Br. J. Pharmac. (1972), **44**, 17–30.

Responses of the isolated, perfused human spleen to sympathetic nerve stimulation, catecholamines and polypeptides

A. B. AYERS,* B. N. DAVIES AND P. G. WITHRINGTON

Department of Physiology, The Medical College of St. Bartholomew's Hospital, Charterhouse Square, London EC1M 6BQ

Summary

1. The responses of the smooth muscle of the capsule and blood vessels of the isolated, perfused human spleen to sympathetic nerve stimulation, adrenaline, noradrenaline, angiotensin, oxytocin, vasopressin, isoprenaline and acetylcholine have been investigated and compared with those of dog spleen.

2. Stimulation of the postganglionic sympathetic nerves to the human spleen at frequencies of 3–10 Hz evoked graded vasoconstriction but very small changes in spleen volume.

3. The injection of adrenaline and noradrenaline in doses of 0·25–25 μg to the human spleen produced graded increases in splenic vascular resistance with very small decreases in spleen volume.

4. Administration of the α-adrenoceptor blocking drug phenoxybenzamine completely abolished or considerably reduced the vascular responses of the human spleen to sympathetic nerve stimulation or the injection of noradrenaline.

5. The vascular action of adrenaline was often reversed to elicit a vasodilatation after phenoxybenzamine suggesting the presence of β-adrenoceptors in the vascular bed. This was confirmed by the administration of isoprenaline which induced a marked reduction in vascular resistance of the human spleen.

6. The polypeptides angiotensin and vasopressin induced a marked vasoconstriction in the human spleen without changes in the spleen volume. These effects were uninfluenced by the administration of phenoxybenzamine.

7. The polypeptide oxytocin caused a slight vasodilatation in the human spleen, an effect almost exactly mimicked by the preservative chlorobutanol.

8. Preliminary experiments suggest that noradrenaline is the transmitter released by the postganglionic nerves to the human spleen.

9. These results provide direct evidence that the normal human spleen, unlike that of the dog, does not have a reservoir function. It is suggested that contractions of the enlarged human spleen may occur in various pathological conditions.

Introduction

Whether the capsule of the human spleen actively contracts in response to sympathetic nerve stimulation and increases in the circulating levels of catechol-

* Present address: Department of Diagnostic Radiology, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W.12.

amines, is still speculative. Most observations have been indirect, including macroscopic observation of spleen size at operation, estimation of peripheral haematocrit values and techniques of X-ray scan (Kryzwanek & Arnold, 1927 ; Yang, 1928 ; Benhamou, Jude & Marchioni, 1929a, b ; Ebert & Stead, 1941 ; Watson & Paine, 1943). The dynamic state of the splenic circulation in man has been most readily studied by injecting red blood cells labelled with ^{51}Cr into the peripheral circulation and recording their entry and flow in the splenic circulation by means of continuous recording external counters (Harris, McAlister & Prankerd, 1957, 1958 ; Bowdler, 1962 ; Prankerd, 1963 ; Toghill, 1964 ; Richards & Toghill, 1967).

The general conclusion of all these studies has been that the normal human spleen does not take up normal red cells from the circulation as an acute store for emergencies. However, the studies with red cells labelled with ^{51}Cr has suggested that the pooling of abnormal cells by normal spleens and the pooling of normal cells by abnormal spleens may occur.

There is little information about the responses of the vascular smooth muscle of the human spleen, apart from the report (Prankerd, 1963) that noradrenaline induces splenic vasoconstriction. In contrast there is a considerable literature, based on direct observation, on the capsular and vascular smooth muscle responses of the spleens of other species including the dog (Green, Ottis & Kitchen, 1960 ; Daly & Scott, 1961 ; Davies, Gamble & Withrington, 1968a, b) and cat (Hertting & Suko, 1966 ; Greenway, Lawson & Stark, 1968 ; Greenway & Stark, 1969, 1970). Some species variation in the responses has been observed.

The present series of experiments is a direct investigation of the capsular and vascular smooth muscle responses of the isolated, perfused spleen of man using fresh organs obtained at operation and not associated with any disease of the spleen. Experiments were also performed on the dog's spleen, isolated and perfused in a similar manner for direct comparison.

Some of these observations have already been communicated to the British Pharmacological Society (Ayers, Davies & Withrington, 1970).

Methods

Experiments were performed on human spleens obtained from patients undergoing major abdominal surgery in a number of different hospitals. The clinical diagnosis and ages are shown in Table 1. Total gastrectomy was performed in most patients when the spleens were removed to facilitate the surgical procedure. All the spleens removed were regarded on macroscopic examination as normal. As soon as the spleen was removed it was placed in heparinized saline solution at room temperature and the splenic vein ligature removed to prevent coagulation ; where facilities were available the spleen was perfused immediately with 50–100 ml of heparinized

TABLE 1. *Data on the patients from whom spleens were obtained and perfused*

Diagnosis	Sex	No.	Age
Carcinoma of stomach	Male	5	51–72
	Female	5	53–73
Carcinoma of colon	Male	1	64
Hodgkins Disease	Female	1	37
Hereditary spherocytosis	Male	1	8

saline (5,000 I.U. heparin, Pularin, Evans Medical Ltd., in 500 ml of 0·9% saline) and transported in this fluid to the laboratory. When the artery and vein were cannulated the spleen was cautiously perfused with McEwens (1956) solution and any patent omental branches which then became apparent were ligated ; dissection of the sympathetic nerves accompanying the splenic artery was then attempted.

Thirty spleens were obtained but only thirteen were suitable for long perfusion because of damage to the capsule or pulp or because short hilar vessels made cannulation impossible. The thirteen patients from whom these spleens were obtained were premedicated and anaesthetized by a variety of methods. Premedication included omnopon and scopolamine (five patients), pethidine and atropine (five patients), promethazine and scopolamine in one patient, whilst one received no premedication. Five of the patients received succinylcholine as muscle relaxant, two had gallamine triethyliodide, one received (+)-tubocurarine and five patients had no record of receiving relaxants. In ten patients anaesthesia was induced with thiopentone and maintained with nitrous oxide and oxygen with halothane administered occasionally ; two were induced and maintained on halothane whilst one patient was induced with cyclopropane and maintained with a mixture of nitrous oxide and oxygen.

