The effect of labetalol on the release of [^{3}H]-labelled noradrenaline and its metabolites from dog isolated saphenous vein

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Recently it has been suggested that labetalol, a competitive antagonist at α- and β-adrenoceptors, can cause the release of noradrenaline from sympathetic nerve endings in the anococcygeus muscle of the rat (Doggrell & Paton, 1978). The possibility of such a releasing action has been investigated further in strips of dog saphenous vein preloaded with [^{3}H-] labelled noradrenaline.

Saphenous veins were removed from anaesthetized beagle dogs, cut into helical strips and incubated in Krebs solution containing ascorbic acid (0.11 mM), EDTA (0.004 mM) and 1-[7-^{3}H] noradrenaline hydrochloride (10 μCi/ml) for 2 h at 37°C. At the end of the incubation the strips were rinsed with fresh Krebs and placed in a 2 ml organ bath under an initial tension of 1 g. The Krebs solution in the organ bath was renewed every 10 min for 90 min to wash out the [^{3}H]-noradrenaline from the extracellular space. At the end of this time the tissue was exposed to various concentrations of labetalol for 10 min and then washed with fresh Krebs (5 × 10 min); this procedure was repeated up to three times. Throughout the experiment the Krebs solution was maintained at 37°C and gassed with 5% CO_2 in O_2. The radioactivity in each 2 ml portion of Krebs solution was determined by scintillation counting.

The repeated administration of labetalol (10^{-5} M) on four successive occasions caused a reproducible 2–4 fold increase in the overflow of radioactivity from the saphenous vein strips. The effect was dose related over the range 10^{-7}–10^{-5} M labetalol. This effect of labetalol was unaffected by omission of Ca^{2+} from, or by the addition of cocaine (3×10^{-5} M) to, the Krebs solution; cocaine itself had no effect on the total release of radioactivity. Exposure of the tissue to phentolamine (10^{-8}–10^{-6} M) or propranolol (10^{-7}–10^{-5} M) (concentrations causing a similar degree of α- or β-adrenoceptor blockade to labetalol) caused only a small increase in release of radioactivity and then only at the highest concentrations used.

In separate experiments the radioactivity released from the saphenous vein strips was analysed by paper chromatography (Muldoon, Vanhoutte & Tyce, 1978). The results indicated that the increase in the release of radioactivity caused by labetalol (10^{-5} M) was due mainly to an increase in 3,4-dihydroxyphenylglycol formation. This suggests that labetalol releases noradrenaline from an intraneuronal storage site and that the noradrenaline is subsequently metabolized, primarily by cytoplasmic monoamine oxidase; the resultant metabolites then diffuse into the extracellular space. This effect of labetalol is not related to its α- or β-adrenoceptor antagonist properties, or to its ability to block uptake$_1$ (Drew, Hilditch & Levy, 1978). Similar findings have been reported for piperoxan (Borowski, Starke, Ehrl & Endo, 1977), phenoxybenzamine (Graefe, Stefano & Langer, 1973) and prazosin (Cambridge, Davey & Massingham, 1977).

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The effect of a high dose of prazosin, and transmural stimulation, on the disposition of transmitter noradrenaline in the rabbit pulmonary artery and the dog saphenous vein

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We have previously reported that high doses of prazosin (>10^{-6}M) cause an increase in [H^3]-efflux from quiescent, [H^3]-noradrenaline labelled strips of

rabbit pulmonary artery (Cambridge, Davey & Massingham, 1977). To identify more clearly the site of action of prazosin we have studied the release of [H^3]-noradrenaline and metabolites induced by prazosin and nerve stimulation in rabbit pulmonary arteries and dog saphenous veins.

Strips of blood vessel were prepared for superfusion (See Vanhoutte, Lorenz & Tyce, 1973; Cambridge *et al.*, 1977) and the superfusate collected for three consecutive 15 min periods. During the second period the tissues were either stimulated transmurally or prazosin (10^{-5}M) was added to the Krebs. For each period total [H^3], as well as [H^3]-noradrenaline, [H^3]-DOPEG, [H^3]-DOMA and [H^3]-noncatechols were separated by column chromatography (Verbeuren, Coen & Vanhoutte, 1977) (See Table 1).

