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EFFECT OF LABETALOL ON THE UPTAKE OF [^{3}H]-(−)-NORADRENALINE INTO THE ISOLATED VAS DEFERENS OF THE RAT

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1 The effects of the combined α- and β-adrenoceptor blocking drug, labetalol, on the uptake of [^{3}H]-(−)-noradrenaline into the isolated vas deferens of the rat have been determined and compared with those of some other α-adrenoceptor blocking drugs and cocaine.

2 Labetalol, like cocaine, produced a simple competitive inhibition of [^{3}H]-(−)-noradrenaline uptake and was about 4 times less potent than cocaine. It is concluded that labetalol is a potent inhibitor of uptake$_1$. Phentolamine and thymoxamine also inhibited [^{3}H]-(−)-noradrenaline uptake, and were respectively 8 and 14 times less potent than cocaine. Tolazoline, piperoxan and yohimbine were inactive in concentrations up to 30 μg/ml.

3 The uptake$_1$ blocking action of labetalol could explain, at least in part, the previously reported difference in its ability to block noradrenaline and phenylephrine vasopressor responses in the anaesthetized dog.

4 The possibility that uptake$_1$ inhibitory concentrations of labetalol could be present in the blood of subjects receiving normal antihypertensive doses of the drug is discussed.

Introduction

Labetalol, in addition to its α- and β-adrenoceptor blocking actions (Brittain & Levy, 1976), is thought to block a cocaine-sensitive inactivation process for noradrenaline. This was inferred from experiments with anaesthetized dogs which showed that labetalol was a potent antagonist of phenylephrine-induced vasopressor responses but was a much weaker antagonist of noradrenaline-induced vasopressor responses; this difference was not apparent in animals pretreated with cocaine. It was suggested that inhibition of a cocaine-sensitive inactivation process for noradrenaline by labetalol resulted in an increase in the plasma concentration of noradrenaline attained after intravenous injection, thereby counteracting the α-adrenoceptor blockade produced by labetalol (Farmer, Kennedy, Levy & Marshall, 1972; Kennedy & Levy, 1975).

There are two cocaine-sensitive sites for the inactivation of blood-borne noradrenaline, the peripheral vascular beds and the pulmonary vascular bed (Vane, 1969). In the peripheral vascular beds, noradrenaline is taken up into adrenergic nerves by the uptake$_1$ process, whereas in the pulmonary vascular bed, noradrenaline is removed by an extra-neuronal process. Both processes are inhibited by cocaine but they are distinguishable by differences in their sensitivity to other uptake inhibitors (Alabaster & Bakhle, 1973; Iwasawa & Gillis, 1974).

Labetalol could increase plasma noradrenaline concentrations by inhibiting either the uptake$_1$ process or the pulmonary uptake process or both. The experiments to be described were undertaken to determine whether labetalol is an inhibitor of uptake$_1$. The effect of labetalol on the uptake of [^{3}H]-(−)-noradrenaline into adrenergic nerves in rat isolated vas deferens has been determined and compared with the effects of some other drugs with α-adrenoceptor blocking activity. The rat vas deferens was chosen because it has a dense adrenergic innervation and, consequently, an avid uptake$_1$ capacity, and because it has been used by other workers to study the kinetics of known inhibitors of uptake$_1$ (Iversen & Langer, 1969).

Methods

The method described by Iversen & Langer (1969) was used with minor modifications. Vasa deferentia were removed from rats (body weight 200 to 400 g) and cut into segments measuring 5 mm by 0.5 mm. Groups of five slices were incubated, with shaking, at 37°C for 30 min in 2 ml of incubation medium of

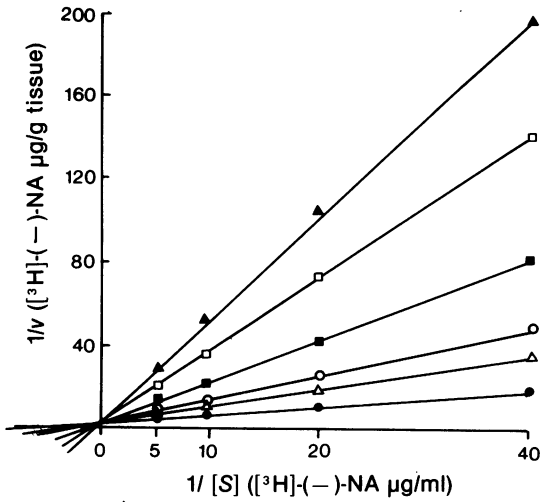

Figure 1 Inhibition of [^{3}H]-(−)-noradrenaline ([^{3}H]-(−)-NA) uptake by cocaine in slices of rat vas deferens. The slices were incubated with various concentrations of [^{3}H]-(−)-noradrenaline in the presence of saline (●), cocaine 1 µg/ml (△), 3 µg/ml (○), 10 µg/ml (■), 30 µg/ml (□), 100 µg/ml (▲). Results from a typical experiment are shown.