Immediately after perfusion of the excised spleen was commenced, test doses of catecholamines (adrenaline or noradrenaline) were administered. No variation in response was observed which could be clearly attributed to any particular combination of premedication or anaesthetic. Apart from this routine premedication and anaesthesia none of the patients were receiving drugs liable to influence the results obtained. The delay between removal and perfusion in the laboratory ranged from 1–4 hours. The weight of the spleens varied from 60–370 g (mean 212 g).

After cannulation the spleen was placed in a Perspex plethysmograph and perfused at constant flow with McEwens solution at 37° C equilibrated with a gas mixture of 95% oxygen and 5% carbon dioxide. Flow was maintained by a Watson–Marlowe Flow inducer (Type MHRE) the output of which was sinusoidal ; the splenic arterial perfusion pressure was measured with a Statham high pressure transducer (P23Gb) (1 mmHg≡1·333 mbar). Splenic venous pressure was maintained constant in each experiment between 0 and 10 cm water. The plethysmograph was filled with liquid paraffin maintained at 37° C by water circulating through the false bottom of the plethysmograph. Whenever possible, changes in spleen volume were monitored by a low pressure Statham transducer (P23Bb) as variations in the height of the liquid paraffin column connected to the plethysmograph. After suitable amplification the perfusion pressure and changes in spleen volume were continuously recorded on a Beckman Type 'R' Dynograph.

The postganglionic sympathetic nerves to the spleen were placed on platinum stimulating electrodes within the plethysmograph with the cathode peripheral. The leads were connected to a stimulator and a pulse counter so that a set number of stimuli of 50 V and 0·5 ms duration could be delivered to the nerves.

Fifteen control experiments were performed on dogs weighing between 9·0 and 16·0 kg. They were anaesthetized with intravenous injection of 3–5 ml of 2·5% methohexitone sodium (Brietal sodium, Lilly) followed by a mixture (5 ml/kg) of 1% chloralose (α-chloralose, Kühlmann, Paris) and 10% urethane (B.D.H.) dissolved in 0·9% saline and filtered. The trachea and an external jugular vein were cannulated. A midline abdominal incision was made and the spleen dissected from its

attachments apart from the splenic artery, splenic vein and postganglionic sympathetic nerve trunk as previously described (Davies & Withrington, 1968). The dog was then heparinized (500 I.U./kg) before the division of the nerve, artery and vein. The spleen was perfused as described above for the human specimens.

Splenic vascular resistance

The mean perfusion pressure was taken as the mean of the measured 'systolic' and 'diastolic' pressures recorded by the arterial transducer since the output of the Watson–Marlowe pump was sinusoidal. Consequently, at constant flow a change in mean perfusion pressure (at constant venous pressure) may be taken as being directly proportional to the change in splenic vascular resistance. Percentage changes in splenic vascular resistance were calculated as the increase in mean perfusion pressure expressed as a percentage of the control mean perfusion pressure.

Noradrenaline assay

The noradrenaline concentration of the effluent was estimated by bioassay on the blood pressure of the pithed rat. Fluorimetric estimation was determined by a modification of the techniques of Häggendal (1963) and Bertler, Carlsson & Rosengren (1968) as described by Allison & Powis (1971).

Administration of drugs

All drugs were injected into the rubber tubing between the flow inducer and the splenic arterial cannula distal to the point at which perfusion pressure was being measured. The following drugs were used: noradrenaline bitartrate (Winthrop), adrenaline bitartrate (Martindale Samoore), isoprenaline sulphate (Savory & Moore), angiotensin (Hypertensin, Ciba), vasopressin (Pitressin, Parke Davis & Co.), oxytocin (Syntocin, Sandoz), oxytocin solvent (Syntocin placebo, Sandoz), acetylcholine (Acecoline, Lematte & Boinot) and phenoxybenzamine (Dibenyline, SKF).

Results

Effects of splenic nerve stimulation on splenic vascular resistance and volume

The splenic nerves were stimulated in ten different human spleens at frequencies of 0·5–30 Hz. Electrical stimulation was usually continued for 30 seconds. As the frequency of stimulation was increased, graded increases in splenic perfusion pressure were obtained at constant flow. In one experiment (Fig. 1) a wide range of stimulation frequencies was applied and the increase in mean perfusion pressure was 7·5, 35, 65, 80 and 105 mmHg at 0·5, 1·0, 3·0, 5·0 and 7·0 Hz corresponding to increases in splenic vascular resistance of 12, 56, 90, 110 and 145 % respectively. In four experiments in which a smaller range of frequencies was applied the mean

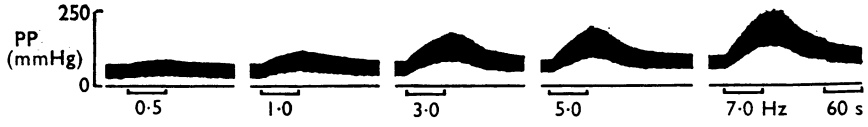

FIG. 1. Vascular responses of the human spleen to sympathetic nerve stimulation. Changes in perfusion pressure (PP) in mmHg caused by electrical stimulation of splenic nerve at 0·5, 1·0, 3·0, 5·0 and 7·0 Hz.

increases in mean perfusion pressure at 1, 3 and 7 Hz were 10, 28 and 111 mmHg corresponding to mean increases in splenic vascular resistance of 16, 37 and 118% respectively. There was generally little vascular response at rates of stimulation below 3 Hz whilst the maximum percentage increases in splenic vascular resistance were observed at frequencies of 7–10 Hz.