During control periods, only a small fraction of the total [H^3] collected from both tissues was due to [H^3]-noradrenaline. Electrical stimulation caused an increase in total [H^3] and absolute release of [H^3]-noradrenaline and deaminated metabolites. Prazosin (10^{-5}M) also caused an increase in total [H^3] but with no change in [H^3]-noradrenaline. Only in saphenous veins treated with the monoamine oxidase inhibitor pargyline, did prazosin cause an increase in [H^3]-noradrenaline that was significantly greater than in untreated strips.

These results concerning the fate of released [H^3]-noradrenaline are consistent with the known morphology and innervation of the tissues studied (Bevan & Su, 1974; Shepherd & Vanhoutte, 1975). DOPEG and noncatechols are the major metabolites of catecholamine uptake at neuronal and extraneuronal sites, respectively (see Osswald, 1978). Preferential formation of deaminated metabolites would therefore be expected from the saphenous vein, where the synaptic cleft is smaller than in the pulmonary artery. In both tissues, however, the release of [H^3] caused by prazosin clearly does not resemble exocytosis and supports suggestions of intraneuronal disruption of noradrenaline storage at high concentrations of prazosin (Vanhoutte, 1978).

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Table 1 Efflux of [H^3]-noradrenaline and its metabolites (10^{-3} DPM/15 ml superfusate)

	[H^3]-*Noradrenaline*	[H^3]-*DOPEG***	[H^3]-*DOMA***	[H^3]-*Noncatechols***
Rabbit Pulmonary Artery (N=5)				
Control Period	1.41 ± 0.23	3.57 ± 0.88	2.80 ± 0.21	4.72 ± 0.88
Stimulation (5 Hz)	8.44 ± 2.05*	7.16 ± 1.27*	3.86 ± 1.15	10.8 ± 3.4
Control Period	0.93 ± 0.19*	2.96 ± 0.64*	1.58 ± 0.22	3.76 ± 0.74*
Dog Saphenous Vein (N=5)				
Control Period	1.70 ± 0.30	25.8 ± 1.8	6.50 ± 0.70	19.4 ± 3.7
Stimulation (2 Hz)	20.6 ± 5.2*	37.3 ± 3.8*	11.3 ± 1.8*	32.3 ± 4.3*
Control Period	1.00 ± 0.20*	22.8 ± 2.1*	6.50 ± 1.0*	18.7 ± 3.6*
Rabbit Pulmonary Artery (N=5)				
Control Period	1.45 ± 0.51	3.28 ± 0.55	2.21 ± 0.40	3.67 ± 0.83
Prazosin (10^{-5}M)	1.74 ± 0.43	13.1 ± 3.1*	4.70 ± 1.30	3.72 ± 1.20
Control Period	1.25 ± 0.31	13.3 ± 3.9	1.42 ± 0.64	4.24 ± 1.45
Dog Saphenous Vein (N=5)				
Control Period	2.70 ± 0.50	30.5 ± 4.2	9.00 ± 1.40	16.0 ± 0.5
Prazosin (10^{-5}M)	3.50 ± 0.70	46.7 ± 4.9*	13.7 ± 2.2*	14.4 ± 0.4*
Control Period	2.20 ± 0.70	59.7 ± 8.0*	16.1 ± 3.4	15.7 ± 1.6

*Value significantly different from preceding value ($P<0.05$ students t-test: paired observations).

**DOPEG = 3,4-dihydroxyphenylglycol; DOMA = 3,4-dihydroxymandelic acid; Noncatechols = normetanephrine (NMN); 3-methoxy-4-hydroxyphenylglycol (MOPEG) and 3-methoxy-4-hydroxy-mandelic acid (VMA).