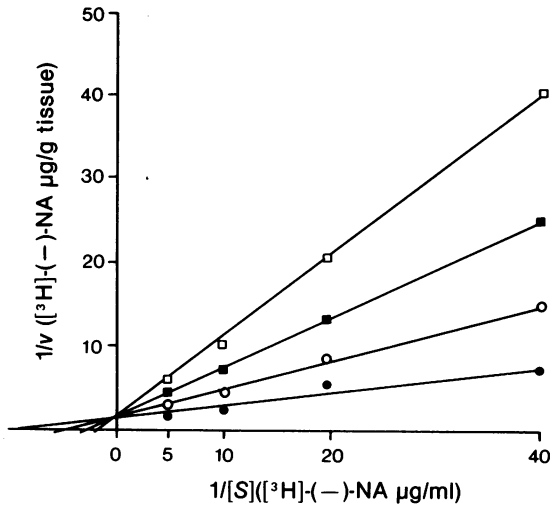

Figure 2 Inhibition of [^{3}H]-(−)-noradrenaline ([^{3}H]-(−)-NA) uptake by labetalol in slices of rat vas deferens. The slices were incubated with various concentrations of [^{3}H]-(−)-noradrenaline in the presence of saline (●), labetalol 3 µg/ml (○), 10 µg/ml (■) and 30 µg/ml (□). Results from a typical experiment are shown.

the following composition (mmol/l): Na^+ 143.4, K^+ 5.9, Mg^{2+} 0.6, Ca^{2+} 1:3, Cl^- 124.5, $H_2PO_4^-$ 1.2, SO_4^{2-} 0.6, HCO_3^- 25.0 and glucose 11.1. Ascorbic acid (20 mg/l), disodium edetate (EDTA) (10 mg/l), pargyline (20 mg/l) and tropolone (5 mg/l) were added to the incubation medium. The medium was gassed with 95% O_2 and 5% CO_2 before use. At the end of the 30 min incubation period [^{3}H]-(−)-noradrenaline, diluted with various amounts of non-radioactive (−)-noradrenaline, was added to give final noradrenaline concentrations of 25, 50, 100 and 200 ng/ml, each concentration containing 0.5 µCi/ml. Incubation was continued for 10 min and the medium was removed and replaced with 5 ml of noradrenaline-free medium for a further 10 min to wash out extracellular [^{3}H]-(−)-noradrenaline. The slices were blotted, weighed, burnt in oxygen (Packard Tri-Carb Sample Oxidiser No. 306) and the resulting tritiated water was assayed in a Nuclear Enterprises liquid scintillation counter. In experiments in which the effects of drugs on the uptake of [^{3}H]-(−)-noradrenaline were examined, the drugs were added to the incubation medium 5 min before the [^{3}H]-(−)-noradrenaline.

Apparent *Km* values for noradrenaline and *Ki* values for the inhibitors of noradrenaline uptake were calculated as described by Lineweaver & Burke (1934) and Dixon (1953) respectively.

The following drugs were used: ascorbic acid (Roche), cocaine hydrochloride (Macfarlan Smith), disodium edetate (EDTA, BDH), labetalol hydrochloride (Allen & Hanburys), (−)-noradrenaline acid tartrate (Winthrop), [^{3}H]-(−)-noradrenaline (sp. act. 10.3 Ci/mmol) (Radiochemical Centre, Amersham), pargyline hydrochloride (Abbott), phentolamine mesylate (Ciba), piperoxan hydrochloride (May & Baker), thymoxamine hydrochloride (Warner), tolazoline hydrochloride (Ciba), tropolone (Ralph N. Emanuel) and yohimbine hydrochloride (Sigma). Concentrations refer to free bases.

Results

Slices of rat vas deferens readily accumulated [^{3}H]-(−)-noradrenaline from the incubation medium. The apparent *Km* for (−)-noradrenaline was 2.71 ± 0.26 µmol/l (mean ± s.e. mean, $n = 20$). This value is similar to that obtained by Iversen & Langer (1969).