In six experiments satisfactory volume recordings were made and a range of frequencies studied in five of these. A similar range of stimulation frequencies was studied in six dog spleens perfused under identical conditions. The volume changes in the human spleen in response to sympathetic nerve stimulation at any frequency was always very small despite the concomitant increases in splenic vascular resistance. In contrast, in the dog, similar vascular responses were always accompanied by considerable reductions in spleen volume. For example, in one experiment in the dog stimulation at 1, 3 and 7 Hz led to increases in splenic vascular resistance of 29, 77 and 91% and reductions in spleen volume of 33, 70 and 68 ml. The corresponding changes in a human spleen at stimulation frequencies of 3, 7 and 10 Hz were 19, 29 and 31% increases in splenic vascular resistance with concomitant decreases in spleen volume of 0, 6 and 8 ml. These experiments are illustrated in Fig. 2.

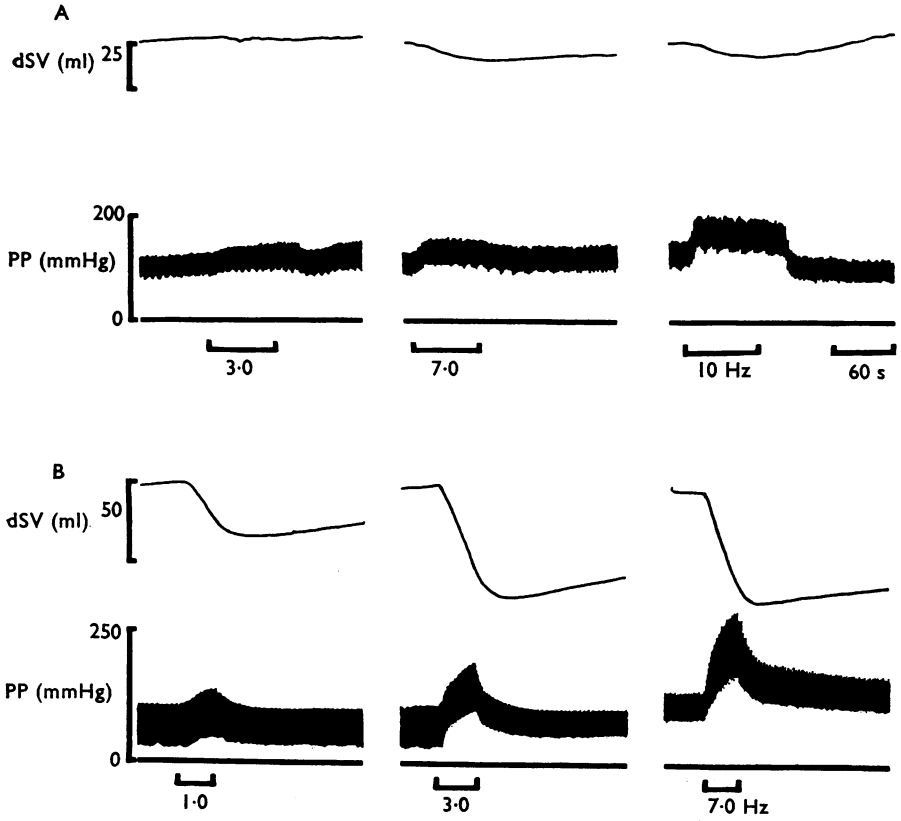

FIG. 2. Responses of the capsular and vascular smooth muscle of the human and dog spleen to sympathetic nerve stimulation. Upper record: human (♂) spleen (A). Lower record: dog spleen (B). Each record consists of a tracing of changes in spleen volume (dSV) and perfusion pressure (PP) in response to stimulation of the splenic nerve at 3·0, 7·0 and 10 Hz in the human and 1·0, 3·0 and 7·0 Hz in the dog.

The mean capsular and vascular responses to sympathetic nerve stimulation at 3 and 7 Hz in five human and six dog spleens are shown in Fig. 3. The mean increases in the vascular resistance in the human and dog spleen at 7 Hz were 79 and 76% respectively. These values are not significantly different ($P>0{\cdot}475$). At 3 Hz, the mean increases in vascular resistance in the two species were 23 and 53% respectively, values which are just significantly different ($P>0{\cdot}025$). In contrast the mean reductions in the volume of the human spleen at 3 and 7 Hz were 0·6 and 3·0 ml; these values are very significantly less ($P<0{\cdot}0025$ at 7 Hz and $P<0{\cdot}0005$ at 3 Hz) than those of the dog's spleen where the mean reductions in volume at the same frequencies were 44·3 and 43·3 ml.

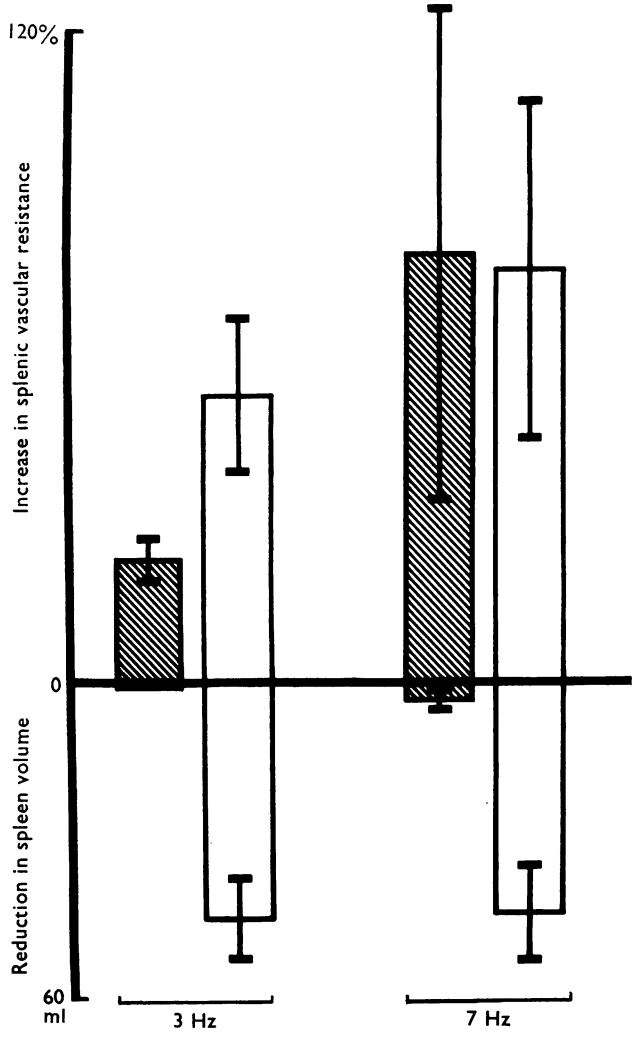

FIG. 3. Mean percentage increases in splenic vascular resistance and mean reductions in volume (ordinates) in five human (hatched columns) and six dog (open columns) spleens in response to splenic nerve stimulation at 3 and 7 Hz. Vertical lines represent the standard errors of the means.

