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THE EFFECTS OF LABETALOL (AH 5158) ON METABOLISM OF ^{3}H(−)-NORADRENALINE RELEASED FROM THE CAT SPLEEN BY NERVE STIMULATION

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Abstract—The competitive α and β adrenoceptor antagonist labetalol, in concentrations up to 10^{-4} M, produced a dose dependent increase in overflow of ^{3}H and [^{3}H]noradrenaline in the isolated blood perfused cat spleen following stimulation of the splenic nerves at a frequency of 10 Hz. Labetalol had no effect on the pattern of overflow of label following stimulation. In experiments in which the metabolism of [^{3}H]noradrenaline released on nerve stimulation was examined, labetalol produced a concentration dependent increase in the percentage of [^{3}H]noradrenaline and a decrease in the percentage of [^{3}H]DOPEG in the venous blood following nerve stimulation. Production of [^{3}H]COMT metabolites and [^{3}H]DOMA was not affected. It is suggested that in the isolated blood perfused cat spleen labetalol produces the elevation of overflow and effects on noradrenaline metabolism by inhibition of neuronal uptake of noradrenaline. The drug has no detectable effects on the enzymes MAO or COMT or on extraneuronal uptake.

Labetalol (AH 5158; 5-{1-hydroxy-2-[(1-methyl-3-phenyl propyl) amino] ethyl} salicylamide) competitively inhibits α adrenoceptors in a number of adrenergically innervated tissues, including guinea pig mesenteric vein, rat vas deferens [1] dog blood pressure [2] and isolated cat splenic capsular strips [3, 4]. In the isolated blood perfused cat spleen labetalol increases the overflow of noradrenaline following stimulation of the splenic nerves at 10 and 30 Hz [4]. Although α blocking agents usually increase transmitter overflow by inhibiting the α feedback loop controlling transmitter release this mechanism is not the principal one utilised by labetalol since addition of a preferentially presynaptic α blocker piperoxan [5] produces a further large increase in overflow [4]. Thus in the cat spleen labetalol appears to act as a preferential post synaptic α blocking agent.

The mechanism whereby labetalol increases overflow probably involves inhibition of uptake since the drug inhibits a cocaine sensitive inactivation process in the dog [1, 2] and increases the venous recovery of label in the cat spleen during arterial infusion of ^{3}H(−) noradrenaline [4].

Labetalol is also known to competitively block β receptors and to possess membrane stabilising properties [1]. Both actions might be expected to reduce transmitter overflow but do not do so in the cat spleen at concentrations of up to 10^{-4} M [4].

The present experiments investigate the mechanism whereby labetalol elevates transmitter overflow by examining its effect on uptake processes and metabolic enzymes. A preliminary account of these results has been published [6].

MATERIALS AND METHODS

Cat spleens were perfused with blood at constant rate *in vitro* (flow rate 6.0 ± 0.3 ml/min) [7, 8].

Stimulation of the splenic nerves. The splenic nerves were stimulated with bipolar platinum hook electrodes. The parameters of stimulation were 200 pulses at 10 Hz, 0.5 ms duration, at a supramaximal voltage (20 V). Periods of stimulation were repeated at 10 min intervals.

Infusion of ^{3}H-labelled (−)-noradrenaline. For infusion of ^{3}H(−)-noradrenaline, a solution of the amine was placed in a 10 ml disposable syringe and delivered to the arterial cannula through 2 ft of fine nylon tubing (Portex gauge 00) by a motor driven pump (McLennan Digital syringe, type DS 201) at a rate of 0.18 ml/min.

Labelling procedure. 0.5 nmole/min ^{3}H(−)-noradrenaline (specific activity 10.3 Ci/mmole) was infused for 10 min. Solution containing ^{3}H(−)-noradrenaline was made up in 0.9% saline containing 5.67×10^{-3} M ascorbic acid and 2.23×10^{-3} M ethylenediamine tetra-acetic acid (EDTA) so that the (−)-noradrenaline concentration was 2.97×10^{-6} M. Following infusion, 1 ml Krebs–Henseleit was injected to wash in the ^{3}H(−)-noradrenaline trapped in the fine polythene tubing.

After labelling, the spleen was perfused with modified Krebs–Henseleit solution (composition $NaHCO_3$ 25 mM; NaCl 120 mM; KCl 4.5 mM; NaH_2PO_4 0.19 mM; Na_2HPO_4 1.83 mM; $CaCl_2$ 1.25 mM; $MgCl_2$ 1.00 mM; Glucose 11.1 mM) at 37° for 30 min to remove the loosely bound label. Samples of the venous outflow were taken every 5 min to examine the pattern of efflux of ^{3}H. The remaining label was collected and counted separately. The difference in amount of tritium infused and the total amount collected in the wash was taken as a measure of the capacity of the spleen to retain infused label.

