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Modulation of the concentration of noradrenaline at the neuro-effector junction in human saphenous vein

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1 We studied the importance of neuronal and extraneuronal uptake and of the pre-junctional α-adrenergic feed-back mechanism for the junctional noradrenaline concentration in the human saphenous vein. All major metabolites of the enzymatic breakdown of noradrenaline were detected in the overflow of superfused veins loaded with [^{3}H]-noradrenaline. The efflux of 3,4-dihydroxyphenylglycol (DOPEG) was drastically reduced in preparations labelled after neuronal uptake blockade indicating its neuronal origin; the other metabolites are formed extraneuronally since they behaved distinctly differently from DOPEG under several experimental conditions.

2 Extraneuronal uptake followed by enzymatic breakdown removes the same amount of noradrenaline from the biophase during nerve activity as that diffusing intact out of the tissue, whereas neuronal uptake appears only half as effective since the overflow of intact noradrenaline increases by only 48% in the presence of desmethylimipramine (DMI). However, in preparations mounted for isometric tension recording, neuronal uptake blockade potentiated contractions to α-adrenergic activation, emphasizing the functional importance of the neuronal disposition mechanism. By contrast, no evidence was found for a hydrocortisone-sensitive extraneuronal uptake compartment, suggesting that extraneuronal removal may have little, if any, functional importance.

3 During nerve stimulation, yohimbine increased the amount of labelled noradrenaline present in the superfusate, while exogenously added noradrenaline decreased it in the presence of cocaine. Thus, prejunctional α-adrenoceptors can modulate the junctional concentration of neurotransmitter in the human saphenous vein.

Introduction

The concentration of noradrenaline at the neuro-effector junction in adrenergically innervated tissues is determined by: (1) the amounts released from the adrenergic nerve endings, which can be modulated by a negative feed-back mechanism through prejunctional α-adrenoceptors and (2) by the activity of the disposition mechanisms for noradrenaline: neuronal and extraneuronal uptake followed by enzymatic degradation. The relative importance of these mechanisms varies from species to species (Vanhoutte, 1978; Bevan, Bevan & Duckles, 1980; De Mey & Vanhoutte, 1980; Vanhoutte, Verbeuren & Webb, 1981a). Few studies have been reported on adrenergic neuro-effector interaction in human isolated blood vessels (Stjarne & Brundin, 1975; 1977a,b; Rorie, Rusch, Shepherd, Vanhoutte & Tyce, 1981) and in particular little attention has been paid to the metabolism of noradrenaline. We therefore studied human isolated saphenous vein to determine the involvement of neuronal and extraneuronal uptake and of presynaptic α-adrenoceptors in the regulation of the junctional concentration of noradrenaline.

Methods

Human saphenous veins were obtained as leftovers from coronary bypass surgery performed predominantly on male patients (aged 35–69 yr). The preparations were allowed to equilibrate in Krebs-Ringer solution (composition, mM: NaCl 118.3, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25, CaEDTA 0.026 and glucose 11.1) for at least 2 h before the actual experiment. The solution was kept at 37°C and continuously aerated with 95% O_2 plus 5% CO_2.

Isometric tension recording

Rings of saphenous veins (approximately 5 mm long) were placed in an organ chamber (50 ml) and connected to an isometric force transducer (Statham UC2) for tension recording. Drugs were added in 0.1 ml aliquots of a stock solution made up with distilled water or ethanol; they were removed by overflowing the organ bath with excessive amounts of fresh Krebs-Ringer solution. Platinum electrodes, placed parallel to the ring were used for electric stimulation. Pulses (square waves, 2 ms, 9 V) were provided at different frequencies by means of a direct current supply triggered by a stimulator. One stimulation frequency lasted until isometric tension had stabilized and then the next higher frequency was applied. All preparations were placed at the optimal point of their length-tension curve using a standard electric impulse. Preparations which at this stage of the experiment did not contract to electric stimulation were discarded.

Measurement of tissue accumulation of noradrenaline

After equilibration in Krebs-Ringer solution, strips were incubated in 2 ml of a solution containing 10^{-7} M tritiated noradrenaline ([7,8 ^{3}H]-noradrenaline, specific activity 13.1 Ci/mmol; Amersham). After 30 min they were repeatedly rinsed in ice-cold Krebs-Ringer solution, blotted with filter paper and weighed. The radioactivity was extracted by placing each strip in 2.5 ml of an ice-cold 1 N acetic acid solution; after 30 min the preparations were transferred to another 2.5 ml of acetic acid. The two acetic acid solutions were pooled and stored for subsequent column chromatography (Verbeuren, Coen & Vanhoutte, 1977; Verbeuren, Janssens & Vanhoutte, 1978).

