Schild plot for the interaction between an antagonist an an agonist with affinity for the saturable uptake system. Depending upon which concentrations of antagonist are used for the analysis, one may obtain a low estimate of pA_2 and/or a regression with a low slope (see Furchgott, 1972 for detailed explanation). The results obtained in this study with the various antagonists and agonists seem explicable in terms of these concepts.

The fact that the pA_2 value for phentolamine against noradrenaline was lower than that against methoxamine is in keeping with the finding that the affinity of methoxamine for uptake$_1$ in rat heart is about 4000 times less than that of noradrenaline (Iversen, 1965). Therefore, when methoxamine is used as the agonist, the effect of uptake$_1$ is negligible. Hence, when the uptake$_1$ process is blocked by cocaine, the pA_2 for phentolamine against noradrenaline is similar to that against methoxamine. The results obtained with labetalol and thymoxamine were similar to those obtained with phentolamine. They were weak antagonists of noradrenaline as judged by their low pA_2 values and the fact that for both labetalol and thymoxamine the slope of the Schild plot was significantly less than unity. However, when the uptake$_1$ process was blocked by cocaine, the pA_2 values for both labetalol and thymoxamine against noradrenaline became similar to those obtained against methoxamine and the slopes of the Schild plots were not significantly different from unity.

These experiments emphasize the need to control the effect of uptake$_1$ in those preparations in which there is an avid neuronal uptake system. The effect of uptake$_1$ is negligible in a large blood vessel like the rabbit aorta and cocaine cuases only a two to three fold potentiation of responses to noradrenaline in this tissue (Furchgott, 1967). Indeed, there is some doubt whether even this small degree of potentiation is due to blockade of uptake$_1$ (Kalsner & Nickerson, 1969; Trendelenburg, 1971). Hence, in the aorta the pA_2 estimate will not be influenced to any greater extent by uptake$_1$. However, complications could arise when pA_2 values are determined using preparations from small arteries and veins which may be more densely innervated and where the effect of the uptake$_1$ process may be more marked. If the assumption is to be made that the pA_2 value corresponds to the affinity constant, then uptake$_1$ is just one important factor among many which needs to be controlled (Furchgott, 1972). When noradrenaline is used as the agonist the effects of uptake$_1$ can effectively be removed by the continuous presence of a suitable concentration of uptake$_1$ inhibitor. Alternatively, it may be more convenient to use an α-adrenoceptor agonist such as methoxamine, which has little or no affinity for the uptake process.

The skilled technical assistance of Mrs Janet Pledger is gratefully acknowledged. I would also like to thank Mr I. Kennedy for helpful discussion concerning adrenergic uptake mechanisms.

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(*Received February 3, 1978.*
Revised March 8, 1978.)