Cocaine (1 to 100 µg/ml) was a simple competitive inhibitor of the uptake of [^{3}H]-(−)-noradrenaline (Figure 1). The highest concentration of cocaine used (100 µg/ml)inhibited uptake by 93%.

Labetalol (3 to 30 µg/ml) (Figure 2), phentolamine (3 to 30 µg/ml) and thymoxamine (3 to 30 µg/ml) were also simple competitive inhibitors of the uptake of [^{3}H]-(−)-noradrenaline. The data from Lineweaver & Burke plots for each drug were used to compile plots of $1/v$ against inhibitor concentration (Dixon,

1953). Ki values, estimated from these plots, are shown in Table 1. Piperoxan, tolazoline and yohimbine did not block the uptake of [^{3}H]-(−)-noradrenaline in concentrations up to 30 μg/ml.

Discussion

The rat vas deferens readily accumulated [^{3}H]-(−)-noradrenaline, and this uptake could be almost completely inhibited by cocaine; it seems reasonable to conclude, therefore, that the uptake process involved was uptake$_1$. Labetalol, like cocaine, produced a simple competitive inhibition of [^{3}H]-(−)-noradrenaline uptake, and was about 4 times less potent than cocaine. We conclude, therefore, that labetalol is a potent inhibitor of uptake$_1$. Blakely & Summers (1977) and Summers & Tillman (1977) recently came to a similar conclusion from their experiments with labetalol in the isolated blood-perfused spleen of the cat.

The inhibitory action of labetalol on uptake$_1$ does not appear to be related to either its α- or β-adrenoceptor blocking actions since a variety of drugs with little or no α- or β-adrenoceptor blocking activity may act as uptake inhibitors (Iversen, 1967a) and, conversely, potent α- or β-adrenoceptor blocking drugs can be devoid of uptake inhibitory activity (present experiments; Foo, Jowett & Stafford, 1968). Furthermore, the adrenoceptor blocking and uptake$_1$ inhibitory actions of labetalol occur over entirely different concentration ranges. Thus, the threshold concentrations for β-adrenoceptor blockade, α-adrenoceptor blockade and inhibition of uptake$_1$ are about 1 ng/ml, 10 ng/ml (Brittain & Levy, 1976) and 1000 ng/ml (present experiments) respectively.

The results of the present experiments could account, at least in part, for the difference in the ability of labetalol to antagonize pressor responses to phenylephrine and noradrenaline in the anaesthetized dog (see Introduction). At present we do not know whether labetalol is capable of blocking the cocaine-sensitive uptake process in the pulmonary vascular bed. Experiments are in progress to determine this and to determine whether the blood levels of noradrenaline attained during intravenous infusion are increased in the presence of labetalol, as would be predicted if the drug was preventing the inactivation of noradrenaline. In preliminary experiments in the anaesthetized dog we have shown that plasma concentrations of intravenously infused noradrenaline are increased in the presence of labetalol, 1 to 10 mg/kg intravenously (Kennedy & Levy, unpublished results).

Finally, in a clinical context, labetalol has been reported to increase levels of free urinary catecholamines in some patients (Harris & Richards, 1977). Increased adrenergic activity in response to the fall in blood pressure caused by labetalol is a likely explanation. However, an uptake$_1$ inhibitory action is an obvious additional possibility although extrapolation from the present results is difficult as uptake processes in different tissues are known to exhibit considerable differences in their sensitivity to inhibition by various drugs (Iversen, 1967b; Iversen & Langer, 1969).

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Table 1 Kinetic constants for [^{3}H]-(−)-noradrenaline uptake and drug inhibition of uptake in the rat vas deferens

Drug		*Apparent Km or Ki value (mol/l × 10^{-6})*
[^{3}H]-(−)-noradrenaline	(20)	2.7 ± 0.26
Cocaine	(5)	14.1 ± 2.2
Labetalol	(5)	51.8 ± 5.1
Phentolamine	(4)	113.5 ± 24.6
Thymoxamine	(4)	195.3 ± 27.4
Yohimbine	(4)	> 85*
Tolazoline	(4)	>187*
Piperoxan	(4)	>129*

Results are mean ±s.e. mean. Figures in parentheses give the number of experiments performed. * No effect on [^{3}H]-(−)-noradrenaline uptake in concentrations up to and including 30 μg/ml.

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