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THE INFLUENCE OF SYMPATHETIC INNERVATION ON VASCULAR SENSITIVITY TO NORADRENALINE

BY

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The central artery of the rabbit ear is much less sensitive to noradrenaline applied to the adventitia than to noradrenaline applied to the intima (de la Lande, Cannell & Waterson, 1966). Cocaine, by selectively enhancing sensitivity to extraluminal noradrenaline, reduces or abolishes the difference in sensitivity (de la Lande & Waterson, 1967). In common with arteries in brain, mesentery and muscle (Carlsson, Falck & Hillarp, 1962 ; Falck, 1962 ; Fuxe & Sedvall, 1965), noradrenergic structures in the ear artery are concentrated in the adventitia and there is no evidence of penetration of sympathetic nerve terminals into the media (Waterson & Smale, 1967). We have proposed therefore that the difference in sensitivity of the artery to intraluminal and extraluminal noradrenaline is related to uptake of noradrenaline into the adventitial noradrenaline stores (de la Lande & Waterson, 1967). Evidence of such a relation has been further examined in the present study by comparing the effects of denervation with those of cocaine. In addition observations have been made on the time course of action of cocaine, and of the effects of cocaine on the response of the artery to nerve stimulation, and to a non-adrenergic stimulant—namely, histamine.

METHODS

Perfusion

The method of perfusing the isolated central artery of the rabbit ear was that of de la Lande & Rand (1965). Small segments of the artery, taken from the base of the ear, were perfused at a constant rate with Krebs solution at 37° C ; to enable drugs to be applied to either the intima or the adventitia, the artery was double cannulated so that the intraluminal and extraluminal perfusion media did not mix. The absence of mixing between the two fluid compartments was tested both by the absence of volume changes in the extraluminal fluid, and by the perfusion of dyestuff at the conclusion of the experiment (de la Lande *et al.*, 1966).

The constrictor response to noradrenaline was measured by the maximum rise in perfusion pressure recorded during sustained contact of the drug and the artery for periods varying from 3 to 10 min according to the nature of the response. As indicated previously (de la Lande *et al.*, 1966) arteries differed in their responses, some displaying a sustained constriction at or near the maximum value while in others there was pronounced fade after the maximum was reached. In addition it was observed that fade was often extremely rapid and followed by a secondary constriction which was better sustained. This latter type of diphasic response was more pronounced with extraluminal than intraluminal noradrenaline. Concentration/response curves to noradrenaline were derived from responses recorded in duplicate or triplicate at two or three concentration levels. Changes in sensitivity to noradrenaline, produced by drugs, or relative sensitivity to intra- and extraluminal noradrenaline were measured in terms of concentrations producing equivalent maximum responses

(sensitivity ratio). Where the two curves being compared were similar in shape and slope, the mean distance apart of the curves was used to estimate the sensitivity ratio; otherwise minimum and maximum values were calculated and the ratio expressed as a range.

Arteries tended to gain in sensitivity during the course of perfusion. In the majority of arteries, the spontaneous increase in sensitivity was not sufficient to prevent quantitative assessment of cocaine's effect on noradrenaline sensitivity. The latter effect was measured in two ways—namely:

Method 1: the responses to intraluminal, and to extraluminal noradrenaline were compared before, and in the presence of cocaine applied to one or both surfaces of the artery.

Method 2: cocaine was applied to the artery by intraluminal perfusion or injection, or by adding to the extraluminal fluid, during the sustained phase of the constrictor response to noradrenaline; the further increase in the response was recorded.

Nerve stimulation

Sympathetic nerves in the artery wall were stimulated by means of field electrodes (the third method of de la Lande & Rand, 1965). In some preparations, the electrodes were placed only in the extraluminal bathing medium, and in others one electrode was placed in each of the intraluminal and extraluminal perfusion media.

Histology

Sections of the artery were examined for the presence of noradrenaline by the method of Falck (1962). The application of this method to the ear artery has been described in detail (Waterson & Smale, 1967).

