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THE INTERACTION OF SEROTONIN AND NORADRENALINE ON THE PERFUSED ARTERY

BY

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The action of serotonin in isolated perfused segments of the central artery of the rabbit ear comprises transient constriction and marked enhancement of the constrictor response to noradrenaline (de la Lande & Rand, 1965). Increased sensitivity to noradrenaline is maintained during infusions of serotonin (de la Lande & Harvey, 1965). In the present study, the mechanism and specificity of this interaction between serotonin and noradrenaline have been examined.

METHODS

Isolated central artery

Vascular changes in the isolated central artery were recorded by the method of de la Lande & Rand (1965), in which the artery was perfused with Krebs bicarbonate solution at 37° C by means of a roller pump delivering a constant rate of flow. Changes in vascular resistance were recorded as changes in perfusion pressure by means of a mercury manometer. Although the rate of perfusion of the artery is unaffected by the level of perfusion pressure, sudden changes in the pressure caused by constriction or dilatation of the artery cause a transient change in the perfusion rate due to the diversion of a portion of the pump outflow into the mercury manometer. Hence constant rate conditions are approached, but not fulfilled, by the method of perfusion. A modification of de la Lande & Rand's procedure was that short arterial segments approximately 1 to 2 cm long and cleaned of adhering tissue were used. The segment was taken from the proximal third of the ear and comprised the portion of the artery lying beneath the muscle layers and extending to the first major lateral branch of the ear vessels. Segments taken from this region did not possess lateral branches of macroscopic dimensions. Two methods of perfusion were used.

Method 1

The segment was cannulated separately at the distal and proximal ends so that there was no contact between the solutions bathing the intraluminal and extraluminal surfaces of the artery (Fig. 1). The absence of fluid paths or leaks was tested periodically by measuring the volume of the extraluminal fluid, and also by infusing dye into the perfusion stream at the end of an experiment and measuring the intraluminal and extraluminal concentrations of dye photometrically.

Method 2

The second method was that of de la Lande & Rand (1965) in which the distal end of the artery was not cannulated, so that the perfusion fluid after contact with the intraluminal surface bathed the extraluminal surface of the artery before being displaced by fresh perfusion fluid.

With both procedures perfusion was maintained at rates of 6 to 8 ml./min. Perfusion pressures were of the order of 10 to 20 mm Hg above the pressure prevailing in the absence of flow, and varied between 2 and 15 mm Hg when referred to the pressure prevailing with flow but without the artery inserted in the perfusion system.

Drugs were dissolved in saline and were injected into the perfusion stream immediately proximal to the cannula, or added directly to the perfusion reservoir, or added to the extraluminal fluid. In arteries perfused by method 1, drugs were washed out of the extraluminal compartment (volume 10 ml.) by successive flushing with 10-ml. lots of fresh perfusate. A similar procedure was used in arteries perfused by method 2 only when rapid wash out was required; otherwise drug wash out resulted from the displacement of the extraluminal fluid by the incoming intraluminal perfusate. Responses to sympathetic nerves were elicited by the methods described by de la Lande & Rand (1965). In this case their technique of employing long arterial segments which were not cleaned of adhering tissue was used.

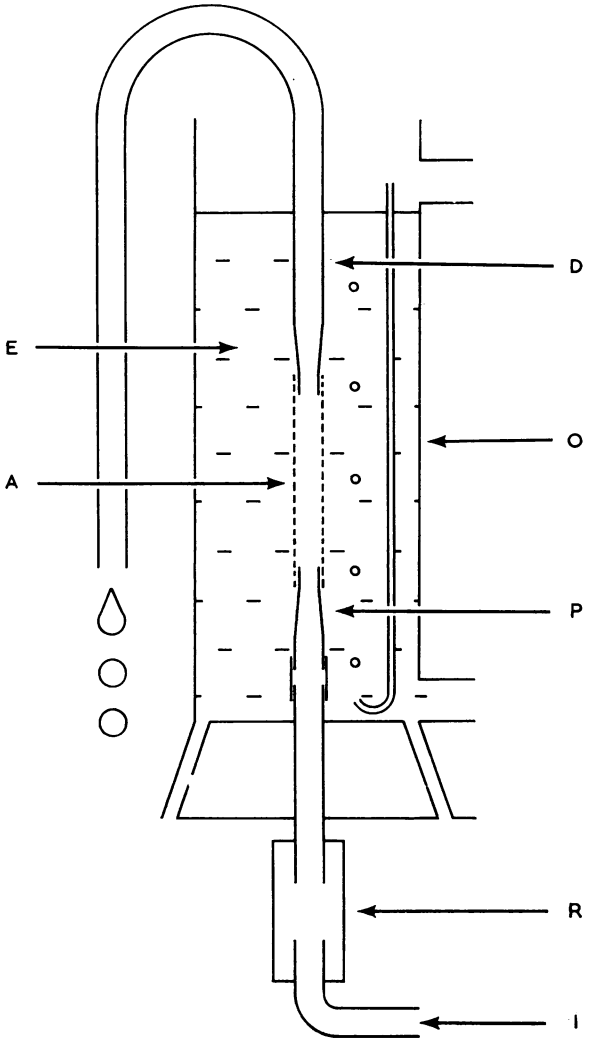

Fig. 1. Perfusion system, method 1. Symbols: A=artery (dotted line); D=distal cannula; E=extraluminal fluid; I=intraluminal fluid; the arrow indicating direction of flow; P=proximal cannula; R=rubber tubing through which drugs are injected into the intraluminal fluid. The extraluminal fluid was gassed with 95% O_2–5% CO_2 at 37° C; the constant temperature jacket of the organ bath (O) is not shown. The distal cannula was not present in arteries perfused by method 2.

Drugs

l-noradrenaline tartrate, l-adrenaline base, cocaine hydrochloride, histamine and phosphate, serotonin creatinine sulphate, bromlysergic acid diethylamide (BOL 148, Sandoz), methysergide (UML 491 bimaleate, Sandoz), angiotensin amide (Hypertensin-CIBA). Weights of drugs refer to the base.

RESULTS

Responses to noradrenaline and serotonin

The responses of the artery to noradrenaline applied intraluminally or extraluminally were similar. During the period of contact of drug and artery there was a rapid rise in perfusion pressure indicating a constrictor action of noradrenaline on the artery. In the majority of arteries the response was sustained at or near its maximum level; in others the response "faded" rapidly. This tended to occur after long periods of repeated applications of noradrenaline, and was observed in each of three arteries which were perfused following overnight storage at 4° C. Each type of response was reproducible and concentration-dependent. The characteristic response to an intraluminal injection of noradrenaline was a rapid transient rise in perfusion pressure which was highly reproducible and dose-dependent irrespective of the nature of the response to sustained contact of drug and artery. The responses to extraluminal additions and to intraluminal perfusions of noradrenaline were concentration-dependent. Comparison of the concentration-response curves of the drug applied intraluminally and extraluminally indicated that noradrenaline was twice to fifteen times more active by the intraluminal route (Table 1).