Experimental plan. After washing, the Krebs–Henseleit solution was replaced with oxygenated blood containing no label, and the spleen allowed to re-equilibrate for 30 min before stimulation of the splenic nerves. During and after stimulation of the

splenic nerves the venous effluent was collected for 4 min in 1′ fractions. Two such 4 min post stimulation samples of blood were collected before addition of drug, followed by one collection after each dose of labetalol. Each dose increment was separated by a period of 20 min to allow the drug to equilibrate with the blood and the spleen.

In general only one or two concentrations of labetalol were tested in each spleen. The limiting factor was the volume of blood that could be collected from a cat and the fact that large blood samples were taken after each period of stimulation.

Blood samples were collected in chilled centrifuge tubes and spun at 3100 *g* for 15 min at 0°. Samples of plasma were removed from the red blood cells and buffy coat.

Estimation of total tritium present. 0.5 ml of plasma was mixed with 0.5 ml H_2O and 10 ml Triton X100/toluene scintillation mixture in a counting vial. Corrections were made for background, quenching and efficiency of counting.

Retention and metabolism of $^3H(-)$-noradrenaline in the spleen. At the end of each experiment, the spleen was blotted on filter paper, weighed and homogenised with a Polytron homogenizer in 10 vol. perchloric acid (0.4 M) containing 3×10^{-3} M EDTA and 5.3×10^{-3} M sodium bisulphite. The homogenate was kept on ice for 1 hr to allow complete precipitation of proteins, and then centrifuged at 3100 *g* for 15 min. The supernatant was removed for metabolite separation and total ^3H estimation. For total tritium estimation, 1 ml supernatant was added to 10 ml Triton X100 scintillation fluid in a counting vial.

Metabolism studies. Aliquots of plasma were adjusted to pH 1–2 with 0.4 M perchloric acid containing 3×10^{-3} M EDTA and 5.3×10^{-3} M sodium bisulphite, and the samples kept at 0° until assayed. In almost every case this entailed overnight storage. Control experiments showed that storage overnight did not affect the composition of the samples.

Aliquots of spleen homogenate supernatant were kept on ice, and assayed with the plasma. A maximum of eighteen samples, including one blank and one standard $^3H(-)$-noradrenaline sample were assayed in one day. The ^3H compounds overflowing in the venous blood were separated by column chromatography on alumina and Dowex 50W × 4 columns into [^3H]noradrenaline (NA), [^3H]-3,4-dihydroxyphenyl glycol (DOPEG), [^3H]-3,4-dihydroxymandelic acid (DOMA) and [^3H]-*O*-methylated metabolites (COMT metabolites) by the method of Graefe *et al.* [9]. In some experiments the [^3H]-*O*-methylated metabolites were further separated into two fractions, one containing [^3H]normetanephrine (NMN) and the other containing the *O*-methylated deaminated metabolites (OMDA)-4-hydroxy-3-methoxymandelic acid (VMA) and 4-hydroxy-3-methoxyphenylglycol (MOPEG).

The following modifications were made to the method. Volumes of eluates were increased so that aliquots could be taken for counting before column separation which allowed corrections for recovery to be made in each sample. The volume of 0.2 M acetic acid used to elute the NA/DOPEG fraction from the alumina column was increased from 2 ml to 3.5 ml, and the 0.2 M HCl used for elution of [^3H]DOMA was increased from 2.5 to 3 ml. The concentration and volume of acid required to elute [^3H]NA from the Dowex 50W × 4 column was increased from 2 M to 3 M and the volume from 2 to 3 ml. These alterations were made to ensure complete recovery of [^3H]NA. 1 ml of each separated fraction was added, to 10 mls Triton X100/toluene scintillant.

Calculation of metabolism results. The amount of radioactivity appearing in each of the metabolite samples compared to that in the sample before separation gave an indication of the recovery and efficiency of each column. The recovery for the alumina columns was 94.8 ± 0.9% ($n = 42$) and for the Dowex columns 95.8 ± 0.9% and 98.7 ± 1.2% respectively ($n = 40$).

The amount of [^3H]NA and ^3H-metabolites present in the pre-stimulation sample was subtracted from the amount in each sample obtained after nerve stimulation.

After subtraction of the venous control the products of nerve stimulation were expressed either as a percentage of the total tritium present in that one minute aliquot, or, as a percentage of the total overflow collected in 4 min. A correction was applied for the small amount of [^3H]NA and [^3H]DOPEG (less than 5 per cent of the total ^3H overflowing) eluted in the 0.5 M Acetic acid fraction.

In spleen homogenates [^3H]NA and metabolites were expressed as a percentage of the total tritium present in the spleen-homogenate supernatant. With every separation, the [^3H]NA used for labelling the spleen was run as a control. This control sample allowed an estimation of the efficiency of the column separation to be made. Results are expressed as mean ± standard error of mean. Significance was assessed by Student's *t*-test. Where appropriate, lines of best fit were obtained by means of regression analysis.