Effects of injections of adrenaline and noradrenaline on splenic vascular resistance and volume

The vascular responses to the administration of adrenaline and noradrenaline in doses of 0·25–25 μg were observed in twelve isolated human spleens. Graded increases in perfusion pressure at constant flow were produced with increasing doses of both catecholamines indicating graded increases in splenic vascular resistance. At doses of less than 1·0 μg, adrenaline was the more potent vasoconstrictor whilst at higher doses the catecholamines were equipotent. For example, in three experiments at a dose of 0·25 μg, adrenaline was 1·4 times as potent as noradrenaline and in five experiments at 0·5 μg the ratio was the same (1·4). However in ten experiments at 1·0 μg, adrenaline was only slightly more active than noradrenaline in increasing splenic vascular resistance (ratio 1·1) whilst in six experiments at 3·0 μg the two catecholamines were equipotent as splenic vasoconstrictors.

In four experiments in which changes in splenic volume could be recorded together with the changes in splenic perfusion pressure, adrenaline was given on twenty-two occasions and noradrenaline on eighteen at various dose levels. In these experiments there were very small reductions in spleen volume accompanying the injections of adrenaline and noradrenaline despite the increases in splenic perfusion pressure (Fig. 4). The maximum reduction in volume occurred in a spleen of 200 g where doses of adrenaline (1·0 μg) and noradrenaline (3·0 μg) induced reductions in spleen volume of 11 and 13 ml respectively. Paired injections of adrenaline

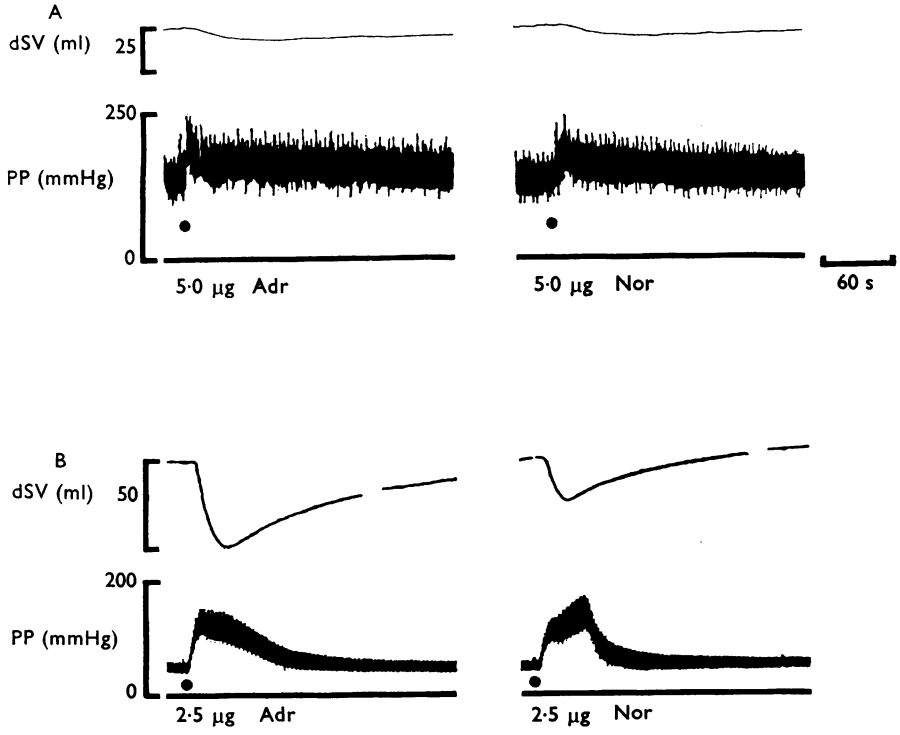

FIG. 4. Responses of the capsular and vascular smooth muscle of the human and dog spleen to close arterial injections of adrenaline and noradrenaline. Upper record (A): human spleen. Lower record (B): dog spleen. Each record consists of a tracing of changes in spleen volume (dSV) and perfusion pressure (PP) in response to 5·0 μg of adrenaline (Adr) and noradrenaline (Nor) in the human and 2·5 μg in the dog spleen.

and noradrenaline over the dose range 1·0–5·0 μg were made in twelve tests and produced small contractions (on fifteen occasions of 5 ml or less) with a range 0–13 ml. The results suggested that, within the limits of accuracy of measurements of the small changes in volume involved and on the changing background, the catecholamines were equipotent on the capsular smooth muscle of the human spleen.

In thirteen control experiments in dogs adrenaline and noradrenaline were administered in doses of 0·25–5·0 μg and the increases in splenic perfusion pressure were similar to those obtained in the human spleen but were accompanied by considerable reductions in volume often in excess of 60 ml (Fig. 4). In the dog adrenaline is more potent than noradrenaline in contracting the capsular smooth muscle but the catecholamines are equipotent in causing increases in splenic vascular resistance (Davies *et al.*, 1968b).

Effects of phenoxybenzamine on the responses of the human spleen to nerve stimulation and injected catecholamines

The α-adrenoceptor blocking agent phenoxybenzamine was administered in doses of 3–10 mg to investigate the receptor spectrum of the smooth muscle of the human spleen. It was observed in six experiments that the increase in splenic perfusion pressure accompanying sympathetic nerve stimulation was reduced in six out of sixteen tests, abolished on nine occasions and in the remaining test an initial increase in perfusion pressure caused by nerve stimulation at 10 Hz was reversed to cause a slight decrease in perfusion pressure indicating a vasodilatation.