TABLE I

THE RELATIVE ACTIVITIES OF NORADRENALINE AND SEROTONIN ADMINISTERED BY THE INTRALUMINAL AND EXTRALUMINAL ROUTES

An activity greater than one means that the drug was more active when applied intraluminally. The activity is derived from concentration-response curves and is the ratio of the extraluminal to the intraluminal concentrations of the drug which produce the same response. The response to noradrenaline is vasoconstriction; the response to serotonin is sensitization, measured by the sensitivity factor. The fourth artery was perfused for three days with periods of overnight storage at 4° C.

Number of experiment	NA vasoconstriction Intraluminal / Extraluminal	5HT sensitization Intraluminal / Extraluminal
1	1·8–3·0	
2	9–10	0·5–0·67
3	20	9·5–15
4 1st day	6·4–8·2	1·2–1·6
2nd day	4·0	1·0–1·8
3rd day	1·9–4·0	1
5	1·8	1

The action of serotonin resembled that of noradrenaline in that intraluminal injections or perfusions caused transient or sustained rises in perfusion pressure. The secondary slower constrictor response to serotonin which was described earlier (de la Lande & Rand, 1965) was observed in the present series of experiments mainly in arteries which were perfused by method 2 and which had also been receiving frequent injections of noradrenaline.

The relative potencies of serotonin and noradrenaline as vasoconstrictors were not compared systematically. However, in each of four arteries the dose response curve

to injected serotonin was much less steep than to noradrenaline. In nine experiments in which injections of serotonin were occasionally interspersed between those of noradrenaline responses to 0.1 to 1 μg serotonin were matched by noradrenaline in one-fifth to one-hundredth of the corresponding dose of serotonin.

Examples of the above responses to noradrenaline and serotonin are included in Figs. 2, 5, 8, 9 and 11.

Effect of serotonin on the response to noradrenaline

Besides causing constriction, serotonin enhances the sensitivity of the artery to noradrenaline (de la Lande & Rand, 1965; de la Lande & Harvey, 1965). The following further characteristics of the interaction between the two drugs were demonstrated in seven arteries.

The constrictor responses to intraluminal injections and perfusions and to extraluminal additions of noradrenaline were increased regardless of whether serotonin was added to

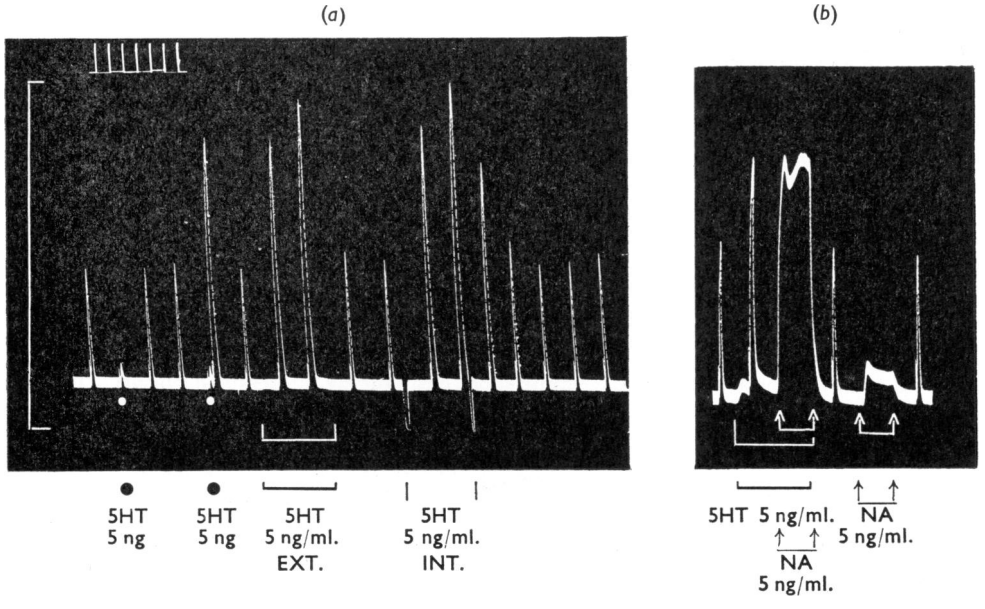

Fig. 2. The effect of serotonin on the arterial responses to noradrenaline (perfusion method 1).

Figure 2 (*a*) shows the responses of an artery during its second day of perfusion to injections of serotonin 5 ng (white dots), and to noradrenaline 10 ng (not marked). The response to noradrenaline injected within 30 sec of the second injection of serotonin is enhanced. The responses to noradrenaline are also enhanced when serotinin 5 ng/ml. is present in the extraluminal fluid for the period shown thus ⌊——⌋ ; and in the intraluminal fluid for the period shown by the interruptions to the perfusion. Note the subsequent transient depression of noradrenaline sensitivity.

Figure 2 (*b*) shows the responses of another artery to injections of noradrenaline 5 ng (not marked) and to noradrenaline 5 ng/ml. added to the extraluminal fluid (shown thus ↑——↑), in the presence and absence of serotonin 2.5 ng/ml. added to the extraluminal fluid. Time scale in minutes. Ordinate is 100 mM Hg.

the extraluminal or intraluminal perfusion media (Fig. 2) and regardless of whether serotonin itself caused constriction (Figs. 2 *a* and *b*).

The sensitizing action of serotonin began about 60 sec after the addition of serotonin and lasted about 120 sec after its removal. The onsets and offsets of action are illustrated by the transient phase of increased sensitivity to injection of noradrenaline following injections of serotonin (Fig. 2*a*) and by the speed with which sensitivity to injected noradrenaline changes, following addition and removal of serotonin from the extraluminal or intraluminal perfusing fluid (Fig. 2*b*). As is evident later in Fig. 9, the offset of serotonin's action was slower in arteries perfused by method 2, where the washout of drug from the extraluminal fluid was determined by the relatively slow rate (8 ml./min) of its displacement by intraluminal fluid.

Following withdrawal of serotonin and recovery of resting tone, there was a period of reduced sensitivity to noradrenaline which lasted for up to 15 min. The reduction in sensitivity was slight compared with the preceding enhancement of noradrenaline (Fig. 1).

Potency of serotonin

The degree of interaction was measured by comparing the dose response curve to noradrenaline (injected or infused intraluminally, or added extraluminally) in the presence and absence of serotonin. The curves were obtained from a minimum of two responses each to noradrenaline at two or more, usually three, dose levels, the doses being restricted to those causing increases in perfusion pressure of between 10 and 150 mm mercury. The means of responses were used to plot dose response curves, since there was normally very little variation in response to a particular dose of noradrenaline. The major effect of serotonin was to shift the curve to the left. There was either no change in the shape and slope of the curve, or a tendency towards an increase in the