Drugs. 7-$^3H(-)$ noradrenaline (10.3 Ci/mmole), Radiochemical Centre, Amersham; (±)3,4-Dihydroxyphenylglycol, (±)4-hydroxy-3-methoxy mandelic acid, (±)normetanephrinehydrochloride, (±)3,4-dihydroxymandelic acid, (±)4-hydroxy-3-methoxy phenyl glycol (piperazine salt), Sigma Chemical Co.; Heparin (mucous), Boots Pure Drug Co. Ltd., Nottingham; PGE_1, Dr. J. E. Pike, Upjohn, Kalamazoo; labetalol (5{1-hydroxy-2[(1-methyl-3 phenyl propyl) amino] ethyl} salicylamide), Dr. G. P. Levy, Allen & Hanburys Research Ltd., Ware, Herts. Triton X100 Scintillant was composed of 5% Scintol 2 (Koch-Light Laboratories Ltd.), 33.3% Triton X100 (Scintillation grade) and 61.7% Toluene (Analar).

RESULTS

Retention of $^3H(-)$-noradrenaline in the cat spleen. $^3H(-)$-noradrenaline (0.5 nmole/min) was infused close arterially from a motor driven syringe into the blood perfused cat spleen preparation. 55 ± 4% ($n = 12$) of the infused [^3H]NA was retained in the spleen after removal of the loosely bound label by washing. Analysis of the efflux rates of ^3H in the wash revealed that over the initial 10 min there was a fast efflux of loosely bound label from the tissue with a rate constant of −5.88%/min ($r = -0.985$; $df = 1$)

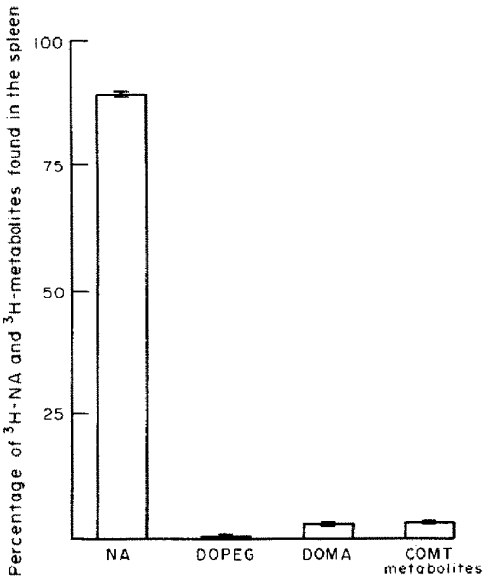

Fig. 1. Percentage of [^{3}H]NA and ^{3}H metabolites found in cat spleens ($n = 3$).

and a second, slow efflux of ^{3}H (rate constant -1.58%/min; $r = -0.995$; $df = 2$) probably related to spontaneous release of [^{3}H]NA from nerve endings.

At the end of each experiment the spleen was removed from the perfusion chamber and homogenised in ice-cold perchloric acid (0.4 M). Comparison of the amount of ^{3}H present in the spleen with that obtained by measuring the arteriovenous difference showed that the spleen contained $89.5 \pm 6\%$ ($n = 3$) of the label estimated that it should contain by the method of collecting the venous outflow.

Separation of the spleen homogenate in 3 experiments as shown in Fig. 1 by column chromatography revealed that $89.3 \pm 0.5\%$ of the total ^{3}H present was ^{3}H(−)-noradrenaline while the remainder consisted of [^{3}H]DOMA ($3.1 \pm 0.1\%$), [^{3}H]COMT metabolites ($3.4 \pm 0.1\%$) and [^{3}H]DOPEG ($0.3 \pm 0.1\%$). Further separation of the COMT metabolite fraction showed that $57.6 \pm 3\%$ of the ^{3}H was [^{3}H]OMDA while $42.3 \pm 3\%$ was [^{3}H]NMN. $3.8 \pm 2.7\%$ of the metabolites were found in the wash. This fraction was discarded in order to minimise contamination of the [^{3}H]DOMA fraction [9].

Release of ^{3}H on stimulation of the splenic nerves. Between the first two periods of stimulation of the splenic nerves there was little difference in the overflow of ^{3}H, the second overflow being $94 \pm 6\%$ of the first. Control experiments showed that the ^{3}H released on stimulation of the splenic nerves decreased slowly with succeeding periods of stimulation. Regression of the points produced by 3 consecutive periods of stimulation revealed no significant change in overflow ($r = -0.179$; $df = 22$) over the period of experiment.

The second stimulation period was used as the control in each experiment against which the effects of addition of drug could be measured.

Effect of labetalol on the pattern of overflow of ^{3}H on stimulation of the splenic nerves. In the absence of drugs $64.3 \pm 4\%$ of the total ^{3}H overflowing in 4 min came over during the first min of collection ($n = 9$). In the next 3 min the percentages of total label appearing were $13.4 \pm 2\%$, $11.2 \pm 1.4\%$ and $11.1 \pm 1.5\%$. Drugs that inhibit uptake of noradrenaline, such as cocaine, tend to prolong the overflow of transmitter [10]. This effect was not seen after labetalol. The percentage of total ^{3}H overflowing in each of the 4 min collection periods did not significantly alter with the concentration of labetalol ($r < 0.475$; $df = 7$) (Fig. 2). Also the mean ^{3}H overflowing in each collection period with labetalol was not significantly altered from the control.