Measurement of the overflow of noradrenaline and metabolites

Helical strips were incubated for 2 h in Krebs-Ringer solution containing [7,8 ^{3}H]-noradrenaline (3×10^{-7} M). At the end of the incubation period, the preparations were rinsed in fresh Krebs-Ringer solution and mounted for superfusion as described previously (Vanhoutte, Lorenz & Tyce, 1973; Janssens, Verbeuren & Vanhoutte, 1981). Briefly, they were suspended in a tunnel-shaped organ chamber maintained at 37°C and were superfused with Krebs-Ringer solution at a flow rate of 3 ml/min. For electrical stimulation of the preparations, two platinum wires (0.5 mm in diameter) were placed parallel to the veins. After a 30 min equilibration period, the superfusate was collected at 2 min intervals for direct estimation of total radioactivity. Preparations in which the overflow of tritiated compounds during electrical stimulation did not increase by at least 50% above basal were discarded.

Column chromatography

To separate [^{3}H]-noradrenaline (NA) from its metabolites 3,4-dihydroxyphenylglycol (DOPEG), 3,4-dihydroxymandelic acid (DOMA), 3-methoxy-4-hydroxyphenylglycol (MOPEG), normetanephrine (NMN) and 3-methoxy-4-hydroxy-mandelic acid (VMA) a column chromatographic procedure was used (Verbeuren *et al.*, 1977).

Drugs

The following were used: acetylcholine chloride (Sigma); cocaine (Bios-Coutelier); desmethylimipramine (DMI, Ciba-Geigy); hydrocortisone (Sigma); (−)-noradrenaline-1-tartrate (Merck); phentolamine (Regitine, Ciba-Geigy); tetrodotoxin (Sigma); yohimbine (Sigma).

Analysis of data

Data are expressed as mean ± s.e.mean. Student's *t* test for paired observations was used to evaluate the data; the values were considered significantly different when $P < 0.05$. Only significant differences are mentioned in the results. The number of preparations in each series is also the number of patients. Data from superfusion experiments were analysed as described previously (Janssens *et al.*, 1981). In short, from unstimulated preparations samples for column chromatography were collected from the 12th until the 18th min. From the 20th min to the 40th min drugs were infused into the superfusate and samples for column chromatography were collected from the 32nd until the 38th min. The preparations were superfused again in control conditions from the 40th to the 60th min and samples were collected from the 52nd until the 58th min. When electrical stimulation was applied the period of stimulation lasted for 60 min and drugs were infused from the 20th until the 40th min of stimulation. Samples for column chromatography were taken from the 12th until the 18th, from the 32nd until the 38th and from the 52nd until the 58th min. The value during the infusion of the drug was compared to the average of the first and third collection period. When no drugs were added to the superfusate, values from the middle time period were not significantly different from the average of the first and third time period. Dose-ratios were calculated as the displacement of the cumulative dose-response curves at 50% of the maximal response.