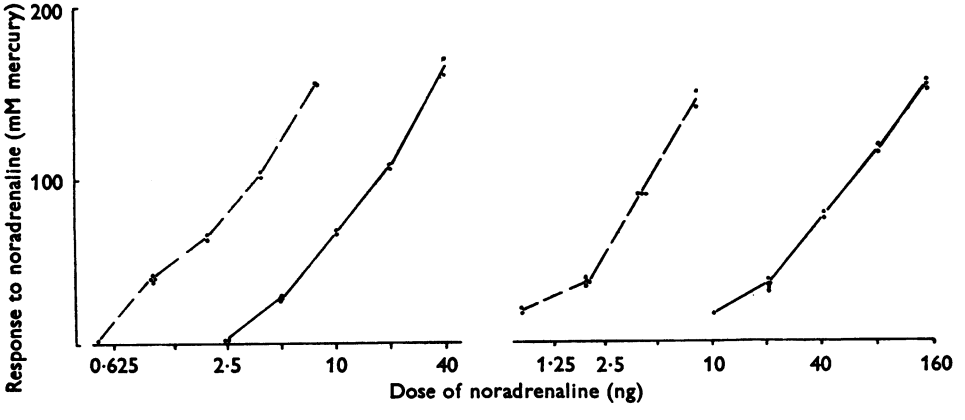

Fig. 3. The effect of serotonin on the dose response curves to noradrenaline. The arteries were perfused by method 2, with rapid wash out of drug from the extraluminal fluid. The curves were derived from the means of two or more responses. At each dose of noradrenaline the individual responses are shown to indicate their high degree of reproducibility. The broken lines are the dose response curves in the presence of serotonin, the concentrations being 5 and 10 ng/ml. in the left-hand and right-hand graphs respectively. The corresponding sensitivity factors expressed as ranges to include the minimum and maximum distance apart of the curves were 4–4.7 and 9.2–16.6.

slope which was minor in comparison with the shift in sensitivity. Curves from two arteries which are representative of those observed in more than 20 arteries are shown in Fig. 3. The degree of sensitization was measured by the shift of the dose response curve to the left and was expressed as the sensitivity factor.

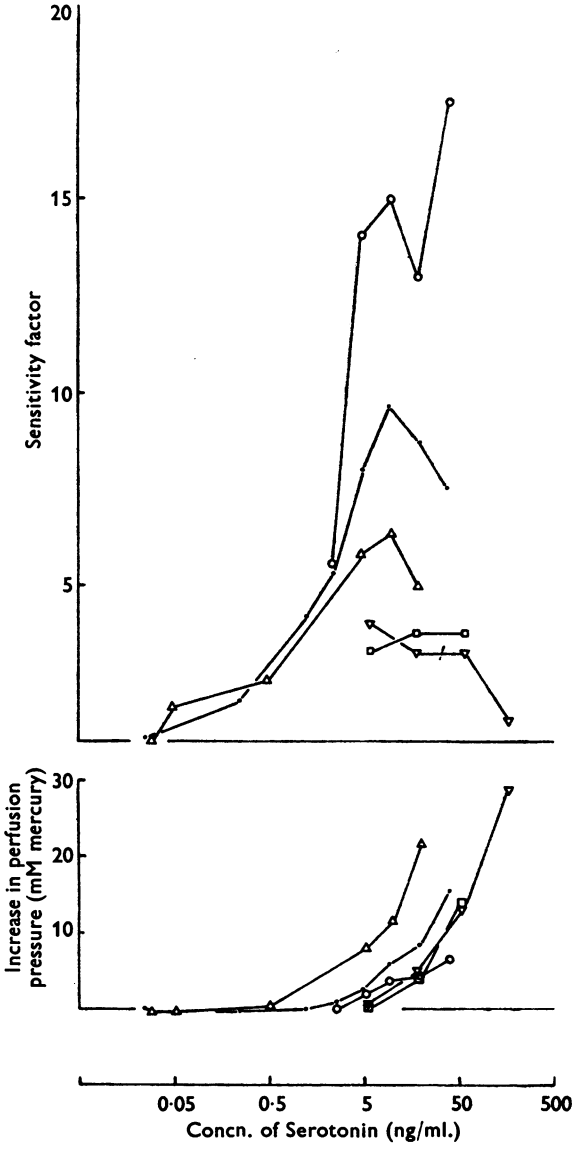

Fig. 4. Dose response curves to serotonin. The upper graph shows in five arteries the relation between the concentration of serotonin, and its ability to sensitize the artery to noradrenaline expressed as the sensitivity factor. The corresponding increases in perfusion pressure produced by serotonin are shown in the lower graph. The arteries symbolized by ● and ○ were perfused by method 2; those symbolized by △, □ and ▽ were perfused by method 1 so that serotonin was present in the intraluminal fluid only.

In two arteries in which the sensitivity factors were measured over a range of concentrations of serotonin the threshold for sensitization was less than 0.5 ng/ml., and in three arteries maximum sensitization was achieved in concentrations of the order of 10 ng/ml. The relationship is shown in Fig. 4, which also includes comparisons with serotonin's corresponding vasoconstrictor action. Fourfold sensitization to noradrenaline occurred in the absence of a vasoconstrictor action of serotonin. In four of five arteries tested, vasoconstriction increased with concentration above levels which were maximal with respect to sensitization. The sensitivity factor for serotonin (10 ng/ml.) in 11 arteries was 8.9 ± 0.87 (mean $\pm$S.E.) (Table 2).

TABLE 2

SOME SENSITIVITY FACTORS FOR SEROTONIN-INDUCED POTENTIATION OF VASOCONSTRICTOR DRUGS AND STIMULI

The figures refer to estimates of sensitivity factors on separate arteries. The figures in brackets refer to the number of arteries when more than three were used; the means and standard deviations are then shown. The effect on stimulation is shown thus, +, since the sensitivity factor is not applicable

Constrictor drug or stimulus	5HT concentration (10 ng/ml.)	5HT concentration (20 ng/ml.)
Noradrenaline	8·9±2·9 (11)	7·3±2·9 (6)
Adrenaline	7·6±2·8 (6)	9·0
Histamine	6·3, 15	9*, 14
Angiotensin	3·6, 100	4·5, 4·9, 13
Stimulation		
(1) Periarterial		+
(2) Nerve		+
(3) Field		+

* After dibenyline, 2 μg.

The relative sensitizing potencies of serotonin applied intraluminally and extraluminally on the action of noradrenaline injected intraluminally were also measured. In three of four arteries there was little difference between the efficacy of the drug applied by either route, and the results, summarized in Table 1, indicated that the sensitizing action of serotonin was less affected by the nature of the arterial surface in contact with the drug than was the case with the constrictor action of noradrenaline.

Effect of noradrenaline on responses to serotonin

The effect of perfusions of noradrenaline on the sensitivity of the artery to the vasoconstrictor response to serotonin was examined. Qualitative features of the interaction which were reproduced in each of four arteries were that the constrictor response of the artery to serotonin, which was injected or perfused intraluminally or added extraluminally, was dramatically enhanced when noradrenaline in concentrations of 1 to 10 ng/ml. was present in either the extraluminal or intraluminal fluid (Fig. 5). The degree of sensitization produced by noradrenaline in concentrations which raised the perfusion pressure by 5 to 15 mm Hg was measured in four arteries. The dose response curves to serotonin injected intraluminally in four arteries were extremely flat, and in one artery there was no response to doses ranging up to 1 μg. In each case the artery became highly sensitive to serotonin once perfusion with NA was started, and the shape of the response curve was altered to a far greater extent than was observed in the opposite type of