Labetalol produced a dose dependent increase ($r = 0.92$; $df = 6$) in the amount of tritium recovered in the 4 min collection period and in the amount of [^{3}H]NA recovered in the first min of collection following nerve stimulation with 200 impulses at 10 Hz (Fig. 3). The maximal increase was obtained with 10^{-4} M, and the dose required to increase the overflow by 50 per cent was 1.4×10^{-5} M. At concentrations greater than 10^{-4} M a decrease in the overflow of ^{3}H was observed. A local anaesthetic action of labetalol [1] may be responsible for this effect.

Responses of the spleen to nerve stimulation. Stimulation of the splenic nerves produced an increase in

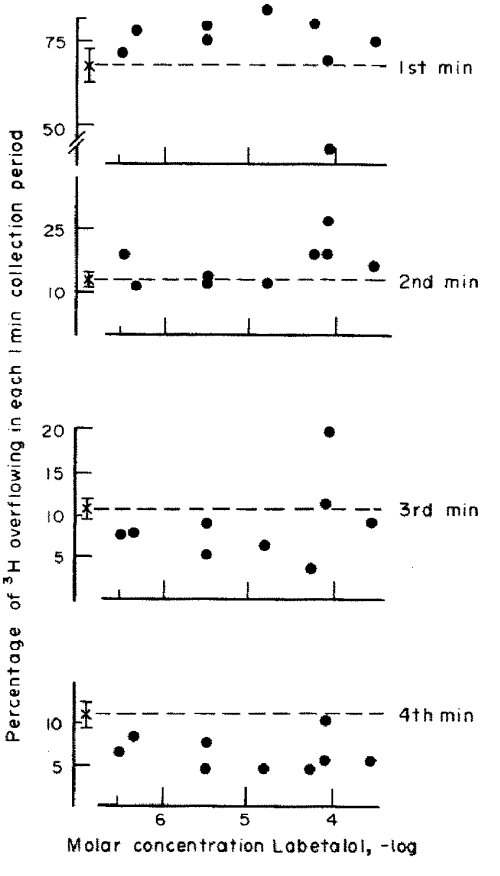

Fig. 2. The effect of labetalol on the pattern of overflow of label. Points marked (×) represent the overflow of label in each minute in the absence of drug ($n = 9$) and those marked (●) the overflow of label in the presence of labetalol. The broken line shows the level of no change.

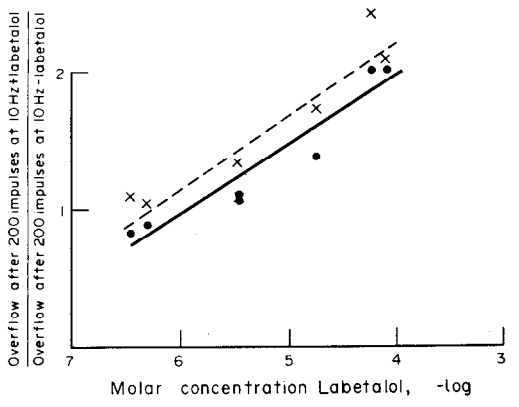

Fig. 3. The effect of labetalol on the overflow of ^3H in the 4 min collection period (●) and on the overflow of [^3H]NA (×) in the first min of collection, following stimulation of the splenic nerves with 200 impulses at 10 Hz. Overflow is expressed as the ratio of ^3H or [^3H]NA overflowing in the presence of labetalol compared to the overflow obtained before addition of drug in the same experiment.

perfusion pressure (vascular response) and a decrease in the spleen volume (capsular response).

The capsular response varied greatly between preparations, and so changes in vascular pressure with nerve stimulation were taken as an index of the splenic response.

Changes in perfusion pressure were measured from peak to peak. Stimulation of the splenic nerves with 200 impulses at 10 Hz increased the base line pressure from 69 ± 12 mm Hg to 198 ± 25 mm Hg ($n = 6$). Concentrations of labetalol up to 10^{-4} M did not alter the base line perfusion pressure, but did potentiate the height of the vascular response. Concentrations above 10^{-4} M depressed the vascular response, confirming results by Blakeley and Summers [4].

Metabolism of ^3H(−)-noradrenaline released spontaneously from the blood perfused cat spleen. In the prestimulation blood samples, [^3H]DOPEG, [^3H]DOMA and [^3H]COMT metabolites represent almost 90 per cent of the total ^3H present (Fig. 4). In 10 experiments [^3H]DOPEG accounted for 36.4 ± 2.4%, [^3H]DOMA 2.7 ± 0.1% while the [^3H]OMDA fraction represented 47.9 ± 2.7% of the total label. The remaining label was found as unchanged [^3H]NA (3.2 ± 0.5%) and the *O*-methylated metabolite [^3H]NMN (7.2 ± 0.7%). 2.6 per cent of the metabolites appeared in the wash.