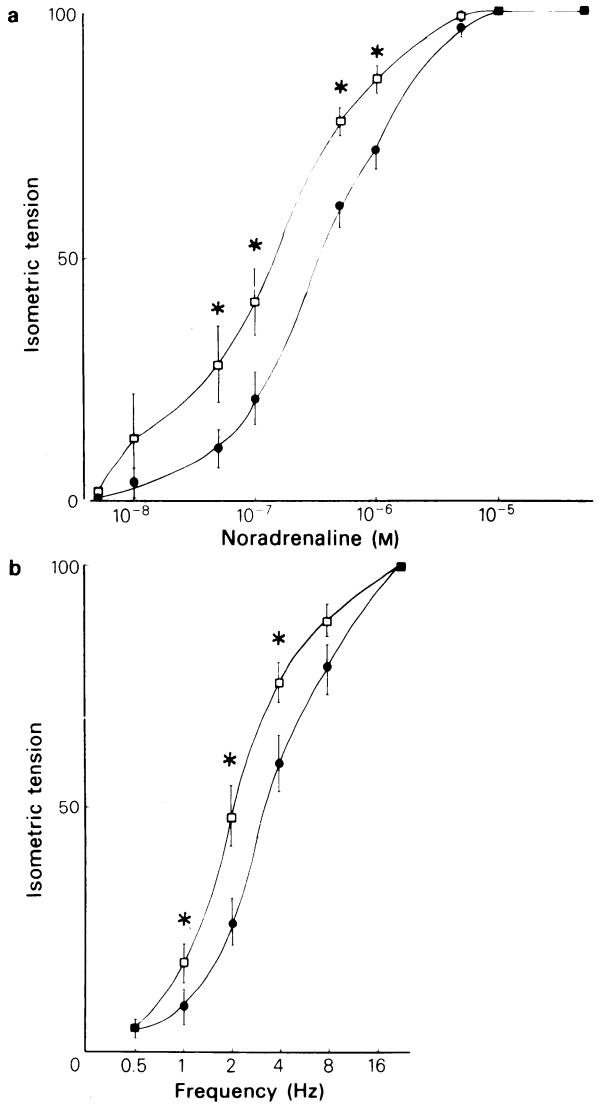

Figure 1 Effect of cocaine on dose-response curves for noradrenaline (a), and on frequency-response curves (b). Control (●); plus cocaine, 3×10^{-6} M (□). Responses are expressed as percentage of the maximal response in control conditions (8.43 ± 1.55 and 4.14 ± 0.89 g respectively). Cocaine caused a significant shift to the left of both curves ($n = 5$ in each series). *Significantly different from control ($P < 0.05$)

Results

Tension studies

In five saphenous veins, cocaine (3×10^{-7}, 3×10^{-6} and 3×10^{-5} M) caused a significant shift to the left of the cumulative dose-response curve to exogenous noradrenaline, the dose-ratio being 1.8, 2.3 (Figure 1) and 1.7, respectively. The same concentrations of cocaine were also tested on the response to electric stimulation in five other saphenous veins. Only with 3×10^{-6} M was the frequency-response curve significantly shifted to the left (Figure 1), the ratio of equieffective frequencies at 50% of the maximum being 1.7. With 3×10^{-5} M the response to low frequencies (1 and 2 Hz) of stimulation was still significantly augmented (from 6.9 ± 2.4 and 22.4 ± 4.2% of the maximal response in control solution to 10.8 ± 2.3 and 27.2 ± 3.6% respectively), but the maximum response was depressed (to 61.5 ± 5.1% of that in control conditions). On the other hand, hydrocortisone (3×10^{-7} to 3×10^{-5} M) had no effect on the dose-response relationship to noradrenaline and electrical stimulation in two series of five saphenous veins.

Accumulation of noradrenaline

Eight strips were cut from each of five veins for incubation in Krebs-Ringer solution containing [^{3}H]-noradrenaline. One strip served as a control, three strips were incubated in 3×10^{-7}, 3×10^{-6} or 3×10^{-5} M cocaine respectively, three other rings with 3×10^{-7} M, 3×10^{-6} M or 3×10^{-5} M hydrocortisone respectively, and the last one with 1.0% ethanol (corresponding to the highest concentration of the solvent reached with hydrocortisone). Column chromatographic analysis revealed that 78.9 ± 3.7% of the total tissue radioactivity in the control group was intact noradrenaline. Ethanol or hydrocortisone did not affect the total amounts of radioactivity accumulated in the tissue; cocaine caused a concentration-related decrease of tissue accumulation of tritiated compounds (Figure 2). This depression with the highest concentration of cocaine was due to a decrease in the noradrenaline fraction (from 6.6 ± 1.7 to $1.5 \pm 0.1 \times 10^3$ d min^{-1} mg^{-1} wet weight) and in the amounts of DOPEG (from 0.20 ± 0.04 to $0.12 \pm 0.01 \times 10^3$ d min^{-1} mg^{-1} wet weight).

Studies on the overflow of noradrenaline

(a) *Origin of evoked overflow of [^{3}H]-noradrenaline and metabolites* Paired rings of saphenous veins were incubated with [^{3}H]-noradrenaline in the absence or presence of cocaine (3×10^{-4} M) and subsequently mounted for superfusion. In the control strips electrical stimulation (2 Hz) caused an increase in tension (1.08 ± 0.66 g) and in the overflow of noradrenaline and the metabolite fractions (with the exception of normetanephrine). In the strips loaded with labelled noradrenaline in the presence of cocaine but superfused in the absence of the uptake