Denervation

The artery in each of 6 rabbits was denervated by removing the homolateral superior cervical ganglion of the rabbit 14 to 24 days previously (de la Lande & Rand, 1965). Denervation was confirmed in each rabbit by the absence of noradrenergic structures in sections of the artery examined by the fluorescent histochemical method. The denervated artery and the artery from the opposite ear which had not been denervated (control artery) were prepared simultaneously under the same conditions; a typical comparison is shown in Fig. 1. The effectiveness of denervation was further demonstrated in 3 rabbits by the absence of a constrictor response of the perfused arteries to field stimulation applied under identical conditions to those which caused massive constriction of the corresponding control arteries. Three arteries were not tested by this procedure.

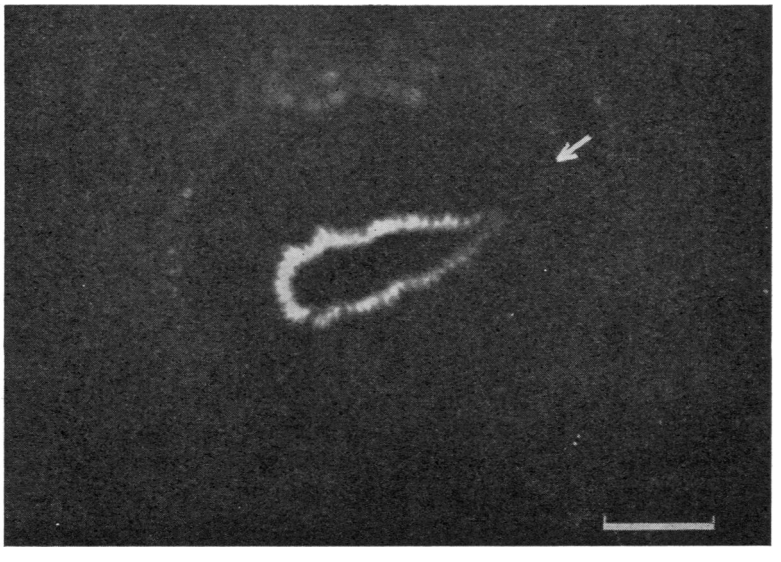

Fig. (1a)

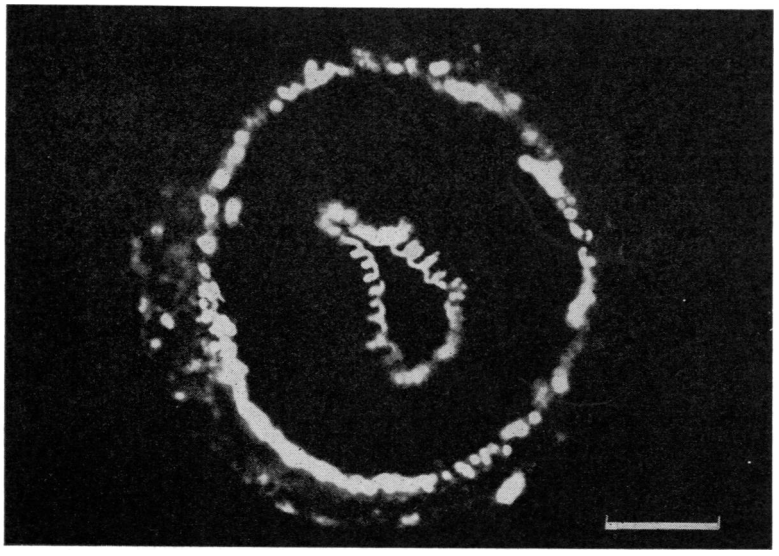

Fig. (1b)

Fig. 1. The appearance of a section of a denervated artery (1a), and of an innervated artery (1b) from the opposite ear of the same rabbit. Both were treated simultaneously by the fluorescence histochemical method. In the denervated artery specific fluorescence is not present in the medial-adventitial border, the position of which is shown at one point by a white arrow. Scale: 100 μ.

Doses of drugs

Dose response curves to noradrenaline were based on rises in perfusion pressure within the range of 10 and 200 mm Hg. In the majority of arteries, the required concentrations of intraluminal noradrenaline were in the range of 0.005 to 0.1 μg/ml., and those of extraluminal noradrenaline in the range 0.02 to 5 μg/ml. All concentrations of noradrenaline refer to the base; those of cocaine refer to the hydrochloride salt, and those of histamine to the acid phosphate salt.