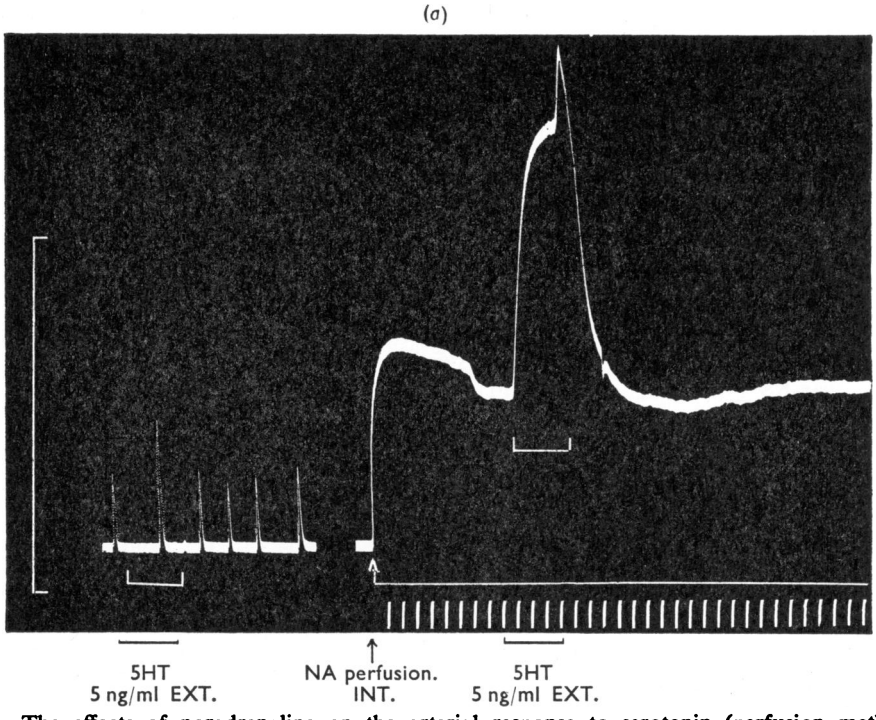

Fig. 5. The effects of noradrenaline on the arterial response to serotonin (perfusion method 1). Figure *5a* shows the responses of an artery to injections of noradrenaline 10 ng (not marked), and to prolonged intraluminal perfusion with noradrenaline 20 ng/ml., starting at the arrow. The massive response to extraluminal serotonin 5 ng/ml., shown ⌊——⌋, contrasts with the absence of a response before noradrenaline perfusion.

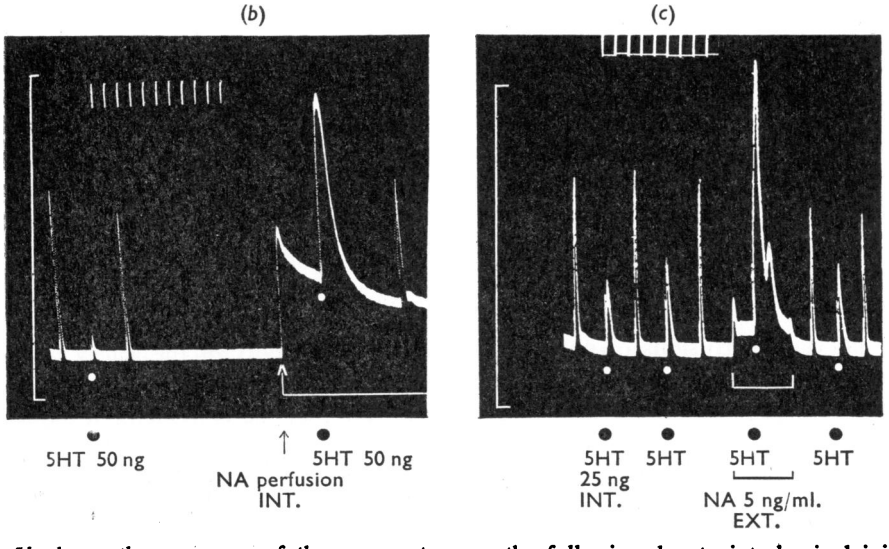

Figure *5b* shows the responses of the same artery on the following day to intraluminal injections of noradrenaline 10 ng (not marked), and serotonin 50 ng, shown by white dots, in the absence of and during prolonged perfusion with noradrenaline 10 ng/ml., which was started at the arrow. The response to serotonin is greatly augmented compared with that to noradrenaline.

Figure *5c* shows the effect of noradrenaline 5 ng/ml. added to the extraluminal fluid shown ⌊——⌋ on the responses of the artery to intraluminal injection of serotonin 25 ng (white dots). Unspecified responses are to noradrenaline 5 ng. Time scale in minutes; ordinate is 100 mM Hg.

