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THE RESPONSES OF THE ISOLATED, BLOOD-PERFUSED SPLEEN OF THE DOG TO ANGIOTENSIN, OXYTOCIN AND VASOPRESSIN

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1 The responses of the smooth muscle of the capsule and blood vessels of the isolated, blood-perfused spleen of the dog to angiotensin, oxytocin and vasopressin have been investigated and compared to the actions of the catecholamines, adrenaline and noradrenaline.

2 Increasing doses of each of the three polypeptides cause graded increases in splenic vascular resistance and reductions in spleen volume.

3 Doses of the polypeptides which evoked increases in splenic vascular resistance not significantly different from increases produced by chosen doses of each catecholamine caused significantly smaller reductions in spleen volume.

4 The time-course of action of the polypeptides on the splenic vascular smooth muscle is different since the time to 50% recovery from vasopressin is highly significantly longer than that for equieffective doses of either angiotensin or oxytocin.

5 Phenoxybenzamine, in a dose which almost blocked the actions of the catecholamines, increased the responses of the vascular and capsular smooth muscle to oxytocin, vasopressin and angiotensin. This increase was not observed with another α-adrenoceptor blocking agent, phentolamine.

6 The significant species variation in the responses of the smooth muscle of the spleen to polypeptides and catecholamines are discussed and the results are considered in the context of the possible physiological roles of the polypeptides in haemorrhage.

Introduction

The actions of the catecholamines, adrenaline and noradrenaline on the splenic capsular and vascular smooth muscle have been established in the dog (Davies, Gamble & Withrington, 1968a,b; 1973), cat (Greenway & Stark, 1970) and in man (Ayers, Davies & Withrington, 1970; 1972). However, although the responses to the polypeptides angiotensin, vasopressin and oxytocin have been investigated in the cat spleen (Greenway & Stark, 1970) and in the human isolated spleen (Ayers *et al.*, 1970; 1972) their actions have not been fully documented in the dog spleen. It is of importance to ascertain the range of smooth muscle responses to these substances so that an evaluation may be made of their possible cardiovascular role in situations when circulating levels are elevated.

In a previous publication, Davies, Robinson & Withrington (1969) analysed the responses of the smooth muscle of the dog's spleen to adrenergic nerve stimulation and to catecholamines after the administration of phenoxybenzamine. In order to demonstrate the continued functional viability of the splenic smooth muscle after adrenoceptor blockade, splenic contractions were elicited by the smooth muscle stimulant angiotensin. However, it was observed that, when the responses to catecholamines and sympathetic nerve stimulation were diminished or abolished, the splenic vasoconstrictor and capsular responses to angiotensin were slightly increased. In the present paper we have specifically studied this observation and confirmed the increased responses to angiotensin; in addition, experiments were designed to answer two questions: firstly, whether these increased responses were specific to this polypeptide, and secondly, whether they were related to some property of phenoxybenzamine other than that due to its α-adrenoceptor blocking action. For this purpose we have tested the splenic responses to two other polypeptides, vasopressin and oxytocin before and after phenoxybenzamine and, in addition, tested all three polypeptides before and

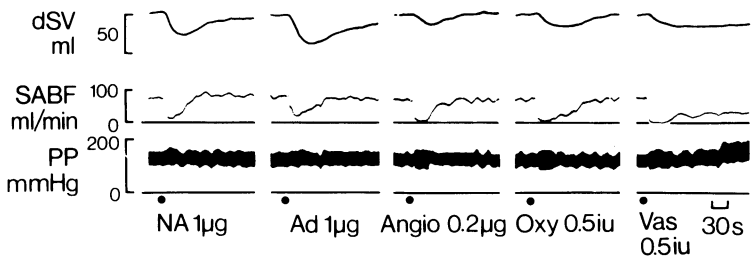

Figure 1 Changes in spleen volume (dSV), splenic arterial blood flow (SABF) and perfusion pressure (PP) in response to close arterial injections of noradrenaline 1 μg (NA), adrenaline 1 μg (Ad), angiotensin 0.2 μg (Angio), oxytocin 0.5 iu (Oxy) and vasopressin 0.5 iu (Vas) in the isolated perfused spleen of the dog.

after inducing adrenoceptor blockade with phentolamine.