RESULTS

Cocaine

The ability of cocaine to cause marked and selective enhancement of extraluminal noradrenaline is illustrated in Figs. 2 and 3, and by quantitative data on nine arteries summarized in Table 1. Figure 2 shows the effects of applying cocaine during the sustained constrictor response to noradrenaline (Methods) and Fig. 3 the effect of cocaine on the concentration/response curves to noradrenaline (Method 1). It will be observed that, in each of the experiments in Table 1, extraluminal cocaine caused only a small increase in sensitivity to intraluminal noradrenaline compared with that to extraluminal noradrenaline, and that intraluminal cocaine, although less active in one artery (number 8), exerted a qualitatively similar action to that of extraluminal cocaine. A tendency for cocaine to be less active by the intraluminal route was also observed in three of a further seven arteries in which the relative sensitizing potencies were estimated solely by the magnitudes of the increased response resulting from the application of cocaine during noradrenaline-induced sustained constriction (Method 2). This tendency was evident, not only by the greater response to extraluminal noradrenaline, but in two arteries by

TABLE 1

EFFECT OF COCAINE ON NORADRENALINE SENSITIVITY

Admin. of noradren.	Concn. of cocaine (μg/ml.)	Ratio of sensitivities: NA during cocaine / NA without cocaine — Experiment No. 1	2	3	4	5	6	7	8	9
Intra-luminal	1	1·1	1–1·5	1·2		1–3·2			1·8–2·2	1·3
	5	1·1		1·7		1–4·2 (1–1·2)	1·8 (2·2–2·5)	1·7–2·0 (1·4)		
	10				1·5–1·8				2·2–2·8 (1·5–1·6)	
Extra-luminal	1	4–9·8	4·8–5·3	6·6–13		3·6–5·1			9·2–9·4	7–10 (7–10)
	5	18				4·5–7·0 (7·8)	10 (10)	6·3–6·6 (8·4)		
	10		9·6–12·0	16·5	(4·7–5·2)				9–12·8 (3·8–4·2)	

	Ratio of sensitivities: NA extraluminal / NA intraluminal								
Cocaine absent	0·03	0·02	0·12	0·2	0·5	0·14	0·23	0·09	0·025
Cocaine present	0·4	0·3	1·2	0·6	1·0	0·8	1·2	1·4	0·15

1. The sensitivity ratios were determined by method (1) for experiments 1 to 8 and by method (2) for experiment 9. A ratio of, for example, 5·0 means that cocaine caused a fivefold increase in sensitivity to noradrenaline. The ratio is expressed as a range where the concentration response curves under comparison differed in shape.

2. Cocaine was applied to the same surface as the noradrenaline; the ratio obtained when cocaine was applied to the opposite surface as the noradrenaline is shown in brackets.

3. The lower table refers to the ratios of the sensitivity of extraluminal to intraluminal noradrenaline in the absence of cocaine (top row) and the maximum ratio observed in the presence of cocaine (bottom row), for each of the experiments 1 to 9. A ratio of less than one means that extraluminal noradrenaline was less active than intraluminal noradrenaline.

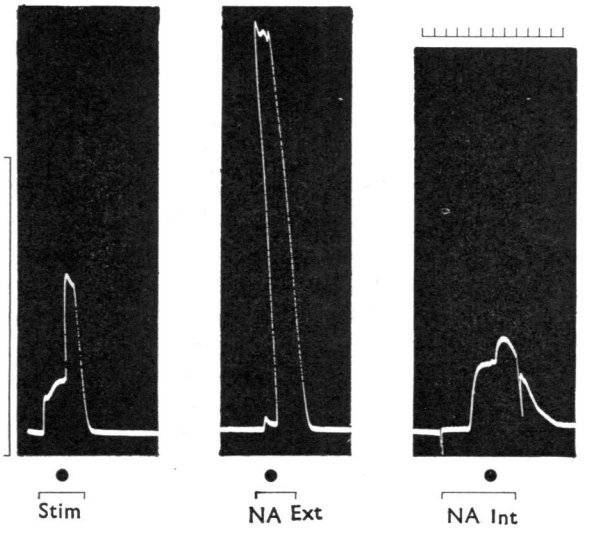

Fig. (2a)