The graded increases in splenic perfusion pressure caused by injections of noradrenaline were abolished or considerably reduced by the administration of phenoxybenzamine in five experiments. In two of these experiments adrenaline was administered on seven occasions in a dose which initially produced an increase in perfusion pressure. The subsequent administration of phenoxybenzamine reversed the vascular action of adrenaline to cause vasodilatation. The actions of phenoxybenzamine on the vascular responses to noradrenaline and adrenaline are illustrated in Fig. 5. This reversal in the vascular response to adrenaline did not always occur for in the three other experiments the vasoconstrictor action of adrenaline was abolished but no evidence of vasodilatation was observed to subsequent injections.

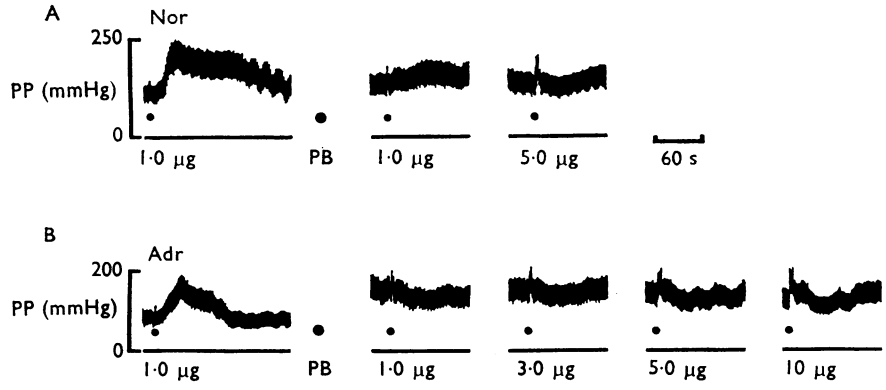

FIG. 5. Effect of phenoxybenzamine on the vascular responses of the human (♂) spleen to noradrenaline and adrenaline. The records A and B show the changes in perfusion pressure (PP) of the human spleen in response to close arterial injections of noradrenaline (Nor) and adrenaline (Adr) before and after a dose of 3 mg of phenoxybenzamine (PB).

The small reductions in spleen volume observed after the injections of catecholamines and sympathetic nerve stimulation were always abolished by phenoxybenzamine.

In three experiments, after the administration of phenoxybenzamine in a dose sufficient to abolish the responses to sympathetic nerve stimulation and injected catecholamines, the venous effluent was collected during and following nerve stimulation at 10 or 30 Hz in order to detect and measure any transmitter released. In two of these experiments little activity could be detected when the effluent was assayed for pressor substances on the blood pressure of the pithed rat. In the other experiment an increase in pressor activity equivalent to 435 pg per stimulus of noradrenaline was detected in the stimulation samples over the control level. A positive identification of noradrenaline was made fluorimetrically.

Actions of isoprenaline on human splenic smooth muscle ; presence of β-adrenoceptors

In two experiments isoprenaline was administered on twelve occasions in doses of 0·5–1·0 μg and vasodilatation was clearly elicited on nine of these occasions since

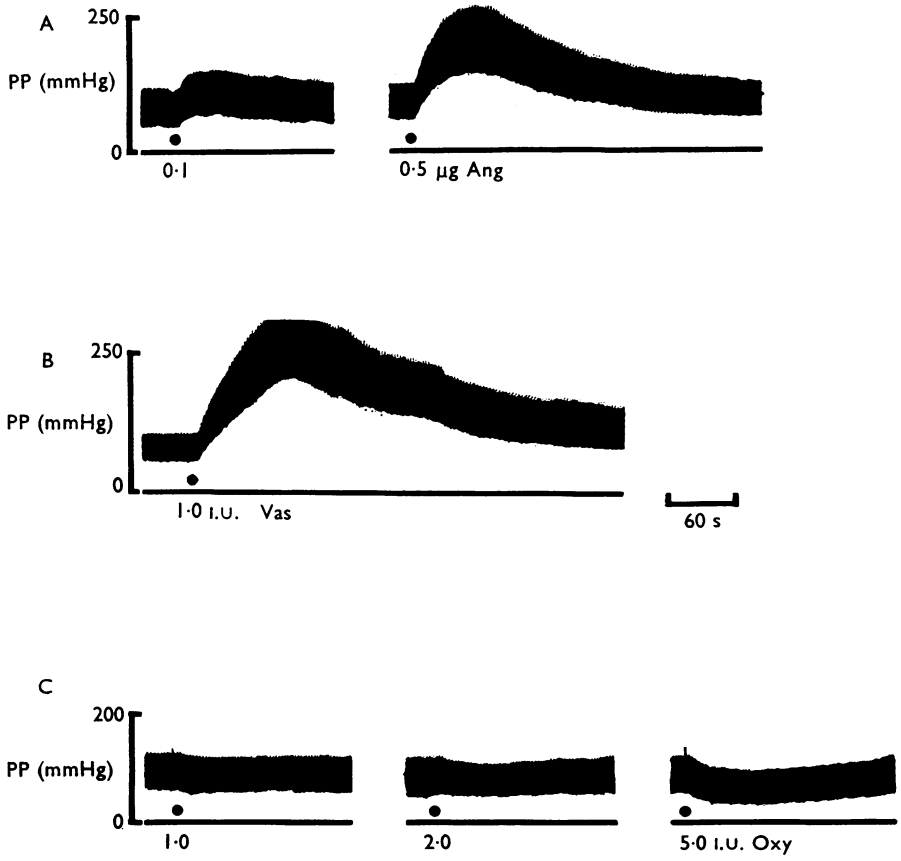

FIG. 6. Vascular responses of the human (♂) spleen to close arterial injections of polypeptides. Changes in perfusion pressure (PP) caused by the injection of 0·1 and 0·5 μg angiotensin (Ang) in A, 1·0 I.U. of vasopressin (Vas), in B and in C the close arterial injections of 1·0, 2·0 and 5·0 I.U. of oxytocin (Oxy).

a reduction in splenic perfusion pressure was observed at constant arterial inflow. These responses together with the reversal in vascular response to adrenaline after phenoxybenzamine suggest the presence of some β-adrenoceptors in the human splenic vascular bed. No changes in volume of the human spleen were observed with isoprenaline.