Methods

Experiments were performed on 17 pairs of dogs (weights 8.1 to 19 kg) anaesthetized with an intravenous injection of 2-5 ml of 2.5% methohexitone sodium (Brietal, Lilly) followed by 5 ml/kg of a mixture of 1% chloralose (α-chloralose, Kühlmann, Paris) and 10% urethane (BDH) dissolved in 0.9% w/v NaCl solution (saline) and filtered.

Preparation of the spleen for perfusion was exactly as described in detail by Davies & Withrington (1968). Splenic arterial blood flow, phasic arterial perfusion pressure, splenic venous pressure and changes in spleen volume were recorded on a direct writing Beckman Type 'R' Dynograph.

Phasic perfusion pressure was recorded with a flexible polythene catheter from a side arm central to the rotameter with a Statham high pressure transducer (P23Gb). The system was at least as good as the present experimental system using a transducer (Type 4-327-0003, Consolidated Electrodynamics division of Bell & Howell) which has an undamped natural frequency of over 110 Hz with an estimated amplitude distortion of less then 5% up to 30 Hz. Mean perfusion pressure was, therefore, taken as the diastolic pressure plus one third of the pulse pressure. Splenic vascular resistance was then calculated as mean splenic arterial perfusion pressure/mean splenic arterial blood flow. Changes in splenic venous pressure, other than the transient increases accompanying the onset of capsular contraction, were negligible and not included in the calculations of changes in splenic vascular resistance. The postganglionic nerves to the spleen were dissected away from the artery and placed on platinum electrodes with the cathode distal. The electrode leads were connected to a stimulator and pulse counter so that a set number of pulses (50 V, 0.5 ms) could be delivered at a known frequency.

All drugs were administered into the rubber tubing, proximal to the cannulated splenic artery. Phenoxybenzamine and phentolamine were administered slowly over a period of 30 seconds. During the period of injection and for subsequent 30 s the venous blood draining the spleen was collected and discarded. This procedure restricted the action of the blocking drugs to the spleen and ensured that little entered the systemic circulation of the donor dog.

The following drugs were used: angiotensin (Hypertensin, Ciba); (−)-adrenaline (McCarthys); (−)-noradrenaline (Winthrop); oxytocin (Syntocin, Sandoz); vasopressin (Pitressin, Parke Davis & Co.); phenoxybenzamine (Dibenyline, SKF); and phentolamine (Rogitine, Ciba).

Results

In the dog spleen, perfused with blood at constant pressure, close-arterial injections of angiotensin, oxytocin and vasopressin caused a reduction in both spleen volume and splenic arterial blood flow. These responses are illustrated in Figure 1 where angiotensin (0.2 μg) and oxytocin (0.5 iu) both caused a reduction in arterial flow from 68 to 3 ml/min and vasopressin (0.5 iu) a reduction from 72 to 2 ml/minute. These reductions in flow represent increases in splenic vascular resistance of 2,190% for angiotensin and oxytocin and 3,520% for vasopressin. Small contractions of the splenic capsule accompanied these vascular effects, the concomitant reductions in volume in response to angiotensin, oxytocin and vasopressin being 12, 16 and 15 ml respectively. In contrast, the two catecholamines, adrenaline (1.0 μg) and nora-