Fig. (2b)

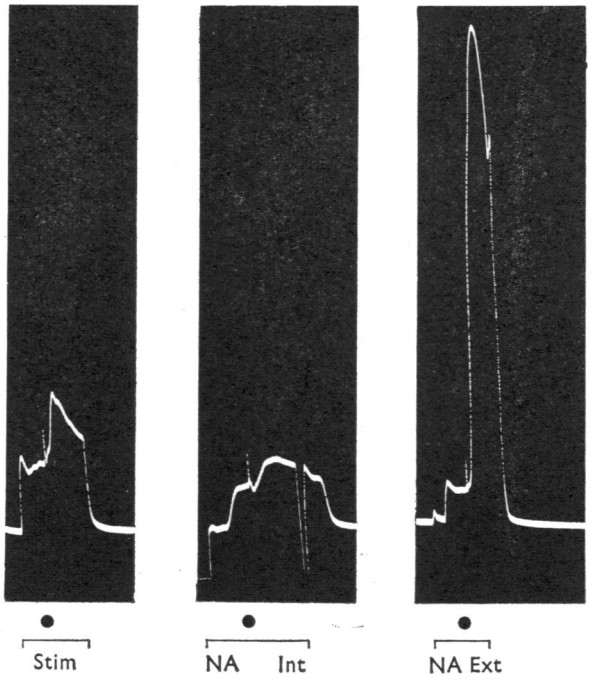

Fig. 2. Comparison of cocaine's action on the constrictor responses to field stimulation (Stim) 0.3 msec, 0.4 pulses/sec, extraluminal noradrenaline (NA Ext) and intraluminal noradrenaline (NA Int), applied for the periods shown by line. The lag between the onset of perfusion and response to intraluminal noradrenaline represents the time required for the noradrenaline to reach the artery from the perfusion reservoir (dead space). (2a). Cocaine 0.5 μg/ml. applied extraluminally at black dot and washed out simultaneously with the noradrenaline, or with termination of stimulation. Note that, although the response to extraluminal noradrenaline is considerably less than those to intraluminal noradrenaline and to stimulation, cocaine produces much greater augmentation of the extraluminal response. Stim 0.4/sec, 0.3 msec. NA Ext 200 ng/ml., NA Int 10 ng/ml. Time scale, minutes. Ordinate, perfusion pressure, 100 mm mercury. (2b). A second artery in which cocaine 5 μg injected intraluminally at black dots. Stim 0.8/sec, 1 msec. NA Ext 50 ng/ml., NA Int 5 ng/ml. Time scale and ordinate as for 3a.

depression of the response to intraluminal noradrenaline by intraluminal but not extraluminal cocaine. However, the striking and consistent feature of cocaine's action on all arteries was the selective enhancement of extraluminal noradrenaline. The net effect was that, regardless of its route of application, cocaine reduced or abolished the difference between the intra- and extraluminal sensitivities to noradrenaline. This effect of cocaine is evident also in the control arteries used in the studies on denervation (Table 3).

Applying the drug during sustained constriction to noradrenaline permitted analysis of cocaine's time course of action. The onset and attainment of maximum sensitization was always rapid (Fig. 2) being complete within 2 to 4 min in all arteries examined. The onset of action of cocaine was slower than that of noradrenaline but the lag was never

TABLE 2

TIME COURSES OF ACTION OF NORADRENALINE AND COCAINE

		Noradrenaline (NA)		Cocaine during intraluminal NA		Cocaine during extraluminal NA		Infused dye	
		Intra-luminal	Extra-luminal	Intra-luminal	Extra-luminal	Intra-luminal	Extra-luminal	Time to reach artery	Interval between appearance of dye and maximum concn.
Expt. 1	Onset of response	17, 17	3, 3	34	15	30 (20)	5		
Flow rate=8 ml./min	Onset to maximum	107 122	11* & 102 12* & 232	102	85	90	65	47	10–20
Expt. 2	Onset of response	21, 23	4, 6	78	37	42 (29)	20		
Flow rate=5·6 ml./min	Onset to maximum	204, 188	12* & 120 20* & 110	170	160	213	125	70	30

1. All numbers are times, in sec.

2. * onset of first peak of diphasic response.