Actions of acetylcholine on the human spleen

Small doses of acetylcholine reduce splenic vascular resistance in many species including the dog (Daly & Scott, 1961 ; Davies & Withrington, 1968). In three experiments in the present series acetylcholine, in doses of 1–25 μg, caused small decreases in perfusion pressure and therefore splenic vascular resistance. No volume recordings were possible in these experiments.

Actions of some polypeptides on human splenic vascular and capsular smooth muscle

Three polypeptides were investigated ; these were angiotensin, oxytocin and vasopressin and their vascular actions are illustrated in Fig. 6.

Angiotensin

In six experiments angiotensin was injected on twenty-one occasions in doses from 0·05–25 μg and graded increases in splenic perfusion pressure were obtained. The results indicated that angiotensin was more potent than noradrenaline in its actions on the splenic vascular bed usually in the ratio of about 2:1 on a weight basis. For example, in one human spleen, angiotensin (0·5 μg) caused an increase in splenic vascular resistance of 74% whilst the same dose of noradrenaline increased the vascular resistance by 41%.

In two experiments the vascular action of angiotensin was not changed by the administration of a dose of phenoxybenzamine which abolished the responses to catecholamines and sympathetic nerve stimulation. These results clearly indicate the direct action of angiotensin on human splenic vascular smooth muscle.

Negligible changes in spleen volume accompanied the increases in splenic perfusion pressure following the injection of angiotensin.

Vasopressin

In six experiments vasopressin (0·5–2·0 I.U.) was injected intra-arterially and caused marked increases in splenic perfusion pressure with no consistent changes in volume. The responses to vasopressin were unaffected by the administration of phenoxybenzamine.

Oxytocin

In six experiments oxytocin (0·5–5·0 I.U.) induced a slight reduction in splenic perfusion pressure which, at constant flow, indicates a reduction of splenic vascular resistance and vasodilatation. This effect was almost exactly mimicked by the administration of the vehicle, chlorobutanol, diluted by an equal amount. It appears therefore that oxytocin has little or no action on the smooth muscle of the human spleen, in marked contrast to angiotensin and vasopressin. The administration of oxytocin or vehicle produced no changes in spleen volume.

Discussion

Investigations on the cardiovascular function of the human spleen have been considerable but indirect. Interest has focussed on the possible role of the spleen as a store of erythrocytes. This suggestion was initially proposed by Stukeley (1723) as a result of his extensive study of the structure of the spleen during which he became impressed by the amount of muscle the organ contained. Barcroft, Harris, Orahovats & Weiss (1925) clearly demonstrated that the spleen of the dog and cat contained a store of red cells which were added to the circulation during exercise or haemorrhage. They calculated that, in these species, splenic contraction increased the circulating blood volume by 10–15%. Further support for this view came from the observations that the increase in red cell count and haemoglobin content of the blood which occurred as a result of fright and exercise, was absent in splenectomized animals (Scheunert & Krzywanek, 1926 ; Abderholden & Roske, 1927 ; Izquierdo & Cannon, 1928).

Inevitably a similar storage function was postulated for the human spleen and Barcroft *et al.* (1925) calculated that the human spleen should be capable of adding 110–258 ml of blood to the circulation. However, whereas it was well established that the dog's spleen was capable of powerful contractions, there was uncertainty over the ability of the human spleen to contract vigorously. Henle (1852) had access to a decapitated human and electrically stimulated the splenic nerve both *in situ* and after removal, 35 min after death. He observed no effect but noted that the spleen which was originally pale and wrinkled became darker and smoother after removal from the body. This, he suggested, showed that the spleen had contracted. Evidence soon accumulated that although some increase in the red cell count and haemoglobin occurred in man, in response to adrenaline and exercise, it was not of the same magnitude as that observed in the dog and cat (Krzywanek & Arnold, 1927 ; Nylin, 1942). Moreover, the small increases that did occur in exercise, haemorrhage and in response to injections of adrenaline were observed in both normal and splenectomized subjects (Dill, Talbot & Edwards, 1930 ; Ebert & Stead, 1941). In contrast however, Yang (1928) observed an increase in the red cell count following the injection of adrenaline in the normal but not in the splenectomized subject, whilst Benhamou *et al.* (1929a, b) by studies with X-ray and red cell counts in normal adult and child subjects, reported contractions of the spleen in response to exercise and the administration of adrenaline.

In the experiments reported here in which changes in spleen volume were continuously monitored, stimulation of the postganglionic splenic nerves at frequencies of up to 30 Hz produced small contractions of the splenic capsule compared with the dog, although increases in splenic vascular resistance were marked. Similarly, negligible changes in the volume of the perfused human spleen could be elicited by close arterial injections of either adrenaline or noradrenaline, both of which are present in the human adrenal gland (von Euler, Franksson & Hellstrom, 1954) and appear in the plasma during stress (Vendsalu, 1960). Nevertheless, both these catecholamines induce large increases in splenic vascular resistance under the same experimental conditions. The present results therefore confirm the general conclusion, previously based on indirect evidence, that in man the normal spleen does not act as a blood reservoir for acute emergencies.

The changes in vascular resistance of the human spleen to sympathetic nerve stimulation were often very striking. The threshold frequency was 2–3 Hz and the

maximum responses were evoked, as in the dog, at 7–10 Hz. We have no knowledge about the postganglionic discharge frequency in man but it seems reasonable to assume that appreciable increases in splenic vascular resistance would only occur under conditions of extreme stress. Our present experiments would indicate therefore that, in the human, the sympathetic nervous supply is a low frequency system.