Labetalol in the concentrations used in this study did not change the pattern of spontaneously released metabolites. However, the fact that the blood is recycled through the spleen every 15 to 30 min means that any change in pattern would have to be large to be detected against a resting background of metabolites.

Release of ^3H(−)-noradrenaline and ^3H-metabolites on nerve stimulation of the splenic nerves at 10 Hz. Stimulation of the splenic nerves produced a large increase in the overflow of ^3H. Examination of the pattern of [^3H]NA and ^3H metabolites overflowing in each of the 4 min (Fig. 5) revealed that in the first collection period, most of the total ^3H overflowing was [^3H]NA and that most of the metabolites in the overflow appeared in the later collection periods. There was no significant change in metabolic pattern with successive periods of stimulation apart from a small change in the percentage of DOMA as shown in Table 1.

To examine the effect of labetalol on the metabolic pattern of the [^3H]NA released on nerve stimulation, the percentages of [^3H]NA and [^3H]NA-metabolites found in each collection period were expressed as a percentage of the total ^3H found in that fraction.

The results shown in Table 2 demonstrate that most of the increase in total ^3H collected in the first collection period after stimulation is unchanged noradrenaline and the remainder released over the last 3 min is found as [^3H]DOPEG and [^3H]COMT metabolites. The percentage of [^3H]DOMA was small and remained a constant percentage in each collection period.

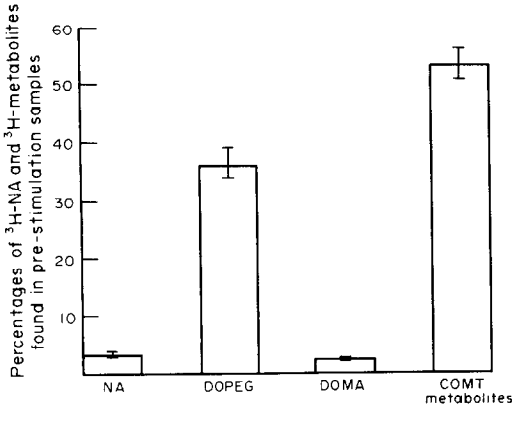

Fig. 4. Metabolism of [^3H]NA released spontaneously from the blood perfused cat spleen. Results are expressed as a percentage of the total ^3H present ($n = 10$).

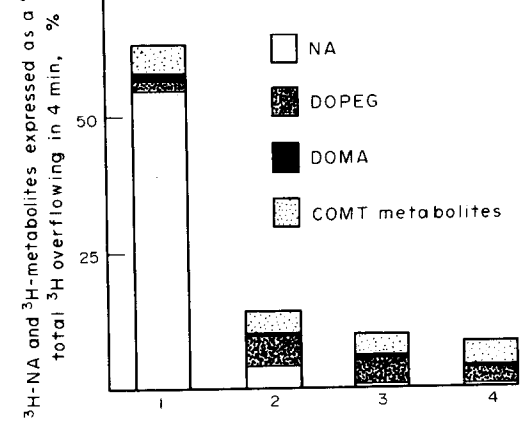

Fig. 5. [^3H]noradrenaline and ^3H metabolites overflowing in each of the 1 min collection periods following stimulation of the splenic nerves with 200 impulses at 10 Hz.

Table 1. Comparison of the metabolite pattern in plasma collected in successive 4 min collection periods after 200 impulses at 10 Hz

	1st stimulation $n = 10$	2nd stimulation $n = 3$	P of difference 1st cf 2nd
NA	60.4 ± 4.3	59.7 ± 2.2	>0.9
DOPEG	14.5 ± 2.7	10.4 ± 3.5	>0.4
DOMA	2.6 ± 0.4	5.5 ± 1.9	<0.05
COMT metabolites	19.0 ± 2.3	22.4 ± 1.1	>0.4

Table 2. Percentage of [^{3}H]NA and ^{3}H metabolites in each control 1′ collection period following stimulation of the splenic nerves with 200 impulses at 10 Hz expressed as a percentage of the total ^{3}H overflowing in that period. The numbers in parentheses refer to the number of observations

	^{3}H metabolite pattern in each collection period			
	1st min	2nd min	3rd min	4th min
NA	83.8 ± 2.4 (9)	32.6 ± 5.6 (9)	7.0 ± 1.4 (9)	4.0 ± 0.8 (8)
DOPEG	2.6 ± 1.4 (9)	31.6 ± 4.7 (9)	41.3 ± 2.5 (9)	35.2 ± 2.4 (8)
DOMA	2.2 ± 0.1 (9)	3.1 ± 0.2 (9)	2.4 ± 0.4 (9)	2.2 ± 0.3 (8)
COMT metab	8.3 ± 1.5 (9)	27.8 ± 4.5 (9)	44.6 ± 1.8 (9)	53.4 ± 2.1 (8)
1st wash	0.1 ± 0.1 (8)	0.3 ± 0.1 (8)	0.5 ± 0.2 (8)	0 (7)
2nd wash	3.0 ± 0.2 (8)	4.6 ± 0.6 (8)	4.1 ± 0.8 (8)	5.2 ± 1.0 (7)