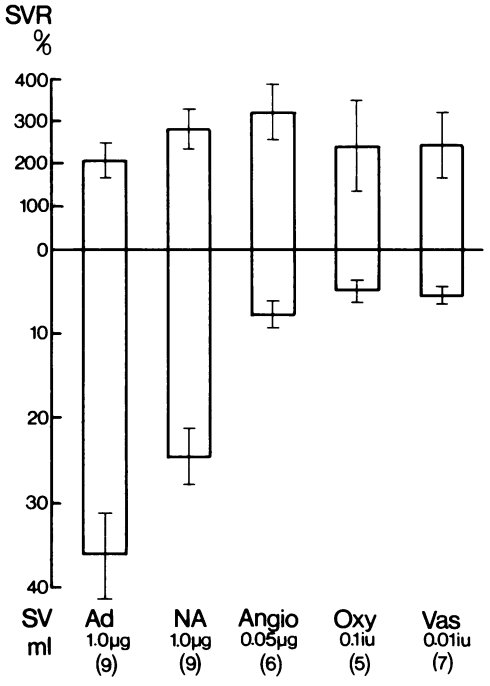

Figure 2 Mean percentage increase in splenic vascular resistance (SVR) and mean reduction in spleen volume (SV) produced by close arterial injection of adrenaline 1.0 μg (Ad), noradrenaline 1.0 μg (NA), angiotensin 0.05 μg (Angio) oxytocin 0.1 iu (Oxy) and vasopressin 0.01 iu (Vas). Vertical lines represent s.e. mean and figures in brackets refer to number of observations.

drenaline (1.0 μg), both caused small increases in splenic vascular resistance of 314% and 450%, but were associated with much larger reductions in splenic volume of 36 and 37 ml respectively.

The essential difference between the responses of the splenic vascular and capsular smooth muscle to these five substances is shown in Figure 2, which contains data from 10 experiments in each of which at least four of the five substances were tested. In each experiment, a dose of each substance was selected which on close-arterial injection would produce approximately the same vascular response. In this series of experiments the mean increase in splenic vascular resistance to adrenaline (1.0 μg), noradrenaline (1.0 μg), angiotensin (0.05 μg), oxytocin (0.1 iu) and vasopressin (0.01 iu) were 214 ± 41%, 293 ± 48%, 337 ± 68%, 258 ± 109% and 255 ± 81% respectively. Statistical analysis revealed no significant difference between the vascular responses to adrenaline and noradrenaline ($P > 0.1$) or between adrenaline and any of the three polypeptides, angiotensin ($P > 0.05$), oxytocin ($P > 0.30$) and vasopressin ($P > 0.30$). However, the concomitant responses of the splenic capsule to the five substances were strikingly different, the mean reductions in spleen volume being 35.9 ± 5.1 ml, 24.4 ± 3.3 ml, 7.3 ± 1.6 ml, 4.6 ± 1.3 ml and 5.2 ± 1.1 ml to adrenaline, noradrenaline, angiotensin, oxytocin and vasopressin respectively. These experiments confirm the previous observations (Davies *et al.*, 1973) that the capsular response to adrenaline is significantly greater than to the same dose of noradrenaline ($P = 0.05$) and in addition show that the reduction in spleen volume in response to the three polypeptides are all highly significantly smaller ($P < 0.0005$). The results clearly demonstrate a difference in the reactivity of the splenic vascular and capsular smooth muscle to catecholamines and polypeptides.

The ratio of potency of these five substances on the splenic capsular and vascular smooth muscle will depend on the dose level. It is not practicable, in the isolated spleen perfused at constant pressure, to establish the complete dose-response curves for capsular and vascular smooth muscle to all three polypeptides in the same preparation. The long duration of action of each polypeptide sets a limit to the number of injections and, in addition, the potent vasoconstrictor properties of each polypeptide prevents their access to capsular smooth muscle at high dose levels. The mean responses of the capsular and vascular smooth muscle to three different dose levels of each polypeptide are illustrated in Figure 3. The doses were chosen to cover the range of vascular effects from just above threshold to almost complete vasoconstriction, i.e. cessation of splenic arterial blood flow. It is clear that the responses of the capsule and blood vessels to angiotensin, oxytocin and vasopressin are dose-dependent but that there is no clear dose-dependent separation of responses as previously reported with the catecholamines. An indication of the relative potencies of the five substances on the splenic vascular smooth muscle may be made at the single dose level for each shown in Figure 2. In this test adrenaline (1.0 μg), noradrenaline (1.0 μg), oxytocin (0.2 μg), angiotensin (0.05 μg) and vasopressin (0.02 μg) were equieffective in increasing splenic vascular resistance (1.0 iu oxytocin or vasopressin is equivalent to approximately 2.0 μg; Goodman & Gilman, 1970).