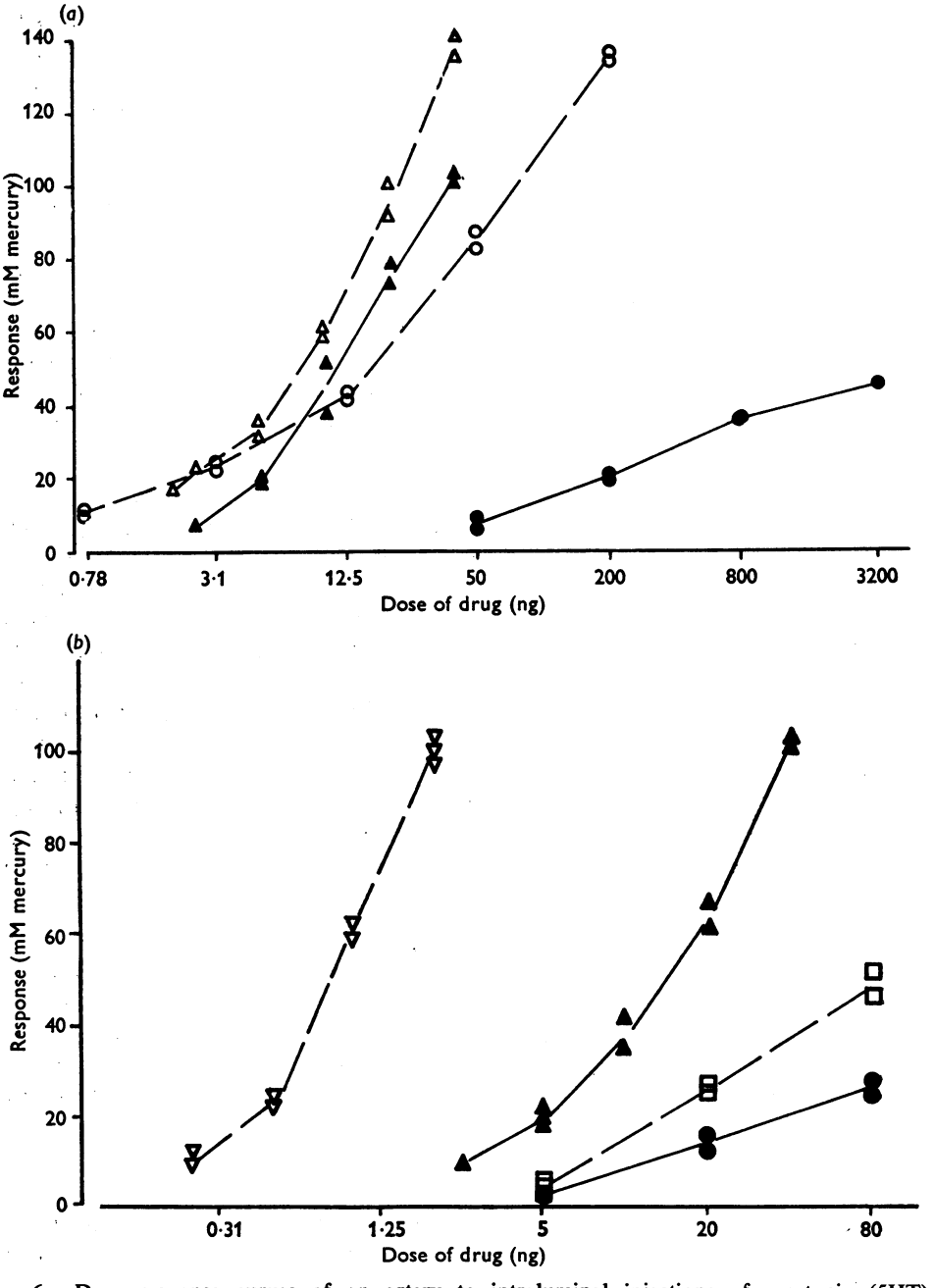

Fig. 6. Dose response curves of an artery to intraluminal injections of serotonin (5HT) and noradrenaline (NA) in the presence of intraluminal noradrenaline 5 ng/ml. (Fig. 6*a*), and, at a later stage, in the presence of intraluminal serotonin 10 g/ml. (Fig. 6*b*). The abscissa refers to the doses of noradrenaline, and serotonin creatinine sulphate. The responses in 6*b* were recorded several hours after those in 6*a*. Symbols: ● 5HT; ○ 5HT, NA present; ▲ NA; △ NA, NA present; □ 5HT, 5HT present; ▽ NA, 5HT present.

interaction—that is, enhancement of action by serotonin. The dose response curves from an experiment in which both types of interaction were compared on one artery are shown in Fig. 6. That the marked interaction was not simply a consequence of the small changes in perfusion pressure produced by the sensitizing drugs was shown by the relatively minor changes in sensitivity to their own constrictor actions produced by each of the drugs. The magnitude of the shifts in sensitivity produced by noradrenaline in the concentrations used (1 to 10 ng/ml.) corresponded to sensitivity factors of the order of 20, 100, 100 and 1,000 in each of the arteries. These values are high compared with the increase in sensitivity to noradrenaline's constrictor action produced by serotonin. Nevertheless, such values are consistent with the preceding evidence that the serotonin's sensitizing action is manifest in concentrations well below those causing constriction, and that the onset and offset of this action are rapid. This combination of properties would lead to an augmented response to serotonin as a result of the transient sensitization of the artery to the vasoconstrictor action of the noradrenaline with which it is being perfused.

Interaction between serotonin and other vasoconstrictors

Other substances or stimuli which were shown by de la Lande and Rand (1965), and de la Lande and Harvey (1965) to constrict the artery were (1) sympathetic nerve stimulation elicited *via* the central auricular nerve or intramural nerves (periarterial or field stimulation) and (2) adrenaline, histamine and angiotensin. As in the case of noradrenaline the vasoconstrictor effects of each of these procedures or drugs were markedly enhanced by serotonin. The results are summarized in Table 2. Only those interactions with angiotensin require comment, since the artery displayed considerable tachyphylaxis to the drug and in a total of nine arteries only four gave reproducible dose response curves before infusion with serotonin. In three arteries vasoconstrictor responses to angiotensin could not be obtained in doses of up to 5 μg, and in two arteries initial sensitivity to angiotensin was not reproducible subsequently. However, in all except one artery, which was at all times unresponsive, a constrictor response to angiotensin which was reproducible and dose-dependent and which reflected a major gain in sensitivity occurred promptly on serotonin infusion.

These findings indicated that enhancement of vasoconstrictor potency by serotonin was not restricted to noradrenaline but was relatively non-specific for vasoconstrictor substances. Lack of specificity for noradrenaline was shown also by demonstrating that sensitization still occurred in an artery which had been treated with dibenyline (2 μg) to reduce sensitivity to noradrenaline by a factor of more than 100. Histamine (0.1 to 2 μg) was employed as the vasoconstrictor substance, its action being unaffected by dibenyline.

Other preparations

The serotonin effect also occurred in the isolated rabbit ear perfused in an identical fashion to the artery, with the exception that the ear is mounted in a plastic warming chamber (de la Lande & Waterson, unpublished).

Two segments of isolated human digital artery, obtained 4–6 hr after death at necropsy and perfused in a similar fashion to the rabbit artery also displayed dramatic enhancement of the vasoconstrictor effect of noradrenaline either immediately following an injection or during an infusion of serotonin (Fig. 7).

(*a*)

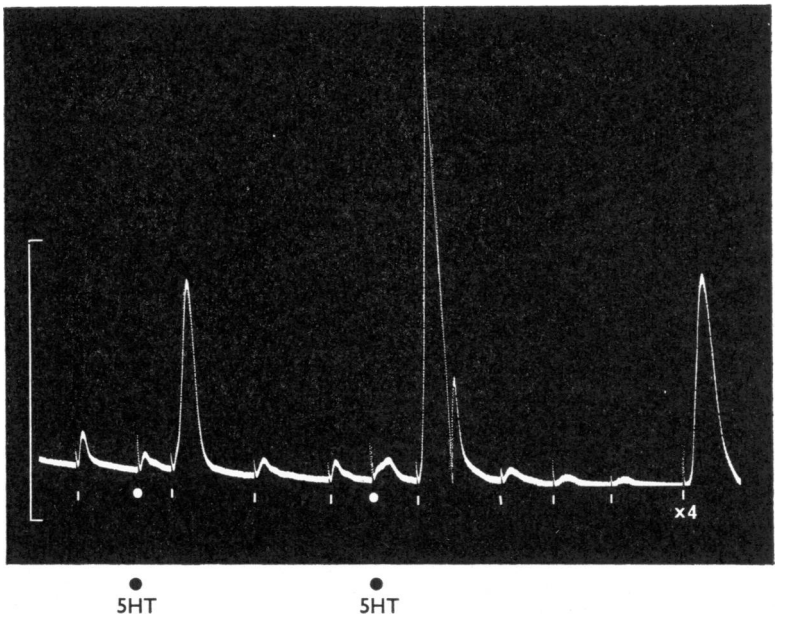

Fig. 7. The responses of human isolated digital artery to noradrenaline and serotonin.

Fig. 7*a*. The responses to intraluminal injection of noradrenaline 0.5 μg are indicated by white lines, and to serotonin 0.5 μg by white dots. Note the marked increase in the response to noradrenaline following an injection of serotonin and the subsequent depression of sensitivity; the latter is particularly marked following the second injection of serotonin. The final response is to 2 μg of noradrenaline.