Effect of labetalol on the metabolite pattern of ^{3}H-noradrenaline and metabolites released by nerve stimulation

(a) *The effect of labetalol on ^{3}H(−)-noradrenaline found in each collection period.* Labetalol had no significant effect on percentage of [^{3}H]NA present in the first min ($r = 0.23$; $df = 7$). as shown in Fig. 6. However the addition of labetalol to the blood perfusing the spleen produced a concentration dependent increase in the percentage of [^{3}H]NA overflowing in the 2nd, 3rd and 4th min of collection. There was a significant correlation ($r > 0.83$; $df > 4$) between the elevation of [^{3}H]NA in each min and the concentration of labetalol. This would indicate that although labetalol has no significant effect on the pattern of overflow of label, the drug does increase the proportion of label which overflows as [^{3}H]NA.

(b) *The effect of labetalol on [^{3}H]DOPEG formation.* The percentage of [^{3}H]DOPEG found in the first min after labetalol did not significantly differ from that found in the controls (2.6 ± 1.4, $n = 9$) However in the following three collection periods, labetalol produced a significant ($r > 0.83$; $df > 4$) dose dependent decrease in the percentage of ^{3}H found as [^{3}H]DOPEG (Fig. 7). Inhibitors of neuronal uptake such as cocaine are known to decrease DOPEG formation following splenic nerve stimulation [11, 12]. Since labetalol produces a similar effect these results provide more evidence that labetalol inhibits neuronal uptake.

(c) *The effect of labetalol on the percentage of [^{3}H]COMT metabolites found in each collection period.* Labetalol had no significant effect on the production of *O*-methylated metabolites in any of the collection periods, as shown in Fig. 8. Separation of OMDA into VMA, MOPEG and NMN showed that labetalol had no significant effect on the formation of either the [^{3}H]*O*-methylated deaminated metabolites (VMA and MOPEG) or on [^{3}H]NMN.

Since these metabolites have been shown to be formed extraneuronally in the spleen [11] labetalol had no inhibitory effect on catechol-*O*-methyl transferase or on extraneuronal uptake.

(d) *The effect of labetalol on the percentage of [^{3}H]DOMA found in each collection period.* Labetalol

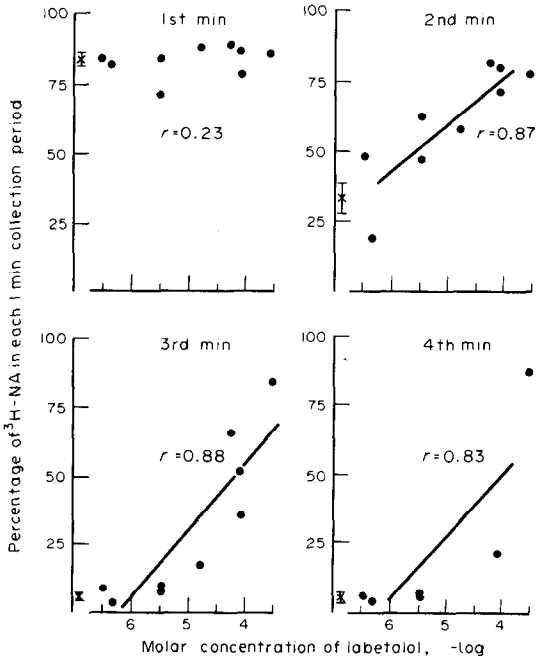

Fig. 6. The effect of labetalol on the percentage of [^{3}H]NA (expressed as a percentage of the total ^{3}H overflowing in each min) in the venous blood following splenic nerve stimulation with 200 impulses at 10 Hz. Points marked (×) [^{3}H]NA overflow in the absence of drugs ($n = 9$); (●) in the presence of labetalol.

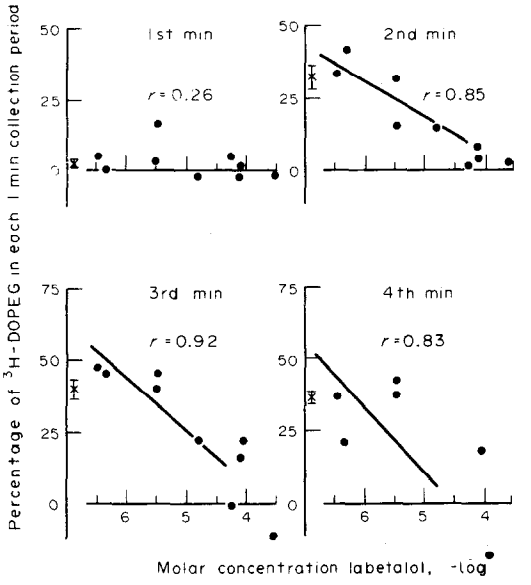

Fig. 7. The effect of labetalol on the percentage of [^{3}H]DOPEG (expressed as a percentage of the total ^{3}H overflowing in each min) in the venous blood following splenic nerve stimulation with 200 impulses at 10 Hz. Points marked (×), [^{3}H]DOPEG overflow in the absence of drug (n = 9); (●) in the presence of labetalol.