While there is little evidence to substantiate the reservoir function of the normal human spleen there is a clearer indication that the pathologically enlarged human spleen may actively contract. Yang (1928) reported a reduction in size, detectable by palpation, after an injection of adrenaline. Saad (1935) investigating the action of drugs on strips of enlarged human spleens, observed contraction in response to adrenaline, which was blocked by ergotamine. Watson (1939) observed a 50% reduction in estimated volume, by X-ray, in response to adrenaline in a patient previously given thorotrast whilst Watson & Paine (1943) observed, during the operation of splenectomy, that close-arterial injection of adrenaline caused a visible contraction and an increase in the red cell count of the splenic venous blood. The experiments described in the present paper were, with one exception, performed on normal human spleens. It may be significant that in the exception, a case of hereditary spherocytosis, the spleen gave some of the largest contractions that we recorded in the series.

The vascular responses of the human spleen to sympathetic nerve stimulation, adrenaline and noradrenaline were profoundly altered by the administration of the α-adrenoceptor blocking drug phenoxybenzamine. The vascular responses to nerve stimulation and noradrenaline were either abolished or significantly reduced. However, in some of the spleens the vascular response to adrenaline was reversed after phenoxybenzamine to cause vasodilatation. These observations present direct evidence of both α- and β-adrenoceptors in the splenic vascular bed as has been previously observed in the dog (Moerman, Scapagnini & de Schaepdryver, 1969; Davies, Robinson & Withrington, 1969). The presence of β-adrenoceptors in the vasculature of the human spleen is further indicated by the vasodilator activity of isoprenaline. Unfortunately, there was no opportunity to administer any β-adrenoceptor blocking agents in the present series of experiments. As we have stated the capsular smooth muscle of the human spleen responds with very small contractions to sympathetic nerve stimulation, adrenaline and noradrenaline. Nevertheless these small contractions were blocked by phenoxybenzamine suggesting a sparse α-adrenoceptor distribution.

The results of the actions of the polypeptides angiotensin, vasopressin and oxytocin in the human spleen provide information suggesting a considerable species variation. In the human spleen both angiotensin and vasopressin have a powerful vasoconstrictor action with little effect upon the capsular smooth muscle. These effects are similar to those obtained in the dog (B. N. Davies & P. G. Withrington, unpublished observations) and in the cat's spleen (Greenway & Stark, 1970). However, in the human spleen oxytocin induced a slight vasodilatation whereas in the dog's spleen it has the same actions as angiotensin and vasopressin. In the human spleen the vasodilatation elicited by oxytocin was largely due to the preservative chlorobutanol used in the preparation of the synthetic form Syntocin. It is probable that oxytocin has little effect on the vascular smooth muscle of the human spleen although preservative-free oxytocin has been reported to have a mild hypotensive action in man (Somlyo & Somlyo, 1970).

Our results on the transmitter release from the sympathetic innervation to the human spleen are preliminary and limited. The supply of intact, normal spleens with a well defined nerve bundle was very small and in these specimens we were reluctant to block the responses at an early stage with phenoxybenzamine so as to collect and assay transmitter by preventing its reuptake. The few experiments we describe are, we consider, worth reporting since they clearly indicate that the transmitter at the postganglionic nerve endings in the human spleen is noradrenaline.

We wish to thank the surgical staff of the many hospitals who kindly cooperated and supplied us with human spleens and Miss Susan Rigby for technical assistance. We are indebted to Mr. D. A. Powis who carried out the fluorimetric estimations of the catecholamines using a Locarte Fluorimeter which was purchased with a grant from the Wellcome Trust. The work was supported in part by a grant from the Medical Research Council.

REFERENCES

ABDERHOLDEN, E. & ROSKE, G. (1927). Die Bedentung der Milz für Blutmenge und Blutzusammensetzung. *Pflüg. Arch. ges. Physiol.*, **216**, 308–321.

ALLISON, D. J. & POWIS, D. A. (1971). Adrenal catecholamine secretion during stimulation of the nasal mucous membrane in the rabbit. *J. Physiol., Lond.*, **217**, 327–339.

AYERS, A. B., DAVIES, B. N. & WITHRINGTON, P. G. (1970). The responses of the isolated perfused spleen of man to sympathetic nerve stimulation, catecholamines and polypeptides. *Br. J. Pharmac.*, **40**, 575*P*.

BARCROFT, J., HARRIS, H. A., ORAHOVATS, D. & WEISS, R. (1925). A contribution to the physiology of the spleen. *J. Physiol., Lond.*, **60**, 443–456.

BENHAMOU, JUDE & MARCHIONI (1929a). La splenocontraction a l'effort chez l'homme normal. *C.r. Soc. Biol., Paris*, **100**, 456–458.

BENHAMOU, JUDE & MARCHIONI (1929b). La splenocontraction a l'adrenaline chez l'homme normal. *C.r. Soc. Biol., Paris*, **100**, 458–460.

BERTLER, A., CARLSSON, A. & ROSENGREN, E. (1958). A method for the fluorimetric determination of adrenaline and noradrenaline in tissues. *Act physiol. scand.*, **44**, 273–292.

BOWDLER, A. J. (1962). Theoretical considerations concerning measurement of the splenic red cell pool. *Clin. Sci.*, **23**, 181–195.

DALY, M. DE B. & SCOTT, M. J. (1961). The effects of acetylcholine on the volume and vascular resistance of the dog's spleen. *J. Physiol., Lond.*, **156**, 246–259.

DAVIES, B. N., GAMBLE, J. & WITHRINGTON, P. G. (1968a). The separation of the vascular and capsular smooth muscle responses to sympathetic nerve stimulation in the dog's spleen. *J. Physiol., Lond.*, **196**, 42–43*P*.

DAVIES, B. N., GAMBLE, J. & WITHRINGTON, P. G. (1968b). Effects of noradrenaline, adrenaline and angiotensin on vascular and capsular smooth muscle of the spleen of the dog. *Br. J. Pharmac. Chemother.*, **32**, 424*P*.

DAVIES, B. N., ROBINSON, B. H. & WITHRINGTON, P. G. (1969). The effects of graded doses of phenoxybenzamine on the vascular and capsular responses of the isolated blood-perfused dogs spleen to sympathetic nerve stimulation and catecholamines. *Archs int. Pharmacodyn. Thèr.*, **180**, 143–154.