3. In order to allow for perfusion of "dead space", the times of onsets of action of intraluminal noradrenaline and of intraluminal cocaine are calculated from the difference between the observed times of onset after commencing perfusion and the time required (shown) for Evan's blue dye 1% perfused by the same route to reach the artery. Nevertheless, the intraluminal times of onset are still probably overestimated, since perfused dye did not attain its peak sustained concentration in the intraluminal outflow for a further 10 to 20 sec, and 30 sec, for arteries 1 and 2 respectively. For this reason, the onset of action of intraluminally-injected cocaine was also estimated (shown in parentheses). The drug injection was made immediately proximal to the artery so that the artery was exposed to a maximum concentration of the drug within 2 sec.

Doses of drugs: Experiment 1: cocaine 1 μg/ml., by injection 2 μg; intraluminal noradrenaline 0·005 μg/ml., extraluminal noradrenaline 0·01·0·2 μg/ml. Experiment 2: cocaine 0·4 μg/ml., by injection 2 μg; intraluminal noradrenaline 0·05 μg/ml.; extraluminal noradrenaline 0·2–1·0 μg/ml.

greater than 60 sec, and was only of the order of 5 to 20 sec in the case of extraluminal applications. These features of cocaine's action are illustrated by data from two experiments shown in Table 2. Attention is drawn to the speed of onset of intraluminal-cocaine's sensitizing action on extraluminal noradrenaline, which in the two experiments was only 9 and 15 sec slower than that of extraluminal cocaine.

Denervation

Six arteries which had been denervated 14 to 24 days previously showed greatly enhanced sensitivity to extraluminal noradrenaline, but a smaller increase in sensitivity to intraluminal noradrenaline. Comparison was made in each case with the artery from the opposite ear which had not been denervated. An example of the concentration/response curves to noradrenaline in the two arteries is shown in Fig. 3. The results are summarized in Table 3, which also shows the effect of cocaine on each control and sympathectomized artery. It will be noted that the denervated artery closely resembles the cocaine-treated artery in its relative sensitivities to intra- and extraluminal noradrenaline—that is, denervation, like cocaine, tends to abolish the difference between these sensitivities. The effect of cocaine on the sensitivity of the denervated arteries to noradrenaline varied between relatively slight enhancement (three arteries) and depression (three arteries).

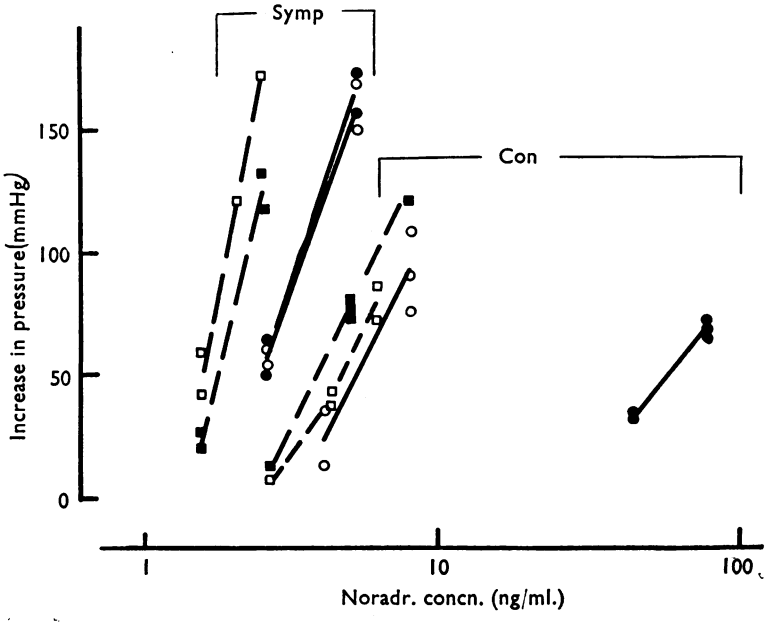

Fig. 3. Concentration-response curves to noradrenaline in a denervated artery (Symp), and a normal innervated artery (Con) from the opposite ear. Closed and open symbols refer to the response to extraluminal and intraluminal noradrenaline respectively. ——=cocaine absent. – – –=cocaine (10 μg/ml.) present.