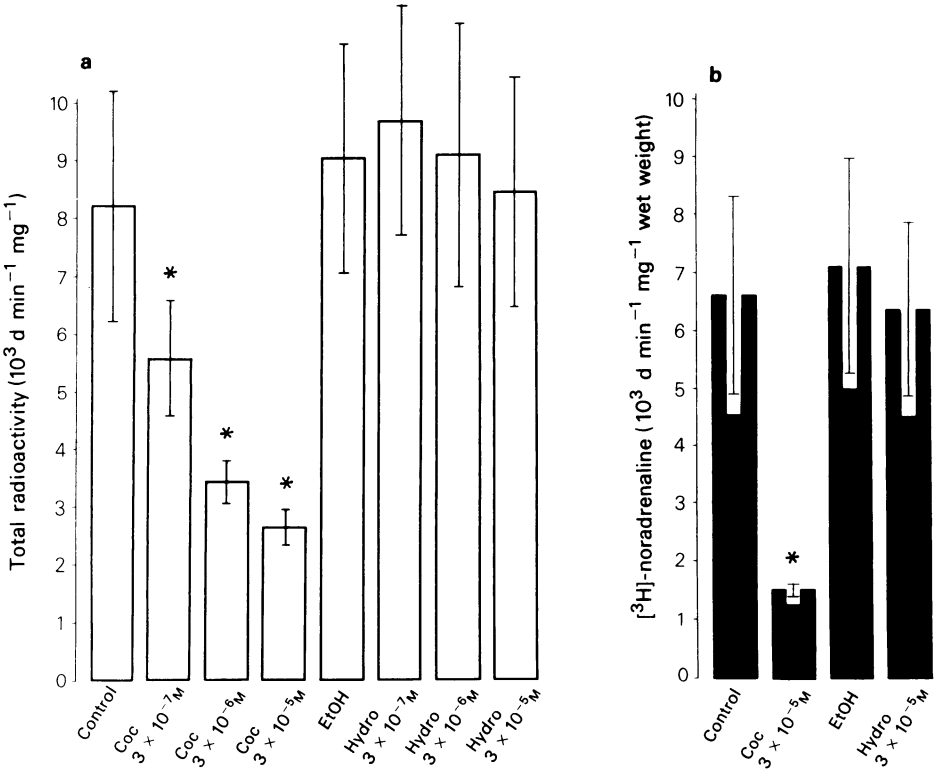

Figure 2 Total radioactivity (a) and amounts of intact noradrenaline (b) stored in human saphenous veins in control conditions, in presence of cocaine (Coc) or hydrocortisone (Hydro) or 1% ethanol (EtOH). Cocaine caused a significant inhibition of tissue uptake at all concentrations tested ($n = 5$ in each group).

blocker, the basal efflux contained almost no DOPEG. Electrical stimulation increased isometric tension in these strips as well (0.67 ± 0.38 g), but did not increase the overflow of triated noradrenaline and its metabolites. Some fractions progressively decreased as is normally observed for basal efflux (Figure 3). Acetylcholine (5×10^{-7} M) superfused during adrenergic nerve stimulation of five vein strips, caused a significant decrease in the evoked overflow of noradrenaline and its metabolites (Figure 4).

(b) *Neuronal and extra-neuronal uptake* (Table 1) Cocaine (3×10^{-5} M) did not change the efflux of [^{3}H]-noradrenaline and its metabolites in unstimulated preparations, but caused a significant increase (19% of the evoked overflow) of intact neurotransmitter when superfused during electrical stimulation (2 Hz). With DMI (5×10^{-6} M) the evoked overflow of tritiated noradrenaline increased by 48%. By contrast, hydrocortisone (3×10^{-5} M) had no effect on the overflow of tritiated compounds during electrical stimulation (2 Hz), whether in the absence or presence of cocaine.

(c) *Presynaptic α-adrenoceptors* (Figure 5). In control solution exogenous noradrenaline (5×10^{-7} M) did not alter the overflow of [^{3}H]-noradrenaline during electrical stimulation (2 Hz); however, it caused a significant increase in the overflow of [^{3}H]-DOPEG from 9.9 ± 2.9 to $14.8 \pm 3.7 \times 10^3$ d min^{-1} 6 min^{-1} of superfusion. In the presence of cocaine (3×10^{-5} M) exogenous noradrenaline (5×10^{-7} M) decreased the nerve stimulation induced overflow of tritiated neurotransmitter. Yohimbine (5×10^{-7} M) significantly increased the overflow of [^{3}H]-noradrenaline. During these experiments, no other changes in the metabolite fractions were observed.