(*b*)

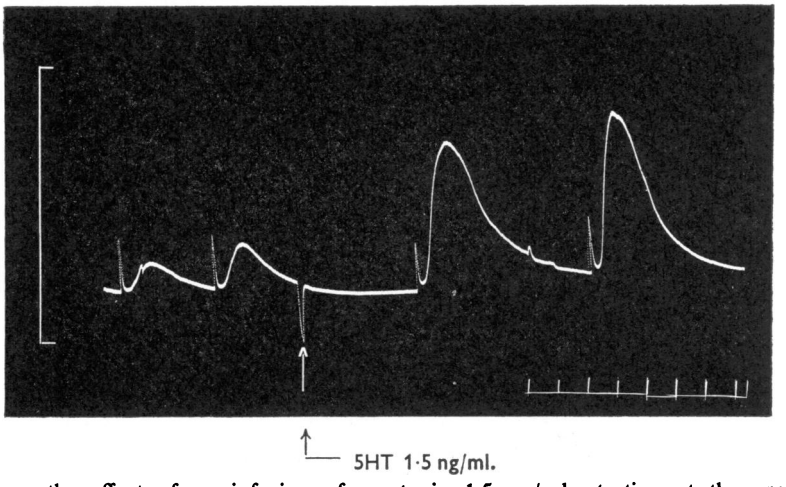

Fig. 7*b* shows the effect of an infusion of serotonin 1.5 ng/ml. starting at the arrow, on the responses to injections of noradrenaline 50 ng. The artery is now in its second day of perfusion, and has increased in sensitivity to noradrenaline. Time scale in minutes: ordinate, 100 mM of mercury.

These findings indicated that the serotonin effect was not specific for the isolated artery, nor for the rabbit.

Cocaine

Cocaine potentiates vasoconstriction produced by sympathetic nerve stimulation of the isolated artery (de la Lande & Rand, 1965). The effects of cocaine and serotonin were compared on arteries which were stimulated at 2-min intervals alternatively by noradrenaline and field stimulation (the third method of stimulation of sympathetic nerves described by the above authors). In each of three preparations, cocaine and serotonin behaved in opposite fashions in that the sensitizing action of cocaine was much more pronounced on the effects of field stimulation, whereas that of serotonin was more prominent on noradrenaline. A typical comparison is shown in Fig. 8.

Antagonists of serotonin

Methysergide and bromlysergic acid diethylamide (BOL), which antagonize serotonin on other smooth muscles, were examined for their ability to modify the sensitization phenomenon. In each of three experiments, methysergide, injected in doses between

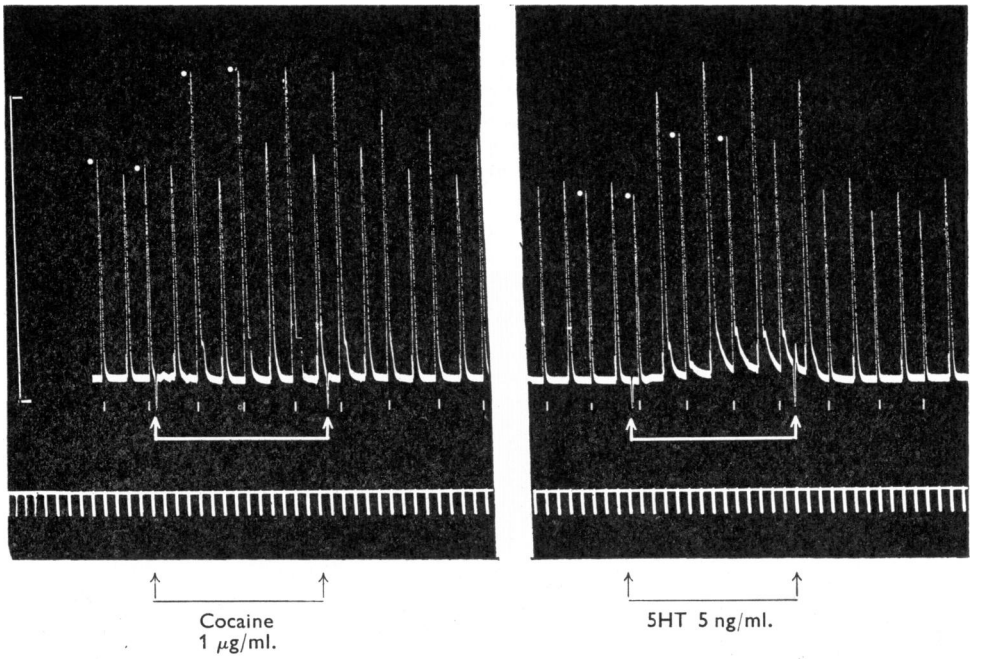

Fig. 8. Comparison of cocaine and serotonin. The effect of cocaine 1 μg/ml. (left-hand record) and serotonin 5 ng/ml. (right-hand record) on the responses of an artery to injections of noradrenaline 10 ng, and periarterial nerve stimulation (white lines) applied alternatively at intervals of two min. Pulses were 1 msec duration and 20/sec for 4 seconds at 30 V. The magnitude of the responses to the periarterial stimuli immediately preceding and following commencement of perfusion of the drugs are designated by white dots. Perfusion method 2 used. Time scale in minutes; ordinate, 100 mM mercury.

0.5 and 2 μg or infused in concentrations of between 10 and 100 ng/ml. caused sensitization to noradrenaline of a similar order to that obtained with serotonin. An example is shown in Fig. 9. Although it was observed that little further increase in sensitivity to noradrenaline occurred when serotonin was added to preparations already sensitized by methysergide, analysis of the interaction of the two drugs was not pursued in view of problems presented by their similar actions.

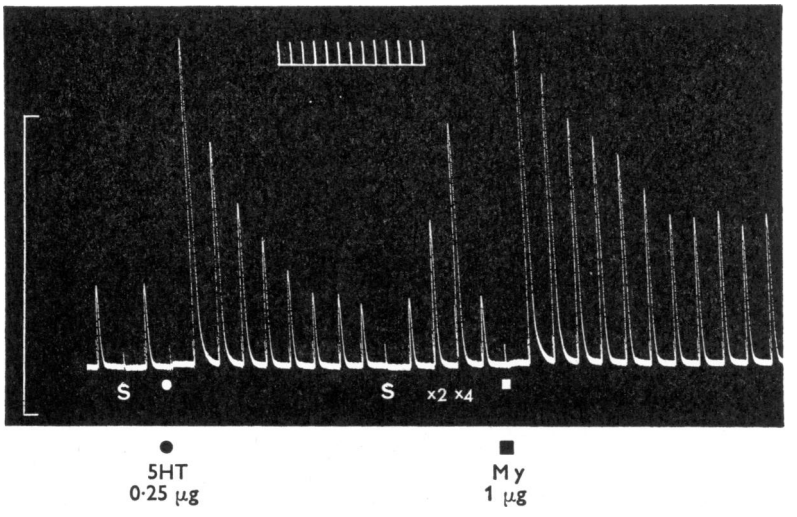

Fig. 9. Comparison of the effects of an injection of serotonin 0.25 μg (white dot) and methysergide 1 μg (white square) on arterial responses to noradrenaline 25 ng; S, injection of saline. Twofold and fourfold increases in the dose of noradrenaline are shown. Time scale in minutes; ordinate, 100 mM mercury.

Bromlysergic acid (BOL) in concentrations between 10 and 100 μg/l. enhanced noradrenaline sensitivity in four of six arteries, but depressed sensitivity in the remaining two. The results of four experiments in which interaction between serotonin and BOL was examined quantitatively are summarized diagramatically in Fig. 10, and show clearly that, irrespective of the change in sensitivity produced by BOL itself, the drug markedly reduced the degree of sensitization produced by serotonin.