TABLE 3
EFFECT OF DENERVATION ON SENSITIVITY TO NORADRENALINE

Artery	Route of application of noradrenaline	Experiment No. 1	2	3	4	5	6
Control	Intraluminal	1·0	1·0	1·0	1·0	1·0	1·0
	Extraluminal	·07	·09	·1	·18	·09	·16
Control + Cocaine	Intraluminal	1·3	2·0	1·8	3·1	4·4	2·3
	Extraluminal	1·4	0·9	1·3	1·8	1·9	0·9
Denervated	Intraluminal	1·9	2·4	9·5	1·3	1·0	5·2
	Extraluminal	2·0	1·5	6·0	0·3–1·0	0·2–0·5	3·8
Denervated + Cocaine	Intraluminal	3·7	—	—	1·8	—	13
	Extraluminal	3·0	—	—	0·8	—	7

Each figure is the ratio of extraluminal noradrenaline sensitivity to intraluminal noradrenaline sensitivity. In each control artery the latter sensitivity is arbitrarily assigned a value of one. Only the mean value of the ratio is shown, except in experiments 4 and 5 where the ratio is expressed as a range to include an increase in sensitivity to extraluminal noradrenaline which occurred spontaneously during perfusion.

Values for cocaine in the sympathectomized artery in experiments 2, 3 and 5 are not shown, since the arteries progressively decreased in sensitivity to intra- and extraluminal noradrenaline once perfusion with cocaine was commenced.

Concentration of cocaine, 10 μg/ml.

Nerve stimulation

In six arteries, responses of approximately equivalent magnitude were elicited by field stimulation, intraluminal noradrenaline and extraluminal noradrenaline. During the sustained phases of the responses, cocaine was applied extraluminally, or intraluminally by injection or perfusion, and its effect measured by the increase in constriction. The results of the six experiments are presented in Table 4. The main feature is that, in five of the arteries, potentiation of field stimulation is much less marked than that of extraluminal noradrenaline, and corresponds more closely to that of intraluminal noradrenaline. These actions of cocaine are also illustrated in Fig. 2.

Histamine

The effect of cocaine on histamine-induced constriction was examined in three arteries. The response to histamine, perfused intraluminally or applied extraluminally, resembled that to noradrenaline in that it was prompt in onset, reached a maximum within 1 to 4 min, and was well-sustained. The ratios of the sensitivity of the arteries to histamine, before, and in the presence of cocaine (1 μg/ml.) were derived from concentration/response curves and are shown in Table 5. The data points to a slight depressant action of cocaine on intraluminal histamine. However, the main feature is cocaine's lack of effect on extraluminal histamine, which contrasts with the marked potentiation of extraluminal noradrenaline. The latter was measured in each artery following the observations on histamine, by applying noradrenaline immediately before, and after, cocaine wash out.

Attention may be drawn also to the relatively small difference between the sensitivity to intra- and extraluminal histamine. The maximum difference was twofold, which may be compared with the five- to tenfold difference commonly observed with noradrenaline.

TABLE 4

EFFECT OF COCAINE ON NORADRENALINE AND ON FIELD STIMULATION

	Experiment No.→	1	2	3	4	5	6
Intraluminal noradrenaline	Int. cocaine	0/3, 0/34		8/12, 20/12	20/30	8/38	10/33
	Ext. cocaine	8/22, 7/13	30/85, 20/55, 8/26		36/45	10/45	
Stimulation	Int. cocaine	32/20		20/18 10/15	15/5 20*/25	105/25	30/30 30/28
	Ext. cocaine	35*/20, 25/5	14/10, 12*/21		59*/20, 40**/36	136*/17	
Extraluminal noradrenaline	Int. cocaine	41/9 115/12		128/12 150/10	170/20	180/10	48/40
	Ext. cocaine	66/4, 136/2	135/5		160/20	180/10	
Stimulation characteristics	Pulse duration (msec)/pulse frequency per sec	0·3/2 00·3/0·4*	1/3 1/8	1·0/0·8	0·3/5 *1/3 **1/1·5	1/1 *1/5	1/0·35

1. Each ratio is the $\frac{\text{Increase in response (in mm) elicited by cocaine.}}{\text{Response (in mm) prevailing immediately before adding cocaine.}}$

2. Stimulation characteristics shown at foot of table marked* refer to the response marked* in the same column.

3. Concentration of cocaine: Experiment 1: 0·5 μg/ml.; 2: 2 μg/ml.; 3: 5 μg injection; 4: 1 μg/ml. (extraluminal), 5 μg injection; 5: 0·5 μg/ml. extraluminal, 5 μg injection; 6: 5 μg injection.