It is apparent (Figure 1) that the polypeptides have different time-courses of action on the splenic vascular smooth muscle. In 10 tests in which doses of angiotensin, oxytocin and vasopressin produced similar maximum increases in splenic vascular resistance, the mean time to 50% recovery was similar for angiotensin and oxytocin (51.8 ± 5.9 s and 67.7 ± 6.2 s respectively,

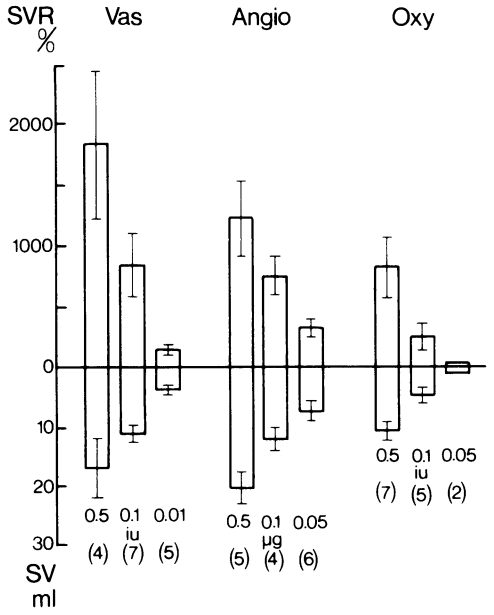

Figure 3 Mean percentage increases in splenic vascular resistance (SVR) and mean reductions in spleen volume (SV) produced by close-arterial injections of graded doses of vasopressin (Vas), angiotensin (Angio) and oxytocin (Oxy). Vertical lines represent s.e. mean and the figures in brackets refer to the number of observations.

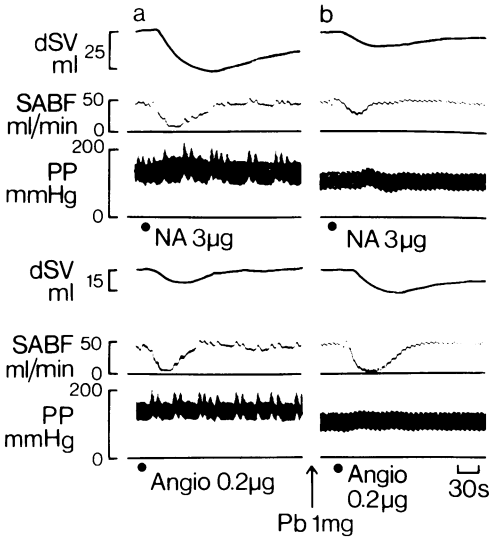

Figure 4 Changes in spleen volume (dSV), splenic arterial blood flow (SABF) and perfusion pressure (PP) in response to close-arterial injections of noradrenaline 3 μg (NA) and angiotensin 0.2 μg (Angio) before (a) and after (b) phenoxybenzamine 1 mg (Pb).

$P = 0.05$). The vascular response to vasopressin was, however, of much longer duration, with a mean time to 50% recovery of 269 ± 46.6 seconds. This is highly significantly longer than for equieffective doses of either angiotensin or oxytocin ($P < 0.0005$).

The effects of phenoxybenzamine

In each experiment, control responses of both the capsular and vascular smooth muscle were obtained to at least four of six standard tests. These were close-arterial injection of adrenaline, noradrenaline, angiotensin, oxytocin and vasopressin, and electrical stimulation of the splenic sympathetic nerves at 3 Hz. Doses of the three polypeptides were selected which produced marked but submaximal effects. Phenoxybenzamine was then given by the close-arterial route, in a dose (1-3 mg) which almost abolished the splenic responses to adrenaline, noradrenaline and splenic nerve stimulation when the standard tests were repeated.