Effect of potassium sulphate

In each of three arteries perfusion with Krebs solution modified by the replacement of the NaCl and $NaHCO_3$ with equi-osmotic amounts of K_2SO4 and $KHCO_3$ (subsequently referred to as K^+ Krebs) caused marked spasm of the artery. This was followed by a slow decline to a resting tone slightly above that prevailing in ordinary Krebs solution, at which stage the effects of vasoconstrictor drugs was examined. In two arteries the vasoconstrictor responses to noradrenaline were characterized by a slow onset and offset of action, and the dose response curves were less steep than those in

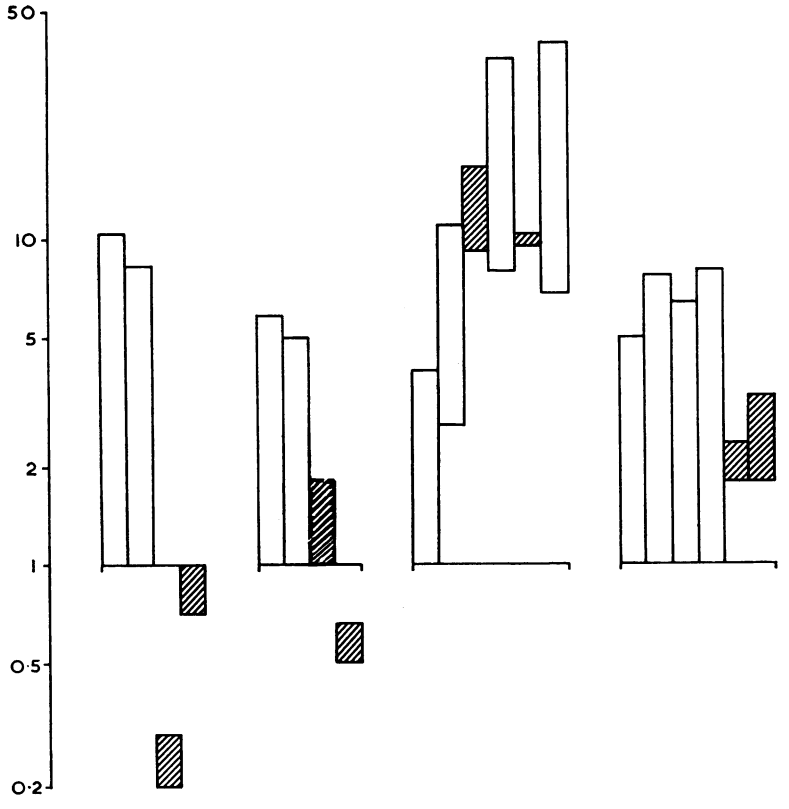

Fig. 10. The effect of bromlysergic acid diethylamide (BOL) on serotonin-noradrenaline interaction. The results of experiments with four arteries perfused by method 2, are shown from left to right; the height of each column represents the sensitivity factor for serotonin plotted on a logarithm scale, and open and shaded columns refer to the absence and presence of BOL. BOL itself altered sensitivity to noradrenaline and in the third artery there was a spontaneous increase in sensitivity to noradrenaline between the first and second applications of serotonin. The base of each column represents the prevailing level of sensitivity to noradrenaline at the time serotonin was added compared with that prevailing at the beginning of the experiment. The sensitivity factors in the first three arteries express the change in sensitivity 2 min after injections of 0.25 μg of serotonin, and, in the fourth artery, during alternative infusions of 1.25 and 5 ng/ml. of serotonin. Reading from left to right the concentrations of BOL were, for artery 1, 120 and 80 ng/ml.; for artery 2, 80 and 120 ng/ml.; for artery 3, 50 and 150 ng/ml.; and for artery 4, 80 and 80 ng/ml.

Krebs solution, while in the third artery the responses were identical in the two media. However, in each artery sensitization of the responses by serotonin no longer occurred; the sensitivity factors in the three arteries being 0.75–1.2, 0.75–1.2, and 1.1–1.2, compared with values of 5.6–6.2, 3.3–3.8 and 5–9 respectively which prevailed before perfusion with the high K^+ Krebs solution. In the third artery the sensitizing action of serotonin returned promptly when the high K^+ Krebs was replaced with Krebs solution. Fig. 11 shows the nature of the responses to noradrenaline and serotonin in high K^+ medium.

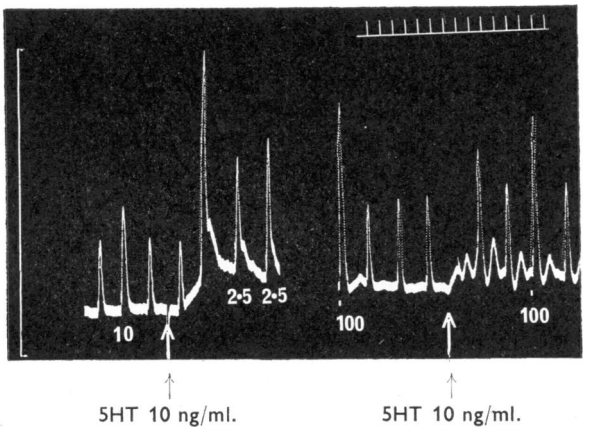

Fig. 11. Comparison between the effects of ordinary Krebs solution (left-hand record) and high K^+ Krebs solution (right-hand record) on the responses of the artery to injections of noradrenaline, and to perfusions of serotonin 10 ng/ml., commencing at the arrows. Unspecified responses are to 5 ng and 10 ng of noradrenaline in the left-hand and right-hand records respectively. Other doses of noradrenaline in nanograms are shown as subscripts. Time scale in minutes ; ordinate is 100 mM of mercury.

DISCUSSION

The interaction between noradrenaline and serotonin on the artery may be explained in terms of either sensitization of the vasoconstrictor action of serotonin by noradrenaline, or sensitization of the vasoconstrictor action of noradrenaline by serotonin. The evidence that interaction occurs in the absence of a constrictor response to serotonin does not distinguish between these explanations, since it is possible that a subthreshold constrictor response to serotonin reaches threshold or greater levels in the presence of noradrenaline. However, despite the much greater magnitude of the sensitization of serotonin's constrictor action produced by noradrenaline, there are two observations which suggest that part at least of the augmented response to noradrenaline is due to a sensitizing action on the part of serotonin. Enhancement of sensitivity by serotonin tended to reach a maximum, while its constrictor response was still submaximal. If the response to noradrenaline is increased by a constrictor component from serotonin, the sensitizing potency of serotonin would be expected to parallel its constrictor potency. Secondly, the changes in the shape of the dose response curve to noradrenaline produced by serotonin are minor compared with the change in sensitivity. This is to be expected if the augmented response to noradrenaline includes very little, if any, of a constrictor component from serotonin. The greater magnitude of the shift in sensitivity to serotonin produced by noradrenaline is also to be expected if it is assumed that the augmented response to serotonin is due to a transient increase in sensitivity of the artery to the noradrenaline with which it is being perfused during the brief contact with serotonin. Such a mechanism takes into account the high potency of serotonin as a sensitizing agent compared with its constrictor potency, and the tendency of some arteries, which did not respond by constriction to serotonin in doses as high as 1 μg, to display a dose-dependent

and sensitive constrictor response to serotonin in nanogram doses once an infusion of noradrenaline was started.