TABLE 5

EFFECT OF COCAINE ON HISTAMINE

Route of application	Ratio of Sensitivities		
	Expt. 1	Expt. 2	Expt. 3
Intraluminal histamine	0·46–0·56	0·6–0·9	·66–1·2
Extraluminal histamine	1·25	·7–1·0	1–1·3
Extraluminal noradrenaline	10	6–10	20
Sensitivity ratio in the absence of cocaine; $\frac{\text{Extraluminal histamine}}{\text{Intraluminal histamine}}$	0·5	0·5	0·9–1·0

The ratios refer to the sensitivity to histamine, or noradrenaline (extraluminal only), in the presence of cocaine, compared with the sensitivity in the absence of cocaine. Concentrations: cocaine 1 μg/ml., histamine 0·1–1 μg/ml.

DISCUSSION

In other tissues, particularly heart and cat nictitating membrane, it has been demonstrated that cocaine prevents uptake of noradrenaline into the storage sites, and that chronic denervation achieves the same effect by causing the storage sites to deteriorate (Trendelenburg, 1963). Hence the ability of cocaine and of denervation to increase the sensitivity of the artery to extraluminal noradrenaline to a level approaching that to intraluminal noradrenaline indicates that the marked differences between these sensitivities in the normal untreated artery is probably related to uptake of noradrenaline into storage sites and not simply to diffusion barriers in the artery. The observations that the sensitizing action of serotonin (de la Lande *et al.*, 1966) and the constrictor potency of histamine (present study) are little affected by their routes of application to the artery are further evidence that diffusion barriers are unlikely to play a major role in the differences in sensitivity to intra- and extra-luminal noradrenaline.

We have shown elsewhere (de la Lande & Waterson, 1967 ; Waterson & Smale, 1967) that the noradrenaline storage sites in this artery are closely packed structures located in the adventitia immediately outside and completely surrounding the outer border of the smooth muscle layer. The position of the storage sites is consistent with the hypothesis advanced previously (de la Lande & Waterson, 1966, 1967) that noradrenaline applied to the adventitia undergoes considerable loss by uptake into the storage sites before it reaches the underlying smooth muscle, and that cocaine and denervation exert their dramatic effect on sensitivity to extraluminal noradrenaline by preventing this loss. The relatively slight potentiation of intraluminal noradrenaline may be explained in two ways, either low uptake or an inability of uptake to influence the concentration of noradrenaline in the smooth muscle. The second explanation is favoured by the speed with which intraluminal cocaine exerts its potentiating and inhibitory effects on extraluminal noradrenaline. The effect of cocaine commences within 15 to 30 sec of application to the perfusion medium, and is maximal or near maximal within a further 2 to 4 min. The time course implies rapid penetration of cocaine from the intima to the storage sites in the adventitia, and it is a reasonable assumption that intraluminal noradrenaline penetrates to the storage sites at a rate at least comparable with that of cocaine. The hypothesis is presented diagramatically in Fig. 4. The storage sites are assumed to represent major sites of loss for both intraluminal and extraluminal noradrenaline, so that loss of intraluminal noradrenaline occurs, but only after it has diffused through the smooth muscle—that is, after it has exerted its physiological action. Hence the extremely high sensitivity of the artery to intraluminal noradrenaline, which has provided the basis for its application to catecholamine bioassay (de la Lande & Harvey, 1965) is explained not by the absence of uptake, but by the inability of uptake to affect this sensitivity ; similarly the sensitivity is little affected when uptake is prevented or abolished by cocaine or denervation.