Discussion

The aim of the present experiments was to determine the importance of neuronal and extraneuronal uptake of noradrenaline, of diffusion of the catecholamine from the junctional cleft and of the prejunctional α-adrenergic feed-back mechanism on the neurotransmitter concentration during adrener-

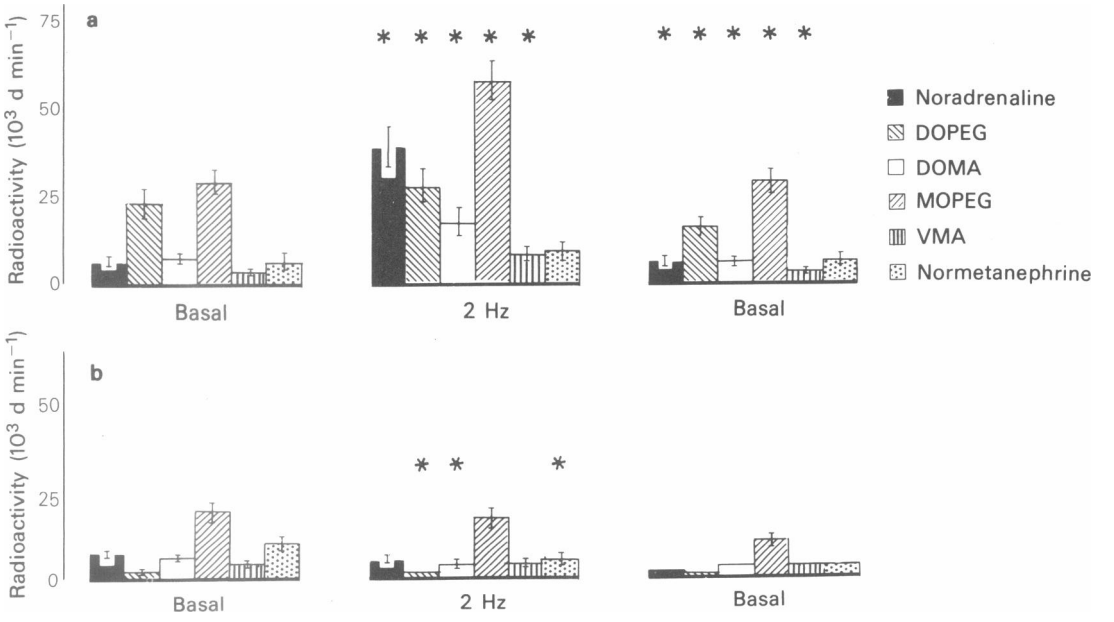

Figure 3 Efflux of tritiated noradrenaline and its metabolites from pairs of saphenous veins in basal conditions, during electrical stimulation (2 Hz) and again in unstimulated veins. One preparation of each pair was incubated with [^{3}H]-noradrenaline in control Krebs-Ringer solution (a) while the other preparation (b) was incubated in the presence of cocaine (3×10^{-4} M) to prevent neuronal uptake. Both groups were superfused with Krebs-Ringer solution without cocaine. *Value significantly different from value in the preceding time period ($P < 0.05$); DOPEG overflow was significantly lower in preparations incubated with cocaine than in the control preparations throughout the experiment ($n = 4$).

gic nerve stimulation in human saphenous veins. Furthermore the enzymatic degradation of the released noradrenaline was studied by determination of the metabolites formed.

In preparations incubated with [^{3}H]-noradrenaline about 80% of the radioactivity stored was intact neurotransmitter; the relatively small amounts of metabolites indicate that they readily diffuse out of the tissue, once formed. The uptake process preceding the storage of neurotransmitter must be mainly neuronal since it was largely inhibited by cocaine. However, a smaller amount of noradrenaline was still stored in the tissue in the presence of a concentration of the neuronal uptake inhibitor which appeared to be maximally effective (Figure 3), which suggests that some extraneuronal storage may also occur. When saphenous veins, previously loaded with [^{3}H]-noradrenaline were superfused, all major metabolites formed by catechol-*O*-methyltransferase (COMT) and monoamine oxidase (MAO), as well as intact noradrenaline, were detected in the superfusate. Most probably these radioactive compounds represent diffusion from extraneuronal sites and leakage of [^{3}H]-noradrenaline from the neuronal storage vesicles. Upon electrical stimulation, the amounts of [^{3}H]-noradrenaline and its metabolites in the superfusion fluid increased. In the rabbit pulmonary artery, electrical stimulation causes release of labelled transmitter from extraneuronal sites (Schrold & Nedergaard 1977). However, this is very unlikely for human saphenous veins studied under the present conditions, since electrical stimulation did not cause an increase in any of the radioactive fractions from preparations loaded in the presence of cocaine. Thus, as in most other blood vessels, electrical stimulation corresponds to adrenergic nerve stimulation (Vanhoutte *et al.*, 1981a). The finding that acetylcholine depresses the evoked overflow of tritiated compounds upon electrical stimulation confirms that it is due to exocytosis (Vanhoutte *et al.*, 1973; 1981a,b).