This procedure was carried out in 11 experiments with angiotensin and part of the results of one experiment is shown in Figure 4, in which the responses to noradrenaline (3 μg) were a decrease in flow, equivalent to an increase in splenic vascular resistance of 354% and a reduction in spleen volume of 29 ml, which were reduced after phenoxybenzamine (1 mg) to 55% and 9 ml respectively. However, in the same experiment, the responses to angiotensin (0.2 μg), which were initially an increase in splenic vascular resistance of 955% and a reduction in spleen volume of 10 ml, were increased after phenoxybenzamine to 1690% and 15 ml respectively. In addition, the vascular response was considerably prolonged. Consideration of the results of all 11 experiments show that the splenic capsular response to angiotensin (0.025-0.2 μg) was increased after phenoxybenzamine in seven tests with no change or a slight reduction in the remaining four. The mean reduction in spleen volume in all 11 tests was 11.2 (± 1.3) ml before, and 14.0 (± 1.7) ml after phenoxybenzamine. The concomitant increases in splenic vascular resistance produced by angiotensin were increased in ten tests after phenoxybenzamine and showed no change in the remaining one. In eight of the ten tests, potentiation was apparent as a greater increase in splenic vascular resistance, the mean increases being 589% (± 133) before and 1018% (± 172) after phenoxybenzamine. In the other two tests, the control vascular response to angiotensin was near maximal

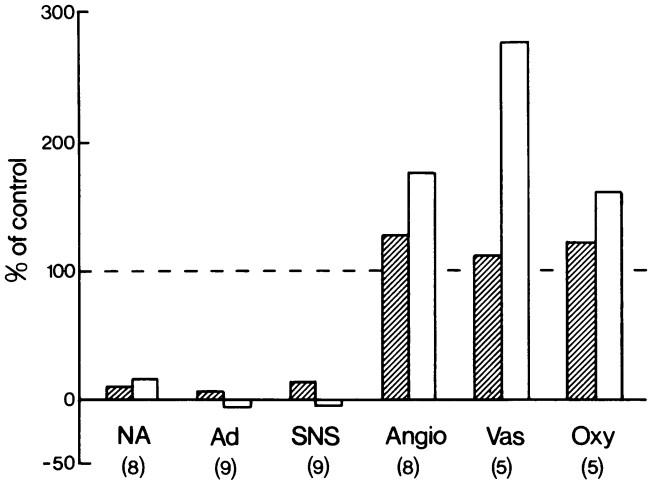

Figure 5 The effects of phenoxybenzamine on changes in splenic vascular resistance (open columns) and reductions in spleen volume (hatched columns) in response to sympathetic nerve stimulation at 3 Hz (SNS), and close-arterial injections of noradrenaline (NA), adrenaline (Ad), angiotensin (Angio), vasopressin (Vas) and oxytocin (Oxy). In each test the response after phenoxybenzamine is expressed as a percentage of the control response before phenoxybenzamine (= 100%). Each column represents the mean of a number of tests which is indicated in brackets.

and the increased response after phenoxybenzamine was apparent as a greatly increased duration. Paired analysis of the responses in each experiment to the same dose of angiotensin, before and after phenoxybenzamine, showed that the increases in both the capsular and vascular responses are significantly greater than zero ($P < 0.0005$ and $P < 0.01$ respectively).

Vasopressin (0.02-0.5 iu) was tested by close arterial injection in 5 experiments and the mean reduction in spleen volume was 18.8 ml (± 8.2). In four of these experiments the capsular response to the same dose of vasopressin was increased after phenoxybenzamine but the capsular response was unchanged in one experiment. The mean reduction in volume to the same doses of vasopressin after phenoxybenzamine was 20.6 ml (± 7.2). The splenic vascular responses to these doses of vasopressin were also potentiated in 4 of the 5 tests and unchanged in one. In one experiment the potentiation was more apparent as a considerably prolonged response. The mean increase in splenic vascular resistance in the four tests was 364% (± 114) before, and 987% (± 266) after phenoxybenzamine. Paired analysis of the capsular and vascular responses to the same dose of vasopressin in each experiment revealed that the increases in responses after phenoxybenzamine were significantly greater than zero ($P < 0.025$ and $P < 0.0125$ respectively).