However, the first explanation—that is, low uptake—is not excluded by our data since, despite rapid penetration of intraluminally applied drugs to the adventitia, it is possible that their concentration is markedly reduced in the outer region of the artery wall by a diluting effect of the extraluminal bathing solution. If such an effect extends to the biophase of the adventitia storage sites, the concentration of noradrenaline in the biophase

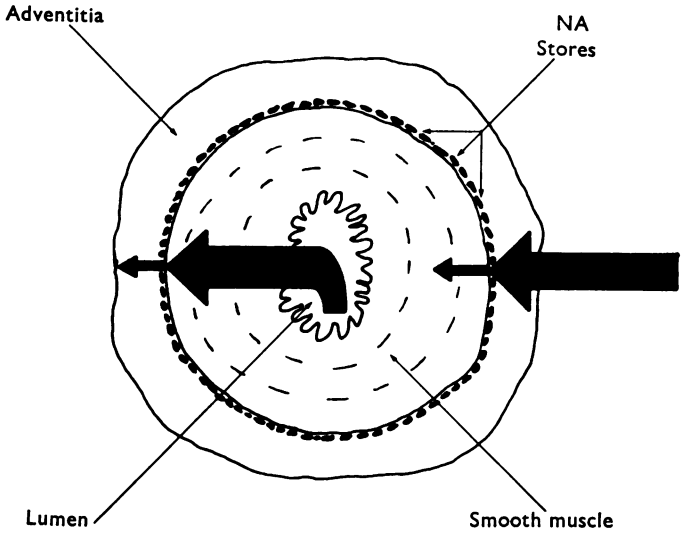

Fig. 4. Diagrammatic representation of the influence of the sites of uptake (shown as NA Stores) on the concentration of noradrenaline in the smooth muscle of the artery. The direction of the arrow indicates the direction of diffusion of noradrenaline, and its thickness the concentration of noradrenaline.

may be considerably less than in the lumen, and uptake of noradrenaline will be correspondingly reduced. Studies at present in progress on the rate of penetration of labelled noradrenaline across the artery wall may assist to distinguish between the two explanations.

An interesting implication of the hypothesis is that the application of noradrenaline into the extracellular space surrounding small arteries may conceivably be a better method of assessing the state of the noradrenaline storage system in vascular beds *in vivo* than the time-honoured procedure of introducing noradrenaline into the lumen by intravenous or intra-arterial application.

Although the potentiation by cocaine of the constrictor responses to stimulation tended to be greater than that to intraluminal noradrenaline, in accord with previous observations (de la Lande *et al.*, 1966), the magnitudes of the increases were much smaller than those to extraluminal noradrenaline. The results were unexpected, since it was anticipated that endogenously released noradrenaline would be exposed to considerable re-uptake in the storage sites before it diffused from the adventitia into the underlying smooth muscle—that is, its response to cocaine would resemble that of extraluminal rather than intraluminal noradrenaline. However, it has been noted that, in guinea-pig and rat vas deferens preparations, where noradrenergic storage sites are distributed throughout the smooth muscle layer, augmentation of nerve-induced responses is nevertheless slight compared with that of responses to exogenous noradrenaline (Bentley, 1966). Furthermore Trendelenburg (1966) has observed that supersensitivity to noradrenaline in the cat nictitating membrane during sustained nerve stimulation and we have observed a similar effect on the artery (de la Lande, unpublished). Hence the possibility emerges

that cocaine's ability to cause supersensitivity by inhibiting uptake of noradrenaline may be modified in some way by the state of supersensitivity prevailing during nerve stimulation. This possibility is being further examined.

SUMMARY

1. The sensitivity of the isolated rabbit ear artery to extraluminal noradrenaline, but not to intraluminal noradrenaline, is greatly enhanced by cocaine. The net effect is that the difference between the intraluminal and extraluminal sensitivity to noradrenaline is greatly reduced, or abolished.

2. The effects of denervation closely resemble those of cocaine.

3. Cocaine also enhances the constrictor response of the artery to field stimulation, but the effect is much less pronounced than that on extraluminal noradrenaline.

4. Cocaine has little effect on the constrictor response to histamine.

5. It is concluded that the position of the noradrenaline stores in the medial adventitial border of the artery determines the low sensitivity of the artery to extraluminal noradrenaline. Cocaine and denervation, by eliminating the effects of uptake, reduce the loss of noradrenaline by uptake into the storage sites as it diffuses from the adventitia to the media.

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