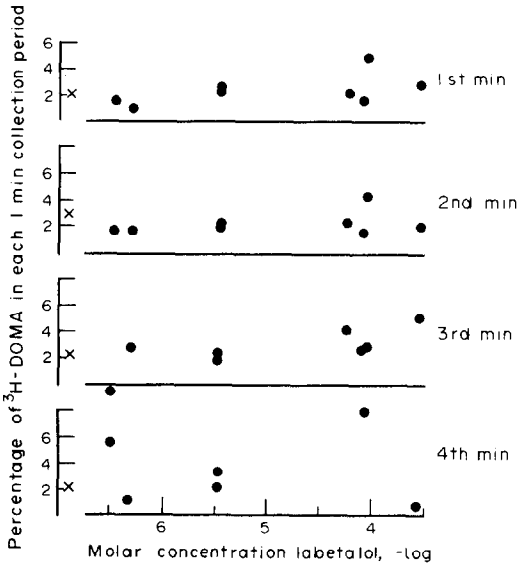

Fig. 9. The effect of labetalol on the percentage of [^{3}H]DOMA (expressed as a percentage of the total ^{3}H overflowing in each min) in the venous blood following splenic nerve stimulation with 200 impulses at 10 Hz. Points marked (×) [^{3}H]DOMA overflow in the absence of drug (n = 9); (●) in the presence of labetalol.

had no significant effect on the percentage of [^{3}H]DOMA (the extraneuronally formed deaminated metabolite) present in any of the four fractions as shown in Fig. 9, showing that the drug has no direct inhibitory effect on monoamine oxidase.

DISCUSSION

The actions of the α and β adrenoreceptor blocker labetalol on the release and metabolism of ^{3}H(−)-noradrenaline have been examined in the isolated blood perfused cat spleen.

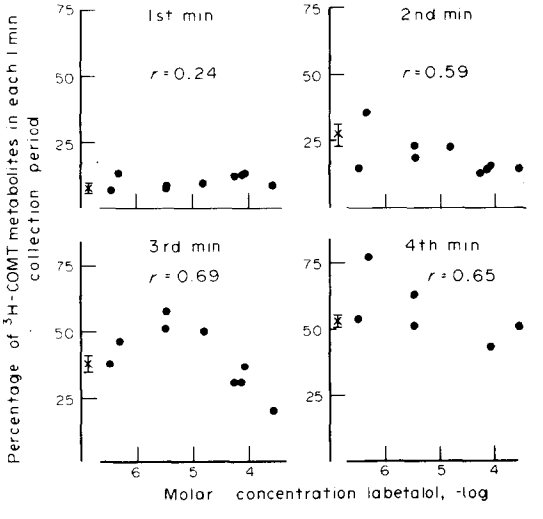

Fig. 8. The effect of labetalol on the percentage of [^{3}H]COMT metabolites (expressed as a percentage of the total ^{3}H overflowing in each min) in the venous blood following splenic nerve stimulation with 200 impulses at 10 Hz. Points marked (×) [^{3}H]COMT metabolite overflow in the absence of drug (n = 9) (●) in the presence of labetalol.

The endogenous stores of noradrenaline were labelled with ^{3}H(−)-noradrenaline and the loosely bound label in the smooth muscle and surrounding tissue was removed by washing with Krebs–Henseleit saline. Measurement of retention of labelled noradrenaline in the spleen by collecting the amount of label overflowing and also by counting the spleen homogenate at the end of the experiment indicated that about 55 per cent of the label infused was retained, largely as [^{3}H]NA. This labelled NA seems to be treated in the same way as endogenous NA, since the effects of labetalol on overflow of label were similar in characteristics to the effects on endogenous transmitter overflow [3, 4]. In order to make a fair comparison between the results of the present experiments and those in which the overflow of endogenous transmitter was measured, the ratio of the overflow of [^{3}H]NA in the first min in the presence of labetalol was compared to that in the absence of the drug. The overflow of label, of [^{3}H]NA, and of endogenous transmitter increased up to about 2-fold with concentrations of labetalol in the range 5×10^{-5}–10^{-4} M. In each case concentrations of labetalol above 10^{-4} M depressed transmitter overflow.