DAVIES, B. N. & WITHRINGTON, P. G. (1968). The effects of prostaglandin E_1 and E_2 on the smooth muscle of the dog spleen and on its responses to catecholamines, angiotensin and nerve stimulation. *Br. J. Pharmac. Chemother.*, **33**, 136–144.

DILL, D. B., TALBOT, J. H. & EDWARDS, H. T. (1930). Studies in muscular activity. VI. Response of several individuals to a fixed task. *J. Physiol., Lond.*, **69**, 267–305.

EBERT, R. V. & STEAD, E. A. (1941). Demonstration that in normal Man, no reserves of blood are mobilized by exercise, epinephrine or haemorrhage. *Am. J. med. Sci.*, **201**, 655–664.

EULER, U. S. VON, FRANKSSON, C. & HELLSTROM, J. (1954). Adrenaline and noradrenaline content of surgically removed human suprarenal glands. *Acta physiol. scand.*, **31**, 6–8.

GREEN, H. D., OTTIS, K. & KITCHEN, T. (1960). Autonomic stimulation and blockade on canine splenic inflow, outflow and weight. *Am. J. Physiol.*, **198**, 424–428.

GREENWAY, C. V., LAWSON, A. E. & STARK, R. D. (1968). Vascular responses of the spleen to nerve stimulation during normal and reduced blood flow. *J. Physiol., Lond.*, **194**, 421–433.

GREENWAY, C. V. & STARK, R. D. (1969). Vascular responses of the spleen to rapid haemorrhages in the anaesthetized cat. *J. Physiol., Lond.*, **204**, 169–179.

GREENWAY, C. V. & STARK, R. D. (1970). The vascular responses of the spleen to intravenous infusions of catecholamines, angiotensin and vasopressin in the anaesthetized cat. *Br. J. Pharmac.*, **38**, 583–592.

HÄGGENDAL, J. (1963). An improved method for fluorimetric determination of small amounts of adrenaline and noradrenaline in plasma and tissues. *Acta physiol. scand.*, **59**, 242–254.

HARRIS, I. M., MCALISTER, J. M. & PRANKERD, T. A. J. (1957). The relationship of abnormal red cells to the normal spleen. *Clin. Sci.*, **16**, 223–230.

HARRIS, I. M., MCALISTER, J. M. & PRANKERD, T. A. J. (1958). Splenomegaly and the circulating red cell. *Br. J. Haemat.*, **4**, 97–102.

HENLE, J. (1852). Versuche und Beobachtungen an einen Enthaupteten. *Ztschr. f. Rat. Med.*, **2**, 299–312.

HERTTING, G. & SUKO, J. (1966). Influence of angiotensin, vasopressin or changes in flow rate on vasoconstriction, changes in volume and (^{3}H)-noradrenaline release following postganglionic sympathetic nerve stimulation in the isolated cat spleen. *Br. J. Pharmac. Chemother.*, **26**, 686–696.

IZQUIERDO, J. J. & CANNON, W. B. (1928). Emotional polycythaemia in relation to sympathetic and medulliadrenal action on the spleen. *Am. J. Physiol.*, **84**, 545–562.

KRZYWANEK, W. & ARNOLD, A. (1927). Der Anteil der roten Blutkörperchen im menschlichen Blut bei Ruhe und Bewegung. *Pflüg. Arch. ges. Physiol.*, **216**, 640–643.

MCEWEN, L. M. (1956). The effect on the isolated rabbit heart of vagal stimulation and its modification by cocaine, hexamethonium and ouabain. *J. Physiol., Lond.*, **131**, 678–689.

MOERMAN, E. J., SCAPAGNINI, U. & DE SCHAEPDRYVER, A. F. (1969). Adrenergic receptors in the isolated perfused dog spleen. *Eur. J. Pharmac.*, **5**, 279–285.

NYLIN, G. (1947). The effect of heavy muscular work on the volume of circulating red corpuscles in Man. *Am. J. Physiol.*, **148**, 180–184.

PRANKERD, T. A. J. (1963). The spleen and anaemia. *Br. med. J.*, **2**, 517–524.

RICHARDS, J. D. M. & TOGHILL, P. J. (1967). The distribution of erythrocytes in the human spleen in health and disease. *J. path. Bact.*, **93**, 653–660.

SAAD, K. (1935). The effect of drugs on the isolated splenic capsule of Man and other animals. *Q. J. Pharm.*, **8**, 31–38.

SCHEUNERT, A. & KRZYWANEK, W. (1926). Über die Beziehungen der Milz zu den Schwankungen der Menge der roten Blutkörperchen. *Pflüg. Arch. ges Physiol.*, **215**, 187–190.

SOMLYO, A. P. & SOMLYO, A. V. (1970). Vascular smooth muscle. II. Pharmacology of normal and hypertensive vessels. *Pharm. Rev.*, **22**, 249–353.

STUKELEY, W. (1723). *On the Spleen, its Description and History, Uses and Diseases, Particularly the Vapours, With their Remedy*. London.

TOGHILL, P. J. (1964). Red cell pooling in enlarged spleens. *Br. J. Haemat.*, **10**, 347–357.

VENDSALU, A. (1960). Studies on adrenaline and noradrenaline in human plasma. *Acta physiol. scand.*, **49**, Suppl. 173, 1–123.

WATSON, C. J. (1939). Haemolytic jaundice and macrocytic haemolytic anaemia: certain observations in a series of 35 cases. *Ann. Int. Med.*, **12**, 1782–1796.

WATSON, C. J. & PAINE, J. R. (1943). A study of the splenic venous blood with particular reference to the haematocrit percentage and the haemoglobin concentration of the erythrocytes before and after splenic arterial injection of adrenaline. *Am. J. med. Sci.*, **205**, 493–501.

YANG, C-S. (1928). The influence of adrenalin on the human spleen and blood picture. *J. chin. Physiol.*, **2**, 163–172.

(*Received July* 26, 1971)