Oxytocin (0.01-0.5 iu) was injected by the close arterial route in seven experiments. In four of these there was an increase in the capsular contraction to oxytocin after phenoxybenzamine, in two there was no change and in one a small reduction. The mean reduction in spleen volume in all seven tests was 11.4 ml (± 1.5) and 13.6 ml (± 2.4) after phenoxybenzamine. The splenic vascular response was potentiated in all seven tests. In five of these the increase in splenic vascular resistance produced by oxytocin was 462% (± 114) initially and 728% (± 212) after phenoxybenzamine. In the remaining two tests, the near maximal vascular responses were prolonged. Paired analysis showed that the increases in both capsular and vascular responses are significantly greater than zero ($P < 0.01$ in both cases).

These differences between the splenic capsular and vascular responses to the three polypeptides, which are all increased after phenoxybenzamine, and the responses to sympathetic nerve stimulation and to the catecholamines, which are all reduced or even reversed, are apparent in Figure 5, where the control responses before phenoxybenzamine are represented as 100% and the subsequent responses after phenoxybenzamine are expressed as a percentage of the control.

Effects of phentolamine

In three experiments control responses to angiotensin, adrenaline, noradrenaline and sympathetic nerve stimulation were obtained prior to the close-arterial administration of a dose of phentolamine (1.0 mg) which blocked the responses to sympathetic nerve stimulation and the injected catecholamines. In these experiments the mean initial responses to angiotensin (0.2 μg) were a reduction in spleen volume of 5.7 ml (± 2.4) and an increase in splenic vascular resistance of 343% (± 191). After the administration of phentolamine the mean responses were not significantly different being 6.0 ml (± 2.3) and 350% (± 226) respectively. Similarly, in single experiments with oxytocin and vasopressin, the administration of phentolamine had no effect on the responses of either the splenic capsular and vascular smooth muscle to either of the polypeptides.

Discussion

The three polypeptides, angiotensin, vasopressin and oxytocin have been shown to possess potent actions on the smooth muscle of the capsule and blood vessels of the dog's spleen. Qualitatively, the predominant action of each polypeptide is on the smooth muscle of the splenic blood vessels causing profound vasoconstriction, although all three substances provoke, in addition, a contraction of the smooth muscle forming the splenic capsule. Angiotensin has been shown to produce similar responses in the cat spleen (Hertting & Suko, 1966; Greenway & Stark, 1970) but vasopressin, in the same preparation caused vasoconstriction with little or no capsular contraction. Experiments on the human isolated perfused spleen (Ayers *et al.*, 1972) revealed that whilst both angiotensin and vasopressin caused vasoconstriction, oxytocin was devoid of any vasoconstrictor properties. There were no accompanying reductions in spleen volume. A considerable species variation therefore exists in the responses of the two splenic smooth muscle systems to these naturally occurring polypeptides (Davies & Withrington, 1973). Furthermore, it is apparent that the relative sensitivity of the two smooth muscle systems to the polypeptides is different from their sensitivity to catecholamines (Davies *et al.*, 1973) since doses of angiotensin, vasopressin or oxytocin which caused constrictions of the splenic vascular bed not significantly different from adrenaline or noradrenaline, provoked significantly smaller reductions in spleen volume than either catecholamine.