As in other adrenergically innervated tissues (Langer, 1974; Vanhoutte *et al.*, 1981a) DOPEG is formed mainly intraneuronally since veins incubated with tritiated noradrenaline in the presence of cocaine contain less labelled DOPEG in the tissue and in the basal efflux. Moreover, when exogenous noradrenaline is infused into the superfusion fluid, the overflow of tritiated DOPEG increases, a finding which can be attributed to intraneuronal displace-

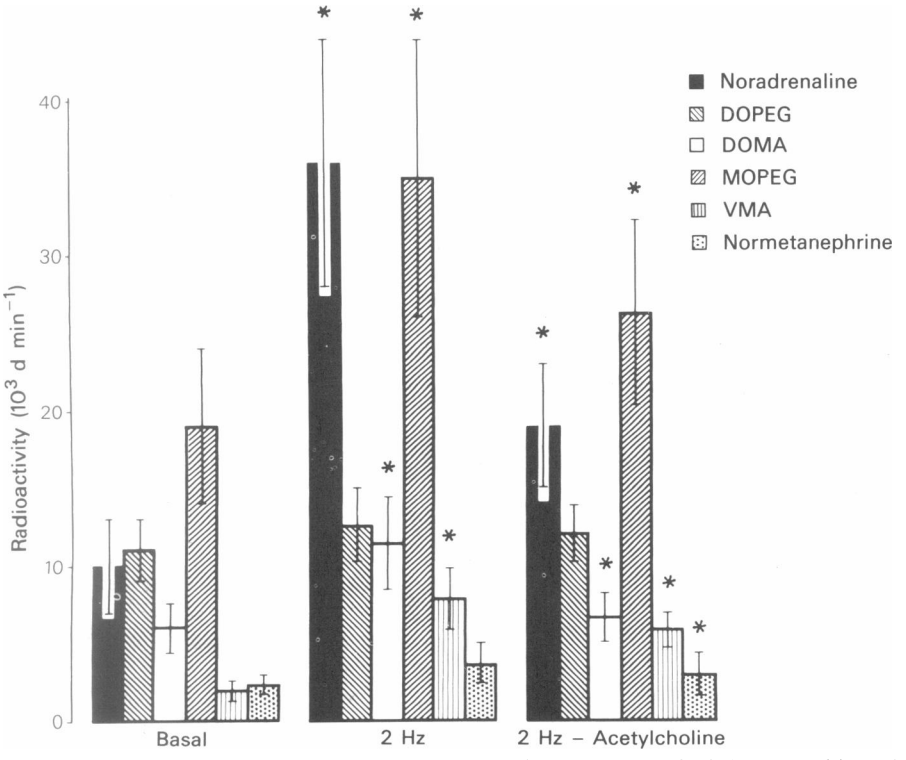

Figure 4 Efflux of tritiated noradrenaline and its metabolites from five saphenous veins in basal conditions, during electrical stimulation (2 Hz) and during electric stimulation in presence of acetylcholine (5×10^{-7} M). Acetylcholine depressed the overflow of all tritiated compounds except for DOPEG. *Significantly different from value in preceding time period ($P < 0.05$) ($n = 5$).

Table 1 Efflux of tritiated noradrenaline and metabolites from human saphenous veins*