Examination of the pattern of overflow following nerve stimulation revealed that 64 per cent of the label comes over in the first min, and of this 80 per cent is found as [^{3}H]NA. The adoption of a 4 min collection period allows the collection of almost all the noradrenaline overflowing. Metabolites overflow much later than [^{3}H]noradrenaline but regression analysis of the amounts of metabolites in each fraction provided curves which suggest that collection over 4 min collects 79 per cent of the total [^{3}H]DOPEG and 77 per cent of the total [^{3}H]COMT meta-

bolites overflowing. As labetalol had no effect on the pattern of overflow it was not necessary to increase the collection period. In this respect labetalol differs from cocaine which causes a considerable prolongation of the overflow following nerve stimulation of endogenous noradrenaline [10, 11] and of [^{3}H]noradrenaline and ^{3}H metabolites from spleens labelled with [^{3}H]NA [11, 12]. This difference may be a flow effect related to the different actions of cocaine and labetalol on the vascular responses of the spleen. The potentiation and prolongation of the vascular response produced by cocaine is much greater than that produced by labetalol. The reason for this difference is probably attributable to the postsynaptic α blocking effect of labetalol which has been reported in the rat vas deferens, rat aortic strip, rabbit aortic strip [13] and in cat spleen strips [4]. The affinity of labetalol for α receptors in the rat and rabbit tissues is greater (pA_2 values with noradrenaline as agonist 7.42–7.45) than in the cat spleen (pA_2 using oxymetazoline as agonist 6.03—from [4]) and this may be part of the explanation for the potentiation of vascular responses seen at low dose levels in the spleen.

After labelling the spleen with [^{3}H]NA the amounts of [^{3}H]NA and [^{3}H]NA-metabolites formed following nerve stimulation can be used to investigate the activity of the degradative enzymes and uptake processes. The amount of [^{3}H]DOPEG formed can be taken as an indicator of the activity of neuronal uptake [11] since inhibition of neuronal uptake leads to a selective decrease in [^{3}H]DOPEG formation because access to the metabolising enzymes MAO and aldehyde reductase is blocked [14]. In addition any inhibitory effect of labetalol on COMT would result in a decrease in the formation of [^{3}H]NMN and [^{3}H]OMDA. Similarly if labetalol had an inhibitory action on the deaminating enzyme, MAO, a decrease in formation of [^{3}H]DOMA, [^{3}H]DOPEG and [^{3}H]OMDA would be expected. Also as most of the [^{3}H]DOMA, [^{3}H]NMN and [^{3}H]OMDA is formed extraneuronally, blockade of $Uptake_2$ would result in a decrease in the formation of these metabolites.

Labetalol produced a dose dependent increase in the percentage of [^{3}H]NA in each fraction and a corresponding decrease in the percentage formation of [^{3}H]DOPEG. This effect was not marked in the first min, probably due to the large amount of [^{3}H]NA and small amounts of metabolites which are found in this fraction. However, when the percent change in [^{3}H]NA from the control in the presence of labetalol is plotted against the per cent change in [^{3}H]-DOPEG, there is significant correlation between the change in NA and DOPEG in each fraction ($r > 0.77$; $df > 3$). This increase in [^{3}H]NA and decrease in [^{3}H]DOPEG is typical of drugs such as cocaine which inhibit neuronal uptake [11] and therefore it is likely that the mechanism whereby labetalol decreases [^{3}H]DOPEG formation is inhibition of $uptake_1$. Other supporting evidence for this conclusion includes the observations that labetalol blocks a cocaine sensitive inactivation process in the anaesthetised dog [1] and increases the recovery of ^{3}H in the venous blood following close arterial infusion of ^{3}H(−)-noradrenaline in the cat spleen [3, 4]. However, the potency of labetalol as an uptake inhibitor is considerably less than cocaine. The concentration of labetalol required to prevent [^{3}H]DOPEG formation following nerve stimulation in the cat spleen was approximately 0.1 mM, whereas a similar effect can be produced by 3 μM cocaine [12]. Labetalol is, therefore, about 30 times less potent than cocaine as an inhibitor of neuronal uptake.

Since labetalol had no effect on the formation of [^{3}H]DOMA or [^{3}H]NMN, this would indicate that the drug does not directly inhibit the degradative enzymes MAO or COMT.

The *O*-methylated deaminated metabolites (MOPEG and VMA) can be formed from [^{3}H]DOMA, [^{3}H]DOPEG or [^{3}H]NMN, so that a small decrease in the formation of [^{3}H]OMDA might be expected if [^{3}H]DOPEG production is inhibited. A small decrease in [^{3}H]OMDA was observed with higher concentrations of labetalol, similar to results reported with cocaine [11, 12]. Since the effect of labetalol on metabolism is largely confined to [^{3}H]DOPEG metabolism, it would indicate that the drug has no effect on extraneuronal uptake.

In conclusion, labetalol increased the overflow of [^{3}H]following nerve stimulation from spleens previously labelled with [^{3}H]NA. The mechanism involved inhibition of neuronal uptake since labetalol selectively decreased the formation of the intraneuronally formed deaminated metabolite, [^{3}H]DOPEG. The drug had no detectable effect on either the degradative enzymes, MAO and COMT or on extraneuronal uptake.

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