The duration of action of angiotensin and oxytocin on the splenic vascular bed is similar but both have a significantly shorter half-life of action than vasopressin. The half-lives of circulating arginine and lysine vasopressin in the dog have been found by radioimmunoassay techniques to be 6.0 and 5.4 min respectively (Biro, Forsling, Martin & Wilmott, 1972). Preliminary experiments (Davies, Forsling & Withrington, unpublished observations) have indicated that the half-life of oxytocin in the dog is slightly shorter. No comparable information exists about the half-life of angiotensin in the dog but superfusion techniques (Ferriera & Vane, 1967) suggest a half-life of 20 seconds. These techniques measure the disappearance of polypeptides from the general circulation but give no indication of their relative disappearance by passage through spleen. Possibly the longer recovery time for the splenic smooth muscle from administered vasopressin is related to a more complete uptake from the blood. No quantitative information is available to verify this point.

The mode of action of angiotensin on smooth muscle has been explained in a number of ways. A central action (Aars & Akre, 1968) may be excluded in the present experiments since the preparation was isolated. Similarly, since the splenic smooth muscle responses were not reduced by doses of phenoxybenzamine, which partially blocked the responses to sympathetic nerve stimulation and injected catecholamines, the possibility that the action of angiotensin on splenic smooth muscle is mediated by the release of stored catecholamines in the nerve endings (Distler, Liebau & Wolff, 1965) may be eliminated. This is in agreement with the observations of Thoenen, Hurlimann & Haefely (1965) who showed that the splenic responses to angiotensin were not reduced by phenoxybenzamine and also with the conclusions of Hertting & Suko (1966) who demonstrated that the responses to angiotensin and vasopressin were unaffected by either chronic sympathectomy or pre-treatment with reserpine. Furthermore, in the present experiments when the responses to stimulation of adrenoceptors were partially blocked by phenoxybenzamine the splenic smooth muscle responses to angiotensin, vasopressin and oxytocin were increased. Paired analysis revealed that the increase in capsular and vascular responses to all three polypeptides after phenoxybenzamine was significant. In each experiment the splenic arterial blood flow before and after phenoxybenzamine was the same so that the increased responses did not arise simply from increases in the arterial concentration of each polypeptide, secondary to reductions in perfusion. However, it

is possible that phenoxybenzamine improves the access of polypeptides to the receptor sites by altering the microcirculation within the spleen. Phenoxybenzamine has a number of well-defined actions including the blockade of α-adrenoceptors, blockade of uptake processes into both nerve and muscle and in addition causes an increase in the release of noradrenaline from nerve endings in response to sympathetic nerve stimulation. All these actions appear at different dose levels of phenoxybenzamine (Enero, Langer, Rothlin & Stefano, 1972) and makes the interpretation of the increased responses observed in the present experiments difficult. However, since in the same experiments the responses to the directly acting polypeptides oxytocin and vasopressin were increased a further action of phenoxybenzamine may be to potentiate the responses of smooth muscle to nonadrenergic stimuli. Furthermore, the increased responses to the polypeptides were not observed after the administration of the α-adrenoceptor blocking agent, phentolamine. This indicates that the increased responses are a specific property of phenoxybenzamine and not related to α-receptor blockade.

Finally the possible physiological significance of the actions of the three polypeptides on splenic smooth muscle must be considered. There is clear evidence in the cat that both angiotensin and vasopressin contribute to the splenic responses in haemorrhage (Greenway & Stark, 1969; Cohen, Sitar, McNeill & Greenway, 1970; Stark, McNeill & Greenway, 1971). The contribution of both polypeptides appears to be restricted to the vascular response. There is no direct evidence in the dog but studies in this species on the release of vasopressin (Schrier, Verroust, Jones, Fabian, Lee & de Wardener, 1968; Rocha e Silva & Rosenberg, 1969; Biro, Forsling & Wilmott, 1972) and angiotensin (Regoli & Vane, 1966; Hodge, Lowe & Vane, 1966) in response to haemorrhage suggest that the arterial concentrations may be sufficient to increase splenic vascular resistance (Davies *et al.*, 1968a). The increased plasma concentrations would have little action of the capsular smooth muscle since this would be almost maximally contracted by the concomitant increase in both direct sympathetic activity and circulating catecholamine levels.

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