	Radioactivity in superfusate (10^3 dmin^{-1} 6 min^{-1})					
	NA	*DOPEG*	*DOMA*	*MOPEG*	*VMA*	*NMN*
Unstimulated veins						
a control solution	13.0±3.7	11.2±2.6	5.0±1.2	15.6±1.2	1.9±0.3	3.1±0.4
cocaine 3×10^{-5} M	12.7±3.5	11.2±2.9	5.1±1.2	14.5±3.3	2.1±0.2	2.2±0.3
Electrical stimulation (2 Hz)						
b Basal	7.8±1.0	14.5±3.4	6.6±1.7	22.0±4.8	2.8±1.0	5.4±1.6
2 Hz	47.5±8.0**	19.7±4.8**	17.8±6.6**	48.4±13.3**	7.9±2.5**	6.5±1.8
2 Hz–cocaine (3×10^{-5} M)	55.1±9.2***	19.8±5.1	17.0±5.6	50.3±12.5	8.3±3.9	6.9±1.6
c Basal	3.9±0.7	5.6±2.4	4.7±2.1	6.1±1.3	1.2±0.7	1.6±0.3
2 Hz	14.2±3.9**	6.6±2.4**	7.2±2.0**	15.3±2.3**	3.8±1.3**	1.7±0.4
2 Hz – desmethylimipramine (5×10^{-6} M)	19.1±3.7***	7.6±1.9	6.7±2.2	14.4±2.6	3.2±1.2	2.3±0.6
d Basal	7.3±1.4	10.4±2.6	4.5±0.8	16.8±5.1	2.6±0.9	4.7±2.0
2 Hz	34.9±8.0**	13.5±3.9	8.3±1.2**	33.3±6.7**	5.8±1.2**	6.9±2.6**
2 Hz – hydrocortisone (3×10^{-5} M)	36.5±7.4	15.9±3.7	9.6±1.2	36.1±7.3	5.7±0.9	8.7±3.5
Electrical stimulation (2 Hz) in presence of cocaine (3×10^{-5} M)						
e Basal	9.2±0.6	8.1±0.4	4.3±0.5	10.0±0.9	0.9±0.1	1.3±0.1
2 Hz	40.6±7.9**	11.4±1.1**	12.6±0.9**	31.4±2.5**	6.4±1.0**	3.2±0.8**
2 Hz – hydrocortisone (3×10^{-5} M)	39.8±6.7	11.5±1.0	11.4±0.9	33.9±2.3	5.6±0.4	3.1±0.7

*Values expressed as 10^3 dmin^{-1} 6 min^{-1} of superfusion and given as mean ± s.e.mean.
**Value during electrical stimulation significantly different from basal efflux ($P < 0.05$).
***Value in presence of the drug significantly different ($P < 0.05$) from value in control conditions.
a, b, c, d, e: Denotes a group of 5 preparations.

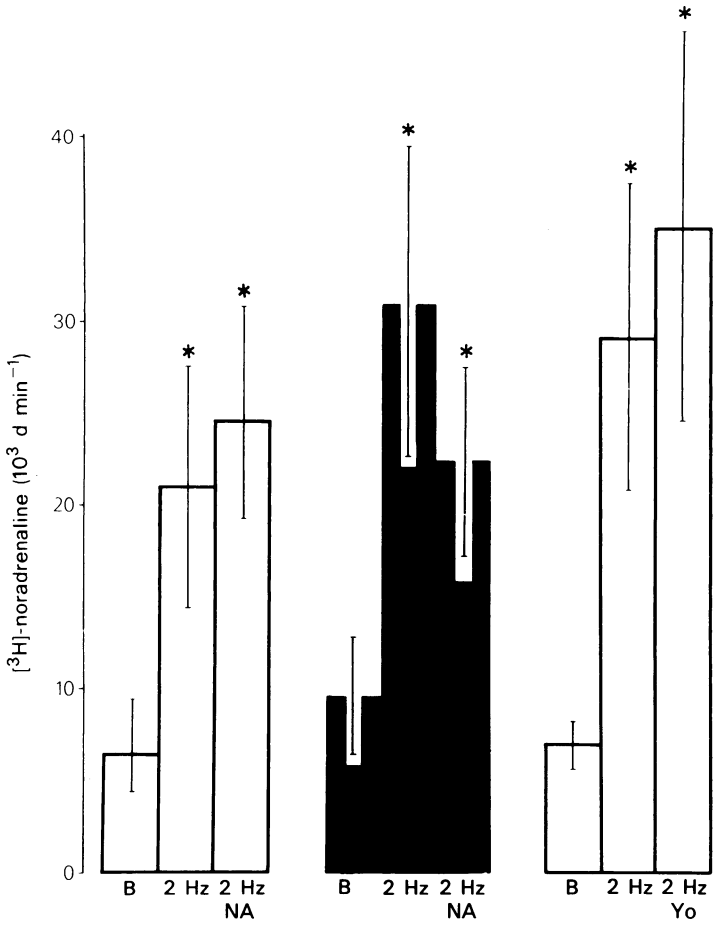

Figure 5 Efflux of tritiated noradrenaline (n = 5 in each group) from unstimulated saphenous veins (B), during electrical stimulation (2 Hz), and during electrical stimulation while superfusing 5×10^{-7} M noradrenaline (NA), or 5×10^{-7} M yohimbine (Yo). Superfusion without cocaine (open columns) and with cocaine (3×10^{-5} M) present throughout the experiment (solid columns). *Significantly different from value in preceding time period.