There is related evidence from an earlier study by Bülbring & Burnstock (1960) on mechanisms of tachyphylaxis and sensitization on the guinea-pig ileum. They observed shifts in sensitivity of the contractile response of the guinea-pig ileum following histamine and acetylcholine which were correlated with the direction of the change in membrane potential. However, serotonin was unusual in that, at a time when complete tachyphylaxis had occurred to its contractile response and its effects on the membrane potential, it was nevertheless capable of sensitizing both the membrane and mechanical responses of the tissue to histamine. These findings point to a sensitizing action on the part of serotonin on this tissue which is not simply a reflection of the changes accompanying its contractile action. Nevertheless, it must be emphasized that the evidence which favours a primary sensitizing action on the part of serotonin on blood vessels is still indirect. Analysis of the concentration rather than dose response curves to serotonin and noradrenaline, separately and in combination, over a wide range of concentrations may provide a more critical test of the identity of the sensitizing agent.

A related question is whether interaction between serotonin and noradrenaline is specific for noradrenaline or sympathetically mediated responses. Our present evidence favours non-specific interaction, since the constrictor responses to histamine and angiotensin are also enhanced by serotonin, and sensitization can occur in the dibenyline treated artery, and also in the chronically sympathectomized artery (de la Lande & Rand, 1965). The evidence is not conclusive, since we do not know whether the constrictor action of angiotensin in the isolated artery may involve sympathetic effector systems, nor is it known whether, in the ear artery, chronic sympathectomy leads to disappearance of noradrenaline. The striking difference between the actions of cocaine and serotonin on noradrenaline and sympathetically induced constriction of the artery provides a further argument against specificity of interaction. The action of cocaine is consistent with its postulated inhibitor action on the uptake of catecholamines into storage sites in sympathetic nerve endings. If the relatively slight effect of cocaine on noradrenaline sensitivity is taken as evidence that uptake into storage sites plays an insignificant role in terminating the action of injected exogenous noradrenaline on the rabbit artery, the much greater augmentation of the action of noradrenaline by serotonin implies that the action of the latter drug is not due to an effect on the catecholamine storage mechanism.

Irrespective of the precise nature of the mechanism of serotonin-noradrenaline interaction, it seems likely from the responses of the artery to methysergide and BOL that the interaction involves serotonin receptors possessing similar structural specificity to those in other types of smooth muscle. However, there appears to be a greater tendency for the drugs to act as partial agonists rather than antagonists, since they not only depress but also mimic the action of serotonin on the artery. The marked enhancement of the vasoconstrictor action of noradrenaline by methysergide is of additional interest in view of the therapeutic use of the drug in the treatment of migraine. Lance, Curran and Anthony (1965) have recently proposed that the beneficial action of methysergide may depend on its stimulating the action of serotonin on cranial vessels, and our findings not only provide experimental support for this suggestion but raise the further possibility that

both drugs may affect the tone of cranial vessels by an indirect mechanism, that of sensitizing them to the vasoconstrictor actions of noradrenaline or other endogenous vasoactive substances.

A feature of the ear artery is its high sensitivity to catecholamines. The sensitivity shows little change in high K^+, despite the assumed absence of a membrane potential under these conditions. Waugh (1962a) demonstrated a similar effect of adrenaline in the perfused mesenteric artery of the dog and concluded that electrical depolarization of the membrane and sodium ion influx was of little quantitative significance in the vasoconstrictor action of the drug. He showed furthermore (Waugh, 1962b) that adrenaline augments calcium-induced contraction of calcium-depleted blood vessels in both normal and high K+ media and has proposed that the adrenergic neurohormone causes vasoconstriction by a membrane action which is basically non-electrical and which increases the availability of calcium ions to the muscle-contracting proteins in the artery. Hence the absence of the sensitizing action of serotonin in the high K+ medium implies both that the function of the receptors through which its action is mediated are linked in some way with the membrane potential, and that the drug does not act on the adrenaline-sensitive phase in the calcium-actomyosin mechanism proposed by Waugh.

Enhancement of vasoconstrictor activity represents an extremely potent action of serotonin, the threshold concentration for the effect in the rabbit artery being of the order of 0.2 ng/ml. Furthermore, it is not species specific, as indicated by the potent action of serotonin on the isolated perfused human digital artery. Hence the question may be asked whether serotonin exerts a similar action *in vivo*. In view of the doubt regarding the presence of serotonin in blood other than in platelets (Erspamer, 1961) it appears unprofitable to consider this question in relation to endogenous circulating serotonin. However, there are many reports of interaction between serotonin and other vasoconstrictor stimuli or drugs in the animal *in vivo*. These include potentiation by serotonin of the pressor and nictitating membrane responses of the cat to adrenaline (Lecomte, 1953), potentiation in veins and antagonism in arteries of the dog limb of the actions of serotonin by noradrenaline (Hurwitz, Campbell, Gordon & Haddy, 1961) and reversal of serotonin's vasoconstrictor action to a vasodilator one in the perfused dog limb during sympathetic nerve stimulation of the lumbar chain (McCubbin, Kaneko & Page, 1962). The last study is the most complete and included the observation that reversal to a dilator action occurred in smaller arteries; larger arteries displayed a vasoconstrictor response to serotonin, but whether the response was different in magnitude is not stated. However, the action of serotonin was unaffected by infusions of noradrenaline. Hence there is no clear relation to our own findings on isolated arteries from rabbit and man, but there is a requirement for further analysis of the interaction in larger arteries under conditions both *in vitro* and *in vivo* from the one species of animal. Such an analysis must take into account evidence that isolated vascular strips are readily sensitized by a variety of agents, including plasma proteins (Wurzel, Bacon, Kalt & Zweifach, 1964). Whether serotonin continues to exert its action in the presence of other sensitizing agents is not known.

Finally, it must be stressed that we have employed only the changes in resistance of the artery to perfusion at a constant rate as the index of changes in its physical properties.

The use of a wider range of indices, including arterial diameter, and arterial length, as well as perfusion at constant pressure rather than constant flow rate, may assist in placing the phenomenon of serotonin-noradrenaline interaction in truer perspective.

SUMMARY

1. The vasoconstrictor action of noradrenaline in the isolated perfused central artery of the rabbit ear is markedly potentiated by serotonin. The same interaction occurs in the human isolated perfused digital artery, and occurs in the absence or presence of a constrictor response to serotonin.

2. The vasoconstrictor action of serotonin, which is weak compared with that of noradrenaline, is greatly enhanced by noradrenaline.

3. Interaction occurs whether the drugs are applied intraluminally or extraluminally. Serotonin also sensitizes the artery to adrenaline, histamine, angiotensin and sympathetic nerve stimulation.

5. The sensitizing action of serotonin is mimicked by methysergide, and reduced by bromlysergic acid diethylamide. It is abolished in a high K^+ medium.

6. The data points to serotonin as the sensitizing factor in its interaction with noradrenaline.

7. It is suggested that serotonin's action is mediated *via* receptors analagous to those in other types of smooth muscle and whose stimulation is linked in some way with the membrane potential of arterial smooth muscle.

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