ment of noradrenaline followed by intraneuronal deamination (Lorenz, Vanhoutte & Shepherd, 1979; Dalemans, Janssens, Verbeuren & Vanhoutte, 1980). All other metabolites behave differently from DOPEG: (1) they are still present in the basal efflux from preparations loaded with tritiated noradrenaline in presence of cocaine; (2) the overflow of these metabolites (with the exception of NMN) markedly increases upon electrical stimulation; (3) when the release of tritiated noradrenaline is reduced with acetylcholine their appearance in the superfusate, unlike that of DOPEG, is significantly decreased and (4) their concentration in the superfusate is not increased upon infusion of exogenous noradrenaline. These observations suggest that NMN, VMA, MOPEG and DOMA, in contrast to DOPEG, are formed extraneuronally as has been demonstrated in a variety of other blood vessels (Vanhoutte *et al.*, 1981). In contrast to dog and rabbit saphenous veins (Verbeuren *et al.*, 1977; 1978; De Mey & Vanhoutte, 1980); there was no, or only a slight, increase on the NMN fraction (Table 1) in the human veins when electrical stimulation was applied. One possible explanation of this difference is that in the human vein, NMN is more easily further deaminated by MAO. The human vein compares well with the rabbit vein but differs from the dog vein in that VMA is present in small amounts in the basal efflux and increases with electrical stimulation (Verbeuren *et al.*, 1977; 1978; Muldoon, Vanhoutte & Tyce, 1978; De Mey & Vanhoutte, 1980; Janssens *et al.*, 1981; Verbeuren & Vanhoutte, 1982). Probably the human vein has a smaller capacity than the dog vein to store this metabolite (Verbeuren & Vanhoutte, 1982).

The increase in extraneuronal metabolites during

nerve stimulation, reflecting the extraneuronal uptake and metabolism of released neurotransmitter, was as great as that of intact neurotransmitter. The stimulation-evoked overflow of intact noradrenaline was increased by only 48% after blockade of neuronal uptake with DMI. This increase represents neurotransmitter that normally would have been taken up by the neuronal amine carrier. Cocaine caused a smaller increase in noradrenaline overflow, probably because of its local anaesthetic effect (Langer & Enero, 1974). Thus, neuronal reuptake normally removes only about half as much of the noradrenaline released during adrenergic nerve stimulation as is lost by diffusion from the junctional cleft and by extraneuronal uptake and metabolism.

The DOPEG fraction in the superfusate hardly increases upon nerve stimulation and does not decrease after blockade of the neuronal uptake in the human vein in contrast to the dog and rabbit vein (Verbeuren *et al.*,1978; De Mey & Vanhoutte, 1980; Rorie, Muldoon & Tyce, 1980). Rapid storage in the vesicles of recaptured noradrenaline where it is protected from deamination by MAO could explain both findings, although a compensatory increase of extraneuronally formed DOPEG (Paiva & Guimaraes, 1978) during infusion of DMI and cocaine cannot be excluded completely.

Extraneuronal uptake of noradrenaline has been divided into a hydrocortisone-sensitive and a hydrocortisone-resistant compartment, the latter being most likely devoid of an effect on the concentration of noradrenaline in the biophase (Graefe & Trendelenburg, 1974; Trendelenburg & Graefe, 1975). In the human saphenous vein the experiments with hydrocortisone (incubations, superfusion, organ bath) do not provide any evidence for a hydrocortisone-sensitive compartment, even after inhibition of neuronal reuptake. Thus, although a considerable fraction of neurotransmitter is metabolized extraneuronally, it could well be that this pathway does not influence the degree of contraction of the smooth muscle cells, both with exogenous noradrenaline or adrenergic nerve stimulation. The physiological role of this mechanism then remains to be established. Although the study of the functional importance of neuronal uptake is partly obscured by the local anaesthetic effects of cocaine (Langer & Enero 1974; Vanhoutte *et al.*, 1981a) the present results suggest that this mechanism may play some role in determining the degree of smooth muscle contraction. The relatively larger potentiation of contractile responses to exogenous noradrenaline when compared to electrical stimulation may be due to the greater sensitivity of the adrenergic nerves to the local anaesthetic effects of cocaine or more likely may indicate that the neuronal amine carrier is not operative during nerve impulses and thus that there is a temporal dissociation between release and recapture of noradrenaline (Verbeuren *et al.*, 1978; Vanhoutte *et al.*, 1981a).

In the present study, yohimbine, a prejunctional α-adrenoceptor antagonist, increases the overflow of labelled neurotransmitter during adrenergic nerve stimulation, while exogenously added noradrenaline decreases it if cocaine is present to prevent intraneuronal displacement of [^{3}H]-noradrenaline, thus indicating that a prejunctional α-adrenoceptor negative feed-back mechanism is operating to modulate noradrenaline release.

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